

New biocompatible tracer particles: use for estimation of microzooplankton grazing, digestion, and growth rates

Astrid Hammer^{1,*}, Cordula Grüttner², Rhena Schumann¹

¹University of Rostock, Department of Live Sciences, Institute of Aquatic Ecology, Freiligrathstr. 7–8, 18055 Rostock, Germany

²Micromod GmbH, Friedrich-Barnewitz-Str. 4, 18119 Rostock, Germany

ABSTRACT: A new class of model food particles is introduced and compared to polystyrene beads and natural food. Made of carbohydrates or proteins, these particles closely resemble natural prey, and are characterised by constant and reproducible quality with respect to C:N ratio, size and efficiency of labelling. Uptake and digestibility by the heterotrophic dinoflagellate *Oxyrrhis marina* were tested and compared to conventional natural (the algae *Synechocystis* and *Chlorella*, FLB) and inert particles (carboxylated polystyrene microspheres and silicate particles). Our present data show that ingestion and digestion rates estimated for starch particles greenF and protein particles greenF were indistinguishable from those estimated for natural food of the same size, while ingestion rate and passage time of polystyrene and silicate spheres are up to 5 times lower. As we can provide unstained particles of a certain quality, it is possible to adapt the dinoflagellates to unlabelled particles of the same quality for a longer time. Thus, our new biocompatible particles may be a useful, simple technique for studies where quasi-natural tracer particles of constant quality are needed. Applications are, for example, studies of zooplankton grazing, growth and reproduction of organisms or general models of the flux of particulate organic matter.

KEY WORDS: Digestible model food · Surrogate prey · Carboxylated microspheres · Food quality · Digestion · Selectivity · *Oxyrrhis marina*

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INTRODUCTION

Estimating grazing losses of microorganism biomass in aquatic systems is central in current research on carbon cycling in pelagic and benthic food webs (e.g. Martinez et al. 1987, Rassoulzadegan et al. 1988, Tranvik 1989, Kuuppo Leinikki 1990, Wong et al. 1992, Sellner et al. 1994). It is thus important to analyse feeding behaviour — predatory preferences and ingestion rates depending on size and chemical characteristics — for a variety of grazers such as heterotrophic protists (e.g. Kuosa 1990, Sherr et al. 1991, Verity 1991a, Epstein & Shiaris 1992, Stoecker et al. 1995), phagotrophic

phytoflagellates (e.g. Porter 1988, Jones et al. 1993), rotifers (Telesh et al. 1995) or copepods (e.g. Berggreen et al. 1988, Paffenhöfer & Lewis 1989).

The results of experiments with labelled food organisms depend strongly on the availability and type of the food organisms used. Food quality may vary with regard to size, C:N ratio, efficiency of the label and presence of particular cell contents, such as toxins. Thus, it is difficult to reproduce the results of feeding experiments. One solution to this problem is the application of model particles such as the common fluorescent polystyrene particles (i.e. McManus & Fuhrman 1986). The advantages of these particles are their extremely narrow size distribution, their high and stable fluorescence (available in a broad spectrum of

*E-mail: astrid.hammer@stud.uni-rostock.de

colours), and their persistence against degradation. This allows a thorough microscopical analysis of ingested particles, and enables the researcher to trace the particles in the environment (Turner et al. 1988, Haugland 1996, Okabe et al. 1997). However, the disadvantages of these organic but indigestible particles are obvious. Studies which employed fluorescent microspheres to measure the ingestion rates of protists (McManus & Fuhrman 1986, Pace & Bailiff 1987, Sherr et al. 1987, Sieracki et al. 1987) provided evidence that some protists, particularly raptorial feeders, discriminate against microspheres. Moreover, artificial particles do not allow growth and adaptation of organisms to prey prior to the experiment.

Consequently, there is great need for model food particles with the following properties: (1) reproducible size distribution; (2) fluorescent stain and ease of counting after ingestion, (3) easy and commercial availability; and (4) edibility, digestibility and close resemblance to natural prey. Organisms should be able to adapt to identical, but unlabelled particles and should not select for or against surrogate relative to natural prey.

