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Whither "biological effects monitoring"?

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The term "pollution" implies biological effects, but until fairly recently, the only readily measurable biological end point was death; while this may be adequate to assess "end-of-pipe" water quality through LC50 determinations, it is not a useful measurement for receiving waters. During the last 20 years, many sublethal bioassays have been proposed or developed for use in marine environments, and several of these have been evaluated during collaborative studies by ICES and other intergovernmental organizations. A few have emerged as being consistently successful and are now used routinely to complement conventional assessments of marine pollution by chemical analyses. These bioassays range from biochemical measurements (such as hepatic mono-oxygenase induction or acetylcholinesterase inhibition) through whole organism changes (e.g., oyster embryo bioassays, measurements of "scope for growth") to measurements of (usually benthic) community structure. Some applications of these will be discussed in detail. Generally, the biochemical bioassays tend to be specific for a limited suite of contaminants, usually of similar chemical structures, and also tend to anticipate possible effects at higher levels of biological organization; at the other extreme, community structure measurements tend to be non-specific, and are retrospective in the sense that they show whether or not significant ecological changes have taken place. "Biological effects monitoring" approaches like these are not likely to replace conventional chemical analyses for regulatory purposes in the near future, but will probably be used increasingly to complement analytical chemistry.

Keywords: biological effects, biomarkers, marine pollution, monitoring.

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Introduction

Our current concerns about marine pollution are usually expressed in terms of chemistry. We regulate the concentrations of polynuclear hydrocarbons (PAH) in material for ocean disposal, we monitor the concentrations of *p,p'*-DDE (2,2-*bis*-[p-chloro-phenyl]-1,1-dichloroethylene, a metabolite of the insecticide DDT) in sea bird eggs, or we limit the concentrations of Hg in edible fish. However, our real concern is with the biological effects of these chemicals: will high PAH concentrations kill benthic invertebrates, or will *p,p'*-DDE affect eggshell thickness, and hence reproductive success in seabirds, or will Hg in the fish we eat poison us? Until about 20 years ago, the only biological "end point" of the effect of pollution was death, usually measured as LC50s (the concentration of a pollutant required to kill 50% of a sample within a fixed time, usually 96 h) with test organisms. LC50s are clearly unsuitable to assess the impact of pollution in "receiving waters" as we want to be warned about effects well before 50% of a population dies. During the last 20 years or so, several methods have been developed to assess sublethal effects of pollution, and with the encouragement of ICES, many of these have been tested, evaluated, and recommended for application. In this paper, I review the present status of these approaches and discuss future needs.

The current status of "biological effects monitoring"

Although many approaches to measuring sublethal effects of pollutants have been proposed or demonstrated under controlled laboratory conditions, few of these have been generally accepted for field use. I have reviewed these approaches elsewhere (Addison, 1996) and discussed there why only a few of those proposed have become widely used. Briefly, the useful approaches fall into four main groups at different levels of biological complexity: a) biochemical changes, b) histopathological changes, including sub-cellular changes, c) whole organism responses, and d) community changes.

Table 1. Some recent examples of cholinesterase inhibition measurements in marine biota.

Location and/or pollution source	Species	Response	Reference
Elbe-Weser "plume" in southern North Sea	*Limanda limanda* (dab)	Approx. 50% AChE inhibition at most contaminated inshore sites	Galgani *et al.* (1992)
French coast	Various teleost spp. and *Mytilus edulis* (mussel)	Detectable AChE activity in all spp., but highly variable, perhaps in response to natural factors	Bocquené *et al.* (1993)
Northwest Mediterranean Sea; sites near municipal and industrial outfalls	*Mullus barbatus* (mullet) and other teleost spp.	Up to 50% AChE inhibition in samples close to municipal or agricultural waste discharges	Burgeot *et al.* (1996)
Pulp mill effluent discharge zone and urban rivers	*Pleuronectes americanus* (winter flounder) and *Salmo trutta* (trout)	Approx. 50% inhibition of AChE in F, but not M fish exposed to pulp mill effluent; approx. 60% inhibition in both M and F fish from urban rivers	Payne *et al.* (1996)
Northwest Mediterranean Sea; municipal outfalls	*Dicentrarchus labrax*	Some reduction in AChE in caged fish held near municipal outfalls at Cannes	Stien *et al.* (1998)

Abbreviations: AChE = acetylcholinesterase; M = male; F = female.

