ECOTOXICOLOGICAL TESTING FOR THE MARINE ENVIRONMENT. 1984.
G. Persoone, E. Jaspers, and C. Claus (Eds). State Univ. Ghent
and Inst. Mar. Scient. Res., Bredene, Belgium. Vol. 2. 588 p.

SYNERGISTIC TESTS ON MARINE INVERTEBRATES. RELATIONS WITH THE NATURAL ENVIRONMENT

G. STORA, G. BELLAN, and R.A. KAÏM-MALKA

Station marine d'Endoume,
Centre National de la Recherche Scientifique
Rue de la Batterie des Lions
F-13007 Marseille, France

ABSTRACT

Most often in perturbed biotopes, the marine fauna is not only submitted to a single type of stress but also to a mixture of deleterious factors acting synergistically. The experiments , described inthis paper were therefore conducted in perfectly controlled conditions allowing the simulation of the principal types of isolated or combined stresses exerted in a perturbed environment on diverse benthic invertebrates. These experiments were based on comparative short- and medium-term studies ofthe sensitivity of the organisms submitted to a single type of stress as a reference, or to a gradient of additional stress. This sensitivity was determined by assessing of the lethal concentration or lethal time. For chosen species the evolution of the sensitivity was determined by "sensitivity indices". These indices were computed as the ratio of the concentration or the lethal time obtained for the diverse stress combinations, to that of the stress used as a reference point. The necessity to obtain a maximum of directly comparable results in each experimental series, led to the development of specific experimental procedures.

Several factors were studied with regard to their effect on various benthic invertebrates representative for the littoral biotope, i.e. the effect of a lethal pollutant in different combinations of temperature and/or

salinity and at different concentrations of dissolved oxygen, the mixture of chemically differing pollutants, and the effect of increasing concentrations of pollutants on physiologically weakened organisms.

The knowledge of the *in situ* and *in vitro* dynamics of a species with regard to similar agressive constraints, permits by comparison, the demonstration of the possibility and the reliability of an extrapolation to the natural environment of experimentally obtained results.

KEYWORDS

Marine ecotoxicology, Methods, *Echinogammarus stocki*, *Venerupis aurea*, *Nereis succinea*, Detergents, Heavy metals, Synergism, Stress, Sensitivity index, Field verification.

INTRODUCTION

In a perturbed ecosystem, the composition and the dynamics of a population essentially depend on the aptitude of the species to resist the stress provoked by the totality of deleterious factors present in the environment. Ecotoxicological studies permit, in perfectly controlled conditions, to determine the action of different stresses and to recognize (according to their nature) the isolated or combined factors capable of inducing the specific mechanisms of faunal degradation and adaptation.

The objectives of the *in vitro* studies and the *in situ* research conducted in the Gulf of Fos and the Berre lagoon (Stora, 1982) were twofold. Firstly, to assess the action of the different combinations of deleterious factors *in vitro* on the species representative for the ecosystem studied. Secondly, to demonstrate the feasibility of the extrapolation of the experimental results, by a comparative study of the *in vitro* and *in situ* species' dynamics, to the natural environment, a crucial problem in ecotoxicological studies.

The *in situ* research conducted in the Gulf of Fos and the Berre lagoon showed that population dynamics were heavily influenced by the rapid mortality of the species. Consequently, short- and medium-term lethal tests on adult specimens were run. These investigations permitted the assessment of the evolution of the sensitivity evolution of the species to various stress combinations. This research allowed Stora (1978) and Stora and Romano (1982) to evaluate the possibility of establishing a relation between in vitro and in situ data.

Research on the evolution of the sensitivity was based essentially on the analysis of the sensitivity of the species submitted to different types of combined stresses with increasing intensity, as compared to their sensitivity to a single type of stress used as a reference. The problem posed by this type of study essentially concerns the possibility of comparing with certainty, the results as a whole and thus to demonstrate the specific action of the reference stress and of the various combinations of stresses. This certainty can only be acquired by following a rigorously identical methodology and by identifying all factors, other than the stresses applied, which could induce a variation in the sensitivity of the organisms.

MATERIALS AND METHODS

MATERIALS

The experiments were carried out in a continuous-flow system (Fig. 1). The pollutant was dosed with a Technicon dosage pump with calibrated tubes and the seawater with Masterflex peristaltic pumps with a variable flow rate. The stock solution of the pollutants was pumped into glass containers with a 2 l capacity and seawater of the chosen salinity was stored into 60 l polyethylene vessels. To ensure homogenous mixing of the test solutions, the tubes containing the pollutant emptied into the seawater tubes prior to the inflow of the test medium into the experimental aquaria. The polystyrene aquaria (length 35 cm, width 19 cm, height 16 cm) contained 8 l of solution. This test volume was sufficient for all test species. Each aquarium could hold three circular polyethylene baskets (diameter 12 cm) with a Nytrel net bottom with a mesh size of 0.5 mm. The pumping system was adjusted to provide a renewal of the experimental media every 16 h. The experimental solution was partially drained by an overflow pipe and also by an unprimed siphon system allowing, the draining of the solution from the bottom of the aquarium.

