The Biophysicist

Using Published Undergraduate Biomechanics Research on Hydra Mouth Opening to Train Undergraduates

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ABSTRACT Biophysics research is exciting because physical approaches to biology can provide novel insights, and it is challenging because it requires knowledge and skills from multiple disciplines. We have developed an undergraduate biophysics laboratory module that teaches fundamental skills such as time-lapse microscopy, image analysis, programming, critical reading of scientific literature, and basics of scientific writing and peer review. The module is accessible to students who are familiar with introductory statistics, cell biology, and differential calculus. We used published research on the biomechanics of *Hydra* mouth opening as a framework because it describes a stunning biological phenomenon: *Hydra*, a freshwater polyp, generates a multicell-wide mouth opening in an otherwise closed epithelium through extreme cell deformations within seconds. This publication was co–first authored by an undergraduate and was featured in the public press, thus providing multiple anchors that make the research accessible and motivating to undergraduates. Students start with a critical reading and discussion of the publication and then execute some of the experiments and analysis from the publication, thereby learning fluorescence time-lapse microscopy and image analysis by using ImageJ and/or MATLAB. Students quantify the kinematics of the tissue deformations during mouth opening and compare their data to the literature. The module culminates in the students writing a short paper about their results following the *microPublication* journal style, a blinded peer review, and final paper submission. Here, we describe one possible implementation of the module with the necessary resources to reproduce it and summarize student feedback from a pilot run. We also provide suggestions for more advanced exercises and for using Python for data analysis. Several students expressed that repeating a published study completed by an undergraduate inspired and motivated them, thus creating buy-in and assurance that they can do it, which we expect to help with confidence and retention.

KEY WORDS biomechanics; image analysis; inquiry-based learning; undergraduate; interdisciplinary; microscopy; scientific literature; peer review

I. INTRODUCTION

The 2022 decadal report on the Physics of Living Systems published by the National Academies of Sciences, Engineering, and Medicine identified that, despite two decades of growth in the number of doctoral degrees awarded for biophysics, the subject remains underrepresented in undergraduate curricula (1). The report calls on physics and biology

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departments to integrate the Physics of Living Systems into their courses (1). Although undergraduate biology curricula have seen major changes in response to the recommendations of the 2009 "Vision and Change: A Call to Action" report to focus on the mastery of core biological principles and skills, biophysics is not an integral part of the curriculum (2–4). Similarly, although introductory physics courses for non-physics majors have been reformed to include more life science applications, which has been found to increase engagement of biology majors with physics (see e.g. (5)), upper-level biophysics courses that build on the introductory course material are sparse. Thus, there is a need and opportunity for biophysics educators to develop upper-level undergraduate biophysics courses that appeal to both physics and biology departments that incorporate pedagogical reforms that are accessible to students from diverse backgrounds.

In physics and biology, pedagogical reforms such as inquiry-based labs have been shown to broadly benefit student learning, improve students' attitudes toward learning science, and increase students' performance in data analysis and interpretation (5–9). Course-Based Undergraduate Research Experiences have been shown to increase confidence and student persistence through a science, technology, engineering, and mathematics degree, especially for students coming from underrepresented backgrounds (10–13). Research has also shown that students who begin the semester with weaker experimental design skills show greater gains than initially higher-performing students (14). Other efforts to incorporate research into undergraduate education have emphasized advanced laboratory techniques, reading and discussing research papers, and laboratory modules based on ongoing research by faculty at the instructional institution (15–17). Moreover, student buy-in, metacognition, and an understanding of learning goals are critical for improving students' perception of their career readiness and persistence in the science, technology, engineering, and mathematics fields (18).

Several courses and/or laboratory modules that fulfill this need for an upper-level biophysics course and implement pedagogical reforms have already been disseminated (19–21). A primary focus of these biophysics modules has been molecular and (sub-) cellular biophysics, focusing on examples drawn from thermodynamics, enzyme kinetics, regulatory networks, and structural biology (21–23). Some recent publications have shown the applicability of tissue mechanics and organismal behavior as tools for teaching biophysics concepts and computational skills, such as image segmentation and object tracking (24, 25). This laboratory module adds to the instructional resources available to instructors to teach biophysics at an organismal level.

Here, we introduce a multiweek, research-based laboratory module focused on tissue biomechanics that is accessible to undergraduate students with familiarity of introductory statistics, cell biology, and differential calculus. The module was developed for and carried out as part of an intermediate-level systems biology course and incorporates pedagogical elements that address student buy-in and skill development. Its content is based on a published biophysics article in the field of tissue mechanics that explores the biomechanics of *Hydra* mouth opening, which is a remarkable, visually striking organismal behavior that students are unlikely to have encountered before (26). In contrast to humans and many other animals, *Hydra*—a small, cylindrical freshwater polyp—lacks a permanent mouth opening, but within seconds, it can form a mouth opening in its head epithelium when it needs to ingest, egest, or regulate osmotic pressure. This deformation can be so extreme that the mouth opening's diameter can exceed that of the animal's body column (26). The popular "How-StuffWorks" video series featured this phenomenon when the original paper was published; the featured video can be used to inspire curiosity in students to understand how these deformations are accomplished (27). The experimental techniques are within reach of most students with previous biology laboratory experience. The experiments to quantify the kinematics of

mouth opening described in the publication use fluorescence microscopy to record video of (spontaneous or chemically stimulated) mouth opening events and track the mouth area as a function of time, thus teaching widely used skills for quantifying biological behavior.

Additional reasons why *Hydra* mouth opening is a great choice for an interdisciplinary undergraduate laboratory module are listed here.

(a) In discussing the kinematics and the mechanism for *Hydra* mouth opening, students are exposed to cellular and organismal biology, as well as to the biomechanics of soft tissues. Thus, the background preparation requires the discussion of both biological and physical principles, which immediately invites students to draw from multiple disciplines when working on this module.
(b) The interdisciplinarity of the system allows for multiple avenues of analysis, allowing the instructor to customize the task to meet the needs and interests of their students. Because of the wide variability in students' comfort with computer programming, which can dissuade some students from engaging with the computational analysis featured in the module, this flexibility is essential for managing student confidence and maximizing buy-in.
(c) *Hydra* are easy to culture in the laboratory at room temperature by using spring water. Their care requires no specialized equipment or chemicals, and excellent protocols exist for maintaining *Hydra* colonies (e.g., (28); see also section III).
(d) Fluorescently labeled transgenic lines are available and facilitate imaging (29–31). Reversible anesthetics, such as linalool, facilitate surgical manipulation and can be applied easily to their aquatic environment (32).
(e) Mouth opening can be induced in a controlled fashion by using reduced glutathione or quinine hydrochloride, thus removing the need for spontaneous openings used primarily in (26), which can be harder to achieve in the limited available time of a teaching laboratory setting (26, 33).
(f) The ImageJ software needed to analyze mouth-opening microscopy data is open source, requires minimal technical expertise, and is widely used in professional research settings across multiple disciplines (34).
(g) Many optional avenues are available for increasing the complexity of the module to accommodate students with more or less experience with computational techniques and image analysis, which we discuss in subsequent sections.
(h) The laboratory module lends itself well to a remote classroom if needed; in this case, the instructor can share the sample data provided in the Supplemental Material with their students.

