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Primary Research Paper

Influence of pulsed inflows and nutrient loading on zooplankton and phytoplankton community structure and biomass in microcosm experiments using estuarine assemblages

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Abstract

Productivity and community structure of phytoplankton and zooplankton are influenced by hydrologic disturbances in many ways. In a recent modeling study it was suggested that pulsed inflows might enhance zooplankton performance, curb accumulation of phytoplankton accumulated biomass, and promote phytoplankton species diversity. We tested these predictions by performing microcosm experiments on natural plankton assemblages from the Nueces Delta, TX, USA. On three occasions (March, June, and September 2001), experiments of semi-continuous and flow-through design were conducted using natural plankton assemblages. We investigated the effect of two different inflow and nutrient loading regimes on zooplankton biomass, and phytoplankton biomass and diversity, i.e., continuous and pulsed inflows of 3 day frequency. Despite differences in initial community structure on these three occasions, as well as the very different communities that arose between experimental designs, our findings showed that pulsed inflows altered plankton dynamics. In all cases, pulsed inflows resulted in greater zooplankton biomass. In most cases, pulsed inflows resulted in lower phytoplankton biomass and higher diversity. We speculate that greater phytoplankton diversity in the pulsed flow treatments favored selectively feeding zooplankton, whose better performance prevented higher accumulation of phytoplankton biomass.

Introduction

Environmental disturbances in aquatic systems, such as nutrient additions associated with inflow events, are known to influence phytoplankton community composition, species diversity, and biomass. This was shown in modeling studies (Ebenhöh, 1988; Roelke et al., 1999, 2003), laboratory experiments (Sommer, 1984; Gaedeke & Sommer, 1986; Roelke et al., 2003), and field exercises (Padisak, 1993; Barbiero et al., 1999; Flöder & Sommer, 1999; Hambright & Zohary, 2000).

In turn, changes in phytoplankton community structure can influence zooplankton community structure (Sommer et al., 1986; Steiner, 2001). For example, after an inflow event to a system where phytoplankton are nutrient-limited, succession from less edible, slower growing, K-selected species to more edible, faster growing, r-selected species might occur (Sommer, 1981; Reynolds, 1984; Sommer et al., 1986; Roelke et al., 1997), which may stimulate secondary productivity. Zooplankton community structure might shift because taxa of small body-size and short generation times, e.g., rotifers and protozoa, will likely be the first to respond to a shift in prey availability, and also the first to recover from flushing losses (Sommer et al., 1986; Reynolds, 1984;

Havens, 1991a, b; Kim et al., 2001). In addition, high phytoplankton species diversity, which can be maintained in systems where the physicochemical environment fluctuates, may result in the proliferation of preferential grazers (Reynolds, 1984, 1989), and again result in a shift in zooplankton community structure.

Zooplankton, however, might mask the effects of nutrient loadings on phytoplankton community structure. For example, strong top-down control exerted by non-selective grazers might prevent additional accumulation of phytoplankton biomass and prevent shifts in community composition (Reynolds, 1984; Sterner, 1989; Cottingham & Schindler, 2000). In addition, through consumer-driven nutrient recycling, strong top-down control might remove nutrient limitation altogether, thereby negating the influence of nutrient additions to the system (Lehman, 1988; Sterner, 1989; Katechakis et al., 2002). Similarly, a well-established population of preferential grazers may control some phytoplankton populations that would have otherwise proliferated following a disturbance (MacKay & Elser, 1998; Saunders et al., 2000; Kagami et al., 2002).

These concepts have direct application to watershed management aimed at restoration of coastal wetland and bay systems (see Roelke & Buyukates, 2001). For example, the Nueces Delta, TX, not unlike many estuaries of the western Gulf of Mexico, has experienced dramatic declines in freshwater inflow over the past 50 years, which have produced deleterious consequences in the delta (Bureau of Reclamation, 2000; Fejes et al., 2005). Temporary pulsed inflows during a river diversion demonstration project corresponded to dramatic increases in net ecosystem productivity, and improved abundance and diversity of intertidal vegetation and benthic communities (Heilman et al., 1999; Alexander & Dunton, 2002; Montagna et al., 2002; Palmer et al., 2002; Ward et al., 2002; Heinsch et al., 2004). It may be that zooplankton and phytoplankton populations were also stimulated following mechanisms discussed in the preceding paragraphs, which have also been observed in theoretical and empirical studies in other similar coastal systems (Hann & Goldsborough, 1997; Roelke, 2000).

We further explored this notion by conducting laboratory experiments using natural plankton assemblages from the Nueces Delta. In these experiments, we focused only on the plankton community, and tested the hypotheses that in a simplified system, i.e., microcosm, pulsed inflows would result in greater accumulation of zooplankton biomass, greater phytoplankton species diversity, and less accumulation of phytoplankton biomass. Our laboratory experiments are meant only as a proof of concept in regards to findings from a previous numerical modeling study (Roelke, 2000), and are not meant to replicate conditions of the natural environment, nor do we infer that results from these experiments can be scaled-up to the ecosystem level. Our experiments are relatively unique in that they include natural phytoplankton and zooplankton communities together, thereby allowing simultaneous direct and indirect interactions between phytoplankton, zooplankton, and the physicochemical environment.

Materials and methods

Laboratory experiments were conducted to examine the influence of pulsed inflows and nutrient loading on zooplankton and phytoplankton biovolume, and phytoplankton diversity on 15 March, 7 June, and 8 September, 2001. Two separate experimental designs were used to accomplish this, semi-continuous and flow-through. We opted to employ two designs because the level of turbulence, which potentially influences zooplankton feeding, was different between the two. That is, semi-continuous experiments experienced turbulence twice daily, while flow-through experiments experienced turbulence once every 45 s (see below).

Natural plankton assemblages were collected from surface waters in 20 l Nalgene carboys from the Nueces Delta, Texas (27° 57′ N, 97° 31′ W). The samples were transported to the laboratory located in College Station, Texas. This process took ~4 h. During this time samples were kept shaded and cool. A portion of the collected water was filtered through 47 mm Whatman GF/F glass fiber filters, and autoclaved at 121 °C and 15 PSI for 30 min. After cooling, media was prepared by dissolving solid standards into the sterilized water to levels of f/2 (Guillard & Ryther, 1962), except for nitrogen and phosphorus concentrations, which

were set according to previous studies that focused on N and P loading into the Nueces River Estuary from a local wastewater treatment plant (N = 900 μM, P = 70 μM, Roelke et al., 1997; Roelke 2000). This process took ~2 h. To avoid bias from larger zooplankton, 200 μm mesh-size plankton net was used to pre-filter the remaining water collected from the delta (Sommer, 1985), which was then used as inoculum for the experiments. The experiments were started ~6 h after water was collected.

Each of the three experiments was comprised of two treatments, with each treatment performed in triplicate. In the semi-continuous design, the treatments were 1 day and 3 day pulsed inflows. In our analyses of the semi-continuous experiments, we assume that volume displacement occurring daily was analogous to continuous flow. In the flow-through design experiments, the treatments were continuous inflow and pulsed inflow (3 day-period). Chamber volumes were constant, so plankton were subjected to flushing losses as a function of the inflow. The incubation design used in these experiments allowed for control of flushing rate and periodicity, nutrient loading magnitude and ratio, temperature, irradiance and photoperiod, and turbulence. A detailed description of incubation design and calculations for flushing rate and periodicity, as well as nutrient loading magnitude and ratio was given in Buyukates (2003).

