

# FREE CHITOBIASE, A MARKER ENZYME FOR THE GROWTH OF CRUSTACEANS

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Copepods are the most abundant animals in the plankton. These crustaceans undergo a series of moults during their development from egg to the adult stage. We developed a new method to measure growth of copepods in the open sea by using the enzyme chitobiase. The enzyme breaks down the old chitin exoskeleton and is released in the ambient water during the moult. Experiments showed that the free chitobiase in water samples can be used quantitatively to calculate copepod production in the sea. Field surveys in the North Sea indicated that copepod growth varied from less than 10% of body weight per day in autumn and winter, up to more than 30% in spring and summer. The chitobiase method could also be a relevant tool to estimate the production by other crustaceans like shrimps and crabs living on the bottom of the Wadden Sea.

The measurement of production is a key variable in ecological research, especially if the focus is on the transfer of carbon and energy through the pelagic food web from algae via animal plankton to fish. The  $^{14}\text{C}$ -bicarbonate incorporation method for estimating primary production and tritium labelled amino acids for estimating bacterial production are used in aquatic studies. To estimate zoo-

plankton production, there is no such method available using the incorporation of a specific substrate during short-time incubations.

Copepods, small crustaceans, representing the most dominant group of the marine zooplankton, have generation times of several weeks to months. Theoretically, the growth of copepods can be deduced from the changes in

abundance and size in time series sampling. However, in the open sea the tracking of populations over sufficiently long time spans is hampered by logistic constraints and by advection, patchiness and vertical migration. Consequently, estimates of secondary production in open marine waters are mainly based on plankton net catches combined with growth rates measured in laboratory experiments.

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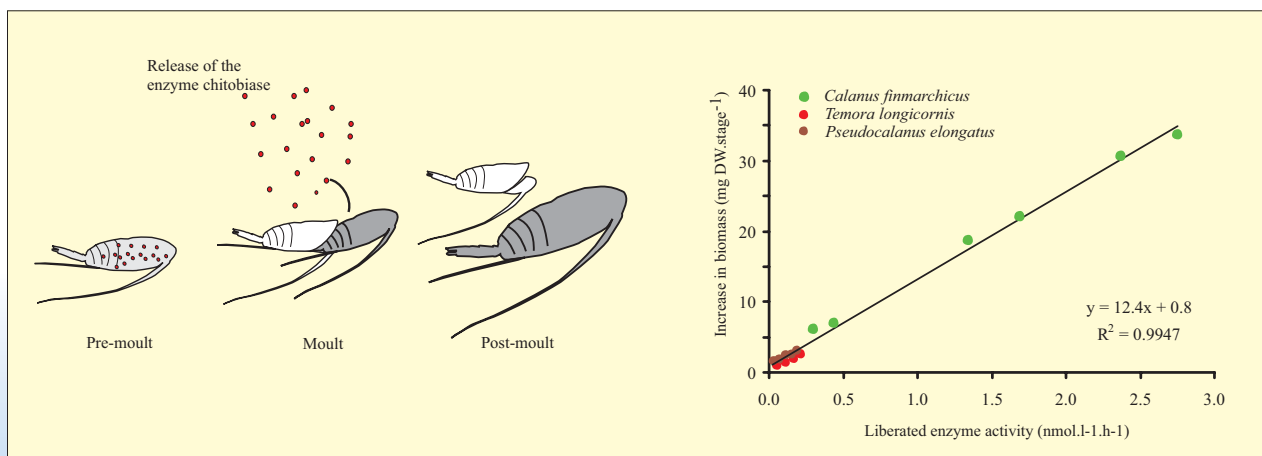


Fig. 1. Left panel: The release of the enzyme chitobiase (red dots) in the ambient water during the moult of a copepodite. Right panel: Relation between the increase in body weight and the released chitobiase activity for moults of different stages of three copepod species. *Temora longicornis* (red), *Pseudocalanus elongatus* (brown) and *Calanus finmarchicus* (green).

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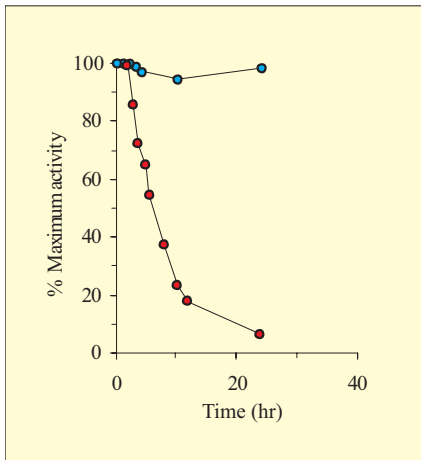


Fig. 2. Free chitobiase remains present in autoclaved, sterile seawater (blue dots) whereas the consumption by bacterioplankton causes an exponential decay of chitobiase in raw seawater (red dots).