The first author of the published work was an undergraduate student, and this interdisciplinary laboratory was designed to provide students a parallel experience to that described in the article. Hence, students feel empowered that they can do similar work and approach the laboratory module with more confidence. This increases student buy-in and resilience in completing technically challenging experimental and computational tasks. The module provides a unique opportunity for undergraduates to experience the research process by performing published experiments, analyzing their own data rigorously and authentically, reconciling their findings with existing literature, and formally presenting and reviewing their work.

II. SCIENTIFIC AND PEDAGOGICAL BACKGROUND

To help understand the mechanics of mouth opening, the instructor should provide students with some biological background on *Hydra*, which we provide here, followed by a description of the pedagogical background that explains the context and teaching framework of the module.

A. Scientific background: biomechanics of *Hydra* mouth opening

Hydra are cnidarian polyps found in freshwater sources around the globe. *Hydra* have a

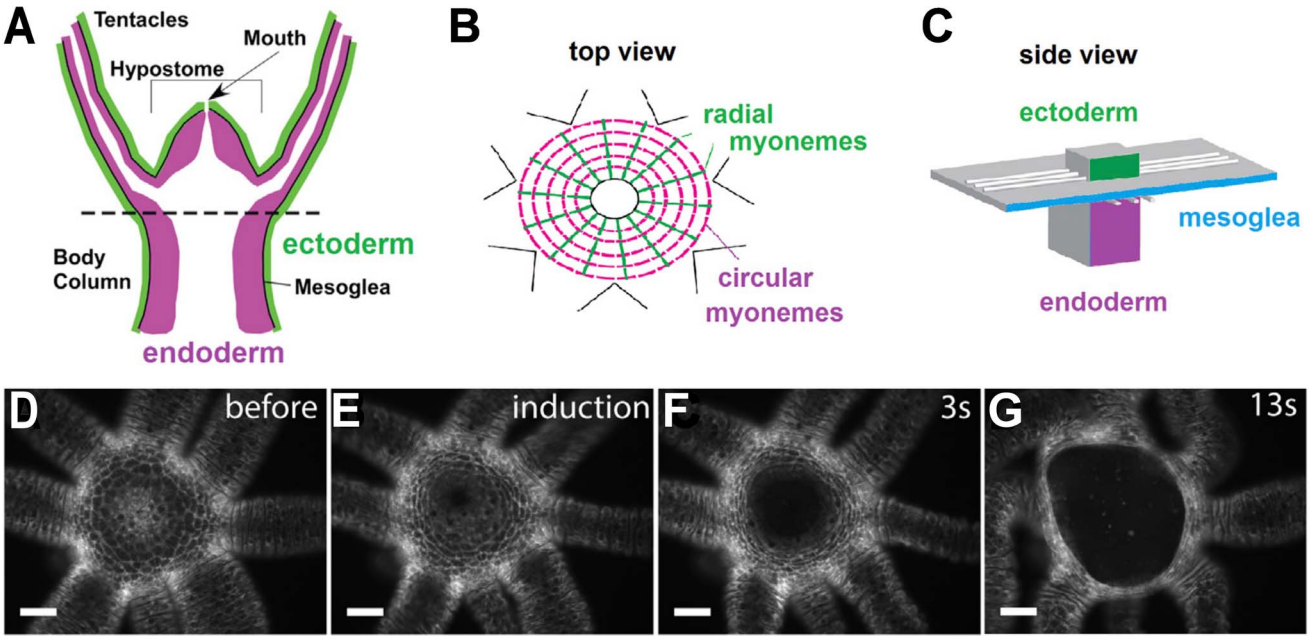

Fig 1. Overview of *Hydra* anatomy and mouth opening. (A) Side-view schematic of the *Hydra* head showing the hypostome and tentacles and the two epithelial layers. (B) Top-view schematic of the *Hydra* head showing myoneme arrangement in the ectoderm (green) and endoderm (magenta) epithelial layers. (C) Side-view schematic showing the perpendicular orientation of myonemes in the endoderm and ectoderm on a cell level. (D–G) Example fluorescence microscopy images of a chemically induced mouth-opening event. The ectodermal cell layer is visible. Scale: 100 microns. (D–G) Reproduced from Carter *et al*. (2016) with permission from authors (26).

cylindrical body column that is a few hundred microns in diameter and a couple of centimeters in length (35). At one end of the body column is an adhesive foot that *Hydra* uses to stick to substrates. At the other end is the head that comprises a conical structure, which is called the hypostome, surrounded by a ring of tentacles (Fig 1A). *Hydra* comprise two epitheliomuscular cell layers, an outer ectoderm and an inner endoderm, connected to each other by an extracellular matrix called the mesoglea (35). Embedded in each epithelial layer is a neuronal network that receives and transmits environmental signals and coordinates behaviors (36). Both epitheliomuscular layers also contain other cell types, such as gland cells (endoderm) and nematocytes (primarily ectoderm) (37). The nematocytes are specialized cells in the tentacles to catch and incapacitate prey. The ectoderm and endoderm (and the other cell types they contain) form continuous cellular sheets. Only when the animal has to ingest, egest, or regulate its osmotic pressure does it rapidly form a temporary opening, the mouth, at the apex of the hypostome (Fig 1A,B). After it is no longer needed, the mouth closes, and the epithelial layers seal back up.

Mouth opening and closing is achieved via contractile forces generated by one-to-two-cell–diameter short filaments called "myonemes" located on the basal side of the epithelial cells (38) (Fig 1C). When stained, myonemes appear arranged as radial spokes that originate in the center of the hypostome in the ectoderm and as concentric circles in the endoderm when looking top-down onto the head (26) (Fig 1B). This arrangement bears similarity to the muscle arrangement in the human iris that controls pupil opening (26); however, it is critical to acknowledge that individual myonemes do not span beyond neighboring cells (Fig 1C); thus, their contractile behavior must be coordinated to achieve organismal-scale mouth opening (33).

After neuronal activation because of environmental chemical signals (e.g., food or certain chemicals, including reduced glutathione and quinine hydrochloride), the ectodermal myonemes in the hypostome contract, causing the mouth opening to form (39–41) (Fig 1D–G). The forces generated by the myonemes necessary to

create the mouth opening act at short range over the millisecond timescale but produce a large symmetric tissue deformation over the seconds timescale, in the absence of centralized neuronal or chemical coordination (33). Mouth opening does not involve any cellular rearrangements; the tissue deformation is accomplished by shape changes of the epithelial cells (26). Thus, mouth opening is a fascinating problem to study—from the biological perspective of control and coordination of behavior and from the physics perspective of large deformations of soft tissue arising from short range, uncoordinated forces.

