Successful production of black tiger shrimp in Australia with new mode of delivery of probiotics

Moving away from delivery via feed to manage bacterial pathogens

By Manuel Poulain, Olivier Decamp, Brad Callcott and Justin Holgate

Shrimp production in Australia is estimated at 5,000 tonnes during 2017-2018, according to official statistics. In Australia, as in all shrimp farming countries, the management of bacterial pathogens is critical. In 2015-2016, outbreaks of acute hepatopancreatic necrosis disease (AHPND) caused by strains of *Vibrio harveyi*, and white spot syndrome virus (WSSV) were recorded in Queensland. Epidemiology studies are ongoing but it has been suggested that WSSV entered the local environment via imported shrimp that had been used as fishing bait. The arrival of these devastating diseases in Australia has prompted farmers to re-examine and improve biosecurity procedures to better protect against further incursions of these diseases. It is well known that proliferation of *Vibrio* species in the farming environment and in the shrimp themselves is a major catalyst for mass mortality associated with these diseases. If we can control *Vibrio* abundance we can reduce the incidence and severity of these diseases.

A well-practised method of *Vibrio* control is via targeted microbial management both in the pond water and soil and in the shrimp themselves. INVE Aquaculture has been working for over 15 years on microbial management in shrimp grow-out, through a combination of biosecurity, quality nutrition, health boosters and probiotics. The approach focuses on the effective management of microorganisms and requires a combination of water treatment and feed treatment with high concentration probiotic mixtures.

Water treatment with probiotics helps to maintain a stable algal bloom, prevents the accumulation of organic waste, and the associated development of anaerobic zones, as well as the release of toxic molecules in the rearing water. Probiotics help to maintain optimal conditions for shrimp culture (Kumar et al., 2016) and make conditions less favourable for opportunistic *Vibrio* growth.

The management of *Vibrio*, especially those associated with shrimp gut colonisation (such as *Vibrio* strains associated with AHPND) is more complex and requires the displacement of *Vibrio* by probiotic strains that are delivered via the feed. The ability of Sanolife Probiotic (INVE) to displace *Vibrio* has been clearly demonstrated in mysis and post larvae (Silva et al, 2013) and juveniles. The reduced abundance of *Vibrio* and increased abundance of *Bacillus* inside shrimp was thus confirmed (Figure 1) (Hostins et al., 2017). Repeated tests in Vietnam confirmed the improved survival of shrimp when challenged with AHPND (Oanh et al., 2015). An additional benefit of these probiotics is their production of enzymes, such as protease, that improve the use of feed, and consequently, improving the growth rate of shrimp (Liu et al., 2009; De Souza et al., 2012).

New mode of delivery

Coating probiotics at the farm is labour intensive and prone to human error. As a consequence, a new product was developed, Sanolife PRO-TAB, for the easy delivery of feed probiotics. Here strains of *Bacillus* are coated on slow-sinking wafers (5mm diameter and 1mm thickness) at a final concentration of $2x10^9$CFU/g. These *Bacillus* strains were selected for their ability



to be metabolically active in a wide range of conditions (salinity, pH, dissolved oxygen). They can inhibit *Vibrio* strains such as those involved in AHPND, and produce enzymes that help the intake of nutrients. The high quality attractants in the wafers make them very attractive to shrimp (Figure 2) and this leads to the fast intake of the *Bacillus* probiotic strains. The benefit is the increasing presence of *Bacillus* in the shrimp gut and the reduced number of *Vibrio*. By displacing *Vibrio* from the shrimp gut, this product reduces the risk of disease associated with *Vibrio*, such as white faeces syndrome and AHPND. In previous field trials in Thailand and India, these wafers were also shown to significantly improve feed conversion ratio (FCR), growth rates and survival.

Farm trials

It was decided to further evaluate the benefit of this product on FCR, growth rate and survival at the Pacific Reef Fisheries Pty. Ltd (PRF). shrimp grow-out farm in Queensland, Australia. This company has a hatchery, a farm and a processing plant. PRF has been successfully producing black tiger shrimp *Penaeus monodon* for decades, with an annual production of 1,000 tonnes. The trial was carried out in seven treatment ponds using Sanolife PRO-TAB and seven control ponds, starting in late November 2016. The average pond size was 8,500m^2. The total area stocked for this trial represents about 12 ha, with about 8.5 million post larvae stocked. As shown in table 1, in the treatment ponds, the wafers were applied as a percentage of the feed, from 20% (days of culture -DOC 0-14) to 10% (DOC 15-28), 3% (DOC 29-56) and 1% (DOC 57-70). Details are given in Table 1.

