PARTICLE FILTRATION IN SOME ASCIDIANS AND LAMELLIBRANCHS [1]

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Suspension feeders in the sea obtain their food by filtering finely particulate organic matter from the surrounding water. This suspended organic material ranges in size from macroscopic particles down to colloidal dimensions. It is of importance, therefore, to know how effectively filter feeders retain particles of different sizes. Previous investigations on the dependency of particle retention in lamellibranch gill of particle size have given varying results. Galtsoff (1928) reported that the oyster *Crassostrea virginica* effectively filtered diatoms and dinoflagellates, whereas 70–90% of *Bacterium coli* escaped the gill filter. According to Loosanoff and Engle (1947), however, even 60 μ cells of the flagellate *Euglena* could easily pass through the gills of *Crassostrea virginica,* only 15–80% being strained from the water. ZoBell and Landon (1937) stated that the gills of *Mytilus californianus* retain bacteria very efficiently. In *Mytilus edulis* Jørgensen (1949a) found an almost complete retention of particles a few micra in diameter. In order to determine whether a difference exists in the straining efficiency between the oyster and the mussel gills, further investigations were made on *Crassostrea virginica* and *Mytilus edulis.*

In a previous paper (Jørgensen, 1949b) it was stated that the ascidian *Ciona intestinalis* strained particles even more effectively than did *Mytilus.* In this paper are reported attempts to determine the efficiency with which different proteins are adsorbed by the feeding mucus of *Ciona.*

The authors wish to express their sincere thanks to Professors Dennis L. Fox and Norris Rakestraw for the interest they have shown in this work.

Technique

The relative retention of particles of various sizes was determined by comparing the rates at which the particles were removed from suspensions. The rate of removal is a function of both the amount of water transported through the animal per unit time and the retentive efficiency. However, it was found that when the animals were adapted to the experimental conditions and were not disturbed, the rate of water transport was rather constant (see Fox, Sverdrup and Cunningham, 1937). In some experiments two types of particles were present in suspension simultaneously. In such experiments, of course, differences in the rates of removal were directly indicative of differences in retention efficiency.

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Suspensions were composed of (1) diatoms (*Asterionella japonica* which form colonies about 200 μ in diameter); (2) colloidal graphite (Aquadag A, obtainable from the Acheson Colloids Corporation, Port Huron, Michigan, U. S. A.); (3) blood hemocyanins (from a crab *Loxorhynchus grandis* and from a gastropod, *Haliotis* sp.); and (4) hemoglobin (from man and a fish *Amphisticus argenteus*).

The colloidal graphite forms stable suspensions in distilled water. Suspensions in sea water were prepared from stock suspensions in distilled water. In sea water, the graphite particles coagulate slowly, and in one or a few days all graphite is deposited as macroscopic particles. The coagulation process proceeds very slowly, however, during several hours following the preparation of the suspension. Measurements have been made of particle sizes in freshly prepared and two hour-old

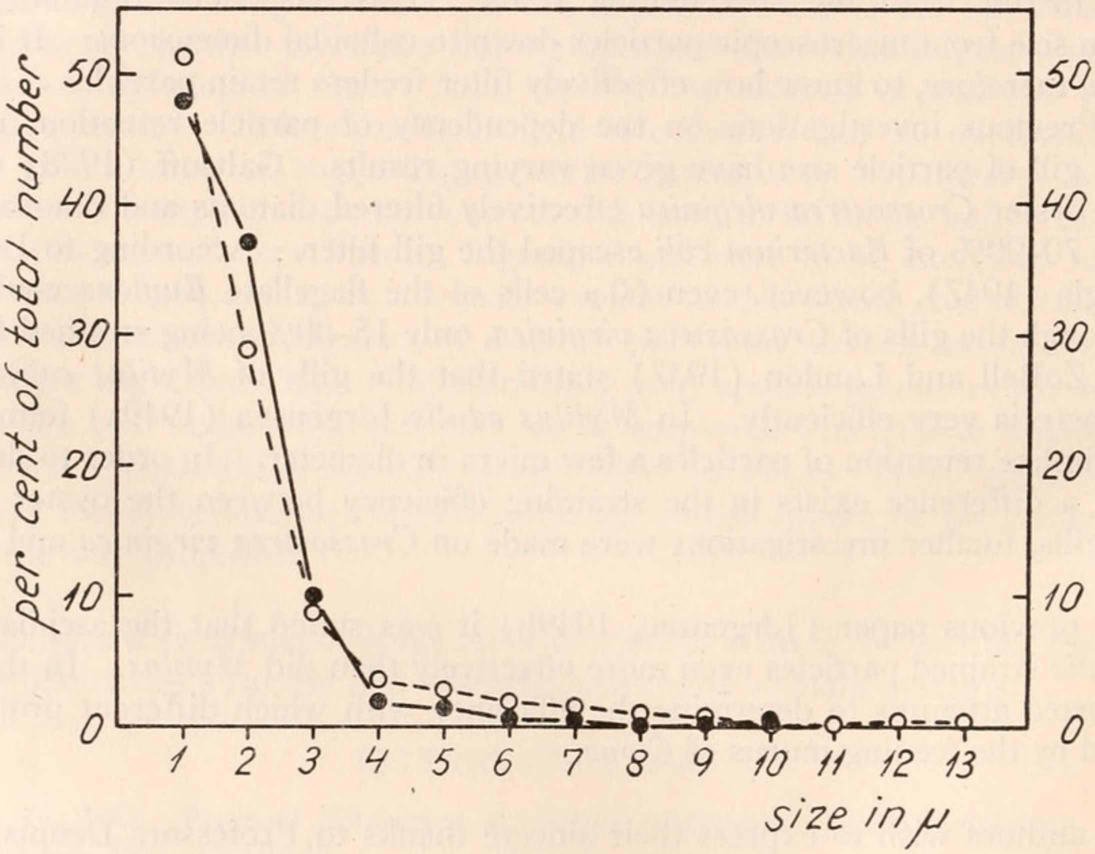

FIGURE 1. Size distribution of graphite particles (Aquadag). ○- - -○, sampled immediately after preparation of the suspension; 505 particles measured. ●—● sampled after 2 hours' agitation with magnetic stirrer; 552 particles measured.

suspensions (Fig. 1). In these examples, it is seen that no, or only small, changes occurred in particle size. By far the greater number were 1–2 μ in diameter. In other instances, however, it was observed that the dominating size increased to 2–3 μ during the first half hour. Thereafter practically no further coagulation might occur during the following 5 hours. The suspensions used in the experiments were never more than some 5 hours old.

