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Particle clearance and selection in three species of juvenile scallops

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Juvenile scallops (< 2 mm shell height) of three species (*Placopecten magellanicus*, *Patinopecten yessoensis*, *Argopecten irradians*) were fed mixed, unialgal cultures. Scallops were fed a total of six algal clones simultaneously and clearance rates were monitored using flow cytometric techniques. In another experiment, scallops were presented with natural assemblages of particulate matter as a food source. Data are presented on differences in clearance rates for the individual algal species as well as size-related differences of algal clones, and uptake of chlorophyll vs. non-chlorophyll cells, both within and between scallop species. Significant differences in clearance rates of individual algal species have been found within and between scallop species. Particle selection does not appear to be based upon size alone and is apparently based on other characteristics of the algae as well. The results demonstrate pre-ingestive sorting.

KEYWORDS: Feeding, Scallops (*Placopecten*, *Patinopecten*, *Argopecten*), Selection

INTRODUCTION

A suitable algal diet is crucial for the development, growth and survival of cultured bivalves. Diets that support larval growth have been determined for several bivalve species, but post-metamorphic dietary requirements are still poorly understood. In addition, very little is known about the functioning of the feeding organs in juvenile scallops (Beninger *et al.*, 1994). Rapid growth and high survival of the early juveniles are critical to the economic viability of bivalve hatcheries. Phytoplankton species cultured for bivalve food are generally selected on the basis of size, nutritive value and amenability to mass cultivation. Recent investigations on the selection of particles by juvenile scallops (Lesser *et al.*, 1991) have demonstrated the importance of understanding species-specific preferences as well, especially when

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providing mixed algal diets. This knowledge is essential for determining the algal species and rations that optimize growth and efficiency in aquaculture systems.

MATERIALS AND METHODS

Juvenile specimens of *Placopecten magellanicus* (Gmelin, 1791) (0.9–1.6 mm), *Argopecten irradians irradians* (Lamarck, 1819) (1.2–1.6 mm) and *Patinopecten yessoensis* Jay (0.8–1.6 mm) were obtained from The Great Maritime Trading Company, New Brunswick, Canada, Shinnecock Tribe Oyster Project, Southampton., New York, and Island Scallops Ltd, British Columbia, Canada, respectively. Animals were maintained in filtered seawater (0.45 μm; 15 °C) and fed *Isochrysis* (TISO) prior to use in experiments. Water was changed daily and animals were used within 3 days of acquisition.

Experiments were carried out at 15°C. Scallops (approximately 5–10 per vial, attached since settlement to monofilament net) were placed in Coulter vials containing varying volumes (10–20 ml) of algal culture mixtures (see below) and gently aerated. Control vials, without animals were run simultaneously to correct for algal cell division during the experiment. Experiments lasted up to 20 h, depending upon the feeding rate (depletion of experimental medium).

Algal cultures (Table 1) were supplied from the Culture Center for Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences. Algal species were chosen based on their size, fluorescence characteristics and prior use as food sources in scallop culture. Cultures were grown in Guillard's f/2 media at 15°C on a 14:10 light/dark photoperiod. For all experiments using cultures, a final concentration of 10^4 cells ml^{-1} was used to avoid any density-dependent changes in clearance rates (Riisgard and Randløv, 1981). Two sets of experiments were run using different mixtures of algal species. The first mixture included: Omega 48–23, PHAEO, PLATY1, 3C and AMPHI; the second contained Omega 48–23, TISO, 3C, PHAEO, CHGRA and AMPHI. Experiments to determine the clearance of natural particulates were set up

TABLE 1. Algal species, clone designations, and approximate sizes of cells used in feeding experiments

Species	Clone	Size range (μm)
Chaetoceros muelleri	CHGRA	4–9
Isochrysis sp.	TISO	3–6
Phaeodactylum tricornatum	PHAEO	12–14 × 2–4 (5.0)
Chroomonas salina	3C	5–12.5 × 6.25–7.5 (6.1)
Amphidinium carterae	AMPHI	12–18 × 9–13 (10)
Tetraselmis levis	PLATY1	10–12 × 6–10 (6.4)
Unidentified prasinophyte	Omega 48–23	3 (2–3)

in the same way as those for cultures, and natural seawater from Boothbay Harbor, Maine, was collected and characterized the day of the experiment.

