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MARINE ECOTOXICOLOGICAL TESTS WITH ZOOPLANKTON

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ABSTRACT

Ecotoxicological tests on existing and new marine pollutants have long included those with zooplankton - both holoplankton and meroplankton. A considerable number of international workers and laboratories work either exclusively with marine zooplankters in toxicity test systems, or include them with other organisms in hazard assessment schemes. A wide variety of test types, and testing exposure regimes and durations are possible, ranging from short-term laboratory assays to longer-term laboratory and field microcosm experiments. The majority of reported studies and current effort have dealt with coelenterates, annelids, molluscs, arthropods (particularly crustaceans), echinoderms, and vertebrates (teleosts). Many workers study the responses of the meroplanktonic life stages (gametes, embryos and larvae) of the benthos and intertidal invertebrates.

Zooplankton assays offer a number of advantages to assessments of pollutants. Among them, the principal ones are small size accommodating large samples per treatment, high sensitivity, the observation and

measurement of fundamental biological responses, wide availability of test specimens, well-known biology of some species and good potential for standardization between laboratories. Disadvantages of zooplankton assays often include the highly specialized techniques and skills for the collection, culture, handling and assaying of organisms, the small volumes of tissues and test solutions for chemical analyses, the labor intensive methods with some species, and probable "physiological hardiness" of some readily cultured species.

In this paper, tests with zooplankters are briefly described for the major groups. The sensitivities to toxicants of some of these species are discussed, with examples. A number of these tests offer a high potential for successfully screening potential long-term toxicants which may negatively affect fundamental biological processes at very low concentrations. Crustacean, echinoid and teleost gamete-embryo-larva tests are of particular importance. This is recognized internationally and is reflected by efforts to apply these tests widely, to refine various techniques and develop new ones with both generic and highly specific applications.

The continued inclusion of zooplankton assays in hazard assessments of chemicals entering marine environments is highly encouraged. Areas of future research with zooplankton assays are : culture of new species and life stages ; comparative physiology and toxicology, especially across salinities and developmental stages ; the derivation of principles underlying sensitivities to common pollutants ; studies of how toxicants affect young stages, i.e. modes of toxic action ; significance of zooplankton sensitivities to success of their populations ; and further development of applied, standardized, rapid screening planktonic assays.

KEYWORDS

Marine ecotoxicology, Hazard assessment, Bioassays, Methods, Zooplankton, Review .

INTRODUCTION

"Ecotoxicology is concerned with the toxic effects of chemical and physical agents on living organisms, especially on populations and communities within defined ecosystems ; it includes the transfer pathways of those agents and their interactions with the environment" (Butler, 1978). Reviews of bioassay procedures by Little and Maciorowski (1979) and Maciorowski et al. (1980, 1981, 1982, 1983) indicated that "there is a need for development and standardization of tests for effects of chemicals on ecological parameters that are indicative of interspecific interactions, community dynamics, and ecosystem function". This paper briefly reviews the current status of toxicological and ecotoxicological tests with marine zooplankton, with particular reference to work in North America and to applications of such tests in the assessment and control of toxic chemicals.

Zooplankton, both holoplankton and meroplankton, have been studied in toxicity tests for many years. Interest in this application of zooplankters has increased markedly in the past 15 years (post-Torrey-Canyon years) in many countries, resulting in a vast literature on their responses to toxic materials. Laboratories, with skilled personnel and facilities to culture, feed, and study specific zooplankters, soon developed specialized toxicity tests with organisms of their choice, typically gametes, embryos, and larvae of molluscs, crustaceans, echinoderms and teleosts. Examples of such laboratories are National Marine Fisheries Service, Milford Laboratory, Connecticut USA, for oyster larvae ; Marine Science Laboratories, Menai Bridge, Wales, UK, for barnacle larvae ; Duke University Marine Laboratory, North Carolina, USA, for brachyuran crab larvae ; Institute for Marine Environmental Research, Devon, UK, for copepods ; St. Andrews Biological Station, New Brunswick, Canada, for lobster larvae ; Doshisha University, Biological Laboratory, Kyoto, Japan, and University of Tromsø, Tromsø, Norway , for echinoid life stages ; and the Biological Station, Nanaimo, British Columbia, Canada, for fish eggs and embryos.

Work in this area was particularly stimulated by the need to control the settlement of barnacle larvae on ship's hulls, the scientific and commercial interest in the physiology and physiological ecology of molluscan and echinoderm larvae, and the concern about oil spill and pesticide impact on invertebrate fisheries. Important contributions included the reviews and recommendations of Sprague (1970, 1971, 1976) and Rosenthal and Alderdice (1976). Attractive features of zooplankton assays and microcosm approaches

are the size, sensitivity and practicality of the tests, the importance of understanding ecological factors influencing survival of young life stages, and the multitude of critical and fundamental biological processes, especially those of reproduction and development, that can be closely examined under toxicant exposures. Both simple chemical toxicity screening and more complex ecotoxicological testing can now be conducted with zooplankton, albeit with nonstandardized procedures for the most part.

The primary theme of this paper is that, if one accepts the concept of using "the most sensitive locally important species" in bioassays, as stated by Benfield and Buikema (1980), together with one or more "standard" species, marine zooplankters provide a most attractive set of species, life stages and responses.

The objectives of this paper are :

- to describe the types of tests, taxonomic groups, and biological responses currently utilized ;
- to discuss the major characteristics of zooplankton tests ;
- to discuss the tests on zooplankton proposed by major organizations and present one test in detail, with particular reference to the criteria that must be met in standardized tests (Hammons, 1980 ; Persoone, pers. commun.) ;
- to discuss the applications of selected tests ; and
- to discuss future trends on methods and applications for marine zooplankton in hazard assessments.

The reader should also refer to other papers in this volume for additional descriptions of current work with zooplankton.

