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EGG LAYING IN TWO POPULATIONS OF *ACARTIA CLAUSI* EXPOSED TO DIFFERENT DEGREES OF POLLUTION

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PLANCTON
COPÉPODES
PONTE
POLLUTION
ADAPTATION

RÉSUMÉ. – La fécondité du Copépode pélagique *Acartia clausi* a été étudiée dans différentes conditions expérimentales au laboratoire. Les *Acartia* appartenaient à 3 générations annuelles différentes de 2 populations, l'une vivant dans la région très polluée de la baie d'Elefsis, et l'autre dans une région relativement propre. La population adaptée aux conditions de pollution a pondu dans tous les cas un nombre d'œufs supérieur. Nous n'avons pas observé de différences significatives dans la fécondation des 3 générations d'*Acartia*. Les différentes conditions de laboratoire (présence-absence de nourriture, photopériode, nature spécifique des Algues servant de nourriture) ne paraissent pas particulièrement influencer la fécondité. Le nombre d'œufs pondus semble être principalement lié au mode de vie antérieur aux expériences, alors que les conditions expérimentales semblent jouer un rôle secondaire.

PLANKTON
COPEPODS
EGG-LAYING
POLLUTION
ADAPTATION

ABSTRACT. – The importance of egg-laying of the planktonic Copepod *Acartia clausi* has been studied under various experimental conditions. The specimens of *Acartia* used belonged to 3 different annual generations and 2 populations : one living in the heavily polluted area of Elefsis Bay, the other living in a relatively clean area of the Saronic gulf. The pollution-adapted population of *Acartia* has a higher reproductive capacity presenting always higher numbers of eggs laid. No significant seasonal variation of the importance of egg laying has been observed between the *Acartia* belonging to different annual generations. Various experimental conditions (presence or absence of food, light conditions, specific nature of food) do not seem to significantly alter the egg output. The number of laid eggs seems to be mainly determined by the previous life history of the animals and to a lesser degree by the « laboratory conditions ».

INTRODUCTION

The fecundity of planktonic organisms constitutes a basic parameter in the estimation of secondary production as it influences recruitment within a population...

The intensity of egg-laying is determined by genetic factors, but it also seems to be influenced by the environmental conditions. For the pelagic Copepods it has often been mentioned that food plays a major role in the induction and intensity of egg-laying (Comita and Anderson, 1959 ; Marshall and Orr, 1952 ; Gaudy, 1971, 1974 ; Valentin, 1972).

Furthermore it seems that not only the quantity but also the quality of phytoplankton present plays a role in Copepod fecundity (Marshall and Orr, 1952 ; Gaudy, 1971).

Finaly, Gaudy (1971) noticed a seasonal variation in the importance of egg-laying in three neritic Copepods (*Temora stylifera, Centropages typicus* and *Acartia clausi).*

As the interaction of all mentioned factors controls the egg production, which is at the basis of production and seasonal variation of a planktonic population, an experimental study on the fecundity of the very common planktonic form *Acartia clausi* was undertaken with animals collected in the Saronic gulf.

The Saronic gulf (gulf of Athens), an extension of the South Aegan sea, is an area of particular socioeconomic importance. The Nort-east part of this gulf (Elefsis-Keratsini bay) is considered as a heavily polluted area because of industrial effluents from a flourishing industry around the bay and of organic matter from the main sewage outfall of Athens and Piraeus.

High concentrations of trace elements in the Elefsis bay sediments have been reported recently (Grimanis *et al.*, 1976).

Research on the content of sediments of the Saronic gulf (Pavlou and Dexter, 1973) and also planktonological studies (Moraïtou-Apostolopoulou, 1974, 1976; Kiortsis and Moraïtou-Apostolopoulou, 1975; Moraïtou-Apostolopoulou and Kiortsis, 1976) have shown that, although the northern part of the gulf is heavily polluted, southwards the influence of pollution sources is reduced and the south Saronic gulf can be considered as a relatively clean area.

Acartia clausi, a common planktonic form all over the Mediterranean and the Aegan Sea, constitutes a basic element of the zooplanktonic community of the Saronic gulf (it constitutes 30 % of the total zooplankton numbers (Moraïtou-Apostolopoulou, 1974)). *Acartia* exhibits a pronounced seasonal variation, being abundant during the cold period and diminishing in numbers with rising sea water temperatures. In the heavily polluted Elefsis bay it becomes very abundant and is the dominant component of the very rich winter zooplankton: In view of its high biomass and rapid generation times, Acartia may be considered as a very important primary consumer.