In all treatments, the photoperiod was 12 h light/dark cycle. Cool white fluorescent bulbs were used as the light source. Depending on the time of day irradiance values change, i.e., low in the morning and evening, and high in the afternoon. To account for this, irradiance was chosen as 200 μE m^{-2} s^{-1} (see Buyukates, 2003). This value was in the range of typical light saturated photosynthesis rates of many phytoplankton (Kirk, 1994). Temperature was held constant at 20 °C in all treatments.

While the two experimental designs, i.e., semi-continuous and flow-through, were very similar in regards to their physicochemical environment, a major difference was the level of turbulence. In the flow-through design experiments, turbulence was controlled using an aerator powered through a time delay relay (5 s on/40 s off). In the semi-continuous design experiments, chambers were gently swirled twice each day. Consequently, turbulence was greater in the flow through experiments.

Periphyton growth on the sides of the incubators was avoided in both experimental designs. In the flow-through design experiments, horizontal surfaces, where periphyton growth was a problem in pilot studies, were covered with aluminum foil, thereby inhibiting growth. The gentle swirling of the semi-continuous design experiments inhibited periphyton growth as well. In all the experiments reported below, periphyton did not accumulate in any of the chambers. Therefore, shading or nutrient uptake by periphyton was minimal.

Samples for microscopic analysis were collected at 3 day intervals and preserved immediately with 5% glutaraldehyde, v/v. Plankton identification and enumeration were conducted using settling chambers and inverted phase-contrast microscopy (Utermöhl, 1958). At least 20 random fields of view were counted at 1000×, 400× and 200× magnifications for different cell-size classes of phytoplankton. This resulted in at least 400 individuals counted for each of the dominant phytoplankton species, and a ±10% counting precision within 95% confidence limit (Lund et al., 1958). The entire settled area was counted for zooplankton. Phytoplankton were identified to the taxonomic level of genus (Prescott, 1978). Zooplankton were categorized into protozoa, rotifers, and copepods (nauplii and copepodids, where the copepodid category included the final adult stage). Phytoplankton and zooplankton volumes were estimated by measuring cell dimensions and using common geometric shapes (Wetzel & Likens, 1991). We assume that for the purposes of this manuscript, changes in biovolume reflected changes in biomass. Phytoplankton diversity was determined according to Shannon & Weaver (1949) using cellular biovolumes.

Differences between mode of inflow among the three experiments conducted on March, June and September were determined by integrating the variables, i.e., bulk phytoplankton and zooplankton taxonomic categories, over the duration of each experiment, then applying a two-factor repeated measures ANOVA (SPSS Inc., 1994). These analyses tested for significant differences between the inflow treatments, i.e., continuous and pulsed inflow, time of year, and the interaction between the mode of inflow and time of year. Statistically significant differences among treatments were assessed at the 5% level of confidence.

Results

Although initial zooplankton community composition within each experiment was similar, as the experiments progressed, it quickly varied with clear effects arising from experimental design, mode of inflow, and time of year. Copepodids and nauplii generally performed much better in the experiments of semi-continuous design, both numerically and in terms of biovolume, while rotifers and protozoa generally performed much better in the experiments of flow-through design (Figs. 1–3). All categories of zooplankton accumulated significantly greater biovolume in the 3 day pulsed inflow treatments of both experimental designs (Tables 1 and 2). In the experiments of semi-continuous design, all categories of zooplankton showed significant time of year effects, with copepodids and nauplii, and protozoa accumulating highest biovolumes in the June experiment, and rotifers accumulating highest biovolumes during the September experiment (Table 1, Figs. 1–3). In the experiments of flow-through design, time of year effects were again significant, but not with all zooplankton categories. Nauplii accumulated highest biomass during the June experiment, and rotifers and protozoa were highest in the March experiment (Table 2, Figs. 1–3). Copepodids showed no significant time of year effect.

As with the zooplankton, phytoplankton community composition and accumulated biovolume varied between the experimental designs, inflow treatments, and time of year. In March, the initial community composition was dominated by a mix of diatoms, with some cyanobacteria and dinoflagellates present (Fig. 4a). At the conclusion of the semi-continuous experiment, *N. closterium* dominated in the 1-day pulsed treatment (Fig. 4b), while a more diverse diatom assemblage arose in the 3-day pulsed treatment (Fig. 4c). At the conclusion of the flow-through experiment, a coccoid green alga was prevalent in both continuous flow and 3 day pulsed treatments (Fig. 4d and e). During the June experiments, an initial assemblage co-dominated by many phytoplankton forms (Fig. 5a) gave way to an assemblage dominated by the diatom *Entemoneis* sp. in both treatments of the semi-continuous experiments (Fig. 5b and c), and an assemblage dominated by dinoflagellates in both treatments of the flow-through experiments (Fig. 5d and e). During the September experiments, centric diatoms (Fig. 6a) dominated the initial community composition. This community gave way to an assemblage again dominated by *Entemoneis* sp. in both treatments of the semi-continuous experiments (Fig. 6b and c). In the flow-through experiments, *Nitzschia* sp. dominated both treatments (Fig. 6d and e).

Despite differences in community structure that emerged in the experiments, similar responses were observed in the phytoplankton as with the zooplankton. In the experiments of semi-continuous design, 1-day pulsed flow treatments consistently resulted in higher total accumulated phytoplankton biovolume compared to the 3 day pulsed flow treatments. This was also true for all the phytoplankton categories except cyanobacteria (Table 1). In the flow-through experiments, pulsed flows did not result in less total phytoplankton biovolume or the green algae taxonomic group (Table 2). Primarily, this was due to the anomalous results from the September experiment. When only the March and June experiments were considered, treatment effects were clear. Total phytoplankton and all phytoplankton categories showed significant time of year effects. Greatest biovolumes occurred in the September experiment (Figs. 4–7).

Finally, phytoplankton diversity showed trends consistent with zooplankton and phytoplankton biovolume. In all the semi-continuous experiments, 1 day pulsed flow treatments consistently resulted in lower phytoplankton diversity compared to the 3 day pulsed flow treatments (Fig. 8a, b and c). In the flow-through experiments conducted in March and June, continuous flow treatments also resulted in lower phytoplankton diversity, but again the September experiment showed anomalous results (Fig. 8d, e and f). Abrupt dips in phytoplankton diversity during the March and June experiments coincided with population shifts.

