Bioinspiration & Biomimetics

PAPER • OPEN ACCESS

Organisms as sensors in biohybrid entities as a novel tool for in-field aquatic monitoring

To cite this article: Wiktoria Rajewicz *et al* 2024 *Bioinspir. Biomim.* **19** 015001

IOP Publishing Bioinspir. Biomim. 19 (2024) 015001 https://doi.org/10.1088/1748-3190/ad0c5d

Bioinspiration & Biomimetics

CrossMark

OPEN ACCESS

RECEIVED
20 September 2023

ACCEPTED FOR PUBLICATION
14 November 2023

PUBLISHED
22 November 2023

PAPER

Organisms as sensors in biohybrid entities as a novel tool for in-field aquatic monitoring

Wiktoria Rajewicz[1,*], Chao Wu[2], Donato Romano[3], Alexandre Campo[4], Farshad Arvin[2], Alexander J Casson[2], Godfried Jansen van Vuuren[3], Cesare Stefanini[3], Joshua Cherian Varughese[1], Barry Lennox[5], Sarah Schönwetter-Fuchs[1], Thomas Schmickl[1] and Ronald Thenius[1]

1 University of Graz, Graz, Austria
2 Durham University, Durham, United Kingdom
3 Scuola Superiore Sant'Anna, Pisa, Italy
4 Université Libre de Bruxelles, Brussels, Belgium
5 The University of Manchester, Manchester, United Kingdom
* Author to whom any correspondence should be addressed.

E-mail: wiktoria.rajewicz@uni-graz.at

Keywords: biohybrid, environmental monitoring, bioinspiration

Abstract

Rapidly intensifying global warming and water pollution calls for more efficient and continuous environmental monitoring methods. Biohybrid systems connect mechatronic components to living organisms and this approach can be used to extract data from the organisms. Compared to conventional monitoring methods, they allow for a broader data collection over long periods, minimizing the need for sampling processes and human labour. We aim to develop a methodology for creating various bioinspired entities, here referred to as 'biohybrids', designed for long-term aquatic monitoring. Here, we test several aspects of the development of the biohybrid entity: autonomous power source, lifeform integration and partial biodegradability. An autonomous power source was supplied by microbial fuel cells which exploit electron flows from microbial metabolic processes in the sediments. Here, we show that by stacking multiple cells, sufficient power can be supplied. We integrated lifeforms into the developed bioinspired entity which includes organisms such as the zebra mussel *Dreissena polymorpha* and water flea *Daphnia* spp. The setups developed allowed for observing their stress behaviours. Through this, we can monitor changes in the environment in a continuous manner. The further development of this approach will allow for extensive, long-term aquatic data collection and create an early-warning monitoring system.

1. Introduction

The Earth has been facing a global ecological crisis for decades. Climate change, oil spills and other anthropogenic catastrophic events have contributed to a water quality crisis and have caused the freshwater supply to diminish [1]. Rapid worsening of the water quality leads to a chain of events that can cause the ecosystem to collapse and eventually, threaten the existence or well-being of many species of animals and plants. The best way to support preventative measures to these destructive processes is extensive water monitoring and global sharing of the data on the water quality [1].

Aquatic ecosystems are complex and affected by a multitude of biotic and abiotic factors [2]. Traditional environmental studies are carried out with the use of 'classical' sensors or surveys based on traditional water sampling techniques [3–5]. While these methods have relatively high precision, they are also time and money-consuming [6–8]. Probes and sensors often require frequent maintenance and large amounts of energy [9]. Moreover, environmental monitoring surveys based exclusively on traditional sampling methods can be too infrequent or inconsistent to accurately determine the water quality [10, 11]. Time limitations also slow down the monitoring process by reducing the amount of data points that

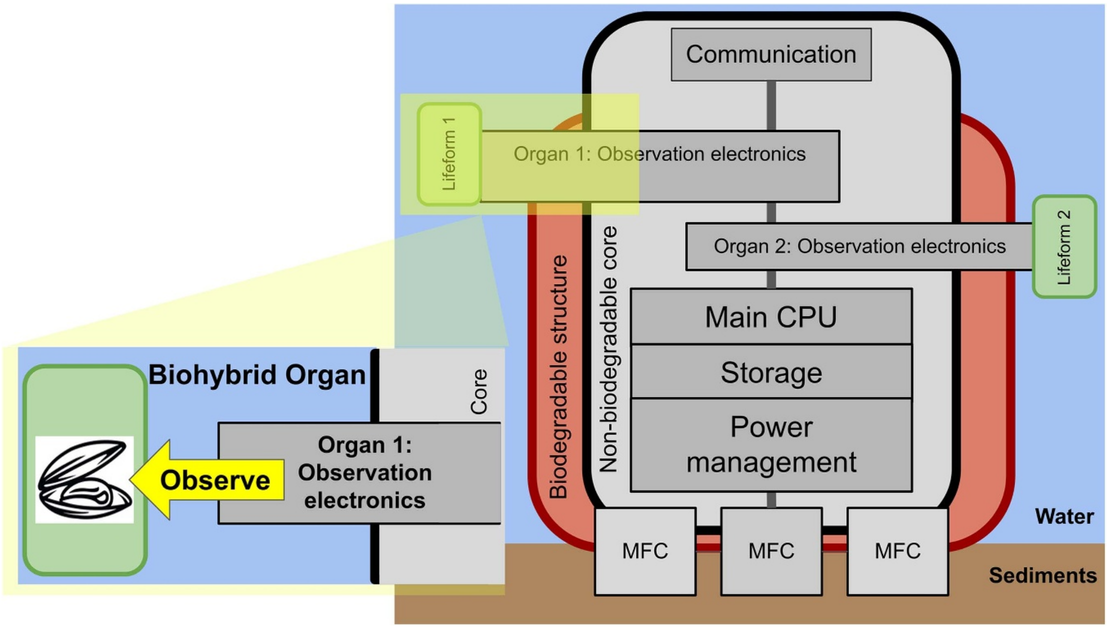

Figure 1. Schematic representation of the concept 'lifeform in the loop'. The biohybrid entity is powered by multiple microbial fuel cells partially covered with mud. Within the non-biodegradable core (light grey), all necessary electronics (dark grey), such as the power management parts, storage devices, the main CPU board and communication devices, are contained. Multiple biohybrid organs (yellow zoom-in box) include an organism hosting part (green) and electronics serving as an observation unit (grey), connected to the main core. The core is stationed and held to the substratum by a biodegradable structure (red).

can be collected at a certain time and make the monitoring less extensive [10–12]. Additionally, due to the complexity of the aquatic ecosystems, tracking individual biotic and abiotic parameters might often result in an incomplete survey as it neglects to account for their mutual interaction [13].

Here, we propose a concept of utilizing organisms (lifeforms) that are adapted by natural selection to the specific ecosystem, as biological sensors in a novel type of robotic entity. Certain organisms have a high sensitivity to changes in the ecosystem. Additionally, they are self-sustaining, which means that the biohybrid entity requires little to no maintenance, in contrast to classical probes which need frequent cleaning and calibration [9]. The main features of these biohybrid entities are their self-actuation and self-powering with the use of low-power devices and energy harvesting. The collection of lifeforms and their responses form an 'artificial organism' that creates a symbiotic relationship with the mechanical parts. Such an active biohybrid agent is currently developed by the project Robocoenosis [14]. This entity uses several living organisms instead of mechatronic components and, thus by definition, is a biohybrid robotic system [15]. This paper presents the concept of underwater biohybrid entities for environmental monitoring and the first results obtained from the built setup. A schematic representation of the biohybrid entity, its structure and the concept of 'lifeform in the loop' can be found in figure 1.

