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Ethics of biohybrid robotic jellyfish modification and invertebrate research

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ABSTRACT

The ethics of invertebrate research have largely been ignored compared to the consideration of higher order animals, but more recent focus has questioned this trend. Using biohybrid robotic jellyfish as a case study, we examine the ethical considerations of invertebrate work and provide recommendations for future guidelines. This paper starts with an overview of philosophical views of animal ethics, the current state of knowledge for invertebrate pain and nociception, and current ethical guidelines. Next, we delve into the case study and analogous precedents. Specifically, in prior studies, we developed biohybrid robotic jellyfish, which modified live moon jellyfish with microelectronic swim controllers for future applications in ocean monitoring. Although jellyfish possess no central nervous system, pain receptors, or nociceptors, we closely monitored their stress responses, using the precautionary and minimization principles in consideration of the 4Rs: reduction, replacement, refinement, and reproducibility. We also discuss ethical considerations related to our studies and suggest that public opinion of invertebrate research relies heavily on repugnance, including fears of 'playing God' or limiting the 'free will' of animals. These issues are also examined for prior bioethics cases, such as the RoboRoach, cyborg beetle, 'microslavery' of microbes, biohybrid robots incorporating tissues from sea slugs (which are known to possess nociceptors), and other tissue cutting experiments involving soft-bodied invertebrates. However, biohybrid robotic jellyfish pose further ethical questions of potential ecological consequences as ocean monitoring tools, such as the impact of electronic waste in the ocean. To conclude these evaluations, we recommend that publishers require brief ethical statements for invertebrate research, which can include the following: a scientific justification for the research, discussion of the 4Rs, and cost-benefit analysis. We also delineate the need for more research on pain and nociception in invertebrates, which can then be used to revise or validate current research standards. These actions provide a stronger basis for the ethical study of invertebrate species, with implications for individual, species-wide, and ecological impacts on animals, as well as for interdisciplinary studies in science, engineering, and philosophy.

KEYWORDS

Ethics, jellyfish, biohybrid robot, modification, enhancement

INTRODUCTION

Although invertebrates constitute over 96% of species in the animal kingdom [1] and are widely used in scientific literature, the ethics of invertebrate research have largely been overshadowed by the focus on mammalian ethics [2]. Most traditional arguments for and against animal research in medical, scientific, or commercial testing do not apply to invertebrates [3]. Invertebrate research is often justified as a more ethical alternative to vertebrate experiments, but more recent scrutiny has questioned this claim [2, 4–7].

To examine and address the ethics of invertebrate research, this paper will use our scientific research with biohybrid robotic jellyfish as a case study, in which natural *Aurelia aurita* are modified using microelectronic systems. The introduction will outline the general philosophical views of animal ethics, pain and nociception in invertebrates, and purposes of the Institutional Animal Care and Use Committee (IACUC), as well as U.S. and international ethical guidelines. Next, the ethical considerations of jellyfish modification will be described, with examples of analogous work in invertebrate literature, followed by the issues posed for this case study on an individual, species, and ecological level. The last section will prescribe recommendations for future work on biohybrid robotic jellyfish and general invertebrate research. We conclude that there is a need for more widespread ethical statements in publications using invertebrate animals and advocate for more invertebrate pain research to refine future ethical guidelines.

Philosophical views of animal ethics

Prominent philosophical views on animal ethics include contractarian or Kantian views, based on anthropocentrism; utilitarian views, based on the best interests of all (including animals) to produce the most good; rights-based or deontological views, based on extending individual rights to animals; and Neo-Aristotelian views, based on animal capability and function [8]. Nussbaum summarizes and critiques these four views in detail [8]. In brief, the contractarian or

Kantian view holds that humans have only indirect moral obligations to animals, insofar as animals can improve the wellbeing of human beings. This ethical framework assumes that the sole value of animals is through their relationships to humans. An anthropocentric view of this kind can still advocate for limited animal welfare in service of human needs and their moral sensibilities, but it fundamentally denies animals any dignity or moral standing. Utilitarian views, in contrast, value the actions that result in the best overall consequences, summing across all individuals. In practice, utilitarianism still favors humans and higher order non-human animals, suggesting that animal breeding for livestock, painless animal death, and other actions are ethically permissible. Right-based (deontological) views offer the alternative perspective that all individual animals have moral rights, regardless of sentience and other psycho-physical characteristics. Finally, Neo-Aristotelian views center on virtue ethics, which values the capability and function of individuals. Aside from the contractarian view, which denies that animals have moral standing, each of these ethical views postulates specific criteria for the moral treatment of animal that we can consider [4, 8]. Furthermore, Neo-Aristotelian virtue ethics draws from both utilitarian and right-based arguments, which is why Mather focuses on utilitarianism and right-based ethics for invertebrates [4].

Utilitarian and rights-based ethical views allow considerations toward invertebrates in two different approaches. Because the former is a holistic stance, utilitarianism primarily regards invertebrates for their ecological value, biodiversity, and other broad impacts. In contrast, the rights-based viewpoint emphasizes individual animals and their wellbeing [4]. These differences aside, both views have problems evaluating the pain and suffering of invertebrates, using physiological responses to stress in disparate animal species as a proxy for pain. This is a issue given that the concepts of pain and nociception, as expounded below, are central to studies of invertebrate ethics.

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Pain and nociception

Pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage," according to the International Association for the Study of Pain (IASP) in its 2020 revision [9]. Although the emotional component highlights an anthropocentric view of pain, this definition is used in the context of both healthcare and pain research, which includes animal studies.

In comparison, nociception is the "physiological response to noxious stimuli that cause or potentially cause tissue damage" [10]. This definition excludes subjective experiences, such as 'unpleasant' sensations or emotions. Sensory receptors known as nociceptors can objectively be examined.

Pain is also considered a central, not peripheral, phenomenon that suggests the need for a brain or centralized nervous system, and that possessing some level of sentience is a necessary condition of experiencing pain [11]. For example, Braithewaite suggests that fish do feel pain, citing the parallel brain development of both fish and mammals [12]. But this has been debated among neuroscientists, who suggest that pain is defined as a product of cortical regions of the mammalian brain and that non-mammalian pain is an anthropomorphic fallacy [13, 14].

Thus, there is a distinction between nociception as sensory information and pain as perception [15]. Non-human pain studies often focus on nociception as a proxy; although it should be noted that nociceptors are nerves that detect noxious stimuli and report information about the state of the tissue, not information about pain [5]. Nociception can activate sensory and motor pathways, including reflexive behavior responses that can, but do not guarantee, higher order responses [5].

Although pain and nociception are understudied in both invertebrates and aquatic animals in general, some results of nociception research in underwater species have been reported [16].

