IN SEARCH OF STANDARDISATION : A COMPARISON OF TOXICITY BIOASSAYS ON TWO MARINE CRUSTACEANS *(PALAEMON SERRATUS* AND *TIGRIOPUS BREVICORNIS)*

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Abstract

In order to mitigate the insufficiency of the number of currently available toxicity tests in marine middle, two studies on the effects of cadmium and zinc were carried through with two marine crustaceans : the prawn *P. serratus* and a copepoda *T. brevicornis*. The comparison of methodologies (rearing conditions - experimental procedure) and results obtained, allowed to show the advantages and the disavantages of these two tests in order to consider a standardisation. We located the CL 50 among larvae at stage IV of *P. serratus* between 250 and 450 $\mu g/dm^3$ for cadmium and between 300 and 750 $\mu g/dm^3$ for zinc. The results obtained with *T. brevicornis* show a more sensitivity of this test since the EC 50 of the larvae production is 78 ± 2 $\mu g/dm^3$ for cadmium and 325 ± 2 $\mu g/dm^3$ for zinc.

Résumé

Afin de pallier à l'insuffisance du nombre de tests de toxicité disponibles actuellement en milieu marin, deux études sur les effets du cadmium et du zinc ont été menées sur deux crustacés marins : la crevette rose *Palaemon serratus* et le copépode *Tigriopus brevicornis*. La comparaison des méthodologies (conditions d'élevage - protocole expérimental) et des résultats obtenus a permis de mettre en évidence les avantages et les inconvénients de ces deux tests afin d'envisager une normalisation. Nous avons situé la CL 50 chez les larves au stade IV de *P. serratus* entre 250 et 450 $\mu g/dm^3$ pour le cadmium et entre 300 et 750 $\mu g/dm^3$ pour le zinc. Les résultats obtenus sur *T. brevicornis* montre une plus grande sensibilité de ce test puisque la CL 50 de la production larvaire est de 78 ± 2 $\mu g/dm^3$ pour le zinc.

Introduction.

There is a real need for more bioassays in order to prevent the negative effects upon the environment of marketed chemicals. One purely practical reason is that manufacturers are required to deliver a « Notice of Specifications » to the authorities before marketing any new chemical ; this document must present the physiochemical, toxicological and/or ecotoxicological properties of the proposed chemical (SMEETS, 1981). The present study will deal only with the toxicological and ecotoxicological aspects, as previously available marine tests concerning aquatic ecotoxicology are inadequate and greatly need improving.

It is here necessary to point out the indispensable conditions for a valid bioassay in toxicology : we have to keep in mind that a test used for calculation of pollution taxes needs a clear experimental procedure in order to prevent any litigation between « polluters » and customers. We have to aim for two conditions.

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- Good repeatability (the same results obtained from the same laboratory).
- Good reproducibility (the same results no matter which laboratory does the assays). For that purpose it is necessary to use : ubiquitous, short life-cycle species, well-represented in local fauna, easy to rear, issuing from genetically well-defined populations, and available all year long (LEPAILLEUR and CABRIDENC, 1981). Test organisms must be produced from standard laboratory-reared populations. Such conditions are the garantee of good stability in sensitivity and behavior of assay populations. In addition, storage and handling of the animals must be realised without any major difficulty.

Two last conditions have to be fullfilled : an intermediary solution is needed to link environmental conditions and low-cost laboratory bioassays conditions, and second methodology should be applied without concern for the period of the year, in any kind of laboratory using standard equipment.

Nowadays, with respect to aquatic ecotoxicology, standard bioassays described in national or international guidelines (AFNOR - OCDE - ISO) are exclusively short-term toxicity tests. Such bioassays, nevertheless, fit quite well with screening procedures aiming to compare the sensitivity of different species to the same pollutant, as well as comparing the toxicity of several chemicals toward a same species. On the other hand, they give no information concerning the impact of pollutants upon aquatic communities in their natural environment. According to different authors, acute bioassays of 96 hours duration are not long enough to evaluate, for instance, the effects of heavy metals upon aquatic organisms (EISLER, 1971 ; PRICE and UGLOW, 1979 ; PESH and STEWART, 1980 ; AMIART-TRIQUET, 1983). Sublethal effects upon life cycles have greater ecological implications (WARREN *in* NIMMO *et al.*, 1978 ; AMIARD, 1983). Thus, bioassays using several of the following life stages can allow us to estimate the highest toxic concentration tolerated by an organism before showing any adverse effects (EATON, 1973). Larval stages represent the most sensitive period during complex marine invertebrates life cycles (PHILLIPS, 1980 ; FRANKLIN, 1983).

Standardisation of ecotoxicological tests and analytical procedures can be considered as proceeding in three steps (QUEVAT, 1981) : preliminary studies, experiments and technical improvements and reporting of methods as guidelines. The present study is restricted to the first step : a preliminary study aiming at a comparison between two tests, their advantages and the specific methods used.

