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# ECOSYSTEM HEALTH: A CONCEPT, METHODOLOGICAL APPROACHES, AND ASSESSMENT CRITERIA

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Increasing anthropogenic load on aquatic ecosystems threatens environmental safety. In this regard, it is important to apply the ecosystem approach to the exploitation of natural resources in order to develop integrated regulatory environmental measures. The term "ecosystem health" is commonly used in assessment of the ecological state of water areas by representatives of foreign scientific communities (HELCOM, ICES, OSPAR, and MEDPOL), but it is not widespread among domestic researchers. The concept of "ecosystem health" is not a new paradigm. Specifically, it is the subject of discussion in the scientific literature since the early 2000s and the issue enshrined in long-term documents of the European Union and the EU Water Framework Directive on environmental preservation strategy. Based on a review of existing literature data, this article presents the principal concepts, approaches, and criteria for assessing the ecological state (health) of aquatic ecosystems. As emphasized, the assessment of the ecosystem health depends on goals and objectives of environmental research, and those are related to applied methodology and, accordingly, to selection of methods and indicators of the ecosystem state. The paper discusses the concept of "organism's health" and several its attributes: homeostasis maintenance, cause-and-effect relationships in the *health–disease* continuum, and functional adaptations. Several approaches for assessing the health of rivers and marine areas are compared. Various indicators, complex indices, and biomarkers of exposure and effects are considered which reflect the susceptibility of aquatic ecosystems to changes resulting from natural and anthropogenic load. Attention is drawn to the need for applying the integrated ecosystem approach in the analysis of the aquatic ecosystem state: this will contribute to holistic assessment of the consequences of human activity on the ecosystem integrity. Based on the experience of the BONUS+/BEAST project, a comprehensive biomarker approach is presented to determining the health of bioindicators with subsequent interpretation of data on the health status of the ecosystems these organisms inhabit. The authors hope that the review will be of interest to both specialists in ecology of aquatic ecosystems and representatives of environmental organizations steeped in ecological expertise.

**Keywords:** ecosystem health, assessment of the aquatic ecosystem state, reference ecosystem states, physiological state, functional adaptations, macrobenthic invertebrates

A rapid deterioration of the state of the ecosystems has exacerbated the need for introducing an integrated management of human activities based on accumulated knowledge on ecosystems and specifics of their functioning. The United Nations proclaimed 2021–2030 the Decade on Ecosystem Restoration. The ecosystem approach to exploitation of natural resources is significant for the analysis and taking actions to establish relationship between human activities and environmental issues, as well as for development of integrated regulatory environmental measures. In this regard, it is important to define some concepts and clarify methodological approaches (Directive 2000/60/EC, 2000); those are often used in foreign scientific communities and expert groups (The Helsinki Commission, HELCOM; Institute for Clinical Evaluative Sciences, ICES WKIMON; Working Group on Ecosystem Effects of Fishing Activities, WGECO; and Study Group for the Development of Integrated Monitoring and Assessment of Ecosystem Health in the Baltic Sea, SGEH) and are not widespread either in domestic biomonitoring studies or in publications on the environmental risk assessment.

Since the early 1990s, the issue of the "ecosystem health" definition and approaches to its assessment has been actively discussed in foreign literature. To a greater extent, this concerns approaches to assessing the aquatic ecosystem state. In the monograph *Ecosystem Health*, Robert Costanza and co-authors (1992) emphasized that this issue is complex: it includes economic, social, and environmental aspects. The concept of "ecosystem health" unites several meanings, even philosophical one – to the same extent as biological and medical ones. This implies the use of different indicators (biological, physical, and chemical) for assessing the ecological state and requires taking into account social and economic consequences of the shifts in "health". Thus, the multiambiguity of the "ecosystem health" definition is embedded in the methods of its cognition and in initial setting of the goal/goals achieved during the research.

When considering the concept of "ecosystem health", we have to start with defining what we mean by the ecosystem.

The ecosystem is a "localized in space and dynamic in time set of various organisms living together and forming communities and conditions of their existence which are in a regular relationship with each other and form a system of interdependent biotic and abiotic processes" (Alimov, 2000). The ecosystem can be characterized by structural and functional indicators that are in certain relationships with each other but can alter the vector of such relationships and the relationship between the flows of matter, energy, and information (Beyers & Odum, 1993). The ecosystem boundaries are mobile; those can vary depending on the research task.

**The concept of "health" is primarily an attribute of living organisms.**

Health as **homeostasis** is the maintenance of the internal stability of an organism aimed at its further survival and prosperity. Homeostasis can be considered as a non-equilibrium system which, however, does not go beyond the "swing". In the late XIX century, Claude Bernard – a French physiologist – introduced the concept of the "stability of the internal environment as the condition for a free life." According to C. Bernard, maintaining the stability of the conditions of the internal environment is the only goal of the organism.

Later, Academician A. Ugolev gave a definition of homeostasis as follows (1987): "In a broad sense, homeostating is maintaining the stability of basic biological, physical, and chemical constants. This concept is the main one in modern interpretations of such various phenomena and conditions as health, disease, and preservation of the environment and the biosphere."

Health as the **absence of disease**. A disease means a significant alteration in an organism, in its functional systems, organs, and tissues, as well as a failure in the regulation of physiological and biochemical processes due to homeostasis-disrupting effects or external and internal changes. The transition from health to disease can be considered as a process of gradual decrease in the organism's ability to adapt to environmental changes which results in a decline in functions.

The assessment of changes in the organism's state is **time-dependent** (in the *organism–ecosystem* structure, it should be time-scaled), and this allows to understand the cause of an alteration in the health of animals (Chernysheva, 2007) or plants and indirectly assess a shift in the habitat quality that could lead to the changes. The "time component" is a continuum, a change in the health of an organism associated with some kind of stressor event (a disturbance, *e. g.* environmental pollution) allowing to link the moment of onset of a stressful effect resulting in deterioration in ecosystem health since sometimes the process has a considerable time lag. However, the time factor has its own "relativity" there: even after industrial accidents or terrorist acts (both heavily affect the biotic component of the ecosystem), the onset of consequences does not begin immediately (it takes time). Sensitive biota components are the first to react. Serious disturbance or even degradation of the affected aquatic ecosystem occurs much later (sometimes in several years) due to ongoing compensatory processes at each structural level of the ecosystem; moreover, it occurs only under imbalance in repair processes (Alimov, 2000 ; Aleksandrov, 2010 ; Ostroumov, 2005). ' Importantly, a chemical or other stressful effect does not always result in deterioration in the health of an organism and its disease. The organism is capable of changing its functioning parameters within certain limits: there is a **functional (physiological) adaptation**.

