

(a) Title of proposal

**Copper Sulfate, Potassium Permanganate & Malachite Green Action on  
*Ichthyophthirius multifiliis* (White Spot) Disease in Food, Ornamental and Pet  
Fish.**

(b) General description

*Ichthyophthirius multifiliis* is a ciliated protozoan that causes “Ich” or “white spot” disease. Ich is the most widely known protozoan parasite on fishes. Adult organisms are oval to round. The adult is ciliated and contains a horse-shoe shaped nucleus which can be seen in older individuals (Floyd & Reed, 2008). The breeding stage of the parasite encysts between two layers of the host’s skin. When mature, it leaves the fish and produces large numbers of free swimming young, called tomites or swarmers. These larvae must find a host within 48 hours (at water temperatures of 75-79°F) or they will die (Floyd & Reed, 2008).

Ich is a protozoan parasite that is easily introduced into a fish pond, tank, or home aquarium by new fish or equipment which has been moved from one fish-holding unit to another. The disease is highly contagious and spreads rapidly from one fish to another. This organism is an obligate parasite which means that it cannot survive for more than 96 hours or “cannot develop.” The parasite has to survive outside of the host in order to be transmitted from one fish to another unless fishes are present. It can cause massive mortality within a short time. An outbreak of ich is an emergency situation which requires immediate treatment; if left untreated, this disease may result in 100% mortality (Floyd & Reed, 2008).

Control of ich outbreaks is often difficult because of the parasites' unusual life cycle and the effect of water temperature on its lifecycle (Floyd & Reed, 2008). Three different solutions can be used to prevent this parasite on fish. Malachite green has been used as a tropical fungicide and antiprotozoal in salmonid farming throughout the world for 50 years (Alderman, 1991). Although effective, disadvantages according to Werth & Boiteaux (1991), include, rainbow trout, *Oncorhynchus mykiss*, showing cell division in eggs treated with malachite green and, more recently, spinal, head, fin and tail abnormalities in trout (Alderman, 1991). Malachite green is a respiratory enzyme poison with effects only reversed by proteins that carry out chemical reactions, known as cytochromes (Alderman, 1991).

The use of copper sulfate for disease and algal control in fish culture has a long history and its efficacy as a healing agent or medicine, for *Ichthyophthirus* has been demonstrated in controlled experiments (Griffon *et al.*, 2007). The chemical is effective and relatively inexpensive, an important consideration when large volumes of water are treated. The disadvantage of copper sulfate is that it is extremely toxic, particularly in water of low alkalinity. Use of copper sulfate may lead to oxygen depletion; therefore emergency aeration should always be available (Griffon *et al.*, 2007).

Potassium permanganate is another widely used chemical compound in aquaculture and can be an effective bactericide against other bacteria and an effective fungicide (Harrell, *et al.*, 1994).

(c) Background/Brief Review of Literature

Alderman, D.J. "*Pathological effects of repeated doses of malachite green: a protein study.*" Journal of Fish Diseases 14 (1991): 521-532.

Floyd, Frances, Reed and Ruth, Peggy. "*Ichthyophthirus multifilis.*"

(<http://edis.ifas.ufl.edu> as viewed on Oct 3, 2008).

Griffon, B.R., Mitchell, A.J. "*Susceptibility of channel catfish, *Ictalurus punctatus* (Rafinesque) to *Edwardsiella ictaluri* challenge following copper sulphate exposure.*" Journal of Fish Diseases 30 (2007):581-585.

Harrell, R.M., Reardon. "*Effects of varying salinities on the toxicity of potassium permanganate to larvae and striped bass, *Morene saxatilis* (Walbaum)*". Aquaculture and Fisheries Management 25 (1994):571-578.

(d) Specific Goals of the project

1. Control of ich in food fishes, ornamental fish and pet fish from 4-6 pairs of each three species to be identified and separated.
2. Spreadsheets listing potential ich cures containing clearly organized information on each fish species, location, type of prevention against ich and what the cures consist of.
3. Scientific article worthy of publication addressing ich management.

(e)

Methods and Timeline

During the research project, I plan to acquire 4-6 pairs of food fish, ornamental fish and pet fish. I will also have a control group of each fish. These fish will not

contain ich. This will allow me to see the difference between fish that contain ich and fish that do not. Each group of fish will have their own separate tank that I will set up at the Biological Field Station in Cooperstown, N.Y. We will dissect wild minnows and put the cysts into each aquarium. We will do some extensive review on experimental infection before introducing cysts into each aquarium. The protozoan will change from an encysted stage to a free swimming stage within 48 hours to find a fish host. The incubation period of these fish will occur within this time frame due to the fast reproductive rate of the parasite. After 1 month, the effected time for fish to become visibly infected with ich will be seen. I will add 1mL potassium permanganate to one tank, malachite green to the second, and copper sulfate to the last tank. I will record the results on how many days each treatment took until there are no more white spots visible. The fish will then be euthanized to obtain skin scrapings. I will collect four slides from each fish. Slides will then be examined with a microscope to tally the number of protozoans present. I will count the number of pustules present in each slide that is taken. I will take two scrapings from the skin and two scrapings from the gill. The data will then be analyzed and recorded for interpretation.

