Application of Biosensors and Artificial Intelligence for Biomonitoring Aquatic Ecosystems Towards Achieving Sustainable Water Utilization

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ABSTRACT

In modern conditions of progressive water pollution intensity resulting from increased discharges of untreated or partially treated waters and frequent technogenic accidents, the development of automated methods and means for operational detection of pollutants and assessment of their danger to aquatic ecosystems and humans has become imperative. This article describes the development of an automated biomonitoring system based on behavioral responses of bivalve mollusks combined with machine learning algorithms for real-time identification of pollutants. The system has been successfully deployed for both marine and freshwater environments, utilizing hall sensors and magnets attached to mollusk shells to detect valve movement patterns. The system incorporates artificial intelligence for anomaly detection using unsupervised machine learning algorithms, including isolation forest (iForest), elliptical envelope, one-class support vector machine (SVM), and local outlier factor (LOF). Laboratory and field tests demonstrate high sensitivity, reliability, and effectiveness in detecting dangerous pollution levels within seconds to minutes. The system represents a significant advancement in continuous automated environmental monitoring for sustainable water utilization across marine and freshwater aquatic ecosystems.

Introduction

In the aquatic environment, we face the simultaneous impact of a complex mixture of many thousands of pollutants on ecosystems (Zhang et al., 2022). While chemical analysis can identify the concentration of pollutants, it cannot assess the actual toxicological threat these substances pose to living organisms. Existing aquatic environment monitoring systems, which rely primarily on physicochemical methods, remain labor-intensive and costly. They generate fragmented data, cover only a traditionally narrow spectrum of pollutants, fail to ensure timely detection of sudden releases, and cannot rapidly assess the associated ecological or human health risks. (Feio et al., 2021; Zolkefli et al., 2020; Kokkali and van Delft, 2014). Furthermore, the number of chemical compounds polluting the aquatic environment is so great that it is difficult to control (Rodriguez-Mozaz et al., 2004). Threshold level indicators, used in modern environmental practice, characterize only quantitative characteristics of substance content, are established for a relatively small number of chemicals, and aimat ensuring the safety of certain categories of water users. They are not aimed at protecting aquatic ecosystems and do not determine the level of danger to the "health" of biota as a whole (Depledge and Galloway, 2005).

Assessing the actual impact of pollution on the aquatic environment is possible using biological monitoring methods. Living organisms respond quickly and accurately to pollution, providing a comprehensive assessment of the state of the aquatic environment in real time, regardless of the composition and origin of xenobiotics. Biological early warning systems (BEWS) are increasingly playing a role in solving the problem of organizing continuous automated biomonitoring of surface waters in environmental practice worldwide (Bae and Park, 2014). These systems utilize living organisms as sensors embedded in electronic circuits and recording various physiological, biochemical, and behavioral indicators (Dvoretsky and Dvoretsky, 2023). These systems, unlike physical and chemical monitoring methods, allow for the acquisition of integrated toxicological characteristics of the environment in real time and the assessment of water quality as a habitat for aquatic organisms (Kramer and Foekema, 2001). Such systems, while not providing information on the content of specific pollutants, provide real-time information on unfavorable aquatic environments. Moreover, given their high reliability, ease of maintenance, high level of automation, and low cost, they are the most suitable means for effective environmental monitoring over large water areas.

One challenge is the correct interpretation of incoming signals with high fault tolerance (Meti and Sangam, 2017). In global practice, BEWS utilizes mollusks, fish, crustaceans, algae, and other organisms as biosensors. Prominent examples of aquatic biomonitoring systems using mollusks include the Musselmonitor (Kramer and Foekema, 2001) and Dreissena Monitor (Borcherding, 1992; Borcherding, 2006).

These systems evaluate the behavioral responses of mollusks, including the extent of valve opening, the nature of their movement, the number of valve movements, and the percentage of open mollusks. The Aqua-Tox-Control and bbe Fish Toximeter systems use fish to assess the ecological state of the aquatic environment by analyzing their behavioral responses (swimming speed, depth of swim in the water column, turning speed, and circling motion) (Mons, 2008; Kuklina et al., 2013). The cardiac activity characteristics of the narrow-clawed crayfish Astacus leptodactylus were used as biomarkers in a bioelectronic system for continuous monitoring of the quality of purified water at the State Unitary Enterprise Vodokanal in St. Petersburg (Kholodkevich, 2007; Kholodkevich et al., 2006).