ZOOPLANKTON TESTS - AN OVERVIEW

TYPES OF TESTS

Four general types of tests with zooplankters exist and they vary greatly in objectives, duration, complexity, cost, reproducibility (precision), and realism.

Acute lethal and sublethal tests

Acute lethal and sublethal toxicity tests in the laboratory are used for basic information on the toxicity of a material, or for data on the comparative toxicity of several materials. They are essentially "screening tests". They may last minutes (e.g. the 30 min echinoid sperm test) or days (1 to 4 day lethality tests with crustacean larvae). They are often static, with single endpoints to measure, and hence are relatively simple tests. Cost per toxicity test is low ($ 100–$ 500 Canadian dollars depending upon the number of tests run simultaneously). Precision is high, but realism (being representative of field conditions) is considered low (NRC, 1981b).

Chronic sublethal tests

Chronic sublethal tests in the laboratory are used to determine the effects of longer low level exposures on key properties of individuals or populations. They are applied when the tested materials are persistent, bioaccumulative, and toxic in the acute tests. The tests may be several weeks in duration, such as with a 30 day lobster-larvae test, or a full life cycle (Calanus spp.). Complexity and logistics are greater – often semi-static or continuous-flow modes of exposure are used, with diluters, and many measurements are taken daily. The cost per trial per chemical could be as high as $ 5–10 x 10^3 Canadian dollars, excluding expensive and detailed analytical chemistry. The precision, with skilled personnel, can be high. The exposure and other test conditions more closely represent field conditions. The concentrations are low and the effects of variables influencing toxicity (temperature, salinity, nutrition, etc.) can be studied.

Chronic microcosm test

The first two types of tests are conducted in the laboratory and measure responses of single species to toxicants. The third type of test is conducted under simulated field conditions, and measures responses of populations of zooplankton to low levels of materials.

The chronic microcosm tests with zooplankton are one to several months in duration, and have as their main objective the measure of population processes and responses (diversity and abundance changes, shifts in predator-prey relationships, physiological-biochemical responses of specific

populations) when exposed to the introduced material(s). They are complex tests to conduct, requiring significant investments of materials, skill and time (see details of CEPEX and MERL tests in Reeve et al., 1976, 1977, Oviatt et al., 1982, and Kuiper, 1984). Schneider (1980) states that "microcosms of intermediate complexity, incorporating zooplankton, may be the best approach for testing chemicals". The complexity and cost (>>10^4 dollars/material) can be reduced with the selection of simpler laboratory-based microcosm tests (see Fowler and Elder, 1980). The precision of the large-scale outdoor tests is low, but measures of impact are probably more realistic.

Acute or chronic in situ tests

The fourth test type, acute or chronic in situ tests, can be deployed at discharge sites, or sites of chronic contamination. An in situ test can either be a cage (container) of organisms being exposed, or a settling plate for meroplankton to settle on and grow. These tests with zooplankton (meroplankton) are in their infancy, but when developed could be a sensitive, realistic test of water quality at selected sites and be linked to other "effects monitoring" approaches. A cage test has been suggested by O'Brien and Kettle (1981). It consists of a simple bioassay chamber for zooplankton culture and testing in the laboratory and in the field. Also, settling plates contaminated with petroleum hydrocarbons have been tested in intertidal and shallow subtidal zones (Anderson, pers. commun.).

The choice of test depends upon the question(s) being asked and the level of confidence about the ecotoxicity of a material that is required (see Davis, 1977 ; Sprague and Fogels,1977 ; NRC, 1981b; and Buikema et al., 1982). The test chosen with zooplankters ultimately depends upon the magnitude and progression of the hazard assessment required to establish reliable toxicity tolerance limits for the specific pollutant.

TAXONOMIC GROUPS

This is a brief overview of the most common zooplankters incorporated into toxicological tests (Table I). This is an introduction to the topic and is not exhaustive in its coverage.

Protozoa

Considerable research has been conducted with protozoans and pollutants (Persoone and Dive, 1978 ; Curds, 1982), with some work on marine species (Gray and Ventilla, 1973 ; Gray, 1974). There has not been wide application of techniques with this group, nor the preparation of standard assay techniques with a marine species. Most of the published studies pertain to toxicant studies with freshwater species (Dive and Leclerc, 1975ab ; Dive, 1982 ; Rogerson et al., 1983). Observations have been made of protozoan survival and population dynamics in several large-scale experimental ecosystem experiments with metals and hydrocarbons (Grice and Reeve, 1981 ; many specific references). There is considerable current interest in more toxicity work with marine Protozoa, especially ciliates (Dive and Persoone, 1984 ; Laake, 1984).

Coelenterates

There have been efforts to develop sublethal assays with colonial hydroids (Stebbing, 1980), and some reports on sensitivities of medusae and coral larvae to hydrocarbons (Percy and Mullin, 1975 ; Loya and Rinkevich, 1980 ; for review, see Stebbing and Brown, 1984). There are no reported standard methods with planktonic life stages of coelenterates as of 1983 (see Standard Methods and reviews of Maciorowski et al., 1980, 1981, 1982, 1983).

Polychaetes

Reish has published many accounts of culture and pollution studies with larval polychaetes, has contributed methodologies to ASTM publications (Reish, 1980) and Standard Methods (APHA, 1980), and has conducted intercalibration exercises (Reish et al, 1978). Many specific studies have been conducted (Chia, 1973 ; Hooftman and Vink, 1980 ; Reish, 1980), particularly with metals. Both Åkesson (1980) and Reish (1980, 1984) extensively reviewed the use of young life stages in bioassays. At the present, regulatory groups underutilize known procedures with these ecologically important organisms.

