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Studies on the influence of plankton on antibacterial activity of sea water

K. Moebus

Biologische Anstalt Helgoland (Meeresstation); Helgoland, Federal Republic of Germany

KURZFASSUNG: **Untersuchungen über den Einfluß von Plankton auf die antibakterielle Aktivität von Meerwasser.** Mit *Escherichia coli, Staphylococcus aureus* und *Serratia marinorubra* wurde der Einfluß erhöhten und verminderten Planktongehaltes auf die bakterizide Wirkung von Meerwasser geprüft. *S. aureus* war der einzige Teststamm, dessen Inaktivation in mit Plankton angereichertem Meerwasser während einiger Experimente deutlich rascher erfolgte als in unbehandelten Parallelproben. Andererseits war dessen Inaktivation in Meerwasser mit stark reduziertem Planktongehalt während derselben Experimente nicht oder kaum geringer als in unbehandeltem Meerwasser. In anderen Versuchen wurde bei diesem Organismus wie auch bei den zwei anderen Teststämmen beobachtet, daß die bakterizide Wirkung von rohem Meerwasser durch Anreicherung mit Plankton entweder nicht signifikant verändert oder aber beträchtlich vermindert wurde. Der für das Überleben der Testbakterien förderliche Einfluß des Planktons war jeweils dann am stärksten, wenn die antibakterielle Aktivität unbehandelten Meerwassers extrem hoch war. Aus den vorliegenden Ergebnissen wird geschlossen, daß mit der Anreicherung von Plankton keine Vermehrung wirksamer bakterizider Substanz, wohl aber eine Erhöhung der Nährstoffkonzentration im jeweiligen Ansatz verbunden war. Auf der Grundlage kürzlich veröffentlichter Befunde über den Einfluß organischer Nährstoffe auf die antibakterielle Aktivität von Meerwasser werden die dargestellten Ergebnisse diskutiert.

INTRODUCTION

The antibacterial activity of sea water is attributed, at least in part, to bactericidal compounds produced by marine organisms among which planktonic algae probably are most important. Numerous phytoplanktonic species are known to form inhibitory matter under natural and/or laboratory conditions. The literature concerning this subject was summarized by Sieburth (1968) and Aubert et al. (1968), and recent information was presented by Aubert & Pesando (1969), Gauthier (1969) and Aubert & Joiris (1971). The importance of planktonic algae was also displayed by the observation of seasonal changes in bactericidal capacity of sea water which were correlated with the life cycles of phytoplankton communities (Sieburth & Pratt 1962, Moebus 1972a).

Generally, untreated sea water is more active against the test organisms used than corresponding sterilized samples (de Gaixa 1889, ZoBell 1936, Vaccaro et al. 1950,

CARLUCCI et al. 1961 and others). Heating of sea water is thought to destroy inhibitory compounds and to result in increased nutrient concentration from killed organisms, thereby causing decreased bactericidal efficacy. Regarding filter-sterilization, retention of dead particles loaded with bactericides, and of viable organisms capable to excrete harmful substances, is assumed to be the reason for reduced antibacterial activity of the filtrate. If so, one should be able to increase the bactericidal capacity of raw sea water by enrichment with living and dead matter present in the sea. This assumption was examined in an investigation reported in this paper.

MATERIALS AND METHODS

Basic materials and methods used were the same as recently specified by MOEBUS (1972a). Sea water always was sampled at station "Kabeltonne" in the channel between the two islands of Helgoland (North Sea). The following procedures were employed for enrichment of sea water with particulate matter:

Enrichment with filter sludge: Two 500 ml portions of raw sea water *(rsw)* were filtered through seperate membrane filters of 0.15 μ mean porosity. The filter sludge of both filter disks was scrubbed off by a sterile loop in 10 ml *rsw* which were added to 100 ml *rsw* to give 10-fold enriched *rsw* ($ersw_{10}$).

Enrichment with viable plankton: Trovidur tubing, about 12 cm in length and 5 cm in diameter, was covered with 10 μ mesh Nylon gauze at one end. This device, stored in ethanol (70 %) for sterilization and rinsed with sterile distilled water before use, was dipped into *rsw* with the gauze in front. Sea water passing the gauze *(10 μ-rsw)* was sucked off by means of a pump until the volume of *rsw* was reduced to $^1/_{20}$ ($ersw_{20}$). The enrichment procedure was finished after 30 to 60 min and warranted viability of even rather sensitive planktonic organisms. $ersw_{20}$ war carefully handled by means of pipettes with open mouth.

Sterile filtrates were prepared at 1 to 2 mm Hg vacuum from each 100 ml *rsw* and *10 μ-rsw* (*fsw* and *10 μ-fsw*, respectively) as well as from 50 ml $ersw_{20}$ ($efsw_{20}$).

Inactivation and growth of bacteria are presented as log (N_x-N_0), where N is the number of colony formers/ml present in the samples after o and x days of incubation at 25° C in the dark, respectively.

