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THE IMPORTANCE OF BACTERIA IN LABORATORY EXPERIMENTS ON REARING THE LARVAE OF *OSTREA EDULIS* (L.)

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Observations made by the writer during the summer of 1955 suggested that the density of the bacterial flora which develops when oyster larvae are reared in small glass vessels is inversely correlated with the number of spat settling (Walne, 1956). From the nature of the observations it could not be determined whether the bacteria were affecting the development of the larvae or whether the properties of the sea water which resulted in a dense bacterial population were unfavourable to larval growth. This was clearly a matter which required further investigation. Investigators rearing marine larvae have commonly found that when a standard rearing technique is employed over a period, considerable differences occur in the proportion of different broods of larvae which grow to metamorphosis. There has been no satisfactory explanation for this phenomenon, although it has been assumed to be due to the presence or absence of minute quantities of various organic compounds in the water (Wilson, 1951; Loosanoff, 1954). The observations recorded in this paper suggest that a partial explanation could be that batches of sea water differ in the number of bacteria which they will support when confined in laboratory vessels, and these bacteria in turn affect the growth of the larvae.

EXPERIMENTAL METHOD

To test the hypothesis, it is necessary to rear larvae in an environment where the bacterial density is low, and to compare the number of larvae which settle as spat with that in control experiments where the normal bacterial population develops. Sea water can be freed of bacteria by various methods. Filtration is perhaps the most satisfactory as the chemical composition of the water is less likely to be altered than by methods which employ heat, but Wilson & Armstrong (1954) have shown that it is very difficult to collect large samples of bacteria-free water by this method. However, although experiments could be set up with bacteria-free water, the larvae could not be freed from bacteria. At the rearing temperature (20° C) marine bacteria divide frequently, and even if only a few are present initially a dense population soon develops. It was therefore decided to use antibiotics, since these would either partially or completely control the bacterial population, without removing

any substance from the sea water which might be required by the larvae. Oppenheimer (1955) has used antibiotics to good effect in hatching marine fish eggs. Some experiments in which lamellibranch larvae were grown for a time in the presence of antibiotics have been reported by Davis & Chanely (1956).

A series of experiments was made in 1956 in which the success of cultures of larvae grown under standard conditions was tested against identical experiments to which penicillin G had been added. The results were promising and a more searching series of experiments, which also included the use of streptomycin and chloromycetin, was made in 1957.

Broods of larvae were obtained from oysters kept in running sea water heated to 20–22° C and enriched with cultures of *Phaeodactylum*. The larvae were liberated naturally and were removed by filtering the water through bolting-silk. After washing in filtered sea water, the larval density was determined by counting samples of an even suspension of larvae. The appropriate volume of larval suspension was then dispensed into hard glass beakers, each containing 1 l. of sea water which had previously been passed through filter-candles to remove silt and the plankton, with the exception of a few bacteria. The average number of larvae added was 1300 per l. The larvae were fed on the flagellate *Isochrysis galbana* Parke, which had been cultured in standard 'Erdschreiber' medium (Gross, 1937). Sufficient was added to give an initial density of about 50 cells per mm^3. Further culture was added daily to keep the food density at about this figure.

In 1956 all experiments of a series stood together in the same water-bath, the temperature of which varied between 20 and 23° C. In 1957 the beakers stood on a bench in a laboratory, the controlled temperature of which varied between 21 and 22° C. All were gently stirred by air bubbling from a glass jet held near the bottom of the beaker. When eyed larvae were observed, spat collectors, in the form of clean mussel shells which had been strung on stainless-steel wire, were suspended in the beaker. When the spatfall was complete, generally 20–30 days after the beginning of the experiment, the total settlement on the mussel shells and the sides and bottom of the beaker was carefully counted. Samples of the larvae which had failed to metamorphose were measured.

The bacterial flora was studied at intervals by plating either 1·0 or 0·1 ml. samples on ZoBell's medium No. 2216 (ZoBell, 1946), and incubated at 22° C for 48 h. When the flora was dense, ten microscope fields were counted on each plate. The size of the field was adjusted where possible so as to count ten to twenty colonies per field. At very low densities (< 1000 per ml.) the colonies were counted directly on the whole plate. The low density populations (0–20,000 per ml.) have thus been estimated fairly accurately; the higher densities, because of the crowding of the colonies on the plates, less precisely.