Molluscs

This group is best known for the standard 48 h oyster embryonic and larval bioassay developed by Woelke and coworkers in the US in the 1960s (Woelke, 1967, 1972). In the US embryos and larvae of Crassostrea spp. are frequently studied under toxicant stress in the laboratory (e.g. Calabrese et al., 1973, Roberts, 1980). Round-robin oyster larval tests have been organized by the Office of Toxic Substances in the USA (Borthwick, 1979), using an ASTM test method. The oyster larval bioassay was considered a viable standard technique for water quality monitoring by an ICES panel report (Stebbing et al., 1980). A number of investigators also work with mussel (Mytilus edulis L.) embryos and larvae (see Wells, 1982a ; Dixon, 1982, among others). Molluscan assays are frequently employed in routine testing in Europe (Renzoni, pers. commun.). A recent review was published in France on the toxicity of ten metals to marine larvae (Deslous-Paoli, 1981), and developmental stages of Mytilus galloprovincialis have been incorporated into toxicity studies on biocides (Luca et al., 1980), metals (Pavicić, 1977), and other pollutants. Calabrese (1984) gives further details of molluscan assays, their applications, and their reproducibility between laboratories.

Crustaceans

Zooplankton from this class, including young life-history stages of benthic and epibenthic species, are widely used as toxicity testing organisms, and are usually the first choice for invertebrates for a hazard assessment of a material. Several organisms have been formally proposed as standard test organisms and are the subject of published or drafted testing protocols, and interlaboratory calibration exercises (see later sections). Organisms were selected for reasons of abundance, ecological importance, well-known biology, culture success, link to commercial fisheries, and sensitivity to specific pollutants. Gentile et al. (1984) also review crustaceans in detail, covering plankton and benthos and many specific questions on testing protocols.

A. Brine shrimp

Artemia eggs, larvae, and adults have been used for toxicity studies for several decades, resulting in a large literature. A relatively simple one or 2- day lethal test with the freshly hatched nauplii has been

developed. It is being recommended by Vanhaecke et al. (1980) in Belgium as a standard "marine" test. An early version of the Artemia test is in some EPA testing protocol documents (e.g. oil spill dispersants). Some concern has been expressed about Artemia's physiological hardiness, hence most workers in the USA prefer planktonic stages of mysids, decapods, molluscs, and fish. Some investigators are comparing sensitivities of Artemia and endemic crustaceans (Wells et al., 1982), using lethal responses, while others are developing new test systems, such as the teratogen test system with Artemia nauplii (Kerster and Schaeffer, 1983). A European and North American round-robin intercalibration has been recently conducted (Persoone et al., 1981 ; Leonhard, pers. commun.). This Artemia nauplii 1-day toxicity test is one of the most developed and practical tests on marine zooplankton to date (Vanhaecke and Persoone, 1984).

B. Copepoda

There is a very large pollution literature with copepods, because of their ecological importance and the ease of maintenance, culture, and testing of a number of genera (Stebbing et al., 1980 ; Corner, Harding, and Conover, pers. commun.). Both acute and chronic tests, especially multi-generation chronic exposures, are possible with calanoid copepods such as Acartia (Ward et al., 1979) and Calanus (Harding et al., 1981). They are useful planktonic test organisms particularly because cultured and endemic populations can be worked with and they offer many fundamental toxicity responses. A review on the use of the calanoid copepod, Acartia, was recently prepared by Heinle and Beaven (1980). Acartia tonsa is being suggested tentatively as a standard test organism (APHA, 1980). Comparative toxicity studies examining the sensitivity of copepods are relatively common now (Lassus et al., 1984).

C. Cirripedia

Toxicity tests with barnacle naupliar larvae were developed several decades ago due to research on settlement-inhibiting paints for steel ships and general marine structures. Studies have recently been reported on heavy metals (Lang et al., 1980 ; Barber and Trefry, 1981), and petroleum hydrocarbons (Blundo, 1978 ; Nelson, 1981 ; Wells, 1982a). Several species of Balanus (balanoides, eburneus, improvisus) have been studied. Larvae can be cultured or are collected from natural habitats seasonally. Measures of survival, molting, behavior and successful larval settlement are most

often used. A computerized behavioral testing system has been developed and tested (Miller, 1980 ; Miller et al., 1982). The settlement assay could prove to be a valuable, predictive laboratory-field test.

D. Mysidacea

Juvenile mysids, particularly the opossum shrimp (Mysidopsis bahia), have been studied in acute and chronic toxicity tests in the past 10 years (Nimmo et al., 1977 ; Breteler et al., 1982 ; Nimmo and Hamaker, 1982a). Nimmo and Hamaker (1982b) recently reviewed mysids in toxicity testing. They have become a routine crustacean test organism in a number of USA laboratories (Anderson, pers. commun.), especially at EPA Gulf Breeze, Florida (Borthwick, 1979). There is an ASTM toxicity protocol subcommittee for mysids. Recently released (24 h) juveniles are used in tests and are sensitive to pollutants (Wells, 1982a ; Carr et al., 1983 ; Gentile, pers. commun.). Their abundance in shallow waters, ease of mass culture, handling and feeding, and apparent sensitivity to a wide range of toxicants make juvenile mysids very attractive for acute tests. The oppussum shrimp "has a great potential as a bioassay organism and may become the marine counterpart of Daphnia" (Benfield and Buikema, 1980).

E. Decapoda

Larval decapods have been studied in many toxicity tests over the past 10 to 20 years, and the applications of specific groups have been recently reviewed (shrimp : Couch, 1979 ; Buikema et al., 1980 ; lobsters : Wells, 1976ab ; crabs : Bookhout and Costlow, 1974 ; Epifiano, 1979). Larvae from these groups are suggested crustacean test organisms in APHA (1980). Epifiano (1982) reviewed their use in the assessment of effects of pollutants.