RESULTS

Effects of filter sludge

Five experiments of this type were performed during May to July 1970 (started on May 29th, June 5th, 12th, 19th, and July 6th, respectively). Samples of 25 ml *rsw* and $ersw_{10}$ were inoculated with 1 ml of test bacterial suspensions (in saline) to attein initial titers of 10^8 cells/ml of *Escherichia coli*, 2×10^7 cells/ml of *Staphylococcus aureus*, and 10^7 cells/ml of *Serratia marinorubra*, respectively. The results obtained after 3 days of incubation are shown in Figure 1. No significant differences between

the bactericidal capacities of *rsw* and *ersw*$_{10}$ were found in regard to *E. coli* (Fig. 1, section A). The inactivation of *S. marinorubra* (section C) in *ersw*$_{10}$ was either the same as or weaker than in *rsw*. Concerning anti-staphylococcal activity (section B) of *rsw*

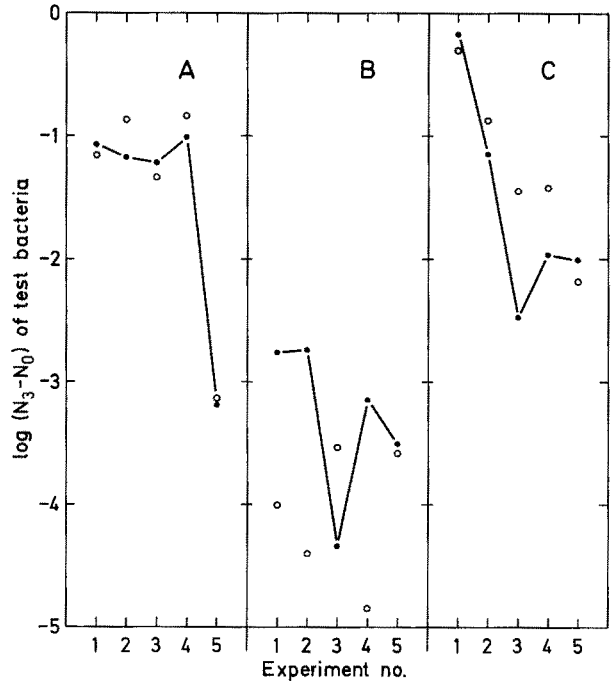

Fig. 1: *Escherichia coli, Staphylococcus aureus* and *Serratia marinorubra* (sections A, B and C, respectively). Inactivation of test bacteria in untreated raw sea water (*rsw*, ●——●), and in *rsw* enriched with filter sludge (*ersw*$_{10}$, o) as observed after 3 days of incubation at 25° C in the dark of 26 ml samples in 200 ml culture flasks. Initial titers of test bacteria were about 10^8 cells/ml of *E. coli*, 2×10^7 cells/ml of *S. aureus* and 10^7 cells/ml of *S. marinorubra*, respectively

and *ersw*$_{10}$, great differences were observed during 4 experiments. Three times, the inactivation of *S. aureus* was considerably stronger in *ersw*$_{10}$ than in *rsw*, in each case correlated with relatively weak inactivation in *rsw*. Once the opposite occurred.

The last-mentioned finding is of special interest. The experiment in question (exp. 3/70) was started on June 12th, one day after the change from a 2-week period of bright sunshine to cloudy and windy weather. The viability of phytoplankton was drastically reduced from one day to the other (DREBES, personal communication). At the same time, an extreme increase in anti-staphylococcal activity of *rsw* was observed: log (N_3-N_0) values obtained during this investigation period were — 3.27 (June 5th), — 2.18 (June 8th), — 5.67 (June 12th), — 5.94 (June 15th), and — 4.86 (June 19th). Dates presented in parenthesis refer to the starts of experiments performed during a 2-year investigation of seasonal changes in antibacterial activity (MOEBUS 1972a). The increased anti-staphylococcal activity of *rsw* might be thought to be due to intensified

excretion of bactericides by planktonic algae present at that time – mainly *Eucampia zoodiacus*, *Nitzschia seriata* and *Skeletonema costatum*. The two last-mentioned species really are known to produce compounds inhibitory for *Staphylococcus aureus* under laboratory conditions (AUBERT et al. 1968). However, there are some findings which throw doubts on this interpretation.

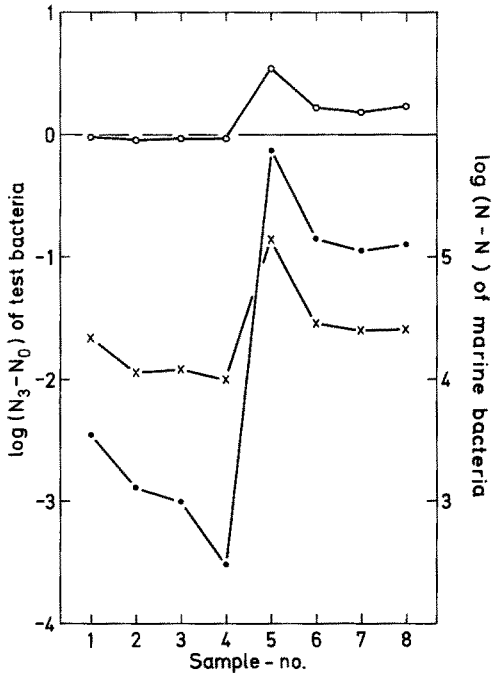

Fig. 2: *Staphylococcus aureus*, *Serratia marinorubra* and marine bacteria. Inactivation or growth of *S. aureus* (●——●) and *S. marinorubra* (o——o) as well as of a mixed population of marine bacteria (×——×) as influenced by matter extracted from filter sludge by sterile filtration. Findings obtained after 3 days of incubation at 25° C in the dark are shown. (For detailed information see text)

