

Population Parameters as Indices of Effects of Mixtures of Chemicals on Aquatic Populations: The Need for Methods

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ABSTRACT

The paper discusses the population as a possible unit of study from a theoretical point of view, a few applications of population studies in ecotoxicology, and finally some field studies on the effects of mixtures of toxicants on fish populations.

From the data taken into consideration it can be concluded that, at least as a first approximation, the concentration-addition models appear to be adequate to describe the effects of mixtures on fish populations. However, there is a need to develop methodologies for the optimization of the information that can be obtained by toxicological tests on aquatic populations and to improve the way of interpreting the results.

1 INTRODUCTION

The greatest difficulty in preparing a review on the effects of mixtures of chemicals on aquatic populations is the scarcity of studies on the subject. However, this could be taken as a useful opportunity to examine the problem from a theoretical point of view and also to discuss specifically the questions related to the assessment of the effects of a substance on a population.

The first remark to be made is that the contemporary toxic substances control practice still has too narrow a theoretical basis and that a number of central questions in ecology have had almost no impact on ecotoxicology (Figure 1).

There are several reasons for this situation: the urgency to find a solution in applied research, the need to predict the effects of a molecule on ecosystems on the basis of limited data, the traditional antitheoretical disposition of people considering themselves to be practical, the need for clear-cut points for legislative or administrative actions and in some countries the tendency toward a rigid approach instead of a flexible one for guidelines, general principles, administrative

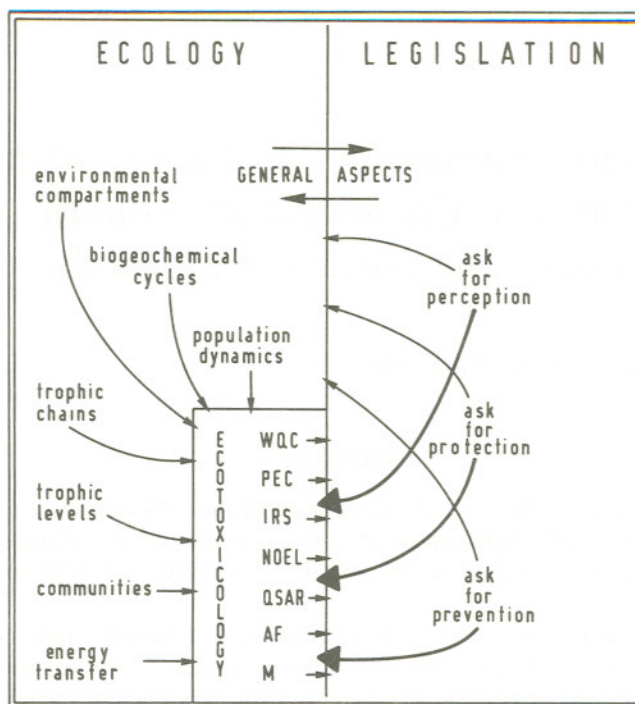


Figure 1 Relationship between ecology, ecotoxicology and legislation. WQC, water quality criteria; PEC, predicted environmental concentration; IRS, integrated rating system; NOEL, no-observed-effect level; QSAR, quantitative structure-activity relationships; AF, application factor; M, monitoring

negotiations, and so on. On the other side there is the preference of ecologists to study natural ecosystems and the prejudice against applied problems in a number of universities.

Biological monitoring of aquatic populations has been widely practised in water pollution control activities. Most of these studies, however, are of limited usefulness in assessing the effects of a single chemical datum and studies have been conducted merely to evaluate the state of health of bodies of water.

Moreover, it has to be remembered that the problem of prediction is now a key point in scientific and regulatory activities and such kinds of study were performed only *a posteriori*.

In fact schematically one can say that the 1950s were the years of the perception of the pollution problems, during the 1960s the activities were directed mainly toward protection, regulating the discharges of effluents or the use of molecules, and the 1970s were the years of prevention. This was based on the assumption

that toxicological and ecotoxicological characteristics of molecules must be known before the chemical is marketed. This is the philosophy of the Toxic Substances Control Act (TSCA) in the USA and the 6th Amendment of the Directive 831/79 on Dangerous Substances in the EEC. Similar approaches have been used in many other countries.

Most ecotoxicological studies are therefore performed in order to enhance the capacity of prediction.

Ideally most ecotoxicological research carried on at the lower level of biological organization should first help in understanding toxicological mechanisms, and second have the ability to predict what will happen at the higher level. Enzymes are studied to predict the effects on cells, and, similarly, organs to predict effects on individuals, and individuals to predict the effects on populations.

For example, the widely accepted definition of a lethal threshold concentration in aquatic toxicology is 'The threshold concentration producing death, for example, the median lethal threshold concentration is the concentration of poison lethal to one half of a test population' (EIFAC, 1983).

Obviously this concept is limited to the 'test population' but implicitly one can say that these data could be used, for example, to predict or to account for a fish kill in the field.

This approach is certainly too approximate. A possible definition of population is 'a group of organisms of the same species occupying a particular space and in time are vague and in practice are arbitrarily fixed' (Krebs, 1978).

