

Accumulation of *Escherichia coli* by the Northern Quahaug¹

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The uptake of *Escherichia coli* by the quahaug, *Mercenaria mercenaria*, was studied to obtain an insight into the environmental parameters significant to the accumulation of bacterial pathogens by shellfish growing in polluted waters and into the kinetics of the uptake process. Experimental uptake was achieved by placing the animals in a flowing water system in which the contamination level of the water and its temperature and salinity could be controlled. Data from periodic assays of individual animals suggested that accumulation of the bacteria by the quahaug proceeds to an equilibrium level which is a function of *E. coli* content of the water and its overall particulate matter. Accumulation takes place in the digestive gland and, to a lesser extent, in the siphon of the animal.

Shellfish are important as vehicles for the transmission of enteropathogenic bacteria to man (5, 10) because of their ability to concentrate organisms from overlying waters polluted by sanitary wastes, storm water runoff, etc. (8). This is due to their normal feeding and elimination processes which have been studied extensively in terms of anatomical and physiological parameters such as pumping rate (4, 7, 11), filtration efficiency (6, 12; J. W. Blake, Biol. Bull., p. 383, Abstr., 1961), pseudofeces production, transport of food particulates through the digestive system, and elimination of feces (1).

In attempting to formulate a general model for expressing the accumulation of bacteria in terms of these physiological parameters, it was necessary first to determine whether and how the level of the organisms in the environmental water, and the interval over which the animals are exposed to this level, affects the rate of accumulation. The present study was designed to answer these two questions and to determine whether, in fact, most of the microorganisms are contained in the "digestive system" of the animal. *Escherichia coli* was chosen as the test organism and the Northern quahaug as the test animal, the former because of its significance as an indicator organism of fecal pollution and the latter because of its availability.

A second objective of the study was to obtain an accumulation factor for the quahaug which would be useful in predicting the maximal levels of indicator or pathogenic bacteria in the animal

from that in the environmental water. A third objective of the study was to obtain information on animal variability, location of organisms, etc., which could be useful in studying the commercial application of the elimination process (depuration) when contaminated animals are placed in "clean" water.

MATERIALS AND METHODS

The organism used for the experimental contamination of seawater and shellfish was a strain of *E. coli* var. i isolated from a seawater sample. It was maintained on nutrient broth incubated at 44.5 ± 0.2 C and stored in a refrigerator when not in use. Nutrient broth cultures incubated at 44.5 ± 0.2 C for 18 to 24 hr were used to contaminate the environmental seawater. Exceptions to this procedure are so noted.

A four-tank experimental system was used to contaminate the quahaugs with *E. coli*. This system (Fig. 1) consisted of two seawater intake lines, one of which passed through a heat-exchange system capable of heating or cooling the water. The water from each intake was passed through a constant head tank, a five-tube ultraviolet light (UV) germicidal treatment chamber (9), and a second constant head tank. By this procedure, the flow through the UV system could be maintained at a constant rate. Although the coliform levels in the intake water system generally were negligible, the UV treatment further decreased the possibility of exogenous coliforms being introduced into the system. Repeated assays of the seawater after UV treatment confirmed the absence of coliforms therein. Four small boxes, each capable of vertical adjustment, were connected to each effluent constant head tank. The effluent water from these adjustable head boxes was carried to the mixing boxes. Thereby, the desired mixture of ambient seawater and cooled or heated sea- or freshwater could be delivered

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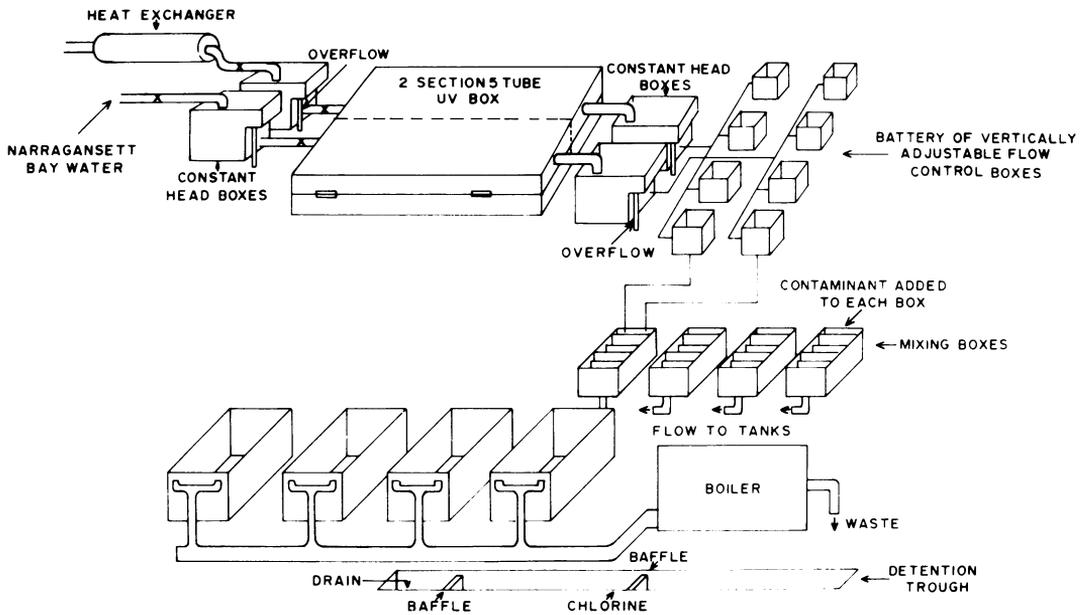


