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Ethics of biohybrid robotics and invertebrate research: Biohybrid robotic jellyfish as a case study

Running head: Biohybrid robot ethics

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ABSTRACT

Invertebrate research ethics has largely been ignored compared to the consideration of higher order animals, but more recent focus has questioned this trend. Using the robotic control of *Aurelia aurita* as a case study, we examine ethical considerations in invertebrate work and provide recommendations for future guidelines. We also analyze these issues for prior bioethics cases, such as cyborg insects and the 'microslavery' of microbes. However, biohybrid robotic jellyfish pose further ethical questions regarding potential ecological consequences as ocean monitoring tools, including the impact of electronic waste in the ocean. After in-depth evaluations, we recommend that publishers require brief ethical statements for invertebrate research, and we delineate the need for invertebrate nociception studies to revise or validate current standards. These actions provide a stronger basis for the ethical study of invertebrates, with implications for individual, species-wide, and ecological impacts, as well as for studies in science, engineering, and philosophy.

KEYWORDS

Animal enhancement, animal modification, *Aurelia aurita,* biohybrid robot, biohybrid robotic jellyfish, jellyfish

INTRODUCTION

Although invertebrates constitute over 97% of species in the animal kingdom (Invertebrates 2018) and are widely used in scientific literature, the ethics of invertebrate research has largely been overshadowed by the focus on mammalian ethics (J. A. Mather 2019). Invertebrate research is often justified as a more ethical alternative to vertebrate experiments, but more recent scrutiny has questioned this claim (J. Mather and Anderson 2007; Robyn J. Crook 2013; Carere and Mather 2019; J. A. Mather 2019; Mikhalevich and Powell 2020), including a recent paper on the ethics of biohybrid robotics research (Mestre et al. 2024).. To examine and address the ethics of invertebrate research, we will discuss the ethical considerations of scientific work on biohybrid robotic jellyfish, in which live jellyfish were modified using microelectronic systems to control their swimming speeds. The development and initial testing of biohybrid robotic jellyfish were conducted by two of our authors, who primarily have backgrounds in science and engineering. Thus, this manuscript uses a collaborative team of research scientists and ethicists to generate ethical concerns and questions of augmenting jellyfish, with a broader discussion about invertebrate research, and considerations for continued work on biohybrid robotic jellyfish. The introduction will outline four general philosophical views of animal ethics, pain and nociception in invertebrates, and current 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. We conclude that research using invertebrate animals requires careful consideration, and call for more invertebrate pain research to refine our understanding and provide evidence to support the creation of new ethical guidelines for research using invertebrates.

Animal ethics have been approached by philosophers through several different frameworks, two of which are utilitarianism and deontology. Ever since Jeremy Bentham founded modern utilitarianism in the 18th and 19th centuries, utilitarian approaches have emphasized the concern for all sentient beings (including animals), defining the right action as that which produces the most good (or pleasure or happiness) and least suffering for all the beings that would be influenced by that action. According to the influential *Animal Liberation* written by well-known contemporary utilitarian Peter Singer, all sentient creatures are equally entitled to having their interests taken into account. Favoring human interests above animal interests is, for Singer, a failure to treating all sentient creatures equally and a form of what he calls "speciesism" (Singer 1975 , Milligan 2015). This is because the only morally relevant characteristic is the ability to feel pain and pleasure, not other kinds of capacities, such as intelligence level, the ability to use language, and so on. In other words, belonging to one species (such as *Homo sapiens*) rather than another is completely irrelevant from the ethical perspective – species membership is morally arbitrary in the same way that hair color or height are. But if a creature can feel pain, we are obligated not to harm it – at least not without a good reason.

However, Singer's principle of equality does not imply that every animal and every human should be treated in exactly the same way all of the time, since species differ in the ways their experience mental states such as anxiety or worry, including the sources of such emotions; hence, different forms of treatment of humans and animals are required (Milligan 2015). This may lead some utilitarians to the view that the interests of some animals may be sacrificed in order to satisfy the interests of some humans. In practice, then, utilitarianism often still favors humans and higher order non-human animals, suggesting that actions such as animal breeding for livestock or painless animal death are ethically permissible.

Deontological or rights-based views are rooted in extending individual rights to animals as 'non-human persons' (Francione 1995; Francione 1996; Regan 2004; Milligan 2015). They offer the alternative perspective that all individual animals have moral rights, regardless of sentience and other psycho-physical characteristics. For Regan – a key contemporary representative of rights-based animal ethics – what matters ethically is not the maximization of outcomes, but acting with respect for the moral standing and value of other creatures (including non-human animals). As a deontological view, Regan's approach to animal ethics is that certain actions (those that would violate animals' rights) are simply impermissible no matter how much overall happiness performing them would bring into others.

