Biomarkers : useful tools for the monitoring of aquatic environments

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SUMMARY

The approaches for monitoring environment quality can be classified in two different categories : (1) the detection and/or quantification of stressors in both physical and biological compartments of the ecosystems (physico-chemical approach), and (2) the evaluation of exposure of living organisms and subsequent effects at the individual, population and/or community levels (biological approach). Indices have been defined for both approaches. Threshold levels for pH, temperature, conductivity, oxidizable matter content, oxygen saturation, nitrogen, phosphorus and ion concentrations, organic contaminant and metal levels are used as physico-chemical indices to assess the quality of aquatic environments and water resources. Similarly, biological indicators have been developed to evaluate the impact of environmental stress on aquatic organisms at various levels of biological organisation. Among these biological indicators, biomarkers are used as tools to assess molecular, biochemical, cellular, physiological or behavioural changes that may reveal exposure of organisms to environmental chemicals. In some cases, biomarkers are able to indicate that chemicals specifically affect metabolic pathways or physiological functions in exposed individuals. Therefore, biomarkers can be used as both diagnostic and predictive tools. A number of case-studies demonstrated that biomarkers are useful tools to detect the presence of xenobiotics in living organisms and to diagnose individual health. However, interference with natural environmental factors or endogenous parameters may complicate the interpretation of biomarker responses, and sampling and measurement procedures must be properly designed and accurately followed. A more recent and fairly promising way to use biomarkers is based on mechanistic links that can be established between effects revealed in individuals and subsequent changes in populations and communities. Though rarely applied to natural ecosystems, the use of such an approach should be able to predict the effects of stress on populations and/or communities from measurements of biomarkers in individuals of selected species sampled in the field.


RÉSUMÉ

Les biomarqueurs, des outils pour le suivi de la qualité des milieux aquatiques. Par L. LAGADIC.

Le suivi de la qualité de l’environnement fait appel à deux types de méthodes (1) la détection et/ou la quantification des facteurs de stress dans les compartiments physiques et biologiques des écosystèmes (approche physico-chimique) et (2) l’évaluation de l’exposition des organismes et de ses répercussions au niveau de l’individu, de la population et de la communauté (approche biologique). Des indices ont été définis pour chacune de ces deux approches. Des valeurs-seuils de pH, température, conductivité, taux de matière organique, taux de saturation en oxygène dissous, concentrations en azote, en phosphore ou en ions, concentrations en polluants organiques ou en métaux sont autant d’indices physico-chimiques utilisables pour évaluer la qualité des milieux aquatiques et des ressources en eau. De façon comparable, des indicateurs biologiques ont été développés afin d’évaluer l’impact de stress environnementaux sur les organismes aquatiques, et ce à différents niveaux d’organisation biologique. Parmi ces indicateurs biologiques, les biomarqueurs sont utilisés en tant qu’outils pour déceler les changements moléculaires, biochimiques, cellulaires, physiologiques et comportementaux susceptibles de révéler l’existence d’un orga- nisme à des polluants chimiques présents dans l’environnement. Dans certains cas, les biomarqueurs peuvent même renseigner sur les atteintes spécifiques d’une voie métabolique ou d’un processus physiologique. De nombreuses études de cas montrent que les biomarqueurs sont des outils performants pour détecter la présence de xénobiotiques dans les organismes et pour effectuer un diagnostic de l’état de santé des individus exposés. Cependant, les interférences avec des facteurs environnementaux naturels ou avec des régulations physiologiques endogènes peuvent parfois compliquer l’interprétation des réponses des biomarqueurs. Les conditions d’échantillonnage et de mesure sont donc déterminantes pour garantir la fiabilité des informations apportées par les biomarqueurs. Actuellement, l’utilisation des biomarqueurs s’oriente de plus en plus vers une approche basée sur les relations mécanistes pouvant être établies entre les effets observés au niveau des individus et les changements au niveau des populations et des communautés. Bien qu’encore rarement appliquée dans les écosystèmes naturels, cette approche pourrait permettre de prédire l’évolution des populations et/ou des communautés à partir de mesures de biomarqueurs réalisées chez des individus directement prélevés dans des milieux soumis à des pollutions chroniques.

