

IN : Chitin and Chitosan, Edit. by SKJAK-BRAEK, ANTHONSEN & SAND-  
FORD, Proceed. 4th. Intern. Conf. Chitin and Chitosan,  
Trondheim, Norway, August 22-24 1988. London and New-York,  
Elsevier, pp. 3-11.

INSTITUT ED. VAN BENEDEN  
LIEGE - Travaux - Fascicule n°

A. 722

## SOURCES OF CHITIN, ESTIMATED FROM NEW DATA ON CHITIN BIOMASS AND PRODUCTION

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### ABSTRACT

On the basis of recent quantitative data on chitin biomass and production, marine surface zooplankton, mainly Copepods and Cladocerans, appears as a valuable source of chitin. Its mean chitin production of about  $1 \text{ g m}^{-2} \text{ year}^{-1}$  is much higher than krill chitin production.

Marine benthic communities growing on naked substrates sometimes exhibit similar chitin production, but chitin proportion with respect to calcified and organic weight of this biological material is far much lower than in krill and zooplankton.

Chitin production is the highest in eutrophic freshwaters; the development of Bryozoan colonies in organopolluted waters seems to offer interesting possibilities, chitin production values of about  $20 \text{ g m}^{-2} \text{ year}^{-1}$  being recorded in some cases.

### INTRODUCTION

The distribution of chitin and its quantitative importance in living beings is now well known. Chitin is synthesized by some unicellular organisms such as diatoms, chrysoflagellates and protozoa, especially ciliates. Chitin and/or chitosan are constituents of cell wall in most fungi, molds and yeasts (1). Chitin is present in cuticular or exoskeletal structures of most Invertebrates, with the exception of all sponges, most Anthozoans and Scyphozoans, and Echinoderms (2, 3). The amount of chitin with respect to total dry weight is the highest in Crustaceans, mainly Decapods. This observation, together with the availability of waste material from canning food industries, may explain the use of Crustacean shells as main source of chitin by most chemical industries.

However, the use of this kind of raw material for chitosan production is criticized by several authors, who also underline some difficulties and uncertainties of supply, stocking and handling (4, 5). An evaluation of the other potential sources of chitin is thus needed. The exploitation of any renewable natural resource on a rational basis requiring preliminary investigations on its productivity, the aim of the present paper is to analyse some recent data dealing with chitin biomass and production in different

natural ecosystems.

## CHITIN PRODUCTION BY INVERTEBRATE COMMUNITIES IN FRESHWATER ENVIRONMENT

### a. Arthropods

From the species composition of the fauna inhabiting lakes, ponds and rivers, it is obvious that the main chitin producers are arthropods. The chitin production and biomass of arthropods in an eutrophic lake was studied by Yamamoto and Seki (6). In a waterchestnut ecosystem in a bay of the lake Kasumigaura in Japan, the population dynamics of the dominant arthropod species was investigated, namely copepods and cladocerans in plankton, freshwater shrimps and larvae of midges (Diptera) among macroarthropods. Although the authors used a non-specific method for chitin determination, they expressed the seasonal fluctuations of the biomass of these arthropod species in grams of Carbon of organic material resistant to HCl and hot KOH treatments, what gives a rough estimation of chitin. According to these results, the total mean "chitin" production ("chitin input") would be  $26 \text{ g C m}^{-2} \text{ year}^{-1}$ , i.e. about  $51 \text{ g "chitin" m}^{-2} \text{ year}^{-1}$ . The highest production was observed in late spring and summer. Decomposition of detritic chitin settled on the bottom of the lake was performed by a rich chitinolytic bacterial flora. The balance between chitin production ("input") and chitin degradation ("output") by chitinoclastic bacteria results in a standing stock of  $\pm 10 \text{ g chitin m}^{-2}$  on the bottom of the lake.

