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# PUBLIC HEALTH ASPECTS OF THE CULTURE OF THE JAPANESE OYSTER *CRASSOSTREA GIGAS* (THUNBERG) IN A WASTE RECYCLING AQUACULTURE SYSTEM

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ABSTRACT

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A study of 24 weeks duration was carried out in which oysters (*Crassostrea gigas*) were grown in four regimes. These were: (i) on phytoplankton cultured in a mixture of secondary treated sewage effluent and seawater for a period of 12 weeks followed by a second 12-week period of feeding on phytoplankton cultured in a "clean", inorganically enriched regime; (ii) as for (i) except that the secondary effluent was sand filtered prior to use; (iii) as for (ii) except that the effluent was charcoal filtered prior to use; and (iv) using "clean", inorganically enriched phytoplankton food for the 24-week duration. At intervals of 2 weeks, populations of oysters were removed for assay for trace metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) and organic contaminants (hydrocarbons, polychlorinated biphenyls). No significant accumulation or depuration of any metal or organic contaminant was evident in any of the regimes. In terms of these contaminants all oysters are within acceptable edible standards as set by the U.S. Food and Drug Administration.

## INTRODUCTION

The chronological development of a pilot scale waste recycling aquaculture system at Woods Hole Oceanographic Institution has been described in a series of recent publications. Subject areas include outdoor mass culture of phytoplankton (Goldman and Ryther, 1975, 1976), comparative growth of various species of bivalve molluscs in the system (Mann and Ryther, 1977), the optimisation of bivalve growth through manipulation of temperature (Mann, 1978, 1979a and b, Mann and Glomb, 1978, Mann and Taylor, 1981), and initial experiments on long term uptake and depuration of trace metal contaminants by cultured organisms (Mann and Ryther, 1979). Following the demonstration of the technical feasibility of culturing organisms in a marine, waste recycling aquaculture system recent efforts have

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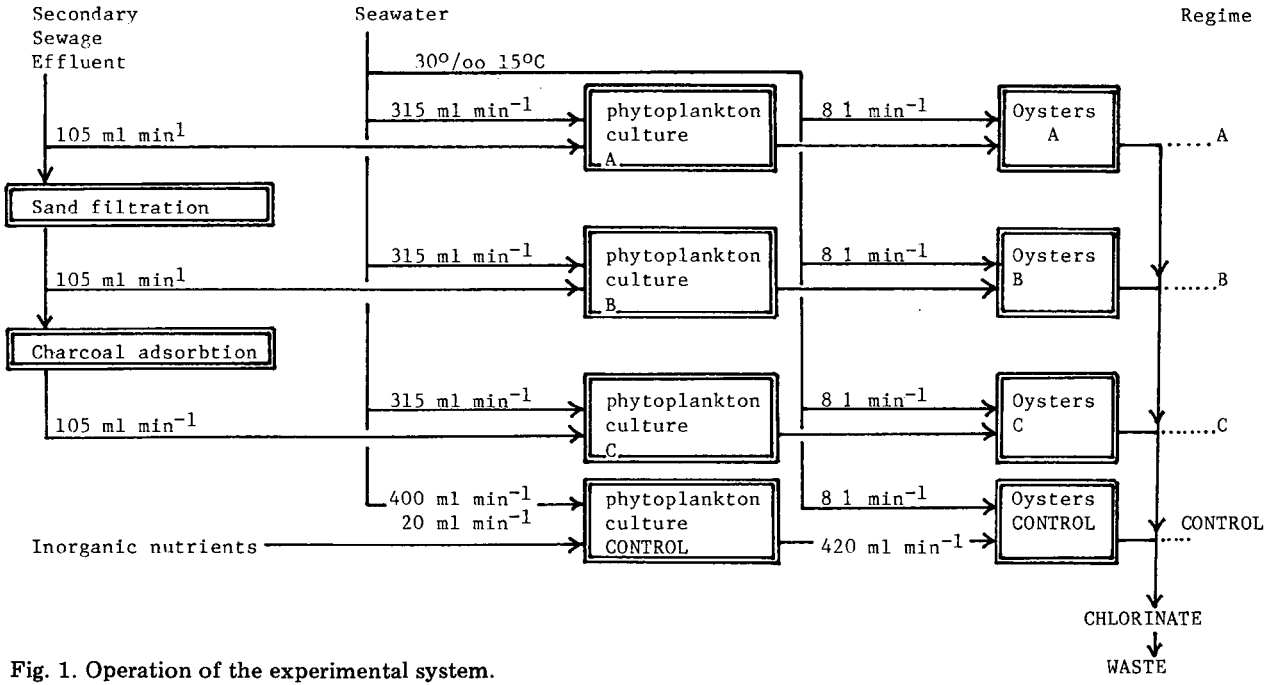


Fig. 1. Operation of the experimental system.

focussed on public health aspects of system operation. The present report describes a series of experiments which examine respectively the accumulation and depuration of trace metal and organic contaminants in a sewage enriched culture system by the Japanese oyster *Crassostrea gigas* in conditions comparable to those previously defined as optimal for culture operation (see Mann, 1978, 1979a and c for discussion of temperature and food control).

The series of experiments represents a logical progression from the study of Mann and Ryther (1979) in which the accumulation and depuration of seven trace metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) by three species of bivalves (*Crassostrea gigas, Ostrea edulis, Tapes japonica*) were observed over an 18-month period. During the course of this study it became increasingly evident that one problem that would be encountered in any onsite application of the waste-recycling process was the change in total suspended particulate load in the sewage effluent. It was not uncommon to obtain a tenfold change in this parameter over a period of only a few days. It is highly desirable to minimize suspended particulate content of effluent in the present application because a considerable proportion of the trace metal, organic and virus content of polluted aquatic systems is associated with the suspended or sedimented particles (Clarke et al., 1956, 1961; Hager and Flentje, 1965; Chen, 1974; Rohatgi and Chen, 1975; Davis and Jacknow, 1975) and due to their filter feeding habit bivalve molluscs will accumulate contaminants associated with particulate material with comparative ease.

