1975

The Effect of Low O2 Levels on the Filtration Efficiency and Pumping Rate of Crassostrea virginica

John F. Quensen
College of William and Mary - Virginia Institute of Marine Science

THE EFFECT OF LOW O_2 LEVELS ON THE FILTRATION EFFICIENCY AND PUMPING RATE OF *CRASSOSTREA VIRGINICA*

A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

John F. Quensen, III

1975

APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts

John F. Quensen, III

Approved, August, 1975

Dexter S. Haven, M.S.

Joseph G. Loesch, Ph.D.

Robert E. Black, Ph.D.

Marvin L. Wass, Ph.D.

Donald F. Boesch, Ph.D.

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ACKNOWLEDGMENTS

The author would like to express sincere appreciation to Dexter S. Haven under whose guidance this study was conducted. Appreciation is also expressed to Robert E. Black, Donald F. Boesch, Joseph G. Loesch, Marvin L. Wass, and J. Ernest Warinner for their helpful suggestions and review of the manuscript. Additional thanks are extended to J. G. Loesch and Frank J. Wojcik for help with the statistics and computer programming and to Bob Bendl for help with the construction of some of the equipment. Thanks also to Mrs. Linda Jenkins for her assistance in the preparation of the final manuscript and to Miss Rosalie Vogel for taking care of the copying and binding.

This study was supported by the Virginia Institute of Marine Science and RANN/NSF grant number GI 38973.

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ABSTRACT

The filtration efficiency (FE) and pumping rate (PR) of the American oyster Crassostrea virginica were determined at five levels of dissolved oxygen: 100%, 50%, 25%, 12%, and 6% saturation. Determinations were made in flowing York River water at temperatures between 18.5 and 26.0°C under naturally fluctuating conditions of salinity and particle concentration. Four determinations of FE and PR were made at each O_2 level on each of seven oysters. FE was calculated for 11 size ranges of particles between 1.00 and 12.6 µm in diameter. The PR was calculated by dividing the FE into the filtration rate.

FE increased with increasing particle size up to four to five µm in diameter. There was little difference in FE between O_2 levels of 100%, 50%, and 25% saturation. At 12% O_2 saturation, the average FE was reduced to 68.5% of the control's FE, and to 59.9% of the control's FE at 6% O_2 saturation. There was no interaction between particle size and O_2 level. But since the FE for smaller particles (<4 µm in diameter) was low at 100% O_2 saturation, the effect of low O_2 levels was to reduce the FE of these smaller particles by a greater percentage.

There was no significant difference in PR between O_2 levels of 100%, 50%, 25%, and 12% O_2 saturation. The PR at 6% O_2 saturation was 65.0% of the control's PR.

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INTRODUCTION

Low dissolved oxygen levels often limit the distribution of aquatic organisms. This is particularly true of benthic species, since their mobility is limited, and the oxygen level is lowest near the bottom. The effect of low oxygen level on the distribution, growth, and survival of commercially important species such as Crassostrea virginica is of particular interest.

There is already evidence that the low levels of dissolved oxygen that often occur in the Chesapeake Bay and its tributaries during the warmer months may adversely affect the survival of molluscs including oysters. Low oxygen levels have been cited as the cause of mass mortalities of oysters and low sets of spat (Haven, personal communication; Hewatt, 1945-47, 1953). More recently, experiments with small oysters have demonstrated that at temperatures between 22 and 24°C and O_2 concentrations of 0.2 ml/l (approximately 2.5% saturation), 50% mortality occurred after 8 to 10 days, and 100% mortality after 10 to 13 days. These experiments also demonstrated that biodeposition rates were reduced by about 40% at O_2 levels of approximately 10% saturation (Haven and Bendl, 1975).

During July, August, and September the bottom waters of some portions of the Chesapeake Bay and its tributaries become nearly depleted of dissolved O_2 (Hires, Stroup, and Sietz, 1966; VIMS Hydrographic Data, 1964-1974, unpublished). At depths below 9M, O_2 levels

as low as 0.2 ml/l are common along the Eastern Shore of the Bay and in the Potomac and Rappahannock Rivers. Oxygen is sometimes undetectable at the same depths north of the Potomac River. Unless careful consideration is given to the future siting of sewage discharges, their added organic loads will cause further oxygen depletion in the Bay and its tributaries. Dredging causes similar hazards when reduced sediments are stirred up into the water column.

Much general knowledge exists, but there is a lack of information relating specifically to the oyster and to sublethal effects of low dissolved oxygen on it. This study investigated the effect of various oxygen levels on two parameters, pumping rate (PR) and filtration efficiency (FE) for particles of different sizes. The pumping rate is the rate of water transport through the gills of the oyster. The filtration efficiency is the percentage of particles in the water pumped through the oyster that is retained by the gills. The reason for selecting these two parameters was that if low oxygen levels reduce either the PR or the FE, then the feeding ability of the oyster is also impaired.

Literature Review

A summary of Nelson's (1960) description of the morphology of oyster gills will aid the understanding of how filtration of particles from the water occurs, and how low oxygen tensions may affect the FE and PR. The gills are made up of rows of filaments that form sheets, or lamellae. In the adult oyster the lamellae are folded into a series of plicae, each containing 8 to 15 filaments. The filaments

connecting adjacent plicae are larger than the other filaments and are known as the principal filaments. They contain blood vessels and chitinous rods that support the gill (Figure 1).

Pairs of lamellae are united along their free edge so that a pair forms half of a gill, or demibranch. Each oyster has four demibranchs, or two gills. The lamellae of a demibranch are connected by interlamellar septae approximately every fourth principal filament. The space between lamellae is thus divided into a series of water tubes which lead to the cloacal or promyal chamber (Figure 2).

Each of the filaments in a plica has five tracts of cilia, four of which are paired (Figure 3). On the outer surface of the filament is the unpaired tract of frontal cilia bordered by tracts of fine frontal cilia. On the sides of the filaments are the large, paired latero-frontal cilia and the lateral cilia. Between adjacent filaments are rows of openings (ostia) to the interior of the plica which is continuous with the water tubes (Figure 1). The lateral cilia create the pumping current, forcing water through the ostia, into the plicae, and then into the water tubes. The water then moves through the water tubes, aided by abfrontal cilia on the principal filaments, and into the cloacal or promyal chamber. The water then exits from the posterior side of the oyster (Figure 2).

The large latero-frontal cilia form a meshwork between the filaments, thus screening out particles which are passed to the fine frontal and frontal cilia. The frontal cilia transport the particles to food collecting furrows which lead to the palps. There particles are sorted and either rejected or passed into the mouth.

The effects of sublethal oxygen levels on molluscs has not been fully investigated. Low O_2 levels may affect molluscs by lowering ciliary activity. Aiello (1960) showed that below an O_2 tension of 37 mm Hg (23% saturation) the rate of ciliary beat on Mytilus edulis gill is directly proportional to the O_2 concentration. Different ciliary tracts in the oyster are responsible for creating the pumping current, straining particles from the water, sorting out potential food particles, and moving ingested material through the digestive tract (Galtsoff, 1964).

Most present knowledge of the effect of O_2 tension on PR is from studies of the regulation of O_2 consumption. The O_2 consumption of Ostrea edulis was found to be 8.42-10.52 cc O_2/hr/gm wet weight at 24.5-24.6°C (Galtsoff and Whipple, 1930). This O_2 consumption is maintained at a constant rate down to a critical O_2 tension (P_c) of 88 mm Hg (Galtsoff and Whipple, 1930) or 100 mm Hg (Prosser and Brown, 1961). Presumably when the O_2 tension is lower than 88 to 100 mm Hg (55 to 63% saturation) the oyster is adversely affected in some way. Mytilus perna and M. edulis were found to increase their PR as the O_2 tension declined to P_c, below which point the PR declined (Bayne, 1967 and 1971). Walsh (1974) found Mercenaria mercenaria could regulate its O_2 consumption the same way. Octopus was found to increase its PR during exposure to low O_2 tensions (Prosser and Brown, 1961). However, Galtsoff and Whipple (1930) found no difference in the PR of O. edulis at O_2 tensions of near saturation and 12% saturation. Since the cilia responsible for creating the pumping current in bivalves require O_2 in order to function (Aiello, 1960; Gray, 1924; Wilbur and Yonge, 1966), it is to be expected that very low O_2 tension would have the effect of reducing the PR.

Several investigators have reported varying FE's in bivalves including C. virginica (Chipman and Hopkins, 1954; Dral, 1967; Hamwi and Haskins, 1969; Jørgensen, 1966; Loosanoff and Engle, 1947; MacGinitie, 1941; Nelson, 1960; Tammes and Dral, 1955). Sometimes these variations have been attributed to the presence or absence of MacGinitie's (1941) mucus sheet (Jørgensen, 1966). It seems reasonable, in light of more recent work refuting the existence of mucus sheets in bivalves (Jørgensen, 1949, 1966; Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970), that the variation in FE must be attributed to other causes. One cause may be changes in the width of the interfilamentary slits of the gills (Rice and Smith, 1958; Wilbur and Yonge, 1966). The size of these slits, or ostia, can be varied by muscular contraction, and probably by the amount of blood filling the interfilamentary junctions (Elsey, 1935). If the ostia become too wide, the latero-frontal cilia would no longer form as effective a mesh in front of them. Dral (1967) explained that the FE of M. edulis may be varied "by shifting the range of the beat of the latero-frontal cilia toward the frontal side...so that these cilia no longer bridge the ostia, and suspended particles have a chance to pass the free space thus formed." Also the beat of the latero-frontal cilia may become uncoordinated or stop in a position that would allow the passage of particles through the ostia (Dral, 1967).

Considering these studies, one might theorize that low O_2 levels could influence FE in two ways. Since O_2 is required for the maintenance of ciliary activity (Aiello, 1960; Gray, 1924; Wilbur and Yonge, 1966), the beat of the latero-frontal cilia may become uncoordinated or stop, thus reducing the FE. However, if the O_2 level is

low enough to have this effect on the latero-frontal cilia, it would be expected to have the same effect on the lateral cilia that create the pumping current, thus causing the PR to decline. The mollusc may also attempt to maintain its PR in low O_2 water in order to maintain its rate of O_2 consumption. One way it may do this is by increasing the width of the ostia so that the gill offers less resistance to the passage of water through it. This would also decrease the FE (Atkins, 1943; Elsey, 1935; Jørgensen, 1955 and 1966; Wilbur and Yonge, 1966).

