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Effects of the "Amoco Cadiz" oil spill on zooplankton

A new possibility of ecophysiological survey

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ABSTRACT: A survey of zooplankton physiology on the northern coast of Brittany (France) was carried out over a one-year period by comparing two estuarine areas, one oil-polluted area (Aber Benoit) following the oil spill by the tanker "Amoco Cadiz" and one non-oil-polluted area (Rade de Brest). A new approach to an ecological survey was made by describing trophic relationships using analysis of digestive enzyme equipment (amylase and trypsin) of zooplankton organisms, mesoplankton populations and some selected species. These measurements allowed determination of (a) groups of populations with homogeneous trophic and faunistic characteristics and (b) groups of species with homogeneous trophic characteristics. The study of the appearance of these groups over a one-year period revealed the succession of populations and their adaptation to the environment on the basis of biochemical analysis. These phenomena observed in the compared areas showed marked differences in the most polluted areas during the productive spring period. Specific treatment of the data using unusual correlations between digestive enzymes is discussed in terms of the immediate effect on the whole population and on a copepod *(Anomalocera patersoni)* living in the upper 10 cm.

INTRODUCTION

Zooplankton composition is partly defined by environmental conditions; thus the measurement of its physiological activity is a possible way to record variations of many factors that are involved in the production mechanisms, such as physical, hydrobiological, primary and secondary production processes, predation and recycling phenomena. The physiological activity of plankton in the field has been recorded by the estimation of cellular regulation (Curl & Sandberg, 1961; Packard, 1971; King, 1972; Boucher & Samain, 1974; Alayse-Danet, 1980). As shown by Boucher et al. (1975) and Samain et al. (1975, 1980), nutrition of planktonic copepods can be estimated by qualitative and quantitative measurements of specific digestive enzymes. We attempted to apply this analysis by studying digestive enzymes (amylase and trypsin) of copepod populations from different areas and of different species during a survey of seasonal variations of planktonic ecosystems. Following the "Amoco Cadiz" oil spill, polluted and non-polluted planktonic ecosystems were compared.

SAMPLING

Zooplankton was collected with a 200-μm mesh net; the sample was split with a Fulsom splitter for collection and measurements of biochemical parameters (amylase,

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trypsin, proteins). Analysis was performed on the total population and on selected copepod species. Samples were ground in distilled water at 4 °C. Amylase, trypsin and proteins were analysed according to methods described by Samain et al. (1977). The sampling strategy has been adapted to the seasonal variations: a monthly survey in spring during the productive phase and irregular collections during winter.

Ecophysiological analysis

Amylase and trypsin are specific for their substrates (starch and proteins respectively). The carbohydrate and protein requirements of different species are not identical; the relative amylase/trypsin levels for each copepod species are characteristic of a herbivorous, omnivorous or carnivorous diet (Boucher et al., 1975). Elsewhere, the two digestive enzymes are regulated by many factors, mainly: the available food characteristics (e. g. quality, quantity, species of phytoplankton, chemical composition, size), the developmental stage of zooplankton organisms, and the species-appropriate diet. These very interdependent factors determine the adaptative processes to the nutritive environmental value. These regulations allow amylase and trypsin levels to vary independently (Samain et al., 1980). Each copepod species in a specific trophic environment and at a certain stage is thus characterized by a particular amylase/trypsin ratio (Samain et al., unpublished). Consequently, the analysis of the amylase/trypsin (A/T) ratio gives information about the trophic orientation of selected species. As different species do not have the same A/T ratio in the same trophic conditions, the measurement of the A/T ratio of a multispecific population will give results in accordance with the faunistic composition of the population and/or the trophic conditions in the sea.

High or low amylase and trypsin values can lead to the same A/T ratio. As algebraical calculations alone could obscure this relation, amylase and trypsin are graphically plotted (Fig. 1). The A/T ratio is the slope of the line. Different protein contents of the organisms may explain the differences in total amylase and trypsin values in the same A/T ratio. This information can be excluded by dividing amylase and trypsin by the corresponding protein content of the organisms.