As shown in Figure 1 (exp. 3, sections B and C), the increased antibacterial activity of *rsw* against *Staphylococcus aureus* and *Serratia marinorubra* was reduced by addition of filter sludge. This probably occurred as a consequence of increased nutrient concentration in $ersw_{10}$, as indicated by the results of an experiment started on June 15th and performed as follows (Fig. 2): 30 ml *rsw* were sterile-filtered to obtain sample 1. 2 further portions of 250 ml *rsw* were sterile-filtered with the same device, each till dryness of the filter disk (samples 2 and 3), without discarding the *fsw* after withdrawal of sample 2. Sample 4 was prepared by sterile-filtration of 500 ml *rsw* with a separate filtering device. Dryness of the filter was avoided until the end of this procedure. Then the filter sludge from both filter disks was scrubbed off in 10 ml *fsw* of sample 4. The suspension obtained was added to further 20 ml *fsw* of sample 4 and subsequently filter-sterilized in a third filtering apparatus (sample 5). Thereupon,

80 ml *fsw* of sample 4 were sucked through the filter sludge and 20 ml of filtrate (sample 6) were withdrawn. With the remaining filtrate, this procedure was repeated twice to obtain samples 7 and 8. Three 5.4 ml subsamples of the 8 sterile filtrates were

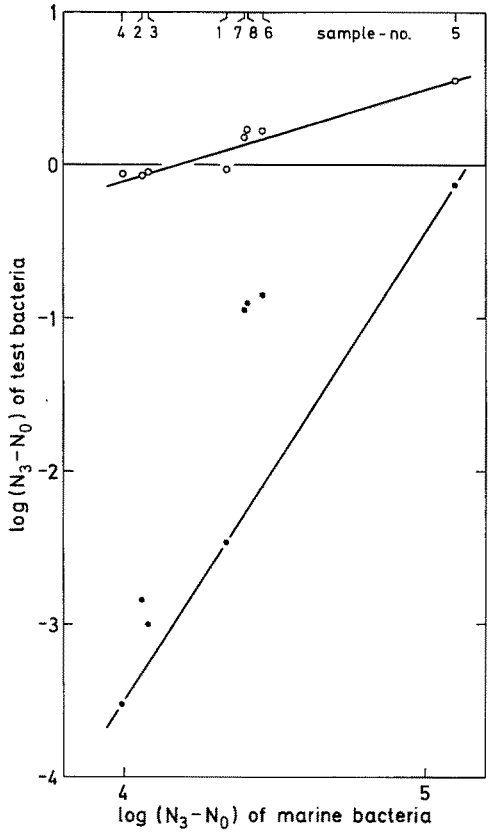

Fig. 3: *Staphylococcus aureus* and *Serratia marinorubra*. Relationships between inactivation or growth of test bacteria and growth of a mixed population of marine bacteria, respectively, in filter-sterilized sea water samples. *S. aureus:* ●——●, *S. marinorubra:* o——o. Same findings as shown in Figure 3. (For further information see text)

dispensed into culture tubes. Two series of subsamples were inoculated with *S. aureus* and *S. marinorubra*, respectively, to about 10^7 cells/ml. The third series was inoculated with a mixed culture of marine bacteria, obtained by storage of *rsw* for 3 days at 25° C in the dark. The number of colony-forming marine bacteria thereby attained was about 10^2/ml. Final volume was 6 ml in each case.

The results of this experiment are presented in Figure 2. Obviously, samples 5 to 8 were enriched with nutrients from the filter sludge as indicated by growth of marine bacteria and even of *Serratia marinorubra*. The decreased inactivation of *Staphylococcus aureus* in these samples certainly also was due to nutrient enrichment. Therefrom

the question arises, whether the observations made from samples 1 to 4 are explainable also on the basis of nutrient concentration. To obtain further information, growth of marine bacteria was plotted versus inactivation or growth of test bacteria, as shown in Figure 3. The straight lines, drawn between extreme values of log (N_3-N_0) found in regard to test bacteria, represent linear relations between the findings concerning both groups of bacteria. Although growth of marine bacteria only can be taken as a rough measure of nutrient concentration, the results shown in Figure 3 support the assumption that inactivation of test bacteria in samples 1 to 4 was caused by lower concentrations of available nutrients.

