Proceedings of the National Shellfisheries Association
Volume 56 — May 1966

EXPERIMENTS WITH OYSTER PURIFICATION IN FLORIDA [1]

John Torpey, Robert M. Ingle, Larry Gillespie and Walter K. Havens
FLORIDA BOARD OF CONSERVATION MARINE LABORATORY
ST. PETERSBURG, FLORIDA

ABSTRACT

Twelve tests on purifying oysters, using ultraviolet light as the sterilizing agent, were conducted. The depuration unit employed was capable of purifying oysters using a low circulation factor at water temperatures of 24 to 26°C. No special cleaning procedure of the bivalve was necessary. In all tests except one, meats were at acceptable bacterial levels within 12 hours. In seven tests the bacterial counts of the water dropped to or remained at zero within four hours and in all tests, within 24 hours. Removal of fecal material did not appear to alter results.

INTRODUCTION

Florida, with approximately 8,000 miles of shoreline, has many acres of actual or potential water bottoms suitable for shellfish culture. In the past, most of Florida's leading estuaries supported thriving shellfish industries. Many of these areas have been rendered unsafe for production due to sewage pollution. Mollusks growing in these waters may carry many diseases such as typhoid, dysentery, and hepatitis. An effective control of pollution is desirable but not always possible. Depuration, or cleansing of shellfish from marginal or polluted waters, could be a solution to this dilemma.

The objectives of this study were to check factors which influence purification and to develop a shellfish purification system suitable for commercial use in Florida.

Depuration is not a new process and has been successfully used for many years to cleanse mussels and clams. In England, Dodson (1928) developed a system using chlorine as the active sterilizing agent and Wood (1957) modified this method for subsequent use on oysters. Several clam depuration plants using chlorine are or have been in operation in the United States, including one at Newburyport, Massachusetts, since 1930.

Use of chlorine in shellfish purification has a serious drawback. Kelly *et al.* (1960) established that chlorine had an adverse effect on the feeding activity of the American oyster, not removed by dechlorination.

Kelly *et al.* (1960) and Wood (1961) tested ultraviolet light as a sterilizing agent and found no adverse effects on feeding activity of oysters. Wood further believes that depuration plants capable of treating 10,000 oysters per operation could be constructed.

The practice of depuration is based on the physiology of shellfish. Briefly, these animals are filter feeders capable of passing several gallons of water per day through their gills. Nutrients are obtained from the water by the ciliary activity and mucus collection which strains off microorganisms and suspended organic materials. Pollutants, including sewage bacteria, can be discarded as pseudofeces or eliminated with the feces. According to ZoBell and London (1936), some of the microorganisms are probably utilized as food by these animals. Shellfish will purify themselves when placed in clean water. The purpose of ultraviolet treatment in a purification system is to keep the water free of harmful bacteria.

MATERIALS AND METHODS

Tests were conducted in a small pilot system with recirculated salt water. The physical structure of the plant (Fig. 1) consisted basically of five parts: sterilizing unit, aeration baffles, holding tank, circulating pump and plumbing, and filter. Most of the component parts were made of

[1] Contribution No. 98, Division of Saltwater Fisheries, Florida Board of Conservation.

