Chapitre 8

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The creation of an augmented jellyfish: Ethical considerations from a scientific perspective

1. Introduction to invertebrate research

Within the animal kingdom, invertebrates constitute approximately 97 % of all species[1,2], and experiments using invertebrate model organisms have provided a wealth of scientific information. For example, research using nematodes[3], fruit flies[4], and squids[5] have had strong impacts in fundamental and translational biology[6], medicine[7], and ecology[8], as well as in physics and engineering[9].

Despite its prevalence, the majority of invertebrate animal work has been unregulated, or conducted without the jurisdiction of formal committees to date. Historically, such uses of invertebrates were justified as more ethical than using higher order vertebrate species because of lack of pain perception and other factors, but more recent discussions have questioned this narrative[10]. To illustrate, the United Kingdom's Animal Welfare (Sentience) Act 2022[11] listed calls for more protections over cephalopod mollusks (e.g., squids and octopuses) and decapod crustaceans (e.g., lobsters and crabs), a subset of aquatic invertebrates. After the bill's introduction in May 2021, both public critique[12] and Parliamentary debates[13] further highlighted the exclusion of most invertebrate species as sentient beings, although the enacted bill explicitly does enable "amend[ments] ... so as to bring invertebrates of any description within the meaning of 'animal' for the purposes of this Act"[14].

1. Rodrigo B. Salvador et al., "Invertebrates in Science Communication: Confronting Scientists' Practices and the Public's Expectations," *Frontiers in Environmental Science* 9 (March 9, 2021): 606416, https://doi.org/10.3389/fenvs.2021.606416.
2. Robert M. May, "How Many Species Are There on Earth?," *Science* 241, no. 4872 (September 16, 1988): 1441-49, https://doi.org/10.1126/science.241.4872.1441.
3. Ann K. Corsi, Bruce Wightman, and Martin Chalfie, "A Transparent Window into Biology: A Primer on Caenorhabditis Elegans," *WormBook*, June 18, 2015, 1-31, https://doi.org/10.1895/wormbook.1.177.1.
4. Michael F Wangler, Shinya Yamamoto, and Hugo J Bellen, "Fruit Flies in Biomedical Research," *Genetics* 199, no. 3 (March 1, 2015): 639-53, https://doi.org/10.1534/genetics.114.171785.
5. Joseph A. DeGiorgis, Marcus Jang, and Elaine L. Bearer, "The Giant Axon of the Squid: A Simple System for Axonal Transport Studies," in *Axonal Transport*, ed. Alessio Vagnoni, vol. 2431, Methods in Molecular Biology (New York, NY: Springer US, 2022), 3-22, https://doi.org/10.1007/978-1-0716-1990-2_1.
6. Norbert Perrimon, Nancy M. Bonini, and Paramindar Dhillon, "Fruit Flies on the Front Line: The Translational Impact of *Drosophila*," *Disease Models & Mechanisms* 9, no. 3 (March 1, 2016): 229-31, https://doi.org/10.1242/dmm.024810.
7. Wangler, Yamamoto, and Bellen, "Fruit Flies in Biomedical Research."
8. R. Rosas-Luis et al., "Importance of Jumbo Squid Dosidicus Gigas (Orbigny, 1835) in the Pelagic Ecosystem of the Central Gulf of California," *Ecological Modelling* 218, no. 1-2 (October 2008): 149-61, https://doi.org/10.1016/j.ecolmodel.2008.06.036.
9. Victoria A. Webster-Wood et al., "Organismal Engineering: Toward a Robotic Taxonomic Key for Devices Using Organic Materials," *Science Robotics* 2, no. 12 (November 22, 2017): eaap9281, https://doi.org/10.1126/scirobotics.aap9281.
10. Michael W. Brunt, Henrik Kreiberg, and Marina A. G. von Keyserlingk, "Invertebrate Research without Ethical or Regulatory Oversight Reduces Public Confidence and Trust," *Humanities and Social Sciences Communications* 9, no. 1 (August 1, 2022): 250, https://doi.org/10.1057/s41599-022-01272-8.
11. United Kingdom Parliament Public General Acts, "Animal Welfare (Sentience) Act 2022," 2022 c. 22§ (2022).
12. Nicola Clayton and Alexandra Schnell, "Why Invertebrates Should Be Included in Animal Welfare Protections," *New Scientist*, July 28, 2021, https://www.newscientist.com/article/mg25133452-200-why-invertebrates-should-be-included-in-animal-welfare-protections/.
13. "Laboratory Animals: Animal Welfare Act" (United Kingdom Parliamentary Debates Volume 703, February 7, 2022), https://hansard.parliament.uk/commons/2022-02-07/debates/E7D8AF2F-9BB3-4475-86D6-39091FB54AC4/LaboratoryAnimalsAnimalWelfareAct.
14. United Kingdom Parliament Public General Acts, Animal Welfare (Sentience) Act 2022.

1.1. Regulations and expert committees overseeing animal welfare

Although the Animal Welfare Act 2022 is the first to recognize sentience in animals in British law, the idea of animal sentience as a potential component of welfare has been established for decades[15, 16]. Similarly, calls to extend humane care to select invertebrate species, both because of and regardless of sentience arguments, have also gained traction in recent years[17, 18, 19]. Within the context of animal experiments, these welfare rights have mostly focused on the treatment of individual test subjects[20].