Samples were analysed by flow cytometry using the differences in fluorescing intensities from cells' respective photosynthetic pigments and/or cell size from forward angle light scatter (Fig. 1) as described previously (Cucci *et al.*, 1985; Shumway *et al.*, 1985).

Statistical analyses

Clearance rates of each group of algal or natural particulate assemblage by the three scallop species were analysed using an analysis of variance (ANOVA) on the fixed effect of clearance rate of total cells. Additionally, an ANOVA was conducted for each species of scallop to determine which species of algae had significantly higher clearance rates. For experiments using natural assemblages of particles a two-way

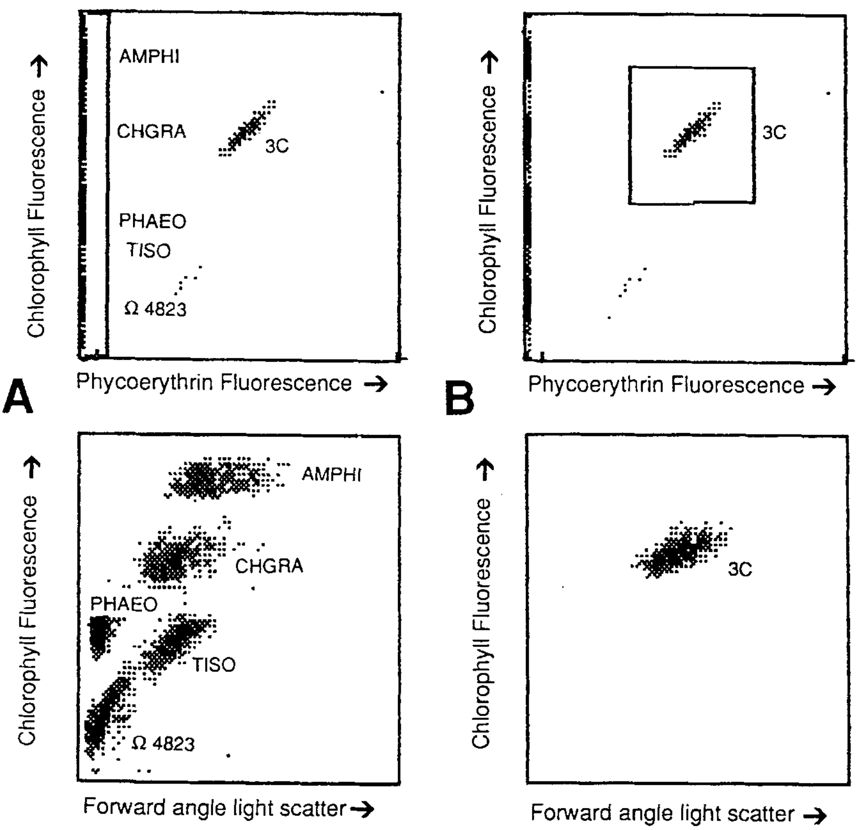


Fig. 1. (A) Bivariate plots of forward angle light scatter (FALS) and chlorophyll and phycoerythrin fluorescence showing the separation of six algal clones by flow cytometric analysis. Clones 3C and CHGRA overlap in their FALS but can be easily separated because only 3C contains phycoerythrin (B). These are actual instrument print-outs.

ANOVA with species and particle size as treatment effects and total particles, non-chlorophyll-containing particles, and chlorophyll-containing particles as measurements was conducted. All experimental data were normally distributed and no unequal variances detected. Significant treatment effects were followed with post-hoc multiple comparison testing using a Student–Neuman–Keuls (SNK) analysis at a significance level of 5%. Results are expressed as clearance rate (cells l^{-1} h^{-1}) per individual.