For each test species (*Palaemon serratus*, a prawn, and *Tigriopus brevicornis*, a copepoda) a specific procedure has been devised. The larval production at 10 days of *T. brevicornis* was compared to *P. serratus* larval mortality levels at stage 4 and at metamorphosis. Two metals have been chosen as pollutants from a consideration of their identical physico-chemical properties and different biological functions : zinc is necessary for marine organism's growth whereas cadmium is not known for any physiological input.

Toxic aspect.

Metal traces exist in the water under ironic form ; compounded or chelated colloidal, or absorbed on particular organic or non-organic matter. The exact metal form can condition not only toxic capacity but also it's ability and absorption rate concerning organisms. The metal polution is only important in terms of bio-availability of the metal. The ironic form shows to be more toxic than the chelated forms and compounds. This seems to depend on the stability and resistance to the separation of the latter ; and in this way their possibility to free metal irons to become available in consideration of the organism (BRYAN, 1971). The bioccumulation results from one at minimum of the following mecanisms : the direct metal solution contact in water by absorption through the body surface, taking in metal by feeding, of water or non-organic particles. It seems that the seriousness of the contamination according to that of feeding or by water are different according to the metal and considered organisms (BRYAN, 1984). It is necessary to note that the situation is different according to whether the study has taken place *in situ* or in the laboratory, with natural or artificial sea water and whether the experiments were long term.

Test organisms.

Palaemon serratus (Pennant).

This species from the Mediterranean and Northern Atlantic coastal waters shows relative abundance and ecological importance in coastal shallow areas. Biology and laboratory rearing procedures from egg incubation to adult are well known (REEVE, 1969 *a* and *b* ; FORSTER, 1970 ; PHILLIPS, 1971 ; WICKINS, 1972 ; LASSUS and MAGGI, 1980). Post embryonnic and larval development of *P. serratus* was described by SOLLAUD (1912, 1916 and 1923) and FINCHAM (1983) : 6 to 8 zoe-like stages are observed before

metamorphosis. Taxonomical features of intermoulting stages were described by DRACH and TCHERNIGOVTZEFF (1967). *P. serratus* inhabits nearshore areas and is directly exposed to pollution sources. According to several authors (EISLER, 1971 ; EISLER and HENNEKEY, 1977 ; PHILLIPS, 1980 ; ...) crustaceans are known for their sensitivity to zinc and cadmium.

***Tigriopus brevicornis* (Muller).**

This shallow water marine species (Copepoda Harpacticoïda) lives mainly in intertidal pools of North Atlantic European coasts. Three larval stages, all variously colored deep orange, have been described by BOZIC (1960) from laboratory populations reared at 20 °C : 5 nauplii sub stages and 5 copepodit stages. Nauplii show a sub circular shape and are characterised by a single red eye ; they develop within 6 to 7 days. Copepodites can be recognized because of their abdominal segment formation, the last sub stage being difficult to distinguish from the adult female at the end of a seven-day development period. Adult sizes range from 0,8 to 1,0 mm (males) and 1,0 to 1,2 mm (females). Sexual dimorphism is only detectable by the pincer shape of antenna 1 wheras ovigerous females can quickly be recognized because of their big egg sacs.

Some hours after the hatching of the eggs a female can produce a new fecund egg sac without needing to be fertilised again. This may be repeated 10 to 20 times. There seems to be a relationship between *T. brevicornis* egg production and its nutrition, according to COMITA (1963). Harpacticoïd copepods have given rise to a lot of works bearing mainly on rearing techniques *(Tisbe, Euterpina, Nictocra* and *Tigriopus)*. Their use as test organisms was also studied : 78 chemicals, including zinc, were experimented on *Nitocra spinipes* (LINDEN *et al.*, 1979), whereas zinc and cadmium 96 h LC 50 were evaluated on the same species by BENGTSSON (1978). The effect of cadmium upon *Tisbe holothuriae* populations has been estimated by HOPPENHEIT (1978), but the widest range of studies is devoted to *Tigriopus* (RANADE, 1957 ; SARAIVA, 1973).

Methods.

Palaemon serratus.

Collection and acclimatization of adults. — *P. serratus* larvae were obtained, after hatching under laboratory conditions, from ovigerous female prawns fished offshore at Roscoff (English Channel) and Le Croisic (Bay of Biscay) between December 1982 and May 1983. Ovigerous females are kept in oxygen saturated sea water during transport between fishing area and laboratory. In such conditions the mortality rate seldom exceeds 5%. In order to avoid any thermal stress, survivors are gathered and kept in the original seawater at 16 °C for 24 hours before being isolated in vessels containing 3 liters of aerated and filtered (0,22 μm) sea water at 20 °C. Thus we can prevent cannibalism or bacterial contamination (REEVE, 1969 *a* ; PHILLIPS, 1971). Only two-year-old shrimps, with an overall size ranging from 90 to 100 mm, are selected. At that stage it is possible to obtain after hatching about 300 larvae which enables selection of healthy live individuals. Every three days, ovigerous females are fed with adult brine shrimp *(Artemia salina)* and the next day the sea water is renewed.