A population needs certain descriptors to be fully defined such as, for example, birth rate, spatial distribution or density, age structure, etc. This paper will discuss the population as a possible unit of study from a theoretical point of view, a few applications of population studies in ecotoxicology, and finally some field studies on the effects of mixtures of toxicants on fish populations.

2 THE POPULATION AS A UNIT OF STUDY IN THE AQUATIC ENVIRONMENT

Rigler (1982) pointed out that although in certain cases limnologists and fishery biologists have common goals, i.e. the prediction of future size of fish stocks, they use completely different approaches to the problem.

For example, in discussing the effects of a pollutant on populations, limnologists tend to apply the classical models derived from population dynamics studies (Lotka, 1925) whilst fishery biologists approach the problem by using theoretical models of exploited fish populations such as those of Russell (1931) or Beverton and Holt (1957).

2.1 Population Models

The principal variable in any population ecological model is the size of the population of the organism under investigation within the habitat, geographical

area or laboratory test-tube under consideration. The relations that connect the size and the age structure of a population provide a more or less realistic life-history pattern of the organism under study, this pattern being synonymous with the fitness characteristics of the organism insofar as they exert direct influence over reproduction. The realism of the models is a function of the ecological variables taken into account, whose changes due, for example, to pollution are reflected by changes of several of the parameters of the models themselves.

The classical theory for population growth and regulation considers that, if population growth is a result of the reproduction characteristics of individuals, then populations either grow indefinitely with constant doubling time or vanish. The rationale behind this model is that the individuals of the population are considered to be identical and independent, to die off at a constant rate d and to reproduce at a constant rate b . If the population is assumed to be so large that its size may be measured by a continuous variable N_t (total number of individuals at time t) through time, then the model may be stated as follows:

$$\frac{dN}{dt} = (b - d)N \quad (1)$$

which integrates to

$$N_t = N_0 e^{(b-d)t} \quad (2)$$

where N_0 is the initial population size at time zero. The birth and death rates are connected in the model in such a way that the population behaviour is completely described by the parameter $r = b - d$; r is referred to as the Malthusian parameter, the intrinsic rate of increase, the reproductive potential or the unlimited growth rate (Christiansen and Fenchel, 1977). These are not general characteristics of natural populations, which are subject to biotic restrictions from predators, pathogens, competitors, parasites, shortages of food, nutrients, space and shelter. Thus, population growth limitation and stability must be the results of inhibitory interactions between the individuals in the population, at high densities, and/or among populations of different species.

The simplest possible model of density-dependent regulation is the logistic model for population growth (Lotka, 1925). The model assumes that both the birth and death rates are linear functions of density such that

$$b = b_0 - \alpha_1 N \quad (3)$$

$$d = d_0 - \alpha_2 N \quad (4)$$

where b_0 and d_0 are the birth and death rates when density restrictions are absent and α_1 and α_2 are density-dependent coefficients. Substituting in equation (1) and rearranging, we obtain

$$\frac{dN}{dt} = rN(K - N)/K \quad (5)$$

K is the carrying capacity of the environment, i.e. the upper limit of the population size, where $b = d$.

Observing that

$$\frac{dN}{dt} = rN^2 \left(\frac{1}{N} - \frac{1}{K} \right) \quad (6)$$

and therefore

$$N^{-2} dN = r \left(\frac{1}{N} - \frac{1}{K} \right) dt \quad (7)$$

equation (5) integrates by substitution to

$$N_t = \frac{N_0 K e^{rt}}{K - N_0 + N_0 e^{rt}} \quad (8)$$

This rearranges to

$$N_t = \frac{K}{1 + \left(\frac{K - N_0}{N_0} \right) e^{-rt}} \quad (9)$$

Thus, if we pose

$$\frac{K - N_0}{N_0} = e^a \quad (10)$$

we obtain the standard form of the integrated logistic equation:

$$N_t = \frac{K}{1 + e^{a - rt}} \quad (11)$$

The three assumptions of this equation are: (1) that r and K are constant; (2) that there are no time lags in the response of actual rate of increase per individual to the change in density; and (3) that all individuals are equivalent in their effect on the reduction of the actual growth rate at each density (Kingsland, 1982).

At various times mathematical ecologists have used the intrinsic rate of increase, the carrying capacity (already defined), the reproductive value, the net reproductive rate and related measures based on mathematical demography as the measure of fitness (Istock, 1982), the ultimate parameter of concern in the evaluation of the impact of toxicants on the biotic phase of ecosystems. The measures are derived as follows.

Consider a cohort of animals (a collection of individuals born at the same time) from birth at time 0 to the maximum lifespan ω . If we change the conditions underlying equations (1) and (5), i.e. that all the individuals of the population are equivalent, and assume that the death rate and the fecundity of the individuals are

dependent on age, then the birth and the death of individuals are no longer independent, but are dependent on the age structure of the population.

Let l_t be the proportion of the cohort that survives from birth to age t , and designate by m_t the number of offspring produced by individuals of age t . Each individual of the cohort on the average at age t contributes $l_t m_t$ individuals to the next generation, i.e. to the cohort born at time t .

The whole reproductive contribution of the cohort born at time 0 is the net reproduction per individual per generation, given by

$$R_0 = \int_{t=0}^{t=\omega} l_t m_t dt \quad (12)$$

If l_t and m_t are unchanged through time, then the population will after some time grow at an exponential rate r and attain a stable age structure, characteristic for the value of r and l_t .

