Duckweed-based Wastewater Treatment Systems:

Design Aspects and Integrated Reuse Options for Queensland Conditions

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Queensland Department of Primary Insustries and Fisheries, 2005

QI05019
ISSN 0727-6273

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1.0 Introduction

Point sources of wastewater pollution, including effluent from municipal sewage treatment plants and intensive livestock and processing industries, can contribute significantly to the degradation of receiving waters (Chambers *et al*. 1997; Productivity Commission 2004). This has led to increasingly stringent local wastewater discharge quotas (particularly regarding Nitrogen, Phosphorous and suspended solids), and many municipal authorities and industry managers are now faced with upgrading their existing treatment facilities in order to comply. However, with high construction, energy and maintenance expenses and increasing labour costs, traditional wastewater treatment systems are becoming an escalating financial burden for the communities and industries that operate them.

For many rural communities, the availability of low-cost land has meant that more extensive, low-energy treatment processes can be a cost-effective alternative, especially for final treatment of effluent. Lagoons are commonly used as a passive means for renovating wastewater following primary treatment, but their efficiency can vary substantially. In particular, disinfection and the removal of nutrients and algal suspended solids by many lagoons is relatively poor and highly variable. Odour production is another limitation of standard passive lagoon systems.

Enhanced lagoon systems, however, which actively promote natural aquatic processes to convert waste nutrients into benign and easily harvested forms, can provide efficient, consistent and economical wastewater treatment — with the added potential for resource recovery. Enhanced lagoon systems have been used extensively overseas. In Australia, however, uptake of these conceptually simple technologies has been slow due perhaps to a lack of local research to demonstrate their usefulness and a cultural preference for mechanical infrastructure. Queensland, in particular, is climatically well positioned to take advantage of lagoon treatment systems that use aquatic plants as productive 'sinks' for wastewater nutrients from a wide range of sources. Of these, duckweed-based treatment systems offer the most promise.

This report was generated, in the first instance, for the Burdekin Shire Council to provide information on design aspects and parameters critical for developing duckweed-based wastewater treatment (DWT) in the Burdekin region. However, the information will be relevant to a range of wastewater sources throughout Queensland. This information has been collated from published literature and both overseas and local studies of pilot and full-scale DWT systems. This report also considers options to generate revenue from duckweed production (a significant feature of DWT), and provides specifications and component cost information (current at the time of publication) for a large-scale demonstration of an integrated DWT and fish production system.

2.0 Biology of Duckweed

Duckweed species are small floating aquatic plants belonging to the botanical family *Lemnaceae*. The family consists of five genera, *Lemna*, *Landoltia*, *Spirodela*, *Wolffia* and *Wolffiella*, among which about 40 species have been identified worldwide (Les *et al*. 2002). Many of these species are cosmopolitan and are found throughout the world, although geographical variations within species have developed in response to local conditions. Several species are endemic to Queensland and can be found occurring naturally in waterways throughout the state when conditions are suitable. Therefore, they should not be considered noxious like problematic exotic species such as salvinia and hyacinth.

The natural habitat of duckweed is free floating on still surfaces of fresh and brackish waters (up to 4000mg/L NaCl according to Leng *et al*. 1995). They prefer full sunlight but can adapt well to low light conditions. While duckweed is reported to tolerate a wide pH range, different optimum pH conditi ons have been quoted that range between 4.5–8 (Skillicorn *et al*. 1993; Zirschky and Reed 1988; Leng *et al*. 1995; Caicedo *et al*. 2000; Cross 2004). A pH greater than 9.5 will inhibit growth. The optimum water temperature range for duckweed growth is between 17°C and 35°C (Iqbal 1999), which corresponds well to Queensland conditions.

Duckweed occurs naturally in water with decaying organic matter that supplies it with a constant supply of growth nutrients and trace elements. Of these growth nutrients, ammonia nitrogen in its ionised form (ammonium NH_4^+) and phosphate are the most critical (Smith and Moelyowati 2001). This preference for ionised ammonium helps explain the optimum pH range, in that at alkaline pH above 8, ammonium is progressively transformed into the unionised state (NH_3). This results in the liberation of free ammonia molecules, which are toxic to duckweed (Caicedo *et al*. 2000). When ammonium concentrations are limited, duckweed is able to utilise other forms of nitrogen (especially Nitrate NO_3) and simple organic molecules to maintain growth (Skillicorn *et al*. 1993).

Duckweed species are capable of exploiting favourable environmental conditions by growing extremely rapidly. Reproduction is primarily vegetative, in which daughter fronds bud from reproductive pockets of the mature frond. Each frond can reproduce as many as 10 times during its lifecycle (Skillicorn *et al*. 1993). Under ideal conditions of nutrient availability, sunlight, pH and temperature, duckweed plants can double their biomass every two days. This is faster than almost any other higher plants, and more closely resembles the exponential growth of unicellular algae than that of higher plants. It is this ability to propagate rapidly by consuming dissolved nutrients from the water that makes duckweed an excellent candidate for wastewater treatment.

3.0 Duckweed-based Wastewater Treatment: Processes

DWT systems operate similarly to conventional lagoon systems that can incorporate deep facultative ponds for solids removal, and stabilisation and polishing ponds for further purification. However, DWT differs from conventional lagoon systems in that they work to prevent rather than encourage planktonic algal growth. This is achieved as a duckweed mat floating on the surface simply out-shades planktonic algae (including toxic blue-green algae/cyanobacteria). The result is greater discharged effluent standards in terms of reduced total suspended solids (TSS) and nutrients. Nutrients contained in phytoplankton are difficult to harvest and are generally released back into the environment, whereas duckweed is easily harvested, which results in direct removal of nutrients from the waste stream. Inhibiting phytoplankton also regulates pH, which shows great diurnal fluctuations in traditional lagoons. In addition, evaporation from the water surface is reduced in DWT systems (Bonomo *et al.* 1997), and the increased efficiency of DWT over conventional lagoon systems means they can occupy less land area (Skillicorn *et al.* 1993).

Duckweed works to purify wastewater in collaboration with both aerobic and anaerobic bacteria. Therefore, the duckweed plants themselves should be considered as only one component of a complete DWT system (see Figure 1).

