

GUEST BLOG- JOHN GUEST'S (AQUATIC ECOLOGIST) MARRON RESEARCH PROGRAM UPDATE

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After more than two years studying and testing many different water bodies I now have strategies and theories that may help marron production and water quality.

My strategies are based on three theories. I have tried to explain my findings as clearly as possible. I have not been in a classroom for nearly thirty years, so please forgive me for any mistakes I have made. I have written this to be used as a discussion paper, please correct and comment on the content.

The reason I am doing this research is because commercial marron farmers cannot get a consistent yield out of multiple ponds/water bodies. The yield difference can be very large (>200%). This is a trend across all farms which have been analysed. The replication of ponds and dams, the hardware (aeration, bird netting, etc), feed (marron pellets) and marron have not eliminated this problem. The production of the marron industry has not increased in the last 15 years and is approximately at 60 tonne per year. I believe one of the main reasons for this is the inconsistent yields, which makes it difficult to estimate income from a marron enterprise.



I have discovered, water bodies/ponds along side of each other which, are feed the same water, have totally different trace element levels and that the different levels of trace elements seems to affect production by effecting the ecology. I believe that a number of factors may be affecting the trace element levels in the ponds, some of which include previous land use, different soil types, different clays/rocks, different nutrient runoff and different types of aeration.

My theories are an attempt to understand the effects the different trace elements levels including carbon has on the ecology of a water body and the effect it has on marron.

Theory 1

Promoting the natural productivity of a water body provides the correct nutrition in the early stages of the life of juvenile marron.

I believe that promoting the natural productivity of a water body will greatly improve a juvenile marron survival and speed of growth to adult size. As with most animals, poor nutrition in the very early stages of life can negatively affect the growth rate and health of the animal as it develops to adulthood.

The promotion of natural productivity is done by first by promoting the microscopic algae called phytoplankton. I believe phytoplanktons are of the most importance in the very early stage of a juvenile's life. The promotion of phytoplankton will then help increase the zooplankton (microscopic animals) which becomes food for the juvenile marron as it grows. Phytoplankton creates oxygen for the water body through photosynthesis. I have found phytoplankton can be promoted by adding micro elements rather than adding phosphorus and nitrogen. Currently there is no feed or systems in place to ensure juveniles receive correct nutrition.

Promote Primary Productivity.

I will use eight micro elements to promote the primary productivity in my marron ponds. Water testing over the last two years of many different water bodies has lead me to come to the conclusion that one or more of these micro elements can be limiting factors in the growth of phytoplankton. There are a number of factors that determine the phytoplankton composition of a water body, one of which is trace elements. A lack of one or more of the elements in the water can also affect other things in the water such as the bacteria. For example, the Nitrobacter which is responsible for the transformation of nitrite to nitrate is heavily dependant on molybdenum.

The total sum of the micro trace elements combined I will add will not add up to more than two parts per million. I am aiming to ensure there is at least the minimum amount of each of the micro elements in the water body. If some of the micro elements I am adding are already present in the water body, adding a small amount it addition to what is already present should do no harm. I will add the trace elements in early spring ready for the new spring growth of phytoplankton. I will do tests during the year to check levels. I expect not to need to add any further trace elements as I think added feed and added humic acid (I will write about later) should maintain sufficient levels in water.



I observed good results from my trials last season in my ponds. A student from Curtin University has started a PhD on the 'Effects of trace elements on natural productivity and the effect in has on the growth performance of Marron'. It is based on my theory and I am sharing my observations with the student.

Theory 2

Lipids and natural oils (triglycerides) are affecting the ecosystem and health of juvenile marron.

My theory is that water bodies with high natural productivity and/or with high feed inputs have the potential for lipids and natural oils to build up to levels under the right conditions, which may have a negative effect on the ecosystem and on the health of juvenile marron.

Lipids and natural oils are naturally produced by the micro/macro algae and added feed pellets decomposing in the water body. Lipids and natural oils can be extracted from micro algae and grains for the production of biodiesel. I believe that if they build up in the water body they can cause health problems and mortalities to juvenile marron due to the lack of development of their digestive system and anatomy compared to adult marron.



The build up of lipids and natural oils may have a negative affect on the some of the important cycles which naturally occur in the water body such as the nitrogen cycle. I suspect this build up is affecting the nitrosomonas and nitrobacter bacteria which are responsible for transforming ammonium into nitrite and then nitrate. If this cycle is interrupted the ammonium will build up in the water and the sediments. The lack of nitrate in the water due to the nitrogen cycle not working correctly encourages Blue Green algae (Cyanobacteria) to become dominate as it can fix nitrogen directly from the atmosphere which enables it to outcompete other life forms. As Cyanobacteria develop it can increase the pH of the water. The increase of pH

by cyanobacteria to levels above 9.6 pH will then cause the ammonium to be converted to ammonia which can become toxic to life forms in the water.