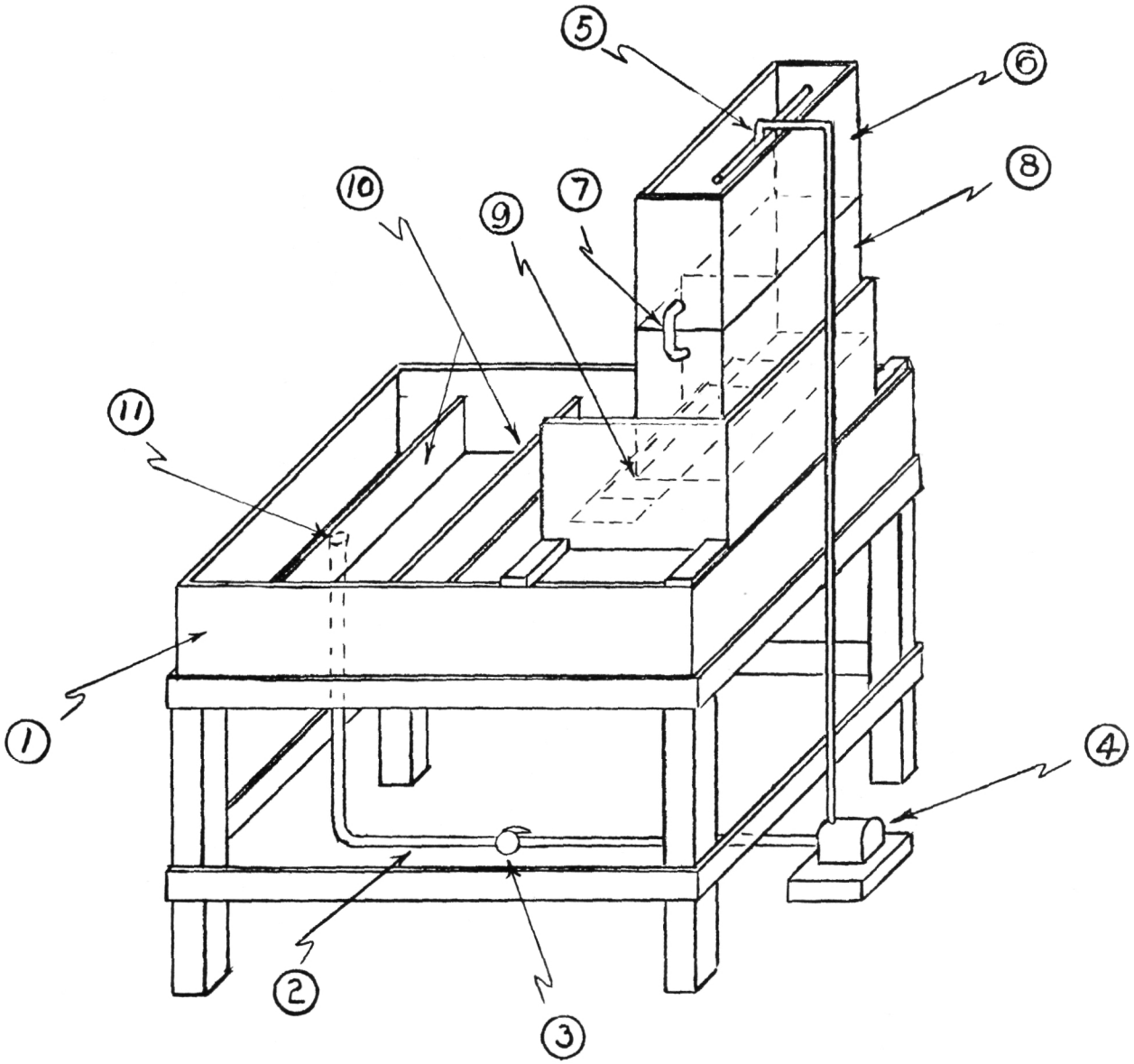

FIG. 1. *Illustration of oyster purification unit: (1) holding tank, (2) plumbing, (3) valve, (4) pump, (5) inlet nozzle, (6) filter unit, (7) connecting pipe, (8) ultraviolet sterilizer unit, (9) aeration baffles, (10) weirs, (11) outlet.*

epoxy-covered plywood and 3/4-inch PVC plumbing fixtures. A polyethylene pump and valve were also used.

The sterilizing unit was 10 inches wide, 24 inches deep, and 22 inches long. It housed three 15-watt ultraviolet germicidal lamps located six inches above the water line. Water from the sterilizer flowed down the aeration baffles into the holding tanks.

The holding tank measured five feet long, three feet wide, and one foot deep. Good circulation was accomplished in this tank by dividing it with three weirs into four compartments. The first three compartments were 18 inches wide and the last com-

partment at the outlet end was 6 inches wide. The first section served as a splash area for incoming water, the middle two held the shellfish, and the last served as an outlet and settling basin. Water flowed from the aeration baffles into the first compartment, over the first weir, under the second weir and over the third, into the last compartment. A slight drop in the water level was maintained at the first and third weirs. Water flowed through a 1/16-inch space under the middle weir. Epoxy-covered plywood racks, one inch from the bottom of the tanks, held the oysters stacked in two or three layers, depending on their size. Two hundred liters of water were maintained in the system.

Water was pumped from the last compartment into the filter box located above the sterilizing unit. The pump was rated at nine gallons per minute head and the rate of flow was controlled by a polyethylene ball and socket valve placed in the line at the inlet of the pump. The filter box was 24 inches by 10 inches by 12 inches and contained a 1-inch layer of replaceable glass wool. Water was allowed to flow by gravity from this box back into the sterilizer to complete the cycle.

Water for these tests was drawn directly from Bayboro Harbor, filtered with a nylon fiber filter, adjusted with deionized water to 17-21 ppt, and tested for coliform bacteria. The filter removed all suspended material larger than 30 μ and most of the smaller particles. No direct control of the temperature was attempted; however, the system was located inside a building and the water temperatures varied from 24 to 26°C. The pH varied from 7.7 to 7.9.

