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I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

'Cloverleaf Blanket Answer' was the only product tested that controlled blanketweed.

Background and expected deliverables

Algal contamination of aquatic plants, in particular with blanketweed, is a major issue on nurseries, most growers wash plants in water and hand-remove visible contamination. However, small amounts of algae can remain and multiply rapidly up during display for sale and cause customer rejection of the plants. Aquatic plants grown in production tanks are sold for aquaria, ponds, swimming ponds, lakes and for biological filtration. Growers can also be held responsible for supplying plants that introduce algae to previously clear water.

The move to conserve water and use rainwater and run-off water, rather than only mains or bore-hole supplies, is likely to lead to algae in water supplied to plants. Algae threads can block filters, while fragments and unicellular algae may pass into aquatic plant trays and tanks. Algae may also increase on pots grown out of water. Algal growth in drainage channels and on pots encourages shore flies which can lead to plant rejection by purchasers; additionally, shore flies transmit fungi such as *Pythium* species.

The herbicide 'Clarosan 1FG' (terbutryn) had its approval revoked in 2007, this product had been used successfully to control filamentous algae and floating weeds without (usually) affecting water lilies. Biocidal products available for algal control in water in the UK are mainly marketed for amateur use, with registration previously under the Health and Safety Executive (now part of the Chemical Regulation Directorate, CRD). Algaecides do not carry claims to protect pond plants, and so do not fall under the remit of the Pesticides Safety Directorate (now part of CRD). These products can thus be used professionally, but an assessment of any health risks needs to be undertaken by users based on the larger volumes likely to be used. The product registration does not consider plant safety and so if used commercially they would also require phytotoxicity tests before widespread use.

Published information is lacking on the efficacy of most of the microbial, enzyme or natural chemical products marketed for algal control. There is information on the use of barley straw and its extracts (published in HNS 145). The Royal Botanic Gardens at Kew have used the dye Dyo-Fix Blue successfully in their water lily ponds, reducing the light penetration to stop photosynthesis by the algae below the water surface.

An EU project has shown the effectiveness of ultrasound in a chemical-free treatment system for fish farms, suppressing algal growth and causing efficient sedimentation of planktonic algae using an LG Sonic device. LG Sonic devices are claimed to help in controlling algal growth, with a difference visible from 24 hours to two weeks after use. (It might be feasible to justify the capital cost of such an ultrasound device if, after a two week treatment period, it was moved sequentially between blanketweed contaminated tanks).

The Centre for Aquatic Plant Management investigated the mode of action of ultrasound on algae, including a blanketweed, *Spirogyra*. Within 14 days there was disruption to the cell contents and walls.. The cells remained buoyant for a few weeks, but were not viable. Some treatments, in particular ultrasound, have been reported to also control fungi, bacteria and biofilms (bacteria and slime). Pathogen control would be an additional benefit from the use of such algal control products.

It is not known if there may be any phytotoxic effect to aquatic plants from the various algal control methods. Although some products and procedures state that they are safe to aquatic plants, observations may have been carried out in ponds where any loss of plant quality may be better tolerated, and where conditions differ from denser packed production tanks.

The specific objectives of the project were:

- To determine the efficacy of some existing UK-marketed microbial and natural aquatic-algae control products.
- To test the concept that ultrasound will control algae in nursery aquatic plant production tanks.
- To check for phytotoxicity to aquatic plant of some algal control treatments.

Summary of the project and main conclusions

Algal control by products added to the water

A list of algal control products available in the UK was compiled. Five powder or liquid products were selected which had varying claimed modes of activity (Table 1). Testing was carried out in summer 2009 at ADAS Boxworth with 50 L tanks (four per treatment) in which filamentous algae (blanketweed), unicellular green algae and Bog Arums obtained from an aquatic plant nursery were introduced. The products were added to the water according to their labels (Table 1). Records of blanketweed water coverage, unicellular algal density, water properties and plant growth were taken fortnightly for eight weeks.

Table 1: Treatments, ingredients and handling precautions, addition rates and frequency of addition for products to 50 L tanks of algae and plants over eight weeks

Product	Ingredients and handling precautions	Dose used (as label)	Application intervals
Untreated	-	-	-
Interpet Blanket weed Buster with Sludge Buster	Probiotic bacteria. No handling precautions.	One spoonful (6.6 g) / 1325 L, mixed first in 1 L warm water.	Repeat every 2 weeks.
TetraPond AlgoFin for blanketweed	Monolinuron 0.75 g / 100 ml. Wash hands after use. May produce an allergic reaction.	50 ml / 1000 L.	Repeat at half-dose every 4-6 weeks.
NT Aquaclear Algae and Blanketweed control	Non-toxic blue dye. Avoid staining clothing.	10 ml / 586 L.	Repeat monthly as the colour fades.
Nishikoi Blanc-Kit Excel	Natural minerals and botanical compounds. Wash hands after use.	5 ml scoop (5.2 g) / 318 L.	Every 2 weeks for 6 weeks, then monthly.
Cloverleaf Blanket Answer	Minerals and enzymes. Wash hands after use.	Level 30 ml scoop / 285 L.	Repeat if required (repeated Day 28).

Outstanding control was shown by the Cloverleaf Blanket Answer with no blanketweed (dead or alive) present after 28 days in any of the four treated tanks. Control was maintained until the end of the experiment (57 days after treatment). After 14 days, the blanketweed broke apart when handled and would have been easy to clean off the plants. In contrast, blanketweed coverage reached 100% in thee out of four untreated tanks over the duration of the experiment. The product powder needs to be mixed with water taken from the tank and then the water sprinkled over the tank surface. The water becomes slightly milky. The product has a retail price of £7.78 for a single treatment of 10,000 L. This was the only product of those tested where a single dose was sufficient to give complete algal control. The product is marketed for fish ponds and so there would not be expected to be concerns about selling plants directly from treated tanks.

There was unexplained variation in the blanketweed cover between tanks receiving the same treatments, such as from the fortnightly doses of the Blanc-Kit Excel, where one of the four tanks had no algae at the finish, while the other three had over 50% cover. Some of the other products such as Aquaclear Algae and Blanketweed control (containing a blue dye to exclude light from the algae) showed promise in two tanks, but possibly could not work against blanketweed floating at the water surface. The AlgoFin herbicide started to give some control, with disintegrated algal strands seen under the microscope after 14 days. However, surviving strands multiplied, and topping up with more AlgoFin after four weeks with the recommended half dose was ineffective. Some blanketweed with broken cells were seen 14 days after treatment with Interpet Blanketweed Buster (containing bacteria), but after eight weeks of fortnightly re-application the blanketweed volume had not been reduced.

All the products were stated to be safe with pond plants. There was no phytotoxicity to Bog Arums in the first weeks of the experiment. The condition of the plants then deteriorated in all the untreated and treated tanks over the eight weeks and so it was not possible to assess phytotoxicity. Growers would be advised to test samples of various aquatic plants in a tank with their selected product before wider use. No treatment effects were detected on unicellular algae (low densities occurred throughout), or on either the pH or EC of the water.

Algal control using an ultrasound device

An LG Sonic SSS 220 ultrasound device was tested against blanketweed in a series of 400 L Bog Arum production tanks on a nursery near Hitchin, Herts. To determine whether one ultrasound device could treat several tanks, the device was moved between four tanks in turn at fortnightly intervals, one tank remained untreated throughout. According to the manufacturer, the device was expected to show effects within two weeks of treatment. Monitoring of algal density, water properties and plant vigour was carried out between 3 June and 12 August 2009. No treatment effects were detected on unicellular algae, with low densities being recorded throughout, or on either the pH or EC of the water.

Blanketweed was disrupted by the ultrasound device leaving brown, broken filaments in three of the four tanks. However, after the treatment period the blanketweed disruption was confined to within 0.3 m of the device. Beyond this distance the floating plants with hanging roots may have impeded the ultrasound waves. The percentage water surface area coverage by blanketweed in two tanks was lower by the end of the experiment, but healthy green blanketweed was still present in all four treated tanks by the end of the experiment. Bog Arum shoot and root growth and appearance were unaffected by the device. It would not be possible to gain effective treatment in plant production tanks with a 14 day treatment;

possibly a more extended treatment would be more effective. Only one ultrasound model was tested, and results might differ with a different manufacturer's product range.

Financial benefit

There are only a few large growers of aquatic plants remaining in the UK, but there are a larger number of smaller ones. Generally businesses have a very diverse customer base including landscape garden companies, garden centres, aquarium, koi specialist suppliers and mail order direct to the public. This makes placing a financial figure on the market very difficult.

Plants growing in pots of soil or compost have to be hosed off and individually manually cleaned before being dispatched (it is not easy to remove all the blanketweed fragments and these can regenerate). Nurseries often differ in the frequency of cleaning of aquatic plant tanks (depending upon the level of contamination and the market the plants are destined for), and some tanks will remain with unsold stock between years which will mean that blanketweed is present to multiply up the following spring.

Therefore, the use of Cloverleaf Blanket Answer to disintegrate blanketweed would mean that plants would not be tangled with weed, be easier to pick out and require much less cleaning time, and tanks might not need to be cleaned out as frequently between crops to remove the blanketweed.

Action points for growers

- Ensure that tanks are treated for blanketweed early in the plant production cycle – not forgetting mother plant tanks.
- Treat blanketweed as soon as any is seen, before it builds up.
- Consider adding Cloverleaf Blanket Answer to tanks to control blanketweed, using manufacturer's recommended rates.
- Check phytotoxicity to Cloverleaf Blanket Answer using a sample range of different plant species before application to whole production areas.
- If testing ultrasound treatment for the control of blanketweed, use it before increasing plant pot density in tanks, and remove any obvious blanketweed before commencing.

SCIENCE SECTION

Introduction

Background

Aquatic plants grown in production tanks are sold for aquaria, ponds, swimming ponds, lakes and for biological filtration. Algal contamination of aquatic plants, in particular filamentous algae (blanketweed) is a major issue on nurseries. Most growers spend time washing plants under running water and hand-removing visible algal contamination prior to plant dispatch. However, small amounts of algae can remain and multiply rapidly either during display for sale or in their final planted location. Growers can be held responsible for supplying plants that introduce algae to previously clear water. Once introduced to these situations algal contamination is difficult to manage.

Algae in water supplied to plants is likely to increase where growers reduce the use of increasingly costly or limited supplies of mains or bore-hole and instead utilise recycled or collected water. Nutrient enriched water from run-off is likely to favour algal growth. Algal fragments and unicells may pass into aquatic plant trays and tanks, including those of marginal plants grown out of water. Additional problems from algae include the blocking of irrigation line and pond filters by algal threads and agglomerations with bacterial slime. Algal growth in drainage channels and on pots encourages shore flies which can transmit rot fungi such as *Pythium* species and the flies are a visible nuisance. Greater reported problems with blanketweed contamination may have been caused by the recent warmer winters which mean the threads do not die back. This causes an earlier build of algae in the following year.

Methods aimed at controlling algae in aquatic plant production were reviewed in HNS 145. These include control of nutrients, mixing of the water column, light reduction, use of fish, manual removal of blanketweed, ultrasound, barley straw, microbial control, and biocides (Table 2). Filamentous algal control was provided by the herbicide ‘Clarosan 1FG’ (terbutryn), which acted without affecting water lilies, but this was lost when UK approval was revoked in 2007 (CEH, 2004). There is limited information on the efficacy of the various other means of control which might be suitable for growers.