Introduction

The general framework of the monitoring of ecosystem quality can be defined on the basis of the following four questions [1]:

How bad is the situation?
Is it getting better or worse?
Why do we have these problems?
What can we do to improve the situation?

The first question refers to the assessment of ecosystem quality, whereas the second implies a dynamic approach which tries to establish how the ecosystem quality is changing. The third question refers to the origin of environmental changes, and may result in a more fundamental approach based on experimental works. The last question has at least two major components: a scientific component which may result in proposals for remediation, and a socio-economic component which involves efforts from public authorities and industries. This last question will not be further developed in the present paper. Instead, emphasis will be placed on how invertebrates can help to answering the first three questions.

Monitoring the quality of the environment: the Biomarker Approach

A) SIMPLIFIED PRESENTATION

Broadly speaking, biomarkers are biochemical or physiological parameters that can be measured in individuals to indicate exposure to environmental chemicals and, for some of them, to detect toxic effects [2-6]. As such, biomarkers describe individual health, and therefore can be considered as diagnostic tools. When individual changes revealed by biomarkers can be connected to actual or potential changes at the population level, those biomarkers can be considered as predictive tools [7, 8]. Both approaches are important components of the assessment of the extent of ecological damage, and of the evolution of ecosystem quality, especially when remediation has been undertaken (Fig. 1).

B) DIAGNOSTIC APPROACH: BIOMARKERS AND ENVIRONMENTAL IMPACT OF POLLUTANTS

A number of biomarkers have been identified [3, 7], and some of them, such as cytochrome P450, acetylcholinesterase, metallothioneins, and DNA damage, which have the advantages of specificity, sensitivity and applicability are at an advanced stage of usage or development as diagnostic tools [9] (Fig. 2).

When higher than natural variability, the response of a biomarker indicates that environmental changes may have affected the individuals in which the biomarker has been measured. Then, the origin of the disturbance must be determined. Various approaches may be used to detect the presence of pollutants and, in the best cases, to identify them or on the contrary, to show that pollutants may not be responsible for biomarker response. This situation describes the most common case, because every biomarker presently used is not specific for one unique type of pollutant.

However, some biomarkers respond to a relatively restricted range of pollutants. Thus, cytochrome P4501A (or related isoforms) is classically recognized as specific for the presence of polycyclic aromatic hydrocarbons, though other compounds such as pesticides are known to cause its induction [10-13]. Similarly, acetylcholinesterase reacts quite specifically to organophosphorous compounds and carbamates, though it also responds to metals [14,15]. Even if they do not unequivocally indicate the nature of the contaminants, such biomarkers allow to limit the analytical screening to a few chemical families, thus significantly reducing the cost of analyses.
Since biomarkers may only reveal a (more or less recent) past exposure to toxicants [16] and considering the specificity of response of some of them, the relevance of chemical analysis is questionable. Basically, two distinct cases may be considered. In the first case, the exact nature of the pollutants is known and the sources of discharges are identified and even controlled. The use of biomarkers allows to ensure that the conditions of discharge (defined using physico-chemical criteria and more rarely toxicological criteria) are respected. In particular it gives the opportunity to ensure that discharge levels do not exceed the capacities of the receiving medium or of organisms which live therein. In the second case, biomarkers give the opportunity to detect accidental, illicit, and/or diffuse discharges. According to the intensity and extent of biomarker response, alert procedures may therefore (or not) be activated to identify the source of pollution without any information on the nature of contaminants.

C) PREDICTIVE APPROACH : BIOMARKERS AND ENVIRONMENTAL RISK ASSESSMENT

Since biomarkers can be used to detect the presence of environmental contaminants and may provide informations on the effects of pollutants on organisms, they may be used for the assessment of environmental quality. Is it possible to predict the effects of pollutants on populations, communities and ecosystems from measurements performed at the individual level? The assessment of ecological risk associated with the release of chemicals in the environment is a key problem in the context of sustainable development and long-term conservation of environmental quality. The relevance of biomarkers for environmental risk assessment has recently been under discussion and some interesting perspectives have been drawn [17-20]. All the arguments that have been presented in this debate are not reviewed here in detail but, it seems interesting to recall the definition and rationale of ecological risk assessment in order to investigate the possible role of biomarkers in such an approach.