The study described here examined the use of simple, inexpensive low-technology methods for controlling quality of effluent originating from an industrial urban area. A combination of a trickling sand filtration system with an activated charcoal column was chosen for investigation in the present study as this offered the following advantages: (i) effective removal of trace metals and organic compounds associated with particles or naturally occurring as particulate entities, (ii) ease of operation, this being restricted to occasional back-flushing of the sand filter, and (iii) low cost. Secondary benefits to this arrangement include the general enhancement of carbon adsorption by prior particulate removal (Anonymous, 1971), and a decreased potential for virus survival due to a decreased dissolved organic carbon level in the effluent used for phytoplankton culture (see Vaughn and Ryther, 1974).

## METHODS

### *Uptake and depuration of trace metals and organic contaminants*

Operation of the experimental system is summarised in Fig. 1. Three levels of sewage treatment were effected (A) none, (B) sand filtration, (C) sand filtration plus charcoal adsorption, with the effluent from each treatment fertilizing a separate food chain. A fourth food chain (CONTROL) en-

riched with inorganic fertilizer (ammonium and phosphate salts) served as a "zero pollution" control. The four regimes are hereafter referred to as A, B, C, and CONTROL.

The system was operated for 24 consecutive weeks. During weeks 0—12 the four regimes were operated as described above and oysters were removed at intervals of 2 weeks for assay for accumulation of selected contaminants. During weeks 13—24 all food chains were operated as per CONTROL to attempt depuration of accumulated contaminants. Again, oysters were removed at 2-week intervals to assay depuration. Details of system components and operation are described below.

*Sewage treatment*

All sewage effluent used in the present study was obtained from the municipal sewage treatment plant at Cranston, RI. This plant serves approximately 65000 people and operates at a capacity of $29.6 \times 10^6$ l/day. $FeCl_2$ is added to the effluent to aid sludge coagulation. An anaerobic vacuum filter sludge digestion process is used. Approximately 15% of the effluent is derived from industrial sources which include five electroplating plants, one screen printing plant, one chemical plant, and one milk processing plant. Effluent was transported to Woods Hole by truck and stored in three 3500-l underground tanks until used.

Sand filtration was accomplished using a filter constructed from a linear polyethylene tank (1 m depth × 0.5 m diameter). The filter outlet, at the base of the tank, was 5 cm below a perforated grid (10 mm diameter perforations) which supported the mixed-bed filter material. The latter consisted of 5 cm of gravel (mean size 8.0 mm), overlain by 5 cm of coarse sand (mean size 1.0 mm), and a 40 cm surface layer of washed fine sand (mean size 0.25 mm). An overflow port was situated 25 mm above the surface of the fine sand layer. Sewage flowed into the filter through a port 5 mm above, and diametrically opposite to the overflow port, at a rate of 250 ml/min. This was greater than the 210 ml/min outflow required for algal culture and ensured a constant head of liquid over the filter bed. The filter operated for 23½ h/day, the remaining 30 min were employed in backflushing the filter with a $\frac{1}{3}$ hp electric pump, operating through a restriction valve, to prevent build up filtered material at the filter bed surface. Except for regular backflushing no operational problems were evident in the filter system throughout a preliminary 4-week test period and the subsequent 12-week experimental period.

Effluent from the sand filter was divided equally into two flows of 105 ml/min. The first of these flowed directly to the phytoplankton culture of regime B. The second passed through a charcoal column (25 cm length, 8.5 cm internal diameter) constructed from PVC and plexiglass, and painted black to exclude light, before flowing into the phytoplankton culture of regime C.

*Phytoplankton culture.* Each regime included one phytoplankton culture contained in a rectangular plywood tank 2.5 m L × 1.85 m W, fitted with a sloping bottom (*D* increasing from 0.3 to 0.9 m) giving a nominal capacity of 4500 l. Phytoplankton cultures were aerated continuously by means of an air line fixed along the deepest edge of the tank. All cultures were operated on a continuous basis. In the sewage enriched regimes influent rates were 315 ml/min of seawater and 105 ml/min sewage. In the CONTROL regime seawater was added at the rate of 400 ml/min, with an accompanying enrichment of $NH_4Cl$ and $NaH_2PO_4$ in the ratio 5 N : 1 P being added at a rate of 20 ml/min to provide enrichment equivalent to that of the sewage (25% sewage = 250 g at /1 N after dilution). "Harvest" phytoplankton overflowed through a standpipe and by gravity to the shellfish tanks.

Flow rates, cell concentrations and culture temperature were all monitored on daily basis.

Throughout the experimental period no washout of cultures was observed at the dilution rate of 40%/day although cultures occasionally failed. In these latter situations cultures were restarted within 24 h from reserve cultures maintained in 120 000 l outdoor ponds. Cultures were maintained at ambient temperature throughout the study (3—18° C range). The diatom *Phaeodactylum tricornutum* was the dominant species in all regimes throughout this period.

*Oyster culture. Crassostrea gigas* were obtained as 5 mm seed from Sea Salter Shellfish Ltd., Whitstable, Great Britain and maintained in flowing seawater, at ambient temperature, at Woods Hole Oceanographic Institution until commencing the present study. Forty-nine populations, each of 20 individuals (mean live weight 25—30 g), were selected from the parent stock. Each population was transferred to a labelled plastic mesh tray (60 cm × 60 cm × 6 cm, Nestier Corp., Cincinnatti, OH). Four groups of 12 populations were selected at random. Each group was transferred to a separate fiberglass-lined, wooden tank (3 × 0.75 × 0.75 m). The remaining population was sacrificed for assay of initial trace contaminant content as described later in the present text. Each of the tanks containing the experimental oyster populations was supplied with unfiltered seawater ($30^0/_{00}$ salinity or greater), heated to 15° C, at the rate of 8 l/min. Seawater flowed through the trays containing the oysters and to waste via a standpipe. Each tank also received the harvest of one of the previously described phytoplankton cultures (420 ml/min). Thus the four tanks containing the experimental oyster populations were equivalent to regimes A, B, C and CONTROL. Mixing of the tank contents was effected by gentle aeration from a perforated air-line fixed along the bottom of the tank. All tanks and associated pipes were cleaned twice per week or more frequently as required.

