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THE FILTER-FEEDING OF *ARTEMIA*

II. IN SUSPENSIONS OF VARIOUS PARTICLES

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The ability of filter feeders to discriminate between particles of different kinds was given an experimental basis in the work of Harvey (1937), who found that *Calanus* feeding in a mixed suspension of *Lauderia* and *Chaetocerus* would take little of the latter. Marshall & Orr (1955) could not confirm these observations and in experiments with mixed suspensions of various plant cells found no clear evidence of any selection mechanism, as also did Ryther (1954) using *Daphnia.*

Corner (1961), analysing the organic and inorganic fractions of total particulate matter in sea water before and after *Calanus* had fed on it, reported that although the fractions were originally present in approximately equal amounts, about 80% of the particulate matter removed by the animals was organic.

The present work is an attempt to study the feeding behaviour of *Artemia* in systems other than those of single species of cells.

MATERIALS AND METHODS

Artemia and the plant cells *Chlorella, Dunaliella* and *Phaeodactylum* were obtained and cultured as described in the previous paper (Reeve, 1963*a*) together with the green flagellate *Asteromonas propulsum* Butcher. These plant cells do not clump or sink out of suspension when healthy. Suspensions of inorganic particles tend to aggregate rapidly and sink. In tests on chalk, alumina, carmine, kieselguhr and many other materials including sand, the last was found to be least unsatisfactory in these respects. When builder's sand was shaken up with sea water the coarse grains rapidly sank, leaving a suspension of fine grains which settled only very slowly. The tendency was overcome by conducting all experiments in screw-top jars which were secured with their vertical axes along the radius of a wheel rotating in a vertical plane and completing a revolution every 2 min. Even the fine grains of sand varied in size from below 3 up to 40 μ in diameter, and a suspension of approximately standard size distribution was obtained by using immediately the supernatant liquid decanted off from a suspension prepared 30 min. previously.

Three series of experiments were set up using adult (9·0 mm. average length) *Artemia.*

The first series of experiments consisted in feeding mixtures of particles to the animals. As far as possible the constituents of the mixtures were present in roughly equal proportions and the particle concentrations of each were of the order of 10 to 100/mm.^{3}. Each experiment was conducted using 350 ml. bottles filled with the particle suspension and containing three male and three female animals. The bottles were slowly rotated for 14–20 hr. Control bottles without animals were treated similarly. In order to reduce the counting errors involved in estimating particle concentration at the end of experiments, mean particle counts were in general based upon the counting of at least twenty fields. Confidence limits (95 % level) were assigned to these mean counts by the method described in detail in Reeve (1962). It can be shown for a series of independent mean particle counts, where the individual particles were distributed randomly in the counting chamber, that these conform to a Poisson model where the mean and variance are by definition equal. By use of the method of Skellam (1960) confidence limits may be set to the mean of the Poisson distribution using only the mean itself and the number of observations from which the mean is calculated.

To test the ability of *Artemia* to select food from mixtures of organic particles, suspensions containing three species (*Phaeodactylum*, *Dunaliella* and *Asteromonas*; *Phaedoactylum*, *Dunaliella* and *Chlorella*) and also suspensions containing two species (*Phaeodactylum* and *Dunaliella*; *Dunaliella* and *Chlorella*) were employed. Mixtures of nutritious and non-nutritious particles, such as mixed suspensions of sand with *Phaeodactylum* and sand with *Dunaliella*, were used to determine whether the animal had any preference for the food cells.

The second series of experiments consisted in finding out more about the reactions of the animals to inorganic particles. The experiments were planned to investigate the effect of the concentration of the sand on the filtration rate of *Artemia*. In all particulars these experiments were identical to those described in the previous paper. Since they were performed using a different population at a different time, a control run was included using *Phaeodactylum*, in order that any differences in feeding behaviour towards organic and inorganic particles should be strictly comparable.

The third series of experiments was undertaken in view of the implications of the preliminary results of the other two series. It was found necessary to ascertain the effect of varying amounts of suspended inorganic material on the ingestion rate of *Artemia* feeding on a plant cell suspension of fixed concentration. This was done by setting up seven replicate feeding experiments each containing a different quantity of standard sand suspension (between 1–100 ml. where 1 mm.^{3} contained 5600 particles), added in such a way that the total volume of medium and the cell concentration were the same in all. Other particulars were as detailed above for mixed suspensions.

RESULTS

In a mixture of *Dunaliella* and sand rotated for 20 hr. and containing no animals the concentrations of both particles remained unchanged. Thus any change in the experimental vessels could be ascribed to the feeding activity of the animals.