EXPERIMENTAL PROCEDURES

Twelve experiments were conducted in this study. The following factors were checked: (1) the ratio of oyster numbers to water volume; (2) the circulation factor (the number of times the whole water volume passed through the sterilizer in one hour); (3) the effect on bacterial counts of the removal of feces and pseudofeces from the water. Observations were also made to determine whether cleaning the animals and sanitizing the tanks and equipment influenced coliform counts.

The experiments were evaluated by the rate of removal of coliform bacteria from the oyster meats and the concentration of coliform bacteria in the water. The lactose presumptive and bile green confirmation tests used in these experiments were conducted under rigid laboratory control and the methods are described in "Recommended Procedures for the Bacterial Examination of Shellfish and Shellfish Waters" (1947) and "Standard Methods for the Examination of Water and Waste Water" (1960). A series of standard agar plate counts, as described in "Standard Methods for the Examination of Dairy Products" (1963), were made on the oyster meats for each experiment in an effort to correlate the progressive decline of the coliform with the non-coliform bacteria.

Three 15-watt ultraviolet lights and 200 liters of water were used in all experiments. The first depuration study involved 195 oysters while 400 were used in all other experiments to gain a better insight of a commercial operation. With the exception of the first experiment, the oyster-to-water ration was two.

Because polluted oysters were scarce, the shellfish used in experiments five through 12 were treated with sewage effluents. A safe level of coliform content for shucked commercial oysters was considered by health authorities to be below the MPN (most probable number) of 16,000 per 100 ml of sample, and for our paper this standard will be used to classify acceptable coliform counts. Pollution was accomplished by adding raw sewage effluent to a circulating tank system for four days. Slightly more than 400 animals were treated each time by adding one quart of effluent to 400 liters of water daily in the tank. Ample aeration was provided.

During the first two experiments a high circulation factor of 4.6 was used and the feces and pseudofeces were siphoned off once a day. The third experiment involved a reduced circulation factor of 2.23. No fecal materials were removed from the tank in this or any of the remaining tests.

In Experiments 4 through 8, the circulation factor was varied from 1.00 to 2.97. In Experiments 4 and 5, before the bivalves were placed in the tanks, the water was treated with ultraviolet light and the coliform content tested zero; also, all tank surfaces, pipes, and valves were sanitized with ethyl alcohol and thoroughly rinsed with tap water. In other experimental conditions, tests 4 through 8 were nearly identical.

In the first eight experiments the bivalves were separated into singles, cleaned of fouling organisms, and scrubbed thoroughly with a stiff fiber brush. In Experiments 9 through 12, the oysters were separated into small clumps only and simply hosed off. The circulation factor was varied from 1.00 to 2.70.

RESULTS AND DISCUSSION

Values for some of the experimental variables and a summary of results are given in Table 1.

Initial MPN of the meats in Experiment 1 was 9,500 per 100 ml, a figure indicating insufficient pollution. The water was free of coliform bacteria after four hours and the count in the meat had dropped to 330 per 100 ml in 24 hours. For the

TABLE 1. *Values for some experimental variables and summary of results.*

Exp. no.	Date	Temp. °C.	Circ. Factor	Sal. ppt		Bacterial Analysis Initial MPN/100 ml	4-hour test MPN/100 ml	24-hour test MPN/100 ml	48-hour test MPN/100 ml
1	10-15-64	24	4.60	20.0	Meats	9,500	2,400	330	58
					Water	4.5	0	0	0
2[1]	10-26-64	26	4.60	18.7	Meats	240,000	1,300	490	79
					Water	5.6	7.8	0	0
3	11-19-64	25	2.23	17.1	Meats	3,300	490	0	0
					Water	170	0	0	0
4	11-23-64	25	2.23	18.5	Meats	11,000	700	9,200	1,400
					Water	0	0	0	0
5[2]	12- 7-64	25	2.97	18.5	Meats	95,000	330	3,300	130,000
					Water	0	0	0	0
6	1- 4-65	24	2.97	20.7	Meats	18,000	700	490	70
					Water	0	0	0	0
7	1-18-65	20	2.00	20.9	Meats	22,000	1,100	230	79
					Water	0	0	0	0
8	2-21 65	26	1.00	18.5	Meats	18,000	790	330	230
					Water	34	22	0	0
9	3-22-65	26	1.00	21.0	Meats	18,000	330	240,000	490
					Water	0	46	0	0
10	4- 5-65	26	2.97	20.5	Meats	17,000	230	330	330
					Water	0	6.1	0	0
11	4-26-65	24	2.00	20.7	Meats	22,000	790	230	0
					Water	2	2	0	0
12	5-18-65	26	1.00	21.0	Meats	18,000	330	230	79
					Water	0	0	0	0

[1] Incidental test at 12 hours: water recorded 0 MPN.