If a population grows at a rate r and has attained a stable age structure, then each age is growing at the same rate r . If such a population gives birth to y individuals, then at time $t - n$ there were

$$t e^{-nr}$$

newborn individuals, of which $y e^{-nr} l_n$ survived at time t .

Then, at time t the total population size including all age classes is

$$N_t = \int_{t=0}^{t=\omega} y l_t e^{-rt} dt \quad (13)$$

individuals, which give birth to

$$y = \int_{t=0}^{t=\omega} y l_t m_t e^{-rt} dt \quad (14)$$

individuals at time t . Taking the factor y away on both sides of the equation (14), we obtain

$$1 = \int_{t=0}^{t=\omega} e^{-rt} l_t m_t dt \quad (15)$$

which connects the demographic parameters l_t and m_t to the exponential growth rate r (Lotka, 1925).

2.2. Applications of Population Models

Toxicological analysis through population growth parameters may proceed in principle along two lines: highly sophisticated multiple modelling of effects or plain single parameter statistical analysis, but in neither case is population growth forecasting considered.

The sample of literature we have chosen, although not exhaustive, can be regarded as representative of the different trends.

Winner and Farrell (1976) devised an experimental design to assess both the acute and chronic toxicity of copper to four species of *Daphnia* and compare their sensitivities. The chronic impact of the toxicant at the population level was measured through the analysis of the following indices:

- the survivorship function l_t , which enters in equation (12);
- the mean number of young per brood, which can be considered an index related to equation (12), but less informative;
- the instantaneous rate of population growth, r , calculated by solving equation (15).

According to the authors' conclusions, the survivorship function is the more sensitive index of copper stress at least in the case of *Daphnia magna*. The instantaneous rate of population growth, r , was, so to speak, disturbed by a 'noise' induced by a stimulating effect of copper on reproduction rate at low concentrations. This effect reduced the statistical significance of the treatments, if analysed through parameter r . In our opinion, although a thorough analysis of all parameters which can be taken into account to describe the effects of a stress on a population is good practice, the more sensitive parameters, l_t in this case, may not be the most important.

The future of a population can be much better predicted by r , which can be considered a synthesis of all facets of reproduction, than by any single parameter of equations (12) or (15) (age-specific survivorship and fertility functions, net reproduction). However, the authors concluded by calculating the application factor and estimating the maximum acceptable toxicant concentration from a chi-square analysis of the survivorship curves, and obtained values much larger than those obtained by other authors for fish.

Calamari *et al.* (1979, 1982, 1983) improved the method in testing the effects of amines and chlorobenzenes on *Daphnia magna*. According to their method, age at reproduction maturity, senility, and parameter r itself could be simultaneously analysed using a model of time dependence of r on mean generation time units.

However, they failed to exploit fully the potentialities of the method by not including the quoted parameters in the evaluation of the effect. The analysis was carried out at a qualitative level and therefore could not give much more information than the common statistical analysis of Winner and Farrell, based on

linear multiple comparison techniques. The only advantage was the visualization of the variation of the instantaneous growth rates (Figure 2).

It is important to point out that Calamari *et al.* (1983) reported the EC_{16} (the concentration reducing the fertility by 16 % after a fixed lapse of time) as the no-effect concentration, since a reproductive impairment ranging from 10 % to 20 % could be considered in the range of natural variability (Canton and Adema, 1978). The population dynamics test results were consistent with the other toxicity indices for all the tested chlorobenzenes, and a QSAR (quantitative structure-activity relationship) equation was defined.

The same type of analysis, for the chronic effects of dieldrin, both on *Daphnia pulex* and on the copepod *Eurytemora affinis*, has been applied by Daniels and Allan (1981): the parameters taken into account are always those of equation (15) (the intrinsic rate of population increase and its individual components). These were, as previously, individually analysed, without further considering combined effects. An interesting suggestion of the authors aims at the reduction of the long duration and expensiveness of the life-table method (demographic parameter analysis). As they demonstrated, both for *D. pulex* and for *E. affinis*, the intrinsic rate of population increase calculated over the entire life-table gave an estimate nearly identical with that calculated over a portion of it. This confirms the validity of the time limit for three broods proposed by the OECD method (OECD, 1981).

On the other hand, Kooijman *et al.* (1983) considered several parameters jointly in modelling the effects of toxicants on aquatic organisms such as algae

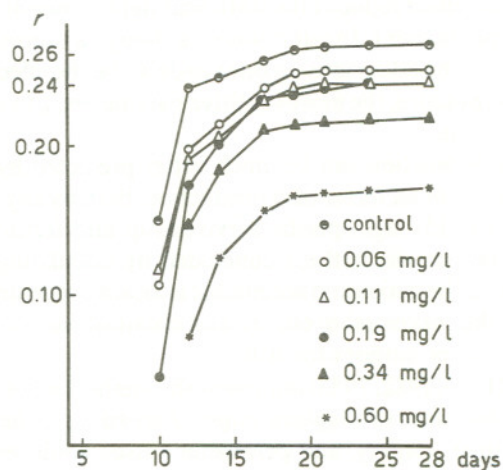


Figure 2 Instantaneous growth rate (r) of *Daphnia magna* population exposed to different 1,2,4-TCB (1,2,4-trichlorobenzene) concentrations. From Calamari *et al.* (1983). Reproduced by permission of Pergamon Press

and bacteria. The model takes into account:

- (a) the number of organisms in the inoculum and their survival to different toxicant concentrations;
- (b) the decrease in the yield of the population;
- (c) the decrease in the intrinsic rate of increase of the population.