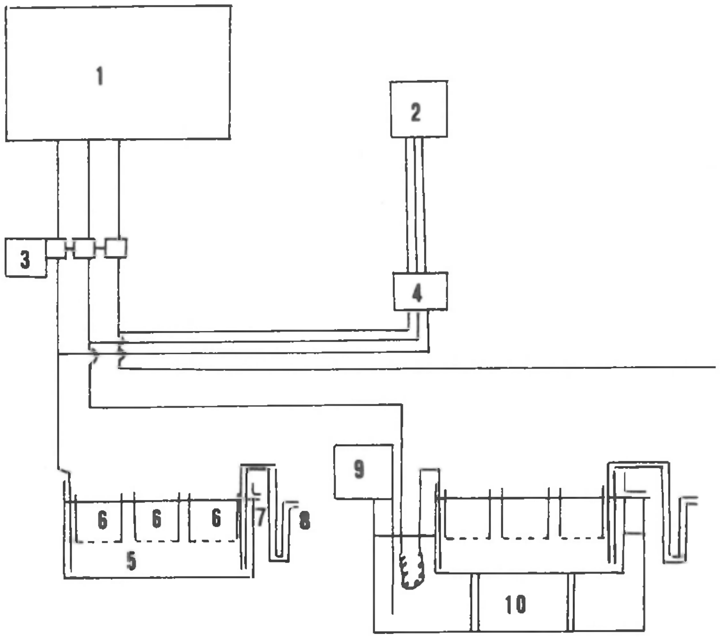

Fig. 1. Scheme of the experimental flow system. 1 = seawater ; 2 = pollutant stock ; 3 = seawater pump ; 4 = pollutant pump ; 5 = experimental aquaria ; 6 = baskets ; 7 = overflow tube ; 8 = siphon ; 9 = thermostat or cryostat ; 10 = water bath.

The experimental temperatures were obtained by placing the aquaria in a thermostatically controlled room. To obtain temperatures higher and lower than the ambient one a water-bath system heated or cooled by thermostats or cryostats were used. A multi-coiled tube placed in the water bath and the aquaria ensured the desired temperature in each experimental setup. The illumination regime room was fixed at a photoperiod of 12 h of light and 12 h of darkness.

EXPERIMENTAL TEMPERATURES

The experimental temperatures were 12, 17, and 22 °C. Although for technical reasons the possible range of the test temperatures was very limited the chosen temperatures were representative for the natural environment. It was considered a favourable coincidence that the intervals between the test temperatures equaled ± 5 or ± 10 °C.

EXPERIMENTAL SALINITIES

The experimental salinities varied according to the species employed. For the species of euryhaline eurytherm biocenoses, sampled from the Berre lagoon, the salinities chosen were 5, 15, and 25 ‰. For species from the biocenosis in muddy sands in sheltered areas the test salinities were 25, 30, and 35 ‰. This range of nonlethal salinities permitted to establish salinity stresses equivalent to ± 5 ‰ or 10 ‰. To maintain an ionic equilibrium close to that found in the natural environment, the different salinities were obtained by mixing seawater of 37 ‰ with tap water. To eliminate any trace of residual chlorine, the freshwater was passed through an activated charcoal filter.

OXYGENATION

A change in the sensitivity of the organisms can be induced by variations in the dissolved oxygen concentration in the medium depending on the test species and the stress applied (Reish 1966, 1970 ; Jones, 1974 ; Thurberg *et al.*, 1974 ; Ritz, 1980). In order to maintain identical oxygen conditions close to 100 % saturation aerators were placed in each experimental aquarium. In the experiments testing the influence of dissolved oxygen concentrations varying between 40 and 100 %, the saturation level was obtained by mixing air and azote in various proportions.

DELETERIOUS FACTORS

A reference stress was induced by exposing the test species to lethal concentrations of a series of pollutants, *i.e.* mercuric chloride and three nonionic detergents, Cemulsol 870, Syntopon C, and Plurafac RA43. Syntopon is an akyl aryl oxyethylene detergent. The two other detergents contain a linear oxyethylene chain in (Plurafac RA43), and a ramified alcohol with 13 carbon oxyethylene groups in (Cemulsol 870).

The choice of the test concentrations of the various pollutants will be discussed elsewhere.

TEST SPECIES

As pointed out by Bellan (1981) the test species must be chosen according to the objectives and must take into account certain criteria into account which depend on the experimental conditions and on the characteristics of the test organisms.

Among these criteria particular attention should be paid to the following :

- the possibility to obtain a large number of animals within a short lapse of time, while avoiding sampling stress ;
- their high degree of resistance to laboratory conditions ;
- their size in relation to experimental conditions and ease of manipulation ;
- their sensitivity to the deleterious factors studied ;
- the degree of representativeness of the ecosystem studied.

Many preliminary experiments have been conducted with the crustaceans *Balanus eburneus*, *Echinogammarus stocki*, the pelecypods *Venerupis aurea*, *Cerastoderma glaucum*, *Mytilus galloprovincialis*, the polychaete *Nereis succinea*. Finally three species *Echinogammarus stocki*, *Venerupis aurea*, and *Nereis succinea* belonging to different taxonomic groups were chosen based on the criteria mentioned above.

During the experimental period the amphipod *E. stocki* was by far the mostabundant species in the environment and also the most practical one to manipulate. Therefore most experiments were conducted with this crustacean. The other species were used to confirm or reject the evolution of the sensitivity observed.

CONDITIONS OF ACCLIMATION

In addition to the specific acclimation linked to experimentation which will be discussed later, the test species were maintained under rigorously controlled acclimation conditions. After collection, the different species were placed in 50 l aquaria in seawater of 17 ± 0.5 °C with a salinity of 15 ‰ for the species sampled from euryhaline and eurytherm biocenosis, and of 30 ‰ for those species from the muddy sand biocenosis in sheltered areas. During the first week the water in the aquaria was changed daily. Later it was replaced once every 3 days. Usually the organisms were used in the 15 days following their collection.

The experiments were invariably started between 16.00 and 18.00 h in order to avoid all problems of sensitivity variation linked to the circadian cycle of the species (Bellan-Santini et al., 1979).

EVALUATION AND PRESENTATION OF THE RESULTS

In short- and medium-term tests, the numerical terms currently used to evaluate the sensitivity of organisms to the various deleterious factors are the lethal concentration, causing mortality in 50 % of the organisms in a given time, generally 48 and 96 h (LC50), or the lethal time necessary to kill 50 % of the organisms at a specific concentration (LT50).