In the middle of the XX century, a new approach was formed defining health as **organism's ability to adapt** to environmental conditions. I. Davydovsky (1950s–1960s) developed the foundations of the medicine of the future, where health is considered as adaptive capabilities of an organism. He defined the disease as the result of a decline in reserves and depletion of the organism's defenses (Davydovskii, 1962).

Later, this direction was developed in space medicine, where the **measure of a person's (operator's) health is defined as the functional capacity** of an organism, the ability to adapt quickly and painlessly to new conditions (challenges) (Baevskii & Berseneva, 1997). In fact, identification and quantitative assessment of this functional capacity is carried out by scientists involved, for example, in pre-flight training of pilots – to minimize possible stressful effects and to detect "defects" in their health at an early stage.

Hans Selye, based on his own long-term experience in neurophysiology and psychoanalysis, created the doctrine of **stress** for isolating a non-specific component in organism's reactions to various effects resulting from the **mobilization of functional reserves** (Selye, 1982). He was the first to note that the stressful effect depends on modality, strength, and duration of the disturbance, as well as on the adaptive capabilities of the organism.

Adaptation is considered as a functional property of biological objects, along with homeostasis. In organisms, the existence of certain cycles of activity which repeat in different time ranges (circadian, ultradian, minute, decasecond, and other rhythms) can be considered as an adaptation as well (Ashoff, 1984 ; Bursian, 2012). A decline in the ability of an organism to change its functional parameters under rhythmic daily shifts in environmental conditions is one of the earliest indicators of deterioration in its adaptive capabilities and, consequently, health.

**The lower the adaptive capabilities, the more uniform the reactions of an organism** and the smaller the arsenal of its probable responses to shifts in the environment. After "heavy" effects, the organism loses lability (in terms of adaptability) due to a shutdown of some functions supporting the plasticity of the transition from one level of regulation of the functional system to another.

The issue of **assessing the ecological state (health) of aquatic ecosystems** is complex and ambiguous (Report of Working Group 28, 2019). In most cases, the criteria are developed for small river ecosystems with a limited set of components and with simple trophic chains and relationships. The term "ecosystem health" was first proposed by David Rapport (1989).

As previously believed, one of the indispensable signs of the ecosystem health is its stability (Costanza et al., 1992). The easiest way to assess it is to analyze the constancy of the population size of key species. Specifically, stable populations determine the favorable ecological status of the system.

This statement also meant that such systems are capable of maintaining a stable biocoenosis determining the stability of their structure, coherence of the functioning of ecosystem components, and sufficient completeness of biodiversity. Healthy ecosystems can maintain their self-purification capacity (Ostroumov, 2005) and, as a result, stability of the water quality acceptable for local biota species.

However, it turned out that the statement about the stability (as a kind of immutability or constancy) of the ecosystem is debatable since the ecosystem might have some lability and be capable of rebuilding its functioning under sudden external effects (The Ecosystem and How It Relates to Sustainability, 2017). "The slight instability is the necessary condition for the true stability of the organism," are the words attributed to Walter Cannon, a famous American psychophysiologist.

Assessing the stability of a system is not an easy task: it implies the ability to predict the dynamics of the system under stress. Michael Mageau and co-authors (1995) identified two components of resilience that can be measured using simulation models – recovery time ($R_T$) and maximum magnitude of stress (MS). $R_T$ can be estimated by measuring the time it takes for a system to recover from a wide range of stressors and to reach previous steady state. MS can be measured by increasing the simulated stress gradually until the system returns to its new steady state (with the strength of the stress causing the shift taken into account). The total resilience score can be derived from $MS/R_T$ ratio. With constant MS value, the system with the shortest $R_T$ is more stable. With equal $R_T$, the system with the highest MS value is more stable.

Importantly, in the early XX century, V. Vernadsky in his doctrine of the biosphere strived to connect the biological component with the geochemical structure of the biosphere, productivity and diversity of living organisms, and energetics. The scientist claimed that complexes of biogeochemical interactions in ecosystems have the property of homeostasis and, therefore, have natural mechanisms for regulating the resistance to affecting factors (Vernadskii, 1989).

In modern reference books, the term "homeostasis" refers to self-regulation, ability of an open system to maintain the internal stability through coordinated reactions aimed at keeping dynamic equilibrium. In biological systems, it can be implemented through adaptive modes associated with the adaptation of the organism's structure and functions, restructuring, or shift in metabolic or energy characteristics of ecosystems (Egorov, 2019). From the perspective of control theory, the ecosystem homeostasis is realized by negative feedbacks between their components according to the Le Chatelier–Braun principle.

In the monograph by G. Polikarpov and V. Egorov (1986), new mechanisms for the formation of ecosystem homeostasis according to a marine environmental pollution factor were identified and mathematically developed, biogeochemical criteria for normalizing anthropogenic load were substantiated, and theoretical basis for studying anthropogenic ecology and biogeochemical cycles of marine ecosystems was formed. Based on the results of long-term research, Academician of RAS V. Egorov (2019) proposed methods for implementing the concept of sustainable development of critical and recreational zones in the Black Sea by regulating the balance between the consumption of water quality resources and their reproduction resulting from natural biogeochemical processes.

The assessment of the sustainability of marine ecosystems to anthropogenic load was developed by several domestic researchers. The issue was generalized in the concept of assimilation capacity, and it served as the scientific basis for the ecological regulation of anthropogenic load on the World Ocean aimed at maintaining the ecosystem integrity (Izrael & Tsyban, 1989).

