

Biomimetic robots promote the 3Rs Principle in animal testing

David Bierbach^{1,2*}, Fritz Francisco^{1,2}, Juliane Lukas^{1,2}, Tim Landgraf³, Moritz Maxeiner³, Pawel Romanczuk⁴, Lea Musiolek⁵, Verena V. Hafner⁵, Jens Krause^{1,2}

¹Faculty of Life Sciences, Division of Biology and Ecology of Fishes, Humboldt Universität zu Berlin, Berlin, Germany

²Department of Biology and Ecology of Fishes, Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

³Dahlem Center for Machine Learning and Robotics, Freie Universität Berlin, Berlin, Germany

⁴Faculty of Life Sciences, Institute for Theoretical Biology, Humboldt Universität zu Berlin, Berlin, Germany

⁵Adaptive Systems Group, Department of Computer Science, Humboldt-Universität zu Berlin, Berlin, Germany

*david.bierbach@gmx.de

Abstract

In order to strengthen animal welfare, many countries require that experimenters follow the ‘3Rs Principle’ when designing animal experiments. The 3Rs call for a reduction in the number of animals used, the refinement of methods to reduce stress as well as the full replacement of animals in experimentation through alternative methods. Biomimetic robots that resemble live animals and allow for natural-like interactions represent a valuable tool to achieve the 3Rs’ objectives. On the basis of our research with a robotic fish that is accepted as a conspecific by live poeciliid fishes, we highlight how biomimetic robots can reduce the number of animals tested by (a) substituting live animals, (b) providing highly standardized cues, and (c) reducing overall stress for live animals during tests through less handling.

Biomimetic robots and animal testing

Technological advances are greatly innovating animal experiments by allowing for new ways of observation (Dell et al. 2014) and how we can manipulate or control an animal’s environments (Ioannou et al. 2012, Butail et al. 2015, Chouinard-Thuly et al. 2017, Stowers et al. 2017, Katsnelson 2018, Romano et al. 2018, Datteri 2020, Landgraf, et al. 2020). This includes new methods like video animations and virtual realities as well as biomimetic robots. However, it is often overlooked that these methods can also help to increase animal welfare during experiments (and husbandry). The 3Rs Principle (first proposed by Russell and Burch (1959), hereafter ‘3Rs’) is now the gold standard for laboratory animal protection policies in many countries. It emphasizes the reduction of the number of animals used, the refinement of methods to decrease animal’s distress and the full replacement of (higher) animals in tests through the use of alternative methods wherever possible (for more details see Tannenbaum and Bennett (2015)). Especially robots built to interact with live animals as either conspecifics (shoal mates, mating partners or opponents during aggressive encounters) or heterospecifics (as prey or predators) are a technique that is advancing to fulfill many of the mentioned measures of the ‘3Rs’.

Robots enable the empirical implementation of some theoretical models of interactions and movements to test

hypotheses from fields like movement ecology (collective movement behavior, Faria et al. 2010, Butail et al. 2014, Butail et al. 2016, Bonnet et al. 2018, Bierbach et al. 2020, Jolles et al. 2020), sexual selection (mate choice and aggression, Phamduy et al. 2014, Romano et al. 2017, Romano and Stefanini 2020) or natural selection (predator-prey interactions, Swain et al. 2012, Abaid et al. 2013, Heathcote et al. 2020, Romano et al. 2020, Polverino et al. 2019). These examples show that especially fishes have been used in experimentation with biomimetic robots and we thus focus on fish to elaborate on the benefits of biomimetic robots for an implementation of the 3Rs.

In general, biomimetic robots consist of a replica, with which the animal interacts directly and a robotic unit that moves the replica. Movement of the replica can be accomplished externally or the robot unit is placed inside the replica and thus makes it movable itself (Romano et al. 2018). Self-propelled robots are often large as all necessary technical equipment has to fit into the robot’s chassis, which can limit their use to interactions with only larger species. Externally dragged robots can be much smaller and thus are also appropriate for smaller species. In addition, an external robot unit allows for moisture-sensitive electronics to be kept away from water more easily. Regarding the steering modes, biomimetic robots can be either interactive (closed-loop), i.e., capable of responding to the actions of live animals, or static (open-loop), i.e., moving and behaving in predefined, non-interactive ways (see Landgraf et al. 2020 for a comparison of both modes). The herein presented examples from our own research – using a biomimetic fish (‘robofish’) to study social behavior in poeciliid fishes (*Poecilia reticulata*, *P. mexicana*) and sticklebacks (*Gasterosteus aculeatus*) – can be considered non-invasive and least harmful or distressing. However, this does not preclude these robots from being used in more invasive and distressing experiments.

