An In-Situ Study on the Influence of Ascidian Suspension Feeding on the Subtidal Nepheloid Layer in the Northern Adriatic Sea

Mark G. J. Hartl & Jörg A. Ott

Institute of Zoology, Department of Marine Biology, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria.

With 8 figures and 2 tables

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Abstract. In order to evaluate the effect of epibenthic suspension-feeding organisms on nepheloid-layer processes, a novel flow-through ‘Benthic Ecosystem Tunnel’ was used on sublittoral marine sediment substrate. Our test organism was the ascidian Microcosmus sulcatus. Particle and chlorophyll a reduction were recorded. The correlation of seasonal fluctuations of chlorophyll a concentrations with chlorophyll a reduction by M. sulcatus indicates the potential for Microcosmus to control local phytoplankton input to the subtidal nepheloid layer. However, the relatively low density of Microcosmus at the study site, the nature of the sediment surface and the susceptibility of ascidians to high turbidity (i.e., high sedimentation rates of total particular matter (TPM) causing frequent periods of siphon closure and inactivity) makes it unlikely that M. sulcatus is an important element controlling the composition of the nepheloid layer on a large scale.

Problem

Recent publications have focused on the influence of filter feeding of densely populated mussel beds (e.g., Mytilus edulis) on particle and chlorophyll a concentrations in eulittoral near-bottom water (Dame & Dankers, 1988; Asmus & Asmus, 1991; Asmus et al., 1992; Prins & Smaal, 1994; Takeda & Kurihara, 1994), whereby the problem of anthropogenic eutrophication has been of particular interest (Officer et al., 1982; Cloern, 1982; Degobbis, 1989, 1990; Asmus & Asmus, 1991; Asmus et al., 1992; Prins & Smaal, 1994; Takeda & Kurihara, 1994), whereby the problem of anthropogenic eutrophication has been of particular interest (Officer et al., 1982; Cloern, 1982; Degobbis, 1989, 1990; Asmus & Asmus, 1991; Asmus et al., 1992; Prins & Smaal, 1994; Takeda & Kurihara, 1994).

1To whom correspondence should be addressed at: School of Ocean & Earth Science, Southampton Oceanography Centre, Waterfront Campus, European Way, Southampton, SO14 3ZH, UK. E-mail: mgjh@soc.soton.ac.uk

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1991; Barmawidjaja et al., 1995). These authors showed that the filtration activity of mussel beds has a strong potential for control of the particle composition of the overlying water in shallow bays. Sullivan et al. (1991) demonstrated that, in connection with vertical mixing in a shallow water column, a suspension-feeding benthic community may effectively control phytoplankton blooms, irrespective of the nutrient input to the system. Petersen & Riisgård (1992) and Riisgård et al. (1995) showed that the colonial ascidian Ciona intestinalis has the retention potential to control phytoplankton in an entire shallow bay.

While most of these studies have investigated eulittoral communities, we attempted to evaluate the potential of Microcosmus sulcatus Heller to control the particle and chlorophyll $a$ concentrations of the nepheloid layer above a sublittoral silty sand substrate in the northern Adriatic Sea. Most publications dealing with the impact of ascidians describe laboratory experiments proposing theoretical models and energy flux budgets (Fiala-Médioni, 1978a, 1978b; Robbins, 1983, 1984; Tito de Morais & Fiala-Médioni, 1985; Koike et al., 1993). In this paper we present the results of experiments conducted in situ under semi-natural conditions using a flow-through benthic ecosystem tunnel of novel design.

**Material and Methods**

1. **Study site**

The northern Adriatic Sea is a shallow basin with a strong east–west trophic gradient (Gilmartin et al., 1990). The western part of the basin is highly eutrophic due to the nutrient input from the river Po and prevailing cyclonic surface currents. During summer stratification, the generally oligotrophic circulation of the eastern part of the northern Adriatic basin becomes strongly isolated from currents in the southern Adriatic Sea, disrupting the trophic gradient and causing the eutrophication of eastern waters (Franco, 1982; Degobbis, 1989). These conditions produce a pronounced seasonal primary production pattern off the Istran west coast with a peak around September (Ahel & Terzic, 1998).