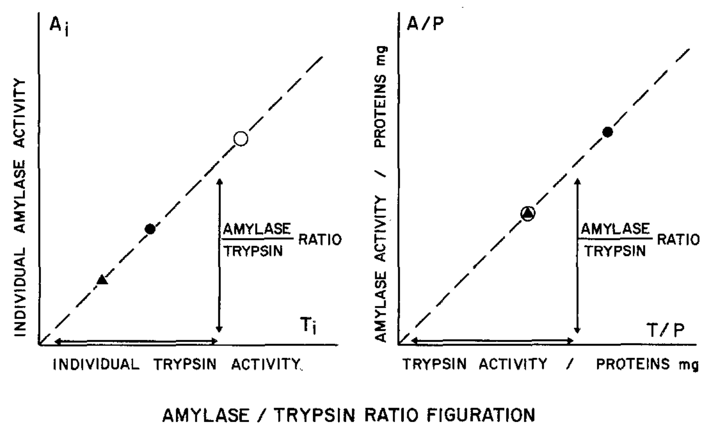


Fig. 1. Geometrical figuration of the A/T ratio using total amylase and total trypsin (left) or amylase/protein and trypsin/protein (right) from the same sample

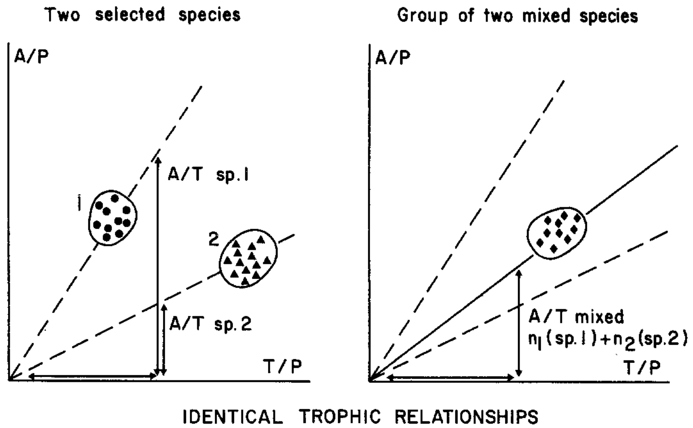


Fig. 2. Theoretical groups of two selected species (left) and of two mixed species of n_1 and n_2 individuals (right) from the same trophic environment

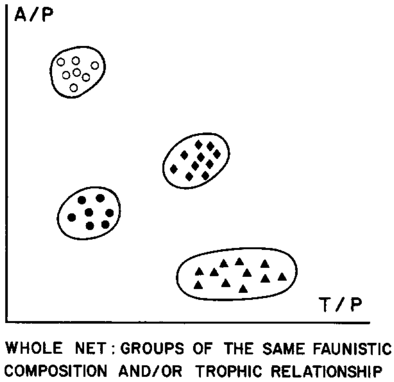


Fig. 3. Theoretical groups of total zooplankton samples with different trophic relationships and/or faunal composition

The amylase/protein (A/P) and trypsin/protein (T/P) ratios are the specific activities of amylase and trypsin. When A/P is plotted against T/P, the slope of the line is again the A/T ratio. When different dots are on the same slope, more or less energy has been expended per mg of proteins to synthesize amylase and trypsin in the same A/T ratio. So we assume that in this case we observe different general physiological levels of the organisms.

As different species are not adapted to the same trophic conditions in the same way, two groups of plots can be obtained of two species in the same trophic environment (Fig. 2). If the sample contains n_1 individuals from Species 1 and n_2 individuals from Species 2, an intermediary group of plots is obtained and is defined by the specific composition of the sample and the relative number of the species. In a more general way, total population samples can be separated by such a method if either their faunistic composition or their trophic adaptation to the environment or the two characteristics are changing (Fig. 3). Changes in trophic conditions can be detected by the study of samples of the different species selected, and a correlated distribution of the data is to be discussed in terms of general physiological variations.

