# ECOLOGIA BALKANICA

2018, Vol. 10, Issue 1

June 2018

pp. 57-84

Synopsis

## Mussels in Ecotoxicological Studies -Are They Better Indicators for Water Pollution Than Fish?

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**Abstract.** EU Member states are required to apply the EU Water Framework and its Daughter Directives in order to achieve Good Environmental Status (GES) for all 11 qualitative descriptors by 2015 in all water bodies for a list of priority and specific pollutants. Therefore, environmental indicators and biological-effect techniques have to be carefully selected for the management of chemicals in the aquatic environment and for developing an integrated framework. The most commonly applied biological-effect tools are measures of the biochemical and physiological state of selected organisms, such as mussels or fish. The present article provides basic information on the EU Water Directive, the essence of biomarkers, and outlines why mussels may be the better choice of indicators in toxicological research and monitoring programs in order to study the impact of contaminants in water ecosystems.

Key words: mussels, contamination, water, indicators.

### Water contamination as a global issue

Water is the key resource required to sustain life on this planet and it is important to both society and ecosystems (PAUL, 2017). In addition, freshwater is the most important resource for mankind, crosscutting all social, economic and environmental activities. It is a condition for life on the Earth, an enabling or limiting factor for any social and technological development, a possible source of welfare or misery, cooperation or conflict (BOUWER, 2002). As stated by LOMSADZE et al. (2017) we depend on a reliable, clean supply of drinking water to sustain our health. We also need water for agriculture, energy production, recreation and

© Ecologia Balkanica http://eb.bio.uni-plovdiv.bg manufacturing. However, many of these uses put pressure on water resources. Therefore, in recent vears, water contamination has been considered as a global issue (CUI et al., 2011; YUAN et al., 2011). Studies conducted in Arabian Gulf (NASER, 2013); Argentina (MARCOVECCHIO, 2004); Bangladesh (RAHMAN et al., 2012); Chile (TAPIA et al., 2009); China (WANG et al., 2005; QIAO-QIAO et al., 2007; YI et al., 2011; LI et al., 2013; ZHANG et al., 2013); Croatia (KLOBUČAR et al., 2008); Egypt (RASHED, 2001a); Ethiopia (YOHANNES *et al.*, 2013); France (SHINN et al., 2009); India (SANKAR et 2006; SIVAPERUMAL *et al.*, al., 2007; RAJESHKUMAR et al., 2013; AROCKIA

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VASANTHI et al., 2013); Malaysia (TAWEEL et al., 2013); Norway (NÆS et al., 1999; BERGE et al., 2011); Pakistan (SHAHBAZ et al., 2013); Serbia (SUBOTIĆ et al., 2013); Slovenia (TOLLEFSEN et al., 2006; MILAČIČ et al., 2017); Spain (OLMEDO et al., 2013); Sudan (PRAGST et al., 2017); Tailand (PEEBUA et al., 2006); Turkey (TÜRKMEN et al., 2005; GÖRÜR et al., 2012); Zimbabwe (BIRUNGI et al., 2007) and even Arctic regions (SONNE et al., 2009) show that contamination is an ecological problem worldwide, suggesting that it could be affecting the aquatic organisms and having a considerable impact on the health of water ecosystems. Thus, the scientific community suggests that urgent actions and strategies need to be taken to prevent and control the pollution.

One of the dangerous types of water pollution is with heavy metals (As, Cu, Cd, Hg, Pb, Zn) (MOISEENKO & KUDRYAVTSEVA, 2001; SHAH et al., 2009; CARRASCO et al., 2011; PAUL & SINHA, 2013; AVKOPASHVILI et al., 2017); persistent organic pollutants (POPs) (HYLLAND et al., 2006; 2008), pesticides (DEVAULT et al., 2009; LOOS et al., 2009; YADAV et al., 2010); crude oil (OLSEN et al., 2007; HOLTH et al., 2009; HOLTH et al., 2011; BALK et al., 2011; VESTHEIM et al., 2012) and plastics (ANDRADY, 2011; LÖHR et al., 2017). Furthermore, it has been reported that in decades the level of recent foreign compounds in aquatic ecosystems such as heavy metals, pesticides and other persistent organic pollutants has increased alarmingly as a result of domestic, industrial and agricultural effluents (SEKABIRA et al., 2010; LUSHCHAK, 2011; ONDARZA et al., 2011; PEREIRA et al., 2013; JÖRUNDSDÓTTIR et al., 2014).

Heavy metals are toxic, nonbiodegradable and persistent environmental contaminants (HAS-SCHÖN *et al.*, 2008). Large quantities of heavy metals have been released into aquatic systems, both fresh and marine worldwide due to a global rapid population growth and intensive domestic activities, as well as expanding industrial and agricultural production (SREBOTNJAK *et*  *al.*, 2012; ISLAM *et al.*, 2014). Thus, they have severely deteriorated the aquatic ecosystems because of their toxicity, abundance, persistence, and subsequent bioaccumulation and biomagnification (ALI *et al.*, 2013).

POPs include many different contaminant groups such as polyaromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs), including dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs) used as flame-retardants, and industrial organochlorines (OCs) such as polychlorinated biphenyls (PCBs) (CIESIELSKI et al., 2017). Since most POPs are lipophilic they accumulate in the lipid-rich tissues of living organisms, and thereby biomagnify in the food web (LETCHER et al., 2010). Under this consideration, the United States Environmental Protection Agency (USEPA) classified 16 of them as priority pollutants (QIAO et al., 2006).