The model specification simply assumes that if the population grows logistically the number of reproducing organisms $M(t, c)$, at time t and concentration c of the compound, is given by:

$$\frac{dM(t, c)}{dt} = R(c)M(t, c) \left(1 - \frac{M(t, c)}{Y(c)} \right)$$

which is the same as equation (5), and where $R(c)$ is the reproduction rate r at concentration c , and $Y(c)$ is the yield or carrying capacity at concentration c of the test compound. The initial conditions are stated as

$$M(0, c) = E(c)$$

where $E(c)$ is the number of reproducing organisms in the inoculum at concentration c of the toxicant; this gives the integrated form of the model

$$M(t, c) = \frac{E(c)Y(c)}{E(c) + Y(c) - E(c) \exp[-R(c)t]}$$

which is a rearranged form of equation (8).

The functions E , R and Y are then set for a series of concentrations of a test compound and for the complete model we have

$$E(c_j) = E_b / 1 + \exp\{E_g(\ln c_j - E_e)\}$$

$$R(c_j) = R_b / 1 + \exp\{R_g(\ln c_j - R_e)\}$$

$$Y(c_j) = Y_b / 1 + \exp\{Y_g(\ln c_j - Y_e)\}$$

If for X one reads E , R or Y ,

- X_b are the parameters in the control (blank),
- X_e are the EC_{50} obtained by probit analysis,
- X_g are the gradient concentration–effect constants.

The advantages of this rather complex model, which in the complete form has nine parameters to be solved, are manifold. First, it is the only one that ‘weights’ the effect of a toxicant on all the relevant facets of the biology of a growing population for which the age structure need not be analysed: survival of the inoculum, i.e. time lag; ultimate equilibrium state of the population, i.e. carrying capacity; dynamic trend toward equilibrium as a function of time, i.e. the intrinsic rate of natural increase. Secondly, appropriate experimental designs can simplify the model. For example, the application of ‘no-effect’ concentration, such that, for example, for reproduction $R(c_j) = R_b$, reduces the problems of estimation.

The model has been tested for two parameters at a time on two strains of algae, *Stephanodiscus hantzschii* and *Scenedesmus pannonicus*, with usually quite a satisfactory fit to experimental data. In our opinion, however, two topics, which have been briefly analysed by the authors as consequences of the model, deserve a deeper analysis in a future extension of the model itself, due to their relevance: the 'no-effect' level estimation from the model and the biomass-dependent toxicity. The former issue, i.e. the estimation of the maximum concentration of toxicant at which the control mechanisms of the organism can resist the effects of the toxic compound, would provide the most valuable information for the model. At present, it is only suggested that, for estimating 'no-effect' levels for any parameter, it is necessary, because of estimating problems, to work at concentrations nearly equal to the 'no-effect' level for other parameters. Lastly, as regards the biomass-dependent toxicity, particularly important in the case of algal tests, the authors propose a modified model for the analysis of the observation that sometimes the reproduction rate is not constant, but increases with the number of cells.

In our opinion, this complex of models represents the most notable effort to fully apply the Lotka model to the analysis of a stress on populations. Unfortunately it deals only with single toxicants, and to find some results of models applied to effects of mixtures we are left with much less sophisticated models.

For example, Gray (1974) studied the effects of a mixture of three metals (mercury, lead and zinc) on cultures of *Cristigera* spp. (a marine protozoan). The growth rates are measured as a constant K assessed as

$$K = (\log_2 N_t - \log_2 N_0)/t$$

easily derived from equation (2). Their analytical device is poor as regards the growth index, yet very informative from a statistical point of view, since through a factorial experimental design and polynomial regressions they tested both the control variables (salinity and temperature) and metal concentrations. A more than additive interaction between metals was found as the method performed rather sensitively.

In contrast to the completeness of analysis of the results of the previous authors, Wong *et al.* (1982) did not try to model the relation between single toxicant and mixture effects. They studied the effects of a mixture of ten metals on physiological and biochemical responses of freshwater algae. Apart from the effect on primary productivity of *Scenedesmus*, *Chlorella*, *Anabaena* and *Navicula*, and on nitrogenase activity of *Anabaena*, the only effect at the population level was reported on two indicators: doubling time, estimated from the logarithmic phase of growth curves of *Ankistrodesmus falcatus*, and carrying capacity. All indicators were affected by the metal mixture, but unfortunately there is no modelling of interrelationship among the effects on different indicators. It is therefore impossible to evaluate the sensitivity of their index of population growth.