Table 1 records the mean cell concentration and number of fields counted to obtain it for control and experimental counts for the first series of experiments using mixtures of plant cells. Usually two experiments (A and B) were run with each prepared mixture (control). The results have been expressed as $(C_t/C_0) \times 100$, using the standard notation of Gauld (1951) where C_0 is the initial (or control) concentration and C_t is the final concentration at the conclusion of the experiment, for each particle type of the mixture. The expression is the percentage of each particle remaining after the animals' feeding activity and has been calculated as a range based on the errors of the counting technique. Reference to Table 1 indicates very little evidence to suggest that *Artemia* discriminates between different plant cells, except in the *Phaeodactylum–Dunaliella–Asteromonas* mixtures, from which a significantly smaller number of *Phaeodactylum* cells have been removed. Lack of success in the culture of *Asteromonas* prevented further investigation of this mixture. No selection is evidenced in any of the other 15 experiments.

Table 1. *Percentage of each food cell of a mixture remaining after feeding*

A, Asteromonas; C, Chlorella; D, Dunaliella; P, Phaeodactylum.

		Control		Expt. A		Expt. B		C_t/C_0 range	
Expt. no.	Plant cell	No. of fields	Mean	No. of fields	Mean	No. of fields	Mean	Expt. A	Expt. B.
1	*P*	80	40	40	24	40	22	54–65	49–58
	C	80	37	40	15	40	13	36–45	30–39
	A	80	11	40	5	40	4	38–43	33–37
2	*P*	40	18	40	16	—	—	75–102	—
	D	40	18	40	9	—	—	43–61	—
	A	40	14	40	5	—	—	33–43	—
3	*D*	40	31	20	15	20	14	38–56	36–52
	C	40	39	20	20	20	20	44–59	44–59
	P	40	36	20	18	20	16	43–58	43–52
4	*D*	40	25	20	16	10	14	55–77	46–71
	C	40	45	20	33	10	28	64–81	52–72
	P	40	41	20	29	10	28	62–80	56–78
5	*P*	10	43	20	24	—	—	46–67	—
	D	10	42	20	25	—	—	49–71	—
6	*P*	40	61	40	2	40	2	3–4	2–3
	D	40	34	40	1	40	1	3–4	2–3
7	*P*	30	40	15	6	15	7	11–18	15–21
	D	30	59	15	9	15	10	12–18	13–20
8	*D*	40	34	20	23	20	25	59–79	65–86
	C	40	40	20	24	20	26	53–69	57–76
9	*D*	40	40	20	25	20	23	55–73	50–68
	C	40	76	20	55	20	50	65–81	60–73
10	*D*	40	39	20	29	20	34	65–85	77–98
	C	40	46	20	34	20	40	65–83	76–96

The results for the 16 sand mixtures have been tabulated in a similar manner (Table 2). In experiment 4B a significantly higher percentage of *Dunaliella* remained and in 5A and B significantly higher percentages of *Phaeodactylum* were ingested. In the other 13 experiments no selection toward either organic or inorganic particle could be proved, and hence the overall conclusion must be that *Artemia* shows no marked selection abilities in this respect.

From the second series of experiments Figs. 1A and B show the effect of increasing concentration of sand upon filtration and ingestion rates respectively. Superimposed are the results of separate experiments using *Phaeodactylum* under otherwise identical conditions. Two significant points emerge.

(1) The maximum filtration rate is independent of the nature of the particle.

(2) The maximum filtration rate is maintained up to a particle concentration of

1000/mm.^{3} for sand, but only up to 50/mm.^{3} for the plant cells; or alternatively the maximum number of plant cells ingested per animal per hour was in the region of 5.5×10^5 and the maximum number of sand particles was 40×10^5.

Table 2. *Percentage of each particle in a sand/plant cell mixture remaining after feeding*

P, Phaeodactylum; D, Dunaliella; S, Sand.

Expt. no.	Particle type	Control: No. of fields	Control: Mean	Expt. A: No. of fields	Expt. A: Mean	Expt. B: No. of fields	Expt. B: Mean	C_t/C_0 range: Expt. A	C_t/C_0 range: Expt. B
1	*D*	40	68	20	43	20	48	57–70	64–78
	S	40	68	20	46	20	47	61–75	62–77
2	*D*	40	49	20	25	20	25	44–58	44–58
	S	40	74	20	35	20	34	42–53	41–51
3	*D*	40	80	20	43	20	38	49–60	42–53
	S	40	84	20	46	20	40	50–61	43–53
4	*D*	40	36	20	23	20	25	55–74	60–80
	S	40	65	20	40	20	30	55–68	42–51
5	*P*	40	56	20	20	20	15	31–41	23–31
	S	40	115	20	63	20	47	51–60	35–45
6	*P*	40	55	20	37	20	34	60–75	55–69
	S	40	54	20	41	20	36	68–85	59–74
7	*P*	40	55	20	34	20	32	55–69	52–66
	S	40	79	20	43	20	39	49–60	44–55
8	*P*	40	72	20	33	20	27	41–52	33–42
	S	40	76	20	43	20	35	51–63	41–51