For this study, it was desirable to use a technique which would allow the simultaneous determination of PR and FE without disturbing the oysters. A literature search showed that none of the techniques described was entirely satisfactory.

Several direct methods of determining PR are described in the literature. Galtsoff's (1964) carmine cone method was judged unsatisfactory since it imposes the unnatural condition of having a glass tube inserted between the valves. This prevents the closing of the valves by which means the oyster can normally regulate the amount of water it pumps. It is also probable that the presence of the tube in the cloacal chamber irritates the animal. The values Galtsoff obtained using this method (a maximum of 3.9 l/hr at 25°C for one oyster) were probably too low (Wilbur and Yonge, 1966). Galtsoff's (1964) rubber dam apron method apparently gives a better estimate of the PR of oysters, but was not used because of the unreliable FE obtained by Loosanoff and Engle (1947) when they used this method in a filtration study. It is possible that the apron irritated the oyster so that a normal FE was not found (Jørgensen, 1966), or that particles of feces collecting in the apron were washed out by the exhalant current and

counted as unfiltered particles. The third direct method of determining PR in molluscs is the dye method (Coughlan and Ansell, 1964). The rate at which a dye solution is introduced into the inhalant siphon is increased until it matches the PR, and the dye curls back from the siphon. This method applies only to siphonate species, and therefore could not be used in this study.

Indirect methods of determining the PR involve measuring the rate at which particles are removed from the water. This method actually measures the filtration rate (FR) rather than the PR. The FR is not equal to the PR unless the particles being counted are retained with 100% efficiency. FR and PR are related by the formula:

$$PR = \frac{FR}{FE}$$

The FR is an unreliable estimate of the PR since the FE can vary unpredictably (Hamwi and Haskins, 1969); moreover, part of this study was concerned with the possible change in FE between O_2 levels. But since the FE and FR could be determined simultaneously, a corrected PR could be calculated from the above relationship. This correction technique has not been used previously.

The FR may be determined in a closed (Jørgensen, 1943) or flowing water system (Walne, 1972), but the latter is preferable since it avoids the accumulation of metabolites and recirculation of filtered water through the mollusc. It is necessary that the rate of flow be greater than the PR before these advantages hold (Walne, 1972).

The majority of filtration studies reported in the literature concern either the FR or the relative FE of different size particles. The determination of the true FE as desired in this study required the

sampling of the exhalant water. VanDam (1954) used siphon tubes to sample the exhalant water of scallops, and a modification of his method was used in this study.

The determination of both FE and FR required a means of determining the concentration of particles in the incurrent, excurrent, and through overflow samples collected. Most of the earlier investigators used photometric means (Jørgensen, 1943), but this necessitated using high particle concentrations. Colloidal graphite, kaolinite, or algae cultures were generally added to the water. The use of radioactive algae allows the use of more natural cell concentrations (Rice and Smith, 1958), but has the disadvantage that cultures of radioactive plankton must be maintained. The Coulter electronic particle counter used in the most recent studies (Haven and Morales-Alamo, 1970; Vahl, 1972a,b; Walne, 1972) is advantageous since the naturally occurring particles in the water may be counted, and was the method used in this study.

MATERIALS AND METHODS

The basic design of this experiment was to hold oysters in troughs of flowing York River water and to measure their PR and FE as influenced by various levels of dissolved oxygen. For each of seven oysters, four determinations of PR and FE were made at each of five oxygen levels (100%, 50%, 25%, 12%, and 6% saturation). For each of these preceeding five levels, FE's were calculated for 11 size ranges of particles between 1.00 and 12.60 μm in diameter.

Since this study was carried out in the fall, winter, and early spring (September, 1974 through April, 1975), the river water was warmed to between 18.5 and 26.0°C. Salinity ranged from 12.6 to 24.0 o/oo. The following variables were monitored throughout the study: temperature, salinity, concentration of particles in the water (ΣV_i, where V_i equals the volume of particles in channel i of the Coulter Counter), rate of flow of water through the troughs, and the percent O_2 saturation. Multiple linear regression was used to determine if any of these variables or the dry weights of the oysters had any effect on the FE or PR. Analysis of variance was used to compare mean PR's and FE's between O_2 levels.

A diagram of the apparatus used is given in Figure 4. Water was pumped from the York River through PVC pipes to a constantly overflowing trough (A) in the laboratory. The water flowed by gravity through a heat exchanger (B) to a second constantly overflowing trough (C). The upper end of this trough was divided into compartments by

three baffles (D). Air stones (E) were placed in each compartment, and vigorous aeration agitated the water enough to avoid supersaturation with gasses. Water siphoned from just below the last baffle flowed into the tops of two PVC O_2 stripping columns (F). The nitrogen, bubbled into the bottoms of the columns by means of air stones, thus moved counter current to the water and stripped the water of dissolved O_2 (Silver, Warren, and Doudofroff, 1963). The rate of nitrogen flow, determined by the flowmeters (G) and valves (H) determined the O_2 level of the water flowing through the valves (I) into the control and experimental troughs (J). The control trough, containing only an oyster shell, was necessary to determine the rate at which particles settled out as the water flowed through the troughs. The average fraction of particles not settling out was used in calculating the FR (cf. page 14). The troughs measured 35 X 8.5 X 12 cm high, and were designed to create a laminar flow of water past the oysters. Water from each trough was siphoned into a 125 ml aspirator bottle where its O_2 content was monitored with a Yellow Springs Instruments O_2 probe and meter.

Oysters between 9.5 and 10.2 cm in length were obtained from the Poropotank River and kept in trays suspended in the York River until needed. They were acclimated to the experimental temperature (18.5-26.0°C) by keeping them in the warm water trough (C in Figure 4) for at least two weeks. Widdows and Bayne (1971) determined that two weeks was sufficient time for M. edulis to become acclimated to such a temperature change.

After the acclimation period, one oyster was placed in each of the four experimental troughs with its exhalant current facing

downstream to minimize turbulence caused by the exhalant current. The flow rate and O_2 content of the water were then adjusted to the desired levels. The O_2 levels used were 100%, 50%, 25%, 12%, and 6% saturation. The O_2 level was changed gradually over a period of one or two hours since it was found that if the O_2 level declined more rapidly the oyster would usually close. After an additional hour to allow the O_2 level to stabilize, determinations of the FE and PR were begun.

To calculate the FE or FR of a mollusc, some means of measuring the concentration of particles in the water before and after filtration was necessary. The Coulter Counter Model T_A used in this study is capable of rapid and accurate determinations of the concentrations of different size particles simultaneously. This allowed the use of naturally occurring particles in the water, and the FE's for different size ranges of particles could still be calculated.

The Coulter Counter distinguishes between particles of different volumes rather than diameters. Thus the ranges of equivalent diameters for the particles in each channel, as listed in Table 1, are actually the diameters of spheres having the same volumes.

The readout on the Model T_A Coulter Counter gives the total number of particles counted and the percentage by volume in each channel. It was therefore necessary to estimate the number of particles in each channel in order to calculate a FE for each particle size range. The equations derived for this purpose are in Appendix I.

The determination of the true FE necessitated the sampling of the exhalant water to determine the concentration of particles in it. Attempts to measure respiration in scallops by sampling the exhalant water with a syringe or siphon tube (VanDam, 1954) suggested

that strict precautions must be taken to insure sampling only the exhalant water. Aquarium water cannot be drawn into the sample without decreasing the accuracy of the determined FE. To insure accurate results in this study, the following technique was devised. A dye (green food coloring) was introduced into the inhalant current of the oyster. The presence of the dye in the exhalant current aided in the precise placement of the siphon tube in the exhalant current from the epibranchial chamber. Judging from the dye pattern, more water passed through the epibranchial chamber than the promyal. The exhalant water sample must be drawn at a rate slower than the PR so that no water other than that from the exhalant current is drawn into the sample (VanDam, 1954). This slow rate was assured by drawing out the tips of the 1.5 mm diameter glass tubing used so that the rate at which the exhalant water was siphoned was only 5-7 ml/min. This meant that it required 15 to 20 minutes to obtain enough sample to count accurately on the Coulter Counter, and thus each determination of FE and PR is actually the oyster's average performance over this length of time.

At the same time the exhalant water was being sampled, water samples from the trough, just above the oyster (inhalant sample), and from the trough overflow were also taken. A few drops of 1% $HgCl_2$ solutionwere added to each sample to prevent the growth of algae and bacteria before the particle concentration could be determined with the Coulter Counter.

The FE was calculated as the percentage of particles retained by the oyster using the formula:

$$FE = \frac{P_i - P_e}{P_i} \times 100\%$$

P_i is the concentration of particles in the inhalant water sample, and P_e is the concentration of particles in the exhalant water. Four replicate FE's were calculated for each of the 11 channels at each of the five O_2 levels for all 7 oysters. The percentage FE was then converted to angles using the arc-sine conversion before statistical analysis. FE was also calculated for all particles larger than 4.00 μm in diameter. This last FE was used in calculating the PR.

The FR is the rate at which particles are filtered from the water. It is expressed as the volume of water from which all particles are removed per unit time, and was calculated by the formula:

$$FR = \left(\frac{cP_i - P_o}{cP_i} \right) F$$

P_o is the concentration of particles in the water leaving the trough, and F is the flow rate through the trough. The correction factor c is the average fraction of particles not settling out in the control trough. The FR was calculated for the total number of particles larger than 4.00 μm in diameter and used to calculate the PR.

The PR, or ventilation rate, is the rate of water transport through the gills of the oyster, and was calculated by the formula:

$$PR = \frac{FR}{FE}$$

The FR and FE used to calculate the PR were for the total number of particles larger than 4.00 μm in diameter. This size range was chosen in advance since Haven and Morales-Alamo (1970) reported that particles larger than 3 to 4 μm in diameter were retained with maximum efficiency.

Since all oysters were about the same size, and the meats of the ones used during the spring had grown much heavier in preparation

for spawning, for statistical analysis the PR's were expressed as ml/min/oyster rather than in terms of wet or dry weight.

Immediately following the collection of inhalant, exhalant, and trough overflow samples, the dissolved O_2, water temperature, and exact rate of water flow through the trough were recorded. The O_2 concentration and temperature were determined with a Yellow Springs Instruments O_2 probe with thermistor and meter. The rate of water flow was determined by collecting the water leaving the trough in a graduated cylinder for 15 seconds. Salinity was determined daily with a Beckman induction salinometer. A relative turbidity index, the total particle concentration expressed in ml/l (ΣV_i), was calculated from the Coulter Counter data for inhalant water samples (see Appendix I).

