final report

Project code: B.NBP.0695

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Date published: 31 January 2016

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

On-farm algal ponds to provide protein for northern cattle

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

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Abstract

Protein-rich feed supplement for grazing cattle leads to increased weight gain during the dry season. Algae had previously been evaluated for intake, digestion and live weight gain responses with positive results. This project was about producing a cheap protein source in the form of microalgae to supplement cattle in northern Australia during the dry season. Major technological advances include (1) the selection of fast-growing, protein-rich microalgae collected from cattle farms, (2) low-cost algae cultivation by using a new pond design and efficient air lift mixers, (3) low-cost harvesting by induced settling. Large-scale cultivation and harvesting was trialled for 1 year with production costs of $781-$2,289 per ton dry matter for different species, with further cost reductions possible for on-farm production. Industry benefits include protein-rich supplement supply all-year-round, improved animal live weight gain and potentially improved animal health.

Executive summary

This project was about producing a cheap protein source in the form of microalgae to supplement cattle in northern Australia during the dry season. Microalgae were proposed as they offer high areal productivities (up to 110 tonnes dry matter (DM) per hectare per year) and can be grown on-farm with almost any water source (including brackish), making farmers independent of price fluctuations for other protein supplements (soybean or cottonseed meal). The project brought together a scientist (Peer Schenk) with extensive experience and knowledge in low-cost algae cultivation and harvesting systems with scientists (Dennis Poppi, Stuart McLennan and Simon Quigley) who are experienced in the nutrition and supplementation of beef cattle with algae and other protein sources in northern Australia. Algae had previously been evaluated for intake, digestion and live weight gain responses with positive results. Other studies report improved animal health/resilience and consumer benefits, as microalgae also provide a mixture of other nutrients that may be beneficial to cattle production and beef products. Most microalgae are high in vitamins and antioxidants and many also contain omega-3 fatty acids. The main issue of this project was to develop ways to cheaply produce algal protein, preferably on-property, to supplement cattle.

New, low-cost technology has been developed by Schenk and his team of 15 scientists and students to significantly reduce the cost of algal biomass production. Major technology advances throughout the project included (1) the selection and adaptation of fast-growing, protein-rich, easy-to-harvest, saline- and heat-tolerant microalgae collected from cattle farms in the NT, (2) a new hydrodynamic pond design that cuts the cost of mixing cultures by half, (3) a new airlift design for efficient culture mixing and CO_2 supply to ensure rapid growth of healthy cultures, (4) a new, low-cost harvesting process that uses gravity for induced settling instead of costly centrifugation, (5) a low-cost solar dryer. All of the above needed to be incorporated in a single large-scale demonstration farm. After substantial testing using smaller pilot facilities, the 250,000 L Algae Energy Farm was constructed and officially opened in August 2014 at the Pinjarra Hills farm of the University of Queensland. Since then, the production of protein-rich cattle feed supplement has been tested and further optimised in routine operation towards low costs and high yields. For more than 1 year, regular yield data have been collected from high-frequency harvesting of microalga *Scenedesmus dimorphus* NT8c (local NT strain with high protein yields), Lemna (a miniature aquatic floating plant) and more recently, *Limnothrix planctonica* (an easy-to-harvest, highly-productive filamentous summer strain). For NT8c, consistent yields have been 54 DM/ha/year (15 g DM/m^2/day) with cell densities of 0.7-1.2 g DM/L. Yields at moderate temperatures around 25ºC during spring and autumn reached 72 t DM/ha/year, but at high water temperatures, ammonium-mediated grazer control restricted further yield increases. Lemna biomass production was only half of that of NT8c (25 t DM/ha/year or 7 g DM/m^2/day), but clear advantages exist for this floating species as harvesting is very simple. Summer strain Limnothrix showed average yields of 72 t DM/ha/year (19.8 g DM/m^2/day), reaching remarkable 110 t DM/ha/year with very high cell densities exceeding 2 g DM/L.

Cost reductions have been achieved by (1) increasing the harvesting frequency to three harvests/week, (2) growth without CO_2 or improved CO_2 dissolving, and (3) the use of low-cost fertiliser combinations that are amenable to grazer control and harvesting by settling. Further cost reductions have been achieved by streamlining standard operating procedures,

resulting in a reduction in labour costs. The option of not using externally supplied CO_2 in combination with the specially designed airlift approximately halved the yields, but reduced operation costs.

Average yield data from routine production showed that 55 tonnes DM per hectare growing area is realistic. Areal protein productivity from microalgae was 72 times more efficient than from soybean cultivation. To supply protein supplement for 1000 250 kg-weaner steers with 3.5 g DM alga supplement per kg W per day over a period of 3 months would require 79 tonnes algae DM. A 2 hectare algae farm should therefore be sufficient to provide protein supplement for 1000 weaner steers, although this can vary depending on the specific requirements for supplementation.

A full techno-economic analysis has been performed based on data collected at the Pinjarra Hills farm that was applied to a 10 ha farm with 8 ha pond surface area (annual production capacity: > 400 tons DM pa). Taken all into account (including costs for operation, maintenance/repair, labour and amortisation of CAPEX), conservatively and without assumption of improvements or at scale operation, the costs were $2,289/ton DM for NT8c. The cost of Lemna production was $781/ton DM and $2,219/ton DM for Limnothrix. Costs for NT8c and Limnothrix production can be cut by >50% if a free CO_2 source is available. Even the combustion of natural gas (e.g. to generate electricity) would provide CO_2 to half the costs of production.

Considering just the production of protein, the production costs of crude protein varied from $2,403/ton for *Lemna* to $5,470/ton and $8,510/ton for *Limnothrix* and *Scenedesmus*, respectively. To make the production of algae competitive compared to other sources of protein (soybean meal), a cost reduction of at least 50% needs to be achieved. The use of low-cost CO_2 would achieve this goal. Alternatively, algae farm operators may co-produce high-value algae-derived compounds, such as carotenoids or omega-3-rich fatty acids. Profit margins for these compounds are remarkable (e.g. omega-3rich algal oil can be sold at >$150/L). If this is a consideration, the processes for inducing and extracting these compounds developed at UQ should be considered and implemented on-farm. The remaining biomass can then be used as a valuable source of proteins and other nutrients as feed supplements for cattle. Additional technological improvements and cost savings are to be expected from the learning curve of this new farming system over the next few years and the economy of scale from on-farm operations. Suggestions are made on processes how costs can be further reduced to reach competitive production (e.g. use of urea for algae growth, establish automation, use of wet algal paste for dairy cows, use of straw for simultaneous algae drying and feed formulation).

Benefits for cattle farmers include convenient on-farm protein-rich supplement supply all-year-round, making them less dependent on price fluctuations and availability of conventional soybean or cottonseed meal. The increased weight gain of weaners can lead to higher farm profitability and potentially a meat product with increased nutritional benefits for the consumer. Following the strong interest from farmers, several large-scale farm proposals are currently being developed (e.g. near Roma, Miles and Goondiwindi). The project leaders wish to further engage with MLA to deploy algae-for-protein on cattle farms.

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1 Background

This project was about developing a novel protein source (algae) for on-property production so as to supplement weaners in particular during the dry season. It is a collaborative project between experienced scientists in algal cultivation **(Schenk)** as well as nutrition and animal production systems in northern Australia **(Poppi, McLennan and Quigley).** The research questions are around developing an algae production system that is robust, easily implemented and maintained and cost-effective by determining yields, fertiliser and water requirements and simple harvesting methods. Commercial aquaculture companies use simple cheap systems to create algal blooms so some of the methodology is available to adapt and develop systems for cattle.

NBP.350 (Poppi, Quigley and McLennan Final Report) showed that supplementing weaner steers with Spirulina microalgae increased intake, digestibility and microbial protein production. The live weight gain responses were similar to cottonseed meal and increased live weight gain by 0.85 kg/d at a level of supplementation of only 3.5 g Spirulina/kg W/d. Algae have a high crude protein (CP) content , Spirulina 706 g CP/kg dry matter (DM) and Chlorella 600 g CP/kg DM. Panjaitan et al (2010) showed that algae could be included in the drinking water but that the concentration required to elicit a significant response in intake and growth rate was much higher than could be achieved through algal blooms in ponds. Nevertheless algae are the first new protein product that has appeared for use in cattle in decades. Poppi and McLennan (2010) outlined that other sources of algae are becoming available as a result of C sequestration programs and biofuel production. However these external sources of algae or algal by-products are likely to be priced at an equivalent protein price to other conventional protein products as companies try to capitalise on their product. In NBP.350 Spirulina was the algal species used but there are many other species which vary in CP and lipid content and composition.

Live weight gain of steers can be markedly improved in beef steers in the dry season up to 1 kg/d depending on the amount of protein supplement (McLennan and Poppi DAQ 100). This has been repeated numerous times and is a consistent observation across northern Australia. The issue is not the biology of the mechanism but rather the source of a cheap protein. Any protein sourced externally to a property inevitably gets priced at an equivalent protein price and means that high levels of protein supplement are not economical. This is particularly so in remote regions of northern Australia where protein is most limiting. A locally produced source of protein at a low price has the potential to completely transform the northern beef industry by markedly increasing live weight gain in the dry season and hence the growth paths (weight for age) and markets which are available to producers. Microalgae are the most promising source of locally grown protein. There are both fresh water and saline water species which can be grown on any land (arable or non-arable) as long as there is a source of water. However, previous microalgae cultivation systems had not been adapted to large-scale on-farm production for animal feed and did not use cost-effective cultivation or harvesting systems, nor did they use strains for high-protein yields that are adapted to northern Australian climates. These aspects have been addressed in this project.

2 Project objectives

The project objectives were

1. to develop a simple pond design and harvest system to generate algal protein

2. to test a range of conditions of design, nutrient supply and water quality (especially bicarbonate level known to vary widely in bores) on algal DM and CP production

3. to test a range of procedures to simply and practically enable harvesting and storing of the majority of the algal DM.

4. to establish yield response data to optimise pond yield and harvest yield (high frequency harvesting, CO_2 supply, fertiliser mixes) so as to address cost per tonne of algae.

5. to establish routine production of both microalgae and Lemna so as to obtain routine production data from ponds under low input and high input systems.

6. to provide new on-farm costing estimates for a 2.5-hectare module able to support 1000 weaners.

3 Methodology

3.1 Isolation of pure microalgal strains

Hundreds of water samples were collected from various environmental samples in Queensland and the Northern Territory. Noteworthy are 13 samples that were collected in October 2012 from the surface and bottom ground of freshwater dams, streams and ponds on NT cattle properties. Sample locations included Brunchilly, Tablelands (GPS 18°52'03"S, 134°30'22"E; sampled from bottom of “Turkey nest”; strain names: NT1x), Katherine Research Station (South Stuart Highway, Katherine; GPS 14°28'21"S, 132°18'17"E; sampled from bottom of “Cooler”; strain names: NT3x); Kidman Springs (Buchanan Highway; GPS 16°07'04"S 130°57'30"E; sampled from surface and subsurface of “Suppleject Dam”; strain names NT5x and NT6x, respectively) and Douglas Daly Research Farm (Jungwa Road, Douglas Daly PMB 105, Winellie; GPS 13°49'59"S 131°11'12"E; sampled from bottom of “Gamba dam”; strain names: NT8x). Samples were preserved in the dark until transferred to the laboratory for analyses.

Single cells were isolated by micropipette on a micromanipulator with an inverted microscope and grown on 96 well-plates before transferred to 100 mL flasks in Bold’s Basal Medium (BBM) for cultivation of pure clonal algal cultures at 25°C, 12 h:12 h light:dark cycle under fluorescent white light (120 µmol photons $m^{-2}s^{-1}$), as described previously (Duong et al., 2012; Lim et al., 2012; Salama et al. 2013).

Lemna minor (duckweed) and *Limnothrix planctonica* were isolated from a pond located at the Pinjarra Hills farm of the University of Queensland.

Details on large-scale algae and Lemna cultivation are provided in the Results and Appendix sections.