Up to now, the only particles with some of these properties were made of alginate (Albright et al. 1987), or radioactively marked starch (Urban & Kirchman 1992) both without reproducible size distribution. For instance, Kivi & Setälä (1995) used pharmacy quality wheat starch in grazing experiments with oligotrich ciliates. They incubated the ciliates with unstained starch particles and used acid Lugol's solution for fixation and simultaneous staining of the ingested starch. The use of dried algae as a food source for zooplankton growth and nutrient release experiments has also been described (Dobberfuhl & Elser 1999).

We hypothesise all of the above properties for a new class of model food particles made of organic substances, such as carbohydrates and protein,

labelled covalently with 5-[(4,6-dichlorotriazin-2-yl)-amino]fluorescein (DTAF). In a series of laboratory experiments, we offered these biocompatible particles to the widely studied species *Oxyrrhis marina* (i.e. Schumann et al. 1994, Flynn et al. 1996, Hansen et al. 1996, Höhfeld & Melkonian 1998), an omnivorous phagotrophic dinoflagellate. We investigated ingestion rate, digestibility and growth rate, and compared the results with those for a number of conventionally used natural and artificial particles. To distinguish the effects of particle quality from size selection we applied particles in 2 size classes of around 1 and 4 µm. Advantages and disadvantages of our biocompatible particles in grazing experiments and additional utilisation are discussed.

METHODS

Grazers. Cultures of *Oxyrrhis marina* Dujardin (strain CCAP 1133/5) were cultivated on autoclaved ASW medium with a base of 33 g l⁻¹ sea salt (Sigma) and 1 boiled wheat grain per 25 ml, as proposed by CCAP. The dinoflagellate had an average length of 15 µm and a calculated biovolume of 950 µm³. Cultures were grown in static flasks at 15°C in complete darkness without aeration. *O. marina* in mid-exponential growth phase was used for all experiments.

Model food particles. The starch and protein particles greenF were provided by micromod Partikeltechnologie GmbH, Rostock, Germany (www.micromod.de). By use of differential centrifugation and controlled sedimentation, the size distribution—determined with the COULTER® Multisizer—could be narrowed (Table 1). Unlabelled and DTAF stained albumin and starch microspheres can be stored for at least 6 mo at temperatures below 4°C.

Table 1. Properties of all particles used. ESD = mean of equivalent spherical diameter ± standard deviation

Particle class	Strain or product code	ESD (µm) ± SD	Fluorochrome	Excitation/emission
1 µm particles				
Bacteria	BA 321	1.00 ± 0.39	DTAF	Blue/green
Albumin particles	Micromod 37-30-103	0.90 ± 0.67	DTAF	Blue/green
Albumin particles	Micromod 37-00-103	0.90 ± 0.67	—	—
Carb microspheres	Polyscience 15702	0.91 ± 0.02	Coumarin	UV/blue
Silicate particles	Micromod 42-02-103	1.00 ± 0.12	Aminofluorescein	Blue/green
4 µm particles				
<i>Chlorella</i>	CCMP 255	3.70 ± 0.52	Pigments	Green/dark red
Starch particles	Micromod 02-30-403	3.50 ± 0.99	DTAF	Blue/green
Starch particles	Micromod 02-00-403	3.50 ± 0.99	—	—
<i>Synechocystis</i>	PCC 6803	2.35 ± 0.46	Pigments	Green/orange
Albumin particles	Micromod 37-30-403	3.60 ± 0.98	DTAF	Blue/green
Carb microspheres	Polyscience 18340	4.00 ± 0.08	Coumarin	UV/blue

We prepared fluorescently labelled bacteria (FLB) as described by Sherr et al. (1987) by DTAF staining. *Pseudomonas fluorescens*, strain BA 321 (Minkwitz 1999), was grown overnight in nutrient broth and harvested by centrifugation before FLB preparation. *Synechocystis* sp. (PCC 6803) and *Chlorella* sp. (CCMP 255) were grown on BG11 medium. Carboxylated microspheres, made of polystyrene, were purchased from Polysciences, Inc., Warrington, PA, USA; green silicate particles (sicastar®greenF) from micromod, Rostock, Germany.