I should note at this point that I have arbitrarily limited this paper to measurements of *biological* response. Although "biomarkers" are sometimes considered to include metabolites such as DNA adducts, I view these as chemical products – admittedly, of biological processes – and I have excluded them from this discussion. I have also limited my discussion to experience with marine organisms or systems, though many relevant studies have been carried out with freshwater biota.

Biochemical changes

These include measurements of enzyme induction or inhibition, or of changes in specific protein concentrations.

Perhaps the oldest of these is acetylcholinesterase (AChE) inhibition, which has had a long history of use as an indicator of the effects of some organophosphorus or carbamate pesticides. AChE is usually measured in fish brain and has been used mainly to assess the zone of influence of pesticide spraying near freshwater systems. Recently, it has been studied in marine biota as a potential indicator of the impact of these, and/or other compounds. Table 1 summarizes some recent observations of AChE inhibition in marine biota. However, no supporting chemical analyses of known AChE inhibitors were made during these studies, although the presence of some pesticides was inferred in studies of samples exposed to agricultural waste streams (e.g., Stien *et al.*, 1998). The precise cause of AChE inhibition, especially in the open sea samples, therefore, remains to be established.

A second such group of assays is based on hepatic mono-oxygenase enzymatic activity catalysed by cytochrome P-450 1A (CYP 1A). This is induced by exposure of the organism to organic compounds with a specific size and shape which allows them to bind to a cytosolic Ah receptor. Such compounds include some polynuclear aromatic hydrocarbons (PAH), some polychlorinated biphenyls (PCB), and some chlorinated dibenzodioxins and furans (PCDD/F). The rationale for measuring CYP 1A is that its concentrations or activity indicate exposure to or accumulation of these chemicals. CYP 1A (protein) concentrations can be measured immunochemically (e.g., Goksøyr, 1985; Park *et al.*, 1986; Lin *et al.*, 1997), or its mRNA can be measured (Renton and Addison, 1992; Courtenay *et al.*, 1993), but the commonest and simplest approach is to measure its catalytic activity directly as the enzymes ethoxyresorufin O-deethylase (EROD: Burke and Mayer, 1974) or benzo(a)pyrene hydroxylase (AHH: aryl hydrocarbon hydroxylase: Nebert and Gelboin, 1968). An extensive literature already exists for this in marine organisms, and some of the more recent examples are summarized in Table 2. Briefly, CYP 1A induction has proved to be a useful indicator of the presence of PAH and PCB (and occasionally PCDD/F), but different components of the system may give different responses (e.g., Goksøyr *et al.*, 1991).

Metallothionein (MT) is a low MW protein rich in -SH groups found in both vertebrates and invertebrates and which sequesters certain heavy metals (usually Zn, Cu, and Cd) and is induced by exposure to them (among others: Depledge *et al.*, 1995). Its use in environmental monitoring has been reviewed by Hogstrand and Haux

Table 2. Some recent examples of CYP 1A-based measurements in marine biota during environmental assessments.

Location and/or pollution source	Species	Response	Reference
NW Atlantic; PCB 8X difference in contaminated v. reference fish	*Coryphaenoides armatus* (rattail)	EROD and AHH 7-9X increase in contaminated v. reference fish; CYP 1A increased	Stegeman *et al.* (1986)
Langesund, Norway; PCB 7X difference in fish between sites in a pollution gradient	*Platichthys flesus* (flounder)	EROD 13X, AHH 3X, and CYP 1A 13X change between sites	Addison and Edwards (1988); Stegeman *et al.* (1988)
Glomma estuary, Norway; PAH 4X and PCB 6X between fish from several sites	*Platichthys flesus* (flounder) *Pleuronectes platessa* (plaice) *Limanda limanda* (dab)	EROD 50X and CYP 1A 2X change between sites	Goksøyr *et al.* (1991)
Sydney Hbr. NS, Canada; PAH 100X difference between sites	*Pleuronectes americanus* (winter flounder)	EROD 6X, AHH 9X, and CYP 1A 5X change between sites	Addison *et al.* (1994)
Miramichi R., NB, Canada; BKME (softwood processing) v. upstream reference site	*Microgadus tomcod* (tomcod)	CYP 1A mRNA 4-11X change from reference sites	Courtenay *et al.* (1993)
NW Mediterranean Sea; sites near industrial or municipal outfalls	*Mullus barbatus* (mullet) *Serranus cabrilla* (comber)	EROD elevated at sites with PAH exposure (indicated by DNA adducts)	Burgeot *et al.* (1996)
NE USA coast; sites near industrial or municipal outfalls	*Pleuronectes americanus* (winter flounder)	EROD, AHH, and CYP 1A generally elevated at contaminated sites	Collier *et al.* (1999)

