**STATE UNIVERSITY OF NEW YORK AT NEW PALTZ**
**2021 Summer Undergraduate Research Experience Application**

*Please keep the current margins and use 11pt font minimum. Submit your proposal in .pdf format.*

Page 1: Cover Sheet

**Principal Co-Investigator 1**

Name: Spencer M Mass, MD (tenure track Instructor) Department: Biology
Campus email: masss@newpaltz.edu
Rank (during award period): ☐ Lecturer ☐ Assistant Prof. ☐ Associate Prof. ☐ Full Prof.

**Principal Co-Investigator 2**

Name: Pamela St. John Department: Chemistry
Campus email: stjohnp@newpaltz.edu
Rank (during award period): ☐ Lecturer ☐ Assistant Prof. ☒ Associate Prof. ☐ Full Prof.

**Student Investigator(s)**

Name: Brianna Flood Major / minor: Biochemistry
Expected graduation date and degree: Spring 2022, BS in Biochemistry

**Project Title***: Quantifying Estrogen Disruptor Retention with Regenerative Phenotypes in Planaria*

Acronym definitions (if not provided in the text of the proposal): BPA (bis-phenol-A); BPS (bis-phenol-S); d8-BPA (deuterated BPA); DMSO (dimethyl-sulfoxide, a solvent)

**Ethical Considerations:**

1. *Does this work involve human subjects?* Yes ☐ No ☒
   *If yes, has the HREB been notified or has the project been approved?* Yes ☐ No ☐
2. *Does this work involve recombinant DNA?* Yes ☐ No ☒
   *If yes, has the IBC been notified?* Yes ☐ No ☐
   *Does this work involve non-human vertebrates?* Yes ☐ No ☒

**Expected start date: May 24, 2021**
(This is needed for budgeting purposes. Provide the most accurate date possible. This information will not affect proposal ranking!)

**Budget** (cannot exceed $1000, including delivery fees). Please provide a preliminary list of items and associated costs. Items may be categorized if you have a long list of items:

Plastic consumables (petri dishes, pipettes, syringes, etc). Fisher Scientific $600
Filters for reagents Fisher $100
Reagents for GCMS and HPLCS Fisher $200
Worms $100
*Budget total: $1,000*
*Does the requested budget cover all expenses related to the proposed work?* Yes ☒ No ☒

*If not, explain the additional sources of funding that will be used.* If we have a shipping overage we have permission from SS&E to cover it with department funds. No anticipated additional expenses.

Pages 2-3: Faculty Statement (no less that 11pt font; keep questions italicized but use plain font for answers)

☒ (We) *I accept responsibility for the conduct of this project and assure that the information in this application is correct. (Indicate your agreement by checking the box.)*

*Question 1: What is the issue you are addressing? Provide a BRIEF and clear review (citations not required) of the prior work that led you to the proposed project.* **Planarians** are flatworms that can regenerate their entire body from mere fragments. Over the past several years the Mass lab has been working to develop the planarian as a sentinel system for environmental endocrine disruptors (EED's) and using ecotoxicology as a method for examining regeneration. These compounds are human pollutants from plastics and other industrial processes that are now ubiquitous in our environment. Previous work in our lab has shown that BPA, BPS and other EED's such as 4-octylphenol have toxic effects on flatworms and interfere with regeneration at moderate doses; interestingly both scientifically and environmentally, at very, very low doses, equally disturbing but opposite effects seems to occur (increasing growth rates, etc.). Work in our lab has also shown that a human breast cancer drug called tamoxifen can significantly lessen the effects of BPA and other estrogenic chemicals on regenerating planaria. We have accumulated significant evidence that these compounds are all working through an estrogen-receptor mediated pathway, including the identification of several associated genes and a hypothesis for a mechanism of action (cytoskeletal disruption).