After four determinations of PR and FE at each O_2 level were made on an oyster, and the associated above measurements recorded, the oyster was removed from its shell, drained, and weighed. The meat was then dried to a constant weight at 100°C.

Experiments were also performed to determine what effects the O_2 stripping columns had on particle concentration and distribution. Water samples were taken before and after bubbling nitrogen through the water and the particle concentrations and distributions determined with the Coulter Counter. In these experiments the nitrogen was introduced into the columns at rates great enough to lower the O_2 content of the water to 6 and 12% saturation.

Multiple linear regression was used to determine if fluctuations in temperature, salinity, total volume of particles in the water (ΣV_i), dry weight of the oysters, O_2 levels, or rate of flow through the trough

had any effect on filtration efficiency. The same test was made to determine if any of these variables other than the rate of flow through the trough had any effect on PR. The inclusion of the flow rate in this analysis would have violated an assumption of the test, since the flow rate and PR were not independently determined. An analysis of variance was performed to test for significant differences in flow rates between O_2 levels.

PR's for different O_2 levels were compared by analysis of variance. When the PR was expressed as ml/min/oyster, blocking on oysters did not remove a significant amount of variation, and so a completely randomized design was used. A two-factor analysis of variance with blocking on oysters superimposed was then used to test for significant differences in the FE's between channels and between O_2 levels. Where significant differences were indicated, the Student-Newman-Keules (SNK) test was used to determine which means were significantly different.

RESULTS

Nitrogen gas bubbled through the water had the effect of reducing the total particle concentration (ΣV_i) 20.2 to 30.1% (Table 2). This was probably due to particles being held to the surface of the bubbles by surface tension. When the bubbles reached the top of the column they burst, throwing the particles against the sides of the column. Accumulation of particles on the sides of the columns above the water line was quite noticeable. Nitrogen bubbles also had and effect on the size distribution of particles. There were always fewer small particles in the water after bubbling nitrogen through it, but in some cases there were more 12.7 to 25.3 μm diameter particles (channels 14-16). Possibly the nitrogen bubbles caused the aggregation of small particles. However, there were so few particles larger than 12.6 μm in diameter that reliable estimates of their concentrations could not be made. For this reason FE's were not calculated for these larger particles.

The change in particle concentration did not have a significant effect on either PR or FE (Tables 3 and 4). Multiple linear regression analyses demonstrated that only the O_2 level had a significant effect on PR and the average FE for particles larger than 4.00 μm in diameter. The effects of fluctuations in ΣV_i, salinity, temperature, flow rate, and dry weight of the oysters (Table 5) on FE were all non-significant over the ranges encountered in this study ($\alpha = 0.05$).

Fluctuations in ΣV_i, salinity, temperature, and the dry weights of the oysters had no demonstratable effect on PR ($\alpha = 0.05$). Flow rate could not be included in the analysis for PR's since PR and flow rate were not independently determined. Therefore an analysis of variance for differences in flow rates between O_2 levels was made. The F ratio for this test was significant ($\alpha = 0.01$), but only the mean flow rates between levels of 25% and 50% O_2 saturation were significantly different ($\alpha = 0.05$) (Table 6). Because the mean PR's for these two O_2 levels were not significantly different ($\alpha = 0.05$) (Table 7), we conclude that differences in flow rate between treatments (O_2 levels) did not influence the PR's between treatments.

The mean pumping rates at each O_2 level ranged from a high of 183 ml/min/oyster at 50% O_2 saturation to 113 ml/min/oyster at 6% saturation (Figure 5). There was a significant difference ($\alpha = 0.01$) between the mean PR's for different O_2 levels (Table 7). The SNK analysis showed that there was no significant difference between the mean pumping rates at 100%, 50%, 25%, or 12% O_2 saturation ($\alpha = 0.05$). The mean PR at 6% O_2 saturation was significantly lower than at 100% or 50% O_2 saturation, but was not significantly different from the PR at 25% or 12% saturation ($\alpha = 0.05$). Thus, even though there was much variation in the PR's, we conclude that the PR at 6% O_2 saturation was about 35% lower than at the control O_2 level (100% O_2 saturation).

Particle size (channels) and O_2 levels had highly significant effects on FE ($\alpha < 0.001$), but there was no significant interaction (Table 8). Thus, O_2 level effect on FE was the same for all particle sizes counted in this study (1.00 to 12.6 μm in diameter). Therefore, within experimental error, the vertical distances between the lines in

Figure 6, in which FE versus particle size is plotted for the five O_2 levels, are equal.

Table 9 gives the average FE for each channel at each O_2 level, as well as the average FE for each O_2 level over all channels and for each channel over all O_2 levels. Average FE's for channels 3 through 9 (1.00-5.03 μm in diameter) were all significantly different ($\alpha = 0.05$) and increased with increasing particle size (Table 9). There was no significant difference ($\alpha = 0.05$) between channels 10 through 13 (5.04-12.6 μm in diameter). This means that particles 5.04 μm or greater in diameter were retained with equal efficiency at each O_2 level. Retention of particles in channel 9 (4.00-5.03 μm in diameter) was marginally significantly lower ($\alpha = 0.05$).

The overall average FE (particles 1.00-12.6 μm in diameter) at 100% O_2 saturation was 73.4% (Table 9). The values at 50% and 25% O_2 saturation were slightly lower at 69.3 and 69.4% respectively and were the only values not differing significantly from each other ($\alpha = 0.05$). The average FE declined to 50.3% at 12% O_2 saturation, and 44.0% efficiency at 6% O_2 saturation.

DISCUSSION

The effects of sublethal O_2 levels on molluscs in general, and C. virginica in particular, have not been fully investigated. An understanding of how low O_2 levels might affect the ecology, distribution, and growth of commercially valuable species is important to the shellfish industry. C. virginica is the most valuable species of mollusc in the Chesapeake Bay, and this study is a significant beginning toward that goal.

While C. virginica can survive O_2 levels as low as 6% O_2 saturation for more than 13 days (Haven and Bendl, 1975), there is little doubt that such low O_2 levels do have significant impact. FE was affected by an O_2 level of 12% saturation, and both FE and PR were lower at 6% O_2 saturation. These O_2 levels commonly occur in the Chesapeake Bay during the summer months (cf. Hires, et al., 1966 and VIMS Hydrographic Data, unpublished).

Filtration Efficiency

The dependence of FE on particle size was expected. Although Loosanoff and Engle (1947) found little correlation between particle size and FE, this was apparently due to their use of the rubber dam apron method to separate the exhalant water (Jørgensen, 1966). Investigators since that time have found that FE is dependent on particle size (Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970). Haven and Morales-Alamo reported that, using naturally occurring particles

in the York River, FE increased with increasing particle size up to 3 to 4 μm in diameter as determined with a Coulter Counter. The findings of this study are essentially in agreement. The FE for Channel 9 (particles 4.00 to 5.03 μm in diameter) was marginally significantly lower than the FE for higher channels. Channel 8 contained particles as small as 3.17 microns in diameter and would thus be expected to show a lower than maximum FE.

It was suspected that if a low O_2 level affected FE, it might have the greatest effect on the FE of small particles since they are normally retained with less than maximum efficiency. The nonsignificant F value for the interaction between particle size (channels) and O_2 level indicated that, within experimental error, the lower O_2 levels reduced the FE of all size particles by equal amounts. However, since the FE for smaller particles (<4 μm in diameter) was already low at high O_2 levels, it was reduced by a greater percentage. For example, the percentage reduction in FE between 100% and 6% O_2 saturation was 60.9% for particles in channel 3 (1.00 - 1.25 μm in diameter) and 28.8% for particles in channel 13 (10.08 - 12.60 μm in diameter). This is an important point, since particles 1 to 4 μm in diameter may be of particular importance to oyster nutrition. Even though they were not retained with maximum efficiency, they occurred in such large numbers as to represent 52% of the total volume of particles (1 to 12 μm in diameter) retained by oysters (Haven and Morales-Alamo, 1970).

How smaller particles (1 - 4 microns in diameter) are retained is not well understood. Atkins (1938) determined that the distance between the latero-frontal cilia in C. virginica is between 1.5 and 3.7 microns. Only larger particles could be expected to be retained with maximum efficiency. Smaller particles may be trapped along with

larger particles (Smith, 1958) or adhere to mucus on the gill in some way other than being caught by a mucus sheet (Haven and Morales-Alamo, 1970). Tammes and Dral (1955) reported that in Mytilus particles adhere to the latero-frontal cilia and are brushed off onto the frontal cilia. Moore (1971) found that the latero-frontal cilia in Mytilus are actually cirri. The component cilia have free ends branching off at 0.6 μm intervals so that a mesh with a porosity of 0.6 to 2.7 μm is formed. It is probable that the latero-frontal cilia of oysters have a similar structure as fixatives tend to separate them into their constituent fibers (Atkins, 1938).

Pumping Rate

For all but the lowest O_2 level (6% saturation), the PR's at various O_2 levels were within the range determined by other investigators. Jørgensen (1955) compared the results of PR studies conducted by several investigators by converting their data to similar terms (Table 10). Comparisons were difficult since different investigators not only used different methods, but stated the size of the oysters in different ways or not al all. For this reason, Jørgensen had to estimate some of the wet meat weights reported in the table. Data from this study, converted to l/hr, have been added to Jørgensen's table for comparative purposes (Table 10). Pumping rates for 100%, 50%, 25%, and 12% O_2 saturation were pooled for this purpose, since no significant difference was found between the mean PR's for these O_2 levels. The variation in the wet meat weights of the oysters used in this study were due largely to seasonal variation since all oysters were picked to be about the same size.

With the exception of Jørgensen's study (1952), all the results he summarized (Table 10) were made by direct methods. Still, the average PR's determined in this study were within the same range. Several of the maximum PR's determined by the direct method were greater than determined in this study. This may be due to differences in the size of the oysters, since some wet meat weights were estimated, or to latitudinal variation. Rao (1953) reported that Mytilus californianus from higher latitudes pumped faster than individuals from lower latitudes when compared at the same temperature. It is also possible that the differences in maximum PR's were due to the differences in technique. Unless the water level was precisely adjusted when using the rubber dam apron method it is possible some water was siphoned through the oyster. On the other hand, in the method used in this study the rate of flow of water through the trough may have limited the calculated FR. The calculated FR cannot be greater than the flow rate since FR = (CE) (flow rate). The flow rate was in excess of the anticipated PR determined from data summarized by Jørgensen (1966), but the results suggest that the maximum PR's approached or may have exceeded the flow rate. This could be corrected in future studies by redesigning the trough to allow a greater flow rate without increasing turbulence.