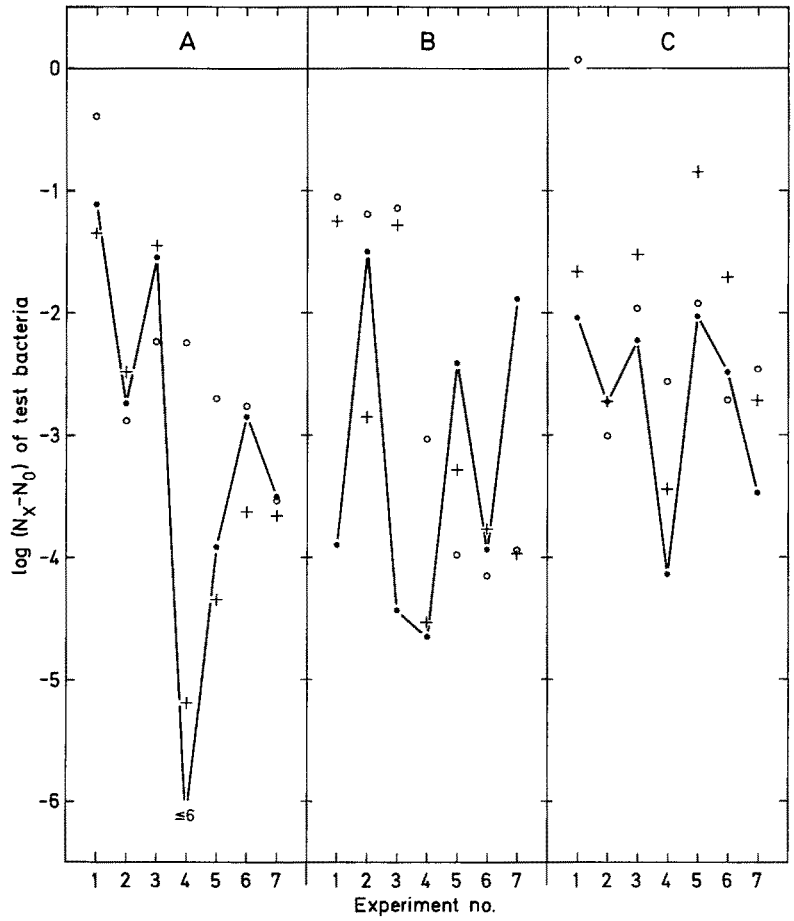

Fig. 4: *Escherichia coli, Staphylococcus aureus* and *Serratia marinorubra* (sections *A, B* and *C*, respectively). Bactericidal capacities of untreated raw sea water (*rsw*, ●——●), *rsw* enriched with viable plankton (*ersw*$_{20}$, o) and *rsw* of greatly reduced plankton content (10 μ–rsw, +), respectively, established after incubation at 25° C in the dark for 2 days *(S. aureus)* or 3 days *(E. coli, S. marinorubra)*. About 10^7 cells/ml of respective test bacteria were initially present in 10 ml samples filled in culture tubes (18 mm ∅)

Effects of viable plankton

In 1971, the experimental procedure was changed to avoid destruction of planktonic organisms and enrichment of marine bacteria. The tests were performed in culture tubes (18 mm in diameter) each containing 10.0 or 9.5 ml of sea water to be tested. In the latter case, 0.5 ml of test bacterial suspension (in *fsw*) were inoculated to attain initial titers of about 10^7 cells/ml.

During July to September 1971, seven experiments were conducted. The results concerning inactivation of test bacteria in the various types of raw and filter-sterilized sea water are summarized in Tables 1, 2 and 3. In Figure 4 with sections A, B and C

Table 1

Escherichia coli. Survival in different types of raw and filter-sterilized sea water, presented as log (N_x–N_0) values established after incubation of about 10^7 cells/ml at 25° C in the dark for x days. Explanations: *rsw* = untreated raw sea water, *10μ–rsw* = *rsw* filtered through 10μ mesh gauze, $ersw_{20}$ = *rsw* 20-fold enriched with viable plankton retained by 10μ mesh gauze; *fsw*, *10μ–fsw* and $efsw_{20}$ = sterile filtrates prepared from corresponding raw samples; n. d. = not determined

No. of experiment	Duration (days)	log (N_x–N_0) found from					
		rsw	*10μ–rsw*	$ersw_{20}$	*fsw*	*10μ–fsw*	$efsw_{20}$
1/71	1	— 1.84	— 1.74	— 1.89	— 1.63	≥ 1.27	— 1.70
	2	— 1.43	— 1.32	— 1.87	— 1.11	— 1.21	— 1.63
	3	— 2.89	— 2.66	— 1.61	— 2.46	— 1.00	— 1.56
	4	— 3.83	— 5.60	— 2.69	— 3.63	— 2.59	— 1.08
2/71	1	— 1.79	— 1.72	— 1.69	— 1.55	— 1.65	— 1.16
	2	— 1.16	— 1.18	— 2.32	— 2.17	— 1.26	— 2.75
	3	— 3.27	— 3.52	— 3.12	— 3.03	— 2.59	— 2.12
	4	— 5.49	— 4.00	— 5.54	— 5.52	— 3.71	— 3.36
3/71	1	— 1.72	— 1.62	— 1.77	— 1.49	— 1.39	— 1.50
	2	— 1.00	— 1.40	— 1.17	— 1.10	— 1.04	— 1.15
	3	— 2.46	— 2.55	— 3.77	— 2.53	— 2.48	— 2.77
	4	— 3.59	— 3.20	— 4.14	— 3.97	— 2.04	— 2.19
4/71	1	— 1.18	— 1.30	— 1.64	— 4.92	— 3.84	— 1.49
	2	— 4.04	— 2.04	— 2.99	≤ 6	— 5.10	— 2.60
	3	≤ 6	— 6.80	— 3.76		≤ 6	— 3.36
	4		n.d.	— 5.57			— 5.98
5/71	1	— 1.54	— 1.43	— 1.58	— 1.49	— 1.60	— 1.17
	2	— 2.41	— 2.37	— 2.92	— 2.10	— 1.07	— 2.70
	3	— 4.08	— 5.65	— 3.30	— 4.54	— 2.66	— 3.88
	4	≤ 6	— 6.87	— 5.74	— 5.30	— 2.06	— 3.13
6/71	1	— 1.67	— 1.62	— 1.61	— 1.66	— 1.13	— 1.62
	2	— 1.02	— 2.59	— 1.14	— 2.90	— 3.99	— 1.06
	3	— 3.15	— 4.38	— 3.24	— 2.32	— 4.96	— 2.48
	4	≤ 6	≤ 6	— 4.24	— 3.52	— 5.98	— 3.87
7/71	1	— 1.73	— 1.65	— 1.69	— 1.77	— 2.01	— 1.66
	2	— 2.95	— 2.65	— 2.87	— 2.96	— 5.63	— 1.04
	3	— 4.50	— 4.34	— 4.47	— 4.75	≤ 6	— 4.83
	4	≤ 6	≤ 6	≤ 6	— 6.60		— 5.83