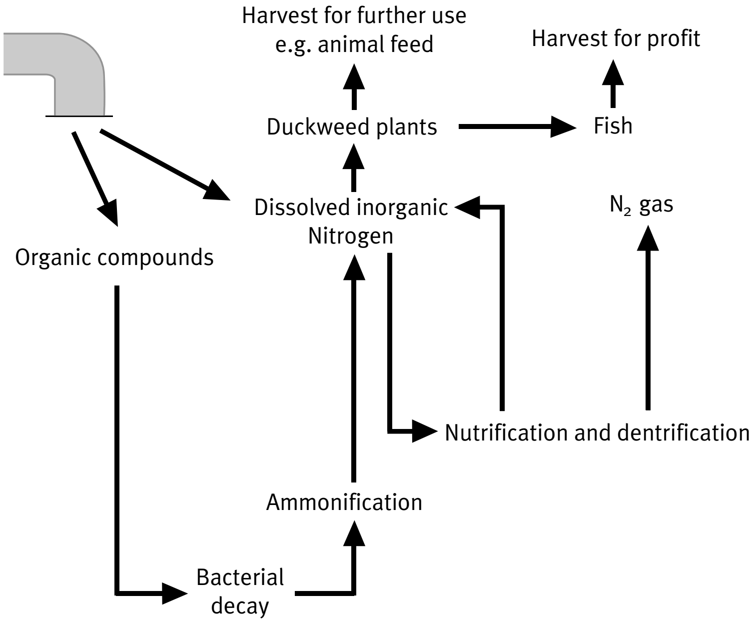

Figure 1. Flow of nitrogenous nutrients within a DWT system utilising bacterial processing and uptake by duckweed plants.

Heterotrophic bacteria decompose organic waste matter into mineral components — specifically forms of ammonia nitrogen and orthophosphates that are readily uptaken by the duckweed plants. Bacterial decomposition consumes oxygen and can cause the mid-water zone to become increasingly anoxic and the bottom of the lagoon to become anaerobic, providing further zones for specialised bacterial processing of organic matter and denitrification (Iqbal 1999; Smith and Moelyowati 2001). The duckweed mat maintains these conditions by inhibiting atmospheric oxygen diffusion at the water surface. However, a 10cm surface layer remains aerobic due to atmospheric oxygen transferred by duckweed roots (Hancock and Buddhavarapu 1993). Bacterial oxidisation of organic matter and nitrification are facilitated here, aided by the additional surface area for biofilms provided by the duckweed roots and fronds.

Other processes that aid nitrogen removal in DWT systems are sedimentation of organic matter and volatilisation of ammonia. Phosphorous is normally reduced in DWT ponds by plant uptake, absorption into clay particles and organic matter, chemical precipitation and sludge removal (Iqbal 1999; Smith and Moelyowati 2001). A dense duckweed mat has also been reported to decrease and control mosquito larvae and odour in a wastewater body by providing an interface between the water and air (Culley and Epps 1973; Iqbal 1999).

Smith and Moelyowati (2001) suggest that pathogen removal is likely to be less effective in DWT ponds than algae-based lagoons due to the absence of very alkaline conditions and less light radiation. However, this can be countered by a sufficient detention time since parasites and parasite ova precipitate with other suspended solids, and suspended pathogens simply die as a function of time (Skillicorn *et al*. 1993; Iqbal 1999). A study comparing faecal coliform counts at the Harrington, NSW, sewage treatment plant (Bell 2003) showed levels exiting the duckweed pond were significantly lower than levels exiting the control pond (46 CFU/100ml compared with 7900 CFU/100ml in the duckweed and control pond, respectively).

3.1 Effectiveness of DWT

DWT has great potential for renovating effluent from a wide variety of sources including municipal sewage treatment plants, intensive livestock industries (including aquaculture), abattoirs and food processing plants. The effectiveness of DWT depends on a system design that facilitates the correct combination of organic loading rate, water depth and hydraulic retention time. These will vary depending on the effluent source and the level of pre-treatment.

In the case where raw sewage (human or livestock waste) is to be processed, the primary treatment objective is to remove solids. This can be achieved in conventional deep anaerobic ponds that encourage the fermentation and breakdown of settled solids by bacterial processes into simple organic and inorganic molecules. Duckweed will enhance primary treatment in these ponds by maintaining anaerobic conditions and reducing odour nuisance (Skillicorn *et al*. 1993). In addition, conventional anaerobic ponds, while effective at reducing BOD, have a negligible effect on total nutrient concentrations (Caicedo *et al*. 2000), so duckweed assimilation will enhance the nutrient removal capacity of these anaerobic systems.

High levels of ammonification occur in primary treatment systems. Cheng *et al*. (2002) found that a range of duckweed species tested could tolerate and grow at high ammonium levels of 240mg/L in swine wastewater — the best performer being a Queensland native, *Spirodella punctata* (recently renamed *Landoltia punctata* — see Cross 2004). Phan (2002), however, found that duckweed may need an acclimatisation period to adapt to the very high N levels in raw agricultural wastewaters.

Most researchers, however, suggest that efficiency gains using DWT are greater in secondary and tertiary treatment of effluent where organic sludge has already been removed or converted into simple organic and inorganic molecules that can be used directly by duckweed (Alaerts *et al*. 1996; Caicedo *et al*. 2000; Smith and Moelyowati 2001; Dalu and Ndamba 2003). In the Burdekin, as with most communities in Australia, primary sewage treatment infrastructure exists to remove solids. The problems currently encountered with municipal wastewater treatment include difficulties in meeting TSS and nutrient (Total N & P, ammonia) discharge regulations. Intensive livestock and other industries that release effluent into natural waterways must comply with similar regulations.

It is in these areas that DWT is highly competitive compared with existing treatment methods. As such, this document will concentrate on system design aspects for effective secondary and tertiary effluent treatment.

3.2 DWT system design principles

There is no single 'off-the-shelf' DWT package that will serve all purposes. Requirements will vary depending on: the effluent source and volume; the level of pre-treatment; the regulated discharge quotas that need to be met; prevailing climate and financial considerations. Large-scale studies from both developing and western parts of the world have been conducted using various DWT system designs and effluent sources, but common recommended design features can be identified.

