



Aquaculture, Pond and Aquaria Consulting

# **Efficacy of MinnFinn to Koi Fish at Predicted Therapeutic Doses**

**Report also includes studies using the solution for algae  
control**

**October, 2007**

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# Efficacy of MinnFinn to Koi Fish at Predicted Therapeutic Doses; Phase II

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## Introduction

The availability of effective medications is an important part of the aquarium and pond fish hobby/industry. If a fish keeper is successful in the treatment of disease, he/she will build confidence in their abilities and will continue to develop in the hobby thus helping build the value of the hobby.

There are many medications available on the market today ranging from chemical solutions to antibiotics. One of the major challenges that the industry may have to face is the regulation of medications by the FDA. The aquaculture industry was regulated approximately 15 years ago making it so there were very few licensed products for use, leaving the industry with a huge deficit. The same may occur to the aquarium/pond industry.

The product MinnFinn (Minntech Corp.) may be a valuable treatment compound for external parasites and bacteria in aquarium fish. Hydrogen peroxide is an effective agent and has been used a great deal in the aquaculture industry. The combination of peracetic acid with hydrogen peroxide in MinnFinn has been well demonstrated as a disinfectant against virus and bacteria. In review of its toxicity to a few fish species, it appeared that freshwater fish are tolerant of the compound and it thus may be a good treatment tool for the reduction or elimination of ecto-parasites and bacteria in tropical fish. With the efficacy against viruses, it may also inhibit the spread of virus in an infected population.

Some preliminary research investigating the effectiveness of MinnFinn on a pathogenic *Vibrio* sp. bacteria *in vitro* showed MinnFinn to be efficient at killing the organism suspended in sea water at or below doses required with hydrogen peroxide. Further investigation is warranted, in both aquaculture and home aquarium systems.

The following provides the methods for the testing of efficacy of MinnFinn at the determined safe levels from the experiment executed in Phase I on Koi. In that experiment both the 25 ppm and 10 ppm doses had little impact on the fish after three treatments with each treatment separated by one day. The fish tested were juvenile Koi with an average size of ~8 grams.

If efficacious, MinnFinn will be marketed for the treatment of external parasites and bacteria. In the treatment of parasites it would be used alone. In the treatment of a bacterial infection however, it may best be done in conjunction with an injection type antibiotic such as Oxytetracycline.

Another possible use for MinnFinn in the Koi industry is as a pond clarifier. Algal blooms are a constant problem for many pond owners. Most of the compounds used to eradicate algae have similar characteristics and medications. Once introduced to the water they are persistent and to be removed a water change must be done or use low enough doses so prolonged exposure does not affect the fish. Many of these compounds are harmful to the environment as well as have a level of toxicity to fish with prolonged exposure. MinnFinn may provide a simple and neutralizable method of eradicating an algal bloom and in conjunction with a clumping agent may clear pond water quickly.

### ***Methods and Materials***

#### *Test Fish*

Testing will be performed on diseased Koi. As there are many retail Koi facilities and commercial Koi farms in the Southern California area, contact will be made with these facilities and attempts to obtain approval to conduct experiments tank side on fish during a disease outbreak. Field testing will provide valuable data as to the efficacy of the compound under every day conditions. Facilities will be contacted until the treatment requirements for the trial can be fulfilled.

Koi are an important species as they are part of an ever increasing sector of the fish keeping industry and it is therefore important to provide a new tool for the growing ornamental pond sector. Fish of any size will be tested but fish numbers should be such that they can provide adequate replicates. If numbers are inadequate for the total number of replicates, testing would still provide good supportive data.

#### *The Treatment System*

Treatments will be done in 80 l plastic bins. The number of animals available for treatment will dictate the number of possible replicates for treatment. A small battery powered aerator will be installed on each bin to maintain adequate oxygenation during the treatment. The system will be made portable for a rapid response to an outbreak.