RESULTS

In 1956 ten series of experiments were made, each series using a different brood of larvae. In each series two treatments were tested. A control treatment, set up with filtered sea water, larvae and food, was compared with a second treatment which was identical, except that sufficient of the sodium salt of penicillin G was added at the commencement to give a concentration of 50 i.u. per ml. In eight of the ten series of experiments made, the two treatments were duplicated. In 1957 a further five series of experiments were made. In the first two series the treatments were triplicated and in the other three quadruplicated. The concentration of penicillin G was selected by reference to the work of Cviic (1953) who showed that which would control many species of marine bacteria. Spencer (1952) had already shown that it was relatively harmless to *Isochrysis* at this concentration.

There was, as expected, a marked difference in the density of the bacterial flora between penicillin experiments and the controls (Table 1). In 1956 the bacterial flora was estimated at the end of the first 24 h and every other day thereafter. In the controls the mean density was about 19,000 per ml. after 24 h, but in the penicillin experiments the density was less than 1000 per ml. On the third day the mean density was 48,000 per ml. in the penicillin experiments, whereas in the controls it had declined slightly to about 16,000. This increase of the bacteria in the penicillin experiments may have been due either to the penicillin breaking down and losing its bacteriostatic properties, or to the development of penicillin-resistant bacteria in the experiments. By the fifth day the numbers in the two treatments were approximately equal.

In the 1957 experiments bacterial estimations were made when the experiments were first set up, but before the addition of any antibiotic, and then every other day (except for series 4 where the initial count was omitted). The water used generally contained a fairly high bacterial population, probably acquired in the day or so which it took to pass through the filtering unit (water was generally used the day after filtering). The average initial bacterial population was 24,000 per ml. in series 1, 2, 5; in series 3 it was abnormally high (230,000). The average of all five series of experiments on the second day was 75,000 in the control, and < 1000 in the penicillin experiments. Thereafter, the density of bacteria declined rapidly in the controls. In the penicillin experiments, the numbers rose a little but not so markedly as in 1956. A general picture of the changes in bacterial density in this type of experiment was given by series 5, 1957 (Table 1), where the bacterial flora was observed daily for the first 6 days. The initial population was fairly high (45,000). In the controls it rose to 88,000 on the first day, and thereafter declined by about half each day, reaching an average of about 2000 per ml. on the sixth day. The penicillin was added after 24 h; the effect was to reduce the bacterial population, which had had time to build up, to only about half that found in the controls.

Experiments were made in 1956 to see whether alterations in the dosage of the penicillin would control this outburst of bacteria. No success was obtained and no improvement in the growth and metamorphosis of the larvae was observed; in many cases the reverse was the case. Apparently many of the bacteria which developed were penicillin-resistant species or strains. Two types of experiments were tried. In one 50 units per ml. were added at the beginning, and a further 50 units every third day. In the second type of experiment 100 units were added at the beginning and a further 50 units every third day. In many experiments of both series very dense bacterial populations gradually developed.

TABLE 1. SUMMARY OF THE BACTERIAL POPULATION IN THE EXPERIMENTS OUTLINED IN TABLES 3 AND 4

(The bacteria are recorded to the nearest thousand per ml. The concentrations of antibiotic used are shown in Tables 3 and 4.)

Day ...		0	1	2	3	4	5	6	7
				1956					
Series 1	Control	—	240	—	5	—	43	—	46
	Penicillin	—	2	—	26	—	39	—	47
2	Control	—	—	60	—	8	—	56	—
	Penicillin	—	—	1	—	24	—	135	—
3	Control	—	84	—	—	23	—	11	—
	Penicillin	—	23	—	—	127	—	19	—
4	Control	—	15	—	14	—	8	—	13
	Penicillin	—	3	—	57	—	3	—	10
5	Control	—	7	—	6	—	5	—	5
	Penicillin	—	1	—	57	—	7	—	24
6	Control	—	7	—	12	—	7	—	—
	Penicillin	—	0	—	95	—	37	—	—
7	Control	—	2	—	24	—	11	—	—
	Penicillin	—	1	—	46	—	129	—	—
8	Control	—	1	—	18	—	16	—	—
	Penicillin	—	0	—	43	—	8	—	—
9	Control	—	—	—	49	—	4	—	9
	Penicillin	—	—	—	51	—	4	—	46
10	Control	—	3	—	6	—	11	—	—
	Penicillin	—	1	—	15	—	30	—	—
				1957					
Series 1	Control	5	—	118	—	6	—	6	—
	Penicillin	5	—	<1	—	1	—	1	—
2	Control	23	—	115	—	6	—	<1	—
	Penicillin	23	—	<1	—	1	—	2	—
3	Control	231	—	77	—	9	—	117	—
	Penicillin	231	—	<1	—	4	—	<1	—
Penicillin and streptomycin		231	—	0	—	0	—	0	—
4	Control	—	—	11	—	<1	—	4	—
	Penicillin	—	—	3	—	<1	—	8	—
Penicillin and streptomycin		—	—	0	—	0	—	0	—
5	Control	45	88	55	20	9	5	2	—
	Penicillin	45	61	23	5	7	4	3	—
Penicillin and streptomycin		45	18	0	0	0	0	0	—