RESULTS AND DISCUSSION

Typical flow cytometric results are presented in Figs. 2 and 5 for scallops fed pure algal clones and natural particulate assemblages, respectively. There was a significant effect (ANOVA: $P = 0.0001$) of species on the clearance rate of total cells (algal cultures) for juvenile scallops (Fig. 3). Multiple comparison testing revealed that all pairwise comparisons were significantly different (SNK: $P < 0.05$). For each species of juvenile scallop a significant effect (ANOVA: $P < 0.05$) of individual algal species on clearance rate (Fig. 4) was observed. Multiple comparison testing for each species revealed a specific pattern. For *Patinopecten yessoensis*, Omega 48, TISO and CHGRA were preferred significantly less than the other clones (Fig. 4, top panel). For both *Placopecten magellanicus* (Fig. 4, middle panel) and *Argopecten irradians* (Fig. 4, bottom panel), Omega 48 was the least preferred while all other clones were consumed at similar rates. Lastly, the experiments utilizing natural particles revealed a significant effect of both species (ANOVA: $P = 0.0001$) and particle size (ANOVA: $P = 0.001$) for the measurement of clearance rates of total particles, non-chlorophyll-containing particles, and chlorophyll-containing particles. *Patinopecten yessoensis* exhibited significantly lower clearance rates of all size ranges tested while *Placopecten magellanicus* and *Argopecten irradians* exhibited significantly higher rates except for the clearance rates of > 8 μm particles for *P. magellanicus*. This species exhibited significantly lower clearance rates of all particle types in this size range.

It is clearly shown in Fig. 2 that two clones, AMPHI and PHAEO, were cleared by *Patinopecten yessoensis* prior to removal of other cells. Both *Placopecten* and *Argopecten* also demonstrated a similar selection of algal species but the differences were not as dramatic. Further, *Argopecten* also selectively depleted the population of TISO. It should be noted that AMPHI and PHAEO are not the same size and, in fact, other clones (CHGRA and TISO) are of similar size to PHAEO and yet were not removed from suspension at the same rate. These choices are probably not, in fact, in the scallop's best interest, as PHAEO is known to be a poor food source for bivalves and AMPHI is suspected to be toxic to shellfish (Guillard, 1958 and personal communications). All three species of scallops appear to clear the larger particles first when presented with natural assemblages of particles (Fig. 5). However, these data are more difficult to interpret and being analysed further, these results indicated that:

- scallops fed on algal mixtures showed clear differences in overall clearance rates (cells l^{-1} h^{-1} individual^{-1}): *Patinopecten* < *Argopecten* < *Placopecten* (Fig. 3);
- there is apparent preference for particular clones (see above) (Fig. 4);