#### *Dosing and treatment levels*

25ppm (5.01 ml in 60 l) and 10ppm (2.0 ml in 60 l) doses will be the doses used in the field testing. All of these doses are considered at 100% active ingredient.

#### *Testing*

Prior to fish being stocked, the test bins will be filled with tank water to a total volume of 60l. Attempts will be made to randomly place five fish in each of the test bins. If fish are available in enough numbers, there will be 2 replicates of each dose and two controls. The treatments will be randomly assigned amongst the bins. Fish will be moved from the tank containing the diseased animals to the test bins directly.

The treatments will be allowed to run for one hour, after which they will be neutralized with a MinnFinn neutralizer. As it will be impossible to follow individual fish post treatment or to repeat treatment, only one treatment will be applied and evaluated in the preliminary trials. If deemed necessary, recommendations will be made on how to design

a setup for multiple treatments. On site resources at some facilities may allow for such a design.

Efficacy will be evaluated through the use of skin scrapes. Once on site, skin scrapes will be done on 6 fish from the infected tank or pond to verify the outbreak of disease and to identify the infecting organism. Of the fish going into treatment, 50% will be scraped prior to treatment and evaluated on the level of infection; mild, moderate or severe. The level of infection will be based on the number of organisms upon scrape and the overall appearance of the fish with regards to behavior and presence of lesions. Post treatment 100% of the fish will be evaluated. The fish examined prior to treatment will be marked, so post treatment evaluation can be compared to prior.

The skin scrape used for determination of infection will be standardized on each fish. Fish will first be anesthetized using MS222. Using a 22mm x 22mm cover slip, a scrape will be done over a 2.5 cm long length of skin and the resulting sample placed on a slide and covered with the cover slip. If a lesion is present, it will be scraped in the same manner. If required, a small amount of fresh water will be used to flood under the cover slip to ensure the sample does not dry up and to allow easier viewing.

Testing will be done on diseased fish which will be initially identified by the owner/manager of the facility. Once the owner/manager has identified a problem, plan is to have myself notified so a test treatment can be applied. Focus will be on primarily parasitic infections, protozoa and flukes. Some of the most common infections are from *Costia (Ichthyobodo necatrix)*, *Trichidina sp.* And *Gyrodactylus sp.* Following confirmation of infection, the set up of the equipment can commence for efficacy testing.

The number of full replicated on site efficacy tests will depend on the quality of the outbreak, i.e. severity, and the number of fish involved. For confidence there should be a total of 5 tests done over at least three facilities which have good replication and readably identifiable disease process underway. Also within these tests would be a good range in fish size. Most likely fish tested will be small as larger fish in large number is not common place at many facilities.

Also included in this portion of the work is continued evaluation of the neutralizing capacity of sodium bicarbonate to MinnFinn in various types of water and various doses. The evaluation will be done through titration in water ranging from deionized to full strength seawater.

## Results and Discussion

Permission was obtained for testing at five facilities, 2 farms, one retail location and two private ponds. The first site visited was Harrison Koi, (Riverside, CA). There were 4 tests done at this location. Two of the trials were in the experimental bins and the other two in production tanks.

*Trial 1, August 1, 2007 Harrison Koi Farm*

The experiment was run in 6 green plastic 80 l bins filled with fresh ground water at 21.2°C a pH of 8.2 and a DO of 7.3 ppm after 30 minutes of aeration. Each bin had a battery operated aerator to remove nitrogen in the new water and maintain oxygen levels during testing. The bins were also placed in a constantly shaded area to avoid water heating and temperature did not increase by more than one degree C over the treatment period.

The protocol for scraping was changed as mentioned above to eliminate the anesthesia of the fish. Fish were placed in a shallow plastic container with approximately 3 inches of water. While one person held the fish upside down, a scrape was taken on the ventral side between the pectoral and pelvic fins moving in the direction of head to tail.