The assessment of the evolution in the sensitivity of the test species submitted to a series of stresses may be difficult to accomplish by using lethal concentrations. Indeed, the research on the evolution of lethal concentrations necessitates the running of preliminary experiments to define the concentration intervals for which a increase in the mortality rate at 48 and 96 h may be expected (Stora, 1972). These intervals vary depending on the synergistic effects of the various deleterious factors and their determination requires numerous experiments. Therefore preference was given to use the lethal time as the criterion allowing a greater number of stress combinations to be tested out simultaneously.

Independent from the value of the lethal concentration or the lethal time, the computation and choice of either the LC50 or LT50 poses problems in all ecotoxicological studies pertaining to the evaluation of the evolution of the species' sensitivity to deleterious parameters (Stora and Bellan, 1977).

COMPUTATION OF THE LETHAL TIME

The lethal time was computed according to the method of Bliss (1938) adapting an earlier method (Bliss, 1935) for experimental work with a small number of test animals. This statistical treatment of data is currently used in toxicology and ecotoxicology (APHA, AWWA, WPCP, 1976 ; Ramade, 1977 ; Möller, 1979).

Along with the LT50, the lethal time necessary to kill 10 % and 90 % of the animals (respectively LT10 and LT90) were calculated for each stress type. In addition to the higher precision derived from determining the LT10 and LT90 along with the LT50 with regard to the sensitivity of the animals and the "margin of action" of the combinations studied (Stora, 1975), it could be considered that death of 10, 50, and 90 % of the animals correspond to a species response at a weak medium or strong stress.

ASSESSMENT OF THE EVOLUTION IN SENSITIVITY

The synergistic effects caused by a mixture of various deleterious factors may be more or less toxic than the effect of each deleterious factor separately. The working party on toxicity-testing procedures of the "Commission européenne consultative pour les Pêches dans les Eaux intérieures" (CECPI, 1983) recognized the following three types of interactions:

- when the toxic effect of a mixture is equal to the sum of the toxic effects of the different deleterious factors considered, the mixture causes an "additive" toxicity ;
- when the toxic effect of a mixture is higher than the sum of the toxic effects of the different deleterious factors considered, the mixture has a "more-than-additive" toxicity, and a synergistic action among the various deleterious factors exists ;
- when the toxic effect of a mixture is lower than the sum of the toxic effects of the different deleterious factors considered, the mixture has a "less-than-additive" toxicity, and an antagonistic action among the different deleterious factors occurs.

Depending on these synergistic effects, the sensitivity of the test species will be decreased or increased by an additional stress. The assessment of the evolution in sensitivity of the organisms is facilitated by the use of the "sensitivity indices" computed as the ratio of the lethal time for a combination of stresses and lethal time obtained for a single stress (reference stress) (Stora, 1978) :

$$IS = \frac{LT_2}{LT_1}$$

in which:

- IS is the index of sensitivity ;
- LT_1 is the lethal time for a pollutant x ;
- LT_2 is the lethal time for the same pollutant associated with another deleterious factor y ;
- $IS = 1$ when there is no evolution ; the additional stress does not modify the sensitivity of the test species to pollutant x ;
- $IS < 1$ there is a positive evolution in sensitivity ; the additional stress provokes an increased sensitivity of the test species to pollutant x ;
- $IS > 1$ there is a negative evolution in sensitivity ; the additional stress decreases the sensitivity of the species to pollutant x.

STUDY OF THE EVOLUTION IN SENSITIVITY OF THE DIFFERENT TEST SPECIES SUBMITTED TO A GROWING GRADIENT OF STRESS

In preliminary studies with Echinogammarus stocki and Venerupis aurea the concentration of each pollutant causing a lengthening of the lethal time in 1 or 2 weeks, in the absence of an additional stress, was determined. Although the experimental time depended upon the evolution of the test species' sensitivity itself depending upon the combination of stresses applied, the experiments could usually be terminated after 3 weeks.

The majority of the experiments were carried out with batches of 16 to 20 animals and in one to three replicates. Irrespective of the test species, this practice yielded reproducible results with respect to the lethal time for animals sampled on the same day and acclimated to identical laboratory conditions.

On the other hand, a certain variation occurred in the results obtained for the same type of stress for different acclimation periods or different collection dates. This variation was linked to the physiological state of the animals. Even if the influence of acclimation can be controlled by using the test animals only a certain time after collection (Stora, 1972), the physiological state of the animals at the sampling moment remains unknown especially in more or less strongly perturbed ecosystems such as the Berre lagoon and the Gulf of Fos. Because the experiments did not aim at determining the sensitivity of the animals for a particular stress, the variability due to varying physiological condition was no problem.

The study of the sensitivity evolution, however, depends on the comparison of the sensitivities of the animals to different types of stresses. The necessity of obtaining a maximum number of directly comparable results which are independent of the variation in species' sensitivity due to experimental constraints, such as the acclimation period or the physiological state of the animals at the moment of collection, led to the perfecting of certain specific experimental procedures.

COMBINED POLLUTANT - SALINITY - TEMPERATURE STRESS

The experiments were conducted with *E. stocki* and *V. aurea*. The sensitivity evolution of *E. stocki*, subjected either to a lethal concentration of 0.4 $mg.l^{-1}$ of mercuric chloride (Stora, 1980a) or to a 10 $mg.l^{-1}$ concentration of the detergent Syntopon C (Stora, 1980b), were studied in relation to increasing salinity and temperature stresses. Similar tests were run with *V. aurea* exposed to a lethal concentration of 0.4 $mg.l^{-1}$ mercuric chloride.