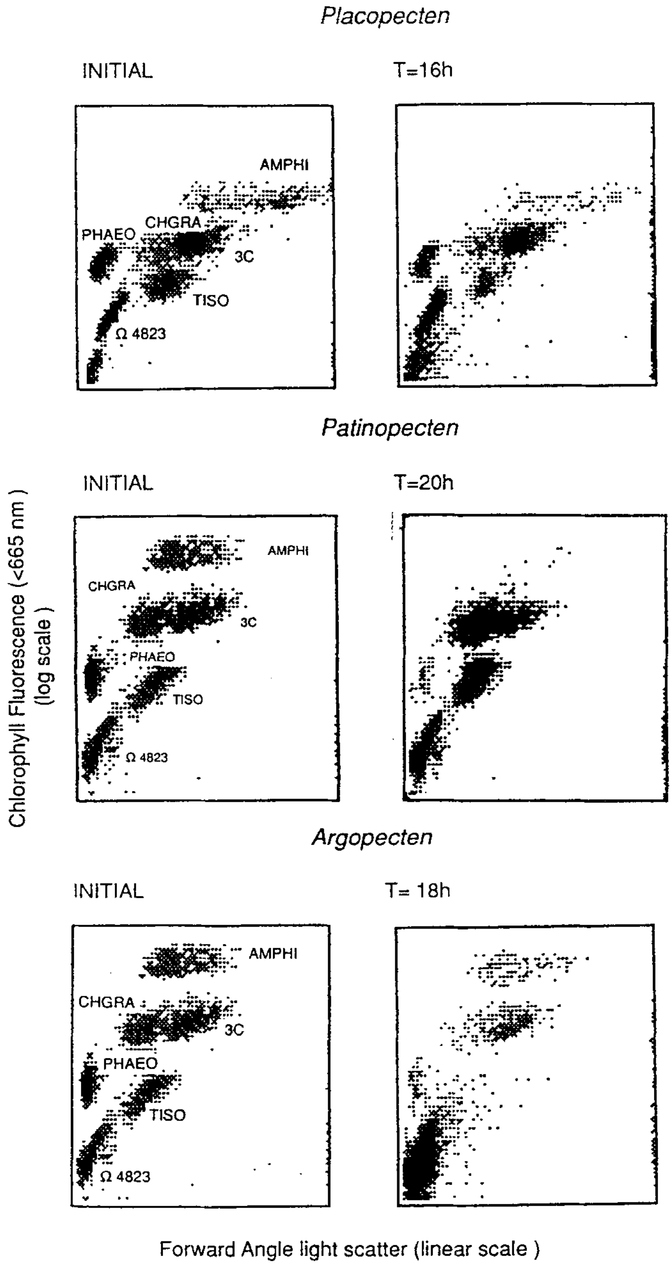


Fig. 2. Bivariate plots of forward angle light scatter and chlorophyll fluorescence showing relative changes in cell numbers within a mixture of six algal clones due to grazing by *Argopecten irradians*, *Placopecten magellanicus* and *Patinopecten yessoensis* at time zero and respectively after 16, 20 and 18 h. Each dot represents a cell. These are actual instrument print-outs.

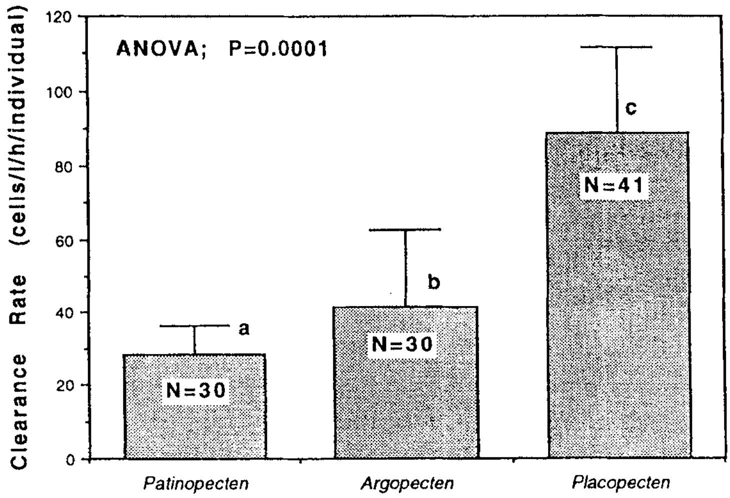
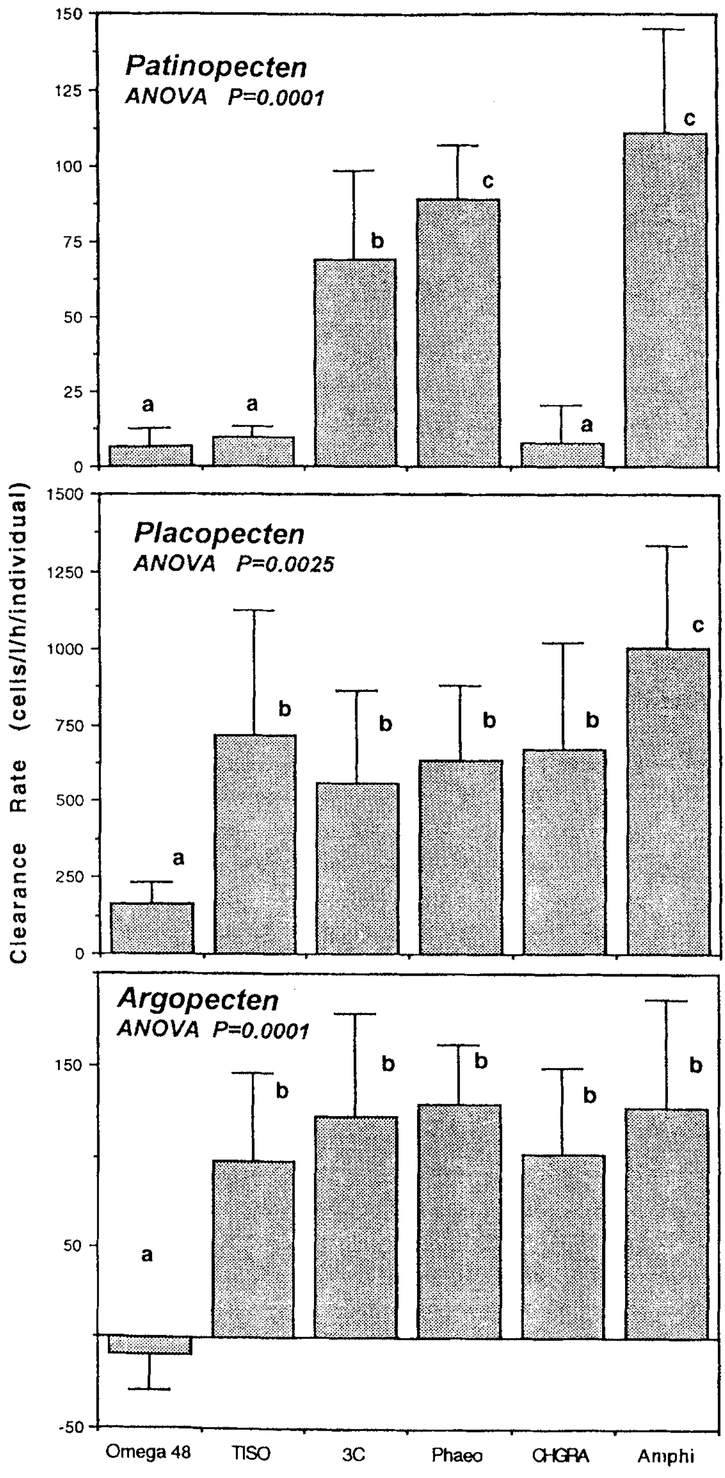