The fish tested in this trial were in the same size range of 15.25 cm to 20.32 cm. In this trial there were two replicates for each treatment and control. Again both 10 ppm and 25 ppm MinnFinn were tested. As with the first trial there were 5 fish randomly assigned to each bin. In this trial each of the five fish from one of the replicates of each treatment was scraped to give a pre treatment view of parasite loading. After the treatment, 3 fish from each bin from those screened previously were randomly selected post treatment and reexamined. Of the bins with fish not prescreened, each of the fish was scraped to monitor for parasite loading after the treatment. Please refer to Table 4 for both Prior and Post treatment parasite loading.

In this trial the fish in both replicates of the 25 ppm treatment started showing signs of stress to the treatment only 20 minutes in. The treatment was immediately neutralized and the fish's behavior returned to normal. There were no mortalities the days following the treatment. A 25 ppm dose appears to have a toxic effect and should not be recommended for use.

There was a significant difference between treated and untreated fish (ANOVA,  $P \leq 0.05$ ). MinnFinn has shown to eliminate the parasite *Trichodina* from Koi effectively at both the 10 ppm and 25 ppm doses. The 25 ppm dose was only present for 20 minutes so the effect was quite rapid. All treated fish had no live *Trichodina* found in skin scrapings. Control fish did however have parasites present. There were also fewer *Dactylogyrus* on the treated fish but starting numbers of the parasite were so low that no determination as to the effectiveness MinnFinn can be made. A true fluke infestation is required to make this determination.

Water pH and temperature were monitored through the treatment. The starting parameters were pH 8.2 and Temp of 20.6 to 21.2. The average levels during treatment were pH 8.55 and 20.8°C for the controls, pH 8.45 and 20.8°C for 10 ppm and pH 8.3 and 20.9°C for the 25 ppm treatments. Again there was a much smaller change in pH when compared to that of the water used in the Phase I experiment.

*Trials 2 and 3, August 7 and 8, 2007 Harrison Koi Farm*

With the results observed in the last trial, the owner of Harrison Koi, Terry Harrison volunteered to have a production tanks of fish treated. For Trial 2 a single tank was selected. The tank was the same origin tank of the fish used in trial 1. As these fish were showing signs of a Trichodina infection, it was selected for testing first. If the treatment proved effective and not harmful, the entire holding facility was to be treated.

Upon inspection of 6 fish from the tank to be treated, it became apparent that a Trichodina infestation was occurring. In each scrape from the six fish there were more than 25 organisms present. The tank was 1287 l and was treated with 10 ppm MinnFinn (42.93 ml) for 1 hour. Prior to the start of the treatment the water was shut off and an air stone was added to ensure adequate oxygen levels.

During treatment the fish acted somewhat agitated in the beginning but settled down to normal behavior after approximately 10 minutes. This is normal when doing static bath treatments with most any chemical. After the 1 hour treatment duration, the neutralizer was added. Post treatment skin scrapes from 6 fish showed a complete elimination of Trichodina from the fish.

The starting pH was 8.3 with a water temperature of 20°C and a DO of 5.9. The pH dropped to only 8.2 during treatment. The DO increased to 6.9 and was most likely due to the hydrogen peroxide component of the MinnFinn. Even with the high buffering capacity of the hard water and small change in pH, the effectiveness of MinnFinn does not seem to be affected much.

With the success of Trial 2, Terry Harrison asked that the whole facility be treated. Please see Table 5 for the scope of the treatments done, doses and neutralization. A total of 21 tanks were treated.