Environmental risk assessment corresponds to an estimate of the probability that an adverse effect will occur in response to the presence of one or several factors of disturbance [18, 21]. Risk assessment for chemicals is based on four successive steps [22]:

- **Hazard identification** is performed using toxicity test data obtained for both target and non-target organisms.
- **Establishment of dose-response relations** is used to experimentally determine the toxic activity of compounds.
- **Exposure assessment** is based on evaluation of the nature and size of populations or ecosystems at risk, as well as the frequency, duration, and intensity of contact with the chemical.
- **Risk characterization** is performed using data from the three preceding steps. It provides a quantitative and qualitative prediction of the probability that a harmful effect will occur.

Most biomarkers fulfil the two first steps but the extrapolation of individual responses to changes in populations or communities is still difficult [23]. *No in situ* study has yet demonstrated that the effect of a chemical on a critical target in an individual was responsible for changes in the population to which it pertains. *A posteriori* studies on the effects of organochlorine pesticides (DDT, DDE) on certain bird populations (peregrine falcon in Great Britain, fish-eating birds in the North American Great Lakes) have shown that population changes were linked with effects of toxicants on individuals [5]. Although interesting from a scientific point of view, this approach is at the opposite of the ecological risk assessment approach since there is no prediction of the effects at the population level but only a reconstruction of passed phenomena.

So far, the use of biomarkers in environmental risk assessment for chemicals still depends on the identification of causal relationships or mechanistic links between the reaction of individuals to exposure and changes in populations and com-
munities. Some studies have already initiated this research for aquatic animals [24], but extrapolation of effects of pollutants across different levels of biological organization still largely depends on theoretical or conceptual links. Although this approach can be justified in the context of research and development of biomarkers, it rarely meets the needs of managers of environmental quality who are generally looking for a diagnostic of the state of the environment. Since one of the future prospects of biomarkers is their use in environmental risk assessment, suitable validation procedures should be rapidly implemented [18, 25, 26].

Extrapolation from the individual to the population and community

A) GENERAL OVERVIEW

The action of environmental pollutants on the individual may result in various internal changes (Fig. 3). Once a chemical penetrates the organism, it binds to various molecules within different tissues. Those molecular interactions result in metabolic changes which may affect different functions at the cellular and tissue levels with consequences at the individual level (e.g. disease development, reduced growth, altered behaviour, impaired reproduction). In this respect, impact on the nervous system usually results in serious disorders in individuals and also in changes in inter-individual relationships.

Most metabolic processes and individual functions are mediated by hormones and require energy. Hormonal control and energetic balance are therefore of primary importance as they both determine eventually the reproductive capacity of the individual, and reproduction is the key-process that links the individual to the population. Successful reproduction is essential for the continuation of the species. The reproductive success of individuals determines population characteristics in terms of structure/composition, density/size, and stability/evolution.

Community level effects depend on both population functions and interactions between populations. Population functions result from both the previous demoeological parameters (structure/composition, density/size, stability/evolution)

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**Figure 3.** Extrapolation of effects from the individual to the population and community: General overview.

Extrapolation des effets de l’individus aux populations et aux communautés : vue d’ensemble du problème.
and individual health, and may induce changes in physicochemical conditions. Interactions between populations are considered as an essential component of community stability and ecosystem function. If the external perception of environmental factors in individuals is affected by neurotoxic pollutants, changes in interspecific relationships may occur, with potential consequences on communities.

