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Rural Industries Research and Development Corporation

Assessing Lotus for Wastewater Bioremediation

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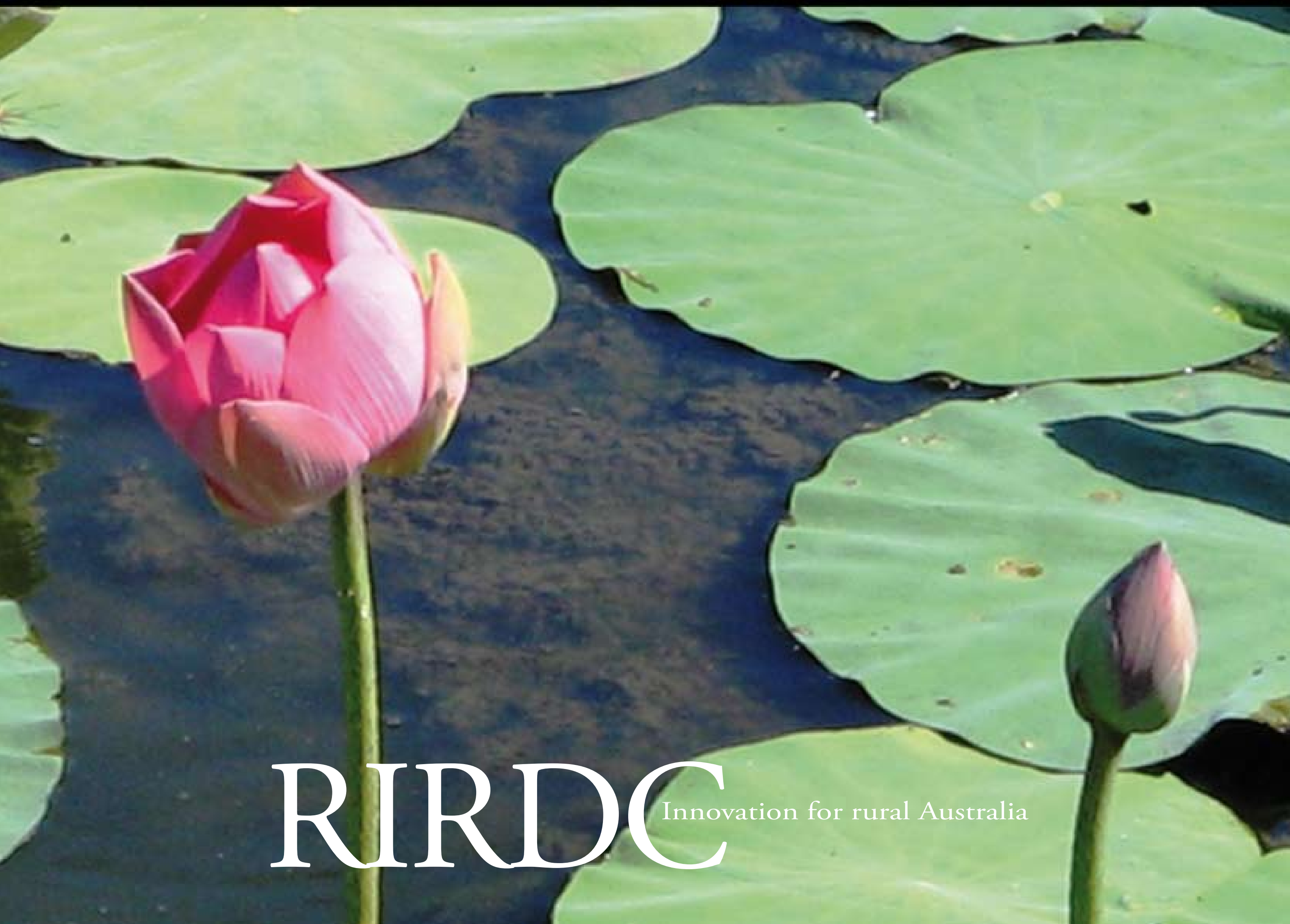

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by Evizel Seymour, Peter Graham, Clarita Agcopra, Karen Willows and Brett Herbert

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Foreword

Aquaculture is under increasing pressure to reduce nutrients in discharge water. Lotus may be a low-cost option for maintaining and improving water quality as well as an additional source of income. This research aims to assess effectiveness of Lotus (Nelumbo nucifera) for wastewater bio-remediation.

This research shows that lotus is very efficient in the capability of reducing the amount of nutrients and suspended solids from the discharge water. The report shows that lotus is effective at reducing the amount of nutrients (ammonia up to 64 percent), total suspended solids (40 percent) and water used within the system (14 percent).

Freshwater aquaculture producers in the tropics will directly benefit from this research. Due to increased efficiencies in stock management and husbandry, improved production due to better water quality in the area of 10 percent could be expected by adoption of these techniques.

Additionally, predator exclusion can be made more efficient through reduced production area and more intensive production.

The lotus industry within Australia is very limited to the exotic food market. The tropical strain of lotus used in this project produces abundant leaves, flowers and pods suitable for the florist industry. A niche industry could develop from this research.

This project was funded from RIRDC Core Funds which are provided by the Australian Government in collaboration with Department of Primary Industries and Fisheries (Queensland) and an industry partner (Daintree River Barramundi Farm).

This report, an addition to RIRDC's diverse range of over 1900 research publications, forms part of our Environment and Farm Management Systems R&D program, which aims to support innovation in agriculture and the use of frontier technology to meet market demand for accredited sustainable production..

Most of our publications are available for viewing, downloading or purchasing online through our website www.rirdc.gov.au.

Peter O'Brien
Managing Director
Rural Industries Research and Development Corporation

About the Author

Evizel Seymour has been involved in aquaculture for over 20 years, working in both research and industry. Her initial introduction into aquaculture was with silver perch and murray cod in central NSW, then moving into developing a goldfish farm in WA. She then ventured into the marine world of mahi mahi, seacucmbers, giant clams, black lip pearl oyster and prawns. She has a thorough knowledge of marine hatchery design, (including live foods), setting up hatcheries in Solomon Islands, Hawaii and Australia in both research and industry fields. A major component of her work has involved on site training for staff to run the hatcheries and facilities.

She is now project leader of the Freshwater Fisheries and Aquaculture Centre (FFAC), DPI&F[1] at Walkamin, where research is directed at using aquatic plant remediation of wastewater of intensive production of Barramundi and developing aquaculture in PNG with native species.

[1] Now known as Queensland Primary Industries and Fisheries, the Department of Employment, Economic Development and Innovation

Acknowledgments

I would like to acknowledge many people; colleagues, friends, acquaintances and foes for making this possible.

I have had tremendous support from all whom I have imposed myself upon for guidance, suggestions and HELP. I think you can all indentify yourselves!

The most instrumental person [other than the fabulous team that made this happen – but I will get to them in a tick] in this project is Brett Herbert. Without his persistence, enthusiasm and words of encouragement when I took over from him, this project wouldn't have made it out the gate. I now have some idea of the challenges he faced.

The other instrumental cogs that made this project work is the amazing team that has been deeply involved all the way through the project: Peter Graham, Clarita Agcopra, Karen Willows and of course David Bull. Thank you for your support and welcoming me to the team. Without you guys, I think I would have walked!

Our industrious industry partner, through sore back, broken bones you swam through all to get amazing results. Also thank you for welcoming the new one 'who doesn't wear heels!'

Other individuals that I am deeply indebted to are [in no particular order]

- Dan Willett
- Richard Knuckey
- The rest of the gang at TMF
- Cathy Hair
- Jes Sammut

Oh, how could I have not mentioned you earlier – the wonderful statisticians that have tried to make sense of all those numbers. Thank you, Bob Mayer and Carole Wright. It was really the guppy effect that we didn't take into account.

Abbreviations

DPI&F	Department of Primary Industries & Fisheries
FFAC	Freshwater Fisheries and Aquaculture Centre
DRB	Daintree River Barramundi
EMS	Environmental Management Systems
ABFA	Australian Barramundi Farmers Association
GBRMP	Great Barrier Reef Marine Park
NH_3-N	Ammonia Nitrogen
TN	Total Nitrogen
NO_2-N	Nitrite
NO_3-N	Nitrate
PO_4^{3-}	Phosphate
TSS	Total Suspended Solids
Ha	Hectare
mg/L	Milligram per litre

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What the report is about

This project assessed the efficiency of lotus reducing the amount of nutrients that are generated in a freshwater aquaculture system. Barramundi were produced at a stocking density similar to industry practices. Lotus was grown to determine if it was capable at reducing the nutrient loading from an aquaculture system.

Who is the report targeted at?

The research is targeted for the freshwater Aquaculture Industry and other industries that are interested in reducing nutrients from wastewater.

Background

There is a real constraint on development of aquaculture in northern Queensland due to restriction of water discharge and extraction. New methods of farming aquatic organisms must be developed to improve water efficiency and permit expansion and economies of scale. Further development of the value added sector of the industry is reliant on expanding production in current commercial systems while maintaining or reducing discharges of wastewater. In a two year production cycle of farms producing 2kg+ fish, there is a high economic loss of crop due to pond crashes in the second summer, when nutrients in bottom sediments are mobilised during the anaerobic conditions following thermocline establishment.

Lotus is identified as an emergent water plant, with capabilities of utilising nutrients from the bottom sediments in fish ponds. There have been few studies conducted into its efficiency in water treatment. Lotus represents a low cost option for maintaining and improving water quality and utilising nutrients in the bottom sediments.

Aims/objectives

The project’s primary aim is to investigate the potential of lotus to improve water quality of wastewater by removal of nutrient and suspended solids. Lotus will be grown in a separate, bioremediation pond, partitioned from the fish production pond.

