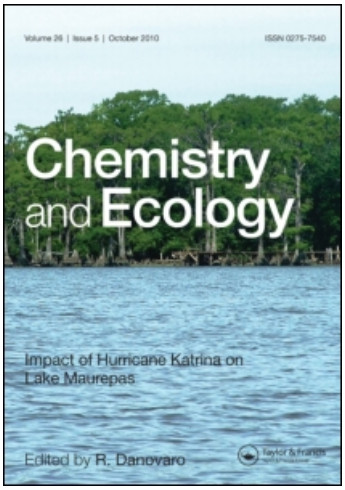

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SELECTION OF BIOINDICATORS OF POLLUTION FOR MARINE MONITORING PROGRAMMES

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The ultimate management concerns related to toxic chemicals in the marine environment are for the magnitude and extent of biological effects, including those on human health, that may result. Over the past several years, the National Status and Trends (NS&T) Program of the US National Oceanic and Atmospheric Administration (NOAA) has supported the development and application of a number of bioeffects measurements, including biochemical indicators of contaminant exposure and of reproductive status in fish, prevalence of histopathological lesions, toxicity bioassays of sediments and water, and benthic community structural features. Some of these measurements have been applied as part of regular sampling at the nationwide network of NS&T sites, while others have been assessed in more intensive, regional studies. Our experience with different indicators is summarized and discussed in relation to a set of monitoring objectives and evaluation criteria. Current conclusions are: (a) iterative application of controlled laboratory experiments and field validation tests are required to verify causality and mechanisms of biological responses to contaminants; (b) contaminant concentrations, indicator species, and local conditions vary considerably in different areas and at different times, requiring the use of different effects measurements oriented toward specific objectives and hypotheses; and (c) tiered, sequential application of a mix of indicators, including direct measurements on indigenous organisms and indirect bioassay approaches, is useful and effective for estimating the magnitude and spatial extent of contaminant bioeffects.

KEY WORDS: toxic chemicals, monitoring, bioindicators, benthic communities

INTRODUCTION

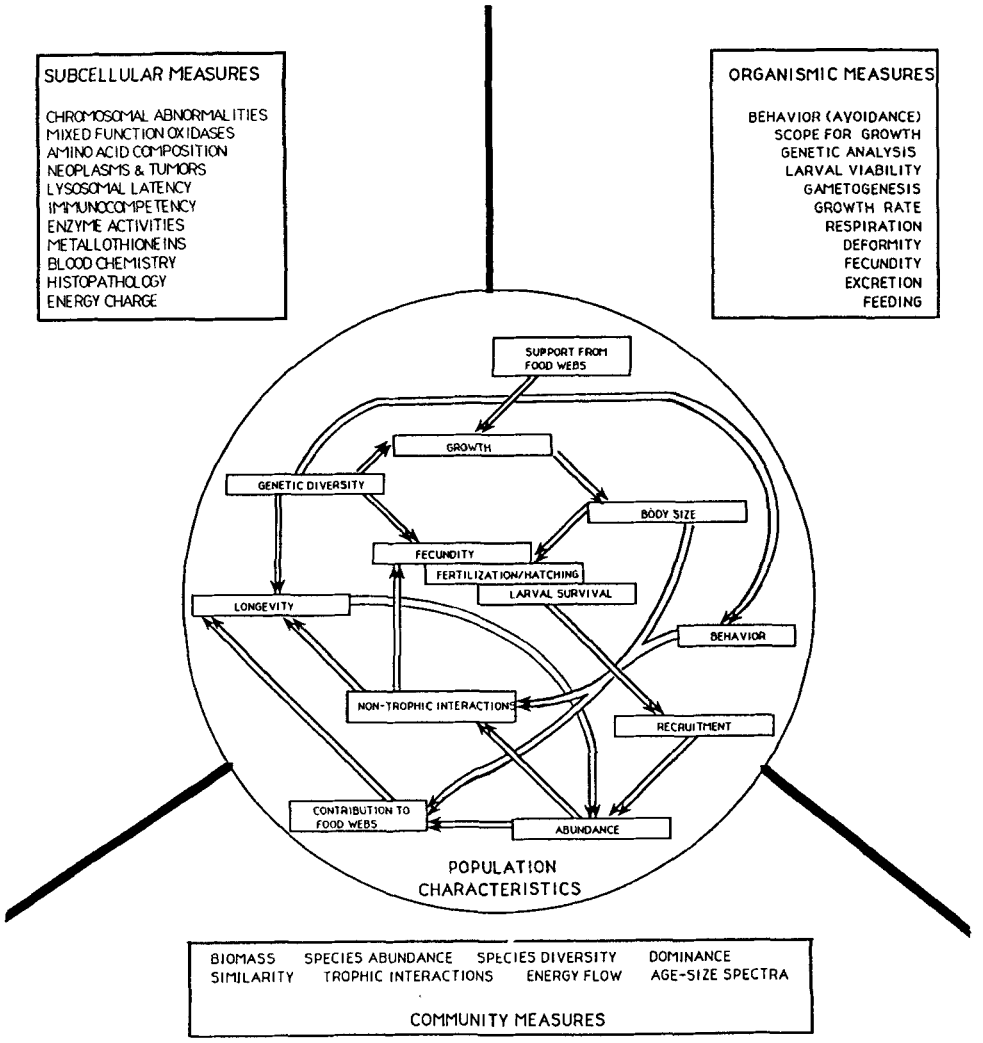
As a result of the variety and intensity of human activities in coastal and near-coastal areas, marine waters receive a wide array of potentially toxic contaminants. These contaminants enter coastal marine waters via direct discharges from municipal and industrial outfalls, runoff from urban areas adjacent to the coast, runoff from agricultural and forested areas, wet and dry fallout from the atmosphere, and in some areas, from dumping of dredged materials, industrial wastes, and sewage sludge (Wolfe *et al.*, 1991). The accumulation of contaminants in coastal areas depends on the total quantities entering a particular coastal water body, and on the volume, flushing, and mixing characteristics of the affected segment. Difficulties in coordinating the regulation of these multiple sources of contaminants in naturally variable ecosystems have resulted in the almost universal requirement in US marine environmental legislation for monitoring of environmental quality (Wolfe, 1988; NRC, 1990). In many areas, such contaminant inputs have led to undesired biological responses and a significant deterioration of environmental quality (O'Connor *et al.*, 1987; Dethlefsen, 1988; McCain *et al.*, 1988; Overstreet, 1988; Hallers and Bijlsma, 1989; GESAMP, 1990). Concerns about the potential long-term consequences of low-level marine contamination (GESAMP, 1989) and about