Nociceptors have been found in the sea slug species *Aplysia californica*, which suggests the conservation of sensory responses between mollusks and mammals [17]. Recent work has also discovered nociceptors in cephalopods, which comprise species of cuttlefish, nautilus, octopus, and squid [5]. These observations demonstrate that noxious stimuli can induce reflexive behaviors in aquatic invertebrates, including withdrawing individual body parts, inducing escape behaviors, and reducing feeding [18, 19].

Although there is a lack of evidence that nociceptors exist in most invertebrates, nociception can be potentially considered an evolutionarily conserved mechanism for animals to interact with the environment. Minimal evidence has shown the existence of nociceptors or even nociceptive responses in lower order animals until the evolution of bilateral symmetry, beginning with annelids [20, 21]. Although invertebrate studies do exist, there is a notable lack of invertebrate pain research to understand the evolution of nociception, compared to other animal models [22].

A better understanding of nociception and its modulation has potential for improved understanding and mitigation of pain and discomfort. Burrell suggests that invertebrates have been underutilized in nociception research despite clear advantages, whereas the detailed characterization of many invertebrate nervous systems and electrophysiology methods that allow individual neurons to be recorded and/or manipulated allows greater insights into nociception research [23].

In spite of lack of evidence of true nociceptors in most invertebrates, many ethicists cite the precautionary principle, or to err on the side of precaution in ethically fuzzy situations. Specifically, researchers should err on the side of compassion by assuming that animals feel pain, if there is any possibility of inducing pain or ethical cruelty [24]. This also ties into the minimization principle, according to which researchers should minimize any pain or harm to the animals, although there remain debates about what minimization truly entails [25], as described in the 'Welfare Interests' subsection of the case study. Therefore, in the absence of clear evidence of pain, proxies such as stress markers or escape responses must be monitored.

Institutional Animal Care and Use Committee (IACUC) and animal welfare guidelines

To ensure animal welfare in research, ethical guidelines exist with recommendations from expert committees and organizations. In the United States, each university's IACUC acts to oversee its animal care and use programs, as described in the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals and the *Guide for the Care and Use of Laboratory Animals*, from the Office of Laboratory Animal Welfare (OLAW) of the National Institutes of Health (NIH) [26–28]. Its overarching goals are to assess and recommend the institution's humane care and use of animals, animal facilities, personnel training; and review and approve animal protocols [26]. Specifically, the IACUC reviews animal use protocols and grants approval prior to such experiments, either by a full committee review or designated member review. Such reviews can offer recommendations or revisions before approval, and experiments are monitored afterwards to ensure proper handling. Major modifications to protocols must be approved separately, and the IACUC must address animal welfare concerns and suspend animal activities if such animal rights are abused [26, 28].

The IACUC serves as an invaluable resource to ensure the humane care and use of animal research subjects, with perspectives from veterinarians, animal scientists, and ethicists or other nonscientific members concerned about welfare. This allows a diverse set of considerations outside of the animal researchers' scientific expertise. However, the IACUC does not protect lower order animals, including all invertebrate animals except cephalopods [29–31]. Even the IACUC's guidelines on cephalopods are defined as recommendations, without required regulations or enforcements for oversight [32]. This means that the IACUC requires knowledge of invertebrate research without formal protocol submissions, which allows cephalopod research to remain unregulated in the U.S. [2].

In contrast, the European Union (E.U.) and other countries do have protective legislation for some invertebrates, as delineated by Browman *et al.* [33]. These include protections to cephalopods in Australia and the E.U.; cephalopods and "some other higher invertebrates" in Canada; octopus, squid, crab, lobster, and crayfish in New Zealand; squid, octopus, decapod crustaceans, and honeybees in Norway; and cephalopods and decapod crustaceans in Switzerland [33]. To highlight one example, the Australian Code of Practice examines four aspects of animal research: well-being, stress, distress, and pain [34]. Although behavioral displays in animals are subject to human interpretation, the National Health and Medical Research Council (NHMRC) revised their code in 2004 to cite that animals have subjective experiences of pain comparable to humans, which include nociceptive reception, transmission, central processing, and memory of stimuli [34].

Thus, further work is needed to examine the conduct and oversight of invertebrate research in the U.S., including jellyfish modification and experimentation. In alignment with current ethical codes, the following will show that invertebrate research on animals is ethical, particularly for invertebrates without evidence of nociceptors or higher order behaviors, such as a sense of self. However, researchers must take care to follow the precautionary and minimization principles, as is described in the case study of jellyfish modification.

CASE STUDY USING BIOHYBRID ROBOTIC JELLYFISH

Summary of research

We will now examine the case study of jellyfish modification, in which a series of experiments were conducted to build a biohybrid robotic jellyfish, composed of an external microelectronic system that controls the muscle contractions of live *Aurelia aurita* (see Fig. 1). The justifications for this research include advances in biology, ecology, and evolution by better understanding the locomotion of jellyfish as a basal organism; advances in robotics by using biohybrid approach to address constraints, such as power consumption and damage tolerance; and broader implications for improved ocean monitoring tools to track climate change.