To ensure the welfare of animal subjects, expert committees and organizations provide ethical guidelines for scientists, approve research protocols, and enforce local regulations, which are dependent on both individual institutions and country-specific guidelines. For example, in the United States, each university establishes its own Institutional Animal Care and Use Committee (IACUC), which typically comprises veterinarians, animal scientists, bioethicists, and other nonscientific members. The IACUC then oversees animal care and use within the university. However, invertebrate animals are not protected under the IACUC, with the exception of cephalopods; even cephalopod-specific guidelines are only recommendations and not enforced regulations[21]. In contrast, the European Union (EU) and countries such as Australia do provide legislation for select invertebrate species[22], with Directive 2010/63/EU of the European Parliament and of the Council[23] touted as "set[ting] amongst the most stringent ethical and welfare standards worldwide" according to EuroScience[24]. Furthermore, the Australian Code of Practice explicitly examines four aspects of animal research: wellbeing, stress, distress, and pain[25].

1.2. Pain and nociception

One nuance within animal welfare discussions is the discernment of pain from nociception. According to the International Association for the Study of Pain, the

15. Andrew N Rowan et al., "Animal Sentience: History, Science, and Politics," *Animal Sentience* 6, no. 31 (January 1, 2021), https://doi.org/10.51291/2377-7478.1697.
16. Marian Stamp Dawkins, "The Science of Animal Sentience and the Politics of Animal Welfare Should Be Kept Separate," *Animal Sentience* 6, no. 31 (January 1, 2022), https://doi.org/10.51291/2377-7478.1708.
17. Claudio Carere and Jennifer Mather, eds., *The Welfare of Invertebrate Animals*, vol. 18, Animal Welfare (Cham: Springer International Publishing, 2019), https://doi.org/10.1007/978-3-030-13947-6.
18. Kelsey Horvath *et al.*, "Invertebrate Welfare: An Overlooked Issue", *Annali Dell'Istituto Superiore Di Sanita* 49, no. 1 (2013): 9-17, https://doi.org/10.4415/ANN_13_01_04.
19. Irina Mikhalevich and Russell Powell, "Minds without Spines: Evolutionarily Inclusive Animal Ethics," *Animal Sentience* 5, no. 29 (January 1, 2020), https://doi.org/10.51291/2377-7478.1527.
20. Marian Stamp Dawkins, "The Science of Animal Suffering," *Ethology* 114, no. 10 (October 2008): 937-45, https://doi.org/10.1111/j.1439-0310.2008.01557.x.
21. *The Institutional Animal Care and Use Committee. Office of Laboratory Animal Welfare* (National Institutes of Health, n.d.), https://olaw.nih.gov/resources/tutorial/iacuc.htm.
22. Howard I. Browman *et al.*, "Welfare of Aquatic Animals: Where Things Are, Where They Are Going, and What It Means for Research, Aquaculture, Recreational Angling, and Commercial Fishing," *ICES Journal of Marine Science* 76, no. 1 (January 1, 2019): 82-92, https://doi.org/10.1093/icesjms/fsy067.
23. "DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the Protection of Animals Used for Scientific Purposes," *Official Journal of the European Union* 276 (September 22, 2010): 33-79.
24. "EuroScience Supports Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes," *EuroScience*, March 13, 2015, ports-directive-201063eu-on-the-protection-of-animals-used-for-scientific-purposes/.
25. *Australian Code for the Care and Use of Animals for Scientific Purposes* (Canberra, ACT: National Health and Medical Research Council Universities Australia CSIRO, 2013).

definition of pain is "an unpleasant sensory and emotional experience associated with ... actual or potential tissue damage"[26]. This describes pain as a central phenomenon, not peripheral, that requires both a centralized nervous system (CNS) and sentience[27]. To compare, the definition of nociception is a "physiological response to noxious stimuli that cause or potentially cause tissue damage"[28]. Nociceptive responses describe objective experiences, which exclude subjectivity, such as emotions and "unpleasant" sensations. Thus, while nociception is only sensory information about the state of the tissue, pain is the perception and interpretation of nociceptive signals[29,30].

Regarding pain and nociception in invertebrates, although some aquatic invertebrates such as cephalopods[31] and the sea slug *Aplysia californica*[32] possess nociceptors, most lower order animals do not have nociceptors or even nociceptive responses[33]. In fact, the existence of nociceptors and responses before the evolution of bilateral symmetry is minimally supported[34]. Among invertebrate animals that do not possess nociceptors and have no regulatory protections is *Aurelia aurita,* the common moon jellyfish[35,36].

1.2.1. Jellyfish nociception and stress responses

Aurelia aurita is a species of true jellyfish (class scyphozoa) that has been studied extensively within biology[37], fluid dynamics[38], and, more recently, robotics[39] for its simple, radially symmetric anatomy and efficient locomotion. Its adult form is composed of a bell-shaped body with flexible tissue (mesoglea) and a singular