Incubation and collecting of newly hatched larvae. — At 20 °C optimal temperature, the incubation period is reduced to 30 days (PHILIPPS, 1971). Because some effects of the rearing medium upon egg incubation have been involved in larvae quality, we have tried to discard larvae issued from eggs incubated for less than 30 days. When the hatching period is approaching, the mother shrimp is transfered into hatching chamber with dark walls and a mesh bottom located in a corner of the 20 l rearing tank. Then, using positive light response of the newly hatched larvae, all stage 1 larvae are collected during their migration from the hatching chamber to a light at the opposite corner of the tank (REEVE, 1969 *a* ; WICKINS, 1972 ; FORWARD and CRONIN, 1978). This way, we may considerably reduce the effects of maternal cannibalism. The more active animals swim near the surface and the weaker animals stay near the bottom. These active larvae from the surface are then isolated in 15 ml of filtered sea water (oxygen saturated, salinity (10^{-3}) 29 ± 1, température : 20 °C, pH : 7,98 ± 0,06) and kept in a thermoregulated room with a 12 h/12 h nycthemeral light, allowing optimal survival and growth conditions (REEVE, 1969 *a*, FORSTER, 1970 ; PHILIPPS, 1971 ; WICKINS, 1972).

Everyday the rearing medium is renewed (sea water, contaminant, food). Larvae are fed on 48 h *A. salina* nauplii (San Francisco Strain) with a diet corresponding to 10/20 nauplii per ml (REEVE, 1969 *a*). Such conditions allow controls to remain below a 10% mortality rate with more than 99% metamorphosis after 45 days. In contrast to some authors who used other *Artemia* strains, it was not necessary to add a complementary food to the diet described here.

Experimental conditions. — One day after hatching, larvae are fed and introduced into contaminated mediums. Toxic agents such as zinc sulfate and cadmium chloride are administered, with concentrations ranging from c (Zn) = 75 to 1 025 $\mu g/dm^3$ and from c (Cd) = 100 to 700 $\mu g/dm^3$. Effects of zinc and cadmium are observed using respectively 10 and 8 hatchings at different periods of the year (tabl. 1). Each experience uses two groups of larvae with 30 individuals per concentration.

Zinc and cadmium solutions. — Two liters of acidified 1 000 mg/dm^3 mother-solutions are prepared in gauged flasks, and kept at 4 °C with pH 2,0. They are used for both *Palaemon* and *Tigriopus*.

		Original Values			2nd day			6th day			10th day		
	Concentrations $\mu g/dm^3$	pH	S . 10^{-3}	O_2 %	pH	S . 10^{-3}	O_2 %	pH	S . 10^{-3}	O_2 %	pH	S . 10^{-3}	O_2 %
	270	7.8	34	90	7.8	34	90	7.8	35	91	7.9	36	94
Z	297				7.9		92	7.9	35	92	7.8	35	91
I	324				7.9		92	7.8	35	93	7.8	35	92
N	351				8		92	7.8	35	91	7.9	36	91
C	378				7.8		90	7.8	35	92	8	36	91
	405				7.9		92	8	35	90	7.8	34	92
	24.48	7.8	34	90	7.9	34	92	7.8	34	92	7.8	35	91
	30.60				7.9		92	7.9	35	92	7.9	35	91
C	36.80				7.8		90	7.9	35	92	8	35	92
A	49.00				8		82	7.8	35	92	7.9	35	91
D	61.20				7.8		90	7.9	34	90	7.8	35	92
M	73.56				7.8		90	8	35	91	7.8	35	91
I	91.95				7.9		92	7.9	34	90	8	34	92
U	97.92				7.8		90	7.8	35	92	8	36	91
M	104.04				8		92	7.8	35	92	7.8	35	92
	110.16				7.9		92	7.9	35	92	7.8	35	92
	122.60				7.8		92	7.9	35	92	7.8	35	92

TABLE 1. — T. brevicornis : *progression of physico-chemical parameters for zinc and cadmium : pH, salinity, dissolved oxygen (nitrite NO_2 < 0,33 mg/dm^3 in any case).*

Tigriopus brevicornis.

Tigriopus population is kept at 20 °C in two liters erlenmeyers filled at 1/3 with sea water. Such stock population remain at an almost constant level (about 50 individuals) : after initial feeding, adults enter a semi lethargic state partially inhibiting larval production.

Mass production is started from erlenmeyer strains filtered on 100 μm mesh membranes in order to obtain only copepods. Common 10 liter glass vessels are used for a 20 °C batch, and after 3 weeks, ovigerous females density is suitable for sampling and test procedures. Because of necessary aeration and subsequent evaporation the water level must be kept constant by adding distilled water. Experimental sea water is made from commercial synthetic salts and adjusted to 30 . 10^{-3} salinity. Intermittent light with a 12 h/12 h photoperiod is provided giving light intensity : 2 500 ± 200 lux. The freshwater Cyanophyceae *Spirulina maxima* is used as dried food. This Nostocale : Oscillatoriaceae commonly produces curly fibres 250 μm long which after being lyophilized provide a suitable food for small live prey used as food for crustaceans.