Thus, there is still need for an easy field method to measure copepod growth more directly.

At the NIOZ, a new approach to estimate crustacean production was developed. The principle is not based on the uptake of a substrate but on the release of a specific substance indicative of growth. In the pre-moult phase of crustaceans, the old chitin exoskeleton is degraded by the chitinolytic enzymes chitinase and chitobiase. The NAG-monomers (*N*-acetyl- $\beta$ -D-glucosaminide) resulting from the cleavage of chitin by this combined enzyme action, are re-used as building blocks for the new chitin skeleton underneath the old exoskeleton. During the moult, the remnant of the old skeleton is shed and the chitinolytic enzymes are released into the ambient water (Fig. 1). In laboratory experiments, the release of chitobiase by juve-

niles during the moult was measured. The amount of enzyme was linearly related to the growth of the juveniles between moults. The relation was similar for three different copepod species, and the regression equation on the combined data (inset in Fig. 1) is used to estimate the total daily growth in mixed copepod populations in the sea. The free enzyme is not accumulating in the water but steadily consumed by bacteria (Fig. 2). Thus, to estimate the total amount of chitobiase released per day, the rate of chitobiase decay over 24 hours has to be measured in addi-

tion to the amount of dissolved chitobiase present in the water.

The spatial distribution of copepod production was mapped with the new technique during surveys in the North Sea. A plume of turbid English Coastal Water with high concentrations of suspended matter and low salinity (see the contours in Fig. 3) crosses the Southern Bight in-between the more saline Central North Sea Water and Channel Water. In September 2000, daily primary production was low inside compared to outside of the plume (0.5

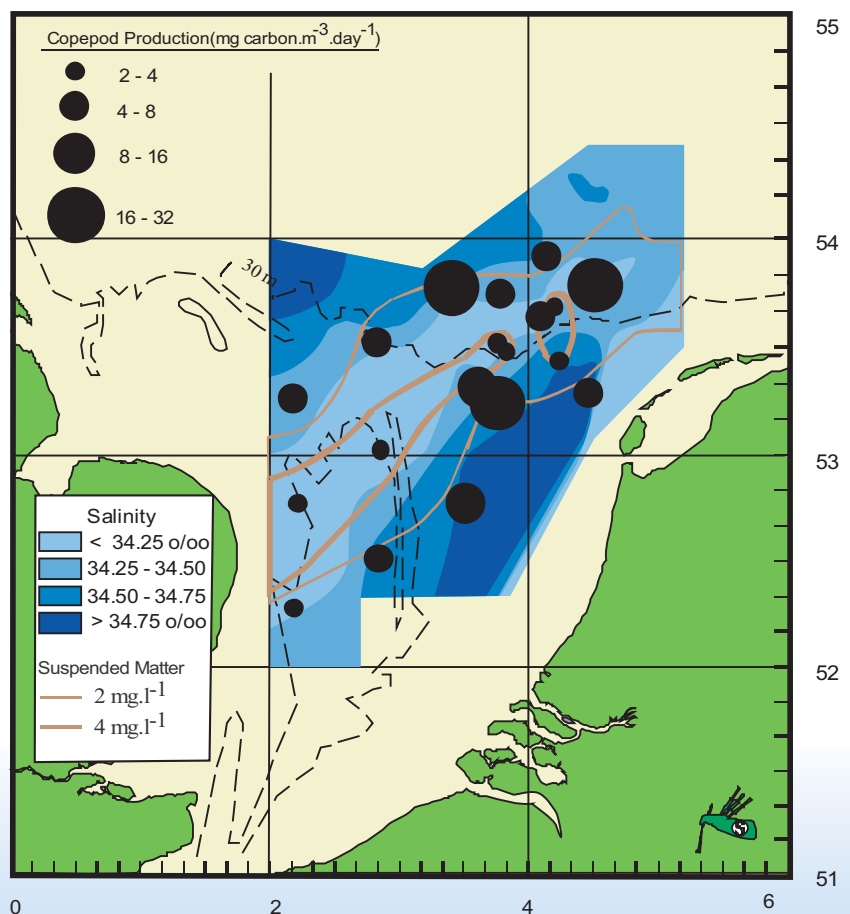


Fig. 3. Copepod production (black dots) estimated by free chitobiase in the southern North Sea plotted on contour maps of surface salinity (blue scales) and concentration of suspended matter (mg/litre, brown lines). Plume & Bloom cruise September 2000.