The hypothesis is that lotus grown in production systems or partition pond systems will act as a nutrient and sediment sink, improving the quality of the water through aeration and shading, while providing a useful by-product. This proposed system can be retrofitted to existing systems with little modification or cost.

Methods used

One component of this project was undertaken at Freshwater Fisheries and Aquaculture Centre (FFAC), at DPI&F, Walkamin. Six replicated fish production ponds were used for the experiment. The replicates systems consist of paired ponds, i.e. one pond for fish production and one treatment pond containing plants or no plants. Water was recycled by pumping from the lower production pond up to the treatment pond and gravity drained back to the production pond. A baffle system made from weed mat was constructed in each treatment pond to maximise retention time and eliminate possibility of channelling of water to the outlet.

The three treatment ponds were planted with lotus (native Australian variety sourced from Ross River, Townsville). A control (also in triplicate) of an unplanted pond with baffles was run concurrently to compare effect of phytoplankton and other pond dynamics on nutrient loads in the system.

To examine the effectiveness of the treatment systems, water quality, water usage, and lotus production were monitored. Water quality [pH, temperature, dissolved oxygen and turbidity was measured twice a day. Water chemistry [nutrient and suspended solids] was monitored fortnightly. Nutrients that were measured were Total Nitrogen [TN], Ammonia Nitrogen [NH_3-N], Nitrite [NO_2-N], Nitrate [NO_3-N], total Phosphate [PO_4^{3-}]) and Total Suspended solids [TSS]. Measuring these water quality parameters was based on the Standard Methods for the examination of water and wastewater (American Public Health Association 1989).

Fish for all experiments were from comparable stock. Every two months a sample of twenty fish were measured and weighed from each experimental unit. Weights were used to calculate growth rates. An initial start weight of the fish stocked and total end weight were measured, as well as survival.

To assess how the system would work in a commercial situation, we collaborated with an industry partner. They modified their existing system to incorporate a bioremediation pond planted with lotus. Water was recirculated from a production pond, through the bioremediation pond back to a production pond. Water samples were collected fortnightly and sent to the laboratory for analysis as for the FFAC component. Initial and final counts of the fish were recorded, as well as survival.

Results/key findings

This experiment has shown that lotus plants grown in production systems or partition pond systems can efficiently reduce nutrients and sediment, improving the water quality. Although, we experienced difficulties in establishing lotus and it grew at different rates in the replicated ponds, the addition of a bioremediation pond greatly improved wastewater generated from a barramundi production pond.

The key findings from this research are that lotus is effective at reducing the amount of nutrients (ammonia up to 64 percent), total suspended solids (40 percent) and water used within the system (14 percent). In the system at Freshwater Fisheries and Aquaculture Centre, the non-plant treatment systems reduced the amount of ammonia (46 percent) which was better than the plant treatment systems. Although the industry partners systems with lotus was more efficient than both of the above systems (64 percent).

Implications for relevant stakeholders for:

Industry

Aquaculture and other water users will benefit from having a practical, realistic water management system that helps to remove excess nutrients and solids from water. A bioremediation system planted with lotus may also reduce the amount of water used by up to 10 percent. A reduced extraction of environmental water and low return of nutrients will help maintain healthy water flows within natural water systems, helping to maintain their natural biodiversity whilst supporting an aquaculture industry.

Additionally, predator exclusion will be made possible through reduced production area and more intensive production. Using lotus for bioremediation means that fish can be maintained at higher densities, which reduces the area of ponds that have to be netted to exclude predators.

There is potential for development of a ‘niche’ floral market utilising lotus flowers and pods.

Policy makers

As aquaculture in Australia has come under greater regulations to reduce the amount of nutrients in wastewater, this report helps to address this issue. Bioremediation systems incorporated into aquaculture facilities can reduce the nutrient loads of wastewater, which in turn reduces the effects of nutrients downstream.

Recommendations

As the aquaculture industry has stricter discharge regulations to follow, the implementation of a bioremediation system would improve their wastewater. Although, lotus are efficient at reducing the nutrient loading and amount of sediment in wastewater, a baffled bioremediation system with high bacterial and phytoplankton loading are also efficient.

Introduction

General background

Limits on the extraction of environmental freshwater and its subsequent discharge with an enriched nutrient load are limiting the expansion of the aquaculture industry in northern Australia. New methods of farming aquatic organisms must be developed that improve the efficiency of water use to allow expansion of the industry. Further development of the value added sector of the industry (fresh boneless fillets in the supermarket sector, and export markets) is reliant on expanding production. This will utilise current production systems but will require a reduction in wastewater discharge. In farms, producing 2kg+ fish, there can be significant loss of crop due to pond crashes in the second summer. Management of these nutrient imbalances is required for the operation of low or no discharge farms. Lotus is proposed as a low-cost option for maintaining and improving water quality by utilising nutrients in the bottom sediments before they can be released, as well as a proposed additional source of income.

The use of plant based water treatment systems could be of immense value to increase productivity and efficiencies in fish farming in the Australian tropics. Research in USA has shown that water hyacinth (*Eichhornia crassipes*) is effective in removing large quantities of Nitrogen (3.4kg/ha/day) from the water (Boyd & Tucker 1998); however water hyacinth is a noxious weed and can cause problems with water quality. Lin (2003) states that an economically viable option to control pond nutrients is to plant rooted aquatic plants that are able to extract nutrients from the mud. Lotus is efficient in removing nutrients (Lin & Yi 2003) and could provide farmers with a solution to wastewater nutrient discharge while also producing a saleable crop of rhizomes, flowers or seed pods used in floristry.

Producers adhering to sustainable production techniques with Environmental Management Systems (EMS) in place will have better market access and economic benefits. The major supermarket chain Woolworths has indicated that farmed fish sold by it should be produced in an environmentally friendly manner, and the EU is increasingly demanding EMS protocols be implemented for commodity access to its markets.

The opportunity existed to turn a problem into a resource, to shift the current paradigms in fish farming by developing Australian innovative approaches to aquaculture. This could reduce environmental impacts while having major efficiency and production improvements. Additionally, exploration and development of niche markets may open the path to the realisation of the market potential of lotus identified in earlier RIRDC projects.

Lotus

Lotus (*Nelumbo nucifera*) grows in wetlands in tropical Asia from Iran to Japan and from China to Queensland (La-ongsri et al in press). It has been introduced and grown in Europe and America, mainly for ornamental purposes (Nguyen 2002).

Lotus is a herbaceous, perennial aquatic plant that grows to 2.5 metres in water. It usually has two different leaf forms; emergent and floating (Nohara & Kimura 1997). The emergent leaves are one of the distinguishing features that separate it from the water lilies, which have only floating leaves. The stalks of the flowers, leaves and pods are woody with small spikes on the side. The flowers are large solitary blooms that have single, double and/or multi petals. The colour can range from single; white, yellow, pink, red or bi-colour of white petals with pink tips (Nguyen 2002). The native Australian variety (same species), found in lagoons and flood plain watercourses of the tropic in Queensland and Northern Territory (Sainty & Jacob 2003) has single blooms of a spectacular deep pink colour [Photo 1 & 2]. The seeds are held in a pithy, conical receptacle known as a seed pod [the central yellow part of the young flower]. Once the seeds are fertilised they enlarge turning the outer pithy covering from yellow, green to a red-orange colour, eventually drying out to a lovely wooden colour [Photo 3, 4 & 5]. The plants send out stolons (runners) [Photo 6] into the water as well as into the mud. In some varieties these stolons, in the mud, can develop into rhizomes. In botany, a rhizome is a characteristically horizontal stem of a plant that is found underground. Lotus rhizomes are sausage in shape with a creamy-white colour. One rhizome can contain up to 4 ‘sausages’ 60-90 cm in length (Nguyen 2002). In contrast, stolons are the thinner stem system running above or below the ground.

In Australia, native lotus is a purely tropical species, and no studies have been conducted into its cultivation for use in water treatment. This project assessed lotus as a tool for wastewater remediation in tropical fish farming and demonstrated its high capacity to reduce nitrogen and phosphorus loadings. These nutrients are responsible for algal blooms and the deterioration of water quality in waterways across Australia.

Photo 1 Native Lotus bud and bloom opening

Photo 2 Native Lotus bud

Photo 3 Seed pod [immature]

Photo 4 Seed pods [notice different ages]

Photo 5 Seed pods with immature fertilised seeds **Photo 6 Lotus stolon showing root mass**

Why lotus?

Lotus was chosen as the primary plant in the trials because of its nutrient stripping qualities (Lin & Yi 2003) as well as its commercial versatility (Nguyen 2002). It is endemic to the region (North Queensland), traps sediments in its fibrous root system, produces high grade flowers and pods, shades the water (reducing evaporation), and grows quickly under favourable conditions. In addition to removing nutrients from the water - such as ammonia, nitrates and phosphates - the plant's rhizomes strip nutrients from the substrate. It also oxygenates bottom sediments, preventing release of toxic gasses and nutrients through anaerobic decomposition processes. Trapping of these nutrients reduces the potential for algal blooms, pond 'crashes' (rapid deoxygenation caused by algae or bacterial abundance), and improves the oxidation and mineralisation processes that aid bacterial nitrification processes in the pond sediments.

Lotus has a particular advantage over many other aquatic plants in that the leaves are held above the water. Most floating aquatic plants leaves cover the air/water interface and heavy cover results in deoxygenation of the water beneath. As lotus leaves are held above the water this does not occur. In addition, the large lacunae in the stolons carry oxygen deep into sediments meaning that sediments with lotus are less likely to be anaerobic. Anaerobic sediments are a factor in mobilisation of critical nutrients including phosphorus.