Experimental procedures. — Temperature, light and salinity conditions and artificial sea water are used identically to mass rearing conditions. *Spirulina* is added as dried powder, at a level of 40 mg/dm^3. Analyses of pH, salinity, dissolved oxygen and nitrites are carried out every two days in order to check the stability of these parameters (table 1). Tests are run in 20 cm^3 pyrex vessels filled with 15 cm^3 of toxic solutions and covered with a glass cap.

Ovigerous females are collected from rearing batches with a Pasteur pipette and washed twice in clean sea water in order to discard nauplii and other particules. Each female is finally introduced into 20 ml vessels and 30 individuals are used for each tested concentration of toxin whereas the controls used × 30 females. Immediately after hatching the females are picked out and separated from nauplii, and after 10 days, the larval production per tested female is estimated. The total count of larvae (copepodits and nauplii) is made using a counting chamber, the larval population being previously fixed with mixture of iodine and potassium iodide. The experiment is stopped at 10 days in order to : avoid new adult stages able to produce a second generation nauplius and obtain all control larval production at copepodit stages. For checking the physiological state of biological material (QUEVAT, 1981) a potassium dichromat control was carried out for each test : as EC 50 values never changed significantly during all the assays, the results are considered as independant from physiological stress.

Results.

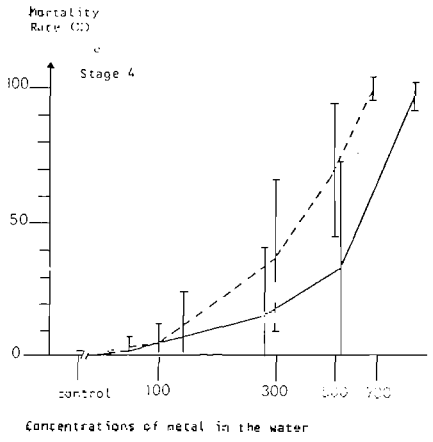
Experiments with *P. serratus* (fig. 1 to 4).

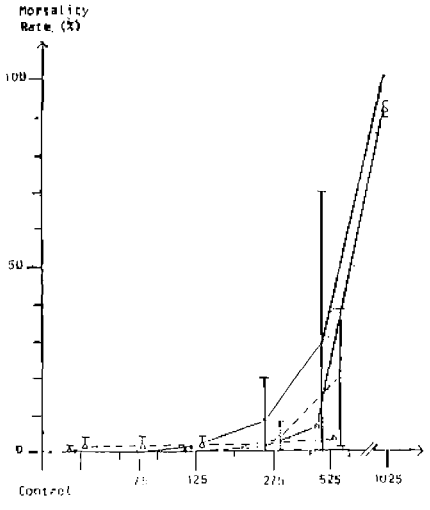
P. serratus moulting frequency is similar for all larvae, controls as well as assays, whatever the metals and/or concentrations tested, and without any apparent relationship to ecological parameters such as season or geographical origin. Larval growth corresponds to a linear relation ship whose slope changes between stages 3 and 4 with the respective equations as follows :

$Y_1 = 3.26\ x - 0.05 \qquad r = 1.00$

$Y_2 = 3.91\ x - 2.15 \qquad r = 1.00$

Reliability in moulting frequency up to stage 4 has already been observed (LASSUS and MAGGI, 1977) and seems to be due to genetic determinism. After stage 4, rearing conditions would be dominant factor regulating moulting. Larval mortality rates were estimated, first during the hatching/stage 4 period (reliability in





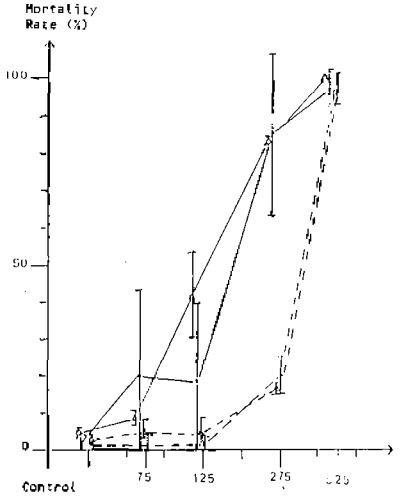


FIG. 2. — P. serratus : *variations of mortality rates according to the geographical origin of larvae reared at different seasons, from hatching through stage 4 inclusive (10 days ± 1) or for 45 days, exposed to different concentrations of zinc in the water (averages for two parts from Channel).*

-▲- Channel winter early -●- Bay of Biscay-winter early -△- Channel, spring summer -○- Bay of Biscay spring summer