Throughout the culture period, partial harvests were carried out from both treatment and control ponds. After an average of 155 days of culture, shrimp were harvested from all 14 ponds. Although this made the detailed analysis challenging, the high number of replicates allowed data to be summarised in Table 2.

Improvements in production

The application of the probiotics as wafers in the first 70 days of culture led to the following improvements: survival increased by 21%; productivity increased by 19% and FCR reduced by 6%.

Although partial harvests make it difficult to analyse difference in growth between treatment and control ponds, an option was to compare the performance of the ponds before the first partial harvest that took place on DOC (Figure 2). It clearly showed that after DOC 50, the growth curves of shrimp in treatment ponds was increasingly steeper than that of shrimp from control ponds, with a difference in body weight reaching 9% after 107 days of culture. An additional point to consider is the timing of the partial harvest. The faster growth in

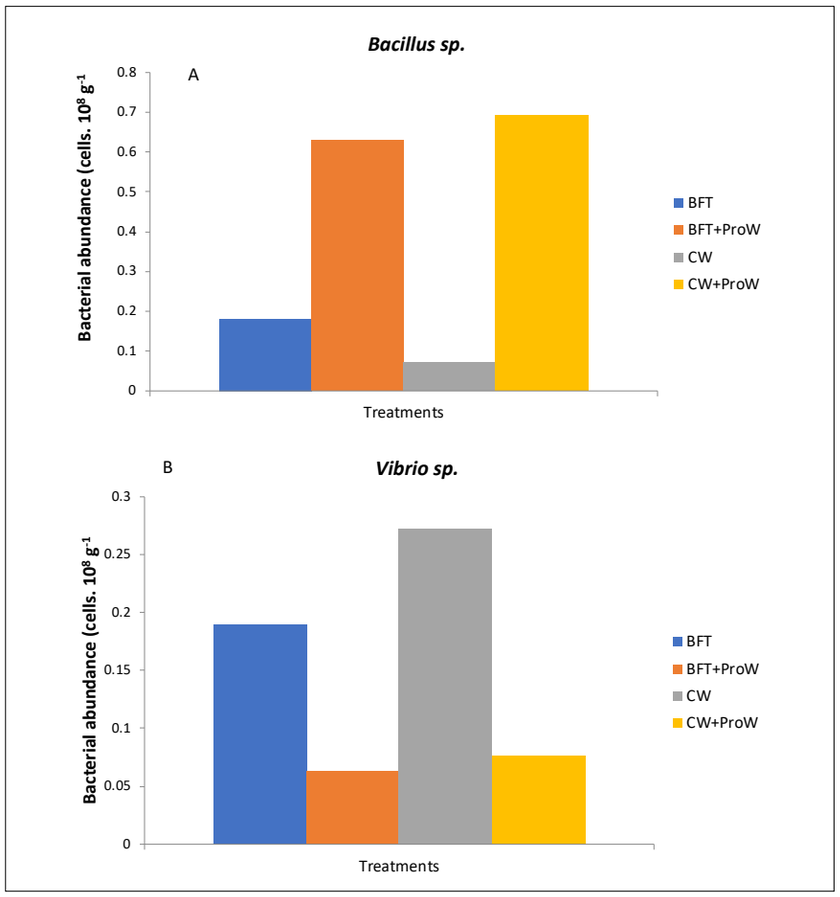


Figure 1. Specific bacterial abundance ± standard deviation (cell 10^8/g) of *Bacillus* (A) and *Vibrio* (B) in *Litopenaeus vannamei* gut in the BFT, BFT+ProW, CW and CW ProW treatments.



Early juveniles "nibbling" on Sanolife PRO-TAB, and ingesting the Sanolife Bacillus strains.

