

CHITIN PRODUCTION BY ANIMALS
AND NATURAL COMMUNITIES IN
MARINE ENVIRONMENT

C. JEUNIAUX, J. C. BUSSERS, M. F. VOSS-FOUCART and M. POULICEK

Oceanographic Research Station STARESO,
Calvi, Corsica

Laboratory of Animal Morphology, Systematics and Ecology
Zoological Institute, University of Liège
B-4020 Liège, Belgium

INTRODUCTION

Since the discovery of the remarkable properties of chitin and of its derivative, chitosan, offering a wide range of possible industrial and agricultural applications 1,2,3, the need of new and trusting sources of chitin for its isolation on an industrial scale was claimed.

At present, chitin is usually isolated from crab and lobster shells, owing to their high chitin content and to their availability in relatively high amounts from fisheries and tinned food industries. However, this kind of raw material offers some disadvantages and the exploitation of alternative sources of chitin has been proposed and investigated. These sources could be, for instance, marine plankton or biological cover of marine rocky shores. As with any prospect of exploitation, these potential sources of chitin require preliminary estimations of chitin availability and renewal, i.e. chitin biomass and chitin production in marine environment.

On a more theoretical and fundamental background, chitin is a highly chemically stable biopolymer widely distributed in animals and fungi, which must certainly play an important part in biogeochemical cycles of carbon and nitrogen. The importance of chitin from this ecochemical point of view relies on its production by natural biocenoses (or communities), on one hand, and on its biodegradation and bioaccumulation in sediments on the other.

Our knowledge of chitin biodegradation and accumulation in sediments has increased considerably, thanks to the works of authors such as Bolin 4, Goffinet 5,6, Poulícek 6-9, Seki 10, Smucker 4,11, Warnes 12, Wirsen 13, Voss-FoucART 15, some of them being able to present the most recent conclusions of their investigations in the present Volume. On the contrary, very little quantitative and experimental data on chitin production are available as yet 14,15.

The aim of the present paper is to try to estimate the biomass and production of chitin at the level of several biological communities living in a single, relatively well defined marine area, namely the bay of Calvi (Isle Corsica), in the Mediterranean Sea.

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POSITION, TOPOGRAPHY AND MAIN ECOLOGICAL CHARACTERISTICS OF THE BAY OF CALVI

The bay of Calvi, situated at the north-west end of the Isle Corsica, in the Mediterranean Sea, is roughly semi-circular in shape, with a maximum width of 6 km. Widely open towards the North, the bay is delimited by a relatively abrupt rocky coast (mainly granitic and granulitic in nature 16), interrupted near Calvi by a sand beach extending over about 3 km. The bay extends over a surface of about 2200 ha (5500 acres). With a mean slope of 2 %, the depth increases rapidly up to 100 meters.

Tidal movements are insignificant. Salinity varies from 37.5 ‰ in winter to 38.1 ‰ in autumn; surface water temperatures fluctuate from 13°C in february to 25°C in summer, with a thermocline located at 20 m in May and 50 m in September 17, 18.

Around the bay, the rocky benthic communities occupy a fringe from 0 to 27 m depth, estimated approximately to 145 ha, which represent an actual available surface of at least 190 ha. The biological benthic communities growing on the infralittoral rocky shore, (identified according to Peres and Picard, 19) are mainly :

- 1) photophilous algae communities characterized by the brown-algae species *Cystoseira stricta*, *C. crinita* and *C. balearica*, in well enlightened conditions and
- 2) sciaphilous communities in crevices and semi-dark caves.

An important surface (about 44 %) of the bottom of the bay is taken up by a *Posidonia oceanica* meadow, from 5 m down to 38 m depth 17,18. The remaining surface (about 50 %) is covered by different kinds of sandy, terrigenous and organo-detritic (bioclastic) sediments 16.

Owing to the poor development of human habitat, industrial and agricultural activities on the coasts of this part of the isle, the bay of Calvi is considered as only slightly polluted (oligotrophic waters), and especially suitable for approaching ecology and ecobiochemistry of non perturbed marine systems.

METHODS FOR QUANTITATIVE ESTIMATION OF CHITIN

In order to estimate the amount of chitin in crude, highly composite materials, a very specific method is needed. The unique highly specific technique allowing a quantitative estimation is the enzymatic method based on the use of purified chitinases 20,21,22. Powerful purified chitinases can be prepared from cultures of bacteria (*Streptomyces antibioticus*, *S. griseus*, *Serratia marcescens*) according to the procedures of Berger et al.23, Jeuniaux 24, 25 or Roberts et al. 26.