RESULTS

The above considerations were applied to the ecological survey of the northern coasts of Brittany after the "Amoco Cadiz" oil spill in March 1978. A general survey was conducted from the Ouessant Islands to St. Brieuc Bay from March 1978 to January 1979 and again in spring 1979 (Fig. 4). A special study was made on the most polluted onshore area, Aber Benoit, and comparisons were made with a quite similar and non-polluted estuarine system, the Bay of Brest, or with other less polluted estuarine areas from the northern coast: Aber Wrach, Bay of Morlaix, Bays of Lannion, Jaudy, Trieux.

Study on two selected copepod species

The two most representative and frequently occurring species, *Acartia clausi* and *Temora longicornis*, were selected for study. Their A/T ratios were noted for the year 1978–1979 using graphical representation. Groups of homogeneous trophic relationships are found to vary with time of year. Comparing *Acartia* from the ecosystems of Aber Benoit and Bay of Brest (Fig. 5), we found similar situations occurring in September and January but observed marked differences in June and to a lesser extent in May. A comparison of *Temora* from the ecosystems of Aber Benoit and estuarine conditions in northern Brittany (Fig. 6) showed marked differences during the spring period (April,

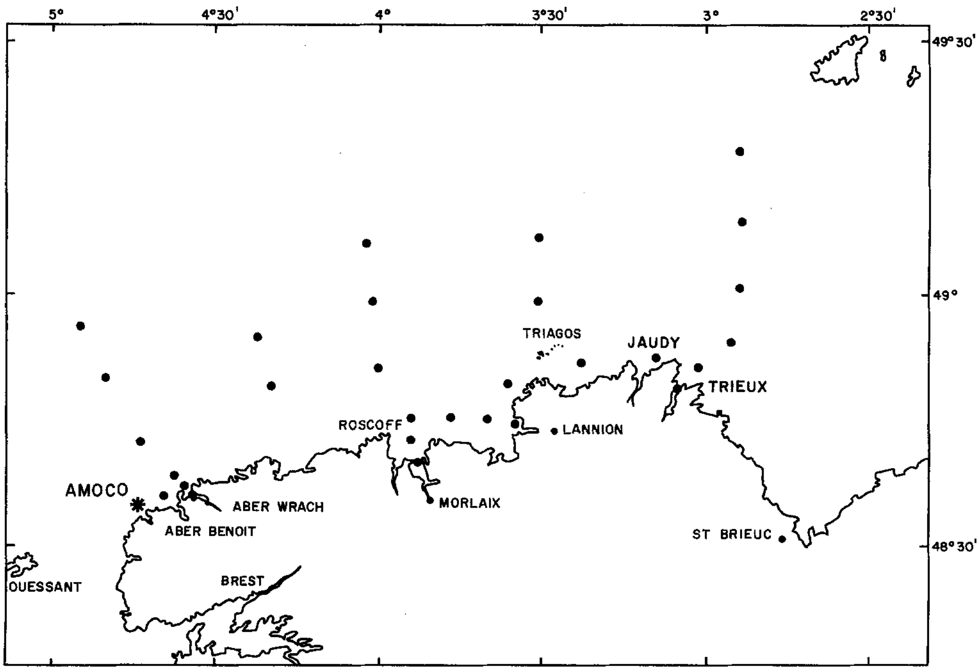


Fig. 4. Geographical location of the stations

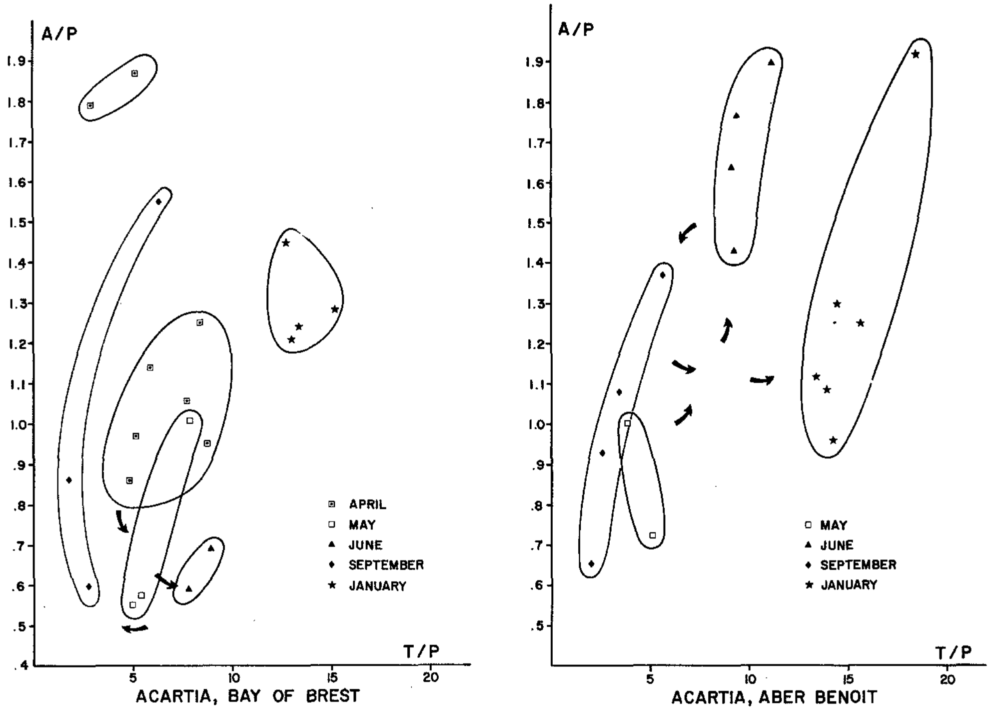


Fig. 5. *Acartia clausi.* Trophic relationships in Aber Benoit and Bay of Brest ecosystems