referring to *Escherichia coli, Staphylococcus aureus* and *Serratia marinorubra,* respectively, findings obtained from *rsw, 10 μ-rsw* and $ersw_{20}$ are demonstrated. (Note: Regarding *S. aureus,* findings ascertained after only 2 days of incubation are shown.) As found by enrichment with filter sludge, *S. aureus* was the only test strain, the inactivation of which sometimes was stronger in $ersw_{20}$ than in *rsw.* The opposite, however, was observed for each test strain during several experiments. Regarding *10 μ-rsw,* the expected decrease in antibacterial activity rather regularly was found only for *S. marinorubra.* Some observations are of special interest.

Experiment 1/71 was performed with a sea-water sample rich in phyto- and zooplankton, the latter mainly consisting of a protozoon (1 to 2 organisms/ml) which dies within about 2 hours of incubation at 25° C. Most abundant planktonic algae were *Chaetoceros socialis* and *Rhizosolenia delicatula* (each up to 10^5 cells/l). *Eucampia zoodiacus, Cerataulina bergonii, Chaetoceros debilis* and *Thalassiosira rotula* were persent in numbers up to 10^4 cells/l. Among these algal species *C. socialis* is known to produce anti-staphylococcal substance(s) (AUBERT et al. 1968). As can be seen from

Table 2

Staphylococcus aureus. Survival in different types of raw and filter-sterilized sea water. (For further information see Table 1)

No. of experiment	Duration (days)	log (N_x–N_0) found from					
		rsw	*10μ–rsw*	$ersw_{20}$	*fsw*	*10μ–fsw*	$efsw_{20}$
1/71	1	— 1.75	— 1.92	+ 0.06	— 1.37	— 1.87	— 1.93
	2	— 4.11	— 2.75	— 2.96	— 2.20	— 1.19	— 1.49
	3	— 5.02	— 6.87	— 5.86	— 4.75	— 2.29	— 2.77
2/71	1	— 1.80	— 1.76	— 1.80	— 1.41	— 1.46	— 1.54
	2	— 2.50	— 3.15	— 2.81	— 4.45	— 3.22	— 2.32
	3	— 5.41	— 5.58	— 5.77	≤ 6	— 5.54	— 4.97
3/71	1	— 1.66	— 1.85	— 1.87	— 1.11	— 1.74	— 1.59
	2	— 5.57	— 2.72	— 2.86	— 4.31	— 2.40	— 2.32
	3	— 6.45	— 4.55	— 5.78	— 6.75	— 3.06	— 4.63
4/71	1	— 1.80	— 1.21	— 1.99	— 2.80	— 4.37	— 1.63
	2	— 5.35	— 5.47	— 4.97	— 4.47	≤ 6	— 3.18
	3	≤ 6	≤ 6	— 6.77	≤ 6		≤ 6
5/71	1	— 1.77	— 1.39	— 1.77	— 1.94	— 1.84	— 1.75
	2	— 3.59	— 4.72	— 4.02	— 2.64	— 2.04	— 1.13
	3	— 5.36	≈ 6.0	— 5.06	— 3.59	— 4.45	— 2.23
	4	— 6.92	n.d.	— 6.86	— 4.04	≤ 6	— 5.84
6/71	1	— 2.86	— 1.60	— 1.75	— 3.95	— 1.90	— 1.74
	2	— 4.07	— 4.23	— 5.85	— 4.68	— 1.65	— 2.79
	3	— 5.08	≤ 6	— 6.83	— 5.64	— 2.83	— 2.00
	4	≤ 6		≤ 6	≤ 6	— 4.31	— 4.49
7/71	1	— 1.79	— 2.81	— 1.86	— 1.12	— 2.35	— 2.74
	2	— 2.12	— 4.03	— 4.06	— 3.51	— 3.46	— 3.67
	3	— 6.85	≤ 6	— 6.70	— 4.42	— 4.59	— 5.29
	4	n.d.		n.d.	— 5.45	— 5.68	≤ 6

Figure 4, enrichment of viable plankton in $ersw_{20}$ greatly reduced the antibacterial activity of *rsw*. *Serratia marinorubra* was able to grow even in $ersw_{20}$ (and in $efsw_{20}$, Table 3).

