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**RESEARCH APPLICATION**

1. **General Information**
   1. **Project Title**

*Note: All text boxes will expand as necessary to*

*accommodate additional text.*

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| **Minimal Effective Concentration on Fluconazole to Eliminate Bryopsis** |

* 1. **Principal Investigator Information**

The Principal Investigator (PI) is the individual responsible for the completion of the project and work product. The PI must ensure that all aquarium guidelines, policies, and procedures are followed. **Note:** A faculty co-PI is required for any proposal submitted by a graduate student.

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| **Ross Cunning (Project Support), Coral Biologist** |

Name Title

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| **Shedd Aquarium** |

Organization

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| **Rcunning@sheddaquarium.org** |

Phone Number E-mail Address

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Address

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| **Bryce Corbett (Primary Project Head), Research Technician** |

Name Title

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| **Shedd Aquarium** |

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| bcorbett@sheddaquarium.org |

Phone Number E-mail Address

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Address

1. **Project Information**
   1. **Summary**

Provide a summary of the project for the lay person not to exceed 250 words. Please comment on the study objectives and the specific question(s) to be answered.

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| Fluconazole is a commonly used treatment to get rid of the green algae, Bryopsis in aquaria. This chemical shuts down the enzymatic pathway necessary to produce ergosterol, which is important to cell wall integrity. Shedd aquarists have been using this treatment and seen promising results in controlling Bryopsis growth, however; acroporid colonies seem to be losing color after the tank is treated with Fluconazole. Thus far, aquarists have been using a standard concentration of 20mg/gal or 5.3 mg/L, but there aren’t any scientific studies to back up this concentration, just word of mouth from seasoned aquarists.  **Question:**  What is the minimum amount of Fluconazole needed to treat Bryopsis?  **Objectives:**   * Identify the minimum concentration of fluconazole that can be used to kill Bryopsis * Identify health metrics to characterize Bryopsis health decline   Additionally, the Veterinary department requested water samples throughout this study to determine how fluconazole can be detected in water over time. |

* 1. **Background**

Describe the type of research to be conducted and the importance of such research. Explain previous studies that have been conducted and the current state of knowledge on the topic, and describe how your project complements this research and/or fills a gap in the literature. Include literature cited as necessary.

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| This study is experimental in nature and will produce quantitative datasets. This research will optimize aquarium practices which becomes increasingly important as our coral collection expands. There is virtually no peer-reviewed literature that documents fluconazole in relation to Bryopsis treatment. It is primarily an anti-fungal drug used by humans to treat yeast infections, oral thrush or urinary tract infections. The concentration used by the Aquarium team at Shedd was purely based on community boards and product information from common manufacturers such as, Bulk Reef Supply, Reef Goods and Saltwater Aquarium. Additionally, it is important to identify the minimal concentration of Fluconazole that still achieves its intended effect of eliminating Bryopsis, while minimizing any potential unintended effects on other organisms in these habitats such as corals.  This research would contribute to not only Shedd’s understanding of Fluconazole’s effect on Bryopsis but to the aquarist community at large. |

* 1. **Methods**

Please describe, in detail, the methods that will be used to accomplish your research goals.

