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TEMPERATURE AND ADAPTATION TO POLLUTION AS FACTORS INFLUENCING THE ACUTE TOXICITY OF Cd TO THE PLANKTONIC COPEPOD *ACARTIA CLAUSI*

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Résumé. –*La température et l'adaptation à la pollution comme facteurs influant sur la toxicité aigue du cadmium chez le Copépode planctonique Acartia clausi.* Des bioessais statiques de toxicité aigue ont été réalisés avec $Cd\,Cl_2, 2H_2O$ en utilisant des femelles adultes du Copépode planctonique *A. clausi.* Les expériences ont été faites à deux températures : 14 ± 0,5 °C et 22 ± 0,5 °C. sur les assemblages naturels de Copépodes de deux milieux différents : l'un pollué par des effluents industriels et domestiques, et l'autre relativement propre. Les résultats des tests de toxicité du cadmium, exprimés comme LC50 48 heures, indiquent une différence significative dans la tolérance au cadmium entre ces deux populations. De plus, chez ces deux populations, l'augmentation de température de 14 à 22° C entraîne un doublement de l'augmentation de sensibilité d'*Acartia* au cadmium.

Summary – Static acute toxicity bioassays were conducted with $Cd\,Cl_2, 2H_2O$ using adult females of the planktonic Copepod *A. clausi.* The experiments were run at two temperatures : 14 ± 0,5° C and 22 ± 0,5° C on natural Copepods assemblages from two different locations : one from an area polluted with industrial effluents and domestic wastes and another one from a relatively clean area. Results of cadmium toxicity tests, expressed as LC 50 48 hours, indicate a significant difference in the tolerance to cadmium between the two populations. Furthermore, for both populations the increase in temperature from 14 to 22° C resulted in a two fold increase in the sensitivity of *Acartia* to cadmium.

The increase in human population and technolical development are putting serious stress on the environment. Information on the nature and degree of pollution damage to the marine organisms is fragmentary, limited mainly to fish and benthic invertebrates. The use of planktonic animals for testing the effects of pollutants is recommended because of their sensitivity, due to their small size, and also the role they play in the marine food chains.

Cadmium salts from industrial and other sources are found in wastes discharged into sea waters (Mckee and Wolf, 1963). The toxicity of Cd to man has been recognised for over a century. There is growing evidence implicating Cd as a potential threat to human health via marine food chains (Mckee and Wolf, 1963 ; Kabota et al., 1968 ; Eisler et al., 1972) and sea food constitutes a major source of Cd in the man diet.

There is a vast amount of literature studying the role of temperature in the physiology and ecology of aquatic organisms. However there is very little evidence on the effects of temperature on chemical toxicity to aquatic organisms.

In order to estimate accurately the impact of polluants to aquatic organisms, one has to check the influence of chemicals in the whole temperature range in which the animals live. The understanding of the relationships between temperature and the response of marine animals to toxic chemicals is also necessary in order to develop water quality criteria or when discharges of toxic substances are located near water heating sources.

The aim of this study was to determine the acute, toxicity of Cd on the survival of the key-planctonic species *Acartia clausi* (Copepoda, Calanoida) as influenced by the temperature and the adaptation of this copepod to a polluted marine environment.

MATERIAL AND METHODS

A. Clausi were collected by using a Wp2 plankton net in two areas of the Saronic gulf (gulf of Athens : Fig. 1). The Northern part of this gulf (Elefsis Bay. Keratsini) is bordered by the most heavily populated and industrialized area of Greece.

High concentrations of trace elements in the Elefsis

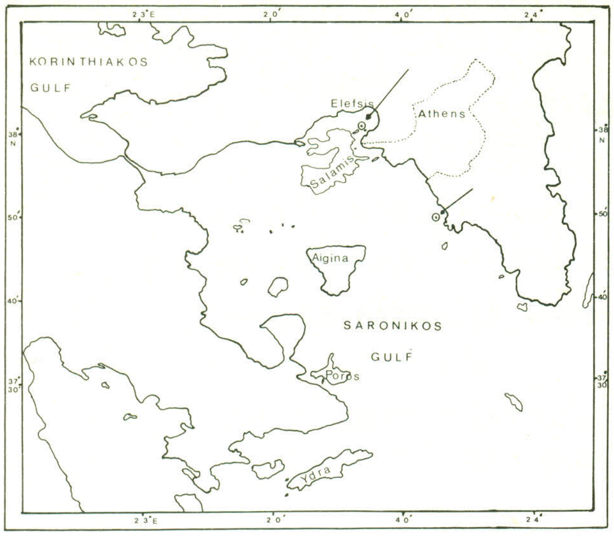

Figure 1 – Saronic gulf, where the study was carried out.

bay sediments have been recently reported (Grimanis et al., 1976). Planktonological studies (Moraitou-Apostolopoulou, in press) also showed that the Keratsini area exhibits the characteristics of primary organic pollution.