B. Pedagogical background

This 6-wk laboratory module was developed for a semester-long course in systems biology (maximum enrollment, $n = 24$) taught at Swarthmore College, which is a highly selective, small residential liberal arts college in the suburbs of Philadelphia, Pennsylvania. In 2022/2023, when this laboratory module was taught, the self-reported ethnic and racial identity of the student body at the college was 32% White, 18% Asian, 14% Hispanic, 10% two or more races (non-Hispanic), and 9% Black or African American (42). Students enrolling in systems biology must meet a few prerequisites, including having taken an introductory biology course in molecular and cellular biology (or have advanced-placement credit), an introductory statistics course, and differential calculus. Historically, students enrolling in the course come from different disciplines and with different levels of preparation in relevant science, technology, engineering, and mathematics fields. During the semester in which this module was first implemented, 15 students were enrolled in the course, with most being second-year biology majors (Fig 2A,B). Entering the course, students reported a high level of proficiency in biology, moderate proficiency in math, and relatively low proficiencies in physics and programming (Fig 2C).

The laboratory module was offered in two sections: Section A was aimed at students with prior computational experience, and section B was for students without said experience. Students could self-select during enrollment, but

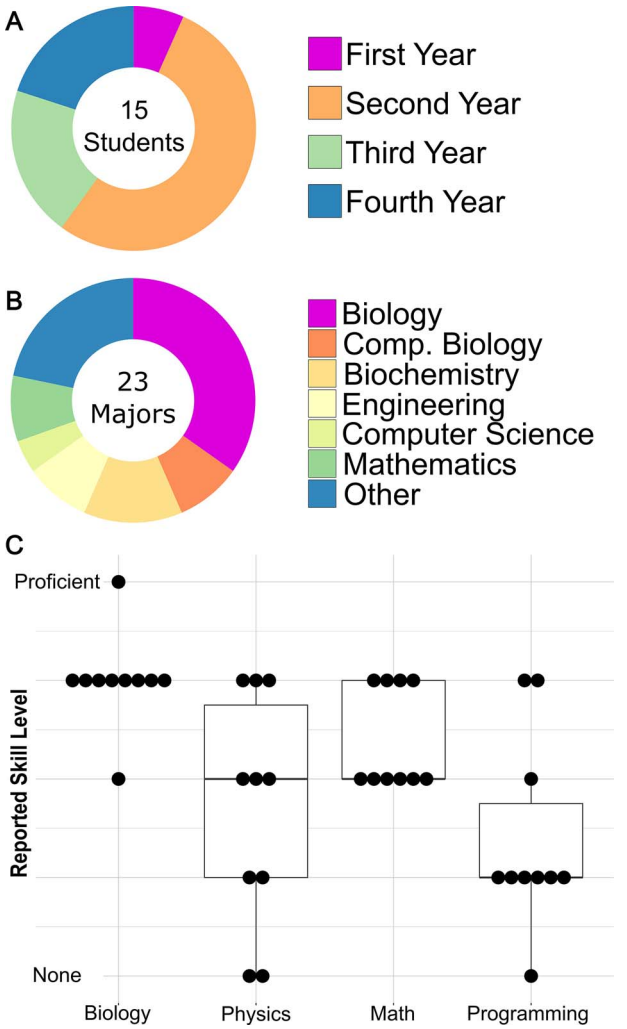

Fig 2. Student demographics and preparation. Breakdown of enrolled students by (A) class year and (B) declared major. Double majors are individually counted, so the total number of majors represented (23) exceeds the enrollment in the course (15). Of the 15 students, only two were not majoring in biology, computational biology, or biochemistry. The Other category includes Spanish, English literature, art, economics, and architecture majors. (C) Student's self-reported proficiency with relevant subject areas before the *Hydra* mouth-opening module ($n = 12$). The vertical axis represents each student's self-assessed proficiency in the given subject area. Each point represents a single student, and the boxes span the 25th and 75th percentile responses.

the faculty member teaching the course would double-check students' prerequisites to ensure appropriate placement. Each laboratory section was provided with the same course materials, allowing students who enrolled in section A to follow the program of section B if they discovered that they were not comfortable with programming on their own. Each laboratory section was

taught by the faculty member and a professional laboratory instructor. Each section met once weekly for 3 h and 15 min. Enrollment in either section was limited to a maximum of 12 students. Students worked in pairs at designated laboratory stations, which were equipped as necessary for the laboratory module. Because the total enrollment in spring 2023 was 15 students, one group consisted of three students.

Most of the students had taken at least one introductory biology course at the college, so they had a basic understanding of laboratory safety. However, because some individuals had not completed biology laboratory safety training, a brief module-specific training was provided to ensure safe working conditions. This training was essential because this module involves working with chemicals, sharps, and biohazardous waste.

An anonymous survey (included as Supplemental Material and reviewed and approved by the Swarthmore College Institutional Review Board [IRB-FY24-25-19]) was administered after course completion to collect students' feedback on the module. The response rate was 67%.

C. Module framework

The overarching goal of this laboratory module is to provide students with an authentic experience of interdisciplinary research. To that end, this module aims to improve student aptitude for performing research with live biological samples, using computation to analyze images from fluorescent microscopy, and reading, writing, and discussing scientific papers. Instead of having an inquiry-based laboratory module wherein students conduct original research, we chose our module on the basis of an undergraduate student first-authored paper. The publication we chose on the biomechanics of *Hydra* mouth opening by Carter *et al.* (2016) (26) is fairly easy to read and does not require a lot of background knowledge in the field, allowing students to connect without having to do much additional reading. To prepare students for the group discussion of the Carter *et al.* (2016) paper (26), the instructor should provide them with some introductory material on mechanics and biology, depending on their level of background preparation. This supplementary instruction will depend on the students' prior coursework in physics and biology but should at a minimum cover a basic description of forces, viscoelastic deformations, and *Hydra* anatomy. Although reproducing published research sacrifices some of the freedom of inquiry-based laboratory modules, it grounds the experience in the context of published research. It also encourages sophisticated reflection and discussion throughout the module by allowing students to compare their data with the published work. We believe three key components are necessary for the success of this kind of laboratory module: framing and metacognition, experimental techniques, and presentation.

During framing and metacognition, students must be encouraged to think about how they might have conducted the research in the paper from the start. This includes engaging students in considering not only the context and the science behind the experiments but also the logistics of the experiment, their ability to analyze the data, and ways of communicating their findings through a scientific paper. Students are asked to read the Carter *et al.* (2016) paper (26) in preparation for this module and to answer a set of reading reflection questions.

The content reflection questions (Table 1) asked students to engage with the scientific ideas presented in the paper. The personal reflection questions asked students to consider their own abilities and confidence in writing a research paper and to brainstorm how they might overcome some of the elements that they might find especially challenging. These reflections are initially individual but are then incorporated into small group discussions and eventually a class-wide discussion. Beginning the entire module with these kinds of prompts encourages students to consider the challenges of writing and publishing academic research and identifies the specific skills that students need to develop to prepare themselves for a research career.

