Effects of pollution on the physiology of *Acartia clausi* and total zooplankton from Saronikos Gulf

Georges VERRIOPOULOS*, Pierre KERAMBRUN** and Maria THESSALOU-LEGAKI*

* Zoological Laboratory, University of Athens, Panepistimiopolis, ATHENS (**Greece**)
** Centre d'Océanologie de Marseille, Faculté des Sciences de Luminy, MARSEILLE (**France**)

A study of the influence of pollution on the physiology of the crustacean copepod *Acartia clausi* and total zooplankton was carried out in coastal polluted and non polluted areas of the Saronikos Gulf (Gulf of Athens). The physiological situation of the organisms was examined by means of a dual approach : metabolic (respiration rate) and enzymatic in order to obtain a pluridimensional view of the effects of pollution on coastal zooplankton at the physiological level. The copepod species, *Acartia clausi*, was chosen as one of the basic elements of the zooplanktonic community of the Saronikos Gulf (MORAITOU-APOSTOLOPOULOU, 1974).

Two sampling areas were chosen : Elefsis bay (eutrophic, one of the most polluted areas in the eastern Mediterranean) and Vouliagmeni bay and Fleves islands (oligotrophic, non polluted area). Several samples were collected in both areas during spring and summer 1991. After transport to the laboratory, one zooplankton sample was used to select two or more sets of mature specimens of alive *Acartia clausi* (100 ind., 200 ind. when possible). The other total zooplankton samples and the sets of selected *A. clausi* were divided into two groups. The first one was preserved in a freezer (- 25°C) and transported by air to Marseille (France) in a dry ice container for electrophoresis and enzymatic tests. Oxygen consumption and dry weight were measured from the second group.

Electrophoresis were carried out on 7,5% polyacrylamide gel using a Tris-Glycine buffer pH 8,5. Several enzymatic activities were revealed. The API-ZYM system (Bio-Mérieux, France) was used as a semiquantitative micromethod to test enzymatic activity of 19 hydrolases. Oxygen consumption measurements were carried out using an oxymeter (YSI model 51 B) according to OMORI and IKEDA (1984). The statistical analysis of the results were based mainly on a multifactor analysis of variance, taking into account the respiration rate and the presence or absence of food during the experiment, the season and the locality.

Figures 1 and 2 show the differences observed in *A. clausi* and total zooplankton zymograms of esterases and amylase between the two sampling areas. Figure 3 gives the comparative activities of 19 hydrolases tested by the API-ZYM technique in the total zooplankton from the two areas.

Mean oxygen consumption rate for *A. clausi* was 5.9 ± 0.7 µl O_2. mg $DW^{-1}.hr^{-1}$ in spring and 3.1 ± 0.9 µl O_2. mg DW^{-1}. hr^{-1} in summer. This seasonal variation was statistically significant (P= 0.02). A geographical variation was also observed : the copepods of the non polluted area (Vouliagmeni-Fleves) had a higher respiration rate (5.6 ± 1.0 µl O_2. mg DW^{-1} hr^{-1} than those of the polluted eutrophic area of Elefsis bay (3.5 ± 0.7 µl O_2. mg DW^{-1} hr^{-1}). But the difference was only significant at the probability level P = 0.06.

No statistically significant variation was observed in the respiration rate of starved and fed *Acartia* collected at different seasons and in the two areas. Nevertheless, the variability induced by the absence of food was greater in *Acartia* from Vouliagmeni-Fleves than in those from Elefsis bay. The seasonal variation was also greater in *Acartia* from Vouliagmeni-Fleves.

The results, either concerning respiration metabolism or enzymatic activities, show that physiological differences exist either in *A. clausi* or total zooplankton according to the area concerned, the very polluted, eutrophic Elefsis bay, and the non polluted oligotrophic area of Vouliagmeni-Fleves.

Enzymes tested are essentially hydrolases acting on substrates provided by the environment. The differences in the activity observed for most of them according to the origin of the organisms, thus may be attributed to the trophic conditions of the sampling areas. Therefore, they appear to be an ecophysiological response to environmental conditions (RIVIERE et KERAMBRUN, 1983). The major tendency is a generally higher enzymatic activity in the zooplankton from Elefsis bay. In *A. clausi*, among the few enzymes whose activity appears to be greater at Fleves, we can only take into consideration alkaline phosphatase and amylase. In the case of *A. clausi*, the pollution of Elefsis bay seems to have an "activatory" effect on most of the enzymatic systems tested.