In assessing the ecosystem health, **biodiversity** of plant and animal species forming the ecosystem is a key component. The quantitative stability of inhabiting species is of great importance for long-term monitoring of water areas. Obviously, water basins inhabited for a long period by stable populations of key species can be classified as environmentally safe. However, there are regions, *e. g.* the Baltic Sea areas, where biodiversity is extremely limited by a set of physical and chemical factors. Those are hypoxia spots, natural sources of sulfur gases emission, critical salinity of some water areas creating boundary conditions for existence (or even survival) of animal and plant species, and thermo- and haloclines as natural environmental factors limiting the ranges of various organisms (Drozdov & Smirnov, 2008). Consequently, in nature, species diversity can be affected by both natural and anthropogenic factors. To analyze the water quality in the ecosystems, various methods for assessing biodiversity are applied, for example, the Shannon, or Shannon–Wiener, index (Strong, 2016). At the same time, there is an opinion that the Shannon diversity index provides a slightly overestimated assessment of water quality under conditions of eutrophication of water basins (Barinova, 2000).

A widely used criterion is O/E ratio. It is the ratio between the observed (O) and expected (E) number of taxa based namely on a taxon richness, not on abundance data.

Importantly, among the approaches to assessing the pollutant effect on the ecosystem state, one of the most significant ones is applying of methods based on determining the physiological response of native organisms (Fig. 1).

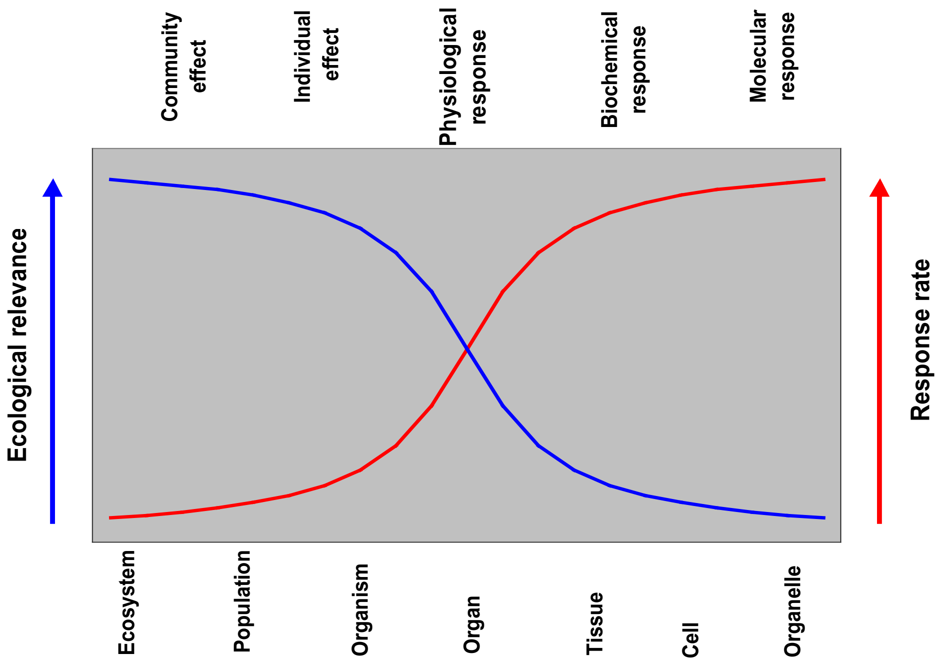

**Fig. 1.** Scheme of the relationship between the response rate at different levels of biological organization and the ecological relevance [from: (ICES, 2010)]

The scheme given (see Fig. 1) shows how limited the approach is when based on the biodiversity index alone. For biodiversity, it takes time to change (from the moment of a polluting factor effect to the onset of clear consequences for the ecosystem). In this regard, there is a problem of timely adoption of cost-effective management decisions aimed at protecting and eliminating the processes of ecosystem health disturbance.

**Functional diversity** is a component of biodiversity describing the diversity of functions that organisms in a community or ecosystem have evolved while interacting. David Tilman (2001) analyzed it in detail.

Usually, studies of the functional diversity assess how organisms affect properties/processes of the ecosystem (Gagic et al., 2015) and what environmental factors and disturbances form the diversity and distribution of functional traits in space and time.

Ecosystem health as a **harmonious unity of the organism and the environment** involves the study of a balanced interaction of environmental components with living organisms. Even V. Vernadsky wrote (1978), "Living matter covers and restructures all chemical processes of the biosphere, and its effective energy is enormous compared to the energy of inert matter. Living matter is the most powerful geological force growing over time." He put forward the hypothesis that "living matter creates for itself an area of life."

Researchers continue to develop this direction. Clive Jones and co-authors (1994) noted that many living organisms act as constructors of environmental elements. The following definition is given: ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species by causing physical state changes in biotic or abiotic materials. Among "ecosystem engineers", there are autogeneic ones (those change the environment with their own living and dead tissues) and allogeneic ones (those transform other's living and dead materials from one physical state to another by mechanical or other effects thus changing the environment). In this case, the vital activity of organisms themselves results in creating a new ecosystem or its components. If the health of an "ecosystem engineer" deteriorates, the ecosystem health is threatened. Specifically, a marine worm *Sabellaria alveolata* (Linnaeus, 1767) is capable of creating extensive bioconstructions that currently form the largest reefs in Europe (Curd et al., 2019). This sedentary colonial polychaete widely distributed in both intertidal and shallow subtidal zones all over the world builds tubes out of sand and shell fragments gluing them together with its mucus (Holt et al., 1998). Such tubes can be inhabited by other animal species. Out of complex relationships between the non-biological and biological components, the most studied are those of a reef community – a multicomponent and structurally and functionally complex "association", with organisms or their communities capable of acting as "engineers" of ecosystem components (Abelson et al., 2016).

In classical domestic publications, **for an integral assessment** of the ecosystem state, it is customary to consider the ratio of production and destruction in the environment (Alimov, 2000). Ecosystem production is the difference between its primary production and total expenditure on metabolic processes for all hydrobionts in the ecosystem. There, the balance of metabolic processes is of key importance. As A. Alimov specified (2000), "if the energy spent on them exceeds the energy contained in primary production, a negative balance of energy in the ecosystem is registered." Usually, the balance in ecosystems is considered as energy flows from accumulator organisms to consumer organisms. It is generally accepted to estimate phosphorus and nitrogen fluxes. "Thus, in a water basin, the key part of the biotic cycle of matter is phosphorus assimilation by autotrophic organisms when creating primary production in aquatic ecosystem," A. Alimov noted (2000).