The graphite concentrations were determined colorimetrically by means of a Beckman Model DU Spectrophotometer or a Klett-Summerson photoelectric colorimeter. The initial concentrations of the graphite were 0.2–0.5 mg./liter. It was found that the light absorption, expressed as a function of the concentration of graphite, followed Beer's Law. In experiments wherein the graphite was completely retained in the feeding organs, the proportion of particles removed from the water could therefore directly be determined from the corresponding decrease in light absorption. This, of course, was not strictly permissible when the graphite particles were only incompletely retained in the filters. Under such conditions large particles were removed more effectively than small particles. Since large particles absorb more light than do small particles, the numerical fraction of particles removed was consequently smaller than might correspond to the decrease in light absorption.

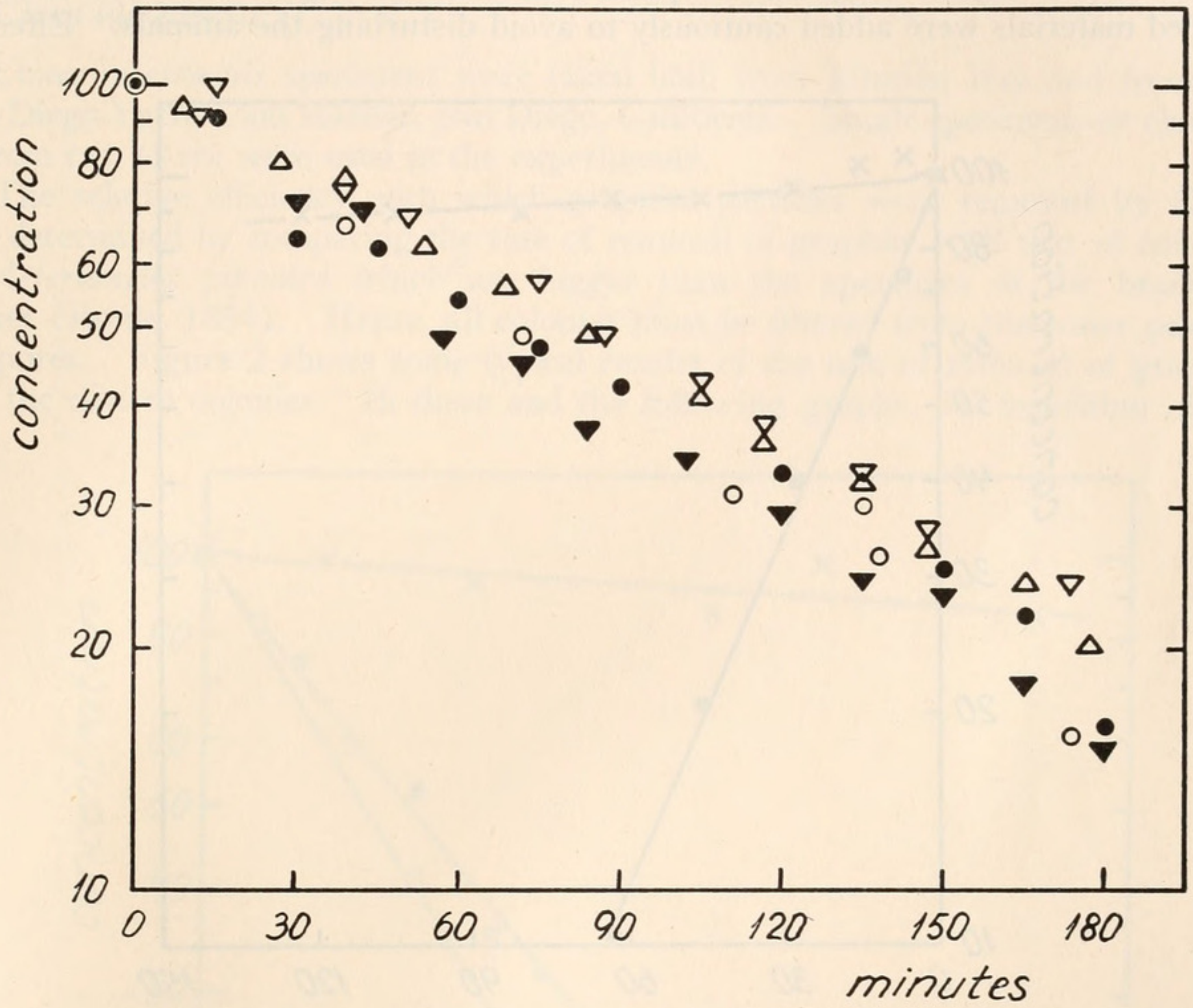

FIGURE 2. *Ciona intestinalis.* Rate of removal of diatoms (● ▼) and colloidal graphite (○ ▽ △). Abscissa: Time in minutes. Ordinate: Concentration in per cent of initial.

The diatoms which were used in the experiments had been cultivated in solutions containing radioactive phosphorus [2] as $P^{32}O_4^{---}$, and had assimilated essentially all the phosphate from the culture media. Samples of the test solution containing the diatoms were evaporated to dryness in planchets under an infrared lamp and assayed for activity under an end-window Geiger-Müller tube with a 1.4 mg./cm.^{2} window thickness. Corrections were made for the small amount of activity leaking into the water from the diatoms.