Grass shrimp (Palaemonetes species) are important toxicity test animals, and static and flow-through studies with their larvae are becoming more common (Buikema et al., 1980). Principles for using grass shrimp larvae are thoroughly described (Buikema et al., 1980), and one protocol is presented in Standard Methods (APHA, 1980, in press for 1984). There are a considerable number of reported studies on specific pollutants and larval shrimp, e.g. oil (Wells, 1982a), that show their sensitivity, especially during molting. Larvae of prawns (Palaemon sp. and Penaeus spp.) and sand shrimp (Crangon spp.) have also been studied (Van Dijk et al., 1977 ; Price

and Uglow, 1979 ; Lassus et al., 1981). Techniques are as yet less standardized, but these genera and Palaemonetes spp. are now suggested test organisms (APHA, 1980). Guidelines for an acute shrimp test have recently been released by the Office of Toxic Substances, EPA (Brungs and Tarzwell, 1984).

Lobster larvae, primarily of Homarus americanus, and Homarus vulgaris, are used in both experimental and screening toxicity tests (Wells, 1976a ; Wells and Sprague, 1976 ; Doe and Wells, 1978 ; Cobb and Phillips, 1980 ; Capuzzo, 1981 ; Capuzzo and Derby, 1982, among others). They offer many advantages as test organisms. Lobster larvae (stages one to three) are sensitive to heavy metals and petroleum hydrocarbons (see Wells, 1976b ; Cobb and Phillips, 1980). They have been incorporated often into specific comparative toxicity studies by regulatory agencies (Environment Canada, Atlantic Region),by research institutes (Sprague and McLeese, 1968) and by industry (Hutcheson, pers. commun.). A tentative protocol for their culture and testing is published (APHA, 1980). In Eastern Canada, there is considerable interest in the effects of persistent pollutants on the lobster fisheries, and several experimental and commercial hatcheries have the capacity to provide larvae for toxicity testing on a year-round basis.

Larvae of true crabs, the Brachyura, have been frequently used as toxicity test organisms (Bookhout and Costlow, 1974 ; Epifiano, 1979), largely stimulated by J. Costlow's group of the Duke University Marine Laboratory, North Carolina. A long-term research program on the identification of larval stages of crabs, together with studies on their culture requirements and basic biology and physiology, resulted in a number of genera being available by the 1970s for acute and chronic tests. One mud crab, Rhithropanopeus harrisii, is used extensively, but other well-studied and either ecologically or commercially important species (e.g. Carcinus maenas, Callinectes sapidus) are incorporated into various toxicity testing schemes. Crab larvae are considered well-suited for bioassays by a recent National Academy of Sciences Committee (NRC, 1981a), and a list of suggested species and a tentative standard bioassay procedure are now published (APHA, 1980). The literature on acute lethal and chronic sublethal effects (growth, development, molting, respiratory physiology, metabolism, behavior) is now very extensive, particularly for dispersed oil to which the larvae are very sensitive (Wells, 1982a).

Other arthropods

Larvae of horseshoe crabs (Limulus spp.) have also been incorporated into bioassays (e.g. Strobel and Brenowitz, 1981). They can be easily obtained in the eastern USA, and cultured and studied as with brachyuran larvae.

Echinoderms

Embryological and larval assays with various echinoderms have been used routinely and experimentally in Japan (Kobayashi, 1971, 1973, 1974, 1977, 1980, 1981 ; Kinae et al., 1981, among others), Norway (Hagstrom and Lönning, 1973 ; Lönning, 1977), Italy (Pagano et al., 1982, 1983), South Africa (Brown and Greenwood, 1978 ; Greenwood and Bennett, 1981), and the USA (Allen, 1971 ; Dinnel et al., 1981, 1982 ; Crawford and Gates, 1981 ; Landrum and Crosby, 1981 ; among others). They have been recommended for use in Canada (Wells, 1982b). The responses of these early stages to petroleum hydrocarbons have been recently reviewed (Wells, 1982a). Fertilization, development to the gastrula stage, and larval development seem to be sensitive to oil exposure. Kobayashi has exposed early stages to many materials, including oils and dispersants, and has written extensively on the topic (see Kobayashi, 1984). Both urchins and sand dollars have been incorporated into studies of oil effects on embryos. Embryological studies with the echinoid, Strongylocentrotus spp., could be conducted year-round with laboratory-held animals, and seasonally with field-caught animals. They require experimental and embryological expertise and a small working space, incubator and microscope. The embryological assays lend themselves to (a) routine screening of contaminants ; (b) study of fundamental reproductive processes affected by pollutants ; (c) study of multiple toxicity due to being able to examine large numbers of treatments ; and (d) responses of young stages, as part of an overall assessment of effects throughout a life history. These applications are mentioned below.

The recent NRC report (NRC, 1981a) stated that "the larval forms most widely used, especially for short-term bioassays, are the developing egg and larva of the sea urchin and sand dollar", and extensively referenced the current methodology. Embryological assays with echinoderms could easily become recommended as "standard marine toxicity protocols" in the 1980s, internationally to join those on species such as Artemia (brine shrimp). Such assays, through the measure of genotoxic and developmental effects, may

prove to be a vital link in marine environmental research between human health and ecotoxicological effects. Some of the sperm bioassays are already being refined and validated "for use as a standard rapid sensitive method for the testing of toxic substances" (Stober et al., 1981). In addition, the sea urchin larval bioassay is one of the recommended tests for monitoring water quality (Stebbing et al., 1980). Such assays with echinoids have now been adopted in Fed. Rep. of Germany (Ernst, 1984), and are continuing in Norway on hydrocarbons (Falk-Petersen and Lönning, 1984), and in Yugoslavia on chlorinated hydrocarbons (Ozretić, pers. commun.).

