International Council for the
Exploration of the Sea.

C. M. 1963
Shellfish Committee
No.25

/This paper not to be cited without prior reference to the Author/

THÜNEN
Digitalization sponsored by Thünen-Institut

Blood Disease of Lobsters (Gaffkaemia)

Results of investigations made in 1962-63.

by

P. C. Wood,

Fisheries Laboratory, Burnham-on-Crouch, Essex.

Since the last report (Wood 1962), the study of the blood disease of lobsters has continued, in co-operation with the Department of Agriculture and Fisheries, Aberdeen, and the Fisheries Division, Department of Lands, Dublin. When possible, investigations have continued along the lines suggested last year, and have included (a) improvement of methods of diagnosis, (b) laboratory infection experiments, (c) survey of natural stocks for incidence of infection, (d) the study of naturally-occurring outbreaks and (e) the possible control of infection in tanks by the use of ultraviolet light.

Further enquiry into the biochemical and cultural characteristics of stock strains has cast doubt on the generic name, and other workers (Deibel & Niven, 1960) have suggested that Gaffkya strains possess enough features to be included in the older genus Pediococcus. In view of this doubt, it is suggested that for the present, the infection is known simply as the bacteraemia of lobsters. In the present report, the term Gaffkya will be used merely for convenience, and without prejudice to any future change of nomenclature.

(a) Methods of diagnosis. The methods employed for the culturing of organisms from infected lobsters were generally similar to those employed last year. The 5% serum nutrient agar was replaced by Oxoid Blood Agar Base No. 3 which supported a heavy growth without the presence of serum. The sparse granular deposit which appeared in nutrient broth was improved by the use of Oxoid Sensitivity test broth, which produced turbid suspensions.

The identification of Gaffkya in heavily infected lobsters presented no problems, blood taken aseptically from the ventral abdominal sinus, or from the membrane at the base of the pincer producing heavy growth on solid media.

The identification of Gaffkya in lobsters in the carrier state was not so simple, as only blood from relatively heavy infections produced growth on direct plating, and often other organisms were present, either from the blood of the lobster itself, or from contamination during the collection of the sample. To improve the sensitivity and selectivity of diagnosis, Dr. J. E. Stewart of the Technicological Station, Halifax, Nova Scotia kindly supplied details of phenyl ethanol media which he has devised for this purpose. Lobster blood was inoculated into an enrichment broth containing 0.25% phenyl ethanol and after 48 hours at 30°C, subcultures were made to phenyl ethanol sheep's blood agar plates. Haemolytic colonies were investigated further; catalase negative greyish-white colonies measuring 1-2mm which were seen as tetrads in smears were identified as presumptive Gaffkya strains, and tested biochemically for final confirmation. Stewart's media were used for surveying the incidence of Gaffkya in natural stocks, and the phenyl ethanol agar for direct cultivation of the blood of dead lobsters, and for counting Gaffkya in water.

(b) Laboratory infection tests. Previous experiments suggested that damage to the integument was a prerequisite for the entry of Gaffkya into the blood stream. Further tests have been made to investigate this more fully. In the first, 14 non-infected lobsters were each held separately in 22 litres of water in plastic tanks with adequate aeration. During the 37 days of the experiment the water temperature

ranged between 15 and 17°C. Lobsters were grouped according to the degree of damage received by the integument:- Group A showed no apparent damage, group B was punctured ventrally when blood was taken 2-3 times each week, and group C consisted of lobsters which were selected because they had received severe, but not recent damage, previous to their purchase. Each lobster was fed with 2-3g. of the meat of infected lobsters on 3 or 4 occasions each week and the water was changed at approximately weekly intervals. From this experiment it was evident

Treatment	Total died	No. of days to death	Survivors infected	Gaffkya first in blood (days)
A. No apparent damage	1/5	31	4	-
B. Punctured	3/5	10,24,29	2	10,10,10,10,17
C. Severe damage	3/4	22,26,29	1	-

that all lobsters became infected, but those with a damaged integument (group B and C) died sooner, possibly as a result of weakness caused by the damage, or more probably because invasion took place earlier, and a higher level of infection was established. Lobsters with severe damage inflicted before the start of the experiment fared no better than those damaged during exposure to infection. At the termination of the experiment all surviving lobsters were placed in a storage tank at 5°C; all died within 4 days.

A similar experiment was continued through the winter when tank-water temperatures were between 3.5 and 9°C. Group C lobsters (severely damaged) were omitted. The results after 38 days storage are shown below, and support the previous experiment, except that the general level of infection was lower, there being no deaths of undamaged lobsters and only 1 being infected at the end of the experiment.