Feeding experiments. All particles were diluted or harvested by centrifugation and resuspended in ASW-medium. Prior to use in feeding experiments, we sonicated the microspheres for 4 s with a Sonopuls HD 60 Bandelin. Microscopic examination confirmed that clumping of particles was rare. Equivalent spherical diameter of particles were measured with an Image Analysis System (CUE 2 [Galai]) and COULTER® Multisizer II. Particles and dinoflagellates were enumerated after filtration onto irgalan black stained, 0.2 µm pore size polycarbonate filters (Isopore) with an epifluorescence microscope (Olympus BH-2 RFCA). For easier enumerability of the slightly less bright albumin particles we recommend the use of glycerol together with an antifading reagent, e.g. propylgallate or the *SlowFade*TM Antifade Kit (Molecular Probes), as an embedding substance between slide, filter and cover slip rather than immersion oil. If the use of an immersion objective is needed, the immersion oil can be applied on top of the cover slip. The following filter sets were used: for the algae BP 545; for FLB and micromod particles BP 490; for the carboxylated microspheres and DAPI counts UG-1 (UV), broad band excitation.

The experimental design followed Sanders (1988) and González et al. (1990). The grazing experiments were performed under the same conditions as those for culturing. To ensure that the presence of bacteria did not affect the ingestion in the experiments, *Oxyrrhis marina* was separated from the culture medium by inverse filtration. Copious amounts of autoclaved ASW medium were used to wash the dinoflagellates. Resuspension in ASW medium was carried out, resulting in dinoflagellate density of approximately 1000 ml⁻¹. Fifty ml aliquots of *O. marina* culture were transferred to flasks and then placed in the dark at 15°C for 1 h to allow the protists to recover from handling shock. Dinoflagellate suspensions were inoculated with particles so that the initial concentration of the particles was 10⁶ ml⁻¹. For the separate selectivity experiment 2 particle types (starch and carboxylated microspheres, 4 µm) were offered simultaneously at concentrations about 0.5 × 10⁶ ml⁻¹ each. A time course started after the addition of particles. Five ml subsamples at *t* = 0 min were immediately fixed with ice-cold glutaralde-

hyde (same salinity as ASW, 1% final concentration) for enumeration of bacteria, particles, dinoflagellates and as a control. A sequence of samples was taken at 10 to 20 min intervals (for 80 to 100 min), immediately narcotised with carbonated water (10% final concentration) and fixed as mentioned above to avoid both preservation-induced egestion of spheres by flagellates and lysis of them.

After the number of particles per dinoflagellate reached a plateau (after the initial 80 to 100 min time course), a 20-fold dilution of the experimental samples with ASW medium (containing only non-stained bacteria) was carried out as preparation for the digestion experiment. We then waited for 24 h to ensure that most of the remaining particles were fed so that the resulting tracer concentration allowed no further uptake. We chose this time lag as pre-experiments had shown that the recommended 20-fold dilution (e.g. González et al. 1990) was insufficient to prevent further ingestion of particles by *Oxyrrhis marina*. A reduction of particle concentration to begin with would not have been feasible either, as it would have implied a sub-optimal tracer concentration during the ingestion experiment. For the digestion experiment, samples of 15 ml were taken at 20 to 60 min intervals over a time course of 1 to 24 h. The decrease in the average number of particles per dinoflagellate cell over time was monitored. It should correspond to the rate of digestion or egestion of particles by *O. marina*.

Within 3 d, fixed samples were stained with 4',6'-diamidino-2-phenylindole (DAPI) (Sherr et al. 1987) for approximately 5 min, filtered onto 0.2 µm pore size, irgalan black stained Isopore filters and examined by epifluorescent microscopy. A minimum of 100 dinoflagellates were inspected for each time point subsample to determine the average number of particles cell⁻¹.

The slopes of increase and decrease of particles cell⁻¹ were determined via regression analysis for each ingestion-digestion experiment. Mann and Whitney tests (GraphPad Prism 2.00) were used for comparing average values of slopes. Ingestion and digestion times (min) were calculated from the *x*-intercept of the increase and disappearance regression lines.