Discussion

With some exceptions, trends observed across our experiments generally supported predictions from a previous modeling study (Roelke, 2000), which

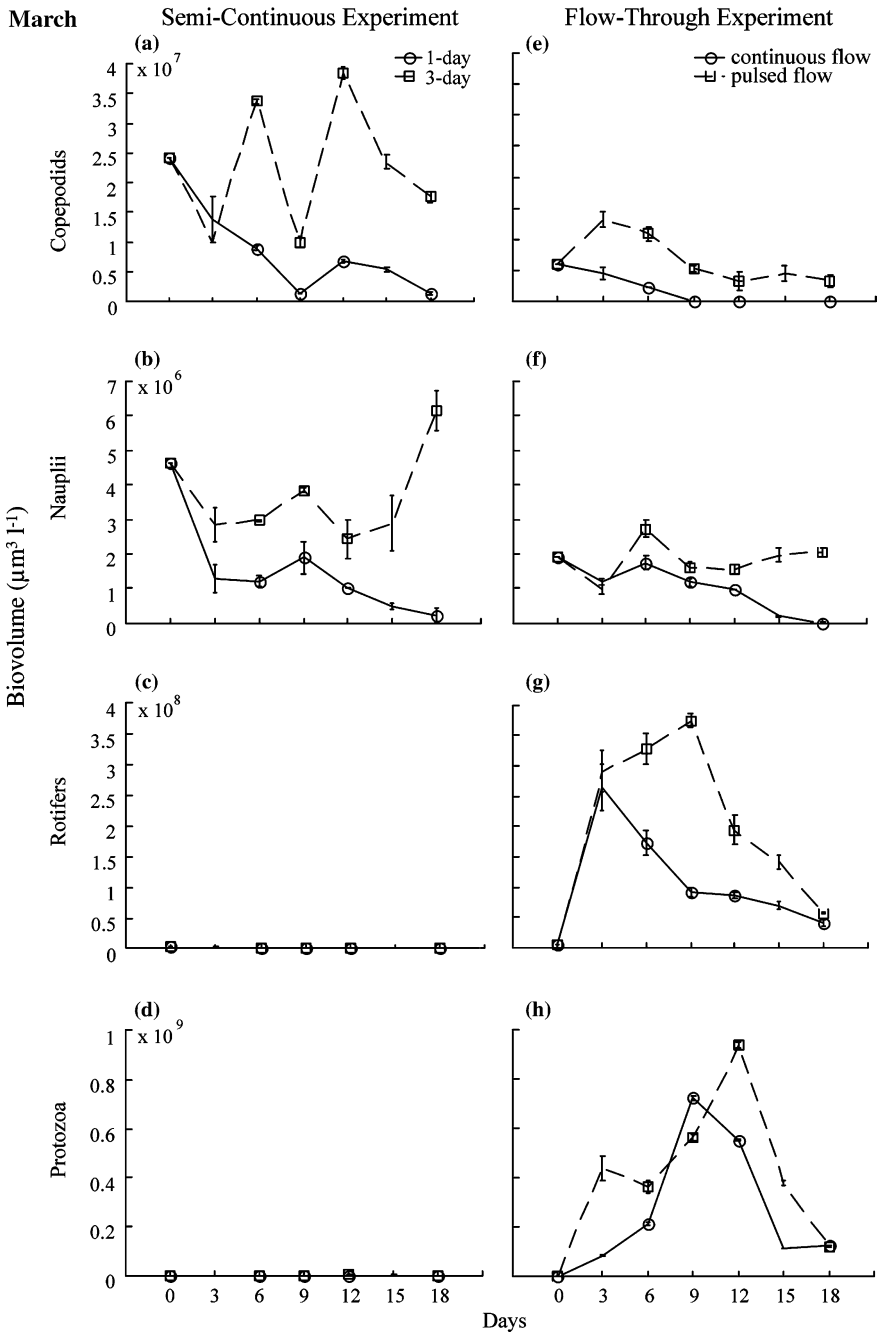

Figure 1. Changes in copepodids (a and e), nauplii (b and f), rotifer (c and g) and protozoa (d and h) biovolume in experiments of semi-continuous (a–d) and flow-through (e–h) design conducted in March. Copepods performed better in the semi-continuous experiments, and rotifers and protozoa performed better in the flow-through experiments. In both experimental designs, zooplankton performed better under conditions of 3 day pulsed inflows. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

concluded that pulsed inflows of 3 day frequency would enhance zooplankton populations, lower phytoplankton biovolume, and promote higher phytoplankton species diversity. Our experimental results, however, are contradictory to previous observations from *in situ* experiments, where

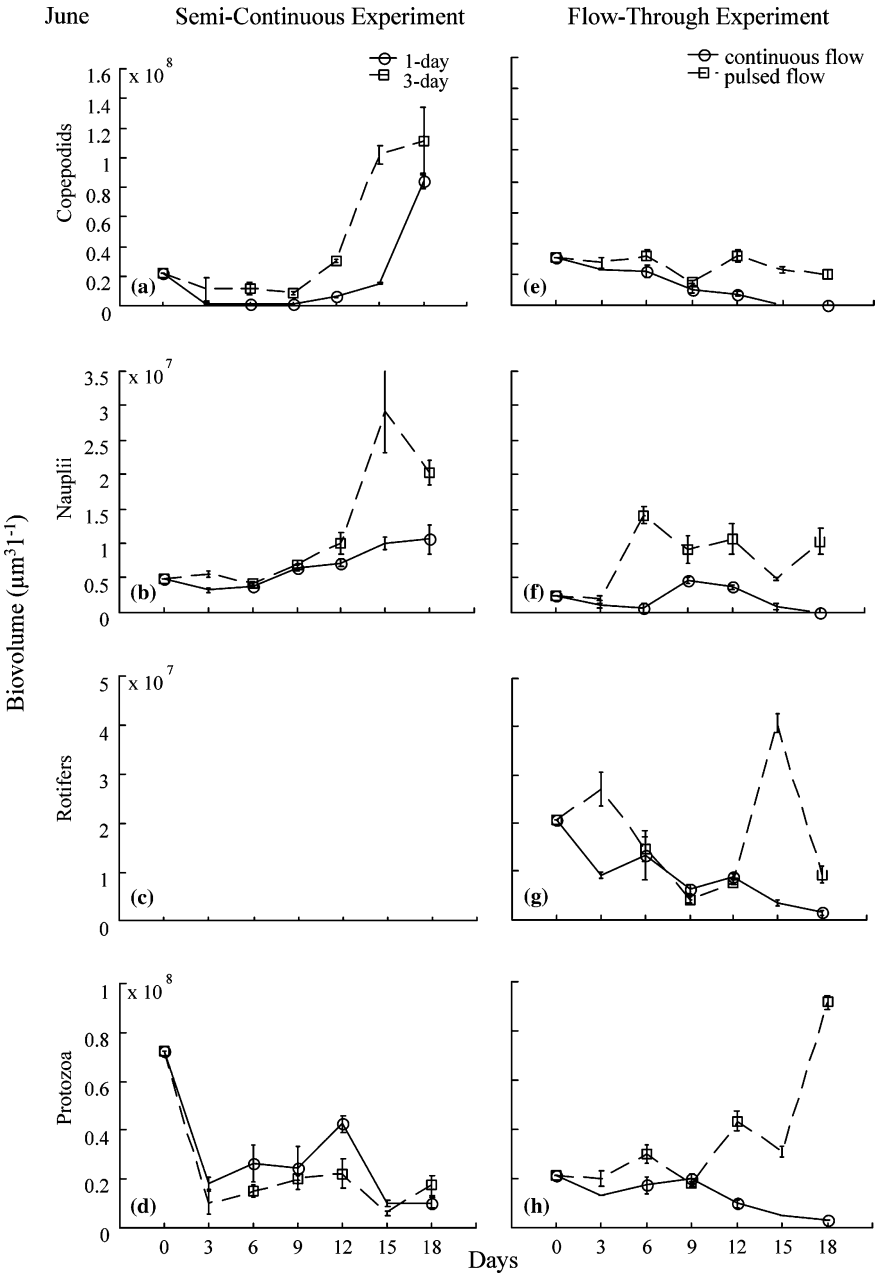

Figure 2. Changes in copepodids (a and e), nauplii (b and f), rotifer (c and g) and protozoa (d and h) biovolume in experiments of semi-continuous (a–d) and flow-through (e–h) design conducted in June. As with the March experiments, copepods performed better in the semi-continuous experiments, rotifers and protozoa performed better in the flow-through experiments, and in most cases zooplankton performed better under conditions of 3 day pulsed inflows. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