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| 18 4-L containers will be used as “tanks” and have a small piece of Bryopsis attached to a 2x2 piece of egg crate per tank (pictured). We will be testing 5 different concentrations of Fluconazole plus 1 control.    Concentrations are: 5.3, 2.5, 1.0, 0.5, 0.1, 0 mg/L. There will be 3 containers per concentration placed in the larger tanks in the Conservation Aquarium System. Each containers will be filled with sea-water to 3800 mL or 3.8 L as to keep concentrations consistent.  To create a 0.2mg/mL stock solution the maximum solubility of Fluconazole in PBS ([Cayman Chemicals](https://cdn.caymanchem.com/cdn/insert/11594.pdf))\*; we use the equation below to calculate the amount of fluconazole necessary to create 700 mL of a 0.2 mg/mL stock solution:  \*Another source states the solubility at 1.39 mg/ml, we will make test solutions of both to determine which concentration is most appropriate.  140 mg of fluconazole dissolved in 700 mL of salt water will provide a .2mg/mL stock solution that can be added to the treatment containers to achieve test concentrations.  To achieve experimental concentrations (5.3, 2.5, 1, 0.5, and 0.1 mg/L ):  To achieve the highest concentration of 5.3 mg/L, 100.7 mL of fluconazole stock solution would be added to 5.3 mg/L treatment containers. Calculations for the volume of 0.2 mg/mL fluconazole stock solution to add to the containers for each other treatment are provided in the table below.  5.3 mg/L = .0053 mg/mL   |  |  |  | | --- | --- | --- | | **Desired Concentration (mg/mL)** | **Volume Needed of .2 mg/mL Stock solution of Fluconazole (mL)** | **Total vol across n=3 replicates (mL)** | | 0.0053 | 100.70 | 302.10 | | 0.0025 | 47.50 | 142.50 | | 0.001 | 19.00 | 57.00 | | 0.0005 | 9.50 | 28.50 | | 0.0001 | 1.90 | 5.70 |   540 mL of stock solution of Fluconazole will be used in total across all treatments.  Sea water in each container will be taken from North West Quad, so there is no difference between the container chemistry and the chemistry of the environment Bryopsis was harvested from other than the addition of fluconazole.  Salinity will be measured using a ThermoFisher Orion Probe daily. When salinity gets higher than 36 ppt, I will add gradually add RO water until the salinity decreases to 35 ppt.  Air will be bubbled into each individual container.  Mobius EcoTech aquarium lights will match the North West Quad settings both by 80-120 PAR and by cycle, so 8 hours of light per day.  Measurements of maximum photochemical efficiency (Fv/Fm) for each Bryopsis fragment will be recorded every day (after dark acclimation) using an Imaging PAM fluorometer during the first week of the experiment. If there is signifcant change in Fv/Fm readings we will continue to take PAM readings until the end of the experiment.  Additionally, visual observations using a Canon Camera will be recorded daily. These images will be loaded into ImageJ and used to monitor color change as a measure of health. ImageJ has a histogram RGB function (pictured below) that assigns numerical values to red, green and blue colors in a selected image. I will use this to quantify the visual observations. The Coral Reef Watch color card will be used as a standard on ImageJ to assign a “healthy” green.    The experiment will be over at the end of of the 14 day treatment period, or when all Bryopsis die, whichever is sooner.  **Veterinary Department Samples:**  300 microliter samples taken before fluconazole addition, then at hour 1, 2, 4, 8, and 12 and then every 24 hours until the end of the experiment. (Total of 20 samples over 14 days)  300 ul taken ~20 times over the course of the experiment represents a total of 6 mL removed from each container. The 6mL removal only represents a .16% decrease of total volume which should have a negligible impact on the study. |
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* 1. **Animal Use**

Please check one of the following:

☐ 1. This research involves the use of live animals (vertebrate or invertebrate) in procedures that may cause more than momentary or slight pain. This includes animals that will be euthanized or sedated for use in the study.

☐ 2. This research does **NOT** use live animals, either vertebrate or invertebrate, in invasive procedures or procedures that may cause more than momentary or slight pain. This includes the use of samples that have been collected previously, and non-contact behavioral observations.

**Please note: If you checked number 1 above, you must complete the additional Animal Welfare protocol form.**

* 1. **Project Outputs**

What are the anticipated outcomes of this project? (e.g. data set, report, publication, presentation)

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| Identification of minimal effective dosage of Fluconazole will inform future treatments and also producing a report or publication for either hobbyist journal and/or scientific publication. |

* 1. **Timeline**

How long is the project expected to take? Include a preferred start and end date. Are there phases?

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| As soon as possible, project will take 14 days. |

* 1. **Budget**

How is the project funded and by what organization? How long does the funding last?

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| Project is funded by Dr. Ross Cunning, minimal expenses, >$50 including: airline tubing, tube splitters and 4-L containers |

1. **Resources Required**
   1. **Department, Aquarium, and Staff Resources**

What aquarium resources will be required? Be specific and detailed. How many aquarium employees will be involved and how much of their time will be required? What data, equipment, and/or samples are needed?

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| Materials used (all currently in hand): airline splitters, airline tubing, 18 4-L containers, egg crate, Imaging PAM fluorometer, camera |

* 1. **Special Shipping and/or Handling Instructions**

If you are requesting samples from the aquarium, please provide specific and detail shipping instructions. For example, “Fill CPT tubes with whole blood to the blue line and chill immediately. Ship on ice but do not allow to freeze.”