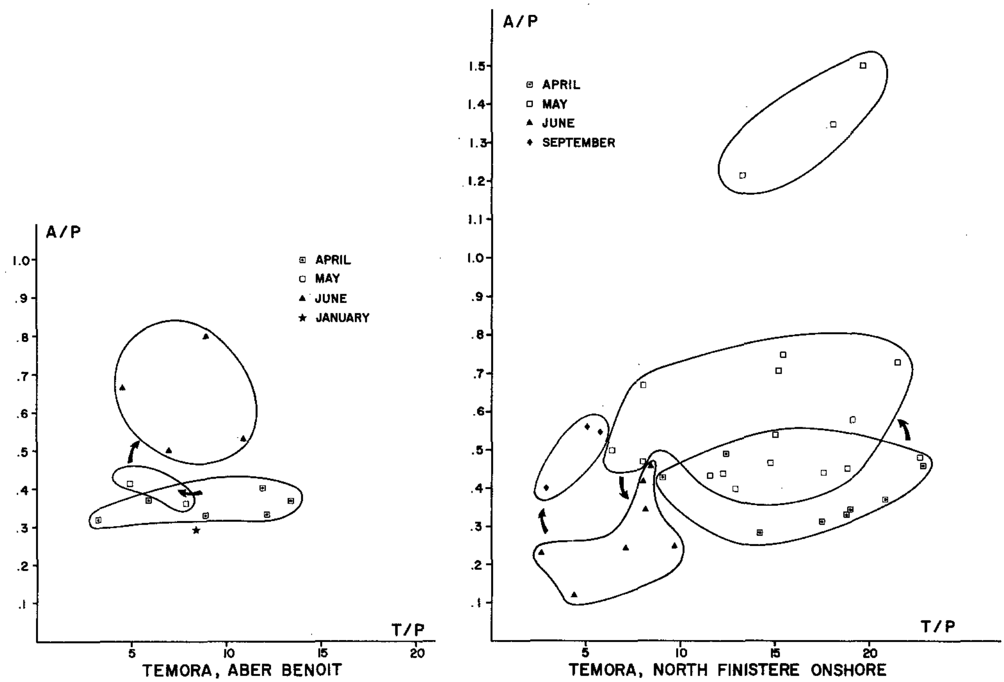


Fig. 6. *Temora longicornis.* Trophic relationships in Aber Benoit and an estuarine area from northern Brittany

May, June). *Temora* individuals were noticeably scarce in Aber Benoit after the oil spill and were completely absent in September.

Study on whole populations

Whole populations from Aber Benoit and Bay of Brest have been compared (Fig. 7). Groups of homogeneous trophic relations and/or faunistic compositions were well defined and related to the season. January conditions were comparable, and June was the most distinct period between the two areas; April, May and September conditions were somewhat similar.

Whole populations from areas of the northern coast of Brittany (onshore and offshore) are plotted in Figure 8. Groups of homogeneous trophic relationships and/or faunistic composition are defined and related either to seasons and/or to geographical area. These