2.3 Application of Models of Fish Populations

In order to be able to discuss how environmental events and pollution can influence fish stocks, it is useful to describe a theoretical model of an exploited fish population. Although much more sophisticated models have been produced, Russell's model remains a convenient and easily understood statement of the factors involved and can be represented in a symbolic form in the following equation:

$$S_2 = S_1 + (R + G) - (M + F)$$

where S_1 and S_2 are the biomass of the exploitable stock of fish at the beginning and end, respectively, of a defined period and R, G, M, F , the four primary factors contributing to the balance in a self-contained fish population (without emigration or immigration). The symbols can be defined as follows: S = biomass of exploitable stock, the quantity of fishes big enough to be caught with the techniques used in the fisheries; R = biomass of recruits, the quantity of younger fishes which during the time of observation have grown to the catchable size range; G = biomass gained by the growth of individuals already a part of the exploitable stock; M = biomass of fish lost to natural mortality from disease, predation or other causes; F = biomass of fish harvested during the period considered for all the factors.

All the terms of the equation in brackets are evidently linked between themselves and interacting in such a way that a variation in one factor can influence one or more of the others at the level of density-dependent response.

Density-independent responses provoked by natural variation of environmental factors such as temperature, water movement, or chemical composition of the water are not considered.

Problems related to the evaluation of the impact of pollutants discharged into the aquatic environment are broad and complex. Until now reliance has been placed on laboratory work since adequate data are seldom available from field observations.

A broad spectrum of laboratory tests has been performed; a synoptic description is given by EIFAC (1983), and for a number of chemicals a safety level has been tentatively indicated (Alabaster and Lloyd, 1982; Train, 1979).

However, the main weakness of the responses of toxicity tests is that the results are related mainly to the individuals or are not easily transformed or transferred into parameters of interest to estimate the effects on a population; yet, from a biological point of view it is the success of the population and not that of the individual that is important.

The best way to understand how pollution can influence a fish population is to start again with Russell's model and consider in which way the factors representing recruitment (R), growth (G), and mortality (M) can be affected by contaminants.

Mortality can be increased both directly and indirectly. The first case is a mass kill of animals due to heavy discharge of a high-level toxin, or a lack of oxygen from organic waste decomposition. The second case is increased mortality resulting from poor health conditions with gradual decline of the population rather than dramatic changes in the quality of the water. This effect can be caused by sublethal chronic poisoning, commonly produced by industrial discharges or poor environmental conditions.

Reduction in R (recruitment) may result from interference on the reproductive cycle and/or developmental stages. It has been demonstrated for several substances that the early life stages of fish are much more sensitive than eggs or adults, sometimes by orders of magnitude (Calamari and Marchetti, 1978; McKim, 1977).

Zooplanktonic crustaceans frequently have very low tolerances to pollutants of various types and a decrease in their populations can cause a reduction in food available for the larvae and in further development of the population. Decrease in available food can be induced also by an algostatic effect which some metals and organic substances can cause. Most of the events that reduce G (growth) have been mentioned above, such as changes in food availability. Possible sublethal intoxication can increase susceptibility to disease, slow growth by decreasing efficiency of food conversion, and increase energy expenditure for maintenance.

An attempt to apply mathematically the described concepts was made by Wallis (1975), who proposed a model for the impact of a waste on a fish population. The basic assumptions were similar to those of Russell's model, but more complex:

$$F_{n+1} = f(F_n, b, E, G_n, G_w)$$

where F_{n+1} is the number of fish in the year $n + 1$; F_n is the number of fish in the year n ; b is a parameter describing the stability of the population; E is the population at equilibrium; G_n is the distribution of variation in environmental conditions due to natural causes; and G_w is the distribution of variations in environmental conditions due to the waste load. Through a number of transformations Wallis (1975) subdivided the basic equation, representing the change of a population over one year, into two parts, the first of which accounts for deterministic processes and the second for stochastic processes:

$$F_{n+1} = F_n + b(E - F_n) + (e^{k-w} - 1) F_n$$

where k is a random variable, while w is the waste impact parameter (i.e. waste concentration in relation to fish mortality). In the absence of waste $w = 0$ and $(e^k - 1) F_n$ accounts only for the stochastic variation of environmental parameters.

After an analysis of the model using simulation and an evaluation of its stability, the author recommended, when applying the model to a particular situation, following a number of steps including an evaluation of the impact of the

waste based on laboratory experiments, which, unfortunately, accounts only for fish mortality.

Waller *et al.* (1971) followed a different approach. They wanted to test the hypothesis that a reduction of 20% in egg production, caused by long-term exposure to 1/200 of the 96-hour LC_{50} of a metal, is biologically insignificant in terms of sustaining a population of fathead minnows (*Pimephales promelas*).

They started from a slight modification of the Ricker (1954) model:

$$R = (\alpha Se^{-\beta S}) e^{\varepsilon}$$

where R = total return from S number of spawners; S = number of spawners in parent year; α and β = parameters estimated from empirical observations; ε = error term associated with the model and log-normally distributed. Parameters α and β , which are constants characteristic of the system, were calculated from field data on the abundance of spawning females and the resulting abundance of female progeny. By means of these data the fluctuations of the population in a 50-generation cycle were simulated.

The calculations were made first in the absence of any environmental variation, secondly with a stochastic term due to environmental influence, by assigning extreme values to error term ε .