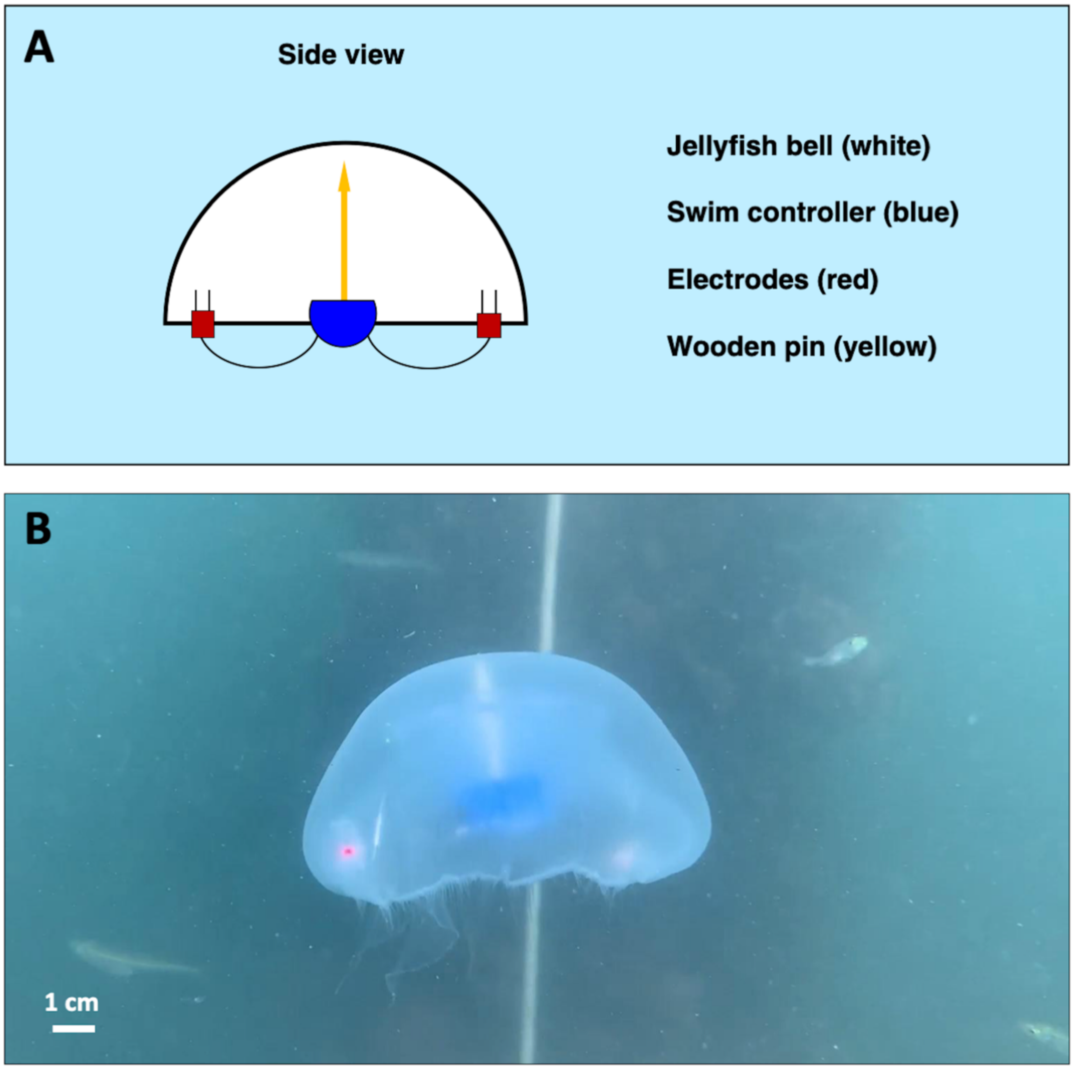

Figure 1. Biohybrid robotic jellyfish. (A) Side view schematic of a biohybrid robotic jellyfish, which shows the jellyfish bell (white) and swim controller components: the microelectronic swim controller (blue), connected via wires to two electrodes (red), and attached to the jellyfish using a wooden pin (yellow). (B) An example of a biohybrid robotic jellyfish deployed off the coast of

Woods Hole, MA. The background contains a rope used during field tests, and natural flora and fauna are visible around the jellyfish. More information about the system and experiments is described in [35] and [36].

This research involved the following experiments, using ten or fewer animals per each experiment, as described in [35] and [36].

1. Muscle excitation experiments (N=10) to determine the spatiotemporal control of jellyfish muscle by placing animals in a dish in the absence of seawater. Electrodes were embedded in the soft tissues.
2. Immunohistochemical staining experiments (N = 6) to visualize muscle striation patterns in excised tissue samples, in which animals recovered post-excision.
3. Vertical free-swimming experiments conducted in the laboratory (N=6) to determine how the external control of swimming frequency affects swimming speeds. Swim controllers were physically embedded into the jellyfish tissue using a wooden pin attachment and wire electrodes.
4. Oxygen consumption experiments (N =7) to calculate the metabolic costs for enhanced swimming speeds.
5. Vertical free-swimming experiments conducted in the coastal waters of Massachusetts (N = 4) to confirm swimming speed enhancements in laboratory results, and as a proof of concept that biohybrid robotic jellyfish could be used in future ocean monitoring applications (Fig. 1B).

The results of (1) and (2) demonstrated a range of effective electrical signals and electrode positions on the jellyfish, which were used to build a self-contained microelectronic system to control jellyfish swimming used in (3) to (5). The results of (3) and (4) showed increased swimming speeds up to 2.8 times, with only a twofold increase in metabolic cost, to reveal the potential for both faster and more energy-efficient swimming in user-controlled live jellyfish. This system also consumed orders of magnitude less mass-specific external power than existing swimming robots. Finally, the results of (5) confirmed enhanced swimming speeds up to 2.3 times in real-world ocean environments.

Nociception and stress responses in jellyfish

No evidence suggests the presence of nociceptors in the Cnidarian class of scyphozoa, or true jellyfish, which include *A. aurita* [16, 21]. As previous ethicists and scientists have claimed, pain is both subjective and potentially constrained to a central nervous system (CNS) [11, 13, 14]. *A. aurita* thus offers advantages because of their lack of brain, CNS, or nociceptors. Furthermore, among different classes of jellyfish, scyphomedusae possess the most diffuse organization of nerves [37–39].

Jellyfish have distributed, non-polarized neuronal networks, which consist of eight sensory structures and two nerve nets: the motor nerve net (MNN) and diffuse nerve net (DNN) [37–40]. The eight sensory structures – also called rhopalia, swim pacemakers, or marginal sensory structures – are equally distributed in indentations along the margin of the bell and directly activate the MNN, which incites muscle contractions or pulses. The DNN also sends sensory signals to induce tentacle contractions and modulate rhopalial activity [37, 39, 41, 42]. The eight rhopalia have a semiindependent relationship that can produce more coordinated muscle contractions [43], with redundant pacemakers to aid the regularity of swimming and improve resilience to tissue damage [44, 45]. Because this distributed nervous structure suggests no mechanisms for pain or nociception, stress responses can be used as a proxy.

The most prominent marker of stress induction in jellyfish is the excess secretion of mucus, which has been reported in literature [46, 47]. This mucus secretion is observed as a defense mechanism from external stimulation, including gentle physical agitation and handling in the species *Aurelia coerulea* [47]. However, jellyfish also secrete mucus for normal behaviors, such as feeding, reproducing and modulating innate immunity [46]. The distinction between normal and stress-induced mucus secretion is only apparent on a proteomic, metabolomic, and transcriptomic level, not observable in behavior. These include tryptamine, other proteins, and metabolites present in stress-induced mucus in *A. coerulea* [46] and gene expression changes using transcriptomic analysis in *A. aurita*, when held with rigid versus soft robotic claws [48].

Ethical considerations

The contents of this case study [35, 36] were presented to the Stanford Benchside Ethics Consultation Service (BECS), a research ethics and regulation committee comprising Stanford University faculty and members of the Stanford Center for Biomedical Ethics. The committee identified the following issues in their resulting BECS report [49], which we will further discuss in the context of biohybrid robotic jellyfish and other analogous work in later sections.

