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Causes and incidence of the "black spot disease" on brown shrimp (*Crangon crangon*)

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Abstract.

Different aspects of the "black spot disease" of brown shrimp (*Crangon crangon*) were investigated : the primary cause of the disease, the eventual influence of chitinoclastic bacteria on the formation and progress of the black spots, the influence of moulting on the progress of the spots, the occurrence of black spots on individual shrimps, the incidence of the disease among the population of shrimps in the Belgian coastal waters and the possible causative factors under natural circumstances. The results of aquarium experiments and of observations on a natural population indicate that physical damage of the exoskeleton, caused by unsuccessful predation attempts or by cannibalistic behaviour, is the primary cause for the formation of black spots. The "disease" seems not to be of pathological origin. Secondary pathological manifestations however may occur.

Résumé.

Différents aspects de la maladie dite "black spot disease" de la crevette grise (*Crangon crangon*) sont étudiés : la cause primaire de la maladie, l'influence éventuelle des bactéries chitinolytiques sur la formation et le dévelopement des lésions nécrotiques, l'influence de la mue sur le dévelopement des lésions, la présence de lésions sur des crevettes individuelles, la répartition de la maladie parmi la population de crevettes dans les eaux côtières belges et les facteurs causatifs possibles sous des circonstances naturelles. Les résultats d'expériences en aquarium et des observations sur la population naturelle indiquent que les blessures de l'exosquelette, dues aux tentatives de prédation ou de cannibalisme, sont à la base de l'apparition des lésions nécrotiques. La "maladie" ne semble pas être d'origine pathologique, bien que des effets secondaires pathologiques peuvent se manifester.

1. Introduction.

The "black spot disease" was first described for the population of brown shrimp (Crangon crangon) in the German coastal waters (MEIXNER, 1967, 1968, 1969 ; SCHLOTFELDT, 1972 ; ICES, 1977). Black areas of destruction were observed on the cuticula of carapax, abdominal segments and appendages.

This contribution reports on the "black spot disease" among brown shrimp in the Belgian coastal waters. Two aspects were investigated :

a. the nature and progress of the disease (observations in vitro) and
b. the incidence of black spots among the shrimp population in the Belgian coastal area (observations in vivo).

2. Observations in vitro.

The aim of the in vitro experiments was to establish

a. the primary cause of the disease,
b. the eventual influence of chitinoclastic bacteria on the formation and progress of the black spots and
c. the influence of moulting on the progress of the spots.

2.1. Material and methods.

In this paragraph the general conditions of the in vitro experiments are described. The specific methodology of each experiment will be given in the following sections.

The shrimps were kept separately in glass jars filled with 3 liters of seawater, in dim light and at a temperature of 12°C. The seawater was natural or synthetic, depending upon the experimental requirements. The laboratory animals were provided every week with fresh seawater and fed with sprat. During the experiments no account was taken of the age or sex of the shrimps.

The bacteria used in the artificial infection experiments (see sections 2.2. and 2.3.) were isolated from black spots. The numbers of bacteria were determined by the dilution culture method. For this purpose bacto marine agar (Difco). enriched with 8% of purified chitin was used (HOCK, 1940).

2.2. Cause of the black spots.

The causative agent of the "black spot disease" has not been established yet. The symptomatology is somewhat similar to that of the "brown spot disease" of Palaemon serratus (MEIXNER, 1969 ; SCHLOTFELDT,1972), where chitinoclastic bacteria are the causative agent (ANDERSON and CONROY, 1968). Physical damage, in consequence of cannibalism or predation, on the other hand was also considered as a possible cause for the black spots (MEIXNER, 1967, 1968, 1969 ; SCHLOTFELDT, 1972). In order to solve the question about the primary cause of the black spots, two experiments were performed.

The first experiment investigated if mere physical damage could induce the formation of black spots. 25 animals were mechanically wounded by amputation of a pereiopod and kept in sterile, synthetic seawater, i.e. in a medium without chitinoclastic bacteria. After maximally 4 days all the animals showed a black spot on the injured appendage.

The second experiment investigated if chitinoclastic bacteria, isolated from shrimps, could cause necrotic lesions on an undamaged cuticula by primary infection. 10 shrimps were artificially infected with these bacteria, which were plated in a thin layer on the intact carapax. After two weeks none of the animals showed black spots on the infected area.

The results of these experiments show that mechanical damage is required for the formation of black spots. Chitinoclastic bacteria seem not to be able to infect an intact, undamaged cuticula and are not needed to cause blackening of the injured areas. This blackening of the cuticula in fact is a consequence of a biochemical process. The enzyme phenol-oxydase, produced by the shrimp's haemocytes, reacts with phenolic substrates, usually tyrosine, present in the haemolymph (PRYOR, 1940 in DENNEL, 1958 ; DECLEIR et al., 1961 ; DECLEIR and VERCAUTEREN, 1965 ; HACKMANN, 1971). The final product is a black pigment, melanine, which forms an impermeable layer on the wound (NOUVEL, 1936 ; HACKMANN, 1971 ; FONTAINE and LIGHTNER, 1975). This mechanism can be considered as a healing after wounding and as a simple means of internal defense against injuries.