In the mediterranean waters the number of annual generations of *Acartia* has been found to fluctuate from 4 at the Adriatic sea (Vucetic, 1957) to 8 for the Black Sea (Porumb, 1968).

This study of the reproductive potential of *Acartia* at the Laboratory contributes to a better understanding of populations dynamics in nature. This paper is part of a larger study on physiology and quantitative biology of *A. clausi*, the influence of pollution on the physiological mechanisms and the seasonal variability of different physiological processes.

MATERIAL AND METHOD

A. clausi was collected using a WP2 plankton net at two areas of the Saronic gulf: a) inside the heavily polluted Elefsis bay and b) in a relatively clean area situated about 25 km S.E. of Elefsis bay.

The sampling was performed at 3 different hydrological periods: a) between January and March 1977, mean surface temperature of 14 °C (cold period); b) between June and July 1977, mean temperature of 22°C (warm period); c) between October and November 1977, mean temperature of 18 °C (intermediate period).

We fixed the sampling periods in order to be able to catch 3 different generations of *Acartia*.

The sorting of *Acartia* (only adult females) was performed immediately after the return to the Laboratory, under a binocular microscope. All experiments were conducted in constant temperature rooms at the following temperatures: $14 \pm 0{,}5$ °C for the cold period, $18 \pm 0{,}5$ °C for the intermediate period and $22 \pm 0{,}5$ °C for the warm period. The experimental temperatures represent the mean water temperature of each experimental period and were chosen in order to exert the minimum thermal disturbance to the animals and facilitate the extrapolation of laboratory data to nature.

The food used in the experiments was a mixture (about 5.10^4 cells/ml) of 4 phytoplanktonic species (*Phaeodactylum tricornutum, Nitzschia clostericum, Skeletonema costatum, Exuviaella baltica*) grown in monospecific cultures. In addition for the *Acartia* of the warm period the four phytoplanktonic species were used separately as food.

The light regime was an alternance of 12 hours of light-dark (5×14 Watt). For the animals of the warm and intermediate period we performed also experiments with continuous light or dark.

For every experimental set 30 *Acartia* were placed individually in glass coppels filled with 50 ml of sea water filtered previously with Millipore filters and autoclaved. The experimental jars were covered with aluminium foil (sheets) and observed every 24 hours. All eggs released were counted under a binocular microscop.

The results were tested statistically by the paired t-test.

RESULTS AND DISCUSSION

Although the mean survival of the females at the laboratory exceeded 10 days, egg-laying was restricted to the first 5-6 days with a usually declining rate.

The egg production of the 3 sampling periods under the different experimental conditions is shown in Tables I, II.

In all cases the number of eggs produced was small. The number of counted eggs must be inferior by about 15 % to the real number of eggs released because of the cannibalism observed in *Acartia*. This percentage was established by preliminary experiments. Furthermore the prior conditions of existence of the females used here are unknown but it is certain that some must have lived out part of their adulthood and already produced a portion of their potential eggs.

To determine experimentally the potential output of females, as it might exist in nature, would require heal-

a) Cold period (January to March), with food (mixture of four phytoplankton species) light-dark.

Exp. days	Mean No of eggs/Cop/day area polluted	non polluted
1	4.0 ± 1.3	3.0 ± 0.03
2	5.39 ± 2.03	1.0 ± 0.04
3	7.0 ± 1.04	3.6 ± 1.07
4	3.0 ± 0.05	1.5 ± 1.08
5	2.5 ± 0.07	1.33 ± 0.05
6	0	0
Total	21,89	10,43

b) Warm period (June to July)

Exp. days	Mixture of food, light-dark Mean No of eggs/Cop/day area		Without food, light-dark Mean No of eggs/Cop/day area		With food, 24 h light Mean No of eggs/Cop/day area		With food, 24 h dark Mean No of eggs/Cop/day area	
	polluted	non polluted	polluted	non polluted	polluted	non polluted	polluted	non polluted
1	10.67 ± 2.03	2.0 ± 0.03	12.33 ± 2.03	2.33 ± 0.05	5.0 ± 1.32	4.0 ± 0.60	11.0 ± 0.60	4.0 ± 1.04
2	4.0 ± 1.82	3.7 ± 0.81	6.0 ± 0.03	2.0 ± 0.03	8.0 ± 0.30	3.0 ± 0.35	1.5 ± 0.07	3.0 ± 1.01
3	4.33 ± 1.02	2.3 ± 0.09	2.66 ± 1.3	1.66 ± 0.57	4.0 ± 0.35	2.5 ± 0.70	3.0 ± 0.03	1.5 ± 0.07
4	2.0 ± 0.03	1.0 ± 0.02	1.0 ± 0.02	1.2 ± 0.03	0	1.0 ± 0.01	2.0 ± 0.02	1.33 ± 0.50
5	0	1.0 ± 0.03	1.0 ± 0.1	0.8 ± 0.01	0	0	1.3 ± 0.01	1.0 ± 0.06
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
Total	21.0	10.0	22.99	7.99	17.0	10.5	18.8	10.83

c) Intermediate period (October to November)