[2] The radioactive P^{32} used in this investigation was supplied by the Oak Ridge National Laboratory on allocation from the Isotopes Division, U. S. Atomic Energy Commission. See also E. D. Goldberg, T. J. Walker and A. Whisenand (1951).

The hemocyanin and the hemoglobin were assayed spectrophotometrically in 5- or 10-cm. cells. The former suspension was dyed with Evans Blue, T 1824, and excess dye was removed by dialysis against sea water. The measurements were made at 6300 Å. Hemoglobin solutions were obtained by hemolyzing the blood which was eventually diluted to one part per 7000 parts of sea water. The measurements were made at 5700 Å.

Experiments were made on from one to six healthy specimens at a time. Prior to a given experiment, the animals were adapted to the experimental conditions for one to several days. They were kept in 1.0- or 3.5-liter glass vessels. The suspended materials were added cautiously to avoid disturbing the animals. Effective

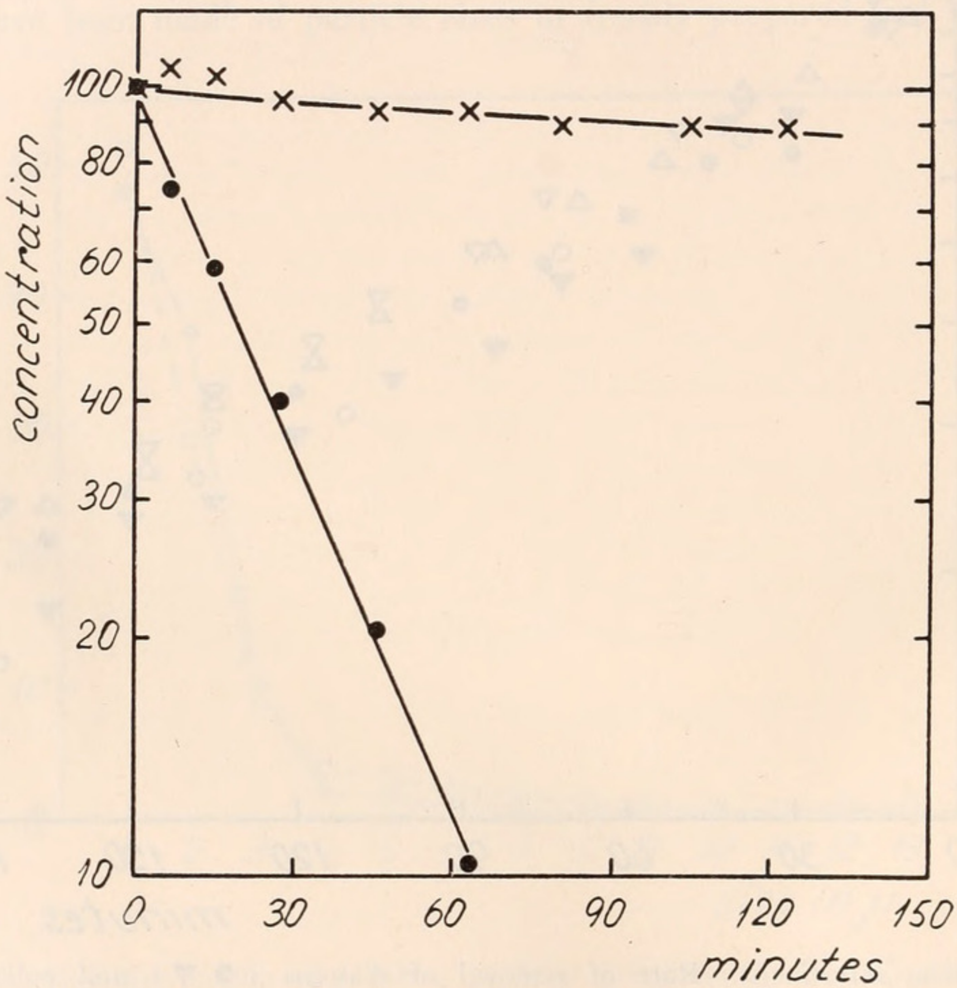

FIGURE 3. *Ciona.* Rate of removal of colloidal graphite (●) and hemoglobin (×) simultaneously present in suspension.

mixing was secured by aeration of the aquaria and aided by the pumping action of the animals. Controls were maintained without animals to ascertain whether any sedimentation or adsorption of the suspended matter occurred during the period of an experiment. In no case did this occur; hence the removal of the suspension was presumed to be due only to the filtering activity of the animals.

Usually the rate of removal of the different substances was determined by separate experiments. In the case of *Ciona intestinalis,* two suspensions, hemoglobin and graphite, were applied simultaneously. The light absorption was measured at 6500 Å and at 5700 Å. At the former wave-length the extinction is almost exclusively due to the presence of graphite, whereas at the latter wave-length the hemoglobin contributes through an absorption maximum. Graphite was found to absorb equally at both wave-lengths. The specific extinction of graphite, which was computed from the readings at 6500 Å, could therefore be used as a "blank." The specific extinction of hemoglobin was obtained by subtracting the extinction of graphite from the extinction at 5700 Å.

Results

Ciona intestinalis (L.):

Ciona intestinalis specimens were taken both from Mission Bay and from the San Diego Yacht Club Harbor, San Diego, California. Single specimens or clusters of from two to six were used in the experiments.