Table 1. Reading reflection questions.

Content reflection	Personal reflection
What were the most significant results or findings?	Knowing your strengths and weaknesses, which parts would be easy for you to write? Which would be especially difficult? Why?
Do you agree with the interpretation of the key results? Why or why not?	Where would you start writing? Which section would you write first?
How did this paper advance the field?	Can you think of strategies to help you write the difficult parts more effectively?

The experimental techniques required for data collection and analysis are examples of current and transferrable skills that may serve students in their future academic and professional lives. This module prepares students to work with aquatic invertebrates, use fluorescence microscopy and video capture, and take measurements from recorded images and video by using computational image analysis. Students must be given ample time to independently engage with every step of the research process to ensure continuity from literature review to data collection and analysis and, eventually, to publication. In the case of *Hydra* mouth opening, this means providing students with the time, materials, and instructions beginning with intact animals, guiding them through sample preparation and image capture, and outlining the necessary steps of image processing (all described in the following section). In addition to ensuring continuity in the students' experience, this approach teaches students skills and techniques that are commonplace in professional research laboratories and provides numerous jumping-off points where motivated or advanced students could extend their analysis to examine the system in more sophisticated ways. In the pilot phase of this laboratory module, we did not explicitly teach students how to maintain a professional laboratory notebook. Best practices for maintaining a laboratory notebook were briefly covered at the beginning of the module, but adherence to these practices was not monitored. We aim to explicitly include teaching this key laboratory skill in future iterations by extending this laboratory module by 1 wk.

Students are asked to present their findings and interpretations in the form of a laboratory report mirroring a scientific publication. We used the short paper format of *microPublication* (43) because of the limited time available for the module (6 wk) and because this course is not a formal writing course. Students were provided example papers from this publisher to have a framework for their own writing and received general guidelines about scientific writing and figure creating. One could easily expand the writing portion of this laboratory and follow a different publication format, extending it into a more intensive, multiweek experience. A critical feature of the writing is that students give and receive peer reviews on their reports to mimic the process of scientific publication. Students then get to incorporate reviewer feedback in generating their final reports. To incentivize students to provide considerate and serious comments on the reports they review, a small portion of each student's grade for the module is based on the level of detail and thoughtfulness of the comments they produce for their peers. This process, of discussion, drafting, feedback, and revision is at the core of the endeavor to publish scientific research.

In summary, the four key components of the laboratory module address basic training that all undergraduate students majoring in the natural sciences should obtain: (a) critically reading a scientific paper, (b) planning and performing an experiment and reconciling results with the published literature, (c) interpreting and presenting results, and (d) writing and peer reviewing scientific papers.

III. MATERIALS AND METHODS

After students have engaged with the primary literature and discussed the experimental protocol, it is useful to break the experiment down into stages, including preparing samples,

mounting samples for imaging, and acquiring and analyzing images. It may also be interesting to students to learn about *Hydra* care and feeding. We briefly cover key aspects of *Hydra* care here and then emphasize steps from the experiment that present learning opportunities for students and highlight possible modifications to suit specific teaching contexts. Protocols for the experimental stages and details about additional resources are provided in the laboratory handout (Supplemental Material). Required materials for implementation are listed in Table 2. Approval for use of human subjects for the post-course survey was obtained from the Institutional Review Board (IRB-FY24-25-19) of Swarthmore College.

A. *Hydra* care

A detailed open-source protocol covers all the essential aspects of *Hydra* care (28). *Hydra* cultures can be maintained in the laboratory by using spring water in food-safe glass or plastic containers at room temperature. Feeding *Hydra* requires live prey, typically brine shrimp (*Artemia nauplii*), which can be grown as needed in the laboratory, as described in Hyland and DeSantis (2022) (28), or purchased live from commercial suppliers (e.g., Instant Baby brine shrimp; Ocean Nutrition, Newark, CA). Commercial brine shrimp can be stored in the refrigerator and used over several weeks. Because brine shrimp live in salt water, they are rinsed with fresh water before adding them to the *Hydra* culture. Feeding is quick and generally completed in the morning to ideally allow for two cleanings in the day. The first cleaning (recommended but not required) happens ∼30–60 min after feeding to remove uneaten prey; the second cleaning (required) takes place 6–8 h later to remove waste material. Feeding once per week is sufficient to maintain a population, whereas growing the population requires more frequent feeding (≥3 d per wk). Because the experiments use only heads and not the whole animals, the body columns can be allowed to regenerate over the course of a few days and then reintroduced to the culture, thus keeping numbers steady.

B. Sample preparation

Students were provided transgenic watermelon (WM) or tricolor *Hydra* (30, 44) and the materials necessary for sample preparation at their workstation (Table 2). Students were provided with 1 mM linalool (anesthetic solution; Sigma-Aldrich, Burlington, MA, catalog number L2602-100G) and 0.5 mM and 1 mM quinine hydrochloride (stimulant that causes mouth opening; Sigma-Aldrich, Burlington, MA, catalog number 22630-10G-F) solutions (32, 33). Students were required to read the safety data sheet and protocols for usage of these chemicals before starting any experiments. Both chemicals are light sensitive and must be kept away from light; additionally, both chemicals are combustible and skin irritants. Therefore, it is critical to wear proper personal protective equipment while executing the experiments and disposing of unused chemicals and contaminated solid materials in the appropriate hazardous waste containers. This provides an opportunity to explain to students why it is indispensable to read protocols and material safety data sheets as part of laboratory preparation. Transgenic *Hydra* are considered biohazardous materials and must be handled and discarded by following state and federal regulations.

If transgenic animals cannot be obtained or circumstances do not allow for work with transgenic animals, brown or green *Hydra* can be obtained commercially. Heads from commercial animals can be labeled with a solution of 1:1,000 (wt:vol) 1-aminoanthracene (Sigma Aldrich, Burlington, MA, catalog number A38606) as described in Goel *et al.* (2024), providing temporary green, fluorescent tissue labeling that can be imaged as described for WM *Hydra* (33). However, the fluorescent signal is weaker and thus may be more difficult to detect, depending on the camera specifications. Cell outlines are less well defined, and thus cell-shape analysis would not be feasible. We provide a sample movie and analyzed data of a stained nontransgenic *Hydra* in the Supplemental Material for comparison.

Students used linalool to anesthetize the *Hydra* before decapitating them and then waited for them to recover from anesthesia

Table 2. Overview of components used in the pilot implementation of the *Hydra* laboratory module. Equivalent items from other vendors can be used instead. The indicated quantities are listed for a single experimental station. In our case, students worked in pairs at each station.