For Regan, animals have certain rights by virtue of being inherently valuable subjects-of-a-life: creatures capable of having experiences and forming attitudes towards the world (Milligan 2015). Their cognitive and emotional capacities are the same in kind, if not degree, as human cognitive and emotional capacities: animals have preferences, desires, beliefs, and memories; they can form expectations and experience happiness or frustration; they care about the quality of their life. This view does not take a single ability of a creature (such as feeling pain) to be decisive when it comes to the question of moral standing. Rather, the view is that many abilities (such as having desires and forming beliefs about the world) contribute to, and collectively constitute, moral standing.

These ethical views postulates specific criteria for the moral treatment of animals (Nussbaum and Wise 2001; J. Mather and Anderson 2007). For example, 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 (J. Mather and Anderson 2007). Differences aside, these views have issues evaluating the pain and suffering of invertebrates, using physiological responses to stress as a proxy for pain. As expounded below, the concepts of pain and nociception are central to considerations of invertebrate ethics.

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) (Raja et al. 2020). In contrast, nociception is the objective "physiological response to noxious stimuli that cause or potentially cause tissue damage" (Magalhães-Sant'Ana et al. 2009). This definition excludes subjective experiences, such as 'unpleasant' sensations or emotions.

Pain is also considered a central, not peripheral, phenomenon; this necessitates a brain or centralized nervous system, and a degree of sentience is necessary to experience pain (Jones 2012). For example, Braithewaite contends that fish do feel pain, citing the parallel brain development of both fish and mammals (Braithwaite et al. 2011). 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 (Rose 2007; Key 2016). Thus, there is a distinction between nociception as sensory information and pain as perception (Kavaliers 1988). Non-human pain studies often focus on nociception as a proxy, although nociceptors are nerves that detect noxious stimuli and report information about the state of tissue, not information about the experience of pain (Sneddon 2015).

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 (Smith and Lewin 2009; Sneddon 2018). In general, pain and nociception are understudied in both invertebrates and aquatic animals. However, some results of nociception research in underwater species have been reported (Sneddon 2015). For example, nociceptors have been found in the sea slug species *Aplysia californica* (Walters and Williams 2019), as well as cephalopods, which comprise species of cuttlefish, nautilus, octopus, and squid (Robyn J. Crook 2013). These observations demonstrate that noxious stimuli can induce reflexive behaviors in aquatic invertebrates, including withdrawing individual body parts, inducing escape behaviors, and reducing feeding (Kandel 2001; R.J. Crook and Walters 2011).

Although invertebrate research demonstrating the presence of nociceptors does exist, there is a notable lack of invertebrate pain research to understand the evolution of nociception, compared to other animal models (Walters and Williams 2019), and the ethical implications of using invertebrates as animal models. A better understanding of nociception and its modulation has potential for improved understanding and mitigation of pain and discomfort, and more information about animal nociception could lead to changes in the regulation of invertebrate research. Burrell suggests that invertebrates have been underutilized in nociception research despite advantages, such as comparative biology approaches to study nociceptive modulation among various species (using afferent nerves specialized for nociceptive versus non-nociceptive stimuli) on cellular and physiological levels. The detailed characterization of invertebrate nervous systems and electrophysiology methods allow greater insights into nociception research (Burrell 2017).

Despite the lack of nociceptors in most invertebrates, it may be prudent to invoke the precautionary principle when considering research on invertebrates; that is to say that we make careful deliberative decisions about how to act, especially where well-being, human or otherwise, is at stake, and when the harms or outcomes of a particular course of action are uncertain. Specifically, researchers could act compassionately by assuming that animals feel pain (Birch 2017). This also ties into the minimization principle, according to which researchers should minimize any pain or harm to the animals (J. Tannenbaum 1999). 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 (*Guide for the Care and Use of Laboratory Animals* 2011; Office of Laboratory Animal Welfare; *The Institutional Animal Care and Use Committee. Office of Laboratory Animal Welfare*). 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 as well as other nonscientific members concerned about welfare. However, the purview of the IACUC does not include the protection of order animals, including all invertebrates except cephalopods (Carruthers 2007; Harvey-Clark 2011; Drinkwater et al. 2019b). Even guidelines on cephalopods are considered recommendations, without required regulations or enforcements (Fiorito et al. 2015), so cephalopod research remains unregulated in the U.S. (J. A. Mather 2019).

In contrast, the European Union and other countries do have protective legislation for some invertebrates *(Browman et al. 2018)*. To highlight an example, the Australian Code of Practice examines four aspects of animal research: well-being, stress, distress, and pain (*Australian code for the care and use of animals for scientific purposes* 2013). Although behavioral displays in animals are subject to human interpretation, Australia's National Health and Medical Research Council 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 (*Australian code for the care and use of animals for scientific purposes* 2013).

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 can be ethical, particularly for invertebrates without evidence of nociceptors. However, researchers must take care to follow the precautionary and minimization principles, as is described in the case study.