Exp. days	With food, dark-light Mean No of eggs/Cop/day area		Without food, dark-ligth Mean No of eggs/Cop/day area		With food, 24 h light Mean No of eggs/Cop/day area		With food, 24 h dark Mean No of eggs/Cop/day area	
	polluted	non polluted	polluted	non polluted	polluted	non polluted	polluted	non polluted
1	7.21 ± 0.3	6.25 ± 0.2	14.0 ± 3.0	8.38 ± 1.7	4.0 ± 1.1	1.33 ± 0.05	5.66 ± 1.05	5.0 ± 1.7
2	6.57 ± 0.2	2.67 ± 0.3	6.0 ± 1.1	6.0 ± 0.9	2.66 ± 0.7	1.0 ± 0.02	3.0 ± 0.9	5.0 ± 1.0
3	5.40 ± 0.7	2.0 ± 0.1	4.0 ± 3.8	2.1 ± 0.8	1.33 ± 0.08	0	5.0 ± 1.6	1.0 ± 0.08
4	5.20 ± 0.6	2.60 ± 0.1	1.0 ± 1.2	1.9 ± 0.1	1.56 ± 0.09	0	1.0 ± 0.07	0
5	2.0 ± 0.1	1.0 ± 0.08	1.66 ± 0.09	0	0	0	1.0 ± 0.2	0
6	0	0	1.0 ± 0.03	0	0	0	0	0
7	0	0	0	0	0	0	0	0
Total	26.38	14.52	27.66	18.38	9.55	2.33	15.66	11.0

a) Warm period (June to July), with food, light-dark.

Exp. days	*Skeletonema costatum* area		*Phaeodactylum tricornutum* area		*Exuviella baltica* area		*Nitzschia closterium* area	
	polluted	non polluted	polluted	non polluted	polluted	non polluted	polluted	non polluted
1	9.0 ± 1.3	4.0 ± 0.2	8.75 ± 1.7	4.0 ± 0.2	10.75 ± 1.7	3.0 ± 1.1	6.25 ± 1.5	2.0 ± 0.1
2	3.7 ± 0.6	3.1 ± 1.1	5.0 ± 1.1	1.5 ± 0.03	2.30 ± 0.08	1.75 ± 0.5	3.0 ± 0.4	1.5 ± 0.02
3	1.0 ± 0.03	3.2 ± 0.2	4.0 ± 0.2	2.0 ± 0.02	2.75 ± 0.06	1.0 ± 0.06	4.0 ± 0.2	1.5 ± 0.01
4	0	0	2.0 ± 0.03	0	0	1.33 ± 0.04	0	1.0 ± 0.04
5	0	0	0	0	0	1.0 ± 0.03	0	1.0 ± 0.02
6	0	0	0	0	0	1.0 ± 0.01	0	0
7	0	0	0	0	0	1.0 ± 0.03	0	0
Total	13.7	10.3	19.75	7.5	15.8	10.08	13.25	7.0

thy animals newly moulted to adulthood and fully inseminated, which can only be provided by laboratory cultures.

In all experiments the number of eggs laid by the *Acartia* adapted to the polluted environment was higher than that of the *Acartia* living in the non-polluted area. These differences proved statistically significant in most cases.

The increased fecundity of Acartia of Elefsis that has also been observed during previous experiments (Moraïtou-Apostolopoulou and Verriopoulos, 1979) reflects either a genetic factor or the response to polluted environmental conditions at which the *Acartia* of Elefsis has grown and is adapted.

The observation that the fecundity of *Acartia* living at the polluted area is increased when small doses of copper are added to the culture medium, provide some evidence that for the pollution-adapted population of *Acartia* the pollution conditions favour an increase in egg laying.

The increased numbers of eggs laid by the *Acartia* of Elefsis bay must constitute an important contribution to the abundance of this form in this bay.

Comparing the egg production of the 3 sampling periods under the same experimental conditions : with food (mixture of the 4 phytoplanktonic species) and alternance of 12 hours light-dark, the *Acartia* of both areas demonstrated very little fluctuations in their egg production and a small increase was observed for both areas during the intermediate period.

