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Augmentation of the food supply for oyster larvae via bacterivorous flagellates: possible implications for larval breeding and oyster pond management

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Abstract. Examination of the protist plankton in the Espevikpollen oyster pond indicates that the size fraction available to the pelagic larvae of *Ostrea edulis* L. includes a substantial amount of heterotrophic flagellates. As these kind of flagellates have been shown to be of good food value to veliger larvae of a related species (*Crassostrea gigas* Thunberg), the heterotrophic flagellates may be of equal importance as the phototrophic flagellates to the veligers. Therefore, the loop via bacterial production and heterotrophic flagellates may play the same role for *Ostrea* veligers under pond conditions as this loop plays for the veligers of *Crassostrea* in certain Japanese aquaculture systems.

Introduction

Natural marine heliothermic ponds are favoured spawning localities for the relict populations of *Ostrea edulis* L. along the Norwegian coast. Since the mild sub-boreal period, the distribution of wild populations of *Ostrea* has declined (Nordhagen 1933), and reproduction can only take place in particularly favourable sheltered localities or in these ponds. Today, *Ostrea edulis* is only found naturally along the Norwegian coast south of the polar circle. Since the 1870s, some of the heliothermic ponds have been exploited for commercial spat production (Friele 1899). The natural Norwegian stock has been reimplanted north of the polar circle, but is maintained there artificially and for commercial production only.

The pelagic larvae of the northern European oyster, *Ostrea edulis* L., are size-selective when feeding, and appear to grow best on flagellates within the approximate size range 3–12 μm. In ponds, when larval densities and grazing pressure are high, the result is that the largest biomass fraction left in the water consists of bacteria-sized particles (Klaveness 1990). This size fraction is not itself an efficient food supply for oyster larvae, but may possibly lead to the development of a microbial food chain including bacterivorous flagellates which could supplement the food of the pelagic veligers of *O. edulis*. A closer examination of the nano- and picoplankton community in a well-stocked pond, in this case the Espevikpoll at the western coast of Norway, may reveal whether heterotrophic flagellates of the right size are a component of the plankton. If so, they may be a significant food source for the oyster larvae.

Materials and methods

The Espevikpoll is a typical heliothermic system (Kirkland, Platt Bradbury & Dean 1983),

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where the main volume of sea water (>25‰) is covered by a thin (approximately 0·5 m) layer of fresh water, fed by small brooks in the watershed. The pond itself is about 200 × 350 m, with a maximal depth of 5 m. The pond is connected to the sea by a 0·5-m-deep channel that may be closed by a gate. The hydrography is outlined in Klaveness (1990), and a simple bathygraphical map may be found in Klaveness & Johansen (1990).

Water samples were taken with a Ruttner sampler at 2 m depth. At this depth, the oyster baskets were located, and the influence of the oysters upon the plankton was expected to be greatest in this stratified system. Water samples from 2 m depth were split between new polypropylene tubes (subsamples fixed with 0·15% electron microscopy grade glutaraldehyde), 100-ml or 250-ml bottles, and 1000-ml bottles (fixed with Lugol's iodine). Additional large samples were fixed with 2·5% glutaraldehyde, to be used for chloroplast monitoring in the fluorescence microscope. All samples were stored in the dark in a refrigerator until counting.

Total procaryote picoplankton (<2 μm, terminology of Sieburth, Smetacek & Lenz 1978) were counted after staining of samples from the polypropylene tubes (method modified from Haas 1982). For the purpose of counting the smallest eucaryotes, autofluorescent particles were recorded in randomly selected areas (ocular squares) on a blackened 0·2 μm Nuclepore™ filter through which 5–10 ml of glutaraldehyde-fixed samples had passed. A very brief staining with proflavine (diluted 1:10, final concentration 0·5–1 μg/l) aided the recognition of aplastidic flagellates. Many of the larger dinoflagellates have previously been screened for the presence of chloroplasts by Lessard & Swift (1986): their lists, together with information from Gaines & Elbrächter (1987) and Dodge (1982), aided in sorting the larger dinoflagellates according to trophic mode.

Finally, micro- and nanoplankton fractions (Sieburth *et al.* 1978) were counted using the classical Utermöhl procedure (Utermöhl 1958) as detailed in Klaveness (1990). At least 30 cells of each species were counted whenever practically possible. A typical cell volume was calculated for each species in each sample by measuring critical dimensions and approximating cell parts to known geometrical figures, or their outlines to functions that could be revolved about an axis and integrated (Klaveness 1990). Whenever practically possible, a large number of cells were measured and means of volumes calculated.