Table 2: Examples of products available in the UK for algal control in water

Product name, and grouping by mode of algal control	Contents, and possible mode of action as stated on the label
Physical activity	
LG Sonic (different models for ranges of 5 m to 186 m)	Ultrasound oscillation causes green algal cells to resonate and the cell membrane ruptures. Gas vacuole ruptures in blue-green algae
Aquasonic	Ultrasound
Indirect activity	
Dyo Fix Pond Blue	Blue dye reduces light penetration to algae
NT Labs Aquaclear Algae and Blanketweed control	Blue dye reduces light penetration to algae
Chemical activity	
Phoslock	Phosphorous absorbed so algal growth reduced
Nishikoi Phos-Kit	Phosphorous absorbed so algal growth reduced
Pond Balance Clears Blanketweed	Ingredients not stated. Adjusts water chemistry
Aqua-Balance stops blanketweed	Ingredients not stated. Restores chemical equilibrium and adds trace elements
Magiclear Clears Green and Cloudy Water	Ingredients not stated. Clumps suspended solids
Waterlife Algizin G	Ingredients not stated. Clears green/brown water by causing the algae to sink
Interpet Green Away	Includes formaldehyde. Clumps algae
Beaver Pond Conditioner	Carbon-based product absorbs nutrients
Chemical algaecides	
Waterlife Algizin P	Benzalkonium chloride & copper sulphate kills blanketweed
King British Algae Control	Copper sulphate pentahydrate kills algae
TetraPond AlgoFin for blanketweed	Monolinuron kills algae
Biological activity - minerals and enzymes and/or bacteria	
Aqualibrium product	Plant extract and fruit oil based
Nishikoi Blanc-Kit Excel	Natural minerals and botanical compounds. Checks molecular structure of blanketweed
Cloverleaf Blanket Answer	Minerals & enzymes.
Interpet Barley Straw Extract	Releases natural chemicals
Pond Pads	Barley straw effective against all algae
NT Labs Barley Straw Pouches	Barley straw
Nishikoi Goodbye Blanketweed	Bacterial culture on barley straw, enzymes. Nutrient competition. Act in conjunction
Nishikoi Goodbye Green Water	Bacteria and enzymes with activated barley straw. Bacteria compete with algae for food
Aquaclean	Bacterial based competition with algae for food
Aqua-Zyme Pond Clarifier	Bacteria and enzymes
Pond Clarifier Tablets	Microbes and enzymes
Interpet Blanketweed Buster with Sludge Buster	Bacteria which compete with algae for available nutrients and consume pond sludge
BioWorld Algae Competitor Microbes	Competitor & organic degrading microbes

Biocidal products available for algae control in water in the UK are mainly marketed for amateur use in ponds or fish tanks. These are HSE registered products (they have no MAPP number) with copper sulphate as the active ingredient. Because they do not carry claims to protect pond plants they do not require registration as pesticides (Andrew Edwards, pers. comm., 2007) and this also means that there is no requirement for efficacy to be proven. These products can be used professionally, but a COSHH assessment of any health risks must be carried out by the user based on the larger volumes likely to be used than by an amateur. The product registration does not consider plant safety, and so growers would need to test the product on samples of the plant species and varieties likely to be treated to ensure there is no phytotoxicity.

There are a significant number of products marketed for algal control in ponds which use a "natural" control method. The ingredients of these are not usually fully detailed, but can include various mixtures of bacteria, plant compounds, minerals or enzymes. These claim to control the algae either by direct activity or by altering the water chemistry to make it less favourable for algal growth. A further means of control is gained by the reduction of light penetration through the use of a coloured dye.

Previous research

Work in France (Deogratias, 2005, 2006) has shown control of algae in reservoir ponds and test tanks after the use of blue dye. Ultrasound using an Algasonic device was also effective in eliminating algae from reservoirs. There was limited success using a microbial product in large ponds, but this product was one of a wide range available (Deogratias, 2006).

An EU project has shown the effectiveness of ultrasound in a chemical-free treatment system for fish farms, suppressing algal growth and causing efficient sedimentation of planktonic algae using an LG Sonic device (Klemenčič *et. al.*, 2008). LG Sonic devices are claimed to help in controlling algal growth, with a difference visible from 24 hours to two weeks after use (Lisa Brand, biologist for LG Sound, pers. comm., 2008).

The Centre for Aquatic Plant Management investigated the mode of action of ultrasound on algae, including a blanketweed, *Spirogyra*. Within 14 days there was disruption to the cell contents and walls, and cytoplasm leakage. The cells remained buoyant for a few weeks, but were not viable (CAPM, 2003).

Some products, in particular ultrasound, have been reported to control fungi and bacteria. The cell wall structure of *Phytophthora* and *Pythium* species means they are more closely related to algae than other fungi, and so it is possible that microbial or enzyme products effective against algae could also be effective against fungi. Biofilms (bacteria and slime) can be controlled by ultrasound as well as algae (Newman, no date). Biofilms can harbour fungi and the mucus may also increase their survival. Pathogen control would be an additional benefit from the use of algal control products.

Published information is lacking on most of the microbial, enzyme or natural chemical products available for algal control. The Royal Botanic Gardens at Kew have used Dyo-Fix successfully in water lily ponds. There is information on the use of barley straw and its extracts (Geiger *et al.* 2005; HNS 145), the barley straw needs to be added several months before bloom conditions are expected to occur. Possibly this is suitable for use in reservoirs provided it can be kept aerated (HNS 82).

Commercial objectives

To evaluate the biological, "natural" chemical and ultrasound methods currently marketed for algal control in water to identify the most effective and cost-effective products, and to check for any detrimental effects on plant growth. These methods should be safe for nursery staff removing plants for sale from tanks throughout the season, and not require a holding period before plant sale. Information on effective products suitable for use in garden centre displays and in customers' ponds would also be gained and increase retail sales.

To determine whether the capital cost of the ultrasound device can be justified by achieving speedy algal kill and thus allowing the device to be moved between production tanks within the same season. Most reported work on ultrasound has been on semi-permanent installations in reservoirs rather than the more shallow production tanks found on nurseries.

Project objectives

Overall aim:

- To control algae in aquatic plant production tanks.

Specific objectives:

- To select from, and determine the efficacy, of some existing UK-marketed microbial and natural aquatic-algae control products.

- To test the concept that ultrasound will control algae in nursery aquatic plant production tanks.
- To check for aquatic plant phytotoxicity to algal control treatments.

Materials and methods

Evaluation of algal control products

This work was carried out in plastic 50 L tanks set up outdoors at ADAS Boxworth, Cambs. There were five algal control products and an untreated control, each replicated four times in randomised blocks, giving a total of 24 plots.

The tanks were set up on 21 May 2009 and left to stand before 45g of blanketweed was added on 3 June. Blanketweed was collected from a number of untreated Bog Arum tanks from a nursery site at Hitchin, Hertfordshire, with a total wet weight of 1.6 kg divided into weighed portions between the 24 tanks. Containers of pond water green with unicellular algae were collected from a Koi fish pond at the same site and 3 L added to each tank to bring the water level to 45 L. Three Bog Arum plants per tank were also purchased from the Hitchin nursery and left unpotted, with the rhizomes floating at the water surface, as at the nursery. The plants were held in another tank before addition to the test tanks on 9 June.

Products to be evaluated were selected to include a range of ingredients and methods of blanketweed control (Table 3). Products were not selected if the water required aeration, as this is not usual in plant production tanks. Where products advised repeat doses these were given, as detailed in Table 4.

Table 3: Algal control products, ingredients and precautions, pack size used and retail pack price with cost to treat 1000L for up to 28 days

Product	Ingredients & handling precautions	Pack size & volume treated	Pack price & cost for 28 days treatment
Nishikoi Blanc-Kit Excel	Natural minerals & botanical compounds. Wash hands after use.	Nominal 262 g for 13,638 L	£14.00 (£2.10 /1000 L)
NT Labs Aquaclear Algae & Blanketweed control	Non-toxic blue dye. Avoid staining clothing	250 ml for 14,200 L	£6.99 (£0.50 / 1000 L)
Cloverleaf Blanket Answer	Minerals & enzymes. Wash hands after use.	800g For 10,000 L	£17.99 (£1.80 / 1000 L)

TetraPond AlgoFin	Monolinuron 0.75 g/ 100 ml. Wash hands after use. May produce an allergic reaction	250 ml for 5000L	£7.78 (£1.60 / 1000 L)
Blagdon / Interpet Blanket weed Buster	Probiotic bacteria. No handling precautions.	25 g for 2273 L for 4 weeks	£8.39 (£7.40 / 1000 L)

Table 4: Treatments, addition rates and application frequency for products used in water volume of 45 L in tanks of algae and plants over 8 weeks at ADAS Boxworth - 2009

T	Product	Packet instructions and quantity used at each application into 45 L	Packet instructions application intervals
1	Untreated	-	-
2	Nishikoi Blanc-Kit Excel	*5 ml scoop (5.2 g) treats 318 L. Mix product granules before removing sample* 0.7 g sprinkled around edge of each tank	*Every 14 days for 6 weeks, then monthly.* Day 1 Day 14 Day 28 Day 42
3	NT Labs Aquaclear Algae & Blanketweed Control	*10 ml per 586L* 3.2 ml to 1 L pond water, then 250 ml of diluted product to each tank	*Repeat monthly as the colour fades* Day 1 Day 28
4	Cloverleaf Blanket Answer	*Level 30 ml scoop per 285 L* 19 ml powder to 1 L pond water, then 250 ml of diluted product over each tank	*Repeat only if required* Day 1 Day 28
5	TetraPond AlgoFin for Blanketweed	*50 ml per 1000 L, then half-dose* 9 ml to 1 L pond water, then 250 ml of diluted product to each tank. 4.5 ml to 1 L pond water then 250 ml of diluted product to each tank.	*Half-dose every 4-6 weeks* Day 1 Day 28
6	Blagdon / Interpet Blanket weed Buster with Sludge Buster	*6.6 g spoonful per 1325 L, mixed first in 1 L warm water* 0.9 g to 200 ml approx 40°C tap water. Stand for 30 mins. Add 50 ml of diluted product to each tank.	*Re-dose every 2 wks* Day 1 Day 21* Day 35 Day 49

(*should have been from Day 14)

Figure 1: Four replicates of treatment tanks at ADAS Boxworth.

Figure 2: Close-up of tank at the start showing floating plants and blanketweed.

Details of the trial set-up at ADAS Boxworth and the records taken are given in Appendix 1. The 24 grey plastic rectangular tanks were initially set up on 1 May 2009 and algae and plants were to be left to establish until mid-June before the addition of treatments. However, because the plants were scorched by the use of the plant stimulant and NPK liquid fertiliser

(Maxicrop Triple as used by the nursery) at the label rate for hydroponics, the experiment had to be re-started on 21 May. Treatments were added after 25 days, 12 days after adding the blanketweed, in order to ensure that the experiment started and ran when weather and light conditions would be favourable for algal multiplication. A dilution rate of 0.5 L Maxicrop Triple per 3000 L water was subsequently used. No soil was added to the floor of the tanks so there was no extra source of nutrient release into the water.

Although some products recommended the removal of blanketweed before treatment and any killed material produced during treatment, this was not done for any treatment as it was decided that growers would not be anticipating combining treatment and manual removal.

Evaluation of ultrasound for algal control

The ultrasound experiment was carried out in a netted tunnel on a nursery near Hitchin, Hertfordshire in five tanks being used to grow floating Bog Arums (Calla lilies). The 400 L black plastic tanks were free-standing with a water surface area 1.5 m x 0.9 m. The tank sides were 0.3 m deep, but with soil and debris in the base (from 0.11 m to 0.21 m deep) the free water was never more than 0.2 m deep. Tap water was used by the nursery to top-up following evaporation. The number of Bog Arum plants in each of five tanks was adjusted to leave 20 plants spread across each. Ten plants per tank were assessed fortnightly for vigour and phytotoxicity, using a “W” sampling pattern across the tank.

The tanks had natural contamination with blanketweed, the amount differing between tanks. The % blanketweed cover across the water of each tank was not adjusted before the experiment commenced, but records were taken for each tank.

Figure 3: Aquatic plant production tanks at the Hitchin nursery, including the five Bog Arum tanks used in tests.

Figure 4: Bog Arum Tanks 1 to 3.