While the market in Australia for lotus products is limited at present, there is a huge potential to develop a "niche" market within Australia or an export market.

All parts of the Lotus plant can and are utilised throughout the world. Australia's indigenous people have eaten the seeds and rhizomes for thousands of years (Stephens & Dowling 2002). In Asia, the flowers and dried pods are used to make exotic displays, the seeds ground for flour or cooked for a snack, the young leaves eaten while the older leaves are used to wrap food such as rice and fish before steaming and the rhizomes can be used as a fresh, dried or frozen a vegetable (Nguyen 2002).

One factor inhibiting the adoption of existing water treatment systems is their high cost. Lotus could be an alternative, low-cost bioremediation water treatment system that also produces a marketable crop.

Varieties

There are a large number of varieties of lotus around the world, ranging from temperate to tropical climates. These varieties can be classed into 3 main market groups based on their main product; flowers, rhizomes or seeds. The varieties in the flower group produce large, beautiful flowers, without rhizomes. The varieties in the rhizome group produce high yields of good quality rhizomes. The varieties in the seed group produce large numbers of flowers with a high fruit set percentage (Nguyen 2002).

The native Australian tropical lotus is classified in the flower group. As the native lotus was selected for this study, eliminating any potential of introducing a weed species, its market potential is mainly in the floral industry; flowers and pods.

The tropical Australian lotus prefers daytime temperatures averaging 25 degrees Celsius, silty loamy soils of a water depth greater than 30 centimetre, good water quality and a pH between 6-6.5 (Nguyen 2002).

Aquaculture

The Australian Barramundi Farmers Association (ABFA) has identified more efficient use of water, particularly regarding re-use and discharge issues, as a major R&D issue. The economic benefits of reduced water usage include greater production of fish from smaller volumes of water, reduced extraction requirements and associated costs and limits, and the ability to comply with Environmental Protection Agency [EPA] discharge requirements.

Barramundi (Lates calcarifer)

Barramundi is a tropical fish widely distributed in the tropical and subtropical areas of the Indo-West pacific region from the Arabian Gulf, through SE Asia to Papua New Guinea and Northern Australia (Greenwood 1976).

In 2006-07 Queensland produced 2,091 tonnes of barramundi in pond-based systems, totalling 80 percent of the aquaculture production of barramundi across Australia (Lobegeiger 2007). Barramundi aquaculture first commenced in Queensland in 1982 with sea-cage systems then expanded into pond based (mostly freshwater) production. Pond-based production now makes up almost 70 percent of Queensland barramundi aquaculture output.

Wastewater

The aquaculture industry is often perceived negatively as a polluter. Improving the sustainability of the industry through reduced consumption of water and nutrient discharge will help address the negative image of aquaculture and enable the continued growth of this industry (32 percent in 2007) that provides significant employment opportunities and cash flow in regional communities.

The main negative environmental impact of fish farming is the release of nutrients and suspended solids to the environment through wastewater discharge. Pond sediments hold most of the nutrient in pond systems, and lotus is an efficient extractor of these nutrients - up to 300 kilogram of Nitrogen per hectare and 43 kg kilogram of Phosphorous per hectare from fishpond mud (Lin & Yi 2003). Turning wastewater into a useful asset (clean, reusable water) will permit expansion of industries requiring water in an age where sustainable water use and disposal is demanded by regulatory bodies, markets and society in general.

Aquaculture facilities are under increasing pressure to reduce the nutrient load in their discharge water. Existing farms wishing to expand production must not increase discharge volume or nutrient loading without licensing approval. Exempting aquaculture facilities that were operating on or before 1 October 1999 it is an offence to discharge aquaculture wastewater into waters in the Great Barrier Reef Marine Park [GBRMP] region. New farms must operate under a zero discharge system or obtain special approval from the Queensland Environmental Protection Agency [EPA] which administers the legislation.

Most barramundi aquaculture facilities are based around zero-discharge systems. Wastewater is contained on-farm and is often diverted to other crops as an irrigation source. While this allows wastewater to be contained, it relies on continued input of new water for the aquaculture operation.

This project seeks to show how wastewater can be utilised more productively in a recirculating system of barramundi production. A recirculating system must be managed to ensure consistent water quality and biosecurity.

Ability to integrate improved water treatment and recycling into existing farm systems was an important consideration in the development and design of the experimental systems. Currently, farms use either settlement ponds to treat water with varying levels of success. Improved design of these, and improvement of water quality which could have uses in water treatment for waste water from other sources, were considered in development of this project.

This project utilises the plug-flow system, where incoming water or "plug" flows from the inlet of treatment area, to the outlet without mixing with any other water in the system. A system of baffles ensures that the entire pond area is used. The nutrient concentrations are higher at the beginning of the effluent stream and lower towards the end, due to the incremental reduction of nutrients in the wastewater (Willett 2003). Managing water in this way ensures that there is maximum contact between wastewater and lotus, minimising the possibility of the water short-circuiting the plants (Smith & Moelyowati 2001). Additionally, the period of no flow between 'plugs' enhances settlement of suspended solids and uptake of nutrients by periphyton on the baffles and other surfaces.

In a farm situation the water supply must be of adequate quality, and available in sufficient quantity, to support the fish farm operation (Rimmer 1995). Barramundi generally have a wide tolerance to a range of physiochemical parameters although even moderate shifts in these parameters can depress growth rates substantially and increase the fishes' susceptibility to infectious diseases. Poor water quality can greatly reduce production of fish within the system. Ammonia is the main nitrogenous compound excreted by aquatic organisms. Free ammonia (NH_3-N) and ionized-ammonia (NH_4^+-N) represent two forms of reduced inorganic nitrogen which exist in equilibrium depending upon the pH and temperature of the waters in which they are found. Of the two, the free ammonia form is considerably more toxic to organisms such as fish and, therefore, we pay considerable attention to the relative concencentration of this particular compound. Ammonia is converted by bacteria to nitrite and nitrate during the nitrification process. Nitrite is toxic to fish at very high concentrations but nitrate is normally non toxic and is readily utilised by plants (lotus/algae).

Industry Partner [Daintree River Barramundi]

Daintree River Barramundi (DRB) is a commercial barramundi farm using integrated farming principles. They have an established aquaculture facility in which wastewater is used to water and fertilise an established mangosteen orchard and taro patch. The mangosteen trees have been solely fertilised from aquatic water plants (*Hydrilla sp.* and *nitella*) and sludge from the aquaculture facility. The water plants are harvested by hand and placed around the tropical orchard trees as mulch. Twice a week, water from the production pond was diverted to water the taro patch.

Daintree River Barramundi is a recirculating system with every second pond a cleansing pond. Water is pumped from the bottom 1 hectare fish pond up to the top bioremediation pond then gravity fed to alternate fish then bioremediation pond until it returns back to the bottom dam again. Periodically, this system is topped up from the creek in the dry season and natural rainfall the rest of the year.

Daintree River Barramundi was keen to investigate the use of lotus as an in-pond treatment option to improve water quality as well as offering an alternative income. Previously in summer months they were harvesting up to 2 tonnes wet weight of *Hydrilla sp.* and *nitella sp*. a week as mulch for the fruit trees (very labour intensive). These plants have a high potential to crash, which in turn depletes the water of dissolved oxygen that is essential for fish growth. One way to manage these potential crashes is to keep fish stocking densities low. To mitigate this problem the industry partner researched other alternative water plants that could be establish in the system. They decided to trial lotus. Lotus was appealing as it is a heavy feeder and an emergent plant rather than submergent. Therefore it does not have the potential to crash overnight compared to the submergent water plants such as *Hydrilla sp.*

Objectives

- To investigate turning the liability of wastewater generated by aquaculture (in the first instance) into an asset.

The project's primary aim was to investigate the potential of lotus to improve water quality by removal of nutrient and suspended solids in wastewater. Lotus will be grown in a separate, bioremediation pond, partitioned from the fish production pond.

The hypothesis is that lotus grown in production systems or partition pond systems will act as a nutrient and sediment sink, improving the quality of the water through aeration and shading, while providing a useful by-product. This proposed system can be retrofitted to existing systems with little modification or cost.

Methodology

Freshwater Fisheries and Aquaculture Centre (FFAC)

Located at Walkamin Research Centre, Department of Primary Industries & Fisheries.

System design

Twelve 320m^2 (0.03 hectare) ponds were used for the experiment. There were three replicates of the control and treatment systems. Replicates consisted of paired ponds, i.e. one pond for fish production plumbed to one treatment pond containing plants or no plants (Fig 1.).