Walne (1972) reported a linear relationship between the log of the flow rate and the log of the FR. This result is questionable since he calculated FR from the flow rate. One assumption made in regression and correlation analyses is that the variables are independently determined. For this reason, in this study flow rate was not included in the multiple linear regression analysis of various factors on PR. Still Walne points out the necessity of having the flow rate

greater than the PR. Otherwise, recirculation of filtered water through the oyster will occur regardless of any baffles used in the trough. Such recirculation will cause the FR to be underestimated.

In spite of the possible limiting effect of flow rate on maximum PR, the demonstrated effect of low O_2 levels on PR is still valid. None of the mean flow rates for low O_2 levels differenc significantly from the control O_2 level (100% O_2 saturation). The mean flow rates differed significantly only between levels of 25% and 50% O_2 saturation, and then there was no difference in mean PR between those two O_2 levels.

The pumping of water through the gills serves two functions in bivalves: feeding and respiration. It has long been generally believed that any regulation of the PR is a consequence of feeding activity, but there is some recent evidence that suggests this may not be the case (Bayne, 1967 and 1971; Hamwi and Haskins, 1969). With the exception of Galtsoff and Whipple's (1930) experiments on two oysters at two O_2 levels, the only information in the literature concerning PR at different O_2 levels comes from studies on the regulation of O_2 consumption in bivalves.

Several molluscs, including C. virginica, regulate their O_2 consumption (Prosser and Brown, 1961). Such regulators are able to maintain the same rate of O_2 consumption even as the O_2 tension declines to some critical limit (P_c). One way that molluscs may maintain the same rate of O_2 consumption as the O_2 tension declines is to increase the PR. Hamwi (1969) found M. merceanria was able to regulate its O_2 consumption by maintaining the same PR and O_2 utilization coefficient down to an O_2 tension of 104 mm Hg. According to Walsh (1974) M.

mercenaria regulated its O_2 consumption down to 40 to 80 mm Hg by any of three means: (1) by maintaining the same PR and O_2 utilization coefficient; (2) by a decrease in PR and an increase in O_2 utilization coefficient such that their product (O_2 consumption) remained constant; (3) by an increase in PR and a trend toward a decrease in O_2 utilization coefficient. Oxygen consumption was more variable in this third mode. Mytilus edulis and M. perna increased their FR's slightly down to an O_2 tension of 80 to 100 mm Hg (Bayne, 1967 and 1971). The increase in FR was probably due to an increase in PR. In all cases, the PR (or FR) declined below P_c.

The reported P_c for oysters is from 50 to 62.5% O_2 saturation (Galtsoff and Whipple, 1930; Prosser and Brown, 1961). Based on the relationship between PR and O_2 tension found in other molluscs, one would not expect this study to show a significant increase in PR with declining O_2 tension since no measurements were made between 100% O_2 saturation and P_c. The PR did show a tendency to decline below 50% saturation, but this decrease was not significant except at 6% saturation. In experiments on two oysters, Galtsoff and Whipple (1930) found no significant difference in PR between O_2 levels of approximately full saturation and 12% saturation.

There is also some evidence that C. virginica may be able to increase its O_2 consumption without increasing its PR. After a period of shell closure, during which time the molluscs accumulated O_2 debts, Mya and Arenaria exhibited PR's several times normal as the debts were repaid (Prosser and Brown, 1961). Collier (1959), however, found that when C. virginica first reopened the PR was lower although the O_2 extraction coefficient was quite high.

It is known that O_2 is required to maintain ciliary activity (Aiello, 1960; Gray, 1924), and it is the lateral cilia that create the pumping current in bivalves. In well oxygenated water between 0 and 30°C, O_2 is consumed at a rate directly proportional to the rate of ciliary beat (Wilbur and Yonge, 1966). Thus it is to be expected that at very low tensions O_2 could become a limiting factor and have the effect of reducing the PR. Aiello (1960) reported that the rate of ciliary activity in M. edulis was dependent on O_2 tension up to 37 mm Hg (23% saturation), but independent of O_2 tension at higher values. However, his data do not include measurements between 7.4 mm Hg (5% saturation) and 37 mm Hg; thus one cannot be sure that the rate of ciliary beat decreased at 12% saturation. Separate experiments conducted in this laboratory (Haven and Quensen, unpublished) indicated that the rate of ciliary beat in C. virginica was independent of O_2 level down to about 12% saturation, but that at 5 to 6% saturation, the rate of ciliary beat was reduced by approximately 50%. It is possible, however, that in these experiments some aeration of the preparations occurred during observation.

Conclusion

The effect of low O_2 levels on the PR and FE may be directly related. The rate of ciliary beat would be expected to decrease somewhere below 23% O_2 saturation, but the oysters could maintain approximately the same PR at 12% O_2 saturation by increasing the interfilamentary distance in the gills. This would reduce the resistance the gill offers to the flow of water through it, since the size of the ostia is increased, and also decrease the FE (Atkins, 1943; Elsey, 1935; Jørgensen, 1955

and 1966; Wilbur and Yonge, 1966). At 6% O_2 saturation the oysters were no longer able to compensate for the reduced ciliary activity in this manner, and the PR began to decline.

It is emphasized that this study made no attempt to measure the length of time oysters pumped at each O_2 level. Other experiments were conducted to determine the survival of oysters at low O_2 levels (Haven and Bendl, 1975). During these experiments the number of oysters open were counted at random times during the day. From these counts it was determined that oysters were open less frequently at low O_2 tensions. At 6% O_2 saturation oysters were open approximately 54% as frequently as controls, and 71% as often as 12% O_2 saturation.

If oysters pump only 65% as fast at 6% O_2 saturation as at 100%, and their FE at 12% and 6% O_2 saturation is only 68.5% and 59.9%, respectively, of that at 100% O_2 saturation, then one may estimate that feeding at 12% O_2 saturation is 48.6% of that at 100% O_2 saturation. Similarly, at 6% O_2 saturation feeding would be expected to be 21.0% of that at 100% O_2 saturation.

We conclude that even though oysters may survive O_2 deficiencies as low as 6% saturation for more than 13 days (Haven and Bendl, 1975), their feeding behavior is impaired at O_2 levels below 12% saturation. The occurrence of such low O_2 levels in many areas of the Chesapeake Bay and its tributaries during the warmer months could therefore have a significant impact on the oyster industry. Glycogen reserves may be depleted, resulting in lower meat quality indexes and consequently lower market value. Lack of adequate food reserves could also have deleterious effects on spawning activity.

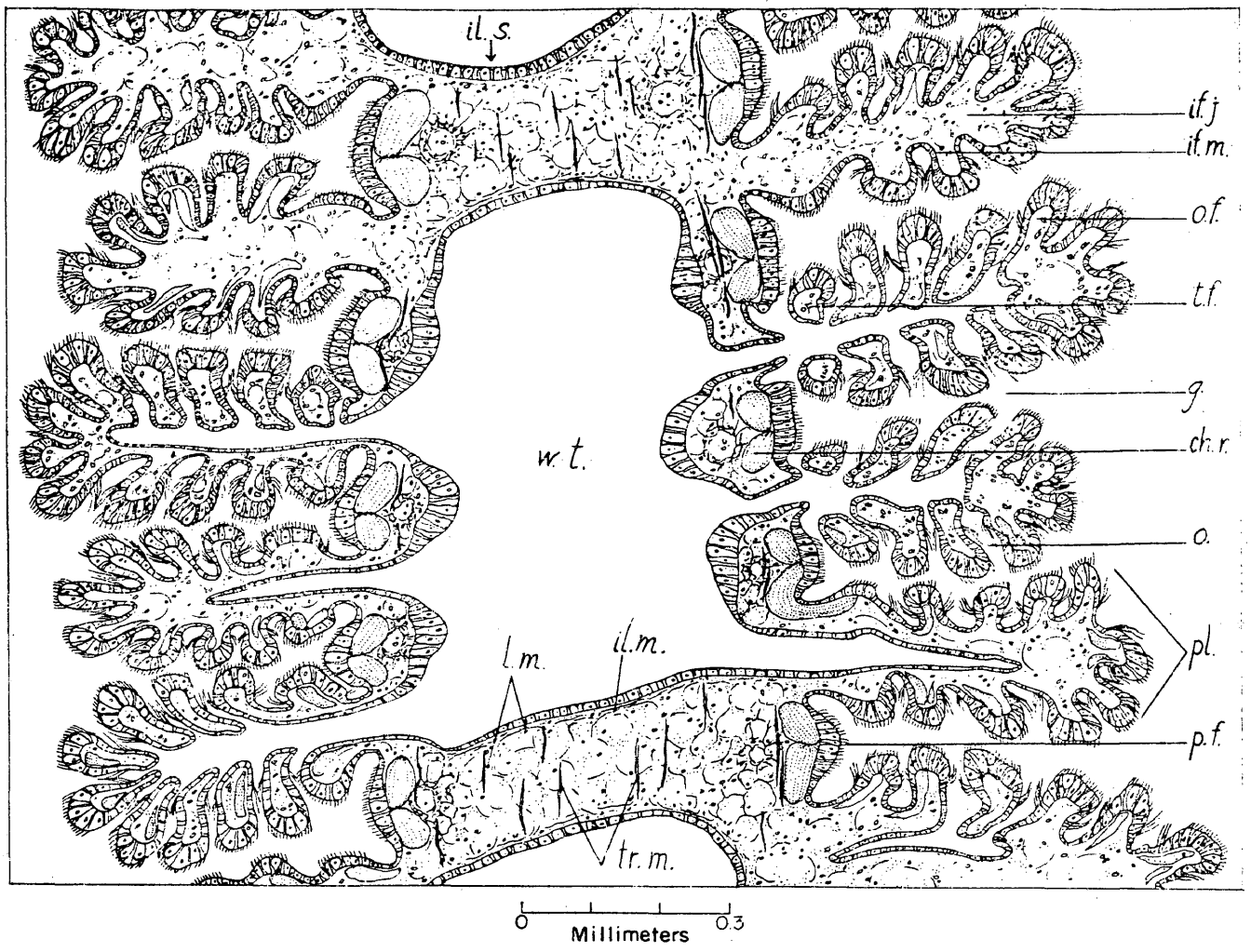

Figure 1: Transverse section of a demibranch of C. virginica. ch.r. - chitinous rods; g. - groove; if.j. - interfilamentary junction; il.m. - interlamellar muscles; il.s. - interlamellar septum; l.m. - longitudinal muscles of the interlamellar septum; o. - ostium; o.f. - ordinary filament; p.f. - principal filament; pl. - plica; t.f. - transitional filament; tr.m. - transverse muscle of the interlamellar septum; w.t. - water tube. (From Galtsoff, 1964.)