### b. Bryozoans

Freshwater bryozoans ("moss-animals") have also been reported to develop dense colonies in some rivers enriched with dissolved or particulate organic matters. In a pond and its outlet, in Belgium, Job (7) was able to measure a biomass of  $128.2 \text{ g m}^{-2}$  (total dry weight) of *Plumatella fungosa*, consisting principally in cuticular material ("ectocysts") made of chitin and non-tanned proteins. These *Plumatella* species generally has two generations a year and the colonies desaggregate almost completely between two successive generations. According to their chitin content of 8.4 % dry weight measured by a specific enzymatic method (8), maximal chitin biomass value can be estimated to  $10.75 \text{ g m}^{-2}$  and chitin production to  $21.5 \text{ g m}^{-2} \text{ year}^{-1}$ . Similar values were obtained for *Plumatella* spp. growing in a danish lake (9) and in the river Meuse in Belgium (10); the exuberant development of bryozoan colonies on the grids of electronuclear power plants sometimes cause troubles for the plant working (11, 12).

### c. Zooplankton in eutrophic stagnant waters

The zooplankton in eutrophic lakes in North Holland is mainly made up of Cladocerans ("water fleas") of the genus *Daphnia*. Their production was estimated between  $3.1$  and  $6.9 \text{ mg m}^{-2} \text{ day}^{-1}$  in the lake Tjeukemeer (13). On the basis of chitin values of 12.22 % of the dry weight determined for marine Cladoceran species (14), the chitin production by water fleas in the lake Tjeukemeer can be calculated to  $136 - 302 \text{ mg m}^{-2} \text{ year}^{-1}$ . In the lake Esrom in Denmark, the annual chitin production by populations of *Daphnia galeata* (15) amounted to  $3.2 \text{ g m}^{-2} \text{ year}^{-1}$ , a value which would represent a total annual production of  $55\,360 \text{ kg chitin}$  for the whole lake extending over  $17.3 \text{ km}^2$ .

## CHITIN PRODUCTION BY ZOOPLANKTONIC COMMUNITIES IN MARINE ENVIRONMENT

Zooplankton is particularly interesting as its species composition generally indicates a clear-cut dominance of organisms with chitinous integuments, namely holoplanktonic Crustaceans (Copepods, Cladocera, Mysidaceae, Euphausiaceae) and meroplanktonic larvae of benthic or pelagic crustaceans. The values of chitin in % of total dry weight range between 2 and 12.2 %, according to species as well as to development stage. A correct estimation of chitin production thus requires careful determination of chitin content and population dynamics of each dominant species. Such data are available for North Atlantic Krill and in the case of surface zooplankton in the bay of Calvi (Corsica).

### a. Krill

The production of krill (Euphausiids) has been thoroughly studied in North Atlantic Ocean and North Sea by Lindley (16, 17, 18). Seasonal and geographic variations of growth, biomass and production at a 10 meter depth were analysed for each of the numerous species of this group. The results are expressed in total dry weight per cubic meter of sampled seawater.

These values can be tentatively translated in terms of chitin production, assuming that the chitin value of 7.08 % of dry weight obtained by Yanase (19) for *Euphausia superba* is representative for the other species of the group. Calculated from Lindley's data (18), the highest krill chitin biomass values are to be found in Norwegian Sea ( $0.62 \text{ mg m}^{-3}$ ), in the North-East of the North Sea ( $0.37 \text{ mg m}^{-3}$ ) and in the Gulf of Maine ( $0.28 \text{ mg m}^{-3}$ ). As far as chitin production is concerned, the highest values were found in the same marine habitats and over Nova Scotia Shelf, where krill chitin production was estimated to about  $0.09 \text{ mg m}^{-3} \text{ year}^{-1}$ .

Antarctic krill (*Euphausia superba*) is already used for the commercial utilization of its edible tail, and the waste material is suitable for chitin isolation and chitosan preparation on industrial scale (20, 21).