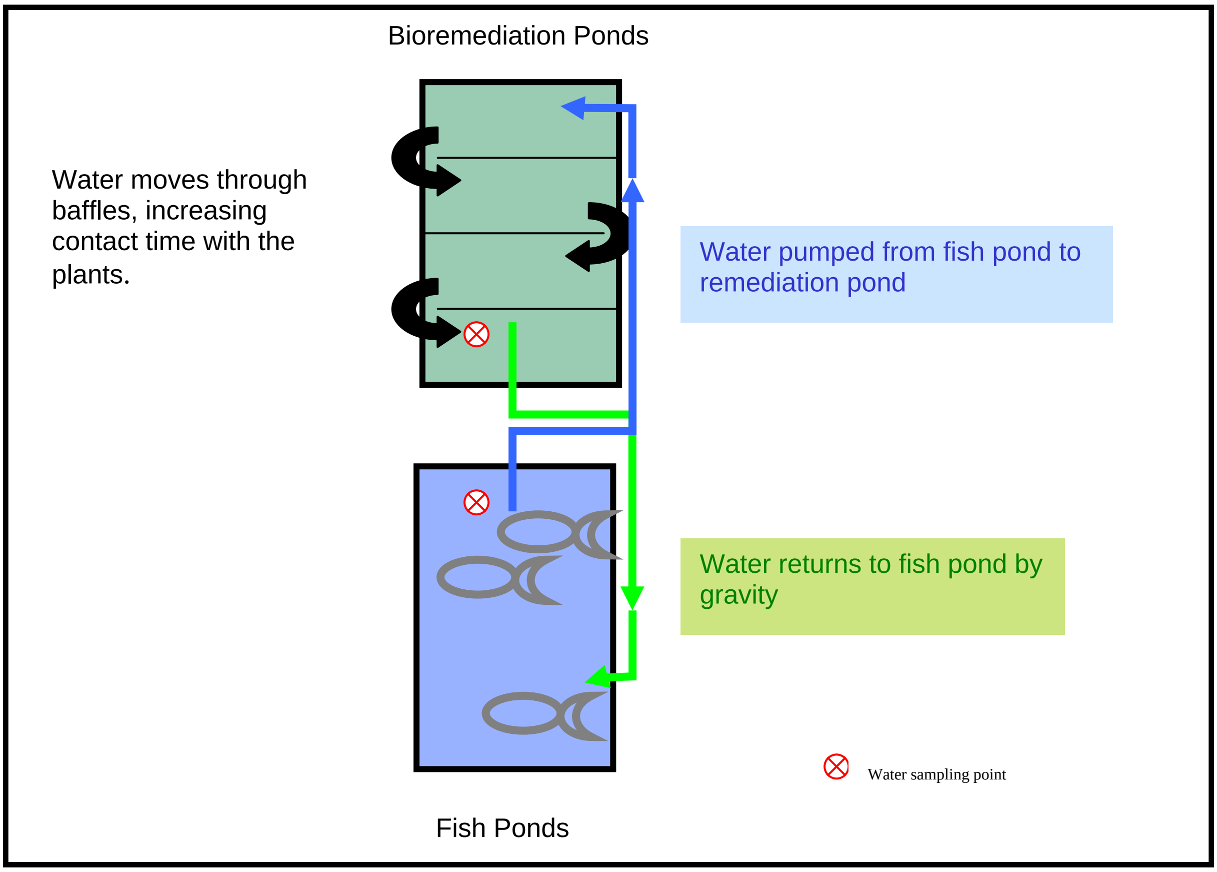


Figure 1 Schematic design of bioremediation system at FFAC

The treatment ponds [plants or no plants] design is the basic unit of a plug-flow system with a shallow rectangular lagoon with baffles. The pond was divided into 4 bays using weed mat to produce the baffle system [Photo 7]. The weed mat was suspended above the water and had a weighted base to allow for variations in water level. The baffles maximise the water retention time and eliminate the possibility of water 'channelling' to the outlet. By eliminating the channelling effect, the water has greater contact time with the plants which remove nutrients from the wastewater in conjunction with both aerobic and anaerobic bacteria. This flow pattern also allows organic solids to settle out from the water column to the bottom of the pond where the lotus can utilise the nutrients. Therefore, while acting as the major nutrient 'sink', the plants themselves should be considered as only one component of each complete treatment system. There is no aeration in the treatment ponds.

A control (also in triplicate) of an unplanted pond with baffles ran concurrently to compare the effect of phytoplankton in collaboration with aerobic and anaerobic bacteria and other pond dynamics on nutrient loads in the system. Baffles had a rich growth of periphyton.

Photo 7 Pond showing baffles [Lotus in background]

All treatment ponds were connected to a production pond below which was stocked with barramundi. The fish production ponds did not have baffles, [Photo 8] had continuous bottom aeration and mechanical aspirators that operated in the morning only.

Water was pumped from the lower production pond (fish) up to the treatment pond and drained back under gravity to the production pond. One third of the water was exchanged daily giving a three day turn over. The plants extract nutrients from the water before the 'filtered' water is returned to the fish pond.

All ponds were under netting and fenced to exclude predators such as birds, rats, cane toads and some insects.

Photo 8 Experimental ponds at FFAC. Bioremediation ponds –top (notice Lotus growth). Fish production ponds – bottom

Lotus

Lotus stolons and seeds were collected from Ross River, Townsville by boat. They were transported carefully to Walkamin, where the stolons were immediately planted into the three treatment ponds at a rate of 40 stolons per pond. The ponds were gradually filled with water over one month to allow lotus to grow to the water depth of 1.5m.

During this study there were two methods of propagation of the lotus in the treatment ponds:

Vegetative

This method produces plants that grow true to type (Nguyen 2002). Lotus plants send out stolons into the water body. These stolons were carefully cut from the plant and transplanted to a new pond. Extreme care was taken not to touch the roots, as this can cause them to die. The mid section of the stolons, between the root nodes, was dug into the mud, leaving the roots exposed to the surrounding water. Plants that were transferred by this method grew quickly, producing large leaves. In Thailand, this in one of the most common methods of planting lotus (La-ongsri et al 2008).

Seedlings

This method produces plants that are not true to type as the seeds hold a high degree of genetic variability (Nguyen 2002). The lotus seed has an extra hard, impermeable seed coat [Photo 9]. This seed coat was carefully scarified until the white endosperm inside is visible. It was then placed into clean water maintained at 25-30^{O}C. Water was changed daily to prevent fungal infection. Once the seedlings were 30cm long they were transplanted to the shallow edge of the treatment pond. Plants that were transferred by this method grew more slowly than the vegetative method with small leaves that gradually enlarged.

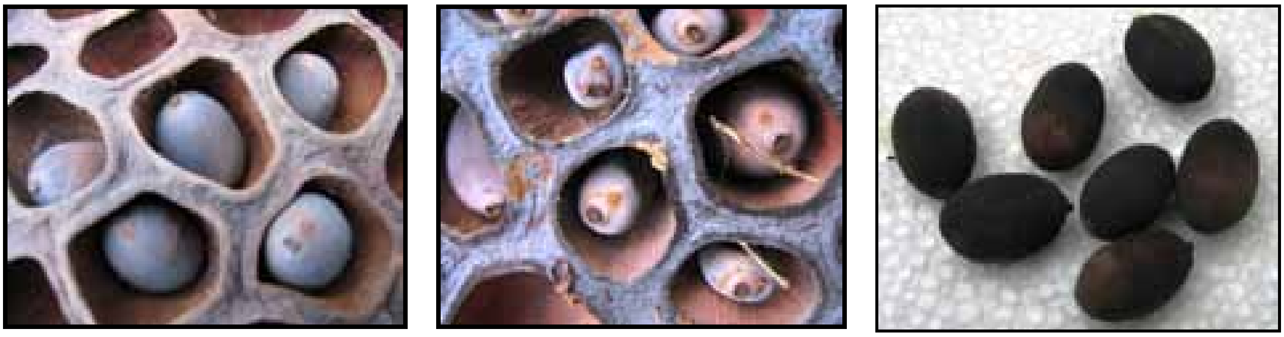


Photo 9 Seeds fertilised in pod Unfertilised in pod Fertilised out of pod

Lotus plants were initially very hard to establish. This could have been due to low nutrient level generated from the fish ponds. They were also attacked by trichoptera larvae (caddis fly) that cuts the leaf off at the base of the stalk, killing the plant. Due to the lotus not establishing in the pond, *Hydrilla sp.*, a native aquatic plant, was trialled [Photo 10]. The *Hydrilla sp.* also suffered attacks from the trichoptera larvae. After closer investigation, it was decided to drain the ponds and dry them out for 2 weeks to eradicate the insects.

Photo 10 Planting *Hydrilla sp.* into the bioremediation pond

Once the ponds were refilled, the ponds were propagated with seedlings which unfortunately struggled through a very cold winter. Once the water temperatures increased, the ponds were propagated with seedlings again which slowly established. Throughout this procedure one lotus plant survived in one of the ponds. Once temperatures increased, this plant rapidly grew covering the whole pond in only 10 weeks.

The main pest that was observed once the lotus was established was *Heliothis sp*. Caterpillars [Photo 11 & 12]. The caterpillars were present on the leaves, flowers and pods. The leaves that were eaten turned brown, exposing the veins [Photo 13]. It is possible to control the caterpillars with Dipel, an insecticide which contains a bacteria *bacillus thuringiensis* that infects the gut of the caterpillar, eventually killing it. However, due to the chemical supplier not guaranteeing the safety of the fish, we erred on the side of caution and decided not treat the lotus.

Photo 11 Heliothis sp. Caterpillar

Photo 12 Heliothis sp Adult

Photo 13 Leaves and pods showing damage from the caterpillars

Aphids were also present towards the end of the study, although they didn't affect the growth of the lotus.

The trichoptera larvae were present throughout the study, but only destroyed the young lotus plants that were planted at the beginning of the project. Once the lotus was well established they weren't a problem.

We harvested flowers and pods for florists on a demand basis. The flowers are very delicate, with a short shelf life (2 days). Development of flower harvesting would need strict procedures to be viable. An alternative to the flowers, is to harvest the buds, then follow the Thai method of folding the petals. This increases the shelf life of the flower, approximately 5 days, while maintaining the beauty of the flower [Photo 14]. Further investigation in handling and harvesting techniques of the flowers, could easily improve on our experience of flower handling.

Photo 14 Lotus buds folded in the Thai method

Following completion of growth of the lotus we searched the mud at the bottom of the ponds to check for rhizomes. No rhizomes were found, agreeing with references that this variety does not produce rhizomes (Nguyen 2002).

Through out the project seeds were harvested and stored for future stock. Restocking of the ponds was from seeds collected from Daintree River Barramundi.