The experiments were conducted during 1994 and 1995 at the Ruđer Bošković Institute, Centre for Marine Research, Rovinj, Croatia, situated on the west coast of Istria, northern Adriatic Sea, about 114 km south of Trieste and 40 km north of Pula (Zavodnik, 1995). A site was chosen to the west of the island of Figarola (45°10' N, 13°60' E), approximately 1 km off the mainland. The position was marked by a buoy fixed to a concrete block which also served as a boat mooring (Fig. 1). The water depth at the site was 26 m. The local sediment is silty sand, interspersed with patches of shell debris. The sediment distribution is caused by a distinct small-scale current pattern originating from a large number of offshore islands. According to Brana & Krajcar (1992), tidal currents turn from a morning north-westerly direction to a south-easterly in the evening. Current speed during a tidal cycle at the study site was measured 20 cm above the substrate with a 585 OEM electromagnetic water current meter and varied between 0 and 18 cm·s$^{-1}$.

Large areas of the northern Adriatic Sea sedimentary plains are dominated by Ophiothrix–Reniera–Microcosmus communities, formed by the brittle star Ophiothrix quinquemaculata, the sponge Reniera sp. and the ascidians Microcosmus spp. (Fedra et al., 1976; Fedra, 1977; Ott, 1981). The active suspension feeders in this system are likely to affect the elemental flux between the benthic and the pelagic compartment, i.e., benthic-pelagic-coupling (Ott, 1990; Prins & Smaal, 1990; Sullivan et al., 1991; Graf, 1992). Two species of the genus Microcosmus, namely, $M.$ sulcatus and $M.$ polymorphus, co-occur on these sediment plains. The dominant $M.$ sulcatus, which can settle on very small shell particles (Costa, 1960), was used for our experiments. It occurs at densities ranging from 0.8 to 32 indiv.·m$^{-2}$, and larvae may settle on already established adults and thus can form clumps of several individuals. In greater depths, and in the absence of competitors for settling space, as many as 200 indiv.·m$^{-2}$ have been reported (Fedra, 1977; Scherübel, 1980). Experimental densities ranging from 45 to 80
individuals $\cdot m^{-2}$ [average 633 g (dry weight) $\cdot m^{-2}$] were necessary in order to achieve reliable measurements. The results were expressed per unit dry weight, which was calculated using the biometrics according to Scherübel (1980). Briefly, the distance between the ingestion and egestion siphon was measured and the weight calculated with the equations $ww = 0.005 \times L^{1.773}$ and $dw = 0.079 \cdot ww$, where $ww$ is wet weight, $dw$ is dry weight and $L$ is the distance between the two siphons [mm].

2. The `Benthic Ecosystem Tunnel'

In order to follow a given body of water in situ over a certain stretch of substrate, a channelling system is necessary to prevent lateral advection and mixing and to ensure that the water sampled has actually passed over the area of interest. Therefore a ‘Benthic Ecosystem Tunnel’, to be operated by a single SCUBA-diver with a back-up crew in a boat on the surface, was constructed based on the design of Dame et al. (1984) (Fig. 2). The tunnel was 5 m long with a trapezoid cross-section measuring 54 cm at the base, 38 cm at the top and 27 cm on either side, enclosing an entrance area of 1243 cm$^2$. It consisted of five slightly conical, transparent polycarbonate segments and covered 2.5 m$^2$ of substrate. The segments were held together by strong rubber bands. At each joint, 2 kg lead weights were used to keep the segments firmly embedded in the substrate. The segments were interlocked and sealed against each other by 1 cm $\times$ 1 cm PVC-rods equipped with a thread of silicone. Fluorescin dye was used to test for any leaks from underneath and at the intersections. No intake of dye was observed at the tunnel intersections or at the base. A square manipulation port was cut into the first and last segment and closed by sealed covers. These could be removed at any given time in order to deploy and recover sediment traps. Ports for water samples were installed at both ends of the tunnel. The whole tunnel could be lifted and laid onto one side. Thus, the collected ascidians could be placed on the substrate and then covered by the tunnel. Prior to experiments, the ascidians were left for 24 h in the tunnel in order to allow them to adapt to their new environment and thereby reduce possible artefacts in behaviour due to handling.
To avoid oxygen depletion inside the tunnel and ensure ventilation during the acclimatization period and after sampling was completed, the lids at the tunnel ends were removed and two additional vents at the tunnel exit were opened. ‘Control’ measurements were carried out by running the tunnel without ascidians. Sedimentation rates and chlorophyll $a$ concentrations at the tunnel entrance did not differ significantly between the runs with and without ascidians (one-way analysis of variance (ANOVA): $P = 0.225$ and $P = 0.551$, respectively).