exposure to a multiplicity of contaminants (Capuzzo and Kester, 1987; Howells *et al.*, 1990) have long provided impetus for inclusion of meaningful biological indicators in monitoring programs for environmental quality. Several recent workshops and symposia have focused on fundamental understanding of biological responses to contaminants and on the identification of those measurements that might be effective as monitoring tools (White, 1984; Bayne *et al.*, 1988; Stegeman *et al.*, 1988; Moore *et al.*, 1989; Malins, 1988; McCarthy and Shugart, 1990; US EPA, 1990).

The choice of bioeffects indicators in marine environmental monitoring programmes, however, still presents the considerable challenge of reconciling the expectations and information needs of environmental managers and decision-makers (who mandate the monitoring), with both the complexity of natural ecological systems and fiscal constraints. A multitude of complex causal interactions, coupled with motion and change at all levels from molecular to ecosystem in the hierarchy of biological organization, lead to patterns of variability that can be exceedingly difficult and expensive merely to detect, much less to understand or predict (Holling, 1985; Wolfe and Kjerfve, 1986; Wolfe *et al.*, 1987). This environmental reality often conflicts with an environmental manager's expectation and need for useful (i.e. simple, accurate, and conclusive) information that can be brought to bear immediately on issues related to the appropriateness of environmental practices or the adequacy of proposed regulations (Bernstein, 1991). Solutions to this generic problem are far from easy: there is increasing recognition, however, that environmental monitoring is an integral component of sound environmental management, and that, where possible, the design of monitoring programmes should be undertaken jointly by scientists and managers to reach carefully specified objectives, including identification of the expected uses for the detailed information to be gathered (Wolfe, 1987, 1988; Wolfe *et al.*, 1987; Bernstein, 1991; NRC, 1990).

From a management perspective, primary interest in contaminant effects is on populations of "important" species, such as those serving as human food resources, key species within a community, or food-web links. Figure 1 illustrates some of the biological and ecological processes that together sustain and regulate population integrity for marine species, and identifies some of the subtle bioeffects measures (at subcellular, organismic, and community levels of organization) that have been applied in environmental assessment and monitoring programs. While it is clear that contaminants can and do affect several of the processes and attributes represented, it must also be accepted that such effects may be extremely difficult to quantify in the face of the many other highly variable sources of population stress (e.g. predation) and compensation (e.g. changes in fecundity or recruitment) inherent within the system (Vaughan and Van Winkle, 1982; Pietrafesa *et al.*, 1986; Schaaf *et al.*, 1987). A fundamental premise, therefore, of monitoring design pertinent to chronic, low-level contaminant inputs is that potentially serious and visible biological consequences (e.g. decreased longevity or survival, massive lesions, population and/or species depletion, community changes, human health effects) will be foreshadowed by subtle (i.e., sublethal) changes. By measuring the subtle, sublethal responses, it may be possible to forecast, even avoid, more serious effects. Although it may never be practical either to predict accurately population-level response to low levels of toxic contaminants in the marine environment or to attribute contaminant causality to changes observed in wild marine populations, it is nonetheless important to choose bioeffects indicators with strong causal implications (i.e, through reproduction, survival, longevity) for resource populations of greatest interest. In

the closely-related context of environmental risk assessment, Suter (1990) distinguishes between "assessment endpoints," or those environmental attributes that are valued ultimately by society, and the actual "measurement endpoints" of response to a hazard. The assessment endpoints related to population numbers and productivity can be highly variable and are rarely diagnostic (except at a massive level). A set of measurement endpoints is desirable, therefore, for documenting contaminant effects, at the same time providing a basis for inferring the potential impact on the assessment endpoints.



Adapted from. Underwood & Peterson (1988) Marine Ecology Progress Series 46. 227-234

Figure 1 Potential measurements of bioeffects encompass several overlapping levels of biological organization, which respond to contaminants over a broad range of time scales, and which exhibit widely different levels of ecosystem or population relevance.

This paper summarizes recent considerations given to the selection of bioeffects criteria in relation to the objectives and needs of NOAA's National Status and Trends Program. (See Turgeon and O'Connor (1991), Turgeon *et al.*, (1991) and NOAA (1989) for general background on the NS&T Program.) Potential criteria for evaluation of bioeffects indicators are identified in relation to specific candidate monitoring objectives, and the merits of several different categories of indicators are discussed according to those criteria. Parallel and coordinated evaluations are underway in relation to the objectives of the Environmental Monitoring and Assessment Program's Near Coastal Component, sponsored by the US EPA (Scott, 1990; Folmar, 1990; Mayer *et al.*, in press).

