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DAPHNIA MAGNA STRAUS LIVING IN AN AERATED SEWAGE LAGOON AS A SOURCE OF CHITIN : ECOLOGICAL ASPECTS

by

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SUMMARY

As a means of determining the suitability of microcrustaceans living in aerated lagoons as a source of chitin, the biomass of these animals was measured twice monthly from October 1993 to November 1994 in a lagoon receiving the waste waters of the town of Differdange (G.D. of Luxembourg). The production and chitin content of *Daphnia magna* Straus, the dominant species of the zooplanktonic community (by number and biomass), were assessed over the same period. From December to February, the microcrustacean biomass was low, varying from 0.035 to 0.440 $g.m^{-2}$. High biomass values were observed in April and June (about 4.3 $g.m^{-2}$) as a consequence of phytoplankton blooms and an increase in temperature. In November, despite the scarcity of algal food, the *Daphnia magna* biomass peaked up to 5.5 $g.m^{-2}$ due to the ability of these organisms to use bacteria and detritus for food. Throughout the sampling period, the *Daphnia magna* biomass far exceeded the *Cyclops* spp biomass.

Fluctuations in the daily chitin production of *Daphnia magna* paralleled the biomass fluctuations of the species, with maximum values measured in April (1.9 $g.m^{-2}.day^{-1}$) and June (4.8 $g.m^{-2}.day^{-1}$). The chitin content of *Daphnia magna* ranged from 2.9 to 7.0 % of the total body dry weight. By the summation of the daily chitin productions of the species, the annual chitin production of *Daphnia magna* was estimated at 11.5 $g.m^{-2}.year^{-1}$. This figure is compared with the chitin production of other crustaceans in the natural environment.

Prospects for using *Daphnia magna* living in the studied aerated sewage lagoon as a source of chitin are discussed.

Key words : *Daphnia magna*, sewage lagoon, chitin, production.

INTRODUCTION

Chitin is the second most abundant biopolymer in the world after cellulose (SANDFORD, 1988). Chitin and its derivative chitosan have found numerous applications in agriculture and industry, principally in the cosmetic, pharmaceutical, and biomedical sectors (SANDFORD, 1988). Consequently there is an increasing interest in chitin in all forms and of all degrees of purity. The current sources of chitin for industry are crab shells (*Cancer magister*, *Chionectes japonica*, and *Paralithodes camschatica*) and shrimp shells (*Pandalus borealis*) (HIRANO, 1988 ; SANDFORD, 1988). This kind of raw material presents, however, some disadvantages, such as (1) a high degree of mineralization which necessitates drastic acid treatment (often resulting in partial hydrolysis of the chitin) (BRINE and AUSTIN, 1981) and (2) variations in the chemical composition according to the sex, moulting stage, or age. Because of these disadvantages, investigators seek alternative chitin sources, such as less calcified crustaceans. In this context, JEUNIAUX *et al.* (1993) have estimated at 2.3 × 10^9 T the total amount of chitin produced by crustaceans each year. This estimate, however, does not take into account the amount produced in artificial ecosystems such as aerated sewage lagoons, believed to be adequate sources of chitinous raw material because they generally support large biomasses of planktonic crustaceans such as copepods and cladocerans (DINGES, 1976 ; SEVRIN-REYSSAC, 1993 ; ULHMANN *et al.*, 1994). Furthermore, the cuticle mineralization rate of such zooplankters is low (BAUDOUIN and RAVERA, 1972). The commonly acknowledged major steps in lagoon treatment are (1) aerobic bacterial decomposition of organic wastes resulting in the release of nutrients, (2) algal utilization of the nutrients (DINGES, 1976). Filter feeding zooplanktonic organisms can develop in these lagoons thanks to a sufficient oxygen concentration (>1 mg/l — SHAPIRO, 1990) and to abundant algal (LAMPERT, 1974 ; GOPHEN, 1977) and bacterial (MCMAHON and RIGLER, 1965 ; PETERSON *et al.*, 1978) food. Zooplankton standing crops are often considerable and are mainly composed of *Daphnia magna*, which is generally the dominant species in lagoons (SEVRIN-REYSSAC, 1993). At the present time, these zooplankters are used as fertilizers in aquaculture, baits for fishing and animal food, mainly in aquaculture (SEVRIN-REYSSAC, 1994). Therefore, their use as a source of chitin for special chemical or medical applications would greatly enhance their commercial value.