Abbreviations: AHH = Arylhydrocarbon hydroxylase; BKME = Bleached kraft mill effluent; EROD = ethoxyresorufin O-de-ethylase; PAH = polynuclear aromatic hydrocarbon.

(1991). MT has been used successfully to indicate metal pollution, but its response is variable, possibly because it may have physiological functions other than the regulation of metal concentrations (Viarengo and Nott, 1993). MT may be measured as the protein by immunochemical or polarographic methods, or as its mRNA. Table 3 lists some recent applications of MT measurements in marine systems.

Environmental estrogens – compounds which interact with estrogen receptors and which, therefore, may disrupt normal endocrine function – have attracted recent interest. These compounds may be natural (e.g., phytoestrogens such as isoflavonoids) or may be present in industrial or sewage treatment plant wastes (e.g., Servos, 1999; Allen *et al.*, 1999). Vitellogenin (Vtg), a protein normally associated with egg yolk production, has been used to indicate the feminising effect of such compounds in male fish and other biota (e.g., Arcand-Hoy and Benson, 1998), as have other proteins associated with egg development (e.g., Arukwe *et al.*, 1998).

These biochemical measurements are attractive for several reasons. First, through experimental studies, the detailed processes of induction or inhibition have been established, and so the mechanistic link between exposure and response is clear. Second, the response is relatively specific because of the constraints imposed by the molecular structures involved in the process. Finally, at least in the case of AChE inhibition and EROD or AHH induction, the measurements are probably cheaper than the cost of chemical analysis of the causative agent. However, the limitations of these approaches must be recognized. Both CYP 1A and MT may have "natural" functions not related to pollutant exposure, and these may confound the apparent relationship between exposure and response. As well, the concentrations or activities – and hence the dose-response relationships – of these systems may be modulated by natural environmental or physiological processes such as temperature or reproduction, which must be recognized or eliminated.

Cellular and histopathological changes

A wide range of histopathological conditions, including gross and sub-cellular lesions (e.g., Myers *et al.*, 1999), neoplasms, and parasitic infections (e.g., Khan and Payne, 1997) have been ascribed to the effects of pollution, but it is often difficult to establish clear cause-effect relationships – at least to limited groups of pollutants – under field conditions (e.g., Couillard *et al.*,

Table 3. Some recent examples of metallothionein (MT) measurements in marine biota during environmental assessments.

Location and/or pollution source	Species	Response	Reference
Bermuda; several sites Tissue Zn 2-3X difference	Tropical reef fish; several spp.	MT up to 4X change between sites	Hogstrand and Haux (1990)
Forth R., UK; tissue Cu 3-6X difference between sites	*Platichthys flesus* (flounder)	MT approx. 4X change between sites	Sulaiman *et al.* (1991)
North Sea; seven sites along a pollution gradient	*Limanda limanda* (dab)	Liver MT in F best correlated with Zn; liver MT in M best correlated with Cu and Cd	Hylland *et al.* (1992)
North Sea; five sites along a pollution gradient	*Limanda limanda* (dab)	Gill MT well correlated with Zn, Cu, and Cd content	Stagg *et al.* (1992)
Patuxent R., USA; tissue Cu, Zn, and Cd varied approx. 3X	*Crassostrea gigas* (oyster)	Complex MT response	Roesijadi (1994)
Fal estuary (UK) and reference sites; tissue Cu and Zn vary 5X(?) between sites	*Carcinus maenas* (shore crab)	Gill MT well correlated with Cu and Zn concs.	Pedersen *et al.* (1997)

Abbreviations: M = male; F = female.