zooplankton populations showed no response to pulsed nutrient additions (Flöder & Sommer, 1999). In those experiments, however, *Daphnia* were prevalent. Large-bodied zooplankton, such as *Daphnia*, are known to buffer plankton communities against nutrient perturbations (Cottingham & Schindler, 2000). Large-bodied zooplankton eventually emerged in our experiments,

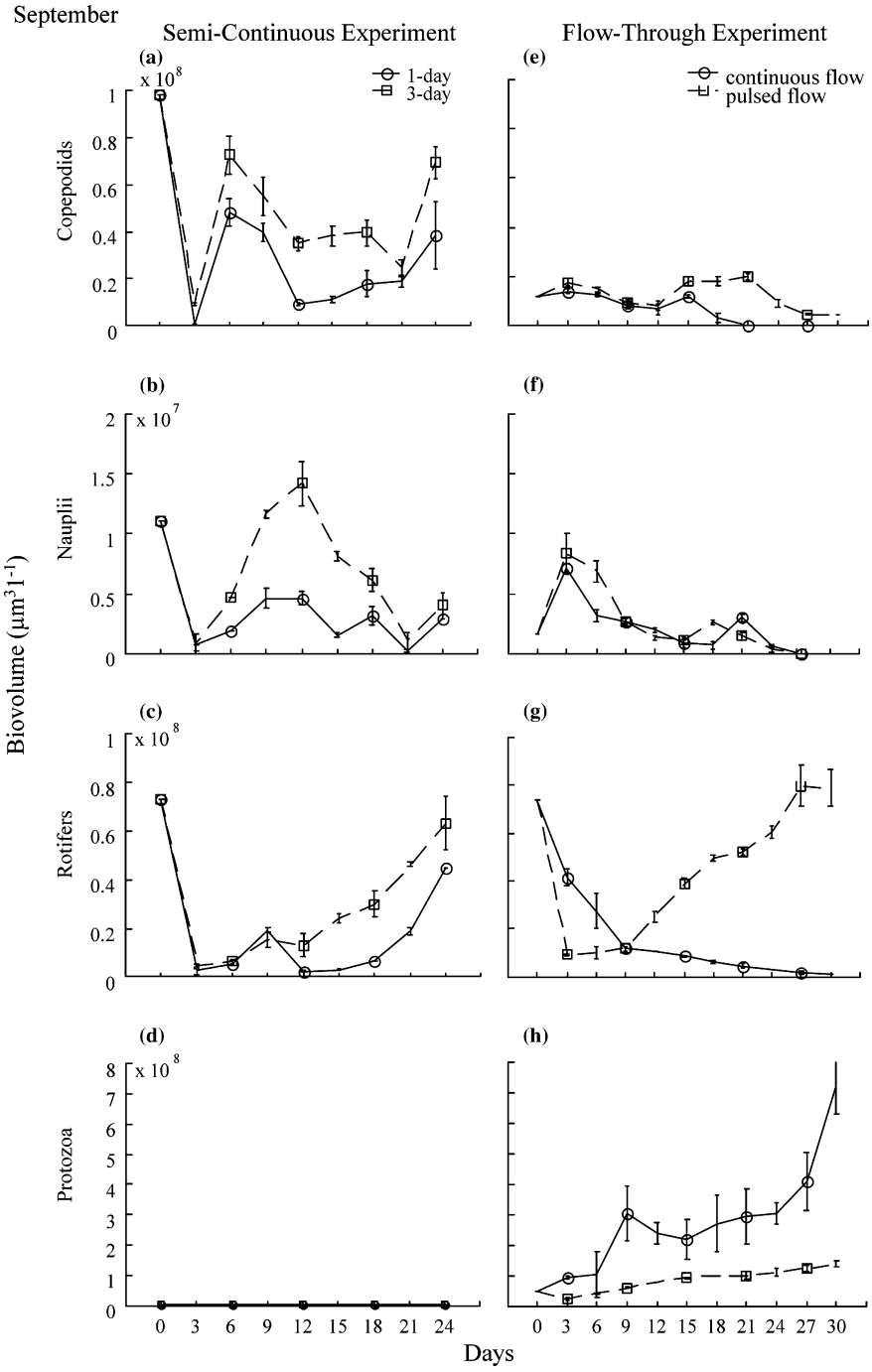

Figure 3. Changes in copepodids (a and e), nauplii (b and f), rotifer (c and g) and protozoa (d and h) biovolume in experiments of semi-continuous (a–d) and flow-through (e–h) design conducted in September. As in previous experiments, copepods performed better in the semi-continuous experiments, and protozoa performed better in the flow-through experiments. In these experiments, rotifers performed equally well in both experimental designs. In most cases, zooplankton performed better under conditions of 3 day pulsed inflows. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

Table 1. Zooplankton and phytoplankton biovolume accumulation in 1 day and 3 day flow treatments in March, June and September experiments. The table lists the zooplankton and phytoplankton groups and results of two-factor repeated measures ANOVA

	Source	SS	DF	MS	*F*	*p*
Zooplankton groups						
Copepodids	BSEP	1.93E + 17	2	9.66E + 16	107.32	0.000
	WSFT	8.85E + 16	1	8.85E + 16	26108.92	0.000
	EPFT	4.63E + 15	2	2.32E + 15	683.33	0.000
Nauplii	BSEP	6.26E + 15	2	3.13E + 15	263.09	0.000
	WSFT	3.31E + 15	1	3.31E + 15	1768.22	0.000
	EPFT	3.44E + 14	2	1.72E + 14	91.87	0.000
Rotifer	BSEP	1.99E + 17	2	9.97E + 16	308.87	0.000
	WSFT	5.49E + 15	1	5.49E + 15	195.04	0.000
	EPFT	9.54E + 15	2	4.77E + 15	169.37	0.000
Protozoa	BSEP	1.28E + 17	2	6.41E + 16	435479.79	0.000
	WSFT	8.54E + 14	1	8.54E + 14	3204056.70	0.000
	EPFT	1.79E + 15	2	8.96E + 14	3360922.00	0.000
Phytoplankton groups						
Cyanobacteria	BSEP	3.27E + 18	2	5.45E + 17	169.23	0.000
	WSFT	4.27E + 17	1	4.27E + 17	0.58	0.474 n.s.
	EPFT	4.17E + 18	2	2.09E + 18	2.58	0.135 n.s.
Green algae	BSEP	1.27E + 21	2	6.33E + 20	294.49	0.000
	WSFT	1.84E + 20	1	1.84E + 20	71.24	0.000
	EPFT	7.14E + 19	2	3.57E + 19	13.82	0.006
Diatoms	BSEP	3.52E + 23	2	1.76E + 23	161.81	0.000
	WSFT	3.37E + 22	1	3.37E + 22	106.27	0.000
	EPFT	3.82E + 22	2	1.91E + 22	60.20	0.000
Dinoflagellates	BSEP	9.98E + 20	2	4.99E + 20	724.76	0.000
	WSFT	3.65E + 20	1	3.65E + 20	269.11	0.000
	EPFT	6.72E + 20	2	3.36E + 20	248.02	0.000
Total	BSEP	3.95E + 21	2	1.98E + 23	194.22	0.000
	WSFT	4.65E + 22	1	4.65E + 22	159.48	0.000
	EPFT	3.24E + 22	2	1.62E + 22	55.59	0.000