A standardized current through the tunnel was created by an airlift, which consisted of a commercially available plumbing pipe, 5 m high, mounted on a concrete base. Sufficient air for a 30-min run of the tunnel was provided by a set of twin 101 SCUBA-cylinders, which could also serve as an alternative air source for the diver in case of emergency. The water flow inside the airlift was adjusted to 20 m$^3$ h$^{-1}$, representing a current of approximately 4 cm $\cdot$ s$^{-1}$ in the tunnel; this was monitored with an ASV flow meter DFM 350, DN 65, installed just below the air intake.
Ascidian suspension feeding in situ

3. Sampling procedures

The ascidians were collected by beam trawl and deposited near the tunnel immediately after dredging, where they were left to recover from the handling procedures. After 24 h, the siphons of all ascidians were wide open and the animals were pumping regularly.

Removal of particles by ascidian filtration as opposed to sedimentation alone was determined by measuring the difference of sedimentation rates at the tunnel entrance and exit using two replicate sediment traps on either end. There was no significant variability between the replicates (Kruskal-Wallis: P = 0.4805). Since studies recommend sediment traps with a diameter:height ratio of 1:3 for optimal sampling efficiency (Kirchner, 1975; Gardiner, 1980a, b; Blomqvist & Härkänsso, 1981), sediment traps made of PVC tubing, 5 cm in diameter and 15 cm high, were used (Hartl, 1996). The sediment traps were placed in PVC tubes with a metal cone at the base, having a diameter of 7 cm and a total height of 20 cm which were inserted into the sediment. The sediment traps could later be inserted into these and also removed without resuspending fine surface sediment particles. In order to prevent the sampling of bed-load transport (i.e., particles rolling along the sediment surface; Moss, 1972), the top edge of the sediment traps protruded 2 cm above the sediment surface. Following the experiment, the sediment traps were sealed with lids and brought to the surface in an upright position. Additional sediment traps measured sedimentation rates outside the tunnel. These traps were made of PVC tubes that were 10 cm in diameter and 30 cm high. Three traps were placed about 30 m away from the tunnel and approximately 1 m apart from each other on the sediment surface, secured with a metal frame and recovered as described above.

The tunnel was run for 20 min, after which water samples for chlorophyll a measurements were taken using 200 ml syringes. Prior to sampling, the syringes were rinsed with ambient water. Samples for each parameter were taken in duplicate, first at the entrance sampling port and then after 2 min, when the same parcel of water was estimated to have reached the end of the tunnel. Variability between the replicates was insignificant (P = 0.954; one-way ANOVA).

4. Laboratory analysis

The whole volume of the traps, except for the traps outside the tunnel, where a 250-ml subsample was taken, was filtered on pre-weighed, pre-rinsed and pre-combusted Whatman GF/F filters. The filters were then washed with distilled water to remove salts, dried at 70°C, weighed for total particulate matter (TPM), combusted in a muffle furnace (4 h at 450°C) and reweighed. In order to estimate the particulate organic matter (POM) fraction, ash-free dry weight (AFDW) was subtracted from the dry weight. Sedimentation rates of TPM and POM without ascidians were expressed in g·m⁻²·h⁻¹. The results including ascidians were expressed in gTPM [g(ascidian dry weight)⁻¹·h⁻¹] and in gPOM [g(ascidian dry weight)⁻¹·h⁻¹], respectively. Chlorophyll a concentrations were measured spectrophotometrically using a Jasco 820 FP fluorometer. Calibration was performed with known chlorophyll a concentrations from a dense culture of Emiliana huxleyi determined with a Hitachi U2000 spectrophotometer.