Growth experiments. For growth experiments, dinoflagellates (final density of about 10² cells ml⁻¹) were added to 50 ml ASW medium. Then either 2 wheat grains per 50 ml or a mixture of unlabelled 4 µm starch particles (final density of 2 × 10⁵ ml⁻¹) and 1 µm albumin particles (final density of 5 × 10⁶ ml⁻¹) or neither were separately added to triplicate 50 ml culture vessels. Periodically, subsamples from each vessel were collected and counted. Growth rates were calculated from the equation: $\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$,

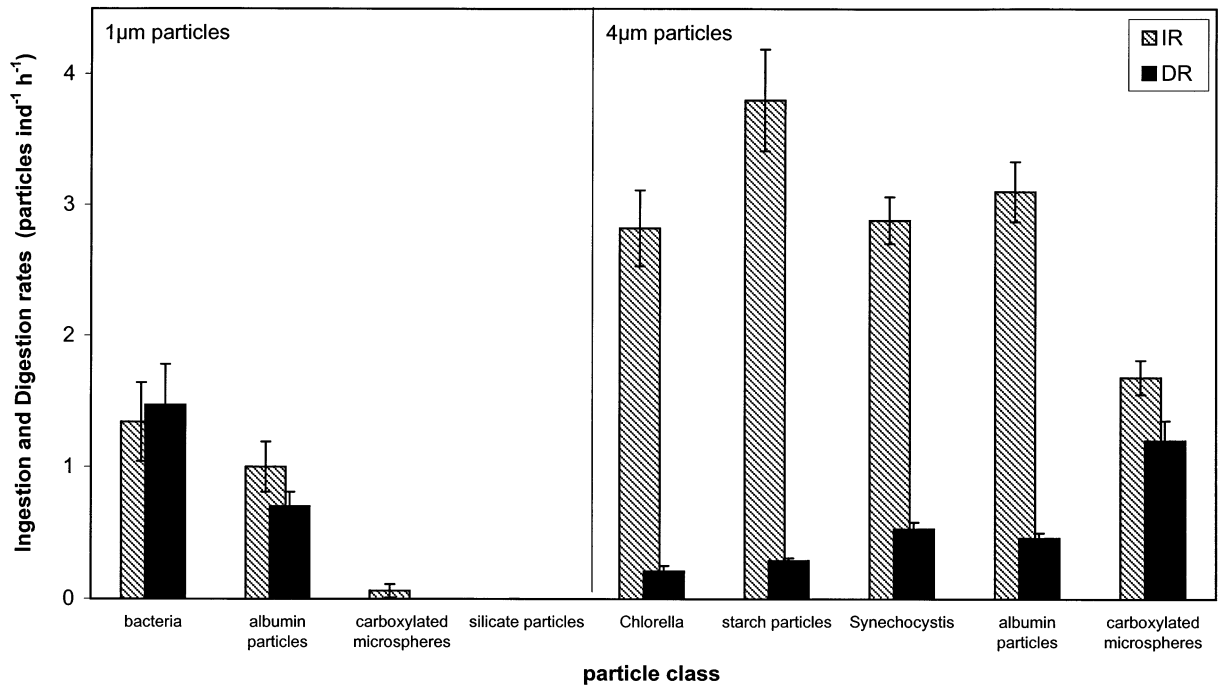


Fig. 1. *Oxyrrhis marina* ingestion (IR) and digestion rates (DR) (mean value \pm standard deviation)

where N_1 and N_2 = average values of dinoflagellate abundance at the beginning and at the end, respectively, of exponential growth; and t_1 and t_2 = the corresponding times for N_1 and N_2 .

RESULTS

Table 1 summarises the food particles we used, i.e. their origin and size. Firstly, we tested larger starch

and albumin particles (around 4 μm) as models for algal food and compared the results with the cyanobacterium *Synechocystis* sp., the chlorophyte *Chlorella* sp. and carboxylated spheres. Then, we tested 1 μm starch and albumin particles, simulating bacteria as food, and compared them with silicate particles, carboxylated spheres and the bacterium *Pseudomonas fluorescens*, DTAF stained.