FIG. 1. Schematic diagram of experimental contamination and depuration system.

to the mixing boxes at the desired flow rates. The four mixing boxes were baffled to mix the water with the suspension of *E. coli* introduced by a metered drip system from a water-jacketed flask maintained at 5 C. The seawater-*E. coli* suspension from each mixing box was then delivered at a rate of 2 liters/min (range 1.8 to 2.1) into a corresponding uptake tank. The quahaugs were placed in a polyvinyl chloride-covered, wire mesh basket within the tank. No more than 100 animals, each weighing between 90 and 110 g, were placed in each basket.

Typically, experimental uptake of *E. coli* by the animals was achieved in the following manner. The required number of animals, preselected for size, were removed from the holding tank, scrubbed clean with running water, and deposited into the basket placed in each depuration tank. A sample of quahaugs was removed to confirm the absence of fecal coliforms. All animals had been obtained from a nonpolluted source and had been maintained in the holding tanks in running seawater for at least 1 week. After introduction of the *E. coli*-seawater mixture into the tank containing the animals, uptake was allowed to proceed for the required period of time. Periodically, samples of the water in the tanks, the animals from each tank, and the UV-treated water were taken and assayed. The animals removed for sampling were replaced by uncontaminated, appropriately marked quahaugs. The water samples from each tank were obtained by pooling equal portions of water removed from each of the four corners and the center of the tank.

To effectively and rapidly sample large numbers of quahaugs for the *E. coli* accumulated from the environmental seawater, a modification of the MacConkey agar roll tube method of Clegg and Sherwood (3) was used. The formula for MacConkey agar as

available from commercial sources was modified by decreasing the bile salts concentration from 0.15 to 0.075%. The sodium chloride eliminated from the medium essentially was replaced by that present in the shellfish and seawater samples mixed with it. The formula for the single-strength medium, as used, was as follows (grams per liter): peptone, 17.0; poly-peptone, 3.0; lactose, 10.0; bile salts #3, 0.75; agar, 13.5; neutral red, 0.03; crystal violet, 0.001. The ingredients of the medium, in an appropriate quantity of distilled water, were boiled for 10 min, and the medium was held in a water bath at 55 to 60 C; it was used within 6 hr of preparation.

Quahaug samples were prepared for assay by homogenizing the required number of animals in a Waring Blendor for 60 sec at 18,000 rev/min and adding 6 g of the homogenate to a 6-oz bottle containing 54 ml of sterile, phosphate-buffered saline (buffered water to which 0.9% NaCl was added; reference 2). A 60-ml amount of double-strength medium was added to the bottle, the contents of which were mixed gently and distributed into six petri dishes (100 by 15 mm). When required, water samples were assayed by adding 60 ml of double-strength medium to 60 ml of the seawater sample in a sterile 6-ounce bottle. The contents were mixed gently and distributed to six petri dishes as above. The medium was allowed to solidify, and the inverted plates were incubated in an air incubator at 45.5 ± 0.5 C for 24 hr. At this temperature, growth of organisms other than *E. coli* present in the quahaug or the seawater was inhibited or reduced, and the *E. coli* grew as characteristic brick red, subsurface colonies. Occasionally, other coliform organisms would grow at the elevated temperature; therefore, only characteristic colonies greater than 0.5 mm in diameter were counted.