*Sampling program*

(i) Sewage effluent. Sewage effluent (secondary treated, sand filtered and

sand-plus-charcoal filtered) was sampled three times per week for both trace metal and organics. Samples for trace metal analysis were collected in an acid-washed, glass beaker and filtered through an acid-leached, 25 mm Gelman A/E glass fibre filter. The filters were stored under desiccation until subsequent analysis of the retained particulate material. The filtrate was stored in L.P.E. bottles at 4° C following acidification (1 ml conc $HNO_3$ per 100 ml filtrate) to await assay.

Four litre quantities of the effluents used in culture regimes A and C were collected three times per week, in hexane washed bottles, and stored at 4° C to await analysis for organic contaminants.

(ii) Shellfish. At intervals of 2 weeks throughout the 24-week duration of the experiment four experimental populations of oysters, one from each of the culture regimes, was removed for contaminant assay. Animals were counted and any mortalities recorded prior to weighing to estimate mean live weight. Of the 20 individuals in each population, five were shucked and freeze-dried to await trace metal assay for Cd, Cr, Cu, Pb, Ni and Zn, five were deep frozen to await assay for Hg, and the remainder were frozen to await assay for organic contaminants. Regime B was not assayed for organic contaminants.

*Contaminant assay: trace metals*

Sewage effluent: Cu, Zn and Cd contents were sufficiently high in the dissolved phase in effluents to be determined directly by flame atomic absorption. Dissolved Ni, Pb, and Cr levels were analyzed using a Perkin-Elmer 303 atomic absorption spectrophotometer equipped with a graphite furnace. Filters containing sewage particulate material were ashed at 550° C for 30 min, boiled with "Ultrex" nitric acid and the leachate volumetrically fixed. Cd, Cr, Cu, Ni, Pb and Zn were subsequently analyzed by the graphite furnace technique described previously.

Oyster tissue: Freeze dried oyster tissue was ashed at 550° C, digested with "Ultrex" nitric acid, filtered and volumetrically fixed for analysis. A dual channel atomic absorption spectrophotometer was used to determine the high salt background effect during analyses by flame. Determinations were attempted using a graphite furnace, but specific salt interferences necessitated the use of salt matched standards which made its use impractical for the freeze-dried samples. Flame analysis showed no background effect for Cu or Zn. Cd, Pb and Ni displayed a signal enhancement with salt. This was corrected by monitoring the background on the secondary channel using a nearby non-absorbing line and electronically subtracting the background from the primary signal. The Cr signal was constantly inhibited above 1000 ppm salt. Since the inhibition cannot be electronically corrected, standards were used containing 1200 ppm of the nitrate species of the major cations.

As Hg is subject to volatilization at low pressure, freeze drying was inade-

quate for sample preservation. Assays were made on fresh or fresh-frozen tissue and results corrected for water content using data collected during freeze drying. Mercury values were obtained using closed system, cold vapor generated atomic absorption preceded by acid—permanganate digestion of the sample. Absolute concentration was measured with digested standards while a two point method of additions was employed to determine any matrix effect.

*Contaminant assay: Hydrocarbons and polychlorinated biphenyls (organics)*

Sewage sample preparation: liquid samples of raw and treated sewage were filtered through precleaned Metrigard superfine glass fiber filters. One liter of the filtrate was then extracted three times with 25-ml portions of hexane. The combined hexane extracts were dried over $Na_2SO_4$, concentrated to about 5 ml and chromatographed on a silica gel column. The solids collected on the glass fiber filters were air dried overnight, weighed and extracted with hexane for 16 h in Soxhlet apparatus. The extract was concentrated to about 5 ml and chromatographed on a silica gel column.

Oyster tissue preparation: about ten oysters were shucked and the soft parts combined and homogenized in a blender. Ten grams of tissue were digested overnight with 4 *N* NaOH at 90° C, and then extracted three times with 15 ml ethyl ether. The combined ether extracts were dried over $Na_2SO_4$, and concentrated to 1 ml. Two ml of hexane were added to the extract and the extract reconcentrated to 1 ml. This hexane extract was chromatographed on a silica gel column.

Silica gel column chromatography: All samples were chromatographed using a 0.9 × 25 cm column filled with 5 g silica (100 mesh, activated to 150° C, and then deactivated with 3% $H_2O$). After the column had been washed with hexane, the sample was added to the top and eluted with 25 ml hexane. The fraction collected (A) contains essentially all the Arochlor 1254 components and the non-aromatic hydrocarbon components. The column was then eluted with 25 ml 6% ethyl ether in hexane. This fraction (B) contained the aromatic hydrocarbon components. Both fractions of each extract were analyzed by gas chromatography.

Gas chromatographic measurements: Determination of polychlorinated biphenyls was performed using a 2-m glass column packed with 3% SE-30 on Chromosorb WHP and a $^{63}Ni$ electron capture detector temperature of 250° C. The carrier gas was 5% methane in argon. Samples were compared with Arochlor 1254 standards to determine concentration.

Petroleum hydrocarbons were determined using a 2-m stainless steel column of 3% Dexsil 300 on Chromosorb WAW and flame ionization detection. Separation conditions were column temperature programming with an inital temperature of 100° C, held for 4 min. The injection port was at 250° C and the manifold at 300° C. The carrier gas was 99.999% helium. Dual columns were used with the flame ionization detector. Samples were concen-

trated 100 times prior to injection and compared with standard concentrations of a normal alkane series $C_{10}$ to $C_{30}$.