Experiments and long-term monitoring missions as well as method development are performed in two Austrian lakes: Lake Millstatt and Lake Neusiedl. Their differences in water quality and biodiversity enable the incorporation of a wide selection of organisms. The lifeforms investigated are taken directly from those lakes and used only for experiments in their respective habitats. In this paper, the following major questions are investigated:

- Q1: Can living organisms be used as complementary sensors for 'classical sensors' for monitoring the overall water quality?
- Q2: Which biotic and abiotic parameters can be measured with the use of organisms as sensors?
- Q3: What are the optimal ways to incorporate lifeforms into the biohybrid entity structure?
- Q4: What are the optimal ways to monitor the organisms' behaviours with the use of an automated setup?
- Q5: Can microbial fuel cells (MFCs) supply enough energy for the functioning of the robot under real-life conditions in long-term aquatic monitoring?

2. Methods

2.1. Organisms investigated

To answer the research questions, several organisms were investigated in the context of being used as living sensors. Lifeforms for the first tests were selected

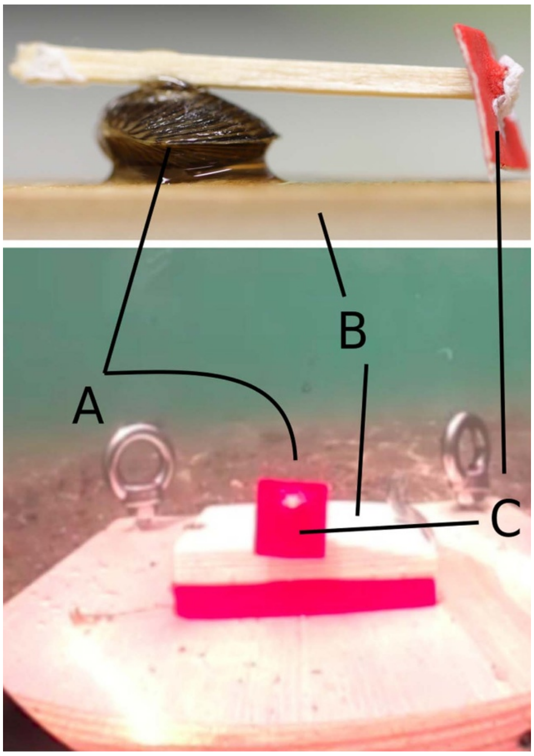

Figure 2. Top: Zebra mussel (*Dreissena polymorpha*) (A) mounted on a wooden board (B). A red tag (C) is placed on the top valve to augment mussel movements and enable visual tracking.

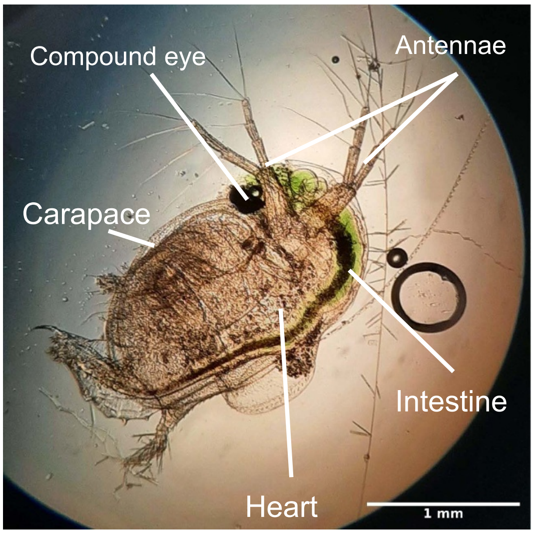

Figure 3. *Daphnia* sp. specimen under a microscope with several internal and external anatomical parts marked (compound eye, antennae, carapace, intestine and heart).

based on the following criteria: (1) sensitivity to one or more of the changing environmental parameters, (2) feasibility of their observation and (3) high availability in the lake of interest. By following these criteria, we constructed biohybrid organs that can track changes in the water and ensure no transfer of lifeforms between habitats.

2.1.1. Zebra mussel

First experiments were carried out using zebra mussels *Dreissena polymorpha* (Pallas, 1771) (figure 2). This species was chosen due to its sedentary lifestyle, quick reaction to external stimuli and abundance in the studied lakes [16]. Their constant filtering of the water makes them highly receptive to various changes in water chemistry. Toxins, changing temperature, fish kairomones or conspecific alarm substances can cause a stress-related response which often shows in valve movements [17]. In the case of *D. polymorpha*, stress reactions include the closing of the valves for a longer period or fluttering movements [18]. *D. polymorpha* is particularly sensitive to low oxygen and irritation caused by the physical presence of small invertebrates [16, 17, 19]. Different environmental cues generate different stress reactions and the cause of stress can be determined through the process of elimination [18].

2.1.2. Zebra mussel—field experiments

The observation was done with the use of video recording and automated movement tracking. Four mussels were attached in front of the camera setup and monitored daily. Due to their relatively small size, generally not exceeding 5 cm, a tag is placed on the top valve to augment the movements and enable easier valve detection (figure 2) [20]. In case the valve gaping decreases significantly over a long period, an alarm is triggered.

Zebra mussels were collected from Lake Millstatt before the experiments took place in May of 2022. Mussels could be found underneath rocks and were gently detached manually. To test the feasibility of the method, four zebra mussels were attached in front of the Raspberry Pi camera using the method presented in figure 2. Pictures were taken every 30 s for 15 min. A sudden disturbance (a dropped rock) was caused once throughout the experiment, to observe the mussels' reactions. In addition to observing the behaviour of an individual mussel, their collective responses were also noted in order to determine their 'group agreement parameter' (the degree to which all individuals react homogeneously to a stressor). This part of the underwater setup corresponds to the 'Biohybrid Organ' presented in figure 1.

2.1.3. Water flea Daphnia spp

Another organism that was tested as the biological element in a biohybrid entity organ was the water flea *Daphnia* spp. (Müller, 1785) (figure 3). This genus of cladocerans has been widely used for toxicological tests by pharmaceutical companies and when studying the effects of various chemicals and heavy metals on animal physiology, e.g. heartbeat [21, 22, 23].