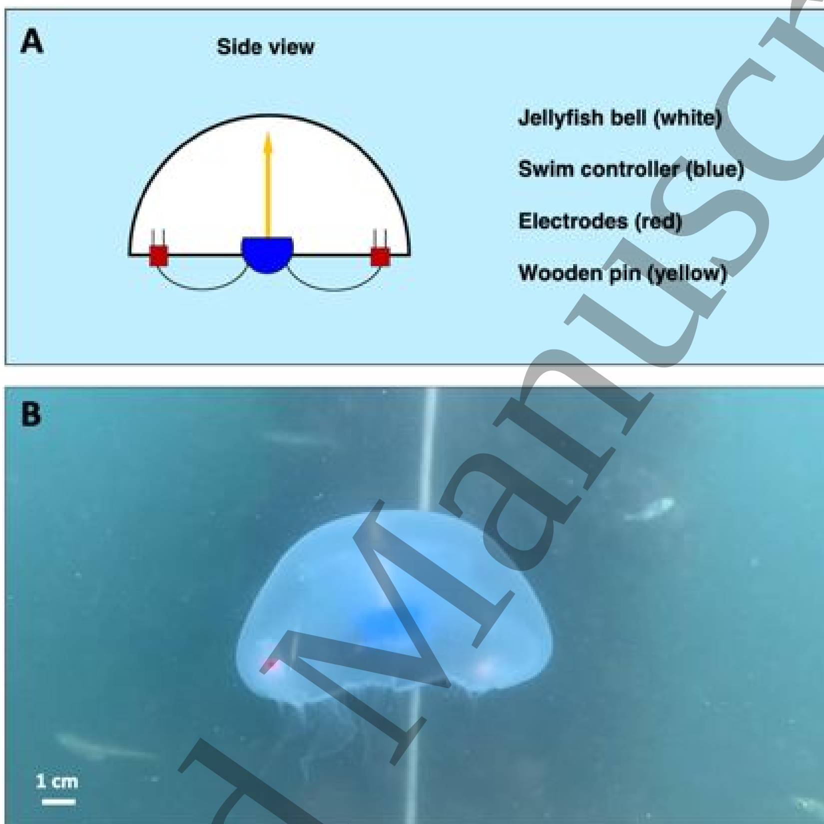

Figure 1. Biohybrid robotic jellyfish. (a) Side view schematic of a biohybrid robotic jellyfish, which shows the jellyfish bell (white) and swim controller components: the microelectronic swim controller (blue), connected via wires to two electrodes (red), and attached to the jellyfish using a wooden pin (yellow). (b) An example of a biohybrid robotic jellyfish deployed in Woods Hole, MA. More information about the system and experiments is described in (N. W. Xu et al. 2020; N. W. Xu and Dabiri 2020; N. Xu et al. 2021).

CASE STUDY USING BIOHYBRID ROBOTIC JELLYFISH

Jellyfish nociception and stress responses

Jellyfish (medusae, the adult form) are invertebrate animals with a bell-shaped body structure composed of flexible mesogleal tissue and a muscle monolayer that lines the subumbrellar surface of the bell. Within the phylum Cnidaria, the moon jellyfish *Aurelia aurita* is a species of scyphozoa, or true jellyfish, which possess eight-fold radial symmetry about the oral-aboral body axis. Other classes of jellyfish exist, such as cubozoa (box jellyfish) and hydrozoa (which include non-jellyfish species). No evidence suggests the presence of nociceptors in the Cnidarian class of scyphozoa, which include *A. aurita (Smith and Lewin 2009; Sneddon 2015)*. As ethicists and scientists have claimed, pain is both subjective and potentially constrained to a central nervous system (CNS) (Rose 2007; Jones 2012; Key 2016). *A. aurita* thus offers advantages because of their lack of brain, CNS, and nociceptors. Even among different classes of jellyfish, scyphomedusae possess the most diffuse organization of nerves (Arai 1996; Satterlie 2011; Katsuki and Greenspan 2013).

Jellyfish have distributed non-polarized neuronal networks, which consist of eight sensory structures (rhopalia) and two nerve nets: the motor nerve net (MNN) and diffuse nerve net (Arai 1996; Satterlie 2011; Katsuki and Greenspan 2013; Byrne 2019). The rhopalia are equally distributed along the margin of the bell and directly activate the MNN, which incites muscle contractions. The eight rhopalia have a semi-independent relationship that can produce coordinated muscle contractions (Hayward 2009) with redundancy (Lerner et al. 1971; Pallasdies et al. 2019). Additional details about the neuronal system of *Aurelia* are available in Pallasdies *et al. 2019*, from a single-neuronal level to organism behavioral level. 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 observed as a defense mechanism from external stimulation (Liu et al. 2018). However, jellyfish also secrete mucus for normal behaviors, such as feeding, reproducing, and modulating immunity (Patwa et al. 2015). The distinction between normal and stress-induced mucus secretion is only apparent on proteomic, metabolomic, and transcriptomic levels, not observable in behavior. These include tryptamine and metabolites present in stress-induced mucus in *A. coerulea (Patwa et al. 2015)* and gene expression changes in *A. aurita* (Tessler et al. 2020).