## RESULTS

Prior to discussing results of contaminant assay on the experimental populations of oysters it is relevant to note the serial dilution of sewage effluent during the culture regime used in order that contaminant levels recorded in the effluent (Tables I, II, IV and VI) can be related to those obtained for the experimental oysters (Tables III, V and VII). Phytoplankton culture regimes A, B and C were enriched in the ratio 25% sewage effluent : 75% seawater. The resultant culture flowed into the shellfish holding tanks at a rate of 420 ml/min and was diluted by an accompanying flow of seawater of 8 l/min. These flow rates were chosen to effect sufficient dilution of the cultured *Phaeodactylum tricornutum* from a concentration of $1 \times 10^6$ — $5 \times 10^6$ cells/ml to a concentration more equitable with optimum feeding concentrations in *C. gigas*; i.e., $5 \times 10^4$—$2.5 \times 10^5$ cells/ml. However, this dilution decreased the amount of sewage effluent in the shellfish holding tanks to 1.25%. These values are lower than those described for previous waste-recycling studies (e.g., Ryther et al., 1975) where concentrations of effluent in excess of 50% were examined for phytoplankton culture. When

TABLE I

Dissolved (D) and particulate (P) metal concentrations in Cranston sewage effluent during the experimental period. All values (ppb) are the mean of at least three samples within the time period. Regimes are A: secondary treated; B: secondary treated plus sand filtration; C: as for B plus activated charcoal filtration. *Indicates contaminated sample

| Weeks | Phase | Metal and regime | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Cd | | | Cr | | | Cu | | |
| | | A | B | C | A | B | C | A | B | C |
| 1—2 | D | 0.43 | — | — | 3.3 | 1.6 | 1.5 | 8 | 26 | 18 |
| | P | 0.28 | <0.05 | <0.05 | 6.6 | <1 | <1 | 60 | 2.0 | <2 |
| 2—4 | D | 0.51 | — | — | 2.2 | 1.8 | 2.0 | 8 | 11 | 28 |
| | P | 1.4 | <0.05 | <0.05 | 30 | <1 | <1 | 332 | <2 | <2 |
| 4—6 | D | 0.68 | 0.18 | 0.16 | 1.7 | 1.6 | 1.6 | 11 | 10 | 11 |
| | P | 0.65 | 0.13 | 0.08 | 5.7 | <1 | <1 | 53 | <2 | 2 |
| 6—8 | D | 0.43 | 0.34 | 0.22 | 1.4 | 1.2 | 1.3 | 6 | 8 | 20 |
| | P | 0.84 | 0.06 | <0.05 | 4.3 | <1 | <1 | 74 | <2 | <2 |
| 8—10 | D | 0.47 | 0.12 | 0.33 | 1.4 | 1.5 | 1.5 | 11 | 11 | 25 |
| | P | 1.2 | <0.05 | 0.15 | 8.1 | <1 | <1 | 46 | <2 | 4.2 |
| 10—12 | D | 0.47 | 0.12 | 0.33 | 1.4 | 1.5 | 1.5 | 11 | 11 | 25 |
| | P | 1.2 | <0.05 | 0.15 | 8.1 | <1 | <1 | 46 | <2 | 4.2 |

phytoplankton cultures are maintained in a light limited regime there is little to be gained from the use of exceedingly high sewage effluent concentrations for two reasons. (1) Excess nutrient passes through the culture system thus necessitating an efficient cleansing mechanism prior to final discharge; e.g., macrophyte culture. (2) Contaminants associated with the influent sewage will be presented to the cultured organisms in greater concentrations thus increasing the depuration requirement.

*Trace metals in sewage effluent.* Trace metal levels in both the dissolved (D) and the particulate (P) phases of the Cranston effluent for each of the three culture regimes are summarized by sampling intervals in Table I. Detectable levels of mercury (Hg) were evident only in the dissolved fraction.

A comparison of regime A versus regimes B and C (Table I) indicates that sand and charcoal filtration was consistently effective in reducing the concentration of particulate metals; however, consistent decreases in dissolved metal concentrations were only evident for Cd and Cr following identical treatments. Summarizing data for both phases in Table II it is evident that marked decreases in overall metal contents were evident for Cd, Cr, Cu, Pb and Zn. Ni levels were consistently high. This is undoubtedly related to the presence of electroplating plants discharging their effluent in the Cranston treatment facility. Metal concentrations in the effluent used in the present study are still well below maximum permissible levels

| Hg | | | Ni | | | Pb | | | Zn | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | B | C | A | B | C | A | B | C | A | B | C |
| <0.3 | <0.3 | <0.3 | 123 | 129 | 136 | 1.2 | 0.83 | 0.76 | 25 | 31 | 24 |
| — | — | — | 20 | 1.3 | 1.5 | 8.6 | 1.7 | <1 | 30 | 3.7 | 2.0 |
| <0.3 | <0.3 | <0.3 | 99 | 145 | 331 | 2.1 | 0.91 | 0.87 | 23 | 22 | 63 |
| — | — | — | 83 | <1 | 1.7 | 23 | 6.1 | 1.2 | 120 | 2.6 | <2 |
| <0.3 | <0.3 | <0.3 | 156 | 147 | 182 | 0.24 | 0.40 | 0.15 | 33 | 38 | * |
| — | — | — | 15 | 2.0 | <1 | 15 | 4.6 | 1.9 | 24 | 5.6 | <2 |
| <0.3 | <0.3 | <0.3 | 149 | 151 | 191 | 0.09 | 0.19 | 0.06 | 32 | 38 | 36 |
| — | — | — | 38 | <1 | <1 | 8.1 | <1 | <1 | 41 | <2 | <2 |
| <0.3 | <0.3 | <0.3 | 156 | 162 | 166 | 0.40 | 0.09 | 0.12 | 4 | 27 | 38 |
| — | — | — | 19 | <1 | 1.8 | 3.9 | <1 | 1.7 | 39 | <2 | 2.8 |
| <0.3 | <0.3 | <0.3 | 156 | 162 | 166 | 0.40 | 0.09 | 0.12 | 4 | 27 | 38 |
| — | — | — | 19 | <1 | 1.8 | 3.9 | <1 | 1.7 | 39 | <2 | 2.8 |