In this perspective, the aim of this study was to evaluate the advantages of exploiting sewage lagoon microcrustaceans as a chitin source. The following criteria were examined : (1) the seasonal availability of microcrustaceans, especially *Daphnia magna*, and some limnological factors affecting its production, (2) the production and chitin content of *Daphnia magna*. The annual chitin production of this species was evaluated and compared with the chitin production in different natural environments.

DESCRIPTION OF THE SITE

The studied lagoon (60,000 m^2; 2.5 m mean depth) is located at Differdange (Grand-Duchy of Luxembourg). It collects the waste waters of the town after primary treatment. The mean resident time is approximately 50 days. The lagoon is continuously aerated. The planktonic crustacean community is composed of the cladoceran *Daphnia magna* Straus and of cyclopoid copepods of the genus *Cyclops* Müller. Rotifers are seldom abundant during the year. The zooplankton is harvested and sold as aquarium food by the company Bioplancton.

MATERIAL AND METHODS

Temperature, oxygen concentration and chlorophyll a concentration measurements

The temperature and oxygen concentration of the water were measured with a probe (WTW Oxi 196, Germany) on each sampling date. The chlorophyll a concentration was determined spectrophotometrically at 663 nm after extraction with 90 % acetone (APHA, 1985).

Sampling, biomass, and daily production of zooplankton

From October 1993 to November 1994, water samples were collected twice monthly at nine evenly spaced stations with a 3-liter van Dorn bottle at a depth of 1.5 m. The samples for zooplankton counts were filtered through a net with a mesh size of 60 µm and fixed in 4 % formalin. The individuals of the various taxon were counted in each sample in order to estimate a mean population density as described by Bottrell *et al.* (1976). The biomass was calculated from the estimated population density and the length-weight relationship of the studied taxon as developed by Dumont et al. (1975). The daily production of *Daphnia magna* was calculated on the basis of the formula of Winberg *et al.* (1971) including 15 size classes (250µm wide) :

$$P = \Sigma(N_x.\Delta W_x) / D_x$$

where P is the daily production (g dry weight. $m^{-2}.day^{-1}$), N_x the number of individuals in size class x (m^{-2}), ΔW_x the weight increase of an individual growing from the size class x to the next size class (g dry weight), and D_x the development time of size class x (in days), determined from a growth curve established by Mitchell *et al.* (1992). The daily secondary production values are expressed in $g.m^{-2}.day^{-1}$ rather than in $g.m^{-3}.day^{-1}$ because of their dependence on primary production which is a surface process. To standardize our results, we express biomass values also on the basis of area. The biomass values per cubic metre can be obtained by dividing the biomass value expressed per square metre by the mean depth (2.5 metres).

Daphnia magna chitin production

On each sampling date, the daily chitin production was evaluated on the basis of the daily production value and the chitin content of the specimens. The annual chitin production of *Daphnia magna* was estimated by summing the daily chitin productions. Between sampling dates, the daily chitin production was estimated by linear interpolation of the results obtained for sampling dates.

To determine the chitin content, we first freeze-dried the raw material (daphnids 1 mm long). Demineralization was performed with 0.5 N HCl at room temperature and proteins were extracted with 1 N NaOH at 100 ° C. The chitin content was assayed by a colorimetric method based on measuring the amount of N-Acetyl-D-Glucosamine monomers liberated after complete enzymatic hydrolysis by purified chitinases (REISSIG *et al.*,1955 ; JEUNIAUX, 1963, 1965). Assays were carried out with commercial chitinases (E.C. 3.2.1.14) and N-Acetyl-D-Glucosaminidase (diluted lobster serum).