Ingestion experiments

Table 2. The correlation between particles/individual and time, and significance of slopes of ingestion (IR) and digestion (DR) rates of particles by *Oxyrrhis marina* (expanded from Hammer et al. 1999)

Particle class	IR slope		DR slope	
	r^2	p	r^2	p
1 μm particles				
Bacteria	0.87	<0.05	0.96	<0.05
Albumin particles	0.93	<0.05	0.83	0.01
Carb microspheres				
Silicate particles				
4 μm particles				
<i>Chlorella</i>	0.97	0.002	0.95	<0.05
Starch particles	0.95	<0.0001	0.98	<0.001
<i>Synechocystis</i>	0.99	0.004	0.97	<0.001
Albumin particles	0.98	0.002	1.00	<0.05
Carb microspheres	0.96	<0.0001	0.95	<0.05

In the first series of experiments, we sampled dinoflagellates every 10 to 20 min to obtain short-term uptake rates of particles. Silicate particles were not ingested. In all other experiments, *Oxyrrhis marina* showed linear uptake of particles to a maximum value, after which particles cell⁻¹ remained constant in an equilibrium between ingestion and digestion or egestion. The correlation between particles/individual and time was given by $r^2 > 0.95$ for all 4 μm particles ($p < 0.01$) and $r^2 > 0.87$ ($p < 0.05$) for 1 μm particles (Table 2). Slopes of ingestion were significantly non-zero in all cases with the exception of silicate particles (Fig. 1). The time between the addition of particles and the leveling off of the uptake curve ranged from 40 to 120 min, depending on particle type.

Comparing 5 particle types of 4 μm diameter revealed significant differences in slopes of ingestion rate ($p < 0.001$, Kruskal Wallis test). Ingestion rates ranged between 1.7 particles $\text{cell}^{-1} \text{h}^{-1}$ for carboxylated microspheres and 4.0 particles $\text{cell}^{-1} \text{h}^{-1}$ for starch particles (Fig. 1). We did not detect significant differences in ingestion rates of 4 μm albumin particles and natural food *Synechocystis* and *Chlorella* ($p > 0.05$). Mean ingestion rates ranged between 2.8 and 3.1 particles $\text{cell}^{-1} \text{h}^{-1}$. Starch particles were ingested at slightly higher rates ($p < 0.01$). Uptake rates of polystyrene spheres (Fig. 1) were significantly lower ($p < 0.001$) than those of semi-natural and natural particles. In a separate series of experiments, we supplied polystyrene spheres simultaneously with starch particles and observed a strict rejection of the inert particles (Fig. 2).

Particles around 1 μm were ingested at rates up to 4 times lower than those for 4 μm particles—between 0 and 1.3 particles $\text{cell}^{-1} \text{h}^{-1}$. Ingestion rates of 1 μm albumin particles and FLB were equal (Fig. 1). Silicate particles were not ingested, with 1 μm carboxylated microspheres at rates near zero.

Digestion experiments

In a second series of experiments, we sampled every 30 min to 1 h to obtain short-term disappearance rates of particles. Results of the experiments are presented in Figs. 1, 3 & 4. Because of low ingestion or absence thereof, the disappearance of silicate and polystyrene particles of 1 μm could not be investigated. In all experiments for the digestion time course, *Oxyrrhis marina* showed linear decline of particles to a minimum value. The correlation between disappeared particles/individual and time was given by $r^2 > 0.95$ for all

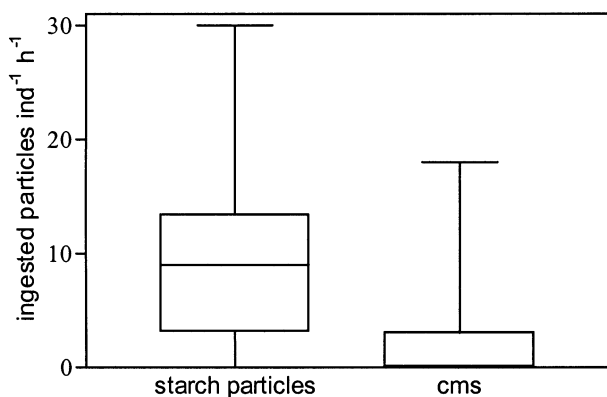


Fig. 2. Simultaneous supply of different particle classes to *Oxyrrhis marina* and selective ingestion. cms: carboxylated microspheres unpaired t -test: $p < 0.001$, box and whisker plots