Plug-flow design

A plug-flow system is the most appropriate for secondary and tertiary effluent treatment using DWT. A plug-flow system will ensure maximum contact between wastewater and duckweed, and minimise the possibility of short-circuiting (Smith and Moelyowati 2001). This will facilitate the incremental reduction of nutrients in the wastewater. Plug-flow systems are also most efficient for pathogen removal (van der Steen *et al.* 1999).

The basic unit of plug-flow systems is a shallow rectangular lagoon. The system can operate singly or as a series of lagoons. The length/width ratio should be as large as possible to encourage plug-flow conditions (Figure 2). Alaerts *et al.* (1996) recommend a ratio greater than 38:1 although this is often difficult to achieve due to practical reasons such as cost. Bonomo *et al.* (1997) suggest a length/width ratio higher than 10:1 will suffice.

Figure 2. A plug-flow lagoon design, which prevents short-circuiting of flow between inlet and outlet, is most appropriate for DWT.

Nutrient uptake

Since duckweed will be the major nutrient sink in these lagoons, a greater biomass will inherently result in greater nutrient uptake. Greater biomass growth will occur at higher nutrient concentrations (up to a tolerance limit), but as duckweed incrementally reduces nutrients from the water, high biomass growth cannot be maintained. Since the ultimate object of treatment is to reduce nutrient concentration, duckweed starvation inevitably will occur at the latter stage in the treatment process.

In a plug-flow system, nutrient concentrations will be higher at the beginning of the effluent stream and lower towards the end. This will facilitate a 'farming' zone (high duckweed production/high nutrient uptake) and a 'polishing' zone (lower overall duckweed growth/lower nutrient uptake).
In the farming zone, where growth nutrients (N & P) are plentiful, duckweed plants are predisposed to absorb them to the exclusion of other elements present in the wastewater column (Skillicorn *et al.* 1993). In the polishing zone, however, duckweed plants starved of N and P nutrients will scavenge for sustaining nutrients. In the process they can absorb toxins and heavy metals if present in the

wastewater. This will have implications on the reuse or disposal of the harvested plants. However, since most agricultural or domestic wastewater does not contain significant concentrations of toxins or heavy metals (Skillicorn *et al*. 1993), polishing zones may simply be considered to be the latter reaches of a continuous duckweed treatment process.

Uptake efficiency

The nutrient uptake efficiency (i.e. the percentage of influent nutrient removed by the treatment) will be determined by the hydraulic retention time. While a short retention time will maintain high nutrient levels (and therefore extend the 'farming' zone), the overall percentage of nutrients removed from the effluent stream is lower. Conversely, a longer retention period will result in a greater percentage of nutrients being removed, but create a relatively less productive 'polishing' zone when nutrients become limiting. For example, the Burdekin pilot trial (Willett *et al*. 2003) tested three effluent retention times, i.e. 3.5 days, 5.5 days and 10.4 days. The relationship between total nitrogen (TN) uptake, uptake efficiency and biomass production by DWT at different retention times from this trial are given in Table 1.

Table 1. Average Total Nitrogen uptake (mg/L/day), uptake efficiency (percentage of influent TN removed by the treatment) and duckweed biomass produced (g/m²/day) at three Effluent Retention Times (E.R.T.). Data derived from Willett *et al*. (2003).

	3.5–days E.R.T.	5.5–days E.R.T.	10.4–days E.R.T.
TN Uptake (mg/L/day)	1.66	1.16	0.77
TN Uptake Efficiency (% removal)	37.55	55.67	70.35
Biomass Produced (g/m²/day)	190	159	151

Overall retention time required in a DWT system will vary depending on a range of factors including the influent nutrient levels, temperature and the discharge standards that must be met. In general, 20 days hydraulic retention time would appear to be a minimum guideline for DWT to achieve acceptable discharge standards and pathogen reduction in municipal sewage treatment (Skillicorn *et al*. 1993).

Retention time is in turn, a function of water depth and flow rate. Shallow ponds are better than deep ponds, but the trade off is the increased land area required and the lack of temperature buffering with shallow ponds. Water depths between 0.6m and 1.5m have been suggested as the most suitable for large-scale DWT systems (Skillicorn *et al*. 1993; Smith and Moelyowati 2001). A horizontal plug-flow velocity up to 0.1m/sec will prevent disturbance of the duckweed mat (Edward 1992). Therefore, based on the daily volume of effluent to be treated, the required retention time, and the above plug-flow and depth specifications, overall pond dimensions can be calculated.

3.3 Recirculating systems

For freshwater and low salinity aquaculture operations, recirculation of water through DWT offers a number of advantages. It reduces the need for recurrent extraction of water from the environment, and helps farmers meet regulated discharge quotas — issues that are currently limiting farm production in some areas of Queensland. Reducing or minimising water discharge, and improving effluent quality through reduction of nutrient levels and suspended solids are now seen as priorities by the larger sectors of the aquaculture industry for maintaining their reputation as 'clean and green' producers.

For aquaculturists, managing ammonia levels is critical for maintaining cultured species in healthy condition so DWT must be able to reduce dissolved ammonia concentration to safe levels prior to recirculation. The treatment area required for effective DWT can be calculated based on the efficiency of ammonia removal. Smith and Moelyowati (2001) have proposed the following formula to calculate ammonium removal efficiencies:

$C_e = C_i 0.64\, e^{-Kn.t}$
with the rate constant (for depths >0.6m) $K_n = 0.137(1.009)^{T-20}$
where C_e is the effluent ammonium level; C_i is the influent ammonium level;
T is temperature (°C) and t is retention time (days)

This formula can be rearranged so that target effluent ammonia levels can be inputted and retention time (t) is unknown:

$$t = \frac{\mathrm{Ln}(C_e / 0.84585\, C_i)}{-K_n}$$

Thus, the retention time required to reach desirable ammonia levels can be calculated. Based on this retention time and the daily volume of water to be treated, treatment pond dimensions can be calculated.

For DWT systems that include primary treatment of raw wastes, Nitrogen levels (ammonia in particular) may be too high for duckweed to grow optimally at the beginning of the effluent stream. This can be remedied by recirculating a portion of the final treated effluent to dilute nutrient levels and achieve greater overall nutrient recovery. This will also have the effect of further reducing pathogen counts in discharged water by dilution. According to Skillicorn *et al.* (1993) recirculation should be considered when BOD_5 is greater than 80mg/L in the first DWT lagoon.