RESULTS

Water temperature and oxygen concentration

In the course of a year, the water temperature varied in sinusoidal fashion (Fig. 1). The temperature was below 10° C from November to May and above 10° C from May to October. The extreme values were recorded in December (1.7° C) and July (25° C).

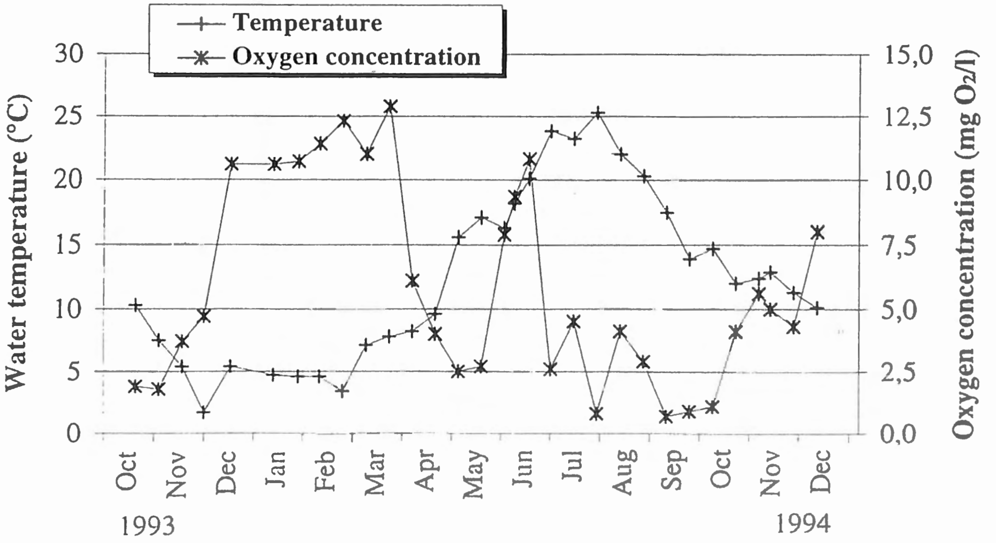

Fig. 1. — Seasonal changes in temperature and oxygen concentration of the water in the sewage lagoon of Differdange from October 1993 to December 1994.

From October to April, the oxygen concentration increased from 1.8 to 12.9 mg $0_2.l^{-1}$ (Fig. 1). Then it dropped to 2.5 mg $O_2.l^{-1}$ at the beginning of May. The oxygen concentration peaked a second time in June at 10.8 mg $O_2.l^{-1}$. From June to November, the oxygen concentration fluctuated between 0.8 and 4.5 mg $O2.l^{-1}$.

Chlorophyll a concentration and crustacean biomass

The chlorophyll a concentration remained generally low (< 1 $\mu g.l^{-1}$); two chlorophyll a peaks were observed, however, one in March (330 $\mu g.l^{-1}$) and one in June (250 $\mu g.l^{-1}$) (Fig. 2). From July to December, the chlorophyll a concentration varied between 2 and 45 $\mu g.l^{-1}$.

The crustacean biomass was low during the winter, varying from 0.035 to 0.440 $g.m^{-2}$ in terms of dry weight (Fig. 2). From March to July, biomass peaks were observed at the beginning of April (maximal value : 4.3 $g.m^{-2}$) and at the beginning of June (maximal value : 4.3 $g.m^{-2}$). Through July and August, the biomass fluctuated sharply between 0.4 and 4.7 $g.m^{-2}$. From September to November, the crustacean biomass increased and reached the highest value measured over the entire year (6.7 $g.m^{-2}$).