Experimental procedure

The animals of each species collected on the same day were divided into three groups kept at three different acclimation temperatures (AT), *i.e.* 12, 17, and 22 °C. For each temperature three subgroups were respectively acclimated to the acclimation salinities (AS) of 5, 15, and 25 ‰ (25, 30, and 35 ‰ for *V. aurea*). After one week of acclimation, each subgroup was divided into six lots of 20 animals. The first three lots were subjected to the same concentration of the pollutant at experimental temperatures (ET) of respectively 12, 17, and 22 °C and a single experimental salinity (ES) of 15 ‰ (30 ‰ for *V. aurea*).The other three were used as controls and kept under the same conditions without being exposed to a pollutant.

Stresses applied to each test species

The methodology allowed the study of the evolution of sensitivity in E. stocki and V. aurea exposed to different types of single or combined stresses, applied successively or simultaneously. For a particular experimental temperature, the species were exposed to :

- the action of the pollutant alone = type 1 stress (Str1) ;
- the combination of a pollutant + salinity shock caused by transferring the animals from the acclimation salinity AS to the experimental salinity ES = type 2 stress (Str2 S) ;
- the combination of a pollutant + temperature shock caused by transferring the animals from the acclimation temperature AT to the experimental temperature ET = type 2 stress (Str2 T) ;
- the combination of a pollutant + temperature + salinity shock = type 3 stress (Str3).

For each experimental temperature, the animals were submitted to the same salinity fluctuations corresponding either to an increase (Str2 S+) or decrease (Str2 S-) in a salinity of 5 or 10 ‰ depending on the species tested. For each experimental temperature, however, the temperature fluctuations to which the animals were submitted depended upon the acclimation conditions and the experimental temperatures, and were of the order of ± 5 °C (Str2 T ± 5 °C), or - 5 °C to - 10 °C (Str2 T - 5 °C, Str2 T - 10 °C) or + 5 °C to 10 °C (Str2 + 5 °C, Str2 + 10 °C). These combinations of experimental stresses are presented in Table I.

The controls were not subjected to a toxicity stress but only to a temperature or salinity stress or to a combination of the two.

Gradients of stress studied

The methodology utilized led in each experimental series, to directly comparable results as a whole. From the results for the different combinations employed, it was possible to follow the evolution in sensitivity of the test species submitted to a increasing stress gradient. This increasing gradient for each experimental temperature as compared to the setups subjected only to a reference stress Str1, may have been due to temperature shocks of ± 5 °C or ± 10 °C or salinity shocks of ± 5 ‰ or ± 10 ‰ or a combination of both. The different gradients are shown in Table II.

Table I. Synopsis of the different combinations of stresses depending upon the conditions of acclimation. Salinity values between brackets are those used for *V. aurea*. (AS = acclimation salinity ; ES = experimental salinity ; AT = acclimation temperature ; ET = experimental temperature)

AS	5 ‰ (25 ‰)			15 ‰ (30 ‰)			25 ‰ (35 ‰)		
ES	15 ‰ (30 ‰)			15 ‰ (30 ‰)			15 ‰ (30 ‰)		
AT / ET	12 °C	17 °C	22 °C	12 °C	17 °C	22 °C	12 °C	17 °C	22 °C
12 °C	Str2 S+	Str3 - 5 °C	Str3 - 10 °C	Str1	Str2 - 5 °C	Str2 - 10 °C	Str2 S-	Str3 - 5 °C	Str3 - 10 °C
17 °C	Str3 + 5 °C	Str2 S+	Str3 - 5 °C	Str2 + 5 °C	Str1	Str2 - 5 °C	Str3 + 5 °C	Str2 S-	Str3 - 5 °C
22 °C	Str3 + 10 °C	Str3 + 5 °C	Str2 S+	Str2 + 10 °C	Str2 + 5 °C	Str1	Str3 + 10 °C	Str3 + 5 °C	Str2 S-

Table II. Increasing gradient of stress obtained in the different combinations of salinity-temperature-pollutant concentration (S+ = + 5 or 10 ‰ ; S- = - 5 or 10 ‰)

Increasing gradient of stress

⟶

Str1 → Str2 T + 5 °C → Str2 T + 10 °C
Str1 → Str2 T - 5 °C → Str2 T - 10 °C
Str1 → Str2 S + → Str3 + 5 °C → Str3 + 10 °C
Str1 → Str2 S + → Str3 - 5 °C → Str3 - 10 °C
Str1 → Str2 S - → Str3 + 5 °C → Str3 + 10 °C
Str1 → Str2 S - → Str3 - 5 °C → Str3 - 10 °C

Combined pollutant - pollutant stress

The benthic population of the polluted environment is most often exposed not to a single pollutant but to a group of pollutants. An experimental series was therefore performed on E. stocki, N. succinea, and V. aurea in order to study the evolution in sensitivity of the species exposed to a mixture of pollutants.

The experiments were at a temperature of 17 °C. The pollutant mixture was prepared by addition of mercuric chloride to different detergents (Syntopon C, RA43, Cemulsol 870). Depending on the species, the animals were subjected to a single concentration of mercuric chloride or detergent, serving as a reference base, and to a increasing concentration of the other pollutant, forming the increasing gradient of additional stress (Table III).

Combined pollutant - dissolved oxygen stress

The perturbation of a polluted ecosystem is often characterized by a depletion of dissolved oxygen levels, which may have an adverse effect on the marine benthic population (Stora, 1982). The influence of the dissolved oxygen level on the toxicity of two pollutants was studied with E. stocki.