Here, flow treatments (1 day vs. 3 day) are repeated measures and different experiment periods (March, June, September) are between subjects measures that used variables of integrated zooplankton and phytoplankton population over the entire period of experiment. The mean difference is significant at the 0.05 level; n.s. = is not significant; BSEP = Between Subjects Experiment period; WSFT = Within Subjects Flow treatments; EPFT = Experiment per. x Flow treatment.

but in the form of copepodids, and they did not appear to exert the same top-down control as previously observed for *Daphnia*. This might have allowed the plankton community to respond to the pulsed inflows.

Copepods tended to perform better in the experiments of semi-continuous design, while rotifers performed better in the experiments of flow-through design, this is quite apparent in our March and June experiments. This result was likely due to the lower turbulence in the experiments of semi-continuous design, which might have favored copepod feeding and growth (Saiz & Alcaraz, 1991; Alcaraz, 1997; Petersen et al., 1998; Quintana et al., 1998). Indeed, in follow-up experiments using the same flow-through chambers but lower turbulence levels, copepod populations flourished (Roelke, unpublished data). The poorer performance by rotifers and protozoa in experiments of semi-continuous design during the March and June experiments likely resulted because of the better performance of copepods. Copepodids are known

Table 2. Zooplankton and phytoplankton biovolume accumulation in continuous and pulsed flow treatments in March, June and September experiments. The table lists the dominant zooplankton and phytoplankton groups and results of two-factor repeated measures ANOVA

	Source	SS	DF	MS	*F*	*p*
Zooplankton groups						
Copepodids	BSEP	5.76E + 15	2	2.88E + 15	2.84	0.136 n.s.
	WSFT	1.74E + 14	1	1.74E + 14	8.36	0.028
	EPFT	6.14E + 15	2	3.07E + 15	147.22	0.000
Nauplii	BSEP	1.72E + 15	2	8.60E + 14	12.07	0.008
	WSFT	1.07E + 15	1	1.07E + 15	30.18	0.002
	EPFT	8.66E + 14	2	4.33E + 14	12.27	0.008
Rotifer	BSEP	5.77E + 17	2	2.89E + 17	687.92	0.000
	WSFT	1.47E + 17	1	1.47E + 17	5019.94	0.000
	EPFT	4.26E + 16	2	2.13E + 16	728.40	0.000
Protozoa	BSEP	1.09E + 19	2	5.46E + 18	6193224.00	0.000
	WSFT	1.00E + 18	1	1.00E + 18	8699585.00	0.000
	EPFT	5.91E + 18	2	2.96E + 18	25697295.00	0.000
Phytoplankton groups						
Cyanobacteria	BSEP	2.80E + 20	2	1.40E + 20	309.37	0.000
	WSFT	7.30E + 18	1	7.30E + 18	18.85	0.005
	EPFT	1.88E + 19	2	9.40E + 18	24.28	0.001
Green algae	BSEP	9.99E + 23	2	3.10E + 22	16.11	0.004
	WSFT	2.28E + 22	1	2.28E + 22	0.76	0.417 n.s.
	EPFT	3.68E + 22	2	1.84E + 22	0.61	0.573 n.s
Diatoms	BSEP	2.85E + 24	2	1.43E + 24	140.01	0.000
	WSFT	1.15E + 23	1	1.15E + 23	15.50	0.008
	EPFT	2.81E + 23	2	1.40E + 23	18.96	0.003
Dinoflagellates	BSEP	2.66E + 22	2	2.12E + 19	627.49	0.000
	WSFT	9.70E + 21	1	9.70E + 21	120.66	0.000
	EPFT	1.91E + 22	2	9.57E + 21	119.01	0.000
Total	BSEP	6.87E + 24	2	3.43E + 24	51.51	0.000
	WSFT	8.11E + 22	1	8.11E + 22	1.35	0.289 n.s.
	EPFT	8.82E + 22	2	4.41E + 22	0.73	0.518 n.s.

Here, flow treatments (continuous vs. pulsed) are repeated measures and different experiment periods (March, June, September) are between subjects measures that used variables of integrated zooplankton and phytoplankton population over the entire period of experiment. The mean difference is significant at the .05 level; n.s. = is not significant; BSEP = Between Subjects Experiment period; WSFT = Within Subjects Flow treatments; EPFT = Experiment per. x Flow treatment.

to graze on rotifers and protozoa (Sterner, 1989; Ingrid et al., 1996).

Rotifer populations were much more prevalent in the September experiments. Heavy rains preceded the field sampling in September. Previous studies have shown that increased performance of rapidly-growing and edible phytoplankton forms, which are often of smaller cell size, typically follow favorable nutrient perturbations (Reynolds, 1984; Sommer et al., 1986; Roelke et al., 1997, 2004; Roelke & Buyukates, 2002), which likely occurred with the elevated freshwater inflows to the delta. Phytoplankton of small cell sizes were much more prevalent in the September experiment. The availability of smaller cell sizes, coupled to the lower salinities, likely created conditions more favorable for rotifers, which reproduce more rapidly than copepods and prefer prey of smaller size (Reynolds, 1984; Sterner, 1989; Horne & Goldman, 1994).