Welfare interests

Restrictions on higher-order animals, including vertebrates and cephalopods, are in place to protect their welfare interests. Because jellyfish do not possess a central nervous system, the BECS committee conceded that it is unclear whether jellyfish have welfare interests that can be harmed through experiments [49]. Debates on the ethics of invertebrate research are ongoing and inconclusive [29–31]. However, to err on the side of caution in accordance to the precautionary and minimization principles, we should apply the 4Rs [50, 51], as defined by the BECS report: *reduction*, the minimization of animals used to answer the scientific question; *replacement*, the use of animal alternatives where possible; *refinement*, procedural changes to minimize pain, suffering, and distress; and *reproducibility*, high quality of research with scientific justification, conducted with rigor [49]. (Note that most ethical considerations use only the 3Rs – reduction, replacement, and refinement – with variations on the potential fourth R, including responsibility.)

However, welfare interests are still subjective, with strong arguments for and against certain interpretations of ethical correctness. In particular, the BECS definition and application of reduction is to reduce the number of animal test subjects; however, instead of a utilitarian framework, Tannenbaum uses a rights-based ethical framework to argue that "fairness to individual animals" sometimes requires using more animals instead of fewer, or causing more total pain to minimize the cost to an individual animal [25]. Thus, applying the minimization principle might favor experiments on more animals for less time, as opposed to fewer animals over longer periods, to reduce the pain load on individuals. Additionally, Dawkins suggests that animal welfare criteria include that animals are in good health and will seek out situations that they 'want' [52], but this view is anthropomorphic and possibly implies some level of autonomy or free will, which also lead to questions of dignity and integrity.

Dignity or integrity interests

Given the ethical issues raised by readers and critics of this research, it is appropriate to apply the aforementioned 4Rs based on an argument from dignity or integrity interests [53]. This argument states that the 4Rs should be applied, even in the absence of sentience, because "animals of sufficient complexity and stability" are afforded protections from the right to dignity, compatible with the rights-based or deontological framework [49]. This brings up similar ambiguities because of the phrase "sufficient complexity and stability," which is again subject to interpretation. On one side, ethicists can argue that invertebrates do not have the required complexity or stability, especially given their lack of 'self.' As an example, planarian flatworms can regenerate from each of 280 individual cut pieces of one parent worm (Fig. 2D) [54]. This principle has also been seen in *A. aurita*, in which partial jellyfish can redistribute their body structure ('symmetrize') to survive (Fig. 2E), as long as these excised animals retain sufficient gastric function [55].

Regardless of these open questions, if all invertebrates are afforded the right to dignity based on their establishment in the animal kingdom, then there are a host of considerations for fruit flies, nematodes, and other common invertebrates, including pests. Only scientific and commercial regulations are covered by ethical guidelines, which also underscores inconsistencies with the relationship between humans and ethical treatment of invertebrates.

Wisdom of repugnance

The 'wisdom of repugnance' or 'yuck factor' states that any intuitively negative response should be interpreted as evidence that such a thing, idea, or practice is intrinsically evil or harmful [56]. Kass states that reflexive revulsion reveals the intrinsic morality of the experiment, with the example of human cloning [57], but critics argue that repugnance is a reaction built upon prejudices. These thoughts should then be scrutinized – whether justified or rebutted – rather than assumed to be a source of moral insight [58]. Thus, although expert and public concern about modification to natural animals prominently features wisdom of repugnance arguments, this reactionary judgement stems from the new and unusual, but warrants further examination beyond the reliance on feelings.

Presumption of restraint

One criticism of animal biotechnology is that the current paradigm is overly permissive of animal experimentation in unclear ethical situations, instead of restrained. Bioethicists, such as Feister, argue that there needs to be a presumption of restraint framework, or "a default position of wariness that must be overcome by morally compelling reasons in order to justify a particular project's moral legitimacy or permissibility" [59]. This requires that research projects involving invertebrates be treated seriously and not for art, novelty, or other sources of entertainment. In essence, the presumption of restraint extends respect and gratitude toward research animals by avoiding hubris in the pursuit of knowledge, participating in the due deliberation of ethical concerns, and avoiding harming animals or viewing them as trivial [49]. This also poses researchers as responsible stewards.

Stewardship

Stewardship is rooted in the responsibility that researchers have to care for animals used for research purposes [60]. This view is based on the idea that animals frequently serve as tools for furthering human interests. This concept should govern our interactions with non-human animals by placing the responsibility for researchers to abide by the 4Rs and use resources appropriately to achieve their intended goals [49]. Furthermore, stewardship requires that animals are used efficiently. As an example, the BECS committee states, “Duplicate experiments are an inefficient use of animals because it wastes the time and effort of researchers” [49].

Environmental impacts

Research in jellyfish modification can also have potential environmental impacts, including additional electronic and plastic waste in the ocean; effects on the animals’ ability to eat and reproduce; and far-reaching implications on other interlinked species [61]. The current state of deploying biohybrid jellyfish robots into ocean environments is limited to short time periods that do not affect animal longevity, and requires researchers to monitor the systems carefully to prevent potential pollution or ecological impacts. Nevertheless, the potential for wider ocean monitoring research warrants further discussion on the environmental impacts, both measurable and immeasurable, in accordance with philosophical environmental ethics, which affirms the value of the environment as a coherent ecosystem with all its diversity [62].

PRECEDENTS AND ANALOGOUS WORK

Before further discussing how we addressed these issues in our case study, precedents on analogous work also provide insight into these ethical considerations. In particular, electrical stimulation is a broad method that has been applied previously in electrophysiology experiments on jellyfish [63–67], robotic control of insect locomotion [68– 71], and human enhancement for rehabilitation [72–74]. Additional work on invertebrates has also included excisions and amputations in aquatic invertebrates, with one application for biohybrid robotic integration using sea slugs [75] despite prior evidence of nociception in the same species [17].

Interestingly, the ethics of scientific research have been largely ignored by the general public and popular media, unless the element of 'playing God' – or human manipulation of the natural world – is introduced. Examples include cyborg insects [76–78], 'microslavery' of microbes [79], and even genetically modified organisms for food and agriculture [80]. (Note that all examples of invertebrate research are provided in the pure pursuit for knowledge and scientific advancement, in accordance with the presumption of restraint, whereas the microbial example states that "biotic games will be played for fun" [79]. However, these microbes are not animals and therefore do not abide under comparable ethical considerations.) Among these disparate examples, one primary criticism is that human control might lead to a slippery slope, in which modification of animals eliminates their freedom to behave the way they wish to. However, as cited previously, human enhancement already exists, and both human and non-human vertebrate experiments are strictly regulated by governing ethical boards [26, 33, 34]. The slippery slope fallacy makes false assumptions and extrapolations about these valid studies.

