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29. FEEDING BEHAVIOUR OF THE GREEN MUSSEL, *PERN A VIRIDIS* (LINN.) IN LABORATORY

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ABSTRACT

The rate of filtration and feeding on six species of diatoms by the green mussel *Perna viridis* Linn In the laboratory has been studied. The number of cells removed per hour depended upon the size and suspension density of the diatom cultures- Generally the mussel was found to eliminate more than 50% of the filtered cells as pseudofaeces. The rate of ingestion was enchanced when the suspension density and cell size were less. Eventhough large quantities of pseudofaeces were produced when suspension density was increased, the actual ingestion of food was not affected by call concentration. The maximum filtration rate [341.43] ml hour -'g m —1 was noted in *Thalassiosira fluviaiillis* suspension. The relation between the rate of filtration and call size and density of the suspension was studied and discussed.

INTRODUCTION

Efficient production of culturable bivalve molluscs in controlled environments require exact information about the rate of feeding and particle transport. Clams and oysters subsist mainly on particles filtered from the surrounding water, which they pump through the gills. An extensive literature deals with the results of microscopical examination of the content of the digestive tract, to estimate the relative significance as food of the various types of organic matter present in bivalves (Allen 1921, Coe 1948 Coe and Fox 1944, Savage 1925, Vervey 1952).

The volume of water from which particles are removed in a given period is termed the filtration rate [Fox etal1937]. Generally the filtration rate and pumping rate are not same, because the retention of prticles by bivalves is rarely 100% efficient [Jorgensen 1955], the pumping rate is usually greater than filtering rate. The ingestion of food by bivalves mainly depend on the filtration rate, but various other factors also control the ingestion rate. Some of the particles filtered by the gills are eaten, while others are rejected as pseudofeces. The ejection of feces and pseudofeces are known as biodeposition.

Although there is considerable information concerning the filtration and pumping rates of bivalves, little consensus regarding rates of feeding or ingestion is available. Under experimental conditions different types of organisms and different mixtures of organisms Qf various size result in different rates of filtration and possibly of feeding (Loosanoff and Engle 1947; Rice and Smith 1958; Smith 1958; Allen 1962; Davids 1964j. Rice and Smith (1958) conducted the major study of *Mercenana mercenaria.* They supplied the clams with the four species of algae tagged with ^^p jn unreplenished suspensions, Allen (i962) studied the filtration rate, ingestion rate and biodeposition of s^p labelled *Pheodactylum* by for species of bivalves. The total biodeposition of three species of bivalves in relation to suspension density was studied by Haven and Morales-Alamo (1966), and found that the density of suspension determines the rate of filtration, pseudofeces formation and rate of food ingestion Walne (1970) investigated the food value of different species of algae in culture. AM [1970] investigated the filtration rate and biodeposition of *Hiatella arctica* by using two species of micro algae in different cell densities. Tenore and Dunstan [1973] studied the rates of feeding and biodeposition of American oyster by using four species of phytoplankton, viz., *Thaiassiosirapseudonana, Skeletonema costatum, Dunaliella tertiolecta* and *Nitzscfiia closterium.* Tenore et al [1973] studied the food chain dynamics of the oyster, clam and mussel in an aquaculture food chain.

Epifanio [1976] studied the nutritional requirements of juvenile and adult bivalves. Foster-Smith [1975] investigated the assimilation efficiency of three species of bivalves by using of *Phaeodactylum* sp and found that the assimilation mainly depend upon the rate of ingestion. The rate of removal of four species of algae from suspension by the oyster *Crassostrea virginica* was determined by Epifanio and Ewart [1977] and found that the filtration rate was influenced by the suspension density and cell size of the algae. Malouf and Breese [1977] measured the effects of algal concentration and larval density on the growth of larval oysters in a flow through feeding system.

Salzwedel [1979] quantitatively estimated the production of pseudofeces and feces by *Tellina jabula.* For long term bioaccumulation studies with suspension feeding mussels, Boetter-Jensen and Dahlgaard [1981] designed an apparatus to maintain phytoplankton cell concentrations at a constant level. Wilson [1983] studied the retention efficiency and pumping rate of *Ostrea edulis* in suspension of *Isochrysis galbana* in relation to cell concentration. Urban and Pruder [i 933] compared the growth of *Crassostrea virginica* at five algal ration levels for a period of 3 weeks Berry and Schleyer [1983] estimated the assmilation efficiency of/'eA/7a/DeA/7a on a natural diet of particles <lOO!*min diameter. Col well et al (1984) has developed microencapaulation techniques for artificial food to rear bivalve molluscs in recirculating system.