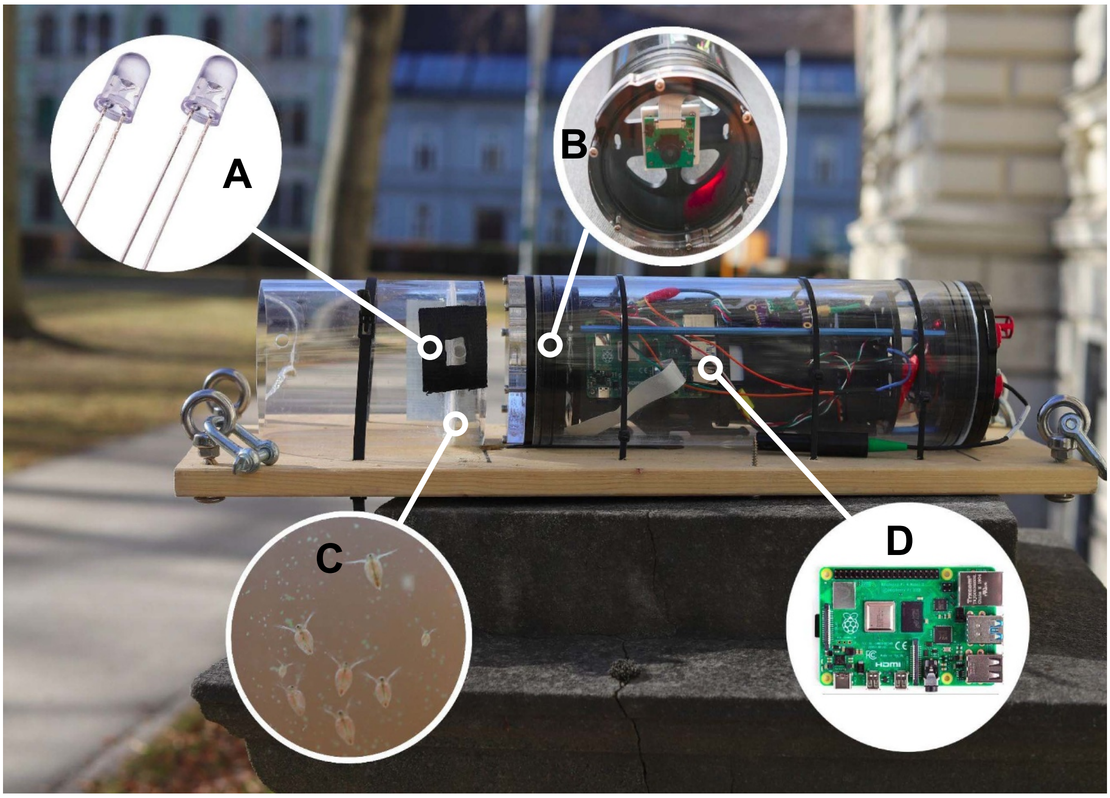

Figure 4. Underwater setup with (A): a light source for background illumination (B): built-in camera piece (C): flow-through chamber with swimming animals and (D): raspberry Pi module as the data storing device.

Numerous studies documented a specific response to certain chemicals, for example, decreased swimming speed when exposed to copper [24–26]. *Daphnia*'s reaction to the changing environment is also instantaneous and apparent to register with an automatic setup.

For the first analysis, various swimming behaviours were chosen for toxicity tests. The behaviour was divided into 'normal', 'spinning' and 'inhibited'. These behaviours can then be treated as degrees of stress with movement inhibition being the most severe. Erratic swimming or 'spinning' is considered to be a continuous and extreme escape behaviour that occurs in response to acute stressors, such as highly toxic levels of certain substances [27].

For experiments with *Daphnia*, a special flow-through cage was built into the underwater monitoring system (figure 4). To allow *Daphnia* to display their normal behaviour, the animals are in constant contact with the surrounding waters. A Raspberry Pi camera facing the cage records *Daphnia*'s reaction to potential toxins present in the environment. This part of the underwater setup corresponds to the 'Biohybrid Organ' presented in figure 1.

The behavioural tests were performed both under laboratory conditions and in the field. Field experiments aimed at testing the feasibility of the method and *Daphnia*'s well-being within the flow-through cage. Laboratory trials were carried out as a calibration of the 'normal' and 'disrupted' swimming behaviour. Here, an increasing salinity was chosen as the stressor.

Firstly, salinity was increased from 0 ppt to 4 ppt over 10 s. This salinity is within the tolerance range of *Daphnia*, however here, the focus is placed on the rapid change, rather than the parameter itself. Further analysis of the swimming patterns was performed. The *Daphnia*'s swimming trajectories were divided into 1: $< 180°$ and 2: $> 180°$. This divide represents 'smooth turns' and 'sharp turns'. *Daphnia* under normal conditions moves in a straight line interrupted by sharp hopping and sinking movements, whereas under sub-optimal conditions, their spinning behaviour results in a smoother and curved trajectory. A Chi-square test was performed on this data to compare the distribution between the *Daphnia* (stressed and unstressed) and turning sharpness (sharp or smooth).

Another series of experiments was performed to calibrate the sensor to an increasing salinity. Five *Daphnia* specimens were placed in Petri dishes with aquarium water and were allowed to acclimate for one hour. Salt solutions were added to each dish while recording continuously. The salinity levels used were 2.5 ppt, 3 ppt and 4.5 ppt ($n = 65$, 50 and 50 respectively with $n = 30$ for control experiments). Acute

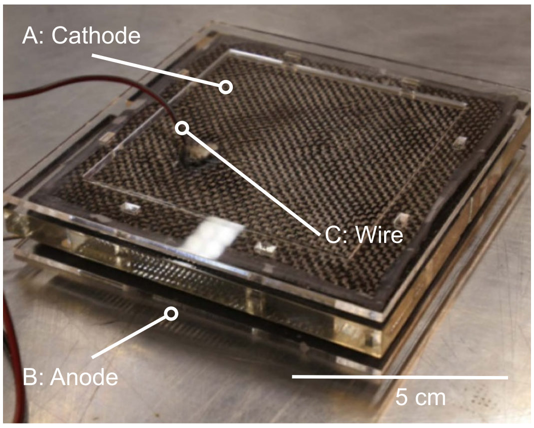

Figure 5. A Picture of the microbial fuel cell module. The MFC consists of a cathode chamber (A) and anode chamber (B) connected to the main biohybrid entity with a wire (C). Figure Reproduced from [28]. CC BY 4.0.

immobilization and spinning behaviour were noted for each dish every hour for 22 h. Movement inhibition was noted for every individual who showed no or close to no movement for over 15 s per recording. In cases where an individual displayed no movement for over 15 s after which short-term spinning was observed, the reaction was treated as a movement inhibition. Spinning was noted for every individual who showed a rapid circular movement at least once throughout the 30 s recording period. The Kruskal–Wallis test was performed on the obtained data to test for differences in immobilisation between different salinities. The results of the behavioural changes after 22 h in different salinity levels are displayed in figure 8.

2.1.4. MFCs

The biohybrid entity is designed for ultra-long runtimes. This is made possible by the use of energy harvesting and low-power electronic parts. Energy is generated using MFCs (figure 5). Here, a modification of the traditional MFC was used, known as the benthic microbial fuel cells (BMFCs) [29]. This type of fuel cell extracts electricity from natural processes occurring in the sediments. They are typically composed of two chambers, one containing an anode and another containing a cathode, separated by an ion-permeable membrane [29]. The anode chamber is buried in the sediments while the cathode chamber is exposed directly to the water layer. Anaerobic respiration processes are carried out by the bacteria present in the anoxic sediments.

Additionally, the MFCs can be used as a biosensor thanks to their continuous dependency on the benthic bacteria. Bacterial activity is reflected in the current obtained by the MFC giving an insight into the bacterial community and the presence/absence of oxygen and other electron acceptors. This concept called 'ecosystem hacking' allows ecosystem monitoring to be carried out by direct observation of the organisms [30].

The main limitation of the MFCs is their low current provided. Here, attempts to scale up the power output have been performed with the burrowing of a power-management system together with the anode connected to multiple cathodes [29]. Six stacked MFCs, connected by a super-capacitor, were constructed. The preliminary tests were carried out under laboratory conditions and in the field, in the Venetian Lagoon. This was to (1) test their scaling up by stacking the cells and (2) test their usability as an autonomous power source for an underwater biohybrid entity. Each cell was controlled by a micro-controller. The current produced by each individual cell and by the collective charge was measured.

2.1.5. Low-power electronics and software

One of the main challenges with constructing biohybrid entities is ensuring their optimum functionality while minimizing energy requirements. In order to simplify and optimise its structure, a dual processor is implemented. An ultra-low-power STM32 (an Arm M4-based platform) serves as the heart of the biohybrid. This operates in connection to an operational module which performs complex computations in a power-efficient manner. For this module, several boards from Speed, equipped with RISC-V processors are used. As these boards consume relatively larger amounts of energy, they are switched on and off by the main board, for short periods of time. Achieving a low-power design must be aimed for by both the electronics side and the software. A proper design of the operating software is crucial for maximising computational capabilities. To reduce the energy consumed, the software is run on the STM32. One of the main challenges is reducing the time it takes to start the computational module, the energy storing and re-conversion. For this reason, the biohybrid entity is programmed to use the energy when and as it is harvested. Ultra low-power deep learning accelerators (such as the Ambiq Apollo) can be used to predict times of the peak power collected. Another task of the power regime is to allow the entity to emerge and submerge using electrical and mechanical energy stored in the device. This approach is energy-efficient as it is only needed to trigger the device.