The relative efficiency with which graphite particles were removed by *Ciona* was determined by comparing the rate of removal of graphite with that of colonies of *Asterionella japonica* which are bigger than the apertures of the branchial basket (Roule, 1884). Hence, all colonies must be filtered from the water passing the pores. Figure 2 shows some typical results of the rate of removal of graphite and the diatom colonies. In these and the following graphs, the logarithm of the

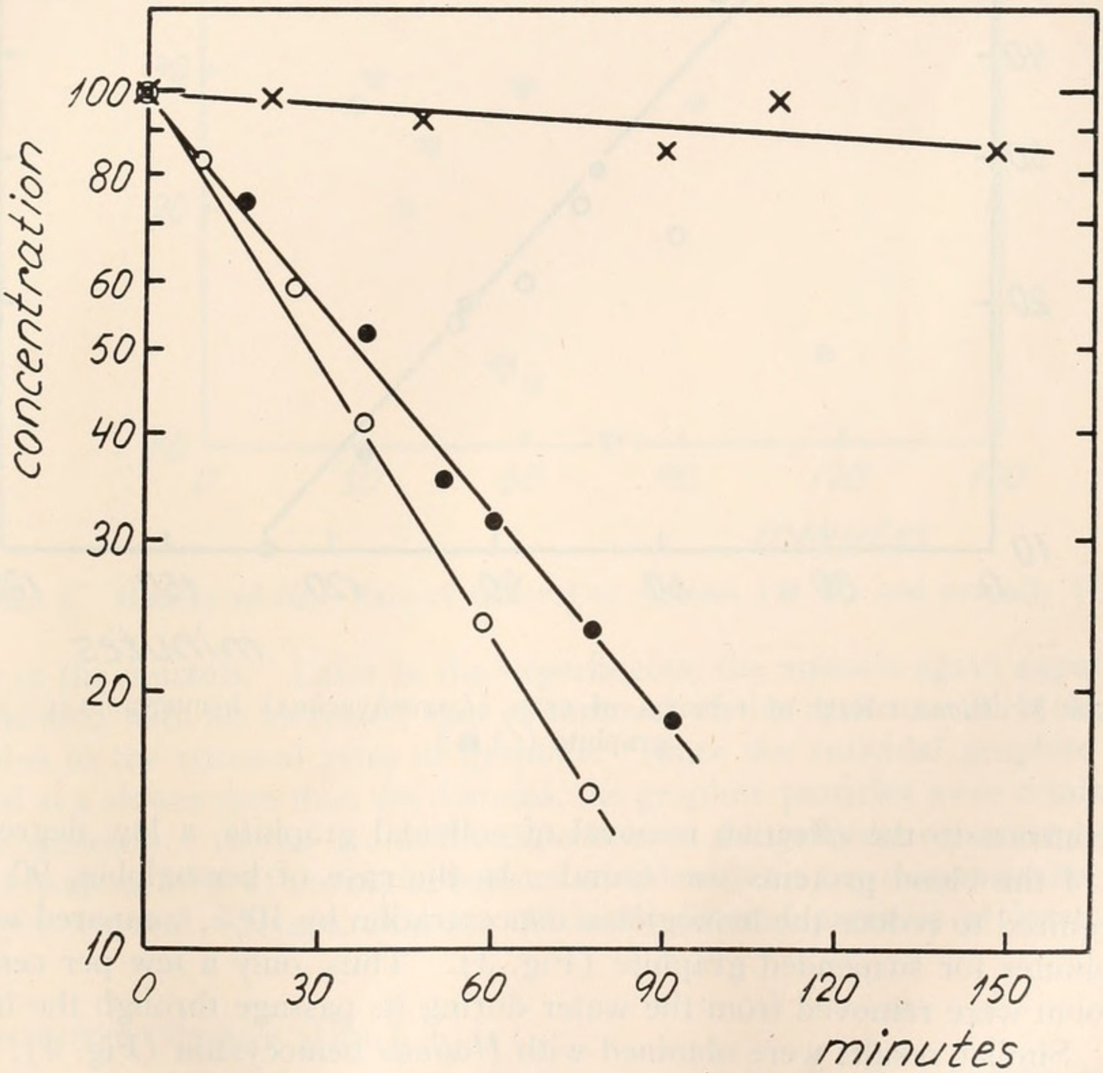

FIGURE 4. *Ciona.* Rate of removal of gastropod (*Haliotis*) hemocyanin (×) and graphite (○ ●).

concentration is plotted against time. The initial concentration is assigned the value of 100. No significant difference was found in the rates of removal of the two materials, even when freshly prepared suspensions of graphite were used. Therefore particles down to 1–2 μ in diameter are effectively removed by *Ciona* from the water passing the branchial basket.

It is also evident from Figure 2 that the curves are nearly straight lines, indicating a constant rate of removal of the suspended material, *i.e.,* a constant rate of water transport throughout the experiments. The relatively constant rate of filtration has always been found in undisturbed, healthy specimens of *Ciona, Mytilus* and *Crassostrea* (see below).