groups are more or less homogeneous: in May data were very dispersed and related to geographical situation (Roscoff, Morlaix, Lannion); for June and September the distribution is better explained by the seasonal variations. But in March and April, just after the wreck, peculiar phenomena were distinguishable: in the first 15 days after the wreck, amylase and trypsin levels were depressed in a correlated way; 30 days later (April), as levels increased on the same correlation axis offshore, they stayed at low values onshore; then later (May), geographical heterogeneity appeared. In spring 1979, the same measurements were taken (Fig. 9). In March, no low levels were detectable. We observed a large group of high amylase and trypsin values which was very distinct from

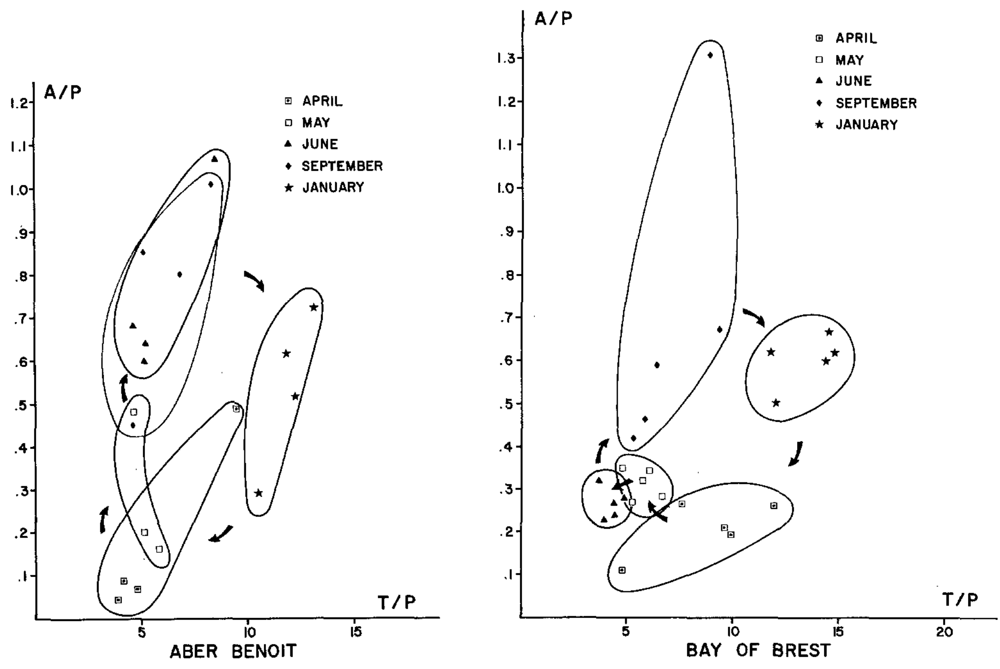


Fig. 7. Total net. Trophic relationships and/or faunal composition changes in Aber Benoit and Bay of Brest