MONITORING OBJECTIVES AND BIOINDICATOR SELECTION

Selection of measurements and design of sampling strategy depend greatly upon the specific objectives of a monitoring program (Wolfe and O'Connor, 1986; Wolfe, 1987, 1988; Wolfe *et al.*, 1987; NRC, 1990a). Source composition and contaminant inputs vary greatly in different locations, as do the dispersive characteristics of the receiving environment and the biological communities and their sensitivities to contaminant exposure. Sampling intensities (in both space and time) must be chosen carefully to optimize the detection of the expected magnitude of differences between areas as well as the expected rates and periods of temporal trends. Great care must also be exercised to ensure that excessive effort and costs are not committed where contaminant levels and associated bioeffects are below meaningful thresholds of concern, or where the detection and quantification of bioeffects is improbable because of other (i.e., non-contaminant) sources of variability.

Table 1 lists an integrated set of general objectives related to trend monitoring of environmental quality in the marine environment. These objectives also represent a tiered logic flow, each step of which is important to the overall assessment. Relations between objectives and approaches for their implementation on different scales of application are discussed in the following text.

The first objective relates to the status and trends of contaminant concentrations: What contaminants are present in the environment? What is their spatial distribution? How are their concentrations changing in time? In a perfect world (where contaminant sensitivities and dose-response relations are understood quantitatively for all contaminants and all organisms under all conditions of exposure), contaminant distributions alone might suffice. Such perfect surrogacy is not foreseeable, however, and we can therefore expect critics to ask questions about contaminant distributions: "Are these contaminants bioavailable?" and "Do they cause effects?" While the exposure and bioavailability issues are addressed directly by measuring either the contaminants (Phillips, 1980; NOAA, 1989) or their

Table 1 Candidate objectives for trend monitoring of marine environmental quality

1. Distribution* of Toxic Contaminants
2. Contaminant Exposure and Bioavailability
3. Distribution* of "Significant" Bioeffects
4. Concordance of Bioeffects with Contaminant Levels

*Distribution, both spatial and temporal

breakdown products (Krahn *et al.*, 1987) in biological tissues, or by measuring contaminant-specific responses such as induction of cytochrome p450E (EROD) in tissues (Hahn *et al.*, 1989), the documentation of effects may not be so straightforward.

Numerous biological and ecological effects of contaminants have been described on the basis of field observation and/or laboratory exposures, and many of these measurements have been successful (in varying degree) in detecting gradients of contaminant effect in areas of moderate disturbance (McIntyre and Pearce, 1980; White, 1984; Bayne *et al.*, 1988; McCarthy and Shugart, 1990). Detection and quantification of contaminant bioeffects in a general monitoring program for environmental quality, on the other hand, is complicated by: the non-specificity of many responses to contaminants; poor understanding of dose-response relationships; synergisms or antagonisms among responses and contaminants; latency of some responses to long-term contaminant exposure; the abilities of individuals, populations, and communities to adapt to, or compensate for, some level of contaminant exposure and stress; and of course, natural patterns of variation associated with environmental variables other than contaminants. In the face of fiscal constraints, these difficulties demand that monitoring programs incorporate elements of hierarchy and adaptability into their basic design, using screening methods for initial detection of response and more stringent methods for detailed characterization and quantification only when and where these are indicated by the results of screening tests (Holling, 1978; Walters, 1986; Wolfe, 1987).

Bioeffects objectives and indicators are not immune from the "So what?" criticism. As suggested previously (Wolfe and O'Connor, 1986), monitoring design is greatly facilitated if levels of concern can be identified in advance for each of the selected endpoints. In this context the National Research Council (NRC, 1990, p. 76) emphasized the need to focus on "meaningful" bioeffects of contaminants, i.e. those with "significant" implications for populations of key species (as distinguished from searches for statistically significant differences in less selective measurements).

Some critics might argue that since biological effects are the ultimate concern, measurements of contaminant concentrations are unnecessary in extensive monitoring programs. Unfortunately, most measurements of contaminant-related effects respond also to other factors, including mixtures of contaminants, other categories of pollution (e.g. nutrient loading, BOD), the physiological state of the organism, and spatially and temporally variable environmental factors, such as freshwater runoff, salinity, temperature, sediment type. Interpretation and comparison of results are particularly difficult for isolated samples or observations. This lack of perfect surrogacy between bioeffects measurements and contaminant distributions necessitates the final objective in Table 1, which addresses another level of "So what?" criticism: "How are the observed bioeffects related to contaminants?" Concordance of observed bioeffects with contaminant concentrations must be demonstrated in any monitoring effort to support any inferences of causality, i.e. to help distinguish contaminant causality from other effects (eg. natural variation, eutrophication, or other human activities). This final objective closes the loop in the monitoring objectives in Table 1, and adds to the value of contaminant data in meeting the first two objectives.

EVALUATION CRITERIA FOR SELECTION OF BIOINDICATORS

Many considerations pertinent to bioindicator evaluation were introduced above. Table 2 reiterates these and adds other criteria important in indicator selection. These criteria are similar to those introduced and applied elsewhere (Wolfe and O'Connor, 1986; Kelly and Harwell, 1989; Scott, 1990; NRC, 1990). These are not listed here in any order of priority or weight and most are qualitative, not quantitative. Their meanings and applications are discussed briefly below.

Significance of a bioindicator refers to its meaningfulness in relation to ecosystem values. Target organisms selected for bioeffects measures should, where possible, be economically or ecologically important species or taxa and should be evaluated in terms of their implications for population-level impacts. The US National Research Council (NRC, 1990) also emphasized this need to focus on "meaningful" bioeffects of contaminants, i.e. on those with "significant" implications for populations of key species (to avoid as much as possible, scientific "fishing expeditions" that merely quantify statistically significant differences).

A useful measurement should also have high sensitivity, or responsiveness, to contaminants. Demonstrated dose-response sensitivity is essential, and usually requires initial demonstration under controlled laboratory conditions, but sensitivity should also be confirmed by field testing and verification in a variety of realistic conditions. Other sensitivity-related factors that must be balanced against dose-responsiveness include low natural variability (relative freedom from geographic or seasonal influences), and acceptable analytical precision (good reproducibility of results).