RESULTS

The initial experiments were carried out between the months of June and August when the ambient water temperature was between 20 and 22 C, and the animals appeared to be very active. The distributions of *E. coli* levels in 24 animals allowed to accumulate the organisms for 24 hr as shown in Fig. 2 are typical of the results obtained in several experiments. The discontinuous nature of the distributions suggested two useful values, the *E. coli* level achieved by the animals which accumulated maximally under the experimental conditions and the number of animals which achieved this level. The former value was obtained as the median of the second segment of the log-probability distribution. Since the number of organisms accumulated by the animals was several orders of magnitude lower than that available during the 24-hr interval, the

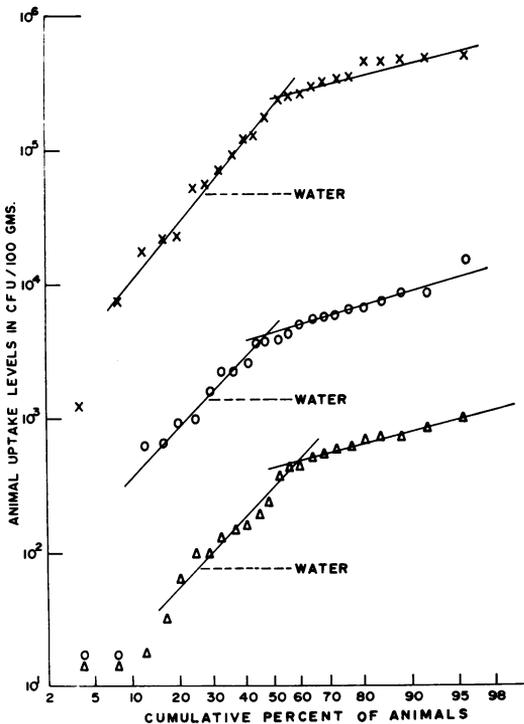


FIG. 2. Influence of the density of *E. coli* in the environmental water on the "steady state" level in the quahaug. Each point represents the *E. coli* level/100 g in a single animal expressed as colony-forming units (CFU). Three experiments, each at a different *E. coli* level in the water, are shown by X, O, and Δ. "Water" designates *E. coli* density in the environmental water. Environmental conditions as follows: seawater temperature, 20 C; salinity, 30‰; flow rate, 2 liters/min; uptake interval, 24 hr.

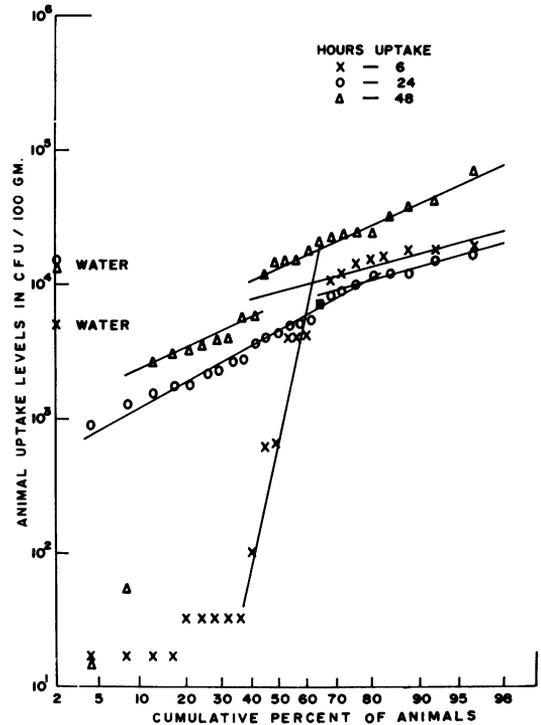


FIG. 3. Influence of the accumulation interval on the *E. coli* "steady state" level in the quahaug. X, O, and Δ indicate levels in the individual animals after 6, 24, and 48 hr, respectively. The *E. coli* densities in the environmental water at each time interval are noted as "water." Environmental conditions as shown in Fig. 2.

possibility was considered that in a given animal an equilibrium condition was achieved wherein intake and removal (biological and physical) were equal. The median of the second segment of the distribution then could be looked upon as the "steady state" level subject to the anatomical or physiological (or both) variability of the individual animals and the variability of the assay method. Those animals whose levels fall within the first segment of the distribution apparently were not active long enough to achieve equilibrium. This premise was examined by investigating the influence of the concentration of *E. coli* in the water and the accumulation interval on the "steady state" level and the numbers of animals which achieve this condition. Figure 2 shows the distributions obtained when the accumulation interval was maintained at 24 hr, and the contamination level of the water was varied as shown. The "steady state" level increased with increasing concentration of *E. coli* in the water but was independent of accumulation intervals between 6 and 48 hr (Fig. 3). The number of animals which