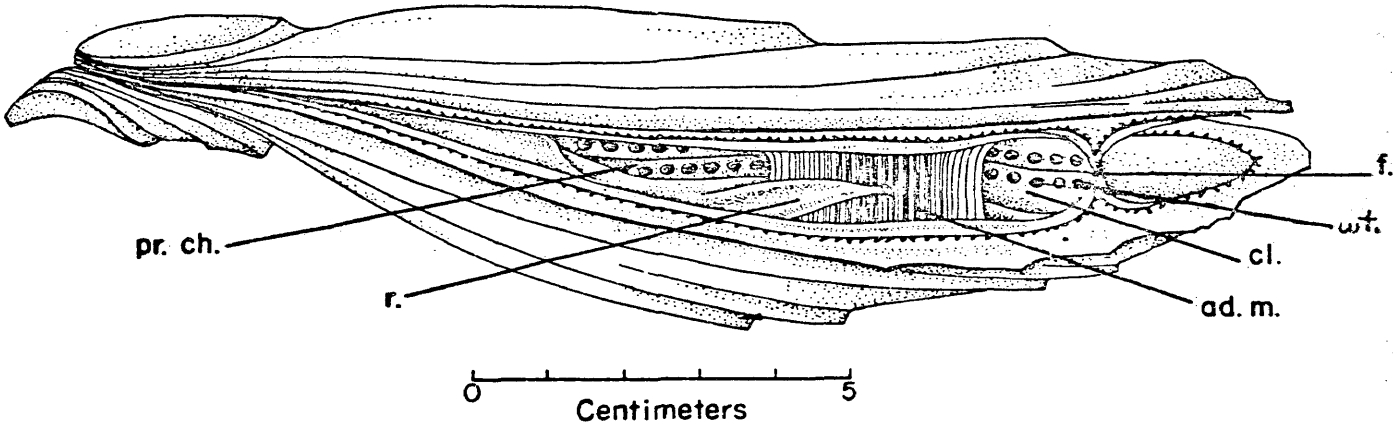

Figure 2: Posterior view of a narcotized oyster. ad.m. - adductor muscle; cl. - cloaca; f. - fusion of the mantle lobes and gills; pr.ch. - promyal chamber; r. - rectum; w.t. - water tubes. (From Galtsoff, 1964.)

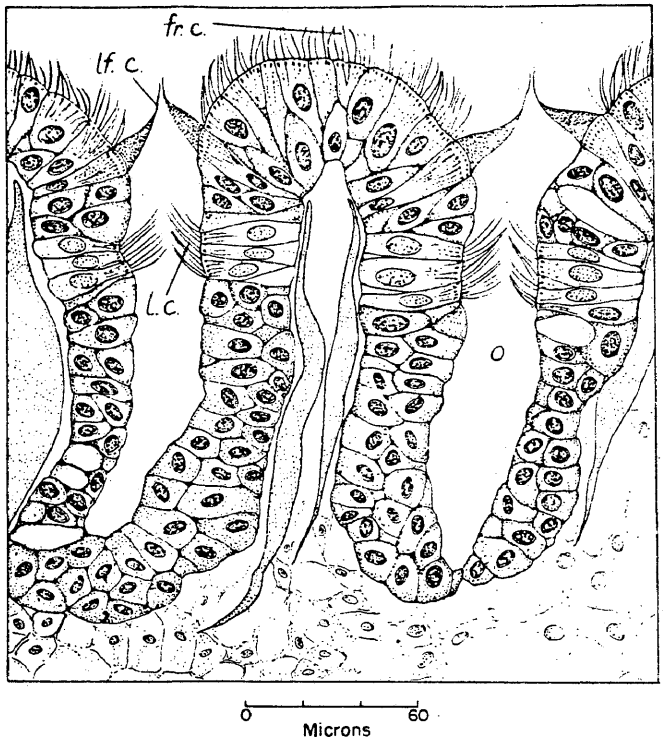

Figure 3. Transverse section of an ordinary filament of C. virginica. Vertical chitinous rods (strippled areas) and blood spaces are at the center. fr.c. - frontal cilia; lf. c. - laterofrontal cilia; l.c. - lateral cilia; o. - ostium. (From Galtsoff, 1964.)

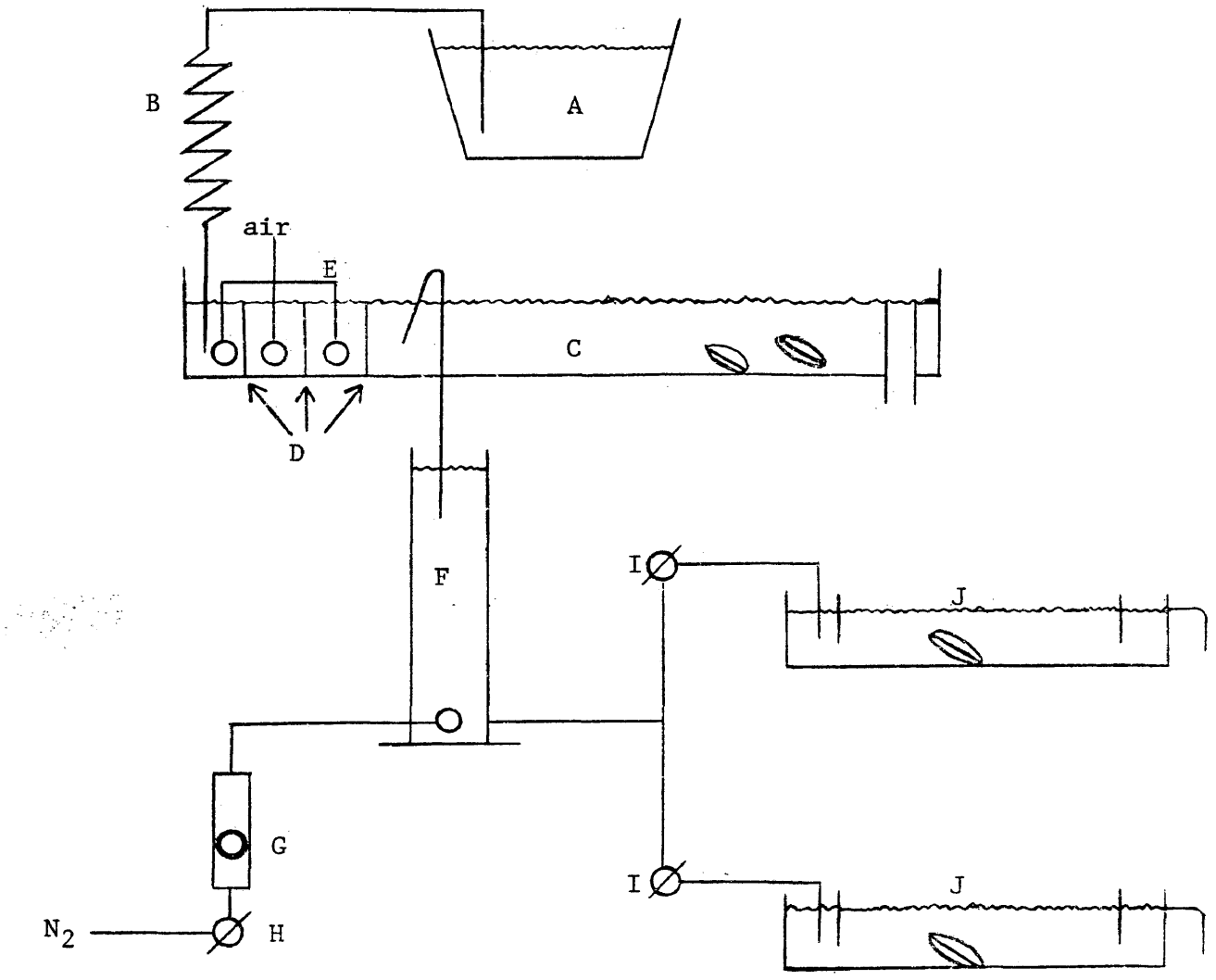

Figure 4: Diagram of apparatus used in this study. For clarity, only one O_2 stripping column and two experimental troughs are shown. A - overhead trough; B - heat exchanger; C - warm water trough; D - baffles; E - air stones; F - O_2 stripping column; G - flowmeter; H and I - valves; J - experimental trough.

Figure 5: Average pumping rates at five O_2 levels. Vertical lines represent 95% confidence intervals.