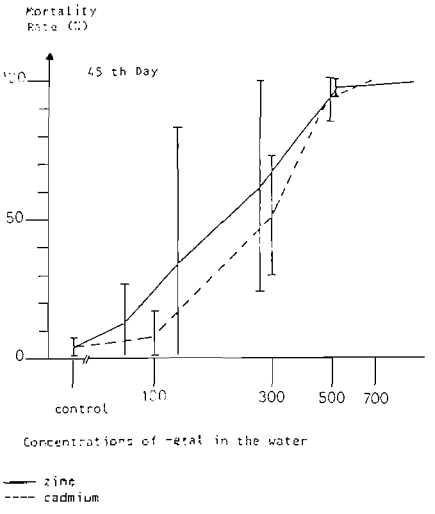


FIG. 1. — P. serratus : *variations of mortality rates of the larvae at stage 4 (10 days ± 1) and at 45 th day, on the all spawning with* zinc *and* cadmium *in the rearing water (averages bearing standard deviation).*

moulting frequency and 10 day development may be assimilated with *Trigriopus* test conditions), and second at the end of the experiment (all control survivors are metamorphosed). *P. serratus* larval mortality rate increase with increasing metal levels in water. When compared 45th day results, stage 4 mortalities show greater differences between zinc and cadmium : a higher mortality rate with cadmium is reliated to a higher sensibility to this metal at the earlier stages (fig. 1). Mortality rate variability is high with zinc and less so with cadmium. We used an « F - test » standard deviation analysis to estimate the importance of the fishing period of the females in relation to the effects of zinc or cadmium upon the larval population.

Stage 4 zinc mortalities in larvae born in late winter are higher than for larvae born in early winter ($\alpha < 0.01$), they, in turn, showed higher mortality rates than larvae born in early spring and in summer ($\alpha < 0.05$). At the end of the experiment, mortalities in each seasonal group are higher and intergroup differences are more marked ($\alpha < 0.01$). Moreover, mortality rates of larvae from the Channel and the Bay of Biscay are not significantly different in any of the seasons considered : $\alpha < 0.05$ (fig. 3). Channel larvae born in early summer, when exposed to cadmium, revealed more sensitivity at stage 4 than larvae born in early or late winter : $\alpha < 0.05$, but no seasonal difference was noticeable at the end of the experiment. On the other hand, Channel larvae are weaker than those fished in the Bay of Biscay ($\alpha < 0.05$) either at stage 4 or at the end of the experiment (fig. 2 et 3).

High variability among different populations (mortality rates) prevent us from determining accurate LC 50. Nevertheless, using figure 1 results, we have estimated extreme value ranges where about 50 % of larvae died :

LC 50 ($\mu g/dm^3$)	*Cadmium*	*Zinc*
Stage 4	300-750	220-450
45th day	90-350	180-360

Experiment with *Tigriopus brevicornis*.

Counting of survivors after 10 days experimentation gives us an evaluation of lethal and sublethal effects.

- Lethal effects : concentration reducing to 50 % the larval production is determined as EC 50.
- Sublethal effects : observation of copepodits/nauplii ratio gives information on toxin effects of toxic substance upon larval development.

We can express this as R = C/LT (1), with R = 1 when tested concentrations show no observable effect upon larval development (C = number of 10th day surviving copepodits ; LT = number of 10th day surviving copepodits and nauplii = total larval production). With low R values we can suggest some direct effects of tested pollutant on nauplii : moulting in copepodit stages has not occured at the same time as in control larvae. However, if comparison of R values for each tested concentration gives some idea of sublethal effects, no information concerning long term behavior of nauplii can be obtained with such a test. A longer experimentation period could eventually reveal delayed moulting or death of larvae.

Zinc. — First tests were carried out in a wide range of concentrations *c* (Zn) : 135 to 405 $\mu g/dm^3$ as screening tests, and gave a larval production EC 50 ranging between *c* (Zn) : 270 and 405 $\mu g/dm^3$. Several concentrations ranging between these two values have been tested in this way (table 2) and also by using the log probits calculation method ; the larval production EC 50 has been estimated as : *c* (Zn) : 325 ± 2. Mortality is complete for 405 $\mu g/dm^3$ and R values ranging from 1 for low concentrations (270 and 297 $\mu g/dm^3$) to 0.69 for 324 $\mu g/dm^3$. However, R values are similar (0.68 and 0.66) for 351 and 378 $\mu g/dm^3$ whereas mortality rates are respectively : 62 and 90%.

Cadmium. — Screening tests were carried out for concentrations ranging from 6.12 to 306 $\mu g/dm^3$. First results situated EC 50 between 61.2 and 122.6 $\mu g/dm^3$, but show some cadmium impact on larval development from 61.2 $\mu g/dm^3$. Another series of experiments has been run with concentrations ranging from *c* (Cd) : 24.5 to 122.5 $\mu g/dm^3$. Theorethical log probit determined EC 50 for larval production results in 78 ± 2 $\mu g/dm^3$. For 110 $\mu g/dm^3$, mortality is complete and R = 0.51 for 49 $\mu g/dm^3$. For 61.2 and 73.5 $\mu g/dm^3$, R = 0.2 and values decrease for higher concentrations : 92.98 and 104 $\mu g/dm^3$ (tabl. 2). Such results emphasize the more obvious impact of cadmium rather than zinc on *Tigriopus brevicornis* larval production at the same concentrations. Inhibitory effects of cadmium on nauplii development can also be observed : significant numbers of 10 day old larvae have not moulted in concentrations lower than the LC 50 value. As soon as cadmium concentration in water reaches 49 $\mu g/dm^3$, larval development is modified whereas no change occurs when *Tigriopus* larvae are exposed to zinc below EC 50 value 325 ± 2 $\mu g/dm^3$).