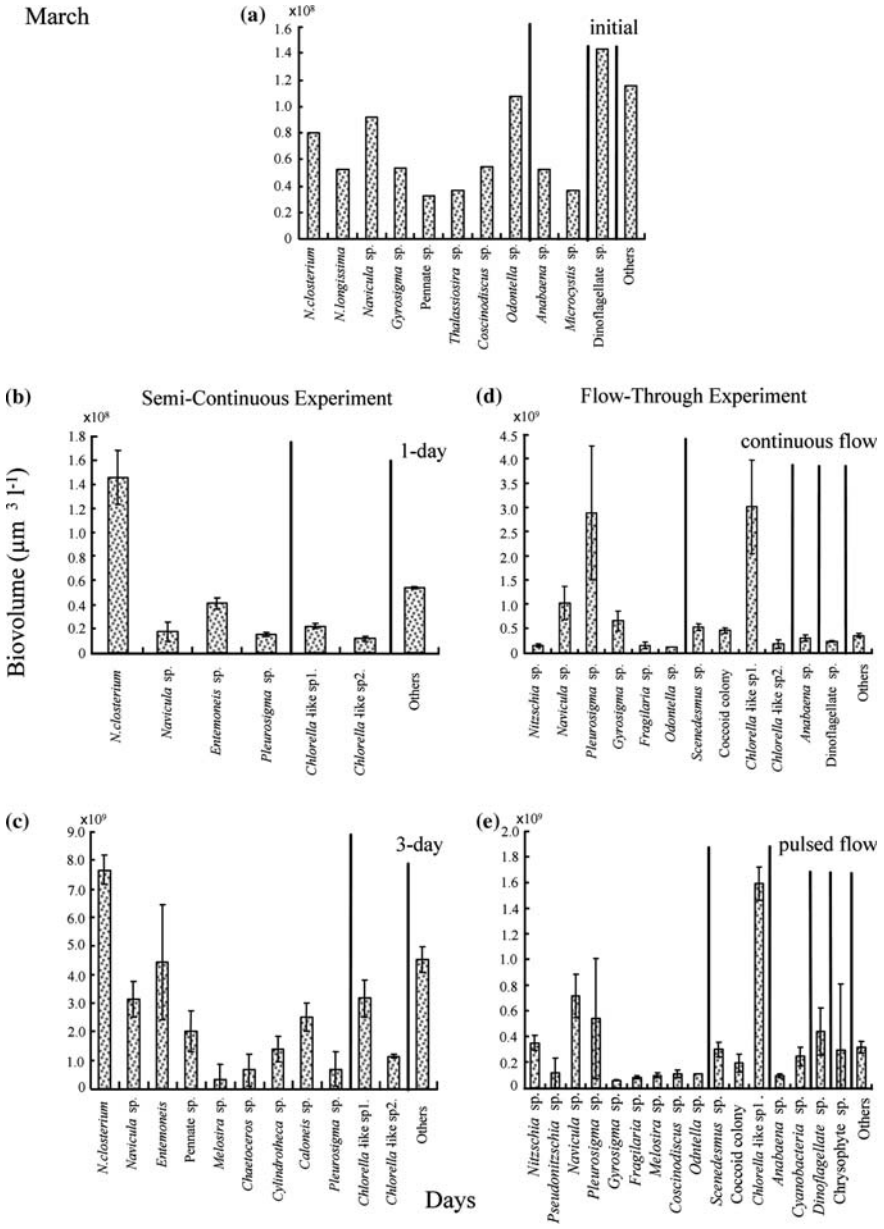

Figure 4. Initial (a) and final phytoplankton community structure in semi-continuous (b and c) and flow-through (d and e) experiments conducted in March. The initial community composition, dominated by a mix of diatoms, with some cyanobacteria and dinoflagellates, gave way to a community dominated by *N. closterium* at the conclusion of the 1 day pulsed semi-continuous experiment, and a more diverse diatom assemblage at the end of the 3 day pulsed treatment. At the conclusion of the flow-through experiment, this initial assemblage gave way to a community rich in diatoms and a coccoid green alga in both continuous flow and 3 day pulsed treatments. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

Despite major differences in phytoplankton and zooplankton community structure between the September experiment and the March and June experiments of semi-continuous design, predictions of the model (Roelke, 2000) were still observed, i.e., zooplankton populations were higher, phytoplankton biovolume was lower, and phytoplankton species diversity was higher under

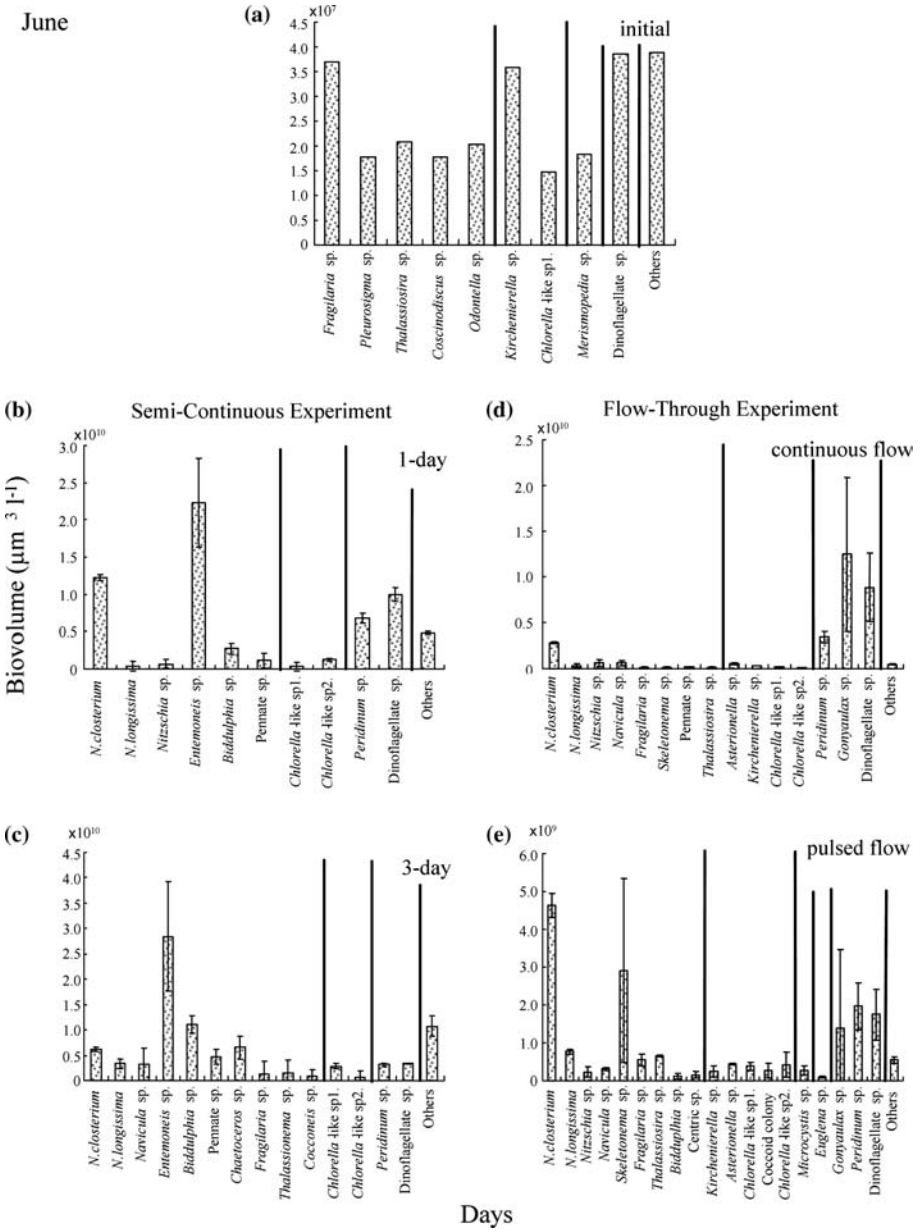

Figure 5. Initial (a) and final phytoplankton community structure in semi-continuous (b and c) and flow-through (d and e) experiments conducted in June. The initial assemblage, co-dominated by many phytoplankton forms, gave way to an assemblage dominated by the diatom *Entemoneis* sp. in both treatments of the semi-continuous experiments, and an assemblage dominated by dinoflagellates in both treatments of the flow-through experiments, with *N. closterium* co-dominating in the pulsed treatment. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

conditions of 3 day pulsed inflows. The flow-through experiments conducted in September also showed greater accumulation of zooplankton biomass in treatments receiving pulsed inflows, but the mode of inflow did not influence phytoplankton biomass or diversity. In this experiment, it was determined using numerical models that the phytoplankton community might have been structured in such a way as to allow complex behavior to arise in the chambers

