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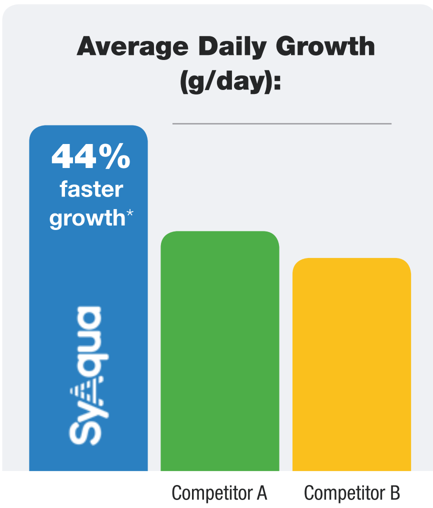

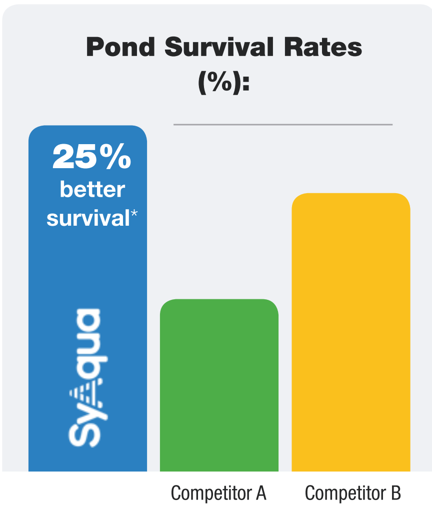

* Based on actual data from 2016 from 606 ponds in Southeast Asia.

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Zuridah Merican

Can the feed segment lead the aquaculture industry again?

Having just completed The Aquaculture Roundtable Series (TARS 2019) where we focused on developing a Fit for Future aquafeed industry in Asia, it is timely that we take the opportunity to honour the feed segment. The feed milling segment is often regarded as a support service for supplying the correct formulated compound feed to ensure optimal growth of the species, but they have been more than that. If we rewind to the 1970s, it was President Enterprise Corporation, Taiwan that produced shrimp feed and promoted monodon shrimp farming in Taiwan and Southeast Asia. In the 1980s, Charoen Pokphand Feeds and San Miguel Foods did the same to supply feed and promote commercial shrimp aquaculture in Thailand and Philippines, respectively. Amongst others, these were the godfathers - companies that brought aquaculture to Asia and catalysed the industry.

What leadership roles did they take? First and foremost, they were more international and able to bring new technology prior to the age of the internet. Most farms were small and focused on day-to-day operations and they were fragmented. There was little exchange of information between farms or learning from mistakes. Feed companies provided a platform for local seminars and farmers to get together. Obviously, these actions were not altruistic as they created markets for their feed and products associated with improving culture conditions.

It is a common business model for farms to spend 80% of their investment on capital expenditure heavily associated with pond infrastructure. With only 20% left for operational expenses, any assistance is valued. As 50% of production costs is attributed to feed, feed companies would extend credit to sustain culture operations until harvest when farmers would get paid for their produce. This credit spurred the industry and the risk burden was shared between the farmer and the feed supplier. Feed companies also knew that good seedstock had to accompany feed for a successful agribusiness and hence, they invested in hatcheries to ensure fry was not a limiting factor in the supply chain, and later added genetics and breeding into the chain.

There seemed to be an unwritten business contract and relationship, and the feed companies played a supporting role, as well as catalysing the growth of the industry with a push and pull effect. These feed companies benefitted as feed sales are intrinsically dependent on the growth of the industry, and if the customer base grows, feed companies grow too.

Enter the 21st century with the rise of the internet and start-up companies – how can feed companies continue to play a similar role and offer farmers a service they cannot access easily?

The aquaculture industry has changed significantly over the past 10 years. Sustainability has overtaken quality as the priority. Not that quality is unimportant but everyone today can produce quality products, so much so that, that it has become a norm and the expectation, instead of a premium. Asia today is focusing on the same feed criteria that Northern Europe did 30 years ago. There is a need for high performance – low environmental impact feed. The past decade has also seen the emergence of new diseases in the shrimp sector while the freshwater and marine fish sector continue to be challenged by existing diseases.