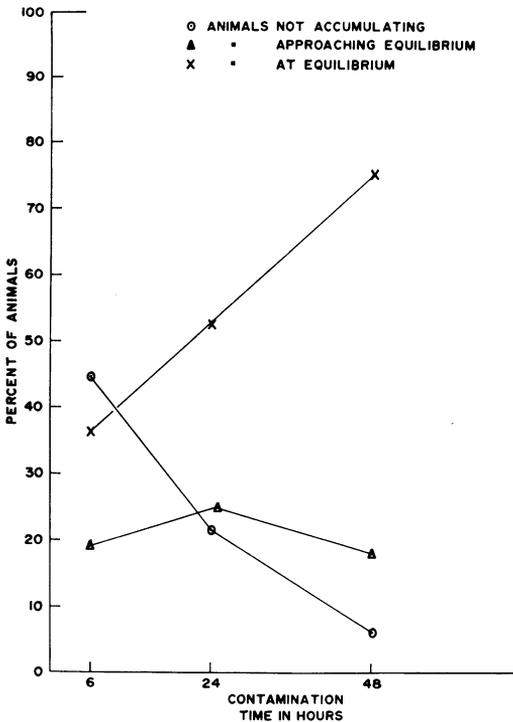


FIG. 4. The percentage of animals accumulating *E. coli* to different stages as a function of the uptake interval. ○, Animals not accumulating are defined as those with *E. coli* counts less than that of the water. △, Animals approaching equilibrium; all those which are not in the other categories. ×, Animals at equilibrium are those animals with bacterial counts at an equilibrium as determined by a log-probability plot of *E. coli* concentration of individual shellfish (Fig. 2 and 3). The points were obtained by averaging the percentages obtained from several experiments.

achieved equilibrium, however, did increase with time (Fig. 4). The values given were obtained by averaging the results of several such experiments. The relationship of the "steady state" level in the animal to the concentration of *E. coli* in the contaminating water was obtained from regression analysis of the experiments in which the accumulation interval or the concentration of organisms in the environmental water was varied (Fig. 5). The formula for the line obtained is $Y = 0.96x + 0.97$, where x is the \log_{10} water concentration and $Y = \log_{10}$ "steady state" level in the animals. Thereby, it was calculated that, with concentrations of 10^1 to 10^5 organisms/100 ml of water, the accumulation factor varied between 6.5 and 8.5, respectively. However, neither the level of the organisms in the water nor the accumulation interval interacted significantly with the accumulation factor.

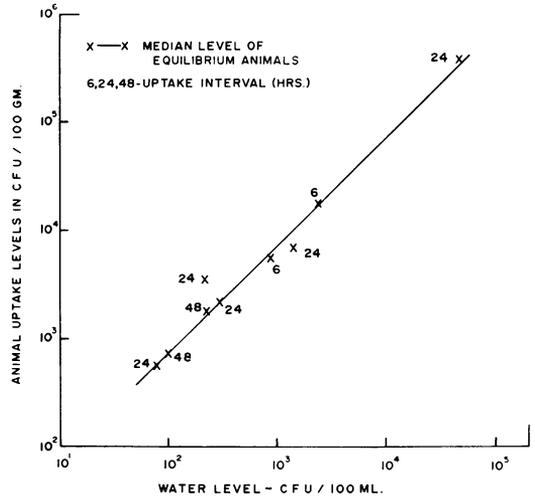


FIG. 5. The equilibrium level of *E. coli* in the Northern quahaug relative to the concentration of the organism in the water. The numbers beside each point denote the accumulation interval over which the trial was conducted. The *E. coli* density in the environmental water varied from trial to trial between 10^1 and 10^5 colony-forming units/100 ml. Other environmental conditions are given in Fig. 2.

The site of accumulation of the organisms within the animal was examined by dissecting the various organs from the contaminated animals and subjecting them to assay for their *E. coli* content. The digestive gland contained most of the organisms, and only in the digestive gland and the siphon tissue did the *E. coli* content per gram markedly exceed that per milliliter of environmental water (Table 1).

DISCUSSION

The premise that the quahaug can accumulate a given bacterium to an equilibrium concentration, which can be related to the concentration of organisms in the water, does permit the formulation of a model to describe the accumulation process in terms of physiological processes such as gill filtration efficiency, pumping rate, transport time, biological decay of the organisms, etc. A tentative model for the accumulation of *E. coli* by the actively functioning quahaugs can be derived as follows:

$$A = \frac{B_a}{B_w} \quad (1)$$

where A is the accumulation factor such as that as obtained from this study and B_a and B_w are the levels of *E. coli* per gram of animal tissue and

TABLE 1. *Escherichia coli* content of various tissues dissected from the quahaug