Pesticides have been widely applied to protect agricultural crops since the 1940s, and since then, their use has increased al., steadily GRUNG et 2015). The organochlorine pesticides (OCPs) became the dominant pesticides after the Second World War. With the publishing of "Silent Spring" by Rachel Carson in 1962, a wider audience was warned of the environmental effects of the widespread use of pesticides. Since 1970s, after evidence of their toxicity, persistence and bioaccumulation in environmental matrices OCPs production, usage and disposal have been regulated or prohibited in most of the developed countries (JAN et al., 2009; MARIN-MORALES al., 2013). In this sense, et organophosphorous pesticides (OPs) in many cases replaced organochlorine pesticides (OCs) in agriculture. Even though they have a short-term degradation and fewer residues, OPs unfortunately lack target specificity and can also cause severe, long lasting population effects on aquatic non-target species (FULTON & KEY, 2001; SUN *et al.*, 2011).

Crude oil consists of variety proportions of hydrocarbons such as alkanes, aromatics and PAHs, asphaltene and resins, as well as non-hydrocarbons namely sulphur, nitrogen, oxygen and traces of metals, particularly copper, iron, nickel and vanadium (BRANDT, 2006; SAKTHIPRIYA et al., 2015; WILBERFORCE, 2016). At present, the petroleum industry has entered into a period of modernization and transition. Due to this industrial development, pollution of seawater by crude oil has been increased and creating a serious issues (BAO et al., 2014). As a result, each year enormous quantities of crude oil are disposed into the environment, either deliberate or accident during crude oil production and transportation. Between 1.7 and 8.8 million metric tons of crude oil are estimated being released in the water environment each year (NRC, 1985), of which >90% as deliberate waste disposal, directly related to human activities (NDIMELE, 2010). According to EFFENDI et al. (2017) oil spills often bring devastated effects on the aquatic organisms and consequently ruin the aquatic ecosystem homeostasis. LENNUK et al. (2015) state that the impact of oil toxicity on various ecosystem elements have been increasingly reported since 1960s and the majority of studies have focused on the oil spill effects on organisms such as plankton, fish, birds and marine mammals. In addition, oil spills incidences all over the world occasionally occur and one of the most devastating ones are linked with the explosion of Deepwater Horizon (WEISBERG et al., 2017) and Exxon Valdez (LINDEBERG et al., 2017).

Last but not least, worldwide there is a growing concern about the risks and possible adverse effects of (micro) plastics because since the 1950s the amount of plastics in the marine environment has increased dramatically (JAMBECK *et al.*, 2015; UNEP, 2016). Furthermore, substantial quantities of microplastics are already present in the global marine ecosystem (ANDRADY, 2011; DESFORGES *et al.*, 2014), from the tropics to the poles, including in

Arctic sea ice and numerous studies have already showed the negative effects of microplastics on marine biota (LUSHER *et al.*, 2013; LUSHER, 2015; ROMEO *et al.*, 2015).

Legislation in the field of water conservation

According to VETHAAK *et al.* (2015) many maritime countries in Europe have implemented marine environmental monitoring programs, which include the measurement of chemical contaminants and related biological effects. Moreover, the European Union (EU) has, over the last twenty years, developed its water policies so that now there is significant European legislation covering marine waters, as well as the lakes and rivers that ultimately flow into our coastal ecosystems.

To maintain the water at an adequate quality for humans and to preserve natural ecosystems and biodiversity, it is necessary to sustainably use, protect and manage the water resources. In the countries of the European Union (EU), national water agencies, which follow EU policy and the requirements of the EU Water Framework and its Daughter Directives (DIRECTIVE 2000/60/EC; DIRECTIVE 2008/105/EC; DIRECTIVE 2008/56/EC; **EUROPEAN** COMMUNITIES ENVIRONMENTAL OBJECTIVES 272/2009; EUROPEAN **COMMUNITIES** TECHNICAL REPORT, 2010), implement regular water monitoring with the aim to control and prevent pollution (MILAČIČ et al., 2017).

Recently, the Water Framework Directive (WFD, DIRECTIVE 2000/60/EC) of the European Union specified monitoring programs required to assess the achievement of good chemical and ecological status for all water bodies by 2015 for a list of specific pollutants (SANCHEZ & PORCHER, 2009). This list currently stands at 41 pollutants (33 priority substances (PSs) and 8 other pollutants) given in Annex I of DIRECTIVE 2008/105/EC; furthermore, the European Commission (EC) proposed recently to include 15 additional PSs on this existing list (BESSE et al., 2012).

More recently, the European Union has implemented the Marine Strategy Framework Directive (MSFD, DIRECTIVE 2008/56/EC). At its heart is the concept of "good environmental status" (GES) for all European waters and the provision of a framework for the protection and preservation of the marine environment, the prevention of its deterioration, and, where practicable, the restoration of that environment in areas where it has been adversely affected (VETHAAK et al., 2015). In addition, the MSFD is a wide-ranging framework directive with the overall objective of maintaining GES in the seas of Europe by 2020 (LYONS et al., 2010).