Fig. 3. Mean clearance rates of *Patinopecten yessoensis*, *Argopecten irradians*, and *Placopecten magellanicus* fed a mixture of six algal clones (initial concentration; 10^4 cells ml^{-1}). Error bars represent 95% confidence intervals and all are significantly different from each other.

- there may be some differentiation between chlorophyll-containing and non-chlorophyll-containing particles (Fig. 6); however, more individual experiments need to be done to establish significance levels;
- no scallop species readily ingested Omega 48–23; this is the smallest of the clones used in experiments;
- when scallops were fed natural assemblages of particles, clearance rates varied between scallop species, with *Placopecten* generally having higher uptake rates for all three size classes of particles (3–5 μm; 5–8 μm; > 8 μm) followed by *Argopecten* and *Patinopecten*. For the 8 μm size class of particles, *Argopecten* had the highest clearance rate.

Results obtained here for *Placopecten* support a previous study of juveniles (6.5–9.5 μm) by Lesser *et al.* (1991), where highest clearance rates were recorded for AMPHI, DUN (*Dunaliella tertiolecta*, 9–11 μm) and UW442 (*Pyraminonas parkeae*, 11–16 μm).

Fig. 4. Mean clearance rates (note differing scales) for each algal clone fed to *Patinopecten yessoensis*, *Argopecten irradians* and *Placopecten magellanicus* in mixtures (initial concentration of cell mixture: 10^4 cells ml^{-1}). Error bars represent 95% confidence limits and like letters indicate no significant difference in clearance rates of individual algal clones. Note that neither *Placopecten* nor *Argopecten* exhibited the same marked preference for particular clones as did *Patinopecten*.