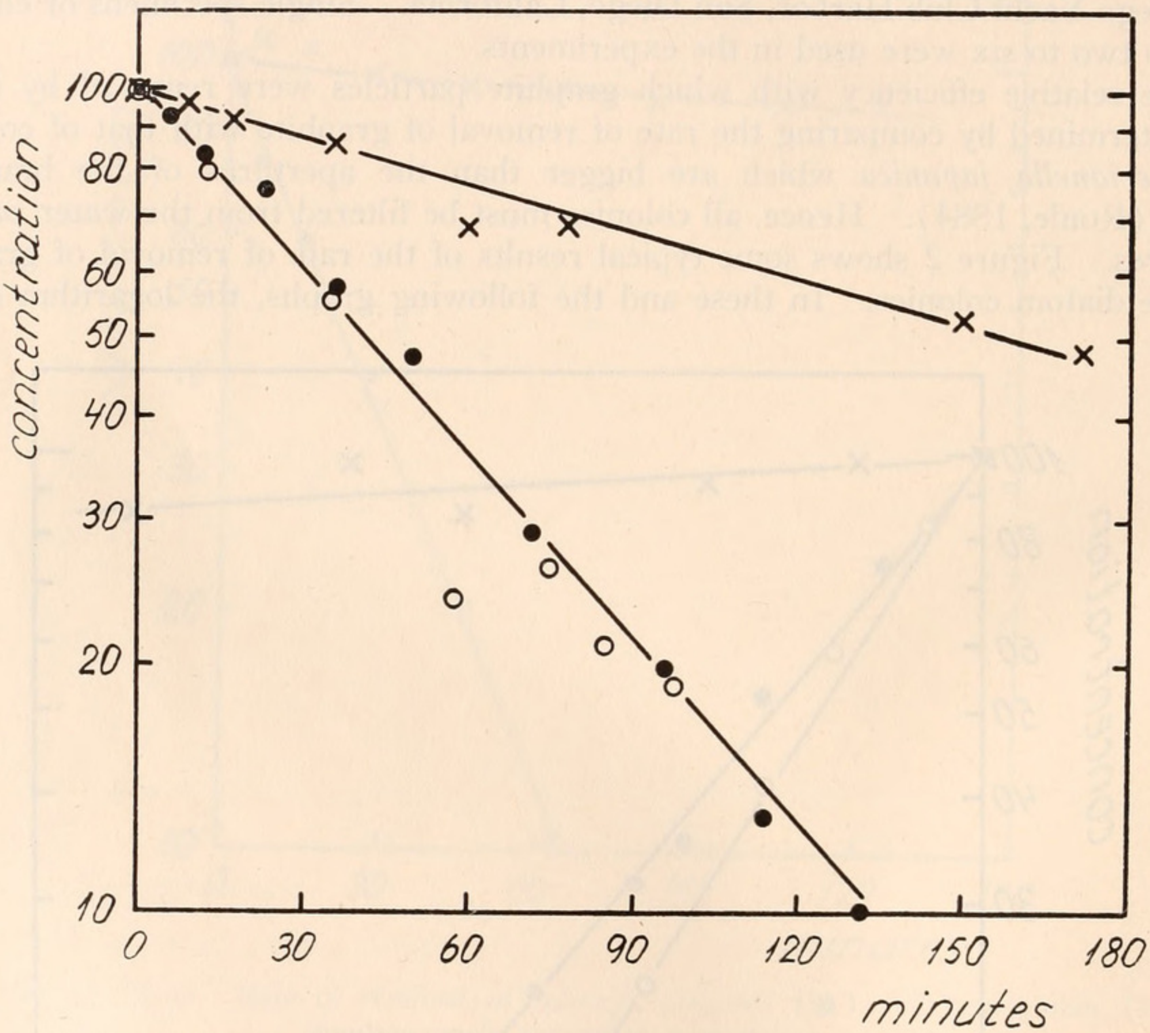

FIGURE 5. *Ciona.* Rate of removal of crab (*Loxorhynchus*) hemocyanin (×) and graphite (○ ●).

In contrast to the effective removal of colloidal graphite, a low degree of retention of the blood proteins was found. In the case of hemoglobin, 90 minutes were required to reduce the hemoglobin concentration by 10%, compared with only three minutes for suspended graphite (Fig. 3). Thus, only a few per cent of the hemoglobin were removed from the water during its passage through the branchial basket. Similar results were obtained with *Haliotis* hemocyanin (Fig. 4), whereas in the only experiment performed with the crab blood, about 50% were extracted in three hours (Fig. 5). More experiments are necessary in order to ascertain whether this difference is significant in the treatment of *Haliotis* and *Loxorhynchus* blood in the feeding organs of *Ciona*.

Mytilus edulis (L.):

Mytilus edulis were taken from the tidal zone off La Jolla, California. All experiments were performed on the same cluster of four specimens, each of which was about 3 cm. in length. Figure 6 shows that the diatoms were removed somewhat more slowly than the graphite. A partial retraction of the mantle edges was observed, however, upon addition of the diatoms, thus suggesting a depression in the

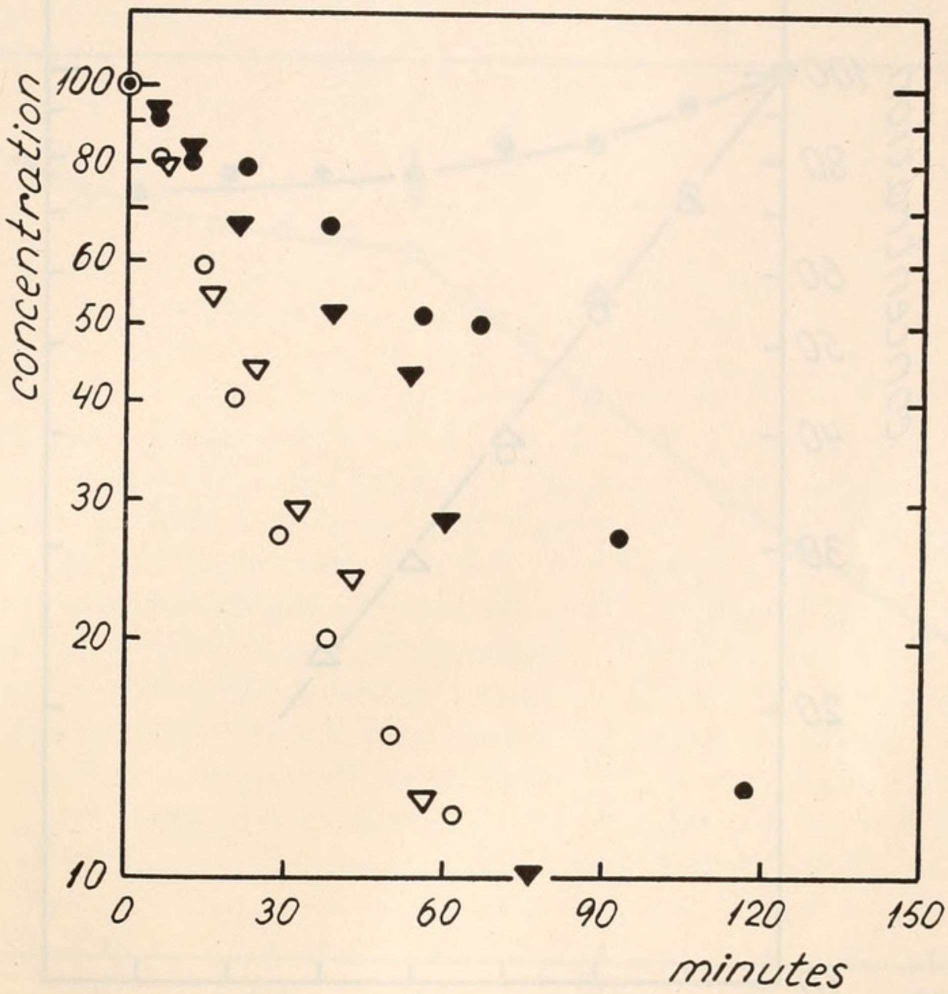

FIGURE 6. *Mytilus edulis.* Rate of removal of diatoms (● ▼) and graphite (○▽).