Item	Vendor	Part number	Quantity
Nikon Ci-L compound light microscope	Nikon	Ci-L	1
EGFP/FITC/Cy2/Alexa Fluor 488 fluorescent filter cube[a]	Nikon	96226	1
K-Cite 120 LED-Mini fluorescent source	Nikon	This item has been discontinued and replaced by K-Cite 120 LED-Mini + fluorescent source	1
FLIR Flea3 USB camera	Edmund Optics	FL3-U3-13E4M-C	1
Dissection microscope (magnification range 6.4×–40×) with reflected and transmitted illumination modes	Leica WILD	M3C	1
Plastic Petri dish (100 mm; one with untreated *Hydra* and one for body column recovery), each filled with 20 ml of *Hydra* medium	Fisher Scientific	FB0875713	2
Glass Petri dish (60 mm) for linalool exposure and cutting	Corning	70165-60	1
20–200-µl pipettor	Sigma-Aldrich	CLS4074	1
Box of 200-µl pipette tips	USA Scientific	1120-8810	1
Dumont style 5 tweezers	Electron Microscopy Services	72700-D	1
Hair Loop[b]			1
Scalpel (#10 blade)	Fisher Scientific	50-109-4381	1
Glass Pasteur pipettes	Avantor	14673-043	2
Pasteur pipette bulbs	Sigma-Aldrich	Z111597-12EA	1
Glass microscope slides	Corning	2949-75X25	1 box
Glass coverslips (22 × 22 mm)	Corning	2845-22	1 box
Double-sided tape	Amazon Basics	00811540031016	1 roll per group
Micrometer	Meiji Techno	MA285	1 for the class
Kimwipes	Fisher Scientific	06-666A	1 box per group
50-ml centrifuge tube (for *Hydra* medium)	Fisher Scientific	14 959 49A	1
15-ml centrifuge tube (for linalool)	Fisher Scientific	12-565-269	1
Aluminum foil	Amazon Basics	131926	to wrap tubes
Transgenic *Hydra vulgaris* (watermelon or tricolor strain)[c]	OpenHydra	https://openhydra.org/strains-database/	10 animals/group/session
Quinine hydrochloride (0.5 mM and 1 mM)[d]	Sigma-Aldrich	Q1125	500 µl each
Hydra medium[e]		https://openhydra.org/	40 ml
Linalool (1 mM)[d]	Sigma-Aldrich	L2602	10 ml
Computer (PC or Mac) capable of running image processing and data-analysis software	-	No specific vendor; minimum 8 GB of RAM	1
Image-processing software	Fiji or ImageJ	https://imagej.net/software/fiji/downloads	1
Data-analysis software	Python or MATLAB	https://www.python.org/downloads/ or https://www.mathworks.com	1
Waste collection[f]	-	Biohazardous sharps and non-sharps; hazardous chemical waste, as per state and federal guidelines	1 each

[a] EGFP, enhanced green fluorescent protein; FITC, fluorescein isothiocyanate; LED, light-emitting diode; PC, personal computer; RAM, random access memory; USB, Universal Serial Bus.
[b] See the Supplemental Material for protocol for making a Hair Loop.
[c] Transgenic animals are not commercially available but can be requested from an established *Hydra* researcher. A list of available transgenic lines and researcher contacts can be found at https://openhydra.org/strains-database/. Transgenic *Hydra* are biohazardous and must be handled by following all state and federal regulations. If transgenic animals are not available, commercial wildtype *Hydra* can be purchased from various vendors and live stained, as described in Goel *et al*. (2024) (33).
[d] Both reagents are dissolved in *Hydra* medium.
[e] For materials and preparation instructions, *Hydra* feeding and maintenance, refer to https://openhydra.org/wp-content/uploads/2019/09/Hydra_Culturing_Protocol.pdf. Deionized water can be used instead of Milli-Q water.
[f] All glass, pipette tips, and scalpels should be disposed of in sharps waste.

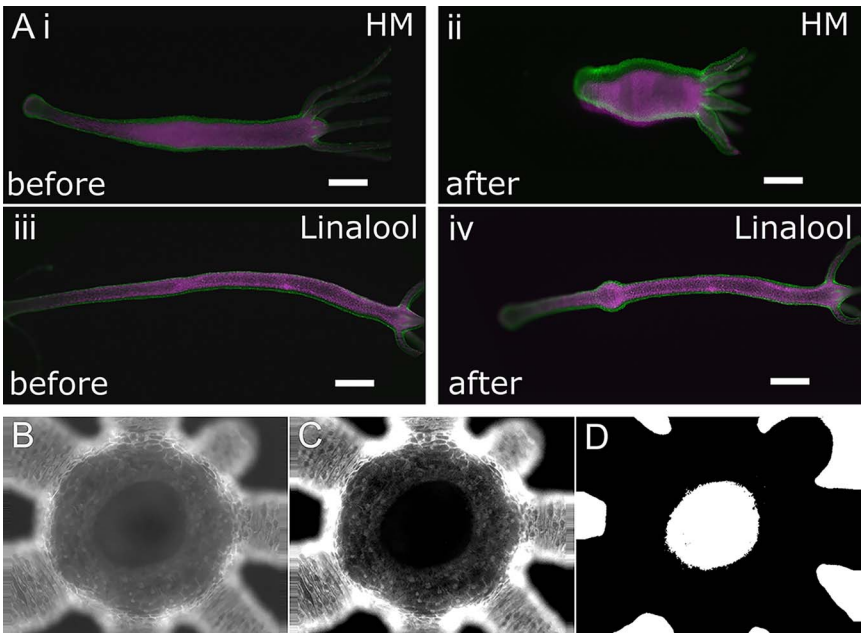

Fig 3. Methods for studying mouth opening. (A) The *Hydra* pinch response is used to test when the anesthetic (linalool) is effective or has worn off. (i) An untreated, elongated *Hydra* in Hydra medium before pinching with forceps near the foot. (ii) The animal contracts when pinched with forceps. Due to the time delay from filter switching (0.6 s), a slight mismatch of the tissue layers occurs. (iii, iv) A *Hydra* incubated in 1 mM linalool for 15 min before (iii) and after (iv) pinching near the foot. The pinch-induced contraction is local (bulging), and no global contraction is observed. Scale bars: 300 microns. (B–D) Illustration of the basic image-analysis steps to isolate the mouth from the image by using image contrast enhancement and thresholding. (B) Raw data, (C) contrast enhanced image, and (D) thresholded image.

before they mounted the *Hydra* heads for mouth-opening experiments. The decapitation step can also be performed without linalool treatment. However, the linalool treatment relaxes the *Hydra*, making it easier to obtain head samples without excessive body column tissue. Too much body column tissue makes it difficult to orient the *Hydra* head for top-down imaging. It is difficult to know when linalool has taken effect and when it has worn off by simply looking at the animals. Because body columns react differently to being pinched by a pair of forceps when they are anesthetized compared with when they are not, the pinch response test can be used to evaluate the anesthesia (Fig 3A). Untreated and fully recovered body columns contract globally in response to a pinch at the lower part of the body column, whereas anesthetized *Hydra* contract only locally (32) (Fig 3A). Students should be encouraged to recognize that if they did not have the pinch test on the intact *Hydra* as a readout, it would be difficult to determine when the effect of the linalool had worn off, and consequently the linalool may affect mouth opening. Thus, this is an opportunity to teach students about the need for designing appropriate controls or markers for whatever treatment they use in experiments.