### b. Surface zooplankton in mediterranean sea

Marine zooplankton in the bay of Calvi (Corsica) was subjected to a detailed analysis during a complete annual cycle (22). This plankton was characterized by the striking dominance of five Copepod species and one Cladoceran genus, the chitin content of which was measured by a specific enzymatic method (14). Chitin values were found to remain relatively constant during larval development and similar to that of adults. Chitin values of Copepods with respect to dry weight ranged from 3.10 % in *Clausocalanus* spp. to 8.58 % in *Acartia clausi*. Higher values (12.22 %) were found in Cladoceran species of the genus *Evadne*.

The mean annual chitin production was calculated to  $1.0014 \text{ g m}^{-2} \text{ year}^{-1}$  (for a water column of 100 m depth). Seasonal variations were found very wide: the highest production of planktonic chitin was found in May, with maximal values of  $20.061 \text{ mg m}^{-2} \text{ day}^{-1}$ , corresponding to a chitin biomass of  $414 \text{ mg m}^{-2}$  (14).

## CHITIN PRODUCTION BY MARINE BENTHIC COMMUNITIES

Quantitative estimations of biomass and production in marine benthic and benthopelagic ecosystems are scarce, owing to the extreme diversity and complexity of most of the marine communities constituting the biological

biological cover of the sea bottom. In such cases, the measure of chitin production need specific experimental and analytical research programs. To this end, we have been studying for 6 years the chitin biomass and production of the biological cover of rocky shores in the bay of Calvi (Corsica, Mediterranean Sea), using a specific enzymatic method with purified chitinase as reagent (23, 24).

In the dominant types of benthic communities so far studied, chitin biomass was mainly due to Crustaceans on one hand, to encrusting colonies of Bryozoa and Hydrozoa on the other hand. Mean values of chitin biomass (excluding Crustaceans) amounted to  $0.71 \text{ g m}^{-2}$  ( $\pm 0.28$ ) in infralittoral communities of photophilous algae, and to  $0.27 \text{ g m}^{-2}$  ( $\pm 0.1$ ) in sciaphilous communities (25). Decapod Crustaceans of medium size contributed in a more variable manner to chitin biomass (from 0 to  $1.8 \text{ g m}^{-2}$ , average  $0.38 \text{ g m}^{-2}$ ).

Chitin production was estimated by the values of chitin biomass elaborated upon naked substrates after given periods of time during the pioneering phase of colonization (25). These experimental substrates consisted in rectangular  $10 \times 20 \text{ cm}$  plates of granite, baked clay, glass and PVC, immersed at different depths. The plates were fastened near the rocky coast and maintained in natural conditions in order to allow the progressive development of biological cover. Sets of plates were removed periodically and the biological cover of each plate was scraped, dried, weighed, powdered by mechanical grinding and aliquots used for the determination of total organic matter and chitin. The results of the chemical analysis of 169 plates (26) can be summarized as follows.

At a depth of 6 m, the development of biological cover is slow, with low chitin proportion with respect to total dry weight and total organic matter (about 1 % total organic matter), due to the abundant development of algae. Maximal chitin production (on granite) during the first 460 days was estimated to  $238 \text{ mg m}^{-2} \text{ year}^{-1}$ . After this period of time, the material was drawn out by waves and other hydrodynamic events (fig. 1).