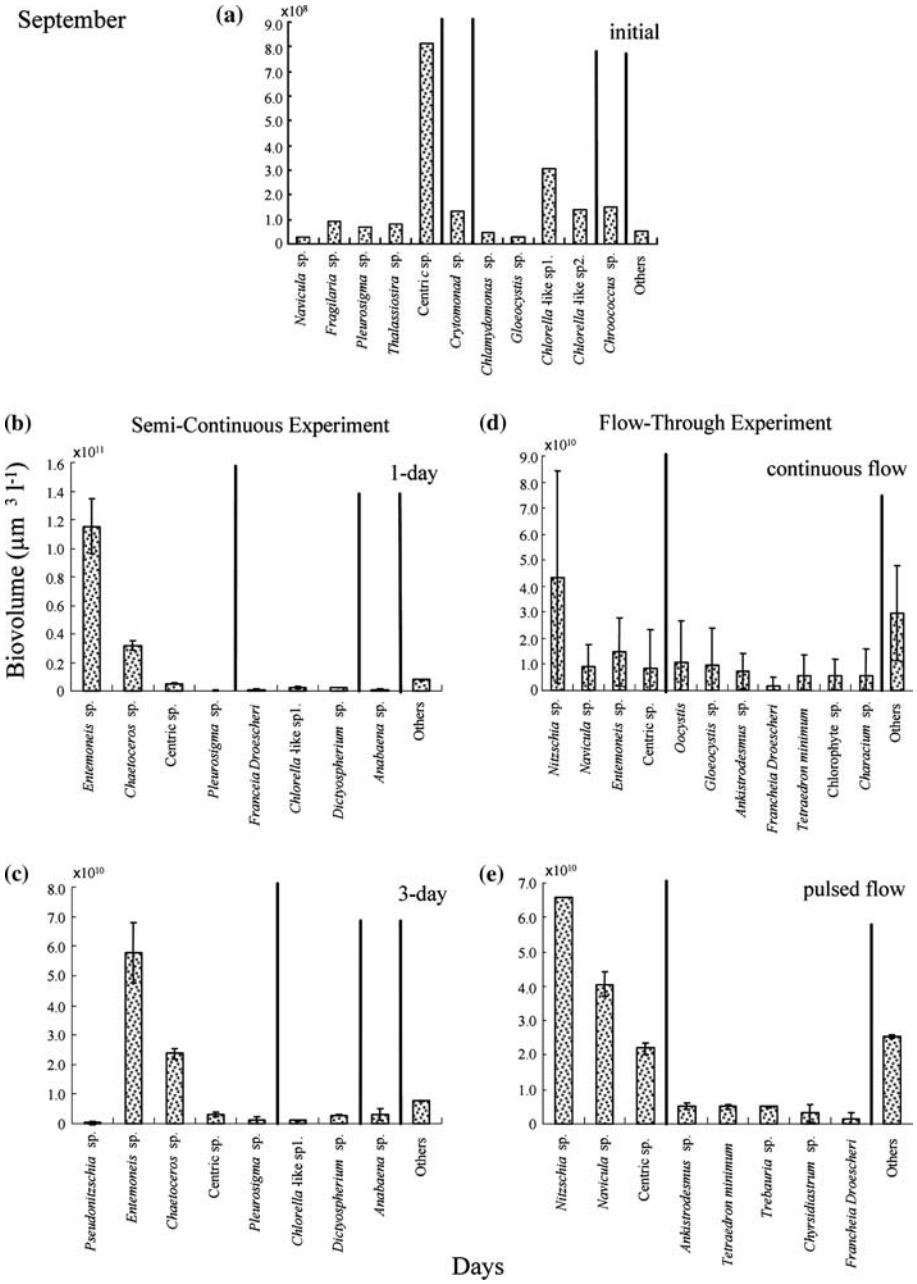

Figure 6. Initial (a) and final phytoplankton community structure in semi-continuous (b and c) and flow-through (d and e) experiments conducted in September. Centric diatoms dominated the initial community composition, and this community gave way to an assemblage dominated by *Entemoneis* sp. in both treatments of the semi-continuous experiments, and *Nitzschia* sp. in both treatments of the flow-through experiments. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

receiving continuous inflow, i.e., low disturbances, while complex behavior was suppressed in chambers receiving pulsed inflows, i.e., large disturbances (see Roelke et al., 2003). This might be a natural phenomena that sometimes occurs in mixed and diverse assemblages, such as phytoplankton communities (see Huisman et al. 2001; Schippers et al., 2001).