GES will be assessed on a regional basis. The Regional Sea Conventions (OSPAR Helsinki Commission Commission, (HELCOM), Barcelona Convention (In 1975, Mediterranean countries 16 and the Community adopted European the Mediterranean Action Plan (MAP), the firstever Regional Seas Program under UNEP's umbrella) and the Black Sea Commission), which aim to protect the marine environment, were required to support the implementation of the MSFD (VETHAAK et al., 2015). According to LYONS et al. (2010) the Joint Research Centre (JCR) and the International Council for the Exploration of the Sea (ICES) were also commissioned to facilitate the preparation of scientific bases for criteria and to propose methodological standards in relation to 8 of the GES descriptors during the course of 2009.

As stated by BESSE et al. (2012) the application of the WFD for the surveillance of chemical contamination of surface waters involves two main objectives: (1) to assess the chemical status of the water bodies, by determining whether contamination levels compliant with the regulatory are Environmental Quality Standards (EQSs); and, (2) to assess the temporal trends of the contamination the different in environmental compartments of aquatic ecosystems. EQSs are defined as "the concentration of a particular pollutant or

group of pollutants in *water, sediment or biota,* which should not be exceeded in order to protect human health and the environment". In addition, the assessment of chemical status under WFD is undertaken in fresh, transitional and coastal waters using EQS, which are derived from toxicological information and used to set acceptable limits for individual priority contaminants (LYONS *et al.,* 2010).

Choice of bioindicator species – fish or mussels

The use of living organisms in the study of environmental quality is widely accepted (STAMENKOVIĆ et al., 2013). However, according to SHERMAN (1994) selecting indicators can be challenging, given the variety of ecosystem characteristics they are intended to track. Furthermore, according to BESSE et al. (2012) there are currently two different strategies chemical for biomonitoring monitoring (i.e. contamination in biota) that can be adopted: passive or active. Passive approaches rely on organisms, indigenous while active approaches rely on transplanted (or caged) individuals from a reference site (see SCHØYEN *et al.*, 2017).

In the review of REES et al. (2008) it is explained the ideal indicator should be: (i) capable of conveying information that is responsive and meaningful to decisionmaking (directly tied to management questions and linked to thresholds for appropriate action relative to designated ecosystem goals); (ii) linked to a conceptual stressor-response framework (with the ability to communicate potential cause-effect relationships); (iii) capable of measuring change or its absence with confidence (robust influences of confounding to environmental factors); (iv) highly sensitive and anticipatory (early warning of potential problems); (v) applicable over a variety of spatial scales and conditions (to support global as well as local comparisons); (vi) desirable operationally (easy to measure, reproducible with minimum measurement error, cost-effective); (vii) integrative (serves multiple indicator purposes); (viii) nondestructive (measurement does not cause ecosystem damage); (ix) easy to understand and communicate (non-specialists need to act on findings); (x) scientifically and legally defensible (robust to peer review and wider challenge).

CAIRNS et al. (1993) encapsulated the issue of single vs. multiple indicators by noting that "... everything is an indicator of something but nothing is an indicator of everything" in a review of ecosystem health indicators. UNESCO identified three classes of ecosystem indicators (slightly modified): (i) ecological indicators: to characterize and monitor change in the state of various physical, chemical, and biological aspects of the environment relative to defined quality targets with thresholds for management action (see also FISHER, 2001); (ii) socioeconomic indicators: to measure whether quality is sufficient environmental to maintain human health, human uses of resources, and favorable public perception (see also CAIRNS et al., 1993); (iii) governance indicators: to monitor the progress and effectiveness of management and enforcement practices towards meeting environmental policy targets. As stated by REES et al. (2008) until recently, ecological indicators have received most attention.

According to WFD, fish represent one of the key elements to evaluate the rivers ecological status (SCARDI *et al.*, 2008; HERMOSO *et al.*, 2010).

BESSE et al. (2012) explain that fish are ideal organisms for checking compliance against biota EQSs, since the protection objectives targeted are human health and/or high-level predators. Hence, out of the 3 biota EQSs defined under Directive 2008/105/EC and the 8 biota EQSs given in the EC proposal to revise this Directive, 10 are based exclusively on a fish matrix. However, BESSE et al. (2012) state that if fish are the test organisms of choice for checking compliance with biota EQSs, they have several characteristics that limit their use for

biomonitoring, while active macroinvertebrates represent good а compromise in terms of feasibility and fulfilling the objectives of the WFD. In addition, the authors add that fish carry disadvantages for long-term trend analysis on contamination patterns, as they are strongly metabolize certain known to contaminants, and that makes them less valuable as indicators for some PSs. For example, fish strongly metabolize PAHs, limiting in this way their relevance as reliable indicators of PAH pollution; indeed, it is only possible to estimate recent exposure to PAH indirectly by determining biliary concentrations of PAH metabolites (VAN DER OOST et al., 1994). Furthermore, caging is generally unsuitable for fish species and the use of fish for active biomonitoring purposes remains difficult to achieve because in logistical terms, the size of these organisms requires large caging systems that are difficult to handle in the field; and, fish are easily stressed, especially in cagingexperiment conditions (which limit their mobility), which risk introducing bias in the responses obtained (OIKARI, 2006).