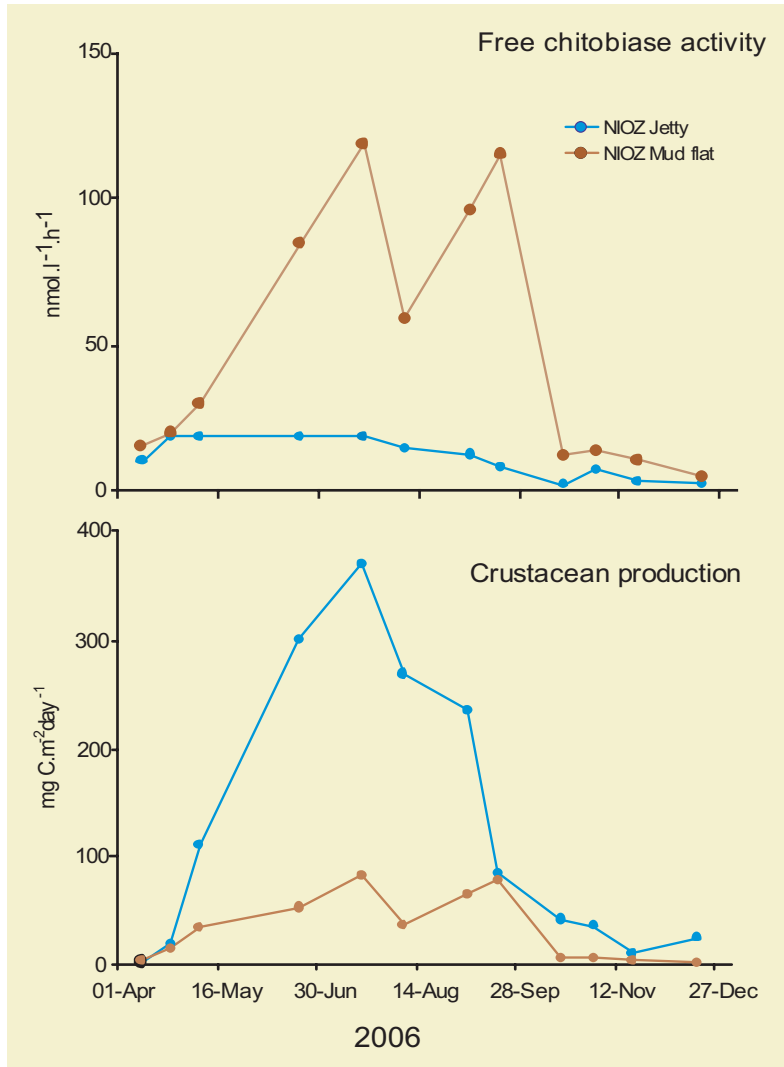


Fig. 4. Seasonal pattern of free chitobiase activity (upper panel) and the calculated crustacean production (lower panel) in the water column at the NIOZ jetty and at the mud flat near the NIOZ harbour, April – December 2006. Enzyme activity (expressed in units substrate cleaved) per litre per hour; production (in mg carbon) per square metre per day.

versus 1 gram carbon per square metre) and mean copepod production showed a similar difference (150 versus 370 mg carbon per square metre). Highest copepod production was measured at the rim and downstream of the plume (Fig. 3) where water masses mixed. One particular station, at the southern, muddy slope of the Oyster Ground – the so-called

Frisian Front – was visited during several cruises through the year. Copepod production was one order of magnitude higher in June and September than in December 2003 and April 2004. The production estimated via the free chitobiase method was compared with the biomass of copepods in the net catches. Production/biomass ratios ranged between 0.30-0.35 during

late spring/summer to 0.05- 0.10 in winter/early spring.

Free chitobiase was frequently markedly higher in near-bottom water samples than in water collected in the upper layer of the North Sea. This phenomenon indicates that copepods or other crustaceans concentrate near the bottom. The benthic boundary layer may form a special, productive habitat for crustaceans due to the constant sedimentation and resuspension of phytoplankton and detritus by the tidal movement. High productivity of the crustacean community of the bottom was measured regularly at a small mudflat near the NIOZ. The free chitobiase signal in the water collected above the mudflat near the end of ebb was significantly stronger than the chitobiase signal in the first flood-water collected at the nearby jetty (Fig. 4 upper panel). The extra chitobiase originates from both the harpacticoid copepods, the crustacean component of the meiofauna in the bottom and from shrimps and crabs living on the bottom. An attempt was made to calculate the square metre crustacean production of the plankton in the mean water column near the jetty and of the mudflat (Fig. 4 lower panel). This pilot study suggests that the chitobiase approach may be a useful tool in field programs to estimate the productivity of all the different crustaceans in the Wadden Sea.