activity of the mussels. Later in the experiments, the animals again expanded and this coincided with an increased rate of removal of the diatoms which closely corresponded to the removal rates of graphite. Since the colloidal graphite was not removed at a slower rate than the diatoms, the graphite particles were retained practically completely. At the end of the experiments, the graphite concentrations were reduced to about 10% of the initial amount added without any demonstrable decrease in rate of removal. Hence, *Mytilus* efficiently filtered particles at least down to 1–2 μ.

Crassostrea virginica (Gm.):

The oysters used in the present experiments were kindly placed at the disposal of one of us (C. B. J.) by Dr. P. S. Galtsoff of the U. S. Fish and Wildlife Service, Shellfish Laboratory, Woods Hole, Mass. The animals had been living for about a month in running water in the tanks of the Shellfish Laboratory. The experiments were performed at the Marine Biological Laboratory, Woods Hole. (See also Jørgensen, 1952).

The oysters readily retained all graphite particles in aged suspensions, that is, suspensions more than about one hour old, but removed incompletely the graphite in fresh suspension. Thus, when fresh suspensions of colloidal graphite were offered to the animals, the initial rate of removal was consistently low and decreased

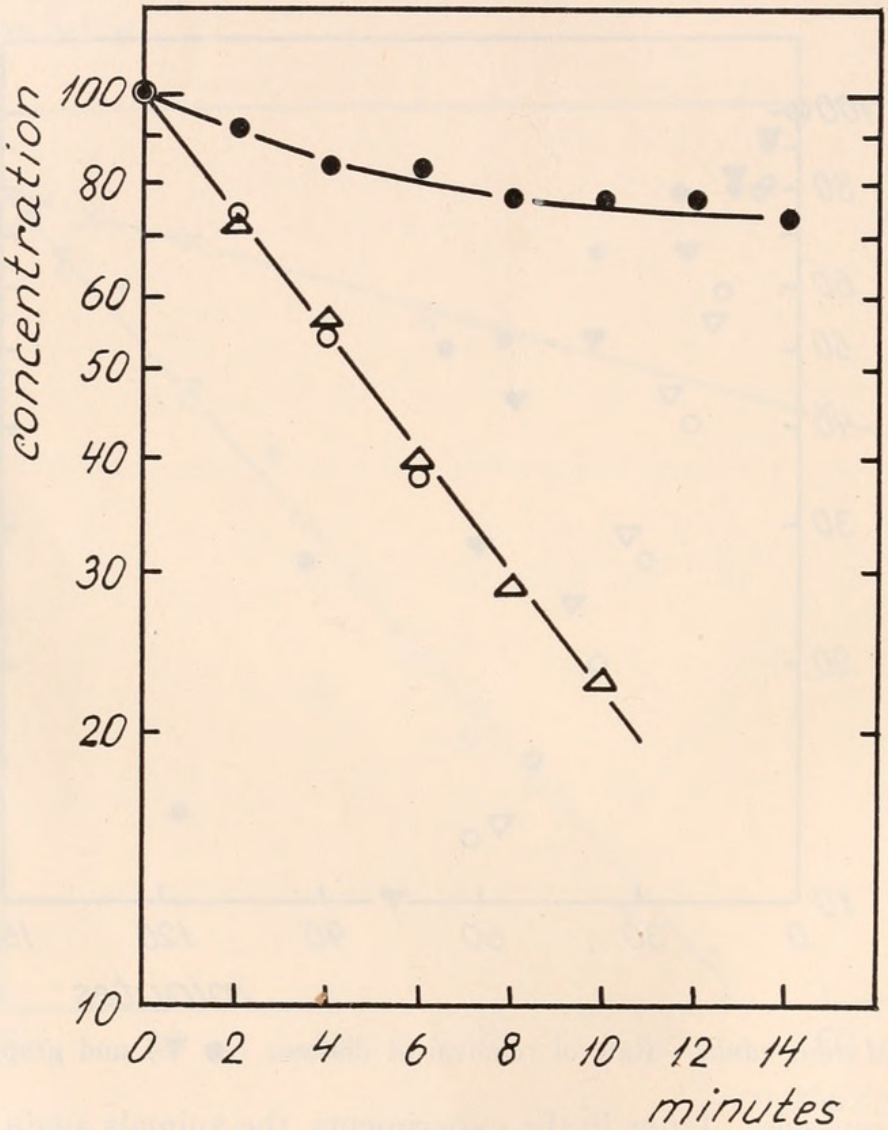

FIGURE 7. *Ostrea virginica*. Rate of removal of graphite from a fresh suspension (●) and from a 4 hours' old suspension (○ △).

with time. Sixteen experiments were carried out, using one oyster at a time. Typical examples are given in Figures 7 and 8. The decreasing removal rates may be due to a preferential retention in the gills of the larger suspended particles causing a decrease in the average size of particles remaining in suspension. Consequently, a less efficient retention in the gill filters resulted. If an aged graphite suspension were exposed to the action of an oyster which was filtering graphite only slowly from a fresh suspension, the rate of removal immediately showed a considerable increase (Fig. 8). In aged graphite suspensions, wherein most particles were about 2–3 μ in diameter, removal rates were high and constant in 26 experiments. Hence, either all the particles or constant fractions were filtered from the water passing the gills. However, it is difficult to understand why a constant fraction of particles of different sizes should be retained when the dominant size was about 2–3 μ but not when the dominant size was about 1–2 μ as in fresh suspensions. Also, the rates of water transport which could be computed from the removal rates of graphite were high in the experiments with aged graphite suspensions, thus indicating a high degree of retention of graphite particles (Jørgensen, 1952). It must therefore be assumed that particles 2–3 μ in diameter and larger were completely retained by the gill filter under the experimental conditions. The graphite in fresh suspension was