The St. John Lab has also been examining the same compounds and developing novel analytical techniques for extracting and quantifying the presence of BPA in planaria after experimental exposure. These methods involve the use of gas chromatography/mass spectrometry (GCMS) and high performance liquid chromatography (HPLC) using deuterated BPA (d8-BPA). After significant protocol and technique development, it is now possible to use our instruments to interrogate this system quantitatively and measure the amount of these compounds present in exposed worms down to levels that are environmentally meaningful and correspond to the dosing we use to interfere with regeneration.

The specific **research question** addressed in this project is to determine if there is a direct relationship between the quantity of BPA retained by worm tissues during experimental exposure and the disruptive phenotypes produced in regenerating worms thus exposed.

*Question 2: How does your project address this issue? Include any hypotheses, if applicable.* Our principal **hypothesis** is that there is a direct relationship between chemical exposure, retention in living tissue and effects on regeneration. Furthermore, we **hypothesize** that we will see an inverse relationship (a so-called *non-monotonic dose response*) between the amount of BPA extracted and quantified and the phenotypic effects on regeneration (moderate doses interfering and low doses augmenting).

In essence, two closely related collaborative projects are being united under one project to simultaneously quantify BPA and determine the biological regenerative effects of this compound. To date we have performed several pilot studies to work out the protocols to do this effectively and with sufficient throughput to generate statistically significant data that combine both aspects of this project.

*Question 3: How will the project be conducted? Summarize any core procedures or activities. Outline your anticipated timeline for each major phase of the project.*

We will be using a method we have devised (with prior RSCA funding) utilizing photo microscopy and computerized image analysis to track regenerating worms over time. Worms will be transected and the regenerating tail fragments will be photographed every 24 hours for 15 days. We will expose worms to various concentrations of BPA and corresponding concentrations of d8-BPA during their regeneration, as well as perform appropriate controls using media (planaria water), and solvent (DMSO plus water). We will then process the images using software we have developed which determines growth of new tissue relative to pre-existing tissue and rates of growth. Data will be analyzed utilizing logistic regression to

plot growth curves and calculate regeneration velocity. Every 48 hours random control and treatment worms will be pulled simultaneously for analysis, which will include extraction of d8BPA or BPA followed by characterization of the extracts using HPLC and/or GCMS.

*Question 4: What is the student's role in the project? What are the student's qualifications? If there are multiple students, address them separately here and justify their separate roles.* **Brianna will be involved directly in all aspects of this project** from surgically transecting the regenerating worms, to making solutions, performing photomicroscopy data collection and extraction and analysis steps using GCMS and HPLC. *She is uniquely qualified to do this work at this level* because she is a member of both of our lab teams and has worked at the bench with us on all aspects of this for three years. She is trained in the husbandry techniques, photomicroscopy, manual and automated image analysis extraction and instrumentation used for characterization. She is also a veteran at National Conferences where she has presented the results of her prior work with us developing many of these protocols. It is important to stress that she will be primarily focused on data acquisition and analysis and will be assisted in these tasks as needed by both professors. There are additional students in both of our labs not funded here that will be assisting in low level animal care and routine maintenance activities such that Brianna, while involved and overseeing her entire project, will be able to devote most of her energy to obtaining and analyzing data.

*Question 5: What are the project's main learning outcomes for the student? Explain <u>how</u> they extend beyond those achieved by normal course activities in your major.* **This is a unique opportunity** for Brianna to take a lead role in an on-going project that she has been involved with over three years doing technique and protocol development that has matured to the point where meaningful science can be conducted. In a capstone-like fashion, this project weaves a multi-disciplinary approach to a relevant set of scientific (and environmental health) issues that would *not have been tractable* prior to her involvement in both of our labs. **Specific Learning Outcomes:** *experimental design; planning & coordinating* multi-operational experiments using a variety of standard and novel techniques; *hypothesis testing* with *statistical analysis; scientific communication* (at least one major conference and very possibly a co-authored paper are expected results).