The food value of unialgal diet and mixed algal diets to juvenile oysters were determined, and mixed algal diet proved to be good for juvenile oysters (Romberger and Epifanio 1981-)

MATERIAL AND METHODS

Six species of diatoms (Table 1) were cultured in the lab. condition by using, f/2 medium (Guillard and Ryther 1962) made up in aged and filtered sea water with the salinity adjusted to 287oo and sterilized by autoclaving. All the six species of diatoms came from clones isolated from Vellar estuary (11°29'N; 79°49'E) and maintained in laboratory. For the feeding experiments, mass cultures of each species were developed. The cultures were shaken periodically to keep the cells in suspension. Continuous illumination at 4000 lux was supplied by 40 W'cool white' florescent lamps for 16 h in a day and temperature ranged from 28-30°C. Cell counts were made with Haemocyrometer (Neubauer improved double ruling' Fein optic. Made in GDR). Algae used in the experiment were from cultures that had reached the stationary phase to avoid the multiplication during the experimental period.

TABLE - 1 . *The algal species used for the experiment and their size in laboratory culture.*

SI. No.	Species of diatoms used	Cell size (A m	Suspension density cells/ml (x10*)
1.	*Chaetoceros gracilis* Schutt non pant	3-5	45 to 500
2.	*The lass iosira fluviatilis* Hust	8-10	7 to 100
3.	*Skeletonema costatum* (Grev.) Cl	7-9	10 to 100
4.	*Thalassionema nitzschoides* Grun	3-5 dia. 15-25 leng,	6 to 55
5.	*Chaetoceros didymus* Ehr	10-18	3.8 to 25
6.	*Streptotheca tamesis* Shrubs	22-30	1.8 to 10

The green mussel *Perna viridis* individuals of same age groups [the wet weight of the whole animals ranged from 2.0 g to 3.1 g] were collected from the field, washed free of epifauna and flora and acclimated in the laboratory aquarium tanks. During the acclimation period the animals were fed with cultured diatoms-mixed form. Prior to the experiment, the animals were allowed to starve for about 12 h. On the day of expeiiment they were removed from the acclimation tank, washed free of adhering debris, blotted free of excess water and weighed. Then the animals were placed in the experimental tank and the algal suspension of known concentration was added. Table 2 shows the cell concentrations of the suspensions used for the experiments. The algal suspension was continuously agitated by mild air bubbles to

TABLE 2. *Rate of filtration and feeding of P.v* iridis *in different suspension density of <aigae.*

1.	C. *gracilis*	2.5	45	232.2	59.00	202.00	104.50	22.60
		3.1	75	241.0	64.40	483.90	180.80	11.68
		2.2	200	250.0	60.00	1040.00	500 00	5.50
		2.4	500	128.0	58.80	1451.50	640.00	3 66
2.	*T. fiuviatilis*	2.5	7	278.5	22.50	26,24	19.50	46.16
		2.8	15	341.4	44.00	101.00	51.10	30.10
		2.1	40	252.0	53.10	141.80	92.80	27,24
		2.0	50	181.40	62.60	118.80	90.70	34.51
		2.0	100	157.50	6500	250.00	157.50	20.63
3.	*S. costatum*	2.7	10	155.5	36.30	5.60	15.55	86.54
		2.5	35	172 0	82.00	68.00	60.20	54.49
		20	60	149 2	81.00	98 80	89 50	45.25
		2.7	100	148.0	136.00	256 20	148.00	34.68
4.	*T. nitzsctioides*	2.4	6	108 0	10 08	5.00	6.48	6702
		2.0	15	122.5	16.60	7.90	12 25	67.76
		2.3	25	128,0	26.50	47.10	32.00	36.01
		25	55	92.2	25 00	102.OJ	50.72	1976
5.	*C, didymus*	2.1	3.8	1179	6.40	3.01	4 48	68.00
		2.3	8	129 9	10 90	12 00	10.39	45.61
		2.5	10	106.4	9.50	17 00	10.64	35.71
		2.5	25	83.5	14.50	37.70	20.83	27.78
6.	*S. tamesis*	2.0	1.8	131.7	3.80	0.94	2.37	80.16
		2.5	5	176.7	10.06	12.00	8.82	45.60
		2.6	7.5	182.0	11.85	24.32	13.65	32.76
		2.1	10	113.3	9.10	14.70	11.33	38.23

avoid diatom settling. The resulting concentration of the diatom suspension was determined by using haemocytometer for each 15 minutes intervel of feeding. In each 15 minutes after sampling, a new suspension of diatom was added to maintain the original concentration.

