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# CURRENT STATUS OF METHODS OF TOXICOLOGICAL RESEARCH ON FRESHWATER CRAYFISH

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"They were dwarfish natures capable of growing into monsters if ill-chance fostered the process...and were highly susceptible to the enroachments of evil. They were...like crayfish who always retreat into shadow, going backwards rather than forwards through life, gaining in deformity with experience, going from bad to worse and sinking into deeper darkness...".

- Hugo, Les Miserables



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# TABLE OF CONTENTS



## LIST OF TABLES



## LIST OF FIGURES

## ABSTRACT

France, R.L. 1986. Current status of methods of toxicological research on freshwater crayfish. Can. Tech. Rep. Fish. Aquat. Sci. 1404: iv + 20 p.

Methods used for toxicological research on freshwater crayfish were reviewed and categorized. The effect of life history variables on test results, and recommendations on experimental protocol and data reporting, are discussed.

The largest proportion (ca 40%) of water quality criteria were derived from laboratory experiments of acute exposure to lethal concentrations, a procedure considered by many to have little relevance to determination of ultimate ecological consequences of pollution upon invertebrates, especially if "standardized" techniques are used. In fact, fewer than half of these experiments incorporated basic life cycle information into experimental design, and only 26.3 and 15.8% investigated temporal effects or were of a sufficient duration to monitor for prolonged mortality, respectively. Failure to a) consider chemistry of the test water, b) utilize statistical analysis to aid in data interpretation, and c) to form ecological predictions from the laboratory test results are serious drawbacks in the assessment of anthropogenic pollution on crayfish when lethality experiments are used for this purpose.

Sublethal measurements formed less than one-quarter of all recommended criteria. Responses such as avoidance, and especially reproduction which may be very sensitive to some contaminants, have been little studied. Only 3.3% of studies considered the ability of crayfish to acclimate to harmful chemicals. Further physiological or mechanistic investigations are required to interpret toxicant effects on crayfish before such traditional indicators as changes in growth become manifest. Studies of tissue residues in crayfish are well represented (17.1%) in the literature. Experimental field enclosure manipulations and observations of life history attributes in polluted natural systems should receive more than their current level of attention (ca 10%) as the final test of laboratory predictions. The most powerful, though rarely performed, water quality criteria are those in which both laboratory and field-derived information are considered together. Integration of laboratory toxicity results with "basic" field life history data into simulation computer modelling has not yet been attempted with crayfish.


Keywords: literature reviews; toxicology; experimental research; methodology.


## RÉSUMÉ

France, R.L. 1986. Current status of methods of toxicological research on freshwater crayfish. Can. Tech. Rep. Fish. Aquat. Sci. 1404: iv + 20 p.

On a étudié et catégorisé les méthodes de recherche en toxicologie utilisée pour l'étude de l'écrevisse d'eau douce (Decapoda, famille des Astacidae). On aborde dans ce rapport l'incidence des variables des antécédents vitaux sur les résultats de tests, et on présente des recommandations sur des façons de procéder et la présentation de données en matière d'expérimentation.

La plus grande partie (environ 40%) des critères servant à mesurer la qualité de l'eau ont été obtenus à partir d'expériences en laboratoire portant sur une exposition importante à des concentrations létales. Un grand nombre de chercheurs estiment que cette façon de procéder n'est pas vraiment utile pour établir les conséquences écologiques finales de la pollution sur les invertébrés, surtout si on a recours à des techniques dites "normalisées". En réalité, moins de la moitié de ces expériences comportaient un plan d'expérimentation qui tenait compte des données fondamentales sur le cycle vital. Et seulement 26,3% et 15,8% d'entre elles étudièrent les incidences temporelles, ou persistèrent suffisamment longtemps pour qu'on puisse observer les taux de mortalité prolongés respectifs. Lorqu'il s'agit d'évaluer les effets de la pollution anthropogénétique sur l'écrevisse et lorsque des expériences sur la létalité sont utilisées à ces fins,les lacunes suivantes constituent une faiblesse certaine: a) ne pas tenir compte de la composition chimique de l'eau devant servir au test; b) ne pas avoir recours à l'analyse statistique afin de faciliter l'interprétation des données; et c) ne pas établir de prévision écologique à partir des résultats des tests de laboratoire.

Les mesures sublétales constituaient moins du quart de tous les critères recommandés. On a peu étudié les réactions comme l'évitement, et plus précisément la reproduction, qui peut être très vulnérable à certains contaminants. Seulement 3,3% des études faisaient état de l'aptitude de l'écrevisse à s'adapter à des produits chimiques dangereux. Il faut d'autres études physiologiques ou mécaniques pour interpréter les incidences des substances toxiques sur l'écrevisse avant que des indices conventionnels comme les changements de taux de croissance ne se manifestent. Les publications sur le sujet comptent un nombre appréciable (17,1%) d'études sur les résidus dans les tissus de l'écrevisse. L'incorporation au domaine expérimental des manipulations et des observations relatives aux attributs des antécédents vitaux dans les écosystèmes pollués devrait susciter davantage l'intérêt des chercheurs (au lieu du taux d'intérêt actuel se situant à environ 10%), au même titre que le test final portant sur les prévisions en laboratoire. Les critères les plus utiles sur la qualité de l'eau, bien que rarement utilisés, sont ceux qui tiennent compte des renseignements conjoints obtenus en laboratoire et tirés des expériences sur le terrain. On n'a pas encore tenté, pour ce qui est de l'écrevisse, de simuler à l'aide de la modélisation informatique l'intégration des résultats sur la toxicité en laboratoire aux données "fondamentales" sur les antécédents vitaux obtenues sur le terrain.


Mots-clés: revues de publications; toxicologie; recherche expérimentale; méthodologie.

## INTRODUCTION

Crayfish (Decapoda, family Astacidae) are the largest and longest-lived members of the freshwater Crustacea. Due to their omnivorous habits, they serve as a key energy transformer between various trophic links and thus help to maintain an efficient energy flow throughout the system (Momot et al. 1978; Crocker and Barr 1968). On an economic basis crayfish may be considered to be a most important inhabitant of many waterbodies (Svardson 1949; Abrahamsson 1972; Avault 1972; Westman 1974; Nolfi and Miltner 1978). The aquaculture potential and rearing methodology have been studied in detail (Westman 1972; Morrissy 1976; La Caze 1970; Mason 1974, 1978; Nelson and Dendy 1978; Leonhard 1981).

That our aquatic environment is currently under stress from an increasing number of anthropogenic pollutants is an fact that cannot be ignored. The use of invertebrates in toxicological testing is essential for protection of these aquatic ecosystems (Maciorowski and Clarke 1980). Crayfish more than amply meet the criteria identified by the United States Environmental Protection Agency (Buikema et al. 1982) for selection of test species for toxicity studies: the organism should (1) represent an ecologically important group; (2) occupy a position within the food chain leading to man or other important species; (3) be widely available (use of indigenous species is preferable), amenable to laboratory testing and easily maintained in the laboratory; and (4) have adequate background data on physiology, genetics, taxonomy and its role in the environment.

The response of crayfish to pollution was reviewed by Hobbs and Hall (1974). Much of the information they give on effects of pollution on crayfish had to be derived indirectly from investigations on comparative physiology, environmental adaptations, or natural history, rather than from studies directly pertaining to impacts of particular contaminants. More recently Leonhard (1977, 1979) proposed the use of stock cultures of crayfish, established and maintained within the laboratory, as "toxicological tools" in the assessment of environmental perturbation. Many studies directly concerned with effects of toxic materials have entered the literature.