[2] Incidental test at 36 hours: meats recorded 17,000 MPN.

second experiment, the initial meat MPN was 240,000, dropping to acceptable levels after four hours and the water was coliform free after 12 hours. In the third experiment, results showed the water count was zero and the bivalve flesh was 330 MPN after four hours. Tests on water and oyster meats showed no increases in coliform count throughout the experiment. This indicated that fecal material did not cause a build-up of coliform bacteria in the meats or water. However, these bivalves were not sufficiently polluted initially.

In Experiments 4 through 8, with the exception of Experiment 5, the coliform counts were reduced within four hours to less than 16,000 MPN. Although in some cases slight increases were noted within the 48-hour test period, the MPN did not exceed the accepted standard. The bivalves tested in Experiment 5 were those used in the preceding experiment. They were re-treated with sewage effluent before use. Coliform counts on the meats fluctuated erratically in Experiment 5 and the final two tests were high. After 36 hours, the coliform count increased to 17,000 MPN and the 48-hour test showed a MPN of 130,000. When compared with the other experiments, no physiological difference was noted in these oysters. However, since they were reused from the prior experiment, the possibility existed that the oysters were induced into inactivity by this treatment. The high coliform count could be attributed to inactivity.

For the last four experiments, the counts made at the end of four hours showed a significant decrease in the coliform MPN count. No appreciable subsequent increases were recorded in Experiments 10, 11, and 12. However, in Experiment 9, a build-up of coliform in bivalve flesh occurred at the end of 12 hours. The MPN ranged from 240,000 at the end of 12 hours to 4.9 million at the end of 18 hours and back to 240,000 at the end of

24 hours. The final 48-hour count had dropped to 490 MPN. During this experiment the oysters spawned extensively and rapid multiplication of coliform bacteria could have been engendered by the spawn. Since the coliform counts in the water for the same period were low, it seems likely that the bacterial growth occurred within the oysters.

Coliform level of the water was low throughout each experiment. In seven tests the bacterial counts of the water dropped to or remained at zero within four hours. No coliform were observed within 24 hours.

Total plate counts showed a progressive decline in noncoliform bacteria that in most cases could be correlated with the decline in coliform bacteria. This indicates that the depuration system is effective against microorganisms other than the coliform groups.

General characteristics common to all the experiments should be mentioned. They include: (1) a loss of water volume from the tanks due to evaporation; (2) the presence of physiological by-products evidenced by numerous frothy bubbles; (3) a mortality of less than one oyster per trial.

Although our operation did not involve more than two bushels of oysters, it would appear that any problems that might be anticipated in a commercial operation could be overcome. More detailed investigations on a commercial scale are needed to ascertain the full potential of depuration utilizing ultraviolet light as the sterilizing agent.

LITERATURE CITED

Anon. 1947. Recommended procedures for the bacterial examination of shellfish and shellfish waters. Amer. J. Publ. Hlth. **37**: 1121-1127.

Anon. 1960. Standard methods for the examination of water and waste water. Amer. Publ. Hlth. Ass., Amer. Water Wks. Ass. and Water Pollut. Control Ass., 11th Ed., p. 494-507.

Anon. 1963. Standard methods for the examination of dairy products. Amer. Publ. Hlth. Ass., 11th Ed.

Dodgson, R. W. 1939. Reports on mussel purification. Fish. Invest., London, Ser. II, **10** (1).

Kelly, C. B., W. A. Arcisz and M. W. Presnell. 1960. Bacterial accumulation by the oyster, *Crassostrea virginica*, on the Gulf Coast. U. S. Dep. Hlth. Educ. & Welfare, Technol. Rep. F60-4.

Wood, P. C. 1957. The cleaning of oysters. Recent changes in the methods used in Brightlingsea. Publ. Hlth., London, **70**: 92-94.

Wood, P. C. 1961. The principles of water sterilization by ultraviolet light and their application in the purification of oysters. Fish. Invest., London, Ser. II, **23**(6).

ZoBell, C. E. and W. O. London. 1936. Bacterial nutrition of the California mussel. Proc. Soc. Exp. Biol. & Med. **36**: 113-116.