Table III. Increasing gradient of stress obtained by mixture of different concentrations of two pollutants (Dgt = detergent)

	Increasing gradient of stress →			
Type of stress	Str1	Str2	Str3	Str4
Pollutant concentrations (mg.l^{-1})	Dgt	Dgt + $HgCl_2$	Dgt + $HgCl_2$	Dgt + $HgCl_2$
	0.1	0.1 + 0.004	0.1 + 0.04	0.1 + 0.4
Pollutant concentrations (mg.l^{-1})	$HgCl_2$	$HgCl_2$ + Dgt	$HgCl_2$ + Dgt	$HgCl_2$ + Dgt
	0.4	0.4 + 0.1	0.4 + 1	0.4 + 10

The experiments were conducted at 17 °C. E. stocki was exposed to a single concentration of 0.3 mg.l^{-1} mercuric chloride or to 10.5 mg.l^{-1} of the detergent Syntopon C at different levels of dissolved oxygen, forming an increasing stress gradient (Table IV).

Combined pollutant - physiological stress

In this series of experiments physiologically weakened animals were used as a reference stress in combination with a stress caused by a toxicant. This type of experiments demonstrated the reaction of animals already subjected to general stress conditions in addition to a critical attack.

To create a reference general stress, the water in the acclimation aquaria was not changed and the aeration shut off 2 weeks before the animals were used. These conditions led to a progressive autopollution of the medium. The experiments were carried out at 15 and 22 °C with E. stocki. An additional gradient was induced by exposure to an increasing concentration of mercuric chloride (Table V).

Table IV. Increasing gradient of stress obtained by the exposure to pollutants at various concentrations of dissolved oxygen (Dtg = detergent)

	Increasing gradient of stress →			
Dissolved oxygen saturation	100 %	70 %	45 %	38 %
Pollutant concentration ($mg.l^{-1}$)	$HgCl_2$ 0.3	$HgCl_2$ 0.3	$HgCl_2$ 0.3	$HgCl_2$ 0.3
Type of stress	Str1	Str2	Str3	Str4
Dissolved oxygen saturation	100 %	90 %	77 %	72 %
Pollutant concentration ($mg.l^{-1}$)	$HgCl_2$ or Dgt 0.3/10.5	$HgCl_2$ or Dgt 0.3/10.5	$HgCl_2$ or Dgt 0.3/10.5	$HgCl_2$ or Dgt 0.3/10.5
Type of stress	Str1	Str2	Str3	Str4

Table V. Increasing gradient of stress obtained by the exposure of physiologically weakened animals (GS = General stress) to increasing concentrations of a pollutant

	Increasing gradient of stress						
concentration $HgCl_2$ ($mg.l^{-1}$)	GS	0.38 + GS	0.79 + GS	1.45 + GS	2.6 + GS	3.8 + GS	7.9 + GS
Type of stress	Str1	Str2	Str3	Str4	Str5	Str6	Str7

RESULTS

The scope of this symposium essentially being directed towards methodology, only a part of the recorded results are given in this paper which are, however, representative for the experiments conducted and may serve as a basis for discussion.

In table VI an overview is given of the recorded lethal times for V. aurea exposed to different combinations of deleterious factors (pollutant - salinity - temperature). In Fig. 2 the evolution of the sensitivity indices computed for the different stress gradients is shown.

In Table VII the lethal times recorded at 17 °C for E. stocki exposed to a general stress and to mercuric chloride are given. In Fig. 3 the resulting evolution of the sensitivity indices are shown. Considering the totality of the experiments it is noted that synergistic effects are responsable for a hypersensitivity or a hyposensitivity to the lethal deleterious factor depending upon the sensitivity of the animals to the considered factor and upon the intensity of the additional stress. In general, the results obtained show that independently of the test species and of the nature of the combinations of deleterious factors, the evolution in sensitivity of the animals along an increasing stress gradient always follows the same general evolution pattern (Fig. 4).

Table VI. Lethal times (TL + confidence limits) in h for V. aurea recorded in different combinations of temperature - salinity stress. (AS = acclimation salinity ; ES = experimental salinity ; AT = acclimation temperature ; ET = experimental temperature)

AS		25 ‰			30 ‰			35 ‰		
ES		30 ‰			30 ‰			30 ‰		
AT / ET		12 °C	17 °C	22 °C	12 °C	17 °C	22 °C	12 °C	17 °C	22 °C
12 °C	TL10	-	-	-	81 ± 42	110 ± 45	89 ± 28	-	-	23 ± 21
	TL50	-	-	-	156 ± 40	199 ± 37	164 ± 29	-	-	78 ± 31
	TL90	-	-	-	302 ± 87	360 ± 33	303 ± 91	-	-	271 ± 91
17 °C	TL10	62 ± 27	98 ± 34	42 ± 10	49 ± 14	84 ± 25	25 ± 15	117 ± 50	137 ± 32	39 ± 16
	TL50	138 ± 30	186 ± 30	64 ± 9	85 ± 12	4 ± 26	68 ± 19	233 ± 46	214 ± 25	88 ± 16
	TL90	306 ± 4	355 ± 143	97 ± 20	147 ± 36	318 ± 83	185 ± 80	466 ± 195	4 ± 53	198 ± 72
22 °C	TL10	78 ± 13	66 ± 19	54 ± 16	53 ± 10	46 ± 8	40 ± 10	53 ± 15	75 ± 10	24 ±
	TL50	114 ± 11	109 ± 15	83 ± 10	76 ± 8	79 ± 5	63 ± 8	90 ± 13	95 ± 7	56 ± 12
	TL90	167 ± 29	179 ± 48	126 ± 29	109 ± 17	89 ± 18	97 ± 18	154 ± 39	122 ± 14	134 ± 47

Table VII. Lethal times (TL + confidence limits) in h for *E. stocki* at 17 °C exposed to a "general stress" (GS) and various concentrations of mercuric chloride