Patinopecten
ANOVA P=0.0001
Placopecten
ANOVA P=0.0025
Argopecten
ANOVA P=0.0001
Clearance Rate (cells/l/h/individual)
150
125
100
75
50
25
0
1500
1250
1000
750
500
250
0
150
50
-50
a
b
c
Omega 48
TISO
3C
Phaeo
CHGRA
Amphi

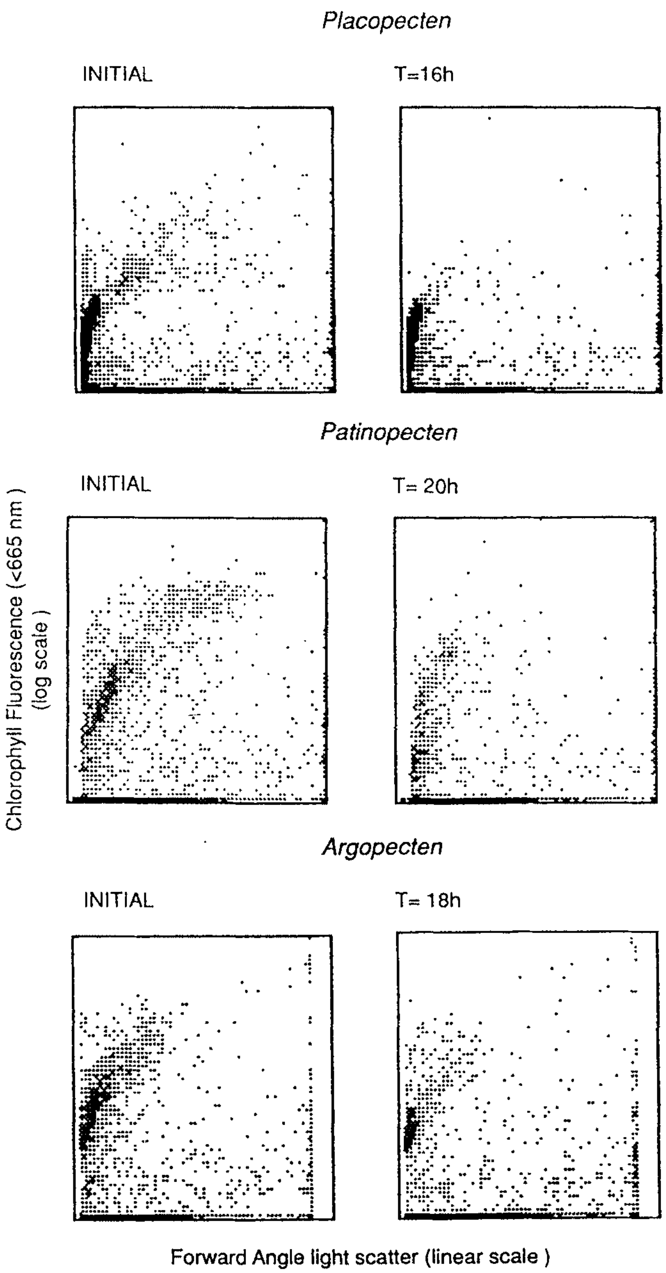


Fig. 5. Bivariate plots of forward angle light scatter and chlorophyll fluorescence showing relative changes in particle numbers within natural assemblages of particulates due to grazing by *Placopecten magellanicus*, *Patinopecten yessoensis* and *Argopecten irradians* at time zero and after 16, 20, and 18 h. Each dot represents a particle. These are actual instrument print-outs.