I suspect that the lipids and natural oils do not evaporate in water, which allows them to build up during summer. I believe a build up of lipids and natural oils gradually cause the collapse of an ecosystem.

Bacteria such as Bacillus produce lipase and other enzymes which break down the lipids and natural oils in the water body and also allowing them to supply the marron with a source of energy. This leads into, and will be better explained in my third theory.

Theory 3

Bacteria affect a marrons energy metabolism.

My theory is that marron are often less active in cold water (<15 degrees Celsius) due to the bacteria such as Bacillus in their digestive system being less active due to the colder temperatures as marron are ectothermic (their body temperature is regulated by the surrounding environment). This reduces the ability of these bacteria to convert lipids and natural oils into energy which is needed to utilize nutrients from their food.

From my studies, I have found that most Bacillus have an operating temperature range of between 15 to 40 degrees Celsius. Lower than 15 degrees Celsius they become dormant in many cases. There are certain strains which are now being sold by some companies which will still be active in water less than 11 degrees Celsius.

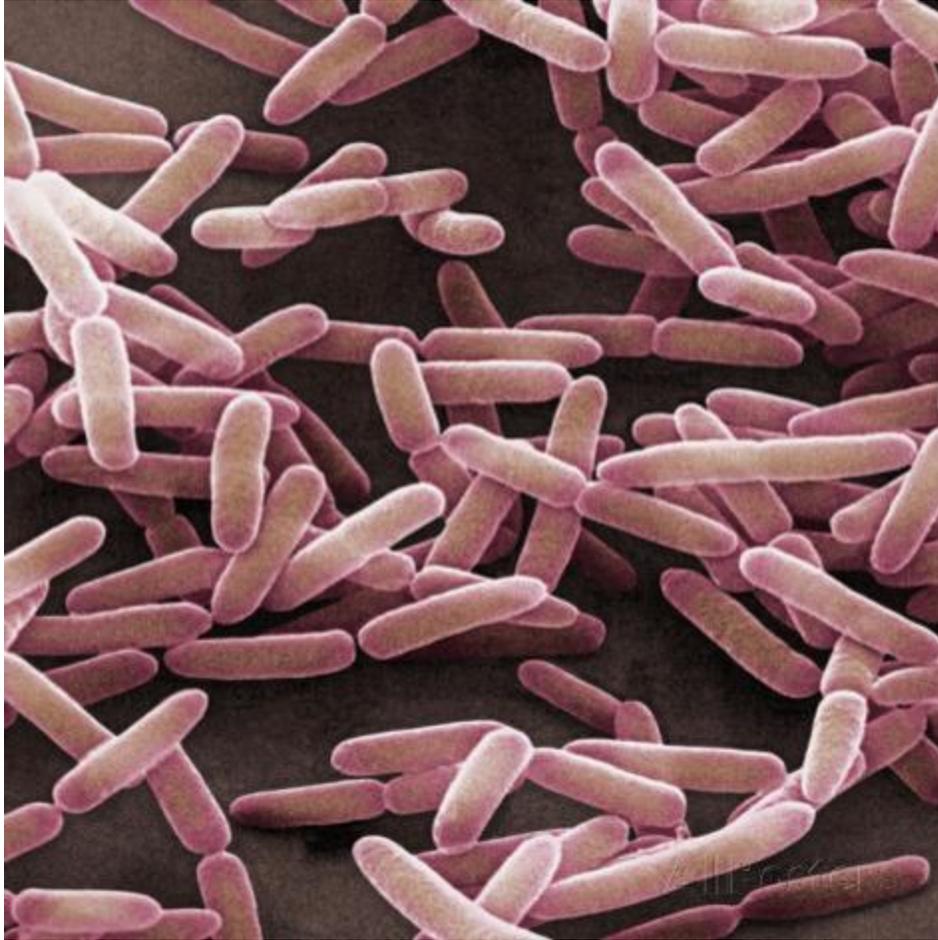
What the bacteria do.

Carbohydrates, proteins and lipids (triglycerides) provide essential nutrients (amino acids) for the construction of living tissue. They also supply the energy to allow the conversion of essential nutrients to living tissue.