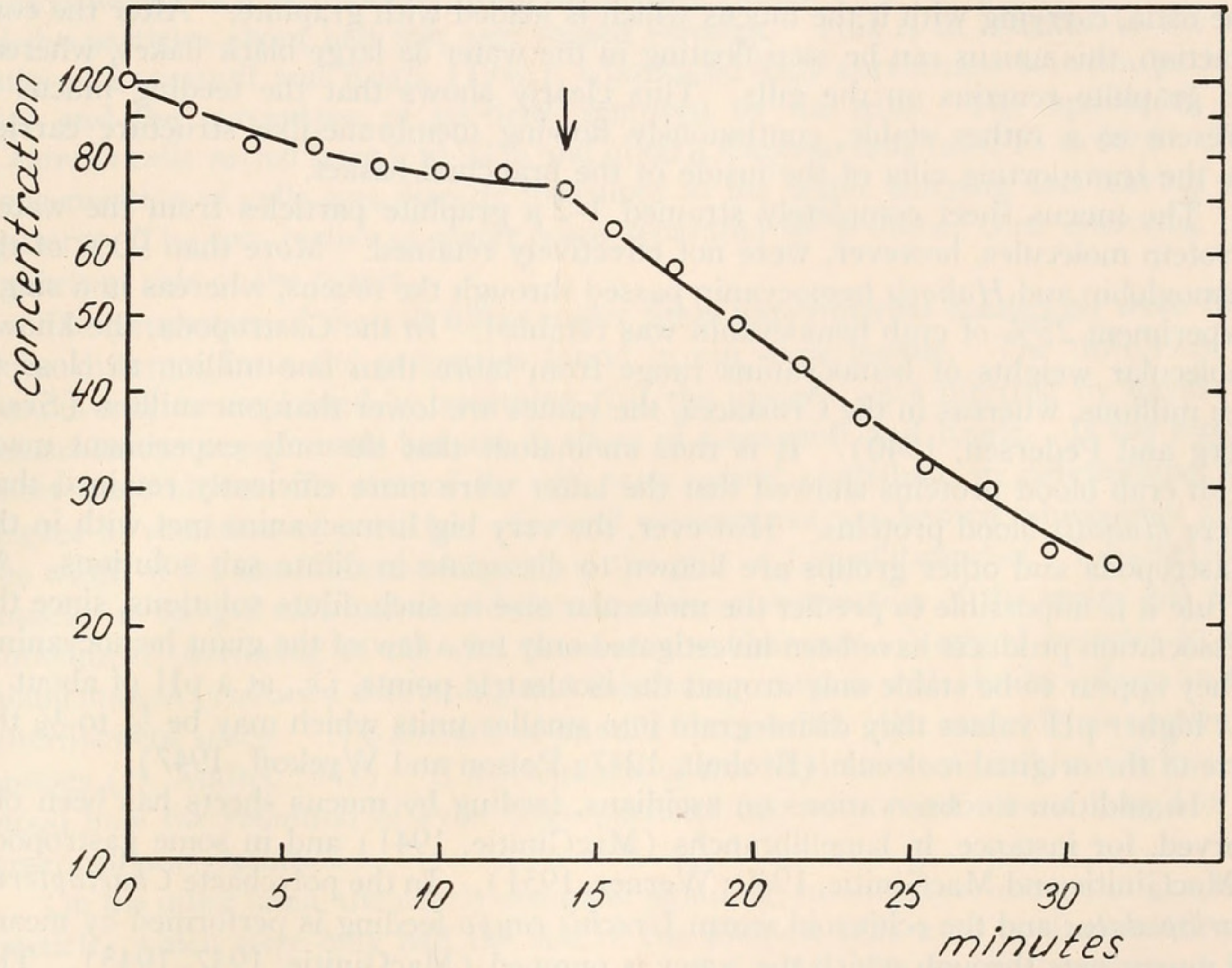

FIGURE 8. *Ostrea.* Rate of removal of graphite from a fresh suspension. At the arrow addition of an aged graphite suspension.

removed about 5 to 10 times more slowly than the graphite in aged suspensions, showing that fewer than 10–20% of the 1–2 μ particles were retained by the mucus of the oyster gills.

DISCUSSION

MacGinitie (1939) observed that when an ascidian is feeding, a sheet of mucus covers the inside of the branchial basket. This mucus is continuously produced by the endostyle and is carried by cilia to the dorsal food groove which conducts the food-loaded mucus toward the mouth (Orton, 1913; Plough and Jones, 1939).

The presence of the mucus sheet can be nicely visualized in transparent *Ciona intestinalis* by addition of colloidal graphite to the water. When *Ciona* is disturbed no feeding mucus is formed and practically no graphite is retained. In the undisturbed animals, however, blackening of the branchial basket results from addition of graphite suspension. The coloring increases in intensity from the region of the endostyle toward the dorsal groove, as should be expected because the mucus sheet is continually carried dorsally. If excessive amounts of graphite, *e.g.*, several milligrams per liter, are added to the water, the animals presently react by closing the exhalent aperture and contracting the body walls violently, thus ejecting water and mucus incorporated with graphite, through the inhalent aperture. The water present in the chamber between the body walls and the branchial basket is forced through the ostia, carrying with it the mucus which is loaded with graphite. After the contraction, this mucus can be seen floating in the water as large black flakes, whereas no graphite remains on the gills. This clearly shows that the feeding mucus is present as a rather stable, continuously flowing membrane-like structure carried by the transporting cilia of the inside of the branchial basket.