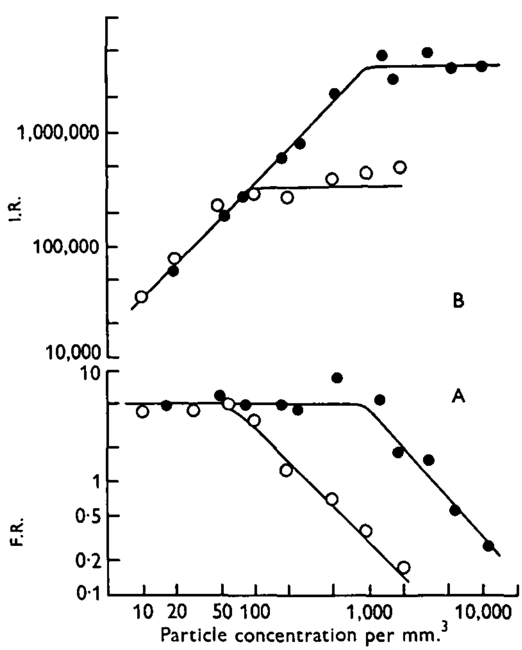

Fig. 1. Filtration and ingestion rates (F.R. and I.R. in Figs. 1 A and B respectively) with increasing particle concentrations, both axes logarithmic. ○, *Phaeodactylum*; ● sand.

The concept of a maximum filtration rate developed in the previous paper is very clearly supported in Fig. 1 A, where it is maintained over so wide a particle concentration.

Attempts to calculate the volume of a sand grain were highly inaccurate because of the wide variation of size and irregular shape of the grains. Finding a mean diameter of 7 μ and assuming a spherical shape, particle volume might be set at 180 μ^3. However, even small particles were generally composed of clusters of smaller ones, which would tend to give the impression of a greater volume than there really was. Bearing this in mind it was probably safe to assume that the average particle volume of sand was similar to that of *Phaeodactylum* (found to be 113 μ^3 in the previous paper). From the above data it may be calculated that *Artemia* was ingesting inorganic particles at the rate of 0·51 mm.^{3} per hour as opposed to 0·045 mm.^{3} for *Phaeodactylum*, or over 12 times more sand by volume.

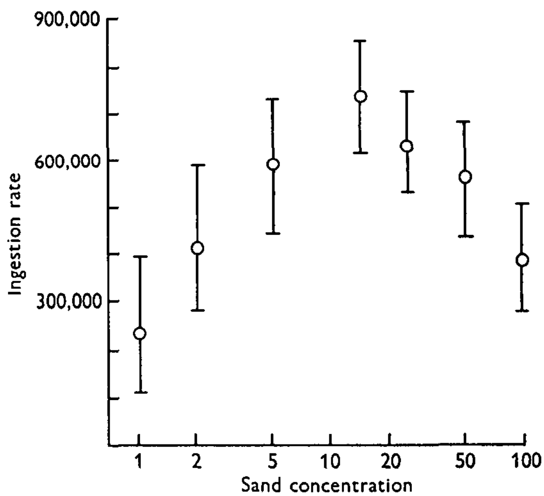

Fig. 2. Rate of ingestion of *Phaeodactylum* (with ±95 % confidence limits based on counting errors) as sand concentration increases.

It is conceivable that the volume estimations of either or both *Phaeodactylum* and sand particles were so inaccurate that the apparently greater rate of sand ingestion could be discounted. In this connexion a knowledge of faecal pellet production rate is not helpful, for even though it was found that many more pellets were produced when feeding on sand (see the following paper, Reeve, 1963 *c*) this could be attributed to its failure to be digested, since digestion has been shown to account for 90 % of the ingested organic material in copepods under certain conditions (Marshall & Orr, 1955). However, from Fig. 2, a typical result of the third series of experiments, the effect of increasing the sand content of the medium on the ingestion rate of *Phaeodactylum* may be seen to lead to an increase in the number of plant cells consumed up to a sand particle concentration corresponding to the addition of 12 ml. of sand suspension. As the total particulate matter was increasing over this range, and as it has been demonstrated that *Artemia* shows little or no preference for the plant cell, the total amount of material ingested must have been considerably more than when feeding in a pure suspension of *Phaeodactylum*. Presumably as the sand reached its highest concentrations the proportion of sand to plant cells was too great for an increase in ingestion of the latter alone to be manifested.