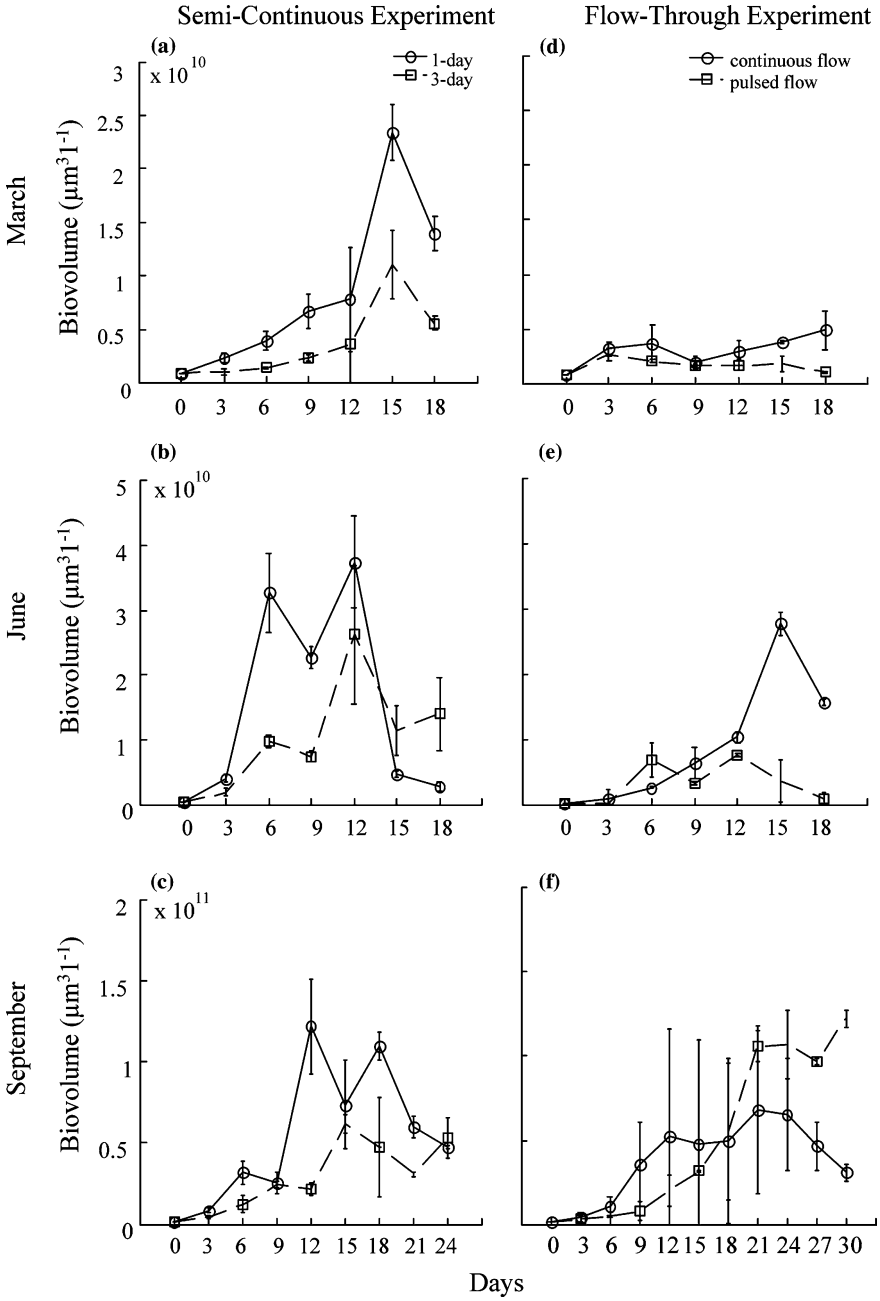

Figure 7. Accumulation of total phytoplankton biovolume in semi-continuous (a–c) and flow-through (d–f) experiments conducted in March, June and September. In most cases, 1 day pulsed and continuous inflows resulted in higher accumulated biovolume when compared to 3 day pulsed inflows. Symbols and error bars indicate the mean ± 1 SD from triplicate chambers.

Our experimental design did not allow for determination of the mechanisms underlying our observations. Nevertheless, we offer some thoughts regarding likely factors. The greater phytoplankton species diversity that occurred in most of our experiments receiving pulsed inflows might have been a result of the fluctuating physicochemical conditions, and the better performance by zooplankton. Under fluctuating conditions competitive exclusion processes are minimized,

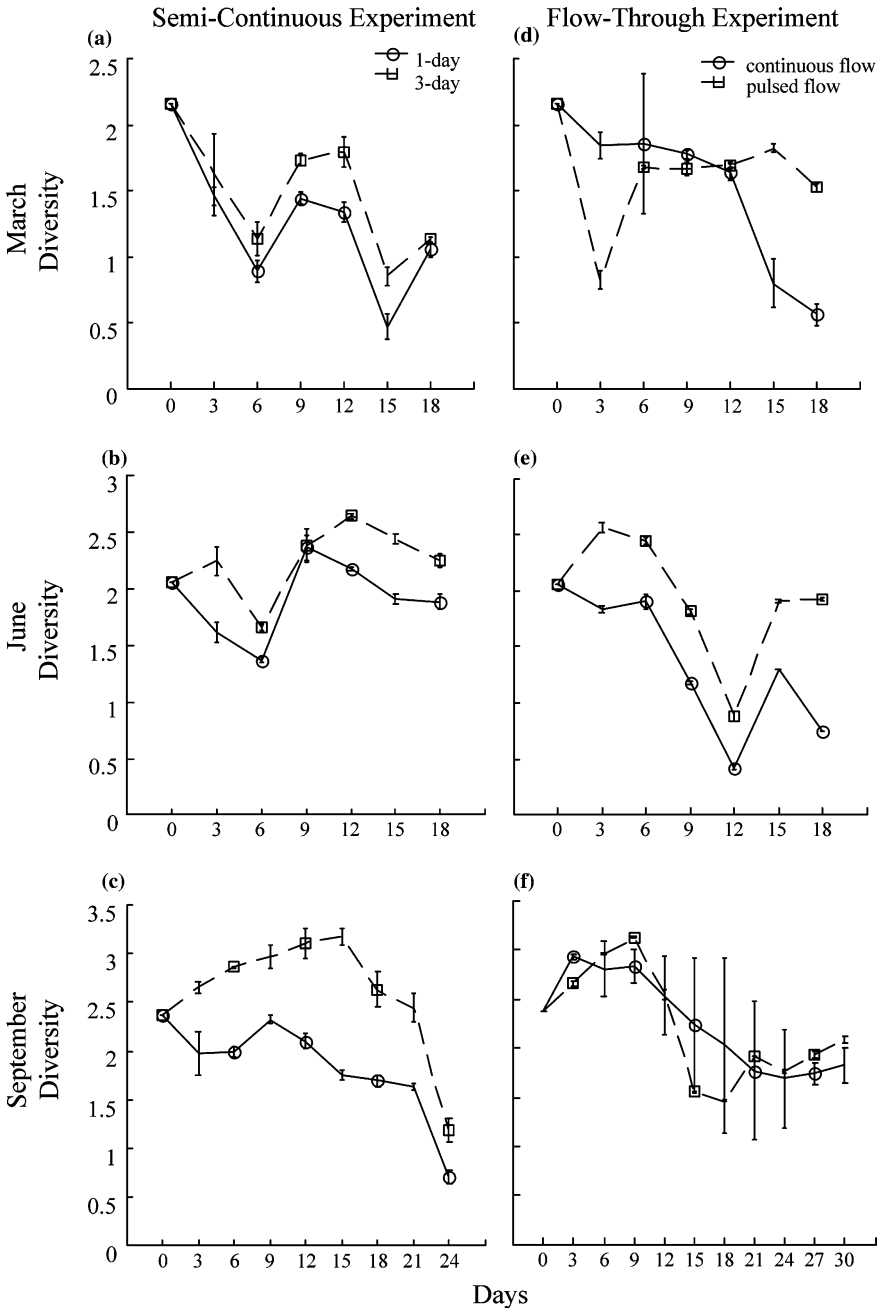

Figure 8. Phytoplankton species diversity in semi-continuous (a–c) and flow-through (d–f) experiments conducted in March, June and September. In most cases, 1 day pulsed and continuous inflows resulted in lower diversity when compared to 3 day pulsed inflows. Symbols and error bars indicate the mean ±1 SD from triplicate chambers.

thereby maintaining diversity (Hutchinson, 1961; Sommer et al., 1986, 1993). Grazing by zooplankton on the most abundant phytoplankton species would also prevent exclusion of slower-growing, less-abundant species (Sommer et al., 1986; Gismervik & Andersen, 1997; Sommer & Stibor, 2002), thereby helping to maintain diversity.

In conclusion, our findings from microcosm experiments indicated that pulsed inflows might alter plankton dynamics in such a way as to stimulate energy transfer up the food web, i.e., through greater zooplankton accumulation of biomass, and prevent excessive accumulation of phytoplankton biomass and maintain phytoplankton diversity. Our experiments did not include predators of zooplankton, as well as many other components of a natural system. Consequently, our findings cannot be scaled-up to the ecosystem-level. In-field mesocosm experiments, where the complexity of the natural environment is better represented, are needed to verify our findings.

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