The purpose of this paper is to review the current status of toxicological research on crayfish, particularly within the last decade, not from a viewpoint of the results, but rather from a methodological perspective. The categorization divisions chosen are adopted from those of Sprague (1976). The importance of life history variables selected from those identified by Buikema and Benfield (1979) that affect test results, as well as recommendations in regard to experimental protocol and data reporting, are also discussed. The studies in this review include those demonstrating important individual methodologies that provide easily accessed information for individuals entering the field of toxicological research on crayfish, in addition to participants concerned with developing a more comprehensive multi-test investigation.

## MATERIALS AND METHODS

For this review 100 publications concerned with the debilitating effects of chemical pollutants on freshwater crayfish were examined. Only those studies in which crayfish were the exclusive or major test organism used were considered. Crayfish experiments pertaining to the bioassay paradigm which stresses inflexible standardization as the means to effect clear comparisons of effluents, are not covered in this review. Those toxicological studies concerned with understanding the effects of environmental perturbation on crayfish biology, are considered. Categorization divisions and methodology were adopted from Sprague (1976). For each paper, the key experimental result for recommending water quality criteria was tabulated under the respective category. In those cases where more than one experimental procedure was used, subjectively weighted fractional values were assigned to each category. Tabulated numerical values represent the relative proportion of total tests utilizing a particular technique. Laboratory experiments were analyzed as to the percentage of the total that incorporated various facets of life history information selected from Buikema and Benfield (1979), as well as several other aspects of experimental protocol and data reporting. As Sprague (1976) cautioned, such procedures are frought with difficulties with respect to the choice of categories and subjective fractionalization of results into these divisions. Nevertheless, the categories provided here form a definable framework from which a clear conceptualization of the "state of the art" of crayfish toxicological research can be reviewed.

## RESULTS AND DISCUSSION

### CHARACTERIZATION OF TOXICOLOGICAL TESTS

#### Laboratory lethal concentrations

The largest number of water quality criteria relied exclusively on lethal levels established in the laboratory (Fig. 1). Acute tests have long been the mainstay of aquatic toxicology and are particularly useful in providing meaningful comparisons between animals, toxicants or test conditions. Other advantages include great utility, simplicity and cost effectiveness, and although perhaps modest in predictive capacity they are still considered by many to be scientifically sound and legally defensible (Buikema et al. 1982). Nevertheless, severe criticism has been directed toward this protocol and considerable evidence now exists that measurement of acute toxicity derived from "standardized" tests has little relevance to determination of the ultimate ecological consequences of pollution (Lehmkhul 1979). The fact that over 40% of all toxicological research on crayfish has dealt with determination of acute mortality at high contaminant concentrations and over only a short period of time, is a serious weakness (this is discussed later).

Sublethal investigations

As a result of dilution and dispersion of pollutants from point source inputs in the natural environment, chronic exposure to sublethal concentrations of such substances is likely to affect a much greater biomass than exposure to acute lethal concentrations (Kleerekoper 1976). Sublethal effects are those defined as subtle changes in an organism's homeostasis induced by environmental stress that produces no immediate short-term damage. Serious repercussions develop, however, at progressive developmental stages, in higher levels of organization (e.g. population structure), or at a later date removed from the immediate exposure.

Physiology: Understanding the physiological actions of pollutants is the key to predicting important sublethal effects that could help prevent incorrect generalizations about toxicity (Sprague 1971). Despite the need to comprehend cause-and-effect mechanisms, only 10.5% of crayfish toxicological studies have utilized this methodology. The development of a clinical (physiological, biochemical or histological) assessment of crayfish health could provide an early warning of adverse effects before such traditional indicators as growth and recruitment failure become apparent.

Malley and Tinker (1979) developed a sublethal test using the uptake of calcium-45 during the critical period of molting, and utilized this measure to interpret the effects of low pH on postmolt exoskeletal synthesis (Malley 1980). Appelberg (1980) arrived at similar conclusions by determination of calcium content following exposure during molting in acid test water. Other studies concerned with toxicity of pesticides have used several application procedures including hemolymph injection (Kaila and Saarikoski 1977; Laska et al. 1978) and leaching or uptake from food (Ludke et al. 1971).

Several clinical measurements are used as biochemical indicators of environmental stress in crayfish. Mills and Geddes (1980) found changes in the osmotic pressure of blood corresponded to the range of salinity which produced behavioral distress. Changes in hemolymph acid-base and ion concentrations were used to assess the sublethal effects of acidity on crayfish (Morgan and McMahon 1982). Increased utilization of metabolic energy in the phosphoademylate system associated with physiological defence mechanisms occurred in response to chronic cadmium exposure (Giesy et al. 1980). Serum protein concentration or ademylate energy change and ATPase activity have been used as rapid measurements of environmental suitability for lobsters (Stewart and Li 1969; Haya et al. 1982) and should be suitable for crayfish studies.

Idoniboye-Obu (1977) found that petroleum hydrocarbons brought about a progressive diminution or irregularity of neurogenic and myogenic biopotentials, indicating a "point of no return" long before the threshold "death response" was reached. In another neurotoxicological study, Saarikoski and Kaila (1977) found that several pesticides diminished the amplitudes and increased the durations of action potentials, sometimes leading to a complete blocking of condution. The calculated effective concentrations (EC50 values) were substantially lower than the 8-day LC50's.

Crayfish often possess compensatory mechanisms toward noxious environmental agents. Detoxification of pesticides and heavy metals may occur via hepatopancreatic enzyme activity, in particular mixed-function oxidases (Lang et al. 1977; Lindstrom-Seppa et al. 1983), or possible metallothionens (Lyon et al. 1983).

Respiration: Respiration has proved to be a sensitive indicator of stress in fish (Sprague 1971), but has received little attention from crayfish toxicologists. Respiratory patterns of crayfish have been affected by exposure to chlorodane and DDT (Daiden and Bennett as cited in Hobbs and Hall 1974; Freeman and Hall 1970) but not to several fungicides (Laska et al. 1978) or to aquashade (Spencer 1984). Anderson (1978) found that Orconectes virilis were able to adjust to Pb concentrations by compensating for decreased gill efficiency through an increase in ventilation volume. Sprague (1971) cautioned that respiratory measurements are of questionable value unless they take account of animal activity, a problem circumvented by the development of methodologies that determine oxygen uptake in crayfish tissues (Hubschman 1967) and individual eggs (Appelberg 1980).

Growth: Growth experiments might be expected to reflect overall integration of sublethal damage and thus be very sensitive to toxicants, but in fact have yielded few regulatory criteria for pollution control (Sprague 1969, 1976). This is consistent with several experiments with crayfish what have found growth of adults to be unrelated to either cadmium (Thorp et al. 1979) or insecticide (Hendrick et al. 1966a) exposure. Alternatively, the growth of recently hatched or juvenile crayfish was very sensitive to sublethal copper concentrations (Hubschman 1967) and elevated salinities (Loyocano 1967). Physiological interference with molting has suggested that crayfish growth could be affected by acidification (Malley 1980; Appelberg 1980). Clearly generalizations regarding the usefulness of growth as a sublethal indicator of toxic stress in crayfish are inappropriate. If such experiments are conducted it is important to be aware of how container dimensions can influence results (Mason 1974, 1978; Goyert and Avault 1978).

Acclimation and acclimatization: Methods of assessing environmental damage have generally depended upon measurements or inferences of short-term lethal effects. Acute toxicity studies test responses to brief shocks and fail to consider the long-term and gradual ability of organisms to adapt physiologically or genetically to a new pollutant (Lehmkhul 1979). The possibility of altered resistance or tolerance of crayfish to high chemical concentrations following previous exposure to sublethal conditions is essential to the comprehensive understanding of any contaminant's effects. However, less than 5% of criteria have been based upon this possibility.