3.2 Classification by DNA sequencing

DNA extraction was conducted at the late exponential phase of cultivation. The cell density at that point was typically $2x10^7$ cells mL^{-1}. Microalgal cells were extracted by using an DNeasy Plant Kit (Qiagen) following the manufacturer's instructions. After extraction, genomic DNA within the 18S rRNA region was amplified on a PCR machine by using the following primers: Forward 5'-GCGGTAATTCCAGCTCCAATAGC-3' and Reverse 5'-GACCATACTCCCCCCGGAACC-3'. The PCR cycling conditions comprised 94°C for 5 min for initialization, 94°C for 30 s for denaturation, annealing at 55°C for 30 s and 72°C for 1 min for elongation. The final elongation step was at 72°C for 10 min. PCR templates were then purified by using a Wizard SV Gel PCR Clean-Up System (Promega). For sequencing preparation, 5 µL of a 25 ng μL^{-1} PCR product were combined with 1 µL of a 10 µM solution of each of the above primers. The reaction was topped up to 12 µL with Millipore water in a 1.5 mL tube and sent to the Australian Genome Research Facility (AGRF) at The University of Queensland for sequencing. The DNA sequencing data were then analysed by MEGA 5.2 and the results were compared by BLAST searches with Genbank entries for classification. All of the strains were registered and deposited in Genbank with accession numbers (as shown in in the Results section). For sequences with >99% identity match, the species name was adopted, otherwise the genus name to the closest match was used. The Maximum Parsimony tree was obtained using the Subtree-Pruning-Regrafting algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). The tree was drawn to scale, with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. The analysis involved 11 nucleotide sequences. There were a total of 463 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

3.3 Standard protocol for growth experiments

After obtaining pure cultures, all isolated strains were grown on BBM medium following a standardized cultivation protocol that used bubbling for aeration and mixing. The standard protocol can be briefly described as follows: All strains were inoculated from a recently grown saturated culture and cultured for 3–4 days to reach the end of the exponential growth phase before starting the standard growth experiment. This culture was used as inoculum at a ratio of 1/10 in volume in 400 mL bottles. The bottles were connected to a bubbling system. Cell density was determined daily by using a hemocytometer. Nitrate concentrations were also monitored daily until the nutrient levels reached zero. by using a colorimetric assay (API test kit; Aquarium Parmaceuticals) and a spectrophotometer following the manufacturer's instructions.

Growth rates were calculated by the following equation (Levasseur et al. 1993)

$$K' = \frac{ln\frac{N2}{N1}}{t2 - t1}$$

where N1 and N2 = cell counts at time 1 (t1) and time 2 (t2), respectively.

Divisions per day can also be calculated once the specific growth rate is known.

$$Division\ per\ day = \frac{K'}{ln2}$$

3.4 FAME analysis

Samples for FAME analyses were collected when lipid accumulation reached its peak, normally after 3-4 days of nutrient starvation. A total of 4 mL of microalgal culture was collected and centrifuged at 8,000 g for 5 min. Biomass was collected and freeze-dried for 30 min. Lipids in the microalgal pellet were hydrolyzed and methyl-esterified in 300 µL of a 2% H_2SO_4 in methanol solution for 2 h at 80°C. Prior to the reaction, 50 µg of heneicosanoic acid provided by Sigma, USA was added as internal standard. After the esterification step, 300 µL of 0.9 % (w/v) NaCl solution and 300 µL of hexane were added and mixed for 20 s. To separate the phase, samples were then centrifuged at 16,000 g for 3 min. A total of 1 µL of the hexane layer was injected into an Agilent 6890 gas chromatograph coupled to a 5975 MSD mass spectrometer. The running conditions were followed using Agilent's RTL DBWax method as described previously (Lim et al. 2012).

3.5 Protein analysis

Protein contents in the algal biomass were measured following the protocol described by González López et al. (2010) with modifications. In brief, freeze-dried biomass (10 mg) was milled and protein was extracted by incubation in 10 mL lysis buffer (containing 5 mL L^{-1} of Triton X-100, 0.3722 g L^{-1} of ethylenediaminetetraacetic acid disodium salt, 0.0348 g L^{-1} of phenyl methyl sulfonyl fluoride) for 20 min. A 0.1 mL portion of this solution was placed in a 1.5 mL Eppendorf tube and 0.1 mL SDS solution was added and the mixture was vortexed. The mixture was then used for measurement of protein concentration following the protocol described in the CB-X Protein Assay Kit (G Biosciences).

The spectrophotometric absorbance was converted to protein concentrations using a calibration curve established with a bovine serum albumin (BSA) standard (2 mg mL^{-1}). The protein content of the biomass was calculated using the following equation:

$$Protein\ (\%) = \left(\frac{CVD}{m}\right) X\ 100$$

Where

C = Protein concentration (mg L^{-1}) obtained from the calibration curve

V = Volume (L) of the lysis buffer used to resuspend the biomass

D = Dilution factor

M = Biomass (mg)

3.6 Design, construction and operation of a 250,000 L algae demonstration farm

The Pinjarra Hills farms (approx. 260 ha) is owned by the University of Queensland and was for all large-scale trials. Construction of the Microalgae Energy Farm in Pinjarra Hills commenced in 2012 and opened for research in middle 2014 (Fig. 1 + 2). The farm contains one 30,000 L and two 40,000 L open ponds, three water storage ponds, four 180 L vertical closed photobioreactors, one 4,000 L closed photobioreactor (a covered raceway pond), one V-shaped harvesting pond, one solar drier, a photovoltaics system with battery backup, a mobile laboratory, a batch centrifuge and other facilities for inoculation, cultivation, harvesting and quality control of microalgae. The farm is not connected to the municipal electricity grid and is located near the Brisbane River that provides sufficient water for microalgae cultivation during the year (Fig. 1). The farm also has an installed water recycling system that ensures re-use of water after cultivation. An overview of the different farm components is shown in Fig. 3 + 4.

Microalgae were initially cultured in 10 L plastic bags to the peak of exponential phase in the laboratory before transferring cultures to outdoor experiments (Fig. 5). Microalgae were then cultured in 180 L bags at Pinjarra Hills before inoculating 4,000 L covered raceway pond to reach enough volume for cultivation in 40,000 L raceway ponds.

Fig. 1. Location of the microalgae farm in Pinjarra Hills in Brisbane (marked as a red oval in the figure) – Queensland, Australia

Fig. 2. Construction of 40,000 L raceway ponds during the first phase of the microalgae farm project in March 2013.

Fig. 3. Cultivation of *Scenedesmus dimorphus* NT8c in raceway ponds (in July 2014).

Fig. 4. Algae Energy Farm technology overview.

Fig. 5. Scale-up of cultivation in 10 L bags in the laboratory before culturing outdoors

Additional details of methods used can be found in publications (see list of publications in appendix) as well as in the published PhD theses of the UQ students involved in this project.

4 Results

4.1 Routine large-scale production of a protein-rich microalgal strains and Lemna

4.1.1 Large-scale production of *Scenesdesmus dimorphus* NT8c

Scenedesmus dimorphus NT8c (Fig. 6) had originally been isolated from the Douglas Daly Research Farm, Winellie, NT, at the beginning of the project and was identified as a superior strain for high protein productivity, salinity tolerance, temperature tolerance and harvesting capability (by settling). Routine large-scale production was established of the NT microalga *Scenedesmus dimorphus* NT8c for more than 1 year (see standard operating procedures in Appendix).

Fig. 6. *Scenedesmus dimorphus* NT8c protein-rich microalgae.

NT8c yields under a high frequency harvesting regime have been consistently at 15 g DM/m^2/day (54 ton DM/ha/year). Routine production under a low-rate cultivation regime varied between 5 g DM/m^2/day and 16 g DM/m^2/day depending on the season (temperature and solar irradiation) and the experiments that were carried out. NT8c yields at moderate temperatures during spring and autumn (around 25ºC) reached 72 t DM/ha/year; but at high water temperatures, ammonium-mediated grazer control restricted further yield increases. Various important optimisation experiments were performed, including testing and comparing of different:

(1) raceway pond shapes (standard vs drop shape; Fig. 7 + 8),

(2) mixing devices (airlift vs paddlewheel; Fig. 9),

(3) CO_2 supply devices (see below details of CO_2 experiments),

(4) inoculation densities (Fig. 10),

(5) secondary dewatering methods (secondary settling, geotextile filtration or centrifugation; Fig. 11),

(6) nitrogen fertiliser supplies (nitrate vs ammonium; see below details of fertiliser experiments).

The results were as follows:

Raceway pond shapes. As predicted by computer-assisted hydrodynamic modelling, a drop-shaped raceway with half the width, but twice the depth at its end was superior to the standard raceway pond shape currently used by the industry (Fig. 7). The advantages compared to the widely-used conventional design are that it (1) cuts mixing costs by half, (2) largely avoids turbulences and "dead settling areas", and (3) has the potential for simple in-pond harvesting by settling at sump points at the deeper ends. Based on this result, all raceway ponds have now been modified to the new more hydrodynamic shape (Fig. 7, right).

Fig. 7. Traditional (left) and improved (right) raceway pond design for the cultivation of *Scenesdesmus dimorphus* NT8c.The improved shape (designed by computer modelling) avoids turbulences by leading water around a tear drop shape in twice the depth but half the width. The new design significantly reduced mixing costs by half and all ponds at the farm have now been modified to this shape. Also shown in the photos is the horizontal airlift (white box), a new design to mix algae and provide CO_2 effectively (for details see Fig. 9).

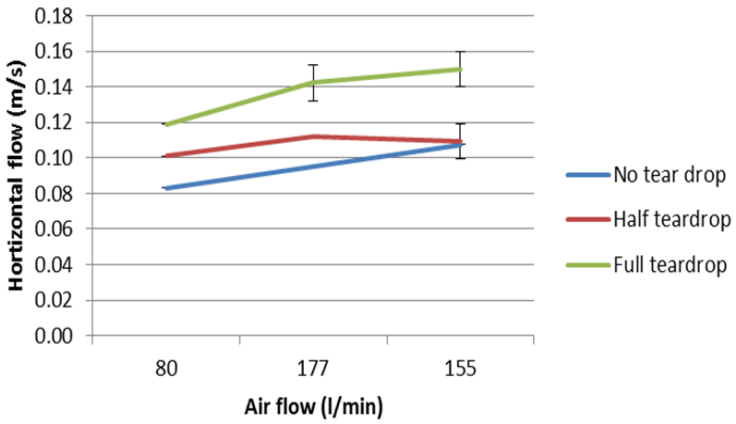

Fig. 8. Flow speed of traditional raceway ponds (no tear drop), a half tear drop design and the superior full tear drop design. Flow speeds improved by 50% and mixing costs were halved.

Mixing devices. Traditional paddlewheels have been compared to especially-designed horizontal air lifts for mixing of algal cultures (Fig. 9). Although approx. twice as energy-intensive compared to standard paddlewheel technology, the airlift:-

(1) supplies higher amounts of atmospheric CO_2,

(2) enables maximal CO_2 dissolving from added sources by using a specially-designed counter-flow ceramic sparger,

(3) deoxygenates cultures to prevent toxic O_2 built-up.

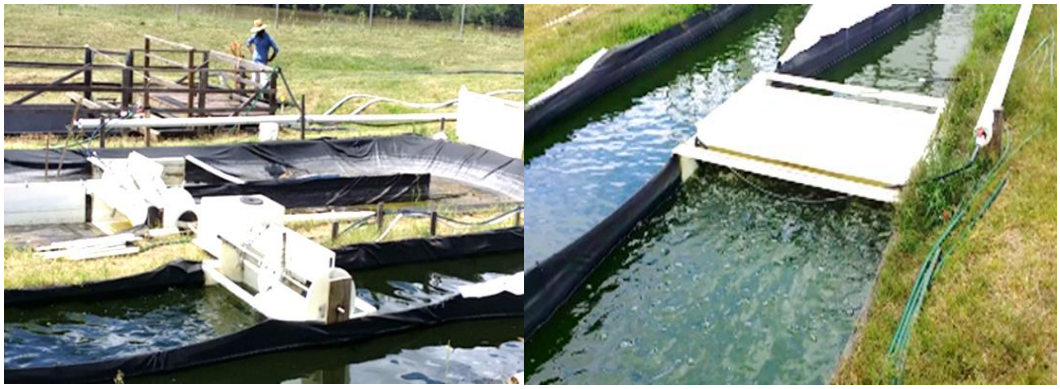

Fig. 9. Traditional paddlewheel (left) compared to new horizontal airlift (right) design. Both circulate the pond, but the airlift (although twice as high in electricity consumption) enables effective CO_2 supply by using ceramic spargers.