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| N/A |

* 1. **Necessary Permits**

Are permits required to conduct this study? If so, please list the agency(s), permit number(s), and expiration date(s). Please note: Copies of all permits must be on file in the Conservation and Research Department prior to the start of the project. It is the responsibility of the project PI to ensure all necessary permits have been obtained.

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| N/A |

1. **Signatures**

Any public presentation of a work product, such as a report, publication, or presentation, identifying Shedd Aquarium as an author, collaborator, or source requires at least one-month lead time to secure necessary approvals. Acknowledgement of Shedd Aquarium is required in any resulting work product(s) and a copy should be submitted to Shedd’s Animal Health Operations Manager via email. Shedd Aquarium staff who provide substantial contributions to the intellectual, theoretical, methodological, and/or analytical development of the research project, and/or who assist in writing or preparing the resulting work product should be listed as coauthors at the researcher’s discretion. All coauthors must agree upon the final draft version prior to publication. Shedd Aquarium staff who provide guidance, assistance with husbandry, or carrying out experimental protocols should be acknowledged.

Depending on the extent of research conducted, a Memorandum of Understanding (MOU) will be drafted and signed by both parties upon project approval. The MOU will outline roles and expectations of the researcher and of Shedd Aquarium. Depending on the extent of Shedd’s involvement, an expectation on the resulting MOU may include coauthorship for Shedd staff as stated above.

All researchers are advised to consult guiding texts concerning the care and use of animals in research while developing methodology. We recommend the following:

* USDA’s Animal Welfare Information Center (AWIC):

<https://www.nal.usda.gov/programs/awic>

* Guidelines for the Use of Fishes in Research:

<https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf>

* American Veterinary Medical Association (AVMA) Animal Welfare Policies:

<https://www.avma.org/KB/Policies/Pages/default.aspx>

*To the best of my knowledge, all information contained in this research application is accurate and true. I accept responsibility for the research project listed in this application as outlined by the terms above.*

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**Principal Investigator Signature Date**

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**Print Name**

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**Faculty Signature** (if PI is a graduate student) **Date**

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**Print Name**

**To submit, please e-mail a copy of this application to the following. The signature page may be scanned and emailed.**

Tanya Salvey

Animal Health Operations Manager

John G. Shedd Aquarium

p 312.692.3232

[tsalvey@sheddaquarium.org](mailto:tsalvey@sheddaquarium.org)

**ANIMAL CARE AND USE PROTOCOL FORM**

The Research Committee at the John G. Shedd Aquarium (hereafter “aquarium”) was established to oversee research conducted at the aquarium and by aquarium staff, and to ensure the welfare of all animals involved in research at the aquarium. Approval by the Research Committee is desirable for all proposals and is required for research that proposes using live animals in procedures that may cause pain, harm, distress, or altering of behavior. This generally excludes samples obtained from routine veterinary or husbandry procedures and non-invasive behavioral observations.

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**Project Title**

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**Principal Investigator (PI)**

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**Phone Extension E-mail Department**

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**Name(s) of Co-PI(s)**

*I certify that, to the best of my knowledge, the information contained herein is accurate and true. As the project PI, I accept responsibility for assuring that all project personnel are familiar with animal welfare policies and are adequately trained in necessary procedures , and I agree to comply with the legal standards for animal care and use established under federal and state laws.*

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**Signature of PI Date**

Once signed, this form may be submitted electronically to the following:

Tanya Salvey

Animal Health Operations Manager

John G. Shedd Aquarium

p 312.692.3232

[tsalvey@sheddaquarium.org](mailto:tsalvey@sheddaquarium.org)

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| ***Please leave blank***  Date Received:  ☐ Accepted ☐ Declined Date:  Proposal #: |

Please complete sections I-III and all appropriate appendices to the best of your ability. You may delete those appendices which are not applicable. Please send the completed form electronically to:

Tanya Salvey

Animal Health Operations Manager

John G. Shedd Aquarium

[tsalvey@sheddaquarium.org](mailto:tsalvey@sheddaquarium.org)

1. **ANIMAL USE**
   1. **Description of Animals**

*Note: All text boxes will expand as necessary to accommodate additional text.*

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| **Species** | **Source (purchased, provided by aquarium, captured in the field, etc.)** | **Total # of animals to be used** |
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* 1. **Justification**

Please justify the use of live animals, specifically the proposed species listed above. Also provide an explanation of how the number of animals was derived.