From these experiments three types of data about the larvae are available —the proportion of the larvae which metamorphosed and settled as spat, the maximum rate of growth of the larvae, and the size to which those larvae which failed to metamorphose grew before dying. These three sets of data will be considered in turn.

The yield of spat from an experiment is of primary interest, since settlement indicates that the larvae have completed their development in the conditions offered. In all, the settlement of fifteen broods of larvae has been compared in control experiments with those to which penicillin had been added. The mean number of spat per litre obtained with each of these fifteen broods with the two treatments is compared in Table 2. It will be seen that, on the average, more spat was always recorded in those experiments to which penicillin had been added and the significance of this difference has been calculated. Because the differences are log-normally distributed, it is necessary to transform the data into logarithms. A 't' test on the transformed data shows that these differences are significant at the $P = > 0{\cdot}001$ level.

TABLE 2. MEAN NUMBERS OF SPAT OBTAINED IN EXPERIMENTS WITH PENICILLIN

(The experimental medium contained 50 i.u. of the sodium salt of penicillin G which the otherwise identical control lacked.)

	1956										1957				
Series ...	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5
Control	258	222	192	0	107	135	218	0	0	0	89	19	107	203	0
Penicillin	300	320	290	48	330	140	411	8	42	0	225	715	573	1155	45
Excess spat in penicillin experiment	42	98	98	48	223	5	193	8	42	0	136	604	466	952	45

Larvae were measured from time to time and their sizes noted. The mean size of the larvae in the different experiments cannot be calculated as it was not practicable to measure sufficiently large samples. The largest size present was, however, probably accurately estimated. There was no tendency for the larvae to be larger in the penicillin experiments than in the controls on the same day. This conclusion applies, of course, only to those controls which were not unduly affected by bacteria. A good example is given by the experiments in series 4, 1957 (Table 4). The mean sizes of about forty larvae measured on the fifth and eighth days are shown below:

Day ...	0	5	8
Control (μ)	183	217	235
Penicillin (μ)	183	217	241
Penicillin/streptomycin (μ)	183	218	243

Growth was slow in this series but the yield of spat from those treated with antibiotics was very good (Table 3). If the bacterial population becomes very dense, then the larvae will eventually stop growing.

An examination of the sizes (Tables 3 and 4) to which those larvae which failed to metamorphose grew before dying shows that in many cases the whole larval population was affected by the addition of penicillin. For conciseness, the sizes at which larvae died have been aggregated into four groups corresponding to the principal stages in larval development:

Group	μ	
1	160–200	Size at liberation, little or no growth
2	210–250	Substantial growth, D-shape of freshly liberated larvae lost
3	260–300	Size at which the anatomical changes which presage metamorphosis take place, including development of eye spots. Many larvae settle at this size
4	>300	Mature larvae. Most larvae settle before reaching this size

The significance of the differences in the proportions of larvae which grew into each size-group can be tested for each series of experiments by reference to the fourfold contingency tables published by Mainland, Herrera & Sutcliffe (1956). In one series (series 10, 1956), no larva grew out of the first size-group; these larvae were probably abnormal and can be disregarded. The difference in the proportion of larvae which grew into the 210–250 μ group between the penicillin experiments and the controls is significant ($P=0{\cdot}01$) in six of the eleven series of experiments with which we are concerned (series 4, 8 and 9, 1956, and series 1, 2, and 5, 1957). In all cases where a difference occurred, the larvae were larger when treated with penicillin. In the next size-group the differences are again significant ($P=0{\cdot}01$) in six of the eleven series of experiments (series 1, 4, 7, 8 and 9, 1956, and series 2, 1957). In one case (series 7, 1956), the larvae were larger in the control, but here many more larvae settled as spat in the penicillin experiments which probably reduced the proportion of larvae dying in the larger size-groups. The numbers in the fourth group, 300 μ, were generally small and considerably influenced by the number settling as spat.