Treatment	Total died	No. of days to death	Survivors infected	Gaffkya first in blood (days)
A. No apparent damage	0/4	-	1	-
B. Punctured	3/5	8, 20, 37	2	All at 5 days

Of the 3 punctured lobsters dying, that which died after 8 days contained so few Gaffkya that there is some doubt whether death was caused by this organism. The generally lower level of infection was probably attributable to the low water temperatures.

In experiments reported previously (Wood, 1962) undamaged lobsters survived when fed infected meat and held at 16-19°C. for 14 days; Gaffkya could not be isolated from their blood. The infection of apparently undamaged lobsters held at 15-17°C. and 3.5-9°C. in the present experiments is believed to be the result of the longer period of exposure, viz. 37 and 38 days respectively.

Damaged lobsters probably became infected through lesions of the integument, the existence of the sinus system immediately beneath this layer, making lobsters particularly vulnerable to systemic infection. Infection of apparently undamaged lobsters could presumably take place through any of the natural pores of the integument, through the soft parts of the gut, or through surface lesions which were not visible to the naked eye. When stored at the higher temperature, the presence of old and of recent wounds render a lobster equally susceptible to rapid infection.

Experiments are now in progress to determine the conditions under which cross-infection takes place among lobsters stored in good and bad conditions. Two tanks were arranged with storage densities similar to those in general use in British short-term storage units /6 lobsters in 22 l./. In each tank, two lobsters were injected with between 0.05 and 1.0ml of an overnight broth culture of Gaffkya. One tank was held in a high ambiant temperature (15-19°C), the other in a cooling unit with water temperature between 6 and 9°C, which is typical of good commercial storage conditions. As lobsters died, they were replaced by lobsters receiving similar treatment, so that the ratio of injected/non-injected lobsters remained the same. The blood of all dead lobsters was cultured, and the numbers of Gaffkya present in the tank water determined at intervals. Water was changed when it appeared to be unsatisfactory.

Lobsters which died in the warm tank were often dismembered and partially eaten by other lobsters before their bodies could be removed from the tank. Thirty-one days after the start of the experiment the conditions of storage in the warm tank were modified so as to prevent dead lobsters from being attacked. Injected lobsters were held in a perforated plastic tray suspended in the tank, permitting a free circulation of water, but preventing infected dead lobsters from being eaten by active ones. The results of this experiment, after a total of 54 days, are shown on Table 1. Cross-infection took place in the warm tank when dead injected lobsters were eaten, 10 untreated lobsters dying between 5 and 25 days after exposure. No cross-infection took place when the injected lobsters were separated, nor in the cold tank, although injected lobsters continued to die. At low temperatures, none of the dead lobsters was eaten by the survivors.

The pattern of cross-infection was interpreted by reference to the numbers of Gaffkya present in the water in the tanks. When dead injected lobsters were eaten, high counts were observed (mean 572 x 10^2/ml) probably as a result of the escape of infected lobster blood, but when no predation took place, the counts were usually low (mean of 83 and 74/ml in the "warm" and "cold" tanks respectively). Large numbers of Gaffkya were present in the water when cross-infection took place, and these bacteria were liberated into the water only after the physical damage of infected lobsters.

Storage in water at low temperature increased the resistance of infected lobsters, as can be seen from the relationship between the volume of broth injected and the length of time that lobsters lived afterwards. Although lobsters stored at low temperatures usually received larger volumes of broth than those stored at

Vol. of overnight broth injected (ml)	Days to death of lobsters held at °C	
	15 - 19	6 - 9
0.05	3	-
0.1	3,3,5,5	12
0.25	2,3,4,4	-
0.3	1	-
0.5	1,2	8,10,13
1.0	2,2,2,3	5,14

higher temperatures, their survival was on average nearly 4 times longer.

The practical implications of these experiments may be summarised as follows:-

(i) Large numbers of Gaffkya organisms must be present in water before infection can take place.

(ii) Damaged lobsters become infected more rapidly than those which show no apparent damage, but even the latter may become infected during prolonged exposure under adverse conditions.

(iii) Under the most adverse conditions (high temperatures and high densities of Gaffkya) death following cross-infection is not likely to take place in under 5 days.

(iv) In water at 6-9°C, cross-infection does not take place among apparently undamaged lobsters either because they are not susceptible, or because in the existing experiments, insufficient Gaffkya organisms were present, but severely damaged lobsters, in the presence of large numbers of Gaffkya, may become infected and die after 8 days.

(v) Artificially infected lobsters do not die in under 5 days when stored at 6-9°C.

(vi) If storage for periods longer than 5 days is required, the chances of deaths due to infection can be reduced by keeping the numbers of Gaffkya in water to a minimum by:- removal of dead infected lobsters; changing the water frequently; lowering the temperature so that dead lobsters are not attacked by others; handling and storing in such a way that lobsters are not mutilated. If it is intended to keep water for long periods without cooling, then water sterilisation equipment may be beneficial.