Trial	Contamination time (hr)	Colony forming units/g						
		Pool ^a	Gills	Mantle	Shell liquor	Siphon	Digestive gland	Contaminating water
1	3.5	1	<1	<1	6	1	110	17
		2	<1	2	9	<1	60	17
	26	1	<2	<1	4	40	80	13
		2	<2	1	25	68	226	13
	48	1	2	1	1.4	62	148	19
		2	<2	1	14	134	137	19
	72	1	46	7	142	8	898	62
		2	18	337	21	4	246	62
2	48	1	21	<1	5	550	1,820	53
3	48	1	26	11	3	99	2,280	74

^a Tissues dissected from three animals, pooled and assayed.

per milliliter of water, respectively. The level of *E. coli* in the animal can be calculated as follows:

$$B_a = \frac{B_w \cdot P \cdot T \cdot K_{fe} \cdot K_s + B_w V_i + B_w \cdot K_{fe} \cdot V_e}{W} \quad (2)$$

where P is the pumping rate for the quahaug in milliliters per minute; T is the transport time in minutes for the organism from intake through elimination in the feces of the animal; K_{fe} is the filtration efficiency expressed as the ratio of *E. coli* retained by the gills to that taken in by the animal; K_s is the survival ratio for *E. coli* over the transport time "T" in the animal; V_i and V_e are the volumes in milliliters of incurrent and excurrent water in the animals; and W is the wet weight of the animal in grams. Since, for the present, the model only considers the accumulation of *E. coli*—a relatively small particle—in low-turbidity water [<3 Jackson Turbidity Units (JTU)], the effect of pseudofeces production has been ignored.

Most of the water in the animal is, in fact, incurrent water, and only a small percentage of *E. coli* cells are filtered out by the gills. Therefore, equation (2) may be simplified as follows:

$$B_a = \frac{B_w \cdot P \cdot T \cdot K_{fe} \cdot K_s + B_w V_f}{W} \quad (3)$$

where V_f is the combined volume of the incurrent and excurrent water in the animal.

Substituting equation (3) in equation (1), we obtain the following relationship which describes the accumulation factor for *E. coli* in terms of physiological parameters responsible for the feeding-cleaning mechanism of the quahaug.

$$A = \frac{P \cdot T \cdot K_{fe} \cdot K_s + V_f}{W} \quad (4)$$

The pumping rate for the quahaug as obtained by Ansel (4) and in this laboratory was about 100 ml/min. The transport time as obtained in this laboratory by using fluorescent particles (median diameter, 2 μ m) was about 45 min (*unpublished data*). The filtration efficiency for *E. coli* by the quahaug has been reported as being between 1 and 30%; therefore, a value of 0.10 appears to be a reasonable approximation of the K_{fe} . The survival ratio (K_s) over the 45-min transport time as obtained by data from this laboratory is about 0.8. The average wet weight of the animals used was 30 g; 15 ml was liquid. By substituting the above values in equation (4), the derived value for A approximates that obtained empirically, 6.5 to 8.5.

$$A = \frac{100 \cdot 45 \cdot 0.10 \cdot 0.8 + 15}{30} = 12.5$$

The model is rather speculative and will have to be examined in the light of more careful measurements of the various parameters, particularly the filtration efficiency.

The small volume of space within the digestive tract occupied by the *E. coli* relative to other particles filtered from the water by the quahaug would suggest that the rate of transport of the *E. coli* within the digestive system, hence the accumulation rate, is dependent not on the *E. coli* concentration in the water but rather on the ratio of the organism to the total ingestible particulates. The net result of this direct effect on the composition of the contents of the digestive system and the indirect effect via the influence on the pumping rate and filtration characteristics of the animal should be a decrease in accumulation with an increasing particulate content in the water. In a fortuitous experiment in which the

turbidity of the intake water was increased to 35 JTU due to a storm at sea, the accumulation factor was observed to be 3.2. This single observation is being extended as part of a study which is considering a variety of environmental parameters such as the quantity and nature of particulates and dissolved materials in the water, temperature, salinity, season, etc., on the accumulation of *E. coli* by the quahaug.

The observations described herein confirm those of previous workers (8, 13) that the concentration of coliforms in shellfish does reflect that of the overlying water during the preceding several hours. Furthermore extended contact with a steady source of pollution only increases the numbers of animals polluted to the maximal concentration for the prevailing environmental conditions. Since the sanitary classification of shellfish growing areas, hence the acceptability of shellfish removed therefrom as a raw food product, is in part dependent on the coliform content of the water, it is important to determine the extent to which the accumulation of *E. coli* and the enteropathogenic bacteria are influenced by the environmental parameters of the growing areas.

ACKNOWLEDGMENT

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