**Table 4:** Parasite Loading of Fish Used for the Testing of MinnFinn Prior and Post Treatment in Trial 2

<b>Prior to Treatment</b>			<b>Post Treatment</b>		
<i>Bin/Treatment</i>	<i>Fish #</i>	<i>Parasite Load</i>	<i>Bin/Treatment</i>	<i>Fish #</i>	<i>Parasite Load</i>
Bin 1/ Control	Fish 1	3 Trichodina	Bin 1/ Control	Fish 1	3 Trichodina, 1 Dactylogyrus
	Fish 2	9 Trichodina		Fish 2	6 Trichodina, 1 Dactylogyrus
	Fish 3	8 Trichodina		Fish 3	3 Trichodina
	Fish 4	3 Trichodina			
	Fish 5	4 Trichodina			
Bin 2/ 10 ppm	Fish 1	14 Trichodina	Bin 2/ 10 ppm	Fish 1	None
	Fish 2	8 Trichodina		Fish 2	None
	Fish 3	10 Trichodina		Fish 3	None
	Fish 4	6 Trichodina			
	Fish 5	29 Trichodina			
Bin 3/ 25 ppm	Fish 1	7 Trichodina, 1 Dactylogyrus	Bin 3/ 25 ppm	Fish 1	None
	Fish 2	5 Trichodina		Fish 2	None
	Fish 3	6 Trichodina		Fish 3	None
	Fish 4	15 Trichodina			
	Fish 5	3 Trichodina			
			Bin 4/Control	Fish 1	3 Trichodina, 1 Dactylogyrus
				Fish 2	5 Trichodina
				Fish 3	4 Trichodina
				Fish 4	1 Trichodina
				Fish 5	3 Trichodina
			Bin 5/10 ppm	Fish 1	None
				Fish 2	1 Dactylogyrus
				Fish 3	None
				Fish 4	None
				Fish 5	None
			Bin 6/25 ppm	Fish 1	None
				Fish 2	1 Dead Trichodia
				Fish 3	None
				Fish 4	None
				Fish 5	None

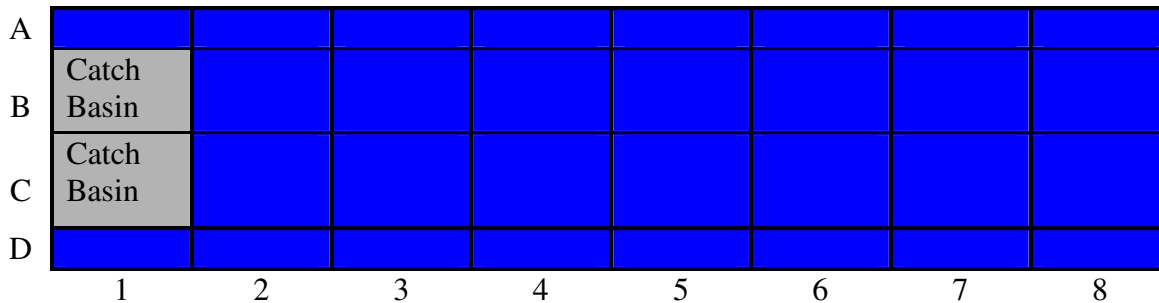
**Table 5:** Tank Specifics for Volume, MinnFinn Dose, Neutralizer Amounts for Complete Holding Facility Treatment at Harrison Koi August 8, 2007

Tank Type	Volume (l)	Number of Tanks	MinnFinn (ml)/Tank	Sodium Bicarb. (g)/Tank	Sodium Thio. (g)/Tank
Small Tanks	1287	8	42.93	138.99	10.68
Large Tanks	3520	8	116.25	380.18	29.34
Gutters	1287	2	42.93	138.99	10.68
Catch Basins	2650	2	88.39	286.16	22.08
Pond	30283	1	1010.17	3270.40	252.34

Sample scrapes from three fish were taken from each of the large and small tanks. The large tanks contained larger fish from 20.32 cm to 45.72 cm. The small tanks contained fish in the 15.25 cm to 20.32 cm size range. The layout had two rows of small tanks along the outside and two rows of large tanks on the inside (Figure 1). Tanks that were not treated were either empty or had recently been treated with another compound. Tank numbering had the rows with a letter designation and the columns a number designation.

The fish in tanks D2 to D8 were all brought into the facility at the same time. These were the fish that demonstrated the infestation of Trichodina. All other fish scraped had no to very few Trichodina. As with most parasitic infections, they can run through the facility fairly quickly once established in one section so Mr. Harrison asked that the entire facility be treated.