Feed companies have rightly focused on preventive solutions as they tend to be more efficient. Furthermore, fish do not consume feed once infected and hence, mode of delivery of a therapy poses a challenge. However, these functional feeds do not seem to be gaining ground. It is often compared with insurance that requires upfront payment whether needed or not. Instead, shrimp farmers are prone to administering their own 'treatments' at the pond side. One must question why there is this trust deficit between farmers and the feed companies. This situation must be resolved in the near future as Asian aquaculture may be losing ground as a cost leader. Asian shrimp provides the best example where survival rates have drastically been reduced due to disease. As a consequence, cost of production has increased and in the impending high supply scenario, Asian shrimp producers may be the losers.

It is the opinion of Aqua Culture Asia Pacific that feed companies still have a leading role to play.

OUR MISSION

We strive to be the beacon for the regional aquaculture industry.

We will be the window to the world for Asia-Pacific aquaculture producers and a door to the market for international suppliers.

We strive to be the forum for the development of self-regulation in the Industry.

A parabiotic positively impacts shrimp production in laboratory and field trials

Cost benefits justify the use of parabiotic as part of a standard operating procedure, particularly late in the hatchery cycle or just before stocking ponds.

By Stephen Newman

A farm in Indonesia

The most widely farmed shrimp species, *Litopenaeus vannamei*, has found its global niche largely as a result of the availability of specific pathogen free (SPF) shrimp. SPF shrimp are not free of all pathogens nor are they resistant or even necessarily tolerant to the pathogens that they are free of. Nonetheless they are valuable tools in many production environments that can lessen the overall impact of diseases (Newman, 2009). SPF shrimp are, however, only one tool out of many that can help farmers.

Vertebrates produce white blood cells that remember the exposure to a pathogen so that they are able to react quickly should they be exposed to the pathogen again. Shrimp do not have this mechanism. As with almost all living organisms, shrimp also have the ability to produce heat shock proteins (chaperone molecules) in response to stress. These proteins are also involved in how the shrimp deal with the presence of pathogens (Junprunga et al, 2019).

Despite the fact that many consider invertebrates to be phylogenetically primitive, they are far from it. Early workers showed that it was possible to exploit penaeid immune systems (Lewis and Lawrence, 1983). However, we now know that their mechanisms of protection are not solely due to the presence of different classes of lymphocytes (Newman, 2009). They have a sophisticated mechanism for dealing with pathogens (Tassanakajon, 2012). The shrimp immune response is complex and while the subject of a great deal of ongoing research, the exact mechanisms remain to be elucidated. It is non-specific in nature although some aspects of it suggest that there may be some specificity. Shrimp appear to have no memory of prior exposure to pathogens and do not form antibodies.