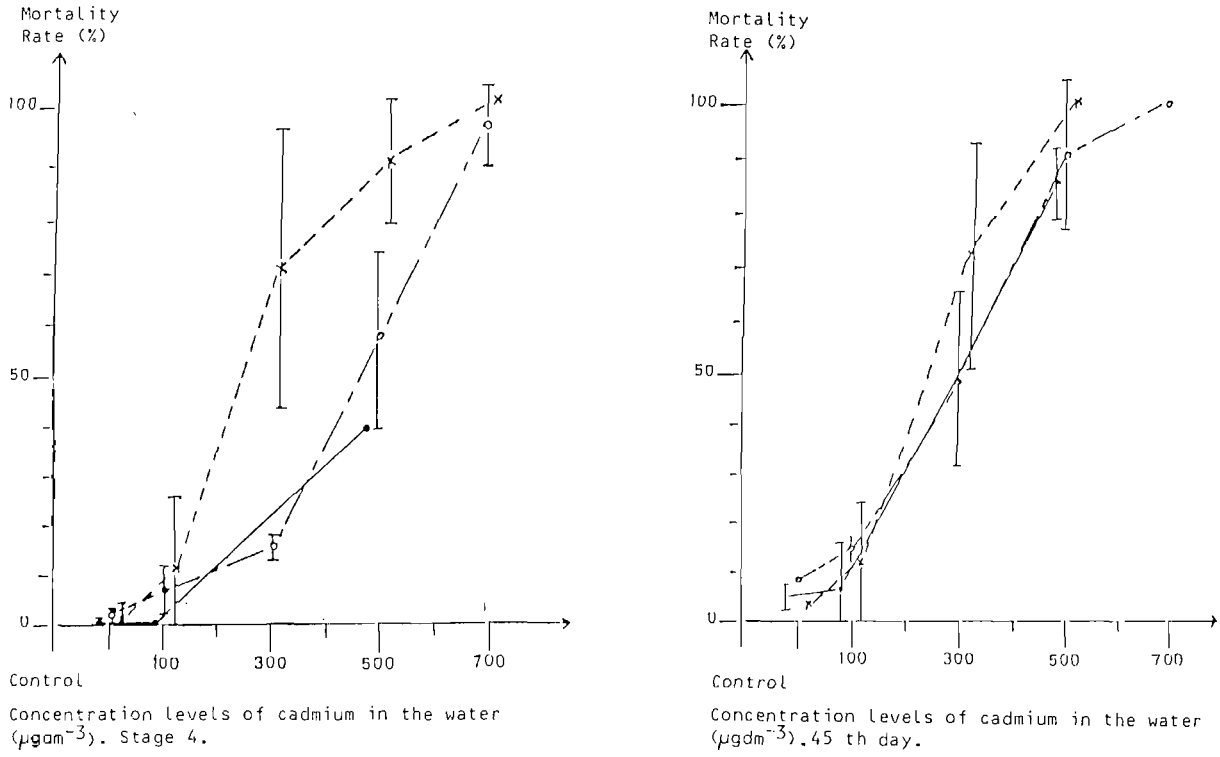


FIG. 3. — P. serratus : *variations of mortality rates of the larvae reared from hatching through stage 4 inclusive (10 days ± 1) or for 45 days, exposed to different concentrations of cadmium in the water (average for two parts from Channel).*