The mucus sheet completely strained 1–2 μ graphite particles from the water. Protein molecules, however, were not effectively retained. More than 90% of the hemoglobin and *Haliotis* hemocyanin passed through the mucus, whereas in a single experiment 25% of crab hemocyanin was retained. In the Gastropoda, the known molecular weights of hemocyanins range from more than one million to close to ten millions, whereas in the Crustacea, the values are lower than one million (Svedberg and Pedersen, 1940). It is thus anomalous that the only experiment made with crab blood proteins showed that the latter were more efficiently retained than were *Haliotis* blood proteins. However, the very big hemocyanins met with in the Gastropoda and other groups are known to dissociate in dilute salt solutions. As a rule it is impossible to predict the molecular size in such dilute solutions, since the dissociation products have been investigated only for a few of the giant hemocyanins. They appear to be stable only around the isoelectric points, *i.e.,* at a pH of about 5. At higher pH values they disintegrate into smaller units which may be ¼ to ⅛ the size of the original molecule (Brohult, 1947; Polson and Wyckoff, 1947).

In addition to observations on ascidians, feeding by mucus sheets has been observed, for instance, in lamellibranchs (MacGinitie, 1941) and in some gastropods (MacGinitie and MacGinitie, 1949; Werner, 1951). In the polychaete *Chaetopterus variopedatus* and the echiuroid worm *Urechis caupo* feeding is performed by means of mucus nets through which the water is pumped (MacGinitie, 1937, 1945). The particle-retaining properties of mucus sheets are apparently different in the different types of filter feeders. MacGinitie (1945) thus found that the mucus comprising the feeding nets of *Urechis* and *Chaetopterus* was unable to retain ovalbumin (molecular weight = 44,000), whereas human serum globulin (molecular weight = 176,000) was partially stopped and *Palinurus* hemocyanin (molecular weight = 450,000) was completely stopped in the mucus nets. These nets are therefore probably less effective than the mucus filter of *Ciona* which only incompletely retained the hemocyanins.

When the gastropod *Crepidula fornicata* is feeding, two sets of mucus filters are formed (Werner, 1951). One is guarding the entrance to the mantle cavity and is mainly removing large particles from the inspired water. Smaller particles pass through, but are strained by the second mucus layer which covers the gills. The dependency of retention on particle size in the two mucus sheets has not been directly measured.

The retention of particulate matter by filter feeding organisms may be a function not only of particle size (reflecting the "porosity of the mucus") but also the charge of the particle relative to the charge of the mucus and possibly of the shape of the particles. Particles with a charge opposite to that of the mucus could be effectively adsorbed. The effect of particle shape and charge on filtering efficiency must await future experimentation.

Lamellibranchia: In *Crassostrea virginica* it was found that graphite particles which were 2–3 μ in diameter were effectively retained by the gills whereas most of the particles about half this size passed through. This is in contrast to the results of Loosanoff and Engle (1947), who found little correlation between particle size and the percentage of particles removed by the gills. The uptake of 5 μ *Chlorella* cells varied from 0 to 92% and of 60 μ *Euglena* cells from 15 to 80%. The concentration of cells was directly measured in the water entering and leaving the oysters. The two bodies of water were separated by a rubber cone enclosing the excurrent side of the oyster.

The properties of the gill filters under these experimental conditions were thus quite different from the properties found in our experiments. The difference in results may be explained by assuming that the oysters were filtering by means of mucus in our experiments but not in those of Loosanoff and Engle. If the mucus sheet were absent, the size of the gill ostia would contribute in determining the degree of retention of particles. Several mechanisms are known which may alter the width of the interfilamental spaces in the gills of Lamellibranchia. Longitudinal muscles in the gill axis and elsewhere can cause a contraction of the entire gill with subsequent narrowing of the slits between the filaments. Vertical muscles in the filaments can produce a shortening and thickening of these with similar effects in the interfilament space. The amount of muscle fibers in the gills varies from species to species (*cf.* Atkins, 1943). If mechanisms of this kind are at work it can be understood how the retention of even large particles can vary from almost complete to practically no retention.

On the other hand, the nearly complete straining, in our experiments, of graphite particles which were only half the size of the smallest cells used by Loosanoff and Engle probably means that mucus sheets, and not the gills proper, performed the straining. Hence, the mucus filtering process in *Crassostrea virginica* effectively retains particles down to about 2–3 μ, only. Similarly, it can be assumed that *Mytilus edulis* was producing mucus sheets in our experiments, and further, that the mucus filter is less porous in *Mytilus* and in *Ostrea* since even 1–2 μ particles were effectively filtered. In previous experiments on *Mytilus edulis* from Plymouth it was observed that the smallest graphite particles generally passed through unadsorbed. This difference between *Mytilus* from California and from Plymouth may be due to differences of specimens from the two localities or due to difference in experimental conditions. From the experiments of Rao (1953) on the variation of pumping rate of *Mytilus californianus* with the latitude, the former explanation appears more reasonable.

Further experiments are needed in order finally to ascertain to what extent formation of mucus filters or adjustment of the size of the interfilamental space without mucus sheet formation are responsible for the effective straining of particles which are only about one or a few microns large.

Summary

1. The efficiency of retention of particles of different sizes has been determined in *Ciona intestinalis, Mytilus edulis* and *Crassostrea virginica* by comparing the rates at which the suspended material was removed from water.

2. The mucus sheet of *Ciona* effectively retained 1–2 μ graphite particles. Only a few per cent of protein molecules such as hemoglobin or gastropod (*Haliotis*) hemocyanin were removed from the water passing through *Ciona*. In one experiment with crab (*Loxorhynchus*) hemocyanin, 25% were retained.

3. *Mytilus* strained practically completely 1–2 μ graphite particles. In *Crassostrea* 2–3 μ particles were effectively filtered from the water, whereas 1–2 μ particles passed through the gills.

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