(f) Role of Student and Faculty Sponsor

██████████ will be working at the Biological Field Station in Cooperstown, NY Tuesday and Thursday afternoons under the supervision of ██████████. There, she will be able to analyze the treatments. Above, is a written time-line for the project, with the relative contributions of the student and faculty guidance.

## FACULTY SPONSOR FORM

*This form must be completed by the faculty sponsor(s), printed and signed, and included in the grant package for submission by the application deadline (noon, 10 November 2008). Additional pages are allowed for narrative comments, which will be strongly considered in the review process.*

STUDENT APPLICANT NAME(S): [REDACTED]

FACULTY SPONSOR NAME(S): [REDACTED]

PROJECT TITLE: Copper Sulfate, Potassium Permanganate and Malachite Green Actions on Ichthyophthirus multifilis (White Spot) Disease in Food, Ornamental and Pet Fish.

*Rate the quality of the project and the ability of the student(s) to carry out and complete the project.*

1 Excellent 2 Very good 3 Good 4 Fair 5 Poor

Project:	<input type="checkbox"/> 1 Originality of project	<input type="checkbox"/> 2 Significance of the project
Student(s):	<input type="checkbox"/> 2 Familiarity with work in the applicable field	<input type="checkbox"/> 1 Ability to organize work
	<input type="checkbox"/> 1 Perseverance in pursuing goals	<input type="checkbox"/> 1 Self-reliance and independence
	<input type="checkbox"/> 1 Potential to complete project	<input type="checkbox"/> 1 Laboratory skills and techniques, if relevant
	<input type="checkbox"/> 1 Ability to work cooperatively with others	<input type="checkbox"/> 1 Ability to communicate effectively

*Rate the student(s)' contribution to this proposal, including originality of ideas, background research, and writing.*

< 25%  25-50%  50-75%  75-100%

*In the space below (and additional pages, if needed), please comment on the following:*

Your role in developing the proposal and how you will facilitate completion of the project.

[REDACTED] has shown a great deal of independence in developing this proposal, which was her original idea. I helped [REDACTED] with some of the organization in the proposal, as well as the experimental design. I look forward to assisting [REDACTED] in carrying out the experiment itself, which will be conducted at the Biological Field Station at Cooperstown. I plan to teach [REDACTED] laboratory techniques, including fish dissections to obtain wild Ich specimens, and experimentally infecting aquarium fishes. I look forward to working with [REDACTED] in processing her results. I will work closely with [REDACTED] on the preparation of parasite whole mount slides and identification of parasites, two key elements of this project.

The applicant(s) potential to carry out the proposed research or creative activity and the relevance of the project to the applicant(s)' chosen field of study; compare the applicant(s) with other students you have supervised or with other successful students you have known.

I believe [REDACTED] is well-equipped to fulfill the goals of this project based on her background, and based on the level of motivation she has demonstrated in developing this proposal. [REDACTED] has a solid academic record, which includes a degree in Veterinary Technology. She is also comfortable in conducting a variety of laboratory techniques, including the preparation of parasite slides. She has demonstrated a level of independent thinking throughout the creation of this proposal.

[REDACTED] has expressed interest in conducting graduate work in the field of veterinary medicine. This project will help her develop skills and gain experience that will help her in her career path.

[REDACTED] has a great deal of initiative, and has shown competence throughout the process of preparing this proposal. I would consider her to be very competitive among her peers.

Additional remarks:

Signature of Faculty Sponsor(s): [REDACTED]

**BUDGET PAGE**

Please refer to BUDGET INFORMATION (page 2) before preparing.

STUDENT APPLICANT NAME(S): [REDACTED]

FACULTY SPONSOR NAME(S): [REDACTED]

**PROJECT TITLE:** Copper Sulfate, Potassium Permanganate and Malachite Green Action on Ichthyophthirus multifilis (White Spot) Disease in Food, Ornamental and Pet Fish.

List all items requested for funding and provide a brief narrative to justify requested expense.