Thus, the following subsections will summarize relevant past scientific studies and their ethical considerations. This will demonstrate that invertebrate animal welfare is primarily valued in the context of human exploitation of the natural world, and undervalued for the invertebrate lives themselves.

Microelectronic stimulation of insect locomotion: RoboRoach, cyborg beetles, and other biohybrid robotic insects

Biohybrid robotic insects to control locomotion, such as RoboRoach [68], are the closest analogous cases to this biohybrid robotic jellyfish study. RoboRoach – a toolkit that allows the wireless control of live cockroach locomotion by electrically stimulating its antenna nerves – has

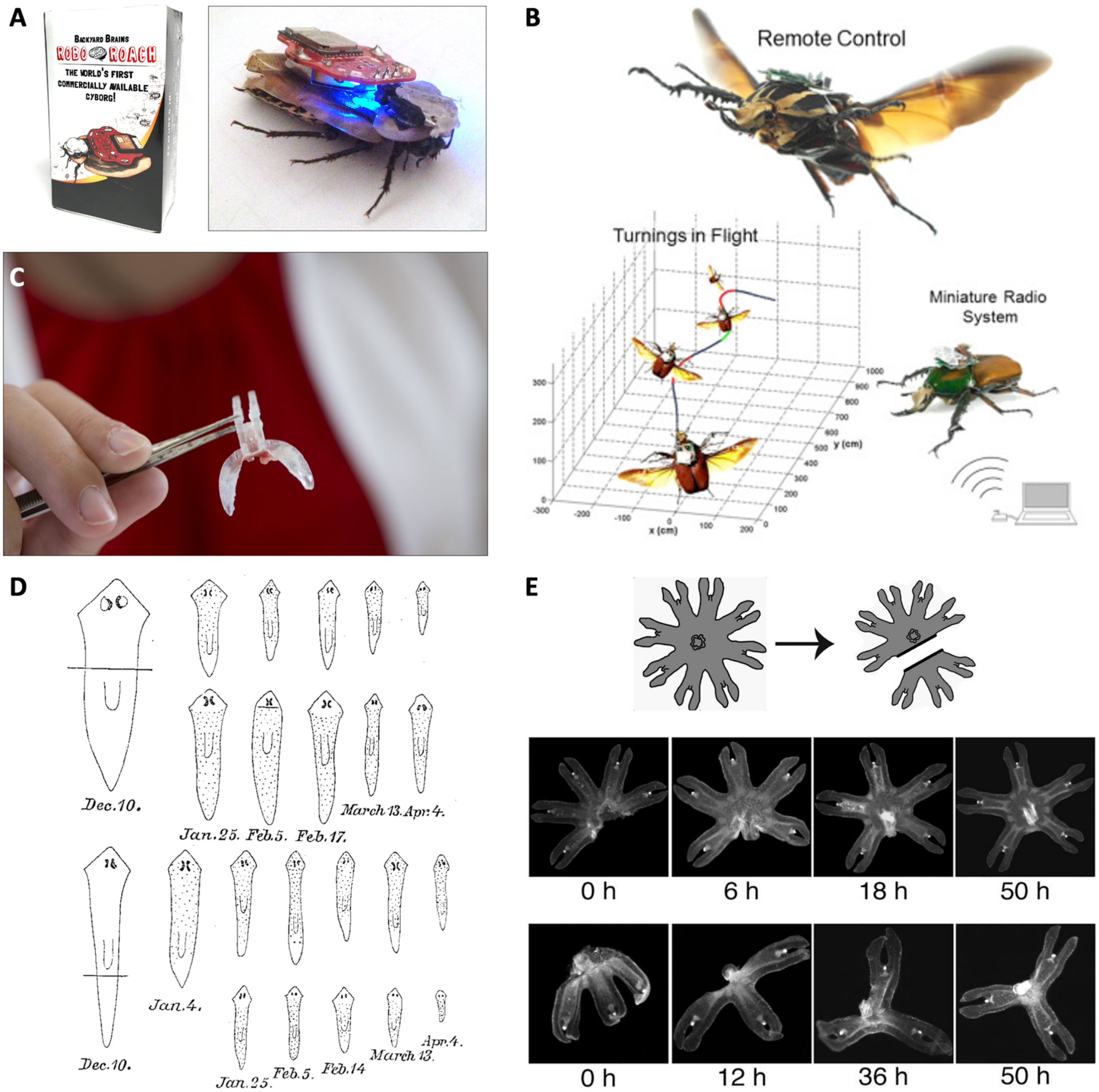

Figure 2. Examples of invertebrate research. A brief selection of comparable invertebrate research, including biohybrid robotic insects and tissue cutting experiments: (A) The commercially available RoboRoach kit and an example of a cyborg cockroach, which users can surgically modify to control animal locomotion [68]. (B) A 'cyborg beetle,' which uses a similar concept of microelectronic stimulation to control its motion [85]. (C) A biohybrid robot incorporating muscle tissue from sea slugs [75], which have been reported to possess nociceptors [17]. Photo credit to

Victoria Webster/Case Western University. (D) A schematic of tissue cutting experiments to observe tissue regeneration in planaria, conducted in 1909 [54]. Planaria were reported to regenerate the entire animal body when cut into 280 separate pieces or fewer [54]. (E) A schematic and two time series of tissue healing experiments [55] using *A. aurita*, the same species of moon jellyfish used to develop biohybrid robots. Jellyfish were excised as shown, with tissue symmetrization occurring over the span of days.

been touted as “the world’s first commercially available cyborg,” as shown in Figure 2A [68]. However, with this title has come criticism from the public, including accusations about animal cruelty and slippery slope fallacies [78]. To address the public questioning whether do-it-yourself (DIY) cockroach surgery is different from burning ants through a magnifying glass, or if this could lead to DIY surgical implants in pets, RoboRoach’s parent company Backyard Brains includes a web page dedicated to ethical issues [77].

First, RoboRoach is posed as a tool for college students, or high school students with adult supervision, to learn about the neural basis of behavior, memory, adaptation, response to stimuli, and animal variability [81]. The website disclaimer reminds students about the utility of RoboRoach as an educational tool and to be respectful toward animals, although it should be noted that this legal disclaimer does not guarantee against misuse, and the mistreatment of cockroaches might arguably be a “reasonably foreseeable misuse.” These cyborg toolkits also include all necessary materials, except for live cockroaches that are sold separately in packs of 3 to account for user errors in initial implantation attempts [81]. Another notable feature is that the website contains information on how to build DIY electrodes, not purchased in the kit, which could potentially encourage misuse.