TABLE II

A comparison of trace metal levels in sewage effluents and seawater from the present study with the data of Jacobs (1973) for Cranston sewage; Mann and Ryther (1979) for Wareham and Cranston sewage, and standards for drinking water and marine waters discharge as set by U.S. Environmental Protection Agency

| | Metal content ppb total | | | | | | |
|---|---|---|---|---|---|---|---|
| | Cd | Cr | Cu | Hg | Ni | Pb | Zn |
| Present study A | 1.42 | 12.36 | 110.3 | <0.3* | 172.7 | 5.46 | 69.0 |
| Present study B | 0.32 | 3.56 | 14.8 | <0.3* | 179.3 | 2.99 | 33.5 |
| Present study C | 0.26 | 2.56 | 23.9 | <0.3* | 196.8 | 1.76 | 46.0 |
| Jacobs (1973) Cranston | — | <20 | 5.3 | — | 358 | <20 | 159 |
| Mann and Ryther Cranston | 0.18 | 7.2 | 14.3 | <0.3 | 61.0 | 3.8 | 15.3 |
| Mann and Ryther Wareham | 0.39 | 3.3 | 58.0 | <0.1 | 9.4 | 1.7 | 56.3 |
| Seawater present study | ** | 0.3 | 2.0 | ** | 3.3 | 1.5 | 3.3 |
| E.P.A. standards 1976 drinking water | 10 | 50 | 1000 | 2 | + | 50 | 5000 |
| Seawater discharge | 5 | + | + | 0.1 | + | + | + |

*Dissolved fraction only.
**Below detection.
— = Not measured.
+ = No standard set.

for drinking water (Table II) suggesting efficient operation of the Cranston sewage treatment in plant.

Examination of the relative metal contents of the seawater and sewage used in the present study (Table II), in conjunction with the relative volumes

TABLE III

Mean metal contents (ppm dry weight) of *Crassostrea gigas* during exposure to sewage enriched food chains (weeks 0—12) and subsequent depuration (weeks 12—24). Regimes: A, secondary effluent; B, as for A plus sand filtration; C, as for B plus activated charcoal filtration; D, CONTROL

| Week | Metal and regime | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cd | | | | Cr | | | | Cu | | | | Hg | | |
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C |
| 0 | | | | 3.7 | | | | 1.2 | | | | 145 | | | |
| 2 | 3.4 | 3.7 | 3.5 | 3.7 | 1.2 | 1.2 | 1.6 | 1.2 | 397 | 131 | 199 | 151 | 0.31 | 0.24 | 0.30 |
| 4 | 4.0 | 3.8 | 3.7 | 4.3 | 1.6 | 1.6 | 1.6 | 1.2 | 272 | 103 | 369 | 379 | 0.32 | 0.49 | 0.31 |
| 6 | 4.6 | 4.1 | 3.5 | 3.6 | 1.7 | 1.3 | 1.6 | 1.3 | 299 | 221 | 74 | 108 | 0.26 | 0.33 | 0.18 |
| 8 | 4.4 | 3.9 | 3.7 | 4.5 | 1.1 | 1.8 | 1.4 | 0.9 | 259 | 84 | 184 | 185 | 0.23 | 0.18 | 0.23 |
| 10 | 3.5 | 2.0 | 4.5 | 4.7 | 1.5 | 1.1 | 1.2 | 1.4 | 159 | 86 | 148 | 212 | 0.22 | 0.18 | 0.24 |
| 12 | 3.2 | 2.3 | 2.6 | 4.5 | 1.2 | 1.0 | 1.3 | 1.6 | 286 | 60 | 156 | 154 | 0.31 | 0.15 | 0.14 |
| 14 | 3.6 | 4.2 | 4.1 | 4.6 | 1.6 | 1.6 | 1.4 | 2.2 | 235 | 110 | 154 | 161 | 0.19 | 0.25 | 0.21 |
| 16 | 3.7 | 3.7 | 3.2 | 4.9 | 0.9 | 2.1 | 1.0 | 1.3 | 300 | 138 | 154 | 182 | 0.36 | 0.28 | 0.60 |
| 18 | 4.0 | 4.4 | 4.5 | 5.6 | 1.8 | 4.3 | 0.9 | 1.4 | 222 | 133 | 171 | 140 | 0.33 | 0.25 | 0.12 |
| 20 | 3.8 | 3.7 | 3.1 | 4.2 | 2.2 | 1.1 | 0.9 | 1.7 | 251 | 108 | 98 | 148 | 0.22 | 0.18 | 0.17 |
| 22 | 3.3 | 3.1 | 3.3 | 3.3 | 1.2 | 1.0 | 0.8 | 0.8 | 317 | 74 | 120 | 110 | 0.28 | 0.17 | 0.14 |
| 24 | 3.8 | 1.9 | 2.6 | 3.0 | 0.7 | 0.9 | 0.8 | 0.7 | 180 | 110 | 77 | 138 | 0.25 | 0.10 | 0.16 |

of each presented to the experimental oysters, reveals that in all instances it was the seawater, not the sewage effluent, that contributed the major proportion of the total quantity of metal in all regimes. Consequently little variation would be expected in the metal contents of oysters from each regime. This is in fact the case in the present study.

*Trace metals in Crassostrea gigas.* The U.S. Food and Drug Administration (F.D.A.) "alert" levels (see Isaac and Delaney, 1972) for Cd in *C. virginica* is 3.5 ppm wet weight (ca. equal to 23.3 ppm dry weight). Levels for *C. gigas* in the present study vary between 1.9 and 5.6 ppm dry weight with no significant differences being evident between the experimental regimes, and either before or during "depuration". Cd exhibits cumulative retention in human liver and kidney tissue, and is causative of neurological disorders; however, the present data suggest no public health hazard from Cd contamination in the present instance.