Inoculation densities. For optimum repeat harvesting and growth cycling, the optimum biomass removal rate had to be determined. This is done so that enough of the biomass was left after harvesting for optimum inoculation of the next cultivation round. Ideally, the culture should always stay in the maximal exponential growth phase. As shown in Fig. 10, there is an optimum window for which inoculation density is in the desirable range. Inoculation densities that are too low result in slow growth uptake of the culture, while inoculation densities too high affect productivity as not enough biomass is removed during the harvest.

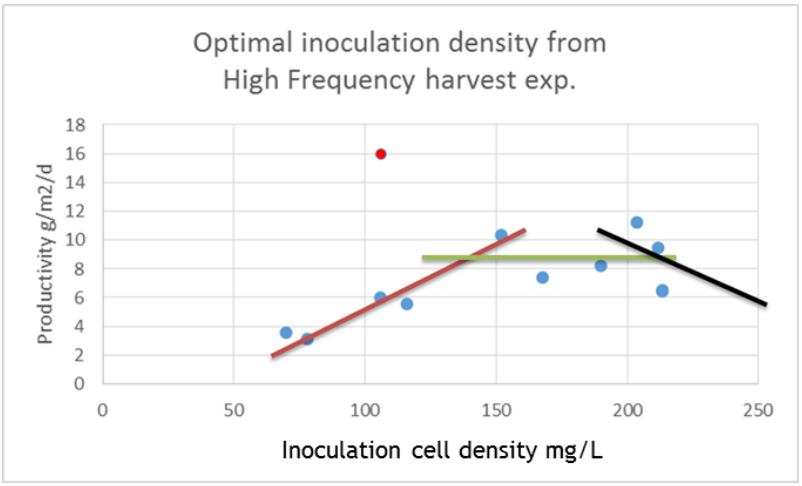

Fig. 10. Optimum inoculation cell culture densities for NT8c should range between 150 and 200 mg/L (experiment carried out during winter months).

Secondary dewatering methods

Primary dewatering (harvesting) of microalgae is carried out by induced settling. However, to ensure rapid drying of algal biomass in the solar dryer, an additional secondary dewatering step is preferable. A comparison of three methods (secondary settling, geotextile filtration or centrifugation; Fig. 11), showed that both, secondary settling in 1000 L tanks and the use of a geotextile, were lengthy procedures that resulted in only partial harvesting with some biofouling occurring. These methods were therefore considered not ideal and a 12 V centrifuge was purchased that can be operated off the solar panels. This resulted in algal paste with approx. 10% dry matter that was amenable to rapid drying in the solar dryer.

Fig. 11. Secondary dewatering devices of concentrated algal slurry. Left: 1000 L settling tank, Middle: Geotextile tube, Right: Centrifuge.

4.1.2 Large-scale production of Lemna

Various local duckweed strains had previously been compared on the farm at Pinjarra Hills. One of these strains (*Lemna minor*) was found to be superior in growth in the water storage ponds on the farm when the correct nutrients were provided (Fig. 12). The arrangement in 1 m^2 floating squares allowed direct comparison of productivity under various conditions. Lemna was relatively easy to grow and harvest routinely. It displayed a wide pH tolerance (Fig. 13). Yields under a high frequency harvesting regime have been consistently at 7 g DM/m^2/day (25 t DM/ha/year). Routine production under a low-rate cultivation regime varied between 3 g DM/m^2/day and 7 g DM/m^2/day depending on the season (temperature and solar irradiation) and the experiments that were performed . Various optimisation experiments were performed.

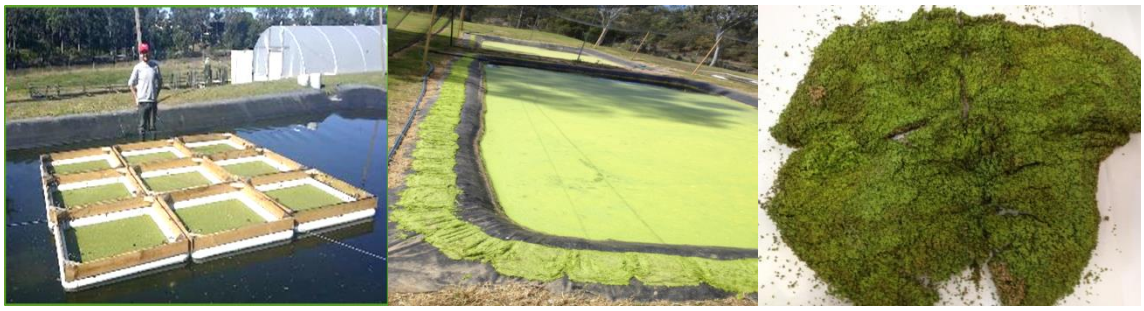

Fig. 12. Cultivation method of Lemna. Left: Growth in 1 m^2 squares, Middle: Routine cultivation and drying, Right: Lemna 'pancake'

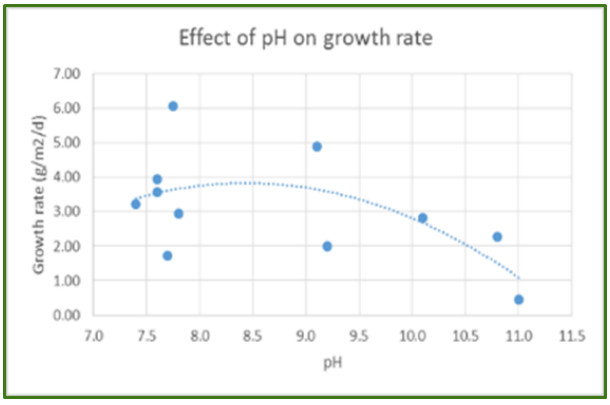

Fig. 13. Lemna displayed a high pH tolerance, but was unculturable at pH 11 or higher and pH 5.5 or lower.

4.1.3 Other high-protein strains for which rapid growth was established

It was apparent that in summer a filamentous alga grew well in storage ponds that could be easily harvested by netting. It started to naturally grow in the ponds during the NT8c experiments during warmer months, but was never more than a minor background contaminant because it was easy to wash out of the system during the routine harvests. This species grows in small to large floc and can settle out very quickly in the large floc form. It is resistant to predation by rotifers as it is too large to be eaten. This dominant strain was further characterised and trialled for large-scale production on-farm to establish whether this alga may be suitable as a potential cattle feed supplement. DNA sequencing identified it as *Limnothrix planctonica* (Fig. 14). This cyanobacterium has no known toxins. Importantly, unlike many other microalgae, it cannot be consumed by rotifers. Its protein contents at 25% was comparable to NT8c and Lemna. Yield data have been determined at a high rate harvesting regime in Nov/Dec 2015 with average yields of 72 t DM/ha/year (19.8 g DM/m^2/day), reaching 95 t DM/ha/year with high cell densities up to 2 g DM/L (Fig. 15). It appears that this strain provides a promising source of protein during summer months when NT8c cultivation is limited by grazer control. Easy harvesting by netting offers additional benefits that result in significantly lower harvesting costs than NT8c (see cost analysis below). However, compared to NT8c, this strain requires warmer water temperatures for growth, external CO_2 supply for good yields and cannot be cultivated effectively in winter.

Other potential algae that looked promising at small- to mid-scale cultivation include two *Desmodesmus* sp. strains isolated from the UQ lakes (Fig. 14). These strains also showed high protein contents, rapid growth rates, good harvesting ability (by settling), and the presence of spikes may prevent the consumption by rotifers.

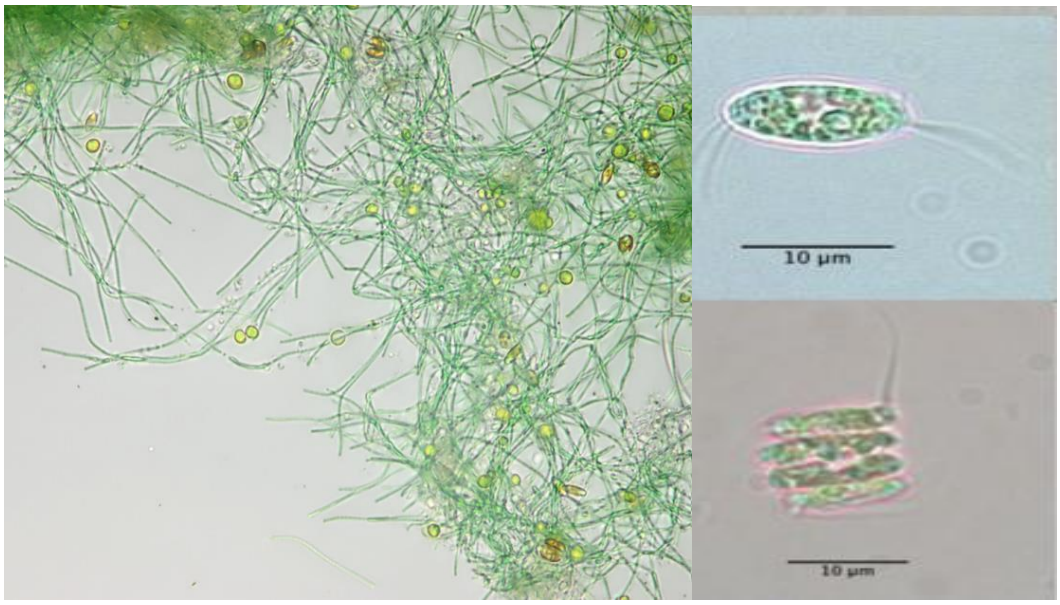

Fig. 14. Other high-protein microalgal strains with potential for cattle feed supplement. Left: *Limnothrix planctonica*, a filamentous cyanobacterium with high protein contents, no known toxins and easy harvesting capability (some other *Chlorella*-type microalgae typically co-exist). Right: Two *Desmodesmus* sp. strains with potential rotifer resistance.

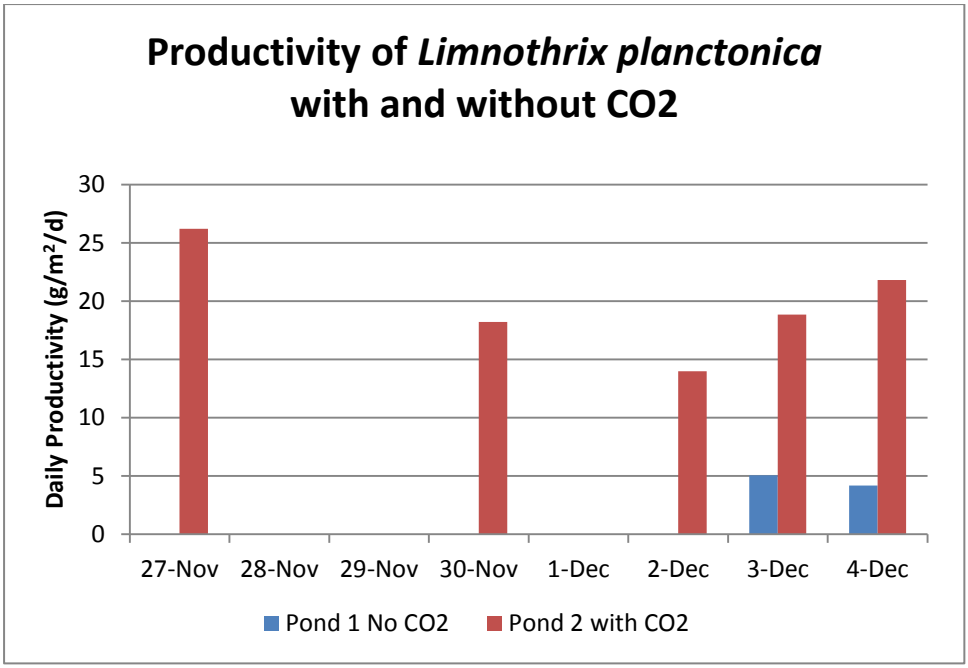

Fig. 15. Sample yields from Limnothrix cultivation at a high-rate harvesting regime with and without externally supplied CO_2.