During the experiment the production of pseudofeces and feces were removed from the tank and collected in a beaker to minimize the chances of reingestion by the mussels. All the feeding in the laboratory at the room temperature of 28°C ± 2°C. The salinity of the feeding suspension was28±1°/oo' and the pH was 7.9 ± 02. The control cultures of diatoms of the same age kept in the experimental condition showed no growth in the experimental duration.

The collected pseudofeces were resuspended in known volume of sterile water and the cell numbers were counted. Then the total number of cells cleared from the suspension was calculated. The rale of filtration was calculated by using the formula F = R/C (Epifanio and Ewart 1977) where 'F' is the filtration rate in ml/h/g whole weight, 'R' is the mean number of cells removed from suspension per gram whole weight per hour and 'C is the number of algal cells per ml in suspension.

RESULTS

Rate of Filtiation

The total number of cells removed from the suspension appeared to be related to the size of the algal cells and the suspension concentration (Fig ') The filtration rate was high in small celled diatom suspensions and decreased towards the increase of diatom cell size. The maximum number of cells filtered in unit time was clearly less for the larger algae, *Streptotheca tamesis, Chaetoceros didymus* and

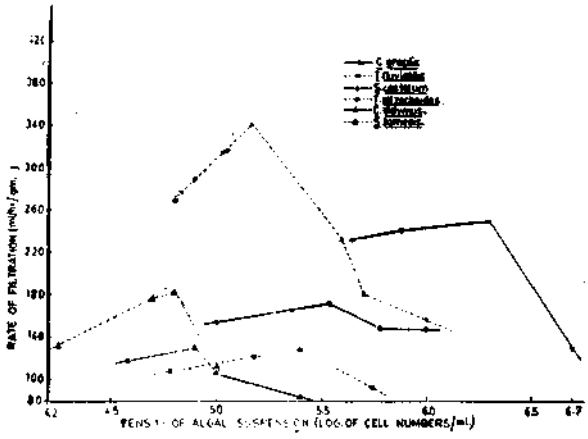

Fig. 1. Mean filtration rate as a function of density of algal suspension in P. *vindis.*

Thalassionema nitzschoides than the other three species (Table 2). The highest value of filtration density of 1.5x106 cells/ml of *Thalassiosira fluviatilis.* Rate of filtration was the lowest (83.5 ml/h/gm) when fed on *C. didymus* at 2.5x10« cells/ml. In all the algal suspensions, the filtration rate was relatively low in low cell densities and gradually incressed up to a particular concentration and then declined with the further increase of suspension concentration. A sudden decline of filtration rate was observed in *Chaetoceros gracHiis* and S. *tamesis* suspensions due to high suspension density [Table 2]. Eventhough fhe filtration rate was reduced with the increase of suspension density in all the experiments the number of cells removed per unit time increased with the increase of suspension density [Fig 2]. The following is the order of filtration rate observed for the six species employed: *T. fluviatilis* > *C. gracilis* > *S.tamesisySkeletonema costatum}C. didymus* > *T. nitzscftoides.* It is interesting to note that the rate of filtration of 5./a/WM/s was slightly higher than that of *S. costatum* eventhough the cell size of the former species is larger than the latter But the mean number of cells of S. *tamesis* removed by the mussel was lower than that of all the other forms.