This article describes the developed aquatic monitoring system using machine learning algorithms and mussel-based biosensors for both marine and freshwater environments and presents the results of laboratory and field tests demonstrating the versatility and effectiveness of the technology across different aquatic ecosystems.

RESULTS

The structure and operation of the system

Continuous and effective environmental monitoring of aquatic areas in the seas, oceans, and freshwater bodies requires the development of an automated monitoring system that would alert to changes in aquatic environmental parameters using the behavioral responses of bivalve mollusks. This system should include a network of biosensor monitoring systems deployed in aquatic environments and a universal monitoring center. Through long-term research in various environments (sea and freshwater), the "ECOBIOMONITOR" system was developed (Shatokhin et al., 2021; Grekov et al., 2020; Grekov et al., 2019). It is based on the behavioral responses of bivalve mollusks, along with software packages that enable real-time identification of pollutants, primary data processing, and internet transmission (Trusevich et al., 2010). This system is an upgrade to a prototype developed in 2008 (Trusevich et al., 2010).

The system operates by recording the activity of bivalve mollusks, specifically the extent to which their valves are open. For bivalves, motor activity is an indicator of normal functioning (Trusevich et al., 2021). Knowledge of the behavioral responses of mollusks in their natural (normal) habitats allows us to assess their reactions under adverse conditions and generate alarm signals. Marine Mytilus galloprovincialis (Lam, 1897) and freshwater Unio pictorum (Linnaeus, 1758) bivalves were used in the tests. The developed system consists of above-water and underwater sections connected by a cable (fig. 1).

The underwater portion of the system consists of a mussel colony unit and a battery, connected to the anchor by a chain. The mussel colony unit is a sealed container designed for a depth of up to 20 meters. It contains: a) a control unit, b) 16 Hall sensors hermetically mounted in the device's housing, c) 16 permanent magnets on remote plates, d) mussel attachment points, e) a light sensor, f) a temperature sensor, and g) a pressure sensor. The above-water portion of the system is a sealed container installed in conjunction with a buoy. The container contains: a) a backup battery, b) a GSM module, c) a control unit, and d) a flashing beacon.

The ends of the cable-rope (KG 3×1.5-70 grade) connecting the above-water and underwater sections are fitted with sealed connectors. This allows for servicing the system even from small vessels. Furthermore, the cable-rope connection between the system components is made through thimbles, allowing for variable installation depth of the underwater section and providing additional reliability during long-term operation. A flashing beacon is integrated into the container's hull and provides visibility of up to 2.5 nautical miles.

The mussels are attached to the underwater section of the system using polymer adhesive (fig. 2). One mussel flap is secured to the device's platform, while the other flap is attached to a freely moving plate with permanent magnets attached to it. The plates are 3D printed using individual templates taken from the mussels. Changes in the distance between the flaps during their movement, and consequently the distance between the Hall sensor and the magnet, lead to changes in the sensor's output voltage.

The underwater module's control software sequentially measures the voltages of all Hall sensors, the light sensor, and the pressure sensor, and also reads the digital temperature sensor. This data, represented as digital codes and combined with a digital marker in a specific sequence, forms a measurement frame. The measurement frame is transmitted to the surface portion of the system via an RS-232 interface and a tethered cable.

The surface module's controller, implemented using the MSP-430FR5994, receives a frame of measurements. After receiving 20 frames of measurements, the controller generates a data packet and transmits it via the GSM module to the system's internet server. The data packet contains data on physical parameters (valve opening distance, water temperature, and illumination) obtained during calibration using codes, the system's serial number, and the current time and date. It also contains additional primary measurement information in the form of digital codes and is used for debugging and diagnostics of the system.

To determine actual mussel activity values, it is necessary to obtain individual calibration coefficients for all the system's measurement channels. This procedure can also be performed in the field immediately before installation. The device's design allows for calibration of the measurement channels without disassembling the device in the field, maintaining its hermetic seal.