The presence of Cr, like Cd, in marine waters is mainly derived from pollution associated with the electroplating industry. Hexavalent Cr is toxic when inhaled, however, the toxicity of ingested Cr (tri or hexavalent) is not known. Recorded Cr levels in *C. gigas* vary in the range 0.7—2.2 ppm dry weight and exhibit no marked differences temporally or between culture regimes. All values are below the F.D.A. alert levels for Cr in *C. virginica* (2.0 ppm wet weight, ca. equal to 13.3 ppm dry weight).

Levels of Cu in *C. gigas* were generally higher in regime A (159—397 ppm dry weight) than regimes B, C and CONTROL (77—221 ppm dry weight with the exception of CONTROL week 4). No temporal pattern was evident suggesting that "depuration" did not occur. The elevated levels in regime A are probably related to elevated Cu levels in the sewage effluent used in this regime (Tables I and II); however, the magnitude of the difference between

| Metal and regime | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hg | Ni | | | | Pb | | | | Zn | | | |
| D | A | B | C | D | A | B | C | D | A | B | C | D |
| 0.20 | | | | 1.2 | | | | <0.02 | | | | 1050 |
| 0.20 | 1.2 | 3.6 | 3.8 | 1.9 | 1.6 | 0.11 | <0.02 | <0.02 | 1351 | 858 | 1152 | 1070 |
| 0.24 | 3.2 | 0.7 | 1.6 | 1.8 | 0.85 | 0.78 | <0.02 | <0.02 | 1160 | 798 | 1577 | 1509 |
| 0.21 | 5.3 | 1.3 | 1.7 | 1.4 | <0.02 | 0.32 | 0.29 | <0.02 | 1463 | 1074 | 938 | 1063 |
| 0.28 | 8.4 | 2.2 | 0.7 | 5.4 | <0.02 | 1.9 | <0.02 | <0.02 | 1250 | 874 | 1290 | 1296 |
| 0.15 | 1.1 | 0.8 | 1.4 | 0.9 | 0.67 | 0.74 | 0.34 | 0.48 | 1005 | 731 | 1053 | 1196 |
| 0.17 | 1.0 | 0.9 | 0.6 | 4.6 | 0.89 | 0.60 | 0.39 | 0.53 | 1138 | 543 | 855 | 1138 |
| 0.37 | 1.3 | 1.2 | 1.0 | 1.6 | 0.98 | 0.53 | <0.02 | 0.85 | 1022 | 809 | 878 | 1089 |
| 0.23 | 7.0 | 3.4 | 0.7 | 3.0 | <0.02 | 0.60 | 0.61 | 0.20 | 1119 | 879 | 825 | 881 |
| 0.17 | 1.3 | 1.4 | 0.7 | 1.0 | 0.28 | 0.35 | 0.73 | 0.29 | 892 | 736 | 810 | 745 |
| 0.26 | 0.8 | 0.9 | 0.6 | 0.7 | 0.11 | 0.23 | 0.23 | 0.05 | 928 | 652 | 570 | 839 |
| 0.14 | 0.6 | 0.6 | 0.3 | 0.4 | 0.60 | 0.79 | 0.36 | 0.23 | 1045 | 551 | 610 | 674 |
| 0.14 | 0.2 | 0.5 | 0.4 | <0.03 | <0.02 | 0.36 | 0.30 | 0.23 | 792 | 549 | 468 | 595 |

the oyster groups is reflective of the considerable dilution of the sewage as previously discussed. All recorded levels for oyster tissues are below the F.D.A. alert level of 175 ppm wet weight (ca. 1167 ppm dry weight).

Alkyl mercury compounds are very toxic to man (lethal dose 20—30 mg). Thus, acceptable Hg levels in foodstuffs are particularly stringent (0.5 ppm wet weight, ca. 3.3 ppm dry weight in shellfish). Levels recorded for *C. gigas* in the present study are in the range 0.10—0.49 ppm dry weight.

No limits have been set for permissible levels of Ni in either drinking water or shellfish as Ni is considered to be of low toxicity to man. A single value of

TABLE IV

Hydrocarbon levels in sewage effluent enrichment in regimes A and C during weeks 0—12. *X*: alkane fraction $C_{20}$—$C_{30}$, *Y*: aromatic fraction $C_{20}$—$C_{30}$, all values in ppb and for *particulate phase only*, liquid fraction consistently below detection limit (0.1 ppb)

| Week | Date | Regime A | | | Regime C | | |
|---|---|---|---|---|---|---|---|
| | | *X* | *Y* | *X* + *Y* | *X* | *Y* | *X* + *Y* |
| 0 | | | | | | | |
| | 9/22 | 0.5 | * | 0.5 | * | * | * |
| | 9/26 | 1.5 | 6.1 | 7.6 | 3.9 | 4.5 | 8.4 |
| | 9/28 | 3.9 | * | 3.9 | 1.4 | * | 1.4 |
| | 9/30 | 36.5 | 19.4 | 55.9 | 3.6 | * | 3.6 |
| | 10/3 | 20.3 | 70.5 | 90.8 | 5.0 | 0.8 | 5.8 |
| 2 | | | | | | | |
| | 10/5 | 16.8 | 11.4 | 28.2 | 0.2 | 0.1 | 0.3 |
| | 10/7 | 0.5 | 1.7 | 2.2 | 0.1 | * | 0.1 |
| | 10/10 | 10.8 | 4.5 | 15.3 | * | 0.1 | 0.1 |
| | 10/12 | 0.1 | 2.2 | 2.3 | * | 0.2 | 0.2 |
| | 10/14 | 2.3 | 8.1 | 10.4 | 4.8 | 0.5 | 5.3 |
| | 10/17 | 0.6 | 7.1 | 7.7 | 0.4 | 0.5 | 0.9 |
| 4 | | | | | | | |
| | 10/19 | 0.3 | 17.1 | 17.4 | 0.8 | * | 0.8 |
| | 10/21 | 0.8 | 12.1 | 12.9 | — | — | — |
| | 10/24 | 0.3 | 2.2 | 1.5 | * | * | * |
| | 10/26 | 0.6 | 1.3 | 1.9 | * | 0.6 | 0.6 |
| | 10/28 | 1.0 | * | 1.0 | * | 3.3 | 3.3 |
| | 10/31 | * | 1.3 | 1.3 | 0.3 | 1.2 | 1.5 |
| 6 | | | | | | | |
| | 11/2 | 1.7 | 4.8 | 6.5 | 0.4 | 1.6 | 2.0 |
| | 11/4 | 2.3 | 3.9 | 6.2 | * | 1.3 | 1.3 |
| | 11/7 | 2.6 | 4.2 | 6.8 | 3.2 | 6.9 | 10.1 |
| | 11/9 | 5.1 | 1.5 | 6.6 | 1.7 | 0.6 | 2.3 |
| | 11/14 | 7.3 | 7.5 | 14.8 | * | 0.2 | 0.2 |
| 8 | | | | | | | |
| | 11/16 | 2.2 | 3.5 | 5.7 | 24.8 | 2.5 | 27.3** |
| | 11/23 | 9.0 | 8.4 | 17.4 | * | 0.3 | 0.3 |
| 10 | | | | | | | |
| 12 | 11/30 | 3.9 | 8.2 | 12.1 | — | — | — |