26. Srinivasa N. Raja *et al.*, "The Revised International Association for the Study of Pain Definition of Pain: Concepts, Challenges, and Compromises," *Pain* 161, no. 9 (September 2020): 1976-82, https://doi.org/10.1097/j.pain.0000000000001939.
27. Robert C. Jones, "Science, Sentience, and Animal Welfare," *Biology & Philosophy* 28, no. 1 (January 2013): 1-30, https://doi.org/10.1007/s10539-012-9351-1.
28. M Magalhães-Sant'Ana, P Sandøe, and Ias Olsson, "Painful Dilemmas: The Ethics of Animal-Based Pain Research," *Animal Welfare* 18, no. 1 (February 2009): 49-63, https://doi.org/10.1017/S0962728600000063.
29. Martin Kavaliers, "Evolutionary and Comparative Aspects of Nociception," *Brain Research Bulletin* 21, no. 6 (December 1988): 923-31, https://doi.org/10.1016/0361-9230(88)90030-5.
30. Lynne U. Sneddon, "Comparative Physiology of Nociception and Pain", *Physiology* 33, no. 1 (January 1, 2018): 63-73, https://doi.org/10.1152/physiol.00022.2017.
31. Robyn J Crook, "The Welfare of Invertebrate Animals in Research: Can Science's next Generation Improve Their Lot?," *Postdoc Journal*, February 21, 2013, https://doi.org/10.14304/SURYA.JPR.V1N2.2.
32. Edgar T. Walters and Leonid L. Moroz, "Molluscan Memory of Injury: Evolutionary Insights into Chronic Pain and Neurological Disorders," *Brain, Behavior and Evolution* 74, no. 3 (2009): 206-18, https://doi.org/10.1159/000258667.
33. Ewan St. John Smith and Gary R. Lewin, "Nociceptors: A Phylogenetic View," *Journal of Comparative Physiology A* 195, no. 12 (December 2009): 1089-1106, https://doi.org/10.1007/s00359-009-0482-z.
34. Edgar T. Walters and Amanda C. de C. Williams, "Evolution of Mechanisms and Behaviour Important for Pain", *Philosophical Transactions of the Royal Society B: Biological Sciences* 374, no. 1785 (November 11, 2019): 20190275, https://doi.org/10.1098/rstb.2019.0275.
35. Lynne U. Sneddon, "Pain in Aquatic Animals," *Journal of Experimental Biology* 218, no. 7 (April 1, 2015): 967-76, https://doi.org/10.1242/jeb.088823.
36. Smith and Lewin, "Nociceptors".
37. Cathy H. Lucas, "Reproduction and Life History Strategies of the Common Jellyfish, Aurelia Aurita, in Relation to Its Ambient Environment," *Hydrobiologia* 451, no. 1/3 (2001): 229-46, https://doi.org/10.1023/A:1011836326717.
38. John H. Costello *et al.*, "The Hydrodynamics of Jellyfish Swimming," *Annual Review of Marine Science* 13, no. 1 (January 3, 2021): 375-96, https://doi.org/10.1146/annurev-marine-031120-091442.
39. Nicole W. Xu and John O. Dabiri, "Low-Power Microelectronics Embedded in Live Jellyfish Enhance Propulsion", *Science Advances* 6, no. 5 (January 31, 2020): eaaz3194, https://doi.org/10.1126/sciadv.aaz3194.

muscle layer on the subumbrellar surface oriented in a ring. The animal contracts and relaxes this muscle ring to swim, expelling a volume of water under the bell in the contraction phase before the subumbrellar volume refills in the relaxation phase[40].

In addition to its simplicity and utility in scientific research, the use of jellyfish has been more ethically justified compared to vertebrate animal counterparts because jellyfish lack of a brain, central nervous system, and nociceptors. Thus, jellyfish do not have the capacity to feel pain, which is constrained to a more centralized nervous structure. Furthermore, within the phylum Cnidaria, composed of aquatic invertebrate animals with stinging cells, the scyphozoan class of jellyfish have the most diffuse organization of nerves (in two distributed nerve nets) compared to other classes, such as cubozoa and hydrozoa[41,42]. This distributed nervous structure suggests that even among its Cnidarian peers, which already do not exhibit nociception, *A. aurita* and other scyphozoa have even less potential for experiencing pain and nociception.

Thus, because of the lack of mechanisms for pain or nociception in jellyfish, stress responses and markers can be used as a surrogate. A prominent marker of stress induction in jellyfish is the excess secretion of mucus, a defense mechanism from external physical stimuli[43]. However, normal behaviors such as feeding and modulating immunity can also induce mucus secretion[44], and the question of whether this mucus production is stress-related can only be distinguished through proteomic, metabolomic, and transcriptomic analyses, not observations of animal behavior[45].

2. Augmented jellyfish for ocean exploration

The motivation driving the creation and use of augmented jellyfish, as defined later in this section, is to add new tools to expand the world's ocean monitoring capabilities. Although the ocean is important for processes such as thermoregulation, carbon sequestration, and food production for both humans and the natural world[46], the majority of the ocean remains unexplored. More knowledge of the physical and biogeochemical processes in the ocean could elucidate important mechanisms of climate change and influence how we humans can act as responsible environmental stewards. To that end, efforts such as the United Nations Ocean Decade 2021-2030

40. Costello *et al.*, "The Hydrodynamics of Jellyfish Swimming".
41. Richard A. Satterlie, "Do Jellyfish Have Central Nervous Systems?", *Journal of Experimental Biology* 214, no. 8 (April 15, 2011): 1215-23, https://doi.org/10.1242/jeb.043687.
42. Takeo Katsuki and Ralph J. Greenspan, "Jellyfish Nervous Systems", *Current Biology* 23, no. 14 (July 2013): R592-94, https://doi.org/10.1016/j.cub.2013.03.057.
43. Wenwen Liu *et al.*, "Stress-Induced Mucus Secretion and Its Composition by a Combination of Proteomics and Metabolomics of the Jellyfish Aurelia Coerulea," *Marine Drugs* 16, no. 9 (September 18, 2018): 341, https://doi.org/10.3390/md16090341.
44. Amit Patwa et al., "Accumulation of Nanoparticles in 'Jellyfish' Mucus: A Bio-Inspired Route to Decontamination of Nano-Waste," *Scientific Reports* 5, no. 1 (June 22, 2015): 11387, https://doi.org/10.1038/srep11387.
45. Michael Tessler *et al.*, "Ultra-Gentle Soft Robotic Fingers Induce Minimal Transcriptomic Response in a Fragile Marine Animal", *Current Biology* 30, no. 4 (February 2020): R157-58, https://doi.org/10.1016/j.cub.2020.01.032.
46. Thomas F. Stocker, "The Silent Services of the World Ocean", *Science* 350, no. 6262 (November 13, 2015): 764-65, https://doi.org/10.1126/science.aac8720.

have proposed major international efforts to generate new scientific knowledge of the ocean and implement sustainable practices[47,48].