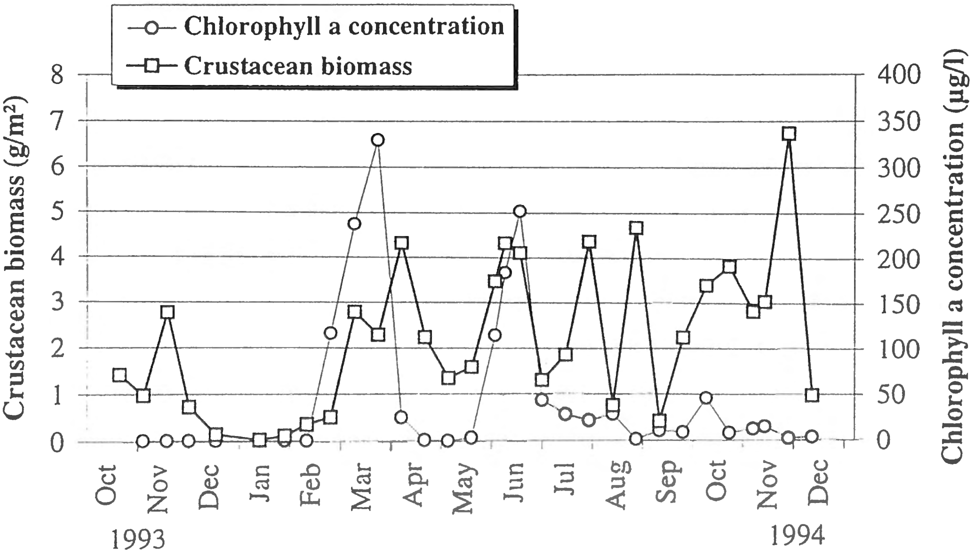

Fig. 2. — Seasonal changes in chlorophyll a concentration and microcrustacean biomass in the lagoon of Differdange from October 1993 to December 1994.

On the whole, the species-related results show that the *Daphnia magna* biomass far exceeded the *Cyclops* spp. biomass (Fig. 3). In winter, however, the biomass of *Cyclops* spp. increased earlier than that of *Daphnia magna*, probably owing to the revival from diapause of copepodits IV overwintering in the sediment (GEORGE, 1973). The observation that adults composed the majority of the population in January and February supports this explanation. Peaks of *Daphnia magna* biomass were recorded in April (3.7 g.m^{-2}), June (3.7 g.m^{-2}), July (4.3 g.m^{-2}), August (4.4 g.m^{-2}), and November (5.5 g.m^{-2}). *Cyclops* spp. biomass exhibited three peaks with values exceeding 0.8 g.m^{-2} : March (maximal value : 1.2 g.m^{-2}), June (maximal value : 1.0 g.m^{-2}), and from mid-September to the end of October (maximal value : 1.4 g.m^{-2}). The lowest biomasses were observed in January (0.035 g.m^{-2}), May (0.070 g.m^{-2}), and August (0.067 g.m^{-2}).