2.3. Secundary influence of chitinoclastic bacteria.

Microbiological examination revealed 100 to 150 times more bacteria on a black spot than on the same surface of an unaffected cuticula. Fungi (mentionned by SCHLOTFELDT, 1972) were never isolated from the black spots. Two different types of bacteria were detected in the affected areas. Chitinoclastic bacteria represent 15% of the total bacterial count on the black spots. On the other hand no chitinoclastic bacteria were found on the unaffected cuticula. For this reason a possible secundary infection of the damaged cuticula by chitinoclastic bacteria, leading to an eventual increase of the spot, was taken into account.

20 animals were experimentally damaged. The upper layer of the cuticula of their carapax was removed over a surface of approximately 0.25 cm^2. 10 individuals were kept in natural seawater, i.e. in a medium with a "normal" amount of chitinoclastic bacteria. The others were kept in natural seawater, supplied with an excess of chitinoclastic bacteria. Every two or three days the surface of each spot was measured and devided by its initial surface. The resulting data reflect the relative surface changes of the spots in relation to time (figure 1).

The surface of the black spots remained about constant in most individuals, whatever were the experimental conditions. Only 2 of the 14 animals, which survived the experiment, showed an increasing black spot (figure 1). This indicates that a surface increase of the black spot, in consequence of a secundary infection by chitinoclastic bacteria, is possible but not evident.

2.4. Moulting and black spots.

Literature is contradictory about the eventual reappearance of black spots after moulting. Meixner observed black spots after moulting only when damage took place just before moulting (MEIXNER, 1967). According to Schlotfeldt however, black spots remain and increase in size from one moult to the next (SCHLOTFELDT, 1972).

The interval between two successive moults can be divided into two periods (PASSANO, 1960). During the first 40% of the interval only the newly-hardened cuticula is present. During the next 60% a new cuticula is formed underneath the old-one. If damage occurs during the first period, the new cuticula remains unaffected and the black spot, which is only present on the old cuticula, will disappear after moulting. If damage however occurs during the second period, the old as well as the new cuticula are injured and the black spot will reappear after moulting. In a group of randomly injured shrimps, 40% of them will not show a black spot after the first moult while 60% of them will show a new black spot on the damaged area after the first moult.

Individual shrimps were physically injured by amputation of a pereiopod and kept in separate jars for about 2 months. After this period 31 animals had moulted at least once. Of these, 13 lost their black spot after the first moult while 18 showed a renewed black spot after moulting. These experimental data are in good agreement with the theoretical expectations (40 and 60% of 31 or respectively 12 and 19). The animals which moulted for a second time during the experiment never showed a black spot after the second moult. The eventual reappearance of a black spot after moulting thus clearly depends upon the moment of damage with respect to the ecdysal cycle.

3. Observations in vivo.

On the basis of quantitative analyses on 31,250 shrimps
a. the occurrence of black spots on individual shrimps,
b. the incidence of the disease in a natural population and
c. the possible causative factors under natural circumstances were investigated.

3.1. Material and methods.

The shrimps were collected monthly during the period 07.1973-06.1976 on two sampling stations in an area 1.5-2.5 miles offshore the Belgian W-coast. For each sample the total number of shrimps and the number of spotted shrimps were counted. All shrimps were measured from the tip of the scaphocerite to the end of the stretched uropods. Affected individuals were analysed upon the number and distribution of the black spots.

3.2. Occurrence of black spots on individual shrimps.

The distribution of the black spots over the different body parts was calculated per 10 mm length class (21-30, 31-40, ..., 71-80 mm). The results are similar for all length classes (figure 2) : pereiopods and pleopods were most affected, followed by abdominal segments, antennullae and antennae. The other body parts were less affected. These observations are almost identical to those for the German coastal waters (MEIXNER, 1969 ; SCHLOTFELDT, 1972). During the analyses we observed that thin appendages, like antennae, antennullae, pereiopods and pleopods often seemed to have been plucked off.

3.3. Incidence of black spots in a natural population.

The density of spotted shrimps shows the same seasonal fluctuations as the density of the shrimp population (figure 3), viz. a large peak in autumn and a less distinct peak in spring, alternated by minimal densities in winter and summer. The percentage of affected shrimps oscillates around an average value of 20% and remains fairly constant throughout the year (figure 4). This is in sharp contrast with the findings for the German waters where this percentage was found to vary seasonally between 0.8 and 8.9 with a clear maximum during summer (SCHLOTFELDT, 1972).