YANCHEVA et al. (2015, 2016a) describe in reviews why in ecotoxicology fish have become the major vertebrate model. Indeed, a tremendous body of information has been (STEINBERG *et* accumulated al.. 1995; BRAUNBECK et al., 1998; MOISEENKO, 2005; RAISUDDIN & LEE, 2008; MURTHY et al., 2013; CZÉDLI et al., 2014). Fish are among the group of aquatic organisms, which represent the largest and most diverse group of vertebrates. They are present virtually in all environments and many species have been found to be susceptible to environmental pollutants (VAN DER OOST et al., 2003). A number of characteristics make fish excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems (LAW, 2003; DE LA TORRE et al., 2010). According to DE LA TORRE et al. (2005) monitoring sentinel fish species is widely used to assess the degree of accumulation of pollutants and the effects on health status. In addition, fish have been found to be good indicators of water contamination in aquatic systems because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates, are also more sensitive to many toxicants (DALLINGER et al., 1987; POWERS, 1989; BARAK & MASON, 1990; WESTER et al., 1991; BURGER et al., 2002) Fish respond to environmental toxic changes with adapting of their metabolite functions (MISHRA & SHUKLA, 2003). They are also preferred in toxicological research because of their well-developed osmoregulatory, endocrine, nervous, and immune systems compared to invertebrates (SONG et al., 2012). In addition, the studies of PÉREZ CID et al. (2001); FISK et al. (2001); RASHED (2001b); MONDON et al. (2001); MANSOUR & SIDKY (2002); USERO et al. (2004); Mendil & Uluözlü (2007); Öztürk et al. (2009); SOUNDERAJAN et al. (2010); ROWAN (2013) show that fish may absorb toxicants directly from the surrounding water and sediments (waterborne exposure), or ingest them through contaminated food in the food chain (dietary exposure), enabling the assessment of pollutant transfer through the tropic web.

The pollutants are accumulated through different organs of the fish because of the affinity between them. In this process, many of them are concentrated at different levels in different organs of the fish body (RAO & PADMAJA, 2000). Therefore, in teleost fish, the gills, liver, kidney and muscles are the tissues most frequently utilized in toxicological studies because they are metabolically active tissues and accumulate toxicants at higher levels (HEIER *et al.*, 2009; JOVIČIĆ *et al.*, 2014).

In their review BESSE *et al.* (2012) present information about different national and international project involving fish as a test organism. The authors provide data about the French "Plan National PCB", which is a public health-oriented program deployed between 2008–2010. It was designed to step up the monitoring of contamination in aquatic environment and fish products, in order to adopt appropriate risk-management measures. Dioxins, furans, dioxin-like PCBs and indicator PCBs, as well as total Hg were investigated within this program. The project framework included sampling schemes on several fish species across more than 300 sites (around 100 sites a year). The fish species were selected for their PCB bioaccumulation geographical profiles, their national distribution profiles, and their history of use as human food. Another program is the "Flemish Eel Pollutant Monitoring Network", which was launched in 1994 in Belgium by the Research Institute for Nature and Forest (Inbo). This monitoring network, public-health oriented, aimed at monitoring the dispersion of pollutants in Flanders for continental waters using European eel (Anguilla anguilla). Over a 10-year period, around 360 sites (streams, rivers, canals, ponds and lakes) across Belgium were sampled for environmental analysis of PCB, OCPs and heavy-metal pollution.

To monitor the health of water ecosystems, other sentinel organisms such as mussels (bivalves) have been identified as suitable candidates to indicate the levels of contaminants and their effects in the aquatic environment and as such, they have been proposed to be suitable "biomonitors" of pollution (NAIMO, 1995; BESADA *et al.*, 2011).

Their filtering habits, low metabolism and ability to bioaccumulate pollutants make them excellent choice an to assess their bioavailability and effects (BAYNE, 1989; FOSSATO et al., 1989; WIDDOWS & DONKIN, 1992). Mussels are also sessile, sedentary, longlived and easy to collect, have a reasonable size for chemical analyses, they are worldwide distributed and present across a very wide geographical areas (basically the entire range of North Atlantic, Baltic, and Mediterranean coastal areas, see THAIN et al. (2008)), particularly abundant in coastal and estuarine waters and often found in large amounts (FARRINGTON et al., 1983; GOLDBERG, 1980; 1986; BOLOGNESI et al., 2006; BELLOTTO & MIEKELEY, 2007; CHANDURVELAN et al., 2015).

The U.S. Environmental Protection Agency (EPA) classifies mussels as biomonitors because they react to changes in the surrounding environment. Additionally, are filter since mussels feeders. thev bioaccumulate heavy metals and POPs and other contaminants in their bodies and shells (RAINBOW, 1993; CAPPELLO et al., 2013). In comparison to fish and crustaceans, bivalves have a very low level of activity of enzymatic systems capable of metabolizing POPs. Therefore, bivalves are widely used as bioindicators of organic pollution in freshwater, marine and estuarine ecosystems because they are known to provide a time indication of environmental integrated contamination, as well as reliable information potential impact of the seafood on consumption on human health (TURJA et al., 2013).