However, Backyard Brains demonstrates due diligence in its online ethics page, noting that its protocols are annually reviewed by an external ethics review board, which is listed as an Institutional Review Board (IRB) [77]. (Note that IRBs are traditionally used for human research, as opposed to IACUCs for animal research, although this could be a minor typographic error.) The experimental protocol instructs the use of cold temperature as ectotherm-appropriate anesthesia, and highlights that cockroaches can adapt to ignore stimuli within minutes, which reportedly cannot be done with painful stimuli [77]. Furthermore, the company conducts a cost-benefit analysis that cites cockroach leg detachment and regrowth after experiments, with a return to normal behavioral responses (locomoting, eating, drinking, reproducing, etc.) within a few hours [82]. Finally, RoboRoach has received accolades from the Society for Neuroscience, NIH, and President Barack Obama. The company concludes that the need for more neuroscience research and public education is more beneficial to society than the potential cost to the cockroaches [77].

In addition to other demonstrations of cockroach turning [69, 83], prior literature also demonstrates the flight control of moths [84] and giant beetles [70, 71, 85]. The 'cyborg beetle' (Fig. 2B) has also come under ethical scrutiny, with some suggestions that such animal modification should be replaced by pure technology or similar slippery slope arguments [86]. Nevertheless, justifications for this research include the overarching goal to build energy-efficient robots for search-and-rescue missions and reconnaissance. Specifically, research using the biohybrid robotic beetle could minimize battery power and focus on control systems, with collaborations at the Defense Advanced Research Projects Agency (DARPA) for both military and civilian uses [76]

These examples illustrate the concern for invertebrate animal welfare, especially in the context of human control and despite how cockroaches are considered pests. Human prejudices and reactions to the aesthetics of certain species are not morally relevant and do not necessarily track the moral value of an animal. The justifications for these experiments are strong, as documented in national recognition and continuation for advancements in these projects. Nevertheless, ethical issues still arise in terms of both the human control of the natural world and whether these experiments incite animal cruelty.

Experiments on entirely soft-bodied invertebrates

In comparison, the integration of muscle tissue from the sea slug *A. californica* into a biohybrid robot [75] (Fig. 2C) has generated less ethical debate, with one article calling slugs "disgusting little wonders of the oceans" before praising the scientific merits of "this creepy robot" [87]. Despite the 'yuck factor,' no specific ethical issues have been raised about harm to the sea slugs, despite the presence of nociceptors confirmed in this species of sea slug [17], or whether muscle tissue could be harvested from higher order animals. This could be because there are either no ethical issues present, or the ethical issues have not yet been recognized or investigated.

The ethical questions raised for the Roboroach and cyborg beetle are also absent in experiments that excised major tissues, including cutting planaria into 280 pieces that each regenerate, as mentioned previously [54] (Fig. 2D). Additional examples include experiments demonstrating the reaggregation of dissociated tissue in Porifera sea sponges, which are multicellular aggregates; this study used chemical and mechanical methods of tissue dissociation, such as extruding sea sponges through a sieve, to show subsequent tissue reaggregation [88].

Tissue cutting and regeneration experiments have also been conducted on *A. aurita*, the species of jellyfish used in the biohybrid robotic studies. Cutting experiments and electrical stimulation to understand conduction of the jellyfish nervous system were reported from the 1881s to 1960s, including excision to form donut-like rings and strips of tissue [63–67]. Aforementioned cutting experiments (Fig. 2E) were also performed in 2015 to show jellyfish symmetrization, or the redistribution of jellyfish tissue into a radially symmetric bell after significant amputation of multiple arms [55]. Jellyfish were anesthetized using menthol and magnesium chloride solutions as both a muscle relaxant and analgesic, and most animals did recover with successful healing processes within days or weeks [55].

These experiments pose interesting ethical questions regarding invertebrate animal welfare and dignity, although no ethical critiques were posed about any of these studies. The researchers provided no ethical statements in their publications, in alignment with journal policies that do not require ethical consideration statements for invertebrate studies. This suggests that the public's ethical dilemmas about invertebrate research primarily focus on the wisdom of repugnance, not invertebrate animal welfare or dignity interests, regardless of the detriment to individual animals. The two preeminent ethical interests are criticism or mistrust of scientists 'playing God,' and humans expunging 'free will' in animals, despite lack of sentience in these lower order invertebrates.

Electrical stimulation of humans and higher-order animals

In addition to the microstimulation of insects and aquatic invertebrates, the integration of electronics has also been used in human enhancement [89] and higher-order animal enhancement [90, 91]. With human enhancement, the first reported studies of direct brain-computer communication were reported in the 1971s [72, 92], and braincomputer interfaces (BCI) were subsequently used to learn about the human CNS and help people regain functions, such as eyesight, hearing, speech, and motion [73, 74, 93]. Regardless of the positives and negatives of enhancement, providing prosthetic limbs [94] and other examples provide benefits to the subject of modification.

To compare to the case study, no such benefit occurs for the jellyfish because faster swimming speeds offer no concrete advantages, such as improved evolutionary fitness. It is possible that faster swimming could potentially improve predator-prey interactions, but more work

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is needed to determine whether jellyfish enhancement offers advantages to the jellyfish themselves. Nevertheless, the primary purpose of jellyfish modification is not for the benefit of the individual animals. Furthermore, it is unclear whether enhanced swimming might cause pain-based or non-pain harm to the animals, such as reducing their reproductive fitness or increasing soft tissue wear over time.

ETHICAL ISSUES ADDRESSED AND OPEN QUESTIONS REMAINING IN BIOHYBRID ROBOTIC JELLYFISH RESEARCH

Stanford scientific ethics expert and BECS committee member [49] Hank Greely noted that biohybrid robotic jellyfish raised new ethical questions [95]. In light of the committee's ethical considerations – welfare interests, dignity or integrity interests, wisdom of repugnance, presumption of restraint, stewardship, and environmental impacts – what are the primary points of criticism against jellyfish modification, and how do they compare to similar precedents?

Ethical critiques of prior studies show inconsistencies, such as biohybrid sea slug robots and excised jellyfish experiments yielding few arguments in public opinion and media coverage. Yet similar modifications of cockroaches, which are typically considered pests and commonly exterminated in households, poses a larger ethical debate.

This incongruity highlights the first overarching consideration, which is that the wisdom of repugnance is key in public opinion. That is, there is unease in research that appears to be 'playing God,' both for welfare issues and for potentially a slippery slope toward moral turpitude, which we have previously addressed and dismissed because of the ethical guidelines in place to prevent this escalation [26, 27, 33, 34]. However, this underscores a potential mismatch between popular opinion and ethical determination, as well as what the scientific community should do to address this gap.