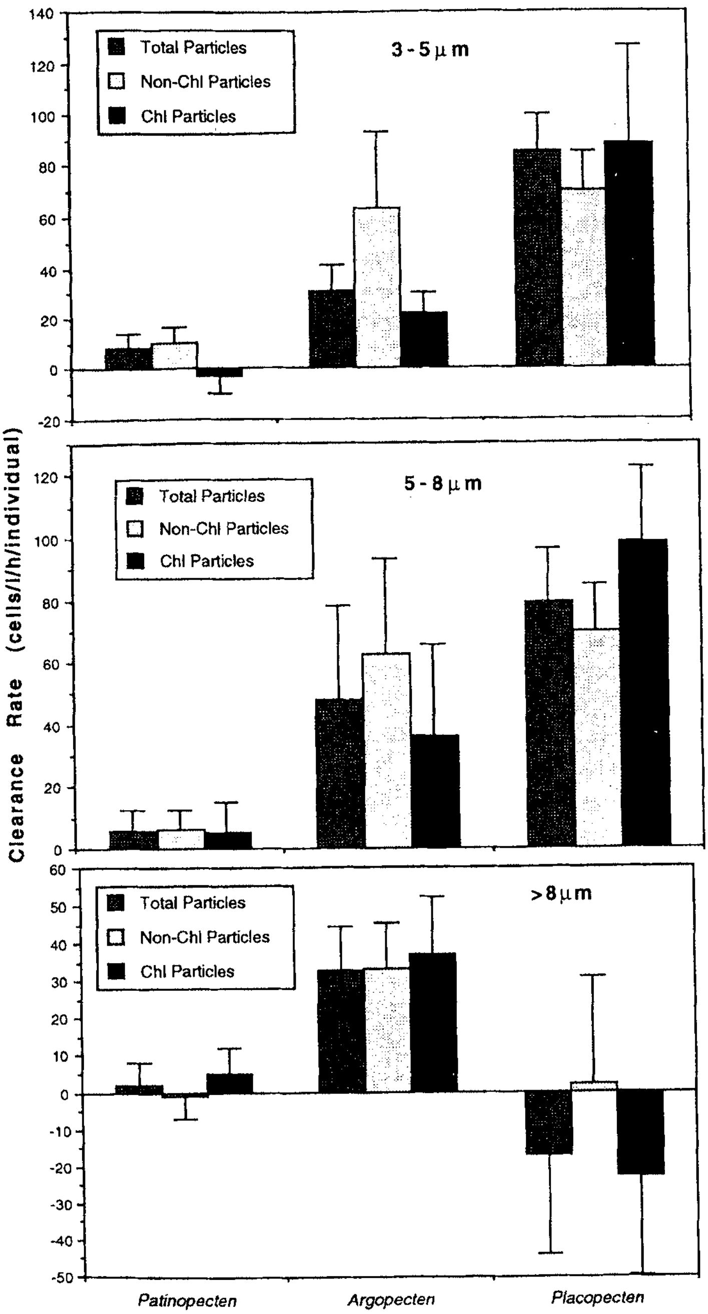


Fig. 6. Mean clearance rates of total particles, non-chlorophyll-containing particles, and chlorophyll-containing particles by each of three species of scallops (*Patinopecten yessoensis*, *Argopecten irradians* and *Placopecten magellanicus*) fed on natural particulate assemblages (initial particle concentration: 10^4 cells ml^{-1}). Data are presented for three size ranges of particles. There was no obvious selection of either chlorophyll-containing or non-chlorophyll-containing particles. Error bars represent 95% confidence levels.

Considerable attention has been paid to the development of 'artificial', non-living diets for larval and post-set bivalves (Langdon and Siegfried, 1985; Langdon, 1989) and efforts are underway to develop such a diet for *P. magellanicus* (J. Kean-Howie, pers. comm.). Most aquaculture facilities, however, still rely on cultured microalgae for the bulk of their feed requirements. Traditionally, culturists used only a few species of algae as 'tried and true' food sources, e.g. *Isochrysis, Thallassiosira, Tetraselmis*, and it is generally accepted that the nutritional effects of two or more species of algae are superior to monocultures as a food source (Epifanio, 1979).

While our experiments do not address the nutritional quality of any of the algal species investigated, it must be remembered that before any alga can be utilized it must be ingested. Particle selection is an obvious advantage to suspension-feeding organisms, not only in sorting organic from inorganic particles, but also in choosing particles of easier digestibility or higher nutritive value over those more difficult or impossible to digest.

Growth rates of scallops need to be determined using various algal diets and it is hoped that these studies, in collaboration with those of researchers working to determine the biochemical requirements for growth of bivalves, will lead to improved selection of new food sources for scallop culture.

CONCLUSIONS

1. Significant differences in clearance rates of individual algal species were found within and between three species of juvenile scallops.
2. Particle selection did not appear to be based upon size alone but was influenced by other characteristics of the algae as well.
3. Pre-ingestive sorting by scallops was demonstrated.

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