State-of-the-art tools for monitoring the ocean include aerial, surface, and subsurface instruments and vehicles[49] capable of remote sensing or collecting in situ data. Of the suite of underwater vehicles available, suech as remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs), bioinspired designs offer an additional approach to create potentially more energy efficient, inexpensive ocean probes to reach more delicate areas where traditional vehicle wakes might perturb some wildlife[50]. Thus, by looking toward nature for inspiration to design aquatic vehicles, bioinspired swimming robots could be used to access more sensitive ocean environments, such as near coral reefs and in deep-sea crevices where animal behavioral data is limited.

Among various options for model organisms, jellyfish offer a unique advantage because of their energy efficiency. To compare, we can examine a metric called the cost of transport (COT), which is an animal's mass-specific energy expended per distance traveled, in which lower COT values translate to higher energy efficiencies. *A. aurita* possess the lowest known COT for all animals, accounting for various modes of locomotion (swimming, flying, and running), and AUVs[51]. Thus, jellyfish-inspired robots could address energy storage and power consumption, one of the main limitations in robotics and ocean technologies to date.

However, within bioinspired design, there is a spectrum of approaches that ranges from purely mechanical robots[52], which do not have animal ethics concerns but often require more power than available for practical applications, to more biological constructs (such as a "medusoid" or artificial jellyfish composed of rat cardiomyocytes seeded on a thin silicone body[53]) which do have ethical considerations from using animal tissues, but the cells on the constructs are limited to specific media and can only survive in controlled laboratory environments. In contrast, the approach that we as researchers chose to pursue is the augmentation of live jellyfish. These biohybrid robotic jellyfish comprise live *A. aurita* and embedded microelectronic systems to control their swimming, including a microelectronic swim controller embedded in the center of the bell and two electrodes embedded into the bell margin for symmetrical activation of forward swimming (Figure 1).

47. Vladimir Ryabinin *et al.*, "The UN Decade of Ocean Science for Sustainable Development", *Frontiers in Marine Science* 6 (July 31, 2019): 470, https://doi.org/10.3389/fmars.2019.00470.
48. UNESCO-IOC, "The Contribution of the UN Decade of Ocean Science for Sustainable Development to the Achievement of the 2030 Agenda. Paris, UNESCO. (The Ocean Decade Series, 34)", 2022.
49. Jim Thomson *et al.*, "The Balance of Ice, Waves, and Winds in the Arctic Autumn," *Eos*, January 23, 2017, https://doi.org/10.1029/2017EO066029.
50. Nicole Xu and John Dabiri, "Bio-Inspired Ocean Exploration", *Oceanography*, 2022, 35-48, https://doi.org/10.5670/oceanog.2022.214.
51. Brad J. Gemmell *et al.*, "Passive Energy Recapture in Jellyfish Contributes to Propulsive Advantage over Other Metazoans," *Proceedings of the National Academy of Sciences* 110, no. 44 (October 29, 2013): 17904-9, https://doi.org/10.1073/pnas.1306983110.
52. Alex Villanueva, Colin Smith, and Shashank Priya, "A Biomimetic Robotic Jellyfish (Robojelly) Actuated by Shape Memory Alloy Composite Actuators", *Bioinspiration & Biomimetics* 6, no. 3 (September 1, 2011): 036004, https://doi.org/10.1088/1748-3182/6/3/036004.
53. Janna C Nawroth *et al.*, "A Tissue-Engineered Jellyfish with Biomimetic Propulsion", *Nature Biotechnology* 30, no. 8 (August 2012): 792-97, https://doi.org/10.1038/nbt.2269.

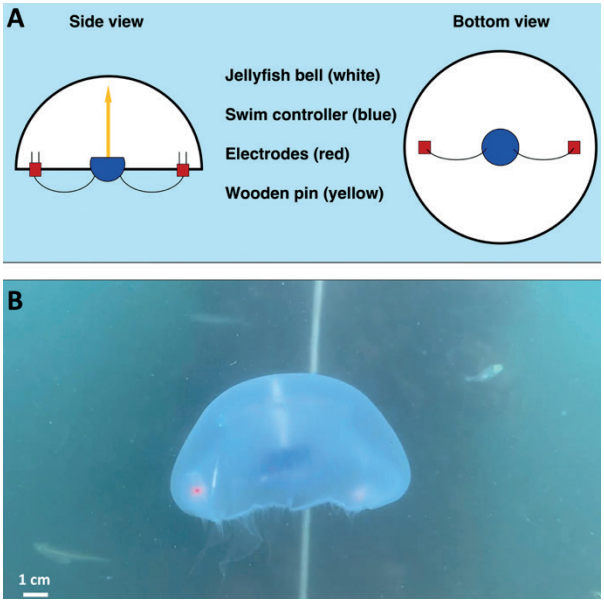

Figure 1. Augmented jellyfish. (A) Schematic to illustrate the jellyfish bell (animal body, shown in white), swim controller (in blue), two electrodes (in red) attached bilaterally at the bell margin, and wooden pin (in yellow) attached to the center of the animal. Two perspectives are shown, including a side view on the left and bottom view on the right. (B) Image of an augmented jellyfish deployed in field experiments off the coast in Massachusetts, USA. Figure adapted from Xu et al., 2021.