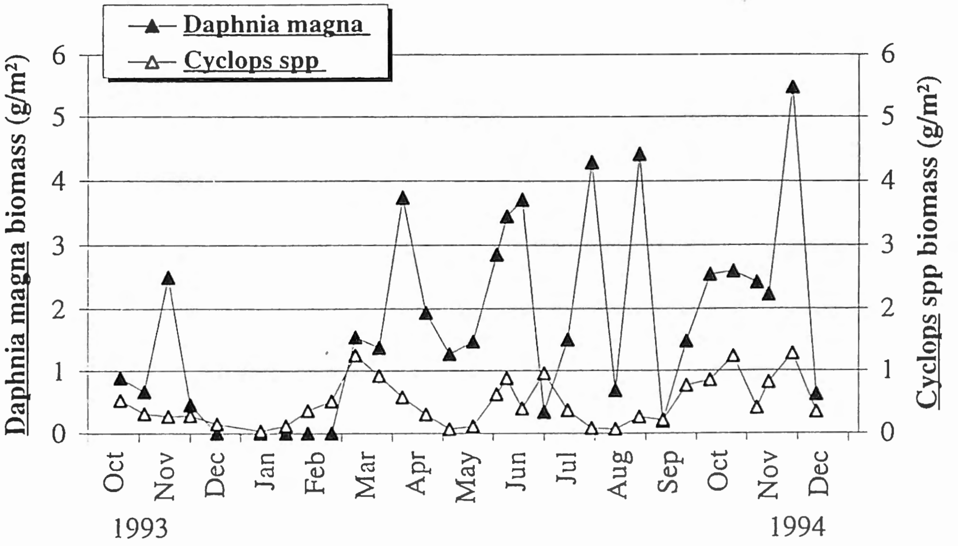

Fig. 3. — Seasonal changes in *Daphnia magna* biomass and *Cyclops* spp. biomass in the sewage lagoon of Differdange from October 1993 to December 1994.

Daphnia magna chitin production

To evaluate *Daphnia magna* chitin production, we first calculated the daily production of *Daphnia magna*, which fluctuated in the same manner as the biomass of the species (Fig. 4). In winter, the daily production was virtually nil. The highest values were recorded in April (maximal value : 1.9 g.m^{-2}.day^{-1}), June (maximal value : 4.8 g.m^{-2}.day^{-1}), and October (maximal value : 1.7 g.m^{-2}.day^{-1}). The calculated annual production of *Daphnia magna* was 234 g.m^{-2}.year^{-1}, *i.e.* 94 g.m^{-3}.year^{-1}. The highest ratio of daily production (P) to biomass (B), i.e. the highest productivity, was recorded in June (P/B = 1.4). Moreover, 35 % of the annual production occurred during this month.

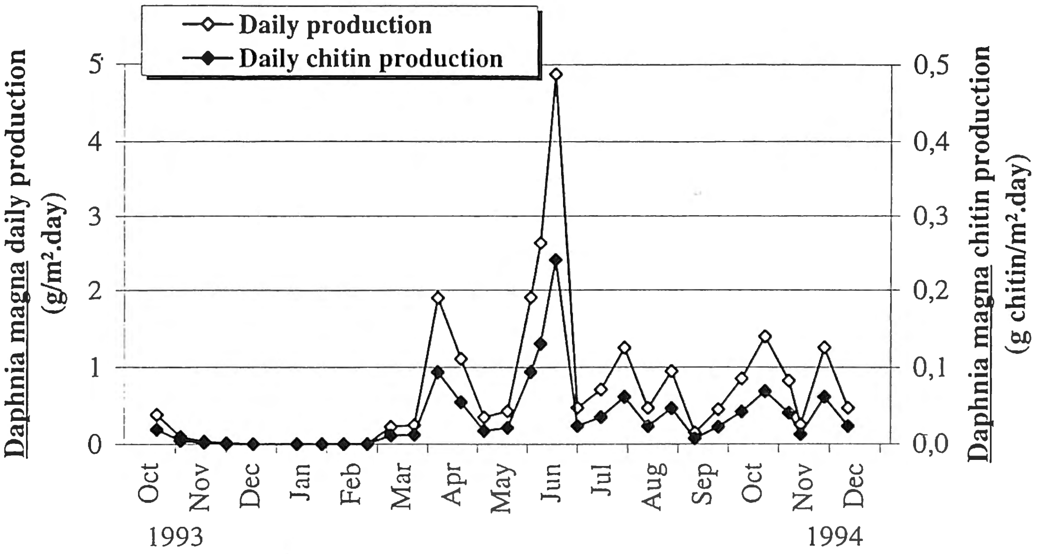

Fig. 4. — Seasonal changes in the daily production and the chitin daily production of *Daphnia magna* in the sewage lagoon of Differdange from October 1993 to December 1994.