Behavioral responses of freshwater mollusks

The freshwater system developed for water supply intake monitoring at Sevastopol's Vodokanal in the Chornaya River (Southern Federal District) provides critical insights into mollusk behavior and system performance in freshwater environments (Trusevich et al., 2010). Prolonged, continuous,

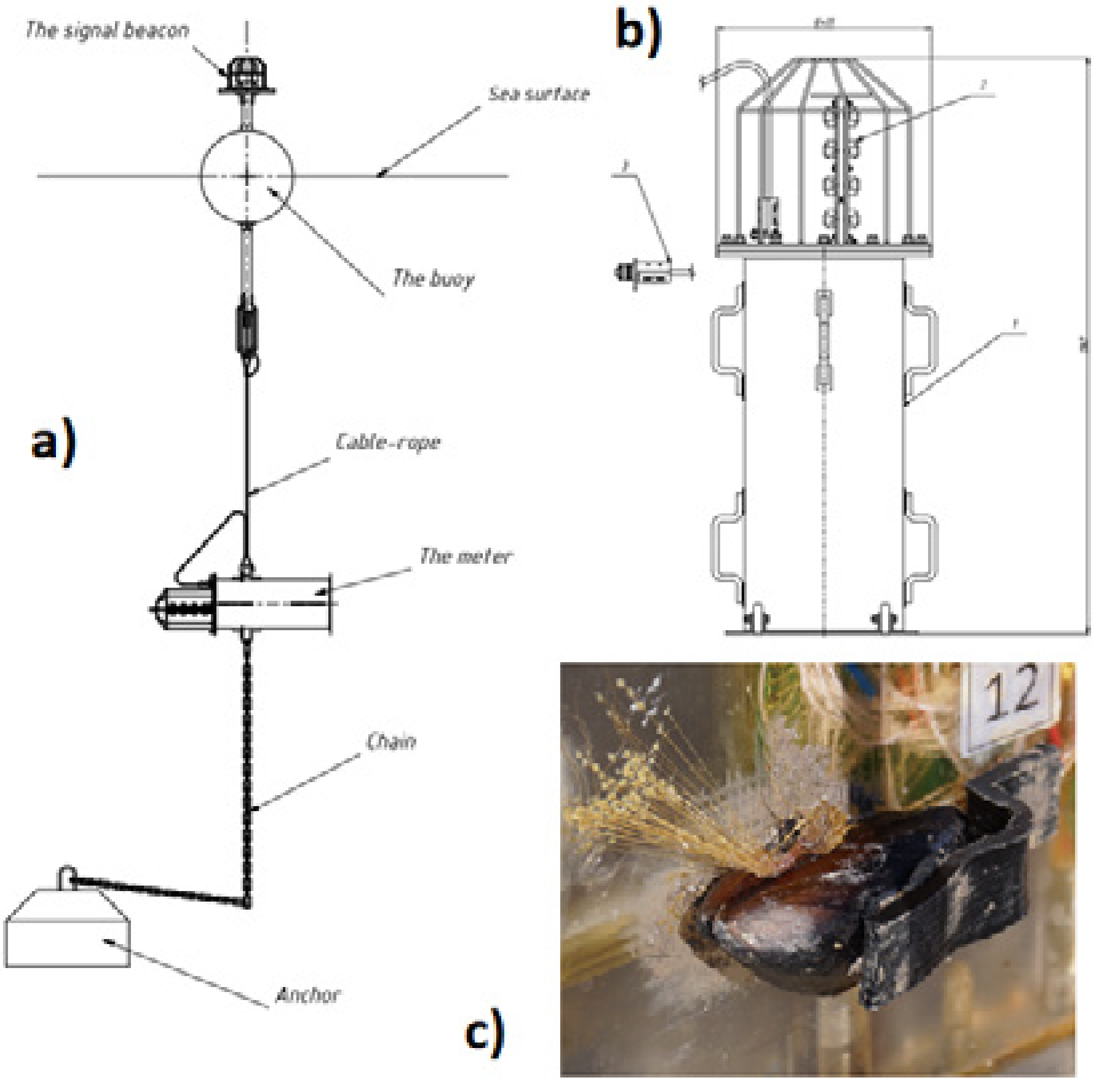

Figure 1. General diagram of the device (a), diagram of the underwater part of the device (b) and attachment of the mussel to the block of colonies (c). 1 – sealed container with batteries; 2 – block of mussel colonies; 3 – watertight connector (Source: Authors)