Cells were also graded according to equivalent spherical diameter (ESD, as recommended by Sheldon (1969), cf. Klaveness 1990) and function (osmotrophs, phagotrophs, phototrophs). In a previous paper (Klaveness 1990), the size category best for the veliger larvae (3–12 μm) was considered approximately equivalent to ESD 2–8 μm (since real plankton cells are rarely perfect spheres). All ciliates except *Mesodinium rubrum* (Lohmann) Hamburger & Buddenbroek were counted as phagotrophs, disregarding the presence of endosymbionts in some species. Klaveness (1984) found that a ciliate carrying endosymbionts (*Coleps hirtus* Nitzsch.) may survive, but does not grow well, without additional food. *Mesdinium* does not take up food (Lindholm 1985) and was counted as a phototroph.

Results

The biomass and the number of recorded taxa of phototrophs, phagotrophic flagellates and ciliates throughout the investigation period are presented in Fig. 1. There is a seasonal trend towards a low number in all categories in the summer and a larger number in the autumn and winter/spring. There is no simple relation to biomass: the number of taxa in all categories is

low in summer when total biomass is also low (cf. May–August 1987), but the same or the opposite may be true both in spring (cf. January and April) and in the autumn (cf. October and November 1987).

For every sample, the plankton may be divided into size categories (micro-, nano-, and picoplankton) and into functional categories (osmo-, phago- and phototrophs), as is shown in Table 1 for the month of May. Within the functional categories, a subcategory (the 2–8 μm ESD fraction) available to the veliger larvae, may be distinguished (Table 1).

The picoplankton size fraction cannot support growth or survival of the oyster larvae. Contrary to the assumptions of Gaarder & Spärck (1932), it has not been possible to demonstrate that the pelagic larvae of *O. edulis* are able to survive or develop on procaryote picoplankton food particles. Imai & Hatanaka (1950) proved that 'bacteria themselves were not suitable for food for larvae' of oyster (presumably *Crassostrea gigas* Thunberg). *Synechococcus* Nägeli. was not suitable as food for *Ostrea* veliger larvae (Walne 1979). In a test performed during the present research programme, 100% of two replicate cultures died within 2 days, when tested upon a *Synechococcus* strain isolated locally (Børsheim & Knutsen 1989). The eucaryote *Micromonas pusilla* (Butcher) Manton & Parke (1·5 μm dia.) may serve as food for *O. edulis* larvae, but may support growth, under laboratory conditions, at very high densities only (Walne 1979).

The accumulated experimental evidence (refs. in Ewart & Epifanio 1981; Klaveness 1990; Yufera & Lubian 1990) indicates that the veligers of oysters thrive upon larger food organisms, approximately within 3–12 μm, that is within the nanoplankton size range. Practical experience from hatcheries where *O. edulis* or other species (like *Crassostrea gigas*) are produced in large quantities has led to the development of mixed food diets for veliger larva consisting of *Chaetoceros tenuissimus* Meunier (=*C. calcitrans*), *Thalassiosira pseudonana* Hasle & Heimdal and *Isochrysis* sp. ('baby food' for *O. edulis* at The Norwegian Oyster Co., Hæreid, personal communication) or 50% *Isochrysis*, 45% *Chaetoceros* and 5% *Chroomonas* (for *C. gigas*, Garland, Cooke, McMeekin & Valentine 1986), i.e. diatoms and phototrophic flagellates about 3–12 μm in size (within the 2–8 μm ESD range).

The phototrophic microplankton is probably not available to the larvae of *Ostrea*. The phagotrophic microplankton mainly consists of colourless dinoflagellates and ciliates. They probably compete with the oyster larvae for the available food.

Under pond conditions, *Ostrea edulis* may spawn once or twice during May–August. The size and quality of the plankton during these months may therefore decide the fate and

Table 1. The plankton on 11 May 1987 as an example of the results, sorted according to trophic mode, size and equivalent sphaerical diameter (ESD). Figures are wet weight, mg/l. A '–' indicates that the category is non-existent

	Functional category					
	Osmotrophs		Phagotrophs		Phototrophs	
Size category	Total	2–8 μm ESD	Total	2–8 μm ESD	Total	2–8 μm ESD
Picoplankton	0·494	0·000	0·000	0·000	0·013	0·000
Nanoplankton	0·000	0·000	0·183	0·076	0·389	0·306
Microplankton	0·000	–	0·016	–	0·000	–