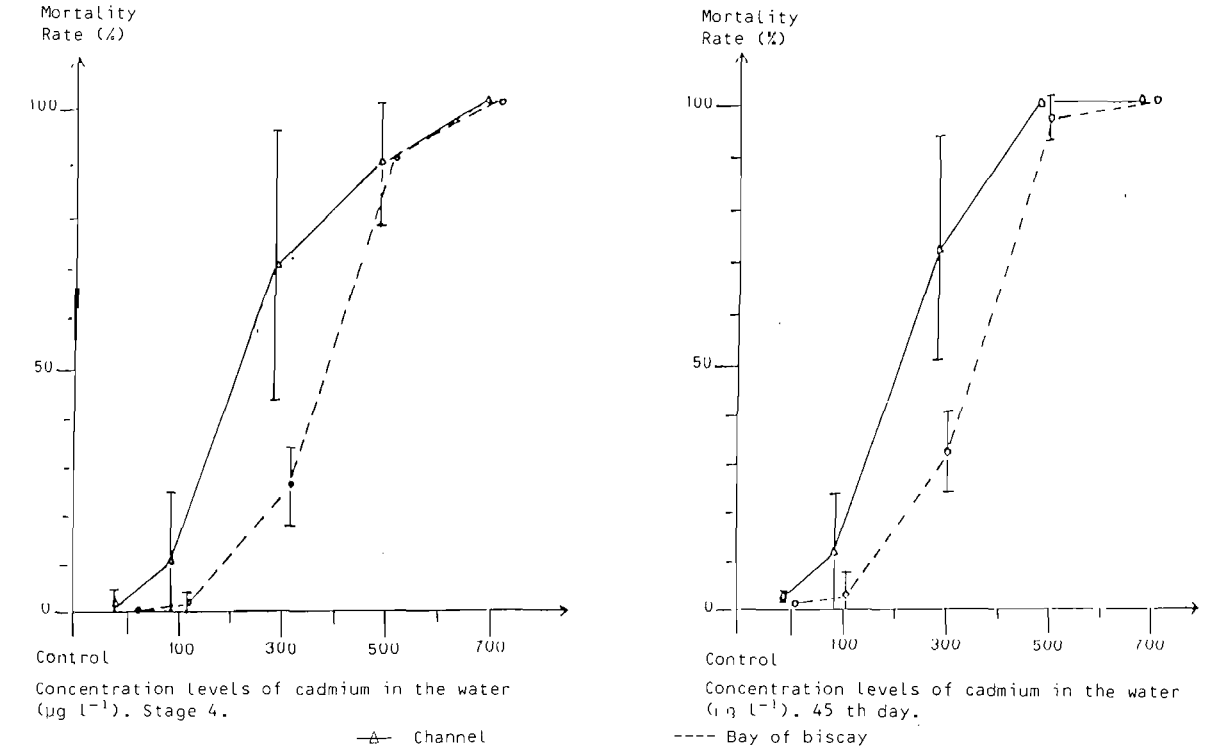


FIG. 4. — P. serratus : *variations of mortality rates according to the geographic origin of the larvae reared at different seasons, from the hatching through stage 4 including (10 days ± 1) or for 45 days, exposed to different concentrations of cadmium in the water (average for two parts from Channel).*

Experiment with *Tigriopus brevicornis*.

CADMIUM screening test

Concentrations µg/dm³	Nauplii	Copepodits	Total larval production	Larval production per female	Copepodits	% death
					tot. larv. prod.	
Control	5	515	520	17.3	0.99	/
6.12	0	510	510	17.0	1.00	2
61.20	335	74	409	13.6	0.18	21.35
122.60	0	0	0	0	/	100
306.00	0	0	0	0	/	100

Concentrations µg/dm³		CADMIUM definitive test				
Cadmium chloride	Cadmium	Nauplii	Copepodits	Total larval production	Larval production per female	Copepodits
						tot. larv. prod.
Control		10	527	537	17.9	0.98
40	24.48	6	509	515	17.2	0.99
50	30.60	15	458	473	15.8	0.97
60	36.80	21	467	488	16.3	0.96
80	49.00	207	215	422	14.1	0.51
100	61.20	342	77	419	14.0	0.18
120	73.56	312	74	386	12.9	0.19
150	91.95	210	32	242	8.1	0.15
160	97.92	138	11	149	5.0	0.07
170	104.04	65	3	68	2.3	0.04
180	110.16	0	0	0	0	/
200	122.60	0	0	0	0	/

TABLE 2. — *Results of screening and definitive tests permissive to determined the LC 50 of the larval production.*

Experiment with *Tigriopus brevicornis*.

ZINC screening test

Concentrations µg/dm³	Nauplii	Copepodits	Total larval production	Larval production per female	Copepodits tot. larv. prod.	% death
Control	8	524	532	17.7	0.98	/
135	0	522	522	17.4	1.00	1.88
189	6	519	525	17.5	0.99	1.32
270	21	480	501	16.7	0.96	5.83
405	0	0	0	0	/	100

Concentrations µg/dm³		ZINC definitive test				
Sulfate zinc	Zinc	Nauplii	Copepodits	Total larval production	Larval production per female	Copepodits tot. larv. prod.
Control		10	527	537	17.9	0.98
1 000	270	18	486	504	16.8	0.96
1 100	297	15	485	500	16.6	0.97
1 200	324	90	198	288	9.6	0.69
1 300	351	66	138	204	6.8	0.68
1 400	378	18	36	54	2.0	0.66
1 500	405	0	0	0	0	/

TABLE 2. — *(Continuation)*.

Discussion.

Comparaison with sensitivity of other tests.