Due to accelerated dynamics of anthropogenic load on aquatic ecosystems, priority in ecological research should be given to the study of the ecological state (health) and ecosystem functioning under modern challenges.

In 2015, in order to create measures mitigating the effects of climate change and allowing to achieve the sustainable use of marine resources, the United Nations adopted the Sustainable Development Goals. Adapting to current and expected changes in marine ecosystems is essential for human society in the context of successful and sustainable use of ecosystem services. Therefore, decision makers need information on the state of regional marine ecosystems, as well as forecasts of their changes based on a comprehensive understanding of the limits of ecosystem variability.

So, the discrepancy between environmental conservation goals and economic development has been and remains the main problem for achieving healthy ecosystem quality (Margules & Pressey, 2000). This has created and continues to create a serious gap in environmental management (Griggs et al., 2013).

To bridge this gap, the **ecosystem approach concept** was developed. Its core is integrated management of human activities based on the best available knowledge on ecosystems, their dynamics, and stress resilience in order to identify and eliminate the main causes of ecosystem degradation (Decisions Adopted by the Conference, 2000 ; The Convention on Biological Diversity, 1993). Such an approach should ensure the use of elements of the ecosystem without loss of its integrity. To a greater extent, this refers to the state of coastal water areas as recipients of insufficiently treated or untreated wastewaters.

Management based on the ecosystem approach should ensure that implementation of economic activities does not interfere with providing ecosystem services. At the same time, its ultimate goal is to preserve and increase the ability of ecosystems to produce services in the long run (Directive 2000/60/EC, 2000 ; HELCOM, 2010, 2014). Moreover, it is important to establish a relationship between an assessment of the ecosystem health and assessment of environmental risks.

**Methodological approaches to assessing the health status of aquatic ecosystems.** To date, several methods have been developed and tested that are in complementarity relations (field research, observation, description, classification, modeling, forecast, *etc.*). Since methods and approaches to assessing the ecosystem ecological state (health) are numerous, we will dwell on only a few.

When implementing the EU directives and the Water Framework Directive (Directive 2000/60/EC, 2000 ; Directive 2006/44/EC, 2006), several assessment criteria were developed – Environmental Quality Standards (EQS) required for water areas to achieve a good ecological status.

These recommended standards should be based on the Background Assessment Criteria and Ecological Assessment Criteria (BAC and EAC, respectively). BAC characterize the variability of the estimated indicators normal for natural systems (variability ranges); EAC are based on the series of values obtained during toxicological experiments (10–100–1000 × $LC_{50}$, LOEC, NOEC, PNEC, *etc.*) which indicate a deterioration in the environmental quality. As a rule, EAC quantitative indicators are obtained in experiments on laboratory animals – test organisms when determining the effect of various toxicants or their mixtures. Nevertheless, the question arises on the environmental relevance of these values for natural conditions.

From an environmental perspective, the environmental quality is defined as a stable state and functioning of all the ecosystem components, “with fluctuations in the values of parameters not going beyond the natural limits registered over a considerable period of time” (Moiseenko, 2009). Thus, the ability of the biocoenosis to maintain physicochemical and other characteristics of the environment (Odum, 1986) and to recover quickly from any effects which are negative in terms of their direction (final result) can serve as a criterion for the good quality of natural waters.

In recent decades, more and more attention has been paid to biological research methods in monitoring of the environmental state.

When beginning assessment, an essential task is the selection of a bioindicator – an animal or plant taken as a key species. This requires a good knowledge of the hydrobiological state of a water basin (in particular, species diversity of its fauna and flora) and a clear understanding of trophic relationships between its inhabitants (Ekosistema estuariya reki Nevy, 2008). Various taxonomic groups – fish, algae, birds, *etc.* – are used as bioindicators characterizing the health of both river and marine ecosystems, but macrobenthic invertebrates are preferred (Dale & Beyeler, 2001 ; Heink & Kowarik, 2010 ; Rosenberg & Resh, 1993 ; *etc.*). The reasons are as follows: those are present in most aquatic habitats; are relatively easy to sample (compared to other biota); are a diverse group; and are long-lived and sedentary organisms (serve as a site sample). Bioindicator species should be well studied biologically. Such animals should respond to stress in a predictable manner and have low variability in their responses (Attrill & Depledge, 1997 ; Depledge & Galloway, 2005). They are known to integrally respond to chronic anthropogenic environmental pollution (Rosenberg et al., 2004). Sampling and analysis of the composition of aquatic invertebrates can be used to monitor continuous or intermittent disturbances, as well as to study the effects of single or multiple stressors and pollutants in their aquatic environment.

However, when selecting bioindicators for biomonitoring, human economic use of certain species comes to the fore in some cases. It is important to apply relevant (acceptable) physiological or behavioral indicators (biomarkers) and their reference limits, as well as to have a possibility of their non-invasive registration.

In the last century, methods and criteria for assessing the ecological state of **freshwater (river) ecosystems** were actively developed due to their relative simplicity, fixed set of components of such ecosystems, and the fact that trophic and functional relationships are properly studied.

In biomonitoring, there are several approaches to assessing the ecosystem health. The main ones are outlined in the publications (Mageau et al., 1995 ; O'Brien et al., 2016 ; Savić et al., 2017) and are reflected in the recommended criteria developed in the course of international environmental projects – Index of Biological Integrity and River Invertebrate Prediction and Classification System (hereinafter IBI and RIVPACS, respectively).

IBI emphasizes the possibility of biota to serve as an integrator of human effects on nature (ecosystem). This indicator allows analyzing the degradation of river ecosystems taking into account the assessment of biological diversity, complexity and rearrangement of trophic relationships, and ecological organization of ecosystem components (Karr, 1999).

RIVPACS aims at determining the composition of animal and plant species for their protection and reproduction. To do this, locations with “fairly good quality” or “free of serious pollution” have to be found – spots free from severe chemical contamination (Wright et al., 1984).

These two approaches differ both in biological data collected and analyzed and in aims of the assessment. River and estuary monitoring and projects based on the proposed indicators have been successfully implemented in Australia according to IBI (AUSRIVAS) and in the UK for 30 years (RIVPACS I, II, and III).