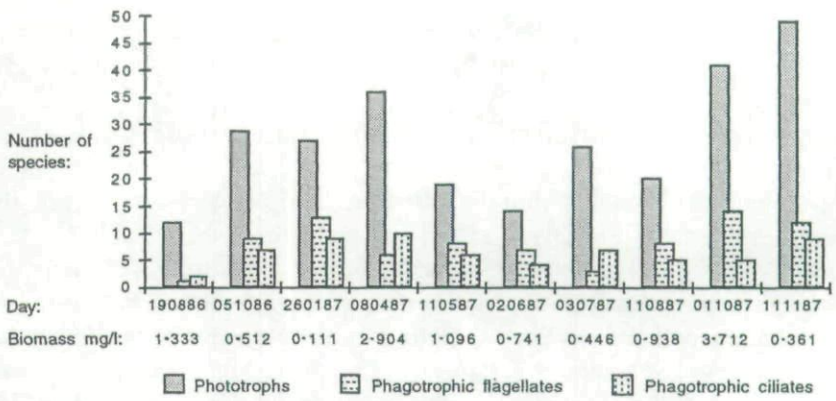

Figure 1. Number of species of phototrophic algae, phagotrophic (heterotrophic) flagellates and phagotrophic ciliates recorded during the sampling period 19 August 1986 to 11 November 1987. The total biomass of monera and protist plankton are recorded (mg/l wet weight) below the day of sampling (format: ddmmyy).

success of the swimming veligers. In a previous paper (Klaveness 1990), the composition of the phototrophic part of the 2–8 μm ESD fraction was described and discussed. During the summer months, it was found that the total stock of plankton is small, and phototrophs of the size available to the veligers (2–8 μm ESD) are too scarce (too heavily grazed), and some are of the wrong kind (coccoid green algae), to bring the veligers to a successful metamorphosis. Fagerland (1945) found that coccoid green algae, 'Chlorella', in an oyster basin passed through the intestine of *Ostrea* veligers undigested; an observation corroborated by the numerous feeding experiments carried out by Walne (1963, 1979).

Since the plankton composition during the summer months was fairly stable (see Klaveness 1990), means and standard deviations for each category were estimated and are here presented in Fig. 2. Picoplanktonic osmotrophs (bacteria) were the category with the largest standing stock. The picoplanktonic phototrophs were blue-green algae; there were very few eukaryotic algae within this size category. The veliger larvae of *O. edulis* therefore find their food within the nanoplankton size range under these conditions. Some of the phototrophic nanoplankton (the 2–8 μm ESD fraction) are of the right size, but less than half of these are suitable food, since coccoid green algae are abundant (Klaveness 1990). The correlation coefficient between the total biomasses of phototrophic nanoplankton and the 2–8 μm ESD fraction during the 4 summer months is 0·96, which indicates that the available fraction follows the variation of the total biomass quite closely. But, about half of the biomass of phagotrophic nanoplankton (Fig. 2) is also available to the veligers at any time (correlation coefficient = 0·84).

Discussion

Of particular interest in this context is the permanent presence of colourless nanoflagellates, with an available fraction during summer of similar magnitude as the 'good-food fraction' of the available phototrophs (about 50% of the 2–8 μm ESD fraction, see Klaveness 1990). The food value of the mixed stocks of phagotrophic flagellates in nature is not well known. There are, however, early reports from Japanese scientists which provide some information in this

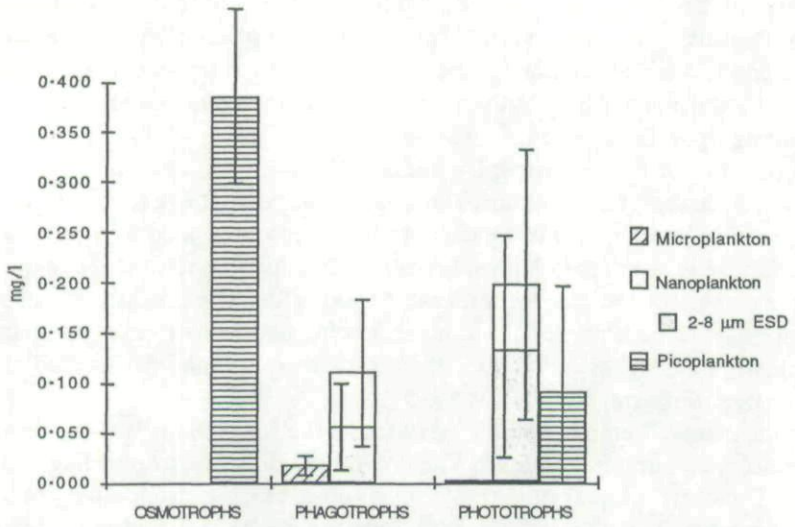

Figure 2. Average biomass of the monera and protist plankton for the summer sampling days, mg/l wet weight. Error bars are ±1 sd, $n = 4$. See test for discussion.