3.4. Causative factors under natural circumstances.

A study was made on the relation between the absolute density of spotted shrimps and the possible causative factors of the "black spot disease" : bottom temperature (as an index of metabolic activity connected with bacterial infection processes), population density (as an index of cannibalism), predation intensity and fishing effort (as an index of injuries inflicted to discarded shrimps). For this study only the data of spotted shrimps belonging to the 41-60 mm length class were used since larger and smaller spotted shrimps were only rarely present in the samples in adequate numbers.

The densities of shrimp and of spotted shrimp were calculated from the results of the monthly samplings in the Belgian coastal waters (REDANT, 1978). Predation intensity was estimated from the densities of the most important shrimp predators and their food preference for postlarval shrimp. The following predator species were taken into account : Odontogadus merlangus, Trisopterus luscus, Gadus morhua, Ciliata mustela, Pomatoschistus species, Trigla species, Agonus cataphractus and Liparis liparis (REDANT, 1978 ; see also C.M. 1980/K : 33). Fishing effort, expressed in numbers of hours fishing, was derived from national fisheries statistics (N.I.S., original data).

The relations were checked by the cross-correlation method. For each sampling station the linear correlations were calculated between the densities of spotted shrimp on the one hand and population density, predation intensity, fishing effort and bottom temperature in the same month, the previous month,... up to four months back (time shifts 0, -1, -2, -3, and -4) on the other hand.

No relation at all was found between the density of spotted shrimps and bottom temperature (figure 5). Also the other types of regression (exponential, logarithmic or power curve fit) did not reveal any apparent relation between these parameters. This indicates that no external temperature dependent processes are involved in the formation of the black spots and that the "disease" is probably not of pathological origin.

A distinct correlation however was found between the density of spotted shrimps and population density at time shift 0. This is completely in line with the findings in the previous section. The other time-shifts gave no significant correlations. The relation between the density of spotted shrimps and predation shows a similar pattern (figure 5). Finally, no relation was found with fishing effort (figure 5). We may conclude that the injuries inflicted to brown shrimp, causing the formation of black spots, are a consequence of cannibalistic behaviour and/or unsuccessful predation attempts. Since also a good correlation was found between the population density and predation intensity, we could not ascertain which value must be attributed to each as a causative factor of the "black spot disease". Fishing activities seem to be of negligible importance as a cause of non-lethal injuries in comparison to predation and cannibalism.

4. Conclusion.

Physical damage of the exoskeleton of brown shrimp, caused by predation attempts and cannibalistic behaviour leads to the formation of black spots, described in the literature as "black spot disease". The phenomenon seems not to be of pathological origin so that the designation "disease" should not be used in this case. Secundary pathological manifestations however may occur as a result of a superinfection by bacteria or parasites invading the damaged tissues.

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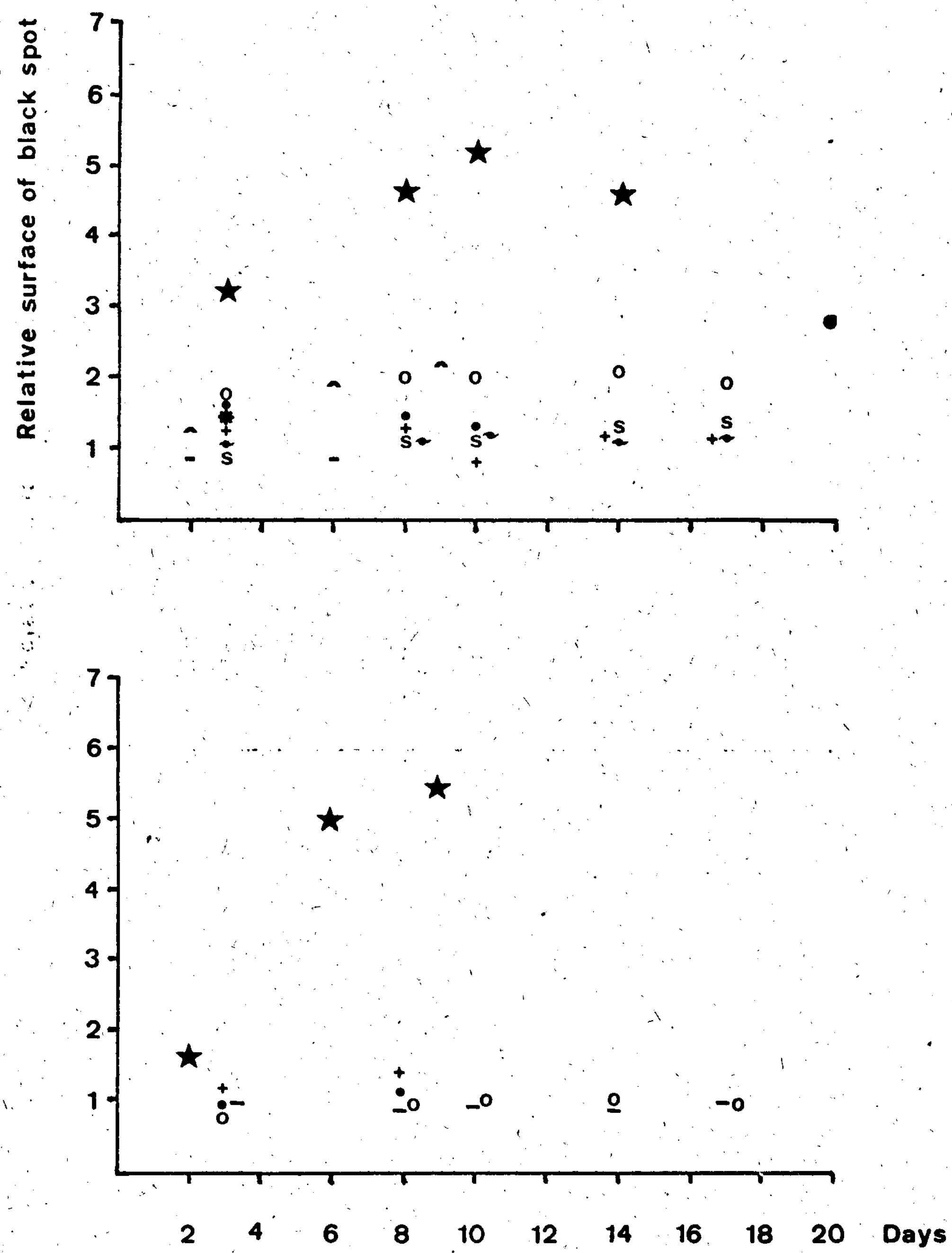