**\$450 Student Fellowship(s) [not to exceed \$500 total for project]**

*Note: A fellowship is a non-wage award related to an academic endeavor and is subject to IRS regulations. Students who are awarded fellowships will be paid only after presentation at Student Research Day.*

Name(s): [REDACTED]

Amount: \$450 \$ \$ TOTAL: \$450 (Not to exceed \$500)

**\$201.44 Supplies/Materials/Minor Equipment:** List and describe below.

- 3-5 ornamental fish (Koi fish) ~\$5 each.
- 3-5 pet fish (gold fish) ~\$2 each.
- 3-5 food fish (cat fish) ~\$5 dollars each.
- Glass microscope slides \$60 a box
- Scalpel blades for skin scrapings \$15.00 a box
- Copper sulfate treatment \$15.00 a bottle
- Potassium Permanganate treatment \$8.95 a bottle
- Malachite green treatment \$7.49 a bottle
- 6 bottles of fish food \$5.00 a bottle
- 1 bag of cat food to feed cat fish \$5.00 a bag

**\$54.00 Travel:** Include air/train fare, mileage (current mileage rate is \$0.585/mile), lodging, per diem (*check [www.gsa.gov](http://www.gsa.gov) for accurate per diem rates by location*), ground transportation, parking, tolls and any other travel expenses required specifically to conduct the research or creative activity (see Budget Information on page 2).

I will drive to the Biological Field Station in Cooperstown, NY from Oneonta, NY which is a 33 minute drive. The mileage is about 23.24. The average mileage rate is \$0.585/mile. I would be driving there two days a week; Tuesday afternoons from 10-12p.m. and Thursday afternoons from 11-12p.m. I estimate the amount of money needed is \$54.00.

\$ \_\_\_\_\_ **Other Expenses:** Describe expense.

**TOTAL REQUEST: \$705.44 (not to exceed \$1,500)**

**TOTAL REQUEST: \$996.44 (not to exceed \$1,500)**

**BUDGET PAGE**

Please refer to BUDGET INFORMATION (page 2) before preparing.

STUDENT APPLICANT NAME(S): [REDACTED]

FACULTY SPONSOR NAME(S): [REDACTED]

**PROJECT TITLE:** Copper Sulfate, Potassium Permanganate and Malachite Green Action on Ichthyophthirus multifilis (White Spot) Disease in Food, Ornamental and Pet Fish.

List all items requested for funding and provide a brief narrative to justify requested expense.

**\$300 Student Fellowship(s) [not to exceed \$500 total for project]**

*Note: A fellowship is a non-wage award related to an academic endeavor and is subject to IRS regulations. Students who are awarded fellowships will be paid only after presentation at Student Research Day.*

Name(s): [REDACTED]

Amount: \$300 \$ \$ TOTAL: \$300 (Not to exceed \$500)

**\$642.44 Supplies/Materials/Minor Equipment:** List and describe below.

6 aquariums (3 fish tanks will be used from the Biological Field Station in Cooperstown, NY). 3 tanks will contain one of the 3 different fish species. 3 tanks will contain control fish, each one a different specie. Aquariums (which includes starter kit supplies) run about \$155.00 each at Wal-mart. I will need 3.

3-5 ornamental fish (Koi fish) ~\$5 each.

3-5 pet fish (gold fish) ~\$2 each.

3-5 food fish (cat fish) ~\$5 dollars each.

Glass microscope slides \$60 a box

Scalpel blades for skin scrapings \$15.00 a box

Copper sulfate treatment \$15.00 a bottle

Potassium Permanganate treatment \$8.95 a bottle

Malachite green treatment \$7.49 a bottle

6 bottles of fish food \$5.00 a bottle

1 bag of cat food to feed cat fish \$5.00 a bag

**\$54.00 Travel:** Include air/train fare, mileage (current mileage rate is \$0.585/mile), lodging, per diem (*check [www.gsa.gov](http://www.gsa.gov) for accurate per diem rates by location*), ground transportation, parking, tolls and any other travel expenses required specifically to conduct the research or creative activity (see Budget Information on page 2).

I will drive to the Biological Field Station in Cooperstown, NY from Oneonta, NY which is a 33 minute drive. The mileage is about 23.24. The average mileage rate is \$0.585/mile. I would be driving there two days a week; Tuesday afternoons from 10-12p.m. and Thursday afternoons from 11-12p.m. I estimate the amount of money needed is \$54.00.

\$\_\_\_\_\_ **Other Expenses:** Describe expense.

**STUDENT GRANT PROGRAM FOR RESEARCH AND CREATIVE ACTIVITY (Fall 2008 round)**

---

I am excited about the proposal [REDACTED] has developed and the opportunity to work with her. I look forward to learning along side with her about the biology of Ich and its potential treatments. This project includes a local component--collection of wild-caught specimens of Ich. Thus, this project fits in nicely with the research program I am beginning to build on parasites of fishes of Otsego Lake.

**Signature of Faculty Sponsor(s):**