Summary of research

A series of experiments were conducted to build biohybrid robotic jellyfish, composed of an external microelectronic system that controls the muscle contractions of live *Aurelia aurita* (see Fig. 1). This research can advance biology, ecology, and evolution by better understanding the locomotion of jellyfish as a basal organism; advance robotics by using biohybrid approach to address constraints, such as power consumption and damage tolerance; and have broader implications for improved ocean monitoring tools to track climate change. Because the bell structure and swimming motion in jellyfish are closely linked to behaviors, such as feeding and predator-prey interactions (Arai 1996), jellyfish are advantageous as a model organism for their evolutionary and ecological insights. It has been hypothesized that their bell structure is beneficial because of the animal's energy efficiency, measured by the cost of transport (COT, a metric of energy efficiency defined as the mass-specific energy per distance traveled).

This research involved the following experiments, using ten or fewer animals per each experiment, as described in (N. W. Xu and Dabiri 2020) and (N. W. Xu et al. 2020), with detailed protocols in (N. Xu et al. 2021).

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. Animals recovered post-excision.
3. 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. Free-swimming experiments conducted in the coastal waters of Massachusetts (*N* = 4) to confirm swimming speed enhancements in laboratory results, as a proof of concept that biohybrid robotic jellyfish could be used in future ocean monitoring applications (Fig. 1B).

Ethical considerations

Welfare interests

Restrictions on higher-order animals, including vertebrates and cephalopods, are in place to protect their welfare interests. Because jellyfish lack a CNS, it is unclear whether jellyfish have welfare interests that can be harmed through experimentation. As already described, debates on the ethics of invertebrate research are ongoing and inconclusive (Carruthers 2007; Harvey-Clark 2011; Drinkwater et al. 2019b). However, to err on the side of caution in accordance with the precautionary and minimization principles, we should apply the 3Rs (Jerrold Tannenbaum and Bennett 2015; Würbel 2017): *reduction*, the minimization of animals used to answer the scientific question; *replacement*, the use of animal alternatives where possible; and *refinement*, procedural changes to minimize pain, suffering, and distress (Jerrold Tannenbaum and Bennett 2015; Würbel 2017).

Nevertheless, the way welfare interests are considered still varies. In particular, the application of the reduction principle – which reduces the number of animal test subjects – is utilitarian in nature; however, instead of a utilitarian framework, Tannenbaum uses a rights-based ethical framework to argue that "fairness to individual animals" requires using more animals instead of fewer, or causing more total pain to minimize the cost to an individual animal (J. Tannenbaum 1999).

Dignity or integrity interests

Given the ethical issues raised by readers and critics of this research, it is appropriate to apply the aforementioned 3Rs based on an argument from interests based in dignity or integrity (Bovenkerk et al. 2002). This argument states that the 3Rs should be applied, even in the absence of sentience, because "animals of sufficient complexity and stability" are owed protections from their right to dignity (deontological framework). This brings up similar ambiguities, in which some ethicists argue that invertebrates do not have the required complexity, given their lack of 'self.' For example, planarian flatworms can regenerate from each of 281 cut pieces of one parent worm (Fig. 2D) (Morgan 1898). This principle has also been seen in *A. aurita*, in which partial jellyfish can redistribute their body structure to survive (Fig. 2E) (Abrams et al. 2015).

Wisdom of repugnance

The 'wisdom of repugnance' or 'yuck factor' states that any intuitive negative response should be interpreted as evidence of intrinsic evil or harm (Midgley 2000). Kass states that reflexive revulsion reveals the intrinsic morality of the experiment, citing human cloning (Kass 1998), but critics argue that repugnance is built upon prejudices. Repugnance responses should then be scrutinized rather than regarded a source of moral insight (Turner 2004). Thus, although 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. Although public perception of ethical concerns may not speak to what is right or wrong from a philosophical perspective, we address public concerns for completeness.

Presumption of restraint

One criticism of animal biotechnology is that the current paradigm is overly permissive of experimentation under unclear ethical situations. Bioethicists, such as Fiester, 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" (Fiester 2008). This requires that research projects involving invertebrates be treated seriously and not as art, novelty, or entertainment. In essence, the presumption of restraint extends respect and gratitude toward research animals, which positions researchers as responsible stewards.

Stewardship

Stewardship is rooted in the responsibility that researchers care for animals used for research purposes (Seamer 1998). 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 3Rs and use resources appropriately, and requires that animals are used efficiently. For example, duplicate experiments are an inefficient use of animals (Chalmers et al. 2014). While the number of animals used in experiments should be minimized, a small sample size may reduce the reliability and reproducibility of the experiments, potentially increasing the total number of animals. For example, if initial studies were underpowered and had inaccurate assumptions, then other studies could need additional time and resources to replicate the studies or obtain more accurate data.