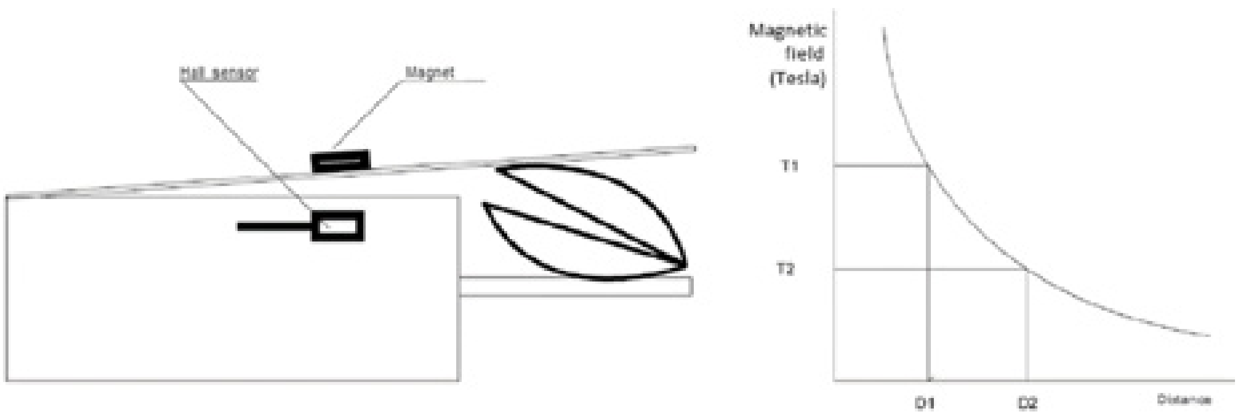

Figure 2. Scheme of attachment of a mussel to a block of colonies (a) and characteristics of the magnetic field in the measurement range (b) (Source: Authors)

year-round research of baseline characteristics of mollusk behavior showed that under normal conditions, valve movements follow a strict daily rhythm. With the onset of dusk, the frequency of adductions (rapid valve closing and subsequent opening within 0.5-1 minute) increases sharply and continuously, with gradually decreasing frequency, until dawn. During daylight hours, the frequency of valve closures ranges from 1 every 2-3 hours to 2-3 per hour. The frequency increases sharply to 10-15 or more times per hour in the initial phase of the nighttime portion of the daily cycle (Trusevich et al., 2021). The ability of the organism to maintain a normal daily rhythm indicates the level of adaptation of the animal to its habitat. Any deviations from normal conditions indicate significant changes in environmental parameters.

In the daily rhythm of freshwater mollusks, two groups of movements can be distinguished: a) adductions (valve closures) – rapid, brief movements of valve closure and opening lasting from 0.5 to 1.5-2 minutes (Trusevich et al, 2021), which mainly facilitate the removal of metabolic products and pseudo-feces, and b) slow, occupying most of the day, shallow oscillations that facilitate filtration and respiration processes. The amplitude and frequency of valve opening are completely individual and vary widely depending on the physiological state of the mollusks and fluctuations in environmental factors.

Mollusks react sharply to many sudden changes in the surrounding environment. Tapping on the aquarium, sudden sounds, vibrations, sudden shadows, and the like cause mollusks to instantaneously (within 2-3 seconds) close their valves for a short time (1-2 minutes) (Trusevich et al, 2021), which appears to be a manifestation of protective reflexes. However, with frequent repetition of these non-damaging effects, the magnitude of the mollusk reaction decreases rapidly and disappears. Such brief synchronized responses of mollusks practically do not affect the overall rhythm of valve movement, and during the operation of automated water environment monitoring systems, they should be excluded by the controlling computer programs as false signals.

Sensitivity of freshwater mollusks to pollutants

Laboratory experiments assessing the sensitivity of mollusks to pollutants in freshwater environments have been conducted since 2017 in 120-liter aquariums installed on the riverbank with continuous water flow from the main riverbed at 4 liters per minute. Experiments were conducted synchronously in flowing and non-flowing variants. In the non-flowing variant, the calculated concentration of the pollutant was introduced into the aquarium through a tube from a distance of 3 meters. Uniform mixing was achieved using an aquarium micro compressor. In the flowing variant, the calculated concentration of the toxicant solution was supplied by a peristaltic pump directly into the flow of incoming water in the aquarium, thus maintaining the necessary concentration of the toxicant throughout the experiment.