Mathews et al. (1977) measured a significant increase in chlorine tolerance in crayfish that had been previously acclimated for three days to sublethal concentrations. France (1983) compared the resistance to low pH of adult crayfish from two populations, one inhabiting a control lake and the other one experimentally acidified over a five year period, and concluded that acclimatization had not occurred. Crayfish collected from an area exposed to intensive insecticide use were 1.4-2.4 times more resistant to pesticides than were crayfish gathered from a control area (Albaugh 1972).

Behavioral responses: Behavioral responses integrate underlying biochemical and physiological processes that are influenced by ambient environmental conditions (Scherer 1977) and, as a result, are essential for comprehensive assessment of any pollutant's effects (Sprague 1976; Buikema and Benfield 1979). Only 3.8% of the selected crayfish toxicology studies have monitored such responses.

Avoidance tests study the ability of organisms to detect the presence of toxicants and to respond by moving away into water more favorable for survival. Chaisemartin et al. (1976) measured the behavioral reactions of young crayfish to heavy metals in solution or contained within sediments, and found a five- to ten-fold interspecific variation in avoidance thresholds. France (1985a) compared the acid avoidance behavior of adult and yearling *O. virilis* from a control population and one inhabiting an experimentally acidified lake. Crayfish from the acidified lake had a noticably reduced avoidance and it was believed that they may have become sensorially acclimatized to the depressed pH conditions. Maciorowski et al. (1980) developed a microcomputer system which used crayfish locomotory activity as a parameter for continuous biological monitoring. Crayfish were observed to perceive sublethal concentrations of copper and respond to them with a general decrease in activity.

Simple reflex reactions of invertebrates to both natural and toxic stimuli have been used to gauge water quality. Sprague (1971), however, cautioned against their wide acceptance, as the ecological significance of many such changes remains uncertain. The abdominal appendages in ovigerous crayfish vibrate to ensure that a current of water is kept circulated about the attached eggs, helping to keep them well-aerated and free of disease. When pleopod movement slows or stops, as might occur from toxicant stress (France 1985b), egg development can be delayed or halted.

Experiments on the effects of crude oil on feeding behavior (Atema and Stein 1974), chemosensory-olfaction in the presence of copper and phosephamidon (McLeese 1975), and burrow construction in petroleum contaminated sediments (Atema et al. 1982), have been performed with lobsters and would be suitable for crayfish studies.

## Reproduction

Reproductive impairment is a good measure of environmental stress because the act of reproduction reflects the product of all factors influencing metabolism (Buikema and Benfield 1979). Sprague (1976) concluded that the most sensitive and productive toxicological experiments are those concerned with chronic effects on the reproductive cycle. However, only 3.1% of the reviewed crayfish toxicology studies have used reproductive impairment as the index of damage.

Brown and Avault (1974) exposed ovigerous females to low levels of antimycin with no resulting egg mortality. Egg development was followed through a post-exposure period and the young showed no morphological or growth differences. Hubschman (1967) reported that embryos were killed by copper concentrations less than one-fourth those which affected maternal females. Similarly, the water concentrations of 14 metals sufficient to diminish reproductive ability by half, were only 30-70% and 40-80% of the 30-d LC50's for *O. limosus* and *Austropotamobius pallipes*, respectively (Boutet and Chaisemartin 1972). Future experimenters should be aware that numerous biological and physical variables can influence crayfish reproductive efficiency in the laboratory, irrespective of addition of chemical agents (Mason 1974, 1977a). Application of the artificial incubation methodology of Mason (1977b) and Rhodes (1981) enables maternal and embryonic responses to experimental perturbation to be separated.

Vey (1977) found that the physiopathological effects of suspended clay particles upon *A. leptodactylus* was to interfere with normal oviposition which caused 20% of the females to resorb oocytes or lay abortive eggs. *O. virilis* recruitment was severely reduced in an artificially acidified lake (France, in prep.). Incomplete cuticular hardening of the glair-cement compound forming egg capsule membranes and stalks resulted in failure of secure pleopod egg attachment. Egg mortality and resorption were also elevated in the acidified lake, but were considered to be of secondary importance. The progression of embryos through seven developmental stages was found to be unaltered by lake acidification, and consequently the basic parameters of egg quality (size and weight) remained unchanged.

## Tissue residues

Certain chemicals can bioaccumulate and produce residues in the body following uptake at even low concentrations. Analysis of tissue residues from crayfish exposed to mobile compounds supplies information useful for biological monitoring of pollutant severity, provides insights into possibilities of biomagnification, and aids in interpretation of mode of toxic action through site-specific measurements.

Studies of tissue residues are well-represented in the literature on toxicology studies with crayfish.

Crayfish, because of their large size, unique polytrophic life habits, and ubiquitous distribution, have served as important indicators of pollution with metals (e.g. Vermer 1972; Bernard and Roy 1977) and pesticides (e.g. Leonhard 1974; Dimond et al. 1968; Hendrick et al. 1966b). Graves et al. (1977) concluded that formulation of water quality criteria based on elevated tissue concentrations of pesticides is ecologically more meaningful than relying upon the obvious limitation of death as the endpoint in most toxicological work. Anderson and Bower (1978) emphasize that the effectiveness of crayfish as biomonitors of metal pollution is dependent on the physiological role of the metal.

The accumulation, retention, sequestation or elimination of chemical agents by crayfish can be influenced by numerous factors. These include the stage of the molt cycle (Wiser and Nelson 1964), pathway of uptake (Bryan 1974; Giesy et al. 1980), basic water chemistry (Chang et al. 1982), interspecific differences (Dickson et al. 1979), and possibility of detoxification mechanisms. Further, the excretion or depuration of accumulated substances once the crayfish is placed in clean water is an important physiological variable that approximates temporal fluctuations of contaminants.

Field studies

The relationships between life histories and environmental disturbances are usually subtle and difficult to interpret. Laboratory studies provide precise dose-effect information concerning the predicted effects of a single pollutant but can never successfully duplicate all the interacting variables characteristic of natural environments. For these same reasons, field studies cannot provide the sensitivity necessary to detect adverse effects before they reach crisis proportions. The failure to assimilate both laboratory and field information in concert, produces studies that may have limited relevance to solving contaminant problems. J. Klaverkamp (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, pers. comm.) has proposed a mechanism for integrating both laboratory and field toxicology data based on reciprocal objectives of increasing or decreasing relevance and identification of mechanisms, thereby producing the "integrated toxicity test design" emphasized by Buikema and Benfield (1979) as being the most valuable tool in toxicological research. Despite the primary importance of field observations in practical studies of pollution only about 10% of the reviewed papers contained such research.

Crayfish held within holding containers placed in situ in contaminated waters constitutes a simple but valuable "field bioassay system" useful for comparison to laboratory derived information (Muncy and Oliver 1963; Hendrick and Everett 1965; Chang and Lange 1967; Leonhard 1977). The transplantation methodology used to compare the ecophysiologies of different crayfish populations (e.g. Bovbjerg 1952) has great potential in ascertaining the effects of contaminants on mortality, growth and reproduction. Although the interpretation of field bioassays may be difficult due to fluctuating toxicant concentrations, the usual situation of mixed pollutants, and physical problems existing in making frequent inspections and chemical measurements, it is essential that laboratory experiments explain the field results reasonably well before the laboratory tests can be considered adequate (Sprague 1971; cf. Cheah et al.'s (1978) comments on Hendrick et al. (1966a)).