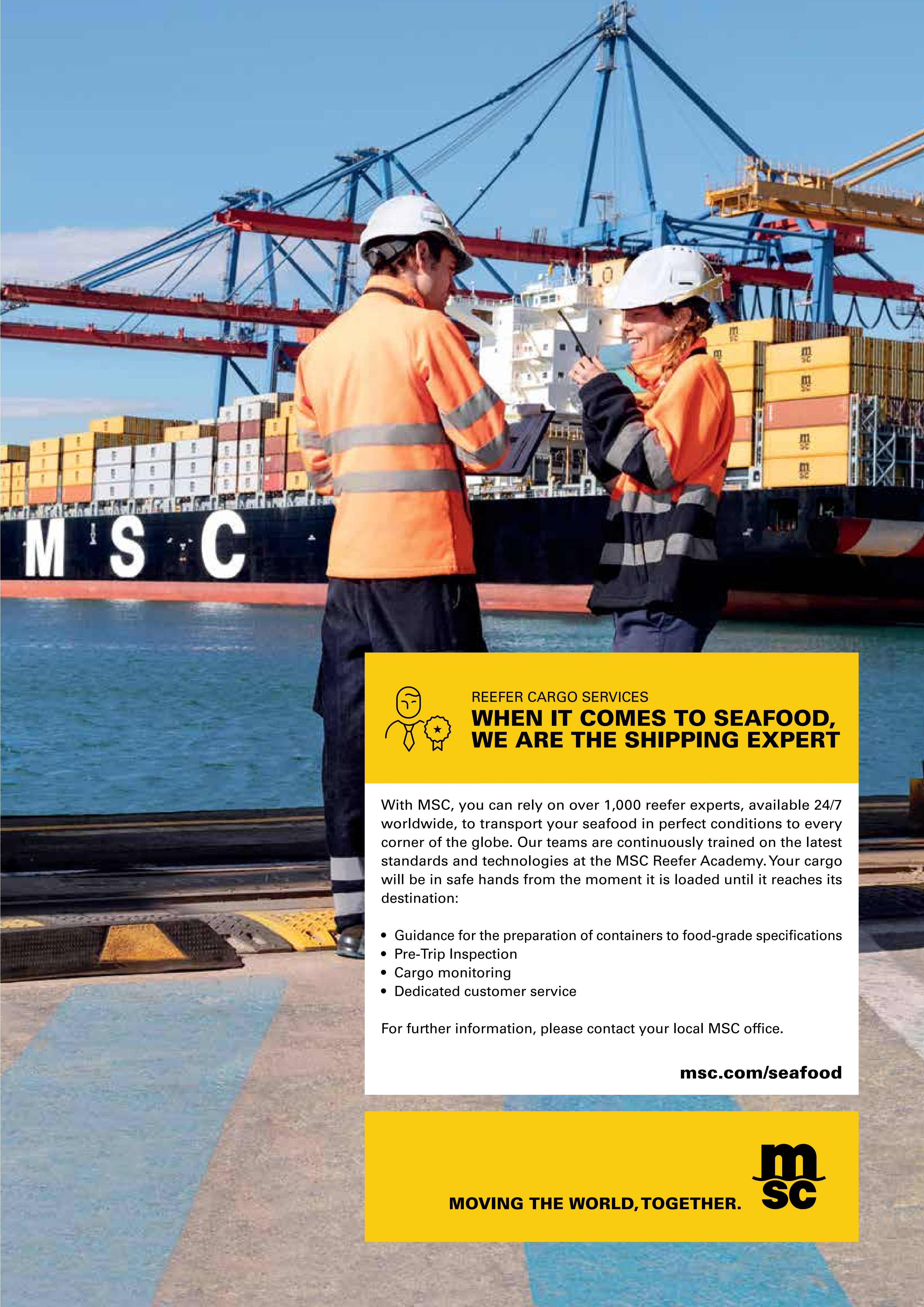
Parabiotic bacteria

Aquaintech Inc (USA) has developed and field tested a parabiotic that clearly benefited shrimp in laboratory trials and large-scale field trials. This parabiotic is a very high density fermentor produced suspension of a proprietary strain of bacteria. The data show a cost benefit and while not all tests were significant at p <0.05, many were.

Extensive laboratory trials were conducted with the parabiotic before it was field tested. The manner in which shrimp are tested can be problematic. Many trials that appear to offer benefits in the laboratory fail to do so in the field. One of the reasons for these failures relates to how shrimp eat (Tacon, 2002). Most products are initially screened for efficacy by direct or indirect addition to feed in aquarium trials.

Shrimp masticate feed before they ingest it. After feed is ingested, it is ground further by the gastric mill before it enters the hepatopancreas and the intestinal tract. The results of aquarium trials can be misleading because of this. As feed is consumed, the shrimp are shrouded in a cloud of particulate materials; a result of grinding any food they ingest to a particle size small enough to pass through the pores in the gastric mill, less than one micron (Pattarayingsakul et al. 2019). This ensures that any material that is tested in an aquarium study is more than likely also being consumed via gill uptake as well as with water that is consumed, etc. Thus, experimental animals may ingest potentially biologically active materials repeatedly. This does not occur in the field where these clouds of particulate materials are rapidly diluted.

The parabiotic is added to post larvae (PL) tanks. PLs are held at high concentrations for the duration of the feeding with supplemental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oxygenation. The parabiotic is fed at a range of dilutions, from 1:500 to 1:5,000, depending on the particular approach used. Typically, water levels in tanks are lowered to facilitate uptake of the concentrated material. High levels of aeration are used to minimise stress and the PLs are held for a minimum of 3 hours. They are then removed, and the process is repeated with naive shrimp.