Teleosts

Extensive biological and toxicological work was conducted in the 1970s with young life stages of herring (Clupea spp.), cod (Gadus morhua), three-spine stickleback (Gasterosteus aculeatus), Killifish (Fundulus spp.), and striped bass (Morone saxatilis), among other species, and effects of many pollutants have been described (Rosenthal and Alderdice, 1976; Wells, 1982a; NAS, 1984). A list of 21 marine and estuarine species, and techniques for their culture and bioassays is presented by APHA (1980), with suggestions for incorporating young life stages. Efforts are being made to reliably culture fish larvae for physiological/toxicological research (Houde and Taniguchi, 1979). There is a "proposed standard practice for conducting toxicity tests with the early life stages of fishes" in preparation with ASTM (Schimmel, pers. commun.), with one objective being to identify test procedures for hazard assessment. Considerable experimental work is underway with fish eggs to identify their cytologic, cytogenetic and embryological conditions in polluted coastal waters (Longwell and Hughes, 1980 ; Longwell, pers. commun.), and standard biological monitoring techniques may evolve from this work. Stebbing et al. (1980) concluded that "fish larval bioassays are needed in view of the ecological and economic importance of these groups of organisms". Some regulatory agencies have responded. The Office of Toxic Substances of EPA in the USA is working on a marine larval fish toxicity protocol (Brungs and Tarzwell, 1984).

Microcosm (Model ecosystem) tests with zooplankton

Over the past 10 - 15 years, a number of microcosm experiments have been conducted and observations made on captured zooplankton populations exposed to low levels of toxicants (Reeve et al., 1976, 1977 ; Lee et al., 1977 ; Davies et al, 1980 ; Kuiper, 1981, 1982, 1984 ; Oviatt et al., 1981).

In North America, the CEPEX (Controlled Ecosystem Pollution Experiment) and the MERL (Marine Ecosystems Research Laboratory) microcosms have demonstrated survival, growth, respiration, behavioral, reproductive, predation, and diversity responses of zooplankters exposed to metals, petroleum hydrocarbons, chlorinated hydrocarbons, and oil-spill dispersants. Sensitive biochemical and physiological responses in fish eggs and larvae exposed to hydrocarbons in large tanks are being measured in the Battelle New England Laboratories (Neff, pers. commun.). Replication of observed responses has at times proven to be difficult (Lawson and Grice, 1977), but probably reflects the "natural variability" components of endemic populations.

Microcosm experiments have been conducted in Scotland (Davies et al., 1980), the Netherlands (Kuiper, 1981, 1982), the USSR (Beletskii et al., 1982) and in Sweden (Linden, pers. commun.). These experiments are a realistic and semi-controlled link between laboratory and full field assessments of the toxicity of materials, and represent the most detailed and expensive assessments available for materials not eliminated in earlier stages of the assessment process. The advantage of such experiments with zooplankton is that "major functional response parameters" with zooplankton under chemical stress can be measured (Adams and Giddings, 1982).

Ecotoxicological tests with field populations

A number of physiological, biochemical and ecological responses of zooplankton have been measured under "stressed" natural conditions (Benon et al., 1975 ; Bamstedt, 1980 ; Samain, et al., 1980 ; Wells, 1982a), but such measurements are relatively rare. For example, zooplankton of the highly contaminated New York Bight do not reflect the suspected effects being caused by waste discharges (Wolfe et al., 1982). A clearer situation apparently exists with spilled oil. Laboratory studies on petroleum hydrocarbons have demonstrated the high sensitivities of certain developmental stages of zooplankton, especially before and during fertilization, and during early embryonic development, hatching, and larval phases. These studies suggest that substantial damage may occur to localized populations of zooplankton during oil spills (Wells, 1982a). Individual organisms at oil spills have been affected through direct mortality (fish eggs, copepods, mixed plankton), external contamination by oil (chorion of fish eggs, cuticles and feeding appendages of crustaceans), tissue contamination by aromatics, abnormal development of fish embryos, possibly

temporary inhibition of feeding by copepods, and altered metabolic rates. Hence, individual zooplankters can be affected under natural conditions. Concern about effects at the population level is leading to quantitative approaches of damage detection that can be applied at sea with individual organisms, i.e. residue measurements in tissues, cytogenetic evaluations, enzyme studies, histopathological examinations and recognition of morphological aberrations.

It is in this area that the development of ecotoxicological tests with zooplankton for hazard assessments of new and existing chemicals overlaps with the need for sensitive and reliable field biomonitoring approaches with zooplankton. The cytogenetic and cytotoxicity techniques of Longwell (USA) and Moore (UK), the enzyme techniques of Samain et al., (1980), the physiological methods of Capuzzo (1981), and the ability to recognize morphological aberrations such as the white-eye syndrome in crustaceans (Minchew et al., 1979 ; Carls, pers. commun.), and intermediate larval stages (Wells, 1976b), and others, require support for further development and application. The general applicability of these zooplankton methods for detecting adverse responses to chemicals in the field, the interpretation of the effects as population responses, and the development of more, generic, sensitive and unequivocal methods clearly warrants further work.

TYPES OF BIOLOGICAL RESPONSES

Some currently measured chemical stress responses among marine zooplankton are shown in Table I. Lethality has been measured in all groups. Developmental effects (rate, success, morphological aberrations), behavior, and biochemical/physiological effects are the most commonly reported sublethal responses, followed closely by feeding behavior, growth and reproduction. To date the uptake, metabolism and depuration of materials has been measured with relatively few groups. Lethal, behavioral, developmental, and reproductive responses are probably simplest to include in zooplankton assays, are sensitive and interpretable, and are of fundamental biological importance.

TABLE 1 SOME OF THE MEASURED RESPONSES OF MARINE ZOOPLANKTON WHEN EXPOSED TO A WIDE RANGE OF COMMON MARINE POLLUTANTS. BASED ON A LITERATURE SURVEY UP TO EARLY 1983.