*Question 6: What is your mentoring plan? Include your meeting schedule. Address safety (laboratory, studio, travel, etc.) or ethical issues too, as appropriate.* Both mentors will be in the lab full time this summer working at the bench on this project, coordinating with Brianna, performing independent replicates of experiments, providing one-on-one mentoring and assistance as needed. In addition, Brianna will keep a formal notebook, make regular presentations at formal weekly lab team meetings where we collectively assess progress and troubleshoot unanticipated issues. We have a full set of standard chemical safety and animal use protocols on file and Brianna is well versed with chemical handling, storage & disposal, humane invertebrate animal husbandry, and safe operation of the equipment.

**Proposed timeline: May 24:** supplies on-hand, initial experimental design finalized; initial analysis techniques (stats) selected; data archiving infrastructure in-place; experimental set-up ready. **June 6** set-up for second round of experiments commences. **June 8:** Initial set of experiments completed and data analysis begins, second round of experimentation begins (different dosing regimens). We expect 4 rounds of experiments and analysis over June and July. Wrap-up and final analysis should begin in mid July.

*Question 7: How do the requested budget items support the project and the student's learning outcomes?* Reagents and disposable materials (dishes, syringes, pipette tips) are required to perform all of this work.
*Question 8: Should campus remain in its COVID-19 protocol during this summer, what steps you will take to adhere to campus guidelines in conducting the project and mentoring the student?* **We are all vaccinated** and routinely tested. Masks & gloves are used in the lab. Doors, surfaces & instruments are routinely sterilized, social distancing in research spaces will be enforced per CDC/SUNY/Campus guidelines.

*Question 9 (if applicable; include additional pages, if needed): What work (whether tied to the current project or not) was accomplished with previous (past 3 years) AYURE or SURE funding? Information on the post-graduation placement of former AYURE / SURE students will be appreciated*

RSCA funding from the last two years (see below) has been used to complete one part of the collaboration between S. Mass and P. St. John and we have submitted our findings for publication in the Journal of Chemical Education. Three of the four student authors on the manuscript are mentioned below and their research projects have been supported through RSCA.

Fall 2020 AYURE with Brianna Flood: This proposal was closely tied to the current one. Brianna exposed planaria to lower doses of deuterated BPA and was able to detect d8-BPA even at these reduced concentrations. This result allowed us to propose the current regeneration study since lower doses are required in order to provide planaria time to regenerate.

Fall 2019 AYURE with Nicole DePaola and Brianna Flood: Nicole DePaola (B.S., 2019, BCM major currently accepted to SUNY Upstate Medical School) and Brianna learned how to care for planaria, carry out exposure experiments and prepare samples for analysis. During this time period, we introduced the use of deuterium labeled BPA as a way to circumvent detection of BPA from environmental contamination.

SUMMER 2018 SURE with Troy Moody: Troy Moody (B.S. 2019, BCM major, currently at TouroCOM Medical School) used HPLC to analyze a number of different potential contaminants in planaria media to better understand variability in signal strength from extracts of worms exposed to BPA. Troy presented this work at the Society for Integrative and Comparative Biology in Tampa, FL in January of 2019.

Fall 2017 AYURE with St John and Mass for Troy Moody: (BS 2019, currently in Medical School at TouroCOM) Developing techniques for using HPLC to detect BPA in planarian tissue extracts. Troy presented this work at the Society for Integrative and Comparative Biology (SICB) in San Francisco, CA in January of 2018.

Other recent but unrelated RSCA funded research in the Mass Lab:

Fall 2019 AYURE with Krystal Yohannan: Further studies comparing locomotion in Ambystomoid Salamanders. Work involved gait analysis of neotenic axolotls with non-neotenic tiger salamanders. This was a continuation of a large scale project funded across several RSCA grants. Krystal presented her work at the Society for Integrative and Comparative Biology (SICB) in Austin, Texas in January of 2020. She is currently in medical school at Touro-COM.