Environmental impacts

Research in jellyfish modification can also have potential environmental impacts, including electronic and plastic waste in the ocean; effects on the animals' ability to eat and reproduce; and far-reaching implications for other interlinked species (Bezanson et al. 2013). The current state of deploying biohybrid jellyfish robots into oceans 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 in accordance with philosophical environmental ethics, which affirms the value of the environment as a coherent and diverse ecosystem (Callicott 1989).

PRECEDENTS AND ANALOGOUS WORK

Before further discussing the case study, we will examine analogous precedents to provide insight into ethical considerations. In particular, electrical stimulation is a broad method that has been applied previously in electrophysiology experiments on jellyfish (Romanes 1876; Romanes 1879; Romanes 1885; Horridge 1954; Horridge 1956), robotic control of insect locomotion (Bozkurt et al. 2009; H. Sato et al. 2009; Hirotaka Sato and Maharbiz 2010; Roboroach), and human enhancement for rehabilitation (Vidal 1973; Flesher et al. 2016; S. W. Lee et al. 2016). Additional work on invertebrates has also included excisions in aquatic invertebrates, with one application for biohybrid robotic integration using sea slugs (Webster et al. 2016) despite prior evidence of their nociception (Walters and Moroz 2009).

The element of 'playing God' – or human manipulation of the natural world – is one major critique of scientific research ethics (Drinkwater et al. 2019a),. Examples include cyborg insects

(Maharbiz and Sato 2010; Underwood 2013; Ethical Issues Regarding the Use of Invertebrates in Education), 'microslavery' of microbes (Harvey et al. 2014), and genetically modified organisms for food and agriculture (Devos et al. 2008). Among these disparate examples, one criticism is that human control might lead to the elimination of the animal's freedom through their modification. However, as cited previously, both human and non-human vertebrate experiments are regulated by governing ethical boards (*Australian code for the care and use of animals for scientific purposes* 2013; Browman et al. 2018; *The Institutional Animal Care and Use Committee. Office of Laboratory Animal Welfare*), although there have been significant criticisms of these ethics committees in both human and nonhuman animal research (Francione 1995; Hansen 2013; Hall et al. 2016). Modification in this manner need not lead to an elimination of freedom, or such a loss of freedom may be justified by other goods which are gained. Thus, the following subsections will demonstrate that invertebrate animal welfare is primarily valued in the context of human exploitation of the natural world, and undervalued for the invertebrates themselves.

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

Biohybrid robotic insects to control locomotion, such as RoboRoach (Roboroach), are the closest analogous cases to biohybrid robotic jellyfish. RoboRoach – a toolkit that allows the wireless control of live cockroach locomotion by electrically stimulating its antenna nerves – has been touted as "the world's first commercially available cyborg," as shown in Figure 2A (Roboroach). To address ethical issues, RoboRoach's parent company Backyard Brains notes in a web page dedicated to ethical issues that its protocols are annually reviewed by an external ethics review board, although it should be noted that this is a commercial entity with vested interests to supports its products (Ethical Issues Regarding the Use of Invertebrates in Education). Regardless, 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 (Devos 2007). 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. 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.

Furthermore, Backyard Brains demonstrates due diligence in its online ethics page. 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 (Ethical Issues Regarding the Use of Invertebrates in Education). 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 (Marzullo 2016; Experiment: Wirelessly Control a Cyborg Cockroach). 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 (Ethical Issues Regarding the Use of Invertebrates in Education), which is a potential model for commercialization or larger-scale applications of other technologies.

Prior literature also demonstrates the flight control of moths (Tsang et al. 2010) and giant beetles (H. Sato et al. 2009; Hirotaka Sato and Maharbiz 2010; Hirotaka Sato et al. 2015). The 'cyborg beetle' (Fig. 2B) has also come under ethical scrutiny, with some suggestions that such animal modification should be replaced by pure technology (Baharudin 2016). Nevertheless, justifications for this research include the overarching goal to build energy-efficient robots for search-and-rescue missions and reconnaissance, where tens of thousands of lives are lost annually in natural disasters (Maharbiz and Sato 2010). Cyborg insects offer exciting advantages for search-and-rescue operations because, currently, no purely artificial systems can match the locomotion and robustness of these insect-machine hybrid systems (Nguyen et al. 2022).