The experiment started on 11 May 2009, with ultrasound use commencing on 3 June. Details of the setting-up of the experiment and the recording intervals are given in Appendix 2. One tank (Tank 1) was left untreated and records taken on six occasions, at fortnightly intervals for 10 weeks, until 12 August. The untreated tank was a tank apart from the first treated tank. Recording commenced in the four ultrasound treatment tanks (Tanks 2 to 5) on the day each in turn received the ultrasound device, with a fortnight's treatment per tank before movement of the device to the next tank. When the ultrasound device was taken out of each tank, recording continued for at least another fortnight.

One mains-powered ultrasound device was used, an LG Sonic SSS 220 with a 10 m range, which was moved between tanks at a fortnightly interval. The transducer was held, in a clamp-stand, 0.1 m under the water in the corner of each tank, pointing diagonally across the tank. Plants were kept 0.3 m away from the device.

Figure 5: LG Sonic SSS ultrasound device, the cylindrical transducer is held horizontally below the water surface with the electronic box connected to a mains electricity supply.

Water quality and algae assessments

The same assessments were carried out fortnightly for the experiments both at Boxworth and at Hitchin. At Boxworth all 24 tanks were checked at each assessment time, but at the nursery an increasing number of the five tanks were monitored as they came into use at the fortnightly intervals used in the experiment. All equipment used in the tanks at both sites was cleaned before being moved between tanks.

Temperature, pH and electrical conductivity (EC) were measured using a combined temperature probe and pH meter (Hanna H1 98128) and an EC meter (Bluelab Truncheon Nutrient Meter). These were used at 10 cm below the surface at Boxworth, in one area of the tank un-shaded by plants or blanketweed. At the nursery, the probe was at half this depth in order to keep it out of the plant debris at the bottom of the tanks. Water properties were measured in case they were altered by the products or any resulting algal decomposition.

The spread of blanketweed across each tank was recorded as a % of the water surface area. The cover by blanketweed as viewed from above was assessed, with blanketweed visible both floating at, or (where there was no blanketweed floating above) below, the surface recorded separately and then totalled. The fortnightly blanketweed cover records were taken in the afternoon when the majority of the filaments had risen towards the surface in the sunlight. It was, however, possible for some algae to remain below the visible depth when the water was cloudy or coloured.

A sub-sample of no more than 20 filaments of the blanketweed was extracted from each tank and placed in a tube with tank water. Observations were made on the appearance of the blanketweed in each tank and a record was made of how easily the strands pulled apart from the main blanket, as dead cells tend to have less cohesion. The cytoplasm condition of 10 filaments per tank, (in particular the arrangement of the chloroplast ribbons), was recorded under a high power microscope, examining about 20 mm of each filament.

A visual assessment of unicellular algal density was made by placing a white ruler into the water and recording the depth at which the 0 cm marking became invisible.

In order to determine unicellular algal content, water samples of 0.5 L were collected in a bottle, then coarse-sieved into another bottle to remove blanket weed. At sampling, care was taken to not disturb the bottom sediment, which would include dead algae. At Boxworth, one sample was taken from one tank per treatment. At Hitchin, one sample was taken per tank. An aliquot was taken in the laboratory from the nursery samples to record light penetration through suspended particles (using spectrophotometry) and for cell/multicell density counts (using a haemocytometer). Samples were assessed within 24 hours of sampling. Spectrophotometry was not used at the Boxworth site, and was discontinued on later dates for the nursery, as the results (not presented) were not useful when water was visibly clear.

After sub-sampling, the remaining water sample was left in the transparent bottle to settle for 24 hours in diffuse natural light during the day. On the next day, an index of the density of live algae was recorded by noting which of the graduated-thickness barlines on a chart was visible when the chart was placed mid-way up behind the sample bottle. Only live algae would be able to keep themselves in suspension after the settling period and so decrease the water clarity.

After eight weeks, all of the blanketweed at ADAS Boxworth was collected from each tank, using a net. The collected blanketweed was gently squeezed until water stopped dripping and then the weight of blanketweed per tank recorded. At the nursery site, the natural starting amount of blanketweed present differed in each tank and was not weighed, and the final weights were not taken as the determination of the presence or absence of the weed was the principle aim and shown by the % cover assessments.

Plant assessments

Plants were assessed for any phytotoxicity (using a 0-10 index, where 10 = dead) with a description of the symptoms, together with a record of plant shoot vigour (using a 0-10 index, where 10 = very healthy). The three Bog Arums at Boxworth, and ten of the Bog Arums from positions across the tank at the nursery were assessed. Root vigour was assessed at the end of each experiment. At Boxworth, plants were removed from the water, but at the nursery site root tips had secured the plants in the tank sediment and so the roots were assessed by feeling under water.

Results and discussion

Evaluation of algal control products at the Boxworth site

The water was alkaline (pH 10.5) before the treatments were added on 15 June (Appendix 3). After product addition, the tanks with Cloverleaf Blanket Answer were slightly less alkaline than all the others (Table 5). The tap water used to fill the tanks was pH 7.8, but it is possible that the alkalinity increased as water evaporated and was topped up.

Table 5 : Fortnightly measurements of mean pH (and standard error) for each treatment- ADAS Boxworth, 2009

Treatment	Day 1		Day 14		Day 28		Day 42		Day 57	
	pH	SE	pH	SE	pH	SE	pH	SE	pH	SE
Untreated	10.5	0.08	9.6	0.27	10.6	0.11	10.7	0.05	10.1	0.22
Nishikoi Blanc-Kit Excel	10.5	0.02	9.2	0.33	10.0	0.48	10.0	0.53	10.2	0.52
NT Labs Aquaclear	10.5	0.03	8.8	0.19	9.9	0.37	10.7	0.12	10.1	0.14
Cloverleaf Blanket Answer	10.5	0.02	8.8	0.27	9.4	0.35	9.4	0.17	9.4	0.17
TetraPond AlgoFin	10.5	0.05	9.1	0.10	10.0	0.50	10.2	0.10	9.9	0.14
Interpet Blanketweed Buster	10.5	0.05	9.0	0.32	9.8	0.45	10.1	0.09	9.9	0.14

Electrical conductivity did not differ between treatments, being around 0.2 EC (Appendix 3). The water temperature favoured blanketweed multiplication, being between 20 to 27°C on most assessment days (Appendix 3). Unusually hot weather between the end of June and start of July (Appendix 4) raised water temperature to 32°C, and probably caused the Bog Arums heat stress as indicated by symptoms of leaf yellowing and poor of growth.

Immediately pre-treatment the blanketweed had spread to give a range of coverage between 20% and 75% of tank surface. The blanketweed was not re-distributed before addition of the treatments. Further variation in the blanketweed occurred because overnight, after treatment on Day 1, birds pulled out and ate some of the weed, particularly in replicate 4. Subsequently the tanks were covered by suspended large mesh pea netting, to prevent interference by birds. Further blanketweed was added on 17 June to replace that loss, with all tanks in replicate 4 receiving 37 g (as loss was greatest from these tanks) and 20 g in all the remaining tanks. A further record of % blanketweed cover was made on 19 June (Table 6) once the algae had spread out over the water surface. Differences in cover were already starting to appear between treatments only 4 days after product addition, with at least one tank from four of the treatments having 5% or less blanketweed cover (Table 6). The blanketweed coverage increased in the untreated treatment over the 57 days of the experiment. Three untreated tanks had 100% cover, but in this and the treated tanks there was some unexplained variation between replicate tanks (Table 6 & Figure 6) Some variation in % cover records between assessments in the same tank (Table 6) may have occurred because the blanketweed rose to the surface when it was sunny and was not always visible when on the bottom of tanks.

Outstanding control was shown by the Cloverleaf Blanket Answer, containing minerals and enzymes, with no blanketweed (dead or alive) present from Day 28. After 14 days the blanketweed broke apart when handled and would have been easy to clean off plants.

Total control of the blanketweed needs to be achieved for a product to be worth using. Images of the coverage following treatment show that, even though in some tanks some products initially reduced the blanketweed, it soon recovered (Figure 7). There was some disruption of the chloroplast ribbons in the cells of all of the treatments after 14 days, in particular by the AlgoFin algaecide (Appendix 5), but only samples from the Blanc-Kit Excel and AlgoFin treatments had a number of strands affected at the final assessment. The Interpet Blanketweed Buster, although re-applied fortnightly, had insufficient activity to stop the blanketweed multiplying quickly to totally cover three of the tanks (Table 6, Figure 6). The AlgoFin algaecide also started to give control, however, surviving strands multiplied, and topping up with more AlgoFin after four weeks with the recommended half dose was ineffective, so that by Day 42 control continued to be lost (Table 6). The Aquaclear Algae and Blanketweed control showed promise in two tanks, but possibly the dye could not work to exclude light once blanketweed floated at the water surface before the dye was replenished. Following fortnightly doses of the Blanc-Kit Excel, one tank had no algae from Day 28, however there was over 50% cover in three tanks. Samples of filaments taken from these tanks did, however, have disrupted chloroplasts and so the treatment might have been continuing to have an effect. Cloverleaf Blanket Answer (Treatment 4) was the only product to give 100% control and blanketweed dispersal (Tables 6 and 7) in all tanks and the only product where a single dose appeared sufficient.

Table 6 : Effect of five algaecide products on control of blanketweed (% blanketweed cover) – ADAS Boxworth, 2009

Treatment	Rep	15/06/2009 Pre-treatment Day 1	19/06/2009 Day 4	29/06/2009 Day 14	13/07/2009 Day 28	27/07/2009 Day 42	11/08/2009 Day 57
1	Rep 1	50	60	50	75	98	100
1	Rep 2	25	30	1	15	97	30
1	Rep 3	25	25	10	50	80	100
1	Rep 4	50*	25	65	95	100	100
2	Rep 1	20	20	15	10	10	70
2	Rep 2	35	65	85	90	100	100
2	Rep 3	40	7	5	0	0	0
2	Rep 4	25*	20	15	60	80	100
3	Rep 1	25	5	5	10	62	10
3	Rep 2	40*	10	1	20	60	25
3	Rep 3	70*	10	5	10	35	85
3	Rep 4	25*	25	50	60	100	100
4	Rep 1	30	2	5	0	0	0
4	Rep 2	75	1	5	0	0	0
4	Rep 3	20*	17	10	0	0	0
4	Rep 4	20*	19	10	0	0	0
5	Rep 1	20	10	5	10	75	40
5	Rep 2	20*	2	20	80	100	100
5	Rep 3	20*	0	5	90	85	100
5	Rep 4	60*	30	50	60	80	100
6	Rep 1	20	2	5	10	80	25
6	Rep 2	30	50	65	85	98	100
6	Rep 3	60	50	55	95	100	100
6	Rep 4	25	35	25	70	90	100

Treatments : T1 untreated, T2 Nishikoi Blanc-Kit Excel, T3 NT Labs Aquaclear
T4 Cloverleaf Blanket Answer, T5 TetraPond AlgoFin, T6 Interpet Blanketweed Buster

* tanks with evidence of blanketweed removal by birds overnight within 12 hours of treatment addition on 15/06/09. More blanketweed was weighed out and added to all tanks on 17/06/09, with more given to all tanks in replicate 4 where most blanketweed was lost.

Table 7 : Effect of five algaecides on the weight of blanketweed at final assessment (Day 57) showing variation between replicate tanks, both untreated (T1) and treated (T2 -T6) – ADAS Boxworth, 2009

Replicate	Final weight of algae (g) following each treatment (T)					
	T1	T2	T3	T4	T5	T6
1	157	75	39	0	98	58
2	90	189	81	0	138	151
3	80	0	67	0	146	189
4	199	170	193	0	172	185

Treatments : T1 untreated, T2 Nishikoi Blanc-Kit Excel, T3 NT Labs Aquaclear
T4 Cloverleaf Blanket Answer, T5 TetraPond AlgoFin, T6 Interpet Blanketweed Buster

The unicellular algae introduced to the tanks attached themselves to the walls of some of the tanks of various treatments, the water being clear in the tanks (Appendix 6) with few cells present (Appendix 7) and so no conclusions could be drawn about treatment effects.