The use of small experimental ponds or large metallic tanks modelled to simulate natural conditions has proven a valuable tool in determining the effects of agricultural manipulations of chemicals on commercially harvestable crayfish in the southern United States (Hendrick and Everett 1965; Chang and Lange 1967; Hendrick et al. 1966a; Ekaneum et al. 1981b). In Canada use has been made of large polyethlene-lined swimming pools with water circulation and sediments (Hamilton 1972), or 1-10 m diameter enclosures placed in situ in the littoral zone of lakes (Chang et al. 1982; Rudd et al. 1980) into which chemicals have been added. Problems arising from elevated mortality (e.g. Ekanem et al. 1981b) could be alleviated by holding the crayfish individually within cages as was done in the two Canadian studies.

The establishment of ecological reserves for long-range studies (including both natural and altered systems) has been recommended as a top priority in unravelling ecotoxicological problems. The experimental acidification of a lake within the Experimental Lakes Area, northwestern Ontario, supplied information on the projected effects of acid precipitation on the resident population of O. virilis (e.g. France and Graham 1985).

Crayfish populations often demonstrate a marked resilience to certain types of stress caused by fishery exploitation or predation (Momot and Gowing 1977; Gowing and Momot 1979; Westman and Pursiainen 1982). Field studies by Hendrick et al. (1966a) with application of pesticides at concentrations more than 30 times those producing laboratory 96 h LC50 values, showed no measurable effect on crayfish survival, growth or reproduction. This demonstrated that even when laboratory tests indicate a direct effect, this result may be hidden in the natural environment. The question of whether crayfish populations exhibit compensatory reactions, such as redistribution of mortality amongst the various factors which normally cause it (Nicholson 1954), to counteract in some degree the adverse effects of pollutants, has not been examined to date.

Environmental scientists must be able to predict and evaluate effects of chemical impacts rather than act on damage that is already present (Rosenberg et al. 1981). The long-term monitoring of natural ecosystems that are already polluted can provide valid insights into chronic impacts, but the opportunities for predictive use of such an approach are limited. Extrapolative methods are needed. The requirement of procedures and approaches for linking laboratory-derived, concentration-effect relationships on the individual, with a measure of ecologically significant pollutant effects in the field on the population, can be met with the development of predictive mathematical models (Rosenberg et al. 1981).

## INCORPORATION OF LIFE HISTORY INFORMATION INTO LETHALITY EXPERIMENTS

Buikema and Benfield (1979) concluded that the reason why so much of our toxicity data are unsuitable for setting water quality criteria is due to failure to incorporate basic life history information into the experimental design and data interpretation. This was especially true "in the case of acute toxicity tests because simple lethality data conducted with 'standard' toxicity tests are intrinsically difficult to interpret and even more difficult to extrapolate to field conditions". Continued use of "cookbook" methods, which fail to consider adequately the test organism's ecological requirements, will produce test results that may be dependent on test conditions rather than the toxicant in question (see reviews by Buikema and Benfield 1979; Maciorowski and Clarke 1980; Buikema et al. 1982).

### Life cycle information

Sensitive life stages: Less than 40% of the reviewed toxicity studies conducted with crayfish have investigated tolerance variability over the life cycle (Table 1). Toxicity studies producing statements of relative sensitivities between different species or toxicants are severely restricted unless the most sensitive life stages are compared (Brown 1973). Immature animals are usually more susceptible to pollutants than adults for a variety of reasons including a larger surface:volume ratio facilitating greater exchange with the environment, a higher weight specific metabolism which could accentuate toxin uptake, and a shorter time to develop physiological compensatory mechanisms. For example, 50% mortality among newly hatched *O. rusticus* and *O. virilis* to copper and low pH, respectively, was reached with an exposure time one fiftieth that required for adults (Hubschman 1967; France 1984). In either case, if the toxicant concentration sufficient to kill 50% of the adult crayfish over an extended period of time, had been accepted as the guide for setting water quality criteria, mortality of the entire juvenile sub-population would have occured within six days and all the young would have expired within a single day. Similarly, Brown and Avault (1974) and Heit and Fingerman (1977) also demonstrated an inverse relationship between crayfish size and toxicant severity.

Molting: Increased sensitivity to toxicants during molting is thought to result from the rapid and non-selective uptake of water and accompanying chemicals during ecdysis, increased penetration of the newly formed integument by toxins, or added physiological demands that may increase rate of oxygen consumption or decrease rate of detoxification of foreign compounds (Buikema and Benfield 1979). However, fewer than one-quarter of toxicity studies with crayfish have monitored the possibility of variable sensitivity in relation to the molt cycle. Postmolt crayfish have been shown to be more susceptible to organophosphates (Baker 1974), low pH (Malley 1980), antimycin (Brown and Avault 1974), and decreased oxygen concentrations (Melanion and Avault 1977). Information on molt-related sensitivity in conjunction with knowledge of regional life history patterns, has immediate practical value to managers for regulating and restricting the application of agricultural toxins or discharge of wastes during specific periods of the year.

Sex differences: Differential responses to pollutants can occur between sexes. Female crayfish were found to be both more tolerant to mercury (Heit and Fingerman 1977) and less tolerant to hypoxic-acid water (Jarvenpaa et al. 1981) relative to males. These studies point to the need to record the sex of test organisms.

### Interspecific relationships

The usefulness of single-species test systems in formulating valid predictions about the impact of chemicals has received harsh criticism (Sprague 1971). Predator-prey interactions, known to be a significant factor in regulating crayfish population structure and abundance (Momot et al. 1978), have not been investigated in crayfish toxicology studies. Sublethal decreases in locomotory activity and escape responses observed in crayfish exposed to contaminants (Maciorowski et al. 1980; Mills and Geddes 1980) suggests that the application of fish predation experiments such as those developed by Stein and Magnuson (1976) and Stein (1977) could provide valuable ecological information.

Although crayfish are susceptible to a wide variety of parasite species (Johnson 1977; Unestam 1972), little is known about how different environmental changes alter infection in the host. Field studies suggest that crayfish weakened by exposure to environmental perturbations may develop an increased sensitivity to pathogenic infection (France and Graham 1985). Such individuals are considered to be under physiological stress and have to exert more energy to maintain normal body homeostasis at the expense of resources needed to resist disease.

The quantal methodology used to estimate crayfish food consumption (Rundquist and Goldman 1981) could have important applications to development of further toxicology protocol which may be directly related to growth rates.

### Nutrition

Contrary to generally accepted protocol that animals be fed sparingly or starved prior to testing, Hubschman (1967) believed that in order to maintain a relatively high metabolic rate comparable to recently collected crayfish and thus conduct ecologically realistic tests, it is essential that they be well fed prior to experimentation. Buikema and Benfield (1979) demonstrated that food quality and availability influences invertebrate behavior and sensitivity to toxicants and stated that "a poorly fed or unfed organism is prestressed before being exposed to a toxicant, and resultant data may be spurious". Test subjects have been supplied with food in only 10% of crayfish toxicology experiments. In one case, the nutritional characteristics of metal toxicity were tested for O. limnosus and A. pallipes (Boutet and Chaisemartin 1972). Crayfish provided with food were more sensitive (based on comparisons of 30-d LC50's) to several metals such as copper and zinc, perhaps due to additional poisoning by consumption of contaminated food. In contrast, increased metabolism due to food supply, reduced toxicity of metals such as lead and nickel.

France (1984) provided hatchlings with a layer of flocculent organic detritus covering most of the aquarium bottom while older crayfish were fed macerated dog food at periodic intervals. Huner et al. (1974) developed a water-stable diet for crayfish culture (proximate analysis is provided in paper) that has great potential as a standardized food source in toxicity studies.

Observations of feeding behavior in laboratory toxicity experiments can supply information useful in interpretation of growth rates observed in field-stressed crayfish populations. The sensory feeding behavior of lobsters has been used to measure sublethal oil effects (Atema and Stein 1974) and could easily be adapted to crayfish studies.