Figure 1 - Relative surface (surface at T_i/surface at T_o) of secundary infected black spots in relation to time (days after damage at T_o) in natural seawater (top) and in natural seawater supplied with an excess of chitinoclastic bacteria (bottom).

Each symbol represents the observations on one shrimp.

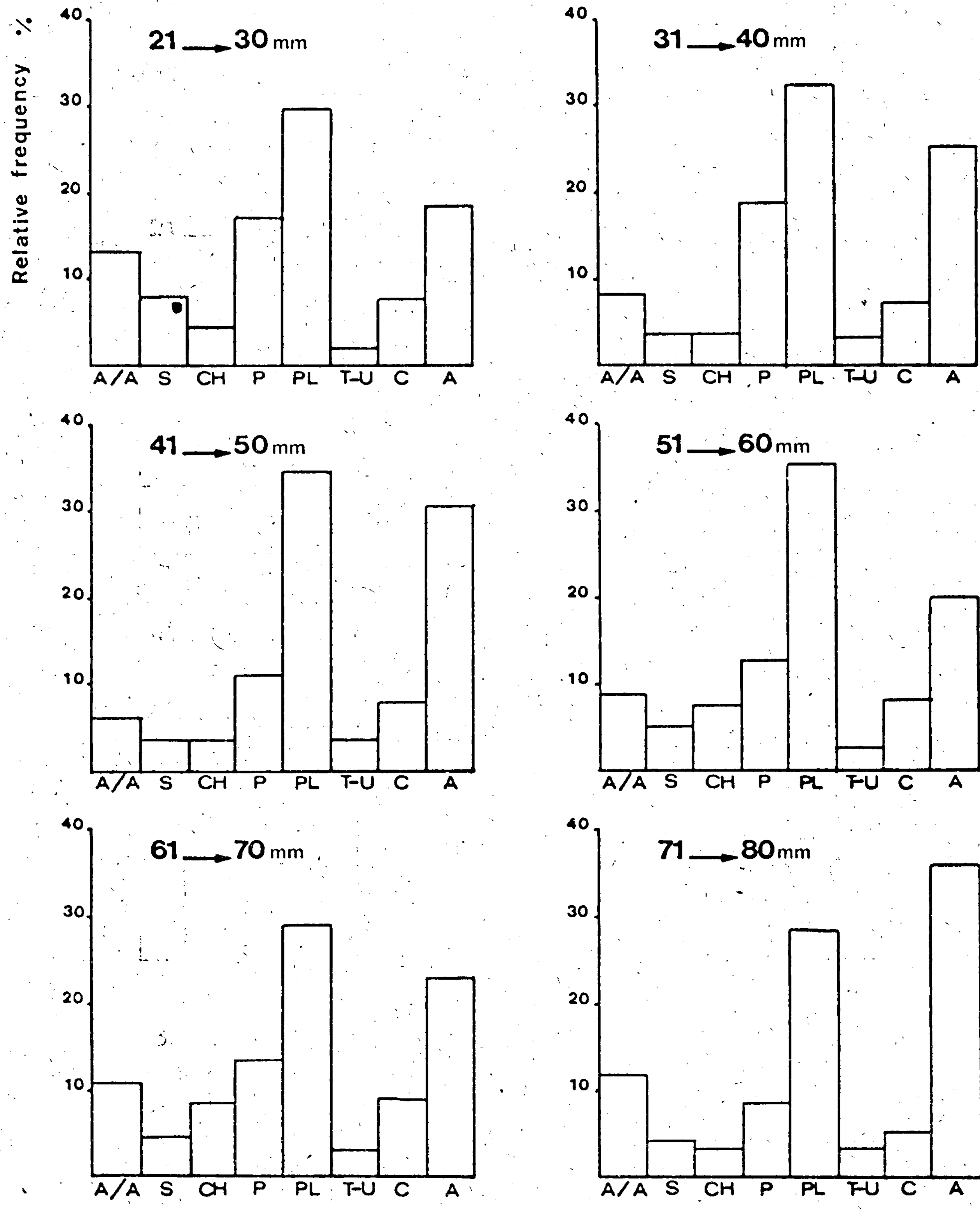

Figure 2 - Distribution of black spots over different body parts of brown shrimp : antennulae and antennae (A/A), scaphocerites (S), chelipeds (CH), pereiopods (P), pleopods (PL), telson and uropods (T-U), carapax (C) and abdominal segments (A).

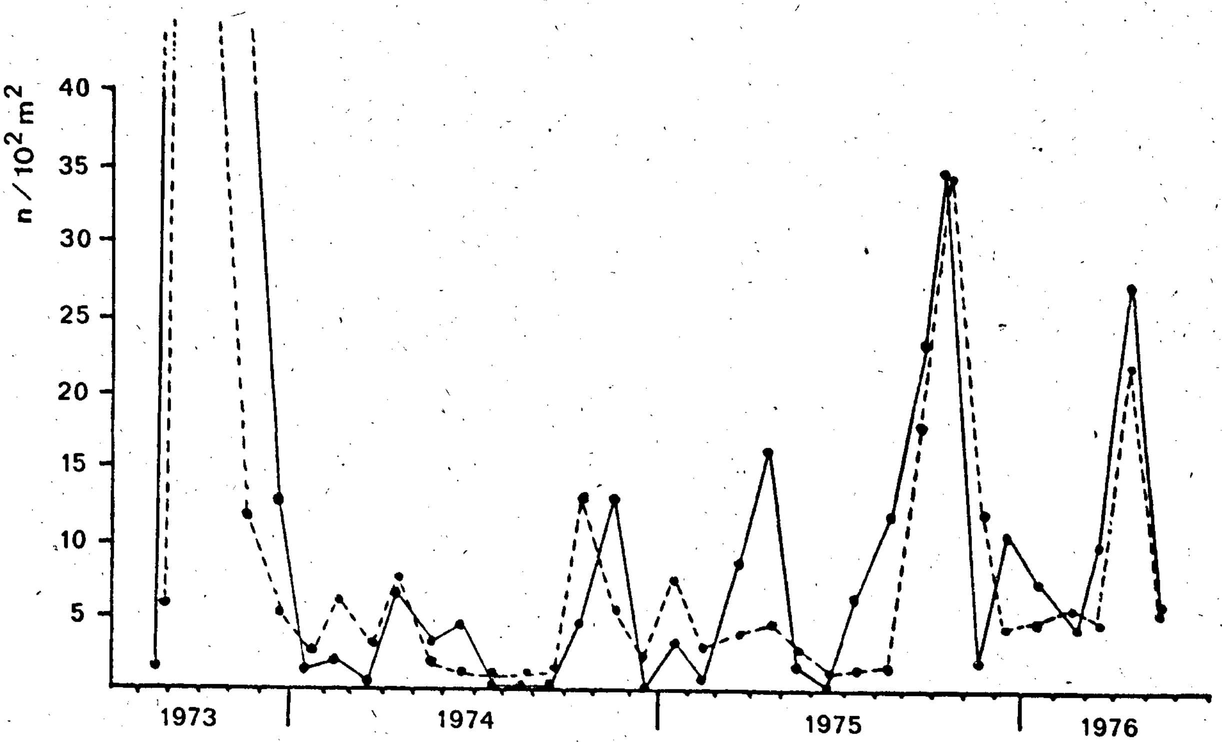

Figure 3 - Density of spotted shrimps (41-60 mm) on two sampling stations in relation to time.