What do IBI and RIVPACS have in common:

- focuses on biological implications in determining river health;
- using the concept of reference states (as fundamental ones);
- subdivision of locations by classes, with a selected set of environmental characteristics;
- assessment of changes and degradation resulting from anthropogenic load;
- requirements for standardization of sampling methods, as well as requirements for technical equipment of a laboratory, used methodology, *etc.*;
- finding of reference environmental standards (EQS).

Moreover, in earlier works involving similarity indices in community studies, *e. g.* Bray–Curtis index, and multivariate analysis techniques [see in particular (Chiu et al., 2011)], it was noted as follows: these indices and techniques are an integral part of predictive modeling approaches that should become the next step in the development of evaluative biological methods. Multivariate techniques compare test sites with reference ones, and this requires an initial model building by means of computer software.

The concept of reference status, or reference condition, was introduced by John Wright (Wright et al., 1984). Reference is fundamental to multivariate bioassessment approaches used throughout the world: it provides a benchmark for comparison for the water basin studied. A commonly used definition of reference condition was given by Trefor Reynoldson and co-authors (1997), "…a condition representing a group of minimally disturbed areas organized according to individual physical, chemical, and biological characteristics." The advantage of the reference condition approach in multivariate techniques is the following: once the reference sites are grouped (based on indicators of biota status), the independent data, *e. g.* physical and chemical indicators, are used to compare the test sites with the reference ones.

In 1999, a special issue of the journal *Freshwater Biology* included detailed analysis of the river health concept [see in particular (Karr, 1999)] which offers assessments based on the state of the biological components of aquatic ecosystems. Changes in species composition of fish communities are often used as an indicator of the consequences of chemical pollution for the environment.

The environmental values associated with the river health are based on preservation of ecological integrity (ecosystem functioning) and sustainability. In some cases, ecological values and human needs are in conflict when determining the river health. As noted (Karr, 1999), one of the reasons for the river bloom was the inability of the river system to regulate the quantity and qualitative composition of the nutrients required – the loss of ecological function; this led to death of several animal species. Accordingly, the ecological "attractiveness" for human recreational use was reduced.

Recent ecological assessments of the health of forest ecosystems and fish communities within the river basin of the Inland Columbia showed a strong relationship between them (Pausas & Parr, 2018): the degradation of fish communities is often associated with significant changes in surrounding forests. Thus, the need for sustainable existence of various natural habitats / spawning grounds of valuable (for humans) fish species for their natural reproduction and protection usually conflicts with the goals of timber or gold mining enterprises. The core is that the loss of forest areas or changes in the quality of natural waters are obligatory consequences of human activity.

Some disorders result from natural processes. Specifically, a fire can be caused by lightning in a prairie or forest. The effect of fires on the change of animal and plant populations can be analyzed from the perspective that those affect the ecosystem state by changing the gene pool of the species included (Pausas & Parr, 2018). Among ecosystem disturbances resulting from anthropogenic load, there are acid rains, deforestation, algal blooms, and introduction of invasive animal and plant species.

In the last ten years, the **Functional Feeding Groups** (hereinafter FFG) approach is actively used. Developed over 30 years ago, it has been modified in some detail [see in particular (Cummins et al., 2005)], but the core of FFG relationship remains fairly simple. FFG are based on a direct correspondence between the categories of food resources in the environment (ecosystem) and populations of freshwater invertebrates adapted to harvest a particular food resource efficiently. Analysis of the trophic structure of benthic macroinvertebrate communities can be carried out for biological assessments of the river ecosystem state. With trophic, or functional, approach applied, the Index of Trophic Completeness was developed (Pavluk et al., 2000). Moreover, FFG coefficients can be used as surrogates when attributing aquatic ecosystems – also based on the reference standards. This approach can be useful in describing the ecological state of freshwater ecosystems. For the Nišava River in southeastern Serbia, Ana Savić and co-authors (2017) showed that surrogate FFG coefficients are consistent with material on observations of ecosystem properties at sampling sites. So, trophic relationships, the nature of the *predator–prey* relationship, the assessment of the physiological state of macrobenthic organisms, and physicochemical data can serve as a measure for attributing a water area in terms of water quality.

The reference condition approach is considered relevant for the environmental assessment of both river and marine ecosystems.

To assess the ecological state of **marine areas**, a number of international communities have been created and are actively working and interacting, *inter alia* HELCOM, OSPAR, and MEDPOL.

HELCOM is aimed at providing up-to-date information for target users of the Baltic Sea region, both at national and international levels, as well as submitting material for pan-European and global forums (European Union; United Nations Environment Programme, UNEP; and International Maritime Organization, IMO). For the Baltic Sea, the main problem is water eutrophication. The comprehensive assessment of eutrophication carried out by HELCOM includes a section on technical/scientific aspects (science for management) and a section on general political actions of the Baltic Rim countries to achieve good ecological status of its water areas. This is necessary to make informed decisions on restoration of the Baltic Sea ecosystem and on achievement and maintenance of the good ecological status of its subregions. Moreover, it is important for achieving HELCOM goals. The quality of the studies is confirmed by the requirements of strict adherence to HELCOM COMBINE recommendations (Manual for Marine Monitoring, 2017). Besides that, indicators of the status of phytoplankton, aquatic vegetation, and benthic invertebrate fauna, as well as physical and chemical characteristics and various types of loads (usually, phosphorus and nitrogen loads) are presented in national, regional, and European reports on the Baltic Sea status assessment (ICES, HELCOM Reports, and PICES Scientific Groups Reports).