## Behavior

Pollutant-induced modifications in behavior are usually manifested in much less time than acute lethality and provide a continual gauge of developing morbidity. Concentrations of lethal toxicants are generally of finite duration. Survival of any species, therefore, depends not just upon its tolerance to the magnitude of toxicant concentration (LC50), but also upon the time course of toxic stress or resistance in relation to duration of perturbation. Although knowledge of median survival times (MST) is essential for predicting the ultimate fate of populations to episodic contaminant pulses, the speed at which an individual goes through the symptoms of impending mortality (ET50) will likewise be of ecological significance. Although 18.4% of researchers undertaking crayfish lethality experiments have reported some observations of behavior, most have only listed symptoms of impending mortality. Few toxicologists have attempted to quantify these responses. Chang and Lange (1967) and Boutet and Chaisemartin (1972) found that the speed of toxic action causing progressive morbidity varied considerably for different pesticides and metals, respectively. Mills and Geddes (1980) developed an important methodology monitoring the threat and escape reactions of adults and general activity level of juveniles, and used the degree to which these responses were elicited as criteria for determining toxic effects. The authors found a more restricted zone of activity within the zone of salinity tolerance. They believed these behavioral results gave a more accurate assessment of stress because field populations were constrained within those salinities in which behavior remained unaffected. France (1984) found that once crayfish become moribund, they varied in their long-term compensation to toxic stress and in the resulting speed at which death occurred. Adults reached their ET50's at 60-87% (mean 77.5%) of the time it took them to die, i.e. MST's; ET50's for juveniles were within 76-93% (mean 86.3%) of their MST values; and once the beginning symptoms of acid toxicity developed the young would quickly expire, with ET50's 81-97% (mean 90.5%) of their MST values. Crayfish toxicologists will produce only a fraction of the total information that is potentially available in lethality experiments if mortality is recorded at interals of only 24 h rather than following Sprague's criticism and make observations much more frequently.

## Physical-chemical parameters

Test vessel size and shape are known to affect invertebrate sensitivity to toxicants (Buikema and Benfield 1979). Container dimensions can strongly influence survival, growth and reproduction of crayfish in culturing systems (e.g. Mason 1974, 1978; Goyert and Avault 1978) but the effects of toxicant stress upon these interactions are not known. France (1983) observed that young were more sensitive to low pH when tested in circular, compared to square aquaria of the same water capacity. Absence of corners may have forced crayfish to expend more energy in the search for refugia thereby increasing their sensitivity whereas animals in square containers become sedentary once a corner was located. Absence of suitable substrate and habitat can increase invertebrate susceptability to chemicals (Buikema and Benfield 1979) as well as affect survival of young crayfish (Mason 1974). Again it is not known how these habitat requirements influence contaminant toxicity with crayfish. To prevent antagonistic pressure and possibly cannibalism, it is essential that adult crayfish be held individually within subdivided compartments.

Light quality and quantity, temperature, and dissolved oxygen effect general metabolism, behavior, and biological cycles, and are recognized as factors which influence invertebrate sensitivity to toxicants. However, they are variables often ignored in favor of "standardized test conditions" with little ecological or physiological basis (Buikema and Benfield 1979). Few crayfish laboratory studies have investigated the significance of these variables. Heit and Fingerman (1977) found that the ability of crayfish to cope with inorganic mercury depended greatly upon the environmental temperature executing changes in metabolic rate. The authors postulated that survival would increase during colder periods of the year due to reduced mercury uptake. Boutet and Chaisemartin (1972) also found increased testing temperatures elevated toxicity of 9 of 14 different metals to crayfish, perhaps due to accelerated body penetration. Thorp et al. (1979) investigated the interaction between chronic cadmium exposure and thermal stress. The central thesis presented was that any chronic stress extracts energy from an individual which must then be borrowed from other functions, thereby decreasing resistance of these functions to additional stress. This explains findings by Jarvenpaa et al. (1981) which show that the effect of acid exposure on A. astacus was much more serious when oxygen concentrations were simultaneously reduced.

The influence of water current on contaminant toxicity to crayfish is not known. Early tolerance experiments by Park et al. (1940) suggest that physiological differences may exist between crayfish stock obtained from either lentic or lotic environments. It is possible that holding of crayfish collected from rivers in static conditions may pre-stress the animals before the toxicant is added. In such cases it would be advisable to utilize a continuous-flow experimental design similar to that of Hubschman (1967).

Modifying chemical factors greatly affect pollutant toxicity. Only 18.4% of reviewed studies reported pertinent chemical data on water used in the experiments. Basic test water chemistry affects speciation of the contaminant, its mode of toxic action, as well as fundamental physiological responses of the organism itself. For example, lowering test water pH from 7.5 to 6.5 increased toxicity of two pesticides to A. fluviatalis (Kaila and Saarikoski 1977). This was due to both shifts in ionic form which promoted transport across biological membranes as well as decreased crayfish tolerance at lowered pH. Differences in water chemistry also dramatically influenced the toxicity of nitrite to crayfish (Beitinger and Huey 1981): increases in water chloride concentration ameliorated nitrite toxicity due to competition at monovalent ion uptake sites. Further, at lower pH (5.6), resistance times decreased as a result of reduction in the protective efficacity of the chloride ions. Studies of crayfish toxicity should incorporate detailed chemical characterization of test water or the interpretation of results will be difficult, if not impossible. Additionally, in order to predict the response of crayfish to pollution, it is essential that the test water utilized be chemically similar to that found in the natural situation to avoid spurious conclusions.

## EXPERIMENTAL METHODOLOGY AND REPORTING OF RESULTS

### Realistic toxicant levels and study purpose

Fewer than half the published studies on crayfish toxicology provide the reader with sufficient background information to judge the relevance of the particular work (Table 2). Because many studies fail to explain the purpose of the investigation it is often difficult to determine the authors' rationalization of particular methodological techniques. Most frequently the choice of concentration and/or chemical form of contaminant used in the toxicity test is left unexplained.

Pollutant realism is needed for a comprehensive hazard assessment strategy. Because most crayfish toxicology studies do not include this, it is difficult to interpret the results, i.e. are the toxicant concentrations used similar to those expected to occur in the natural environment (e.g. Brown and Avault 1974; McLeese 1976; Ekanem et al. 1981b) or are they magnitudes higher than those predicted under even the most noxious conditions? The widespread use of high element concentrations in laboratory tolerance experiments led Thorp et al. (1979) to state that "although striking differences between treatments and controls are frequently evident in those experiments, the results, unfortunately, provide little insight into the more realistic condition of chronic low level pollution". Supportive evaluations and estimations of maximum toxicant concentration in the field based on expected use or disposal patterns is an essential step in the sequential assessment of potential risk to aquatic life (Duthie 1977; Saunders 1979). As Duthie explains, "the key factor in the decision on whether a material can be utilized with appropriate acute risk level, is the ratio of concentrations found to be toxic for test species to the concentration expected in the environment". Until the crayfish toxicology literature provides references to pertinent chemical data or includes direct measurements by researchers themselves of contaminant levels in impacted regions inhabited by the species in question, they will remain far from the goal of protecting such organisms from local extinction. Additionally, the toxicity of mixtures of chemicals expected to occur together naturally, e.g. copper and mercury, may be greater than the sum of the toxicities of each component individually, i.e. synergism (Boutet and Chaisemartin 1972; Ekanem et al. 1981a). Such attempts at ecological realism have been seriously understudied.