The highest energy source comes from Lipids 9.5 kcal/g, then protein 5.6 kcal/g and then carbohydrate 4.1 kcal/g. The lipids are broken down by enzymes such as lipase and protease which are produced by the Bacillus bacteria. They are broken into a form which allows the energy to be utilized by life forms. Due to the high energy levels produced by lipids it is important that they are utilized as an energy source. If lipids are not converted to a usable form it can have a negative effect on the nutritional value of feed whether natural or artificial (added) due to the lack of energy to convert the essential nutrients to living tissue.

Lipids and natural oils need to be broken down so that they can be used in the marrons' energy metabolism in the Krebs cycle, which is how cells get their energy to grow.

The enzymes can also be produced by other bacteria like Pseudomonas bacteria but Bacillus is the main bacteria used in aquaculture. Many, if not all, of the probiotic/microbe products which are sold by companies to improve the health of an aquatic animal and to reduce sludge in a water body contain Bacillus bacteria. They have different strains which have been developed to work under different conditions like temperature, acidity and salinity. The different strains still produce the same enzymes.



In humans, *Lactobacillus* is one of the sources of Bacillus. Products like Yakult, yogurt and many of the probiotics for human digestion contain Bacillus bacteria.

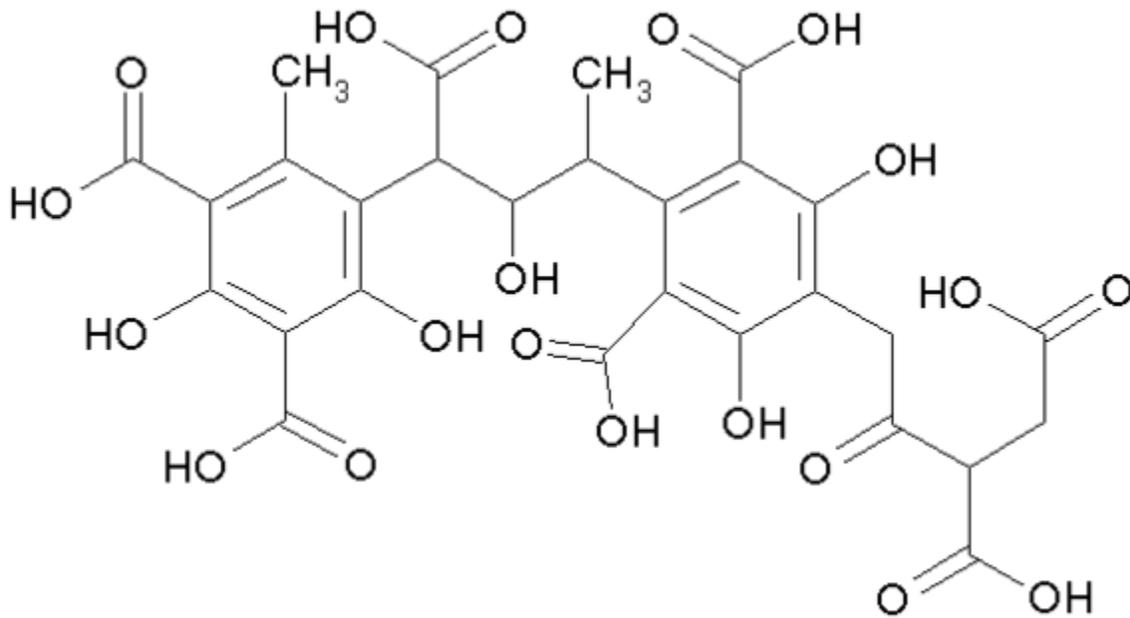
Bacillus bacteria need organic carbon as a food source. They do not feed on lipids or natural oils; they produce enzymes (products) that break them down. This means that you can have a water body high in lipids and natural oils with little Bacillus if there is not a source of the right type of organic carbon. Different sources of carbon seem to be more effective at promoting bacillus. Hydrocarbons are very effective at promoting Bacillus. Bacillus is used to help clean up oils spills in the ocean; they appear naturally or can be added. The hydrocarbons are a food source.

Promote Bacillus Bacteria.

I am using a number of methods to promote and maintain Bacillus in my water bodies.

Humic acid.

The humic acid I am adding to my ponds is extracted from brown coal from South Australia by a company called Omnia. The humic acid is called S90. It is granular and is made to be broadcasted onto pasture or dissolved and sprayed on. There are many different types of Humic acids which are made by different companies from different materials such as peat. I have tested a number of them and believe the S90 is the best for my purpose. It is also rich in trace elements and low in cadmium.