The enzymatic method for chitin determination 21, was applied as summarized as follows. The analysed material was washed, weighed ("calcified weight"), then decalcified by HCl 0.5 N at room temperature. After washing and drying ("decalcified weight"), the material was treated by 1N NaOH at 100°C during 3 h, in order to remove proteins, to liberate most of the bound chitin and thus to facilitate its enzymatic hydrolysis. After washing, the chitin in the insoluble residue was hydrolysed by a solution of purified chitinase (Koch-Light, 1 mg/ml, or Serva, 3 mg/ml) at pH 5.2 and 37°C, during 6 h, then incubated in dilute buffer at pH 5.2. These incubations in chitinase and buffer were repeated if necessary until "complete" hydrolysis was achieved. After addition of a solution of N-acetyl-D-glucosaminidase (or chitobiase) from lobster serum, the total N-acetyl-D-glucosamine liberated during the successive incubations was measured by colorimetric method 27.

The values given by this enzymatic method are systematically lower than the actual ones, due to the facts, that some chitin, strongly asso-

ciated to other components, is able to withstand prolonged enzymatic action and that some acetylglucosamine residues in the chitin chain are replaced by non-acetylated residues, which are not taken into account in the colorimetric measure of the final hydrolytic products. The mean error is estimated to about 10 %.

When the studied material is made of Crustaceans only, chitin may be more readily estimated by weighing the residue after decalcification and hot alkali treatment. The values given by this simpler procedure are somewhat higher than the actual chitin amounts.

RESULTS

Chitin Biomass in Rocky Benthic Communities

In infralittoral communities of photophilous algae characterized by *Cystoseira* spp., the chitin biomass is principally due to sessile and encrusting colonies of Bryozoa and Hydrozoa, living on brown algae, on one hand, to vagile and crawling species of Crustaceans on the other hand. The whole benthic biological cover, excluding Decapod Crustaceans, contributes to a chitin biomass of $0.71 \text{ g.m}^{-2} (\pm 0.28)$. Decapod Crustaceans of medium size (taller than 5 mm. long) contribute to more variable chitin biomass values (from 0 to 1.8 g.m^{-2} ; average $0.38 \text{ g.m}^{-2} \pm 0.51$)²⁸.

In sciaphilous communities inhabiting semi-dark caves²⁸, the chitin biomass of encrusting colonies is lower and amounts to $0.27 \text{ g.m}^{-2} \pm 0.1$. The contribution of Decapod Crustaceans is very variable and may be sometimes very high (big species inhabiting holes and crevices).

Chitin Production by Infralittoral Rocky Benthic Communities of Photophilous Algae, estimated by a Study of Pioneering Communities Growing on Naked Substrates.

The rate of chitin production by infralittoral communities living on the rocky shore was estimated by measuring the amount of chitin accumulated after given periods of time by pioneering communities allowed to settle and grow on naked substrates submerged in sea water in the vicinity of the coast of the bay of Calvi.

Several sets of rectangular plates of granite, baked clay, glass and PVC, of the same size (10 x 20 cm) were submerged and fastened by Scuba divers at different depths (6 and 18 meters) and maintained in natural conditions. At given intervals (from 1 to 15 months), sets of plates were removed, and the whole biological cover of each face of each plate was separately scraped, dried, weighed and powdered, if necessary, by mechanical grinding ("Ika-werk" grinder). Aliquots of the powder were decalcified and used for determination of total organic matter and total chitin. The results are given in table I, in which individual values obtained for both faces of the same plates are pooled, and are calculated per square meter.

The species composition of the biological cover corresponds roughly, after a few months, to that of an infralittoral community of photophilous algae.

At a depth of 6 meters, the development of benthic pioneering communities (total dry matter production) was relatively similar on the different types of experimental substrates during the first 6-10 months, but was found to be much better on granite and baked clay thereafter.

Table I. Chitin production by pioneering communities growing on naked substrates, at 6 m depth (from June 1981 forward).

Substrate	chitin biomass, mg. m ⁻²				Calculated chitin production mg. m ⁻² . month ⁻¹	
	Sept. 81 3 months	Jan. 82 7½ months	May 82 11 months	Sept. 82 15 months	winter	spring- summer
PVC	2.5	33.9	36.7	46.7	-	-
Glass	1.3	-	7.0	7.4	-	-
Baked Clay	1.7	15.8	19.2	136.	0.97	29.2
Granite (1)	2.3	12.8	22.1	99.0	2.65	19.2
(2)	2.3	12.8	22.1	298.0	2.65	69.0

(1) whole biological cover, Decapod Crustaceans excluded.

(2) whole biological cover, including Decapod Crustaceans.

As far as chitin is concerned, the quantitative data in Table I indicate that, when the naked substrates were immersed in June at a depth of 6 meters, chitin production proceeded slowly during the first 10 months, then increased considerably on granite and clay substrates. This difference may be explained either by season influence, or by the progressive constitution of the biological cover, including enhancement of colonization rate by the presence of a pioneering community. It is likely that the faster chitin production rate measured during the last 6 months in this experiment is a better estimation of the actual chitin production of a well settled community. As granite is a natural substrate more similar to those building the rocky shore of the bay than the other experimental substrates, chitin production by photophilous communities of the first 12 meters depth can be evaluated to 19.2 mg.m⁻². month⁻¹ from May to September, and to 69 mg. m⁻². month⁻¹ when Decapod Crustaceans are taken into account (table 1). The chitin production of pioneering communities varies to some extent with the season and seems to be more important from september to december. This point will be discussed more fully elsewhere.