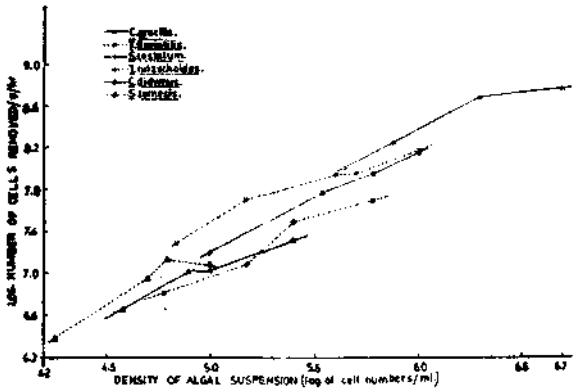

Fig. 2. Rate of removal as a function of algal suspension in *P. viridis*

Food Ingestion and Pseudofeces Production

The rate of food ingestion also varied with respect to the algal cell size and suspension density. In low Suspension density, most of the filtered cells were ingested and the vice versa was observed in high concentrations, The filtered cells rejected were eliminated as strings of pseudofeces. But in most of the cases the actual amount of cells ingested was "ot affected, by concentration. The least amount of filtered cells were ingested when fed on C. *gracilis.* More than 75% of the filtered cells were eliminated out as pseudofe. c^s even in the lowest suspension density [4.5 xlO"] and only 226% was ingested. The ingestion rate gradually decreased and only 3.66% was ingested at the highest suspension density [5 x 10» cells/ml]. The mean number of cells ingested by the mussel was more or less same (60.55 ± 2.6 x 10^ cells h) in all the suspension densities. More than 50% of the filtered cells of *T. fluviatilis* were ejected as pseudofeces and 46 16% was ingested at the lowest suspension density used. The pseudofecal production increased with the increase of suspension density and 20.63% was ingested at the cell density of 1x10^ cells/ml. The actual number of cells ingested by the mussel

was 22.5 X 10»/h and SSxIOo/hper animal a the lower and higher cell densities respectively.

The highest ingestion rate was observed when fed on *S. costatum.* In the lowest suspension density [1 x 10» cells/ml] 86.54% of the filtered cells were ingested. The remaining was [13.46%] ejected out as pseudofeces. But the percentage of ingestion was decreased to 34.68% in the highest concentration of alga, cells (1 X 10« cells/ml.) The actual number of cells ingested was more or less same in the concentrations 3.5x106 and 6 x 10^ cells/ml. But the values were slightly lower and higher in the extreme suspension densities.

The cells of the other 3 diatoms, *T. nitzschoides, C. didymus* and S. *tamesis* were effectively ingested at the lowest concentrations. Eventhough the cell sizes of these diatoms are larger than the other three, the ingestion rates were relatively high. As in other cases, the pseudofecal production was enhanced when the suspension density increased. Eventhough the number of cells removed from the suspension was increased with the increase of suspension density, the percentage of filtered cells ingested decreased. The mean number of cells ingested shows a complicated pattern with respect to the suspension density. The number of cells ingested by the mussel ranged from 10.08 x 10^ to 26.5 X 10« cells/h per animal in *T. nitzschoides,* 6.4 X 10« to 14.5 x 10« cellsyh per animal in *C. didymus* and 3.8x108 to 11.85 x 10" cells/h per animal in *S. tamesis.*

DISCUSSION

Rate of Filtration

As many workers have emphasized, the pumping rate in bivalves is a function of the concentration of particles present in the suspension. The present study reveals that the filtration was very much decreased due to the increase of suspension density and cell size. Mostly the diatoms of small cells were efficiently cleared by the mussel. When the cell concentration was low, the efficiency of retention was high. The retention efficiency mainly depend upon the cell size, suspension density and the relative effect of the bivalve. Loosanoff and Engle (1947) observed that the cells of flagellate *Euglena* of 60M. could easily pass through the gills, sometimes only 15% were strained from the suspension, and maximum retention was 80%. But the cells of *Chlorella* [5 (*] were retained by the gills, varyingly from 0 to 85%. Tammes and Oral [1955] observed widely varying retention of particles less than 30-40 *v*-by mussels. The retention efficiency of the mussel *I\/lytiius edulis* on blood corpuscles of 7-8/x varied from 0 to 98%. Jorgensen (1966) reported that very high concentrations can evoke supression or deterring responses in suspension feeders. Davids [1964] found a reduction in particle retention of mussels at concentration of */V/'fz5c/?/a* above 1 x 106 cells/ml. Epifanio and Ewart [1977] reported that the total number of cells removed from suspension was related to the size of the algal cells. They observed that the maximum number of cells filtered was clearly less for the larger algae than for the smaller ones. Further, they found that the rates of filtration was inversely related to the concentration of particles in suspension. However Ballantine and Morton [1956] claimed that *Lasaea rubra* cleared suspensions of *Chromulina pusiHi* (1 to 2 ^) and *Prorocentrum micans* (30-40(A) with equal rates independent of the size. The results of the present study closely agrees with the results of Epifanio and Ewart [1977].