4.2 Cost reduction through variation of harvesting frequency, CO_2 supply, fertiliser mixes

In previous reports, it was noted that there is potential to significantly reduce the costs of on-farm production by testing different harvesting frequencies, CO_2 supply and fertiliser mixes. In particular it had to be established whether high frequency harvesting compared to low frequency harvesting increases yields. Furthermore, although the external supply of CO_2 was considered favourable for algae cultivation, it also comes with a high cost. Therefore, algae cultivation in the absence and presence of CO_2 had to be compared, considering that lower areal yields may not pose a problem for cattle farms that have enough land. The use of various nitrogen fertiliser mixes had to be tested to potentially identify more cost-effective fertilisers. Results are shown below for *S. dimorphus* NT8c, Lemna and Limnothrix).

NT8c: A comparison of high vs low frequency harvesting for *Scenedesmus dimorphus* NT8c is shown in Fig. 16. Frequent harvesting (2-3 x/week) resulted in higher yields compared to low frequency harvesting (1 x/week). Similarly, the comparison of external CO_2 supply vs no CO_2 supply for *S. dimorphus* NT8c cultivation, showed a near doubling in productivity , although variation was quite high (Fig. 17). These experiments were carried out under a low frequency harvesting regime. CO_2 experiments under high-frequency harvesting regime at higher temperatures were hampered by a high pressure from rotifers but confirmed that yields were halved without the addition of externally supplied CO_2.

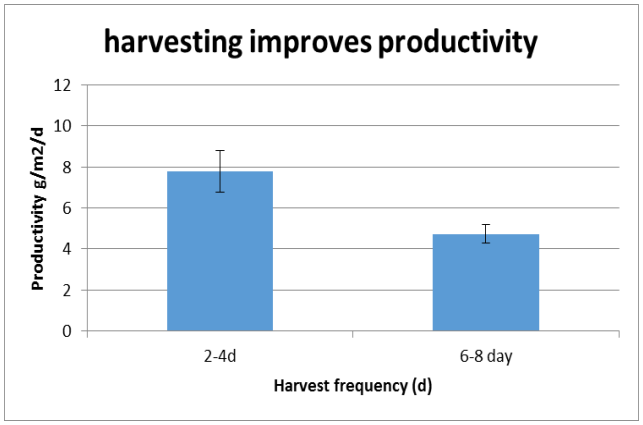

Fig. 16. Frequent harvesting significantly improved productivity of NT8c. Experiments were carried out during winter time.

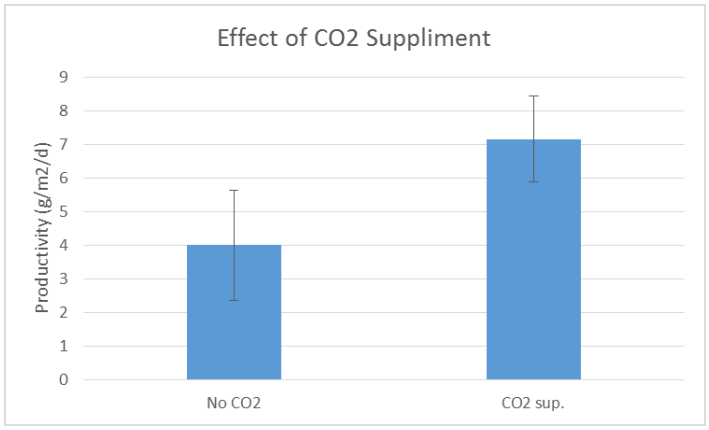

Fig. 17. CO_2 supplementation of NT8c cultivation led to higher yields. Experiments were carried out during winter.

As it can be expected that losses occur during the external supply of CO_2, different CO_2 transfer methods were directly compared to each other under different gas flow rates using different devices. As shown in Fig. 18, the deep ceramic sparger stone was identified as the most effective CO_2 dissolving device at a low gas flow rate of 0.2 L/min.

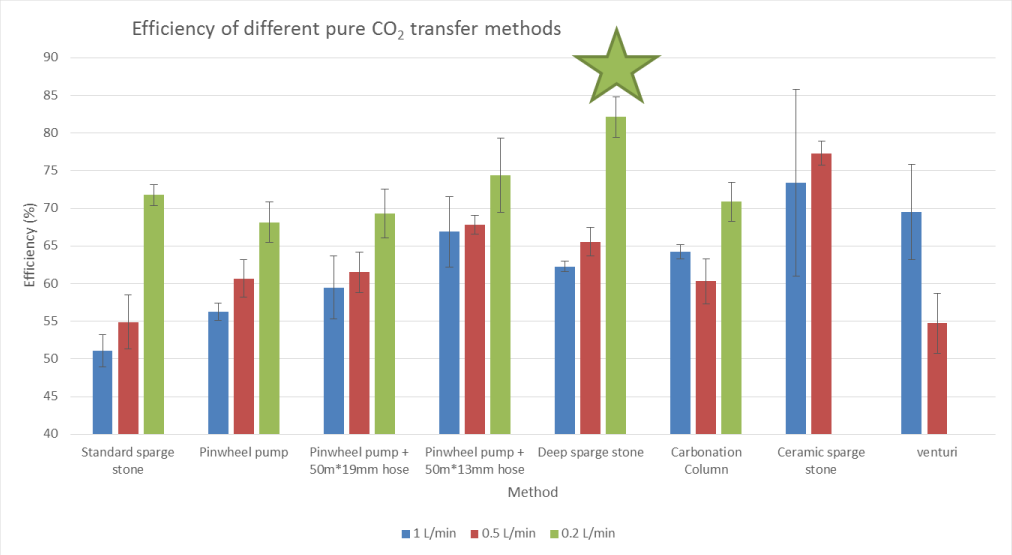

Fig. 18. Comparison of different CO_2 delivery devices. The star indicates the preferred option for the algae farm at Pinjarra Hills.

It was considered that different nitrogen fertiliser sources may lead to different productivities and potential cost savings. However, a comparison of nitrate vs ammonium fertiliser did not reveal any significant differences (Fig. 19), therefore the more cost-effective ammonium fertiliser was preferred. It should be mentioned though that the use of high amounts of ammonium fertiliser for NT8c (2-3 mM at a pH of 9.2) to control rotifers during summer months also inhibits growth. Another, yet unexplored, option is the use of urea, the cheapest nitrogen source. However, the main difficulty with the use of urea is that it cannot be easily measured in the culture and it was unclear how to provide the right dosage during routine cultivation. Therefore, an indirect method for measuring urea was developed by providing both nitrate and urea as N sources. As urea is considered the preferred N source for algae, the depletion of nitrate also indicates that urea was depleted and can therefore be added again without overdosing on urea. The amount of N in the medium did not have a major effect on protein levels.

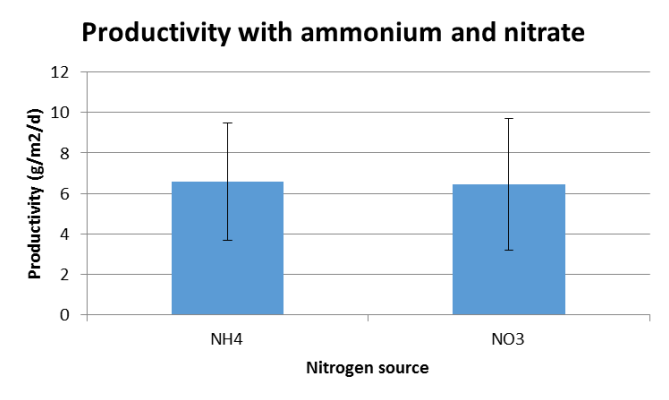

Fig. 19. Comparison of ammonium vs nitrate as nitrogen fertiliser for NT8c cultivation. No significant difference in productivity could be measured. Experiments were carried out during winter.

The costs for fertilisers required for NT8c cultivation are listed in Table 1. Collectively, costs for fertiliser are approx. $82/t DM (8 cents/kg algae).

Table 1. Costs for nutrients and chemicals used for *S. dimorphus* NT8c cultivation.

Fertiliser				
	kg(nut)/kg(alg)	$/kg(nut)	$/kg(alg)	
Micronutrient mix	0.004	3	0.012	
MAP	0.04	0.7	0.028	
$MgSO_4$	0.02	0.1	0.002	
NH_4SO_4	0.2	0.2	0.040	
			Subtotal	0.082
Chemicals				
	kg(nut)/kg(alg)	$/kg(nut)	$/kg(alg)	
Chlorine	0.0015	1	0.0015	
KOH	0.02	1	0.0200	
			Subtotal	0.022
			Total	**0.104**

Lemna: Not much was known about the nutrient requirements of Lemna (*Lemna minor*) at the beginning of the project. Compared to the published literature, we noticed that the addition of higher iron contents dramatically improved growth in the ponds. Another large noticeable effect was found when calcium was added in higher amounts (Fig. 20). This not only lead to higher yields, but also to higher protein contents and a visible colour change towards a greener product (Fig. 21). The frequency of harvesting (1x/week vs 2-3x/week) did not significantly affect productivity of Lemna in ponds.

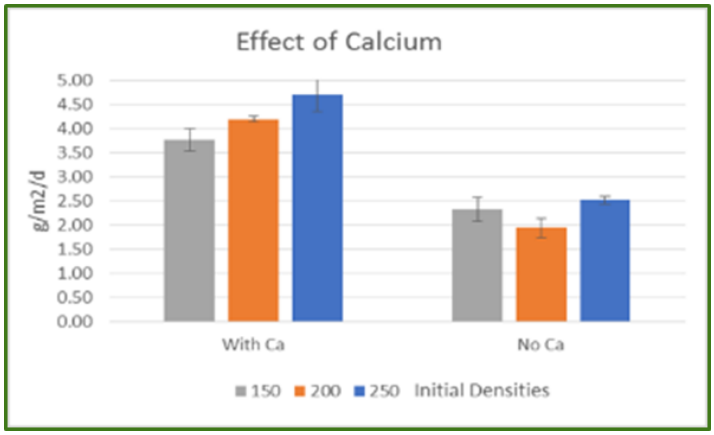

Fig. 20. Lemna yields significantly improved with the addition of calcium to the ponds. Higher initial inoculation densities also led to higher yields under high calcium regimes.

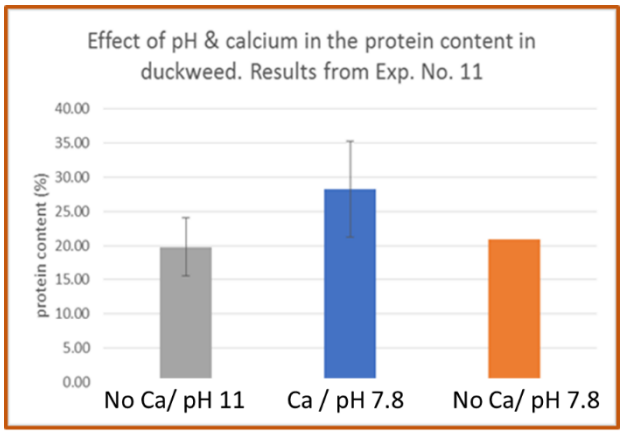

Fig. 21. The addition of calcium to Lemna growth medium in ponds led to higher protein contents (top) and a greener product (bottom).