The water samples used in experiments 2/71 and 3/71, respectively, were extremely poor in planktonic organisms. However, the latter was rich in detritus and sediment particles. In both samples, *Ceratium fusus* and *C. furca* were the most abundant algae. Diatom species were present in numbers below 10^3 cells/l. The most important observation made during these 2 experiments refers to inactivation of *Staphylococcus aureus*, which was stronger in *fsw* than in *rsw* during experiment 2/71.

Experiment 4/71 was performed with a sea-water sample containing extraordinarily large amounts of phytoplankton which was in excellent condition. The enrichment procedure required about 1 hour (the longest time ever needed). Unfortunately, no quantitative information upon planktonic algae is available in respect to this and

Table 3

Serratia marinorubra. Survival in different types of raw and filter-sterilized sea water. (For further information see legend to Table 1)

No. of experiment	Duration (days)	log (N_x–N_0) found from					
		rsw	*10μ–rsw*	$ersw_{20}$	*fsw*	*10μ–fsw*	$efsw_{20}$
1/71	1	— 1.98	± 0	+ 0.29	± 0	± 0	+ 0.12
	2	— 1.77	— 1.88	+ 0.27	— 1.96	— 1.95	+ 0.11
	3	— 3.97	— 2.34	+ 0.07	— 1.91	— 1.91	+ 0.07
	4	— 4.46	— 3.27	+ 0.07	— 1.87	— 1.86	+ 0.04
2/71	1	— 1.94	— 1.87	— 1.91	— 1.95	— 1.95	— 1.88
	2	— 1.27	— 1.27	— 1.36	— 1.85	— 1.89	— 1.89
	3	— 3.28	— 3.28	— 4.99	— 1.64	— 1.93	— 1.86
	4	— 4.43	— 4.98	— 4.55	— 1.39	— 1.92	— 1.84
3/71	1	— 1.97	— 1.94	— 1.98	+ 0.10	— 1.95	± 0
	2	— 1.83	— 1.78	— 1.82	+ 0.10	— 1.93	± 0
	3	— 3.78	— 2.48	— 2.04	+ 0.13	— 1.96	— 1.98
	4	— 4.79	— 3.47	— 4.62	+ 0.11	— 1.88	— 1.94
4/71	1	— 1.90	— 1.95	— 1.92	— 1.95	— 1.88	— 1.73
	2	— 1.36	— 1.68	— 1.56	— 1.92	— 1.39	— 1.32
	3	— 5.87	— 4.56	— 3.44	— 1.82	— 2.98	— 2.98
	4	— 5.19	— 5.05	— 4.00	— 1.74	— 2.65	— 2.74
5/71	1	— 1.95	— 1.98	— 1.90	± 0	— 1.97	— 1.94
	2	— 1.75	— 1.91	— 1.69	— 1.91	— 1.96	— 1.90
	3	— 3.98	— 1.15	— 2.08	— 1.80	— 1.92	— 1.88
	4	— 3.45	— 3.22	— 3.81	— 1.70	— 1.84	— 1.86
6/71	1	— 1.95	— 1.96	— 1.90	— 1.97	— 1.93	— 1.95
	2	— 1.49	— 1.87	— 1.59	— 1.93	— 1.93	— 1.91
	3	— 3.52	— 2.29	— 3.29	— 1.87	— 1.90	— 1.88
	4	— 4.44	— 3.39	— 4.43	— 1.84	— 1.86	— 1.84
7/71	1	— 1.90	— 1.95	— 1.97	— 1.95	— 1.96	± 0
	2	— 1.20	— 1.56	— 1.50	— 1.89	— 1.92	— 1.92
	3	— 4.53	— 3.28	— 3.54	— 1.81	— 1.66	— 1.78
	4	— 5.27	— 5.80	— 5.82	— 1.59	— 1.51	— 1.73

the following experiments. According to own observations, *Chaetoceros socialis* and several *Rhizosolenia* species were most frequent, but *Asterionella japonica, Eucampia zoodiacus* and *Bidulphia* spec. were also numerous. At least *C. socialis* and *A. japonica* are capable of producing bactericides. During this experiment, the inactivation of each test strain was considerably weaker in $ersw_{20}$ than in *rsw* and *10 µ-rsw*. However, more important are the observations made from the respective sterile filtrates (Tables 1, 2 and 3). Against *Escherichia coli* and *Staphylococcus aureus*, the filtrates were at least as effective as the corresponding raw waters. *Serratia marinorubra* was inactivated to lesser degrees but its kill in *10 µ-fsw* and $efsw_{20}$ was the strongest ever observed during this investigation.

Experiments 5/71 to 7/71 were performed with sea-water samples considerably poorer in phytoplankton than the sample used in experiment 4/71. The plankton enrichment procedures required only about 35 min. *Rhizosolenia shrubsolei* was by far the most predominant in sample 5/71, whereas samples 6/71 and 7/71 contained no clearly dominant algal species. The most striking observation made during these experiments concerns identical inactivation of *Escherichia coli* and *Serratia marinorubra*, respectively, in all 3 types of raw sea water during 4 days of incubation (Tables 1 and 3, exp. 7/71).