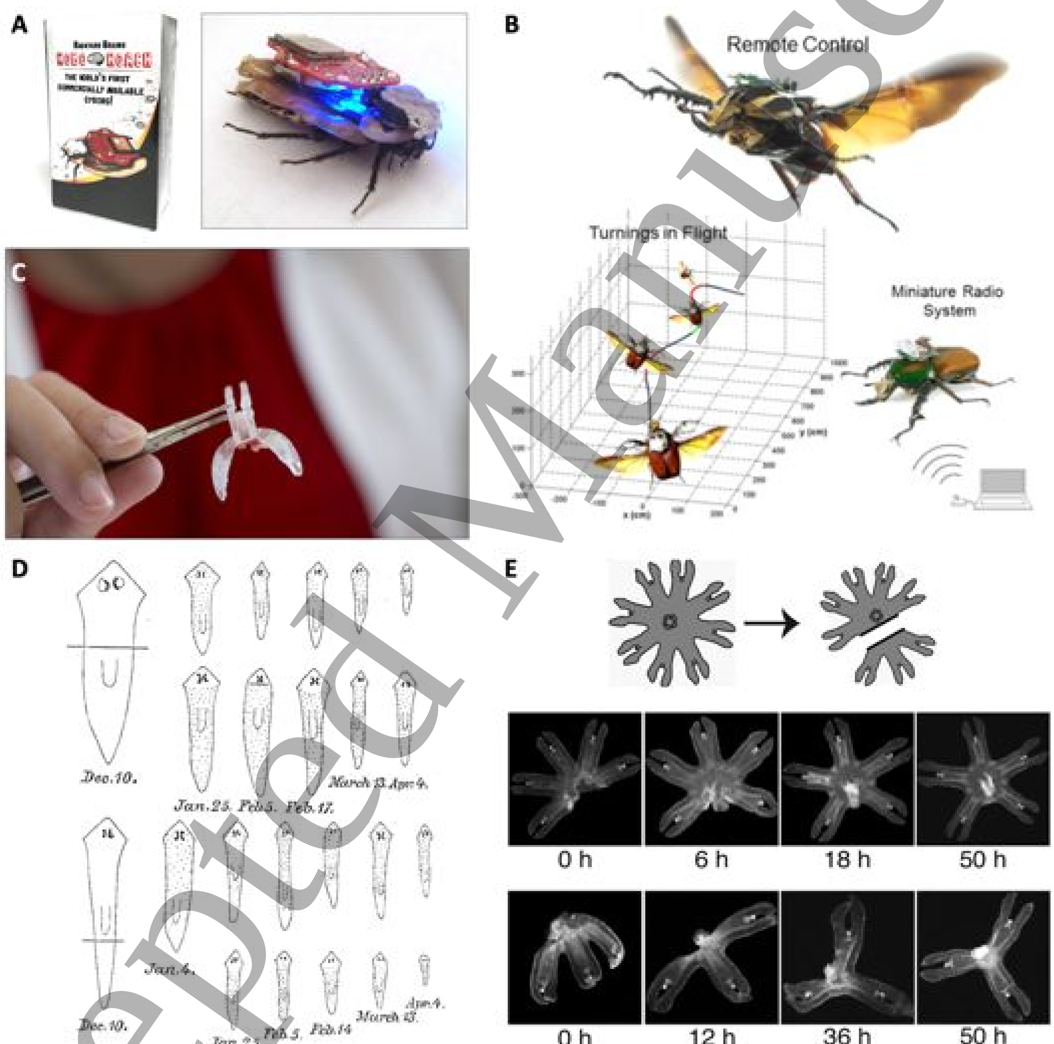

Figure 2. Examples of invertebrate research. A brief selection of comparable invertebrate research, including biohybrid robotic insects and tissue cutting experiments: (a) The commercially available RoboRoach kit and an example of a cyborg cockroach, which users can surgically modify to control animal locomotion (Roboroach). (b) A 'cyborg beetle,' which uses a similar concept of

microelectronic stimulation to control its motion (Hirotaka Sato et al. 2015). (c) A biohybrid robot incorporating muscle tissue from sea slugs (Webster et al. 2016), which have been reported to possess nociceptors (Walters and Moroz 2009). Photo credit to Victoria Webster/Case Western University. (d) A schematic of tissue cutting experiments to observe tissue regeneration in planaria. Planaria were reported to regenerate the entire animal body when cut into 281 separate pieces or fewer (Morgan 1898). (e) A schematic and time series of excised jellyfish and tissue healing experiments (Abrams et al. 2015) using *A. aurita*, the same species of jellyfish used to develop biohybrid robots.

These examples illustrate the concern for invertebrate animal welfare in the context of human control, despite the fact that cockroaches are considered as 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 involve or encourage acts of animal cruelty. It is also noteworthy that up to millions of individual fruit flies, mosquitoes, and nematodes are dissected for neuroscience and healthcare research, yet these practices rarely face the public criticism that cyborg invertebrate robots encounter.