Second, Mather suggests that humans selectively decide which animals deserve welfare rights based on concepts such as utility (i.e., honeybees) and aesthetics (i.e., butterflies) [2], based on Kellert's survey of the public perception of invertebrates [96]. This hierarchy concept can be extended to the idea of jellyfish as visually beautiful or calming, which is why a biohybrid robotic sea slug is less controversial than a biohybrid robotic jellyfish. However, the lack of ethical concern in the same jellyfish species in symmetrization experiments [55] suggests that the wisdom of repugnance is still the primary ethical concern. Furthermore, this is incongruous with the perception of jellyfish and cockroaches as nuisances, which should lower their perceived value by humans.

Third, the question of ecological consequences introduces an additional complexity to the ethics of this case study, not relevant to the prior examples. In particular, the long-term goal to use biohybrid robotic jellyfish as ocean monitoring tools raises questions about anthropogenic environmental and ecological impacts. Questions of whether sea turtles would be harmed if they swallowed a bionic jellyfish, or how harmful the potential addition of electronic waste (e-waste) to the ocean would be, all warrant further discussion to improve stewardship and reduce negative environmental impacts. We will discuss our approach to addressing these ethical considerations of biohybrid robotic jellyfish, with respect to three levels: the ethics of jellyfish as individuals, as a species, and as part of an ecosystem.

Considerations to A. aurita as individuals

The primary role of IACUC in overseeing protocols is to ensure the welfare of individual animals. Thus, the ethical considerations of individual animal rights focus primarily on specific experimental procedures, in accordance with the 4Rs: reduction, replacement, refinement, and reproducibility. These 4Rs also address the relevant issues of animal welfare, rights to dignity or integrity, presumption of restraint, and stewardship in individual animals.

The scientific consensus is that jellyfish do not have sentience or pain because of their distributed nervous system structures. This is corroborated by the previously posed question of whether jellyfish lack a sense of 'self,' given jellyfish halves can subsist separately [55]. Regardless of these points, the 4Rs provide the best practice for conducting research on biohybrid robotic jellyfish:

1. *Reduction:* No more than 10 animals were used for each experiment, and riskier experiments (including freeswimming tests of biohybrid robotic jellyfish, tissue excision, and oxygen depletion) used fewer animals. However, sufficient animal test numbers were needed for statistical significance. One instance of animal reduction occurred within the field experiments, in which video data for 2 out of the 4 total animals could not be used for image analysis; however, additional experiments in coastal waters were not conducted, and these videos were still used as observational data to retain their utility.
2. *Replacement:* In addition to experimental work, a theoretical model of jellyfish hydrodynamics was also developed to show good agreement between experimental and theoretically predicted swimming speeds, with errors less than 1 cm s^{-1}. However, animal experiments were still required to validate these models.
3. *Refinement:* Refined protocols included procedures in alignment with the precautionary principle to minimize any potential pain, suffering, and distress. In accordance, different methods of attaching the swim controller onto the jellyfish were tested before the final design using a physical wooden pin. The final pin design minimized tissue damage compared to measures using superficial Histoacryl Flexible (B. Braun Medical Inc., Bethlehem, PA, USA) and mussel-inspired adhesives [97]. These methods caused larger areas of tissue damage after removal, compared to a small hole that healed within a day after removing the wooden pin.

We used behavioral stress responses as a proxy for pain and observed no excess mucus secretion in these biohybrid robotic jellyfish swimming experiments. Animals were allowed to rest between subsequent experiments. Furthermore, the jellyfish did not show any negative side effects after the robotic devices were removed; animal behavior returned to its normal state, including typical feeding behaviors.

Finally, another aspect of refinement included anesthetics. Although we tested menthol and magnesium chloride solutions as known jellyfish anesthetics [55], these chemicals arrested jellyfish motion and negatively impacted studies of both animal locomotion and oxygen consumption. Therefore, although no anesthetics were used in these studies, we conducted due diligence to first address these issues before determining the best protocols for ethical scientific pursuit.

4. *Reproducibility:* The value of reproducibility requires experiments to be done with scientific rigor, especially given the determination that duplicate experiments are not a good use of animal subjects [49]. However, to show reproducibility, similar experiments can perhaps be conducted to test for additional parameters. For example, the vertical swimming experiments ($N = 6$) on biohybrid robotic jellyfish showed speed enhancements of up to 2.8 times under quiescent laboratory conditions [35]. From the first series of experiments alone, it is unclear whether enhanced jellyfish swimming speeds could also occur under natural conditions in the ocean, particularly in the presence of surface currents. Therefore, similar experiments were conducted on $N = 4$ animals in the coastal waters of Massachussetts to show speed enhancements up to 2.3 times [36]. These two studies confirm the same results under new conditions, which provide further knowledge using similar experimental protocols.

In summary, all animals recovered post-experimentation with minimal instances of long-term effects, and were subsequently able to swim, feed, and reproduce. Even experiments done in the absence of seawater, in a 2-L seawater environment to measure oxygen depletion over 6- to 8-hour intervals, or with embedded electrodes and microelectronic devices into the gelatinous tissue did not negatively impact the animals' ability to thrive. For example, all 10 animals in muscle excitation experiments, 7 animals in oxygen consumption experiments, and 4 animals in field tests had no resulting side effects; all 6 animals in the immunohistochemical staining tests recovered after tissue excision within a few days, with minor bell deformations that did not impact feeding or other behaviors in; and only 2 out of 6 animals experienced temporary abnormal muscle wave propagations that returned to normalcy after free-swimming laboratory experiments, with 2 other animals acquiring minor bell deformities that also did not impact survival. These rare instances of jellyfish bell deformation usually resulted from animals being constrained in the corner of tanks, which also occurred in normal animal husbandry and have been reported in aquariums, from personal communications.

Care was taken to ensure that a minimum number of animals were used in total, and that all animals used were allowed to recover in between and after experiments. Furthermore, behavioral stress responses were monitored during the experiments to minimize mucus secretions. Aside from pain-based harm, enhancement with the swim controller could also cause unintended consequences to the individual animal, such as potentially reducing its reproductivity or increasing tissue wear and breakdown, which would otherwise occur naturally at different rates. More research should be conducted to determine the impact of such non-pain harms, if evident. However, a limitation of this case study is that no proteomic, metabolomic, or transcriptomic analyses were conducted to determine if the animals were stressed on a molecular level. The presence of stress markers in a similar scyphozoan species when taken out of seawater [47] suggest that stress markers might also be observed in these spatiotemporal muscle response experiments in the absence of seawater. Nevertheless, all of these animals recovered immediately after the experiments. Previous work has also shown stress-induced differences in the transcriptome of *A. aurita* when handled more roughly [48], so further work should be done to analyze biohybrid robotic jellyfish versus natural animals to determine molecular stress responses.