Values for plates immersed at a depth of 18 meters are so far incomplete, but seem to indicate a higher chitin production during winter season, around 33.1 mg. m⁻² month⁻¹ on baked clay and upto 51.7 mg. m⁻². month⁻¹ on granite.

Chitin Biomass and Production by Epiphytic Communities growing on Posidonia Leaves.

The primary and secondary production of epiphytic communities growing on the leaves of *Posidonia oceanica* in the *Posidonia* meadow of the bay of Calvi was studied by Mesureur^{29,30}, at a depth of 10 meters.

A study of the seasonal foliar renovation allowed consideration of leaf as a new substrate available for epiphytic communities, the age of which was identified by its position in the foliar bundle, and confirmed by experimental markings. The epiphytic biological cover of leaves was scraped, pooled according to age, weighed and powdered. Besides carbonates, organic matter and proteins, chitin was identified and measured using our enzymatic method.

The main producers of chitin were found to be Hydrozoa and Bryozoa. The chitin production by the epiphytic biological cover was estimated to 1.25 mg per m² of leaf surface per month during autumn and winter, and 0.5 mg per m² of leaf surface per month during spring and Summer. According to the numerical density of *Posidonia* foliar bundles (407 bundles per m² at a depth of 10 meters) ^{17,18} and to the number and age of leaves in each bundle, epiphytic chitin production was calculated with respect to the surface of sediment occupied by the *Posidonia* meadow on the ground of the bay. The annual chitin production was thus estimated to 75 mg. m⁻². year⁻¹.

Chitin Production by Zooplankton

The biomass and production of zooplankton in the bay of Calvi was studied by Dauby ^{31,32} during two annual cycles, and the results expressed with respect to a square meter of surface water, for the whole water column. The most striking characteristic is that Copepod Crustaceans contributed to more than 90 % of the total zooplankton biomass, except during short periods, when Pteropod Mollusks and larvae of benthic Crustaceans became abundant.

The Copepod fauna is dominated by five species or groups of species belonging to the genus *Clausocalanus*, *Centropages*, *Oithona*, *Acartia* and *Calanus*. The Copepod production was estimated to about 20 g (dry matter). m⁻².year⁻¹.

On the basis of a mean proportion of chitin of about 5 % with respect to Copepod dry matter, the chitin production was roughly evaluated during a period of twelve months from March 1983 to February 1984 (Dauby, ³²). The Copepod chitin production was low (2 mg. m⁻². day⁻¹) at the end of the winter, than increased considerably in spring (up to 17 mg.m⁻². day⁻¹); a further but lower peak (7 mg. m⁻². day⁻¹) was observed in July; the production was low (from 0.5 to 2 mg. m⁻². day⁻¹) during the rest of the year. The average annual chitin production was thus estimated to 1 g.m⁻². year⁻¹.

DISCUSSION AND CONCLUSION

The bay of Calvi can be taken as an example of a relatively well delimited and explored marine area from an ecochemical point of view. The contribution of different biocenotic compartments to chitin production can be tentatively calculated on the basis of the data reported or gathered in this paper, as summarized in Table II.

Crustaceans, benthic Decapod species as well as planctonic Copepods, appear as the main contributors to chitin production per surface unit and per year. Moreover, it must be recalled that larger species inhabiting holes and crevices, such as lobsters and big crabs, have not been taken into account, nor nectonic Crustaceans such as shrimps. Despite the abundance of other animal groups with chitinous exoskeletons, such as Hydrozoa, Bryozoa, Annelids and Mollusks, most of the chitin production in this marine system is accounted for by Crustaceans.

This is even more evident when chitin production is expressed for a whole marine area, such as the bay of Calvi in its entirety. In this case (table II), planctonic and benthic Crustaceans account for about 95 % of total chitin production in the bay, which is estimated to 24.8 Tons per year.

Table II. Chitin production by several communities in the bay of Calvi.

Community	chitin production g.m ⁻² . year ⁻¹	Surface covered ha	Chitin production (whole bay) kg. year ⁻¹
<u>Benthic</u>			
- photophilous algae on rocky shores			
* 0-12 m (1)	0.23	76	175
* 12-27 m (2)	0.62	114	707
* Small Decapod Crustaceans	0.60	190	1 140
- <i>Posidonia</i> meadow (epiphytes)	0.075	1 056	792
<u>Planctonic</u>			
- Zooplankton (Copepods)	1.0	2 200	22 000
Total	-	2 200	24 814

(1) On basis of values obtained for pioneering communities growing on granite plates in spring and summer, after 1 year's immersion at a depth of 6 m.

(2) idem, for communities growing on granite plates in autumn and winter, after a 6 months' immersion at a depth of 16 m.

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