As the larvae in the penicillin experiments were not observed to grow more rapidly than in the controls, the larger size at death of the larvae in many of the penicillin experiments must have been due to the larvae living longer.

In 1957 some experiments were made using streptomycin and chloromycetin. Cviic (1953) has shown that a mixture of penicillin and streptomycin is more effective for controlling marine bacteria than either antibiotic on its own. In series 1 and 2 (Table 4) a mixture of 50 units of penicillin G and 0·25 mg streptomycin sulphate per ml. was tried. This completely suppressed the bacteria for over 9 days but the effect on the larvae was variable. In the first series none of the larvae in the three replicates grew to more than 250 μ and they speedily died, which suggested that the streptomycin was harmful. In the second series, the larvae were killed by mistake in one experiment when they had grown to 280 μ. In the replicate there were 395 spat. The larvae, however, behaved abnormally in the early stages of the experiment. Their ciliary activity was extremely rapid and they did not close up as rapidly as

TABLE 3. SUMMARY OF THE RESULTS OF TEN SERIES OF EXPERIMENTS (1956) TESTING THE EFFECT OF ADDING 50 I.U. OF Na PENICILLIN G TO CULTURES OF OYSTER LARVAE

(C = control experiments. P = experiments to which penicillin was added.)

Series	Origin of parent oyster	Date started	No. larvae per litre	Type of expts.	No. of expts.	% of larvae which achieved each size group (μ) 210–250	260–300	>300	No. of spat
1	Isle of Lewis	19. iii. 56	1500	C	2	89	65	25	175, 340
				P	2	95	91	44	556, 43
2	1954, Brittany	4. iv. 56	1300	C	1	—	—	—	222
				P	1	—	—	—	320
3	Isle of Lewis	9. iv. 56	1600	C	1	—	—	—	192
				P	1	—	—	—	290
4	Brittany	3. vii. 56	1200	C	2	84	35	1	0, 0
				P	2	100	89	6	50, 45
5	1954, Brittany	3. vii. 56	1400	C	2	98	74	0	125, 89
				P	2	98	81	21	355, 305
6	River Colne	7. vii. 56	1500	C	2	93	73	16	75, 195
				P	2	91	58	12	230, 50
7	River Colne	7. vii. 56	1700	C	2	93	70	2	365, 70
				P	2	85	40	0	425, 397
8	1954, Brittany	7. vii. 56	1050	C	2	79	4	0	0, 0
				P	2	100	75	14	5, 10
9	River Colne	12. vii. 56	1300	C	2	80	12	0	0, 0
				P	2	96	32	0	8, 75
10	Brittany	5. viii. 56	1400	C	2	0	0	0	0, 0
				P	2	0	0	0	0, 0

TABLE 4. SUMMARY OF THE RESULTS OF THE 1957 SERIES OF EXPERIMENTS

(The parent stock was always from the river Colne. In series 5 the penicillin was not added until the experiment had been running for 24 h.)

Series	Date started	No. larvae per litre	Antibiotics added per ml. Penicillin G (i.u.)	Streptomycin (mg)	Chloromycetin (mg)	No. of expts.	% larvae which achieved each size group (μ) 210–250	250–300	>300	No. of spat
1	27. iii. 57	1100	0	0	0	3	86	26	—	111, 73, 82
			50	0	0	3	94	42	—	292, 61, 323
			50	0·25	0	3	28	—	—	0, 0, 0
2	29. iii. 57	1300	0	0	0	3	78	8	—	0, 0, 58
			50	0	0	3	93	45	—	809, 798, 538
			50	0·25	0	2	69	8	—	0,* 395
3	1. iv. 57	960	0	0	0	4	68	12	—	230, 2, 197, 0
			50	0	0	4	Not recorded			612, 629, 332, 720
			50	0·05	0	4	Not recorded			545, 282, 387, 394
4	3. iv. 57	1400	0	0	0	4	96	51	—	749, 14, 29, 22
			50	0	0	4	91	58	—	1091, 1211, 1137, 1182
			50	0·05	0	4	Not recorded			1259, 992, 1292, 1082
5	7. v. 57	1400	0	0	0	4	65	1	—	0, 0, 0, 0
			50	0	0	4	97	9	—	0, 0, 0, 179
			50	0·05	0	4	95	31	—	752, 183, 404, 74
			0	0·05	0	4	51	—	—	0, 0, 0, 0
			0	0	0·025	4	98	28	—	0, 0, 0, 0