1999). However, one response which has been well investigated both experimentally and in the field includes changes to lysosomal membranes, particularly in digestive tissues of bivalves. Lysosomal stability can be assessed by measuring the activity of associated enzymes or by the permeability of the membrane to lipid soluble dyes. Like many other biological responses to pollution, bivalve digestive gland lysosomal stability is affected by natural factors such as seasonal (Tremblay *et al.*, 1998) or even tidal changes (Tremblay and Pellerin-Massicotte, 1997), but several studies have shown some correlation between declining lysosomal stability and pollution stress (Table 4).

Whole organism responses

These include imposex, "scope for growth", and the oyster embryo bioassay (OEB).

Imposex describes the masculinization of female neogastropod molluscs following exposure to organotin compounds, usually the tributyltin cation (TBT), a component of some marine antifouling paints. The condition leads to a partly developed penis and vas deferens which may obstruct the female oviduct to prevent reproduction. Measurements of vas deferens development, relative penis size, or imposex frequency, are often well correlated with TBT exposure (see references in Table 5). These anatomical changes seem to be restricted to neogastropods (though not all of them show the condition: Gibbs *et al.*, 1997), but if the "biochemical lesion" caused by TBT results from inhibition of cytochrome P-450 mediated aromatase (Matthiessen and Gibbs, 1998), evidence of more widespread endocrine disruption in other organisms exposed to TBT could be expected. Some recent applications of imposex measurements are summarized in Table 5.

"Scope for growth" measures energy partitioning over a short period under standard conditions. Its use is based on the premise that an organism experiencing stress will use ingested energy less efficiently than a healthy organism and so will have less energy available for growth or reproduction (Widdows and Johnson, 1988). "Scope for growth" can, in principle, be applied to any organism, but is usually measured in sessile animals so that the "noise" in energy use created by movement is reduced. Briefly, organisms are sampled in the wild, or from caged transplants, and their energy accumulation and excretion is measured under standard environmental conditions, including food supply. Table 6 shows some examples of the use of "scope for growth" in assessing pollution impacts.

The OEB measures anatomical deformities in the developing oyster (usually *Crassostrea gigas*) embryo and is arguably a lethality test since severely deformed embryos may not fully develop. It has been used mainly in regulatory work, which may explain why there are relatively few "open" literature references to its application. However, the bioassay has been compared with other tests, usually of acute lethality. It is quite sensitive and gives results consistent with other bioassays (Butler *et al.*, 1992; Thain, 1992; Matthiessen *et al.*, 1993, 1998; Clarkson *et al.*, 1999).

Table 4. Some recent examples of the use of lysosomal stability measurements in environmental monitoring.

Site and species	Pollution source	Response	Reference
Sullom Voe oil terminal, Scotland; various bivalve spp.	PAH	Lysosomal stability declines with increasing PAH tissue burdens	Widdows *et al.* (1985)
Langesundfjord, Norway; *Mytilus edulis* and *Littorina littorea*	Industrial pollution gradient	Generally decreasing lysosomal stability with increasing contaminant tissue burdens	Moore (1988)
Halifax Hbr. Canada; *Mytilus edulis*	Municipal discharges including metals	Generally declining digestive gland lysosomal stability associated with high metal exposure, notably Zn	Ward (1990)
Southern North Sea; *Limanda limanda* (dab) hepatocytes	Elbe-Weser plume pollution gradient	Increasing lysosomal stability with increasing distance offshore	Lowe *et al.* (1992); Köhler *et al.* (1992)
Italy, polluted v. reference sites; *Mytilus galloprovincialis*	Heavy metals	Declining lysosomal stability in digestive gland associated with heavy metal exposure	Regoli (1992)
Puget Sd., USA; *Mytilus edulis* digestive gland	Mainly sediment-bound PAH	Declining lysosomal stability assessed by various measures associated with sediment contaminants and independent of "natural" stresses	Krishnakumar *et al.* (1994)

Abbreviations: PAH = polynuclear aromatic hydrocarbon.

Table 5. Some recent examples of imposex and organo-tin measurements in marine biota during environmental assessments.