(c) The survey of natural stocks (Table 2). A total of 444 lobsters from several areas have been examined by blood culture into Stewart's media. Only one lobster has yielded a strain similar to Gaffkya homari, although 12 others were sufficiently similar to require further investigation. The continued absence of Gaffkya from these samples shows that this organism was not common in the British lobster stocks examined between 1962 and 1963.

(d) Studies of naturally occurring outbreaks. Only two cases of heavy mortalities in commercial lobster storage units were reported during the year and these were reported too late for investigation. Owing to the shortage of British lobsters, quantities of Canadian lobsters were imported, and of 3 consignments examined, 2 were shown to be heavily infected. One consignment of several hundred pounds was held satisfactorily at 10°C in a commercial storage unit, but of 19 which were removed to water at 17°C, 15 were overwhelmed with infection within 4 days. All lobsters had evidence of severe damage to the claw caused by the use of plugs. In addition to this enquiry, 371 preserved pleopods from 16 consignments of British lobsters from various sources, which died in commercial tanks were examined by nigrosine smears. None was positive for Gaffkya.

In view of the severity of the winter and the prolonged period of low water-temperatures followed by a cool summer, it is possible that the incidence of Gaffkya in natural stocks and in storage tanks may have been lower than in normal years.

(e) The possible control of numbers of Gaffkya in water held in storage tanks. Previous experiments have shown that mortalities due to cross-infection can best be controlled by storing lobsters at water temperatures between 6-9°C. However, where it is desired to store lobsters for 5 days or more in units having no refrigeration apparatus, and where there are infrequent changes of water, it may be possible to reduce mortalities due to cross-infection by means of water sterilisation equipment.

Whilst it is not possible to elaborate fully here, preliminary experiments have suggested that high Gaffkya concentrations in seawater in storage units may be controlled by simple ultra-violet equipment. Using data obtained from a pilot u/v plant similar to that devised for oyster purification (Wood 1961), it was calculated that in a tank of 1,000gal (4540 l) of seawater, in which the water is being recirculated at 1,000gal/hr (4540 l/hr) beneath a light source consisting of 5 lamps of 30 watts /total u/v output 36 watts/ a 99% reduction of the Gaffkya population is achieved in approximately 5 hours. Only practical tests can show whether this speed of destruction is rapid enough to prevent cross-infection of lobsters held under commercial conditions. In the present state of our knowledge

it is suggested that the reduction of water temperature to 6-9°C is a more suitable method of reducing both mortality and cross-infection, thus confirming the view of Roskam (1958).

References

Deibel, R. H. & Niven C. F. 1960. Comparative study of Gaffkya homari, Aerococcus viridans, tetrad forming cocci from meat curing brines and the genus Pediococcus. J.Bact., 79, 175.

Roskam, R. T. 1958. A short note on Gaffkaemia in lobsters. ICES. Shellfish Committee, No. 59.

Wood, P. C. 1961. The principles of water sterilisation by ultra-violet light, and their application in the purification of oysters. Fish.Invest.Lond.,Ser.2, 23, No. 6.

Wood, P. C. 1962. A preliminary note on Gaffkaemia investigations in England. ICES. Shellfish Committee, No. 31.

TABLE 1

Summary of results of cross-infection experiments 1963

Water Temp. °C	Treatment of lobsters		Totals	Died	Days exposed to Gaffkya at time of death	Gaffkya in water/ml.			
						No. of counts	Max.	Min.	Mean
15–19	All lobsters in direct contact	Injected with Gaffkya	10	10	1,1,2,2,2,2,2,3,3,3	8 +	2,325 (x 10^2)	1.6	572
		Not injected	13	10	5,5,7,7,11,12,12,12,22,25				
15–18	Injected lobsters in perforated tray	Injected with Gaffkya	7	7	1,3,3,4,4,5,5	3 ⁑	175	25	83
		Not injected	4	0	-				
6–9	All lobsters in direct contact	Injected with Gaffkya	6	6	5,8,10,12,13,14	11 z	250	16	74
		Not injected	5	0	-				

\+ Excluding 1 count of 42.5 x 10^5/ml.

⁑ Excluding 1 count of 172.5 x 10^3/ml.) These high counts probably the result of physical damage to dead lobsters.

z Excluding 1 count of 64 x 10^3/ml.)

TABLE 2

Incidence of Gaffkya in British Lobster Stocks. 1962-63

Origin of Lobsters	Number of consignments	Lobsters examined	Present	Absent	Suspicious +
Yorkshire	13	212	1	206	5
South Coast	6	102	0	99	3
South West Coast	1	6	0	6	0
Scotland	9	124	0	120	4
Totals	29	444	1	431	12

+ These strains sufficiently like Gaffkya homari to require further examination.