The mollusks were tested for sensitivity to: ammonia – 1; 2; 10 MAC; copper sulfate – 0.062; 0.125; 0.25; 0.5; 1; 2 MAC; detergent sodium dodecyl sulfate (SDS) – 1; 2; 3; 5 MAC; lead acetate – 10; 20; 30; 50 MAC; cadmium sulfate – 20; 30; 100 MAC; potassium bichromate – 10; 20; 100 MAC; nickel sulfate – 20; 100 MAC (in terms of metal ions), in accordance with MAC values established by Russian Hygienic Standards (Trusevich et al., 2017).

Results showed that signs of mollusk reaction to the appearance of polluting chemical agents in water depend on the nature and concentration and manifest in increased frequency and duration of adductions, decreased amplitude of valve opening, and/or complete closure of valves during negative influences. Negative effects are detected for several hours after the removal of polluting components from aquarium water. Mollusks react sharply to ammonia and detergents even at relatively low concentrations – 2 mg/l (2 MAC) according to Russian Hygienic Standards (SanPiN 1.2.3685-21). Copper ions present the highest stress factor for mollusks, with sensitivity thresholds as low as 0.006 mg/l (Trusevich et al., 2017). In the waters of the Chornaya River, which have a high content of humic substances and other components of organic and mineral suspension, the toxic action of heavy metals on mollusks is greatly reduced. The first signs of lead impact at 2-hour exposure are only detected at 100 MAC (0.6 mg/l), nickel at 100 MAC (2 mg/l), cadmium at 20 MAC (0.02 mg/l), and chromium at 20 MAC (1 mg/l). This is explained by the fact that salts of heavy metals and other chemically active substances, upon entering water environments with high humic acid content, practically instantaneously form complex compounds of varying stability levels and are largely converted to sediment in bottom silts, while the greatest danger to living organisms comes from free ions of chemically active elements (Trusevich et al., 2017).

It is important to note that mollusk reaction in the flowing water variant of the experiments is significantly lower than in the non-flowing variant. Consequently, when assessing the toxicity of a substance, significant discrepancies can arise between flowing and stagnant water conditions. These conclusions are fully consistent with data from numerous foreign researchers.

Organization of a system for monitoring the aquatic environment

Active research, which began in 2020, is currently underway to develop sensor networks for water quality monitoring, including those based on the principles of the Internet of Things (Trusevich et al., 2010). The creation of a unified automated aquatic environment monitoring system with a single monitoring center is a critical requirement for implementing the operational control program, as it will ensure the detection of emergency situations and the adoption of measures to prevent negative impacts on aquatic ecosystems. The proposed system consists of three elements: a server with a database, the biosensor systems described in the previous section, and user or operator information panels (fig. 3).

The system server is implemented using scripts stored on remote computers in the Data Center. These scripts enable the following capabilities:

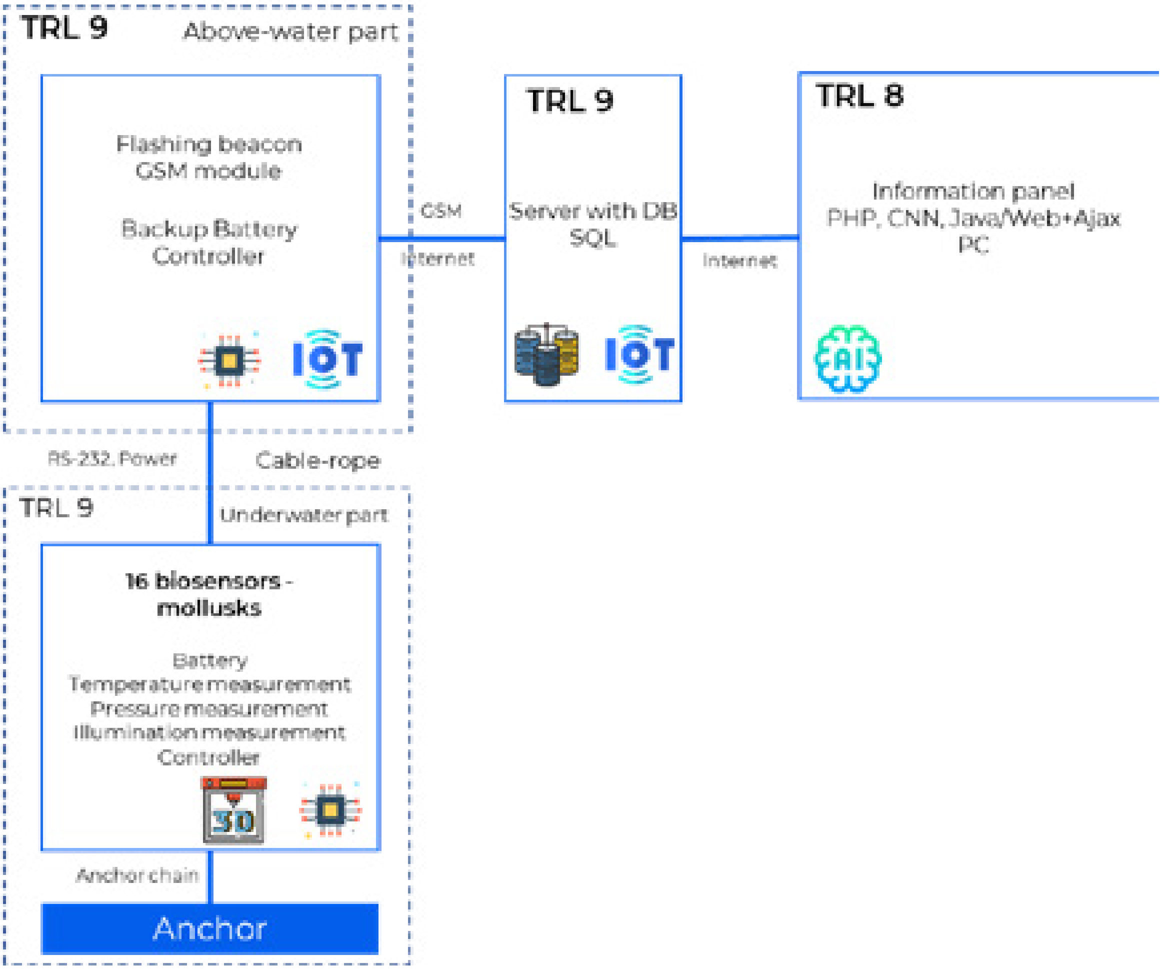

Figure 3. Diagram of the aquatic environment monitoring system (Source: Author)

importing current calibration coefficients for each system to the server; receiving data from the system and storing this data on the measurement data server; providing system operators with access to measurement data, primary data, calibration coefficients, and data received from the system online. The server software, responsible for processing incoming measurement data, extracts data from the POST request received from the system. Based on the received system serial number, this software finds the corresponding data on the server and appends the new data to the primary measurement data files and physical characteristics files.

The systems transmit primary and preprocessed measurement data to the server and upload calibration coefficients. Using specialized software on the dashboard, the operator can access the server and monitor the status of any operating system (or determine why one is currently down). The software automatically monitors physiological parameters and behavioral responses of mussels, such as valve opening range, closure frequency, and duration of closure, among other things. After analyzing these parameters, the program can identify significant deviations from normal values and generate alerts that may indicate pollution in the monitored aquatic area.

To manage the system, the operator must periodically (but no more than once every 24 hours) monitor and analyze the system's status: to identify unusual behavior patterns; to detect measurement channels that are not functioning properly; and to study long-term degradation patterns (possible responses to long-term, low-level pollution).

Laboratory and field tests of the device

Before long-term trial operation, the system's functionality was verified in laboratory and field conditions for both marine and freshwater environments.

Laboratory tests (marine environment): During the first stage, a prepared mussel colony block with attached marine mussels was placed in a tank containing seawater, where the mussels adapted for 24 hours, and data on their normal valve activity was transmitted to the server. The seawater was enriched with oxygen by blowing air through it using a micro-compressor. The lighting was not changed during the tests, and no impacts on the tank were allowed to confirm the "NORMAL STATE" signal in the program interface. At the beginning of the tests, 5 grams of a 10%

ammonia solution were added to the tank using a syringe. Within 10 minutes, a "POLLUTION" warning signal appeared on the operator's computer monitor. A second portion of the prepared solution, 5 grams at a 10% concentration, was then added to the tank. Within 10 minutes, a "DANGEROUS POLLUTION" warning signal appeared on the operator's computer monitor. Changes in motility parameters were monitored on the operator's computer monitor using a valve activity analysis program. After the warning signals were generated, the water in the tank was replaced with clean water, and the time it took for the mussels to regain motility was recorded.