respect. Imai & Hatanaka (1949, 1950) isolated a strain of colourless flagellate identified as '*Monas* sp.', numbered '34', and found it to be a 'favourite food of larvae of various marine animals' (Imai & Hatanaka 1950; Imai, Hatanaka, Sato, Sakai & Yuki 1950). It had already proved useful in large-scale propagation of oyster larvae in tanks (presumably *Crassostrea gigas*, cf. Imai & Hatanaka 1949; Imai *et al.* 1950), and was used with success for the same purpose with *Ostrea edulis* L., which had recently been introduced in Japan (Imai, Sakai & Okada 1953). The size of this *Monas* sp. was 2–10 µm, depending upon the availability of food bacteria for the flagellate (Imai & Hatanaka 1950).

During a survey of a natural oyster farm (Mangokura-ura), Imai *et al.* (1950) found this flagellate to be very common. It was assumed to play an important role in the natural production of seed oysters, and the supply of *Monas* was maintained (via bacteria) by decomposition of eel grass, *Zostera marina* L., during the breeding season of the oysters. These early observations on the dependency of flagellates upon the decay of *Zostera*, and thereupon the success of *Crassostrea* veligers, have later been corroborated in Sendai Bay (Koganezawa 1972; ref. from Ventilla 1984). These observations may well be compared with the conditions in the Espevikpoll during the summer months. In the Espevikpoll system, bacterial substrates may enter the system from the extensive belts of benthic vegetation (Klaveness & Johansen 1990), and secondarily from the catchment area, via the freshwater brooks. The standing stock of bacteria in the Espevikpoll is quite high but constant during the summer months, and provides favourable conditions for bacterivorous phagotrophs.

Colourless flagellates have also been observed to develop in oyster ponds elsewhere. Fagerland (1945) recorded *Bodo marina* Braarud (=*Leucocryptos marina* (Braarud) Butch.) and *Gymnodium lohmanni* Paulsen in abundance during the summer, without discussing their possible food value for oyster larva.

The smaller phagotrophs in the Espevikpoll are either 'naked' dinoflagellates, such as *Gymnodinium wulffii* Schiller, colourless cryptomonads such as *Leucocryptos marina*

(Braarud) Butch. or *Goniomonas* Stein, choanoflagellates (like *Monosiga marina* Grøntv. var. *minima* Paasche), or chrysophytes. None of the aplastidic chrysophytes in the Espevik poll were identified as belonging to the genus *Monas* (a genus open for discussion; cf. Bourrelly 1968; Starmach 1980), but were recorded as aplastidic '*Ochromonas*' or '*Chromulina*', depending upon flagellation.

Some properties of the phagotrophic nanoplankton appear to be favourable for grazers: they lack an indigestible (f.ex. cellulose) cell wall, few species have spines or processes which make them hard to handle, and few unite into larger colonies or form mucilage or slime (cf. discussion of these properties in Klaveness 1984). In addition, their close relationship to the chromophyte algae may indicate a comparable food value. The productivity of phagotrophic flagellates appears to be high due to efficient feeding and growth rates comparable to their food particles (Fenchel 1986). In fact, their growth rates may far exceed those of their phototrophic counterparts.

The importance of heterotrophic flagellates in the food chain leading up to the *Ostrea* veligers should not be underestimated. The biovolume of heterotrophic flagellates may be as high as that of phototrophs of comparable food value, and the productivity of heterotrophic flagellates may be higher. Dynamic experiments are needed to shed more light upon these possibilities.

Walne (1979) imported a strain of colourless flagellates from Japan in 1953, and tested its feasibility as food for oyster larvae. Although the strain grew fast under laboratory conditions, a problem arose when the food flagellates were led into larval culture. The high number of bacteria that inevitably came along with the flagellate medium impeded the development of the larvae. Therefore, unless a different technique is developed, it may be more promising to modify bacterial production under natural conditions (cf. the effects of decaying *Zostera*, see above). For the experimentally inclined oyster producer this may be a promising but also a risky challenge, requiring both a good understanding of the microbial food web as well as skills in hydrography.

A recent report on the food preferences of the planktotrophic larvae of *Crassostrea virginica* (Gmelin) (Baldwin & Newell 1991) shows, by dual radioisotope methods, that heterotrophic flagellates may possibly play an important supplementary role in the nutrition of the larvae of this species also.

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