The most significant group to cadmium is crustaceans (EISLER, 1971) as confirmed by the results recorded in table 3. If we compare the CL 50 obtained with *P. serratus* larvae at stage IV with other crustaceans results compiled in table 6, we note homogeneity of values. Thus PRICE and UGLOW (1979) indicate a CL 50 (5-6 days) of 350 µg/dm³ for *Crangon crangon*, EISLER and GARDNER (1973) locates the CL 50

(96 hours) for *Crangon septemspinosa* and for *Pagurus longicarpus* at 320 $\mu g/dm^3$ and for *Palaemon vulgaris* at 420 $\mu g/dm^3$. The two experiments that we undertook reveal a more important impact of cadmium than zinc, at the same time on *P. serratus* larvae and on *T. brevicornis* larval production. This corresponds to the results usually obtained on the crustaceans. It is necessary to take into account that the results compiled in the tables 3 and 4 confirm the sensitivity of the tests using *T. brevicornis* : EC 50 of the larvae production 78 ± 2 $\mu g/dm^3$ for cadmium, 325 ± 2 $\mu g/dm^3$ for zinc. This was confirmed by HOPPENHEIT (1977) and HOPPENHEIT and SPERLING (1977) who situated the lethals values for the copepode *Tisbe holothuriae* to higher doses : between 148 and 1 125 $\mu g/dm^3$ over 30 weeks ; the sublethal effects have a value of 148 to 222 $\mu g/dm^3$ (49 $\mu g/dm^3$ for *T. brevicornis*).

Species	CL 50 $\mu g/dm^3$	Period	Authors	Class
Palaemon serratus (larvae)	220-450	10 days		Crustacea
Tigriopus brevicornis (larvae)	78 ± 2	»		»
Crangon crangon	350	5-6 days	PRICE *et al.*, 1979	»
Crangon septemspinosa	320	96 hours	EISLER, 1973	»
Pagurus longicarpus	320	»	»	»
Palaemon vulgaris	420	»	»	»
Panaeus nerguiensis	460	48 hours	SOMMANI, 1980	»
Fundulus majalis	21 . 10^3	96 hours	EISLER, 1973	Fish
Cyprinodon variegatus	50 . 10^3	»	»	»
Agonas cataphractus	33 . 10^3	»	PORTMANN and WILSON, 1971	»
Mya arenaria	2.2 . 10^3	»	EISLER, 1973	Mollusc
Mytilus edulis	25 . 10^3	»	»	»
Nereis virens	25 . 10^3	24 hours	»	Annelid
Ctenodrilus serratus	2.5 . 10^3 - 5 . 10^3	96 hours	REISH and CARR, 1978	»
Ophryotracha diadema	2.5 . 10^3 - 5 . 10^3	»	»	»

TABLE 3. — *Effects of cadmium on differents species.*

Species	CL 50 $\mu g/dm^3$	Period	Authors	Class
Palaemon serratus (larvae)	300-750	10 days		Crustacea
Tigriopus brevicornis (larvae)	325 ± 2	»		»
Palaemonetes varians	38.4 . 10^3	96 hours	AMIARD-TRIQUET, 1981	»
Crangon crangon	6.3 . 10^3	»	»	»
Artemia salina	3.1 . 10^3	»	»	»
Palaemon serratus	80 . 10^3	48 hours	FRAIZIER, 1973	»
Palaemon serratus	50 . 10^3	96 hours	»	»
Nitocra spinipes	4.3 . 10^3	»	LINDEN *et al.*, 1979	»
Albarnus albarnus	41.9 . 10^3	»	»	Fish
Anguilla anguilla (juvenile)	63 . 10^3	»	AMIARD-TRIQUET, 1981	»
Salmo irideus	4 . 10^3	48 hours	BROWN, 1970	»
Nereis diversicolor	49.4 . 10^3	96 hours	AMIARD-TRIQUET, 1981	Annelid

TABLE 4. — *Effects of zinc on differents species.*

Comparison procedures and unfolds two tests.

P. serratus exposure period is long (45 days at 20 °C) and because of that, handling must be painstaking : accuracy of results depends upon the frequency of renewing the rearing medium (every day). On the other hand, experimental pressures are not so great for *Tigriopus* : after the hatching of the last larvae (2^{nd} or 3^{th} day of the experiment) observations are not necessary until the 10^{th} day. Moreover, about one

month is needed between adult prawn collection and larvae hatching in experimental conditions ; whereas the fishing period is more or less extensive according to geographical area : September to July in the Channel (CAMPILLO, 1979) and December to June in the Bay of Biscay (KURC *et al.*, 1965). Such experimental preconditions are detrimental in comparison to all year availability of adults in the Copepod test.

Another point is the variation in sensitivity of *P. serratus* larvae according to geographical origin or fishing season. As a consequence repeatability of results is unsatisfactory even when experiments are carried out by the same operator using a strictly defined method. The *Tigriopus* test does not show such disavantages : at any time equally sensitive copepods are available for toxicant testing. To prevent the degeneration of the initial strain, it is strongly advised to check tests by a toxicant control reference (e.g. : potassium dichromat).

To conclude, it would appear that *P. serratus* is not really appropriate for use in the case of a standard test procedure, at least not unless rearing conditions are clearly controlled for several generations, and such conditions lead to high cost procedures. In the case of long term studies on real acceptation capacities of natural waters such experimental procedures can be proposed because ecological parameters are taken into account. On the other hand *Tigriopus brevicornis* seems to be easier and cheaper to use for *in vitro* standard bioassays aiming to present a classification of different chemicals on the basis of their toxicity. Easy rearing conditions assures low-cost test procedures, and the use of synthetic sea water enables a wide range of laboratories to participate in a ring-test.

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