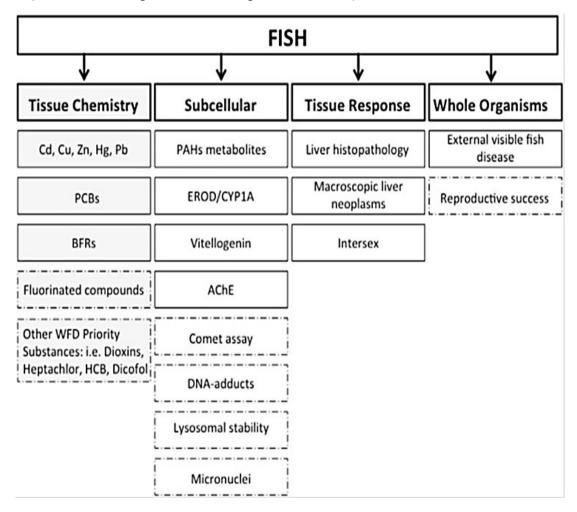
To monitor the nature and extent of coastal pollution, a Mussel Watch Programme (MWP) was developed by GOLDBERG (1975; 1986) in an attempt to quantify the levels of pollutants in coastal systems. BESSE et al. (2012) describe in their review that in marine waters, the NOAA "Mussel Watch" represents the oldest, continuous running contaminantmonitoring program in US coastal and Great Lakes waters. This project has been running since 1986, but the original "US Mussel Watch" program was initiated in 1975 as shown above. The program covers 300 coastal sites and monitors over 100 organic and inorganic contaminants (PAHs, PCBs, DDTs, TBT, OCPs and toxic trace elements) in sediment and biota tissue. It is based on yearly collection and analysis of oysters and mussels. Since there is no species of mussel or oyster common to all coastal regions, a variety of collected species are (Mytilus species, Crassostrea virginica and Dreissena species) in order to gain a national perspective (BESSE et al., 2012).

As stated by BESADA *et al.* (2011) the use of mussels to monitor coastal pollution is now widely accepted and supported by many international organizations (see APETI *et al.*, 2010; MARUYA *et al.*, 2014; MELWANI *et al.*, 2014; SCHÖNE & KRAUSE JR., 2016; BEYER *et al.*, 2017). According to SCHØYEN *et al.* (2017) most commonly the mussel watch studies involve collection of samples from natural blue mussel populations, but the adoption of an active biomonitoring alternative bv using transplanted blue mussels has gained considerable popularity in ecotoxicology research and monitoring.

*Mytilus sp.* have been widely used since the 90s, and have been shown to be one of the most successful model organisms for time-integrated responses to complex mixtures of pollutants (UNEP/RAMOGE, 1999). Furthermore, the blue mussels such as Mytilus edulis and Mytilus galloprovincialis are widely used as sentinels in coastal pollution monitoring (mussel watch) programs, mainly because their biological characteristics make them very suitable as bioindicators for assessing the quality status of coastal waters (ZATTA et al., 1992; HAGGER et al., 2008; WEPENER & DEGGER, 2012; SPARKS et al., 2014; ROUANE-HACENE et al., 2015; FARRINGTON et al., 2016).

In this sense, in France, Ifremer (French research institute for exploration of the sea) has been running the "RNO" network (National observatory network on marine environment quality) since 1974 and since renamed "ROCCH" - National chemical contamination network. This approach is based on monitoring natural populations of three bivalve mollusks - one oyster species (Crassostrea gigas) and two mussel species (Mytilus edulis and Mytilus galloprovincialis) in order to ensure national-scale coverage. About 50 chemical contaminants (PAHs, PCBs, OPs and metals) are currently being monitored at 80 sampling sites around the French coastline. Organisms that have spent at least 6 months seeded in the zone are sampled semi-annually for metal pollutants and annually for organic pollutants (BESSE et al., 2012). In addition, in 1996, Ifremer developed RINBIO ("Re'seau Inte'grateurs Biologiques") as a methodology built around caging marine mussels (Mytilus galloprovincialis). Indeed, using transplanted organisms made it possible to compensate for the scarcity of wild-mussel colonies in certain sections of the French Mediterranean coastline

as explained by BESSE et al. (2012). The authors also mention the recent "Projet Mytilos" initiative, based on the same structural principle RINBIO, as was developed to study heavy-metal contamination (Cd, Hg, Ni and Pb) in caged mussels (Mitilus galloprovincialis) at over 120 sites spanning the entire Mediterranean Basin. Another monitoring program in which mollusks were used is the National Water-Quality Assessment (NAWQA) program in the USA, which was launched in 1993 by the United States Geological Survey (USGS) to monitor spatial and temporal trends of contamination for a selection of micropollutants (PAHs, OPs, PCBs, dioxins and metals). This monitoring program encompasses several species: (1) a bivalve mollusk (*Corbicula fluminea*); (2) three insect genus (*Trichoptera, Chironomidae, Plecoptera*); (3) six fish species; and, (4) vascular plants (*Potamogeton sp.* and *Elodea sp.*). Chemical analysis in resident biota was integrated as part of the first cycle of assessments (1993–2001), but was dropped from the second cycle due to the high costs involved and to data-interpretation difficulties (see BESSE *et al.*, 2012).



**Fig. 1.** Determinants and measurements included in the fish component of the ICES/ OSPAR integrated monitoring framework. *Legend:* solid lines - core methods; broken lines additional methods; PCBs - polychlorinated biphenyls; BFRs - brominated flame retardants; AChE - acetylcholinesterase; WFD - Water Framework Directive, WFD - priority substances are required in biota under DIRECTIVE 39/2013/EU

(see THAIN *et al.* (2008) and VETHAAK *et al.* (2015)).