The little seasonal fluctuation in the numbers of eggs produced by *Acartia* was surprising enough because, in nature, during the warm period, *Acartia* is scarce in the plankton samples, while during the intermediate period a clear quantitative decrease of the very abundant winter population is observed.

The scarcity of *Acartia* in nature during the warm period could be related to the observation that during the warm period the lowest hatching rate of the 3 experimental periods was observed. During the cold period a relatively high hatching percentage was noticed (75 % for the *Acartia* of the polluted area and 70 % for the *Acartia* of the non polluted area). During the warm and intermediate period an abrupt decrease of egg hatching was observed (about 30 % for the *Acartia* of the warm period and 45 % of the intermediate). It seems possible that in nature the unhatched eggs sink to the botton where they remain in a state of dormancy.

Resting eggs of Copepods are known in freshwater. Cooley (1971); Zillioux and Gonzalez (1972); and Kasahara *et al.* (1974) discovered abundant numbers of marine Copepods (including *A. clausi*) resting eggs in sea botton muds. The resting eggs must play an important role in the repopulation of sea areas (especially for the fairly enclosed Elefsis bay) after the seasonal dissapearence of planktonic species. Gaudy (1971) noticed a seasonal variation of the importance of egg laying of *A. clausi* with a maximum during May but the restricted numbers of observations made this author doubtful about the validity of these observations. The fact that the egg laying was carried out in the Laboratory at the same temperature for all seasons also complicates the extrapolation of Laboratory data to nature.

Starvation experiments were carried out with females collected during both the warm and intermediate period, in order to determine the effect of food on egg production. The absence of food does not seem to exert a net influence on egg laying. The numbers of eggs laid with and without food presented small fluctuations ; for the pollution-adapted population a small increase of the eggs laid was noticed. All differences in egg production proved statistically unsignificant. It has often been referred to in the literature that the induction and importance of egg laying is related to phytoplankton abundance (Marshal and Orr, 1952 ; Edmonson *et al.*, 1962). Gaudy (1971, 1974) noticed an increase in egg production of *A. clausi* placed in various phytoplankton concentrations, at the lower range of food concentrations.

The observed slight differences in the egg production between fed and starved animals may be attributed either to the relative independance of this omnivorous form from the phytoplankton, or to the hypothesis that egg laying is mainly determined by the previous history of the organism in nature and not to the conditions of the laboratory.

There are few studies on the effects of light, other than photoperiod on population dynamics. Buikema (1973a) demonstrated that the light intensity significantly affects both the number of young per brood and the total offspring of *Daphnia* sp. Sorgeloos (1975) noticed that *Artemia salina* has a significantly higher reproductive capacity in darkness than under light conditions.

For the animals of the intermediate period and for both sampling areas the alternance dark-light seems to be the ideal light condition for egg production and a decrease of the importance of egg laying was observed when the animals were placed in continuous dark or continuous light. The differences proved significant only for the polluted area : between dark-light and dark : 95 % level, $t = 2{,}77$ d.f. = 4 and between dark-light and light : 99,99 % level $t = 22{,}07$ d.f. = 3.

The warm-adapted *Acartia* seems less influenced by the light conditions and presents small differences in egg laying under different light conditions.

The importance of egg laying of the warm-adapted *Acartia* was studied when fed with qualitatively, different food. The *Acartia* of the unpolluted area presented small differences in egg laying when fed with the 4 monospecific cultures. *Acartia* of the polluted area presented greater, but still insignificant variations : reduction when fed with *Skeletonema, Exuviaella* and *Nitzschia,* and an increase when fed with *Phaeodactylum.* Thus the *Acartia* of the polluted area seems more influenced by the quality of food than the *Acartia* living at the unpolluted area. Gaudy (1971) also states that the specific nature of algal cells used as food influences the importance of egg laying.

Although some differences in the importance of egg laying has been observed under various experimental conditions in the laboratory, it appears that the importance of egg laying in *Acartia* is mainly determined by the previous history of the organism in nature and to a lesser degree by the laboratory conditions.

Results of this study indicate that the two populations of *Acartia clausi* adapted to different pollution conditions present differences in their reproductive potential. These differences could be the result of a slow physiological adaptation or have a genetic base suggesting that there may be two physiological races.

It is important to notice that during previous studies the *Acartia* of the polluted area was found to present higher longevity, higher feeding rates, increased thermal tolerance (Moraïtou-Apostolopoulou and Verriopoulos, 1980, 1981 a, b), as well as increased resistance to heavy metal stress (Moraïtou-Apostolopoulou, 1978, Moraïtou-Apostolopoulou and Verriopoulos, 1979; Moraïtou-Apostolololoulou *et al.*, 1979).

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