the phenomena observed just after the wreck in 1978. In April, these values were comparable with the offshore data from the preceding year; in June, the 1978 and the 1979 groups were comparable. The results obtained from the copepod *Anomalocera patersoni* are not comparable with those from other species (Fig. 10). Data were not distributed into marked groups; their correlation coefficient is $r = 0.66$. The lowest values correspond to the immediate wreck area – Aber Benoit, Portsall – and to the most immediately polluted other parts of the coast in April – Bay of Lannion, Triagos. In the following year (1979), two groups of data were observable (Fig. 11): one was homogeneous, the amylase and trypsin values were in accordance with the general observations; the other group with lower values was on or under the lower part of the 1978 correlation axis and corresponded to the onshore stations near Aber Benoit and Aber Wrach or located offshore during March in the general current and wind direction. It is also noticeable that in 1979 *Anomalocera patersoni* was about 1.5 times more abundant than during the same period in 1978.

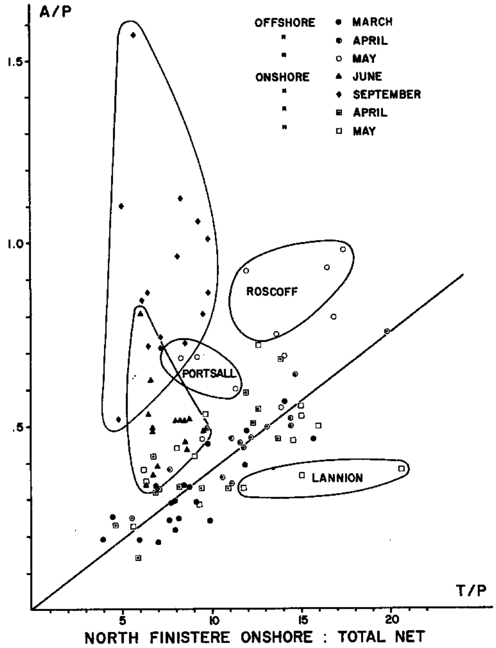
DISCUSSION

Considering either the species or the total population of copepods from the non-polluted area (Bay of Brest), we can distinguish several groups over a one-year period. The amylase and trypsin levels vary seasonally with trophic relations (diet, food characteristics, related species and faunistic composition). In the same way, different groups are observable for the more or less polluted aquatic ecosystems of northern Brittany. We will attempt here to compare these groups in terms of seasonal, geographical and abnormal situations. The A/T of the groups and their homogeneity will be analysed; eventual unusual correlations between amylase and trypsin will be discussed.

Comparing *Acartia* from Aber Benoit and the Bay of Brest (Fig. 5), we found groups corresponding to homogeneous trophic relationships very distinct in June, usually the most productive period in Brittany. Less marked differences in May, and comparable situations in September and January, indicate that similar trophic conditions occur for *Acartia* during these other months in the two areas. Some characteristics in the trophic relationships of *Acartia* are markedly different in June. A comparison of *Temora* from the oil-polluted Aber Benoit and from other similary less or non-polluted estuarine areas (Fig. 6) also revealed a discrepancy in June, and in the other spring months as well. The differences observed in the trophic relationship seemed to be lethal for *Temora* (Rojat, 1979), indicating that some characteristic of the ecosystem was highly modified in June. Possibly the phytoplankton concentration or its floristic composition or nutritive value was altered directly by the effect of oil on the phytoplankton production or indirectly in relation to nutrient modifications.

When the whole populations of zooplankton from Aber Benoit and Bay of Brest were compared (Fig. 7), marked differences were also visible during the June period. Trophic relationships lead to this phenomenon, but faunistic modifications cannot be excluded.