3. Results

Here, the results are focused on testing the feasibility of the methodology and the biohybrid design. The setups constructed were tested both in the field and under laboratory conditions. Through them, we were able to gather data on the organisms' behaviour and the applicability of the MFCs for underwater biohybrid entities.

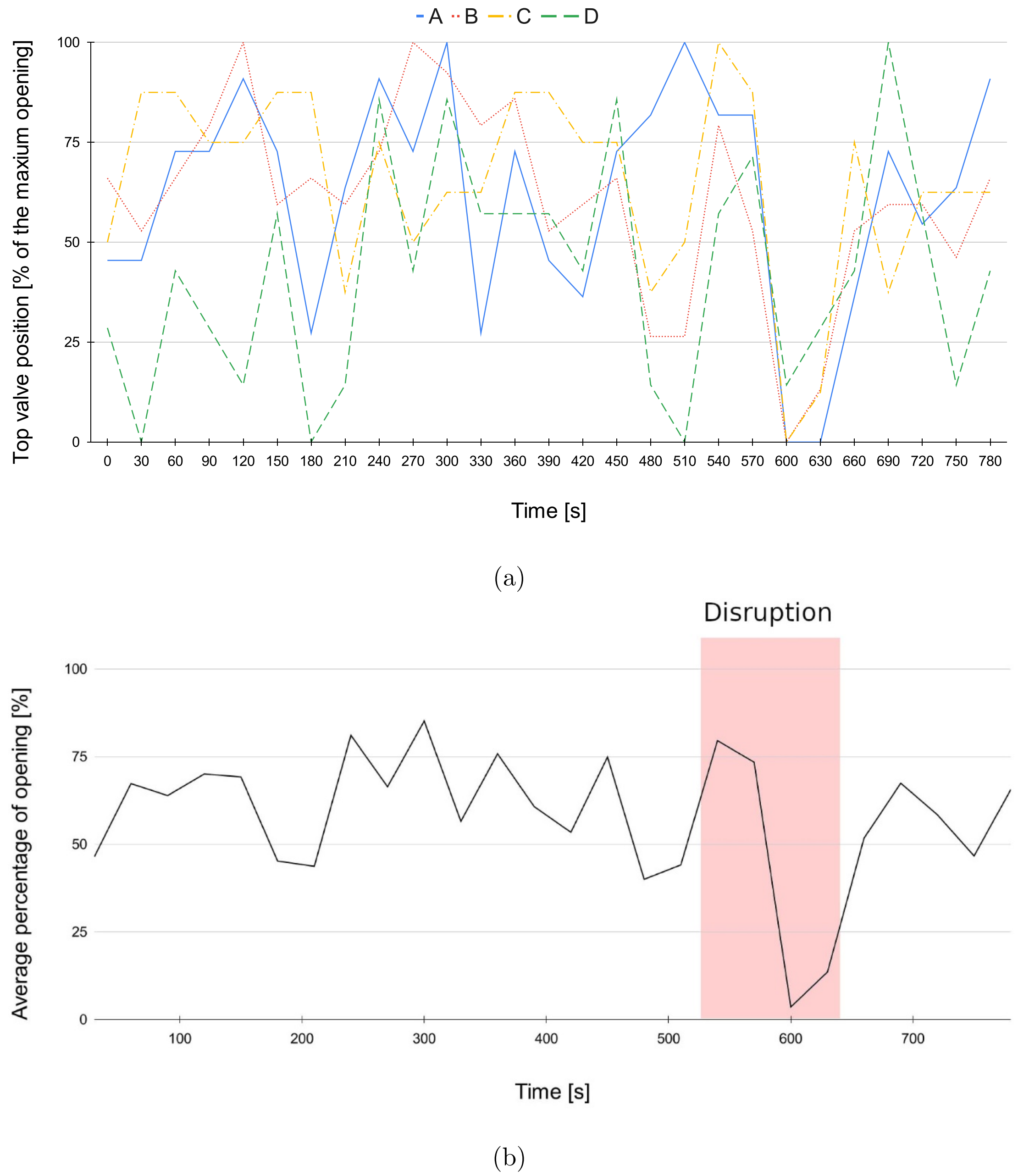

Figure 6. Results of an experiment performed with an underwater monitoring setup. Four zebra mussels were attached to a wooden board in Lake Millstatt. (A) The distance between the top valve and the wooden board was tracked for each mussel separately (1–4) for 13 min, taking a photo every 30 s. (B) Average degree of valve opening, taken as a percentage of the maximum opening. After 500 s (approximately 8 min) a disturbance was caused in the surrounding water.

3.1. Zebra mussel

To test the feasibility of the zebra mussel as a sensor in the biohybrid entity, a series of field experiments were carried out. The movement tracking for the zebra mussel was carried out by calculating the distance between the top valve and the wooden platform (figure 2). By using this approach, the biohybrid entity was able to measure the valve movements, i.e. the well-being of the mussel.

The measurements of the valve movements (as taken from the top valve coordinates) from each of the mussels are presented in figure 6(A). The degree of the opening was then converted to percentages, with the maximum degree of openness representing 100% and an average was taken (figure 6(B)). The results showed simultaneous and quick closing movements (high group agreement parameter) to the sudden disturbance in the environment (a rock dropped in the proximity of the experiment location). This shows that this bioinspired setup is able to detect the distress of the mussel with simple valve tracking. It also confirmed that the quick valve movements are a short and immediate reaction to physical stress.

In this particular experiment, a specific reaction was expected, therefore it was of merit to use an average opening as the monitor of stress. In case of an

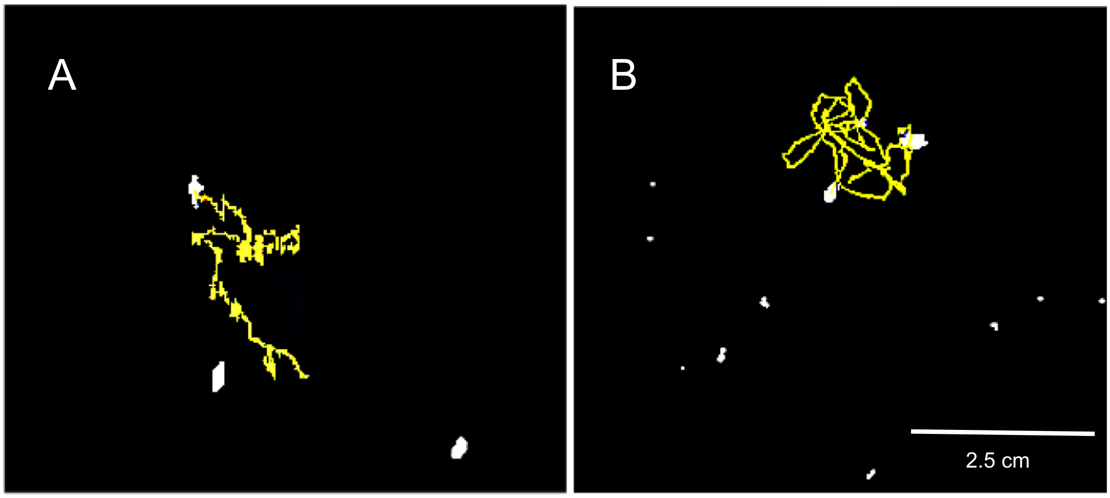

Figure 7. Post-processed footage of live *Daphnia* inside the underwater setup. White shapes indicate *Daphnia*. (A): Processed footage of *Daphnia*'s swimming trajectory under normal conditions (B): processed footage of *Daphnia*'s swimming trajectory immediately after exposure to a stressor, here, increased salinity. The trajectories shown are a 30 s-long sample of footage taken every 5 min over an 8 h period. The scale bar shown in (A) and (B) corresponds to both images. The analysis of the data obtained during these experiments can be found in section 3.2.

unknown stressor, various statistical approaches will be used and applied to the group of mussels which are expected to show similar valve patterns.