For treatment systems that require recirculation, an additional design aspect is that start and end points of the plug-flow should be at a proximate location so that pumping distance is minimised (e.g. U-shaped or serpentine plug-flow. See Figure 2).

Recirculation is also recommended in systems where the primary aim of DWT is to produce duckweed biomass for further use. These systems would incorporate multiple inlets designed to distribute high nutrient levels along the lengths of ponds to increase the 'farming zone' area. Nutrient removal efficiencies (overall percentage of nutrients removed) are decreased, however, in these systems and a polishing zone would still be required if it is necessary to discharge low nutrient water. Therefore, land requirements would be greater than for a plug-flow DWT system.

3.4 Crop management

Managing the duckweed crop for optimal production and nutrient recovery is aimed at maintaining a steady state system at the highest productive duckweed density. An optimum standing crop density is a complete cover (to block phytoplankton) that still provides enough space to accommodate further growth before over-crowding reduces the biomass production. Skillicorn *et al.* (1993) used a base stocking density of 600g/m² (wet weight) in their Bangladesh DWT system to yield daily incremental growth of between 50 and 150g/m^2/day. In the pilot DWT system in the Burdekin (Willett *et al.* 2003), a base density of 1kg/m^2 equated visually to a complete coverage of the surface by a single layer of duckweed fronds. This yielded on average 175g/m^2/day of duckweed biomass.

Note: duckweed biomass can be measured by placing a known-size floating quadrat (e.g. 0.25m^2) on the culture plot, and dip netting out all of the duckweed within the quadrat. Standardised wet weights of harvested duckweed can then be measured by hand-squeezing out excess water within the net, weighing and converting to g/m^2.

Seeding, distributing and containing duckweed plants

Seed stock should be taken from all available native species of duckweed growing in the same region as the DWT system, to ensure the plants will be well adapted to the local climatic conditions. Polyculture of duckweed species is an advantage to maintain optimum production because seasonal variations can favour growth of one species over another at different times. A newly seeded crop may take some time (one week or more) to acclimatise to the new nutrient conditions in the DWT lagoon before significant growth is visible.

Since duckweed plants float on the water surface, they are susceptible to movement by the wind. High winds can pile duckweed into thick mats on the downwind side of lagoons, reducing the effectiveness of DWT. Floating containment grids on the lagoon, however, will prevent this and ensure the efficient distribution of plants across the entire growing surface (see Photo 1). The size of the grid system is determined by the ambient wind conditions and the projected flow rate, but common cell sizes range between 16m^2 to 50m^2 (Skillicorn *et al.* 1993; G. Pollard pers comm.; R. Bell pers comm.).

Photo 1. Harrington Sewerage Treatment Works, NSW: 0.7 ha DWT lagoon showing floating grid network for containing duckweed.

A number of suggestions have been made on the choice of containment system, ranging from bamboo, sealed PVC or polyethyline pipes, to purpose-built UV resistant extruded plastic baffles. The grid is anchored at certain points in the lagoon and individual baffles are loosely connected to each other by wire (or similar) so that duckweed plants can slowly disperse between grids where they intersect. This ensures the even distribution of biomass across the pond. Choice of containment system is a function of material cost, robustness and its compatibility with harvesting equipment.

Harvesting

Biomass production is positively correlated with crop density up to a limit when overcrowding starts to inhibit reproduction. Harvesting is required to return the crop to the optimal base density prior to overcrowding. The frequency of harvesting will therefore depend on how fast the duckweed plants are growing. This will be site specific and dependant on localised climatic conditions, nutrient levels and species. Theoretically, daily harvesting of incremental growth is ideal but not practical in terms of labour requirements and cost.

The pilot trial in the Burdekin (Willett *et al.* 2003) demonstrated that the duckweed biomass could more than double from the base density (1kg/m^2) before overcrowding significantly slowed growth. On average, biomass doubled every 5.7 days. The weekly scheduled harvests in the Burdekin study appeared to be an appropriate compromise between labour constraints and biomass production. In cooler climates, the doubling rate is likely to be less, so less frequent harvests would be required.

Because duckweed floats, harvesting can be done by netting or scooping duckweed from the pond surface, or by pushing duckweed over a spillway onto a collecting pan using booms or mechanical devices. Netting and scooping is labour intensive and more suitable to smaller operations or plug-flow ponds that are narrow enough to provide access to the whole duckweed crop from the perimeter of the pond. Larger, broader ponds require a self-propelled craft that essentially 'bulldozes' the duckweed plants towards the pond perimeter where it piles and can be scoop-harvested from the pond (as per current practice), or theoretically pushed over a spillway onto a collecting pan.

A mechanical harvester developed by Bio-Tech Waste Management is shown in Photo 2. The harvester is manufactured with aluminium to keep the weight to a minimum. It has two hulls, both of which are 3.2m in length and 0.3m in width. The harvester is 1.8m in width and is powered by a 5.5-hp petrol engine and uses four ventures to propel it through the water. There are no propellers, and the harvester is able to ride over, or rather submerge, the floating barriers as it passes. The duckweed is collected between the two hulls and moved to the holding area at the point where the duckweed is removed from the pond. A hand-held remote control unit controls the harvester. Bio-Tech Waste Management estimate that four to six hours would be required to harvest the duckweed from a pond with a surface area of 5000m^2 (R. Bell, BTWM, pers comm.).

Photo 2. Remote controlled and venturi propelled mechanical duckweed harvester by Bio-Tech Waste Management (*photo courtesy Robert Bell*).

Preventing duckweed escape

Restored effluent should be released from treatment lagoons without inadvertently releasing duckweed into receiving waterways. A discharge pipe that incorporates a barrier skirt will prevent the duckweed escaping from the pond by evacuating water from mid-way in the water column (see Figure 3).