Animal reproduction, as expressed through fecundity and fertility, can be impaired as a result of both direct impact of toxicants on the endocrine system, and indirect effect on energy allocation. Besides alterations in courtship and mating behaviour, changes in reproduction can be caused by changes in maturation of gametes, fertilization process and age of sexual maturity, or by morphological abnormalities in adults. Reduction of fertility result from the effects of contaminants on egg viability, larval development, or hatching of youngs. Hormonal and energetic biomarkers can be used to detect changes in fecundity and fertility, and can also be related to individual growth [7, 23, 27, 28]. Such biomarkers are therefore considered as potential predictive indicators of changes in populations, with possible consequences on communities. However, only a few studies have so far demonstrated causal links between hormonal or energetic alterations in individuals and changes in populations and communities. This requires a good knowledge of the succession of pollutant effects across levels of biological organisation, as illustrated by the following examples.

B) EFFECTS OF ENDOCRINE DISRUPTORS IN INVERTEBRATES: CASE-STUDY OF TRIBUTYL Tin (TBT) IN COASTAL ECOSYSTEMS

The effect of pollutants on the endocrine system have received considerable attention over the past five years [29]. Endocrine-disrupting effects of environmental chemicals have long been demonstrated in invertebrates. A number of plant allelochemicals are known to affect growth and reproduction of insects by acting as hormone mimics [30]. Synthetic hormone mimics have also been developed as insecticides against insect pests (e.g. diflubenzuron, tebufenozide) [31-33]. More recently, endocrine-disrupting effects of environmental chemicals have been identified in freshwater crustaceans [34-37] and marine molluscs [38, 39]. In these latter, tributyltin (TBT) was identified as the compound acting on the reproductive system of individuals, and from a twenty year long field survey, the progression of effects from the molecular level to communities has been established, as summarized below.

TBT is an organotin compound used in antifouling paints applied onto boats. Long-term studies on marine gastropod populations have demonstrated that TBT induces a phenomenon named ‘imposex’ which results in the development of male characteristics in females. This change was first identified in Nucella lapillus [40] and Nassarius obsoletus (= Ilyanassa obsoleta) [41] and has been reported in more than 40 species of marine mollusc exposed to TBT [42]. The dogwhelk Nucella lapillus and the oyster drill Ocenebrina aciculata are highly susceptible to TBT and are being largely used for investigating its effects at sub-cellular, individual and population levels [reviews in 38, 39, 43]. In females, TBT may inhibit cytochrome P450-dependent aromatase which converts testosterone to 17β-oestradiol. Inhibition of sulphur conjugation and excretion of testosterone and its metabolites is another putative pathway of TBT mode of action. In any of those situations, the level of male steroids increases, leading to anatomical changes in the reproductive system. Following the development of the penis and vas deferens, the affected females become sterile and eventually die. As a result of reduced reproductive output and individual death, populations decline. Extinctions of populations of N. lapillus and O. aciculata have even been reported [43, 44].

In spite of the role of N. lapillus in shore communities as a necrophagous species, as investigated by experimental manipulations of field populations, no community level effects were clearly demonstrated as a consequence of dogwhelk population decline. This may result from the lack of long-term observations of dogwhelks in the context of the whole community on such shores, and also from compensatory mechanisms which are able to maintain the communities functional (e.g. replacement by other necrophagous species such as decapod crustaceans), except in the case of catastrophic events such as oil spills [38].

Since the use of TBT in antifouling paints has been restricted in 1982 in France and in 1987 throughout Europe, North America, Australia and Japan [45-47], the degree of imposex in some dogwhelk populations has decreased [48], but the process of recovery of all affected populations and communities is likely to be slow as bioaccumulation still occurs in some organisms that now recolonize the contaminated areas [38, 46, 47].

This case-study demonstrates that the effects of reproductive impairment on marine molluscs populations and consequences on communities can conceivably be predicted from individual measurements of biomarkers which represent potent early-warning indicators of environmental pollution by endocrine-disrupting chemicals.

C) EFFECTS OF POLLUTANTS ON INVERTEBRATE BIOENERGETICS: CHANGES IN THE PHYSIOLOGICAL ENERGETICS OF FRESHWATER CRUSTACEANS

The demonstration of mechanistic linkages between effects at different levels of biological organisation has been achieved using the freshwater amphipod Gammarus pulex in various experimental contexts [49]. Physiological energetics assessed through measurements of scope for growth have been used to predict the concentrations of pollutants that impair growth and reproduction as well as the magnitude of this impairment. Reduced scope for growth which results from lower individual feeding rate have been shown to be correlated with decreased reproductive patterns, through reduced offspring size and brood viability [50, 51].