3.2. Daphnia

The first experiments were conducted using an underwater setup. Setup testing was done in the lakes of interest, while trials including stress reactions were conducted under laboratory conditions. The results from both tests are presented in figure 7. Swimming trajectories of *Daphnia* under normal conditions in their natural habitats form a semi-straight line with sharp turns (figure 7(A)). Shortly after conditions become unfavourable, i.e. the salt is added, the trajectories changed visibly (7(B)) with the swimming being relatively smoothly curved and having a circular trajectory. The Chi-square test was performed on the data ('stressed' and 'unstressed' against 'sharp turns' and 'smooth turns'). The results of the Chi-square tests showed a statistically significant difference in the swimming trajectory $X^2(1, N = 898) = 4.66, p < 0.5$. Stressed *Daphnia* are more likely to present smooth turning than *Daphnia* under normal conditions.

Next, in order to further calibrate the sensor, the reaction to three salinity levels (2.5, 3 and 4.5 ppt, also 'low', 'medium' and 'high') was tested. The behaviour change was observed for each salinity over 22 h. Both the movement inhibition and spinning were at their lowest levels at the lowest salinity. At the highest salinity *Daphnia* showed increased spinning within the first 3 h of exposure after which the animals became immobile (figure 8).

Various time steps (1 h, 5 h, 10 h and 22 h) were compared regarding the spinning and movement inhibition. The Kruskall–Wallis test showed significant differences in movement inhibition between each group and the control at all of the time steps. There was no significant difference in the movement inhibition and spinning behaviour between the low and medium salinity at the 22 h mark. Regarding the spinning behaviour, significant differences were found between low and high salinity at 5 h and between medium and high salinity at the 22 h mark ($p < 0.5$). The differences in movement inhibition were significantly different between all groups at the 5 h and 10 h mark ($p < 0.001$). At the 22 h mark, only the difference between low and medium salinity was not significant.

3.2.1. MFC

Preliminary experiments on the MFCs were carried out in the field. The voltage obtained from each individual cell and their collective voltages are presented in figure 9. The voltage was observed to rise gradually as the bacterial activity intensified. Once it reached a limit of 500 mV or the bacterial activity stabilized, the discharge of the capacitor was triggered into the super-capacitor which was then able to power the biohybrid. This was a sufficient amount of energy to perform 10 measurements per day, store them and allow data transmission. This shows that the MFCs are a sufficient energy source for low-power electronics and are able to trigger periodic measurements over long experiment runtimes.

4. Discussion

In this paper, the first results of the development of underwater bioinspired entities are presented. It was shown that living organisms give promising

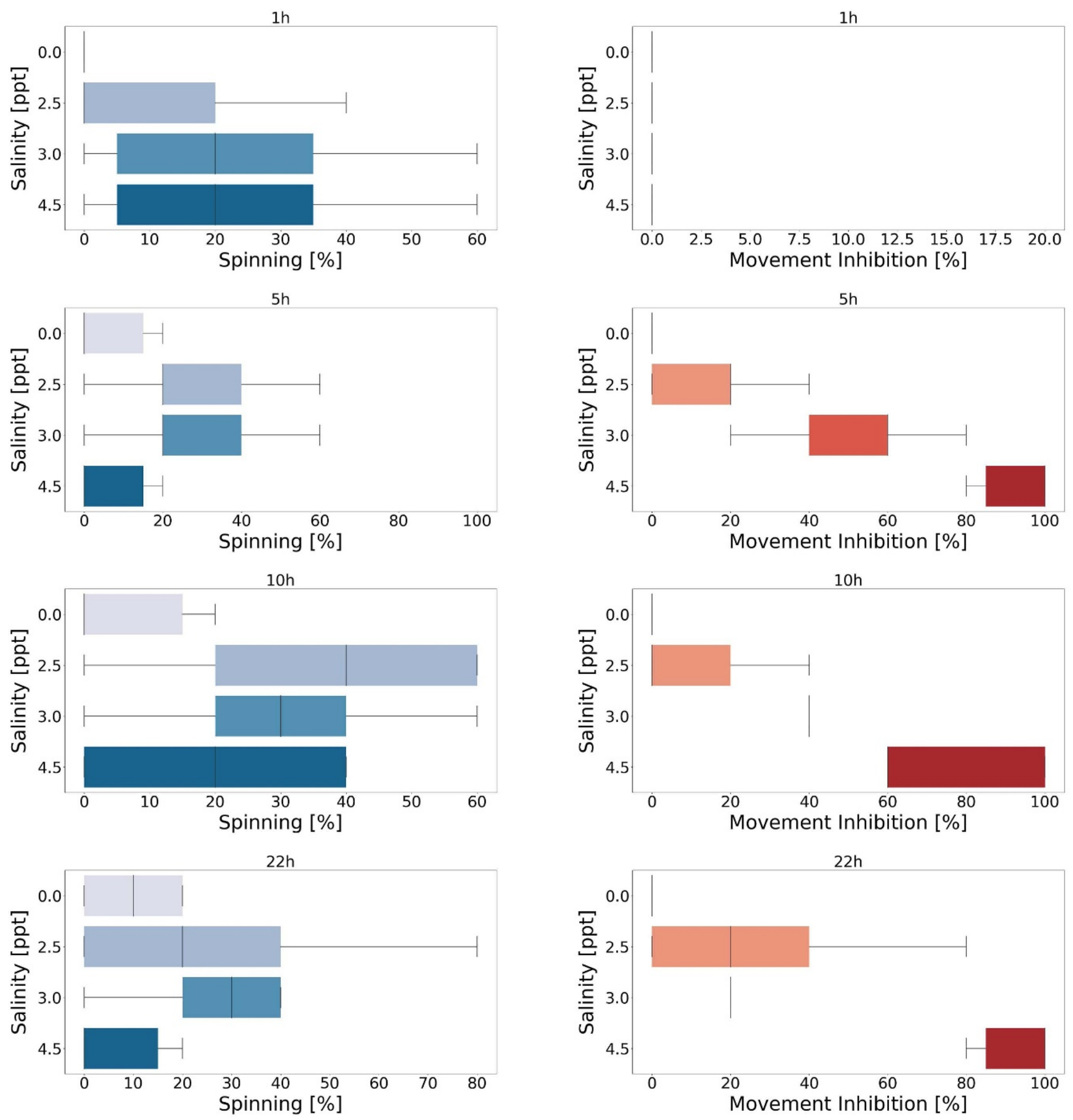

Figure 8. Behavioural responses of *Daphnia* to varying salinity levels (0, 2.5, 3 and 4.5 ppt) with time. The behaviours investigated were spinning and movement inhibition. The whiskers of the box plots represent Q1 (lower whiskers), Q2 (box with the median line) and Q3 (upper whiskers reaching maximum).

results for being extensions of traditional sensors for environmental monitoring. We also show that MFCs are a sufficient energy source and have the potential to become an environment-friendly power supply.

The concept of a 'lifeform in the loop' has been successfully used in research investigating the use of bio-inspired robots with terrestrial plants, fish swarms and honeybees [31, 32]. This concept introduces a methodology for 'ecosystem hacking' where insight is gained through observation of the natural processes occurring between the organism and its environment [30, 33]. This leads to the creation of an early-warning system which aims to successfully detect sudden changes in the environment. Additionally, other features of the biohybrid entity, such as its biodegradability and autonomy can be transferred to other fields such as passive monitoring, classical robotics etc [2, 34]. The overall contribution of this project to various branches of science and industry is presented in figure 10.