**Figure 1:** Harrison Koi Sales Room Tank Layout



For experimental purposes, 4 tanks were examined post treatment to determine the treatments effectiveness. Tanks D3, D4, D5 and D6 all had heavy infestations of Trichodina prior to treatment and were selected for exam post treatment. Another factor that was requested by Mr. Harrison was to leave the water flowing in two of the tanks and determine if the treatment was still effective. The water in D5 and D6 was therefore left on at approximately 3.75 lpm.

After one hour of treatment, the skin scrapes from all fish, 3 per each of the 4 tanks, showed an elimination of the infestation. There was one live Trichodina found on one fish. All of the fish showed no impact from the treatment. A report 7 days after



treatment from Mr. Harrison stated there were no mortalities and the feeding response in the fish has been very good. He is anxious to add this tool to his treatment regime.

*Trial 4, August 15, 2007 Laguna Koi*

The retail location where testing was done was Laguna Koi in Laguna Beach California. Laguna Koi is an upscale retail establishment selling Koi and goldfish as well as supplies and feed. The owner Ben Plonski invited me to come and look at fish to see if there were any individuals that may make good candidates for testing. When shipments come from Japan, they often will have some parasite loading.

Sampling was done using scraping as described in Trial 2. All equipment between tanks was sterilized in a bleach bath on site.

Upon examination of Koi from two tanks, there were no parasites found. Three fish were inspected from a recent Japan shipment and two from a tank that was thought to have a problem with *Costia*. Two black moors were also examined as they were showing clinical signs of parasitic infection. The copepod *Argulus* was found on one fish which would definitely cause scratching but was in such low numbers that a test treatment would not give an indication of efficacy.

Mr. Plonski noted two Koi showing severe signs of Dropsy in two different tanks. These animals were caught and examined. Both fish had severe ascities and hemorrhaging around the scales. Fish 1 (20.32 cm) had a much more distended abdomen and exophiala than Fish 2 while Fish 2 (25.40 cm) had more severe hemorrhaging and ulcerative lesions. They both also had severe *Costia* infestations. Fish 1 had a single *Gyrodactylus* in the initial scrape while Fish 2 had 5.

Both fish were placed in an 80 l bin and treated in 60 l of water at 10 ppm. Repeated skin scrapes were done at 0.5 hr, 1 hr, 1.5 hr and 2 hr. These fish were to be euthanized due to the extent of disease so it was opportune to do an extended treatment and monitor the effects. Due to the extent of the disease in Fish 1, the stress of handling and treatment induced mortality. At 1.75 hrs into treatment Fish 1 was dead.

At 0.5 hrs into the treatment, the level of *Costia* on both fish was reduced. Many were dead and far fewer live organisms. There were also no live *Gyrodactylus* in either fish's sample. Fish 2 did have one dead *Gyrodactylid* in the sample. After 1 hr the majority of *Costia* were dead. In the sample from Fish 1 there was one section of the scrape where live *Costia* occurred. It was odd that the majority were dead. It was hypothesized that with the severe distension of the scales on these fish, organisms may have been harbored in the scale pocket within the mucus layer protecting them from the treatment. The action of scraping removed these and they show up live in the sample. The sample from Fish 2 had no live *Costia*. Both fish had no *Gyrodactylus*.

After 1.5 hrs samples from both fish showed no live *Costia*. Fish 1 had one live very large *Gyrodactylus*. This was also the last scrape from this fish as it died shortly after the

last handling. At 2 hours Fish 2 was behaving normally and the last scrape sample showed mostly dead *Costia* with a small patch of live organisms. These may have originated from a protected area as described earlier. It appears that the extended duration did not have any immediate effects on the fish.