*Below detection limit.
**Suspected contamination.

8.4 ppm dry weight was recorded in *C. gigas* in regime A at week 8. Excepting this one instance all values were below 5.4 ppm dry weight, recorded in the control population at week 8.

Pb levels in *C. gigas* varied in the range 0.02—1.9 ppm dry weight but exhibited no obvious temporal pattern or differences between experimental regimes. The F.D.A. "alert" levels for oysters is particularly stringent at 2.0 ppm wet weight (13.3 ppm dry weight) as Pb is notably toxic causing gastro-intestinal problems, anorexia, abdominal pain, paralysis and anaemia.

Zn is considerably less toxic than Cd, Cr, Hg or Pb. Adult humans require 10—15 mg/day. Recorded levels for *C. gigas* of 468—1577 ppm dry weight are well below the shellfish alert level of 2000 ppm wet weight (13 300 ppm dry weight).

*Hydrocarbons in sewage effluent.* Hydrocarbon concentrations in the liquid fraction were consistently below detection. In the particulate fraction (Table IV) it is evident that a consistent and considerable decrease in concentration in both the alkane (*X*) and aromatic (*Y*) fractions is evident following passage of the effluent through a sand filter and activated charcoal column. Temporal variability in the secondary effluent (regime A) is related to changes in suspended sediment loading; however, this variability is damped by the supplementary treatment applied to regime C. Overall levels are generally low, rarely exceeding 20 ppb in regime A and, with one exception, 10 ppb in regime C.

TABLE V

Hydrocarbon levels in *C. gigas* in regimes A, C and CONTROL. Week 0—12, sewage enrichment in regimes A and C. Weeks 12—24, sewage free regime. *X*: alkane fraction $C_{20}$—$C_{30}$; *Y*: aromatic fraction $C_{20}$—$C_{30}$. All values in ppb wet weight

| Week | A | | | C | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|
| | *X* | *Y* | *X+Y* | *X* | *Y* | *X+Y* | *X* | *Y* | *X+Y* |
| 0 | 4.4 | 7.8 | 13.2 | | | | | | |
| 2 | 2.0 | 5.0 | 7.0 | 0.8 | 1.0 | 1.8 | 0.6 | 1.9 | 2.5 |
| 4 | * | 7.2 | 7.2 | * | 0.7 | 0.7 | 2.4 | 8.0 | 10.4 |
| 6 | 0.3 | 2.3 | 2.6 | * | * | * | 2.5 | 1.0 | 3.5 |
| 8 | * | 1.2 | 1.2 | 1.5 | 2.4 | 3.9 | 6.8 | 2.0 | 8.8 |
| 10 | * | * | * | 0.9 | 1.6 | 2.5 | 1.9 | 16.5 | 18.4 |
| 12 | 0.4 | 43.1 | 43.5 | 1.1 | 6.2 | 7.3 | * | 0.1 | 0.1 |
| 14 | 2.4 | 2.8 | 5.2 | * | 1.5 | 1.5 | 0.3 | 0.5 | 0.8 |
| 16 | 4.3 | 13.8 | 18.1 | 0.2 | 7.0 | 7.2 | 0.2 | 0.3 | 0.5 |
| 18 | 8.9 | 4.8 | 13.7 | 2.9 | 7.3 | 10.2 | 4.4 | 5.3 | 9.7 |
| 20 | 0.5 | 9.7 | 10.3 | 9.2 | 3.1 | 12.3 | 8.0 | 17.4 | 25.4 |
| 22 | 2.8 | 3.7 | 6.5 | 1.7 | 3.0 | 4.7 | 2.8 | 5.3 | 8.1 |
| 24 | 0.4 | 5.9 | 6.3 | 1.9 | 0.7 | 2.6 | 1.3 | 4.4 | 5.7 |

*Below detection (0.1 ppb).

*Hydrocarbons in Crassostrea gigas.* Three major conclusions can be made from the data given in Table V. They are: (i) no trends are evident to suggest increasing hydrocarbon content in a sewage enriched regime or depuration of hydrocarbon content on transfer of oysters from a sewage-enriched to a clean regime; (ii) temporal fluctuations in all the regimes examined (A, C and CONTROL) appear to be random events; (iii) hydrocarbon levels are comparable in oysters grown in all three regimes suggesting that the measured quantities are, in fact, background levels inherent to the experimental stock and are not related to accumulation of hydrocarbon introduced with the sewage effluent enrichment.