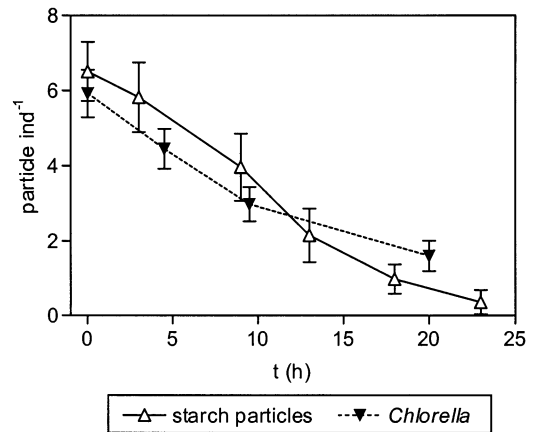


Fig. 3. Digestion of different particle classes by *Oxyrrhis marina* (mean \pm 95% CI)

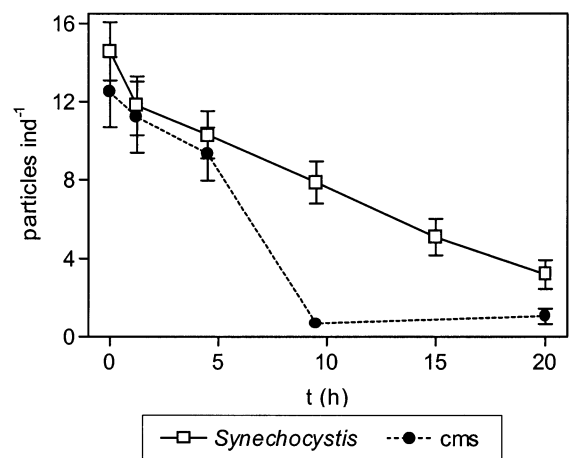


Fig. 4. Digestion and egestion, respectively, of particles by *Oxyrrhis marina*. cms: carboxylated microspheres (mean \pm 95% CI)

particles ($p < 0.05$), except for 1 μm albumin particles (0.83, $p < 0.01$) (Table 2). Slopes of digestion were significantly non-zero in all cases (Fig. 1).

We did not detect any significant differences between slopes of digestion rate of 4 μm starch particles and natural food *Chlorella* ($p > 0.05$) (Fig. 3). Mean digestion rates ranged between -0.21 and 0.29 particles $\text{cell}^{-1} \text{h}^{-1}$. The 4 μm albumin particles and *Synechocystis* were digested at slightly higher (0.5 particles $\text{cell}^{-1} \text{h}^{-1}$) but also at similar rates ($p > 0.05$) (Fig. 1). In contrast, carboxylated microspheres rapidly disappeared from the dinoflagellates. Their egestion rate amounted to 2 to 6 times the digestion rate for semi-natural and natural food (Fig. 4).

For a size of 1 μm , the digestion times of albumin particles and FLB were equal (Fig. 1) and with no difference to their ingestion rates ($p > 0.05$). However,

when uptake-disappearance slopes for 4 μm particles were compared, significant differences appeared in all cases ($p < 0.05$). Digestion was from 5 to 13 times slower than ingestion (Table 3).

Growth rate

Results from the growth experiments showed that the maximum cell density and specific growth rate of *Oxyrrhis marina* in medium with new particles were similar ($p > 0.05$) to those measured in conventional medium with wheat grains supplied. However, the growth rates of *O. marina* exposed to medium without particles was significantly lower (Fig. 5).

DISCUSSION

Our principal hypotheses about the applicability of the new biocompatible particles were met in the experiments with *Oxyrrhis marina*. Thus, the model particles presented here will almost certainly be a starting point for experiments with a whole new class of tracer particles.