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* 1. **Duplication**

Does the study unnecessarily duplicate any previous work? If yes, please justify below.

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* 1. **Animal Evaluation**

Please explain how animals will be monitored for pain and stress. What specific criteria will be used to determine if an animal is under distress? (e.g. loss of appetite, unusual behaviors, etc.) How often will animals be monitored for these symptoms?

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* 1. **Removal from Study**

What criteria will be used to determine when and if an animal must be removed from the study before anticipated? Please be as specific as possible and include a specified endpoint. For example, “animals will be removed from the study if no feeding is observed for 48 hours.”

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* 1. **Disposition**

How will animals be disposed of at the conclusion of the study?

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1. **Euthanasia**

Regardless of whether it is an intended part of the study design, an appropriate method of euthanasia must be identified in case the need arises. Please check one of the following:

☐ Euthanasia is a part of the study design

☐ Euthanasia is **NOT** a party of the study design

The most appropriate method of euthanasia will vary based on the animal, environment (laboratory, field, etc.), and other factors, but the need to minimize animal pain and distress must be considered. The American Veterinary Medical Association Guidelines on Euthanasia (<https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>) provide information and suggestions for euthanasia procedures.

Please describe the method of euthanasia that will be used.

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1. **Animal Procedures**

Please check all procedures that will be used. For each checked box, please complete the corresponding appendix.

☐ **A.** Substance(s) collected from **live** animals (blood, body fluids, tissue collection, etc.)

☐ **B.** Substance(s) administered to **live** animals

☐ **C.** Dietary manipulation

☐ **D.** Environmental manipulation, including temperature, light, salinity, etc.

☐ **E.** Wildlife field studies

☐ **F.** Surgical procedures

☐ **G.** Use of harmful/hazardous agents (biological, radioactive, or chemical)

☐ **H.** Other procedures not outlined above

**Appendix A- Substances collected from live animals (blood, bodily fluids, tissue, etc.)**

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| **Substance Collected** | **Site of Collection** | **Method of Collection** | **Amount Collected** | **Frequency of Collection(s)** |
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**Appendix B- Substances administered to live animals**

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| **Substance Administered** | **Method of Administration** | **Dose** | **Concentration** | **Frequency** |
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**Appendix C- Dietary manipulation**

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| **Substance Manipulated** | **Amount Manipulated** | **Duration** | **Frequency** |
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**Appendix D- Environmental Manipulation**

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| **Environmental Parameter Manipulated** | **Amount Manipulated** | **Duration** | **Frequency** |
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**Appendix E- Wildlife Field Studies**

1. **Permits Required**

Please list all permits required, including the name of the authorizing agency, the permit number, and the expiration date. Please note: Copies of all permits must be on file in the Conservation and Research Department prior to the start of the project.

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| **Name** | **Number** | **Expiration Date** |
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1. **Capture**

Describe how target species will be captured in the field. Be as specific as possible. Please include the collection method, handling time, expected mortality rate, methods used to reduce by-catch, and endpoint for collected animals (transport to another location, release in field, etc).

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1. **Handling**

Describe how animals will be handled in the field. If data or samples are collected in the field, these methods must be described below. Include length of handling time prior to release or transport.

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1. **Release**

☐Animals will be released in the field

☐ Animals will be transported to another location

1. **Transport**

Describe methods for transporting collected animals from the field to their destination, including approximate duration of travel.

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**Appendix F- Surgical Procedures**

Please describe all surgical procedures that will be performed. Include information on pre-operative and post-operative preparation of animals, where applicable.

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**Appendix G- Use of Harmful/Hazardous Agents**

Please list all harmful/hazardous agents that will be used. Include information on required personal protective equipment, hazards associated with use, proper storage procedures, and appropriate methods for treatment/clean-up. Please note: If hazardous agents are to be used in the aquarium, a material safety data sheet (MSDS) must be on file in the Conservation and Research Office.

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**Appendix H- Other Procedures**

Please describe any other procedures involving live vertebrate animals that have not been described above. Please provide as much detail as possible outlining the type of procedure, frequency, and duration.

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