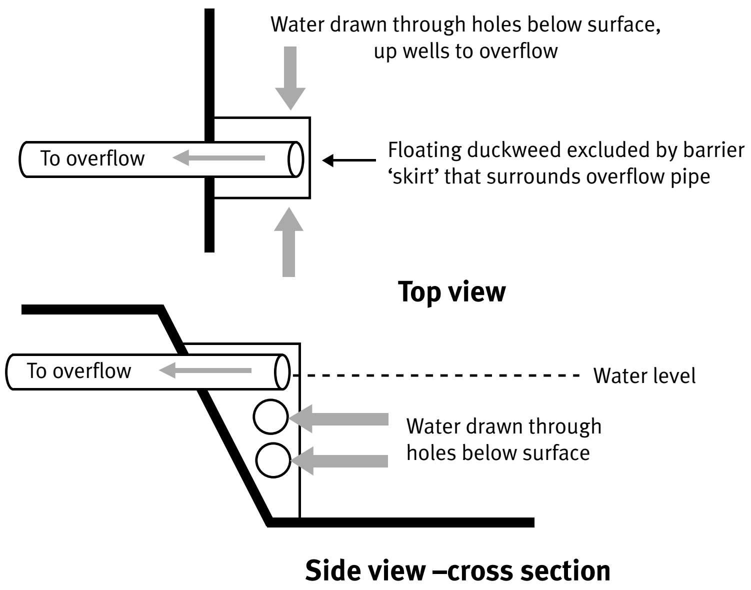

Figure 3. Floating duckweed plants in the DWT lagoon are excluded from entering the discharge pipe by a barrier 'skirt'.

3.5 Retro-fitting existing lagoons with DWT

Since many small communities and intensive livestock and processing industries currently use passive lagoon systems for wastewater treatment (either primary and/or subsequent treatment), the existing infrastructure can be retrofitted for DWT for greater efficiency. Most existing lagoons would already be of an appropriate depth and capacity. While these existing lagoons may not have been constructed to the ideal plug-flow specifications, baffles placed in the lagoon can direct current and prevent short-circuiting. Floating containment grids can be configured to suit any shape pond. Modifications may be required to the spillway or discharge point to facilitate harvest of the duckweed and prevent inadvertent release of duckweed to the environment.

3.6 Existing DWT installations in Australia

Harrington Sewage Treatment Works

Harrington township is situated on the mid-north coast of NSW. The Harrington Sewage Treatment Works (STW) uses a Pasveer Channel system for primary effluent treatment followed by secondary treatment through two clarifiers. This system is very efficient at removing organic solids and nitrifying inorganic ammonia. Therefore, discharged TKN (including ammonia nitrogen NH_3) is low from this system, and most of the remaining nitrogen is in the form of nitrate (NO_3). A DWT system (by BioTech Waste Management) has been installed on two existing maturation ponds with the intention of reducing discharged total nitrogen (TN), total phosphorous (TP), suspended solids (TSS) and faecal coliforms to state standards. The two maturation ponds are 0.75ha and 0.65ha, respectively, with a 1.5m depth (Photo 1).

With the lack of ammonium in the effluent, the duckweed successfully uses nitrate as the chief growth nutrient. Monitoring of this system has demonstrated that DWT restores effluent beyond the standard required by EPA for all measures (Bell 2003). Because of the combination of low NH_3 in the effluent and the cool winter climate at Harrington, duckweed growth is slow through most of the year and as such, is rarely harvested from the STW. However, the duckweed mat inhibits algae growth and maintains low TSS discharge, which was unable to be achieved prior to DWT.

Ventura Vineyard Lagoon

The Ventura Vineyard is located in the Adelaide suburb of Virginia. It utilises a water storage dam (0.2 hectare; av. Depth 4.0m) for crop irrigation. The dam draws water from the discharge of the Bolivar STW some 20 kilometres away. The water generally has a TN level of 10mg/L and a TP level of 0.5mg/L when it leaves the STW.

The vineyard dam has a history of dense algal blooms, which impede irrigation by blocking the filters delivering water to the drip irrigation system. A DWT system was installed to eliminate the algae in the water, which it successfully did within several weeks of being installed. The water was analysed in late December 2003 and the suspended solids were <3mg/L and the water was crystal clear (Bio-Tech Waste Management data). Since the objective of this DWT installation is to maintain very low levels of suspended solids, the duckweed is not harvested. Another objective is to reduce evaporation, which is achieved by the maintenance of the duckweed mat over the surface of t he water.

The level of water in the dam can rise and fall by up to a metre during a 12-hour irrigation period, so the DWT system was installed allowing the floating barriers and duckweed to rise and fall with the water level (Photo 3).

Photo 3. Ventura Vineyard irrigation lagoon, Adelaide. The objective of DWT on this lagoon is to maintain very low levels of suspended solids for the drip irrigation system and to minimise evaporation (*photo courtesy Robert Bell*).

4.0 Integrated Reuse Options for DWT

One of the most important benefits of DWT is that the harvested duckweed can be a valuable by-product. Nutritionally, duckweed can be substituted for soy meal and fish meal in a variety of products. Duckweed plants grown in the 'farming' zone of a treatment lagoon will have the highest nutritional value and can contain up to 45% crude protein by dry weight, with higher concentrations of essential amino acids than most plant proteins. Duckweed meal not only has a low fibre content but high levels of vitamin A and pigment, particularly beta-carotene and xanthophyll. This make it an especially valuable and proven food source for cultured animals such as fish, ducks, chickens, pigs and ruminants (see Skillicorn *et al*. 1993; Ogburn and Ogburn 1994; Bio-Tech Waste Management 1998; Iqbal 1999; Landesman *et al*. 2002, Willett *et al*. 2003).

While it is evident that duckweed has the potential to become a major protein commodity, this may not be fully realised until it can be economically reduced to a dried, easily handled product. This is because the fresh harvested material is difficult to store and transport. In addition, since fresh duckweed contains around 92% water, the relative concentration of nutrients available to grazers is lower than in the dried meal. If duckweed could be economically dried to preserve its nutritional value, and pelletised into a stable form that could be easily stored, it would offer a locally produced, environmentally friendly, fishmeal-free Ag-feed that could be applicable to a wide range of intensive livestock systems.

Various desiccation methods have been used on experimental scales to validate the performance of dried duckweed meal in livestock diets, ranging from convection and solar drying to microwave technologies (Skillicorn *et al*. 1993; Bio-Tech Waste Management 1998; and others). However, to date no large-scale drying facility has been developed that is both economical and able to preserve the nutritional value of the duckweed (lengthy exposure to direct sun-drying degrades beta carotene concentration — Skillicorn *et al*. 1993). Further research in this area is warranted.