Specificity is a double-edged criterion. General contaminant monitoring programs, such as NS&T, are typically concerned with potential effects of any and all contaminants that may be present. In that respect, generality of response is desirable, while simultaneously seeking specificity with respect to non-contaminant sources of stress (such as nutritional state, reproductive state, dissolved oxygen, and osmotic or temperature stress). This combination of characteristics is difficult to find, however, and measurements are therefore frequently selected for their responsiveness to only a particular contaminant. This criterion can lead to some responses, such as induction of cytochrome P-450E and EROD (ethoxyresorufin-O-deethylase) activity, specific to particular contaminant classes (e.g. PAHs).

A useful bioeffects measurement will also be broadly applicable over large scales of time and space. This criterion is closely related to the previous two, and especially

Table 2 Evaluation criteria for bioeffects measurements in marine environmental quality monitoring

- Significance (Relation to Valued Ecosystem Components)
- Demonstrated Contaminant Sensitivity
  - A. High Response/Dose
  - B. Low Natural Variability
  - C. Precision: (Low Analytical Variance)
- Specificity
  - A. Independence from Non-Contaminant Causality
  - B. Responsiveness to Multiple Contaminant Types
- Scales of Applicability
- Representativeness (Surrogacy for Other Variables)
- Cost Effectiveness (Sample Size/Collection/Storage/Analysis)

to non-contaminant sources of variability. It is important that a measurement can be used at any period over several months without significant seasonal effects (e.g. arising from changing temperature, food availability, or reproductive status). It is also important to select indicator species that have defined patterns of distribution, making use of species that are both sessile (or at least territorial or non-migratory) in order to represent the local scale, and widespread to ensure comparability of measurements over broad geographical areas.

Representativeness reflects how well a measurement represents, or serves as a surrogate for, others which may be of greater overall concern or interest in terms of resource values or long-term effects, but which may suffer from poorer contaminant sensitivity or higher cost. Another feature of representativeness is the ability of a measurement to forecast subsequent effects. The final criterion, cost effectiveness, requires little discussion, but consideration should be given to the overall costs of collecting the samples and making the measurements, including options for sample size and storage requirements, analytical simplicity, etc. These cost considerations must be weighed against the value of the expected information: i.e. "How well defined are the applications for this information?" and "What decisions depend on it?"

Figure 2 (Adams *et al.*, 1989) illustrates relationships among some of the selection criteria for various categories of bioeffects measurements at different levels of biological organization. The diagram illustrates how these categories relate to each other in terms of ecological relevance (or perhaps significance to resource values) on the vertical axis, and the temporal frame of the contaminant response on the horizontal axis. Biochemical and subcellular measurements, at the lower left, lead into organismic responses near the centre, and finally at the upper right, with the highest ecological relevance (but the longest response times), are the population and community level responses. Of course, a responsible environmental manager would like a short suite of inexpensive but sensitive measurements with high contaminant specificity (most of which tend to be in the lower left sector) that accurately forecast potentially important effects at the population level (for which most measurements tend to be expensive, relatively insensitive, and not contaminant-specific).

DIRECT VERSUS INDIRECT MEASURES

Up to this point, the discussion has been oriented mainly toward *Direct Field Measurements*; that is measurements made on indigenous organisms sampled directly from a contaminated environment. These can be contrasted (Figure 3) with *Indirect Measurements*, such as bioassays of sediment or water toxicity using lethal or sublethal endpoints with test populations of organisms. Direct and indirect measurements of effects fare quite differently under the various criteria identified above. The major advantages of bioassays are: [1] that the population of test organisms is controlled, thereby reducing some of the variation that arises from sampling field populations; and [2] that the bioassays are conducted under controlled laboratory conditions (e.g. constant salinity and temperature. Other advantages of bioassays are that they are relatively inexpensive, they exhibit good precision, and they offer possibilities of screening relative contaminant effects with a high degree of spatial resolution.

Limitations of the bioassay approach have been discussed elsewhere (Swartz, 1988; Spies, 1989). Their main disadvantage is that results are not readily