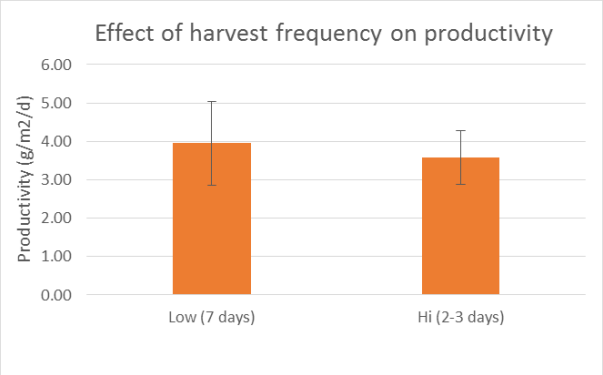

Fig. 22. Frequency of harvesting (1x/week vs 2-3x/week) did not significantly affect productivity of Lemna in ponds.

Experiments with Lemna performed in late winter found average yields to be around 15 t/ha/year (4 g/m^2/d; Fig. 22). Productivity during late spring averaged at 23 t/ha/year (6.1 g/m^2/d), with peaks above 34 t/ha/year (9 g/m^2/d). Recent experiment utilised the optimal conditions identified from previous experiments, pH 7-8, addition of Ca and the optimal initial stocking density of 250 g (wet weight). The optimal stocking density experiment was repeated to confirm that this had not changed with the warmer weather. When comparing stocking densities of 250 g, 300 g and 400 g wet weight inoculation per 1 m^2 there was no significant difference in the productivity, 25 t/ha/year (6.6 g/m^2/d) measured after 3 days growth. The optimal stocking density is essentially complete surface coverage within the growing space; 250 g wet weight was equivalent to around 22 g DW.

The main focus of the recent experiments was to identify the maximum stocking density for Lemna that does not inhibit production and thus recommend harvest frequency. Ponds were harvested between 1 to 21 days. Plotting these data found there was an unexpected lag phase (Fig. 23); we attribute this to the damage that occurs during the harvest/dewatering and subsequent re-inoculation of the pontoon. The comparison of harvested dry mass relative to growth time found that peak density occurred after 15 days and was on average 121 g/m^2, around six times that of the recommended inoculation density. There was no significant increase in biomass with longer growing times and thus we recommend that harvests should occur at least once every 2 weeks. The highest productivities were observed between days 2 and 9 with yields often well above 30 t/ha/year (8 g/m^2/d), peak biomass was close 100 g/m^2 at day 9 in some samples (Fig. 24). With this in mind, a safe harvesting strategy would be to harvest 50-75% of the biomass once every week. This would ensure the biomass load was never too high (above 120 g/m^2), the lag phase due to harvest/inoculation would not occur and initial biomass after harvest should always be above 22 g/m^2. With this strategy in mind yields above 38 t/ha/year (10 g/m^2/d) might be achieved.

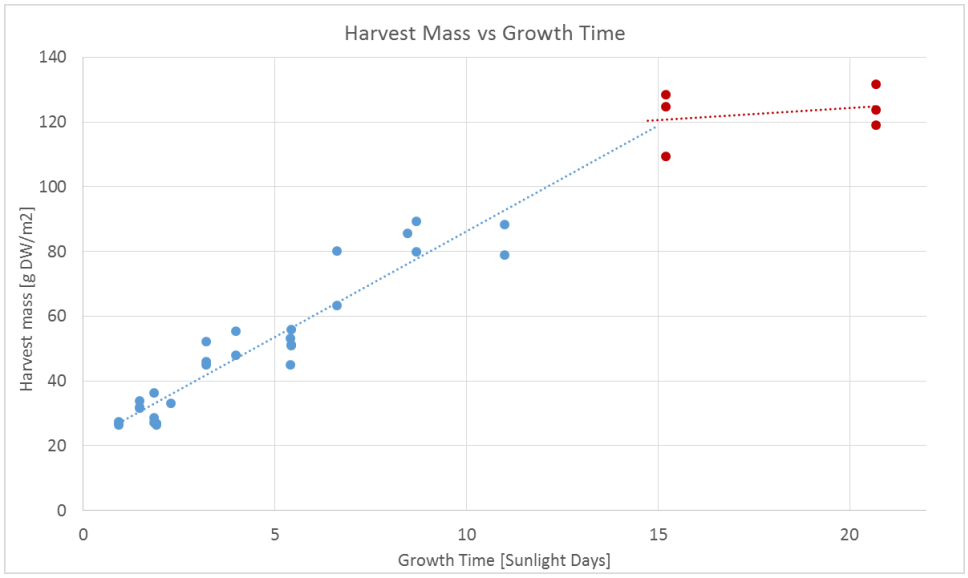

Fig. 23. Dry weight yields of Lemna with varied harvest times.

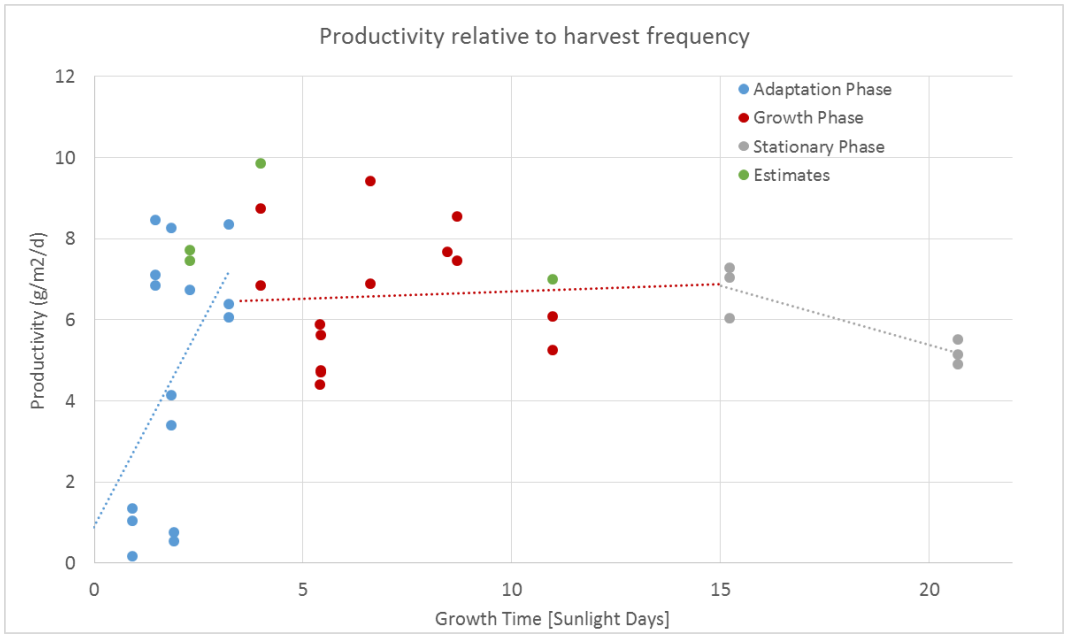

Fig. 24. Productivity of Lemna relative to harvest frequency.

Limnothrix: As mentioned above, NT8c was found to be ideal for winter, spring and autumn months, but required grazer control during the hotter summer months which limited its yields. Limnothrix was identified as a promising summer strain that was not affected by rotifer attack. It has a high protein contents (40-45%) and was cultured at a high frequency harvesting regime with KNO_3 as its nitrogen source, with average yields of 72 t DM/ha/year (19.8 g DM/m^2/day), reaching 95 t DM/ha/year with high cell densities up to 2 g DM/L (Fig. 15). However, its average yields without external CO_2 supply was only about one quarter of that at 17 t DM/ha/year (4.7 g DM/m^2/day). The pH without external CO_2 supply climbed from 8.2 (CO_2 addition set point) to 11 in the first day, where it remained for the rest on the

experiment. On the other hand, Limnothrix growth with CO_2 was so rapid that the CO_2 sparger system had to be modified to keep up with its CO_2 demand. At peak production, Pond 2 was using a 30 kg CO_2 cylinder every 2-3 days, suggesting productivity over 112 t/ha/year (30 g/m^2/d). The average productivity of 72 t DM/ha/year (19.8 g DM/m^2/day) is likely to be an underestimate as there was significant built-up of algal biomass on the pond sides which could not be included in the productivity measurement. Figs. 25 and 26 show examples of daily productivity and harvesting regimes at different times.

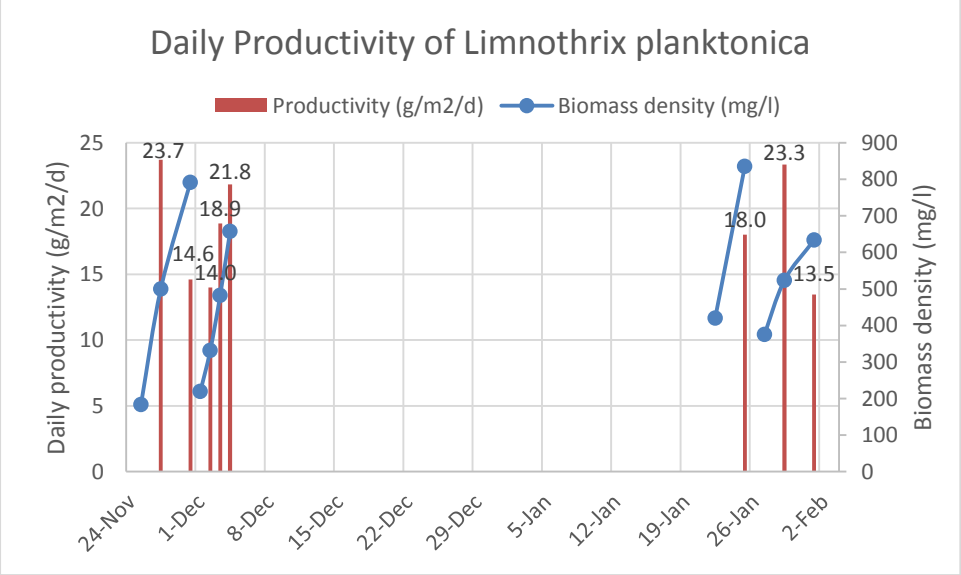

Fig. 25. Daily productivity calculations. Blue dots represent culture density in the pond (mg/L), lines represent individual production runs. Orange bars represent daily productivity measurements, values presented on top of each bar (g/m^2/d). Average daily productivity for the Nov/Dec and Jan/Feb production run was 18.6 and 18.3 g/m^2/d respectively.

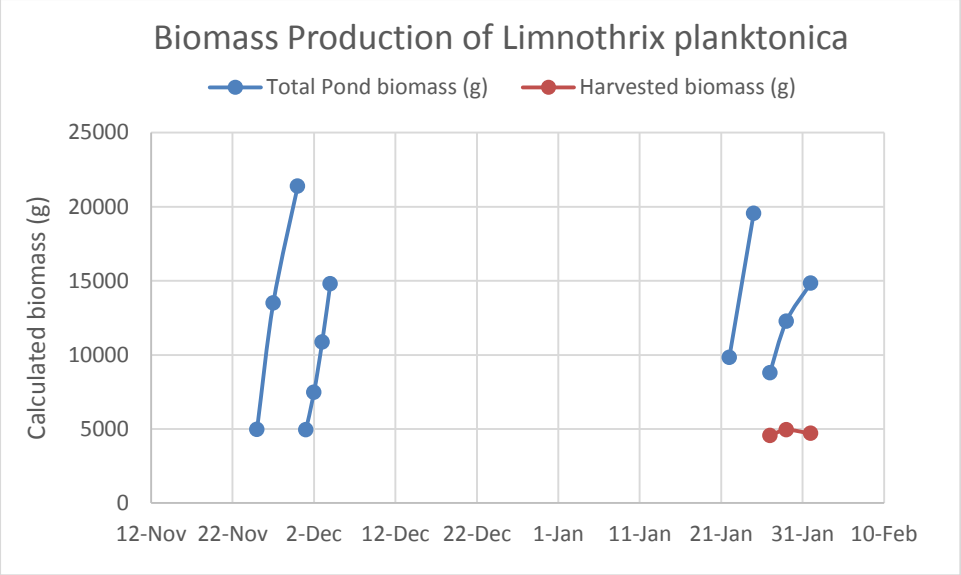

Fig. 26. Calculated pond biomass from 4 production runs. Blue dots represent total pond biomass, very similar to the culture density measurement presented above, except the pond volume is additionally taken into account (15 cm for Nov/Dec and 13 cm for Jan/Feb). The forth production run had biomass continually removed by the automated harvester (orange dots).