4.1. MFCs

For this biohybrid entity for environmental monitoring, the MFCs are used as an environmentally friendly and autonomous power source. Harvesting electricity from electrogenuous bacteria has been previously researched and tested extensively in the laboratory and non-aquatic fields [35–37]. Their usability in underwater projects has also been recognised in recent studies [2, 38]. As mentioned previously, the main challenge with using the MFCs is their low currents produced. Studies showed that MFCs are

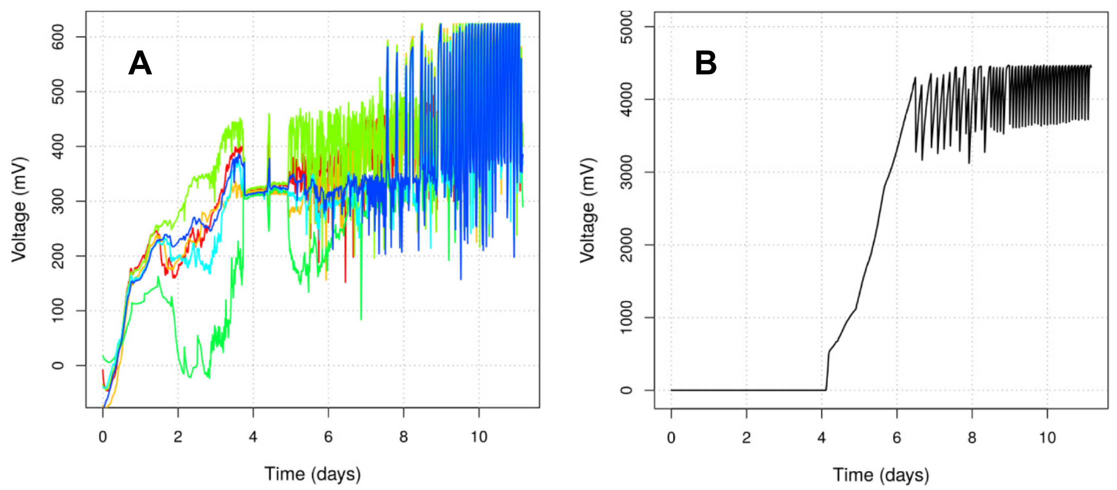

Figure 9. Results of the experiments testing the voltage produced by the microbial fuel cells. (A): Voltage produced by each of the six microbial fuel cells (various colours) and (B): Voltage gathered by the super-capacitor charged by microbial fuel cells (black). Figure Reproduced from [14].

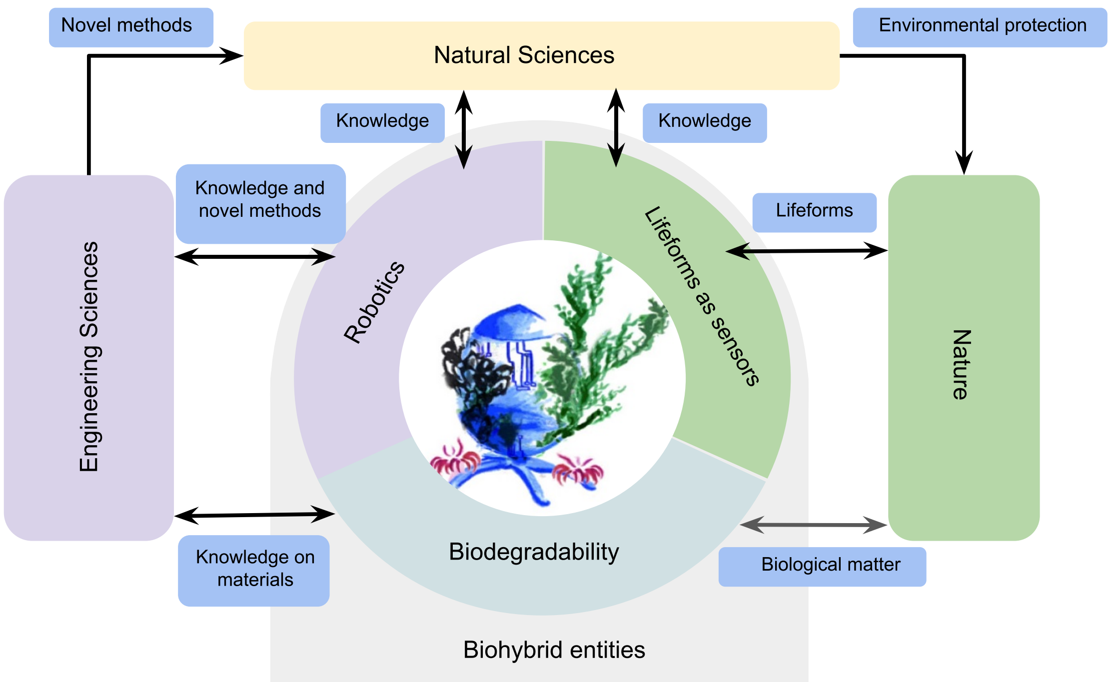

Figure 10. A schematics illustrating the overall impact of the concept of biohybrid entities as tools for environmental monitoring and fields it draws inspiration from. The grey box represents the field of biohybrid entities, which here are built by combining (1) robotics (purple), (2) using lifeforms as sensors (dark green) and (3) biodegradability (light green). Blue boxes specify ways in which our research draws from and influences other fields. Dark green fields represent the ongoing research regarding the usability of lifeforms as sensors and it leads further to gain more information on the organisms used (knowledge in the field of nature). Purple fields represent the research on robotics and it further leads to engineering sciences as a broader field. We draw knowledge on various materials from the field of engineering, and in turn, provide research on novel methods that can be applied to traditional robotics. This research gains provides a new type of data to the natural sciences (yellow box) and draws the knowledge needed to identify the changes in the environment.

difficult to scale up and when connected together, a voltage reversal might occur [39]. Here, we show that stacking multiple MFCs, connected by a super-capacitor provides enough energy for experimental runtimes with the use of low-power electronics. Previous studies and experiments have also found this to be a successful method of scaling up benthic MFCs [29].

4.2. Organisms as sensors

The preliminary results obtained from experiments with mussels showed that they are a good indicator of a disruption in the environment. Their valve movements are quick responses, of which the frequency can potentially specify the stressor. More tests regarding this behaviour will be performed. As this species has been recognised as particularly sensitive to low oxygen levels [16], it is worth investigating this and other parameters in relation to the aforementioned behaviour.

For the final design of the biohybrid entity, a larger number of mussels is recommended to account for any organism-specific differences in behaviour. However, for the experiment described in this study, four specimens were sufficient to observe the disrupting effect of the disturbance. Additionally, various statistical analyses will be applied to read other possible stress behaviours. In terms of a water pressure change, such as the one in this study, it is sufficient to observe the average behaviour across all animals, as their reaction is predictable and the stressor is known. For future monitoring, individual measurements will be considered additionally to the group behaviour to account for any differences in the responses.

In the case of *Daphnia*, the swimming patterns gave a clear indication of a change in the environment. Here, spinning, also described as 'smooth turning', and movement inhibition were investigated as a stress response. Results indicated that *Daphnia* under stress is more likely to present smooth turns that are a disruption to their normal hopping and sinking pattern. It was also shown that movement inhibition is directly correlated to increasing salinity and can be used as one of the sensor calibration parameters. Based on these results, it can be advised to divide *Daphnia*'s swimming pattern into 'mobile' and 'immobile' and then further to 'normal', 'spinning' and others.

Detecting spinning behaviour opens the door to faster detection of disruption as it often occurs immediately after the exposure and with lower stress levels. This creates degrees of stress on which the biohybrid entity can base decisions, such as sounding an alarm, increasing the frequency of the analysis, relocating etc. These and other behaviours, such as sinking, decreased speed or swarming, are worth further investigation to determine the levels of *Daphnia*'s reactions. The sensor will also be calibrated against other stressors such as heavy metals, pesticides and Diesel.