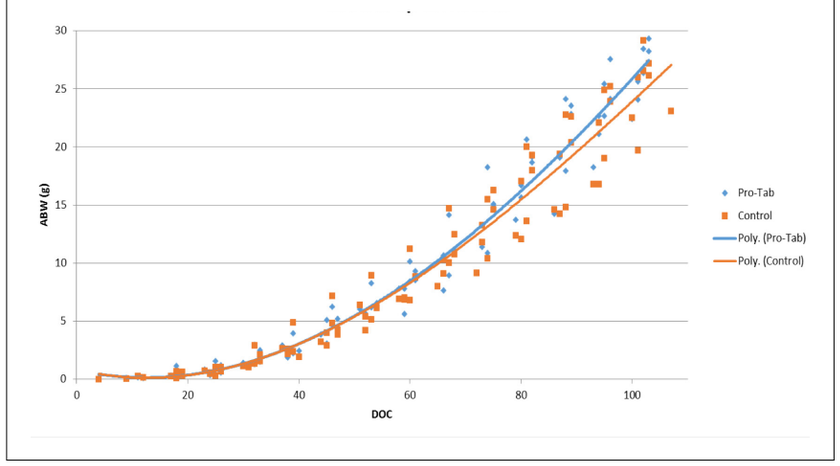


Figure 2. Growth curve of shrimp reared in treatment vs control ponds, during the first 107 days of culture. A trend line of polynomial order 3 was selected, with a R^2 value of 0.94.

the treatment ponds led to more frequent partial harvest in these ponds (28 versus 24 for control ponds). Also, the first partial harvest in treatment ponds occurred after DOC 108 whereas the first partial harvest in control ponds took place after DOC 117, 12 days later. As a consequence, biomass was harvested at earlier dates from treatment ponds than control ponds. For example, after 21 weeks of culture 48% of the total biomass from the Sanolife PRO-TAB ponds were harvested with partial harvest, compared to 38% for the control. Final harvest took place in week 22 and 23.

The combination of faster growth (as seen by the earlier partial harvest), increased survival (as estimated at the final harvest) and improved FCR led to a higher profitability for the farmer: more harvested biomass produced by relying on reduced quantity of feed (115 kg less feed per tonne of shrimp produced).

This product fits into the holistic program being presented to farmers around the world, together with products for biosecurity (Sanocare PUR*), health boosters (Sano S-PAK and Sano TOP-S) and water probiotics (Sanolife PRO-W).

DOC	Shrimp biomass (kg/ha)	PRO-TAB (kg/ha/day)
0-7	5.5	0.9
8-14	71	1.7
15-21	379	1.3
22-28	766	1.7
29-35	1,651	2.5
36-42	2,033	2.7
43-49	2,762	3.3
50-56	3,276	3.4
57-63	3,775	1.1
64-70	4,260	1.1

Table 1. Application rate of Sanolife PRO-TAB in treatment ponds

Treatment	Pond ID	Pond area (m^2)	DOC at harvest	Harvest ABW (g)	Total biomass at harvest (kg)	Productivity (tonnes/ha)	FCR
Control	7	8,400	155	37	6,775	8.1	2.22
	10	7,600	155	38	8,590	11.3	1.88
	16	9,200	161	43	7,480	8.1	1.83
	18	9,300	160	37	11,840	12.7	1.76
	19	9,200	154	38	10,230	11.1	1.76
	87	8,500	147	41	6,310	7.4	1.83
	97	9,000	157	34	7,490	8.3	2.11
Average			**156**	**38**	**8,388**	**9.6**	**1.91**
Pro-TAB	6	6,000	149	36	7,140	11.9	1.77
	13	7,700	159	43	7,770	10.1	1.90
	15	8,600	160	36	11,900	13.8	1.69
	17	9,500	160	34	16,240	17.1	1.63
	20	9,000	156	42	7,560	8.4	1.85
	86	8,000	148	41	8,850	11.1	1.92
	98	9,500	152	34	7,200	7.6	1.83
Average			**155**	**38**	**9,523**	**11.4**	**1.80**

Table 2. Summary of results of 7 control ponds and 7 treatment ponds





Manuel Poulain is Technical Support Manager and **Olivier Decamp**, PhD is Segment Director, Farm, at INVE AQUACULTURE, Thailand.

Brad Callcott is Operations Manager at Pacific Reef Fisheries, Australia.

Justin Holgate is Manager, Primo Aquaculture-Ridley, Australia.

Email: m.poulain@inveaquaculture.com (Manuel Poulain)
(Note: * Sanocare PUR*is not available in Australia).