Summer 2018 SURE with Michelle Pirrone: Comparing movements of locomotion in Ambystomoid Salamanders. Work involved measuring forces generated during locomotion in tiger salamanders and metamorphosed axolotls. Michelle was an engineering student with a minor in biology. She presented the results of this work at the Society for Integrative and Comparative Biology SICB in Tampa, FL in January of 2019. She is currently pursuing a PhD in Biomedical Engineering.

Page 4: Student Statement (should not be copied from the mentor's section; no less that 11pt font; keep questions italicized but use plain font for answers; for a multiple-student statement, you can extend up to 1.5 pages, taking care to address each student separately for Qs 1, 3, & 5)

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*Question 1: Describe your role in achieving the goal(s) of proposed project. If multiple students are involved, describe your roles and workload division here.* I will be the primary student researcher on this project, and will be responsible for carrying out all aspects of the project: planaria husbandry, worm surgery for regeneration, photo microscopy, digital image analysis, biological/behavioral research, BPA extraction, and BPA quantification via HPLC/GCMS analysis. There will be additional students working in the lab to assist in worm care and colony maintenance, so that I may focus on the data acquisition aspect of the project.

*Question 2: Describe the specific methodology you will employ in carrying out this role.* The BPA/deuterated BPA retention studies will begin with surgically transecting 10 worms per experimental Petri dish. The regenerating worm fragments will then be incubated in 30mL of either a control media (artificial pond water or a DMSO/water solvent), or different concentrations of BPA or d8-BPA in media. These concentrations will vary from 1 uM-20 uM. The regenerating worms will be imaged via photo microscopy every 24 hours for a total exposure period of 15 days. The images will be analyzed using manual and automatic image analysis software, which will be used to ratio the area of regenerated blastema to the area of the whole worm. This data will be analyzed by generating growth curves using logistic regression. BPA and d8-BPA retention of control and experimental dishes will be quantified every 48 hours via BPA/d8-BPA extraction, and HPLC detection and GCMS derivatization/detection. The main goal of this research project is to determine whether or not there is a correlation between the retention of environmentally relevant BPA/d8-BPA concentrations by exposed planaria (which will be quantified and calculated using HPLC and GCMS detection) and observed phenotypic effects during tail regeneration.

*Question 3: Describe your qualifications to participate in the proposed project.* I am a biochemistry major in my third year at SUNY New Paltz, and I have been working with Dr. Mass and Dr. St. John on BPA/d8-BPA retention studies for three years. I am well versed in the HPLC/GCMS analytical protocols I assisted in developing over my time with this project, as well as worm colony maintenance and care. I have extensive experience with photomicroscopy and the manual analysis of these images. I have also attended several conferences at which I presented data obtained earlier in my studies.

*Question 4: Describe your plans to meet with your mentor for guidance.* I will be working closely with Dr. Mass and Dr. St. John throughout the course of the project, and I am expected to present all obtained data to them weekly in order to formulate possible solutions to any problems that arise. I will be keeping a detailed lab notebook in which I will explicitly outline all protocols and procedures conducted on a daily basis. Both Dr. Mass and Dr. St. John will be present in the lab as well throughout the project, and will be available in the event of any imminent questions or issues.

*Question 5: Describe your plans after graduation and how your involvement in the proposed work relates to these goals.* After graduation, I hope to further my biochemical studies at either graduate school or medical school. I am extremely interested in stem cell regeneration, as well as the harmful effects of various chemicals on organisms with regenerative capabilities. I hope to be involved in graduate research and post-doctoral clinical studies regarding pathology and human cancer studies involving chemical exposure. My involvement in this current research project will supply valuable laboratory and data analysis experience, and my current work with EED's will provide crucial background knowledge for the work I plan to do in my future studies.

*Question 6: Describe your plans for presenting the results of your research, including but not limited to the required SURE presentation in Fall* **I will present** a poster at the SURE presentation in the Fall semester. I plan to attend the Society for Integrative & Comparative Biology conference in Phoenix, Arizona in January of 2022. I also plan to present a poster at the Science Research Symposium (SRS) as well as at the American Chemical Society (ACS) conference at the end of the academic year.