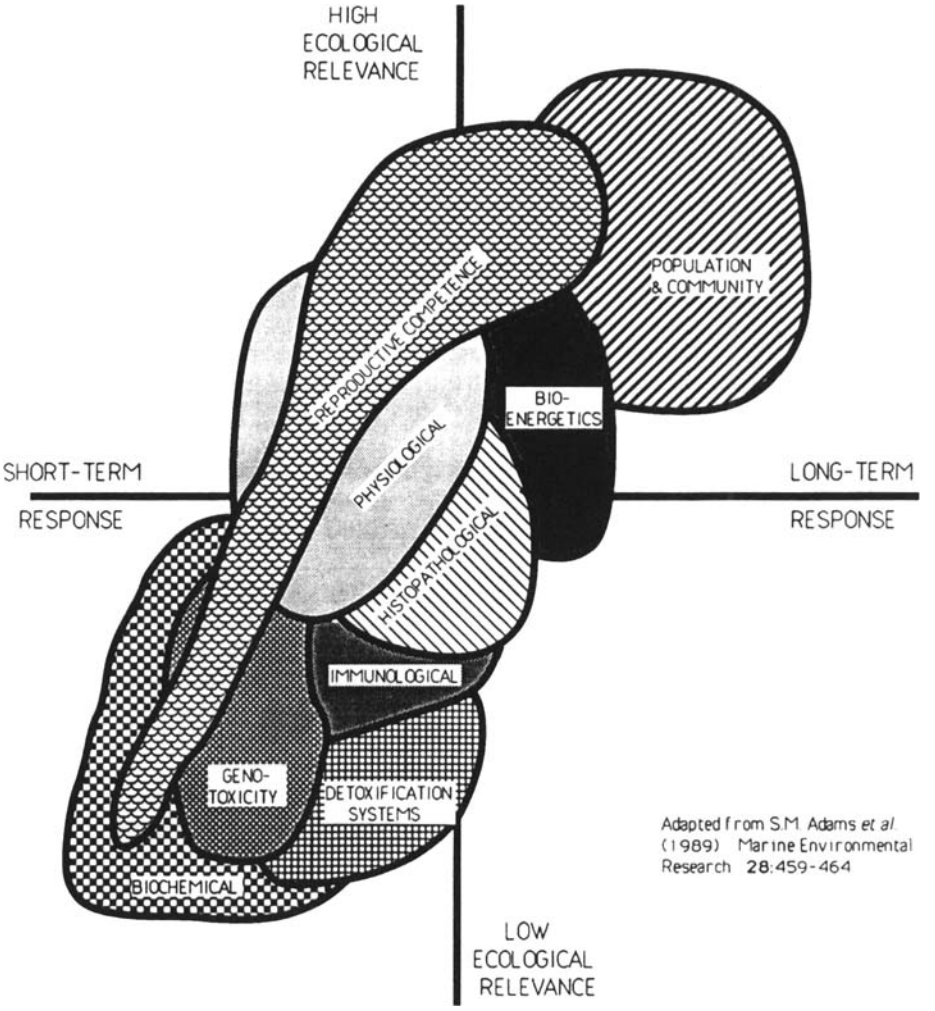


Figure 2 Potential measurements of bioeffects encompass several overlapping levels of biological organization, which respond to contaminants over a broad range of time scales, and which exhibit widely different levels of ecosystem or population relevance

translatable to effects in the field; this problem may be avoided by using indigenous species as test organisms in "realistic" conditions. The realism of a one-litre beaker, however, is obviously limited regardless of whether the test is static or flow-through, or whether the medium is aerated or not. Field verification of laboratory bioassays can be achieved through iterative, hierarchical tests in successively more complex systems (including microcosms, mesocosms, test ponds, etc) until the investigators are satisfied that the functions of the natural system are adequately simulated (Wolfe, *et al.,* 1987). Used in concert with a selection of direct measurements,

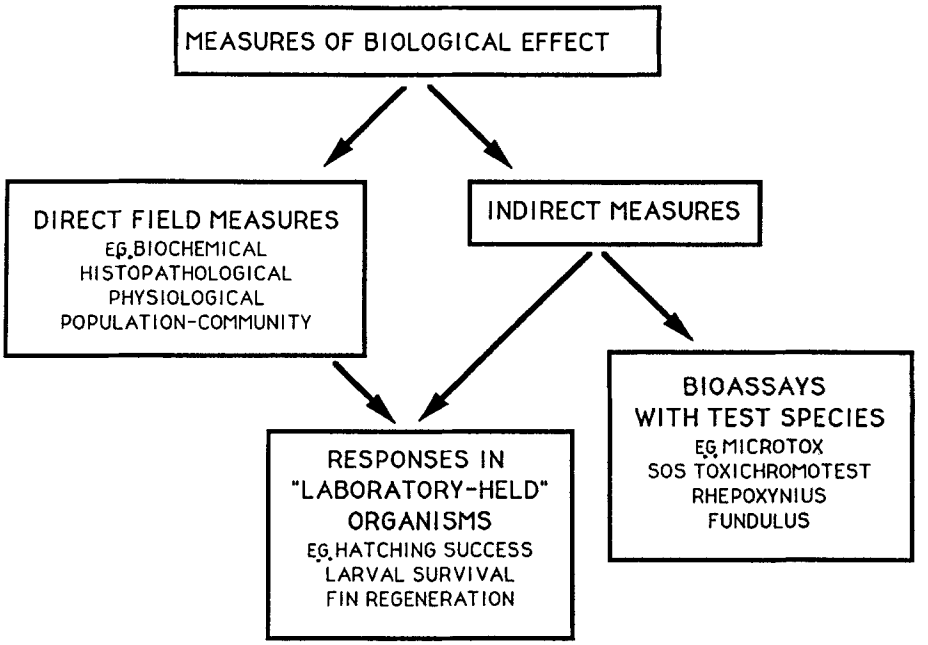


Figure 3 Direct and indirect measurements of biological effect. Responses or characteristics determined in field-collected samples provide a direct measure of effects. Testing of field samples under artificial, but controlled laboratory conditions can provide improved precision of estimates of relative bioeffects, but may lack environmental realism

however, bioassays can none the less provide valuable screening information on relative toxicity potential, including the delineation of small-scale spatial gradients of toxicity.

A variation of the indirect bioassay strategy (Figure 3) is to collect resident organisms from the environments of interest, and then to test their ability to respond under standardized test conditions. It should be noted that whenever this involves bringing live organisms from the field to the laboratory, and holding them for some time (for example, prior to induction of spawning), the protocol may suffer some of the interpretational problems and disadvantages associated with bioassays.

Another disadvantage of sediment toxicity bioassays is that different tests can give different results, possibly leading to different conclusions. Some results from a comparative study of bioeffects measurements (Long and Buchman, 1989) are shown in Tables 3 and 4. A suite of 15 sediment samples taken from different locations in San Francisco Bay was tested by a variety of bioassays using different test organisms and endpoints, and different methods of sediment preparation and testing (Long *et al.*, 1990).

A principal components analysis was performed on the combined toxicity test results, with the first factor accounting for 45% of the variation in the data, the second 36%, and the third 19%. Factor 1 accounted for most of the variation in survival and development of *Mytilus* larvae and, to a lesser extent, survival of

Ampelisca (Table 3). The first three of these endpoints showed high correlations with each other and (in the order listed) with a suite of contaminants in the sediment samples. Factor 1 was associated most with mercury, total organic carbon, percent clay, and a number of metals in the samples. Survival of *Ampelisca* was not as strongly correlated with those endpoints, shown in Table 3 by the different rank ordering of correlation coefficients.