No obvious phytotoxicity occurred from any of the treatments at the start of the experiment, however, the conditions which favoured algal growth were possibly unsuitable for the plants so that, unrelated to treatment effects, old leaves yellowed, were lost, and then not replaced. This was recorded as phytotoxicity, but as it was seen in the untreated it was not strictly so. Loss of vigour was also shown but not related to treatment or otherwise (Appendices 8 & 9).

Figure 6: Fortnightly assessment of the % blanketweed cover per replicate tank for Treatments 1 to 6 from pre-treatment on 15 June 2009 to Day 57 – ADAS Boxworth, 2009

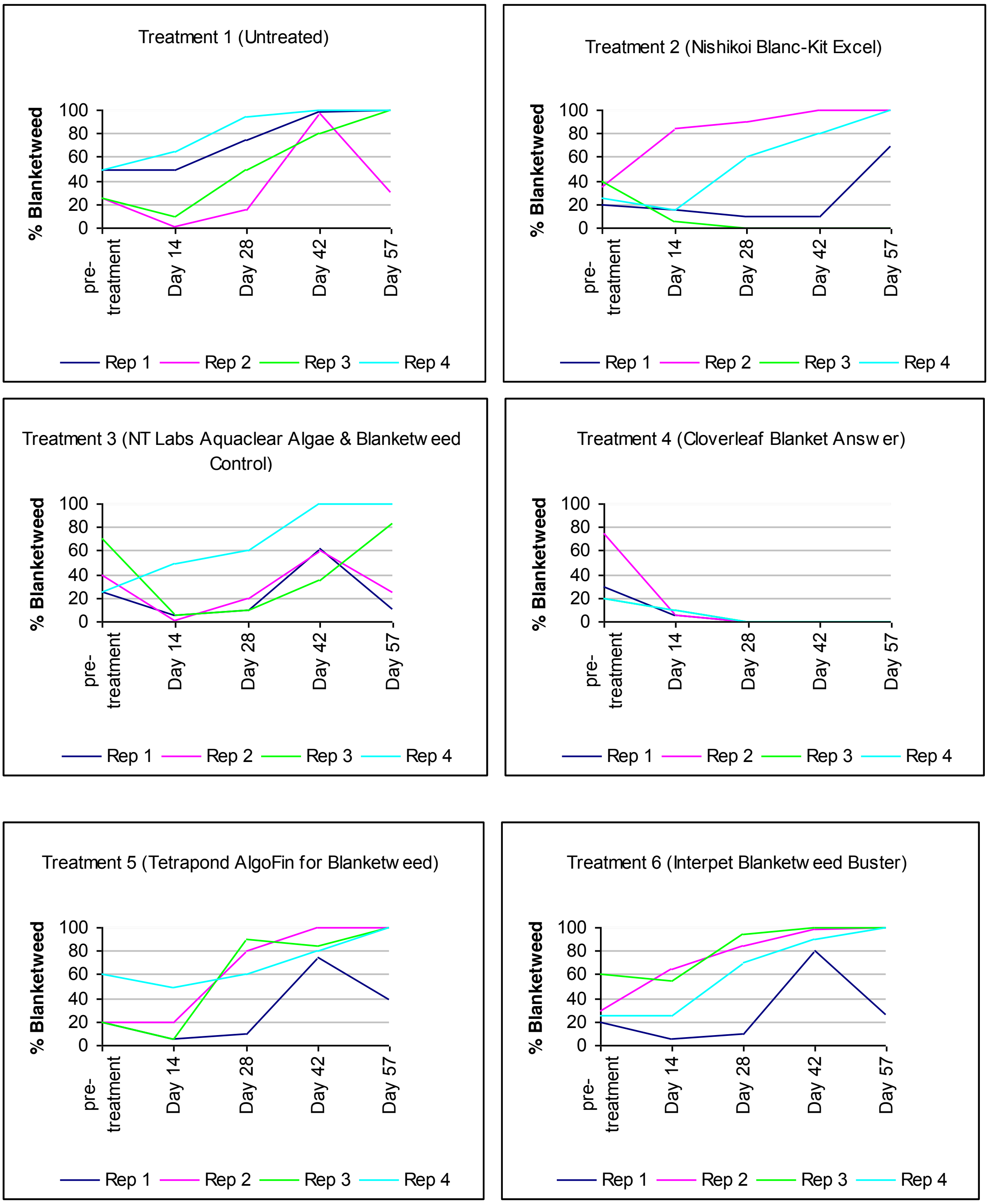

Figure 7: Example tanks of blanketweed and plants at final assessments of Treatments 1 to 6 on Day 57 – ADAS Boxworth, 2009

Treatment 1
Untreated

Treatment 2
Nishikoi Blanc-Kit Excel

Treatment 3
NT Labs Aquaclear Algae & Blanketweed Control

Treatment 4
Cloverleaf Blanket Answer

Treatment 5
TetraPond AlgoFin for Blanketweed

Treatment 6
Interpet Blanketweed Buster with Sludge Buster

Evaluation of ultrasound treatment at the Hitchin nursery site

There was no consistent pattern of either increase or decrease in electrical conductivity or pH in tanks following ultrasound treatment (Appendix 10). The conductivity tended to decrease from 0.5 to 0.2 in all the tanks (including the untreated) over the 70 days, as did that of the site's tap water used to top-up the tanks. The pH was slightly alkaline, mainly around pH 8. Water temperatures were between 16 and 20 °C. During the experiment there were several heavy downpours of rain (Appendix 11) which raised the water level in the tanks. The residual current device (circuit breaker) on the mains supply to the ultrasound device in Tank 5 was found to be tripped out on 24 July after one thunderstorm.

Bog Arum tanks on the nursery were utilised and labelled for the experiment, but the plants in the tanks were not purchased for the experiment. It was agreed with the nursery that other Bog Arum tanks would be the first choice for plant sale by the nursery. Unfortunately, it was not possible to keep the experiment tanks untouched, and plants were seen to have been taken from, or added to, the test tanks throughout the experiment as orders were made up by nursery staff (Table 8). Blanketweed was thus lost in some tanks, but it was still possible to see whether the blanketweed was able to increase after ultrasound treatment of the tank.

The ultrasound did not affect the vigour of the plants. All ten plants recorded per tank had a consistently good foliage score (index 10) at each recording date, although later some older leaves were yellowing (including in the untreated). Similarly, there was good root vigour (index 10) at experiment termination on 12 August when three plants per tank were assessed (results not presented).

Although water samples were taken to assess the unicellular algal density by haemocytometer reading (Appendix 12) and also initially by spectrophotometry (results not presented) the amount of this type of algae was too low throughout the experiment to be able to record any effect by the ultrasound device. The clarity in the settling vessel (Appendix 12) was as a result of colouration in the water, not the suspension of live algae in the water.

Brown, dead, blanketweed was recorded in the treatment tanks Tanks 2 and 4 after the 14 day treatment period, but was only seen again at the next recording for Tank 2 (Table 8 & Appendix 13), possibly because it subsequently sank. The brown blanketweed appeared gel-like and slimy and broke into small pieces when handled (Figure 10). In contrast, the healthy green blanketweed had long filaments (0.4 m long or more) with a strand quality which was smooth and silky and hard to detach from around the plant roots (Figure 9). In Tank 2 there were still green healthy strands below the water surface across the tank and right up to the ultrasound. In Tank 4 the brown floating strands were only found within 0.3 m of the ultrasound device, i.e. up to the margin of the nearest plants and mass of blanketweed (Figure 8). One small floating blob of dead broken filaments was retrieved from Tank 5 on Day 14. Treated Tank 3 never had any brown floating blanketweed. Blanketweed in the untreated tank (Tank1) was only ever green.

Under the microscope, the random 10-filament samples from Tank 2 at 14 and 28 days after treatment, Tank 4 after 28 days and also the specifically sampled brown blob in Tank 5 when the ultrasound was removed, had disrupted blanketweed cell contents as well as the healthy filaments that were counted (Table 8 & Appendix 14). There were wiry loose threads, the same diameter as the chloroplast ribbons which normally criss-cross around the cells of the blanketweed filaments. The wiry strands were thought to be tangles of chloroplast ribbons released from inside the cells by the ultrasound treatment (Figures 11 & 12). No brown blanketweed was seen or sampled in either the untreated tank or Tank 3 after treatment.

At the start of the experiment, Tanks 3 and 5 had below 30% blanketweed cover, and the other three had 90% or more, and so differences after ultrasound treatment were not intended to be compared directly between the tanks (Table 8 & Appendix 13). However, of the tanks with near total initial cover, by Day 42 the untreated (Tank 1) had 80%, whereas the treated tanks Tanks 2 and 4 had 10% and 30% cover, respectively. Tank 3 (where no brown blanketweed was seen) increased from 5% to 35% by Day 42. Tank 5 showed a small reduction at Day 14, but the cover then returned by Day 28.

The reduction in % cover by Day 28 in Tanks 1 and 2 may be misleading because it followed removal of some plants (with blanketweed) by nursery staff. However, in Tank 2 of the 15% blanketweed cover at this time, 12% was brown and broken, not green and healthy. By the final assessment on 12 August for an unknown reason the water had become dark and so only the blanketweed floating near the surface could be assessed, therefore the further decrease shown in Table 8 for Tanks 2 and 3 could be misleading. In general, although the ultrasound was sometimes able to kill the blanketweed within the two weeks, there was never total control of the high % cover present and so the healthy filaments left would be able to multiply to fill the space.

It would not be possible to gain effective treatment in a series of plant production tanks with each in turn utilising the device for a fortnight in order to share the cost of the device (£800 retail) between tanks. Longer treatment might give greater control, but this would need to fit within the relatively short aquatic plant sales period. Only one ultrasound model was tested, and results might differ with a different model or another manufacturer's product.

Table 8 : Effect of 14 days treatment with the LG Sonic SSS ultrasound device on % blanketweed water surface coverage (at or below the surface) in Tanks 2, 3, 4 and 5 compared with their % cover pre-treatment and the untreated Tank 1 – Hitchin, 2009

% blanketweed without ultrasound 3 June -12 August	Monitoring intervals in each treated tank	% blanketweed cover for Tanks 2– 5 until final assessments on 12 August			
Tank 1		Tank 2	Tank 3	Tank 4	Tank 5
>95	Day 1 (installed)	100	5	>90	25
10+	Day 14 (removed)	B■ 15+	5	B 80	B■10
25	Day 28	■ 15	10	■ 20	30*
80	Day 42	10	35	30*	
60	Day 56	35	10*		
50*	Day 70	15*			

Ultrasound installation dates: 3 June, 17 June, 1 July and 16 July in turn for Tanks 2 to 5

+ Blanketweed was probably removed by the nursery when some plants were taken out.
* On 12 August the water was too dark to accurately record blanketweed below the surface.
B Brown broken strands floating on surface. ■ Disintegrated blanketweed filaments present

Figure 8: Bog Arums in Tank 4 with ultrasound device(bottom left) after two weeks installation.