RESPONSES TO POLLUTANTS	GROUPS OF ORGANISMS IN LABORATORY														LAB. FIELD	FIELD
					CRUSTACEA				DECAPODA							
	PROTOZOA	COELENTERATES	POLYCHAETES	MOLLUSCA	ANOCOSTRACA	COPEPODA	CIRRIPEDIA	MYSIDACEA	SHRIMP	LOBSTERS	CRABS	MISCELLANEOUS	ECHINODERMS	TELEOSTS	MICROCOSM TESTS	FIELD ECOTOXICITY TESTS
LETHALITY	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
SUBLETHALITY																
Behavior						X	X	X	X	X	X		X	X		X
Feeding behavior	X			X		X	X			X				X	X	
Uptake, depur.						X			X		X				X	X
Biochem., Physiol.					X	X			X	X	X		X	X	X	X
Development		X	X	X	X			X		X	X	X	X	X	X	
Growth	X			X	X			X		X	X			X		
Reproduction	X			X		X					X		X	X	X	
Abundance, diversity															X	X

TABLE II CHARACTERISTICS OF ZOOPLANKTON TESTS IN THE LABORATORY

	Practical	Biological
Advantages	Many species, some available throughout year	Well-known biology
	Minimum space	High sensitivity.
	Minimum test volumes	Clear and fundamental responses
	Some simple and rapid procedures	Endemic vs. cultured comparisons possible
		Suitable for interlaboratory comparisons
Disadvantages	Static test solutions	Simplicity/rapidity may be a trap
	Uncontrolled exposures	Considerable culture/food facilities
	Labor-intensive procedures	Radioisotope techniques required
	Seasonal availability of some species	Commonly used species "hardy"
	Skilled personnel needed	Mostly "single-species" tests

CHARACTERISTICS OF ZOOPLANKTON TESTS IN THE LABORATORY

There are a number of practical and biological characteristics of zooplankton toxicity tests in the laboratory (Table II). These are grouped as advantages and disadvantages, for convenience.

There are four practical advantages :

1. Many species of zooplankton suitable for testing can be cultured almost year-round, or can be obtained from field populations during long parts of the year. Hence availability of many of the currently most favored species for standard tests (i.e. polychaete larvae, oyster and mussel larvae, copepods, mysids, various crustacean larvae, echinoid larvae) is high, and some of these (specific copepods, Artemia, mysids, decapod larvae, echinoids) are widely available and can be easily shipped. Notable exceptions are barnacle larvae, some decapod larvae, and most if not all fish eggs and larvae.
2. Minimum space for testing (exposures, microscopic examination, chemical preparation) is required, making zooplankton testing especially ideal for mobile laboratories and ship-board studies.
3. Test volumes needed for individual organisms or stages are small, usually less than 50 ml, allowing large numbers of samples per treatment and large numbers of treatments to be tested simultaneously.
4. Many of the response measurements, from microscopic observations of dead or incapacitated organisms to sublethal measurements (biochemical to growth) are relatively simple and rapid to perform, have high precision, and can be competently conducted by careful, well-trained technicians.

There are several important biological advantages to conducting assays with zooplankton :

1. The species most commonly used or with a potential for greater use have well-known culture requirements and biology (see NRC, 1981b on this point).
2. Many zooplankters are very sensitive to the common pollutants (e.g. petroleum hydrocarbons, see Corner, 1978 ; Wells, 1982a). This point is well recognized. Reasons for the higher sensitivities may be the high metabolic rates, high surface to volume ratios, many growth and

developmental processes occurring simultaneously, lack of detoxification enzymes, high permeability, frequent molting and water uptake, continuous filtering of water, etc. The reasons are in most cases little studied, yet are basic to understanding the applicability of data from specific zooplankton tests.

3. Zooplankton organisms offer many unequivocal stress responses of a fundamental nature, i.e. ability to fertilize, develop, hatch, molt, feed, or swim.
4. There are many opportunities to work with both cultured and endemic populations, for comparisons of sensitivities and reproducibility, e.g. Cirripedia larvae, calanoid copepods, so as to calibrate and interpret the laboratory tests.
5. A number of species have wide geographic distributions, or can be shipped readily, allowing successful interlaboratory toxicity comparisons, e.g. *Artemia* cysts, *Mytilus edulis*, copepods, mysids, echinoids.

There are, however, a number of practical and biological disadvantages to the conduct of toxicological and ecotoxicological toxicity tests with marine zooplankton (Table II).

The first practical problem with most tests is that static or semi-static dosing regimes are employed, due to the extreme handling/jostling sensitivity of the species or life stages or for practical reasons of conducting continuous-flow assays with many very small compartments. Hence, the test solutions may change in composition over time, often in an unknown manner. The high temperatures of some recommended tests accentuates this problem, e.g *Artemia* at 25 °C. This problem can be corrected with considerable chemistry, especially with labelled compounds, and the employment of small-scale diluters, if these are affordable. Much criticism has been directed towards this problem and the effects on the interpretation of the data. The very short tests, such as those less than 1 - 2 h, would not be greatly affected at low temperatures and with highly soluble substances. However, there still may be a problem with losses to walls, into air spaces (Rogerson et al., 1983) or into the organisms themselves.

Other practical problems include the labor-intensive nature of many tests, especially if time-concentration-response relationships are required; the seasonal availability of some organisms, e.g. *Pseudocalanus* sp. and barnacle larvae in Nova Scotia coastal waters or barnacle larvae, the

frequent difficulty of keeping some of the preferred plankters alive, e.g. decapod and fish larvae ; and the requirement of having very careful, skilled technical staff for each phase of the culture, maintenance, and testing.