Table 4 shows the endpoints associated with Factor 2 for the same suite of samples, illustrating that the *Ampelisca* and sea urchin *Strongylocentrotus* endpoints (production of the pigment echinochrome and normal development) were better correlated with a different set of samples and contaminants. In this case, the list is headed by chlorinated organic compounds (DDTs, other pesticides, PCBs), lead (Pb), and PAHs, while the other metals and sediment characteristics show lower correlations. Factor 3 (not shown) accounted mainly for another endpoint (egg production in the polychaete *Dinophilus*), which was most strongly correlated with yet another sequence of samples, where PAHs headed the list. Two bioassay endpoints, urchin development (Table 4) and *Rhepoxynius* avoidance (Factor 3, not shown), produced results that were counter-intuitive. These endpoints showed inverse relations with contaminants (greatest effect at lowest contaminant concentrations). For both of these endpoints, however, there was a high negative correlation with sediment characteristics (TOC and percent clay) and that effect may have precluded any meaningful interpretation of other contaminant relationships. These data serve to re-emphasize that different tests may be suited to different areas, and that choice of a test, whether an indirect bioassay approach or a direct measurement on an indigenous organism, depends on the specific objectives.

Table 3 Rank correlations between selected toxicity results and concentrations of chemicals in fifteen sediment samples from San Francisco Bay. Abbreviations follow Long *et al.* (1990), for example "tDDT" refers to the sum of DDT and its metabolites, "o-pesticides" refers to sum of other pesticides analyzed.

FACTOR 1	*.936*	*.932*	*.922*	*.600*
	Mytilus edulis		*Rhepoxynius*	*Ampelisca*
	% Normal	% Survival	% Survival	% Survival
Hg	−.77	−.72	−.67	−.54
TOC	−.68	−.64	−.70	−.13
% Clay	−.65	−.50	−.57	−.06
Cd	−.56	−.60	−.54	−.49
Ag	−.48	−.54	−.25	−.52
Cu	−.46	−.43	−.15	−.27
Ni	−.44	−.47	−.50	−.25
Cr	−.39	−.39	−.39	−.26
Bay PAHs	−.39	−.46	−.15	−.31
Zn	−.37	−.40	−.25	−.43
o-PAHs	−.34	−.41	−.12	−.29
tDDT	−.31	−.34	.02	−.65
LW PCBs	−.30	−.33	−.07	−.71
HW PCBs	−.29	−.36	−.01	−.40
Pb	−.28	−.24	−.10	−.55
tPCBs	−.27	−.33	.03	−.44
o-pesticides	−.26	−.32	−.04	−.71
tPAHs	−.25	−.33	.10	−.29
% silt	.37	.37	.58	.41

Table 4 Rank correlations between toxicity endpoints for sea urchins (*Strongylocentrotus*) and amphipods (*Ampelisca*) and the concentrations of chemicals in San Francisco Bay Sediments (See Table 3 and text)

FACTOR 2	*.954*	*(–).876*	*.752*	*–.735*
	Strongylocentrotus echinochrome	avoidance	*Ampelisca abdita* % Survival	*Strongylocentrotus* % normal
tDDT	–.79	.70	–.65	.69
o-pesticides	–.77	.80	–.71	.57
LW PCBs	–.71	.79	–.71	.50
Pb	–.61	.70	–.55	.34
tPCBs	–.50	.43	–.44	.43
tPAHs	–.44	.33	–.29	.24
HW PCBs	–.43	.38	–.40	.40
Ag	–.39	.55	–.52	.13
Zn	–.37	.56	–.43	.03
o-PAHs	–.34	.29	–.29	.04
Cd	–.34	.34	–.49	–.07
Bay PAHs	–.29	.27	–.31	.02
Cu	–.27	.09	–.26	–.21
Hg	–.20	.37	–.54	–.26
Cr	–.08	.09	–.26	–.21
Ni	–.01	–.01	–.25	–.32
% silt	.09	–.30	.41	.14
TOC	.37	–.07	–.13	–.78
% Clay	.38	–.10	–.06	–.66

BIOEFFECTS MEASURES WITHIN NOAA'S NS&T PROGRAM

Within the past 4 years, the NS&T Program has undertaken intensive bioeffects surveys in San Francisco Bay, Boston Harbor, Long Island Sound, Hudson-Raritan Estuary, and Tampa Bay. Studies in some of these areas are continuing, and cooperative studies with the State of California are being planned for coastal areas of the Southern California Bight. Preliminary reports are available for the San Francisco Bay work (McCain *et al.*, 1989; Long *et al.*, 1990; Spies *et al.*, 1990; NOAA, 1991), and papers on the work in Long Island Sound, (including Gronland *et al.*, 1991 and Turgeon and O'Connor, 1991).

NOAA's NS&T effort is how focused on three different classes of indicators related to contaminant exposure and bioeffects: direct measurements of effects in fishes (Table 5), sediment toxicity bioassays (Table 6), and direct measurements in molluscs (Table 7). Some are regularly applied in conjunction with the chemical monitoring at the full nationwide suite of NS&T coastal sites (Turgeon *et al.*, 1991), while others are used only in intensive study areas or are undergoing preliminary testing and evaluation.