The chitin content of *Daphnia magna* varied from 29 to 70 mg chitin per gram whole body dry weight, *i.e.* from 2.9 to 7.0 % of the total dry weight. Therefore, as the chitin content of *Daphnia magna* appears to be relatively constant over the year, a mean value of 4.9 % was used to calculate the daily chitin production of *Daphnia magna*. The daily chitin production is thus a constant part of the daily production. Consequently, daily chitin production and total daily production vary similarly (Fig. 4). Maximal values were recorded in April (94 mg chitin.m^{-2}.day^{-1}), June (241 mg chitin.m^{-2}.day^{-1}), and October (82 mg chitin.m^{-2}.day^{-1}). The annual chitin production of *Daphnia magna* reached 11.5 g chitin.m^{-2}.year^{-1}, *i.e.* 4.6 g chitin.m^{-3}.year^{-1}. The annual chitin production for the whole lagoon was thus estimated at 690 kg.

The annual chitin production of *Daphnia magna* at Differdange is compared in Table 1 with the chitin production of crustaceans in both fresh and salt water (JEUNIAUX *et al.*, 1993). The figure obtained in this study is four to eighty times higher than the annual chitin production of other Daphnia species living in eutrophic lakes, whatever the unit used (g.m^{-2}.year^{-1} or g.m^{-3}.year^{-1}). On the whole, the amount of chitin produced annually by marine crustacean communities, expressed per square metre, is on the average ten times lower than the amount produced by *Daphnia magna* at Differdange. The differences are greater when the data are expressed per cubic metre.

TABLE 1

A comparison of the chitin production of some Crustacean community in freshwater and in marine ecosystems

Community	*Annual chitin production ($g.m.^{-2}.year^{-1}$)*	*Annual chitin production ($g.m.^{-3}.year^{-1}$)*
Fresh water		
Daphnia hyalina and *D. cucullata* (Tjeukermeer — Holland) (1)	0.14-0.30	0.092-0.204
Daphnia galeata (Lake Esrom — Denmark) (2)	3.2	0.160
Daphnia magna (sewage lagoon) (3)	11.5	4.6
Salt water		
Surface zooplankton (Calvi Bay — Corsica) (4) (Copepods, Cladocerans)	1.0	0.010
Euphausiacea (South Pacific) (5)	5.3	0.021
Euphausiacea (North Atlantic) (6)	0.01-0.04	0.00024-0.00085
Large benthic Decapods (7)	1.5	-

(1) Calculated by GERVASI *et al.* after VIJVERBERG (1981).
(2) Calculated by GERVASI *et al.* (1988) after PETERSEN (1983).
(3) The present study.
(4) GERVASI *et al.* (1988).
(5) Calculated by JEUNIAUX *et al.* (1933) after RITZ and HOZIE (1982).
(6) Calculated by JEUNIAUX *et al.* (1933) after LINDLEY and (1982).
(7) After JEUNIAUX *et al.* (1993).

DISCUSSION

In the absence of fish or invertebrate predators, the biomass and production of microcrustaceans depend mainly on the temperature and food availability (*e.g.* WEGLENSKA, 1971; WATTIEZ, 1979; HART, 1990). In spring, the two microcrustacean biomass peaks are clearly related to the chlorophyll a peaks. Yet as the algal resources decline, strong competition is liable to take place between daphnids and the phytophagous juvenile stages of *Cyclops* spp. This might be the cause of the drop in the biomass of *Daphnia magna* observed at the end of June.

From July to December, the microcrustacean biomass peaks did not correlate with the chlorophyll a concentration. Over the summer, the low availability of algae may have prevented the biomass of copepods from reaching high values, but despite the lack of algal food during the autumn of 1994, the biomass of microcrustaceans reached its highest value of the year (6.7 $g.m^{-2}$). This may be due to the ability of cyclopoid copepods and daphnids to feed on bacteria and suspended organic matter (*e.g.* McMahon and Rigler, 1965 ; Porter, 1977 ; Nilssen, 1978 ; Peterson *et al.*, 1978).