2.1. Contributions to science and engineering

The strategy of combining an electronic swim controller with live jellyfish themselves offers advantages by leveraging the animal's own body for actuation, efficient hydrodynamics, regenerative tissue properties, and natural survivability in a wide range of ocean environments, including hypoxic and deep-ocean areas where many other species cannot survive and adapt[54]. As such, these augmented jellyfish have the potential to explore more extreme environments, requiring only hardened microelectronic systems and sensors that can withstand high pressures instead of an entire underwater vehicle.

With the choice of designing augmented jellyfish as future ocean measurements tools, we built and tested biohybrid robotic jellyfish to determine their swimming capabilities in both laboratory[55] and field environments[56]. To summarize, augmented

54. Katherine Bell and Nicole Raineault, "New Frontiers in Ocean Exploration: The E/V Nautilus, NOAA Ship Okeanos Explorer, and R/V Falkor 2016 Field Season", ed. Ocean Exploration Trust, Joanne Flanders, and Amy Bowman, *Oceanography* 30, no. 1 (March 1, 2017): 1-94, https://doi.org/10.5670/oceanog.2017.supplement.01.
55. Xu and Dabiri, "Low-Power Microelectronics Embedded in Live Jellyfish Enhance Propulsion".
56. Nicole W. Xu *et al.*, "Field Testing of Biohybrid Robotic Jellyfish to Demonstrate Enhanced Swimming Speeds," *Biomimetics* 5, no. 4 (November 21, 2020): 64, https://doi.org/10.3390/biomimetics5040064. Xu, N. W., Townsend, J P., Costello, J. H., Colin, S. P., Gemmell, B. J. and Dabiri, J. O. (2021). Developing Biohybrid Robotic Jellyfish (Aurelia aurita) for Free-swimming Tests in the Laboratory and in the Field. Bio-protocol 11(7): e3974. DOI: 10.21769/BioProtoc.3974.

jellyfish exhibited swimming speeds up to three-fold faster than their non-modified counterparts at only a two-fold increase in the energetic cost to the animal (COT), instead of the estimated nine-fold increase[57]. One of the main advantages of studying jellyfish swimming is its comparative energy efficiency; this biohybrid robotic approach demonstrated the potential for even faster and more efficient swimming. By externally controlling live jellyfish, we can achieve these enhancements by bypassing other considerations, such as filter feeding and reproduction, which are wired for individual survival and evolutionary fitness. In addition to advances in biological capabilities, our engineered jellyfish also consumed 10 to 1 000 times less external power per mass than other existing swimming robots, including both traditional AUVs and bioinspired constructs[58].

Thus, augmented jellyfish have applications in biology, ecology, and evolution by understanding and modifying how jellyfish naturally swim; applications in robotics by using biohybrid techniques to address current challenges, such as damage tolerance and high power consumption; and potential applications in oceanography as monitoring tools to track markers of climate change.

3. Ethical considerations of augmented jellyfish

As scientists and engineers involved in creating augmented jellyfish, we sought advice from bioethics experts at the Stanford Benchside Ethics Consultation Service to begin discussions about the ethical considerations and implications of our work. An extended discussion of the ethical considerations of augmented jellyfish in the context of invertebrate research can be found in[59], and additional discussions with ethicists are ongoing as *A. aurita* research continues.

3.1. Summary of research components

To provide context before delving into ethical considerations, our previous research using augmented jellyfish included the following components:

Muscle excitation experiments to determine the electrical signals needed to stimulate jellyfish muscle, in which electrodes were embedded into live animal tissue in the absence of seawater (using 10 individual animals).

Free-swimming experiments in a laboratory tank to determine augmented jellyfish swimming speeds (using 6 animals).

Metabolic experiments that measure oxygen consumption over time to calculate the COT (using 7 animals).

Free-swimming experiments off the coast of Woods Hole, Massachusetts, USA to confirm augmented jellyfish swimming speeds in real-world environments and as a proof of concept for future ocean applications (using 4 animals).

During all experiments, the animals were monitored carefully in accordance with the minimization principle[60] and precautionary principle[61] whenever possible, and

57. Xu and Dabiri, "Low-Power Microelectronics Embedded in Live Jellyfish Enhance Propulsion".
58. Xu and Dabiri.
59. Xu *et al.*, "Ethics of Biohybrid Robotic Jellyfish Modification and Invertebrate Research".
60. J. Tannenbaum, "Ethics and Pain Research in Animals," *ILAR Journal* 40, no. 3 (January 1, 1999): 97-110, https://doi.org/10.1093/ilar.40.3.97.
61. Jonathan Birch, "Animal Sentience and the Precautionary Principle", *Animal Sentience* 2, no. 16 (January 1, 2017), https://doi.org/10.51291/2377-7478.1200.

the test subjects were allowed to rest and recover after the experiments, including extra food provisions. We also observed that most animals continued to thrive and even reproduce, which are less likely to occur if the animals were overly stressed. For field experiments, we also took care to prevent leaving behind animals or electronic waste in the ocean post-experimentation.

3.2. Categories of ethical considerations for this case study

Regarding the research ethics of augmented jellyfish as a case study, six main considerations to address are welfare interests, dignity or integrity interests, wisdom of repugnance, presumption of restraint, stewardship, and environmental impacts. These can be considered for jellyfish as individuals, as a species, and as influencing the environment and ecology. Welfare interests for individual test subjects are the basis of animal research restrictions and regulations. Because *A. aurita* lack a centralized nervous system or brain, it is unclear whether jellyfish have welfare interests that could be harmed during experiments, and the topic of invertebrate research ethics is still debated[62, 63, 64]. Regardless, we applied the 4Rs—reduction, replacement, refinement, and reproducibility[65]—and precautionary and minimization principles, as further explained in section 3.3 ("Considering the 4Rs for individual jellyfish"). The application of the 4Rs could also be argued from the basis of dignity or integrity rights. That is, even in the absence of sentience, individual animals are still owed protections from a deontological framework[66]. However, for *A. aurita,* the definition of an individual jellyfish is more complicated because during asexual reproduction, a sessile polyp transitions from a single individual to a stack of individual juvenile jellyfish that shear off during strobilation[67]. Furthermore, partial adult jellyfish can also survive as separate individuals[68].