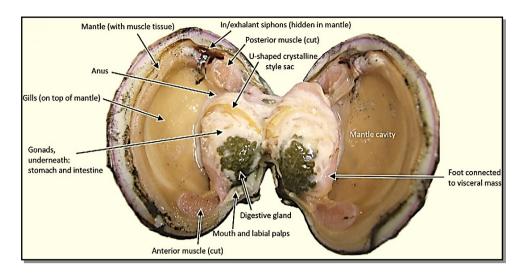


Fig. 2. Overall bivalve anatomy (after SCHÖNE & KRAUSE JR., 2016).

However, other freshwater bivalves such as zebra mussel (*Dreissena polymorpha*) can be used as a freshwater substitute of *Mytilus* sp. (LEPOM *et al.*, 2012) and has already been successfully used in ecotoxicological studies and monitoring programs (CAMUSSO *et al.*, 1994; JOHNS & TIMMERMAN, 1998; DE LAFONTAINE *et al.*, 2000; GUERLET *et al.*, 2007; BINELLI *et al.*, 2010; FARIA *et al.*, 2010; YANCHEVA *et al.*, 2016c; d; 2017).

It is important to outline that the organisms used to cover all three main trophic levels in monitoring programs are bryophytes, macroinvertebrates, and fish. Furthermore, the most commonly applied species are Fontinalis antipyretica for bryophyte species, invasive bivalves Dreissena polymorpha and Corbicula fluminea for invertebrate species, and Cyprinus carpio for fish species (see BESSE et al., 2012).

According to BINELLI et al. (2015) one of the fundamentals in the ecotoxicological studies is the need of data comparison. In their study the authors showed the advantages of Dreissena polymorpha application in biomonitoring, as well as for the evaluation of adverse effects induced by several pollutants, using both *in vitro* and *in* vivo experiments. Since Directive 2013/39/EU establishes EQS for biota because it has been demonstrated that pollutants bioaccumulate in aquatic organisms, BENITO et al. (2017) evaluated bioaccumulation of inorganic elements in the soft tissues of zebra mussels in order to assess the usefulness of this species as a bioindicator of contaminant presence in superficial waters along the Ebro River Basin authors (Spain). The concluded that Dreissena polymorpha not only supplies information about current water quality but also acts as a witness of past water quality by bioconcentrating toxic elements present in the environment and providing relevant results about historical water contamination. BESSE et al. (2012)explain that in Switzerland, the International Commission for the Protection of the Waters of Lake Geneva (CIPEL), established in 1962, has monitored trends of contamination in biota, notably fish, for 30 years, with the dual purpose of public health and the environment. For example, in 2004, organic micro-pollutants (mercury, PCBs and organotins) and trace metals in aquatic ecosystems and biota (fish and mussels) of Lake Geneva were investigated. Metals and organotins were monitored in Dreissena polymorpha, as were mercury and PCBs in

several fish species (*Perca fluviatilis, Lota lota, Coregonus sp.* and *Salvelinus alpinus*).

Although, zebra mussels is a harmful and among the top 100 most dangerous invasive species in aquatic habitats, its pervasiveness means that it can be also used as a bioindicator to assess current and past presence of elements in water. Moreover, according to BESSE et al. (2012) for active strategies, the use of invasive species should be avoided, meaning that the use of zebra mussels and Asian clams (Anodonta) (which are among the most widely-used species) should be limited to sites that these species have already colonized. However, MERSCH et al. (1996) and BOURGEAULT et al. (2010) successfully used zebra mussels in caging experiments dealing with the effects of different pollutants.

Lastly, the most commonly used bivalve tissues in toxicological studies are gills and digestive gland (Fig. 2) because they play major part in the process of bioaccumulation of toxicants. Thus, they are used in biomarker analyses to the study the subsequent negative effects.

# *Choice of biomarkers in integrated monitoring*

Biomarkers are defined as responses to any exposure evidenced in histological, physiological, biochemical, genetic and behavioral modification (FOSSI & MARSILI, 1997; FOSSI, 1998). Furthermore, as stated by VIARENGO & CANESI (1991) the effects of pollutants on living organisms can be evaluated at different levels of organization (molecular, cellular, individual, population and community.

More recent, VAN DER OOST *et al.* (2003) defined biomarkers as biological indicators from an exposure to a stressor responding in various ways. PICADO *et al.* (2007) add that biomarkers, which act as early warning signals of the presence of potentially toxic xenobiotics, are useful tools for assessing either exposure to, or the effects of these compounds providing information about the toxicant bioavailability.

A range of techniques (Fig. 1 and 3) has been developed to quantify or indicate the effects of pollutants on aquatic organisms from cellular to community levels (ICES, 2004). In addition, THAIN *et al.* (2008) explains that the most widely used biological-effect tools are measures of the biochemical and/or physiological state of selected organisms, such as mussels or fish.

According to HYLLAND *et al.* (2006) over the past decade there has been evidence of effects at low exposure levels. The authors state that many chemicals are metabolized or cause effects at very low levels (e.g. endocrine disrupting substances, PAHs and OPs). Therefore, descriptor 8 in MSFD defines that "concentrations of contaminants are at levels not giving rise to pollution effects" (Table 1).