Field testing (marine environment): Tests in the marine environment were conducted by exposing the mussels to a measured level of pollution in the immediate vicinity of a mussel colony block located at the operating depth. An environmentally safe solution of table salt, detergent, and white clay was first prepared. The underwater module of the complex was installed at a depth of 3 meters, where the mussels adapted for 2 hours, and data on their normal valve activity was transmitted to the server. The prepared solution was poured through a 40 mm diameter plastic pipe at a distance of 1 m from the underwater module. The operator monitored the mollusks› motor activity using a valve activity analysis program. Within 15 minutes, the «DANGEROUS POLLUTION» warning signal was automatically generated.

Additionally, the system was tested for mechanical impacts, leakage, and hydrostatic pressure, as well as for measuring mussel valve opening, temperature, illumination, and pressure, and for monitoring battery voltage.

Field trial operation (marine environment): From August to December 2021, the first unit of the system was deployed in the coastal waters of Sevastopol at the mariculture aquafarm for trial operation. From June to October 2022, the system was reinstalled. After 120 days, 14 of the 16 mussels remained in the operating position (two mussels died).

Freshwater system performance: The freshwater system deployed for two years in continuous functioning at water intake monitoring points in Sevastopol demonstrated high sensitivity, reliability, and efficiency in detecting dangerous water pollutants in real time. The system successfully operated under natural conditions in the Chornaya River, showing the ability to continuously monitor freshwater quality while maintaining operational stability over extended periods.

Anomaly detection using machine learning

Anomaly detection in our system is implemented using machine learning algorithms. The study assessed the feasibility of anomaly detection in bivalve data using unsupervised machine learning algorithms (Grekov et al., 2023). Four machine learning algorithms were tested for the anomaly detection procedure: elliptical envelope, isolation forest (iForest), one-class support vector machine (SVM), and local outlier factor (LOF).

By tuning the hyperparameters of the four algorithms, their performance estimates were obtained, and the response time of the methods for anomaly detection was assessed. Since an F1 score of one was obtained for three algorithms with different hyperparameters, the methods were compared in terms of response time and anomaly detection. The F1 score is the harmonic mean of precision and recall and provides a better estimate of misclassified cases than the accuracy score.

For three anomalies, the iForest machine learning algorithm demonstrated the best anomaly response rate when averaging data over 15 minutes. The same set of averaging times was tested for all models: no averaging, 1 minute, 5 minutes, 15 minutes, and 30 minutes. The elliptical envelope and one-class SVM algorithms also demonstrated good performance, but their anomaly detection rates were lower than those of the iForest algorithm. This artificial intelligence component significantly enhances the system's ability to identify subtle environmental changes and distinguish genuine pollution events from natural behavior variations, thereby improving the reliability and responsiveness of the monitoring network.

Conclusion

The developed system enables early detection of dangerous levels of pollution in both marine and freshwater bodies. The system automatically and in real time receives digital data on mussel movements using Hall sensors and magnets attached to their shells. Individual responses of 16 mussels are simultaneously recorded. A synchronized response by a group of mussels (at least 70% of the total) triggers an alarm. The developed software analyzes mussel movement trends, identifies synchronized mussel responses to environmental influences, automatically triggers an alarm, and transmits data online (all in real time). The system's latency to respond to pollution ranges from a few seconds to several minutes, depending on its nature and concentration.

10 years of use of the system in both marine and freshwater environments confirm its high effectiveness in assessing water quality and pollution levels in seas, freshwater bodies, and rivers. The design features of the developed system allow it to be used both as a permanent water monitoring station and as a portable option for active monitoring in specific regions and areas. The system's demonstrated performance in diverse aquatic ecosystems – from seawater to river freshwater – underscores its versatility and robustness for sustainable water utilization applications.

The key advantages of the proposed system include: a highly automated monitoring process, the ability to create large automated aquatic monitoring networks, enabling real-time detection of pollution sites and assessment of their hazard to humans and other living creatures, high reliability, ease of operation, durability, and a relatively low cost. The use of native mollusks allows for the scalability of the complex and the entire system to other regions. Integration of artificial intelligence and machine learning algorithms further enhances the system's capability to detect anomalies with high precision and minimal false positives, supporting effective environmental management and the achievement of sustainable water utilization goals.

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