C. Data collection

Students were given a demonstration on how to use the fluorescence microscope and collect data by using a camera mounted on the microscope by one of the instructors. Depending on the level of familiarity students have with fluorescence microscopy, instructors can decide what level of detail to share with the students about the physics behind fluorescence, how it is used as a tool in biology, and the optics behind fluorescence imaging. Data collection presents an opportunity to discuss the trade-off between collecting data at high temporal resolution versus the storage space available. There are two major limitations to collecting high-resolution data: physical storage and computing power to process large, single videos at a time. It is also valuable to emphasize how imaging the micrometer (or some object of known size) is key to get the scale factor of the microscope at the given magnification.

Students in both laboratory sections were provided a tutorial on how to use ImageJ (National Institutes of Health, Bethesda, MD) to extract the *Hydra* mouth areas from the image data, plot the mouth area as a function of time in MATLAB (version 2016a; Mathworks, Natick,

MA), and use the MATLAB curve-fitting toolbox to fit the mouth area curves to the logistic model (Eq. 1) that was provided in the Carter *et al*. (2016) paper (26) (Fig 3B–D). Students with prior computational experience in section A were provided with the main steps for analyzing the images but were expected to write their own code to do so. However, all students were introduced to ImageJ (34, 35), a freely available graphical user interface–based software commonly used for image analysis in biological and medical contexts, and students in section A could choose to follow the same handout as students in section B.

The instructors explained to students how digital images can be treated as matrices of numbers, how to manipulate image brightness and contrast, and how to use binary thresholding to isolate image features (Fig 3B–D). Our students used the MATLAB curve-fitting toolbox to fit the data to the model, but the analysis could also be performed by using Python (Python Software Foundation, Wilmington, DE). The Supplemental Material explains how to perform the curve fitting in MATLAB and Python and provides a scaffold Python code that could be given to students with limited experience. Depending on availability of time and prior student knowledge, one might want to discuss curve-fitting methods more broadly.

To increase the quality and size of students' final dataset without dramatically increasing the time needed for the module, the laboratory instructor prepared and mounted samples ahead of time for the subsequent sessions in weeks 3 and 4 (after all groups had prepared their own samples in the second week) so that students could focus on imaging and collecting data. The third and fourth weeks of the module were dedicated to the collection of more polished data, which was shared among the whole class to increase the collective sample size. Each student then analyzed the entire class's dataset independently.

D. Presentation

Students were provided with resources on how to present experimental data, create figures, and draft a report that contained their findings, comparisons to the literature, and their interpretation of the data. Although the rest of the laboratory module was a group effort, students were required to complete this last part individually. After students had drafted their reports, they were asked to review the work of two of their peers. Peer review was conducted in a double-blind manner. We first discussed how to peer-review others' work, emphasizing the significance of being thorough, compassionate, and constructive. 10% of the laboratory module's participation points were based on peer reviewing other students' reports. In the Supplemental Material, we provide a summary of how points were assigned to the different laboratory module components. The students then received their two reviews so that they could incorporate the feedback and submit a final report. This exercise accomplishes several goals. It exposes students to the peer-review process and helps them learn both how to provide and how to receive feedback. It gives students a chance to think critically about material they are reading. It also exposes students to different ways of presenting and interpreting data.

IV. RESULTS AND DISCUSSION

In this section, we describe essential experimental parameters, expected results that students should obtain from the experiment, some fruitful points of comparison with published findings (26), and some common points of struggle that students encountered during the module. We also discuss the results of the survey, investigating changes in students' self-reported comfort and confidence across the module to assess the efficacy of this laboratory module in achieving its goals.

A. Experimental results

On the basis of the final written reports, students largely confirmed the observations in Carter *et al.* (2016) that claimed the rate of mouth opening is consistent among *Hydra* despite variation in maximum mouth area between individuals (26). Students were able to successfully fit

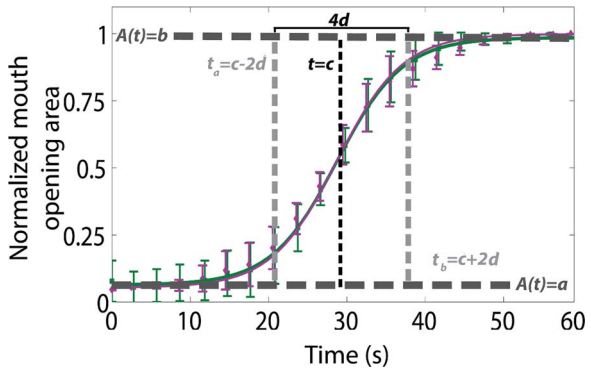

Fig 4. Definition of fit parameters. The normalized mouth opening area ($A(t)$) was fit according to Eq. 1. Data show mean and standard deviations of the mouth opening for the two epithelial layers, with ectoderm in green and endoderm in magenta. Figure adapted from Carter *et al*. (2016) with permission from authors (26).

their recorded data to Eq. 1, the modified logistic equation, where $A(t)$ is the normalized area of the mouth as a function of time, t is the time from the initiation of the opening process, a is the lower asymptote, b is the upper asymptote, c is the inflection point, and d is the rate of mouth opening (26), with R^2 values > 0.90:

$$A(t) = a + \frac{b}{1 + e^{\left(\frac{-(t-c)}{d}\right)}} \quad (1)$$

In Eq. 1, a, b, and c are experimentally constrained and correspond with the initial mouth-opening area, maximum mouth-opening area, and time at which the mouth-opening area is 50% of the maximum, respectively. After curves are normalized to the maximum opening, a is expected to be equal to 0 and b to be equal to 1. Parameter d is related to the length of time in which most of the mouth opening occurs and is inversely proportional to the rate of opening (Fig 4). Thus, faster openings correspond to smaller d values.

Students found that their raw data collapsed to the characteristic sigmoidal shape after normalizing the opening area by the maximum area for each opening sequence, as observed in the published paper (Fig 5A,B). For some data, the mouth opening was more gradual, wherein the mouth first opened a small amount and then paused before opening wider. This can be seen in Figure 5A, where one of the curves is longer and has a bump before the typical S-shaped curve. For data that were suboptimal, students truncated the data as needed and set a to 0 and b to 1 instead of having them be fit parameters to improve the quality of the fit.