Necropsies were done on both of the fish. Both had a very similar presentation with a large amount of fluid in the peritoneum which looked to contain some blood. The major organs looked pale and the kidneys were enlarged. It looked most like an infection of the bacteria *Aeromonas hydrophila*. A common organism that can cause severe septicemia with a presentation as noted in these animals. Mycobacteriosis can have similar gross clinical signs but internally the presentation is much different.

*Trial 5, August 31, 2007, California Koi*

The fifth trial was executed at California Koi in Fallbrook California. The owner Takemi Adachi had experienced an elevated mortality in one tank of approximately 500, 7.5 cm to 10 cm koi juveniles. He allowed for an inspection of the fish and testing on a subsample using MinnFinn. The infected tank was 6000 l with a flow rate of approximately 7 to 15 l/min.

The fish were extremely fragile and in an advanced state of disease. Morbid fish were dying due to the handling from the tank to the transport bucket and holding. The owner recovered 30 fish for screening and testing. The initial sampling was through skin scrapes and was carried out as described in other trials. At the sampling of the skin scrape, it was noted that the mucus level on the fish was thick and it was thought that there was a parasite load. Other clinical signs included lethargy with mild hemorrhagic lesions on 2 of the 5 fish examined. 3 of the fish also had some mild abdominal swelling.

Upon initial screening of skin scrapes, no ectoparasites of any consequence were noted on any of the fish examined. Only 2 had very mild levels of *Costia* and one with a single dactylogyrid fluke. The owner agreed to terminal sampling so gill samples were taken and examined.

The gill samples were taken from the right side on all of the animals from the first two arches. A small portion of the gill tips was taken and placed on a slide. A cover slip was then placed on the sample and water applied to the edge. Surface tension carried the water under the cover slip to help suspend the sample and prevent rapid drying.

The gross examination of the gill clips revealed a severe infection of the gills most likely bacterial in origin. There was a severe level of epithelial hyperplasia and fusion with sloughing of cells and mucus. The impact on the fish was severe and was causing severe mortality of about 3%-4% per day.

A test was set up using five of the test bins with 60 l of water. Two of the bins were used as controls and three were treated at 10 ppm. Five fish were placed in each bin. The treatment was run for one hour and all of the surviving fish were examined by skin

scrape. After the termination of the treatment there were 4 control fish alive and 11 test treatment fish. The control fish again had very congested gills with significant cellular and mucus debris. There also was a fair degree of epithelial proliferation on the gill.

The fish from the treated group still showed significant proliferation but much of the debris had been eliminated and congestion was reduced. With this it was decided to collect gill tissue from five more fish from the infected main tank for histology. The histological sample was cut from the fish with the arch so the filaments would remain attached and be better for sectioning. All samples were taken from the first two arches on the right side of the fish.

In order to compare the animals after treatment, enough chemical was left for the owner to do a single 10 ppm treatment and neutralization on the affected tank. Application was as described in Trial 4. The treatment was conducted on September 1, 2007. Due to the advanced state of disease in the tank, approximately 50% of the fish were lost at treatment which is common with any treatment during an advanced state of disease. The fish that survived the treatment however had no mortality the following 14 days. On September 9, 2007, five fish were examined through a gill clip. The gills were in much better condition with less congestion and more definition returning to the lamellar architecture. The owner reported that he was amazed at how much better the fish were doing and was pleased with the treatment.

From the fish sample for whole gill observation, samples of gill were also taken for histology. Sampling was as described above except the sample was taken from the left side, opposite from where the whole mount sample was taken.

### *Pathology*

The gills from the pre-treatment fish demonstrated extreme secondary lamellar fusion. Samples were collected from six fish and all showed severe fusion. A presentation like this is often due to bacterial and parasitic infections. In this case a bacterial infection is most probable as no parasites were seen in whole mounts. See Figures 2 and 3 for examples from these samples.