Analysis of the groups' homogeneity or heterogeneity of the whole zooplankton population from the northern coast of Brittany revealed that seasonal groups were larger in March and April than in May and June. These results are in accordance with the general observations of the production mechanisms on this coast (Samain et al., 1978). In March and April, the production of each onshore subecosystem (bays or abers) begins as



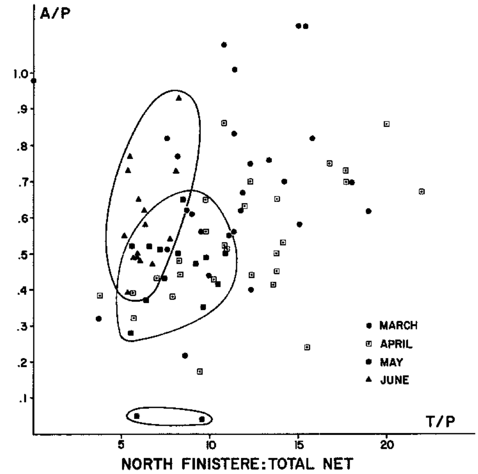
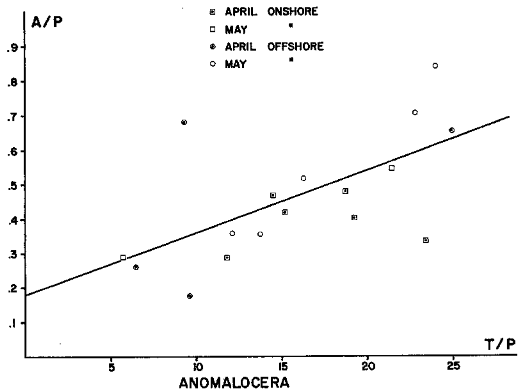


Fig. 8. Trophic relationships and/or faunistic composition changes on the northern coast of Brittany; samples as a function of seasonal or geographical characteristics (1978)

Fig. 9. Trophic relationships and/or faunistic composition changes on the northern coast of Brittany; samples as a function of seasonal or geographical characteristics (1979)



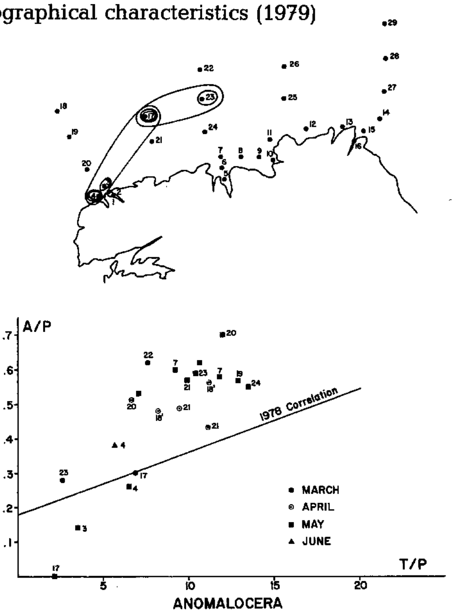


Fig. 10. *Anomalocera patersoni* (1978). Anomalous correlation between specific amylase and trypsin activities. A/P = 0.174 + 0.018 · T/P (r = 0.66, n = 17)