### Duration of experimental exposure

Crayfish toxicology continues to suffer from the dilemma of "96-hour complacency" that plagued experiments with fish during the 1950's (Macek 1980). Although Sprague (1969) stated that preconceived ideas of "best" procedure for experiments should not be carried too blindly from fish toxicology to invertebrate tests, few researchers on crayfish seem to be aware of quantitative differences between acute and chronic toxic thresholds. The ecologically more meaningful asymptotic or incipient lethal level, recommended by Sprague (1969) as the single most useful criterion of toxicity, should always be employed. The incipient LC50 is defined as that level of environmental perturbation beyond which 50% of the population cannot live for an extended or indefinite period of time, i.e. the ultimate toxicant level at which the LC50 becomes effectively constant. Remarkably, only 15.8% of lethality tests with crayfish have utilized exposures for prolonged periods of time and attempted to establish such chronic thresholds.

The demonstration of delayed mortality with exposure of crayfish to several natural and anthropogenic chemicals (Fig. 2) indicates that testing periods must be extended until treatment mortality has stablized rather than simply choosing an arbitrary time for termination. It is of little use in terms of water quality criteria, to state that crayfish can survive at a given concentration for such a fixed interval of time (Hubschman 1967). Brown (1973) stated that "data not wanted are virtually meaningless lists of LC50 values for different poisons for arbitrary periods of exposure or such things as statements of the relative toxicities (based on LC50 values at such times) of two poisons, as these may well be reversed with prolongation of exposure... As a principle, recommendations for arbitrary or 'standard' periods of exposure are best ignored". The increase in LC50's from 96 h to incipient exposures, as a result of protracted mortality (France 1984; Boutet and Chaisemartin 1972), substantiates the unreliability of conducting short-term experiments with crayfish.

## Temporal effects

The failure of most test methods to take into account the time-concentration interaction makes it difficult to apply laboratory results to field situations where episodes of extreme toxicant concentration are usually of a finite duration and can be expected to undergo dramatic fluctuations. Only one-quarter of crayfish lethality experiments utilize methodologies associated with temporal effects. Most studies even fail to report slopes of concentration-effect curves, percent survival curves or cumulative mortality tables and especially median survival times ±95% confidence intervals.

Few studies have addressed the problem that exposure to constant toxicant levels is an inadequate characterization of a changing natural environment. McLeese (1976) simulated the gradual decrease in fenitrothion concentration following the observed pattern reported in stream conditions, by a progressive flushing of treatment containers with clean water. France (1984) used a schematic technique to relate laboratory mortality at stable concentrations to temporal fluctuations observed at several polluted sites. Hubschman (1967) held crayfish in a discontinuous copper regime and found that animals exposed for three 8-h periods interrupted one week apart demonstrated a similar mortality pattern and MST to crayfish exposed for a single 24-h period. He deduced that copper toxicity was cumulative for *O. rusticus*. Thorp and Wineriter (1981) compared survival of crayfish exposed to continuous, rhythmic and arhythmic elevated temperature regimes, and developed ecologically realistic statements concerning intermittent thermal pollution.

The ability of crayfish to recover from a moribund state induced by chemical stress, once the toxicant concentration begins to decrease again, will strongly influence the eventual percentage of the population that will be affected. Maciorowski and Clarke (1980) and Buikema et al. (1982) recommend a post-exposure period be included in toxicity tests with aquatic invertebrates to study phenomena of recovery or delayed mortality, but this has only been incorporated into a few crayfish investigations. Hubschman (1967) found that mortality of crayfish following a one-day exposure to copper was extended over a period of two weeks so that concepts of acute toxicity were meaningless in such cases. Transfer of crayfish to clean water resulted in delayed toxicity for crayfish exposed to mirex (Ludke et al. 1971) and nitrite (Beitinger and Huey 1981) but not low pH (France 1984).

## Interspecific differences

Hobbs and Hall (1974) have cautioned that inferences should not be drawn from a single species with regard to predicting the effects of pollution upon crayfish in general. For example, those species which burrow into sediments are probably better able to tolerate contamination of their habitat than are those species living in open water beneath rocks and debris. It is also probable that physiological differences exist between crayfish that could influence species sensitivity to toxicants. There were considerable differences in both the survival resistances and concentrations of 14 metals necessary to reduce reproduction by 50%, between *A. pallipes* and *O. limosus* (Boutet and Chaisemartin 1972). As well, *A. pallipes* generally had an avoidance threshold five to ten times lower than that of *O. limosus*, possibly due to variations in sensory perception (Chaisemartin et al. 1976). The higher survival of *Procambarus clarki* compared to that of *Faxonella chypeata* at similar mercury concentrations may be the result of smaller sizes of the latter species (Heit and Fingerman 1977).

## Statistical analysis

Less than one-quarter of crayfish lethality experiments utilize even the most basic statistical analysis to aid in data interpretation. For sound evaluation and comparison of toxicity data, the concentration-effect curve must be accurately defined by its 95% confidence interval and slope function, and goodness of fit estimated by the appropriate test (Hodson et al. 1977; Sprague and Fogels 1977; Buikema et al. 1982; Brown 1973). Sprague (1969) argued the need for standardization in statistical interpretation of toxicological data and recommended the log-probit transformation procedure of Litchfield (1949) and Litchfield and Wilcoxon (1949) which were mathematically more conservative than other computer techniques because of larger confidence intervals. Baker (1974) compared the LC50 values determined by three methods, one graphical and two computational, and found they were in close agreement for a variety of different experimental circumstances. In the final selection of a method, it is important that practical considerations be given as much attention as the usual theoretical and statistical considerations (Stephan 1977).

## Ecological interpretation and conclusions

Many researchers on crayfish toxicology problems consider their work complete with presentation of an LC50 value or series of survival times. The ecological assessment of laboratory results in the formulation of recommendations for agricultural or industrial managers are rarely undertaken. Although several investigations of crayfish-pesticide interactions have formed, to varying degrees of usefulness, ecological conclusions for rice field managers (Hendrick et al. 1966a; Ludke et al. 1971; Cheah et al. 1978), the attempt by Brown and Avault (1974) to relate laboratory mortality to field toxicant application rates, is exemplary. The percentage of three size classes of P. clarkii which would be expected to expire by different application levels of antimycin designed to eradicate fish pests, were compared. From this, tentative conclusions were drawn regarding the feasibility of pesticide application under a variety of different managerial scenarios. Similarly, France (1984) interrelated results obtained from life cycle tolerance experiments, knowledge of crayfish life history patterns, and data concerning the magnitude and duration of chemical pulses, to form a prediction concerning the response of O. virilis populations to acidification.

## CONCLUSIONS

The prediction of biological damage in the environment, gained from toxicological research on crayfish, is at a "state of the art" similar to that achieved by experimentation with fish during the early 1960's. The failure to make use of innovative techniques already tried and proven in the field of fish toxicology, or to develop tests specific to crayfish, has meant that much of present day data may ultimately provide little in the way of predicting deleterious anthropogenic effects on crayfish stocks. More optimistically, however, it should be noted that of those toxicity studies on crayfish incorporating protocols other than a 96 h LC50, most have been undertaken only within the last five years.

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# APPENDIX

## A. GENERIC INDEX

### EUROPE

Astacus
Airaksinen et al. 1977; Appelberg 1980, 1981; Jarvenpaa et al. 1981; Kaila and Saarikoski 1977; Lang et al. 1977; Lindstrom-Seppa et al. 1983; Saarikoski and Kaila 1977.

Austropotamobius
Boutet and Chaisemartin 1972; Chaisemartin et al. 1976; Lyon et al. 1983.

### AUSTRALIA

Cherax
Mills and Geddes 1980.