However, in most reports, it is customary not to use specific numerical data, but to apply generalized coefficients. Thus, the Ecological Quality Ratio (EQR) is actively used in assessing the ecological status of water areas. The EQR is recommended in the Water Framework Directive aimed at achieving or maintaining a good ecological status of surface waters by 2021. Therefore, the values of biological quality elements should be taken into account when attributing water basins to any class of ecological status or ecological potential. The EQR scale was adopted as a generalized criterion for comparative regular monitoring of the status of different ecosystems, especially in assessments based on indicators of phyto- and zooplankton state. In this case, for each category of surface water quality (from high down to poor ecological status), it is proposed to calculate boundaries by assigning a numerical value for each category and establishing borders between quality classes. In practice, high EQR values (close to one) indicate a status with no deviation, with unsignificant or little deviation from the reference values; therefore, such values are a sign of an acceptable status corresponding to "areas not affected by eutrophication." Low EQR values indicate moderate, significant, or severe deviations from baseline and unacceptable status corresponding to "eutrophicated areas," with moderate or poor ecological status. As a rule, EQR values are comparable with those of other complex indices. But in the case of the Benthic Quality Index (Blomqvist et al., 2006 ; Rosenberg et al., 2004), significant deviations of indicators (up to 86 %) were allowed; therefore, even extremely low EQR values can sometimes be considered as exceeding the boundary of the good/moderate water class.

To date, the status of benthic invertebrates is assessed for the high sea alone. It can vary significantly between Baltic subregions due to wide distribution of hypoxic and anoxic zones in the Baltic Sea and the Gulf of Finland. Currently, the Baltic Proper – from the Bornholm Deep to the basin northern area and the Gulf of Finland – is in a disturbed state.

The assessment of water quality by various indicators can differ significantly. These discrepancies highlight the issue of using different indices in different countries (regional specifics) and the obvious need for careful intercalibration of methods. Moreover, integral methodological approaches should be developed. Only the use of several indicators will reduce the risk of erroneous assessment of the ecosystem state and increase the reliability of the final conclusions on its health.

To assess environmental quality and health status of marine organisms more accurately, European researchers proposed several approaches and developed complex indices based on biomarkers. Those are the Integrated Biomarker Response, IBR (Beliaeff & Burgeot, 2002); Metal Pollution Index, MPI (Usero et al., 1997); Biomarker Response Index, BRI (Hagger et al., 2008); and Bioconcentration Factor, BCF. The latter one assesses the bioavailability of heavy metals for tissues of living organisms (Mendoza-Carranza et al., 2016). These approaches involving integrated indices (for effect and exposure biomarkers) have been successfully applied for assessing ecological state in many marine areas throughout the world, especially in European coastal zones [see in particular (Biomarkers, 1992 ; Turja et al., 2014 ; Yeats et al., 2008)].

To assess the ecological state of several locations in the eastern Gulf of Finland (the coastal Baltic Sea), N. Berezina and co-authors (2017) proposed a set of well-known biotic indices and methods, including a Saprobity System (based on phytoplankton), Raffaelli and Mason index (meiobenthos), and two macrobenthic derived indices (Goodnight–Whitley index and Benthic Quality Index). As a non-widely implemented index, the authors of this work used the embryo malformation frequency in benthic amphipods (Sundelin & Eriksson, 1998).

In the development of modern methods for analyzing biological effects of anthropogenic load on aquatic ecosystems, one of the key aims is to determine the criteria for assessing their health based on certain biological indication methods. One of the benefits of their applying is that the indicators recorded (biomarkers) reveal themselves at the organism level when the aquatic environment is exposed to sublethal concentrations of pollutants. This allows detecting shifts in the functional state of individual animal species long before the onset of serious changes, degradation of populations and communities, and disturbances of ecosystems they inhabit (Kholodkevich et al., 2018 ; Depledge & Galloway, 2005 ; Kuznetsova & Kholodkevich, 2015). Based on the data obtained, it is possible to develop scientifically grounded methods for assessing the environmental risk for ecosystems. This approach is based on the classical works of the representatives of the British scientific school analyzing the biological effects of environmental pollution and methods for assessing the aquatic ecosystem health. The approach is postulated on the statements substantiated in a number of publications [see in particular (Depledge & Galloway, 2005)]:

- healthy ecosystems are inhabited mainly by healthy animals;
- by measuring the health status of key animal species in the ecosystem, it is possible to assess the environmental consequences of pollution of their habitat.

In most developed countries, this approach is currently a priority for analyzing pollution and its effects on the ecosystem health. The approach allows to carry out an objective assessment of the functional parameters (health) of animals and the ecological state (health) of the aquatic ecosystems these animals inhabit.

Integrative index approaches to monitoring and assessing marine pollution are still under development and improvement. This allows to create a holistic approach to understanding the marine ecosystem health.

The most commonly used biochemical markers of exposure are:

- detoxification enzymes activity – EROD and GST;
- effects of the antioxidant defense system – expression level of mRNA for SOD and SOD activity;
- biomolecular damage levels – DNA breaks (F value);
- lipid peroxidation (LPO) and protein carbonyl (PC) content;
- assessment of the microbiome state of bioindicators.

In this review, it is not possible to list all the indicators used or recommended.

When assessing the ecosystem ecological state (health), an approach involving multi-integrated biomarker indices is actively used: this lays a solid foundation for multiple assessment of marine pollution. The data obtained in the course of integrated studies can serve as a reliable base for a timely and adequate assessment of the state of marine ecosystems, *inter alia* estuarine ones, allowing to predict their changes and to plan environmental measures.

**Experience of participating in the pan-Baltic BONUS+/BEAST project.** The strategic HELCOM Baltic Sea Action Plan (BSAP) defines the main long-term objectives and the need for appropriate management decisions to achieve “good ecological status” and “healthy wildlife.” Hazardous substances were marked as one of the main threats to the Baltic Sea ecosystem and its biota. Accordingly, the BSAP pointed out the need for developing monitoring of the biological effects of pollutants and their mixtures on biota aimed at reliable assessment of ecosystem health. The BEAST (Biological Effects of Anthropogenic Chemical Stress) project contributed to achievement of these goals and solution of several problems (Lehtonen et al., 2014).

The BONUS+/BEAST project involved 16 partners – European institutions – from all the Baltic Rim countries, including the St. Petersburg Research Centre for Ecological Safety of the Russian Academy of Sciences (RFBR grant No. 08-04-92424-BONUS_a). In 2008–2011, within the framework of this project, biomonitoring studies were carried out in several subregions of the Baltic Sea which differ significantly in water salinity, biodiversity, oxygen conditions, *etc.* Field and experimental studies were carried out, with both long-settled and new methods applied in selected water areas of five Baltic Sea subregions; so far, information on the biological effects of hazardous substances there was limited. To establish a relation between the organism’s reactions associated with anthropogenic chemical pollution of the environment and the effects observed at higher biological levels, the participants of the BEAST project formed integrated multi-box tools, with biomarkers as sensitive diagnostic tools included. To date, the biomarker approach in assessing the ecosystem state is widely used: this can provide valuable data on suitable methods for effect assessment (Rudneva & Roshchina, 2008 ; Handy & Depledge, 1999 ; Soldatov et al., 2014) and thereby reduce uncertainties related to extrapolation of biological effects to studied species, endpoints, and chemicals.