* This experiment was terminated early by accident.

usual. This behaviour seemed to be characteristic of experiments to which streptomycin had been added. In the next three series of experiments a mixture of 50 units penicillin G and 0·05 mg streptomycin sulphate per ml. was tried. Once again the bacteria were completely suppressed for at least 9 days. In two of the three series of experiments (3 and 4) the larvae did well, much better than the controls, but not significantly different from those to which only penicillin G had been added. In series 5 the result from the penicillin/streptomycin mixture was much better than in the control, or in that to which penicillin alone had been added, both in respect of the number of spat produced and in the size reached by the larvae before death. It seems probable that when the bacterial flora is very vigorous, or perhaps when certain species are present, then the penicillin/streptomycin mixture will give better results than penicillin alone.

One experiment was tried using chloromycetin; this is a wide spectrum antibiotic. The larvae grew well until there was an outburst of bacteria apparently resistant to this antibiotic, when they died. Chloromycetin may not, therefore, be as useful as the penicillin/streptomycin mixture and, as it costs at least two-thirds as much again, it may not have a place in large-scale rearing of larvae.

DISCUSSION

The proportion of larvae which develops to metamorphosis differs, under standard conditions, in different broods. An example of this is shown by the results of series 6, 7 and 8, 1956. These experiments were all started on the same day with the same batch of water, but the three broods differed considerably in the proportion of larvae which metamorphosed. One of the factors affecting their survival is described in this paper, where it is demonstrated that the bacteria which develop when sea water is confined in the laboratory are sufficient to arrest the growth and development of otherwise healthy larvae. The almost complete suppression of bacteria by the penicillin/streptomycin mixture does not, however, make all larvae do equally well. Other factors in the larvae, and perhaps in the water itself, are still at work. At one time the writer thought that the glycogen food reserves at the time of liberation might be of importance, but the investigations of Collyer (1957) showed that there was little difference between various batches of larvae examined.

The way in which the larvae and bacteria interact is unknown but there seem to be two possibilities: either certain bacteria may cause a disease in the larvae, or some or all of the normal bacterial constituents of the sea water may irritate the larvae either directly by settling and multiplying on the shell or flesh, or indirectly by the secretion of 'external metabolites' into the water.

Bacteria are sparse in the sea because, amongst other factors, of the relative absence of solid surfaces. A surface immersed in the sea, unless protected in

some way, speedily becomes covered by bacteria. It has been suggested that because of the active physical properties of solid surfaces it is only at a surface that the dissolved organic matter, which is present in sea water in only very small quantities, reaches a high enough concentration to support a vigorous population (ZoBell, 1943). When the water is confined in a small vessel the area of surface becomes relatively large and the bacteria become very abundant. The smaller the containers the larger the bacterial population (ZoBell & Anderson, 1936). It is probable that many bacteria are associated with the larva both on the shell and perhaps on the surface of the living tissue. If the bacteria on the larvae are the important ones then this would give a mechanism whereby bacterial effects would be produced in the sea.

It can be seen from the results given in this paper and elsewhere (Walne, 1956) that batches of water differ considerably in the bacterial population which they will support when kept under standard conditions in the laboratory. If these differences are reflected in the bacterial population on the larvae in the field, then this could be a factor influencing the successful growth and development of marine larvae in the sea.