Biological disadvantages are numerous (Table II). The primary one is that the apparent simplicity and rapidity of a recommended "standard" zooplankton test may be a trap. As for all organisms for experimental work, they require care, specialized knowledge and considerable experience for accurate and reproducible toxicity estimates. An example is the Artemia nauplii test, which can produce results influenced by the hatching, maintenance and exposure methods and times because the nauplii are developing so rapidly. Uniform training and routinely conducted intra- and inter-laboratory calibrations should be mandatory. It is not by accident that the reliable contributions on zooplankton responses to pollutants have been generated by laboratories with vast culture and biological expertise and experience, e.g. Artemia Reference Center, State University of Ghent, Belgium, for Artemia ; Duke University Marine Laboratory, Beaufort, USA, for decapods ; Chesapeake Biological Laboratory, USA, for copepods ; Kobayashi's laboratory at Doshisha's University, Kyoto, Japan, for echinoid gametes ; EPA, Gulf Breeze Laboratory, USA, for mysids, etc.

Another biological limitation is that the common zooplankton tests are conducted with readily cultured or easily maintained species, hence the "hardy species" are being used to establish threshold toxicity values, and water quality objectives. The species worked with must be successfully caught, held, fed and tested, with minimum control mortalities or other signs of stress (e.g. color changes in lobster larvae and copepods). Many groups of zooplankton are rarely or never worked with for these reasons, yet may be very sensitive to persistent materials and potentially useful in hazard assessment schemes, e.g. chaetognaths.

The last and most serious biological limitation is that most recommended and currently available tests with zooplankters are single-species tests, which do not measure effects on ecological processes. Hence most zooplankton tests are not true ecotoxicological tests. Cairns (1983) and the recent NRC report on test methods (NRC, 1981b) have discussed this problem in detail. Toxicants can be successfully screened with simple, single-species tests. However, many materials may need to be studied further with microcosm and field ecophysiological techniques which are for key

TABLE III SOME PROPOSED TEST PROCEDURES WITH MARINE ZOOPLANKTON. ACUTE TESTS DENOTED BY A AND CHRONIC TESTS DENOTED BY C. (cont'd)

ORGANIZATION	SPECIES AND LIFE STAGES	TYPE OF TEST	RESPONSES
ICES (Stebbing, et al., 1980) (suggested techniques)	Viable techniques for effects monitoring		
	(1) Oyster larval bioassay	A	48-h, embryo development
	(2) Sea-urchin larval bioassay	A	fertilization and development assay
	Potentially useful bioassays		
	(1) calanoid copepods	A, C	survival, growth, faecal pellet production
	(2) mysids	A, C	survival growth life cycle tests
	(3) other invertebrate larvae - polychaetes - shrimps - brachyurans	A, C	lethality fertilization frequency of abnormal larvae swimming activity rate of metabolism developmental rate
	(4) fish larvae	A, C	embryonic or larval heart rate growth survival percent viable hatch

TABLE III cont'd SOME PROPOSED TEST PROCEDURES WITH MARINE ZOOPLANKTON. ACUTE TESTS DENOTED BY A AND CHRONIC TESTS DENOTED BY C.

ORGANIZATION		SPECIES AND LIFE STAGES	TYPE OF TEST	RESPONSES
APHA (1980) (tentative methods)	(1)	Protozoa - Tetrahymena pyriformis (Strain W)	C	growth rate of population maximum population density accumulation and concentration of toxicant
	(2)	Adult calanoid copepods, especially Acartia tonsa	A	lethality, moribundity
	(3)	Larval shrimp, crabs, lobsters (Palaemonetes, Penaeus, Homarus, 5 spp. crabs)	A	egg hatchability rate and success of molting swimming ability tendency to lose appendages meta morphosis lethality
	(4)	Molluscs - embryos of 10 spp. of oysters, clams, scallops and mussels	A	normal vs. abnormal larvae
	(5)	Fish - 21 spp. - cultured and field-caught larvae e.g., sheepshead minnow, Atlantic silverside	A	egg viability egg hatchability lethality growth
ASTM (Subcommittee E47-01)	(1)	Mysids - Mysidopsis spp. - juveniles and adults	A, C	survival growth reproduction
(methods under review)	(2)	Fish - early life stages - Opsanus, Cyprinodon, Menidia	A, C	survival, growth abnormal development behavior time required for hatching

groups of zooplankton in an early stage of development (Samain et al., 1980; Conover, pers. commun.).

REVIEW OF SELECTED ZOOPLANKTON TESTS

It is evident from the overview given and from previous recent reviews (Hart and Fuller, 1979 ; Buikema et al., 1980 ; Hammons, 1980 ; Stebbing et al., 1980 ; NRC, 1981ab ; Leclerc and Dive, 1982) that a number of toxicity and ecotoxicological test procedures with zooplankton are being proposed as standard protocols, and others are being developed by organizations and individuals. The overall objective is to have a suite of nationally and internationally acceptable protocols with marine zooplankton, to supplement those with algae, bacteria, other invertebrates, and fish.

TABLE IV SUMMARY OF PROPOSED TEST PROCEDURES WITH MARINE ZOOPLANKTON. PROPOSED DENOTED BY X; POTENTIAL DENOTED BY O.

ORGANISMS	ORGANIZATION		
	APHA (1980)	ASTM (1983 a,b)	ICES (1980)*
Protozoa	X		
Polychaetes			O
Mollusca	X		X
Copepoda	X		O
Mysidacea		X	O
Decapoda	X		O
Echinoids			X
Teleosts	X	X	O

* See Stebbing et al. 1980.