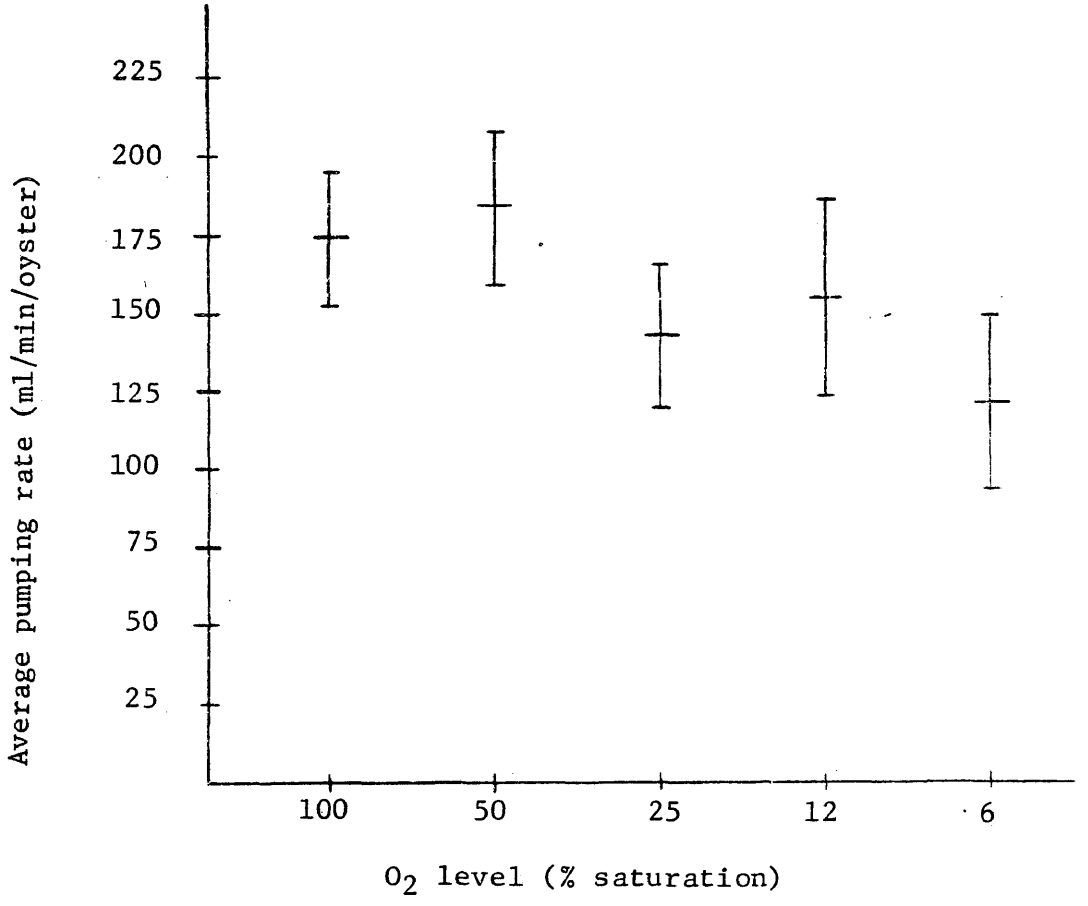

Figure 6: Average filtration efficiency (%) versus particle diameter (μm) at five O_2 levels.

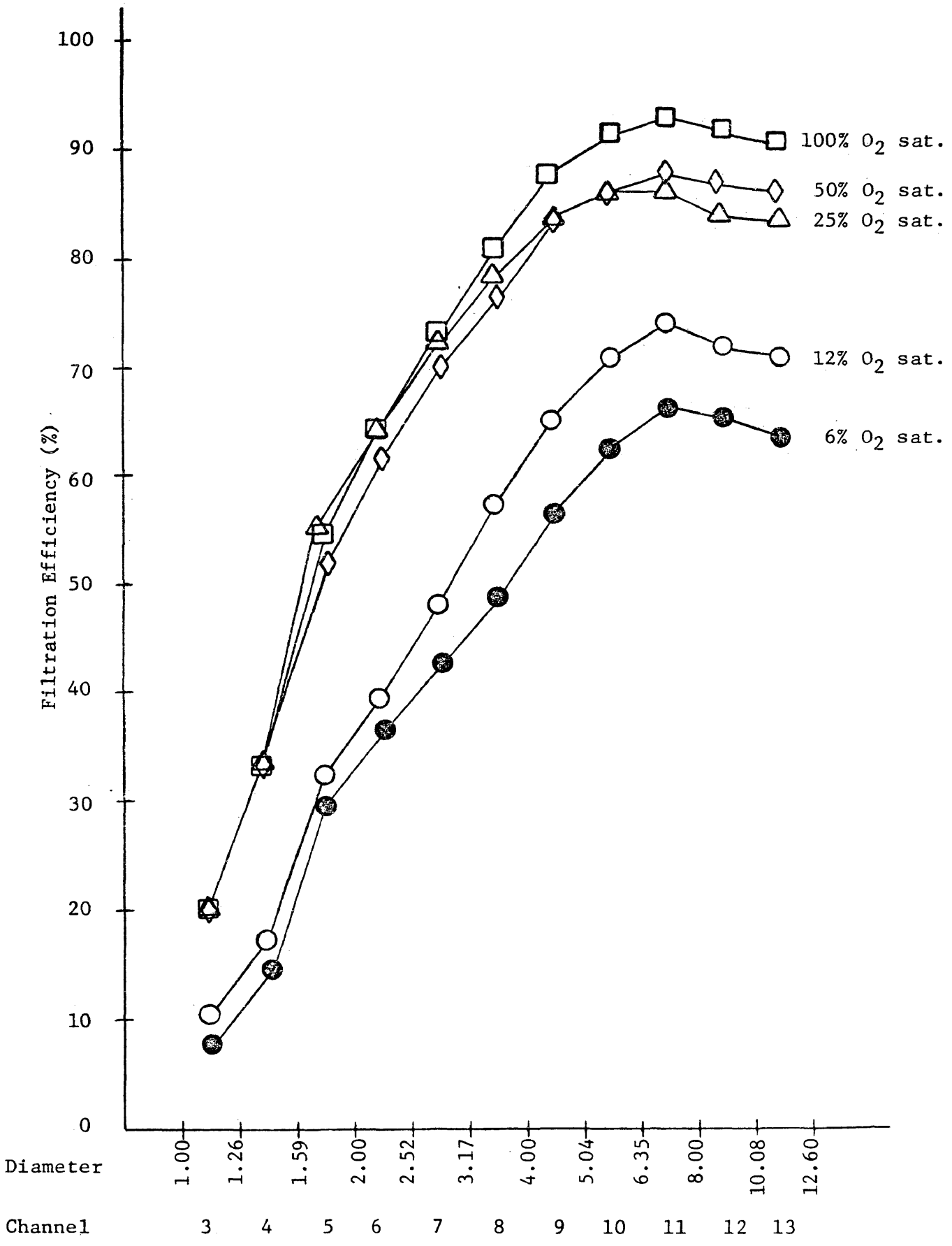

TABLE 1

SIZE RANGE OF PARTICLES IN EACH CHANNEL OF THE COULTER COUNTER.

Channel	Size range in μm (equivalent diameter)
3	1.00-1.25
4	1.26-1.58
5	1.59-1.99
6	2.00-2.51
7	2.52-3.16
8	3.17-3.99
9	4.00-5.03
10	5.04-6.34
11	6.35-7.99
12	8.00-10.07
13	10.08-12.60
14	12.7 -15.9
15	16.0 -20.1
16	20.2 -25.3

TABLE 2

THE PERCENTAGE REDUCTION BY VOLUME OF PARTICLE CONCENTRATION CAUSED BY USING N_2 TO LOWER THE OXYGEN CONCENTRATION IN THE WATER.

	6% O_2 saturation		12% O_2 saturation	
Channel	I*	II	I	II
3	11.7	34.2	8.5	27.9
4	14.9	40.2	8.8	34.7
5	13.9	38.7	15.9	30.1
6	13.9	36.7	17.3	31.8
7	13.9	30.2	18.4	31.0
8	11.8	25.6	19.4	30.1
9	12.8	33.4	21.2	31.1
10	21.8	35.8	25.6	24.5
11	21.1	27.0	36.2	27.6
12	17.6	23.6	27.9	18.3
13	20.2	20.6	26.6	20.6
14	6.0	-35.5	25.7	41.0
15	48.7	-61.5	46.1	-18.6
16	47.0	-119.8	40.8	65.7
Total	20.2	25.6	24.7	30.1

*The Roman numerals designate different experiments.

TABLE 3

RESULTS OF THE MULTIPLE LINEAR REGRESSION ANALYSIS OF VARIOUS FACTORS ON PUMPING RATE.

VARIABLE		RANGE
(X_1)	Temperature	18.5 - 24.0°C
(X_2)	Salinity	12.6 - 24.0 o/oo
(X_3)	Total volume of particles	0.536 - 9.347 ml/l
(X_4)	Dry meat weight	1.068 - 4.869 gm
(X_5)	Oxygen	4.0 - 100.0% saturation

ANALYSIS OF VARIANCE

Source	SS	df	MS	F
Regression				
X_1 thru 5	54303.19	5	10860.64	2.329*
Deviation	624939.81	134	4663.73	

$R^2 = 0.0794$

Regression				
X_1 thru 4	18558.55	4	4639.69	.948 n.s.
Deviation	660684.44	135	4893.96	

$R^2 = 0.0273$

Regression			
X_1 thru 5	54303.19	5	10860.64
Regression			
X_1 thru 4	35144.57	4	8786.14

Table 3 (continued).

Source	SS	df	MS	F
Regression				
X_5 after				
X_1 thru 4	19158.62	1	19158.62	4.108*
Deviation	624939.81	134	4663.73	

n.s.: Not significant

*: Significant when $\alpha = 0.05$

TABLE 4

RESULTS OF THE MULTIPLE LINEAR REGRESSION ANALYSIS OF VARIOUS FACTORS ON FILTRATION EFFICIENCY.

VARIABLE		RANGE
(X_1)	Temperature	18.5 - 26.0°C
(X_2)	Salinity	12.6 - 24.0 o/oo
(X_3)	Total volume of particles	0.536 - 9.347 ml/l
(X_4)	Dry meat weights	1.068 - 4.869 gm
(X_5)	Flow rate thru trough	164 - 312 ml/min
(X_6)	Oxygen	4.0 - 100.0% saturation

ANALYSIS OF VARIANCE

Source	SS	df	MS	F
Regression				
X_1 thru 6	5750.14	6	958.36	9.99***
Deviation	12765.04	133	95.98	

$R^2 = 0.3106$

Regression				
X_1 thru 5	749.15	5	149.83	1.13 n.s.
Deviation	17766.04	134	132.58	

$R^2 = 0.0405$

Regression			
X_1 thru 6	5750.14	6	958.36
Regression			
X_1 thru 5	749.15	5	149.83

Table 4 (continued).

Source	SS	df	MS	F
Regression				
X_6 after X_1 thru 5	5000.99	1	5000.99	52.1***
Deviation	12765.04	133	95.98	

n.s.: Not significant

***: Significant when $\alpha < 0.001$

TABLE 5

WET AND DRY WEIGHTS OF OYSTERS USED IN THIS STUDY.

Oyster	Wet meat wt. (gm)	Dry meat wt. (gm)
1	6.580	1.068
2	11.914	2.154
3	11.430	2.031
4	11.703	2.487
5	17.608	3.633
6	22.105	4.869
7	15.716	3.970

TABLE 6

ANALYSIS OF VARIANCE (ANOVA) AND STUDENT-NEWMAN-KEULES TEST (SNK) FOR DIFFERENCES IN MEAN FLOW RATES BETWEEN O_2 LEVELS.