There remains scope for utilising fresh harvested duckweed as a livestock feed (or feed supplement) in Australia, however, this will generally be restricted to operations in close proximity to where the duckweed is farmed. Such integrated operations could include dairies, piggeries, cattle and sheep feedlots, and aquaculture farms. A RIRDC study in Australia has already confirmed the palatability of fresh duckweed by a range of livestock and the production gains that can be achieved using duckweed as a protein substitute (Bio-Tech Waste Management 1998).

4.1 Integrated DWT and fish production

Of particular interest to the current study is the integration of DWT with fish production. This is because fish production can be integrated with existing water treatment infrastructure without significant extra land requirements, and the Queensland climate is favourable to both DWT and fish production. Such integration may contribute to cost recovery of the wastewater treatment process or help glean environmental benefits using aquaculture. Conversely, dedicated aquaculture operations can utilise DWT to minimise the environmental impacts of the operation and improve profits by substituting duckweed for commercial diets.

The value of duckweed as a commercial food source for Australian cultured fish has not been realised despite ample overseas experience in feeding duckweed to cultured fish, as a low-cost supplement in commercial diets for both tilapias (Hasen and Edwards 1992; Skillicorn *et al*. 1993; Fasakin *et al*. 1999; El-Shafai *et al*. 2004) and channel catfish (Robinson *et al*. 1980). It is also a sole source of feed for carp polyculture (Skillicorn *et al*. 1993).

The Burdekin trial (Willett *et al*. 2003) identified a local aquaculture species, Jade Perch (*Scortum barcoo*) that may be reared on duckweed-supplemented diets. In that trial, jade perch were grown in-line of the municipal effluent stream, post tertiary treatment. The fish actively consumed and gained weight (average weight gain: 0.7g/day/fish over 102 days) solely on fresh harvested duckweed. Survival was 100%. These results were supported by a local aquaculturist who rears jade perch on feed supplemented by 60% homegrown fresh duckweed with no reduction in growth rate (G. Pollard, pers comm.) (Photo 4). This significantly reduces grow-out costs. These findings suggest a more formal feeding trial is warranted to quantify the value of duckweed as a substitute or supplemental food source for jade perch. In addition, Fletcher and Warburton (1997) have found that decomposed *Spirodela* duckweed is as effective as commercial pellets as feed for cultured redclaw crayfish, *Cerax quadricarinatus*. This has positive implications for the local redclaw industry.

Photo 4. Jade perch and redclaw crayfish production pond in Mackay. Duckweed is cultivated over the pond surface to reduce nutrient levels and regulate water quality. Fresh duckweed is harvested daily and placed into floating fish pens (far end of the pond) to feed jade perch. Redclaw are grown underneath the duckweed mat (*photo courtesy of G. Pollard*).

The greater potential for duckweed-based diets, however, is beyond only those aquaculture species that will consume it unprocessed. For example, large aquaculture industries in Queensland include barramundi and penaeid shrimp; however, duckweed would need to be processed into a more suitable form since these species will not consume fresh plant matter. For crustaceans in particular, the abundance of carotenoids and pigments in duckweed can stimulate growth (in Landesman *et al*. 2002). Little work, however, has been done to test the effectiveness of duckweed as a feed ingredient for marine and freshwater species that do not accept the fresh plant matter. This is again owing to the lack of processing technology.

Fish production criteria

If fish production is to take place in the final stage of an effluent stream, i.e. post DWT, water quality parameters must be maintained within the tolerances of the cultured species. Poor water quality can result in loss of production by impairing development and growth, with the most extreme result being death. The effect of water quality will vary from species to species, and larval and juvenile stages may have lower tolerance levels than adult stages. Besides fish health, assured safety of the cultured stock for human consumption is critical. Low levels of water contamination may cause no obvious adverse effects but gradually accumulate in the fish to the point where it poses a potential health risk to human consumers.

The ANZECC (2000) Guidelines for Fresh and Marine Water Quality have provided general water quality values for physio-chemical parameters and toxicants to be applied to aquaculture operations. These will provide a guide to water quality levels that need to be achieved if fish culture is to be incorporated into the treated effluent stream. EPA Wastewater Reuse Guidelines (2004) have designated classes of recycled water for use in Queensland based on health risk. Non-human food chain aquaculture (for ornamental or pet food products) could use Class C recycled water, while human food chain aquaculture should only take place using Class A+ recycled water. Reference to these guidelines should be made to ascertain whether treated effluent meets these water quality criteria before an in-line aquaculture operation should be considered.

The above criteria refer to contamination levels in the actual water. If these criteria are unable to be met, fish production is still feasible at adjacent sites where clean water is available, using duckweed harvested from the effluent stream as a food source. While public health considerations are still critical, the threat of transferring pathogens or other toxins from the effluent is greatly reduced by dilution. Further, the best quality duckweed (highest nutritional value) comes from the 'farming' zone of the DWT. The predilection of duckweed in this zone, to exclusively uptake growth nutrients over other contaminants, enables their safe utilisation (Skillicorn *et al.* 1993). Haustein *et al.* (1987) have shown that repeated testing of duckweed samples harvested from nutrient rich urban wastewater has consistently failed to find any heavy metals or known toxins in concentrations approaching USFDA (United States Food and Drug Agency) food standards prohibiting human consumption.
Likewise, in the Burdekin study (Willett *et al.* 2003), semi-quantitative analyses of fresh harvested duckweed showed no residual elements from the effluent stream that were greater than ANZECC (2000) toxicant guidelines proposed for aquaculture.

Duckweed plants, as a function of their surface area, accommodate attached pathogens from the wastewater. As such, pathogens will inevitably be harvested along with the crop. When fresh plants are fed to fish, dilution of pathogens will occur in the fish pond. Surviving pathogens consumed by fish will be digested in their guts (Skillicorn *et al.* 1993). In instances where plants are processed and dried, desiccation will further destroy pathogens. Haustein *et al.* (1987) found that no viable human pathogens could be cultured from dried sewage-grown duckweed in four years of testing.

While this demonstrates potential for large-scale fish production using effluent-grown duckweed, public assurance is paramount for wide acceptance of food cultured using by-products from the effluent treatment process.

5.0 Proposed Demonstration System Design — Integrated Treatment and Fish Production

Wastewater managers in the Burdekin Shire Council (BSC) are, at the time of this publication, investigating options for tertiary treatment of their municipal effluent, as part of an on-going regional effort to reduce environmental nutrification and comply with new EPA regulations. Results from the Burdekin pilot trial (Willett *et al.* 2003) suggest that a DWT system may be one viable option based on local climatic conditions, land availability and the cost-effectiveness of installation and management.