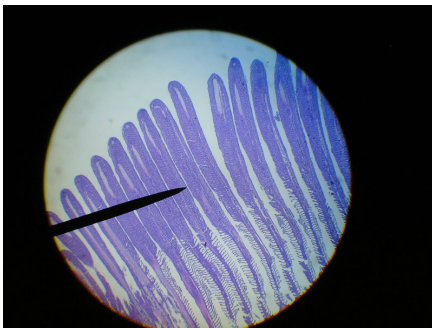


Figure 2: Pre-treatment gill sample displaying severe secondary lamellar fusion 40x



Figure 3: Pre-treatment gill sample displaying severe secondary lamellar fusion 100x

In the six post-treatment fish sampled there appears to be a healing process occurring. In each of the fish there was notable reestablishment of normal architecture with individual secondary lamellae returning. One of the samples was almost normal in appearance. See Figures 4 and 5 for examples of two of the samples.



Figure 4: Post-treatment gill sample displaying improved secondary lamellar fusion 40x

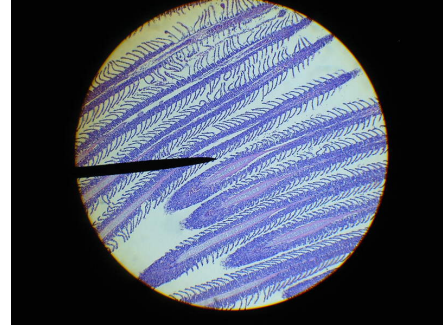


Figure 5: Post-treatment gill sample displaying improved secondary lamellar fusion 100x

*Trial 6 and 7, September 14 and 27, 2007, Tom Hawthorn*

Mr. Hawthorn is a private pond owner with a 50,000 gal pond. He has very little filtration with waterfowl living on the pond. The influx of nutrients induced a series of algae blooms throughout the summer. Mr. Hawthorn allowed some water to be sampled for MinnFinn testing. The water was very heavily loaded with algae and an estimated secchi depth of 2 to 6 inches was made.

Two gallons of water was sampled from the pond in clean one gallon water bottles on each September 14, 2007 and September 27, 2007. On both sample days the pond was in full bloom with a suspended phytoplankton. The water was examined microscopically using 400 x magnification. Each sample was made up from three 10  $\mu$ l sample placed on a slide and covered with a glass cover slip.

Upon examination of sample from both sample days a unicellular algae (a) was found to be the most dominant. The identification is difficult. The organism appears to be spherical with rigid spines on the outer surface (Figure 6). Another species found (b) was believed to be the green algae *Scenedesmus sp.* (Figure 6). Also present in the sample were several species of diatoms and some invertebrates including a rotifer species.

The following procedure was used for both test days unless otherwise stated. For testing the sample water was measured into one liter subsamples in 6 1 liter beakers the same day as collection. Two of the beakers were left untreated as controls. Two were treated at a 5 ppm dose of MinnFinn and the last two treated at 10 ppm. The beakers were left in the sun so photosynthetic activity could be observed with the formation of bubbles on the side of the beaker due to oxygen production.

*Trial 6*

After 30 minutes of treatment, the 5 ppm samples appeared to have little impact. Microscopic examination revealed mostly healthy cells with a few clear ones. The 10 ppm however had approximately 25% of the cells observed with contents starting to clear or cells completely clear.

Forty minutes into the treatment, the beakers were observed for bubble coverage inside the beaker wall. The control beakers had approximately 80% of the wall covered while the 5 ppm beakers had 60% to 70% and 10 ppm 50% to 60%. There appeared to be decreased photosynthetic activity in treated samples.

After 1 hour of treatment the sample at 10 ppm was showing a good effect with most of the cells clearing and appearing dead. The 5 ppm sample still showed approximately 75% of the cells looking normal.

The beaker with the control sample had the same appearance prior to the start of the experiment, still with live protozoa. Both of the test beakers had no living protozoa in the microscope samples.