Survivals post challenge

Laboratory trials have been conducted by many different groups and the results demonstrated that fed shrimp were able to tolerate exposure to both viral and bacterial pathogens at higher levels than naive shrimp.

Figure 1 describes the results of aquarium trials in which PL15 fed the parabiotic at mysis 3 (M3) were challenged with by a waterborne exposure of 10^5 CFU/mL of *Vibrio parahaemolyticus*. Each group consisted of 30 PLs. Experiments with a natural challenge showed a similar impact. The test results showed that under the challenge conditions, the parabiotic fed shrimp were less susceptible to this strain of *Vibrio*. The control groups experienced 60, 50 and 80% survivals with an average of 63%. The parabiotic fed animals experienced 70, 100 and 90% survivals, respectively, with an average of 87%.

Another series of experiments involved exposing parabiotic fed shrimp to tissues containing high levels of the Taura Syndrome Virus-TSV (Figure 2). The results clearly demonstrated that shrimp fed the parabiotic were better able to tolerate exposure to TSV. In replicate studies, 98% of the control shrimp died, whereas in the parabiotic fed groups, one had a 98% survival and the other a 28%. The differences in the results are a reflection of differences in the viral loads in the infective tissues. Other tests confirmed that shrimp fed the parabiotic required much higher exposures to TSV to produce the same level of mortality as in the control group. A similar observation was noted with white spot syndrome virus (WSSV).

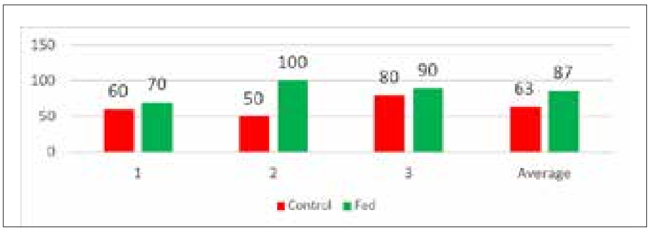

Figure 1. Challenge against *Vibrio parahaemolyticus* (Thailand).

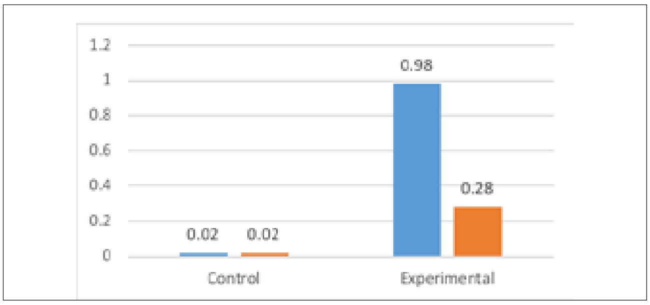

Figure 2. Survival of parabiotic fed shrimp in a TSV challenge.

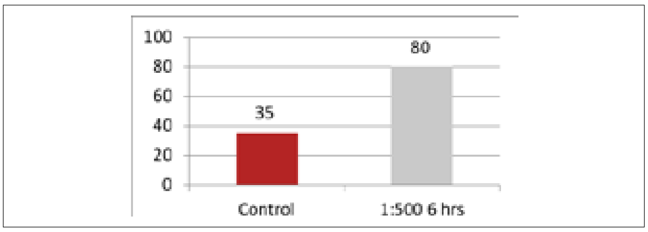

Figure 3. Post exposure survival (%) to EMS/AHPND causing pathogen.

Another test of the parabiotic involved exposing fed PLs to a *V. parahaemolyticus* strain that causes early mortality syndrome/ acute hepatopancreatic necrosis disease-EMS/AHPND (Figure 3). These results were the average of three replicates. There was a clear-cut impact. PLs fed the parabiotic at a 1:500 dilution for 3 hours were largely refractory to infection with the bacteria that causes EMS/AHPND, with 80% of the animals surviving, compared with 35% in control group.