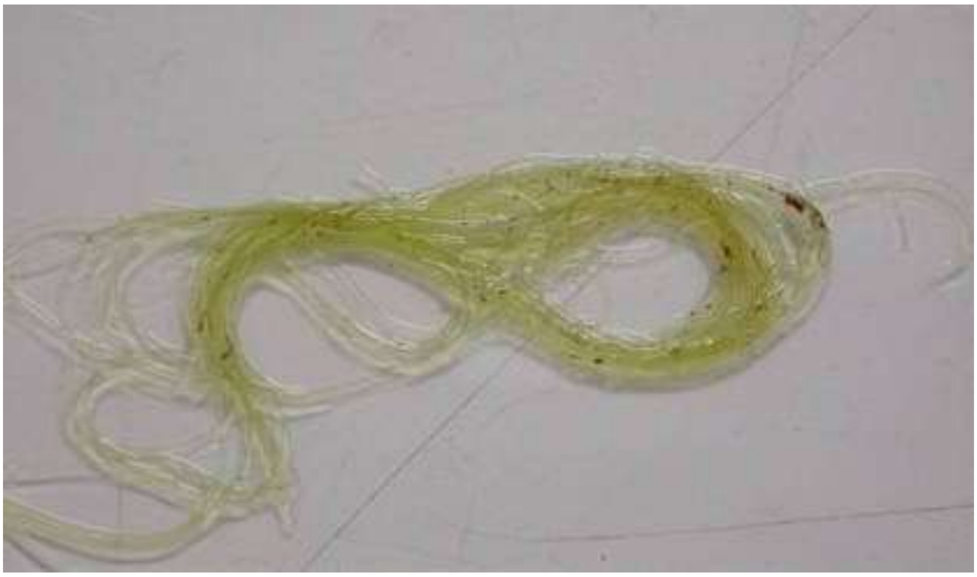

Figure 9: Healthy blanketweed filaments from nursery Tank 3, before ultrasound treatment – Hitchin, 2009.

Figure 10: Broken and brown blanketweed filaments from nursery Tank 2 after two weeks of ultrasound treatment - Hitchin, 2009.

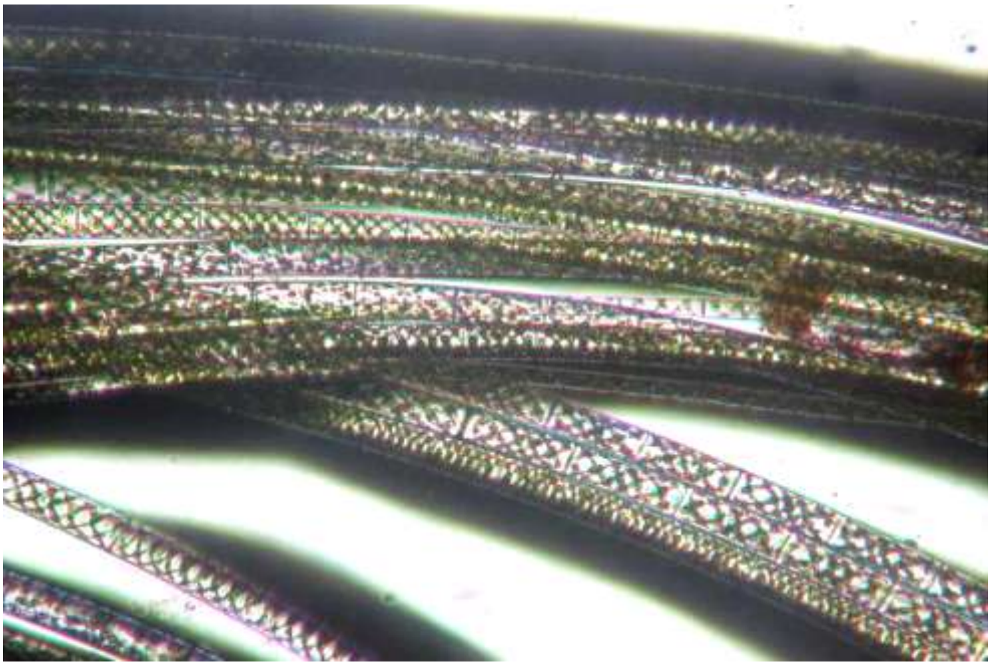

Figure 11: Microscope examination of healthy blanketweed strands showing intact chloroplast spirals.

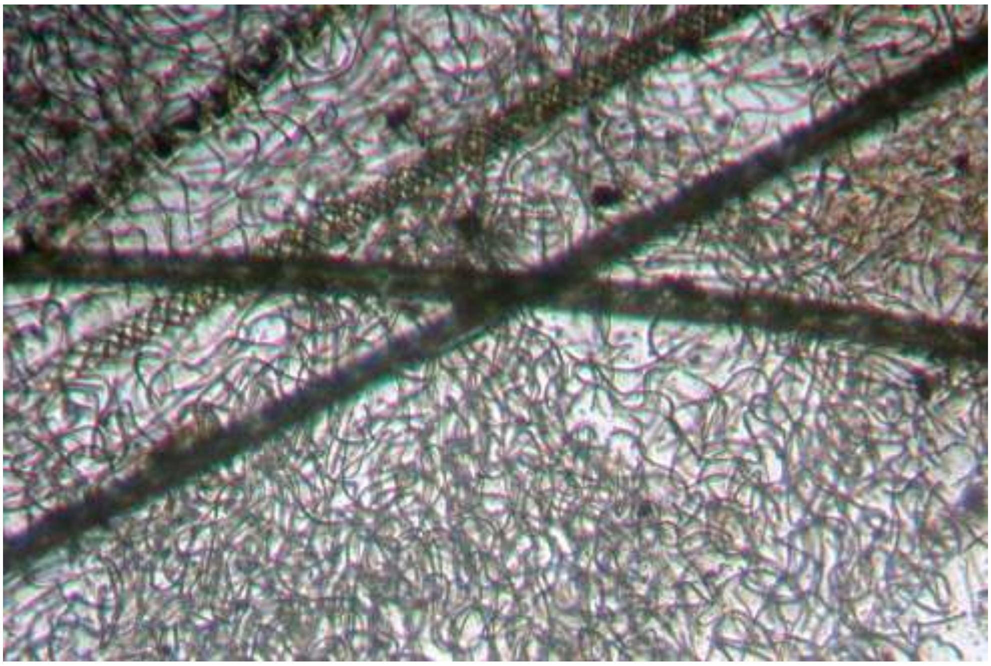

Figure 12: Microscope examination of blanketweed strands following two weeks of ultrasound treatment, showing ruptured cell contents (chloroplast stands). In the tank, the blanketweed mass near the ultrasound device became brown and lost cohesion.

Conclusions

Of five product additives evaluated, only one (Cloverleaf Blanket Answer) cleared the blanketweed totally from the tanks within four weeks of being added. One dose was sufficient, even with a high coverage of blanketweed, and so treatment on a nursery would cost £1.80 per 1000 L tank. There was no phytotoxicity.

Some of the other added products had partial success, in particular Blanc-Kit Excel; however only consistent and complete removal of blanketweed within a relatively short period would be an acceptable option for nursery use.

The ultrasound device used in the nursery tanks, although shown to lead to the destruction of filamentous algal cells by the disruption of their contents, was unable to clear the tanks of blanketweed following a two week installation period. Blanketweed survived from beyond about 0.3 m from the ultrasound device and was able to multiply. It was not known whether a longer installation period would be more effective. Future research is needed to determine whether the disintegration of cell contents by ultrasound could be used against water-borne fungal or bacterial plant pathogens that cause diseases such as *Phytophthora* crown rot of water lily and iris bacterial rot *(Pectobacterium carotovorum*).

Successful testing of ultrasound devices by other researchers has used more open bodies of water, such as reservoirs and commercial fish pens. The target treatment area of ultrasound is where the algae are found photosynthesising (within 0.2 m of the water surface), but aquatic plant leaves, petioles or floating roots are also within this area. For in-use plant production tanks the high density of plant tissue at the surface may thus impede the penetration of the ultrasound waves, so that the destructive resonance caused by the wave oscillation cannot be set off within the algal cells. The ultrasound manufacturers recommend removal of obvious blanketweed, and if this was possible on a nursery the penetration of the ultrasound through the water might be improved.

Technology transfer

Article

Sound treatments for the wrong sort of blooms. *HDC News,* June 2009, p7.

Wedgwood E. F. Control of blanketweed in aquatic ornamentals. *HDC News,* in press.

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References

CAPM, (2003). Annual Report Summary 2003. Centre for Aquatic Plant Management. http://www.nerc-wallingford.ac.uk/research

CEH (2004). Information sheet. Chemical Control of Algae. Centre for Ecology & Hydrology. http://www.capm.org.uk

Deogratias, J-M. (2005 & 2006). Lutte contre les algues dans les basins de stockage d'eau G.I.E. Fleurs & Plantes du Sud-Ouest (unpublished research station report).

Geiger, S., Henry, E., Hayes P. and Haggard, K. (2005). Barley straw – algae control literature analysis. On http://www.barleyworld.org/barleystraw/

HNS 82 (1997). Control of aquatic weeds in nurseries and water storage systems: a literature review.

HNS 145 (2007). Guidelines for integrated pest and disease management for ornamental aquatic plants.

HSE registered products. http://webcommunities.hse.gov.uk

Klemenčič A., Bulc, T. & Istenič, D. (2008). Development of a Chemical-free water treatment system for fish farming. CHEM-FREE, a Co-operative Research Project funded within the EU 6th Framework Programme contract COOP-CT-2006-032719. http://www.chem-free.eu

Newman J. (undated). The use of ultrasound to control bacteria, fungi and algae in aquatic systems. (includes published references) http://www.water-land.co.uk

Appendix 1: ADAS Boxworth site crop diary: May 2009 - August 2009

Date	Task
1st May	• Tanks (x25) washed with detergent and then left to soak (24 trial tanks and one spare tank).
5th May	• Tanks rinsed out and then filled with water & left for the chlorine to evaporate.
11th May	• Collected 75 Calla Lilies from Hitchin Nursery and water from the Koi pond. Lilies cleaned of blanket weed and 3x put into each tank along with 3L of Koi pond water to make 45L per tank. First set of blanket weed collected in April to use in trial in poor health so discarded.
15th May	• Trial run of water samples from Hitchin carried out on the spectrophotometer for absorbency variation.
18th May	• 15ml Maxicrop added to each tank (dilution as advised by nursery).
	• 50g fresh blanket weed collected from Hitchin and added to each tank along with an extra 50ml/tank of algae water and mud from the bottom of the nursery tanks.
19th May	• Electrical Conductivity (EC) readings taken in each tank.
20th May	• Leaves turning brown on lilies, rhizomes weak and rotting. Plants removed from tank, rinsed and put into a holding tank. Possible effect of the Maxicrop added 18th May.
21st May	• Spoke with Debbie at Hitchin Nursery to confirm Maxicrop dilution – incorrect dilution rates originally provided to us in error.
	• All tanks at Boxworth emptied out and re-filled with water, then left for the chlorine to evaporate.
28th May	• Cleaned off the dead leaves from the Calla Lilies in the holding tank.
3rd June	• Fresh blanket weed collected from the nursery and 45g added to each tank along with 3L of Koi pond water/tank to make 45L of water per tank. No mud added to the tanks.
9th June	• 3x lilies added to each tank.
12th June	• pH, EC and Temperature readings taken (Pre-treatment).
15th June	• Pre-treatment assessments carried out on all tanks.
	• Treatments added to each tank.
16th June	• Majority of blanket weed missing from 10 tanks principally in replicate 4 – suspect birds have been feeding overnight – green droppings around tanks.
	• Tanks covered with pea netting.
17th June	• Estimated % blanket weed left in each tank and added an extra 20g to reps 1-3 and 37g to rep 4 where most had gone.
	• Replaced smallest lilies with fresh ones collected from the nursery.
19th June	• % blanket weed cover assessed.
29th June	• Day 14 assessments. Snails seen and removed from tanks.

1st July	•	Treatment 2 repeated (2nd dose) as per instructions.
	•	Further snails seen and removed from tanks.
	•	Spectrophotometer readings omitted from trial as inconclusive.
6th July	•	Treatment 6 repeated (2nd dose) as per instructions.
13th July	•	Day 28 assessments. Snails seen and removed from tanks.
	•	Treatment 2 repeated (3rd dose) as per instructions.
	•	Treatment 3, 4 & 5 repeated (2nd dose) as per instructions.
21st July	•	Treatment 6 repeated (3rd dose) as per instructions.
24th July	•	Maxicrop added to each tank as plants looked starved. (Stock 0.05 ml in 300 ml water then 6ml stock per tank).
27th July	•	Day 42 assessments.
	•	Treatment 2 repeated (4th dose) as per instructions.
3rd August	•	Treatment 6 repeated (4th dose) as per instructions.
	•	Maxicrop added to each tank at same rate as 24 July.
11th August	•	Day 57 assessments (final). Fish found in three tanks. All Calla Lilies in poor health.