Whatever the mechanism, the results show a way by which the performance of a given batch of sea water can be improved in the laboratory. A number of workers in recent years have suspected that the dissolved organic matter in sea water plays an important part in the activity of marine animals. The observations of Collier, Ray, Magnitzky & Bell (1953) on the filtration of *Crassostrea* and of Loosanoff (1954) on rearing the larvae of *Crassostrea* and other species of lamellibranchs, may be cited. Of particular relevance are the papers of Wilson (1951) and Wilson & Armstrong (1952, 1954), in which the growth of *Echinus* larvae in waters of different origins are compared. If the results obtained by Wilson are viewed in the light of experiments reported in this paper, it seems possible that the bacterial floras, which waters from different areas will support, provide an explanation for Wilson's results. The flora will, in turn, depend on the nature and quantity of the organic compounds present in the water; little is known about these, but it is certain that they will vary considerably.

SUMMARY

Preliminary experiments had suggested that the extent of the development of the bacterial flora which develops when sea water is confined in small vessels is an important factor in the laboratory culture of oyster larvae. In the experiments reported in this paper the growth and settlement of oyster larvae was compared in controls of normal sea water with those in which the bacterial flora was controlled with antibiotics.

A concentration of 50 i.u. of the sodium salt of penicillin G per ml. suppressed bacterial growth for at least 2 days and, in a series of experiments using fifteen different broods of larvae, significantly ($P > 0{\cdot}001$) more spat

were obtained in those to which penicillin had been added than in the controls.

A mixture of 50 units of penicillin G and 0·05 mg streptomycin sulphate was tried in three series of experiments. This completely suppressed the development of bacteria for at least nine days. In all three series many more spat were obtained than in the controls, but in only one series was there more spat than in the comparable penicillin experiment. It is suggested that when the bacterial population is very vigorous, or perhaps when certain species are present, this mixture will be more useful than penicillin alone. Experiments with other concentrations of penicillin, and with streptomycin and chloromycetin alone were not successful.

REFERENCES

COLLIER, A., RAY, S. M., MAGNITZKY, A. & BELL, J. O., 1953. Effect of dissolved organic substances on oysters. *Fish. Bull. U.S.*, Vol. 54, No. 84.

COLLYER, D., 1957. Viability and glycogen reserves in the newly liberated larvae of *Ostrea edulis* L. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 335–7.

CVIIC, V., 1953. The bactericidal and bacteriostatical action of antibiotics on marine bacteria. 1. Penicillin and streptomycin. *Acta adriat.*, Vol. 5, No. 7, pp. 135–66.

DAVIS, H. C. & CHANLEY, P. E., 1956. Effects of some dissolved substances on bivalve larvae. *Proc. nat. Shellfish. Ass.*, Vol. 46, pp. 59–74.

GROSS, F., 1937. Notes on the culture of some marine plankton organisms. *J. mar. biol. Ass. U.K.*, Vol. 21, pp. 753–68.

LOOSANOFF, V. L., 1954. New advances in the study of bivalve larvae. *Amer. Scient.*, Vol. 42, No. 4, pp. 607–24.

MAINLAND, D., HERRERA, L. & SUTCLIFFE, M. I., 1956. *Tables for Use with Binomial Samples*. New York.

OPPENHEIMER, C. H., 1955. The effect of marine bacteria on the development and hatching of pelagic fish eggs, and the control of such bacteria by antibiotics. *Copeia*, Vol. 1, pp. 43–9.

SPENCER, C. P., 1952. On the use of antibiotics for isolating bacteria-free cultures of marine phytoplankton organisms. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 97–106.

WALNE, P. R., 1956. Bacteria in experiments on rearing oyster larvae. *Nature, Lond.*, Vol. 178, p. 91.

WILSON, D. P., 1951. A biological difference between natural sea waters. *J. mar. biol. Ass. U.K.*, Vol. 30, pp. 1–26.

WILSON, D. P. & ARMSTRONG, F. A. J., 1952. Further experiments on biological differences between natural sea waters. *J. mar. biol. Ass. U.K.*, Vol. 31, pp. 335–49.

—— —— 1954. Biological differences between sea waters: experiments in 1953. *J. mar. biol. Ass. U.K.*, Vol. 33, pp. 347–60.

ZOBELL, C. E., 1943. The effect of solid surfaces upon bacterial activity. *J. Bact.*, Vol. 46, pp. 39–56.

—— 1946. *Marine Microbiology*. Waltham, Mass.

ZOBELL, C. E. & ANDERSON, D. Q., 1936. Observations on the multiplication of bacteria in different volumes of stored sea water. *Biol. Bull., Woods Hole*, Vol. 71, pp. 324–42.