ANOVA

Source	SS	df	MS	F
O_2 levels	7207.000	4	1801.750	4.264**
Error	57046.393	135	422.566	

**Significant when $\alpha = 0.01$.

SNK

Average flow rates for each O_2 level arranged in ascending order (lines indicate no significant differences when $\alpha = 0.05$):

O_2 saturation (%):	25	12	100	6	50
Average flow rate:	240.1	248.6	249.0	250.9	261.6

TABLE 7

ANALYSIS OF VARIANCE (ANOVA) AND STUDENT-NEWMAN-KEULES (SNK) TEST FOR DIFFERENCES IN PUMPING RATES BETWEEN O_2 LEVELS.

ANOVA

Source	SS	df	MS	F
O_2 levels	85,248.83	4	21,312.21	4.79**
Error	600,482.64	135	4,448.02	

**Significant when $\alpha = 0.01$.

SNK

Mean pumping rates (ml/min/oyster) for each O_2 level arranged in ascending order (lines indicate no significant differences when $\alpha = 0.05$):

O_2 saturation (%):	50	100	12	25	6
Mean PR:	183.0	174.2	155.1	142.9	113.0

TABLE 8

2-FACTOR ANALYSIS OF VARIANCE OF FILTRATION EFFICIENCY WITH BLOCKING ON OYSTERS.

Source	SS	df	MS	F
O_2 levels	74,458.65	4	18,614.66	241.02***
Particle size	293,859.74	10	29,385.97	380.48***
Interaction	2,964.77	40	14.12	0.18 n.s.
Blocks (oysters)	16,765.26	6	2,794.21	
Error	114,227.81	1479	77.23	
Total	502,276.23	1539		

***Significant when $\alpha < 0.001$.

n.s.: Not significant

TABLE 9

AVERAGE FILTRATION EFFICIENCY (PERCENTAGES) FOR EACH CHANNEL AT EACH OXYGEN LEVEL, OVER ALL OYSTERS. LINES INDICATE NO SIGNIFICANT DIFFERENCE BETWEEN MEANS (α = 0.05).

	Oxygen Saturation (%)					
Channels	100	50	25	12	6	Average
3	20.2	20.1	19.9	10.3	7.9	15.2
4	34.0	34.1	34.1	17.1	15.1	26.5
5	54.1	52.5	55.5	32.8	29.9	44.8
6	64.1	61.2	64.2	39.6	36.6	53.2
7	73.1	70.4	72.5	48.4	42.7	61.8
8	80.9	76.9	78.6	57.6	48.9	69.2
9	87.9	83.6	83.7	65.2	56.5	76.3
10	91.3	86.3	86.6	70.9	62.3	80.5
11	92.9	87.7	86.5	74.3	66.4	82.6
12	91.8	86.4	83.9	72.1	65.4	80.8
13	90.8	86.1	83.8	71.0	63.6	78.5
Average	73.4	69.3	69.4	50.3	44.0	

TABLE 10

COMPARISON OF AVERAGE AND MAXIMUM PUMPING RATES WITH THOSE MEASURED BY OTHER INVESTIGATORS.

RESULTS AS SUMMARIZED BY JØRGENSEN (1955):

Source*	Wet wt. (gms.)	Locality	Temperature °C	Pumping rate 1/hr Avg.	Max.
(1)	c. 20	New England	---	6	26
(2)	20	New England	19-26	15.3	34
(3)	c. 20	New England	19-23	8.1	21
(4)	---	New England	28-32	---	37
(5)	13	York River, Va.	9-30	9.6	16
(6)	c. 20	New England	22-23	11	15.5
		RESULTS OF PRESENT STUDY:			
	6.6	York River, Va.	18.5-24	9.5	18.0
	11.9			8.8	18.0
	11.4			9.5	17.9
	11.7			9.2	16.2
	17.6			9.5	16.7
	22.1			11.8	15.5
	15.7			10.6	18.6

*(1) Nelson, 1935 and 1936; (2) Loosanoff and Nomejko, 1946; (3) Loosanoff and Engle, 1947; (4) Loosanoff, 1950; (5) Galtsoff, 1947; (6) Jørgensen, 1952.

APPENDIX I

For this study it was necessary to estimate the number of particles in each channel of the Coulter Counter as well as the total volume of particles counted since the Coulter Counter readout gives only the total number of particles counted and the per cent by volume in each channel. First the Model T_A Coulter Counter was calibrated according to the manual instructions and the work sheet filled out. The particle size limits for each channel and the geometric mean volume of a particle in each channel were then r tad from the work sheet. The derivation of the following equations assumes that the geometric mean volume is a reliable estimate of the volume of a particle in a particular channel.

$$\sum_{3}^{16} V_i = \frac{\sum_{3}^{16} n_i}{\sum_{3}^{16} \frac{f_i}{\overline{V}_i}}$$

$$V_i = (f_i)(\Sigma V_i)$$

$$n_i = \frac{V_i}{\overline{V}_i}$$

f_i = fraction by volume of particles in channel i, and is read from the Coulter Counter

$\sum_{3}^{16} n_i$ = the total number of particles counted in channels 3-16 inclusive, and is read from the Coulter Counter

$\overline{V}_i$ = the geometric mean volume of one particle in channel i (in μm^3) and is read from the Coulter Counter work sheet

V_i = the volume of all particles in channel i (in μm^3) and is unknown

n_i = the number of particles in channel i and is unknown

$\sum_{3}^{16} V_i$ = the total volume of particles in channels 3-16 inclusive and is unknown

APPENDIX II

Included in this appendix is a summary of the data collected on each of the seven oysters at each of the five oxygen levels. The means ($\bar{x}$) and standard deviations (s) given are for sample sizes of four. The filtration efficiencies for each channel of the Coulter Counter were converted from percentages to angles using the arcsine transformation.

Explanations of column heading abbreviations that may not be readily interpretable follow:

S o/oo	salinity in parts per thousand
ΣV_i	total volume of particles in the water
flow	flow rate of water through the trough
PR	oyster's pumping rate

	O_2 % sat.	O_2 mg/l	Temp °C	S o/oo	ΣVi ml/l	flow ml/min	PR ml/min	Filtration Efficiency in Degrees for Each Channel 3	4	5	6	7	8	9	10	11	12	13
Oyster 1																		
$\bar{x}$	95.58	7.41	22.5	19.0	3.163	229.0	98.0	12.10	26.57	36.87	42.21	49.03	57.82	65.65	72.51	77.65	79.85	80.46
s	2.95	0.09	1.0	0.0	0.662	3.8	17.9	6.55	5.36	4.23	7.88	7.60	7.52	5.96	4.82	3.65	3.39	7.76
$\bar{x}$	50.55	4.12	20.4	18.0	2.068	265.0	161.7	18.59	29.61	41.31	45.96	51.70	56.91	62.60	65.35	70.27	67.25	70.54
s	1.61	0.24	1.9	1.2	1.233	7.6	31.5	17.68	12.98	8.17	7.08	6.41	3.90	2.82	3.00	3.90	6.24	11.06
$\bar{x}$	24.15	1.96	20.6	18.3	2.231	248.0	146.4	24.12	33.73	44.04	48.19	53.82	59.91	65.38	70.28	72.48	68.09	69.12
s	1.10	0.06	0.5	1.4	1.389	27.8	76.6	11.18	8.24	6.69	8.24	8.52	8.41	6.62	6.21	7.30	3.27	4.46
$\bar{x}$	12.50	0.97	21.0	23.5	3.214	232.0	228.4	8.46	8.31	19.72	17.46	22.71	29.81	34.15	37.92	43.77q	45.04	45.90
s	0.00	0.00	0.0	0.0	0.378	4.6	56.6	3.50	7.61	4.37	9.48	8.59	6.44	5.05	5.22	3.78	5.17	8.70
$\bar{x}$	5.63	0.45	20.9	18.3	1.364	256.0	81.4	6.63	9.42	21.71	26.59	32.22	40.10	47.67	50.95	56.48	50.79	53.22
s	1.11	0.09	0.3	0.5	1.277	10.8	38.6	6.68	7.62	12.84	11.71	10.05	7.90	5.95	5.56	5.32	5.22	3.81
Oyster 2																		
$\bar{x}$	100.00	7.61	23.8	18.3	0.822	272.00	156.5	30.69	35.53	44.11	50.33	54.62	58.80	63.11	65.62	67.51	68.15	66.88
s	0.00	0.05	0.3	0.0	0.120	5.66	34.4	14.54	13.20	9.93	9.54	9.61	8.19	8.68	8.43	9.31	7.35	6.92
$\bar{x}$	48.45	3.77	22.0	20.0	1.464	284.25	235.7	26.63	36.62	46.15	51.00	55.32	57.21	62.63	64.79	66.29	66.06	65.40
s	1.20	0.09	0.6	0.0	0.330	8.96	51.3	5.25	2.40	2.61	2.96	1.91	5.11	3.44	3.64	2.59	2.97	2.05
$\bar{x}$	25.63	2.06	20.5	19.7	1.466	234.00	90.2	30.43	37.34	46.75	52.15	58.99	61.52	65.47	67.63	68.25	68.51	65.34
s	1.25	0.14	1.0	0.1	0.241	13.27	12.1	2.87	2.78	2.26	2.85	1.67	2.17	2.76	3.49	3.84	6.19	5.77
$\bar{x}$	12.51	0.98	22.1	19.0	2.286	256.00	104.6	21.28	28.36	37.92	42.14	27.03	51.57	59.06	63.83	67.21	68.50	67.21
s	1.78	0.15	1.5	1.0	0.731	14.24	82.6	7.16	7.13	8.93	9.74	11.22	11.10	9.03	6.68	6.24	3.57	3.05
$\bar{x}$	5.56	0.46	20.0	18.3	1.790	250.00	179.7	23.70	30.50	38.43	40.42	43.70	47.65	54.10	57.79	61.77	64.06	59.28
s	0.66	0.06	0.0	1.3	0.561	9.52	58.9	2.10	3.21	5.94	9.24	11.41	10.78	7.49	5.34	4.88	2.48	5.77