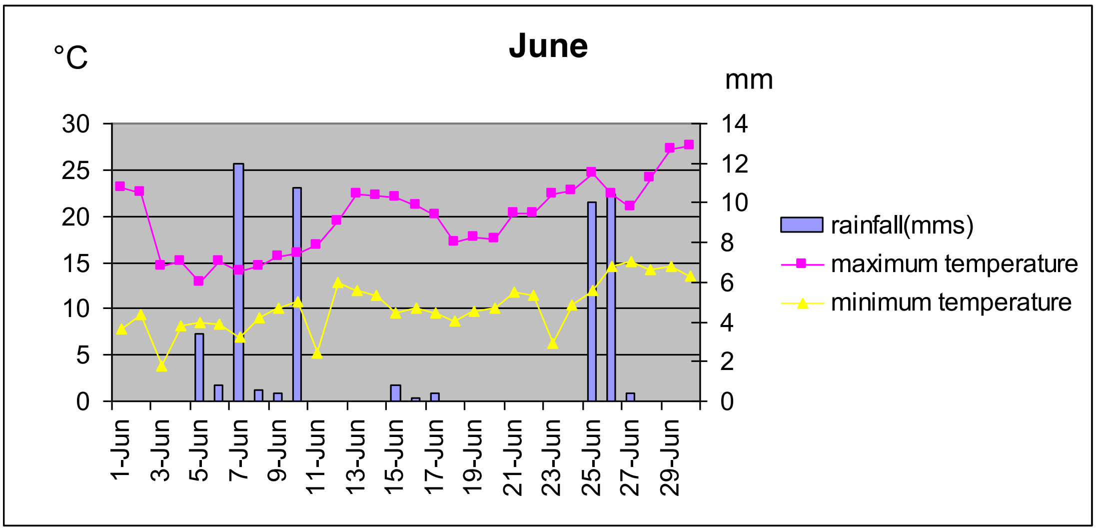
Appendix 2: Hitchin nursery site crop diary: April 2009 - August 2009

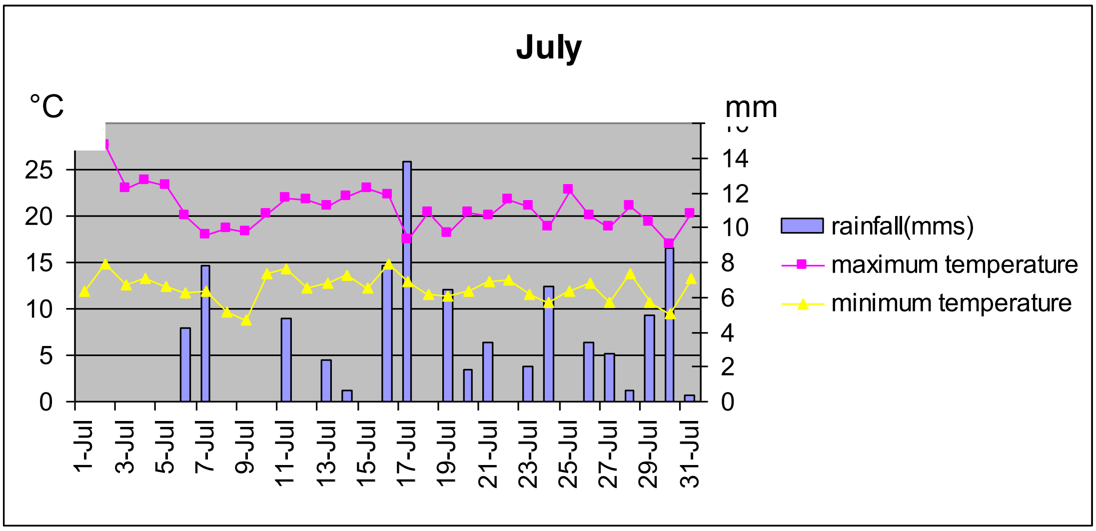
Date	Task
20th April	• Nursery site visited to select tanks for ultrasound.
11th May	• Finalised recording techniques and equipment required for algal density.
	• Set up x 5 Calla tanks with 20 plants each.
18th May	• Tanks & plants checked prior to start of assessments.
3rd June	• Algal, water & plant records taken in Control (Tank 1) & Tank 2. Ultrasound installed in Tank 2.
4th June	• Algal density in settling vessels scored & haemocytometer & spectrophotometer readings taken.
17th June	• Algal, water & plant records taken in Control (Tank 1) and Tank 2 & Tank 3. Ultrasound installed in Tank 3.
	• Some plants missing from trial tanks (no longer 20 / tank).
18th June	• Algal density in settling vessels scored & haemocytometer & spectrophotometer readings taken.
1st July	• Algal, water & plant records taken in Control (Tank 1) and Tank 2, Tank 3 & Tank 4. Ultrasound installed in Tank 4.
2nd July	• Algal density in settling vessels scored & haemocytometer & spectrophotometer readings taken.
16th July	• Algal, water & plant records taken in Control (Tank 1) and Tank 2, Tank 3, Tank 4 & Tank 5. Ultrasound installed in Tank 5.
17th July	• Haemocytometer readings taken, no algae seen and so spectrophotometer readings not taken. Settling vessel not scored in error.
30th July	• Algal, water & plant records taken in Control (Tank 1) and Tank 2, Tank 3, Tank 4 & Tank 5. Ultrasound removed from Tank 5.
	• RCD for Ultrasound tripped out in storms whilst in Tank 5.
31st July	• Algal density in settling vessels scored. Haemocytometer readings taken, no algae seen and so spectrophotometer readings not taken.
12th August	• Algal, water & plant records taken in Control (Tank 1) and Tank 2, Tank 3, Tank 4 & Tank 5.
	• Ten plants removed from each tank (Control & all treatment tanks) at nursery for phytotoxicity and root and shoot vigour assessments
13th August	• Algal density in settling vessels scored. Haemocytometer readings taken, no algae seen and so spectrophotometer readings not taken.

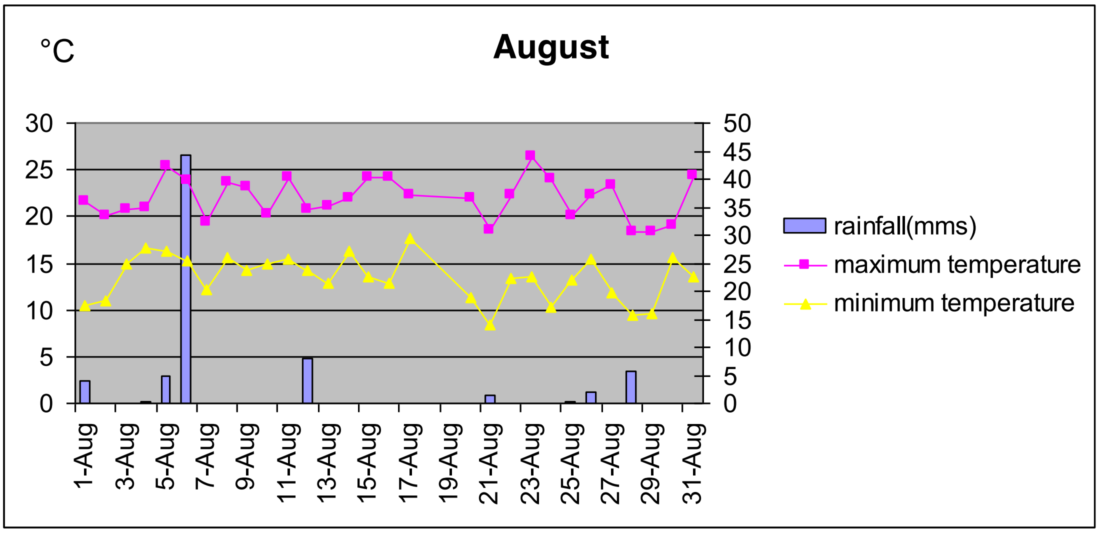
Appendix 3: In-situ assessment of electrical conductivity (EC), pH and water temperature at 10 cm depth – ADAS Boxworth, 2009

Rep	Treatment	Pre-treatment			Day 14			Day 28			Day 42			Day 57		
		EC	pH	°C	EC	pH	°C	EC	pH	°C	EC	pH	°C	EC	pH	°C
1	1	0.2	10.4	25.9	0.3	10.0	31.7	0.3	10.6	24.8	0.2	10.8	20.6	0.2	9.6	21.3
	2	0.2	10.5	26.4	0.3	8.9	31.8	0.3	9.4	24.9	0.3	9.6	20.4	0.2	10.7	21.5
	3	0.3	10.4	26.1	0.4	8.7	31.8	0.3	9.3	24.8	0.2	10.3	20.7	0.2	9.9	20.9
	4	0.2	10.5	26.8	0.3	9.1	31.9	0.3	10.0	24.7	0.2	9.7	20.1	0.2	9.5	20.9
	5	0.3	10.5	26.7	0.3	8.9	31.9	0.3	8.6	24.8	0.3	10.4	20.0	0.2	10.3	21.6
	6	0.2	10.5	26.4	0.3	8.8	31.9	0.4	8.9	24.8	0.2	10.4	20.3	0.2	10.3	21.3
2	1	0.3	10.6	26.3	0.3	9.1	31.6	0.3	10.3	24.6	0.2	10.8	20.3	0.2	10.2	20.9
	2	0.2	10.5	25.9	0.3	10.1	31.8	0.3	10.8	24.6	0.2	10.9	20.6	0.2	10.7	20.6
	3	0.3	10.5	26.0	0.4	8.4	31.9	0.3	9.2	24.4	0.2	10.8	20.0	0.2	10.3	21.3
	4	0.2	10.6	26.0	0.4	8.0	31.5	0.3	8.9	24.6	0.2	9.3	19.6	0.2	9.3	20.7
	5	0.2	10.5	25.7	0.3	8.9	31.8	0.3	10.5	24.7	0.2	10.8	19.2	0.2	9.8	20.9
	6	0.2	10.5	26.0	0.3	10.1	32.3	0.3	10.7	24.6	0.2	10.5	20.2	0.2	10.8	21.0
3	1	0.2	10.5	26.3	0.3	9.2	31.7	0.3	10.6	24.3	0.2	10.6	20.3	0.2	9.8	21.0
	2	0.3	10.6	26.4	0.3	9.2	31.3	0.4	9.0	24.1	0.3	8.7	20.2	0.2	8.6	21.3
	3	0.2	10.6	26.0	0.4	8.9	31.8	0.3	10.2	24.3	0.2	10.7	20.3	0.2	10.4	20.7
	4	0.3	10.5	26.2	0.3	8.7	31.8	0.4	8.7	24.3	0.3	9.0	20.8	0.2	9.0	21.2
	5	0.2	10.3	26.1	0.3	9.1	31.4	0.3	9.7	24.4	0.2	10.8	19.2	0.2	10.4	21.0
	6	0.2	10.6	25.5	0.3	10.2	31.3	0.3	10.4	24.5	0.2	10.8	20.2	0.2	10.6	20.7
4	1	0.2	10.6	26.2	0.3	10.2	31.9	0.3	10.8	24.7	0.2	10.7	20.1	0.2	10.6	21.6
	2	0.2	10.5	26.5	0.4	8.6	31.1	0.4	10.9	23.9	0.3	10.8	20.4	0.2	10.6	21.4
	3	0.3	10.5	26.5	0.3	9.3	31.0	0.3	10.7	23.8	0.3	10.8	21.1	0.2	10.0	21.3
	4	0.2	10.5	26.3	0.4	9.2	30.5	0.3	10.0	23.8	0.2	9.7	20.8	0.2	9.7	21.1
	5	0.3	10.5	26.1	0.3	9.3	31.4	0.3	10.8	24.1	0.3	10.8	20.7	0.2	10.3	21.7
	6	0.2	10.4	26.7	0.3	9.5	31.5	0.3	10.9	24.3	0.2	10.7	20.8	0.2	10.1	21.2