Finally, a third simulation was performed adding a superimposed mortality, step by step from 1% to 99%, applying a fixed mortality to the population of females in each generation and subtracting the calculated number before the next spawn cycle.

It was shown that the population goes to extinction when superimposed mortality reaches 78% for the lower level of environmental variation and 71% for the higher level of environmental variation. Therefore a 20% reduction in egg production does not theoretically cause an irreversible damage in the population under study.

2.4 Field Studies on Fish Populations

As already mentioned in the Introduction there are a large number of studies on biological monitoring of aquatic organisms, but most of them lack both chemical analysis and toxicological information. Obviously there are exceptions, among them a number of papers describing work carried on in the United Kingdom that were recently reviewed by EIFAC (1980). The research started with the comparison between predicted and observed toxicity of effluents, containing different poisons, in laboratory experiments (Lloyd and Jordan, 1963, 1964). The assumption was that toxicants were additive and the toxicity could be calculated by adding the toxic unit (TU) of each poison. According to the EIFAC (1983) definition, a toxic unit is the concentration of poison expressed as a proportion of its asymptotic threshold concentration, commonly or conveniently expressed as a proportion of some approximation (e.g. 48-hour LC_{50} , 96-hour LC_{50}) of this

concentration. The research project was successfully developed into field studies. Most of the papers quoted refer to mixtures composed of metals, ammonia, phenol and cyanide.

Edwards and Brown (1967) attempted to find the Σ TU (the sum of concentration of toxicants expressed as a fraction of their respective concentration lethal to 50% of fish for a defined period) above which a fish population would not exist. The concentrations of chemical substances polluting a river were analysed in 100 stations and the values of Σ TU 48-hour LC_{50} were correlated to the status of the fish population at the place of sampling. They found that coarse fish populations could exist when Σ TU 48-hour LC_{50} was less than 0.3–0.4 for rainbow trout. However, in a later paper Brown *et al.* (1970) suggested that the absence of fish could be predicted when Σ TU exceeded 1.1–1.4 of the 48-hour LC_{50} for salmonids, but criticized previous results on the basis that analytical data referred only to a limited number of poisons; therefore the predictions tended to underestimate the toxicity.

Moreover, laboratory studies showed that relevant mortalities were recorded after long-term treatments with 0.2 TU of the 48-hour LC_{50} . Fish can also move along the studied part of the stretch of river and in doing so not be continuously exposed to the toxicants.

Alabaster *et al.* (1972) improved the described framework of research, evaluating also the influence of the annual frequency distribution of various toxicants. These distributions tended to be log-normal.

Data indicated that a coarse fish population was present when Σ 48-hour LC_{50} for rainbow trout was 0.28 of the 50 percentile and 0.6 with the corresponding 95 percentile. A percentile is the value below which the stated percentage of a series of measurement falls (EIFAC, 1983). However, the information on fish population was reported in a synthetic way and not analytically displayed or quantified, i.e. from eight to ten species were present, the age structure of the sample of roach appeared abnormal, etc.

A better description of fish population was given by Solbé (1973), who studied a river polluted mainly by zinc and ammonia. Fish were caught by electrofishing in the river and by nets and traps in the small lakes. He found that a good non-salmonid population exists where the values of the 50 percentile of Σ TU 48-hour LC_{50} for rainbow trout were less than 0.25 and 0.9 was the 95 percentile, over a period of three years. The distribution of fish species was given in detail, along the river and in the small lakes, together with the percentage of each species caught by number. Size-class distribution was also studied for the roach (*Rutilus rutilus*) but the data were not further elaborated. The growth rate of this species has also been considered and it was demonstrated that it was lower in the stream than in the small lakes.

A wide study on fish and fisheries in an area with a number of lakes polluted by copper and zinc was performed by Norway (EIFAC, 1977). The sum of the concentrations of copper and zinc slightly exceeded the water quality criteria

proposed by EIFAC (1973, 1976) in Lake Ringvatnet and Lake Hostovatnet, where there was a good fishery, while Lake Bjørra, presenting copper and zinc concentrations much higher than EIFAC standards, was fishless.

Acclimation, concentration distribution in time and chemical characteristics of water (the presence of a certain quantity of chelating substances, e.g. humic acids) could account for the presence of good fisheries in the two lakes.

A quantitative survey of fish population was not made; however, the status of a fish population at a fishery was estimated by means of several parameters. Condition factors and growth rates of the fish were normal and population density and parasites appeared to be the major factors in controlling the growth rate.

Data for catch/effort were also presented and were constant in the years 1969–1975; the total yield estimated by the catch data of sports fishermen indicated a figure of 3–4 kg per hectare per year, which is quite good for Norwegian trout and char lakes.

These findings were indirectly confirmed from the data presented by Van Loon and Beamish (1977), who found fish absent in Ross Lake with high levels of copper and zinc, and a slight reduction in fish population in Hammell Lake, where during limited periods in the year the sum of metal concentrations exceeded the 95 percentile of water quality criteria indicated by EIFAC while the 50 percentile of the sum of the two metals was 0.5. On the other hand, in Cliff Lake, where copper and zinc were present but at lower concentrations, fish were flourishing.