Direct measurements in fish (Table 5) encompass chemical measures of exposure, reproductive status, histopathology, genotoxic effects, and immune response. Elevations of fluorescent aromatic compounds in fish bile (Krahn *et al.*, 1986) indicate recent hydrocarbon exposure and metabolism, while elevated levels of MFO activity indicate more prolonged exposure. Levels of aryl hydrocarbon hydroxylase (AHH) in English sole (*Parophys vetulas*) are correlated with sediment contaminant levels at the sites where fish are collected, and the enzyme activity can be induced by intramuscular injection of organic extracts of contaminated sediments

Table 5 Direct measurements of exposure and bioeffects in fish different species in different regions)

- Fluorescent Aromatic Compounds in Bile[1]
- MFO (Aryl Hydrocarbon Hydroxylase) in liver[2]
- Ovarian maturation (Vitellogenesis)[3]
- Plasma Estradiol[4]
- Prevalence of Liver Lesions[5]
- Xenobiotic Adducts of DNA[6]
- Macrophage Aggregates[7]

References: 1. Krahn *et al.*, 1986; 2. Collier *et al.*, 1989; 3. Johnson *et al.*, 1988; 4. Stein *et al.*, 1988; Johnson *et al.*, 1989; 5. Myers *et al.*, 1987; 6. Stein *et al.*, 1989; 7. Gronlund *et al.*, 1991.

(Collier *et al.*, 1989). Hepatic AHH activity was also correlated negatively with levels of plasma estradiol in vitellogenic female English sole (Johnson *et al.*, 1988, 1989). Fish from contaminated areas exhibit genetic damage in the form of covalent binding of genotoxic compounds to DNA (Stein *et al.*, 1989; Varanasi *et al.*, 1989). The prevalence of many histopathological lesions is also higher in fish from contaminated sites (McCain *et al.*, 1989); some lesions, including megalocytic hepatosis and nuclear pleomorphism, are considered to represent early pathological stages in the formation of liver neoplasms (Myers, *et al.*, 1987). Winter flounder (*Pseudopleuronectes americanus*) from contaminated sites in Long Island Sound had higher indices of liver macrophage aggregates than those from an uncontaminated site (Gronlund *et al.*, 1991). This index may prove useful in future assessments of stress response in fishes in contaminated environments. While none of these measurements alone is sufficient to characterize the scale of effects in relation to contaminant exposure, collectively they establish a basis for assessing the biological effects of complex mixtures.

Because these observations are made on indigenous fish, background conditions and sensitivities to existing conditions need to be established in each new area and for each species. Because of the mobility of many fish, measurements may show variation among individuals, providing only coarse resolution of the spatial scale of effects.

To supplement direct observations on fish, one or more sediment toxicity bioassays (Table 6) are used in each area selected for intensive study. To date, we have relied most heavily on bioassays of bivalve larval development and survival and amphipod survival (Long *et al.*, 1990; NOAA, 1991), but the Microtox assay has been tested extensively (Shiewe *et al.*, 1985; NOAA unpublished). The sublethal endpoints (growth of polychaetes and urchins) listed in Table 6 show promising sensitivity to contaminated sediments (Casillas *et al.*, 1989), and are undergoing field tests this year in the NS&T Program. Cytogenetic endpoints with urchin embryos (Hose, 1985) are also promising rather than the more established endpoint of survival (Long *et al.*, 1990; NOAA, 1991). As illustrated by the data in Tables 3 and 4, considerable caution is required for the interpretation of bioassay data (Swartz, 1988; Spies, 1989). None the less, synoptic surveys employing toxicity bioassays provide valuable screening data and improved spatial resolution of the extent of possible contaminant effects within a region (e.g. Meador *et al.*, 1990; NOAA, 1991), especially when consistent with independently observed effects in indigenous organisms.

A stronger NS&T focus on bioeffects in indigenous bivalve molluscs would allow a more robust analysis and interpretation of chemical data collected in the Mussel Watch Project, and would avoid some of the interpretation problems associated with sediment toxicity bioassays. While the use of bivalves as bioeffects sentinels could

Table 6 Indirect measurements (bioassays) of relative bioeffects

ACUTE TOXICITY
• Bivalve Larval Development & Survival[1]
• Amphipod Survival[2]
• Urchin Larval Development & Survival[3]
SUBLETHAL
• Microtox (*Photobacterium phosphoreum* Inhibition)[4]
• *Neanthes arenaceodentata* Growth & Egg Production[5]
• Growth of *Armandia brevis*[6] and *Dendraster excentricus*[7]
• Cytological Abnormalities/Anaphase Aberrations in Urchin Embryos[8]

References: 1. Chapman & Morgan, 1983; Chapman & Becker, 1986; 2. Scott & Redmond, 1990; Swartz *et al.*, 1985; 3. Dinnel *et al.*, 1982, 1987; Meador *et al.*, 1990; 4. Shiewe *et al.*, 1985; 5. EPA Region 10 test protocol; 6. Plesha, Casillas *et al.*, unpublished; 7. Casillas *et al.*, 1989; 8. Dinnel *et al.*, 1982, 1987; Hose, 1985.

Table 7 Direct measurements of exposure & bioeffects in bivalve molluscs (different species in different regions)

• Gonadal Index & Follicle Development[1]
• Histopathology[2]
• Xenobiotic Adducts of DNA[3]
• Stress Proteins[4]
• Glutathione Metabolism[5]
• Lysosomal Stability[6]

References: 1. Capuzzo, NOAA, unpublished data; 2. Auffret, 1988; NOAA, unpublished data; 3. Kurelec *et al.*, 1990; 4. Sanders, 1987; Steinert & Pickwell, 1987; Veldhuizen-Tsoerkan *et al.*, 1990; 5. Wenning and DiGiulio, 1988, Wenning *et al.*, 1988; 6. Moore *et al.*, 1987a,b; Moore, 1988; Lowe & Clarke, 1989.

overcome many of the difficulties of interpretation arising from variation in fish, contaminant bioeffects are generally not as well understood in invertebrate species as in vertebrates. Table 7 lists some promising endpoints as possible bioeffects measurements in bivalve molluscs. The usefulness of many of these endpoints is not yet well-established.