**Figure 6:** Algal Cells in Suspension from a Water Sample Collected from the Pond of Mr. Tom Hawthorn



The treatment was terminated 90 minutes after start by adding the sodium bicarbonate and sodium thiosulphate neutralizer. The control beakers were still as in the beginning and contained approximately 10% dead algal cells. The 5 ppm sample looked to have approximately 25% dead cells with no invertebrates and the 10 ppm beakers contained approximately 75% dead cells and again no invertebrates. The 10 ppm dose appeared to be quite effective.

The beakers were left overnight to see what longer term impact may look like. After 20 hours the control beaker still looked as it did on treatment day with heavy bubble formation on the side of the beaker and a healthy green color. Some settling occurred and the sample was stirred prior to subsampling for microscopic exam. Microscopic inspection demonstrated many healthy cells.

The sample treated at 5 ppm had somewhat less bubbles on the side of the beaker and was not as brilliant in color. The 10 ppm had no color and was only turbid with no bubble formation.

A dissolved oxygen and pH reading was taken from each beaker. The control had a Dissolved Oxygen of 14.5 ppm which is 170% of saturation at the 26.4°C temperature. The photosynthetic activity producing oxygen super oxygenated the water. In the 5 ppm treatment beaker the oxygen level was 11.7 ppm. Again this is above saturation at 142% indicating that algal activity is still present. The Dissolved oxygen of the 10 ppm dose was 6.9 ppm which is only 80% of saturation. With this level it is apparent that the algae was deactivated and possibly rotting and consuming oxygen dropping levels below 100%.

The pH also reflected a similar trend as in water that contains highly photosynthetic plants will be higher than other water. The control sample had a pH of 10.4, 20 hours after the termination of treatment. The 5 ppm beaker was at 9.7 and the 10 ppm sample at 9.4.

### *Trial 7*

The difference between trial 6 and trial 7 was that the termination of the treatment occurred at 60 minutes. The cross section of the algae in the sample was the same as in Trial 6. The pre-treatment dissolved oxygen levels were 14.6 ppm to 15.6 ppm in all of the beakers and pH between 9.4 and 9.8.

The observations of bubble formation in the beakers were similar to the previous trial. The appearance of the beakers also followed suit with the control sample being very green, the 5 ppm slightly less so and the 10 ppm having no green color. The oxygen levels post-treatment were 13.9 ppm for controls, 10.0 for 5 ppm and 6.1 ppm for 10 ppm. These results are similar to the previous trial.

The microscopic examination was also similar to that of Trial 6. The pre-treatment samples contained large numbers of live cells with few dead. After treatments were completed, the control looked as the pre screen did, the 5 ppm treatment had a mild to moderate increase in cells that appeared dead (~40%) and finally at 10 ppm the majority of cells (>90%) appeared dead or dying.

Trial 8, September 17, 2007, Jim Migliaccio

Mr. Migliaccio is the owner of a 7570 l pond. He had called about a water clarity problem. Upon arrival at the pond site it was apparent that an algal bloom was in process. The bottom of the pond at a depth of 106.7 cm depth. The pond is a concrete in ground pond with a neoprene paint painted liner. Mr. Migliaccio consented to a test treatment to terminate the algal bloom.

A microscopic examination revealed a mild bloom with few very small cells. The algae may be a nanocloropsis like organism due to its small size.

The temperature was 20.6°C, dissolved oxygen 7.6 ppm and pH 8.4. A treatment of 10 ppm was prescribed giving a dose of 259.78 ml MinnFinn and for neutralization 837.93 g of sodium bicarbonate and 64.56 g of sodium thiosulphate. The treatment was set for one

hour. The application of the treatment and neutralizer was done by first diluting in a 20 l bucket with 15 l of pond water and distributed across a large part of the pond surface.

The following day Mr. Migliaccio reported a drastic improvement in water quality. It appeared that the treatment was effective. With the small size of the cells, it is difficult for the filter to remove the organisms. With this a clumping or clarifying agent was added to the water to assist with final clarification.

It appears that MinnFinn is effective at deactivating phytoplankton in suspension. If MinnFinn is used for algal suppression, a clarifying agent should be recommended for final particulate removal.