Appendix 4: Daily weather for June to August 2009. Records of rainfall, and maximum and minimum air temperature – ADAS Boxworth







Appendix 5: Microscope examination of 10 blanketweed strands per tank – ADAS Boxworth

T1 untreated, T2 Nishikoi Blanc-Kit Excel, T3 NT Labs Aquaclear
T4 Cloverleaf Blanket Answer, T5 TetraPond AlgoFin, T6 Interpet Blanketweed Buster.

		pre-treatment 15/6/09 Day 1		29/6/09 Day 14		13/7/09 Day 28		27/7/09 Day 42		11/8/09 Day 57	
		Number of blanketweed strands either healthy or not healthy out of 10 sampled per tank									
Replicate	Treatment	Healthy	Non-Healthy	Healthy	Non-Healthy	Healthy	Non-Healthy	Healthy	Non-Healthy	Healthy	Non-Healthy
1	1	10	0	10	0	10	0	10	0	0	10
2	1	10	0	4	6	10	0	10	0	8	2
3	1	10	0	10	0	10	0	*	*	10	0
4	1	10	0	10	0	10	0	10	0	9	1
1	2	10	0	0	10	10	0	9	1	3	7
2	2	10	0	10	0	10	0	10	0	0	10
3	2	10	0	0	10	*	*	*	*	*	*
4	2	10	0	6	4	10	0	10	0	0	10
1	3	10	0	0	10	10	0	10	0	10	0
2	3	10	0	10	0	10	0	10	0	10	0
3	3	10	0	10	0	10	0	10	0	10	0
4	3	10	0	0	10	10	0	10	0	10	0
1	4	10	0	0	10	*	*	*	*	*	*
2	4	10	0	0	10	*	*	*	*	*	*
3	4	10	0	5	5	*	*	*	*	*	*
4	4	10	0	10	0	*	*	*	*	*	*
1	5	10	0	0	10	*	*	*	*	10	0
2	5	10	0	1	9	10	0	10	0	6	4
3	5	10	0	1	9	10	0	10	0	9	1
4	5	10	0	0	10	10	0	10	0	0	10
1	6	10	0	0	10	10	0	10	0	10	0
2	6	10	0	8	2	10	0	10	0	9	1
3	6	10	0	10	0	10	0	10	0	10	0
4	6	10	0	0	10	10	0	4	6	10	0

* No blanketweed was present to sample on this date

Non-healthy strands had disrupted chloroplast strands inside the cells with sparse or absent chloroplasts and/or broken, short, filaments. Unhealthy filaments were usually still green. The sample taken from each tank could only be a tiny proportion of the total number of filaments and so should only be used as an indication of the health of the majority of strands.

Appendix 6: In-situ visual assessment of unicellular algae density, and water clarity (depth an immersed mark became invisible) - Boxworth

		Pre-treatment		Day 14		Day 28		Day 42		Day 57	
Rep	Treatment	Depth in cm to 22cm	Clarity	Depth in cm	Clarity	Depth in cm	Clarity	Depth in cm	Clarity	Depth in cm	Clarity
1	1	8.5	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	2	6	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	3	10.5	semi-clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	4	6	cloudy	11.5	cloudy	10	cloudy	10	cloudy	16	cloudy
	5	6	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	6	15	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
2	1	5	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	2	20	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	3	bottom	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	4	11.5	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	5	20.5	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	6	20	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
3	1	7.5	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	2	4	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	3	6	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	4	19.5	clear	bottom	clear	bottom	clear	10	cloudy	14	cloudy
	5	19.5	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	6	12	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
4	1	9	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	2	5	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	3	6	cloudy	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	4	5.5	cloudy	12	cloudy	10	cloudy	15	cloudy	bottom	cloudy
	5	17.5	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear
	6	12	clear	bottom	clear	bottom	clear	bottom	clear	bottom	clear

Appendix 7: Unicellular algal assessments (one tank per treatment) – ADAS Boxworth

		Pre-treatment			Day 14			Day 28			Day 42			Day 57		
Rep	Treatment	No. Algae Species	No. Cells / mL (out of 6 grids)	Settling Vessel Algal Density	No. Algae Species	No. Cells / mL (out of 6 grids)	Settling Vessel Algal Density	No. Algae Species	No. Cells / mL (out of 6 grids)	Settling Vessel Algal Density	No. Algae Species	No. Cells / mL (out of 6 grids)	Settling Vessel Algal Density	No. Algae Species	No. Cells / mL (out of 6 grids)	Settling Vessel Algal Density
1	1	x	x	9	0	0	10	0	0	x	1	20,000	x	0	0	10
	2	x	x	7	x	x	10	x	x	x	x	x	x	0	0	10
	3	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
	4	x	x	9	x	x	9	x	x	x	x	x	x	0	0	10
	5	x	x	4	x	x	10	x	x	x	x	x	x	0	0	10
	6	0	0	9	x	x	10	x	x	x	x	x	x	1	1667	10
2	1	x	x	5	0	0	10	0	0	x	0	0	x	0	0	10
	2	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
	3	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
	4	x	x	8	x	x	10	x	x	x	x	x	x	1	6667	10
	5	0	0	9	x	x	10	x	x	x	x	x	x	1	1667	10
	6	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
3	1	x	x	8	0	0	10	0	0	x	0	0	x	0	0	10
	2	0	0	3	x	x	10	x	x	x	x	x	x	0	0	10
	3	0	0	7	x	x	10	x	x	x	x	x	x	0	0	10
	4	x	x	9	x	x	10	x	x	x	x	x	x	0	0	9
	5	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
	6	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
4	1	x	x	8	0	0	10	0	0	x	0	0	x	0	0	10
	2	x	x	8	x	x	9	x	x	x	x	x	x	0	0	10
	3	x	x	8	x	x	10	x	x	x	x	x	x	3	6667	10
	4	1	1667	9	x	x	9	x	x	x	x	x	x	0	0	10
	5	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10
	6	x	x	9	x	x	10	x	x	x	x	x	x	0	0	10

Appendix 8: In-situ assessment of plant phytotoxicity (index 10 = severe) and vigour (index 10 = most vigour) per tank – ADAS Boxworth

		Pre-treating 15/06/09 Day 1						29/06/09 Day 14						13/07/09 Day 28						27/07/09 Day 42					
		Plant 1		Plant 2		Plant 3		Plant 1		Plant 2		Plant 3		Plant 1		Plant 2		Plant 3		Plant 1		Plant 2		Plant 3	
Rep	Treatment	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour	Phytotox.	Vigour
1	1	0	10	0	10	0	10	2	8	2	8	2	8	0	10	0	10	2	9	0	2	0	2	0	1
2	1	0	10	0	10	0	10	0	10	1	9	1	9	2	8	0	10	2	8	0	4	0	4	0	4
3	1	0	10	0	10	0	10	1	9	1	9	1	9	0	10	1	9	1	9	2	4	2	2	2	2
4	1	0	10	0	10	0	10	0	10	1	9	1	9	5	6	5	6	5	6	3	1	3	1	3	1
1	2	0	10	0	10	0	10	0	10	0	10	1	9	0	10	0	10	0	10	0	4	0	4	0	4
2	2	0	10	0	10	0	10	1	9	1	9	1	9	8	1	6	5	3	7	0	2	0	2	0	0
3	2	0	10	0	10	0	10	1	9	1	10	1	10	0	10	0	10	0	10	0	5	0	4	0	4
4	2	0	10	0	10	0	10	0	10	0	10	1	9	0	10	4	8	0	10	0	4	0	2	0	1
1	3	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	10	0	4	0	2	-	0
2	3	0	10	0	10	0	10	0	10	0	10	1	9	0	10	0	9	0	9	1	4	1	4	1	4
3	3	0	10	0	10	0	10	0	10	0	10	1	8	0	10	0	10	0	10	0	4	0	2	0	2
4	3	0	10	0	10	0	10	1	8	2	8	3	7	2	9	0	9	0	10	1	4	3	2	0	1
1	4	0	10	0	10	0	10	0	10	1	9	1	9	0	10	0	10	0	10	1	4	1	3	0	3
2	4	0	10	0	10	0	10	0	10	1	9	1	9	0	10	0	10	0	10	1	5	0	5	0	3
3	4	0	10	0	10	0	10	1	10	1	10	1	10	0	10	0	10	0	10	0	6	0	3	0	2
4	4	0	10	0	10	0	10	0	10	0	10	1	9	0	10	0	10	0	10	0	6	0	4	0	2
1	5	0	10	0	10	0	10	0	10	0	10	0	10	2	9	2	9	2	9	0	4	0	4	0	4
2	5	0	10	0	10	0	10	2	8	1	9	1	8	0	10	0	10	0	10	0	4	0	4	0	4
3	5	0	10	0	10	0	10	0	10	1	9	1	9	0	10	0	10	0	10	0	7	2	2	0	2
4	5	0	10	0	10	0	10	2	8	2	9	1	9	2	9	2	9	5	5	3	5	0	1	3	1
1	6	0	10	0	10	0	10	1	9	1	9	1	10	0	10	0	10	0	10	0	6	0	4	0	4
2	6	0	10	0	10	0	10	1	9	1	9	1	9	8	3	0	10	1	9	0	5	0	2	0	1
3	6	0	10	0	10	0	10	1	9	1	9	1	9	2	9	3	7	3	6	2	2	3	1	3	1
4	6	0	10	0	10	0	10	0	10	1	9	1	9	2	8	1	8	0	10	1	6	1	2	0	1

Appendix 9: Final assessment of three Bog Arum plants per tank for shoot and root vigour (Index 0=dead to 10=strong vigour) on 11 August – ADAS Boxworth, 2009

Treatment	Plant 1 vigour		Plant 2 vigour		Plant 3 vigour	
	shoots	roots	shoots	roots	shoots	roots
1	1	1	1	1	1	1
1	1	1	2	3	2	3
1	1	0	5	5	1	1
1	2	2	0	2	0	1
2	1	1	1	4	3	5
2	0	0	0	0	1	1
2	6	8	4	5	2	5
2	1	1	0	1	1	4
3	3	6	3	3	4	6
3	1	2	1	2	1	1
3	4	3	2	3	1	1
3	2	4	3	4	1	0
4	1	1	1	1	3	3
4	1	3	2	3	3	5
4	2	3	5	5	2	2
4	1	3	2	3	5	7
5	1	2	1	1	2	1
5	1	2	3	2	2	2
5	6	8	1	1	2	2
5	1	1	2	1	1	1
6	3	3	2	2	3	5
6	3	2	1	1	0	0
6	1	1	0	1	1	1
6	2	2	1	1	4	3

Treatment	Shoots		Roots	
	Mean vigour	SE	Mean vigour	SE
1	1	0.38	2	0.39
2	2	0.53	3	0.74
3	2	0.34	3	0.54
4	2	0.42	3	0.49
5	2	0.42	2	0.56
6	2	0.37	2	0.39