The BEAST project uses biochemical markers of oxidative stress, lipid metabolism, acetylcholinesterase content, disruption of lysosome membrane integrity, cardiac activity (heart rate recovery time after a standardized load), presence of genetic disorders (occurrence of micronuclei in cells of various tissues), violations of the early stages of ontogenetic development, presence of parasites in the organisms of bioindicator species of hydrobionts, *etc.* In detail, the issue is discussed in the monograph *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress* (1992). The peculiarities were taken into account of changes in physical and chemical characteristics of the environment, *inter alia* involving passive samples, for different study areas. Historically established conditions were considered for the exploitation of certain water areas in connection with economic activity, *e. g.* use of water areas as ports. The results of the BEAST project were published in several papers and articles, with the main ones being (ICES, 2010 ; Lehtonen et al., 2014 ; Turja et al., 2014).

The project was focused on detecting deterioration in health parameters of bioindicator species (fish, molluscs, and crustaceans) applying biomarkers to compare the ecological state of the areas studied with conditionally reference water areas with similar hydrological and temperature regimes, as well as with similar natural populations of local bioindicator animals. As a result, Integrated Biomarker Response was determined for different study stations.

In the course of the project, a principal component analysis was carried out as well. This allowed to link some indicators of the organism's susceptibility to environmental pollution (Turja et al., 2014).

**Experience in developing a methodological approach to assessing the health of key species of aquatic ecosystems.** Extremely high dynamism of anthropogenic processes imposes special requirements on the speed of detection of ecosystem disturbances and on the speed of taking adequate environmental measures. This necessitates creation and implementation of express methods for diagnosing the current ecological state of surface waters in order to promptly identify the areas of "environmental trouble". In the BEAST project, there was a "novelty": non-invasive recording of the heart rate (hereinafter HR) and analysis of the peculiarities of cardiac activity of local mollusc species from water areas with different anthropogenic load in order to determine possible differences in their functional state were proposed. As shown earlier [see in particular (Depledge & Galloway, 2005 ; Kholodkevich et al., 2017 ; Kuznetsova & Kholodkevich, 2015)], an effective assessment of the ecosystem state (health) can be based on the long-term monitoring of any vital function of the bioindicator – motor, cardiac activity, respiration, *etc.* Specifically, the reaction of the cardiovascular system can be considered as an integral response of the organism to shifts in environmental factors. At the same time, we can apply HR as an ecotoxicological biomarker since it reflects the intensity of physiological processes; moreover, in many cases, it allows to draw a conclusion on the organism's functional state (Kuznetsova & Kholodkevich, 2015).

The pulse is one of the key indicators of the cardiovascular system functioning. The rate may change under various factors (physical activity, stress, and nutrition), but in the absence of pathologies, heart contractions should quickly return to normal. This well-known and verified statement served as the basis of our methodology for testing the functional state of bioindicators taking into account HR recovery time in molluscs and crustaceans after removing the functional load (Kuznetsova, 2013).

As a functional test, it is proposed to consider **reflex actions** under standardized test loads:

- change in water salinity;
- change in water temperature;
- change in lighting;
- effect of vibration.

According to these test stimuli causing test reactions, we propose to assess the adaptive capabilities of the organism – its measure of health.

As mentioned above, we proposed to use a rapid **change in water salinity** in the range of physiological tolerance for a certain animal species as one of the test stimuli (Kuznetsova, 2013).

In a number of our studies on bivalves, it was shown as follows: molluscs responded to a rapid **change in water salinity** (freshwater influx) with a **characteristic behavioral reaction** – valve closure; this resulted in isolation of mussels (their mantle cavities) from the unfavorable environment (Kholodkevich et al., 2009). The process was accompanied by an initial sharp increase in HR, and this can be considered as the primary non-specific response of the mollusc cardiovascular system to stress. Thus, a generalized response to a change in salinity (as a non-damaging osmotic stress effect, within the tolerance range of the species studied) can be used as a standardized stimulus. For freshwater molluscs, we recommend increasing salinity for 1 or 2 hours (up to 10–12 ‰, according to the results of T. Kuznetsova's personal experiments in 2012–2019). For marine molluscs, we recommend halving salinity of their natural habitat.

A **change in temperature** can also serve as a test and can be used to analyze changes in HR in populations of littoral *Patella caerulea* Linnaeus, 1758 differing in the settlement horizon (Santini et al., 1999). Importantly, a change in temperature of the environment necessarily affects the level of metabolism. As known, with a change (increase) in temperature of the environment by 10 °C, the level of metabolism of hydrobionts rises by 2–3 times. The Q10 coefficient has been experimentally determined for different molluscs and crustaceans, and it varies slightly between species due to peculiarities of their biology (Braby & Somero, 2006).

Previously, it was found that molluscs sampled in environmentally safe water areas differ from animals sampled in polluted water areas: those show a higher adaptive ability expressed in a significantly shorter HR recovery time ($T_{rec}$) after removing the functional load (Kholodkevich et al., 2009 ; Kuznetsova & Kholodkevich, 2015). Moreover, animals from environmentally safe water areas, compared with those from polluted water areas, show higher uniformity of reactions expressed in a low coefficient of variation of individual HR values ($CV_{HR} \leq 0.1$) for this group of animals after the test exposure and recovery of initial water salinity. After a raw of investigations, a methodological approach was proposed (Kholodkevich et al., 2018, 2009 ; Kuznetsova et al., 2010, 2018) to a comparative assessment of the ecological status of water area based on the analysis of adaptive capabilities of bioindicators. It was successfully applied not only in laboratory studies, but also in several field ones (Kholodkevich et al., 2015 ; Kuznetsova et al., 2018 ; Turja et al., 2014); those revealed a relationship between anthropogenic pollution of the aquatic environment and the proposed physiological indicators (biomarkers). A peculiarity of this method for assessing the organism's functional state is the absence of subregional limits. This makes the method more accessible during intercalibration.