Barramundi Production

Barramundi (*Lates calcarifer*) was the finfish species used in the experiment. It is widely grown in the region with well established production parameters that can be used to compare with the data collected from this project. Growout of barramundi followed standard protocols for feeding and maintenance, to market size of approximately 2-3 kilogram. Rimmer (1995) states that fish greater that 400 gram should be feed 1 percent. Food conversion ratios for commercial producers range from 1.3:1 to 2.0:1 during the warmer months and increasing during the colder months.

The six fish production ponds were prepared with 40 kilogram of agricultural lime that was spread over the base of the empty pond. The ponds were filled with filtered (350 micron) channel water, from Tinaroo Lake, 14 days prior to taking delivery of the fish.

Fish were received from the supplier, and held in quarantine for 10 days. Immediately on arrival 6 fish were sent to the Oonoonba Veterinarian Laboratories (OVL) for a routine health check. Before stocking, 50 fish from each of the six ponds were weighed and measure (standard & total lengths). Prior to stocking in the ponds, the fish were given a salt bath to remove parasites and disease. They were held for 1 hr at 10ppt salinity.

Fish used for all ponds were from the same cohort, ensuring that the stock used in each trial had the same parentage and minimising the risk of misleading results due to genetic differences. The fish (total length 100 millimetre) were evenly distributed (n = 410) between the 6 ponds.

Growout of barramundi to market size (approximately 2-3 kilogram) followed standard industry procedures. We also had to follow animal ethics regulations.

Initially, when the fish were less than 20 grams, fish were feed in the morning (9-10 am) and afternoon (3 -4 pm). Once the fish were greater than 100 grams, they were only feed in the afternoon (3 -4 pm), to satiation. All amounts of food consumed were recorded for each pond.

Every two months a sample of twenty fish were weighed and measured (standard & total lengths) from each production pond. Weights were used to calculate growth rate and food conversion ratios. An initial start weight of the fish stocked and total end weight was also measured. [Photo 15 & 16]

Photo 15 Measuring Barramundi

Photo 16 Weighing Barramundi

Barramundi grow best in water temperatures greater than 23 degrees Celsius (Rimmer 1995). When water temperatures dropped below 23 degrees Celsius, all handling of fish ceased to avoid the high risk of opportunistic bacterial and fungal infections.

At the end of the study the fish were sold after a tender process. We assisted with the purchaser in the final harvest. For harvest, the water level of the pond was lowered. Aqui-S, an anaesthetic, was added to the water, anaesthetising the fish and allowing ease of handling while reducing stress on the fish. The fish were collected by hand, washed, collectively weighed and then placed directly in an ice slurry.

At final harvest we also weighed and measured twenty individual fish from each production pond.

Water Chemistry

To examine the effectiveness of the treatment systems, water quality, water usage, and lotus production were monitored.

Sampling procedures and analysis methods for water sampling are an important part of the water testing regimen.

To determine the best time to recirculate the water, we conducted a 24 hour profile of the nutrients in the production pond. Once the nutrients became detectable in the system, for example; Ammonia, samples were collected every two hours for a 24 hour period. Once these samples were tested, we could determine if there were any nutrient peaks, in a daily cycle. We then adjusted the pumping time to correspond with the peak. It was established that pumping time should commence approximately 2 hours after feeding. Water was pumped nightly during the off peak tariff [7 pm – 12 am]. Water flow meters were used to record the volume of water pumped each night, and to ensure consistency between all 6 systems.

Water quality was monitored twice daily by measuring pH, temperature, salinity, dissolved oxygen, and turbidity using a multi- parameter meter, TPS brand 90-FLT. [Photo 17 & 18]. The meter was calibrated weekly or when required.

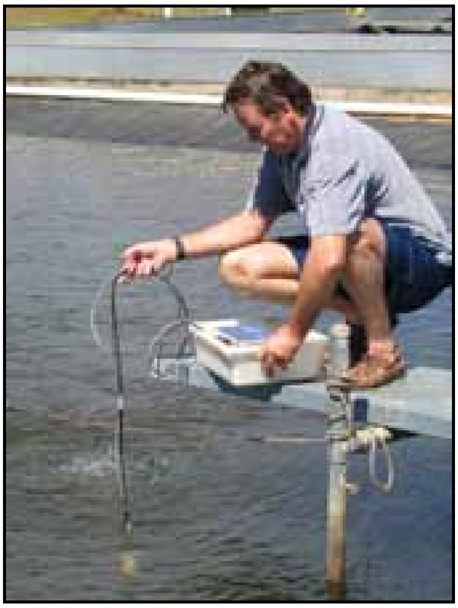


Photo 17 TPS meter **Photo 18 Technician using TPS meter**

Water chemistry samples, were collected fortnightly between 8 – 9am (after pumping has ceased) from the treatment ponds and the fish production ponds. All water samples were collected from at least 200 millimetre from the water surface. The water samples taken from the fish production ponds were collected approximately 0.5 metre away from the pump intake foot valve while the samples for the treatment pond were collected about 0.5 metre away from the outflow point. As a reference sample, a water sample was also collected from the irrigation channel at the same time as all the other samples were collected. All samples were analysed immediately after collection.

The samples were analysed for the following chemical parameters; Total Nitrogen [TN], Ammonia Nitrogen [NH_3-N], Nitrite [NO_2-N], Nitrate [NO_3-N], total Phosphate [PO_4^{3-}]) and Total Suspended solids [TSS]. Measurement of these water quality parameters was based on the Standard Methods for the examination of water and wastewater (American Public Health Association 1989).

Samples for ammonia, nitrite and nitrate were pre-filtered (0.45 micron). The chemical analyses were carried out on an Aquamate visible spectrophotometer using Hack reagents. [Photo 19 & 20]. The Aquamate is serviced every year.

Photo 19 Technician using Hach meter **Photo 20 Hach vials testing for Ammonia**

Alkalinity, total hardness and calcium hardness were measured every four weeks using Palintest reagents and analysed on a Palintest Photometer 6000. [Photo 21]. This machine is serviced every year.

Photo 21 Palin Photometer 6000

Total Suspended Solids [TSS] were also measured for each water sample. [Appendix for procedure] TSS was calculated based on 1 litre of sample water.

Water Usage

All water usage, discharge or top up volumes were measured. Water from the irrigation channel was passed through a 500 micron bag to top up the water lost through evaporation.

The only discharge throughout the entire project was when the ponds were drained to remove the infestation of invertebrates. Since this incidence was not due to water quality issues we believed that the entire project could have run without any water discharged from the system.

Industry Partner [Daintree River Barramundi]

Located at the Daintree River Barramundi, Daintree River, North Queensland.

System design

The system design at the Daintree River Barramundi (DRB) involved integrating the bioremediation system into an already existing aquaculture facility. A practical approach was taken, ensuring that a working farm could easily modify its system to include bioremediation.

The industry partner already had a large production dam, so it was decided to purpose build a bioremediation pond into the existing system. The bioremediation pond was divided into 4 with weed mat to fabricate the baffled system similar to the ones at FFAC, following the plug-flow design of a shallow rectangular lagoon. This pond differed from the FFAC ponds by the addition of bottom aeration through out the bays.

Water was pumped from the large production dam (1 hectare) [Photo22] to the top sediment pond (0.3 hectare). Water then gravity fed to the experimental production pond (0.2 hectare) [Photo 22], then flowed from the surface of this pond via an open concrete spillway to fall 15 centimetre into the bioremediation pond [Photo 22] that was planted with lotus. From this pond, water returned to the storage dam under gravity [fig 2]. Pumping was initiated daily, depending on a needs be basis. At dry times, water was pumped on a continuous basis, while during the wet season water was only pumped at night. During periods of heavy rain pumping was not necessary.

Water from the creek was used to replace losses through evaporation and water used for the mangosteen and taro crops.

Photo 22 Production dam (left), fish pond (middle) and bioremediation pond showing Lotus and Hydrilla sp. (right)

