Turbidity triggers larval release by the intertidal barnacle *Semibalanus balanoides*

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ABSTRACT:

Gravid adults of the common intertidal barnacle *Semibalanus balanoides* (L.) brood fully developed larvae until individuals perceive some cue from the environment that triggers synchronous larval release. The prevailing hypothesis has been that phytoplankton blooms trigger release because they provide a food source for nauplius larvae. Through observations and field experiments, we tested the hypothesis that turbidity from any source, not just phytoplankton blooms, can trigger release. We documented five larval release events at three sites in the northeastern United States. Two events coincided with chlorophyll increases, and all five coincided with turbidity increases. In experiments, the larval release response was equivalent when adults were exposed to diatoms or inert synthetic beads, and it was significantly higher than under exposure to filtered seawater. We also tested the hypothesis that turbidity can decrease the risk of cannibalism for newly-released nauplii. Under experimentally manipulated conditions, adults consumed significantly fewer nauplii in a high-turbidity environment. We suggest that reproduction in this species may have evolved to coincide roughly with the local onset of winter/spring phytoplankton blooms, but the timing of larval release may have been fine-tuned further

by cannibalism and predation pressures. The potential for turbid conditions to serve as a refuge for planktonic larvae of other marine organisms merits further investigation.

KEY WORDS:
Synchrony · Turbidity · Reproduction · Larvae · Cannibalism · Barnacles

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INTRODUCTION

The plankton community in coastal waters of the temperate North Atlantic Ocean changes considerably with the seasons. In late winter or early spring, dense diatom blooms appear, and they are often followed shortly after by pelagic nauplius larvae of the common and widespread intertidal barnacle *Semibalanus balanoides* (Fish 1925). In some areas, *S. balanoides* larvae account for up to 15% of zooplankton individuals (Frolander 1955), but remain in the water column for only 3-6 weeks (Barnes and Barnes 1958). Nauplii feed on phytoplankton and are themselves prey for carnivorous zooplankton and planktivorous fish (Lockhead 1936; Bousfield 1955). Therefore, the timing of *S. balanoides* larval release relative to the seasonal population dynamics of other species could have an important effect on coastal marine food webs.

Gravid *Semibalanus balanoides* adults brood their larvae for days to months after the developmental sequence is complete until individuals encounter environmental conditions that prompt larval release in mass synchrony (Moore 1935; Barnes 1962). The synchronous release of nauplii often coincides with phytoplankton blooms (Barnes 1956, 1957, 1962), presumably to ensure a plentiful food supply for the larvae. However, in the field, larvae are sometimes released in the absence of diatom blooms (Barnes 1962), and adults in the laboratory often release when exposed to high concentrations of many kinds of plankton, including brine shrimp nauplii (Starr et al. 1991). Additionally, we (Gyory and Pineda 2011) found that the abundance of first-stage nauplii was strongly correlated with the passage of storms that increased water turbidity. We therefore suggested that larval release may be triggered by high turbidity (caused by phytoplankton blooms or other sources) because the weakly-swimming, newly-released larvae are better protected from cannibalism when the filter-feeding appendages of adults are temporally clogged by particles; the "turbidity hypothesis."

In the present study, we tested three predictions of the turbidity hypothesis: (1) Larval release in the field should coincide with periods of high phytoplankton abundance or high turbidity from other sources. (2) Adult barnacles should release larvae when exposed to high concentrations of phytoplankton or inert synthetic beads. (3) High turbidity should decrease the rate of cannibalism on newly-released barnacle larvae.

MATERIAL AND METHODS

Field observations of larval release patterns

We tracked the larval release patterns of barnacles at three sites along the northeastern coast of the United States to determine whether release was related to changes in turbidity, chlorophyll concentration, or various abiotic variables (water temperature, salinity, or depth). The three field sites were (1) a dock in Little Harbor, Woods Hole, Massachusetts (41° 31.366’ N, 70° 40.008’ W); (2) the University of Rhode Island pier in Narragansett, Rhode Island (41° 29.524’ N, 71° 25.145’ W); and (3) the University of New Hampshire pier in New Castle, New Hampshire (43° 04.316’ N, 70° 42.707’ W) (Fig. 1). Larval release of *Semibalanus balanoides* is known to occur sequentially, in this order, in these three regions (Fish 1925; Pineda et al. unpub.). All sites had an abundance of *S. balanoides* adults distributed vertically in the intertidal zone from approximately high water to low water spring tide levels, which is the usual range for this species (Stubbings 1975).

From November 21, 2009 to February 25, 2010, we sampled barnacle adults to determine what proportion of the population was gravid and what proportion had empty mantle cavities. When a *Semibalanus balanoides* individual releases its larvae, all larvae leave the mantle cavity, usually in 24 hours or less (Barnes 1955). Thus, a rapid increase in the proportion of adults with empty mantle cavities signaled a larval release event. We randomly sampled at least 31 adult barnacles (mean = 60, SD = 19) daily whenever possible. On a few occasions, severe weather impeded sampling efforts.