The scheme of this methodological approach is shown in Fig. 2.

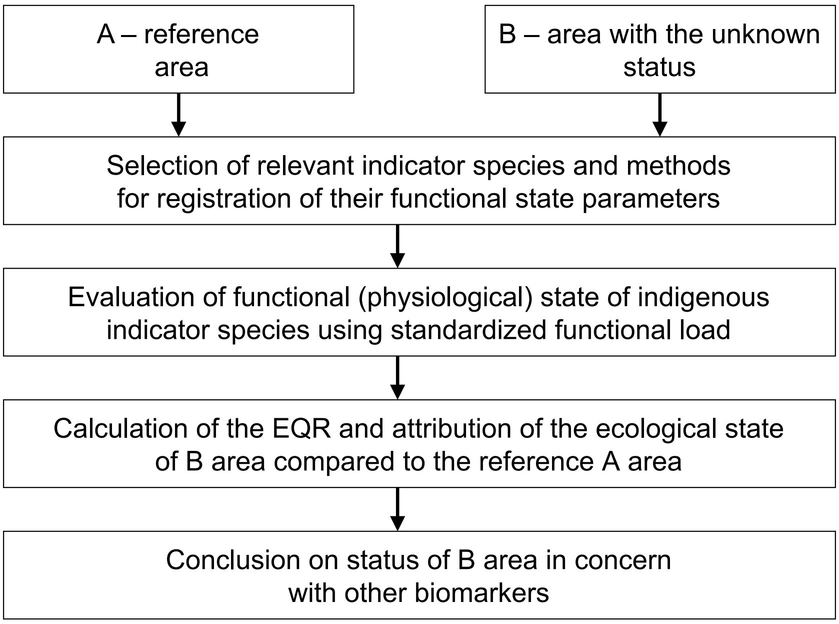

**Fig. 2.** Scheme of the proposed methodological approach to the comparative assessment of the ecological status of water areas

Thus, an experimental possibility was shown to study the adaptive capabilities of various organisms from various ecosystems and, therefore, according to I. Davydovsky, to draw a conclusion on their physiological health (Kholodkevich et al., 2017 ; Kuznetsova & Kholodkevich, 2015 ; Kuznetsova et al., 2018). This is necessary both for practical purposes (the use of animals with stable biomarkers to analyze the quality of natural waters as a habitat for aquatic organisms) and for assessing the state of natural populations in which, due to natural variability, there are individuals of different health. Molecular genetics, biochemical, and behavioral biomarkers of selected key population species (prevailing in abundance and biomass), the degree of animal infestation with parasites, *etc.* will help to establish a grounded conclusion on the population health and, possibly, to represent the health of the studied ecosystem with certain degree of reliability.

All the data obtained on the assessment of the state of local invertebrates can be supplemented with material on the bioaccumulation of heavy metals in the tissues of local animal species (for example, bivalve molluscs, gastropods, and crustaceans). This integrated information allows us to extrapolate the results of studying several indicators of individual animals on the assessment of the population health and the ecosystem state in various water areas where water, sediments, and animals were sampled. The analysis helps in ranking water areas by the level of their pollution taking into account the peculiarities of the operation of vital functional systems in biological objects studied – living “biomonitors” of the habitat quality. It can be concluded that in locations with signs of shifts in the functional state of the bioindicator (with significant changes in the operation of its main functional systems), we should expect changes in the ecosystem state. This can also be identified and proved by applying various approaches and assessment criteria for the environmental safety of natural waters.

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# ЗДОРОВЬЕ ЭКОСИСТЕМ: ПОНЯТИЕ, МЕТОДОЛОГИЧЕСКИЕ ПОДХОДЫ, КРИТЕРИИ ОЦЕНКИ

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Всё возрастающая антропогенная нагрузка на водные экосистемы создаёт угрозы экологической безопасности, и в этой связи важным является экосистемный подход к эксплуатации природных ресурсов с целью разработки комплексных регулирующих мер в природоохранной сфере. Термин «здоровье экосистемы» широко используют в оценке экологического состояния акваторий представители зарубежных научных сообществ (HELCOM, ICES, OSPAR, MEDPOL), однако нечасто применяют отечественные исследователи. Концепция «здоровье экосистемы» не является новой парадигмой: она не только активно обсуждается с начала 2000-х гг. в научной литературе, но и закреплена в долговременных документах Евросоюза и в Водной рамочной директиве ЕС по стратегии сохранения окружающей среды. В статье на основе обзора существующих литературных данных представлены основные понятия, подходы и критерии оценки экологического состояния (здоровья) водных экосистем. Подчёркнуто, что оценка здоровья экосистем зависит от целей и задач экологических исследований, с чем связана применяемая методология и, соответственно, выбор методов и показателей, характеризующих состояние экосистемы. В обзоре рассмотрены понятие «здоровье организмов» и некоторые его атрибуты: поддержание гомеостаза, причинно-следственные связи в континууме здоровье — болезнь, функциональные адаптации. Представлен сравнительный анализ ряда подходов к оценке здоровья рек и морских акваторий. Рассмотрены различные показатели, комплексные индексы, биомаркеры экспозиции и эффектов, указывающие на подверженность водных экосистем изменениям в результате природных и антропогенных воздействий. Отдельное внимание обращено на необходимость применения комплексного экосистемного подхода в анализе состояния водных экосистем, что будет способствовать интегральной оценке последствий деятельности человека для целостности экосистем. На основе опыта выполнения международного проекта BONUS+/BEAST представлен комплексный биомаркерный подход к определению здоровья биоиндикаторов с последующей интерпретацией данных о состоянии здоровья экосистем, в которых эти организмы обитают. Авторы надеются, что обзор будет интересен как специалистам в области экологии водных экосистем, так и представителям природоохранных организаций, ответственным за проведение экологических экспертиз.

**Ключевые слова:** здоровье экосистемы, оценка состояния водных экосистем, референтные состояния экосистем, физиологическое состояние, функциональные адаптации, макробентосные беспозвоночные