According to MARIA *et al.* (2009) connections must be established between external levels of exposure, internal levels of tissue contamination and early adverse effects and determining the extent and severity of such contamination only by the result of water chemical analysis is insufficient and often overestimates the proportion and duration of exposure to the Therefore, integration toxic agent. of chemical analyses with biomarker responses in organisms has been recommended for monitoring anthropogenic activities (HYLLAND et al., 2008). According to BAYNE (1986) good interpretation of the data can be obtained by studying the effects of pollutants in individuals, with the aim of understanding and eventually predicting the possible consequences at higher levels.

In the past 25 years, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring and risk assessment programs (NRC, 1987; HINTON *et al.*, 1992; HINTON, 1994; SCHMITT *et al.*, 2007; PANDEY *et al.*, 2008; ARDESHIR *et al.*, 2017). PINTO *et al.* (2009) suggest that the biomonitoring process should include analyses at different levels of biological organization, from subcellular and cellular analysis of tissues and

organs, to the of population and community levels. Therefore, studies using biomarkers are essential to complement environmental monitoring in order to control pollution effects on the animals that inhabit the water bodies (AU, 2004).

The usefulness of any biological-effect method will depend on how well it is able to separate anthropogenic stressors from the influence of environmental or host-related processes (LYONS et al., 2010). According to HYLLAND et al. (1998) it is well established that there are seasonal variations, most commonly coordinated with reproduction, for most biomarkers in temperate organisms. Furthermore, according to HYLLAND et al. (2006) the methods must be able to separate between effects from contaminants and natural variation or the effects from other factors. environmental For example, KAMMANN et al. (2005) found that certain biomarkers such as hepatic EROD activity in fish are influenced by a number of noncontaminant related factors including ambient water temperature and stage of sexual development. As explained bv HYLLAND et al. (2006) European flounder (Platichthys flesus), which is a common test species in monitoring programs in Europe and numerous toxicology studies, shows natural variability through a year (HYLLAND 1998); response to individual et al., contaminants (BEYER et al., 1996); combination of contaminants (SANDVIK et al., 1997) or response in caging experiments (BEYER et al., 1996). On the other hand, there is enough evidence for already established cause-and-effect relationships between the presence of contaminants and biologicaleffect responses in the organisms. A good example here would be tributyltin (TBT)induced imposex in dogwhelk as THAIN et al. (2008) explain. Once population effects directly could be related that to concentrations of organotins in the marine environment were observed (GIBBS & BRYAN, 1996), management actions were taken to reduce TBT emissions and introduce

international policies through the EU and International Maritime Organisation (IMO), which has resulted in a decrease in the prevalence and severity of imposex (BIRCHENOUGH *et al.*, 2002).

WHO (1993) state that biomarkers have been classified into three separate categories that correspond to three major parameters conduct ecological necessary to risk assessments. To perform such an accurate ecological risk assessment, ecological effects, as well as exposure and susceptibility to contaminants must be well-characterized following identification or formulation of a problem. In each of these processes, welldefined biological indicators can be used in certain cases to help make inexpensive predictions regarding the bioavailability (exposure), mechanism of action (effect) and uncertainty of response (susceptibility) elicited by various anthropogenic substances (SCHLENK, 1999). According to VETHAAK et al. (2015) the concept of a background level of response (residual noise of the measurement found from responses of animals in relatively clean waters) is applicable to all effects measurements. Assessment criteria analogous to the Environmental Assessment Criteria (EAC) (i.e. representing levels of response below which unacceptable responses at higher, e.g. organism or population, levels of biological organization would not be expected) are applicable for some biological effects measurements, and these have been termed "biomarkers of effect". In other cases, the link to higher level effects is less clear, and these measurements have been termed "biomarkers of exposure", in that they indicate that exposure to hazardous substances has occurred.

Biological effects measurements and chemical methods have been selected for the *biota matrix* (*separated as fish, mussels and gastropods*) using different criteria – general designs for integrated monitoring of fish are presented in Fig. 1 and of mussels in Fig. 3. Mussels in Ecotoxicological Studies - Are They Better Indicators for Water Pollution Than Fish?

**Table 1.** Qualitative descriptors for determining Good Environmental Status as defined in Annex I of the MSFD (see LYONS *et al.*, 2010).

 Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic and climatic conditions.
 Non-indigenous species introduced by human activities are at levels that do not adversely alter ecosystems.

3. Populations of all commercially exploited fish and shellfish are within safe biological limits exhibiting a population age and size distribution that is indicative of a healthy stock.

4. All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.

5. Human-induced eutrophication is minimized, especially adverse effects, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.

6. Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystem are safeguarded and benthic ecosystems, in particular, are not adversely affected.

Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.
 Concentrations of contaminants are at levels not giving rise to pollution effects.

9. Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.

10. Properties and quantities of marine litter do not cause harm to the coastal and marine environment.

11. Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.

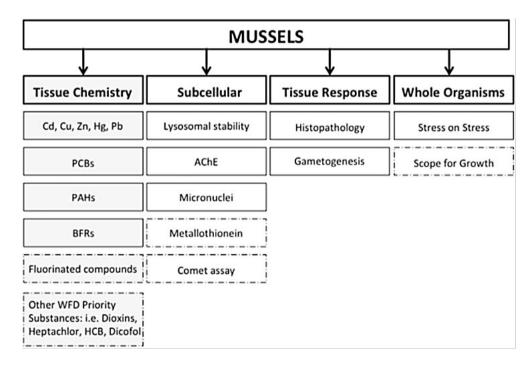


 Fig. 3. Determinants and measurements included in the mussel component of the ICES/ OSPAR integrated monitoring framework. *Legend:* solid lines - core methods; broken lines additional methods; PCBs - polychlorinated biphenyls; PAH - polycyclic aromatic hydrocarbon; BFRs - brominated flame retardants; AChE - acetylcholinesterase. WFD - Water Framework Directive. WFD priority substances are required in biota under Directive 39/2013/EU (see THAIN *et al.* (2008); VETHAAK *et al.* (2015)).