*Polychlorinated biphenyls in sewage effluent.* The particulate phase consistently contributed the greater proportion of the total polychlorinated bi-

TABLE VI

P.C.B. levels in sewage effluent enrichment in regimes A and C during weeks 0—12. All values in ng/l effluent as Arochlor 1254

| Week | Date | Regime A | | Regime C | |
|---|---|---|---|---|---|
| | | Particulate | Aqueous | Particulate | Aqueous |
| 0 | 9/22 | 13 | <8 | 11 | <10 |
| | 9/26 | 122 | <1.2 | 22 | <1.3 |
| | 9/28 | 71 | <0.4 | <4 | <1.7 |
| | 9/30 | 1204 | <1.0 | <7 | <1.2 |
| | 10/3 | 1155 | <1.6 | <7 | <1.6 |
| 2 | 10/5 | 1575 | <9 | 22 | <2 |
| | 10/7 | 50 | <4 | — | <2 |
| | 10/10 | 94 | <4 | 33 | <8 |
| | 10/12 | 57 | <8 | 40 | <8 |
| | 10/14 | 118 | <9 | 21 | <8 |
| | 10/17 | 22 | <2 | 10 | <2 |
| 4 | 10/19 | 42 | <3.4 | 14 | <9 |
| | 10/21 | 60 | <5 | — | — |
| | 10/24 | 154 | <5 | 20 | <10 |
| | 10/26 | 74 | <5 | 14 | <9 |
| | 10/28 | 61 | <9 | 20 | <9 |
| | 10/31 | 44 | <9 | 25 | <9 |
| 6 | 11/2 | 48 | <8 | 14 | <2 |
| | 11/4 | 71 | <4 | 17 | <2 |
| | 11/7 | 115 | <2.1 | <6 | <1.7 |
| | 11/9 | 229 | <3.1 | <7 | <1.7 |
| | 11/14 | 91 | <1.2 | — | <2 |
| 8 | 11/16 | 88 | <6 | 20 | <5 |
| | 11/23 | 187 | <10 | 40 | <2 |
| 10 | 11/30 | 97 | <5 | — | — |
| 12 | | | | | |

TABLE VII

P.C.B. levels in *C. gigas* in regimes A, C, and CONTROL. Weeks 0—12; sewage enrichment in regimes A and C. Weeks 12—24; sewage-free. All values are given as ppb wet weight Arochlor 1254

| Week | A | C | CONTROL |
|---|---|---|---|
| 0 | 51 | | |
| 2 | 27 | 28 | 17 |
| 4 | 19 | 27 | 20 |
| 6 | 14 | 22 | 23 |
| 8 | <2 | 27 | 49 |
| 10 | <2 | 17 | 25 |
| 12 | 24 | 34 | 9 |
| 14 | 51 | 89 | 29 |
| 16 | 63 | 48 | 66 |
| 18 | 79 | 66 | 40 |
| 20 | 22 | 72 | 33 |
| 22 | 47 | 17 | 78 |
| 24 | 48 | 56 | 11 |

phenyls in the sewage effluent used in both regimes A and C throughout the study (Table VI). Polychlorinated biphenyl concentrations were consistently higher in the particulate phase in regime A, but concentrations in the aqueous phase were comparable for both regimes. The U.S. Environmental Protection Agency 1976 drinking water standards' maximum permissible level of 1 mg/l is consistently exceeded by effluents used in both regimes A and C.

*Polychlorinated biphenyls in Crassostrea gigas.* (Table VII). The three conclusions stated with respect to hydrocarbons in *C. gigas* are equally applicable to the results of polychlorinated biphenyl assay in the experimental *C. gigas.* There is no evidence of any accumulation and/or depuration throughout the time course of the experiment, random temporal variation occurs in the recorded levels, and no consistent differences are evident between the three regimes. Recorded polychlorinated biphenyl concentrations are well below the proposed maximum tolerance of 2 ppm wet weight (U.S. Federal Register April 1, 1977).

## DISCUSSION

The data of the present study indicate that the oysters cultured in a waste recycling aquaculture system under the regimes described herein consistently exhibit low, sewage effluent derived contaminant levels. This is perhaps, particularly interesting in that bivalve molluscs have often been selected as environmental pollution indicators due to their supposed ability to accumulate pollutants. It should, however, be noted that, unlike in natural sys-

tems, the bivalves in the present study were not cultured at a sediment—water interface. In natural seawater systems subjected to heavy metal pollution sediments often form an important sink for contaminants (see Chen et al., 1974; Rohatgi and Chen, 1975). Consequently the elevated levels of metal contaminants recorded in polluted environments may be more representative of the bivalve—sediment relationship than the bivalve—water column relationships.

The U.S. Food and Drug Administration "alert" levels for trace metal contamination are derived from data collected in surveys of trace metal contaminants in selected shellfish species collected from both coasts of the United States. Data are compounded by species with emphasis being placed on *Crassostrea virginica* and *Mercenaria mercenaria* as these are major commercial products. Neither of these species was examined in the present study as both had been previously examined for growth potential and found to be of little value in waste recycling systems (Mann and Ryther, 1977). Throughout the preceeding text the assumption has been made that data derived for these East Coast species, *C. virginica*, could be comparable to those for *C. gigas* as their ecological niches are very similar.

The data of the present study are considerable and consistent in suggesting that public helath hazards directly attributable to the trace metal and organic contaminants in oysters grown in a waste recycling—aquaculture system of the type described herein are minimal. However, this should not be taken to mean that in certain circumstances metal contamination would not be severe, or that other contaminant-related, biological or physical problems would not be encountered which would present major obstacles to the eventual on-site application of waste recycling technology. For example, the present data indicate that the Cranston sewage treatment plants are operating efficiently and producing effluent that is well within acceptable standards as defined by the U.S. Environmental Protection Agency; such a situation is undoubtedly not universal. The consistently low contaminant levels in both effluents and cultured organisms indicates the importance of maintaining high quality, low contaminant effluent for application in the present food chain.

Aside from the public health problems associated with trace metals and organic contaminants there remains the question of survival of human enteric viruses in the present culture system. Preliminary studies suggest that this problem is surmountable (R. Mann, J.M. Vaughn and E.F. Landry, unpublished data, 1980); however, a definitive statement on virus survival cannot, as yet, be provided.

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