Comparison with polystyrene particles

To begin with, the starch and albumin particles greenF have all the following advantages of microspheres. Because the production process and the commercial availability guarantee reproducible particle classes, size and shape are removed as complicating and selectivity factors (e.g. Goldman & Dennett 1990,

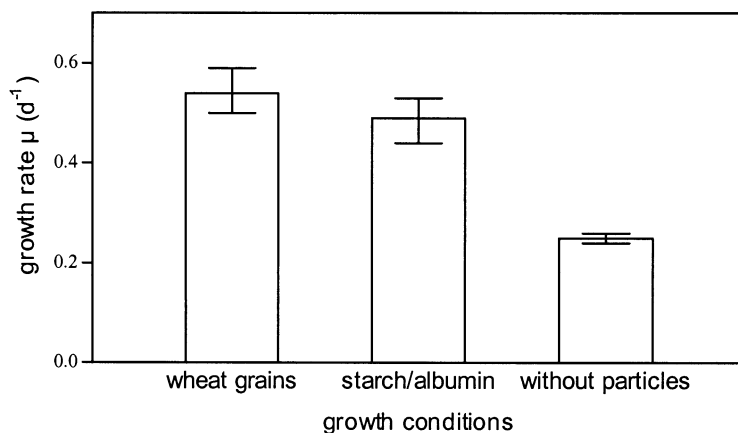


Fig. 5. Growth rates of *Oxyrrhis marina* at different food conditions

González et al. 1990, Hansen 1992). Representative and repeatable analyses can be carried out. The development of even narrower size classes is currently in progress. Individual particles could easily be counted within the organisms if fluorescence is increased by use of antifading agents, as recommended for albumin particles. The particles can be stored for a longer period.

Comparison with natural food particles

For natural food, selectivity has often been recognised, as depending on different parameters. For example, Verity (1991b) and Flynn et al. (1996) identified nutritional value as an important criterion for prey selection by ciliates, with poor prey being rejected or being digested more slowly and only incompletely. In contrast to this, our new particles preclude variations in food quality.

Ingestion experiments

The ingestion experiments within this study gave the same results as for natural food of equal size, such as albumin particles and the same-sized algae *Chlorella* or 1 μm albumin particles and FLB (Fig. 1). Selectivity against DTAF staining as assumed by Putt (1991) could not be shown. No discrimination occurred, as it would for artificial particles (Fig. 1). The uptake of carboxylated microspheres was especially low in dinoflagellates given both, inert and starch, particles (Fig. 2). Dolan & Simek (1998) found comparable results for the flagellate *Bodo*, when offered inert microspheres with *Synechococcus*. Other work confirms the selection bias against

Table 3. Times of ingestion and digestion by *Oxyrrhis marina* (mean \pm SD), and ratio of digestion/ingestion for microspheres egestion

Particle class	Ingestion time (min part ⁻¹)	Digestion time (min part ⁻¹)	DR/IR
1 μm particles			
Bacteria	45 \pm 0.10	41 \pm 0.09	0.91
Albumin particles	60 \pm 0.11	86 \pm 0.13	1.43
Carb microspheres	1000 \pm 8.33	–	–
Silicate particles	–	–	–
4 μm particles			
<i>Chlorella</i>	21 \pm 0.02	286 \pm 0.54	13.4
Starch particles	15 \pm 0.02	207 \pm 0.14	13.9
<i>Synechocystis</i>	21 \pm 0.01	113 \pm 0.11	5.4
Albumin particles	19 \pm 0.01	130 \pm 0.11	6.7
Carb microspheres	36 \pm 0.03	50 \pm 0.06	1.4

artificial particles (e.g. Nygaard et al. 1988, Jones et al. 1993). For example, various bodonid species did not ingest microspheres, and ingestion rates for chryso-monads were far lower than measured with labelled bacteria (Pace & Bailiff 1987). The authors conjectured that the organisms recognise these inert particles as non-food.