4.3. Challenges

As shown before, the concept of 'lifeform in the loop' is worth further investigation. We show that information on the ecosystem can be drawn, from a simple observation of the movement. This, however, comes with new challenges that must be overcome to provide reliable results. Organisms' behaviour is a low-precision sensor which creates certain difficulties when analysing the data. Certain parameters can cause various responses when acting separately or combined, showing the complexity of the aquatic ecosystems [13]. This makes it difficult to identify the source of stress precisely. For example, the valve movements of the zebra mussel are influenced by a variety of factors, some of which might not be relevant to the monitoring mission (physical irritation, predator presence etc) [18]. One of the ways to overcome this challenge is by performing detailed data analysis comparing the behaviour of multiple organisms and identifying whether the behaviour is caused by chronic or acute stress. For this approach, a thorough understanding of the species biology and ecology is essential. Here, well-researched species were purposefully chosen in order to focus on biohybrid entity development, rather than purely biological research. Once the method is established, more organisms will be incorporated into the setup. Besides the two organisms described previously, several other ideas will be investigated, such as the swan mussel *Anodonta cygnea*, *Hydra* and representatives of the group Chironomidae.

It is important to mention that this concept does not aim to replace the traditional monitoring usually performed with the use of sampling and classical sensors. It offers a complementary method to already established monitoring programs, in order to provide different, additional types of data on the investigated water body. Sample-based surveys and sensor measurements are reliable and effective methods for monitoring water quality. Sensors are a widely available and easy-to-obtain source of data on a specific set of parameters of interest. They can also be used continuously which is of merit in case of a sudden environmental change and gathering large amounts of long-term data. However, due to their narrow spectrum, it is unfeasible to use them for all environmental factors, some of which may be crucial to the overall water quality. Similarly, sample taking provides precise information on the investigated substance (i.e. heavy metals), however, each substance requires a specific methodology (i.e. burning) which must be planned and prepared and usually prevents drawing other information from the sample. However, the overall state of the ecosystem can often include factors unaccounted for during traditional monitoring surveys. Parameters for which there are no sensors, for example, unknown toxins or diseases, can affect the entire water body without being detected by routine measurements.

Here, we proposed an early-detection system whose main role is to detect a disruption in the environment early enough to induce additional measurements from the scientists in the management of the respective water bodies. In case the alarm is set off by the biohybrid entity, samples or sensor data still must be taken in order to confirm the source of the disruption. Analysing specific stress responses and comparing them between the observed organisms can help to narrow down the pool of potential stressors, although the need for human intervention is not completely disregarded. Therefore, it is important to stress that the proposed system provides a different type of data that will complement that obtained from sensors and sample taking.

Certain steps are taken to increase the robustness of this approach. The biohybrid entity can draw conclusions and assumptions about the ecosystem by combining the responses of multiple organisms. Different species will exhibit different reactions to certain stimuli, thanks to which we can detect a broad range of environmental changes.

4.4. Similar initiatives

The concept of using organisms as natural sensors has been previously investigated in other contexts [18, 40]. Similar approaches have been implemented in recent years for laboratory-based monitoring. To mention one prominent example, a product designed by *AquaDect* uses various species of mussels as an early-warning system based on their valve movements [41]. Similarly, the design of another organism-based piece of equipment, *Daphtox*, provides early detection of various changes in the water quality [42]: up to 10 living *Daphnia* swim in a water chamber constantly supplied with treated water. Using image analysis, a deviation in their swimming pattern can sound an alarm informing about a potential toxin in the water. Also, *Lab-on-a-Chip* systems have been recently used to develop complex integrated biosensors with trained aquatic arthropods (e.g. ostracods) with interesting potential for biomonitoring tasks [43].

While these approaches are highly effective, we offer to develop those methods into novel, ultra-low power, autonomous biohybrids for in-situ observations. This will help to reduce the cost of the monitoring missions and reduce significantly the need for maintenance. Usually, traditional sensors require frequent and careful up-keeping, as well as calibration [9]. Organisms are able to self-calibrate and self-sustain which greatly reduces the need for human interference with the system.

To avoid the transport of species to a new habitat and to prevent changes in the environment, only local organisms are investigated [44, 45]. The well-being of the organisms used has to be considered at all times to accurately perceive their natural reactions during experiments. The concept of biohybrid entities as devices for long-term environmental monitoring has shown promising results worth further investigation.

4.5. Outlook

Project Robocoenosis aims to develop the methodology for creating biohybrid entities (biohybrids) for environmental monitoring. Large segments of this bioinspired entity are meant to be biodegradable and remain in the environment after the mission is terminated. This minimizes the negative impact of the biomonitoring missions on the habitat. This part of the project is under development and is not shown in this work. The system powered by the MFC allows the creation of a self-sustaining monitoring system. Paired with organisms that are adapted to surviving in their habitats, it has the potential to provide an extensive long-term and continuous database. Thanks to the long lifespan of mussels and the continuous breeding of *Daphnia* within the experimental setup (data not shown) we can create an entirely self-reliant system. Further research will be focused on these aspects within the next years.

Data availability statement

The data that support the findings of this study are openly available at the following URL/DOI: https://10.5281/zenodo.8375648.

Acknowledgment

This work was supported by EU-H2020 Project Robocoenosis, Grant Agreement No 899520. Furthermore this work was supported by The Field of Excellence COLIBRI (COmplexity of LIfe in Basic Research and Innovation) at the University of Graz. We further thank the 'Österreichische Bundesforste AG', the 'Burgenländische Landesregierung, Abt. 4 (Natur- und Klimaschutz)' and the 'Biologische Station Illmitz' for their support.

ORCID iDs

Wiktoria Rajewicz https://orcid.org/0000-0001-6328-2023
Alexander J Casson https://orcid.org/0000-0003-1408-1190
Joshua Cherian Varughese https://orcid.org/0000-0002-3250-0742
Ronald Thenius https://orcid.org/0000-0002-0420-4444

References

[1] Damania R, Desbureaux S, Rodella A-S, Russ J and Zaveri E 2019 *Quality Unknown: The Invisible Water Crisis* (World Bank) pp 77–87
[2] Thenius R *et al* 2018 *Subcultron-Cultural Development as a Tool in Underwater Robotics* (*Springer International Publishing* vol 732) ed P Lewis and C Headleand (Springer) pp 27–41
[3] Hirota Y 1994 Effects of dissolved oxygen in a de-ionized water treatment on GaAs surface *J. Appl. Phys.* **75** 1798–803
[4] Lee W and Westerhoff P 2006 Dissolved organic nitrogen removal during water treatment by aluminum sulfate and cationic polymer coagulation *Water Res.* **40** 3767–74
[5] Li D, Xu X, Li Z, Wang T and Wang C 2020 Detection methods of ammonia nitrogen in water: a review *Trends Anal. Chem.* **127** 115890
[6] Volkov A G and Ranatunga D R A 2006 Plants as environmental biosensors *Plant Signal. Behav.* **1** 105–15
[7] Banna M H, Imran S, Francisque A, Najjaran H, Sadiq R, Rodriguez M and Hoorfar M 2013 Online drinking water quality monitoring: review on available and emerging technologies *Crit. Rev. Environ. Sci. Technol.* **44** 1370–421
[8] Pamanji D, Sagar P S and Datta D 2021 Water quality survey on potable water through analysis of physicochemical