Evidence of the status of fish populations was given examining the species composition in the lakes, the year classes of captured fish and the status of the ovaries of the females. Moreover, by comparing the catch per unit of effort, Lake Hemell appears to have smaller densities of some species in respect to other nearby lakes, thereby confirming the effects of the occasional occurrence of high concentration peaks.

3 DISCUSSION

Laboratory studies on life-cycles and early life stages of fish are considered important in defining water quality criteria for fish (Calamari and Marchetti, 1978) and the existing literature is relatively abundant (see, for example, McKim, 1977). However, no attempt has been made to correlate the results of these toxicological tests in the laboratory with the possible effects on a population, the only theoretical attempt being that of Waller *et al.* (1971).

Most of the considerations on the results of the tests on life-cycles or on early life stages of fish have followed a logical pathway internal to the discipline: comparisons have been made among the results of life-cycle tests and those considering only developmental stages; water quality criteria have been proposed

stressing the importance of these kinds of data; maximum acceptable toxicant concentrations have been derived from life-cycle tests, etc.

Similar consideration could be made also for invertebrate life-cycles or reproduction studies; for example, Blank *et al.* (1978) state only that 'the ratio between adult 3-wk LC_{50} and EC_{50} for reproductive impairment is generally close to 1; i.e. effects on reproduction were generally not more sensitive than long-term lethal effects on adults.' On the other hand, Canton and Adema (1978) underlined the theoretical importance of a test on reproductive potential of a group of animals but quite arbitrarily indicated 20% as a limit to be considered in a range of natural variability. Moreover, Calamari *et al.* (1983) reported EC_{16} as the 'no effect' concentration, considering only the parameter σ , one standard deviation, below the EC_{50} in probit analysis.

To the writers' knowledge no attempts have been made to account for the relationships between the response to stress and the normal parameter of population biology of the species under study, e.g. whether the organism is an *r*- or a *K*-strategist.

This review tends therefore to show the developing state of the art in the field of the assessment of toxic effects on aquatic populations. The existence of only a limited number of papers on the subject and the reluctance to exploit existing population models confirm the scarce theoretical basis of work carried out.

It has also been shown how far in practice fish population models are from the research already performed on fish populations in the field. However, although not going deeply into modelling the structure of fish populations, the short review of the methods and the results of field studies made in section 2.4 show a promising framework for the assessment of the effects of mixtures on fish populations and introduces the conclusions of the EIFAC report on mixtures of toxicants (EIFAC, 1983).

Finally, it can be concluded that in order to study the effects of mixtures of chemicals on aquatic populations, at least to a first approximation, the concentration-addition models appear to be adequate. However, the approach based on population studies is weaker. There is a need to develop methodologies for the optimization of the information that can be obtained by toxicological tests on populations.

4 REFERENCES

- Alabaster, J. S., and Lloyd, R. (1982). *Water Quality Criteria for Freshwater Fish*, 2nd edition. Butterworths, London: 361 pages.
- Alabaster, J. S., Garland, J. H. N., Hart, I. C., and Solb , J. F. de L. G. (1972). An approach to the problem of pollution and fisheries. *Symp. Zool. Soc. Lond.*, 87-114.
- Beverton, R. J. H., and Holt, S. J. (1957). *On the Dynamics of Exploited Fish Populations*. United Kingdom Ministry of Agriculture and Fisheries, Fish. Invest. Series 2, Vol. 19, 533 pages.