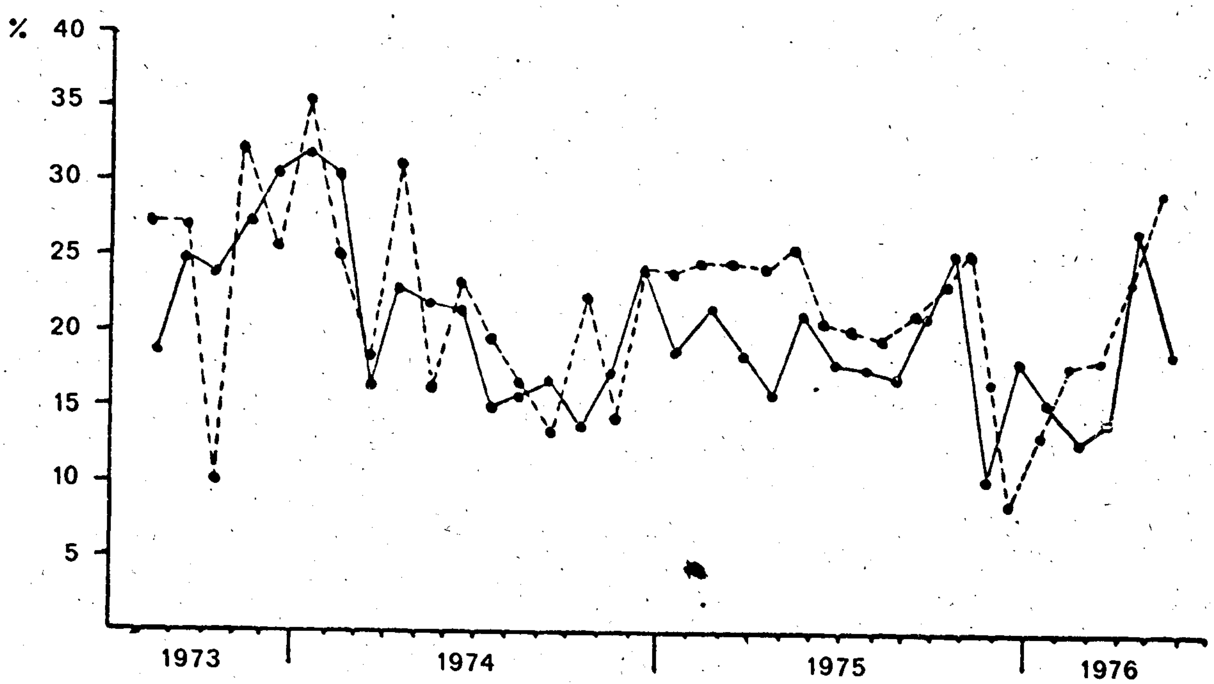

Figure 4 - Percentage of spotted shrimps on two sampling stations in relation to time.