parameters from various sources in Rajam *AIP Conf. Proc.* **2417** 020010
[9] Kotamäki N, Thessler S, Koskiaho J, Hannukkala A O, Huitu H, Huttula T, Havento J and Järvenpää M 2009 Wireless in-situ sensor network for agriculture and water monitoring on a river basin scale in southern Finland: evaluation from a data user's perspective *Sensors* **9** 2862–83
[10] Sommerfield C K 2016 Qualities and limitations of fluvial suspended sediment data United States Geological Survey *J. Coast. Res.* **32** 719–24
[11] Roussel J-M, Paillisson J-M, Tréguier A and Petit E 2015 The downside of eDNA as a survey tool in water bodies *J. Appl. Ecol.* **52** 823–6
[12] Dilger T, Melzl H and Gessner A 2018 *Legionella* contamination in warm water systems: a species-level survey *Int. J. Hyg. Environ. Health* **221** 199–210
[13] Fernández N, Gershenson C *et al* 2014 Measuring complexity in an aquatic ecosystem *Advances in Computational Biology* (*Advances in Intelligent Systems and Computing*) vol 23 ed L F Castillo and M Cristancho (Springer International Publishing) pp 83–89
[14] Thenius R *et al* 2021 Biohybrid Entities for Environmental Monitoring *ALIFE 2021: The 2021 Conf. on Artificial Life* (*Online, July 2021*) (MIT Press) p 33
[15] Sun L, Yu Y, Chen Z, Bian F, Ye F, Sun L and Zhao Y 2020 Biohybrid robotics with living cell actuation *Chem. Soc. Rev.* **49** 4043–69
[16] Alexander J J E and McMahon R F 2004 Respiratory response to temperature and hypoxia in the zebra mussel *Dreissena polymorpha Comp. Biochem. Physiol.* A **137** 425–34
[17] Kobak J and Ryńska A 2014 Environmental factors affecting behavioural responses of an invasive bivalve to conspecific alarm cues *Animal Behav.* **96** 177–86
[18] Dzierżyńska-Białończyk A, Jermacz Ł, Zielska J and Kobak J 2019 What scares a mussel? Changes in valve movement pattern as an immediate response of a byssate bivalve to biotic factors *Hydrobiologia* **841** 65–77
[19] Kobak J, Poznańska M and Kakareko T 2012 Behavioural changes of zebra mussel *Dreissena polymorpha* (Bivalvia) induced by Ponto-Caspian gammarids *Biol. Invasions* **14** 1851–63
[20] Matthews J, Van der Velde G, Bij de Vaate A, Collas F P L, Koopman K R and Leuven R S E W 2014 Rapid range expansion of the invasive quagga mussel in relation to zebra mussel presence in the Netherlands and Western Europe *Biol. Invasions* **16** 23–42
[21] Ortells R, Reusch T B H and Lampert W 2005 Salinity tolerance in *Daphnia magna*: characteristics of genotypes hatching from mixed sediments *Oecologia* **143** 509–16
[22] Siciliano A, Gesuele R, Pagano G and Guida M 2015 How *Daphnia* (Cladocera) assays may be used as bioindicators of health effects? *J. Biodivers. Endangered Species* **5** S1:005
[23] Noss C, Dabrunz A, Rosenfeldt R R, Lorke A and Schulz R 2013 Three-dimensional analysis of the swimming behavior of *Daphnia magna* exposed to nanosized titanium dioxide *PLoS One* **8** e80960
[24] Hansen L R and Roslev P 2016 Behavioral responses of juvenile *Daphnia magna* after exposure to glyphosate and glyphosate-copper complexes *Aquatic Toxicol.y* **179** 36–43
[25] Ren Z, Li Z, Ma M, Wang Z and Fu R 2009 Behavioral responses of *Daphnia magna* to stresses of chemicals with different toxic characteristics *Bull. Environ. Contam. Toxicol.* **82** 310–6
[26] Roozen F and Lürling M 2001 Behavioural response of *Daphnia* to olfactory cues from food, competitors and predators *J. Plankton Res.* **23** 797–808
[27] Dodson S, Hanazato T and Gorski P R 1995 Behavioral responses of *Daphnia pulex* exposed to carbaryl and *Chaoborus* kairomone *Environ. Toxicol. Chem.* **14** 43–50
[28] Rajewicz W, Romano D, Varughese J C, Van Vuuren G J, Campo A, Thenius R and Schmickl T 2021 Freshwater organisms potentially useful as biosensors and power-generation mediators in biohybrid robotics *Biol. Cybern.* **115** 615–28
[29] Babauta J T, Kerber M, Hsu L, Phipps A, Chadwick D B and Arias-Thode Y M 2018 Scaling up benthic microbial fuel cells using flyback converters *J. Power Sources* **395** 98–105
[30] Stefanec M, Hofstadler D N, Krajník T, Turgut A E, Alemdar H, Lennox B, Şahin E, Arvin F and Schmickl T 2022 A minimally invasive approach towards "ecosystem hacking" with honeybees *Front. Robot. AI* **9** 791921
[31] Schmickl T *et al* 2013 ASSISI: Mixing animals with robots in a hybrid society *Biomimetic and Biohybrid Systems* vol 8064, ed N F Lepora (Springer) pp 441–3
[32] Wahby M, Heinrich M K, Hofstadler D N, Neufeld E, Kuksin I, Zahadat P, Schmickl T, Ayres P and Hamann H 2018 Autonomously shaping natural climbing plants: a bio-hybrid approach *R. Soc. Open Sci.* **5** 180296
[33] Ilgün A *et al* 2021 Bio-hybrid systems for ecosystem level effects *ALIFE 2021: The 2021 Conf. on Artificial Life (Online, July 2022)* pp 41–51
[34] Albaladejo C, Sánchez P, Iborra A, Soto F, López A J and Torres R 2010 Wireless sensor networks for oceanographic monitoring: a systematic review *Sensors* **10** 6948–68
[35] Walter X A, You J, Winfield J, Bajarunas U, Greenman J and Ieropoulos I A 2020 From the lab to the field: self-stratifying microbial fuel cells stacks directly powering lights *Appl. Energy* **277** 115514
[36] Ieropoulos I A, Greenman J, Melhuish C and Horsfield I 2012 Microbial fuel cells for robotics: energy autonomy through artificial symbiosis *ChemSusChem* **5** 1020–6
[37] Tsompanas M-A, You J, Philamore H, Rossiter J and Ieropoulos I 2021 Neural networks predicting microbial fuel cells output for soft robotics applications *Front. Robot. AI* **8** 633414
[38] Buitrón G and Cervantes-Astorga C 2013 Performance evaluation of a low-cost microbial fuel cell using municipal wastewater *Water Air Soil Pollut.* **224** 1470
[39] Oh S-E and Logan B E 2007 Voltage reversal during microbial fuel cell stack operation *J. Power Sources* **167** 11–17
[40] Kirsanov D, Legin E, Zagrebin A, Ignatieva N, Rybakin V and Legin A 2014 Mimicking *Daphnia magna* bioassay performance by an electronic tongue for urban water quality control *Anal. Chim. Acta* **824** 64–70
[41] Bownik A and Wlodkowic D 2021 Advances in real-time monitoring of water quality using automated analysis of animal behaviour *Sci. Total Environ.* **789** 147796
[42] Soldán P 2021 Improvement of online monitoring of drinking water quality for the city of Prague and the surrounding areas *Environ. Monit. Assess.* **193** 758
[43] Romano D, Rossetti G and Stefanini C 2022 Learning on a chip: towards the development of trainable biohybrid sensors by investigating cognitive processes in non-marine Ostracoda via a miniaturised analytical system *Biosyst. Eng.* **213** 162–74
[44] Ren J *et al* 2021 An invasive species erodes the performance of coastal wetland protected areas *Sci. Adv.* **7** eabi8943
[45] Havel J E, Kovalenko K E, Thomaz S M, Amalfitano S and Kats L B 2015 Aquatic invasive species: challenges for the future *Hydrobiologia* **750** 147–70