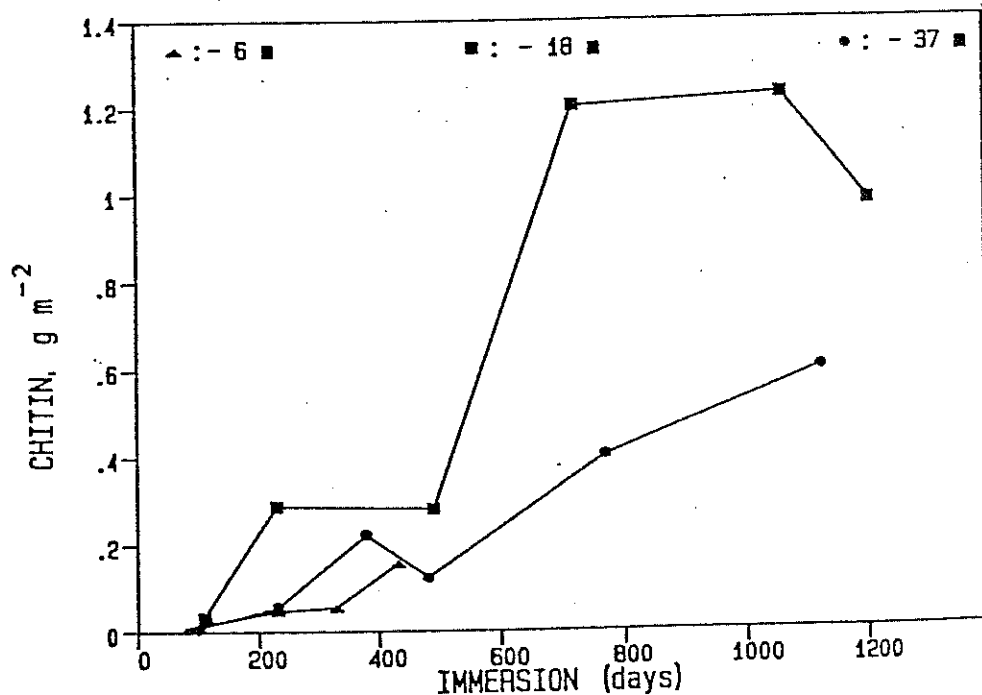


Figure 1 : chitin biomass by pioneering benthic communities growing on naked plates at different depths in the bay of Calvi (Corsica) (mean values for 3 plates of granite, baked clay and PVC).

At a depth of 18 m, near the rocky shore, the development is slow during the first year, then much quicker, a maximum being attained after three years. The proportion of chitin with respect to total organic matter reached a mean value of about 1%, except on glass (2%). The chitin production was estimated to  $300 \text{ mg m}^{-2} \text{ year}^{-1}$  during the first year, and to  $1 \text{ g m}^{-2} \text{ year}^{-1}$  during the second year of the pioneering colonization process (fig. 1). At the same depth, but inside a *Posidonia oceanica* meadow, the chitin production did not exceed  $0.12 \text{ g m}^{-2} \text{ year}^{-1}$ .

At a depth of 37 m, the development of the biological covering seemed to proceed slower, particularly on glass substrates. The proportion of chitin with respect to total organic matter increased slowly during the first year, to reach about 1%. The production of chitin was more variable than at - 18 m: it was estimated to  $340 \text{ mg m}^{-2} \text{ year}^{-1}$  during the two first years of pioneering development (fig. 1).

All these values of chitin production must be considered as minimal ones, the simultaneous biodegradation by consumers having not been taken into account. Moreover, this type of experimentation, suitable for the study of production by sessile colonial organisms, is less favourable for that of free vagile organisms such as crabs and other crustaceans inhabiting crevices and holes which are missing in our experimental substrates.

The importance of chitin production by populations of large Crustacean species can be appreciated by the production values of a natural population of the spiny Lobster *Panulirus homarus* inhabiting a small isolated reef off the Natal Coast in South Africa (27). Taking into account the production of total fresh tissues, the mean proportion of cuticle, the percentage of chitin in the cuticle, and the production rate of castskins (exuviae), the chitin production by this Lobster population can be estimated to about  $1.5 \text{ g m}^{-2} \text{ year}^{-1}$ , a figure of the same order of magnitude as the highest chitin production values obtained so far for the whole pioneering biological cover.

## SEDIMENTS

Bearing in mind the well known resistance offered by chitin to most chemical and physical agents, the quantitative importance of chitin production by planktonic, pelagic and benthic animal communities would allow to predict the accumulation of detritic chitin in sediments, and the possible use of such a stock of "dead" chitin as a source for industrial supplying. However, the abundance and diversity of chitinolytic microorganisms in most sediments insure quick biodegradation of "dead" chitin and the recycling of its elements, so that sediments generally contain minute traces of chitin. This question is more fully examined in another chapter in this volume (28).