To address public criticism of this work, in almost every scientific presentation or media interview, we have been asked about whether the swim controller harms the jellyfish, in accordance with the wisdom of repugnance or "yuck factor," in which intuitive negative responses are interpreted as a sign of harm or evil[69]. Although Kass states that the intrinsic feeling of revulsion highlights the actual morality of the experiments, as with reflexive reactions to human cloning[70], critics state that

62. C. Harvey-Clark, "IACUC Challenges in Invertebrate Research", *ILAR Journal* 52, no. 2 (January 1, 2011): 213-20, https://doi.org/10.1093/ilar.52.2.213.
63. Peter Carruthers, "Invertebrate Minds: A Challenge for Ethical Theory", *The Journal of Ethics* 11, no. 3 (September 2007): 275-97, https://doi.org/10.1007/s10892-007-9015-6.
64. Eleanor Drinkwater, Elva J. H. Robinson, and Adam G. Hart, "Keeping Invertebrate Research Ethical in a Landscape of Shifting Public Opinion", ed. Aaron Ellison, *Methods in Ecology and Evolution* 10, no. 8 (August 2019): 1265-73, https://doi.org/10.1111/2041-210X.13208.
65. Hanno Würbel, "More than 3Rs: The Importance of Scientific Validity for Harm-Benefit Analysis of Animal Research", *Lab Animal* 46, no. 4 (April 2017): 164-66, https://doi.org/10.1038/laban.1220.
66. Bernice Bovenkerk, Frans W. A. Brom, and Babs J. van den Bergh, "Brave New Birds: The Use of 'Animal Integrity' in Animal Ethics", *The Hastings Center Report* 32, no. 1 (January 2002): 16, https://doi.org/10.2307/3528292.
67. Mary N. Arai, *A Functional Biology of Scyphozoa* (Dordrecht: Springer Netherlands, 1996), https://doi.org/10.1007/978-94-009-1497-1.
68. Michael J. Abrams et al., "Self-Repairing Symmetry in Jellyfish through Mechanically Driven Reorganization," *Proceedings of the National Academy of Sciences* 112, no. 26 (June 30, 2015), https://doi.org/10.1073/pnas.1502497112.
69. Mary Midgley, "Biotechnology and Monstrosity: Why We Should Pay Attention to the 'Yuk Factor,'" *The Hastings Center Report* 30, no. 5 (September 2000): 7, https://doi.org/10.2307/3527881.
70. Leon R. Kass, "The Wisdom of Repugnance: Why We Should Ban the Cloning of Humans", *Valparaiso University Law Review* 32, no. 2 (1998): 679-705.

repugnance is built upon personal biases and deserves more scrutiny than immediate trust[71]. Thus, this reflexive judgement warrants more examination about whether its roots lie in aversion to immorality or if the technology is just new and unusual. It is also worth noting that other studies of invertebrates have also drawn criticism. One example, RoboRoach[72], is a commercially available surgical toolkit to teach the public about neural circuits by embedding an electronic system into live cockroachs to externally control their locomotion, a similar concept but different implementation and application to augmenting jellyfish. These two research avenues share the same wisdom of repugnance by the public, including a concern about scientists "playing God" and effacing "free will," both related to animal welfare issues and the slippery slope argument that this could work escalate into higher order animals.

One critique of current animal research is the overly permissive view of experiments under unclear ethical considerations, such as invertebrate research even with evidence of nociception. For example, Fiester argues that there needs to be a framework with the presumption of restraint, "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"[73]. To do this, researchers should treat invertebrates with respect and solemnity, not as a source of novelty or entertainment. Similarly, the idea of treating research animals with respect and gratitude extends into the idea of stewardship, in which researchers are responsible for caring for the animals used in their research[74]. Because animals are often used as tools for furthering human interests, researchers need to act as stewards and using animal resources appropriately and efficiently, e.g., duplicate animal experiments would be wasteful[75].

Finally, augmented jellyfish can also have potential environmental impacts when used in ocean applications. These include plastic and electronic waste, inhibiting the animal subjects' ability to eat or reproduce, and other far-reaching implications for other species in the ecosystem[76]. Because our field experiments were limited to short time periods (one to two hours) that did not affect animal longevity and required researchers monitor the animals and experiments at all time, we reduced the potential for environmental impacts thus far, but wider ocean monitoring efforts warrant further discussion regarding environmental ethics[77]