Observations concerning marine bacteria

Some findings of general interest were obtained in regard to colony-forming marine bacteria as summarized in the following.

(1) By enrichment of *rsw* with filter sludge, the number of colony formers was increased in $ersw_{10}$ by 100 % to 600 %. The smallest increase in bacterial numbers was observed from $ersw_{10}$ used in experiment 3/70 (Fig. 1). Since the enrichment procedure was always the same, inactivation of marine bacteria during preparation of filter sludge must be taken into account.

(2) The preparation of $ersw_{20}$ by inverse filtration was accompanied by enrichments of marine bacteria ranging from 50 % to 200 % of colony formers present in *rsw*. The rise in bacterial numbers was independent of the phytoplankton mass contained in *rsw*. Increases by 200 % were found in experiment 4/71 and 6/71.

(3) The bacterial content of *10 µ-rsw* mostly was the same as, or 20 % to 40 % smaller than, found from *rsw*.

(4) After incubation of raw sea-water samples, the relative number of marine bacteria forming colonies of at least 1.5 mm in diameter during 5 days of incubation at 25° C on 2216 E-agar was found generally to be greater in $ersw_{10}$ and $ersw_{20}$ than in *rsw* or *10 µ-rsw*. (1.5 mm is an arbitrarily chosen measure.) According to JANNASCHS (1968) findings, predominance of large colonies of marine bacteria can be ascribed, at least in part, to increased nutrient concentration supporting selective enrichment of respective bacterial species during growth of mixed populations. Therefore, it is of interest that bacteria forming such large colonies were found to dominate in each of the different types of raw sea water used during experiments 3/70 and 4/71. Both

experiments are characterized by strong antibacterial activity of *rsw* but considerably weaker kill of test bacteria in $ersw_{10}$ and $ersw_{20}$, respectively.

(5) After incubation of the various types of raw sea water, largest numbers of colony-forming marine bacteria/ml generally were found from $ersw_{10}$ and $ersw_{20}$. In $ersw_{20}$, maximum numbers mostly were attained after one day. Decrease in colony formers/ml of at least one order of magnitude during the following 3 days of incubation was a common observation. It occurred independently of the presence or absence of test bacteria as well as of the bactericidal effect of $ersw_{20}$. In 1970 (Fig. 1), only three times were similar observations made from $ersw_{10}$, however, each in correlation with a beneficial effect of filter sludge enrichment for *Staphylococcus aureus* (exp. 3/70) and *Serratia marinorubra* (exps. 3/70 and 4/70), respectively. Growth of marine bacteria in *rsw* and *10 µ-rsw* lasted for 2 or 3 days and was followed by relatively small decreases in bacterial numbers/ml. After 4 days of incubation, mostly rather similar numbers of colony formers/ml were established for *rsw*, *10 µ-rsw* and $ersw_{20}$. Finally, ist must be mentioned that, from nearly each tested sample of the 3 types of raw sea water during experiment 4/71, larger numbers of marine bacteria were found than during the other experiments of the 1971 series. This was essentially independent of the presence of test bacteria and is also indicative of increased amounts of nutritive matter present in sea water at that time.

(6) During experiments 1/71 to 6/71, marine bacteria were observed which developed giant colonies spreading over the whole surface of 2216 E-agar. Sometimes the number/ml of these bacteria was increased after plankton enrichment. During incubation they failed to grow in *10 µ-rsw* but multiplied most rapidly in $ersw_{20}$. In the presence of *Serratia marinorubra*, growth of these organisms was considerably repressed in *rsw* but scarcely in $ersw_{20}$.

DISCUSSION

Numerous planktonic algal species are known to produce bactericidal compounds under natural and laboratory conditions. Findings referring thereto are based essentially on enrichments of algae either from their natural habitats or in uni-algal cultures. Therefore, our knowledge regarding the actual ecological relevance of biogenic bactericides is limited. In this respect, the observation of seasonal changes in antibacterial activity of sea water (SIEBURTH & PRATT 1962, MOEBUS 1972a), correlated with the life cycles of phytoplankton communities, is of special importance. Increased bactericidal capacity of sea water at times of phytoplankton blooms or break-downs is generally thought to be indicative of effective concentrations of compounds a priori harmful for the test organisms used. However, this conclusion is doubtful for several reasons.

The antibacterial activity of sea water as observed by laboratory tests cannot be put on a level with the bactericidal capacity really existing at the time of sea-water sampling, since gross changes in water quality occur during the tests. These variations are due to metabolic activities of living beings, to lysis of dead or dying organisms, and

presumably to some other processes. Therefore, it seems reasonable to base any conclusions regarding effects of bactericides on findings obtained by tests of sterile filtrates. These contain the soluble fraction of organic matter present at any time, at least a part of which may be bactericidal. As reported by MOEBUS (1972a), considerable variations in antibacterial activity of *fsw* were found, likewise correlated with the development of phytoplankton, but the changes in bactericidal effects of *rsw* and *fsw* often were of opposite tendencies. By these observations (and others) the antibacterial activity of *rsw* was demonstrated clearly to be influenced by living and/or dead particulate matter during the tests themselves. However, by the same findings, the importance of bactericidal products of marine organisms was called in question. This becomes evident by the fact that sometimes the kill of *Staphylococcus aureus* was stronger in *fsw* than in *rsw*. Meanwhile similar findings were obtained for *Escherichia coli*.