	TL10	TL50	TL90
GS	24	140	282
GS + 0.38 mg.l^{-1} $HgCl_2$	31 ± 13	101 ± 18	331 ± 126
GS + 0.79 mg.l^{-1} $HgCl_2$	24 ± 9	48 ± 8	95 ± 25
GS + 1.43 mg.l^{-1} $HgCl_2$	33 ± 10	68 ± 9	142 ± 34
GS + 2.6 mg.l^{-1} $HgCl_2$	24	260	283
GS + 3.8 mg.l^{-1} $HgCl_2$	32 ± 16	52 ± 9	82 ± 22
GS + 7.9 mg.l^{-1} $HgCl_2$	21 ± 6	48 ± 7	91 ± 18

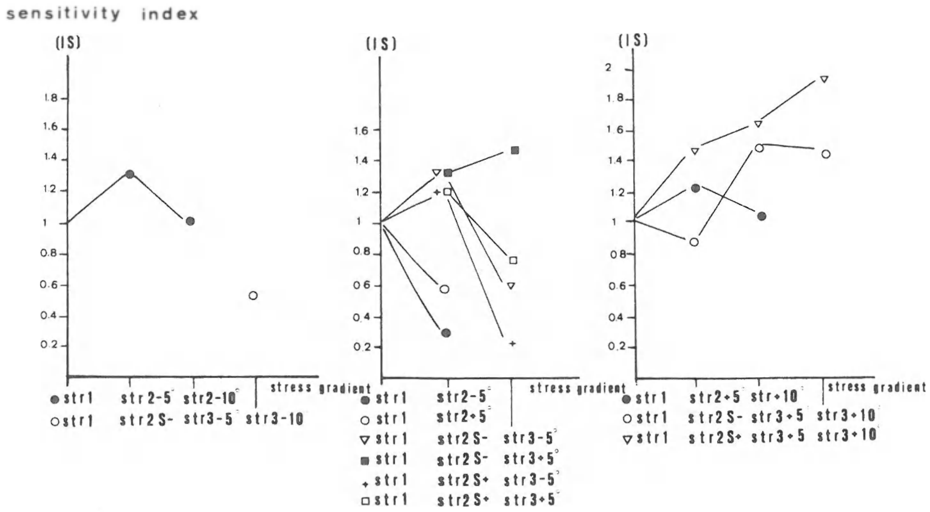

Fig. 2. Evolution of the sensitivity indices (IS) computed from the LT50 values for *Venerupis aurea* exposed to a increasing stress gradient.

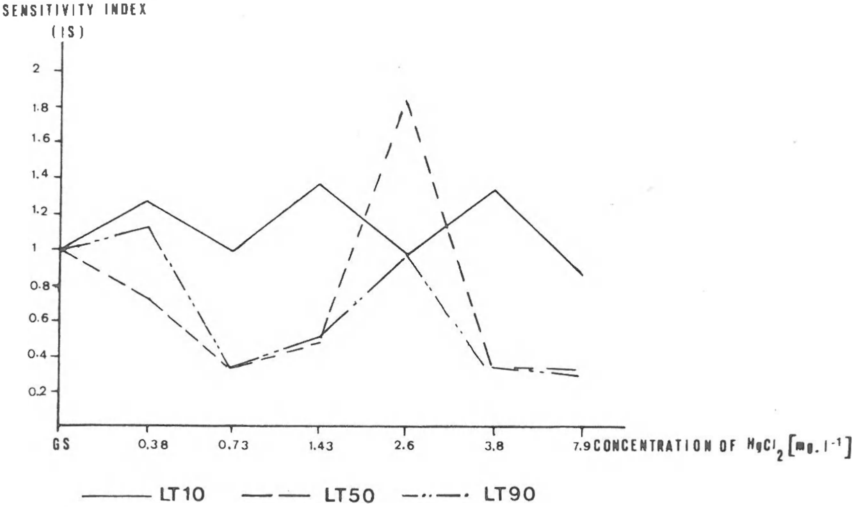

Fig. 3. Evolution of the sensitivity indices obtained for Echinogammarus stocki exposed to increasing concentrations of a detergent.

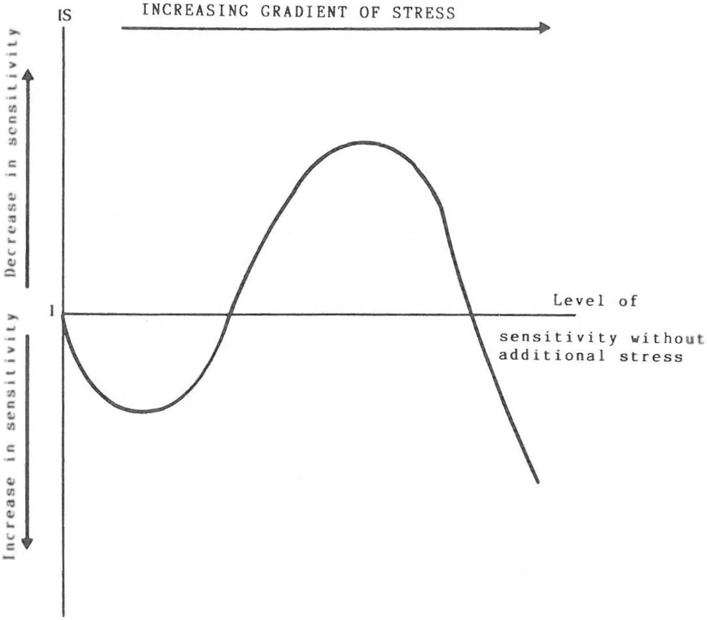

Fig. 4. General pattern of the evolution in sensitivity.