Stress-induced reductions in G. pulex feeding rate have been shown to be correlated to reductions in the rate of incorporation of leaf organic material into freshwater food webs [51]. Field trials further indicated that between-site differences in G. pulex feeding rate were correlated to differences
in community structure, but this correlation did not result from causal relationships between G. pulex energetics and community structure [49]. Similarly JONES et al. [53] have indicated that changes in the feeding behaviour of Daphnia catawba induced by sublethal concentrations of toxicants may have more rapid and pronounced effects at the community (i.e. algae populations) than on the cladoceran population itself.

Energetic biomarkers certainly have a real potential for predicting effects of pollutants on invertebrate population structure and dynamics [23, 27]. On the basis of experimental and field investigations, a conceptual framework has been elaborated, which supports modelling approaches [54, 55]. However, compensatory population mechanisms and natural variations of the parameters related to individual bioenergetics may act as confusing factors so that the regular use of these latter as predictive biomarkers still requires further investigations.

These two examples show that mechanistic links can be established between biomarkers measured in individuals and changes at the population level, the key-process that links the individual to the population being reproduction. In invertebrates, biomarkers related to reproduction through hormone metabolism and energetic balance appear as highly relevant to predict effects at the population level. Prediction of further consequences on communities and ecosystems relies on a sound knowledge of the functional role of the population and of the interactions between populations.

Assessment of the ecological impact of chemicals through the use of biomarkers: concluding remarks

A large amount of data show that environmental contaminants may exert deleterious effects on organisms and ecological processes. However, even if responses of organisms or processes to pollutants can be demonstrated in controlled conditions (e.g. laboratory, microcosms or mesocosms), this does not necessarily imply that the same responses (or any response) will be observed in the field. Intrinsic unpredictability of the natural environment generally impedes the identification of precise causal relationships between effects of toxicants on individuals and subsequent consequences on populations and communities. The demonstration of such relationships implies that the component of biological responses due to background fluctuations (e.g. natural variations) and to confounding variables (e.g. natural factors) is accurately known [56].

Physiological compensation in individuals and/or natural heterogeneity within populations, that usually ensure survival of species within a community [57], frequently make it difficult to extrapolate from biochemical and physiological changes to population-level effects of exposure [58-59]. Compensatory mechanisms have themselves a cost which can, in turn, interfere with the performances of individuals (Darwinian fitness). Even adaptation or genotype selection may have a cost in term of population performances (e.g. decrease of tolerance to other stresses in tolerant populations with reduced genotype variability) [60-62].

Surprisingly, population biology is rarely considered in ecotoxicological studies, even though modelling approaches of population dynamics, which take into account the effects of pollutants on various biological responses, have been proposed [63-66]. Population-level effects are sometimes measurable only after several generations when affected endpoints are long-term parameters such as juvenile recruitment or age to first reproduction [67, 68]. The age-structure of natural populations may also result in age-specific stress that will not necessarily affect the whole population survival and dynamics [69]. On the other hand, field observations suggest that populations are frequently interconnected, especially in large-scale ecosystems (e.g. sea shore) or spatially complex landscapes so that the real object of interest for ecotoxicologists should consist in metapopulations rather than in isolated populations [70].

Therefore, even when laboratory experiments or model outputs predict either population decrease or survival, various natural fluctuation processes (e.g. immigration, food availability, etc) may considerably modify actual demographic parameters, especially in unpredictable or short-lived systems (e.g. temporal ponds and rivers, coastal ecosystems) or when pollution is spatially restricted and that juvenile organisms may colonise from surrounding non-contaminated areas [56, 71]. Conversely, populations living in uncontaminated environments may be affected by the recruitment of juveniles from areas which can be polluted. In the case of such a metapopulation approach, the impact of human activities on habitat fragmentation and/or conservation is of primary importance.

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