Food Ingestion

Although there is considerable information concerning the filtration rates of bivalves, less is known about their actual feeding rates. In the present study, the results presented in the Table 2 shows that considerable amounts of filtered cells were ingested in low suspensions concentrations [Fig 3]. The ingestion rate was found to be high in S. *costatum* and low in C. *gracilis.* Considerable amounts of *S. tamesis* also was ingested. Matthiessen and Toner [1966] calculated that adult oysters growing near Martha's vineyard, Massachusetts could not possibly eat more than 1.1 xlO' algal cells/ animal/day. Tenore and Dunstan (1973) showed that both clams and oysters fed most efficiently at food concentrations of 2 x 10^ cells.ml.

Eventhough the filtration rate was affected by high suspension concentration, the actual

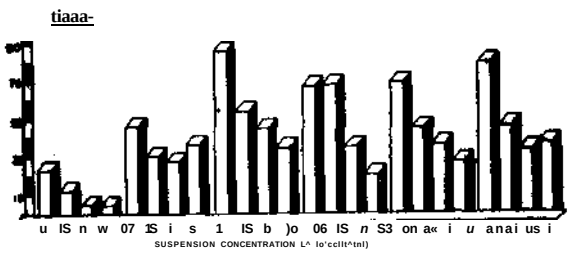

Fig 3 Number of cells ingested in relation to percentafle of total ceils filtered in *P. viridis.*

number of cells ingested was not affected (winter, 1973). The findings of the present study closely agrees with Winter's (1973) concept. The table 2 shows that the percentage of filtered cells ingested decreased with the increase of suspension, concentration, since copious amounts of pseudofeces were produced when the suspension density increased. It is evident that *Perna viridis* apparently have a maximum rate of ingestion in low cell concentration.

Some other factors also govern the ingest on rate of bivalves. Loos^noff and Engle(19l7) and Floyd (1953) reported that th"? depressed feeding rate of oyster on *Chlorella* was due to the external metabolites present in the filtrate. The absence of silt with the algal diet reduced the clearance rate and ingestion rate in *l\/lytHus edulis* (Kiorbe et al 1980) Walne (1970) found that the higher the concentration of *Dunaliella,* the lower the growth rate of oysters i e. the food ingestion was reduced The results of the present study shows that the number of cells ingested was slightly increased when the suspension concentration increased. In *T. nitzschoides,* the number of cells ingested was found to be doubled in high concentration. Navarro and Winter (1982) also observed similar results in *l\Aytilus chilensis.* In these cases, the digestion would be partial, and the high ingestion rate however counter balanced by a significant decrease in assimilation efficiency (Navarro and Winter 1982).

The differences in ingestion rate of various diatoms may be due to the nutritive value of the species and sorting mechanism of the bivalve. Loosanoff (1949) and Menzel (1955) described ciliary mechanisms which they believed may be responsible for sorting certain particles containing perhaps the more nutritious material in the food strained off the gill. Jorgensen (1949) found that when the rate of uptake of graphite suspensions of 4 to 5 (^ particle size was compared with that of flagellate cultures of *D. inornata* and */ galbana* of similar particle size, the filtration rate of flagellate cell was greater. However, the ingestion of palatable or nutritious particles may stimulate filtration. Epifanio (1979 b), and Romberger and Epifanio (1981) reported that the difference in growth rate in oysters when fed with various species of algae was due to the relative digestability of the algae. So it is believed that the mussel *P. viridis* is havir.g some selective mechanism to avoid less nutritive materials. The least ingestion rate of C. *gracilis* might be due to the poor palatable or nutritive quality of the species

Generally S. *costatum* and *T. fluviatilis* are considered to be good sou'ce of food to bivalves. The high feeding rate of *Perna viridis* on *S. costatum* proves the same. Tenor and Dunstan (1973 argued that the oysters fed on *Ttialassiosira pseudonana* a nd *Isoctirysis galbana* have grown well. This was due to the high rate of ingestion and assimilation. Ukeles and Wikfors (1982) reported that the growth of oysters was rapid when fed with *Thalassiosira.* Dean (1957) compared the food value of S *costatum* which field observations had indicated to be a good food to bivalves The present investigation also proved *S. costatum* as a good source of food to *P. viridis.*

The three species of diatoms 7'. *nitzschoides, C. didymus* and S. *tamesis* aslo seem to be equally good food source to *P. viridis,* fjnce the ceils of these forms also ingested considerably. Walne (1970) stated that with some variations, that algae which were good or bad foods for one species of bivalve ware of similar value to the other species of bivalves also.