DISCUSSION AND CONCLUSIONS

Considered from a purely methodological point of view, the experiments demonstrated that the variability of the results depend mainly on the physiological state of the animals. This is particularly clear for the experiments testing the combined effects of "pollutant - temperature - salinity" where the animals subjected to a different pre-experimental phase are subsequently placed in identical experimental conditions at each test temperature. For example at 12 °C (Table VII), depending on the acclimation conditions, the same concentration of pollutant can either provoke the death of 50 % of the animals at the end of 78 h or not be lethal at all within the experimental period. This variability, which is a function of the physiological state of the animals, demonstrates the impossibility of assessing a direct relation between experimental data on the sensitivity of one species to specific deleterious factors and the data recorded in the natural environment. However, numerous concurrent points exist between the species dynamics recorded *in vitro* and the species dynamics studied *in situ* in the Berre lagoon and in the Gulf of Fos. Similar evolutions in sensitivity of different species, independent of their taxonomic group and the nature of the stress applied, have been recorded *in vitro* which correspond to *in situ* observations made in the studies of the dynamics of benthic populations of the coastal fringe of the Berre lagoon. In this lagoon the benthic populations are exposed to erratic salinity variations witha high amplitude, to sediment discharges, and to diverse forms of pollution linked to the discharge of domestic and industrial effluents. The predominant influence of each of these deleterious factors is not manifested by the mortality of particular species, but affects all taxonomic groups which all react in a similar manner. The faunal dynamics are much more influenced by the intensity of each type of stress than by its quality. This finding confirms the experimental results.

The observed evolutionary pattern in sensitivity demonstrates that changes in the living conditions and mortality of a species not necessarily arise from exposure to pollutant concentrations. In the southern zone, where the Berre lagoon presents the highest levels of pollutants, the natural population is most abundant and the predominant effects of the pollutants are less manifested (Stora and Arnoux, 1983). In the northern zone on the contrary, the population, maintained in a permanently unstable state by the influx of fresh water and sediments, is proven to be hypersensitive to much lower pollutant concentrations. The addition of stressing agents provokes significant mortalities and a state of permanent impoverishment of the population.

In general, it may be true that this phenomenon of species hypersensitivity, as a result of the exposure to low pollutant concentrations in combination with stressing environmental factors (salinity, temperature, other pollutants etc.), is responsible for the rapid and marked degradation processes which are often merely attributed to the presence of particular pollutants in the natural environment.

One of the principal criticisms of short-term experimentations is based on the utilisation of very high concentrations of a pollutant which are never observed in situ. It must be noted, however, that even if this is very often the case, the concentrations measured in the sedimental phase of the ecosystem, are often close the experimental concentrations. In fact the use of these high short-term test concentrations prove that the species may be able to resist in the absence of specific perturbations high levels of pollution present in their environment or in their own organs due to toxicant accumulation (Bryan and Hymmerstone, 1973 ; Catsiki, 1980). Vernberg and Vernberg (1972) were able to maintain the crab Uca pugilator, exposed to a concentration of 0.18 mg.l^{-1} of mercuric chloride, alive for about 2 months. Similary Kaïm-Malka (1981) utilised a concentration of 30 mg.l^{-1} of an alkyl aryl oxyethylene detergent in long-term experiments. The concentration of each pollutant found in the natural environment might not be limiting but the organisms, by being subjected to an accumulation of stresses, might temporarily be in a critical physiological state which renders them sensitive to weak concentrations, provoking massive mortality.

The results of the in vitro tests show that the combination of various deleterious factors might increase the resistance of the test species to the lethal deleterious factor. This favourable aspect of a stressful situation was demonstrated in the study on the distribution of pollution indicator-species in the channel of Caronte. The peculiarity of the channel of Caronte connecting the Gulf of Fos to the Berre lagoon is the presence of a double pollution gradient. The first gradient is linked to the augmentation of the amount of organic matter associated with salinity variations. The second gradient is strictly due to an increase of pollutant concentrations. The evolution of the populations of species such as V. aurea, Mediomastus cf californiensis, Capitella capitata, Lumbrineris latreilli, along these two different gradients passes through a maximum phase. In other words, it could be deduced from the in vitro studies and the previous observations that for these species, a certain "stress intensity" corresponds to one of their ecological requirements providing optimal living conditions. In general, this reaction of particular species to the stress intensity might be a way of explaining the formation of the facies of a polluted environment.

It is interesting to note that the evolutionary tendencies in the reaction of a species as a function of an in vitro stress gradient are very similar to the evolution in the specific richness and the total abundance of a population exposed to increasing gradient of perturbation (Fig. 5) (Pearson and Rosenberg, 1978 ; Stora, 1982). This similarity between the

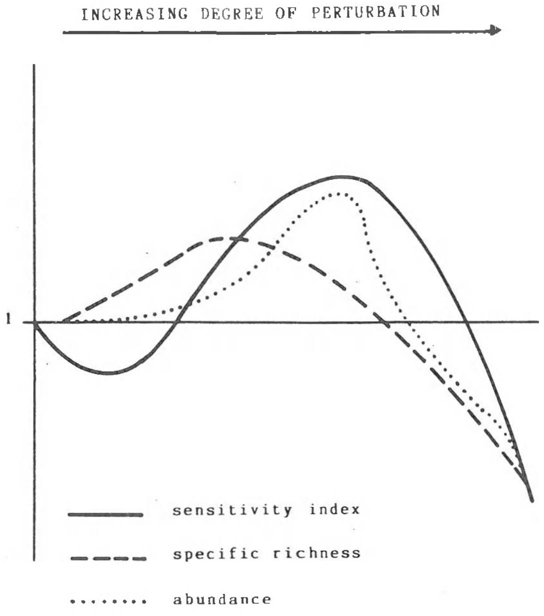

Fig. 5. Comparison between the evolution of the in vitro sensitivity indices and the species richness and the abundance of a population in function of an increasing degree of perturbation.

responses of the in situ population and the in vitro species, which are the basic elements of this population, leads to the hypothesis that the evolutionary pattern demonstrated might be a basic part of a descriptive and preconceived model of the dynamics of a littoral ecosystem exposed to perturbation. The phase of irremediable hypersensitivity, where all additional stress results in an accelerated degradation of the population, represents the most serious danger for an ecosystem.