As Limnothrix naturally settles, it was possible to develop an automated continuous harvesting system, rather than the batch system that was used for *Scenedesmus*. .So rather than try to send the culture from the pond to the V-tank for overnight settling, a “dead zone trap” was trialled. Water was pumped from the pond to a 1000 L tank (only when the paddlewheel was operating, 12 h a day). The majority of the algae settled out and the water was allowed to overflow back into the pond (Fig. 27).

Fig. 27. Automated continuous harvesting system developed for Limnothrix using settling in 1000 L tanks. Shown are the start of filling up a 1000 L tank, the 1000 L tank once filled to the overflow, the collected sludge after 2 x 12 h runs, and secondary dewatering via draining onto shade cloth.

After 2 full days of operation the 1000 L tank was full with algal sludge to overflowing back into the pond. Excess water was siphoned off leaving about 500-600 L of thick algal sludge. After several trials we found the sludge could be poured onto shade cloth to remove additional water (estimated 30-50% further reduction in water). The final algal paste had a jelly-like consistency, it could be rolled together on the shade cloth and easily picked up by hand. This was bucketed and placed into the solar drying stations. The effect this harvest had on the pond was significant. Biomass flows much more evenly and it does not appear to be overstocked. There is no easy way to accurately measure biomass productivity whilst the algae is so flocculated and benthic. The best we could do is measure dry weight harvested, which is the best measure of actual production. Measuring Nitrogen draw down rates is a rapid measure to monitor production. We observed N consumption rates of > 0.16 M of N/m^2d which is faster than anything we have ever observed before.

4.3 Estimated on-farm costing for a module able to support 1000 weaners

A techno-economic model was developed based on protein-rich stockfeed production through algae and Lemna cultivation, as well as a cross analysis of production with and without utilising purchased CO_2 and with and without the use of solar panels for electricity generation. Details of the calculation under these scenarios are shown in Table 2 for *S. dimorphus* NT8c, Table 3 for Lemna and Table 4 for Limnothrix. A list of constants used for the calculations is shown in Table 5 and a comparison to previous costings from other studies is shown in Table 6.

The techno-economic analysis has been performed based on data collected at the Pinjarra Hills farm that was applied to a 10 ha farm with 8 ha pond surface area (annual production capacity: > 400 tons DM pa). A larger area needs to be used if no external CO_2 is used. Taken all into account (including costs for operation (OPEX), maintenance/repair, labour and amortisation of capital costs (CAPEX)), conservatively and without assumption of improvements or at scale operation, the costs were **$2,289/t DM** for *S. dimorphus* NT8c, **$781/t DM** for Lemna and **$2,219/t DM** for Limnothrix.

For *S. dimorphus* NT8c, the model predicts a biomass production cost of $2.56/kg ($2,559/t DM) and $2.29/kg ($2,289/t DM) with and without addition of externally-supplied CO_2, respectively. The use of solar panels weighted favourable for a reduction in production costs when no external CO_2 was supplied. This is because airlifts are the preferred option to supply atmospheric CO_2 under these conditions which consume approx. twice as much electricity compared to paddlewheels (Fig. 9).

Significant additional cost savings maybe achieved by implementing the following:

A. Reduced construction costs based on

(1) simpler regulatory approvals
(2) use of competitive contractors/suppliers
(3) much simpler earthworks (e.g. use of flat land)
(4) larger-scale earthworks
(5) use of on-farm machinery and labour
(6) bulk pricing for pond liner (e.g. re-inforced polyethylene (RPE) with expected life span > 20 years from Colorado Lining International @ <$3/$m^2$).

B. Reduced operation costs by

(1) using less or no CO_2,
(2) using cheaper commercial fertiliser (incl KOH) from bulk supply,
(3) scaling up production in larger 1-acre ponds (economy of scale),
(4) implementing the learning curve expected for new technologies/farm practices,
(5) establish automation,
(6) use of wet algal paste for dairy cows (avoids drying costs),

(7) use of straw for algae drying (accelerates drying and provides a palatable known product).

The following tables summarise operating (OPEX) and initial capital costs (CAPEX) or set-up costs) for NT8c, Lemna and Limnothrix. OPEX include amortisation of CAPEX. Scenarios with and without external CO_2 supply is shown for the microalgae but not for Lemna (as it does not require external CO_2).

Costs with and without CO_2 supply: The use of externally supplied CO_2 allows more frequent harvesting of microalgae with yields at least twice as high than without CO_2 addition. It also requires approx. half the land use and therefore the initial CAPEX are significantly lower (Table 2 right columns). However, the cost of microalgae production (OPEX) without CO_2 supply is slightly lower due to the relatively high costs of CO_2. This cost is likely to go down in the future. Lemna cultivation does not require any external CO_2 supply (Table 3). Therefore the plus CO_2 supply option for microalgae and Lemna cultivation appear to be more attractive as much lower initial CAPEX need to be raised.

Role of on-farm labour and level of automation: A minimum of a biologist and labourer full-time, as well as a half-time engineer are considered for the farm. The role of the biologist include regular quality control of cultures (health checks) and dried product. The role of the labourer includes all regular farm operation steps. A half-time engineer is required to look after maintenance and repair of equipment and installations and to perform technical upgrades as required. A reduction of harvesting frequency (e.g. for Lemna) would reduce labour costs, but on a 10 ha farm, harvesting would still occur at least 3 times a week (as different ponds mature), therefore would still require a full-time labourer and biologist. Automation is envisaged for water top-up, pH maintenance via CO_2 addition (+CO_2 scenario only) and culture optical density monitoring. A conveyor belt will be installed to link algal paste to solar drying.

Table 2. Techno-economic analysis of *S. dimorphus* NT8c production

NT8c with CO_2 addition

Stockfeed NT8c	no solar	solar			
	OPEX* ($/kg)	Solar OPEX ($/kg)		**CAPEX** (tot$)	**Energy** (kWh/day)
Cultivation	0.108	0.104		1,005,500	22.4
Dewatering	0.145	0.127		40,000	85.5
Drying (conv belt)	0.014	0.000		171,300	67
CO_2	1.455	1.455		171,800	0
Solar				144,584	
Labour	0.366	0.366			Total energy
Maintenance	0.200	0.218			174.9
Total	2.288	2.269	Total	1,388,600	
Amortisation of CAPEX	0.265	0.290	Total (incl solar)	1,544,184	
Total with amortisation	**2.553**	**2.559**			

*Operating costs

Table 2 (continued). Techno-economic analysis of *S. dimorphus* NT8c production

NT8c without CO_2 addition

Stockfeed NT8c no CO_2	**no solar**	**solar**			
	OPEX ($/kg)	Solar OPEX ($/kg)		**CAPEX** (total $)	**Energy** (kWh/day)
Cultivation	0.361	0.104		3,351,667	74.7
Dewatering	0.484	0.423		133,333	285
Drying (conv belt)	0.014	0.000		171,300	67
CO_2 (no CO_2)	0.000	0.000		0	0
Solar				352,711	
Labour	0.524	0.524			Total energy
Maintenance	0.481	0.525			426.67
Total	1.864	1.590	Total	3,656,300	
Amortisation of CAPEX	0.640	0.699	Total (incl solar)	4,009,011	
Total with amortisation	**2.504**	**2.289**			

Table 3. Techno-economic analysis of Lemna production

Stockfeed Lemna

	OPEX ($/kg)	Solar OPEX ($/kg)		CAPEX (total $)	Energy (kWh/day)
Cultivation	0.108	0.104		1,021,500	22.4
Drying	0.060	0.060		50,000	0
CO_2 (no CO_2)	0.000	0.000		0	0
Solar				144,584	
Labour	0.152	0.152			Total energy
Maintenance	0.198	0.216			174.9
Total	0.518	0.531	Total	1,071,500	
Amortisation of CAPEX	0.263	0.263	Total (incl solar)	1,216,084	
Total with amortisation	**0.781**	**0.794**			

Table 4. Techno-economic analysis of Limnothrix production (with CO_2)

Stockfeed Limnothrix	**no solar**	**solar**			
	OPEX ($/kg)	Solar OPEX ($/kg)		**CAPEX** (tot$)	**Energy** (kWh/day)
Cultivation	0.108	0.104		1,005,500	22.4
				40,000	85.5
Drying (conv belt)	0.100	0.000		171,300	67
CO_2	1.455	1.455		171,800	0
Solar				144,584	
Labour	0.152	0.152			Total energy
Maintenance	0.200	0.218			174.9
Total	2.015	1.929	Total	1,388,600	
Amortisation of CAPEX	0.265	0.290	Total (incl Solar)	1,544,184	
Total with amortisation	**2.270**	**2.219**			

Table 5. Values and assumptions used in economic model

Description			Value	Units	Reference
Cultivation	Construction Costs		12	$/m2	a
	Growth rate		15 (NT8c) 4.5 (Lemna) 15 (Limnothrix)	g/m2	b
	Nutrient demand	NH_4SO_4 = 0.2 Mono ammonia phosphate = 0.04 Micronutrient mix = 0.004		Kg nutrient / kg algae	a
	Nutrient cost	NH_4SO_4 = 185 Mono ammonia phosphate = 730 Micronutrient mix = 2500		$/t	Xiamen Vastland chem. Co. (c) [1]
CO_2 Transfer	Efficiency		77	%	b
	Apparatus costs		152,180	$ total	(Twaomey and Labett, 2013) and DongQiang EPE Co. (c) [2]
	CO_2 cost		0.80	$/kg	BOC (d)
Dewatering	Concentration at harvesting		0.1	% algae	(Sharma et al., 2013), (a)
	Concentration after pH assisted settling		3	% algae	
	Concentration after centrifugation		40	% algae	
	Base demand		0.33	g/L media	a
	Base cost		350	$/t	Shengxinhai Chemical Co., Ltd (c) [3]
	Cost of centrifuge		40,000	$/each	Liaoyang Tianxing Pharmaceutical Machinery Factory (c) [4]
	Energy demand of centrifuge		7.5	kW	
Drying	Areal demand		1.5	m^2/kg algae	A
	Apparatus costs		95	$/$m^2$	LongJian Machinery (c) [5]
	Energy demand		2.2	kW	
	Heat demand		7.9	kW	
Economic assumptions	Cost of electricity		0.256	$/kWh	(McArdle, 2014)
	Cost of water		200	$/ML	(BDO, 2014)
	Pumping demand		45	W/kg harvest/ day	(Kaya et al., 2008) (a)
	Maintenance		5	% of total CAPEX	(Zamalloa et al., 2011)
	Biologist wage		60,000	$/year	Payscale.com
	Engineer wage		80,000	$/year	
	Labourer wage		45,000	$/year	
	Lifetime of project		20	Years	Assumption
	Interest rate		3	%	

a = adapted from performance at pilot farm, b = data collected during this study, c = prices from advertising material, d = personal communication

[1] http://vastland.en.alibaba.com/
[2] http://donggiang.en.alibaba.com/
[3] http://www.sxhchem.cn/
[4] http://txzyjx.en.alibaba.com/
[5] http://cqlongjian.en.alibaba.com/
[6] http://towinmachine.en.alibaba.com/
[7] http://healthchemical.en.alibaba.com/

5 Discussion

5.1 Benchmarking algae as a protein supplement for cattle

This project was about producing a cheap protein source in the form of microalgae to supplement cattle in northern Australia during the dry season. Microalgae were proposed as they offer high areal productivities and can be grown on-farm with almost any water source (including brackish), making farmers independent of price fluctuations for other protein supplements (soybean or cottonseed meal). Algae had previously been evaluated for intake, digestion and live weight gain responses with positive results. Other studies report improved animal health/resilience and consumer benefits if algae contained omega-3. The main issue of this project was to develop ways to cheaply produce algal protein to supplement cattle. To benchmark costing for algae supplement production from this project against previous studies, a comparison was made. As shown in Table 6, other technoeconomic analyses determined the cost of microalgae production to range between $1,790 to $10,200 per ton.