An example of student-reported d values based on the class data for individual mouth openings within a 95% confidence interval is (1.86, 4.94; $n = 5$), which was much wider than the 95% confidence interval of (4.00, 4.40; $n = 19$) reported in Carter *et al*. (2016) (26). However, the confidence intervals for the rate of mouth opening between student recordings and published data overlapped. Justifications for inconsistencies between the observed and published data included the following: differences in *Hydra* strains (Carter *et al*. (2016) used

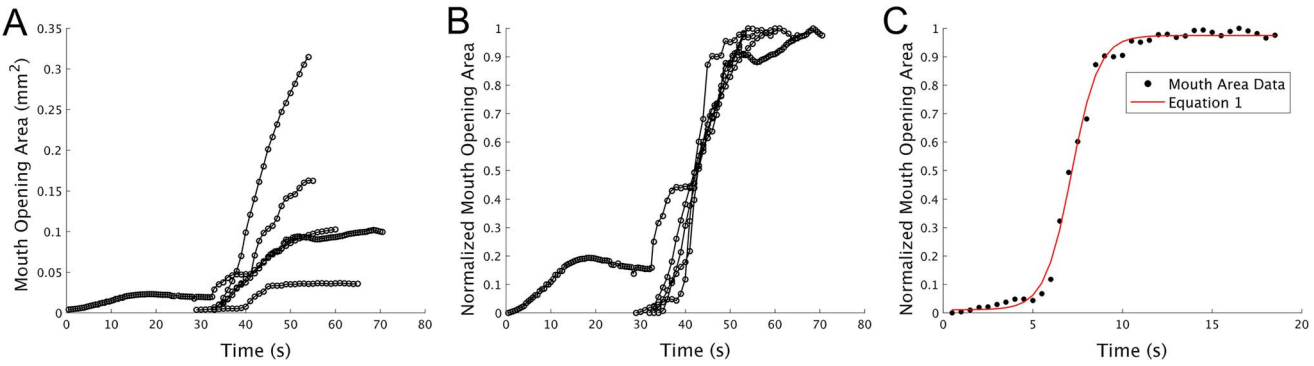

Fig 5. Example data of *Hydra* mouth-opening dynamics from a student generated by using MATLAB. (A) Raw ($n = 5$) data aligned at the point in time at which the mouth opening reached 50% of its maximum value. (B) Normalized mouth-opening data. (C) Student-generated normalized *Hydra* mouth-opening curve fitted to a modified logistic equation (Eq. 1). The data follow the expected S-shape for mouth opening. Because students independently decided the frame rate of their recordings, data were recorded at either 1 or 2 frames per s. Mouth area cannot be detected before opening, so recordings do not start at 0 s.

WM *Hydra* (26), whereas students used both WM and tricolor *Hydra* (30) due to animal availability) and differences in using spontaneous versus chemically induced mouth opening and type of inducer. Students used primarily quinine hydrochloride to induce the *Hydra* feeding response and observed only a few spontaneous openings, whereas Carter *et al*. (2016) analyzed primarily spontaneous openings and some that were induced by using reduced glutathione (26).

Common challenges students faced during their data analysis included using pooled image data from the class that had inconsistent or missing labels. Some students were unsure of their videos' frame rate, which would affect the *d* value they calculated. This taught the students the value of standardized naming conventions and detailed notes on experiments. Overall, students largely suggested that their observed chemically induced mouth opening occurred at a faster rate than the spontaneous mouth openings used in Carter *et al*. (2016) (26).

B. Student confidence and comfort

This module aimed to improve student aptitude for performing authentic interdisciplinary research by providing experience with live biological samples, fluorescent microscopy, image analysis, and computation and with reading, writing, and discussing scientific papers. The results of the student survey show that the module was successful in increasing student comfort and confidence with these areas. Figure 6A shows the students' self-reported comfort with various skills aligned with these goals and how this comfort changed after the completion of the module. The elements that showed the greatest increase in student comfort (i.e., working with live animals, fluorescent microscopy, image analysis, programming, discussing scientific papers, and writing scientific papers) were the six elements for which students' premodule comfort was the lowest. We see the greatest increase in comfort (and the second lowest initial comfort) with image analysis. We speculate that these gains may be attributed to the inclusion of authentic and interdisciplinary content and to the inquiry-based approach to data analysis, which allowed students to more thoroughly engage with the computational techniques at hand. We also note that programming shows the lowest initial level of comfort, reflecting the low incoming proficiency that students reported with programming, suggesting that their lack of comfort is correlated with a lack of training and exposure (Fig 2C). This result supports the claim that this module addresses shortcomings in the traditional laboratory curriculum by building comfort in categories where the students were initially most apprehensive and inexperienced.

To control for different initial comfort levels, we also examined the normalized gain for each element (Supplemental Table S1), which once again is the highest for image analysis, followed by working with live animals and interdisciplinary research (notably the element with the highest initial comfort). This shows that the module is also effective at amplifying students' existing interests, as well as increasing their comfort with elements where they initially lack confidence.

Although students reported higher levels of comfort with reading scientific papers than with writing or discussing them (2.9 versus 2.6 and 2.6, respectively) before the module, their reported levels of comfort after the module were essentially equal (3.4 for reading and 3.5 for writing and discussing). This result, coupled with the substantial increases in comfort with discussing and writing scientific papers, suggests that the module meaningfully improves student comfort with writing scientific papers. Additional research is required to identify how each of the individual writing-based reforms in this module affected student comfort and could possibly be achieved by interviewing students as to what they think this could be attributed.

Students were also asked about how their confidence with the learning goals of this module changed (Fig 6B). All student respondents to the survey ($n = 10$) reported that this module made them more confident in their ability to read and discuss scientific papers and to accomplish interdisciplinary research, and most students reported