Experiments on entirely soft-bodied invertebrates

In comparison, the integration of muscle tissue from the sea slug *A. californica* into a biohybrid robot (Webster et al. 2016) (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” (Bond 2016). Despite the ‘yuck factor,’ no specific ethical issues have been raised about harm to the sea slugs, despite the confirmed presence of nociceptors (Walters and Moroz 2009). 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 281 pieces that regenerate (Morgan 1898) (Fig. 2D). Additional examples include experiments demonstrating the reaggregation of dissociated tissue in Porifera sea sponges, using chemical methods of tissue dissociation, as well as mechanical methods such as extruding sea sponges through a sieve (Lavrov and Kosevich 2016).

Tissue cutting and regeneration experiments have also been conducted on *A. aurita*, the species of jellyfish used in biohybrid robotic studies. Cutting experiments and electrical stimulation to understand conduction of the jellyfish nervous system were reported from 1891 to 1971, including excision to form donut-like rings and strips of tissue (Romanes 1876; Romanes 1879; Romanes 1885; Horridge 1954; Horridge 1956). 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. Jellyfish were anesthetized using menthol and magnesium chloride solutions as a muscle relaxant and analgesic, and most animals recovered within days or weeks (Abrams et al. 2015).

These experiments pose 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 such statements for invertebrate studies. This suggests that the public's ethical dilemmas about invertebrate research may primarily focus on the wisdom of repugnance, not animal welfare or dignity interests, regardless of the potential 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 lower order invertebrates. Although public and media perception of ethical concerns may not speak to what is right or wrong from a philosophical perspective, addressing public concerns is crucial to build public trust in science and promote scientific literacy through scientific communication.

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

Biohybrid robotic jellyfish may raise new ethical questions (Rogers 2020). In light of the ethical considerations presented here, 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, pose a larger ethical debate. This incongruity highlights the first overarching consideration, which is that the wisdom of repugnance is key in public opinion. There is unease in research that appears to be 'playing God,' both for welfare issues and for potentially a slippery slope toward moral turpitude, although ethical guidelines exist to prevent this escalation (*Australian code for the care and use of animals for scientific purposes* 2013; Browman et al. 2018; Office of Laboratory Animal Welfare; *The Institutional Animal Care and Use Committee. Office of Laboratory Animal Welfare*). However, this underscores a potential mismatch between popular opinion and ethical determination. Among philosophical discussions, ethical animal research still focuses largely on the minimization and precautionary principles and broader issues than public perception.

Second, Mather suggests that humans selectively decide which animals deserve welfare rights based on concepts such as utility (e.g. honeybees) and aesthetics (e.g. butterflies) (J. A. Mather 2019), based on Kellert's survey of the public perception of invertebrates (Kellert 1993). 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 excision experiments (Abrams et al. 2015) suggests that the wisdom of repugnance is still the primary broadly recognized ethical concern.

Third, the question of ecological consequences introduces an additional complexity to the ethics of this case study. The long-term goal to use biohybrid robotic jellyfish as ocean monitoring tools raises questions about 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 to the ocean would be, warrant further discussion to improve stewardship and reduce negative environmental impacts. We will discuss our approach to addressing these ethical considerations, 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 3Rs. The scientific consensus is that jellyfish do not have sentience or pain because of their distributed nervous systems. This is corroborated by the question of whether jellyfish lack a sense of 'self,' given jellyfish halves can subsist separately (Abrams et al. 2015). Regardless, the 3Rs 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 free-swimming 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 insufficient numbers of tests occurred within the field experiments, in which video data for 2 out of the 4 total animals could not be used for image analysis. For statistical significance, we were originally set to obtain usable data for 4 animals, which would have required testing an additional 2 animals at the very least. However, instead of conducting additional field experiments we used only 2 animals without statistical significance, and the two videos unsuitable for image analysis were still used as observational data to retain their utility despite criticism from reviewers about the lack of field data. Although reduction is meant to occur during the planning stage, we did not want to increase the number of animals after our consideration of reduction in the planning stage. Thus, these numbers were chosen after power analyses considering the effect size, sample size, variability, and statistical significance to generate pilot data appropriate for our studies.
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, so this model can be used as an instance of replacement for future research.
3. *Refinement:* Refined protocols included procedures in alignment with the precautionary principle to minimize any potential pain, suffering, and distress. In accordance with this principle, 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 adhesives (B. P. Lee et al. 2011), which caused larger areas of tissue damage after removal, compared to a pin hole that healed within a day after removal.

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, animal behavior returned to baseline after the robotic devices were removed.

Finally, another aspect related to refinement included anesthetics. Although we tested menthol and magnesium chloride solutions (Abrams et al. 2015), these chemicals arrested jellyfish motion and negatively impacted studies of both locomotion and oxygen consumption (i.e., these chemicals inhibited animal motion entirely and affected respiration rates). Therefore, we were unable to use anesthetics for refinement; 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.

In summary, all animals recovered post-experimentation with minimal instances of long-term effects, and were subsequently able to swim, feed, and reproduce. For example, all animals in muscle excitation experiments, oxygen consumption experiments, and field tests had no resulting side effects; all 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; 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 bell deformation usually resulted from animals being constrained in the corner of tanks, which also occurred with typical animal husbandry, including in aquariums.