	O_2 % sat.	O_2 mg/l	Temp °C	S o/oo	ΣVi ml/l	flow ml/min	PR ml/min	Filtration Efficiency in Degrees for Each Channel 3	4	5	6	7	8	9	10	11	12	13
Oyster 3																		
$\bar{x}$	100.00	7.82	21.4	21.4	1.084	247.0	168.4	30.92	38.94	47.87	52.66	59.09	64.19	69.68	73.15	72.26	72.94	69.94
s	0.00	0.20	1.0	1.2	0.385	35.7	91.1	4.54	4.94	6.39	8.10	9.10	9.09	8.08	6.26	5.39	7.59	7.88
$\bar{x}$	43.57	3.63	22.5	21.5	1.921	247.0	168.3	29.97	38.51	46.84	49.69	58.49	63.95	69.15	70.02	68.10	70.35	67.32
s	0.00	0.00	0.0	0.0	0.187	3.8	33.9	3.01	1.84	1.47	5.82	1.10	1.15	1.25	1.60	2.03	1.94	3.43
$\bar{x}$	25.00	1.92	22.4	21.8	1.523	234.5	127.0	24.76	34.63	42.89	47.92	53.36	58.30	61.60	64.65	64.50	65.92	62.72
s	0.00	0.01	0.3	0.0	0.087	15.8	63.8	2.43	1.09	1.93	3.67	5.91	5.41	5.46	5.20	5.65	4.34	2.87
$\bar{x}$	12.92	0.98	23.3	21.4	1.301	253.0	172.0	23.63	30.10	37.79	42.63	47.99	51.64	55.90	58.56	60.07	60.70	56.51
s	1.39	0.14	1.5	0.9	0.284	39.4	97.9	6.62	7.91	10.00	11.01	11.76	12.76	12.40	12.14	9.81	8.32	5.47
$\bar{x}$	6.13	0.50	20.6	18.6	1.400	246.0	103.0	14.35	19.12	27.48	30.40	32.80	35.30	38.72	44.39	46.60	50.24	43.19
s	1.03	0.07	1.3	1.6	0.276	12.0	92.8	4.06	3.95	4.61	8.04	10.62	10.72	7.61	4.42	5.20	9.00	2.73
Oyster 4																		
$\bar{x}$	99.38	7.62	22.3	22.0	1.025	243.0	213.8	25.65	34.55	42.11	46.18	50.48	56.39	63.69	67.89	70.93	75.00	75.08
s	1.25	0.17	0.5	0.0	0.176	7.6	37.0	7.44	7.96	7.03	7.99	7.58	9.33	9.08	9.31	6.81	6.28	6.66
$\bar{x}$	48.13	3.70	21.8	23.0	0.796	256.0	116.1	29.23	38.76	48.17	55.00	59.59	62.90	66.00	66.85	65.70	65.71	65.79
s	1.25	0.95	0.5	0.0	0.317	19.0	67.1	5.19	6.11	5.87	6.21	6.34	6.53	5.77	5.56	3.24	4.10	5.18
$\bar{x}$	25.00	1.90	22.0	22.8	0.754	261.0	175.1	28.50	36.09	45.52	50.97	55.29	58.36	6.273	64.83	62.33	62.80	64.63
s	0.00	0.01	0.7	0.3	0.216	10.4	80.3	7.26	7.60	6.93	6.05	5.27	5.11	3.08	2.81	4.31	5.47	7.07
$\bar{x}$	12.50	0.97	21.6	22.8	1.341	234.0	97.4	24.42	30.05	38.79	41.51	46.03	52.86	59.03	64.36	64.25	65.44	65.32
s	1.02	0.08	1.1	1.0	0.342	5.2	21.4	6.70	7.39	6.04	12.45	12.98	11.18	9.34	8.17	7.18	6.37	6.55
$\bar{x}$	7.66	0.59	22.5	21.0	1.397	264.0	152.2	10.20	15.02	20.89	24.00	26.18	28.79	33.00	37.68	39.15	43.31	43.10
s	0.31	0.03	0.0	0.0	0.223	8.0	69.1	6.45	4.85	5.78	5.95	8.92	10.64	9.61	9.54	11.12	8.27	7.62

	O_2 % sat.	O_2 mg/l	Temp °C	S o/oo	ΣVi ml/l	flow ml/min	PR ml/min	Filtration Efficiency in Degrees for Each Channel 3	4	5	6	7	8	9	10	11	12	13
Oyster 5																		
$\bar{x}$	90.63	7.55	19.6	16.6	5.087	247.0	193.8	30.69	41.64	60.88	67.15	72.99	72.98	80.89	82.65	82.63	81.32	81.14
s	1.25	0.12	0.3	0.0	1.288	2.6	18.3	6.83	4.69	2.24	2.14	1.46	1.76	1.40	1.35	1.57	2.13	2.16
$\bar{x}$	47.93	4.02	19.1	17.0	1.159	292.0	152.8	21.92	31.23	41.66	48.19	55.12	60.13	64.48	64.42	64.84	61.16	59.67
s	0.05	0.02	0.3	0.0	0.087	0.0	107.8	5.65	4.07	5.11	6.16	5.95	6.17	6.19	5.59	3.65	6.14	4.89
$\bar{x}$	25.94	2.17	20.0	14.6	8.393	227.0	117.7	17.48	29.64	48.97	54.52	59.71	65.21	66.81	67.58	67.17	65.94	65.70
s	3.13	0.26	0.6	2.4	1.687	49.8	62.3	4.22	5.84	6.59	6.23	6.90	7.34	8.96	8.33	8.34	8.16	4.09
$\bar{x}$	12.25	1.01	20.1	16.9	3.247	254.0	168.9	18.99	26.33	39.02	45.69	49.01	52.56	54.03	54.91	54.10	49.37	54.07
s	1.84	0.15	0.9	0.9	1.171	14.8	87.2	8.76	13.55	12.14	10.69	12.21	11.81	13.77	14.92	15.46	10.58	14.10
$\bar{x}$	5.32	0.44	20.6	16.2	4.261	257.5	151.9	27.72	34.94	48.24	54.16	58.08	59.77	62.53	62.79	62.01	59.52	57.69
s	0.63	0.05	0.8	0.0	3.528	3.0	96.0	7.08	5.32	4.68	8.33	10.75	12.23	13.07	10.24	8.37	6.42	6.23
Oyster 6																		
$\bar{x}$	93.75	7.34	22.6	17.1	1.107	257.5	219.9	25.73	33.38	48.27	55.93	61.37	64.92	69.53	71.39	72.43	66.49	64.21
s	4.63	0.17	2.3	0.7	0.434	21.8	38.3	5.57	5.99	7.93	9.69	11.42	11.89	8.11	6.13	4.31	5.21	5.37
$\bar{x}$	50.48	3.96	22.1	18.1	1.256	239.0	230.1	24.61	35.43	47.70	54.83	61.30	65.29	67.61	69.79	71.12	69.34	67.86
s	3.36	0.24	1.3	0.5	0.473	3.8	8.7	4.51	1.33	1.51	3.17	3.37	4.93	6.47	7.84	7.89	7.43	6.69
$\bar{x}$	24.70	1.92	23.1	16.7	1.581	241.5	172.9	26.74	35.62	47.76	57.74	49.87	62.91	67.41	70.55	70.93	63.42	64.46
s	1.13	0.07	1.8	0.5	1.162	9.2	33.2	9.26	8.02	5.59	6.77	3.30	4.52	4.53	3.77	2.58	3.80	4.65
$\bar{x}$	12.75	1.03	21.0	16.7	1.287	237.0	164.9	19.87	27.05	40.63	48.03	55.86	63.44	69.13	71.78	74.57	66.03	63.41
s	0.29	0.01	0.7	0.0	0.207	3.8	44.1	6.86	7.03	5.99	6.41	4.90	1.95	1.74	0.25	2.75	1.21	5.18
$\bar{x}$	5.75	0.48	20.3	16.2	2.833	265.5	91.8	18.63	26.09	37.96	44.00	49.23	53.29	55.16	56.95	57.65	55.09	57.23
s	1.19	0.01	0.7	0.0	0.672	8.5	54.6	9.04	8.77	9.56	8.55	7.76	7.30	7.05	7.48	7.98	9.30	12.20

	O_2 % sat.	O_2 mg/l	Temp °C	S o/oo	ΣVi ml/l	flow ml/min	PR ml/min	Filtration Efficiency in Degrees for Each Channel 3	4	5	6	7	8	9	10	11	12	13
Oyster 7																		
$\bar{x}$	92.88	6.82	25.3	16.5	0.977	247.5	169.1	31.07	38.96	51.52	57.69	63.56	68.70	74.76	76.37	78.60	69.89	68.40
s	1.92	0.16	1.2	0.0	0.296	5.3	47.8	4.52	2.84	3.50	5.59	5.70	7.04	7.85	7.63	5.83	2.38	3.08
$\bar{x}$	50.48	3.87	23.3	18.3	2.528	248.0	216.3	35.53	44.0	53.31	55.67	57.59	62.52	70.19	76.69	79.90	79.47	81.49
s	4.19	0.32	0.3	0.0	0.709	3.3	21.8	5.27	4.06	4.39	6.43	7.90	8.44	6.99	5.55	5.98	5.09	4.34
$\bar{x}$	24.06	1.88	22.9	16.7	1.524	235.0	171.1	33.47	43.22	61.14	62.39	67.56	70.89	74.03	74.17	74.14	68.90	72.08
s	0.63	0.07	1.7	0.5	1.108	47.4	40.7	7.57	7.39	2.10	4.38	3.29	2.72	2.84	2.60	4.13	6.21	7.73
$\bar{x}$	12.81	1.04	21.0	17.1	2.773	274.0	149.8	14.51	20.59	30.75	35.40	39.97	43.58	47.31	50.16	57.87	51.65	49.31
s	0.36	0.04	0.7	0.5	1.536	4.0	117.6	5.60	8.80	11.80	13.62	14.88	15.42	13.97	12.08	10.34	10.87	7.38
$\bar{x}$	5.90	0.50	19.6	12.8	7.274	247.0	88.5	12.73	24.82	37.26	40.81	43.49	45.83	50.09	54.07	58.15	54.94	56.59
s	0.66	0.05	0.3	0.0	1.108	2.6	57.8	8.43	4.44	6.12	9.94	10.90	12.08	11.49	12.44	9.87	11.02	12.43

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VITA

John F. Quensen, III

Born in Richmond, Virginia, September 13, 1949. Graduated from John Randolf Tucker High School, Henrico County, Virginia, June, 1967; B.S., Virginia Commonwealth University, June, 1971, with a major in Biology. NSF-URP participant at the Virginia Institute of Marine Science, Summer, 1970.

Entered the School of Marine Science of the College of William and Mary, September, 1972 as a graduate assistant in the Department of Applied Biology. Married Miss Janet M. Murphy on July 15, 1973.