Biomimetic robots reduce number of live animals in testing

Obviously, substituting live animals with robots reduces the number of live animals used in testing. To illustrate the magnitude of these reductions, we compared our recent

studies involving the robofish system to similar studies that did not use robots. For example, one classical design to measure an animal's sociability is a dichotomous choice test where a focal individual can choose to associate with one or more (stimulus) conspecifics or not (Wright and Krause 2006). In two recent publications, we used the robofish as a social stimulus to measure live guppies' sociability (Bierbach et al. 2018, Lukas et al. 2021). As our tests only involved the live focal fish with the robofish substituting any stimulus individuals, we reduced the number of animals in this setup by more than 50% (assuming a stimulus group of 'one') to over 80% (assuming stimulus groups comprising 5 individuals, see for example Jolles et al. 2017).

However, it is noteworthy that to first establish a biomimetic robot for a specific animal species, additional testing with live animals will be necessary. This comprises observations of live animals to extract their movement and appearance as well as initial acceptance tests. The latter have to be repeated for every new species to interact with the robot. Nevertheless, we argue that by using existing behavioral data from the new target species, this number can be kept low. Moreover, for closely related species with similar behavioral repertoires, often only minimal modifications to the replica are necessary to keep this overhead low (e.g., see Bierbach et al. 2018 where we used our guppy-proofed robofish system with the related but slightly larger species *Poecilia mexicana* by increasing replica size).

Biomimetic robots provide highly standardizing (social) cues

Open-loop and (to a lesser extent) also closed-loop robots provide live focal individuals with highly standardized social cues. Robots can move with little variation and mutual interactions can be controlled or fully removed and therefore allow for testing of all individuals with an almost identical set of social stimuli (Bierbach et al. 2018, Lukas et al. 2021). As a result, individual differences in response are then inevitably caused by focal individuals' intrinsic differences and not by mutual influences between focal and stimulus animals. This fact helps to analyze data using less data-hungry statistical approaches. Also, by reducing the signal-to-noise ratio, it allows experimenters to further decrease the necessary sample sizes to find given effects (Sneddon et al. 2017). Despite their usefulness, open-loop robots can have lower acceptance rates as compared to closed-loop ones (see Landgraf et al., 2016) yet are more easily built and controlled.

Biomimetic robots reduce handling stress

When investigating leadership phenomena, researchers often train a certain set of animals to act as leaders, for example by repeatedly feeding them on a prominent location or object thus making use of classical conditioning approaches (Reebs 2000, Swaney et al. 2001, Couzin et al. 2011, Ioannou et al. 2015). In a test situation, pre-trained individuals are then assumed to lead the naïve ones to the learnt location/object. While some training procedures may be seen as valuable enrichment measures (depending on species and context), there is potential to cause distress to the animals as training is

highly handling-intensive (i.e., individuals have to be placed repeatedly into a training situation for conditioning). Stress experienced during training could be completely circumvented, simply by forgoing training and using robotic animals as leaders instead.

In a recent publication, we used differentially-sized robotic leaders and found a bigger-is-better pattern as guppies regardless of own size followed larger robofish replicas more readily (Bierbach et al. 2020). Focal individuals were only tested once and the robot leaders did not require training. In another study, robofish led live guppies towards certain goal areas inside a test aquarium (Musiolek et al. 2020). The same experiment without robofish would have required extensive training of multiple sets of live leaders for each of the five goal areas (rather than switching goal areas with the 'press of a button'). In another study, we used an interactive closed-loop robofish that acted as a pure follower towards a live guppy (Jolles et al. 2020). As outcomes were strictly driven by the speed of the single live fish in the pair, we could show the importance of swimming speed for the emergence of leadership and other collective properties. Training seems unfeasible to turn live animals into "pure followers" and it would require instead high numbers of tests (involving many different live individuals) to get the same number of "pure follower" cases by chance.

As a point of caution, we appeal that measures are taken with regard to injuries through collisions. Robots that are much bigger and heavier than the animals they interact with may represent a risk and will need specific algorithms to counteract this (see PoulBot, Gribovsky et al. 2018). In contrast, animal-sized robots made of soft materials that move at low speeds pose low danger for the live animals.

Conclusions

As every experimental method, robotic animals have several shortcomings for the study of (social) behavior in animals (e.g., questionable acceptance as conspecifics, weaker social attraction if open-loop steered, complicated operation of those systems, cost-intensive design and development). Nevertheless, we argue that these systems not only advance scientific knowledge but also help design animal experiments in the spirit of the 3Rs. Tannenbaum and Bennett (2015) noted that "Russell and Burch clearly want scientists to practice reduction now, so that the number of animals can at least be reduced progressively as statistical and experimental techniques are improved" and biomimetic robots that interact with live animals are one promising way how this reduction can be achieved.

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