Pseudofeces Production

All the particles filtered on the gill surface are carried by ciliary currents to the labial palps and so to the mouth or to the mantle edge to be rejected as pseudofeces, Table 2 shows that

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HAVEN, D. S. AND R. MORALES ALAMO-1936. Aspects of biodeposition by Oysters and other invertebrate filter feeders. In relation to body size and different algal concentration *Mar. Biol.,* 67 (3): 255-266.

RICE. T. R. AND R. J. SMITH 1958. Filtering

the production of pseudofeces increased with the increase of suspension concentration. Loosanoff and Engle (1947) using a continuous flow system, added *Chlorella* suspension to seawater and estimated that the pseuodfeces

ALLEN, W. R. 1921. Studies on the biology of freshwater mussels. Experimental studies on the food relations of certain Unionidae. *Biol: Bull.,* 40 : 210-241.

determined with radioactice phytoplakton. U. S. Fish Wildlife Serv., *Fisheries Bull.,* 68:73-82.

ROMBERGER, H. P. AND C. E. EPIFANIO. 1981: Comparative effects of diets consisting of one or two algal species upon assimilation efficiencies and growth of juvenile oysters, *Crassostrea virgmica* (Gmelin). *Aquaculture,* 25 :*11-11.*

SALZWEDEL, H. 1979. Reproduction, growth, mortality and variations in abundance and biomassof *Tellina jabula* (Bivalvia) in the German Bight in 1975-76; Veroff. Inst. Meeresforsch. Bremerh., 18:111-202.

SAVAGE, R. E. 1925. The food of the oyster. *Fish. Invest. London Ser,* II, 8 (1)

SMITH, R. J. 1958. Filtering efficiency of hard clams in mixed suspensions of radioactive phytoplankton. Proc. Natl, shellfish. Assoc, 48:115-124.

TAMMES, P. M. L. AND A. D.G. ORAL 1955. Observations on the straining of suspensions by mussels. *Arch, neerl. Zool.* 11 :87-112.

TENORE, K. R. AND W. M. DUNSTAN 1973. Comparison on feeding and biodeposition of three bivalves at different food levels. *Mar. Biol.,* 21 .190-195.

TENORE, K. R., J. C. GOLDMAN AND C J. PHILLIP. 1973. The food chain dynamics of the o/ster, clam and mussel in an aquaculture food chain. *J, exp. mar. Biol. Ecol.,1 2:* 157-165.

UKELES, R. AND G H. WIKFORS. 1982. Design, construction and operation of a rearing chamber for spat of *Crassostrea virginica {Gmelin). J. Shellfish Res.,* 2(1): 35-39.

URBAN, E. R. AND G. D. PRUDER. 1983. Comparison of the growth of *Crassostrea virginica* (Gmelin) at five algal ration levels with specific reference to predictive feeding equations. *J. Shellfish Fas.,* 3(1) : 102 p.

VERWEY, J. 1952. On the ecology of distribution of cockle and mussel in the Dutch Wadden Sea. Their role in sedimentation and the source of their food supply. With a short review of the feeding behaviour of bivalve mo< Muses. *Arch, neerl. Zool.,* 10:171-239.

WALNE, P. R. 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus. Fishery Invest., London Ser.,* 2; 25 (5) : 62 pp.

WILSON, J. H 1983. Retention efficiency and pumping rate of *Ostrea edulis in* suspensions of *Isochrysis galbana. Mar. Ecol. Prog. Ser..* 12(1): 51-58.

WINTER, J. E. 1973. The filtration rate of Mytilus edulis and its dependence on algal concentration, measured by a continuous automatic recording apparatus. *Mar. Biol., Biol.,* 22: 317-283.