Testing in the field in cages

Figure 4 shows the average survival of shrimp in two cage studies. Four control cages and four experimental cages, each containing 40 PL12 (20/m^2) were stocked into a single pond at two different sites on the same farm (sites A and B). At 56 days, the experiment at site A was terminated. Only 16% of shrimp in the control group were alive compared with 44% of the parabiotic fed shrimp. This 28% difference was a 175% increase in survival. After 59 days, the experiment at site B was terminated, 32% of the control group shrimp were alive compared with 40% of the parabiotic fed shrimp. This 8% difference was a 25% increase in survival.

In another series of experiments (Figure 5), a single cage was placed into each of six ponds. PL12 placed in the cages of three of these ponds were naive controls and three in other ponds were fed the parabiotic prior to stocking. At the end of the experiment, there was a significant difference in survivals with the parabiotic groups consistently outperforming the controls. These tests demonstrated once again that shrimp fed the parabiotic prior to stocking had increased survivals.

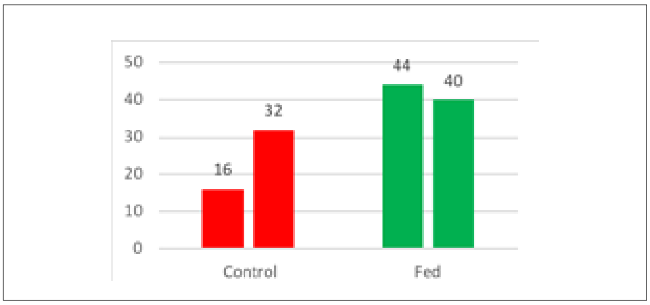

Figure 4. Shrimp survival (%) at termination of study (GMSB-Honduras).

These results highlighted an important observation. There must be something occurring in the shrimp population such that exposure to the biogenic parabiotic had an impact, i.e. there must be a pathogen present that was affecting the shrimp negatively. When survivals in control groups were high, which indicated little or no problems due to the presence of pathogens, it was expected that survivals should be slightly higher or about the same in the fed shrimp. Conversely if the controls had a very high shrimp mortality, which could be indicative of a highly virulent pathogen or the presence of any number of mitigating factors affecting susceptibility, any beneficial impact could be overwhelmed.

The results from extensive experiments in the field corroborate that feeding the parabiotic to PLs is beneficial. Additionally, we observed a wide range of impacts on fed shrimp which were clearly cost-beneficial but were not always related to any overt health issues. The mechanism of action is likely to be complex.

Field trials

This parabiotic has been used on PLs in the field, in shrimp farms in many different countries. For the most part, there were significant cost benefits that justified the use of the product as part of a standard operating procedure.

Table 1 describes the results of one such trial. There were three control and three fed ponds in the trial as described in Table 1. PL12s were stocked at 8/m^2. The cost benefit was significant. For every dollar spent on the use of the parabiotic the farmer saw more than an USD9.00 increase in profit. This was calculated using a

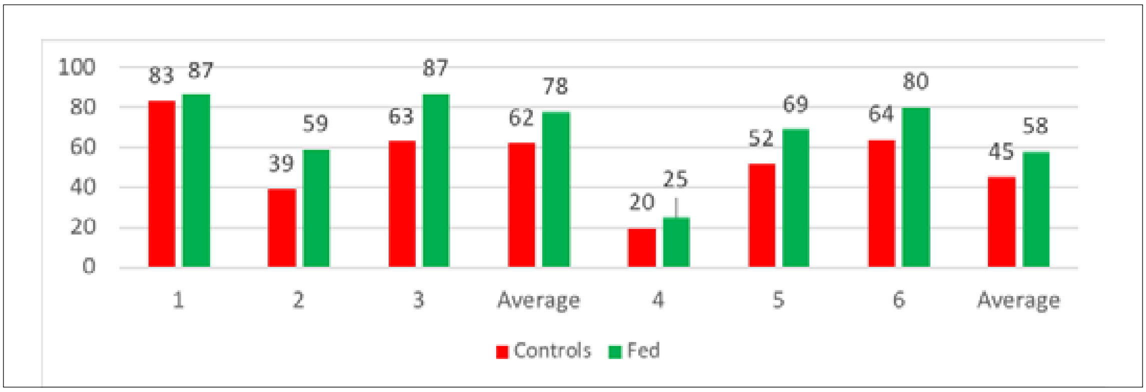

Figure 5. Shrimp survival (%) in field trails in cages (GMSB-Honduras).