71. Leigh Turner, "Is Repugnance Wise? Visceral Responses to Biotechnology", *Nature Biotechnology* 22, no. 3 (March 2004): 269-70, https://doi.org/10.1038/nbt0304-269.
72. Roboroach, "The RoboRoach Bundle", Backyard Brains, n.d., https://backyardbrains.com/products/roboroach.
73. Autumn Fiester, "Justifying a Presumption of Restraint in Animal Biotechnology Research," *The American Journal of Bioethics* 8, no. 6 (August 25, 2008): 36–44, https://doi.org/10.1080/15265160802248138.
74. J.H Seamer, "Human Stewardship and Animal Welfare", *Applied Animal Behaviour Science* 59, no. 1-3 (August 1998): 201-5, https://doi.org/10.1016/S0168-1591(98)00134-8.
75. Iain Chalmers *et al.*, "How to Increase Value and Reduce Waste When Research Priorities Are Set", *The Lancet* 383, no. 9912 (January 2014): 156-65, https://doi.org/10.1016/S0140-6736(13)62229-1.
76. Michelle Bezanson, Rochelle Stowe, and Sean M. Watts, "Reducing the Ecological Impact of Field Research: Ecological Impact of Field Research", *American Journal of Primatology* 75, no. 1 (January 2013): 1-9, https://doi.org/10.1002/ajp.22086.
77. J. Baird Callicott, *In Defense of the Land Ethic: Essays in Environmental Philosophy*, SUNY Series in Philosophy and Biology (Albany, N.Y: State University of New York Press, 1989).

3.3. Considering the 4Rs for individual jellyfish

Regarding welfare interests for individual animal test subjects, the current consensus based on scientific research is that jellyfish do not have sentience or experience pain because of their distributed nervous systems (including two distinct nerve nets, rather than a centralized system) and lack of nociceptors[78]. This is also supported by the question of the whether jellyfish have a sense of "self," given that halves and quarters of this jellyfish species can survive separately as individuals, as demonstrated in previous work. In a series of excision experiments, separated jellyfish parts were able to survive and resymmetrize (i.e., redistribute their body to regain body symmetry) over a recovery period of days to weeks. These resymmetrized jellyfish were able to feed and continue living in laboratory conditions[79]. Thus, it is arguable as to whether individual jellyfish even possess welfare interests that can be harmed through experiments. Nevertheless, we conducted protocols to err on the side of caution, using both the precautionary and minimization principles to apply the 4Rs – reduction, minimizing the number of animals used to address the scientific research; replacement, using alternatives to animals such as theoretical or physical models when possible; refinement, minimizing pain and suffering with procedural changes; and reproducibility, conducting high quality research with scientific rigor to justify the use of animals – in consideration of jellyfish as individual animals within our experiments[80].

Regarding reduction, our experiments used between four to ten individual animals, which was minimal but high enough to account for statistical significance and natural animal variation, especially in more involved free-swimming experiments, which included six animals in the laboratory and four in the field. In addition to minimizing the number of animals, we also used a rights-based principle by Tannenbaum, in which out of "fairness to individual animals," using more animals can minimize the cost to an individual animal[81].

Regarding replacement, we developed a theoretical model of jellyfish swimming based on prior a hydrodynamic model in literature, but animal experiments were needed to validate these models. Because both laboratory and field experiments showed good agreement with the theoretical model[82, 83], future experiments can use results from the model as a replacement strategy.

Regarding refinement, we allowed the animals to rest and feed before and after experiments, and these experiments were designed to minimize stress on the animal when possible (such as monitoring their mucus secretions, although molecular analyses were not conducted at this stage and warrant further work in the future), such as minimal handling to insert the swim controller and keeping shorter experimental durations. Furthermore, although the device is attached using a wooden pin and electrodes through the mesoglea, which appears harsh, note that we first tested a variety of adhesives, such as medical-grade flexible cyanoacrylates and mussel-inspired adhesives for saltwater use[84]. However, these superficial adhesives were less

78. Arai, *A Functional Biology of Scyphozoa*.
79. Abrams *et al.*, "Self-Repairing Symmetry in Jellyfish through Mechanically Driven Reorganization".
80. Würbel, "More than 3Rs".
81. Tannenbaum, "Ethics and Pain Research in Animals".
82. Xu and Dabiri, "Low-Power Microelectronics Embedded in Live Jellyfish Enhance Propulsion".
83. Xu *et al.*, "Field Testing of Biohybrid Robotic Jellyfish to Demonstrate Enhanced Swimming Speeds."
84. Bruce P. Lee *et al.*, "Mussel-Inspired Adhesives and Coatings", *Annual Review of Materials Research* 41, no. 1 (August 4, 2011): 99-132, https://doi.org/10.1146/annurev-matsci-062910-100429.

tractable to apply onto the mucus-covered animals and caused more tissue damage after removal, whereas the wooden pin created a hole with minimal damage that healed within a day after removal[85]. These quick healing and regenerative abilities are in accordance with the literature[86].

Moreover, regarding scientific reproducibility, an additional consideration to the traditional 3Rs[87], we believe that these experiments are justified with strong data and scientific rigor as peer reviewed publications. The fact that our field experiments supported the same conclusions and swimming speed enhancements as our laboratory experiments, even with additional researchers to conduct these experiments, suggests reproducibility in various environments. And as a final cost-benefit analysis, this research has broad applications in biology, robotics, and environmental science.

3.4. Considering jellyfish as a species and potential impacts to the environment

Although modifications to *A. aurita* apply to individual animals, the augmentation of individual jellyfish has potential implications for the welfare of the species as a whole if future research involves extended ocean deployment. First, there is an open question of whether the swim controller device affects the individual's feeding, longevity, and reproduction, which can then affect its evolutionary fitness. Because our previous tests have been limited to the order of hours, with only a few extended tests up to 48 hours, whether this question is relevant to the species is still unknown.

Moreover, *A. aurita* is considered an invasive species[88], and the overpopulation of jellyfish blooms has negative impacts on the environment[89,90]. In fact, jellyfish blooms are affected by factors such as natural cyclical variation, anthropogenic causes, and climate change, but jellyfish have high evolutionary fitness due to their multi-phase life cycle (both a free-swimming sexual phase and a sessile asexual reproduction phase)[91]. Thus, it is unclear whether modifying only their adult form is capable of provoking species-wide changes. And further, as a hypothetical scenario, even if augmenting adult jellyfish does decrease their evolutionary fitness, it is unclear if this species-level welfare is a negative considering their classification as a nuisance species.