The stage of gonadal development and selected histopathologies have been routinely recorded in bivalve molluscs collected in the Mussel Watch Project since its inception. While the gonadal stage and lipid content of the bivalves have provided important information on the comparability of contaminant concentrations in different samples, no meaningful correlations have been observed between bivalve reproductive status and site contamination levels. The combined influences of season, temperature, salinity, and other natural variability may damp out detection of any contaminant effects over th nationwide set of Mussel Watch sites. In other work (Capuzzo and Leavitt, 1988; Capuzzo *et al.*, 1991), however, changes in lipid class distributions and lipid: protein ratios in *Mytilus edulis* were correlated with body burdens of aromatic hydrocarbons and PCBs along a pollution gradient. Such shifts in lipid metabolism may have sigificant implications for the timing and overall success of reproduction in mussels.

Neoplasia has been detected in *Mytilus edulis* from only a few Mussel Watch sites on both the east and west coasts of North America. Contaminant concentrations in mussel tissues were compared by a two-way analysis of variance at the sites with and without incidence of neoplasia; significant differences were found for alpha-

chlordane, pesticides, and combustion-related PAHs on the east coast, and for only combustion-related PAHs on the west coast (Battelle, 1991). Auffret (1988) noted that the incidence of granulocytomas was greater in mussel tissues from a polluted site than in those from a reference site. These possible relationships will be investigated further in future studies.

Several other bioeffects measurements in bivalves show promise for large-scale monitoring applications, but further testing is required to demonstrate causal and dose-response relationships and sensitivity in a wide range of field conditions (Table 7). Genotoxicity of contaminants under some conditions has been indicated in *Mytilus* through ^{32}P-postlabelling detection of DNA adducts (Kurelec *et al.*, 1990) and through measurement of DNA strand breakage and incidence of micronucleated cells (Bolognesi *et al.*, 1991). Many organisms, including bivalves, produce specific proteins in response to different sources of stress, including contaminant exposure. Analytical refinements in recent years permit the separation and identification of a multiplicity of stress proteins and their isomers (Sanders, 1990; Veldhuizen-Tsoerkan *et al.*, 1990). Similarly, glutathione (GTH) is involved in the metabolism of electrophilic xenobiotics (DiGiulio *et al.*, 1989). Glutathione-S-transferase (GST) catalyzes the conjugation of contaminants with glutathione, and both GST and GTH are higher in invertebrates exposed to contaminants (Lee *et al.*, 1988; Boryslawskyj *et al.*, 1988; Wenning and DiGiulio, 1988; Wenning *et al.*, 1988).

Histopathological changes in the composition, structure, and stability of lysosomes and vacuoles have also been demonstrated following exposure of bivalve molluscs to a variety of chemical contaminants (Fowler *et al.*, 1975; Wolfe *et al.*, 1981; Moore *et al.*, 1987a,b; Moore, 1988). Decreased lysosomal stability was observed in several molluscan species exposed to PAHs (Moore *et al.*, 1987a; Moore, 1988), although the response varies also with a number of natural environmental factors. Destabilization of lysosomal membranes leads to enlargement of lysosomes and increased permeability, and the changes in permeability can be quantified enzymatically (Moore, 1988). More research will be required, therefore, to refine these techniques (i.e., related to stress proteins, glutathione metabolism, and lysosomal stability), and to demonstrate meaningful contaminant specificities and sensitivity in different organisms and acceptability for widespread field application.

CONCLUSIONS

The evaluation and selection of bioeffects measurements are continuing within the National Status and Trends Program. Some important conclusions to date can be summarized, both with respect to the NS&T Program *per se* and relative to other monitoring programmes.

Monitoring design and associated research

Present understanding of biological effects and environmental assessment capability are still at a stage where iterative applications of laboratory experimentation and field validation are required. Rigorously controlled laboratory exposures are required to elucidate causality, mechanisms of action, dose-response relationships, and multifactorial interactions. Field validation tests should be designed to verify detection thresholds and sensitivities for the measurements in variable field conditions. Field validation may involve hierarchical testing on different spatial

scales (i.e., from microcosms to mesocosms to ponds) and by design of sets of embedded system models representing successively larger and more complex systems (Wolfe *et al.*, 1987).

Environmental Monitoring Programmes

Environmental monitoring programmes have been criticized (Wolfe, 1987; NRC, 1990) for failing to specify clear objectives and to match their design and implementation to those objectives. Measurement programs designed to determine the magnitude of biological effect, its spatial extent, and temporal trend, need to focus on specific objectives and hypotheses and to employ sampling approaches appropriate to the dimensions of the system being studied and scales of variability. They need also to incorporate flexibility or adaptability in their design so as to respond to changes in objectives as problems change and as the level of understanding and state-of-the-art evolve.

Bioeffects Measurements

The selection of such methods depends on the detailed objectives, which will vary in different areas. In different geographic areas, different indicator species and responses may be required. Each response and each species will require separate calibration and testing, both in the laboratory and the field, to demonstrate comparability and utility of the results. No single bioeffects measurement is likely to satisfy the requirements of any large-scale assessment. A suite of measurements, encompassing aspects of exposure and interrelated effects, may provide the desired level of understanding where a single measurement alone would be ambiguous. In some cases, simultaneous application of multiple measurements is required for valid interpretation, and cost savings can often be achieved through the use of a tiered, sequential approach. The mixture of direct and indirect measurements is useful; direct measurements demonstrate that significant effects are occurring in the ambient field conditions at the study site, while indirect measurements (i.e. bioassays) can screen potential effects over large and diverse areas, and can provide high resolution estimates of the spatial extent of effects.

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