	With parabiotic	Control	Difference (%)
Survival (%)	57.6	48.4	19
Weight at harvest (g)	9.2	9.6	-5.2
Yield (kg/ha)	733.6	641.8	14.3

Table 1. Nursery pond trial in Ecuador.

	With parabiotic	Control	Difference	Increase (%)
Survival (%)	30.7	29.04	5.8	1.6
Weight (g)	10.6	10.4	0.2	1.7
Yield (kg/ha)	569.9	530.6	39.3	7.4
FCR	1.89	2.04	0.15	7.35

Table 2. Field trial in production ponds (463 ha, 24 ponds, 83 million PL, Naturisa, Ecuador).

	With parabiotic	Control	Difference	Increase (%)
Survival (%)	57.3	56.7	0.6	1.1
Weight (g)	14.24	13.29	0.95	7.1
Yield (kg/ha)	785.5	731.36	54.1	7.4

Table 3. Field trial in production ponds (181 ha, 18 ponds, 18 million PLs (Naturisa, Ecuador).

computer program that plotted a regression curve based on the costs of all inputs and that predicted the time at which the profit from the harvest was maximised.

Cost benefit through harvest

Results from many field trials showed that there was a cost benefit when the parabiotic was fed late in the hatchery cycle or immediately before stocking the ponds. It also showed a strong benefit in the hatchery (data available). Moreover, the field trials also showed a number of other results as well. No two shrimp farms are the same. The benefits varied. Feed conversion ratios (FCRs) improved in a number of tests. Animals were sometimes larger. Sometimes, whatever was impacting the shrimp did not seem to be affected by consuming the parabiotic. There were trials in which there was no apparent difference between the groups. Usually this was a result of the presence of pathogens accompanied by serious stressors overriding any benefits that can be derived from the parabiotics.

Based on the accumulated data from laboratory, cage and full cycle tests, we postulate that the parabiotic affected the shrimp in a short-term effect. Cage studies and early harvested field trials showed a fairly consistent effect and the laboratory studies demonstrated that the shrimp were stronger in some way. Exposure to the parabiotic appeared to strengthen the PLs, in a manner that is not yet clearly understood. This increase in fitness gave the fed shrimp an advantage under some culture conditions.

Table 2 shows results from a very large field trial in production ponds. Shrimp did poorly in terms of survival rates, although the shrimp in the parabiotic fed group averaged slightly better and their average weight was better at harvest. These small differences resulted in a 7.4% increase in harvest yields between the groups. Even if one assumes that survivals and weights are all basically the same, the 7.35% difference in FCR was significant across 12 ponds.

The results in Table 3 demonstrated that the final average weight of shrimp at harvest was almost a gram greater when they were fed the parabiotic than naive controls. A significant cost benefit was demonstrated.

Cage used in studies

Conclusions

Shrimp farming is constantly plagued by production challenges. It is largely practised by small scale farmers often with little understanding of what is required to be truly sustainable. Failure to consider the role of maturation and hatcheries in the transmission of pathogens combined with a failure to ensure that shrimp are not produced under highly stressful conditions are serious profit limiting issues. Pathogens that can be controlled in some manner, such as lower overall loads in the environment, can impact shrimp that are weakened by these stresses. There is evidence suggesting that by not controlling stress, there is a negative impact on shrimp physiology.

A proprietary parabiotic was tested in the laboratory and in the field in both short term and full cycle trials in several countries in South America and Southeast Asia. The short-term benefits were consistent in terms of improving resistance to a variety of pathogens. Cage experiments and short-term field trials suggested a benefit lasting at least 60 days. Full cycle use showed that there were a variety of possible impacts on the final outcome of the crop. The use of the parabiotic was cost effective and frequently resulted in increased profits from improved feed conversions, better growth and higher survivals.

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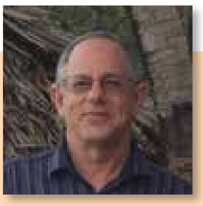

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