## DISCUSSION

Some recent quantitative data are now available concerning chitin biomass and production in a limited number of representative aquatic ecosystems; they are compiled in table I. No data are available for terrestrial ecosystems. The numerical abundance and the species diversity of insect communities are reliable indications of the ecological importance of chitin production in terrestrial ecosystems. However, terrestrial insect populations could hardly constitute an appropriate source of chitin, owing to the difficulties encountered for the isolation of chitin in pure state from

quinone-tanned insect cuticles.

TABLE 1  
Chitin production by some natural communities

Community	Chitin production $\text{g m}^{-2} \text{ year}^{-1}$	Authors
<u>SEA WATER</u>		
Krill, Atlantic Ocean and North Sea	0.0045	Lindley, 1982 <sup>(1)</sup>
Zooplankton (Calvi Bay, Mediterranean Sea)	1.0014	Gervasi, Jeuniaux and Dauby, 1988
Pioneering benthic communities (Calvi Bay, Mediterranean Sea)		
Depth : - 6 m	0.23	Jeuniaux, Voss-Foucart, Gervasi, Bussers and Poulicek, 1988 and present paper
- 18 m first year	0.30	
second year	1.00	
- 37 m	0.34	
Spiny Lobster population (Natal Coasts)	1.5	Berry and Smale, 1980 <sup>(1)</sup>
<u>FRESH WATER</u>		
Arthropods, eutrophic lake, Japan	51.0	Yamamoto and Seki, 1979
Plankton ( <u>Daphnia</u> ) eutrophic lakes		
- Holland	0.13 - 0.30	Vijverberg, 1976 <sup>(1)</sup>
- Denmark	3.2	Petersen, 1983 <sup>(1)</sup>
Bryozoans ( <u>Plumatella</u> )	21.0	Job, 1976 <sup>(1)</sup>

(1) values calculated from total dry weight production.

Marine animal communities are important chitin producers. Chitin production by pioneering benthic communities growing on naked substrates may reach values of  $1 \text{ g chitin m}^{-2} \text{ year}^{-1}$  equal to those obtained with zooplankton, but the proportion of chitin is very low with respect to total organic matter and, a fortiori, to total calcified material.

Zooplankton seems to be a better source of chitin, with mean chitin production values of  $1 \text{ g m}^{-2} \text{ year}^{-1}$  and important chitin biomass, especially in Spring. Taking into account the relative extent of rocky shores and open sea waters, chitin annual production by zooplankton in Calvi bay was at least 10 times higher than that of benthic communities (25, 26). Zooplankton appears also to have a much higher chitin production than krill. Moreover, chitin processing from zooplanktonic material, mainly made up of copepods, would be facilitated by the very low degree of calcification and sclerotization of the cuticles. Marine zooplankton thus appears as an appropriate renewable chitin resource, but its spatial dispersion and seasonal biomass variations could hinder paying industrial exploitation

Chitin production is the highest in eutrophic freshwater ecosystems. Zooplankton, macroarthropod and freshwater bryozoan communities of some organopolluted waters have shown chitin production values 3 to 50 times higher than those of marine communities. The exploitation of such natural sources of chitin would however be rapidly hindered by the limited extension of these waters.

The case of freshwater bryozoans deserves further attention : these sessile animals are able to produce quickly important colonies embedded in thick cuticular envelope made up of chitin and protein, neither calcified nor quinone-tanned, allowing easy extraction of chitin. It is not irrational to imagine the industrial intensive culture of such organisms in organopolluted waters.

Industrial culture of chitin producing organisms is another way to solve problems of chitin supply. Chitin and chitosan production by selected mold species in culture could probably be more rewarding than the exploitation of any natural chitin biomass. Recent tentatives are in progress in this field and seem to be promising.

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