- Blank, H., Dave, G., and Gustafsson, K. (1978). *An Annotated Literature Survey of Methods for Determination of Effects and Fate of Pollutants in Aquatic Environments*. Report from the National Swedish Environment Protection Board. 398 pages.
- Brown, V. M., Shurben, D. G., and Shaw, D. (1970). Studies on water quality and the absence of fish from some polluted English streams. *Water Res.*, **4**, 363-382.
- Calamari, D., and Marchetti, R. (1978). Relevance of studies on developmental and young stages of *Salmo gairdneri* in establishing water quality criteria for fisheries. *Ber. Umweltbundesamt*, **10**, 201-210.
- Calamari, D., Galassi, S., and Da Gasso, R. (1979). A system of tests for the assessment of toxic effects on aquatic life: an experimental preliminary approach. *Ecotoxicol. Environ. Saf.*, **3**, 75-89.
- Calamari, D., Galassi, S., and Setti, F. (1982). Evaluating the hazard of organic substances on aquatic life: the paradichlorobenzene example. *Ecotoxicol. Environ. Saf.*, **6**, 369-378.
- Calamari, D., Galassi, S., Setti, F., and Vighi, M. (1983). Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere*, **12**, 253-262.
- Canton, J. H., and Adema, D. D. M. (1978). Reproducibility of short-term and reproduction toxicity experiments with *Daphnia magna* and comparison of the sensitivity of *Daphnia magna* with *Daphnia pulex* and *Daphnia cucullata* in short-term experiments. *Hydrobiologia*, **59**, 135-140.
- Christiansen, F. B., and Fenchel, T. M. (1977). *Theories of Populations in Biological Communities*. Springer-Verlag, Berlin.
- Daniels, R. E., and Allan, J. D. (1981). Life table evaluation of chronic exposure to a pesticide. *Can. J. Fish. Aquat. Sci.*, **38**, 485-494.
- Edwards, R. W., and Brown, V. M. (1967). Pollution and fisheries: a progress report. *Water Pollut. Control*, **66**, 63-78.
- EIFAC (1973). *Working Party on Water Quality Criteria for European Freshwater Fish. Report on Zinc and Freshwater Fish*. EIFAC Technical Paper 21. European Inland Fisheries Advisory Commission, Rome: 22 pages.
- EIFAC (1976). *Working Party on Water Quality Criteria for European Freshwater Fish. Report on Copper and Freshwater Fish*. EIFAC Technical Paper 27. European Inland Fisheries Advisory Commission, Rome: 21 pages.
- EIFAC (1977). *Working Party on Biological Monitoring. Report on the Effect of Zinc and Copper Pollution on the Salmonid Fisheries in a River and Lake System in Central Norway*. EIFAC Technical Paper 29. European Inland Fisheries Advisory Commission, Rome: 34 pages.
- EIFAC (1980). *Working Party on Water Quality Criteria. Report on Combined Effects on Freshwater Fish and Other Aquatic Life of Mixtures of Toxicants in Water*. EIFAC Technical Paper 37. European Inland Fisheries Advisory Commission, Rome: 49 pages.
- EIFAC (1983). *Working Party on Toxicity Testing Procedures. Revised Report on Fish Toxicity Testing Procedures*. EIFAC Technical Paper 24 (Rev. 1). European Inland Fisheries Advisory Commission, Rome: 37 pages.
- Gray, J. S. (1974). Synergistic effects of three heavy metals on growth rates of a marine ciliate protozoan. In Vernberg, F. J., and Vernberg, W. B. (Eds.) *Pollution and Physiology of Marine Organisms*, pp. 465-485. Academic Press, New York.
- Istok, C. A. (1982). Some theoretical considerations concerning life history evolution. In Dingle, H., and Hegmann, J. P. (Eds.) *Evolution and Genetics of Life Histories*, pp. 7-20. Springer-Verlag, Berlin.
- Kingsland, S. (1982). The refractory model: the logistic curve and the history of population ecology. *Q. Rev. Biol.*, **57**, 29-52.
- Kooijman, S. A. L. M., Hanstveit, A. O., and Odgersma, H. (1983). Parametric analyses of population growth in bio-assays. *Water Res.*, **17**, 527-538.

- Krebs, C. J. (1978). *Ecology. The Experimental Analysis of Distribution and Abundance*, 2nd edition. Harper and Row, New York: 678 pages.
- Lloyd, R., and Jordan, D. H. M. (1963). Predicted and observed toxicities of several sewage effluents to rainbow trout. *J. Proc. Inst. Sewage Purif.*, **1963**, 167–173.
- Lloyd, R., and Jordan, D. H. M. (1964). Predicted and observed toxicities of several sewage effluents to rainbow trout: a further study. *J. Proc. Inst. Sewage Purif.*, **1964**, 163–186.
- Lotka, A. J. (1925). *Elements of Physical Biology*. Williams and Wilkins, Baltimore: 460 pages. (Reprinted 1956 as: *Elements of Mathematical Biology*, Dover Publications.)
- McKim, J. M. (1977). Evaluation of tests with life stages of fish for predicting long-term toxicity. *J. Fish. Res. Board Can.*, **34**, 1148–1154.
- OECD (1981). *Guidelines for Testing of Chemicals*. Organization for Economic Cooperation and Development, Paris.
- Ricker, W. E. (1954). Stock and recruitment. *J. Fish. Res. Board Can.*, **11**, 559–623.
- Rigler, F. H. (1982). The relation between fisheries management and limnology. *Trans. Am. Fish. Soc.*, **111**, 121–132.
- Russell, E. S. (1931). Some theoretical considerations on the 'overfishing' problem. *J. Cons. Cons. Int. Explor. Mer*, **6**, 3–20.
- Solbé, J. F. de L. G. (1973). The relation between water quality and the status of fish populations in Willow Brook. *Water Treat. Exam.*, **22**, 41–61.
- Train, R. E. (Ed.) (1979). *Quality Criteria for Water*. Castle House, London: 256 pages.
- Van Loon, J. C., and Beamish, R. J. (1977). Heavy-metal contamination by atmospheric fallout of several Flin Flon area lakes and the relation to fish populations. *J. Fish. Res. Board Can.*, **34**, 899–906.
- Waller, W. T., Dahlberg, M. L., Sparks, R. E., and Cairns, J., Jr. (1971). A computer simulation of effects of superimposed mortality due to pollutants on populations of fathead minnows (*Pimephales promelas*). *J. Fish. Res. Board Can.*, **28**, 1107–1112.
- Wallis, I. G. (1975). Modelling the impact of waste on a stable fish population. *Water Res.*, **9**, 1025–1036.
- Winner, R. W., and Farrell, M. P. (1976). Acute and chronic toxicity of copper to four species of *Daphnia*. *J. Fish. Res. Board Can.*, **33**, 1685–1691.
- Wong, P. T. S., Chau, Y. K., and Patel, D. (1982). Physiological and biochemical responses of several freshwater algae to a mixture of metals. *Chemosphere*, **11**, 367–376.