Manifold observations reported by MOEBUS (1972a, b) point to the involvement of nutritive matter in test bacterial kill in sea water. Results of further investigations support this view (MOEBUS 1972c). The bactericidal effect of sterile sea water could be increased by addition of low amounts of organic nutrients. Peptone was most effective at final concentrations below 10^{-4} mg/ml. Changes in concentration of useful nutrients (amino acids, sugars and others) even at these low levels must be expected to occur under natural conditions, as indicated by the findings of RILEY & SEGAR (1970) concerning amino acids. Therefore, seasonal changes in antibacterial activity of *fsw* may be due to variations in concentration and nature of nutritive substances, which on their part greatly depend upon phytoplankton development. In *rsw*, the influence of nutrients on the survival of test organisms certainly is superimposed, mainly by activities of marine bacteria as food consumption and excretion of harmful substances.

The present findings support this view. Although the inactivation of *Staphylococcus aureus* was increased sometimes by addition of filter sludge or viable plankton, these findings cannot be indicative of increased concentrations of bactericides caused by such additions. Otherwise, removal of plankton should have produced decreased anti-staphylococcal activity of *10 µ-rsw* during respective experiments. This was not the case. During other experiments, the effects of filter sludge or viable plankton on survivability of *S. aureus* were either negligible or even favourable. Such findings were common for both other test strains.

It is of special interest that beneficial influences of enrichments were most pronounced at times of greatly increased antibacterial activity of *rsw*. During experiment 4/71 this was found to be correlated with likewise increased activity of *fsw* against *Escherichia coli* and *Staphylococcus aureus*. Inactivation of *E. coli* even was stronger in *fsw* than in *rsw*. At first sight, the latter findings may be thought to suggest large amounts of bactericides present. However, such an assumption is inconsistent with the findings obtained from the 3 types of raw sea water. Apart from the fact that plankton enrichment resulted in decreased bactericidal capacity of $ersw_{20}$, observations regarding marine bacterial growth are indicative of increased nutrient concentration at that time. Marine bacteria forming large colonies on 2216 E-agar were dominant in each type of raw sea water tested, and growth of marine bacteria in *rsw*, *10 µ-rsw* and $ersw_{20}$ mostly exceeded that found from analogous samples during the other experiments of the 1971 series. The strong antibacterial activity of sterile filtrates observed

during experiment 4/71, therefore, is attributed to increased concentration of available nutrients produced by the large mass of phytoplankton present in the sea-water sample used. This statement is in agreement with findings obtained from experimentally enriched sterile, natural and synthetic sea water (MOEBUS 1972c).

If this interpretation corresponds to reality – and this is my opinion – the different results established for the 3 test bacterial species after incubation in sterile filtrates must be attributed essentially to strain-specific properties such as food requirements, capability to use nutrients from sea water and sensitiveness to inorganic constituents of this medium. The importance of both last-mentioned factors is suggested by recently reported observations (MOEBUS 1972c). In raw sea waters, several other parameters, such as release and consumption of nutrients as well as competition for food, are additionally involved in test bacterial kill. Competition for nutrients between marine and test bacteria was found to be considerable importance, at least in regard to *Serratia marinorubra* (MOEBUS 1972a, c).

Interpretation of antibacterial activity of sea water solely on the basis of biological findings is always an extremely difficult task since, with absolute certainty, various factors are involved simultaneously in inactivation of non-marine bacteria. With the exception of salinity, no important parameter remains nearly constant, neither under natural conditions nor during the tests lasting a few days. Therefore, one can only attempt to estimate the relative importance of the different factors. According to recent results (MOEBUS 1972a, b, c). I think that the importance of biogenic bactericides, present at the time of water sampling, in regard to self-purification of the sea is considerably overestimated. This statement is supported by the findings reported in this paper. Although co-operation of bactericidal products of planktonic algae in inactivation of test bacteria cannot be excluded, most results obtained during this investigation suggest dominant effects of available nutrients, which are superimposed by activities of marine bacteria, at least under laboratory conditions.

SUMMARY

1. Antibacterial activity of sea water against *Escherichia coli, Staphylococcus aureus* and *Serratia marinorubra,* as influenced by enrichment with filter sludge or viable plankton as well as by removal of plankton, was investigated. Inactivation of *S. aureus* only was sometimes increased by the enrichments employed.
2. During several experiments, inactivation of the 3 test strains was considerably decreased by enrichment with dead or living particulate matter. Most striking effects of this kind were observed at times of greatly increased bactericidal capacity of untreated sea water.
3. Sometimes, the inactivation of *Escherichia coli* and *Staphylococcus aureus* in sterile filtrates, prepared from the different types of raw sea water used, was the same as or even stronger than in corresponding samples of raw sea water.
4. From the present findings, it is concluded that bactericidal products of planktonic algae in regard to antibacterial activity of sea water are less important than available nutrients.

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Author's address: Dr. K. MOEBUS
Biologische Anstalt Helgoland (Meeresstation)
2192 Helgoland
Federal Republic of Germany