DISCUSSION

Harvey (1937) and Fuller (1937) found low filtration rates with *Nitzschia* (= *Phaeodactylum*, see Lewin, 1958), the latter suggesting these cells were too small to be effectively filtered, although Harvey found that, in a mixed suspension of *Lauderia* and *Chaetocerus*, *Calanus* preferred the former. *Chaetocerus* was probably considerably larger than *Nitzschia* (Gauld, 1951) and may even have been too large (Riley, Stommel & Bumpus, 1949) though *Calanus* will feed on it (Raymont & Gross, 1942). A possible explanation of this selection might be that *Lauderia* tended to sink from suspension, which resulted in an apparent filtration rate being recorded. In this context Marshall & Orr (1955) found no selection by *Calanus* in mixtures of *Lauderia* with *Skeletonema*, *Syracosphaera* or *Cryptomonas* and reported an experimental mixture of *Prorocentrum* and *Coscinodiscus* in which the flagellates swam freely and the diatoms sank to the bottom of the dish. They concluded that in *Calanus* if selection takes place it is to no marked degree, which is very well in agreement with the findings here presented for *Artemia*, and with the views of Provasoli and his associates (personal communication) based on experiments they are currently conducting.

However, the question has been reopened by the recent work of Petipa (1959), who observed that females of *Acartia clausi* would choose the larger solitary round cells from a mixture containing solitary and chain varieties of varying size and form. Mullin (1962) confirmed this tendency to prefer larger cells, and Anraku (1962) found that *Acartia tonsa* would eat equally readily *Thalassiosira fluviatilis* and *Artemia* young in a mixed suspension of the two, though *Calanus finmarchicus* definitely preferred the plant food. He was able to correlate this divergence of behaviour with the morphologically different structure of their mouthparts.

Corner (1961), analysing the organic and inorganic fractions of total particulate matter in sea water before and after *Calanus* had fed on it, found that of a total of 505 mg. (dry wt.) of particulate matter, after feeding, 461 mg. remained, of which 215 mg. was inorganic and 246 was organic. The animals had removed 46 mg. of material, 80 % of which was organic, although the medium contained only 56 % organic material. The reliability of the analyses was not fully worked out, so that it was not possible to be sure how real were the differences in the small amounts actually consumed. Furthermore, the faecal pellets might be expected to contain a high percentage of the non-digestible inorganic fraction. These were prevented by a sieve from being swept out of the apparatus with the flow of the water. If this agitation caused a certain loss of material from the pellets, relatively more inorganic material would be returned to the water to create the impression of selective feeding rather than selective digestion. It is also possible (Gauld, personal communication) that some of the inorganic particles might have been too small for the filtering setules (said to be no closer than 5·7 μ by Ussing, 1938).

The apparent automatic filtration of sand over a much wider range of concentrations than would be expected from its particle volume may have several possible explanations which will be touched upon in the following paper. That it is taken at all may be adduced as further evidence that the feeding and ingestion apparatus of *Artemia* is not capable of any degree of discrimination. Whether this special behaviour towards inorganic material is of any functional advantage to the animal is a matter of speculation, as also is whether any other filter-feeding zooplankton show it. It is doubtful that such high concentrations of inorganic particles as were used in some of the experiments reported here would ever be encountered in nature, but in small quantities inorganic particles have been found to stimulate a tripled intake of organic particles. This may be valuable in natural waters where the inorganic fraction can equal or exceed the organic (Corner, 1961); the ingestion rate would be increased so that the amount of organic material ingested per unit time was at least as great as if the total particulate matter were organic. However, a faster ingestion rate might result in a lowered digestion efficiency. Another implication is that laboratory determinations of filtration rates using pre-filtered water could under certain conditions considerably underestimate those to be expected under natural conditions in which some inorganic particles were present.

SUMMARY

1. The ability of *Artemia* to select particles from mixed suspensions has been studied.

2. The animal shows no appreciable ability to discriminate between plant cells presented in mixtures containing two or three different types.

3. The animal shows no appreciable ability to discriminate between nutritious and non-nutritious particles.

4. When presented with mixed suspensions of *Phaeodactylum* and sand particles the animal ingests much greater volumes of sand, over a wide range of concentration of sand particles. Low concentrations of inorganic particles appear to promote increased ingestion of plant cells.

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