Method	In JAMP	CEMP category	Rec. by WGBEC	QC
Mussels				
Whole sediment	Yes	II	Yes	В
bioassays				
Sediment pore water	Yes	II	Yes	В
bioassays				
Sediment seawater	Yes	II	-	-
elutriates				
Water bioassays	Yes	II	Yes	В
In vivo bioassays	No	-	Yes	B (some)
In vitro bioassays	No	-	Yes	-
Lysosomal stability	No	-	Yes	B-a
Multidrug resistance	No	-	Yes	-
(MXR/MDR)				
Scope for growth	No	-	Yes	B-a
AChE	No	-	Yes	-
Metallothionein (MT)	No	-	Yes	B-a
Histopathology	No	-	Yes	-
Imposex/Intersex in	Yes	I (M)	Yes	Q
gastropods				
Benthic community	Yes	-	Yes	В
analysis				
Fish				
AChE in muscle	No	-	Yes	-
Lysosomal stability	Yes	II	Yes	B-a
Externally visible	Yes	I (V)	Yes	B-a
diseases				
Reproductive success	Yes	II	Yes	B-a
(eelpout)				
Metallothionein (MT)	Yes	II	Yes	B-a
ALA-D	Yes	II	Yes	B-a
Oxidative stress	Yes	II	-	-
CYP1A-EROD	Yes	II	Yes	Yes
DNA adducts	Yes	II	Yes	B-a
PAH metabolites	Yes	II	Yes	Q
Liver	Yes	I (V)	Yes	B
neoplasia/hyperplasia		~ /		
Liver nodules	Yes	I (V)	Yes	В
Liver pathology	Yes	I (V)	Yes	В
Vitellogenin in cod	No	_	Yes	Yes
Vitellogenin in flounder	No	-	Yes	-
Intersex in male	No	-	Yes	B-a
flounder				

**Table 2.** OSPAR status of biological-effect techniques for invertebrates and fish (JAMP) (see THAIN *et al.*, 2008).

CEMP category: II, method suitable for marine monitoring purposes; I, method suitable and analytical-quality control (AQC) is available; M, mandatory method in place, with AQC and assessment criteria established. Quality control: V, voluntary method in place, with AQC but conducted voluntarily. Recommendations for inclusion by WGBEC (ICES, 2007a) and information on existence of quality control [QC: B, Biological Effects Quality Assurance in Monitoring Programmes; B-a, available (BEQUALM, 2009); online Q, Ouality Assurance of Information for Marine Monitoring Environmental in Europe (QUASIMEME, 1992).

WGBEC (ICES, 2007a) has reviewed the biological-effect of techniques status regularly and recommended in its reports those techniques for fish and invertebrates that are in the research phase, look promising, and require development and analytical-quality control, or are available for use and take-up in national and international monitoring programs. Some of the recommended methods have been included in OSPAR guidelines for contaminant-specific or general monitoring (JAMP, 1998a; b) and have, after a process of quality assurance, been included in CEMP (JAMP/CEMP, Joint Assessment Programme/Co-ordinated Monitoring Monitoring Programme). Environmental The updated list (Table 2) includes information on the current position of each technique relative to these guidelines.

### Conclusions

In summary, in 2006 Working Group on Biological Effects of Contaminants (WGBEC; ICES, 2006) made a preliminary proposal, endorsed by Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open-Sea Areas (WKIMON) for combining methods in a program of integrated chemicaland biological-effect monitoring that contains ecosystem components: three water, sediment, and biota, restricted so far to fish, bivalves, and gastropods (THAIN et al., 2008). Moreover. according to Directive 2008/105/ EC Member States are now required to check compliance with EQSs and to monitor the contamination trends for PSs, and they strongly recommended to are use integrating matrices, e.g., biota in order to meet these objectives for bioaccumulative substances (BESSE et al., 2012). Overall, we can conclude that it is especially important

to choose fish as a test species when the research concerns human health, but mussels are also commercial products in terms of aquaculture and are consumed extensively in some areas of the world. From our case studies (GEORGIEVA et al., 2014a; b; 2015; 2016; YANCHEVA et al., 2014a; b; 2016b; 2017) we can confirm that mussels are easier to collect on the field compared to fish, but fish are particularly sensitive to contamination, which is essential in the field of ecotoxicology. Therefore, we would recommend that both, fish and mussels are used in monitoring programs on water contamination, along with combined biomarkers which can be applied on vertebrates and invertebrates for better results.

#### Acknowledgements

This paper is supported by the NPD-Plovdiv University under Grant No SP17-BF003 "Integrated biomarkers for priority toxic substances in aquatic ecosystems by using zebra mussel (*Dressena polymorpha* Pallas, 1771) as bioindicator".

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Received: 07.12.2017 Accepted: 10.04.2018

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