The respiration rate of *A. clausi* showed a clear geographical and seasonal variation in the metabolism. Copepods from the polluted, eutrophic area (Elefsis bay) have a lower respiration rate than those of the non polluted, oligotrophic area (Vouliagmeni-Fleves). Respiration rate also decreases by about 50% from spring to summer in *Acartia* from Vouliagmeni whereas this decrease is lower in polluted waters.

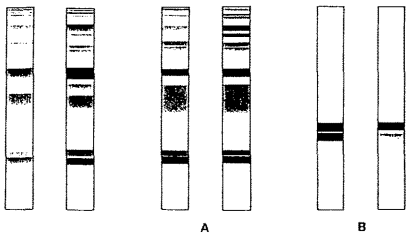

Fig. 1. Acartia clausi. Zymograms of esterases. Sample from Fleves on the left, from Elefsis on the right.

Fig. 2. Total zooplankton. A, zymograms of esterases; B, zymograms of amylase. In both cases, sample from Fleves on the left, from Elefsis on the right.

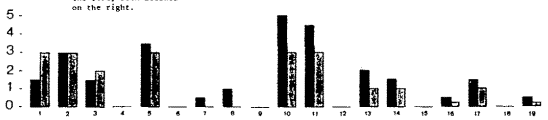

Figure 3. *Acartia clausi*. Comparative enzymatic activities mean obtained from API- ZYM (arbitrary units from 0 to 5). Dark: Elefsis bay samples; Light: Fleves islands samples.
1, acid phosphatase; 2, esterase (C4); 3, esterase lipase (C8); 4, lipase (C14); 5, leucine arylamidase; 6, valine arylamidase; 7, cystine arylamidase; 8, trypsine; 9, α-chymotrypsine; 10, acid phosphatase; 11, phosphoamidase; 12, α-galactosidase; 13,β-galactosidase; 14,β-glucuronidase; 15, α–glucosidase; 16,β–glucosidase; 17, N-acetyl-β-glucosaminidase; 18, α-mannosidase; 19, α-fucosidase

Literature data indicate that the population of *A. clausi* of Elefsis bay is adapted to pollution (MORAITOU-APOSTOLOPOULOU et VERRIOPOULOS, 1979, 1981 a, b). Our results, both concerning enzyme activity and respiration rate, show that the differences observed between sampling areas seems to be directly linked to the degree of water pollution, which influences ecophysiological relationships, thus affecting general metabolism.

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Heavy metal concentrations in the Kerkyra Strait (N.E. Ionian Sea)

F. VOUTSINOU-TALIADOURI, E. GEORGAKOPOULOS-GREGORIADES and R. PSYLLIDOU-GIOURANOVITS

National Centre for Marine Research, Hellinikon, ATHENS (**Greece**)

Kerkyra Island is situated in the uppermost Northeastern part of the Ionian Sea. The study area is located between the Kerkyra Island and the Northwestern part of Greece. It communicates with the Ionian Sea through two straits: 1 n.m. wide in the North, 6 n.m. in the South; maximum width of the area 16 n.m. (Fig.1). The depth contours generally follow the coastal lines, and maxima of around 70 m are attained in the central part of the region. There is a shallow (<20 m) embayment north of the Kalamas River Delta.

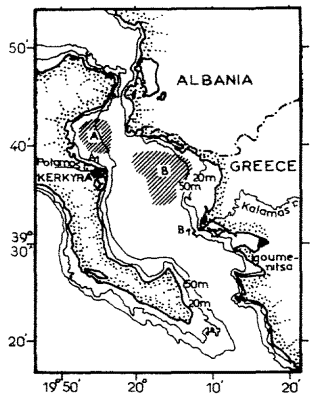

Table I : The study area, the bathymetry and the areas mentionned in the text.

The main interest of the study is focused in the marine environment around the city of Kerkyra, with a permanent population of 40.000 inhabitants and over 100.000 tourists a year. The study area receives agricultural and industrial wastes brought by the Kalamas River and some by a small torrent north of the city of Kerkyra. The goal of this study is to assess the environmental state of the region, as far as heavy metal pollution is concerned, and to answer to questions related to the impact of the domestic waste disposal through a central sewage outfall system, which will be constructed soon.