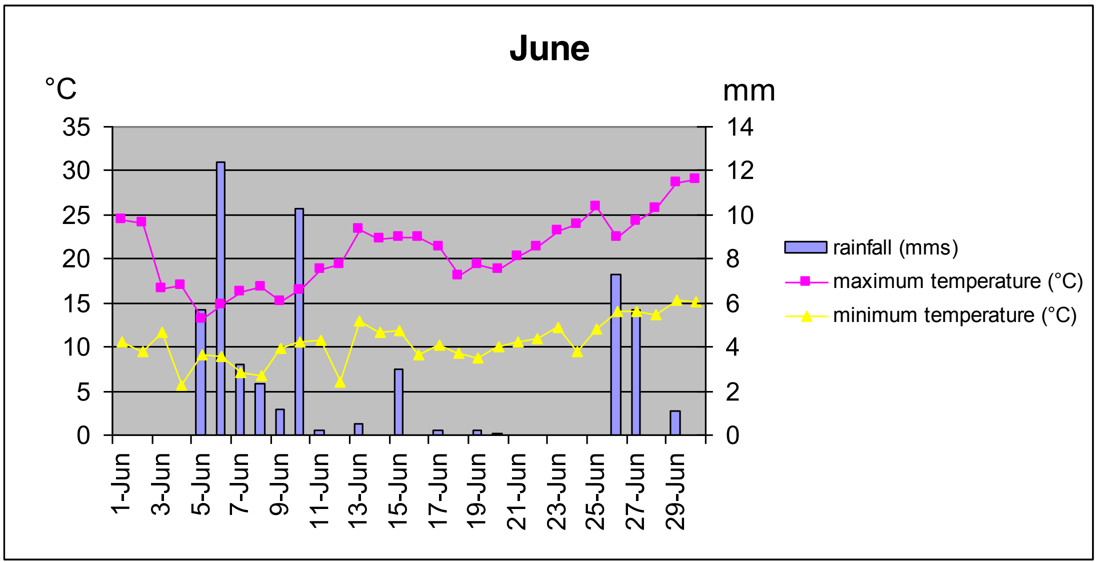
T1 untreated, T2 Nishikoi Blanc-Kit Excel, T3 NT Labs Aquaclear T4 Cloverleaf Blanket Answer, T5 TetraPond AlgoFin, T6 Interpet Blanketweed Buster

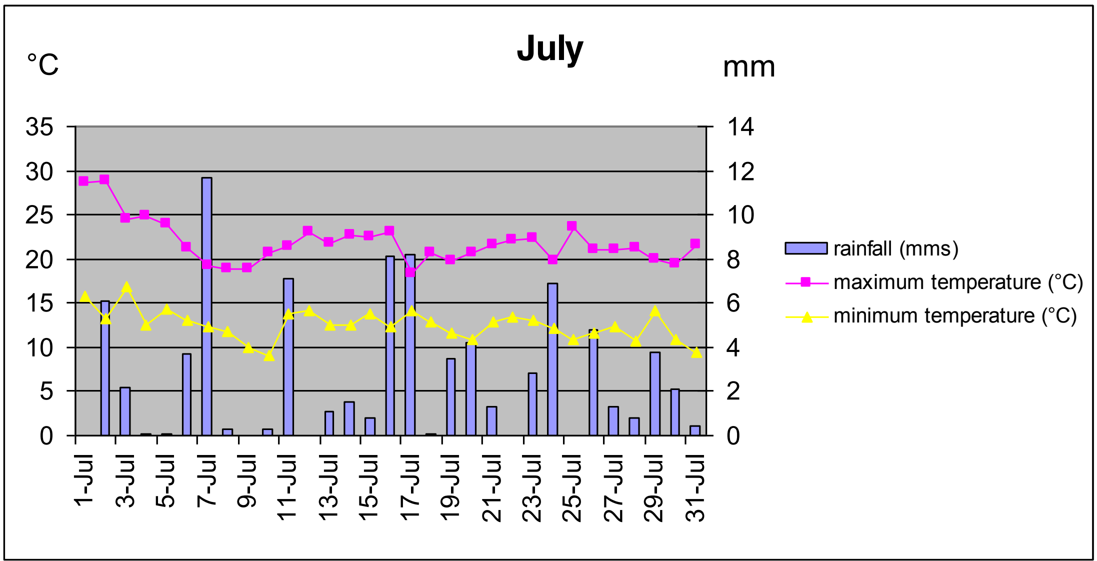
Appendix 10: In-situ assessment of water properties – Hitchin, 2009
(◊ ultrasound removed after 14 days operation)

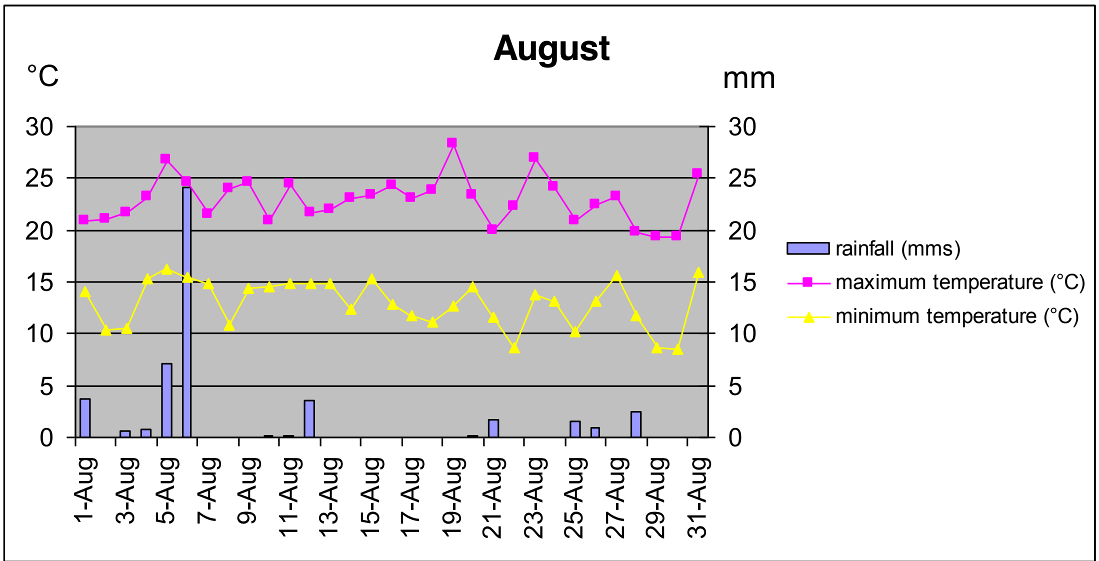
Date	Tank	EC	pH	Temp °C
03/06/09	1	0.5	8.8	17
Day 1	2	0.5	9.3	17
	3	n/a	n/a	n/a
	4	n/a	n/a	n/a
	5	n/a	n/a	n/a
	Tap Water	0.5	9.2	n/a
17/06/09	1	0.2	8.55	16.2
Day 14	2 ◊	0.4	8.39	16.3
	3	0.3	7.84	15.8
	4	n/a	n/a	n/a
	5	n/a	n/a	n/a
	Tap Water	0.5	7.44	17.3
01/07/09	1	0.3	8.86	20
Day 28	2	0.4	8.23	19.8
	3 ◊	0.4	7.79	19.6
	4	0.4	7.59	19.5
	5	0.3	8.13	19.5
	Tap Water	0.4	7.28	16.7
16/07/09	1	0.2	10.0	19.5
Day 42	2	0.4	8.36	19.2
	3	0.3	7.86	18.6
	4 ◊	0.4	7.61	17.4
	5	0.2	9.11	18.2
	Tap Water	not taken	7.34	19.6
30/07/09	1	0.2	8.92	15.6
Day 56	2	0.3	8.57	15.6
	3	0.3	7.92	15.3
	4	0.2	7.89	14.8
	5 ◊	0.3	8.43	15.1
	Tap Water	0.2	7.38	16.2
12/08/09	1	0.2	8.84	18.5
Day 70	2	0.3	7.89	18.1
	3	0.3	7.91	18.2
	4	0.3	8.09	18.1
	5	0.2	8.13	18.1
	Tap Water	0.2	7.41	18.6

- recording not commenced in these tanks. * records not taken

Appendix 11: Daily records of rainfall, and maximum and minimum air temperature from June to August – Hichin, 2009







Appendix 12: Unicellular algal density (cell count, and water clarity after settling) and blanketweed strand condition – Hitchin, 2009. No ultrasound used in Tank 1

Date	Tank	Haemocytometer number. cells / mL	Settling vessel algal density index (10 = clear)
03/06/09	1	0	6
Day 1	2	0	8
	3	-	-
	4	-	-
	5	-	-
17/06/09	1	0	9
Day 14	2 ◊	0	9
	3	0	10
	4	-	-
	5	-	-
01/07/09	1	0	9
Day 28	2	0	10
	3 ◊	0	10
	4	0	9
	5	-	-
16/07/09	1	0	*
Day 42	2	0	*
	3	0	*
	4 ◊	0	*
	5	0	*
30/07/09	1	0	8
Day 56	2	0	9
	3	0	9
	4	0	9
	5 ◊	0	9
12/08/09	1	0	5
Day 70	2	0	9
	3	0	9
	4	1667	8
	5	0	8

- recording not commenced in these tanks. * records not taken
- ◊ ultrasound removed from tank after 14 days operation

Appendix 13: In-situ assessment of blanketweed and unicellular algae – Hitchin, 2009

(◊ ultrasound removed after 14 days operation)

Date	Tank	% cover Blanketweed at surface	% cover Blanketweed under water	Strand quality	Cm depth of clear water	Other plants present at the water surface
03/06/09	1	85 - 90	10 - 15	Green	2.5	duckweed
Day 1	2	~80	~20	Green	3	
	3	n/a	n/a	n/a	n/a	
	4	n/a	n/a	n/a	n/a	
	5	n/a	n/a	n/a	n/a	
17/06/09	1	~5	~5	Green	4	
Day 14	2 ◊	~15	0	Brown Green	2.5	duckweed
	3	0	~5	Green	to bottom	
	4	n/a	n/a	n/a	n/a	
	5	n/a	n/a	n/a	n/a	
01/07/09	1	25	0	Green	5.5	duckweed + other
Day 28	2	15	0	Brown Green	4.5	duckweed
	3 ◊	<5	0	Green	7.5	duckweed
	4	90 - 100	0	Green	5	
	5	n/a	n/a	n/a	n/a	
16/07/09	1	60	20	Green	4.5	duckweed
Day 42	2	0	10	Green	to bottom	duckweed
	3	0	10	Green	to bottom	duckweed
	4 ◊	80	0	Green Brown	6	other weed
	5	25	0	Green Brown	to bottom	duckweed + other
30/07/09	1	40	20	Green	4.5	duckweed + other
Day 56	2	5	~30	Green	to bottom	duckweed + other
	3	<5	~30	Green	to bottom	duckweed + other
	4	<5 dead	~15	Green	7	duckweed + other
	5 ◊	10	0	Green	13	duckweed + other
12/08/09	1	50	can't see	Green	3	dark, pea-green water
Day 70	2	15	can't see	Green	5	dark water
	3	10	can't see	Green	6	dark water
	4	30	can't see	Green	9	brown, cloudy water
	5	30	can't see	Green	8	brown, cloudy water

Appendix 14: Microscope examination of 10 blanketweed strands – Hitchin 2009.
Ultrasound removed ◊ after 14 days.
In some samples ■ , in addition to the 10 strands examined, the contents of some other strands were also present but they could not be counted

Date	Tank	Healthy	Non-healthy (disrupted chloroplasts, intact strand walls)
03/06/09	1	10	0
Day 1	2	10	0
	3	n/a	n/a
	4	n/a	n/a
	5	n/a	n/a
17/06/09	1	10	0
Day 14	2 ◊	10	■
	3	10	0
	4	n/a	n/a
	5	n/a	n/a
01/07/09	1	10	0
Day 28	2	10	■
	3 ◊	10	0
	4	10	0
	5	n/a	n/a
16/07/09	1	10	0
Day 42	2	10	0
	3	10 (thin)	0
	4 ◊	10 (some thin)	0
	5	10	0
30/07/09	1	10	0
Day 56	2	10	0
	3	10	0
	4	10 (thin)	■
	5 ◊	10	0
12/08/09	1	not recorded in error	not recorded in error
Day 70	2	not recorded in error	not recorded in error
	3	not recorded in error	not recorded in error
	4	not recorded in error	not recorded in error
	5	not recorded in error	not recorded in error