Species and site	Response	Reference
Nassarius reticulatus; SW England	Imposex frequency related to tissue TBT; declines in TBT over time not reflected in changing imposex frequency	Bryan *et al.* (1993)
Thais clavigera, Thais bronni; 32 sites around Japan	Imposex frequency high and strongly correlated to both tissue TBT and TPT	Horiguchi *et al.* (1994)
Littorina littorea (periwinkle); Estuaries of R. Crouch and Hamble (UK)	Declining TBT concs. in water, sediment and tissues since 1987; parallel increase in O-group individuals, but no evidence of imposex	Matthiessen *et al.* (1995)
Nucella emarginata (whelk); Vancouver Is. Canada sites	Declining imposex frequency and RPS associated with declining environmental TBT	Stewart and Thompson (1994); Tester *et al.* (1996)
Nucella lapillus (dogwhelk); Eastern Canada, various sites	Widespread imposex (or spp. absent) generally associated with environmental TBT concs.	Prouse and Ellis (1997)
Thais clavigera; Japan Inland Sea	Up to 50% RPL; correlated with tissue TBT + TPT concs.	Horiguchi *et al.* (1994)
Nucella lapillus (dogwhelk); NW Spain	Significant correlation between RPS and tissue TBT	Ruiz *et al.* (1998)
Morula granulata (whelk) and *Saccostrea cucullata* (oyster);	Up to 57% imposex frequency depending on site; imposex correlated with TBT in oysters and DBT in whelks	Reitsema and Spickett (1999)

Abbreviations: DBT = dibutyltin; TBT = tributyl tin; TPT = triphenyltin; RPS = relative penis size; RPL= relative penis length.

Table 6. Some recent examples of the use of scope for growth (SFG) to assess marine pollution.

Site and species	Pollution source	Response	Reference
San Francisco Bay USA; *Mytilus edulis*	Municipal sewage	Decline in SFG with increasing chlordane, dieldrin, Cr, Cu, Hg, Ag, and Al concentrations	Martin *et al.* (1984)
Langesundfjord, Norway; *Mytilus edulis*	Industrial wastes	Decline in SFG with increasing PAH and PCB	Widdows and Johnson (1988)
Hamilton Hbr., Bermuda; *Arca zebra*	Municipal wastes and TBT	Decline in SFG with increasing PAH and TBT	Widdows *et al.* (1990)
Southampton Water, UK; *Cerastoderma edule*	Mixed contaminants	Decline in SFG correlated with increasing Cu in sediments	Savari *et al.* (1991)
UK coastal sites; *Mytilus edulis*	Industrial and municipal wastes	Decline in SFG from north to south, correlated with increasing PAH and TBT exposure	Widdows *et al.* (1995)
Venice Lagoon, Italy; *Mytilus galloprovincialis*	Municipal waste	Decline in SFG correlated with tissue PAH, PCB, DDT, and HCH, but not metals	Widdows *et al.* (1997)

Abbreviations: PAH = polynuclear aromatic hydrocarbons; DDT = 2,2-bis(p-chlorophenyl)1,1,1-trichloroethane; HCH = hexachlorocyclohexane; TBT = tributyltin; PCB = polychlorinated biphenyl.

Community responses

Species abundance and diversity has been widely used to assess the status of a community, and it is generally accepted that a healthy community should be both productive (leading to abundance of individuals of a species) and diverse, in that a range of species confers stability on the community. In a grossly polluted environment, diversity and abundance may be reduced (e.g., Kingston, 1992). Various diversity indices have been developed, but during the past 20 years or so, the most obvious evolution in this area has been the application of multivariate (as opposed to univariate) statistics to abundance-diversity measurements. The multivariate statistical approach allows more information from these measurements to be used, so that much more subtle changes in community structure can be detected than from univariate analyses (e.g., Gray *et al.*, 1990). The approaches have shown that benthic meio- or macrofaunal community structure may change in response to the impact of offshore oil production facilities (Gray *et al.*, 1990), municipal waste (Warwick *et al.*, 1990), mine wastes (Olsgard and Hasle, 1993), and general sewage pollution (Zmarzly *et al.*, 1994). The structure of coral reef communities may change in response to local mining, or to El Niño events (Warwick and Clarke, 1993). Provided sampling is appropriate, temporal changes indicating the gradual recovery of benthic communities from pollution events may also be recorded (e.g., Clarke, 1993). There have also been modifications to this basic approach, e.g., the computation of k:r ratios within a community to identify, e.g., recent colonization by opportunistic species.