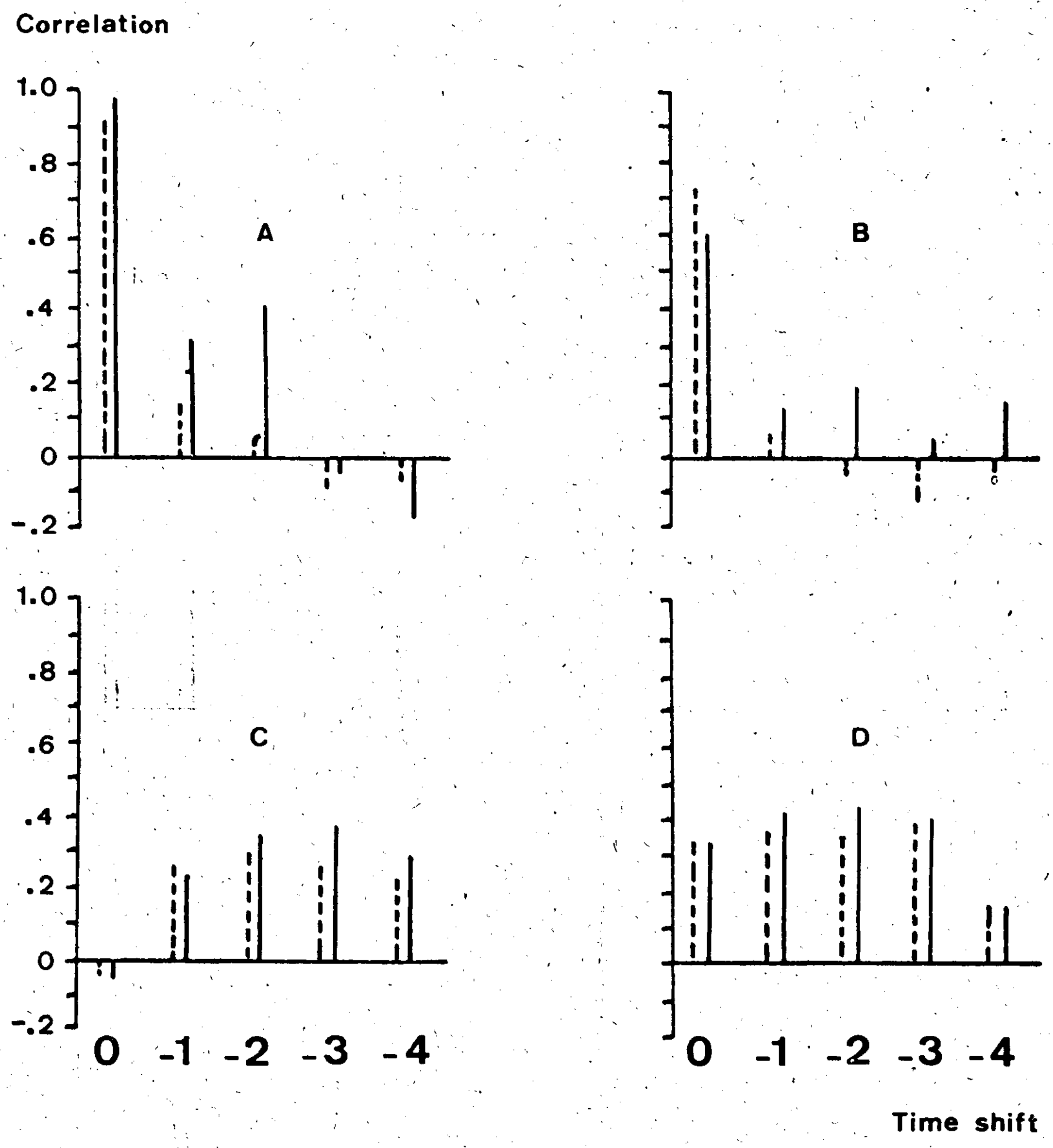

Figure 5 - Cross correlation between the density of spotted shrimps (41-60 mm) and population density (A), predation intensity (B), fishing effort (C) and bottom temperature (D) on two sampling stations.

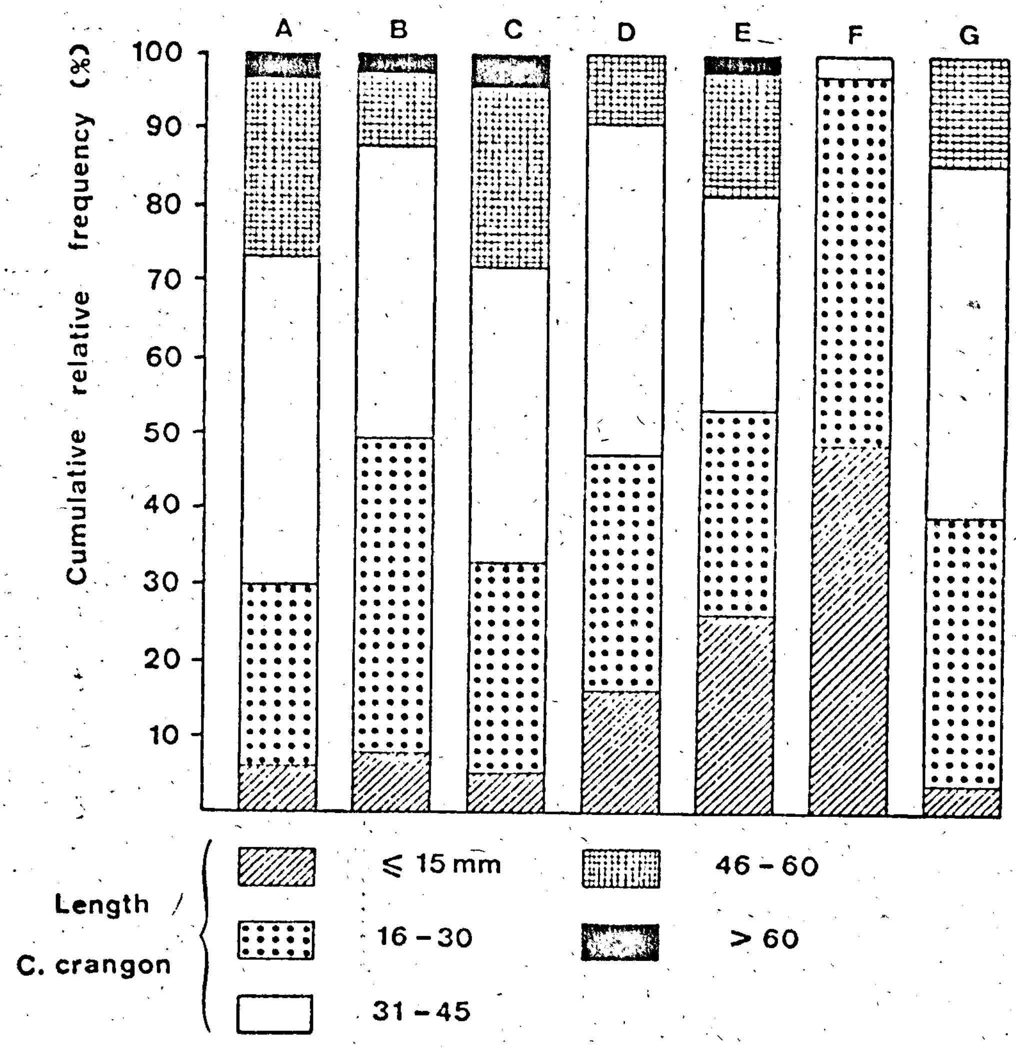

Figure 1 - Cumulative relative frequency distribution of length classes of shrimp in the stomachs of
A : Odontogadus merlangus,
B : Trisopterus luscus,
C : Gadus morhua,
D : Ciliata mustela,
E : Trigla species,
F : Agonus cataphractus,
G : Liparis liparis.