TABLE V MAIN FEATURES OF A ZOOPLANKTON TEST IN THE LABORATORY

FEATURES	ORGANISM: Decapoda (Homarus spp.)
Life Stage	Larvae, stages 1-4
Toxicological Criteria	Lethality, rate of development, molting success, feeding, respiration, among others
Test Conditions	Static; semi-static
Sensitivity	High-see Wells 1976b, 1982
Degree of Standardization	High for static, probably low for others
Reproducibility	Intra-good Inter-good for oil, otherwise unknown
Rapidity	L*-fast; SL**-up to 5 wks at 20C
Cost/Test	L-<$500/test; SL->>$10^3$/test
Training - Expertise	High
Usefulness	High; commercial species
Major Advantage	Clear, easy to measure responses
Major Disadvantage	Labor-intensive
Documentation	Large-see Cobb and Phillips (1980)
Main References	Wells (1976b); APHA (1980); Capuzzo (many references)

* L-lethal; ** SL-sublethal.

Tests proposed by three major organizations concerned with standard toxicity and monitoring methods are shown in Table III. This table should not be interpreted as a review of all procedures being developed worldwide, simply some of those in North America and Europe. In addition, the APHA methods are being revised for 1984. The proposed tests cover eight groups of organisms and a wide variety of suggested acute and chronic responses. Table IV shows clearly that molluscan and teleost tests are favored, followed by

the crustaceans (copepods, mysids, decapods). Somewhat surprisingly, the two big US organizations, APHA and ASTM, have apparently not yet drafted standard protocols for polychaetes and echinoids, yet an ICES working panel recognized the potential of both groups. To date the OECD has only considered freshwater testing protocols as they are addressing premarket testing of chemicals (MacGregor, pers. commun.). This reviewer is unaware of current activities in FAO on standard toxicity protocols. It is interesting, and perhaps significant, to note that the organizations in Tables III and IV have not yet included the Artemia naupliar test promoted and standardized by Persoone et al. (1981), yet this test together with others is being used by the US EPA in standardized oil dispersant testing. The Artemia test also has great potential as a reference zooplankton technique for laboratories working with other endemic species e.g. copepods (Wells et al., 1982).

Table V gives the main features of a typical laboratory zooplankton test. Such tables could be prepared for a variety of the most promising zooplankters based on information in this volume, and would be useful to a rapid selection of state of the art, standardized tests.

APPLICATIONS OF SELECTED TESTS

There are a number of testing protocols and toxicological approaches with marine zooplankton that can be applied in specific ways to describe effects of new and existing chemicals, formulations and materials. A few of these protocols are relatively well standardized (see above) ; most are not, as the tests are still under development (e.g. larval fish bioassays) or are used only by a few laboratories or agencies (e.g. echinoid assays).

The immediate need for standardized protocols and approaches with zooplankton, so as to have a standardized and comparable toxicity data base on chemicals, should not discourage the continued research and work to develop new tests and improve on existing ones. However, there is an immediate need internationally for commonly accepted test procedures with marine zooplankton. There are four reasons :

1. They are sensitive to persistent pollutants and should be included in estimates of hazards.

2. There are some standard procedures (e.g. with Artemia, oyster larvae, echinoid eggs and sperm) and others that with little additional effort could be worked with routinely, in many laboratories.
3. Hazard assessments should always consider responses of major phyla and trophic levels.
4. New chemicals or formulations that may impinge upon marine systems should be screened for toxicity and hazard uniformly from one country to another, following the example of the OECD toxicity testing protocols. These points have been stated earlier by others but are worth repeating.

Tests with marine zooplankton can be used in at least four ways :

1. Acute and chronic testing of chemicals and formulations during their development to ensure safer products or products with known effects. Examples : paints (barnacle larvae), oil spill dispersants (copepods and Artemia nauplii).
2. Acute screening tests for new chemicals, prior to use or recommendations for use. Examples : insect hormone mimics, components of drilling muds.
3. Incorporation into detailed hazard assessment schemes, for nondegradable and bioaccumulative compounds that were not identified as "environmental chemicals" by 1 and 2.
4. Biomonitoring of industrial effluents and ambient water quality (Gruber, pers. commun. ; Stebbing et al., 1980 ; Capuzzo and Lancaster, 1981, with copepods in the New York Bight).

These applications require considerable interaction between marine toxicologists specializing in one or more of the zooplankton procedures and the regulatory agencies responsible for assessing and controling toxic chemicals, or the industries that are producing new chemicals. Chemicals entering or suspected of entering marine waters, from estuaries to offshore waters, may already have been screened with sensitive freshwater assays (i.e. the OECD premarketing tests), but should have their toxicities verified with the most sensitive marine tests possible. Some of these could include zooplankton as detailed above.

CONCLUDING TRENDS WITH MARINE ZOOPLANKTON

In the past 15 years, considerable attention has been given to the development and application of toxicity tests with holoplankton and meroplankton. There is particular concern about the impact of pollutants on young life stages of commercial fisheries species (Waldichuck, 1979). This effort and concern can now be translated into the routine inclusion of zooplankters in marine hazard assessments, where the test procedures can be chosen by national and international groups.

These are many procedures available, crossing all applications, screening to predictive tests to monitoring in polluted areas. There is a need to direct effort now at the choice of a few international tests that can be rigorously standardized and applied. These tests should not just be single-species (Cairns, 1983). Some should be multispecies, microcosm tests (Hammons, 1980, 1981) or multispecies, process-response laboratory tests (Vandermeulen and Hemsworth, 1977). Some of the available choices and centers of development are described above, and in other papers in this volume. There is considerable activity with zooplankton testing protocols but much remains to be done. In particular, the principles behind the sensitivities of particular species and life stages, research on modes of toxic action, and the applications in effects-monitoring programs need to be pursued. Perhaps most importantly, the value of zooplankton tests at the regulatory level needs to be translated to the responsible technocrats and bureaucrats, so that sensitive tests are indeed applied on a routine basis, and the results translated into actions to prevent continued pollution. This paper attempted to describe the current scientific activity on marine zooplankton toxicity tests with this urgent application as the primary goal.

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