Surface sediment samples were collected during 3 cruises in 1990-91 over a grid of 102 stations, using a 0.1 m2 van Veen grab. The sample network was particularly dense in the sea area off the city of Kerkyra.The samples were examined for organic carbon and the metals Fe, Cr, Ni, Mn, Zn, Co, Cu and Pb.

The extraction of the metals was achieved with 2N HCl and the determination of the metal content in the leachates was performed on a Perkin-Elmer 305 B A.A.S. (SATSMADJIS & VOUTSINOU-TALIADOURI 1981). Organic carbon was obtained according to GAUDETTE, *et al.*, 1974. Analyses were performed in triplicate. The reliability of the whole process had been ascertained in Intercalibration Exercises (I.A.E.A., 1978). The analyses indicated the following average standard deviations and coefficient of variations: Fe (%o) 0.85, 4.4; Mn ($mgkg^{-1}$) 38, 5.0; Zn ($mgkg^{-1}$) 4.3, 7.2; Cr ($mgkg^{-1}$) 3.8, 3.4; Ni ($mgkg^{-1}$) 3.3, 4.1; Co ($mgkg^{-1}$) 0.9, 8.2; Cu ($mgkg^{-1}$) 1.1, 4.8; Pb ($mgkg^{-1}$) 0.8, 6.7.

The mean values of the analyses are depicted in Tables I & II. Most organic carbon values range from 0.4 to 1.1% and do not indicate any anthropogenic input.

Heavy metal concentrations are generally low and depict almost the same ranges as those obtained, with similar methodology, in other Greek coastal unpolluted regions (Table I).

Table I : Heavy metal concentrations in Greek coastal unpolluted regions (Data for East Aegean Sea, Amvrakikos Bay, South Euboekos Gulf, Lesvos Island, Messolonghi Lagoon, Milos Island, Navarino Bay and Pagassitikos Gulf from VOUTSINOU-TALIADOURI, 1988)

Area	Fe (‰)	Cr	Ni	Mn	Zn	Co	Cu	Pb
		(ppm)						
Kerkyra Isl.	**0.70-3.40**	**35-257**	**65-190**	**200-1400**	**21-94**	**2-24**	**7-30**	**8-24**
East Aegean S.	1.40-3.00	52-157	39-291	280-2640	25-55	8-24	4-29	11-22
Amvrakikos B.	0.49-3.05	27-177	33-188	323-3820	12-80	4-30	2-31	7-21
S.Euboekos G.	0.60-1.50	37- 90	25-144	165- 555	25-44	4-15	0-40	12-27
Lesvos Island	0.32-2.10	40-247	20-315	172-1126	18-43	0-19	3-12	10-39
Messolonghi L.	1.00-2.80	56-112	40-112	470-1380	30-80	6-16	8-34	6-17
Milos Island	0.30-0.60	10- 19	6- 21	113- 251	15-18	2- 4	2- 4	2- 7
Navarino Bay	0.20-3.00	12-251	8-123	243- 600	7-81	4-15	0-32	2-28
Pagassitikos G.	1.30-3.00	50-186	32-228	290-2790	38-72	8-22	9-25	19-30

In general, heavy metal concentrations display the same distribution pattern: four regions with slightly enhanced concentrations.
i) area A: (Fig.1) the enrichment factors of the metal concentrations in this area range from 1.47 to 2.07 (Table II); **ii) area B:** the enrichment factors of the metal concentrations (except Pb) range from 1.10 to 1.70; **iii) area A1** (at the mouth of the torrent Potamos): the concentrations of Pb and Cu are slightly elevated and **iv) area B1** (at the mouth of the Kalamas River): the concentrations of Cu, Co and Cr are slightly elevated.

Table II: Heavy metal enrichment factors in the study area.

	Fe	Cr	Ni	Mn	Zn	Co	Cu	Pb
Area A	1.85	1.62	1.47	1.75	1.70	1.60	2.07	1.50
Area A_1	-	-	-	-	1.10	-	1.23	1.33
Area B	1.22	1.10	1.10	1.70	1.10	1.20	1.24	-
Area B_1	-	1.32	-	-	-	1.60	2.00	-

The slight enrichment in heavy metal concentrations encountered in areas A and B (covered by fine-grained sediment) is attributed to the physicochemical processes of the material supplied by the nearby rivers. In conclusion, surface sediments of the study area show heavy metal concentrations similar to those reported for other unpolluted Greek areas, although a slight anthropogenic enrichment of the concentrations of Cu, Co, Cr and Pb at the mouth of the rivers is sustained.

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