5. Data analysis and statistics

The tunnel performance was tested for variability using one-way ANOVA. The averaged data of each series of experiments are presented separately to illustrate variable sedimentation rates outside the tunnel. With these varying conditions in mind, the data were tested for significant difference between experiments with and without ascidians using a one-way repeated measurement analysis of variance (one-way RM-ANOVA) (Fry, 1993).

Results

Sedimentation rates outside the tunnel were variable and reflected varying seasonal particle concentrations (Table 1). Increased TPM sedimentation rates were usually
Table 1. Comparison of the average seasonal total particulate matter (TPM) sedimentation rates at the tunnel entrance and outside the tunnel.

<table>
<thead>
<tr>
<th>month</th>
<th>TPM sedimentation rates at the tunnel entrance [g·m⁻²·h⁻¹] ± SD</th>
<th>TPM sedimentation rates outside the tunnel [g·m⁻²·h⁻¹] ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>14.29 ± 17.89</td>
<td>1.55 ± 0.22</td>
</tr>
<tr>
<td>June</td>
<td>12.50 ± 6.02</td>
<td>1.49 ± 0.19</td>
</tr>
<tr>
<td>Sept</td>
<td>27.14 ± 14.30</td>
<td>3.98 ± 1.22</td>
</tr>
</tbody>
</table>

Fig. 3. a. Average sedimentation rates of total particulate matter (TPM) plotted against the average sedimentation rates of particulate organic matter (POM) at the tunnel entrance expressed in g·m⁻²·h⁻¹; b. TPM sedimentation rates plotted against the percentage of POM, indicating the quality of seston entering the tunnel.

accompanied by higher POM fractions (Fig. 3a) and independent of season, the percentage of POM decreased with increasing TPM, indicating reduced food quality (Fig. 3b; Table 2).
Table 2. Seasonal variability of the POM fraction of TPM collected in the traps and chlorophyll a concentrations sampled from the water passing through the tunnel.

<table>
<thead>
<tr>
<th>month</th>
<th>percentage POM of TPM at the tunnel entrance [%] ± SD</th>
<th>one-way ANOVA</th>
<th>chlorophyll a concentration at the tunnel entrance [μg l⁻¹] ± SD</th>
<th>one-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>13.14 ± 3.2</td>
<td></td>
<td>10.79 ± 6.97</td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>16.60 ± 6.68</td>
<td>P = 0.470</td>
<td>9.96 ± 5.11</td>
<td>P = 0.0244</td>
</tr>
<tr>
<td>Sept</td>
<td>15.75 ± 7.15</td>
<td></td>
<td>14.24 ± 6.11</td>
<td></td>
</tr>
</tbody>
</table>

In both runs, with and without ascidians in the tunnel, the TPM sedimentation rates between the tunnel entrance and tunnel exit differed significantly (P < 0.0001; Fig. 4a,b). There was, however, no significant difference in reduction between the two groups (44% with ascidians and 43% without ascidians; P = 0.253).

The TPM sedimentation rates increased with higher initial TPM, probably because of enhanced particle aggregation. Introduction of ascidians showed a similar trend. However, a decrease in TPM retention was revealed by plotting the TPM sedimentation rates at the tunnel entrance against TPM reduction as a percentage of ascidian biomass (% [kg (ascidian dry weight)⁻¹ · m⁻² · h⁻¹]) (Fig. 5).

The removal of POM increased after introduction of ascidians compared with the reduction of POM solely due to sedimentation. On average 40% of POM was removed from the water column due to sedimentation (Fig. 6a). Introducing *M. sulcatus* brought about a nonsignificant (P > 0.05) increase in POM reduction to about 43% (Fig. 6b). Furthermore, sedimentation of particles with a low POM content at the tunnel entrance generally lead to a low POM reduction by ascidians. In such cases the percentage POM of TPM at the end of the tunnel was often higher than at the entrance (Fig. 7).