The effect of temperature on microcrustacean biomass is highlighted by the differences observed between the maximum biomass values recorded in autumn 1993 and autumn 1994. In November 1993, the mean temperature was 6.6° C lower than in November 1994 and the biomass value (2.8 $g.m^{-2}$) was less than half that recorded in November 1994 (6.7 $g.m^{-2}$).

In order to estimate accurately the daily production of *Daphnia magna*, it is necessary to take into account how the development rate of this organism varies in relation to environmental factors. Our study being limited in time and scope, however, we used a growth curve (Mitchell *et al.*, 1992) established under laboratory conditions at constant temperature (20° C) and with a constant food supply (*Chlamydomonas reinhardii*, 0.5 $mg.l^{-1}$). Consequently, our estimate of the production may differ somewhat from the actual value because of the deviation of laboratory conditions from the prevailing natural conditions.

The maximum daily production was higher in June (4.8 $g.m^{-2}.day^{-1}$) than in April (1.9 $g.m^{-2}.day^{-1}$). This is due to the faster embryonic development and faster growth of juveniles at higher temperatures. In June, the mean temperature of the water was 20° C and embryonic development would take 2 to 3 days (Bottrell, 1975). Several generations could thus benefit from the temporary high availability of algae. In March and April, on the other hand, the mean temperature was only 7.5° C. Embryonic development would take 11 to 12 days. The daphnid offspring thus did not appear until April, when algae were scarce. This reduced their production.

As expected, winter was the least productive period. Only 2.1 % of the total production occurred during December, January, and February. This is due to the low availability of algal food and to the low water temperature. Daphnids can survive this period by producing long-lasting eggs (ephippia) that hatch in spring when the temperature and photoperiod increase (Larsson, 1991).

The annual chitin production calculated for the *Daphnia magna* population living in the sewage lagoon of Differdange is higher than that of any of the freshwater and marine communities with which it was compared (Table 1). This is probably due mainly (1) to the small number of competing species in the zooplankton community of this kind of sewage lagoon (at Differdange, the zooplankton community is composed solely of *Daphnia magna*, *Cyclops* spp., and *Brachionus* spp.), (2) to the high availability of food in the lagoon (algae in spring and summer, bacteria and suspended matter throughout the year), and (3) to the absence of fish and macroinvertebrate predators of zooplankton (Shapiro *et al.*, 1975). When the annual chitin production is expressed per cubic metre, large differences appear between *Daphnia magna* and the marine zooplankters. The reason for this is that marine zooplankters are distributed over a deeper water column than freshwater zooplankters. The low annual chitin production values reported for North Sea euphausids probably reflects the fact that Lindley (1982) did not evaluate the production of exuviae for these organisms.

CONCLUSION

In choosing the raw material for chitin production, one must consider four main criteria : (1) the seasonal and geographic availability of the source, (2) the chitin content of the organism, (3) the accessibility of the chitin in this organism, (4) the quality of the extracted chitin. Our aim is to assess the advantages of using cladocerans and copepods living in a sewage lagoon as a chitin source. So far, we have examined the first two criteria.

In terms of biomass, *Daphnia magna* appears as the dominant species of the lagoon. Because this organism is omnivorous and the food availability high (algae, bacteria, and suspended organic matter), chitin production by this species appears, in the limits of the accuracy of our estimation, very high as compared to other potential sources. The daily production, however, does depend on the temperature and food supply and is therefore subject to major seasonal variations. Winter, especially, is a critical period when the daily production is very low.

Another advantage of lagoon crustaceans is that, unlike pelagic marine *crustaceans* which are distributed over a deep water column, they are easy to harvest (with a pump-and-net system, for instance). These preliminary results suggest that microcrustaceans living in lagoons, especially *Daphnia magna*, are an excellent raw material for producing chitin.

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