This section was, in the first instance, prepared for the BSC to build on knowledge gained from the pilot research, to develop a demonstration system design for large-scale duckweed polishing of secondary treated effluent at the Ayr Sewage Treatment Plant using commercially available DWT system components (supplied by Bio-Tech Waste Management, Armidale, NSW. BTWM are sole commercial suppliers of these components in Australia). Relevant aspects of this section have been retained in the current general access publication to provide guidelines for those considering DWT for other industrial applications as it provides lagoon specifications and component costs (accurate at the time of publication). The proposed research addresses the feasibility of integrating fish production within the treatment protocol, by evaluating water quality and the use of harvested duckweed as both a fresh and dried feed supplement for jade perch (*Scortum barcoo*) and redclaw crayfish (*Cerax quadricarinatus*).

5.1 Objectives

1. Determine on a large scale using DWT, the quantity of Nitrogen and Phosphorous that can be removed from secondary treated wastewater at the Ayr Sewage Treatment Plant in a dedicated treatment lagoon. Implicit in achieving this objective is to build on pilot-scale results to determine (a) the most appropriate effluent retention time required to meet EPA discharge standards and water quality guidelines for in-line aquaculture, and (b) nutrient uptake rates and efficiencies.

2. Demonstrate an effective large-scale duckweed harvesting protocol from the treatment lagoon. This includes evaluating harvest frequencies, the use of a mechanical harvester and a harvest spillway system built into the banks of the pond. This spillway system has not been used before because DWT systems installed to date in southern states have not required frequent harvesting; however, the use of spillways is thought to be a more cost effective method of removing the duckweed from the pond surface.

3. Demonstrate the use of harvested duckweed as both a fresh and dried feed supplement for jade perch (*Scortum barcoo*) and redclaw crayfish (*Cerax quadricarinatus*). This includes determining (a) the protein content of dried duckweed, and (b) its suitability and the best inclusion rate of duckweed in diets of these species based on growth and survival rates, and cost of production. Harvested duckweed will also be analysed to evaluate its safe use (e.g. for elemental residues and microbial contamination).

5.2 Methods

Tertiary Treatment Lagoon Specifications

The dimensions of the proposed duckweed wastewater treatment pond are 100m × 50m (total surface area = 5000m^2 = 0.5 hectares) × 1.5m deep. The total holding capacity of the pond will therefore be 7.5 ML. The inlet and outlet will be at opposite ends and a HDPE baffle with an opening on one side will divide the pond in half to prevent short-circuiting of flow. This will effectively create two smaller interconnected ponds of 50m × 50m (See Figure 4). The HDPE baffle will have a large sleeve at the top to house floating logs while the bottom will have a smaller sleeve to hold steel weights. The floating logs and steel weights will ensure the baffle sits upright in the pond, and a nylon rope through the top sleeve will be anchored on opposite banks to secure the baffle.

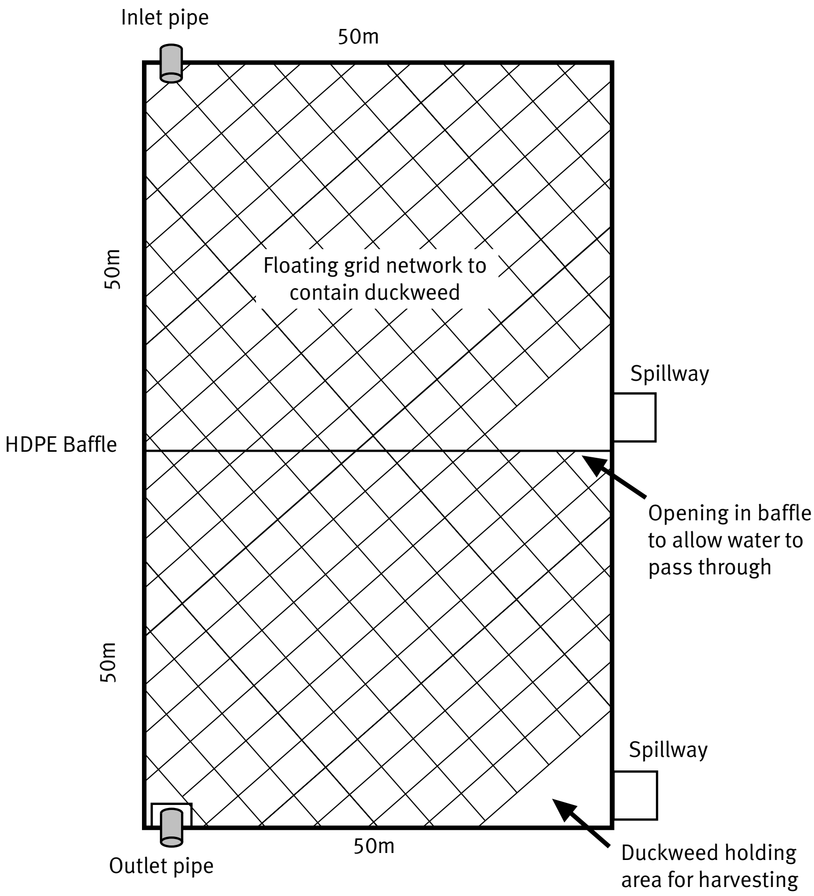

Figure 4. Schematic of proposed DWT lagoon at the Ayr Sewage Treatment Plant.

Apart from preventing short-circuiting of flow, the HDPE baffle will divide the 'farming' zone from the 'polishing' zone, so evaluation of nutrient uptake, duckweed growth and quality can be compared between treatment areas. Based on the Burdekin pilot trial (Willett *et al.* 2003), an average duckweed harvest of 6150kg/week (wet weight) could be expected from a treatment lagoon of this size. Floating barriers will be installed on the pond in a grid-pattern to contain and distribute duckweed. Local species of duckweed will be stocked to achieve a base density of 1kg/m^2.

Effluent Retention Time

An initial effluent retention time (ERT) of 21 days will be trialled, based on recommendations in the literature for nutrient and pathogen removal. With the pond's holding capacity of 7.5ML, the flow of the treated effluent from the sewage treatment plant should be 357 000L per day. This equates to the quantity of wastewater produced daily by a population of 1785.