Some studies showed that cell surface compounds or surface charge may be involved in particle selection. Gerritsen & Porter (1982) reported that the retention of small particles by the filter feeding zooplankter *Daphnia* increased when the particle surface charges were neutralised. Sanders (1988) found that surface properties of differently coated microspheres seemed to affect grazing of the suspension feeding ciliate *Cyclidium*. The efficiency of particle capture by *Oxyrrhis marina* also seems to be a function of the magnitude and polarity of the electrostatic charge on particles (Hammer et al. 1999). As reported from this study, artificial model particles, i.e. polystyrene and silicate particles, had surface charges (≤ -107 mV) rather distinct from those of natural (algae and bacteria) and surrogate particles made of starch and albumin (≥ -17 mV). This curbed particle capture by the microorganisms mostly of negative charge (Smith et al. 1998). The new food particles, especially those made of albumin, are characterised by surface properties (measured as surface charge) very close to those of natural food of the same size, which could be an important reason for the comparable ingestion rates.

Digestibility and growth studies

There was no egestion of the starch and albumin particles, as proven by a slow and continuous particle decline similar to that of natural food (González et al. 1990). 4 μm starch and albumin particles exhibited the same digestion pattern as same-sized algae (Fig. 3). In contrast, the egestion rate for algae-sized carboxylated microspheres cell^{-1} was significantly greater, indicating a rapid disappearance from the dinoflagellates (Fig. 4). The organisms seem to be able to detect this non-food. Dubowsky (1974) and González et al. (1993) also reported significantly faster processing rates of inert microspheres as compared to natural prey. These findings are in contrast to investigations from Dolan & Simek (1997, 1998), which described no differences between inert, heat-killed and natural prey in digestion rate or residence time for the ciliate *Strombidium* and the flagellate *Bodo*. Prey analogues were processed like natural prey.

In our experiments, similar digestion rates were also obtained for *Oxyrrhis marina* being grazed on FLB and on bacterial-sized albumin particles (Fig. 1). Sherr et

al. (1988) reported the same rates of ingestion and digestion for mixed species assemblages of flagellates grazing on FLB and, as we do, a significant linear relationship between rates of ingestion and digestion. As found by Schumann et al. (1994) and Davidson et al. (1995), algae-sized particles were digested more slowly than they were ingested. A relatively short ingestion phase (up to 21 min) is followed by a longer digestion phase (up to 5 h). This large DR/IR ratio of 13 markedly contrasts with the small DR/IR ratio of 2 for inert microspheres (Table 3). Investigations about the disappearance of particles from organisms should constitute one part of the edibility and usability test of the new particles.

Furthermore, we estimated the growth rate of *Oxyrrhis marina* on different food sources, and found no differences between natural food and a mixture of unlabelled starch and albumin particles (Fig. 5). These results let us assume that it is now possible to adapt the animals to the new food with unlabelled particles of the same quality and size without the problematic pre-starving period (e.g. used by Monger & Landry 1991, Head & Harris 1994).

Outlook

The manufacture of particles made of other carbohydrates, such as dextrane, chitosane and cellulose, and of particles composed of carbohydrates and protein is in progress. This opens further possibilities for various experiments. For instance, knowledge about digestibility of different prey items is rather scarce (Sherr et al. 1988, González et al. 1990, 1993, Dolan & Simek 1997, 1998). The new particles can be used to study processing by the organisms of different-sized particles or those of different composition. Further, important food components or extracts from prey species of great ecological significance can be integrated into the model particles ('microencapsulations') to investigate their influence on feeding behaviour and growth and reproduction.

One problem, however, cannot be solved with such model particles. The particles do not move and do not mimic living cells. Therefore, raptorial feeders responding to movement of the prey or organisms with known preferences for living prey (Dolan & Coats 1991, Landry et al. 1991, Putt 1991, González et al. 1993, Li et al. 1996) will not be satisfied with these model particles. Landry et al. (1991) have conducted experiments showing that marine flagellates and ciliates select living over heat-killed bacteria by a factor of 4 when presented with both prey simultaneously. Thus, use of non-motile prey could lead to underestimation of actual grazing rates if some fraction of bacte-

rioplankton in a water sample were motile. However, this problem arises for all surrogate food items.

The observations in the present study confirm the important role for tracer particles which are representative of natural prey and which are simultaneously characterised by constancy in different parameters. Follow-up research involves testing the advantages of the new particles in a number of different species including proto- and metazoa. All the suggested fields provide a broad scope for applications.

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