I put 3kg in a 1000 meter square pond when first filled and then add approximately 500grams a week when feeding. I have not tried adding it to large dams yet. Adding the Humic acid turns the water a dark tannin colour. Humic acid is high in Lignins which is a form of organic carbon and hydrocarbons. Other sources of carbon used in aquaculture are barley straw and molasses. I suspect that the reason why barley straw may help the health of a water body when added is because it produces a type of organic carbon which promotes Bacillus and molasses may do the same. Another effect of having a source of lignin/humic substances in water

is that hydrogen peroxide can be produced in the presence of sunlight. Hydrogen Peroxide is used in water to control algae. I suspect it breaks down the lipids and natural oils.



Bacteria.

A company has just started to sell microbes which work below 11 degrees Celsius which cleans water and prevents build up of excess nutrients. The product is called Aqua-Tron cold water formula. I have spoken to one of the suppliers (who gave a presentation at the MGA last field day) and they said the microbes are a Bacillus strain which works in cold water. I believe if we use and promote Bacillus in cold water the same Bacillus will help marten break down the lipids and natural oils in their digestion system, producing energy for the marten to remain active for longer.

I believe some water bodies already promote Bacillus bacteria naturally in cold temperatures. This might explain why I have seen marten that are still active in water which is lower than 11 degrees Celsius. In most cases the marten would be very inactive at this temperature.

I am not sure if we need to add new Bacillus strains or just promote the natural Bacillus in the water body.

Trace elements.

The micro trace elements I will be adding to promote phytoplankton will also help provide essential elements to bacteria.

Marine Easy Clean. Product; The Water Cleanser.

I am also using a substrate which has been developed to promote and maintain Bacillus in a water body. The product is called The Water Cleanser. It is wax with a source of organic carbon added which promotes and maintains Bacillus bacteria on its surface. The Bacillus grows on the block because the organic carbon, which has been added to the wax block, feeds the Bacillus. The Bacillus bacteria will be promoted for at least 12 months until all the organic carbon has been consumed by the bacteria. The wax block will either enhance the natural bacteria in the water or it can be a substrate for added Bacillus to grow on. The combination of The Water Cleanser and the humic acid I am using will, I hope, promote the Bacillus bacteria in the water body so it can produce the lipase and protease enzymes which then break down the lipids and natural oils (triglycerides) both in the water and in the digestive systems of the life forms in the water (I suspect the lipids and natural oils may have a negative effect on Zooplankton).

The Water Cleanser product was a chance discovery. A Pearl farmer in Western Australia was experimenting with different coatings which he could apply onto his pearl shells to stop the build up of marine growth on his shells. He found putting wax on his shells did not have any negative effects, unlike many other coatings. He then added different things to the wax to try to make it naturally slippery in water so things would not stick to it. He eventually succeeded in making the wax become slippery in water. What he then noticed was that the water quality improved as well. It was then he discovered that it was Bacillus bacteria on the surface of the wax which was making the wax slippery. He had promoted Bacillus which then produced the enzymes which clean the water. He had added a natural unprocessed form of organic carbon to his wax which did not leach into the water. It was the organic carbon in the wax which promoted the Bacillus. The wax is full of microscopic holes which the bacteria can live in.

The Water Cleanser possible effects.

I believe I have seen this product in action in two outdoor tanks. The tanks are 4 metres across and 1 metre deep and in direct sunlight. They have had a healthy population of marron living and breeding in them for approximately three years with no water exchange except evaporation replacement. They both have their own small bio-filters. The only aeration is a small umbrella fountain in the centre. The water is a tannin colour caused from the logs and timber put in the tanks as hides/homes. I have tested these tanks a couple of times over the last two years because the marron and the ecosystem look very healthy. I was very surprised to find levels of total phosphorus as high as 2.6mg/L and available phosphorus 1.8mg/L in one of the tanks. The other tank had levels of 0.28 mg/L phosphorus. It is widely recommended that phosphorus levels should be less than 0.1mg/l



At the beginning of this spring one of the tanks started turning a soupy green. It is the first time this has happened since they were filled. The interesting thing is the tank that started turning green was the tank with the low levels of phosphorus. As there was a chance it could get worse in summer, the tank was drained and refilled. It was around this time I was sent some samples of Marine Easy Clean products for the first time. The owner of the tanks saw the samples of The Water Cleanser and then told me he had put some of the product in the bio-filters of the tanks three years ago. He brought the product at a Marron Growers Association field day in Northcliffe three years ago from a lady who did a presentation to growers. He had not thought to tell me about them as he did not think the product was doing anything. He had taken the blocks/balls out of one of the tanks bio-filters about 12 months ago. It was the tank that now had gone green even though it had much lower phosphorus levels than the other tank The Water Cleanser wax product was in the form of small balls. One of

the balls from the good tank was tested and it was found to be covered in Bacillus bacteria. Larger wax blocks are used in bigger water bodies.