ACKNOWLEDGEMENTS

We are grateful to Dr. Sheila Jacob for translating this paper into English.

LITERATURE CITED

APHA/AWWA/WPCF. 1976.
Méthodes normalisées pour l'examen de l'eau et des effluents liquides. 13ème Ed. Washington, DC 20036, USA.

Bellan G. 1981.
Manuel des méthodes de la recherche sur l'environnement aquatique. 7ème partie : Bioessais selectionnés pour la Méditerranée (tests utilisés dans le projet commun coordonné FAO (GPPM) PNUE/PNUE sur la pollution en Méditerranée). FAO Doc. Techn. Pêches 208. 37 p.

Bellan-Santini D., G. Bellan, and D.J. Reish. 1979.
Variations de l'influence d'altéragènes suivant le cycle d'activité jour/nuit de certains organisms marins. C.R. Hebd. Séanc. Acad. Sc. Paris, Sér. D, 288(1):139-141.

Bliss C.I. 1935.
The calculation of the dosage mortality curve. Ann. Appl. Biol. 22:134-167.

Bliss C.I. 1938.
The determination of the dosage mortality curve from small numbers. Quart. J. Pharmacol. 2:192-216.

Bryan G.W. and L.G. Hymmerstone. 1973.
Adaption of the polychaete Nereis diversicolor to estuarine sediments containing high concentrations of zinc and cadmium. J. mar. biol. Ass. UK 53:839-857.

Catsiki A. 1980.
Contribution à l'étude de la contamination des peuplements benthiques de l'étang de Berre par les métaux (mercure, cuivre, zinc, plomb). Thèse 3ème Cycle, Univ. Aix-Marseille II. 181 p.

Commission européenne consultative pour les pêches dans les eaux intérieures, Groupes de travail sur les systèmes de tests de toxicité. 1983.
Rapport révisé sur les tests de toxicité sur les poissons. Doc. Techn. CECPI (24) Rév. 1. 41 p.

Jones M.B. 1974.
Survival and oxygen consumption in various salinities of three species of Idotea (Crustacea, Isopoda) from different habitats. Comp. Biochem. Physiol. 48A:501-506.

Kaïm-Malka R.A. 1981.
Altérations biologiques chez Idotea balthica basteri (Isopoda). Rapp. Comm. int. Mer Médit. 27(2):209-210.

Möller F. 1979.
Manual of methods in aquatic environment research. Part 5 - Statistical tests. FAO Fish. Techn. Pap. 182. 131 p.

Pearson T.H. and R. Rosenberg 1978.
Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16:229-311.

Ramade F. 1977.
Ecotoxicologie. Collect. d'Ecologie 9. Masson Publ., Paris. 201 p.

Reish D.J. 1966.
Relationship of polychaetes to varying dissolved oxygen concentrations. 3rd Int. conf. water pollut. res., Munich. Sect. 3, Paper 10. 10 p.

Reish D.J. 1970.
The effects of varying concentrations of nutrients chlorinity and dissolved oxygen on polychaetous annelids. Water Res. 4:721-735.

Ritz D.A. 1980.
Tolerance of intertidal amphipods to fluctuating conditions of salinity, oxygen and copper. J. mar. biol. Ass. UK 60:489-498.

Stora G. 1972.
Contribution à l'étude de la notion de concentration léthale limite moyenne (CL_{50}) appliquée à des invertébrés marins. 1. Etude méthologique. Téthys 4(3):597-644.

Stora G. 1975.
Etude des peuplements benthiques littoraux du golfe de Fos. Rapport SPPPI. 25 p.

Stora G. 1978.
Evolution comparée de la sensibilité de deux polychètes soumises à l'action de détergents en fonction d'une augmentation de la température; notion d'indice de sensibilité. Rev. Inst. Océanogr. méd. 51-52:101-113.

Stora G. 1980a
Etude de l'évolution de sensibilité d'un crustacé amphipode (Echinogammarus stocki Kar.) soumis aux effets synergiques de diverses combinaisons d'altéragènes. Vèmes Journ. étud. pollut., Cagliari, CIESM. 761-768.

Stora G. 1980b.
Influence des variations combinées de et de salinités sur la sensibilité d'un crustacé amphipode soumis à un altéragène léthal. J. franç. Hydrol. 11(3),No. 33:225-240.

Stora G. 1982.
Recherches de bionomie descriptive et expérimentale (in vivo at in vitro) dans quelques biotopes littoraux soumis à des variations naturelles ou artificielles des conditions du milieu (notamment dans l'étang de Berre et le golfe de Fos). Thèse Doctorat es-Sciences, Univ. Aix-Marseille II. 200 p. + annexes 119 p.

Stora G. and G. Bellan. 1977.
Utilisation de la notion de concentration léthale en toxicologie d'invertébrés marins. Rev. inst. Océanogr. méd., 7ème collect. 48:125-129.

Stora G. and J.C. Romano. 1982.
Comparative study in situ and in vitro of the mortality rate of two polychaetes submitted to an increase in temperature and pollutants. (in press).

Stora G. and A. Arnoux. 1983.
Effects of large freshwater diversions on benthos of a Mediterranean lagoon. Estuaries 6(2):115-125.

Thurberg F.P., A. Calabrese, and M.A. Dawson. 1974.
Effects of silver on oxygen consumption of bivalves at various salinites. p. 67-78. In : Pollution and physiology of marine organisms. Vernberg F.J. and W.B. Vernberg (Eds). Academic Press. 492 p.

Vernberg W.B. and F.J. Vernberg. 1972.
The synergistic effects of temperature, salinity and mercury on survival and metabolism of the adult fiddler crab Uca pugilator. Fish. Bull. 70:415-420.