Finally, one of the biggest open questions is how this research could impact the environment and ecology, which also relates to the idea of being responsible environmental stewards. As future tools for ocean monitoring, possible issues include

85. Xu and Dabiri, "Low-Power Microelectronics Embedded in Live Jellyfish Enhance Propulsion".
86. Sosuke Fujita, Erina Kuranaga, and Yu-ichiro Nakajima, "Regeneration Potential of Jellyfish: Cellular Mechanisms and Molecular Insights", *Genes* 12, no. 5 (May 17, 2021): 758, https://doi.org/10.3390/genes12050758.
87. Würbel, "More than 3Rs".
88. William M Graham *et al.*, "Linking Human Well-Being and Jellyfish: Ecosystem Services, Impacts, and Societal Responses", *Frontiers in Ecology and the Environment* 12, no. 9 (November 2014): 515-23, https://doi.org/10.1890/130298.
89. Robert H. Condon *et al.*, "Questioning the Rise of Gelatinous Zooplankton in the World's Oceans", *BioScience* 62, no. 2 (February 2012): 160-69, https://doi.org/10.1525/bio.2012.62.2.9.
90. Robert H. Condon *et al.*, "Recurrent Jellyfish Blooms Are a Consequence of Global Oscillations", *Proceedings of the National Academy of Sciences* 110, no. 3 (January 15, 2013): 1000-1005, https://doi.org/10.1073/pnas.1210920110.
91. Mariko Takao, Hiroko Okawachi, and Shin-ichi Uye, "Natural Predators of Polyps of Aurelia Aurita s.l. (Cnidaria: Scyphozoa: Semaeostomeae) and Their Predation Rates", *Plankton and Benthos Research* 9, no. 2 (2014): 105-13, https://doi.org/10.3800/pbr.9.105.

plastic and electronic waste from the swim controller devices, as well as if other animals were to ingest these components. Future iterations of these controllers could include biodegradable electronics[92] and plastics[93]. However, this is still an open question because our current technology has not reached this level yet. Previous field experiments[94] were conducted off the shore with multiple scientific SCUBA divers observing the augmented animals to ensure that no components were left in the ocean after the tests. For future studies, we want to be conscious of potential issues as the technology advances for more autonomous experiments.

4. Recommendations for future jellyfish experiments

For future work with augmented jellyfish, the minimization and precautionary principles should drive scientific protocols, in accordance with the 4Rs and incorporating additional tools to track animal responses, such as molecular stress markers and longer-term tests for survivability. More extensive laboratory experiments could be used both to assess individual animal behavior and to extrapolate evolutionary fitness for the species if, in the future, augmented jellyfish are deployed autonomously in the ocean.

Regarding ethical considerations for individual animals, scientists should conduct a cost-benefit analysis prior to each research project that involves jellyfish subjects, including the number of test subjects required, justification of the research, and discussion of the 4Rs, such as why non-animal replacements are not possible in the proposed work or how procedural refinements can minimize stress. Despite the lack of systemic ethical oversight on jellyfish and similar aquatic invertebrates in scientific studies, such ethical considerations can be outlined similarly to IACUC protocols for vertebrate research.

Regarding ethical considerations for species-wide and ecological consequences, future work with augmented jellyfish should continue to be done with input from bioethicists. Multidisciplinary teams featuring bioethicists can address multiple ethical frameworks, which provide a more comprehensive discussion that can highlight open questions, possible scenarios, and unintended consequences about deploying modified jellyfish as real-world tools. For example, as a hypothetical situation, if augmenting jellyfish causes harm to a few individual animals but allows researchers to track markers of climate change with the potential to reduce ocean acidification, which would take precedence: the utilitarian value of the potentially positive outcome for the entire ocean or the deontological value of the actual animals involved? With input from both ethicists and the scientists involved in this work, the continuing research of designing, building, and implementing biohybrid robotic jellyfish can grow in a safer and more ethical manner. Perhaps future directives and legislation can also offer more standardized guidance for augmented jellyfish and other invertebrate research.

92. Ting Lei *et al.*, "Biocompatible and Totally Disintegrable Semiconducting Polymer for Ultrathin and Ultralightweight Transient Electronics", *Proceedings of the National Academy of Sciences* 114, no. 20 (May 16, 2017): 5107-12, https://doi.org/10.1073/pnas.1701478114.
93. Christian Lott *et al.*, "Field and Mesocosm Methods to Test Biodegradable Plastic Film under Marine Conditions", ed. David Hyrenbach, *PLOS ONE* 15, no. 7 (July 31, 2020): e0236579, https://doi.org/10.1371/journal.pone.0236579.
94. Xu *et al.*, "Field Testing of Biohybrid Robotic Jellyfish to Demonstrate Enhanced Swimming Speeds".

5. Acknowledgements

The authors gratefully acknowledge Olga Lenczewska, Sarah E. Wieten, and Carole A. Federico for their expert contributions as bioethicists and coauthors of an extended discussion regarding the ethics of augmented jellyfish and invertebrate research[95]. We also thank Mildred Cho, David Magnus, and Hank Greely for their contributions through the Stanford Benchside Ethics Consultation Service, a research ethics and regulation committee within the Stanford Center for Biomedical Ethics that provides a document of potential considerations for scientists who seek ethical advice about their research.

95. Xu *et al.*, "Ethics of Biohybrid Robotic Jellyfish Modification and Invertebrate Research".