Care was taken to ensure that a minimum number of animals were used, and that all animals were allowed to recover 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 (Liu et al. 2018) suggest that stress markers might also be observed in these spatiotemporal muscle response experiments in the absence of seawater. Previous work has also shown stress-induced differences in the transcriptome of *A. aurita* when handled roughly (Tessler et al. 2020), 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 species. First, there is an open question of whether the microelectronic swim controller affects the feeding, longevity, livelihood, and reproduction of individuals, which can affect the evolutionary fitness of the species. The current microelectronic system is limited to tests up to a few hours at maximum. 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 behaviors that might impact species-level survivability.

However, an important consideration is whether decreased species-level survival is even relevant, given the overpopulation of jellyfish blooms that can negatively impact the environment(Condon et al. 2012; Condon et al. 2013; Graham et al. 2014). *A. aurita* have been considered an invasive species (Malej et al. 2007; Manzari et al. 2015).

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. As noted, *A. aurita* and other jellyfish species are considered nuisances, with ecological consequences from increases in jellyfish blooms (Condon et al. 2012; Graham et al. 2014; Fossette et al. 2015) and invasive takeovers of coastal lagoons (Malej et al. 2007; Manzari et al. 2015). Such blooms can also negatively impact human industries, such as fisheries and tourism (Condon et al. 2013). For field experiments in which *A. aurita* were tested in the Atlantic Ocean, biohybridic robotic jellyfish were closely monitored 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 (Condon et al. 2013). The specific causes of these blooms have ranged from natural cyclical variation to anthropogenic causes and climate change (Condon et al. 2012; Condon et al. 2013; Graham et al. 2014), but the high evolutionary fitness of jellyfish is likely due to its multi-phase life cycle (Takao et al. 2014), which includes sessile polyps (asexual) and free-swimming medusae (sexual) (Arai 1996). Variations in environmental conditions could favor dense populations of either phase or reproductive type (Duarte et al. 2013).

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 (Thevenon et al. 2015; Boucher and Friot 2017), 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 (Im et al. 2020) and amphipods (Hodgson et al. 2018). This requires investigation into using more environmentally friendly materials, such as biodegradable electronics (Irimia-Vladu et al. 2012; Lei et al. 2017; Li et al. 2018) and plastic (Lott et al. 2020).

Because the current technology for biohybrid robotic jellyfish is not at this level, it is difficult to make assessments of long-term ecological impacts. Regarding the case study, care was taken to ensure that no components were left in the ocean after the field tests in (N. W. Xu et al. 2020).

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 3Rs 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 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, if biohybrid robotic jellyfish could detect and prevent coral bleaching, does this benefit to the environment outweigh sacrificing small numbers of jellyfish or marine animals that ingest the jellyfish? Using live jellyfish as ocean drones could also reduce our overall environmental impact, compared to traditional underwater vehicles. For instance, the failure of one underwater vehicle could introduce orders of magnitude more plastic, metal, and electronic waste than an entire swarm of biohybrid robotic jellyfish. The energy efficiency of live jellyfish also reduces the power consumption required for operation, thus enabling longer ocean monitoring expeditions, which could allow us to track markers of climate change over a longer spatiotemporal scale. The more natural wakes of biohybrid robotic jellyfish could lead to more observations of natural animal behaviors in the deep sea. Finally, using the animal's living tissue as natural sensors could enable us to detect more subtle changes in water quality. These hypothetical situations could be helpful in determining whether to use live jellyfish for various applications.

These open questions and critiques about the long-term effects of deploying biohybrid robotic jellyfish 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. In the meantime, the presumption of restraint principle stands.

General invertebrate research

A significant stakeholder in this case study is each jellyfish test subject, which compels further examination into general invertebrate ethics. Although the majority of ethical guidelines do not require protocol submissions and welfare checks, researchers should use minimization and precaution, and follow the 3Rs. 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 why alternatives to animal research cannot be done with comparable information.
- Number of animals used and a discussion of the 3Rs.
- 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.
- Potential benefits of invertebrate research and rationale for pursuing these innovations, as a method of educating the general public.

The lack of systemic ethical oversight on invertebrate research also underscores the lack of knowledge about invertebrate pain. Therefore, we also recommend further 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, with examples of ethical issues and policy strategies for biohybrid research listed in Mestre et al 2024. To illustrate, biohybrid robots offer a promising alternative to fully artificial systems, which possess substantial challenges and limitations. It is unclear whether the investment of time and resources to build robots that match the agile locomotion of animals at similar scales is warranted – or even possible – when biohybrid systems can already achieve these goals. 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.

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The authors N.W.X. and J.O.D. conducted research on biohybrid robotic jellyfish.

AVAILABILITY OF DATA AND MATERIAL

Not applicable

CODE AVAILABILITY

Not applicable

AUTHORS' 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.

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