I believe having bacillus constantly in the ecosystem provides a constant supply of enzymes like lipase which keep the lipids and natural oils from building up and then damaging the ecosystem and it also helps provide the enzymes for the marrons digestive system. I think the tannin in the water, which is a form of humic acid, may have also helped the Bacillus bacteria. I have recently become a Distributor for Marine Easy Clean for marron growers who wish to try The Water Cleanser because of what I have been seeing and learning. Trials of the The Water Cleanser have just started in some marron ponds which are part of a PhD which is being carried out on the product. The trials are being completed on the same farm as the trace elements phytoplankton PhD.

I suspect that it is not phosphorus that causes Cyanobacteria (blue green algae) and damage to ecosystems; instead it is the production of lipids and natural oils which come from the increased natural primary productivity which phosphorus causes. If bacteria such as Bacillus are not promoted in the water body by organic carbon the lipids and natural oils build up to levels which cause the problems which we often blame on phosphorus.

Marron growers have been standing along side of their ponds for many years saying they cannot see why one pond is producing differently to another. I believe this is because you cannot actually physically see the trace elements and bacteria which are the cause of the difference.

Farm Management Strategies.

Management strategies I will implement on my marron farm as the result of these three theories.

1. In winter drain water body if possible and wash out. If it cannot be drained, lower the water body as low as possible to let the banks be exposed to the air and sunlight for a couple of weeks. Doing this I believe will either wash the lipids and natural oils out or help the sun and air them break them down.

1. Treat water with 50% Hydrogen peroxide. In the pond which has been washed out I would treat it with 5 litres of hydrogen peroxide per 1000 square meters. If it is not feasible to treat all water bodies at least treat the water bodies which are going to have brood marron or juveniles in them. In a pond which has not been washed out I would treat the water left in the pond before I refill it and then treat the pond as above when refilled. I believe the hydrogen peroxide will break down the lipids and natural oils.

1. Add trace elements. I will then add trace elements; I am working on developing a mix of micro elements which will have everything required to bring the water up to the levels needed. Only a small amount will need to be added. At the moment I add the trace elements separately.

1. Add humic acid. I will then add 3kg of S90 humic acid for an initial dose and after that add 500grams a week maintain a tannin colour in the water.

1. Add the Marine Easy Clean blocks. I will add 3 blocks per 1000 square metres water body. I will suspend them above diffusers if possible.

1. Add microbes/bacteria. I am still undecided whether I need to add them. I will possibly give one or two treatments. One at the beginning of summer when the water temperature reaches 20 degrees with a warm water bacillus and one treatment in winter containing the cold water Bacillus when the water drops below 15 degrees.

1. Unless my ph is below 7 in will not add lime.

All of the above will be done in addition with other normal farming practices. I suspect the wax blocks will remain active for a number of years and can be reused each year, only adding one new block a year. I will treat all of my ponds. If I had a large number of ponds and it was too expensive I would treat the ponds which are

poor performers each year. I have found on most farms that certain pond/dams will do poorly year after year. I am hoping the above strategies will help improve poor performing ponds and water bodies.

I also hope to learn what the biological indicators are in our water, which will tell us whether the ecosystem is working correctly. Things like swimming beetles and different macro algae for example may be used to indicate the health of an ecosystem. This may save doing expensive water testing.

Another interesting development which may help marron farmers is, in recent trials at the South Metropolitan TAFE Fremantle. Novacq which has been developed by the CSIRO to replace fish meal and fish oil in feed and has improved the growth of prawns in ponds by 30% to 40% has caused nearly a 100% increase in growth of 50 to 80 gram marron in initial tank trials. Even though this needs further trials before any firm predictions are made, it shows Novacq has the potential to increase marron growth. Below is a link that explains what Novacq is. It is interesting to see they use bacteria/microbes and a food source for them is Carbon. If I were to guess I would say they are using Bacillus or similar bacteria to produce the enzymes like Lipase and protease, which when dried out and feed to the prawn allows them to get the energy out of their food to then convert the essential nutrients to living tissue.

<http://www.abc.net.au/landline/content/2014/s3984247.htm>

Please be aware that my theories have not been proven correct and my methods of managing my water have never been tested. Every thing I have written could be incorrect. If anyone applies any of my management strategies they do so at their own risk. Only time will tell if they help me grow marron. I have written this document as a discussion paper not as a scientific paper. Let me know your comments and suggestion.

I have been lucky enough to receive a scholarship from the Premier's Agriculture and Aquaculture Entrepreneurship Program which will allow me to travel interstate and internationally to further my understanding of water ecology.

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