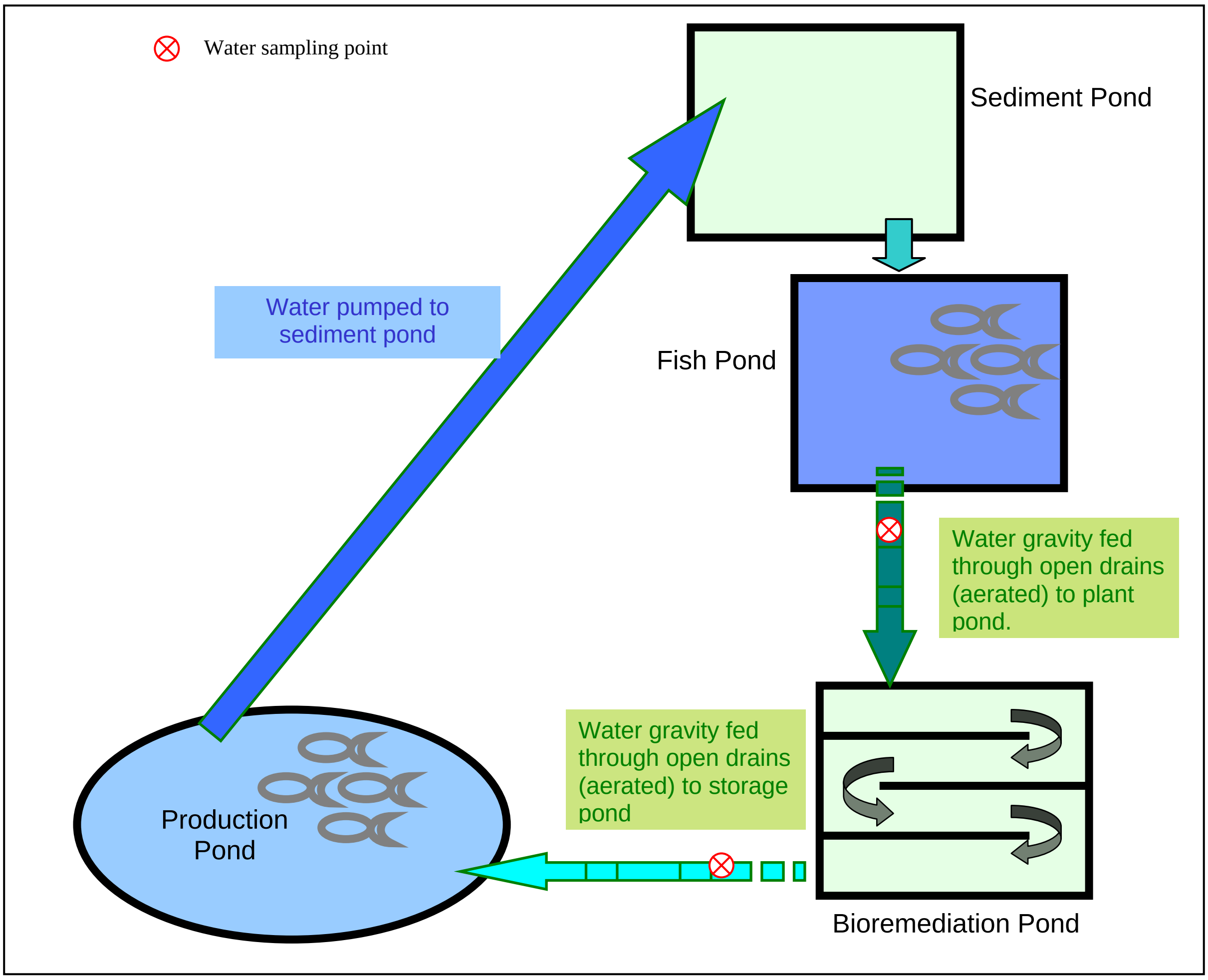


Figure 2 Schematic design of bioremediation system at Daintree River Barramundi

Lotus

The source of lotus was as for the FFAC study. DRB only propagated from seeds.

Lotus was planted in the ponds from seedlings collected from Ross River, Townsville. They were propagated as for FFAC. Plants establish quickly in the pond without the issues that FFAC experienced.

Pests were similar to those experienced at FFAC but as lotus were not in an enclosed area; birds had access to feed on the pests keeping numbers low.

Barramundi Production

The fish pond (0.2 hectare) was stocked with 1100 fish at 800 grams. The fish were harvested from cages in the large production pond and transferred to free range in the fish pond.

The fish were fed to satiation once a day in the evenings.

The fish were harvested at approximately 3 kilogram.

Water Chemistry

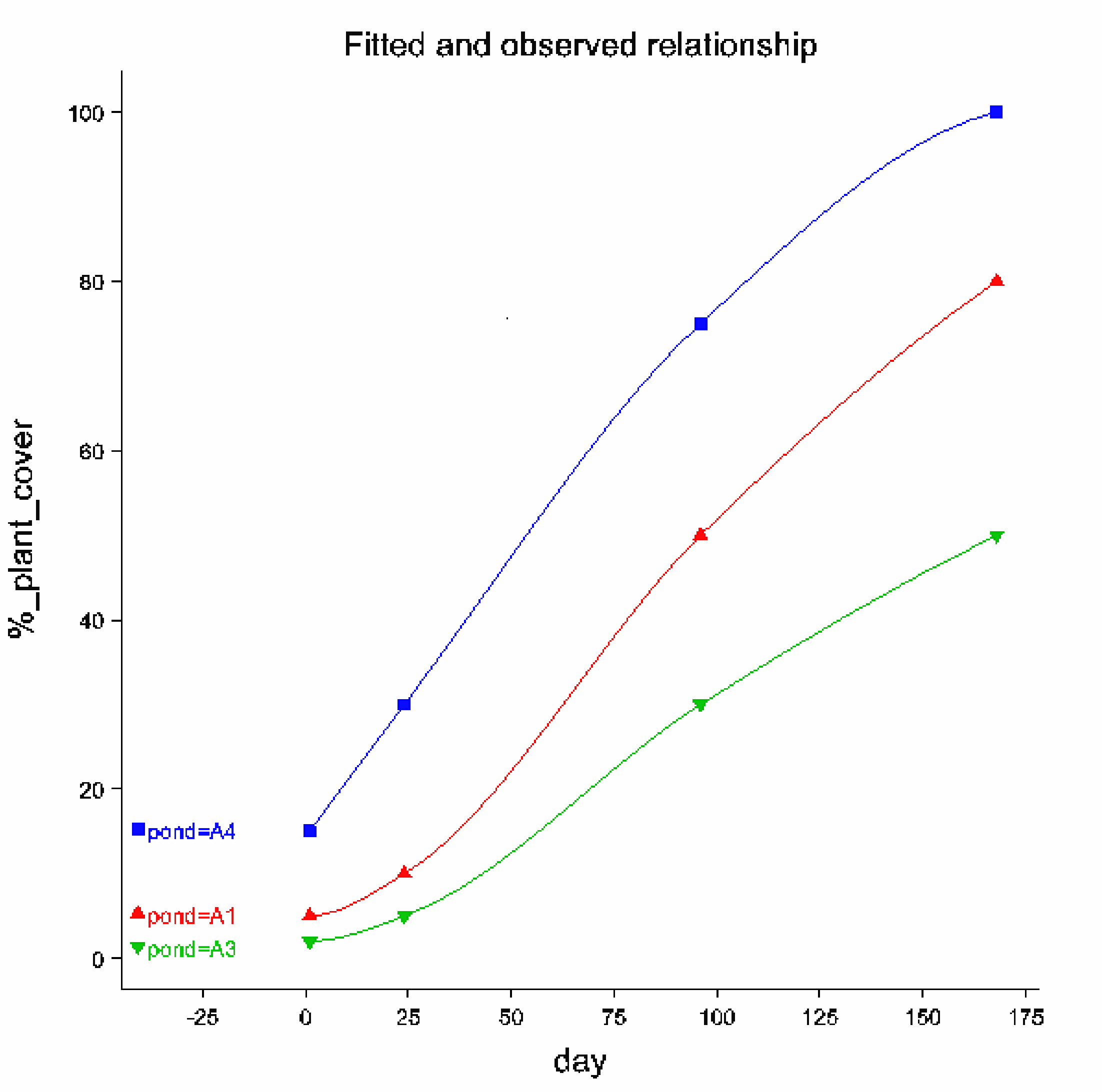
Water samples were collected fortnightly in the morning (between 7 – 9 am) from the drain exiting the fish pond, and at the drain exiting the lotus pond. Samples were collected in 2 litre bottles. They were couriered to FFAC in eskies with ice blocks to keep them cool. The samples would mostly arrive within 24 hours.

All samples from DRB were processed as for the FFAC samples (see methods for FFAC).

Results

Freshwater Fisheries and Aquaculture Centre

The growth of the lotus was initially very slow. Because of this, *Hydrilla sp.* was planted as an alternative plant to uptake nutrients and settle suspended solids from the wastewater. Both plants struggled to establish during the winter but as nutrient and water temperatures rose, both plants grew rapidly. The *Hydrilla sp.* established faster than the lotus. The lotus sent out stolons into the main water body and into the *Hydrilla sp.*, eventually shading the *Hydrilla sp.* causing it to die. The observed rate of plant growth was different between all plant treatment ponds [Graph 1].



Graph 1 Establishment of lotus cover in three treatment ponds

The regression graph above shows the growth of the lotus from the treatment ponds. It can be seen that the 3 ponds grew at different rates. After 175 days, one pond had 100 percent cover, while the other 2 pond had 80 and 50 percent coverage in the same duration.

Each pond of the plant treatment had lotus growing at different rates. Due to the differences in growth rates between ponds, and the ability of the no plant treatments efficiency of nutrient uptake there were no statistically significant differences between the treatments with or without plants.

Although there was no statistical significance between the treatments; plant or no plants, the project shows that an addition of a bioremediation pond improves the quality of the wastewater from the fish production ponds. Table 1 below, shows the non-plant systems reduced the ammonia by 46 percent while the systems with plants (lotus & *Hydrilla sp.*) removed only 28 percent of the ammonia. This could be due to the amount of phytoplankton in the water. Although, the treatment ponds with lotus removed a greater amount of total N, 32 percent compared to only 17 percent from the non-plant treatment. This could be due to the efficiency of the plant taking up the nitrogen. The plant treatment ponds also removed slightly more of the nitrite and nitrate from the fish wastewater (59 percent and 43 percent reduction) compared to the non-plant treatments (44 percent and 38 percent).

Both the treatment ponds (non plant and plant) were equally efficient in reducing the amount of phosphate (38 percent and 35 percent respectively) in the system.

Total Suspended Solids was reduced by 44 percent in the plant treatment while in the non-plant treatment it slightly increased (2 percent).

This research shows that the addition of a bioremediation pond, improves the quality of the wastewater by removing sufficient nutrients (mainly ammonia, nitrite and nitrate) to enable it to be re-used within the system. The post-bioremediation return water to the production pond was of a good enough quality to maintain production of fish. The project shows that a production run can be completed with very little water discharge maintaining the nutrient levels within the acceptable range for fish health and growth.