Table 6. Summarised outcomes of previous TEAs and comparison to MLA project technology.

Cost	Scale	Growth rate	CO_2 cost	Notes	Reference
$1,790-2,020/ton Biomass		10 g/m^2/d	$110/ton	Includes European carbon credit subsidies	(Slade and Bauen, 2013)
$10,200-9,300/ton and **$7,200-6,400/ton** Biomass	1.15 and 115 MT/yr Biomass (50 ha and 5000 ha)	12 g/m^2/d	$46/ton		(Amer et al., 2011)
$2,219/ton	438 t/yr biomass	15 g/m^2/d	$800/ton	10 ha module with 8 ha cultivation area	UQ-Algae Energy Farm
With free CO_2 **$764/ton**	438 t/yr biomass	15 g/m^2/d	$0		
Lemna biomass **$781/ton**	204 t/yr	7 g/m^2/d	n/a		

The low cost of $1,790/ton was determined by incorporating significant European carbon credit subsidies using the assumption that costs for CO_2 would only be $110/ton and without providing the scale that was used for the calculation. The costs determined for microalgae production from large-scale cultivation data in this project (without carbon subsidies and with current high CO_2 costs of $800/ton) were $2,219/ton. This calculation is very sensitive

to the proce of CO_2 as it was by far the highest cost for algae dry matter production. By comparison, if CO_2 would be available for $110/ton as stated in the cited study, the cost of algal biomass production using MLA/UQ technology would only be $964/ton DM. The cost of Lemna production using UQ/MLA technology was already relatively low with $781/ton. Hence, the costs of on-farm protein supplement becomes interesting but further cost reductions need to be targeted to compete with soybean or cottonseed meal.

Remarkably, the protein productivity from microalgae on a per hectar basis was 72 times more efficient than from soybean cultivation. Considering just the production of protein, the production costs of crude protein varied from $2,403/ton for *Lemna* to $5,470/ton and $8,510/ton for *Limnothrix* and *Scenedesmus*, respectively (Table 7). But to make the production of algae competitive compared to other sources of protein (soybean meal), a cost reduction of at least 50% needs to be achieved.

Table 7. Comparison of costs and productivity on a crude protein basis

	Scenedesmus NT8c	Lemna minor	Limnothrix	Soybean meal	cottonseed meal
Crude protein	20-40%	30-35%	38-45%	38-48%	30-50%
tons/h/y	54	29	73	3	1450
DW Tons h/y	54	29	73	0.96	?
proteinTons /h/y	16.2	8.7	29.2	0.4	0.45?
Cost/ton	$2,553.00	$781.00	$2,270.00	$410.00	$295.00
DW in final product	100%	100%	100%	88%	92%
protein $/ton	$8,510.00	$2,403.08	$5,469.88	$1,096.26	$801.63

5.2 Suggestion for further cost reductions

As shown in the Results section, significant additional cost savings can be achieved by implementing the following on cattle farms which were not part of the current study:

For capital costs:

1) simplified construction approvals
2) use of competitive contractors/suppliers
3) much simpler earthworks (e.g. use of flat land)
4) larger-scale earthworks
5) use of on-farm machinery and labour
6) bulk pricing for pond liner (e.g. RPE from Colorado Lining International @ <$3/$m^2$).

For operating costs:

7) using low-cost or no CO_2,
8) using cheaper commercial fertiliser (incl. KOH) from bulk supply,
9) scaling up production in larger 1-acre ponds (economy of scale),
10) implementing the learning curve expected for new technologies/farm practices,

11) establish automation (e.g. nutrient control; conveyor belt for dewatering/drying)
12) consider use of wet algal paste for dairy cows (avoids drying costs),
13) use of straw for algae drying (accelerates drying and provides a palatable known product; e.g. in the form of pellets).

The use of low-cost CO_2 would achieve a significant reduction of >50% of the production costs. The current cost of CO_2 (food-grade) was deterimined to be $0.80/kg for the TEA. However, clean sources of CO_2 can also be easily obtained from flue gases from combustion of natural gas or biogas, and this should be considered for on-farm production. This has the added benefit of generating electricity and nitrous oxides which (when added to algae cultures) will provide an additional nitrogen source. Table 8 shows a comparison of prices for CO_2 from different sources. The lowest price is paid for flue gases from gas-fired power plants (last row), but this would not be an option for most cattle farms. Instead the use of natural gas for power generation can be considered.

Table 8. Price and cost comparison of various sources of CO_2

Element	MW	Price/ kg	CO_2 yield	CO_2 cost/kg
CH_4 (Natural gas)	16	$0.14	2.75	$0.05
C_3H_8 (Propane)	44	$0.55	3	$0.18
Commercial compressed CO_2	44	$0.80	1	$0.80
Concentrated CO_2 source *	44	$0.005-0.05	0.13-1	$0.005-0.05

* https://hub.globalccsinstitute.com/publications/accelerating-uptake-ccs-industrial-use-captured-carbon-dioxide/2-co2-market

Alternatively, algae farm operators may co-produce high-value algae-derived compounds, such as carotenoids or omega-3-rich fatty acids. Profit margins for these compounds are remarkable (e.g. omega-3-rich algal oil can be sold at >$150/L). If this is a consideration, the processes for inducing and extracting these compounds developed at UQ should be considered and implemented on-farm. The remaining biomass can then be used as a valuable source of proteins and other nutrients as feed supplements for cattle.

5.3 Other potential benefits of using algae as feed supplement

Additional benefits from using microalgae or Lemna as protein-rich supplement that are mentioned in other studies include:

1) reduced methane emissions
2) improved animal health
3) a higher quality more nutritious meat product (if omega-3 containing algae are used)
4) a reduced footprint for animal production

The last point has been modelled in detail in a recent paper by CSIRO authors using figures from this project (Walsh et al., 2015). It proposed that algae if used at large scale for feed supplement, significant areas of farm land could be freed up leading to a reduction in carbon emissions.

5.4 Achievement of specific project objectives

1. to develop a simple pond design and harvest system to generate algal protein

A new pond shape has been developed based on hydrodynamic modelling that reduced mixing costs by half. A new mixing technology using air lifts has been developed that improves CO_2 supply to algae for increased yields. A new harvesting system has been developed that uses induced settling, rather than centrifugation, leading to 98% reduction in costs for harvesting.

2. to test a range of conditions of design, nutrient supply and water quality (especially bicarbonate level known to vary widely in bores) on algal DM and CP production

Cultivation methods for microalgae and Lemna have been optimised at large-scale using new nutrient supply formulas based on commercial fertilisers. Various calcium and carbonate levels, as well as different salinities have been tested and microalgae and Lemna that display a wide salinity and temperature tolerance have been isolated and used for large-scale cultivation.

3. to test a range of procedures to simply and practically enable harvesting and storing of the majority of the algal DM.

Harvesting by induced settling has been implemented as a routine procedure for primary dewatering of microalgae followed by water recycling. Secondary dewatering has been implemented by centrifugation of small culture volumes and drying has been implemented by using a new solar dryer design to enable long-term storage of algal DM.

4. to establish yield response data to optimise pond yield and harvest yield (high frequency harvesting, CO_2 supply, fertiliser mixes) so as to address cost per tonne of algae.

High frequency harvesting, CO_2 supply and fertiliser formulas have been tested at large-scale and optimised for more than 1 year to achieve high yields of microalgae and Lemna.

5. to establish routine production of both microalgae and Lemna so as to obtain routine production data from ponds under low input and high input systems.

Rotine production has been implemented for two microalgae (Scenedesmus and Limnothrix as summer strain) and Lemna over many months and over different seasons. Low input and high input systems (e.g. with and without CO_2 supply or low and high frequency harvesting) have been compared side by side to enable the collection of realistic data for a techno-economic analysis under various scenarios.

6. **to provide new on-farm costing estimates for a 2.5-hectare module able to support 1000 weaners.**

   Average yield data from routine production showed that 55 tonnes DM per hectare growing area is realistic. To supply protein supplement for 1000 250 kg-weaner steers with 3.5 DM alga supplement per kg W per day over a period of 3 months would require 79 tonnes algae DM, although this can vary depending on the specific requirements for supplementation.

   A full techno-economic analysis has been carried out that includes all capital and operating costs for a 10 ha farm with 8 ha growing area with 438 tonnes DM annual production. This showed that costs of production varied from $784 to $2,219 per ton of DM, depending on the production system and alga used. It showed that the major expense was the use of CO_2 and that, if a free or low-cost CO_2 source is available, costs can be further reduced by at least 40%. Further significant cost savings are achievable with the expected learning curve when implemented in northern cattle farms.

6 Conclusions/recommendations

6.1 Recommended on-farm operation modes

The results from this project encourage the implementation of on-farm cultivation of microalgae as protein supplement for weaners. Although protein contents were comparable, high yielding seasons and harvesting methods differed for different algae. Therefore it is recommended that at least two different algae should be cultivated on-farm if grown over the entire year. For example, Lemna or NT8c could be grown over 6-9 months, while Limnothrix or another grazer-resistant alga could be grown during the hotter months. Assuming that Lemna is grown for 9 months at $781/t DM and Limnothrix at $2,219/t DM, the average cost of production over the whole year (including) would be $1,141/t DM. These actual costs are still relatively high (compared to other protein-rich feed currently available) but cost savings due to upscaling/automation, the expected on-farm learning curve and the independence from feed supplement price fluctuations make microalgae and Lemna attractive for cattle farmers. Depending on local conditions, farmers may opt for certain strains over others and may consider seasonal production. Plans for larger on-farm production modules and a brief operation mode are shown in Appendix 2.

6.2 Recommendation to do a small feeding trail

The algae analysed in this study have proten contents between 27-45% and are therefore comparable to previous trials carried out with Chlorella, cottonseed meal and soybean meal. These show that the main factor affecting weight gain is the amount of crude protein in the feed. Other factors, such as pallatability and methane emissions are still of interest and could be examined in a small feeding trial. The combining of algae with other stockfeeds, such as straw and hay is considered advantageous and this (1) offers practical solutions for drying of the product and (2) may enable a more accurate dosing of the algae. It would also be of interest how animal feeding behaviour is affected. For example, it would be good to determine if a large amount of feed can be provided without causing overfeeding. This could be enabled by providing the right balance of feed attractants (e.g. molasses) as opposed to repellents. Lemna was identified as the cheapest source of protein in this study, but is known to have a high calcium content. There is a number of Lemna feeding trials with desirable outcomes reported in the literature but this aspect may require further investigation.

6.3 Plans to deploy larger on-farm algae production systems

Following the interest from farmers, several large-scale farm proposals are currently being developed (e.g. at Roma, Miles and Goondiwindi). These are still in the planning phase and require further input and agreements before construction commences (including consultation of MLA). Details of planned modules on these demonstration farms is summarised in Appendix 2. In Roma, an emphasis will be placed on organically-produced algae protein in combination with straw as feed for organically produced beef. In Miles, planned algae farm production aims to provide feed for both meat and dairy cattle in the vicinity with the possible use of CSG water that is abundant in this area. In Goondiwindi plans have been discussed to pilot-test microalgae cultivation to grow nutritious feedstock for piggeries, poultry and possibly human consumption. Several overseas companies are currently in negotiation with UQ/MLA to deploy the algae cultivation and harvesting technology developed in this project.

The project leaders wish to further engage with MLA, cattle farmers and other interested parties to develop a preferred deployment strategy for algal protein feed supplement on cattle farms in Queensland and the Northern Territory.