### NORTH AMERICA

Orconectes
Anderson and Bower 1978; Boutet and Chaisemartin 1972; Chaisemartin et al. 1976; Chang et al. 1982; Dickson et al. 1979; France 1984, 1985a, 1985b; France and Graham 1985; Hamilton 1972; Hubschman 1966, 1967; Leonhard 1974, 1977, 1979; Malley 1980; Malley and Tinker 1979; Mathews et al. 1977; McLeese 1976; Morgan and McMahon 1982; Rudd et al. 1980; Spencer 1984; Vermer 1972.

Cambarus
Dickson et al. 1979; Dimond et al. 1968; Maciorowski et al. 1980; Thorp et al. 1979; Thorp and Wineriter 1981; Wiser and Nelson 1964.

Faxonella
Hiet and Fingerman 1977.

Procambarus
Albaugh 1972; Baker 1974; Beitinger and Huey 1981; Bernard and Roy 1977; Brown and Avault 1974; Chang and Lange 1967; Cheah et al. 1978; Ekanem et al. 1981a, 1981b; Giesy et al. 1980; Graves et al. 1977; Hendrick and Everett 1965; Hendrick et al. 1966a, 1966b; Hiet and Fingerman 1977; Idoniboye-Obu 1977; Laska et al. 1978; Loyacano 1967; Ludke et al. 1971; Melanion and Avault 1977; Morgan and McMahon 1982; Muncy and Oliver 1963.

## B. POLLUTANT INDEX

Table 3. Hydrogen ion: Appelberg 1980, 1981; France 1984, 1985a, 1985b; France and Graham 1985; Malley 1980; Morgan and McMahon 1982. Salinity: Loyacano 1967; Mills and Geddes 1980. Nitrite, chlorine: Beitinger and Huey 1981; Mathews et al. 1977. Copper: Boutet and Chaisemartin 1972; Bryan 1967; Hubschman 1966, 1967; Lyon et al. 1983. Cadmium: Boutet and Chaisemartin 1972; Giesy et al. 1980; Lyon et al. 1983; Thorp et al. 1979. Mercury: Boutet and Chaisemartin 1972; Chang et al. 1982; Hamilton 1972; Hiet and Fingerman 1977; Rudd et al. 1980; Vermer 1972. Zinc: Boutet and Chaisemartin 1972; Bryan 1967; Chaisemartin et al. 1976; Giesy et al. 1980; Lyon et al. 1983. Lead, Nickel: Anderson 1978; Boutet and Chaisemartin 1972; Chaisemartin et al. 1976. Mirex: Graves et al. 1977; Ludke et al. 1971. DDT: Airaksinen et al. 1977; Albaugh 1972; Dimond et al. 1968; Freeman and Hall 1970; Lang et al. 1977. Other hydrocarbons HCB, HCBD, PCP, TCP (6+): Idoniboye-Obu 1977; Kaila and Saarikoski 1977; Laska et al. 1978; Saarikoski and Kaila 1977. Pounce: Jolly et al. 1977. Antimycin: Brown and Avault 1974. Fenitrothion: Leonhard 1974; McLeese 1976. Other organo-phosphates (aldrin, methylparathion, toxaphene, guthion, azodrin): Albaugh 1972; Baker 1974; Hendrick and Everett 1965; Hendrick et al. 1966a, 1966b.

Table 1. Percentage of toxicity studies with freshwater crayfish incorporating selected life history information (cf. Buikema and Benfield 1979) into experimental methodology.

| Characteristic | Percentage |
|---|---|
| Life cycle | |
| Sensitive life stages | 39.5 |
| Molting | 21.1 |
| Sex differences | 21.1 |
| Ovigerous females | 1.3 |
| Interspecific relationships | 0 |
| Nutrition | 10.5 |
| Behavior | 18.4 |
| Physcial-chemical parameters | 18.4 |

Table 2. Percentage of toxicological research on freshwater crayfish utilizing selected facets of experimental protocol and reporting.

| Characteristic | Percentage |
|---|---|
| Realistic toxicant levels and study purpose | 41.6 |
| Long-term experimental exposure | 15.8 |
| Temporal effects | 26.3 |
| Interspecific differences | 8.3 |
| Statistical analysis | 21.1 |
| Ecological interpretation and conclusions | 50.0 |

Table 3. Index of methodological variables utilized to assess the effects of various pollutants on crayfish.*

| | Ions | | | Metals | | | | | Hydrocarbons | | | Organo-phosphates | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Testing procedure | $H^+$ | o/oo | N,Cl[1] | Cu | Cd | Hg | Zn | Pb,Ni | Mir.[2] | DDT | A[3] | P.,An.[4] | Fen[5] | B[6] |
| acute | • | • | • | • | • | • | • | • | • | • | • | • | • | • |
| chronic | • | • | | • | • | • | • | • | • | | | | • | • |
| physiology | • | | • | • | • | | • | | • | • | • | | | |
| growth | • | • | | • | • | | | | | | | • | | • |
| respiration | • | | | • | | | | • | | • | | | | |
| acclimation | • | • | • | | | | | | | • | | | | |
| behavior | • | • | | • | | | • | • | | | | | | |
| reproduction | • | | | • | • | • | • | • | | | | • | | |
| accumulation | - | - | - | • | • | • | • | • | • | • | | | • | |
| field studies | • | • | | | | • | | | • | • | | | • | • |
| population | • | | | | | | | | | | | | | |

* Studies listed in Appendix B
1 N = nitrate
2 Mir. = mirex
3 A = other hydrocarbons: HCB, HCBD, PCP, TCP (6+)
4 P = pounce; An. = antimycin
5 Fen = fenitrothin
6 B = other organo-phosphates: aldrin, methylparathion, toxaphene, quithion, azodrin