Fig. 11. *Anomalocera patersoni* (1979). Plot of specific amylase and trypsin activity; geographical position of anormalous values

a function of local hydrobiological conditions, but the main production seems to be offshore; thus in May or June, all the coastal areas are overgrown by the offshore production, and all the northern Brittany ecosystem studied is homogeneous in trophic and faunistic characteristics. In May 1978, high heterogeneity was attributable to distinct geographical characteristics, particularly in Lannion Bay (Fig. 8). One year later (Fig. 9), homogeneity characterized the area. Only two aber stations were distinguishable. As in 1978 and 1979, spring meteorological conditions (temperature) were the same; these results are in accordance with the hypothesis that there is a correlation between the heterogeneity of the onshore ecosystem in May and the oil-spill pollution (Samain et al., 1978). In May 1978, two mechanisms could have emphasized the initial heterogeneity: (a) the toxic properties of the oil could have prevented the offshore production overgrowing the coastal ecosystems until June, and (b) each local ecosystem polluted at different levels could have resulted in trophic and faunistic characteristics. This phenomenon disappeared in June, so the direct effects of the oil spill seemed to operate till May 1978 on the general onshore ecosystems.

Theoretically, variations of the digestive enzyme synthesis in the same A/T ratio were analysed in terms of general metabolic variations. In this case, two cellular parameters that are usually independent became correlated. We have also demonstrated that experimental intoxication of *Artemia salina* by copper or zinc depressed specific amylase and trypsin levels (Alayse-Danet et al., 1979). In 1978, the general correlation and the low values found in the first 15–30 days after the wreck were supposed to correspond to a general metabolic depression (Samain et al., 1978). But in 1979 this correlation disappeared for most data, and remained only for some severely oil-polluted stations. The faunistic composition analysis (Lefèvre, personal communication; Rojat, 1979) indicates that very high mortality occurred during the first month. The correlated low amylase and trypsin data seem, therefore, to provide a good approach for estimation of physiologically perturbated zooplankton.

In April and May 1978, *Anomalocera patersoni* was the only species with amylase and trypsin correlated on the whole northern Brittany coastal area (Fig. 10). It was found until May and disappeared in June. During this period, all areas that had been directly oil polluted were submitted to natural or human cleaning, and high values of hydrocarbons were recorded on the first subsurface layer (Marchand, 1978). As this species lives in the upper 10 cm, we attributed the existing correlation to a physiological perturbation. This hypothesis seemed to be improved one year later. A lot of data were not correlated, and *Anomalocera* did not disappear after May but was 1.5 more abundant. Only a few of them with low values, near the 1978 axis, corresponded to onshore and offshore areas of Aber Benoit and to the back of the Bay of Lannion. The sediments of the back of these two estuarine areas were still oil polluted by March 1979. Hydrocarbons in the upper layer of the sea are significantly higher (Abers 2.6 μg/l, Bay of Lannion 2.0 μg/l) than in the less or non-polluted area: Jaudy estuarine (0.8 μg/l), Trieux (0.7 μg/l) (Marchand & Caprais, 1979). The other observed lower values of amylase and trypsin were located in the direct axis of dominant wind for this spring period. They could be related to the slow decontamination of the aber area with storms. Thus as the upper group would be in accordance with normal trophic relationships, the lower one would indicate that *Anomalocera patersoni* is very oil sensitive, probably because of its upper position in the seawater column.

CONCLUSION

There is evidence that an ecophysiological survey of a planktonic ecosystem is possible by measuring two digestive enzymes, amylase and trypsin from zooplankton, because trophic relationships, population variations and physiological perturbations are observable in such a way. The copepod *Anomalocera patersoni* seems to be useful for studying light hydrocarbon pollution with this approach.

In the "Amoco Cadiz" case, three periods of pollution effects are detectable on the planktonic ecosystem: an immediate effect for 15 days offshore and 30 days onshore when the metabolism of the zooplankton was affected and high mortality occurred; a secondary effect until June when it was not possible for exogenous species to settle in the extremely toxic or much modified (probably in their trophic structure) coastal ecosystems, Lannion Bay and Aber Benoit; and a tertiary effect one year later on subsurface-living organisms when oil was released from the very polluted area continuously or with occasional storms.

As no reference state was performed on the northern coasts of Brittany before the "Amoco Cadiz" was wrecked, a pluriannual survey using such an ecophysiological approach would be necessary to estimate whether long-term faunistic changes were occurring.

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