Experiments including ascidians showed low POM reduction at low POM sedimentation rates at the tunnel entrance (low quality, but high TPM). Higher POM sedimentation rates at the tunnel entrance lead to more POM being removed from suspension due to sedimentation alone. Whereas the reduction of POM by ascidians in September was moderately correlated with POM entering the tunnel (r = 0.60), the data for May and June showed no correlation at all (r = 0.04 and r = 0.2, respectively).

Average chlorophyll a concentrations in the water entering the tunnel ranged from 17 to 50 μg · l⁻¹. The concentrations in September were significantly higher than those measured in May and June (Table 2). The filtration activity of the introduced ascidians removed significantly more chlorophyll a from the overlying water than during experiments without ascidians, averaging 44 and 22%, respectively (P < 0.05).

Separate analysis of each run revealed that chlorophyll a reduction was positively correlated with the incoming chlorophyll a concentration, both with and without ascidians. At low chlorophyll a concentrations at the tunnel entrance, runs with
Fig. 4. Average TPM sedimentation rates at the tunnel entrance [g·m⁻²·h⁻¹] plotted against the reduction of TPM [%] after passage through the tunnel due to (b) sedimentation and ascidian filtration and (a) sedimentation alone.

Fig. 5. Average reduction of TPM sedimentation rates expressed as percentage kg (ascidian dry weight)⁻¹·m⁻²·h⁻¹.
Fig. 5. Average POM sedimentation rates at the tunnel entrance [g·m⁻²·h⁻¹] against average POM reduction [%] after passage through the tunnel with (b) and without (a) ascidians.

Fig. 6. Average ascidian reduction of POM sedimentation rates at the end of the tunnel expressed as g·m⁻²·h⁻¹ against the percentage POM of TPM.
Fig. 8. Average reduction [%] of chlorophyll a during passage through the tunnel against the chlorophyll a concentration [mg l$^{-1}$] at the tunnel entrance with (b) and without (a) ascidians.

and without ascidians showed similar reduction patterns. Higher chlorophyll a concentrations during runs including ascidians, however, enhanced chlorophyll a reduction (Fig. 8a,b).

**Discussion**

Fluctuating sedimentation rates illustrate the strong influence seasonal, oceanographic and geomorphologic conditions have on shallow aquatic ecosystems. It is here that coupling of benthic and pelagic processes are best visible (Graf, 1992). The benthic boundary layer is characterized by a distinct vertical gradient caused by a natural sorting effect of heavy inorganic particles from lighter organic particles, which is due to varying current speed in the overlying water (Muschenheim, 1987).

Not only did the sedimentation rates in the surrounding water vary during our experiments, but also the relationship between inorganic and organic seston components. This indicates a constant change in the quality of particles transported...
to the benthos. Increasing total particulate matter (TPM) brought about an increase of inorganic as well as organic particles, although the percentage of POM (expressed as percentage of TPM) decreased with increasing TPM (Fig. 3a,b). This leads to the conclusion that during periods of high TPM sedimentation rates the seston entering the tunnel contained more resuspended inorganic material and was therefore of lower nutritional value to ascidians.

During runs without ascidians, TPM reduction at the tunnel exit was directly proportional to TPM sedimentation rates at the tunnel entrance. This may be partly due to increased particle aggregation and partly due to the reduced current velocity inside the tunnel (4 cm · s⁻¹), at which the resuspended particles resettle (Fig. 4a).