If the quality of the wastewater exiting the duckweed pond exceeds standards required to meet EPA quotas and water quality guidelines for in-line aquaculture, the flow at the inlet will be increased thereby reducing the ERT. Likewise ERT will be increased if the quality of the water exiting the pond does not meet the required standards.

Nutrient Monitoring and Duckweed Evaluation

Effluent will be sampled weekly according to standard methods (APHA, 1989) for dissolved and total nitrogen and phosphorous content. Samples will be taken from incoming and discharged effluent from the lagoon. Samples will be stored at –18°C until collected and analysed. Samples of duckweed from 'farming' and 'polishing' zones will be dried to determine moisture content and analysed for dry-weight nutrient content (Total N and P). All nutrient analyses will be conducted on a Lachat QC8000 flow injection analyser at BIARC according to standard methods (QuikChem methods, 1996).

Samples of duckweed will be sent for analysis to evaluate its safe use (elemental residues by Australian Government Analytical Laboratories; microbial analyses by Centre for Food Technology).

Other water quality variables such as water temperature, pH and dissolved oxygen (DO) will also be recorded routinely from the treatment system.

Harvesting Protocol

Developing a harvesting protocol includes calculating duckweed nutrient uptake rates and efficiencies in order to determine the most appropriate harvest frequency. Guidelines will also be documented for the use of a mechanical harvester and a harvest spillway system built into the banks of the pond. The duckweed pond will have two spillways: one in each zone (see Figure 4). The two spillways will streamline harvesting, as the duckweed will not have to be moved as far to a harvesting area. Each spillway will be connected to a sealed collecting pan where the duckweed can stand for a day or two to start to dewater. The spillways will each have a sliding 'gate', which will stop the water in the pond escaping while the duckweed is not being harvested. Duckweed is 92% water and tests have shown that it loses up to 50% of its water content in the first two days of being out of water (R. Bell, BTWM, pers comm.). After the second day from harvesting it will be much lighter and easier to handle and transport.

Integrated Fish Production

The fish production component of the trial will be conducted at the Bribie Island Aquaculture Research Centre (BIARC). This will allow us to first determine whether the DWT in Ayr can meet specific water quality conditions required for aquaculture, prior to installing the in-line infrastructure for fish production. It will also allow us to cost-effectively conduct and closely monitor feeding trials on jade perch and red claw crayfish at the dedicated aquaculture facilities at BIARC.

Duckweed will be regularly harvested from the DWT lagoon at the Ayr STP, dewatered on the collecting pan for two days, and small quantities will be packed in polystyrene boxes for courier transportation to BIARC. Investigating feasible drying methods will be part of this research, and focus on low-cost solar radiation. Dried meal will be pelletised at various sizes using a commercial extruder and established techniques.

Jade perch fingerlings will be purchased and housed within cages in the existing freshwater recirculation tank at BIARC. Diets (fresh and dried duckweed and commercial pellets as control) will be assigned to cages in a replicated randomised design, and after a two-week acclimatisation/weaning period, feeding trials will commence. Growth, survival and feed conversion rates and general fish health will be assessed during an 8-month trial. This information will be used to establish (and report on) the feasibility of duckweed-based aquaculture diets using wastewater-grown duckweed. Feeding trials on redclaw crayfish will follow a similar protocol.

Pending positive results from the feed trials and water quality analyses from the DWT lagoon, jade perch and redclaw production could then be integrated in-line at the Ayr STP, in an aquaculture production pond that receives water from the DWT lagoon. This has not been budgeted for in the current proposal; however, the suggested specifications for the aquaculture pond are 50m × 40m (surface area: 2000m^2) × 2m deep. Commercial floating raceways installed into the pond are the most efficient units for fish grow-out, and each 14m^3 unit has a production capacity of up to 1200kg of fish. The redclaw will live on the bottom of the pond in appropriate artificial habitats (hides).

5.3 Required budget

The following budget (Table 2) incorporates costs (where known) to build and operate the demonstration DWT lagoon system according to the above specifications at the Ayr STP, and to conduct feeding and growth trials of jade perch and red claw crayfish at BIARC. The project will be scheduled to run for 24 months, which includes system construction, evaluation and reporting. QDPI in-kind salary and staff travel expenses have been omitted from the budget in this current document.

Table 2. Budget details.

	BSC In-kind	New Funds
Salaries	0.4 staff required for weekly duckweed harvest and handling, collection of water samples	**$26 370** (TO2-4 temp — 50% for 12 months. To conduct feed trials at BIARC)
Operating		**$32 500** BIARC feeding trials and water quality analyses (extruder hire; drier materials; transport of Ayr duckweed to BIARC; fingerlings/control feed purchase, nutrient analyses, elemental residues and microbial analyses, facility costs)
Capital	Construction of lagoon and spillways; plumbing the lagoon in-line with existing infrastructure at Ayr STP	**$45 000** BTWM System: includes supply and install floating barriers; seed pond with duckweed; instruction manual on cultivation and harvest; includes initial on-site instruction by BTWM staff. Additional on-site visits by BTWM staff to work with BSC staff (only if required) @ $4000/trip **$12 000** BTWM remote controlled harvester with off-road trailer
Total		**$115 870**

6.0 Acknowledgements

This report is Stage III of a collaborative effort to improve water quality in Burdekin waterways by the Department of Primary Industries and Fisheries (DPI&F), the Burdekin Shire Council (BSC) and the South Burdekin Water Board. Funding for the current stage was through the DPI&F Profitable Aquaculture Program and the Burdekin Rangeland Reef Initiative. The BSC provided in-kind support, thanks to Neil Hansen, Merv Pyott and Mark Allpress.

Robert Bell, of Bio-Tech Waste Management (BTWM), provided advice on the design and costing of the proposed large-scale demonstration DWT system at the Ayr Sewage Treatment Plant. He also provided company data on the performance of other systems that operate using BTWM components. Thanks to Wayne Knibb for his initiation and supervision of bioremediation research at the Bribie Island Aquaculture Research Centre, and for endorsing the current project within the broader bioremediation portfolio. Dr Paul Palmer, Catriona Morrison and Bruce Rutherford have provided valuable assistance in the development of DWT research at BIARC, and their efforts are appreciated.

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