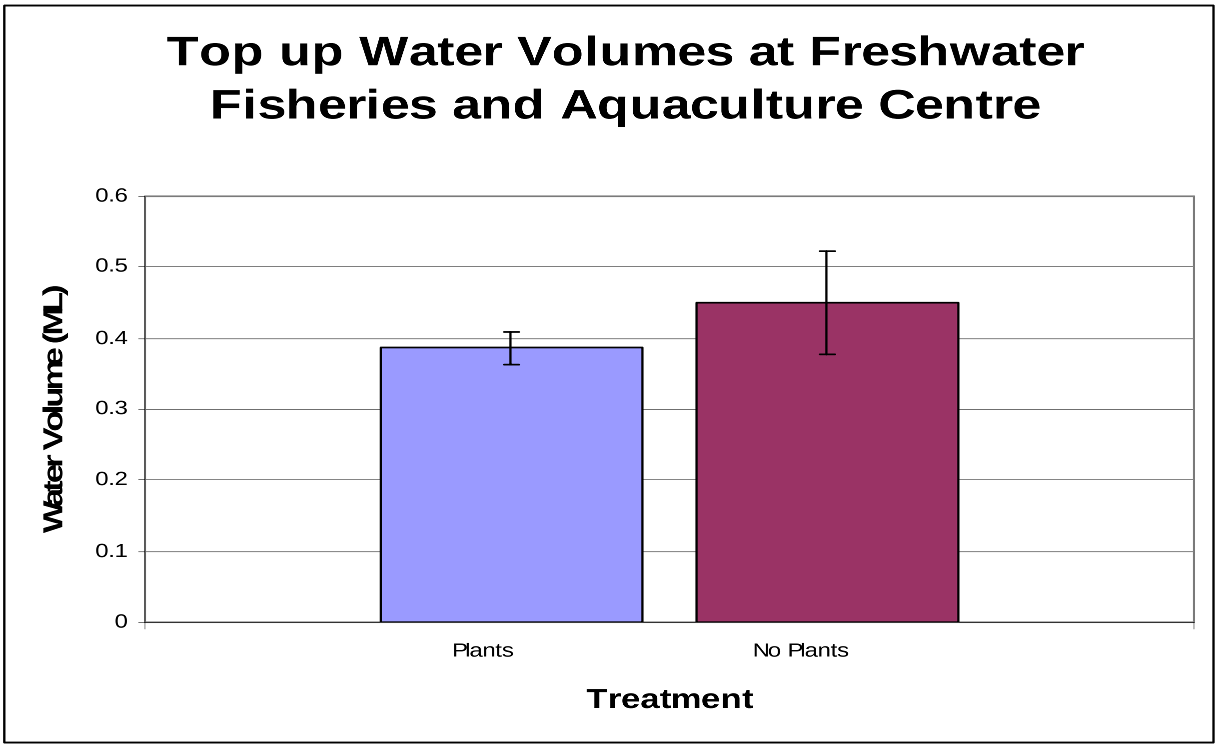
Table 1 Nutrient levels from FFAC system

	No Plant Treatment			Plant Treatment		
Parameters	Fish Pond	No Plant Pond	% RE	Fish Pond	Plant Pond	%RE
NH_3-N (mg/L)	0.4834 ± 0.64	0.2627 ± 0.48	46	0.4592 ± 0.40	0.3309 ± 0.37	28
NO_2-N (mg/L)	0.0142 ± 0.02	0.0080 ± 0.01	44	0.0551 ± 0.07	0.0275 ± 0.04	50
NO_3-N (mg/L)	0.2091 ± 0.24	0.1294 ± 0.18	38	0.6213 ± 0.58	0.3547 ± 0.35	43
N (mg/L)	5.3610 ± 3.59	4.4423 ± 3.84	17	5.9171 ± 3.79	4.0211 ± 2.75	32
$PO4^{3-}$ (mg/L)	1.4362 ± 0.51	0.8899 ± 0.47	38	1.6762 ± 0.49	1.0891 + 0.48	35
TSS (mg/L)	28.3072 ± 24.28	28.9803 ± 20.30	-2	30.8756 ± 25.62	17.4240 ± 17.61	44
Alkalinity (mg/L)	76.6667 ± 19.58	47.6000 ± 10.42	38	74.2105 ± 19.46	51.7307 ± 14.76	30
Ca Hardness (mg/L)	45.7143 ± 10.28	35.2000 ± 6.53	23	46.0526 ± 11.97	41.7308 ± 12.64	9
Total Hardness (mg/L)	72.1429 ± 19.72	38.8000 ± 9.16	46	65.5263 ± 24.03	44.4231 + 14.38	32

Note: % RE = Removal Efficiency (%)

The top-up water volumes for the lotus experiment were analysed to investigate whether the systems with no plants required higher water inputs due to increased evaporation. The ANOVA found no significant differences in water use between the 6 pond systems (p-value = 0.094), nor when plant systems were compared to non-plant systems (p-value = 0.315).

Although there was no statistically significant difference between the amount of water used to replace evaporation, raw data shows [graph 2] that the plant ponds required 14 percent less water than the ponds without plants. For a farmer this would translate into a cost saving of over 10 percent of the water bill. However, it would need to be confirmed, as the statistical test showed no difference.



Graph 1 Total water volumes used for topping up treatment systems at Freshwater Fisheries and Aquaculture Centre

There was no statistically significant difference in fish harvest yield (P>0.05) between treatments [Table 2]. The biomass from the ponds ranged from 28 tonnes per hectare to 38 tonnes per hectare after 24 months with weight ranging from 3 kilograms to 6 kilograms. The average yield at FFAC for this project was 33 tonnes per hectare. The fish from both treatments were of similar biomass, size and quality (Table 2). This is equivalent to the best performing farms in the industry.

Table 2 Final Barramundi harvest showing the average biomass in each treatment

Treatment	Total Harvest (kg)
Plant	3289 $\pm$ 160
non Plant	3493 $\pm$ 143

The fish from both treatments were sampled and tasted by buyers. The buyers who sampled the barramundi from the program were impressed with the quality of the flesh and the taste. It was commented that the flesh was white, clean and firm with a slight pink tinge, equivalent to wild caught fish. This is a positive result indicating that the fish were of an acceptable quality for market.

The food conversion rate was 1.1:1 to 1.2:1, which are equivalent to the better industry rates.

The market aspect of the lotus was a minor part of the project, and not explored to its full potential. The flowers and pods have a local potential market with the florist industry. [Photos 23 & 24] There is also potential for the sale of dried leaves. As this was secondary, we did not pursue this avenue.

Photo 23 Lotus floral display for table setting

Photo 24 Floral display using only Lotus

Field Day at FFAC

Once the project was nearing completion a field day was held at FFAC to demonstrate the application of the technologies develop within the project. The day was designed for attending farmers to interact with researchers from DPI&F and management from EPA. A tour of the project was followed by a short presentation from the project leader, a presentation from the District Manger of EPA and discussion time. An informal approach was taken to encourage discussion from all people involved. (Appendix E)

Daintree River Barramundi

Immediately after the initial planting, lotus grew rapidly in the bioremediation pond. The pond had established *Hydrilla sp.*, which was regularly harvested for mulch for the tropical fruit orchard. Once the lotus was established it out-competed the *Hydrilla sp.*, by shading it with the emergent leaves, similar to at FFAC. The lotus did not establish close to the aerated area of the pond.

From the Table 3 below it is apparent that the lotus affected the nutrient levels in the fish effluent water. The ammonia was reduced by 64 percent indicating that the system was more efficient than the systems at FFAC (28 percent), although the ammonia levels were lower than at FFAC. This could be due to the lower stocking rate. The planted pond reduced the amount of suspended solids (TSS) by 47 percent. This is compared to the TSS reduction in ponds with plants at FFAC (44 percent reduction).

Table 3 Nutrient levels from the Industry Partner system

Parameters	Fish Pond	Plant Pond	% RE
NH_3-N (mg/L)	0.1268 $\pm$ 0.104	0.0456 $\pm$ 0.044	64
NO_2-N (mg/L)	0.0040 + 0.007	0.0029 $\pm$ 0.0052	27
NO_3-N (mg/L)	0.1828 $\pm$ 0.165	0.1478 $\pm$ 0.1418	19
N (mg/L)	1.4478 $\pm$ 1.79	1.2416 $\pm$ 1.5614	14
PO_4^{3-} (mg/L)	0.4764 $\pm$ 0.150	0.3674 $\pm$ 0.133	23
TSS (mg/L)	17.2379 $\pm$ 11.623	9.0999 $\pm$ 8.772	47
Alkalinity (mg/L)	20.3571 $\pm$ 12.004	17.5000 $\pm$ 11.889	14
Ca Hardness (mg/L)	16.0714 $\pm$ 14.829	17.8571 $\pm$ 12.2025	-11
Total Hardness (mg/L)	25.7143 $\pm$ 16.392	27.1428 $\pm$ 18.157	-6

Note: % RE = Removal Efficiency (%)

From discussion with the Industry partner, less water was used in the production run with lotus bioremediating the water than in previous runs with no bioremediation. From the results of this project, they are developing a series of smaller ponds (0.25 hectare) to plant lotus in to treat the water. The development will allow a lotus pond to be taken off line for harvest while the others continue to treat the water.

The harvest of fish resulted in a first grade quality fish. Stocking density was lower than at FFAC.

The food conversion rate was 1.3:1.

The industry partner received many enquiries for lotus flowers and pods from local florists. Although no sale of lotus was achieved during the project, a lot of interest was generated from local florists and floral artists. This indicates that a niche market could be established for fresh lotus flowers and pods sales. This, however, was only a secondary aim to the primary aim of water quality improvement.

Due to the lotus not producing rhizomes, they were not analysed for heavy metals.

Implications

Industries directly benefiting from this project will be the freshwater fish production sector in the tropics. The value of freshwater pond based barramundi in Queensland was $18.5 million in 2006-07 producing 2091 tonnes. Since 2004-05 the barramundi industry has been steadily increasing.