In pollution monitoring and assessment, most community structure analyses have focused on benthic communities presumably because they are more stable in space and time than pelagic communities, though, in principle, there is no reason to exclude the latter.

An overview of current methods for biological effects monitoring

Figure 1 illustrates some of the strengths and weaknesses of the biological effects monitoring approaches discussed here. Generally, the strength of the biochemical approaches is that they are usually specific to a limited suite of chemical stresses, and there is a clear causal or mechanistic link between the stress and the response. Their weakness is that their ecological relevance is low, i.e., it is difficult to predict that a biochemical adaptation will lead to a population or community level response. At the other end of the scale, community structure analyses have high ecological relevance, but it is usually difficult to assign differences in community structure to a specific stress, though the recovery of benthic communities from the "Amoco Cadiz" spill (Clarke, 1993) is an exception. The development of imposex in neogastropods as a response to a fairly specific group of chemicals (organo-tins) is an anomaly in

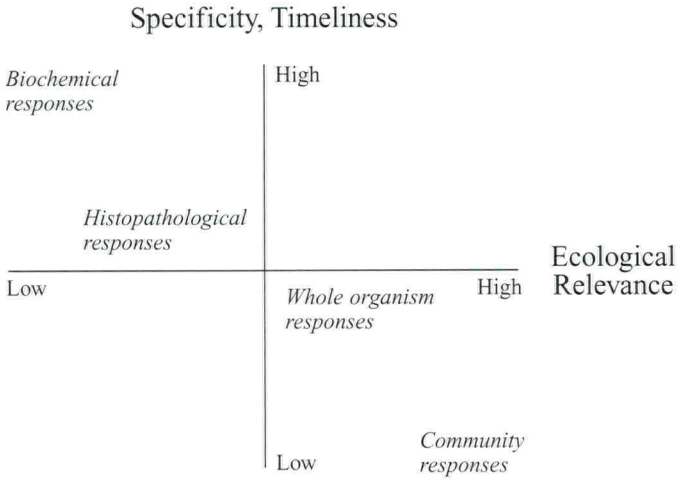

Figure 1. Ecological relevance and specificity of the biological effects measurements discussed in the text.

this trend. Other whole organism measurements such as scope for growth tend not to be specific to a single stress. Finally, the biochemical measurements tend to be prospective – they may provide an early warning of later changes – while community structure changes tend to be retrospective – they show that a change has occurred.

Biological effects measurements at present, therefore, represent a range of "tools" which can be used to assess the state of the marine environment, and as is the case in most other areas of science, the challenge is to ask the right questions to be answered by using these tools.

The role of ICES in biological effects measurements

Immediate needs

In principle, the application of biological effects measurements to environmental problems is no different from the use of analytical chemistry. In both cases, there is a "sample" (usually from the environment) and an "analyte" – either a chemical residue, or a biological response. The analogy can be carried even further: in CYP 1A induction, the selective binding of the inducer to the cytosolic Ah receptor to trigger induction parallels the "clean-up" steps in analytical chemistry to isolate analytes of interest. As in chemical analysis, the detector response must be calibrated (i.e., a dose-response relationship established). A few such "calibrations" have been described in the open literature, such as those of CYP 1A induction in fish by PAH (Addison and Payne, 1986) and the SFG - TBT relationship in some bivalves (e.g., Widdows *et al.*, 1990, 1995).

The variability of the "detector" in biological effects measurements must also be defined. In general, biological responses are likely to be modulated by factors such as age, sex, condition, and reproductive status in individual organisms, and these confounding variables have to be eliminated. (It is worth emphasizing that they also should be eliminated during *chemical* analyses of contaminants in biota for environmental monitoring.) Again, the most comprehensive attempts to define this kind of variability comes from CYP 1A-based and MT measurements in North Sea monitoring species such as flounder (Eggens *et al.*, 1996; Hylland *et al.*, 1998) and dab (Lange *et al.*, 1998).