The influence of pollution sources decreases southwards so that the south Saronic gulf can be considered as a relatively clear area (Pavlou and Dexter, 1973 ; Moraitou-Apostolopoulou, 1974, 1976 ; Kiortsis and Moraitou-Apostolopoulou, 1975 ; Moraitou-Apostolopoulou and Kiortsis, 1976).

A. Clausi, a common planktonic form in the Aegean Sea (Moraitou-Apostolopoulou, 1972) increases in numbers in the Saronic gulf, representing 50 % of the total number of Copepods (Moraitou-Apostolopoulou, 1974). Inside the heavily polluted Elefsis Bay, *A. Clausi* forms very dense populations being the almost exclusive component of zooplankton during winter.

A similar proliferation of *A. Clausi* in polluted waters has also been mentioned by others (Platt et al., 1970 ; Guglielmo, 1973 ; Citarella, 1973) so that this species is now considered as a pollution adapted or even pollution indicator species.

In previous experiments (Moraitou-Apostolopoulou and Verriopoulos, in press) we found significant differences, in the impact of copper to *A. Clausi* between populations living : a) inside the heavily polluted Elfsis Bay and b) in a relatively clear area of the Saronic gulf about 25 km southwards of Elefsis Bay. In order to check if these two populations present also differences in the tolerance to cadmium we have performed comparative experiments with animals belonging to those populations.

All experiments were conducted in constant temperature rooms and at two temperatures : a) 14 ±0,5° C b) 22 ± 0,5 °C. The sea water used was previously filtered in millipore filter and autoclaved. In preliminary tests a range of concentrations was used to obtain the appropriate concentrations for the final tests.

The test solutions were prepared by diluting a stock solution (20 mg l^{-1}) of hydrated cadmium (CdCl2 . 2H20), freshly prepared in order to obtain the final concentrations for the cadmium ions (0-controls 0,1 ; 0,2 ; 0,4 ; 0,6 ; 0,8 ; 1,1 ; 1,2 ; 1,4 ; 1,8 ; 2,2 mg l^{-1}). For every concentration and for each one of the two populations, 20 mature and apparently healthy females were put individually in glass coppels containing 50 ml of solutions. The coppels were covered with aluminium foil sheets.

Survivors and dead animals were counted every 24 hours and the dead animals were removed. An animal was thought to be dead when failed to respond to mechanical stimulation.

Forty-eight hours LC50 values (concentration of a toxicant lethal to 50 of the test animals after 48 h of exposure) were determined. The experimental results give two limits values between which the real lethal concentration is placed. In order to obtain an accurate result, we used Bliss's modified method (1938) for small numbers of test animals.

RESULTS – DISCUSSION

Table 1 presents the mortality of the two populations exposed at the different concentrations of cadmium and at the two tested temperatures.

The calculated (according to Bliss's method) LC 50 48 hours values are given in Table 2.

Cadmium prooved to be much less toxic to *Acartia* than copper. In previous experiments (Moraitou - Apostolopoulou and Verriopoulos, in press) the calculated LC 50 48 h values for copper (at

Tableau 1. Percentage of mortality of *Acartia clausi* in different concentration of cadmium.

Concentr. mg.l^{-1}	t = 14°C				t = 22°C			
	24 h		48 h		24 h		48 h	
	clear	pollut.	clear	pollut.	clear	pollut.	clear	pollut.
0	0	0	20	5	0	0	20	15
0,1	.	.	.	.	5	.	35	.
0,2	.	.	.	.	10	.	50	.
0,4	5	0	40	10	.	0	.	45
0,6	5	5	45	30	20	5	55	60
0,8	20	5	50	25	.	15	.	70
1,0	15	10	55	35	30	10	70	75
1,2	25	10	70	55	.	15	.	75
1,4	30	10	85	55	35	25	80	85
1,8	38	20	88	60	35	30	75	95
2,2	35	25	90	65	.	30	.	90

18° C) were : 0,034 ± 0,0044 mg l^{-1} Cu for the clear area and 0,082 ± 0,026 mg l^{-1} Cu for the animals of the polluted region.