By implementation of these water improvement techniques, a 10 per cent increase of production could be expected. This would equate to an increased value in production by 209 tonnes.

Environmental benefits from adoption of cleaner aquaculture techniques will be significant, particularly as the industry is under greater regulatory pressure to reduce water usage and discharge. The prime benefits will be from increased environmental flows and greatly reduced discharge both in terms of overall volume and nutrient outputs. Aquaculture and other water users will benefit from having a practical, realistic water management system that helps to remove excess nutrients and solids from water, reducing the overall requirement of water, by up to 10 percent, to be extracted from natural sources through ability to recycle, and provides a by-product with demonstrated value. A reduced extraction of environmental water and low return of nutrients will help maintain healthy water flows within natural water systems, helping to maintain their natural biodiversity whilst supporting an aquaculture industry.

Additionally, predator exclusion will be made possible through reduced production area and more intensive production. Currently, over 30 percent of stock is lost to bird predation alone, even when mitigation measures and management (eg stocking of large fish, net cage production) are employed.

Improved viability of fish farming enterprises through increases sustainability will enable further employment generation and cash flow in rural communities in tropical Australia. Improvement of the environmental image of aquaculture will also have a positive social impact on society’s view of the industry.

Recommendations

As this project had difficulties in establishing the lotus, the market for the flowers and pods was not investigated in detail. There was great interest from the local florists with much discussion on the potential of the flower market. The flower could be marketed to a niche clientele, for example; weddings as a single stem centre piece arrangement. The flowers are stunningly beautiful and extremely fragile. As the flower is very delicate with a very limited life (1 day) before all the petals drop off, techniques need to be developed in the handling of the flower, all the way through from picking to presentation. Initial trials indicate that under certain conditions the flower is robust and not as delicate as when roughly handled.

There could also be potential for the sale of dried leaves to Asian stores.

Establishing lotus in the ponds has been a lengthy process therefore; extension of this research would be advantageous. As there are many different varieties of lotus, comparing other varieties for example; rhizome varieties, to the tropical variety would develop more information on the efficiency of lotus to remediate nutrient loaded water. If the rhizome variety was cultured, then research in to the development of the rhizome industry could be undertaken.

Discussion with people in the aquaculture industry shows that there is an interest in looking at other types of water plants, for example; water chestnuts (*Eleocharis dulcis*), kang kung (*Ipomea aquatica*) and other Asian foods to remove nutrients

Other opportunities for new projects developing from this project would be to compare the nutrient uptake of lotus to algae eating fish such as Bony Bream (*Nematalosa erebi*) which in turn could be used as fish meal or to compare the nutrient uptake of lotus to other Asian water plants for example; water chestnut.

This type of water improvement system could be implemented into other industries that generate ‘nutrient rich’ wastewater, for example; dairy, pig, feedlots.

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Appendix A Water quality standards for Barramundi

Table A-1: Water quality standards for Barramundi

Water variants	Acceptable levels for Barramundi culture	Levels in water where fish kills have occurred
Temperature	23 – 30°C	< 18°C, (depending on age)
Oxygen	> 6ppm, up to 100%	< 3ppm,
PH	7.5 - 8.6	< 4-5, > 9-10
Ammonia (NH_3)	< 1.2 ppm	>1.2 ppm
Nitrate (NO_3)	< 1.0 ppm	>110 ppm
Nitrite (NO_2)	< 0.2 ppm	> 2.0 ppm (fresh)
Total hardness	20 - 200 ppm	> 200 ppm (CO_2 excess)
Total suspended solids	< 80 ppm	> 5000 - 100,000 ppm
Total dissolved solids	< 400 ppm	> 5000 - 20,00 ppm

Modified from Rimmer (1995) and Department of Fisheries, Government of Western Australia (2005)

Appendix B Chemicals used for Hach Meters

Total Nitrogen (mg/L)

Hach Method 10071

Nitrogen Total, Test 'N' Tube Persulphate Digestion Method (0 to 25mg/L N)

Total Phosphorus as PO4 (mg/L)

Hach Method 8190

Phosphorus, Total, PhosVer3 with Acid Persulphate Digestion, Test 'N' Tube Procedure (0.00 to 3.50mg/L PO_4)

Nitrate (NO3-N mg/L)

Hach Method 8192

Nitrate, Low Range, Cadmium Reduction Method (0 to 0.50mg/L NO_3^-–N)

Nitrite (NO2-N mg/L)

Hach Method 8507

Nitrite, Low Range, Diazotisation Method (0 to 0.300mg/L NO_2^-) Powder Pillows

Ammonia (NH3-N mg/L)

Hach Method 8155

Nitrogen, Ammonia, Salicylate Method, powder pillows (0 to 0.80mg/L NH_3–N)

Palin Test

Alkalinity (mg/L)

Ca Hardness (mg/L)

Total Hardness (mg/L)

Methodolgy for TSS

TSS (mg/L).

Glass microfiber filter papers [1 micron pore size] for each water sample was placed into a constant temperature drying oven for at least 3hrs at 105^0C, weighed. These were then cooled in a vacuum sealed container, then weighed. The water samples, up to 1L, were filtered through the paper. The filter papers with the suspended solids were then returned to drying oven for at least 4hrs. They were then removed, cooled in a vacuum sealed container and re-weighed.

Appendix C Summary of the water quality results

TableC-1: Summary of the water quality results at Freshwater Fisheries and Aquaculture Centre

Treatment	NH3-N (mg/L)			NO2-N (mg/L)			NO3-N (mg/L)			N (mg/L)			PO43- (mg/L)			TSS(mg/L)		
	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average	Max	Min	Average
Fish Pond (No Plant)	3.31	0.01	0.48	0.08	0.00	0.01	1.17	0.03	0.21	16.25	0.00	5.36	2.93	0.61	1.44	133.00	0.37	28.31
Bioremediation (No Plant)	2.40	0.00	0.26	0.06	0.00	0.01	0.94	0.02	0.13	17.16	0.00	4.44	2.22	0.30	0.89	77.00	2.50	28.98
Fish Pond (Plant)	1.67	0.01	0.46	0.29	0.00	0.06	2.55	0.02	0.62	20.44	0.00	5.92	2.95	0.75	1.68	94.00	0.24	30.88
Bioremediation (Plant)	1.44	0.00	0.33	0.22	0.00	0.03	1.93	0.02	0.35	9.15	0.00	4.02	2.26	0.23	1.09	53.33	0.33	17.42

Appendix D Lotus Field Day

Lotus Field Day at Freshwater Fisheries and Aquaculture Centre, Walkamin Research Station


Photo D-1 [left – right] Field Day at FFAC with interested farmers attending. DPI&F Fisheries Biologist and EPA manager talking with interested farmer

Photo D-2 Display board at field day

Photo D-3 Invitation to field day at FFAC

Appendix E Conferences attended

AAQ conference 2007, Childers. Presenting “Can we really produce fish with a zero discharge?”

Australasian Aquaculture Conference 2008. Presenting ‘Assessment of Lotus (Nelumbo nucifera) for wastewater bioremediation’ (Abstract below)

Title: ASSESSMENT OF LOTUS (Nelumbo Nucifera) FOR WASTEWATER BIOREMEDIATION.

Summary/Description:

There is a real constraint on further development of aquaculture in northern Queensland due to restriction of water discharge and extraction. New methods of farming aquatic organisms must be developed to improve water efficiency and permit expansion and economies of scale. Further development of the value added sector of the industry is reliant on expanding production in current production systems while maintaining or reducing discharges of wastewater. This system aims to reduce or eliminate the need to discharge water and increase the carrying capacity of farms through quantum increases in management, feeding, and harvesting efficiencies. In two year production cycle farms (those producing 2kg+ fish) there is a high economic loss of crop due to pond crashes in the second summer, when nutrients in bottom sediments are mobilised during the anaerobic conditions following thermocline establishment. Development of these farms is inhibited by inability to prevent this occurring in some situations. Lotus represents a low cost option for maintaining and improving water quality and utilising nutrients in the bottom sediments, while providing a useful secondary crop.
Lotus is identified as an Asian vegetable with export and domestic market potential. In Australia, native lotus is a purely tropical species, and no studies have been conducted into its cultivation or use as an Asian vegetable or in water treatment. This project to assess lotus as a tool for wastewater remediation in tropical fish farming has demonstrated its high capacity to reduce nitrogen and phosphorus loadings. These nutrients are responsible for blue green algal blooms and the deterioration of water quality in waterways across Australia.

It was found that established Lotus plants are very efficient at removing suspended solids and nutrients in wastewater of barramundi production ponds. The nutrients that were monitored were: Total Nitrogen, Ammonia, Nitrite, Nitrate, Total Phosphorus, Total Suspended solids and alkalinity. All nutrients were shown to be reduced from the Lotus.

This experiment has shown that lotus plants grown in production systems or partition pond systems can act as nutrient and sediment removal agents, improving the water quality through aeration and shading, while providing a useful by-product.

Assessing Lotus for Wastewater Bioremediation

by E Seymour, P Graham, C Agcopra, K Willows and B Herbert

RIRDC Publication No. 09/089

Lotus is identified as an emergent water plant with capabilities of utilising nutrients from the bottom sediments in fish ponds. This RIRDC publication assesses the efficiency of lotus to reduce the amount of nutrients generated in a freshwater aquaculture system.

Lotus represents a low cost option for maintaining and improving water quality and utilising nutrients in the bottom sediments.

The research is targeted for the freshwater Aquaculture Industry and other industries that are interested in reducing nutrients from wastewater.

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Pond showing baffles with lotus in the background

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