Finally, biological effects measurements deserve laboratory "intercalibration" for the same reasons as do chemical analyses. A few such intercalibrations have already taken place, focusing on CYP 1A-based measurements, MT, and lysosomal stability (Stagg and Addison, 1995; Viarengo *et al.*, 2000), and more are planned as part of the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) and BEQUALM (Biological Effects Quality Assurance in Monitoring) programmes.

The future role of ICES

In the past, ICES has functioned at two levels: a general level, in which it has provided general scientific advice and/or promotion of approaches to assessing marine environmental quality, and a rather specific level, in which it has conducted intercalibration exercises, or produced well-validated methods, e.g., through the TIMES (*ICES Techniques in Marine Environmental Sciences*) series. There is a continuing need for both of these activities.

At the "general" level, there is increasing awareness among monitoring agencies that biological effects measurements can be usefully applied to complement chemical analyses in pollution assessments [see, e.g., the (former) North Sea Task Force and OSPARCOM programmes]. ICES should continue to encourage the use of these approaches, since, in the long run, it is the biology with which we are most concerned. Furthermore, ICES should encourage, in a general way, the development and improved understanding of the links between effects at various levels of biological complexity.

More specifically, ICES has, in the past, run intercalibration exercises, usually for chemical analyses, which have been extremely useful for analysts to identify steps or approaches in their analyses which have given rise to errors. Recently, ICES, through its Working Group on the Biological Effects of Contaminants, and others have begun to develop such exercises for biological effects measurements (e.g., Stagg and Addison, 1995; Viarengo *et al.*, 2000). ICES should continue to encourage the development of such exercises, e.g., via the QUASIMEME and BEQUALM programmes, and should encourage active participation in them.

More generally, and as a "research" issue, the relationships between biological effects measurements at different levels of complexity need to be better understood. The best characterized biological effects measurements, in the sense that there is usually a fairly specific cause-effect relationship with pollutants, are biochemical assays. One outstanding question is how to develop these to increase their predictive value for possible impacts at higher levels of biological complexity. Biochemical changes may be *correlated* with higher order responses, even though there need be no causal link between them. Thus, at Langesundfjord (Norway) during the IOC-GEEP (Intergovernmental Oceanographic Commission Group of Experts on the Effects of Pollution) practical workshop, flounder CYP 1A-based measurements showed the same spatial trend in response to pollution as did scope for growth in mussels (Bayne *et al.*, 1988). In the southern North Sea during the IOC-ICES Bremerhaven Workshop, dab hepatic CYP 1A-based measurements showed generally similar spatial trends to those of oyster embryo bioassays on sediment elutriates, or benthic macrofaunal abundance or biomass (Stebbing *et al.*, 1992). Usually, this kind of correlation need not imply a cause-effect relationship – after all, why should a biochemical adaptation in fish cause a change in the distribution of benthic invertebrates? One example of a situation where there may be a causal link between biochemical and higher order responses is in the PAH-contaminated Puget Sound (USA). Here, the presence of environmental PAH has led to CYP 1A induction in liver of English sole, which is associated with DNA adduct formation and the subsequent development of neoplastic foci. In other words, a "whole organism" response in the sense that health of individual fish is affected, and this may lead to population and/or community changes (Stein *et al.*, 1990). More studies of this sort linking biochemical to higher-order responses should be encouraged.

Will biological effects measurements replace chemical analyses in assessing marine environmental quality? I think not, at least over the short term. But we are already seeing such measurements used in conjunction with, and to complement, chemical analyses, e.g., in the North Sea/OSPARCOM programmes (North Sea Task Force, 1993). In such cases, the combination of chemical and biological data is more easily interpreted than either set by itself would have been. Furthermore, taken together, they provide a "weight of evidence" that may be more convincing than either data set alone. There are also situations where biological measurements "lead" chemical analyses. The observation that AChE activities vary with site in the North Sea and Northwest Atlantic implies the presence of unexpected chemical contaminants or, at least, that the conventional wisdom that known AChE inhibitors have short environmental half lives may be wrong. Similarly, the distribution and frequency of imposex is being used as a "screening" approach to identify sites in which high concentrations of TBT may be sought using chemical analyses. This trend to use chemical analyses and biological effects measurements in a complementary approach should continue.

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