Considerations to A. aurita as a species

Modifications to live jellyfish also have implications for the welfare of the entire jellyfish species. First, there is an open question of whether the microelectronic swim controller affects the feeding, longevity, livelihood, and reproduction of the individual animals, which can affect the evolutionary fitness of the species. The current microelectronic system is limited to tests up to a few hours at maximum because of the chosen battery design, but energy-dense batteries provide the potential for longer term jellyfish modifications. Future experiments over longer periods, up to days or weeks with the swim controller attached, should be done to determine whether the swim controller negatively affects feeding or other behaviors that might impact species-level survivability.

However, an important consideration is whether decreased species-level survival is even an issue, given the overpopulation of jellyfish blooms that can negatively impact the environment [98–100]. *A. aurita* have been considered an invasive species [101, 102], although the same argument with cockroaches as pests has had no bearing against criticisms of RoboRoach [77, 78].

Considerations to the environment and ecology, influenced by A. aurita

Evolutionary fitness and jellyfish blooms

The environmental and ecological implications of this work tie into broader welfare interests and the idea of stewardship, or responsibility to the planet and its living beings. As noted, *A. aurita* and other jellyfish species are considered nuisances, with ecological consequences from increases in jellyfish blooms [98, 100, 103] and invasive takeovers of coastal lagoons [101, 102]. Such blooms can also negatively impact human industries, such as fisheries and tourism [99]. For field experiments in which *A. aurita* were tested in the Atlantic Ocean, we closely monitored the biohybridic robotic jellyfish to ensure no animals were left in the ocean after experiments, even though this species is endemic to the area.

In light of these considerations, both species-level and ecological-level consequences from altering *A. aurita* fitness should either be negligible or offset with other periodic changes in jellyfish populations [99]. The specific causes of these blooms have ranged from natural cyclical variation to anthropogenic causes and climate change [98–100], but the high evolutionary fitness of jellyfish is likely due to the multi-phase life cycle [104], which includes sessile polyps (asexual) and free-swimming juvenile and adult medusae (sexual) [39]. Variations in environmental conditions could favor dense populations of either polyps or free-swimming phases, as well as either asexual or sexual reproduction [105].

Microplastics and e-waste pollution in the ocean

Perhaps a larger concern is the potential introduction of more plastic and e-waste to the ocean [106, 107], which stems from the proposed application of biohybrid robotic jellyfish as ocean monitoring tools. Possible issues include other aquatic wildlife ingesting the microelectronic components, which might cause bodily harm, as was reported in prior incidents of plastic ingestion in fin whales [108] and amphipods [109]. This requires investigation into using more environmentally friendly materials, such as biodegradable electronics [110–112] and plastic films [113].

Because the current technology for biohybrid robotic jellyfish is not at this level, it is difficult to make assessments of long-term ecological impacts, but regarding the case study, care was taken to ensure that no swim controller components were left in the ocean after the field tests in [36].

RECOMMENDATIONS

Future work on biohybrid robotic jellyfish

The existing work to modify live jellyfish brings considerations on an individual, species, and ecological basis. To summarize, the minimization and precautionary principles using the 4Rs are paramount for individual animal welfare. More extensive experiments in the laboratory are needed to assess animal behavior and fitness for the species, including measurements of molecular stress markers and determining if microelectronic swim control negatively affects their survivability. More discussions with ethics experts are also needed to predict unintended consequences and determine recommendations for future research. Even if jellyfish modification does cause some harm to this or other species, researchers and ethics experts must conduct a cost-benefit analysis to weigh the potential benefits. As a hypothetical example, if biohybrid robotic jellyfish could detect and prevent coral bleaching in a method that scientific SCUBA divers or underwater vehicles cannot, does this benefit to the environment outweigh sacrificing small numbers of jellyfish or marine animals that ingest the jellyfish?

These open questions and critiques about the long-term effects of deploying biohybrid robotic jellyfish in ocean environments are entirely reasonable. However, these concerns need not be answered within the scope of the current research, which is still limited to careful surveillance of these biohybrid systems. We will continue to reflect upon possible environmental impacts, including plans for ongoing assessments and mitigation of those impacts before future applications are introduced; this includes discussions with more scientists and ethicists. In the meantime, the presumption of restraint principle stands. Biohybrid robotic jellyfish must remain supervised if deployed in ocean environments until we can address these ecological issues first, such as incorporating biodegradable plastics and electronics [110–113].

General invertebrate research

A significant stakeholder in this case study is also each jellyfish test subject, which compels further examination into general invertebrate ethics. Although the majority of ethical guidelines do not require protocol submissions and animal welfare checks based on lack of evidence of nociception, researchers should follow the 4Rs, minimization, and precaution. We recommend that journals require ethical statements for invertebrate research, and in the absence of such requirements, that researchers should include a brief ethics statement of the following:

- Scientific justification for the research, including justifications for why alternatives to animal research cannot be done with comparable resulting information.
- Number of animals used and a discussion of the 4Rs.
- Cost-benefit analysis to compare the cost to the individual animals versus the benefits to others, including but not limited to other individuals within the same species, other species, humans, and broader environmental impacts. This analysis should also consider the moral interests and needs of the invertebrates.

The lack of systemic ethical oversight on invertebrate research also underscores the lack of knowledge about invertebrate pain. Therefore, we also recommend further scientific studies of pain and nociception using invertebrates as model organisms. Although this appears to be a catch-22, pain research on invertebrates is justified by the potential to revise or validate current research standards. The seeming contradiction of inducing potentially painful stimuli in more animal experiments to understand animal nociception is a lesser evil, compared to the continuation of unregulated invertebrate research. Finally, the inconsistent public responses toward comparable invertebrate studies highlight two gaps: a mismatch between public opinion and ethical evaluation, and a question of how the scientific community should approach these ethical boundaries. This suggests a need for scientists to discuss careful communication of their research to the public and reflect upon their work when there are apparent gaps between public opinions and ethical conclusions.

Thus, this evaluation acknowledges and agrees with the expert opinion that "there is something disconcerting about mechanically changing animals for our utility … Is it wrong, is it right? I don't know, but I am confident we will face these kinds of questions more and more often" [94]. We need to be prepared to answer these questions by engaging in further ethical discussions with both experts and the general public about environmental impacts and animal welfare, which we can only do with additional research on environmentally sound technology and invertebrate-based nociception, as well as precautionary action.

AUTHOR CONTRIBUTIONS

N.W.X. and J.O.D. conceived the idea; N.W.X. wrote the initial manuscript, after early conversations with O.L, S.E.W, and C.A.F.; N.W.X, O.L., S.E.W., C.A.F., and J.O.D. edited the manuscript.

DISCLOSURE

The authors N.W.X. and J.O.D. conducted research on biohybrid robotic jellyfish [35, 36].

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