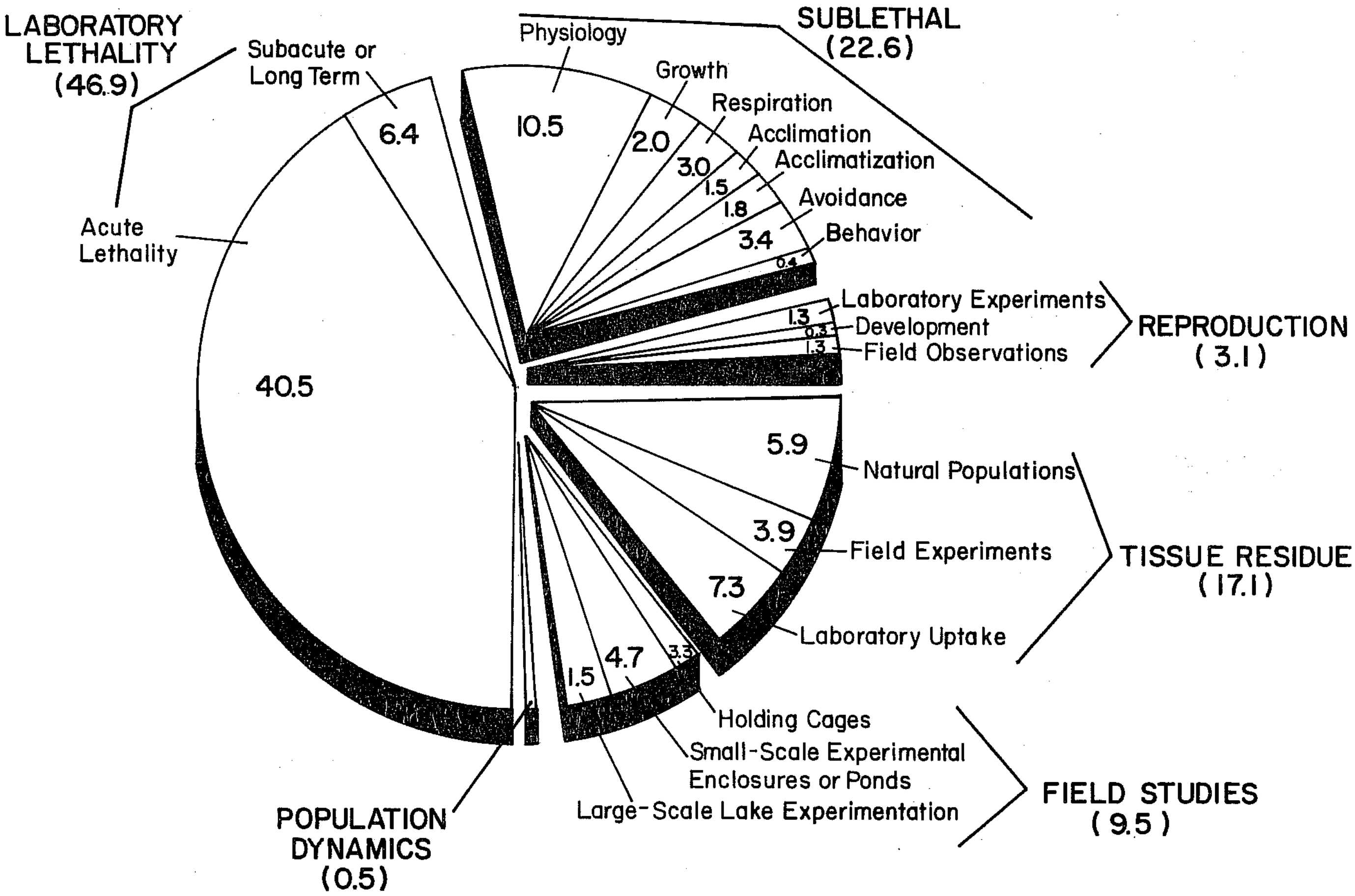


Fig. 1. Schematic representation of toxicological methodology formulating water quality criteria with freshwater crayfish. Numerical values represent relative percentages of total (N=100) tests utilizing particular technique. Studies using more than one experimental procedure were given fractional values in respective divisions. Categorization adopted from Sprague (1976).

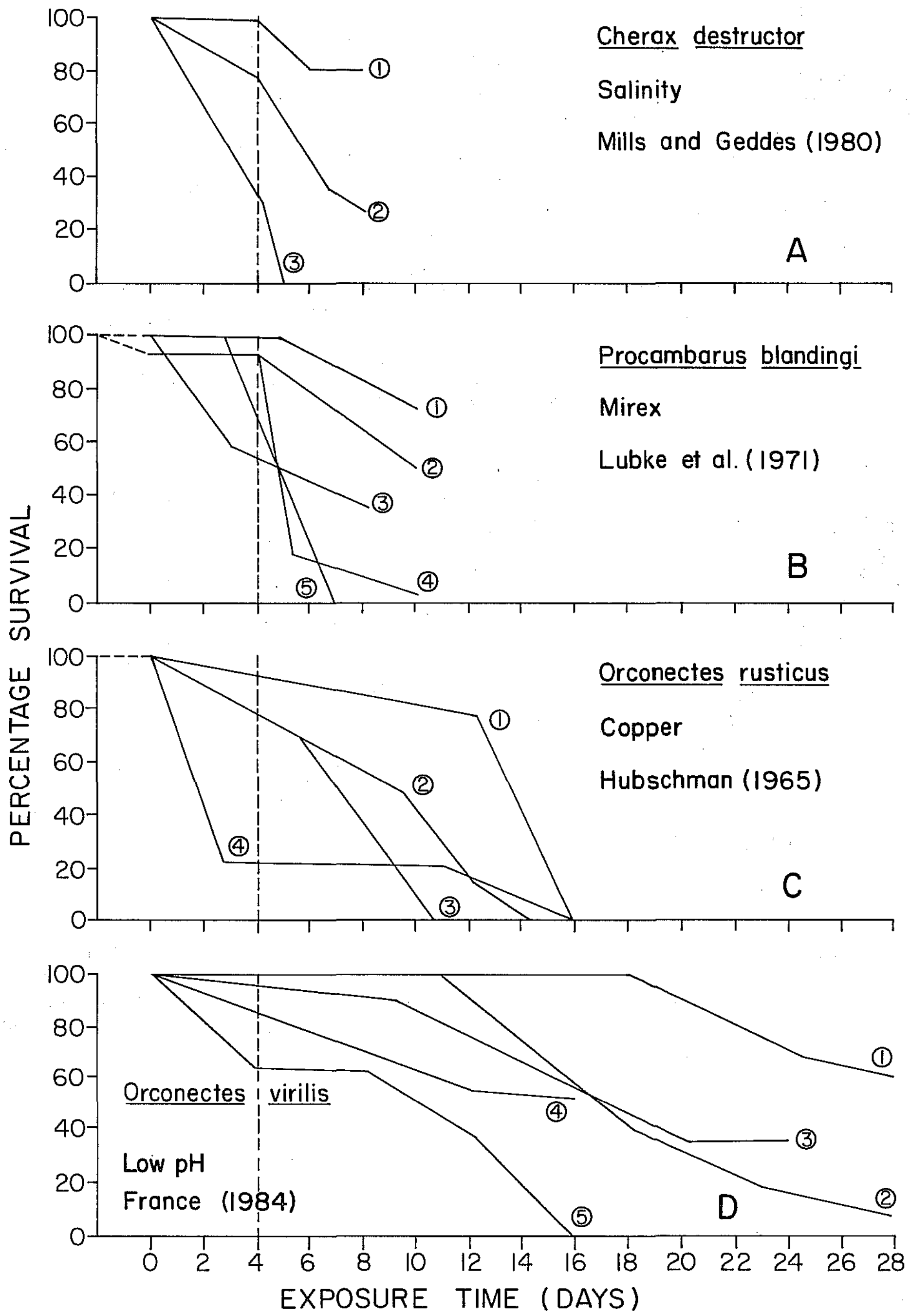


Fig. 2. Demonstration of the unsuitability of experimental duration of exposure for 96-h (4-d) exposure for estimation of toxicity with crayfish due to prolonged mortality.

a) Mills and Geddes. 1980. Aust. J. Mar. Freshwater Res. 31: 667-676.
   1) continuous exposure of adults to 24.5% salinity.
   2) continuous exposure of juveniles to 26.6% salinity.
   3) continuous exposure of juveniles to 30.0% salinity.

b) Ludke et al. 1971. Bull. Environ. Contam. Toxicol. 6: 89-96.
   1) initial 6-h exposure to 5 ppb Mirex in concentration followed by transfer to clean water.
   2) initial 24-h exposure to 5 ppb Mirex.
   3) exposed to 2 granules of Mirex bait (feeding effect).
   4) initial 58-h exposure to 5 ppb Mirex.
   5) exposed to 10 granules of Mirex bait enclosed in wire (leaching effect).

c) Hubschman. 1966. Crustaceana 12: 34-42.
   1) continuous exposure of adults to 1 $mg \cdot L^{-1}$ Cu.
   2) initial 24-h exposure of adults to 2.5 $mg \cdot L^{-1}$ Cu followed by transfer to clean water.
   3) initial 24-h exposure of adults to 6 $mg \cdot L^{-1}$ Cu.
   4) continuous exposure of young to 0.125 $mg \cdot L^{-1}$ Cu.

d) France 1984. Can. J. Zool. 62: 2360-2363.
   1) continuous exposure of adults to pH 3.0 water.
   2) continuous exposure of adults to pH 2.6 water.
   3) continuous exposure of juveniles to pH 4.0 water.
   4) continuous exposure of young to pH 5.0 water.
   5) continuous exposure of young to pH 4.0 water.