Concerning the mode of action of Cd, it is well established that in mammals and fishes, even at very low concentrations, it disturbs central functions of the organisms by, affecting various basic biochemical and physiological processes (Larson et al., 1976 ; Berlin and Ullberg, 1963). This action of cadmium is mainly due to its high affinity for sulphydryl groups, but also for hydroxyl groups and ligands containing nitrogen (Nilson, 1970).

Tableau 2. LC 50 48 h values of Cd (mg . l^{-1}. *Acartia clausi)*

Temperature	area clear	polluted
14°C	1,20±0,028	1,50±0,038
22°C	0,6 ±0,043	0,74±0,023

Results of cadmium toxicity tests expressed as LC50 48 h values show significant differences in the tolerance between animals from the two differently, polluted areas. The tolerance of *A. clausi* from Elefsis Bay was higher than that of the animals from the clear region.

A similar higher tolerance to copper of the pollution-adapted *A. clausi* concerning the impact of copper in lethal and even sublethal concentrations has been observed previously (Moraitou-Apostolopoulou in press ; Moraitou- Apostolopoulou and Verriopoulos, in press). An increased tolerance to the toxic effects of some metals can be obtained under laboratory conditions by previous exposure to sublethal concentrations of these metals (Loyd, 1960 ; Sprague, 1970 ; Saliba and Absanullah, 1973).

In other respects, metal-resistant strains of organisms has been collected in contamined areas (Whitton, 1970 ; Bryan and Hummerstone, 1971 Russel and Morris, 1972 ; stokes et al., 1973 ; Moraitou- Apostolopoulou and Verriopoulos, in press). The organisms of an area may presumably evolve physiological mechanisms to either moderate the stress and tolerate it or perhaps compensate artificial stress produced by man's activity.

On the point of view of the organisms, the adaptability to metals seems to be a favourable mechanism. However, it should be remembered that many of these tolerant organisms contain greater concentrations of metals than normal animals and these may be transmitted to non-adapted predators, including man (Bryan, 1976).

Bryan (1974, 1976) found that copper was absorbed more rapidly by tolerant than non-tolerant polychaetes and thought that this may be a future of copper tolerant worms reflecting their greater capacity for binding and detoxicating copper in epidermal cells. The same author concludes that circumstantial evidence suggests a genetically determined metal tolerance which, for the most of metals, develops separately. Russel and Morris (1972) also indicate that the tolerance to copper of the brown alga *Ectocarpus siliculosus* is genetically determined.

A two fold increase was observed in the sensitivity of *A. clausi* to cadmium by an increase in temperature from 14 to 22° C.

Eisler (1971) found also a two to three fold decrease in the 98 h TL_m for cadmium with *Fundulus* in sea water when the temperature was raised from 5 to 20° C.

The effects of temperature upon the acute toxicity of heavy metals may be direct or indirect. Within the tolerance range of an organism, a metabolic rate increase, due to the temperature elevation, causes numerous biochemical and physiological changes. Tissue anoxia at high temperatures has been observed in aquatic animals, thus the effects of any toxicant that either increases metabolic demands or blocks oxygen uptake may be rendered more active by increased temperatures. Temperature also causes alterations in cellular enzymes, with implication where the toxicant inhibits or accelerates specific cellular enzymes. Temperature also changes permeability of cellular membranes.

However it must also be emphasized that detoxification mechanisms and excretory processes also increase at higher temperatures.

Indirect effects of temperature to toxicity of metals include solubility and diffusion rate changes (mainly availability of dissolved oxygen).

The observed marked increase of the sensitivity of *A. clausi* to cadmium with a temperature increase from 14 to 22 ° C is of particular interest in temperate regions, marine animals encounter similar temperatures during summer. The tests of metal toxicity on aquatic organisms are usually run under ecological favourable conditions (artificial oxygenation, low temperatures). Our results support evidence that at least in some less favourable conditions (eg. increased temperature) some marine organisms are less resistant to metals. In order to obtain a more accurate idea of metal influence on aquatic organisms, toxicity bioassays should include the range of environmental conditions in which a toxicant acts.

On the other hand, it must be remembered that most planktonic animals develops more than one generation during the year and the different generations could exhibit different resistance capacities towards toxicants. *A. clausi* lives in the Saronic Gulf in an environment, the temperature of which fluctuates from about 13 ° C to 24,5° C.

Research in course in our laboratory indicates that there is a significant differenciation concerning various physiological processes between the animals of the different generations. It seems probable that the generation of summer tolerates better Cd than the winter generation (our experiments were run between January and March 1978).

All the quoted concentrations of Cd are nominal because the complexing capacity of sea water and the environmental conditions change significantly the nominal values (Doudourof and Katz, 1957 ; Mckee and Wolf, 1963).

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