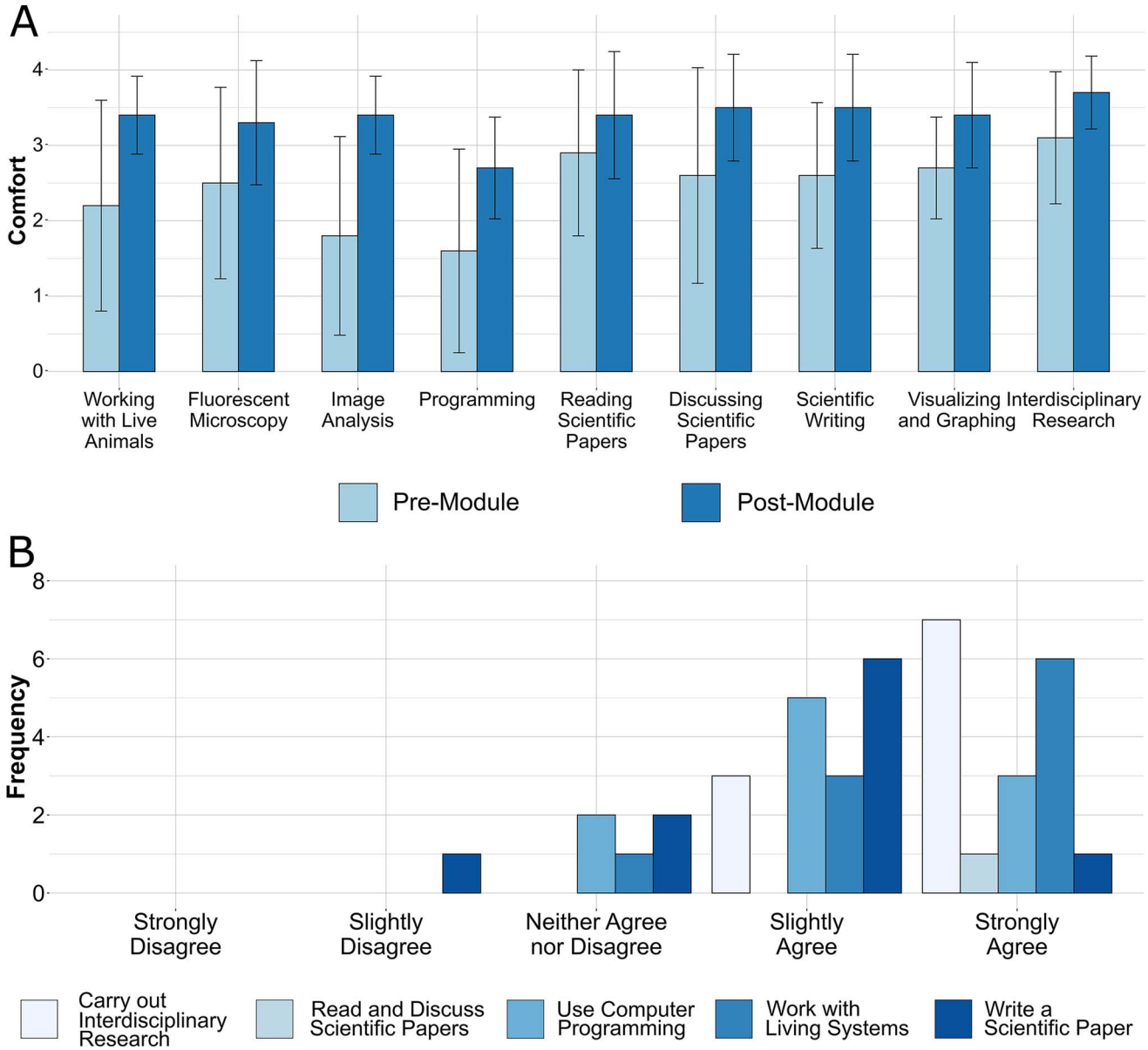

Fig 6. Self-reported comfort and confidence with laboratory module skills ($n = 10$). (A) Student-reported comfort with various elements of the laboratory experience before (light bars) and after (dark bars) the *Hydra* mouth-opening module. The score on the vertical axis represents an average comfort level, where 0 means Very Uncomfortable and 4 means Very Comfortable. Error bars represent the standard deviation. (B) Stacked histograms of students' agreement with the statement "The *Hydra* mouth-opening module made me more confident in my ability to [blank]."

confidence gains for the three other learning goals. Although no direct assessment of student aptitude with each learning goal before and after the module was made, the marked increase in self-reported confidence shows the value of this module to empower students to engage in the research process. Moreover, 60% of students surveyed found the fact that the central paper was first-authored by an undergraduate to be inspiring, citing it as a source of engagement, interest, and confidence. Specifically, in their open-ended response, one student said that "the *Hydra* mouth opening paper was inspiring because it served as an example of an undergraduate student (like myself and my classmates) having the opportunity and skills to make scientific contributions." Another student said that "the fact that an undergraduate student wrote the paper made me

more interested and engaged. It made me feel inspired and like I could be capable of doing this unit."

C. Optional advanced exercises: curve fitting and viscoelastic models of biological tissues

In our implementation of the laboratory module, students with a background in programming were encouraged to write their own code to analyze the image data. Building on this first step, one could expand this laboratory module to teach classical image processing, including denoising images, thresholding binary images, and segmentation. Students can then try extracting features of the mouth shape beyond its area, such as symmetry and circularity. Alternatively, students who do not have a background in programming can learn these same operations in ImageJ. They can further try to build an automated pipeline for image processing by using the Record feature in ImageJ that generates ImageJ macro code based on Java language for the steps students perform by using the ImageJ graphical user interface. The students can then make minor tweaks to the generated code and directly run it in ImageJ to analyze mouth-opening movies.

For a more biophysical focus, students could be introduced to the concept of viscoelasticity in tissues and try to extract tissue relaxation times by fitting the latter half of the mouth-opening area curve to an exponential function, as in the Carter *et al*. (2016) paper (26). To further explore the mechanical behavior of viscoelastic tissues in response to forces, students could be asked to simulate a single-spring dashpot system subject to an external force. Students can start by writing down the equation of motion for a spring dashpot system subject to an external force. They can then use numerical ordinary differential equation solvers to observe how the spring dashpot system deforms in response to a constant external force or a sharp, short-lived impulse or kick. In both cases, students should be encouraged to analyze the qualitative response of the system to these forces and test how changing the spring stiffness and/or dashpot viscosity affects the response time of the system. This provides a useful opportunity to introduce the notion of viscoelastic relaxation time. Then, students can be asked to simulate other external force profiles, such as sinusoidal forces or exponentially decaying forces, and then analyze the response of the system. Links should be made to how the spring dashpot system models soft tissue and the different force profiles model a range of mechanical conditions that different soft tissue experience. Students can be encouraged to think about what kinds of force profiles might generate a deformation similar to what they observe in mouth opening. Students can also try to further expand first to a linear chain of spring dashpots and then to a two-dimensional network (e.g., (33)). Note that these exercises can become a semester-long stand-alone module on modeling biological systems.

V. CONCLUSION

This undergraduate *Hydra* mouth-opening biomechanics laboratory module teaches fundamental skills, such as time-lapse microscopy, image analysis, programming, critical reading of scientific literature, and basics of scientific writing and peer review. By using a research paper first-authored by an undergraduate student as the basis of this module, undergraduates can identify with the research and feel empowered and are thus motivated to determine how to do the research themselves. By offering two sections with different computational requirements, the module is broadly accessible to all students with introductory level biology and some classical mechanics knowledge. The additional exercises and suggestions for learning opportunities that we discuss can be expanded to stretch this module to more weeks or to engage more advanced students.

SUPPLEMENTAL MATERIAL

All Supplemental Material is available at: https://doi.org/10.35459/tbp.2024.000285.

AUTHOR CONTRIBUTIONS

E-MSC developed the laboratory module and designed and executed its implementation in the pilot described herein. JP

was a student participant and produced the sample data shown in the results. SH and E-MSC developed the student survey, and E-MSC administered the survey. SH and E-MSC analyzed survey results. TG and EMSC designed the advanced exercises. SH, TG, and E-MSC wrote the initial draft of the manuscript. SH, TG, and JP generated the figures. All authors edited the final draft.

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