Data on Agonus after GABRIELS (1977).

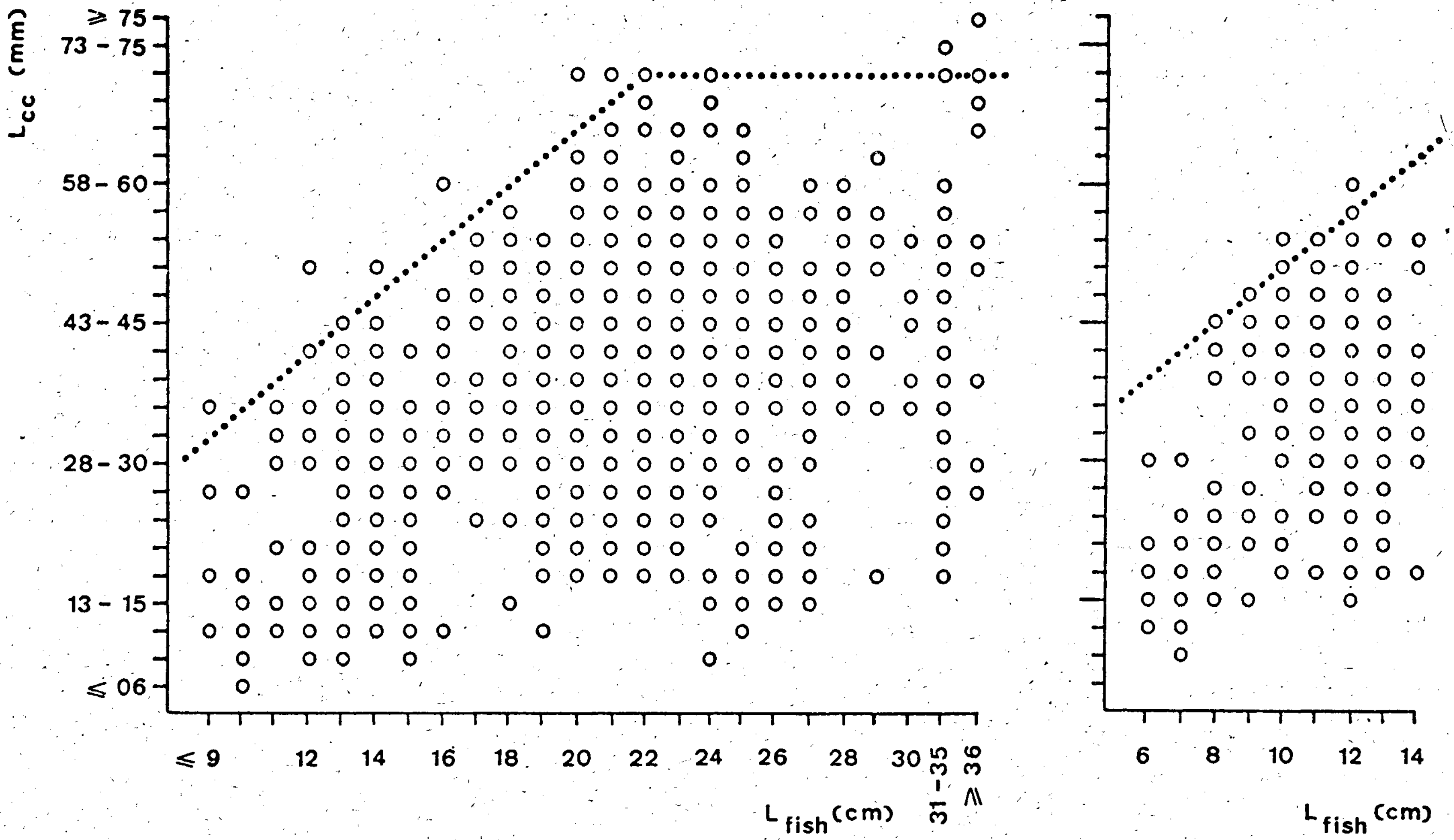

Figure 2 - Length distribution of shrimps in the stomachs of a large predator (Odontogadus merlangus, left) and in the stomachs of a small predator (Liparis liparis, right) in relation to the size of the predators.