7 Key messages

- This project developed microalgae as a protein supplement for cattle weaners, suitable for the dry season.
- New microalgal strains have been collected from northern cattle farms that display high growth rates, high protein contents, high salinity and temperature tolerance and that can be cost-effectively harvested by induced settling.
- Lemna, a small floating plant, has been developed as a cost-effective alternative to microalgae.
- A new low-cost microalgae pond cultivation system has been developed at farm-scale that, based on hydrodynamic modelling and a new mixing technology, provides high yields at minimal capital and operating costs.
- A cheap harvesting method has been implemented for microalgae, based on induced settling.
- A 250,000 L demonstration farm has been constructed and tested in routine operation that combines all new technology and the isolated strains from this project in one farm that serves as a prototype for low-cost algae production systems adapted to northern cattle farms.
- Costs of production varied from $784 to $2,219 per ton of dry matter for a projected 10 ha farm with 438 tonnes annual production, depending on the production system and alga used. Further significant cost savings are achievable with the expected learning curve.
- Industry benefits include protein-rich supplement supply all-year-round, independence from price fluctuations of other protein sources, improved animal live weight gain and potentially improved animal health.
- The increased live weight gain of weaners can lead to higher farm profitability and potentially a meat product with increased nutritional benefits for the consumer.

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9 Appendix

9.1 Appendix 1: Refereed publications (with MLA acknowledgements)

1. Garg S, Wang L, Schenk PM (2015) Flotation separation of marine microalgae from aqueous medium. ***Separation and Purification Technology*** 156:636-641. **(IF 3.1)**

2. Ahmed F, Fanning K, Netzel M, Schenk PM (2015) Induced carotenoid accumulation in *Dunaliella salina* and *Tetraselmis suecica* by plant hormones and UV-C radiation. ***Applied Microbiology and Biotechnology*** 99:9407-9416. **(IF 3.8)**

3. Ghasemi Naghdi FG, Schenk PM (2015) Protocols for lipid extraction from wet algal biomass. *IN:* ***Hydrocarbon and Lipid Microbiology Protocols.*** T.J. McGenity, K.N. Timmis, B. Nogales Fernández (eds). Springer Berlin Heidelberg.

4. Duong VT, Thomas-Hall SR, Schenk PM (2015) Growth and lipid accumulation of microalgae from fluctuating brackish and sea water locations in South East Queensland – Australia. ***Frontiers in Plant Science*** 6:359. **(IF 3.6)**

5. Duong VT, Ahmed F, Thomas-Hall SR, Quigley S, Nowak E, Schenk PM (2015) High protein- and high lipid-producing microalgae from northern Australia as potential feedstock for animal feed and biodiesel. ***Frontiers in Bioengineering and Biotechnology*** 3:53.

6. Ghasemi Naghdi F, Thomas-Hall SR, Durairatnam R, Pratt S, Schenk PM (2014) Comparative effects of biomass pre-treatments for direct and indirect transesterification to enhance microalgal lipid recovery. ***Frontiers in Energy Research*** 2:57.

7. Adarme-Vega TC, Thomas-Hall SR, Lim DKY, Schenk PM (2014) Effect of salinity on long chain fatty acids synthesis and associated gene expression in *Tetraselmis* sp. ***Marine Drugs*** 12: 3381-3398. **(IF 4.0)**

8. Sharma KK, Garg S, Li Y, Malekizadeh A, Schenk PM (2013) Critical analysis of current microalgae dewatering techniques. ***Biofuels*** 4:397-407.

Selected results were also presented at SICBENS (Seoul International Conference on Biological Engineering & Natural Science, South Korea, 29-31 August 2014), and at the Algae Biomass Summit in San Diego, USA, 29 Sept – 3 Oct 2014) where MLA's contribution was duly acknowledged.

Conference presentations (with MLA acknowledgements):

1. Schenk PM (2015) Algae Energy Farms for Biodiesel and Cattle Feed. The Australasian Bioenergy and Bioproducts Symposium, Brisbane, 12 October (Invited Speaker and Panellist).

2. Schenk PM (2015) Algae for Food, Feed and Fuel. UQ-TUM Research Symposium on Water, Environment and Sustainability, Munich, Germany, 11+12 June (Invited Speaker)

3. Schenk PM (2015) Low-Cost Algae Farming for Food, Feed & Biodiesel. Global Forum for Innovations in Agriculture, Abu Dhabi, UAE, 9-11 March (UAE Hosted Scientist and Invited Symposium Speaker).

4. Schenk PM, Thomas-Hall SR, Nowak E, Lim D, Adarme-Vega TC, Sharma K, Garg S, Narala R, Ahmed F, Duong VT, Malekizadeh A, Ghasemi Naghdi F, Britten A, Alsenani F, Abd Halim N, Al-Amery A, Zhou L, Tannock S (2014) **Development of low-cost high-efficiency algae energy farms in Australia.** 8th Annual Algae Biomass Summit, San Diego, USA, September 29 – October 2 (Invited Speaker).

5. Schenk PM (2014) **Low-cost high-efficiency algae energy farms in Australia.** Seoul International Conference on Biological Engineering & Natural Science, Seoul, South Korea, August 29-31 (Invited Symposium Chair and Speaker).

6. Narala RR, Garg S, Sharma K, Malekizadeh A, Tannock S, Thomas-Hall SR, Pratt S, Schenk PM (2014) **Low-cost microalgae cultivation systems for large-scale feed and fuel production.** 8th Annual Algae Biomass Summit, San Diego, USA, September 29 – October 2.

7. Schenk PM (2014) **Algae energy farms.** Inaugural Centre for Marine Science TalkFest, Brisbane, February 28 (Invited Speaker).

8. Sharma KK, Ahmed F, Schenk PM **(**2014) **Have you got your anti-oxidants from microalgae?** 8th International Algae Congress, Florence, Italy, 1-3 December

9. Malekizadeh A, Sharma K, Garg S, Narala R, Ghasemi Naghdi F, Ahmed F, Nowak E, Duong VT, Britten A, Tannock S, Thomas-Hall SR, **Schenk PM** (2014) **Development of low or no-energy-input microalgae harvesting techniques.** 8th Annual Algae Biomass Summit, San Diego, USA, September 29 – October 2 (Invited Speaker).

10. Duong VT, Ahmed F, Thomas Hall S, Nowak E, Schenk PM (2014) **Lipid and protein accumulation profiles of freshwater microalgae collected in the Northern Territory – Australia.** International Conference on Life Science and Biological Engineering. Sapporo, Japan, 22- 24 July. (Invited Symposium Speaker)

11. Schenk PM, Thomas-Hall S, Nowak E, Lim D, Adarme-Vega TC, Sharma K, Garg S, Narala R, Ahmed F, Duong VT, Malekizadeh A, Ghasemi F, Britten A, Medina-Cabrera E, Abd Halim N, Chunxiao M, Gao Z, Pratt S, Tannock S (2013) **Development of low-cost high-efficiency algae energy farms.** International Marine Biotechnology Conference, Brisbane, November 11-15 (Invited Keynote Speaker).

12. Garg S, Wang L, Schenk PM (2013) **Effects of algal hydrophobicity and bubble size on flotation separation of microalgae from aqueous medium**. Chemeca 2013, Brisbane, 29 September - 2 October.

13. Duong VT, Nowak E, Lim DKY, Carvalhais LC, Schenk PM (2013) **Effects of environmental conditions on lipid accumulation and diversity of microalgae at the South East coast of Queensland – Australia.** International Marine Biotechnology Conference, Brisbane, 11-15 November (Invited Symposium Speaker; presented by PhD student Duong Van Thang).

14. Schenk PM (2012) **Microalgae Energy Farms for durable production of fuel and livestock feed.** 2nd Algae World Australia, Perth, April 16-17 (Invited Opening Session Speaker).

15. Li Y, Garg S, Duong VT, Ahmed F, Adarme-Vega TC, Sharma K, Narala RR, Lim DKY, Rincón-Flóres VA, Nowak E, Malekizadeh A, Ghasemi F, Schuhmann H, Tannock S, Schenk PM (2012) **The Future of Microalgae Farms: a low cost module for agriculture adoption.** 8th Asia-Pacific Conference on Algal Biotechnology, Adelaide, 9-12 July (Poster).

16. Schenk PM (2011) **Development of microalgae energy farms for adoption by agriculture.** Bioenergy Australia, Sunshine Coast, November 23-25 (Invited Speaker; first speaker in keynote session).

Further publications are in preparation on the following topics:

- A simple, yet effective off-grid farm design for low-cost microalgae cultivation and harvesting
- Effective control of rotifers for *Scenedesmus dimorphus* NT8c production
- Development of a new solar drier suitable for large-scale microalgae production
- Large-scale *Scenedesmus dimorphus* NT8c production
- Induced flocculation of *Scenedesmus dimorphus* NT8c using KOH
- Development of a hydrodynamically improved raceway pond.
- A new airlift design for algae culture mixing in raceway ponds.

9.2 Appendix 2

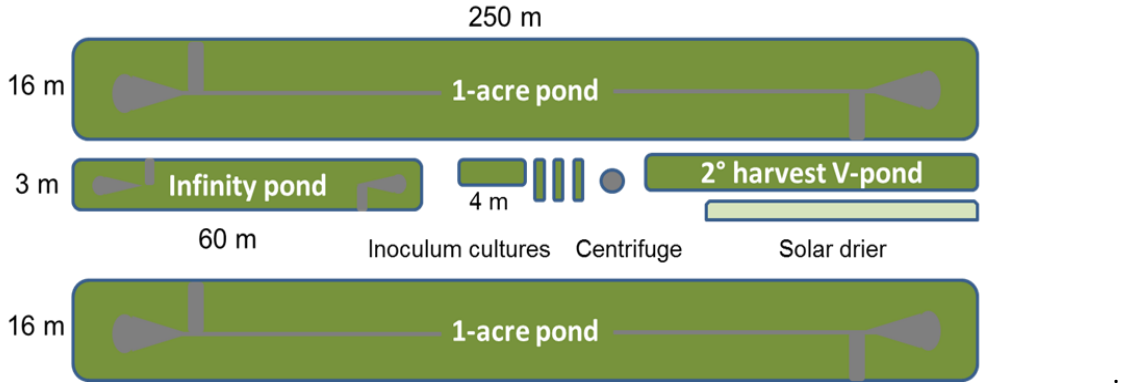
Algae Farm Modules for future on-farm deployment

The minimum recommended size for 1 module is two 1 acre-ponds on a 1 ha site.

FARM SET-UP:

The 2-acre algae farm comprises:

- 2 large 1-acre raceway ponds (16 m wide x 250 m long) to hold 800 kL at a depth of 0.2 m
- 1 pond as backup-culture/inoculation pond (3 m x 60 m) to hold 36 kL ("Infinity pond")
- 1 small protected backup/inoculation pond (4 kL) and small bag cultures (initial inoculum)



Proposed design of a 2-acre Algae Farm Module for cattle farms

FARM OPERATION:

INITIAL SETUP:

The initial inoculation stage consists of small volumes of pure microalgae culture. Typically, 20 litres inoculum is diluted into 160 litres of water and nutrients. After this setting phase the diluted inoculum and the water are pumped into a covered 4,000 litres pond where the algae can grow in a protected environment. This culture provides the inoculum and backup culture for an open raceway pond of 36,000 L. This pond serves as the initial inoculum and backup culture for a full-size 1-acre raceway pond.

REGULAR OPERATION:

1. Cultivation: Microalgae are continuously kept in exponential growth phase, by adding fertiliser every 2-3 days. Microalgal grazers are controlled by fertiliser addition. Regular (min 2x/week) quality control checks include nutrient measurements, pH measurement and microscopic analysis of the culture. Harvesting is typically carried out 3 times a week, depending on temperature and sunshine that influence algal growth.

2. Harvesting: The culture is subjected to induced overnight settling through flocculation in its pond. Typically 50-80% of the algal culture settle and the remaining culture is used for regrowth. The concentrated sludge is removed by pumping into as separate V-pond (capacity 40 kL). After secondary settling, a basket centrifuge can be used for further de-watering and if drying is required, the resultant algal paste is transferred into a (solar) dryer to get dried storable biomass. Optionally an industrial drier can be used for cloudy days.