The combined filter-feeding activity of all the ascidians in the tunnel significantly reduced the TPM sedimentation rates at the end of the tunnel (P < 0.05). However, increased TPM at the entrance decreased particle reduction when the reduction was expressed as a percentage of the ascidian biomass (dry weight) (Fig. 5). This suggests, in agreement with Robbins (1983, 1984), that *M. sulcatus* reduces filtration rates with increasing TPM, thus maintaining a constant ingestion rate at satiation level. The low-quality character of such particle loads (low POM content at high TPM) may make high filtration rates energetically uneconomical. The consequence of filtering vast amounts of inorganic particles is the accumulation of nondigestible material that has to be processed. Ascidians deal with high sedimentation by incorporating the particles into mucus, which bypasses the gut and is excreted as pseudofaeces (Tito de Morais & Fiala-Médioni, 1985), eventually reducing filter-feeding activity (Robbins, 1983, 1984). Regardless of season (Table 2), high TPM sedimentation rates at the tunnel entrance were typically concomitant with a low POM fraction (Fig. 3a,b). In addition, during runs with ascidians, a low percentage of POM in TPM sedimentation led to an increase in POM at the end of the tunnel (Fig. 7). This observation suggests that the POM increase could be caused by mucus expelled by the ascidians as a reaction to high sedimentation, thereby partly masking POM retention from ascidian filter feeding.

During phases of high primary productivity, considerable amounts of chlorophyll *a* are transported to deeper water layers by means of aggregation and successive sinking of phytoplankton. Filter-feeding benthic organisms may significantly deplete chlorophyll *a* concentrations in near-bottom layers by ingesting large amounts of cells (Asmus & Asmus, 1991; Cloern, 1992; Herman & Scholten, 1990; Officer et al., 1982; Petersen & Riisgård, 1992; Takeda & Kurihara, 1994). The increased chlorophyll *a* depletion during periods of high chlorophyll *a* concentrations observed during this study (Fig. 8b) suggests that ascidians possess some kind of particle quality control mechanism and may be able to respond to seasonal changes in primary productivity, ensuring that filtration activity is highest when food abundance is at its maximum.

Pumping rates for *Microcosmus sabatieri* reported by Fiala-Médioni (1978a) reached up to 31 · h⁻¹ · g⁻¹ ascidian dry weight. At this rate during the present experiment the ascidians would have filtered 1952 liter · h⁻¹ · m⁻², representing approximately 10% of the water passing through the tunnel. Jørgensen (1949) measured filtration rates reaching 28 liter · h⁻¹ per individual for *M. edulis*. According to Prins & Smaal (1994), densely populated *M. edulis* beds with a biomass of up to 2190 g (AFDW) · m⁻² could potentially clear the whole Oosterschelde Basin.
in 4 to 5 days and thus play an important part in controlling material fluxes to and from the sediment. Obviously, the filtration activity of the ascidian biomass at our study site cannot compete with such clearing rates. Nonetheless, because of the water depth and dynamic sediment conditions, with little or no substrate for mussels to settle on, *M. sulcatus* was the dominant epibenthic megafaunal element. Becerro & Turon (1992) suggest a link between temperature, food availability and reproduction for *M. sabatieri*, which spawns between September and November. Ribes et al. (1998) reported a correlation between temperature and filtration rates for *Halocynthia papillosa*, another pyurid ascidian species in the Mediterranean, but in that case temperature did not correlate with food availability. The correlation of seasonal fluctuations of chlorophyll *a* concentrations with chlorophyll *a* reduction by *M. sulcatus* indicates the potential for *Microcosmus* to control local phytoplankton input to the sublittoral nepheloid layer. Whether this increase in filter-feeding activity is a reaction to the increased food availability or simply due to increased water temperatures in September remains unclear.

**Conclusions**

The reduction of chlorophyll *a* concentrations during passage through the tunnel was significantly greater with ascidians than without ascidians. Chlorophyll *a* reduction with ascidians increased with seasonal increases in primary production, which suggests that ascidians react to fluctuating chlorophyll *a* concentrations. However, the susceptibility of ascidians to high turbidity, *i.e.*, high TPM sedimentation rates causing frequent periods of siphon closure and inactivity (Fiala-Médoni, 1978a), and the relatively low density of *Microcosmus sulcatus* at the study site makes it unlikely that this ascidian species is an important element controlling the composition of the nepheloid layer in the northern Adriatic Sea on a large scale.

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**References**

Ascidian suspension feeding in situ