At the three field sites, we measured water salinity, temperature, depth, turbidity, and chlorophyll fluorescence. A logger (model XR-420, RBR Ltd., Ottawa, Ontario, Canada) recorded temperature and salinity every five minutes. A fluorometer (dual-

wavelength, single-angle sensor) measured turbidity and *in vivo* chlorophyll fluorescence simultaneously (model *ECO* FLNTU, WET Labs, Philomath, Oregon, USA). The instrument took a "burst" of measurements (one per second for five seconds) every five minutes. In Massachusetts and Rhode Island, we strapped the instruments to pier pilings 0.5 m above bottom. The water depth was 1.5-2 m during the highest tides. In New Hampshire, it was not possible to strap the instruments to pier pilings, so the instruments were attached to a floating dock instead, where they remained 0.5 m below the surface at all times. We obtained tide and water level data from the United States National Oceanic and Atmospheric Administration (station ID numbers: 8447930, 8452660, and 8423898).

Instrumentation problems at the Massachusetts site caused loss of salinity data and required that we eliminate some bad values from the turbidity and chlorophyll data. A piece of macroalga wrapped itself around the *ECO* fluorometer, and every time the blades of the alga swept past the sensors, the readings were unrealistically high. We removed the bad values from the chlorophyll and turbidity data (in Massachusetts only) as follows: (1) Since the instrument sampled once per second for 5 seconds every 5 minutes, we computed the median for each 5-second sampling burst. This eliminated bad data in situations when only some of the values in the sampling burst were affected by the alga. (2) When all five values in a sampling burst were bad, we divided the sampling period into 2-hour bins and calculated the mean and standard deviation of the values in the bins. If the standard deviation of the mean was equal to or greater than half of the mean, we eliminated the highest 1/3 of the values from the 2-hour bin. (3) We calculated the median values for each 1-hour bin, and those are the values used in the analyses (see

Electronic Supplement 1 for figures of filtered and un-filtered data). After these corrections, the effective sampling rate for the instrument became 1 hr^{-1}.

Larval release in response to phytoplankton or turbidity

We conducted experiments to test whether the larval release response was different when gravid adult barnacles were exposed to unfiltered seawater, seawater with diatoms added, or seawater with particles added. The diatom was *Skeletonema marinoi* Sarno et Zingone (strain CCMP 1332 from the National Marine Phytoplankton Collection [NMPC] at Bigelow Laboratory for Ocean Sciences) added at 10^7 cells L^{-1}. Although many previous studies on barnacle feeding reported using the diatom *Skeletonema costatum* (Greville) Cleve, a recent study discovered that *S. costatum* is actually a species complex made up of previously unrecognized species, including *S. marinoi* (Sarno et al. 2005). The strain we used from the NMPC had been identified initially as *S. costatum* when it was collected in 1956, but has been re-classified since then. It has a cell length of 6-14 μm, cell width of 6-8 μm, and forms chains of 2-45 cells. The particles we used were neutrally-buoyant Dynoseeds® 40-μm polystyrene beads (Microbeads AS, Skedsmokorset, Norway) added at 10^7 beads L^{-1}.

At each field site during low tide, we gathered barnacle-covered rocks that were small enough to fit inside a one-liter clear plastic jar. We placed one rock inside each jar and immediately filled it with one of the three treatments listed above. After sealing the jars with lids, we placed them inside plastic cages that floated at the water surface and were tethered to the sampling dock. We assume that the floating cages maintained the

jars at ambient water temperature and ambient light levels, and the slight to moderate wave action around the docks kept the phytoplankton and beads suspended inside the jars. After 24 hours, we recovered the jars, filtered the water through 100 µm mesh, and counted the number of nauplii swimming in the water and the number of adults on each rock. We ran experiments twice in Rhode Island and twice in New Hampshire. Experiments contained multiple replicates of each treatment (Table 1).

The statistical analysis for these experiments tested the null hypothesis that there is no difference in the larval release response of adults when exposed to beads, *Skeletonema marinoi*, or unfiltered seawater, versus the alternative hypothesis that there is a difference among the three treatments. There were two complicating factors. First, not all the adults were gravid at the beginning of the experiments, and it was impossible to determine how many were gravid without sacrificing the animals. Second, the number of nauplii produced can be highly variable among individuals. To address these uncertainties, we developed a statistical model relating the observed number of nauplii in each jar to the unknown number of gravid adults and the distribution of the number of nauplii released by each of them. Based on this model, we performed a likelihood ratio test of the null hypothesis that the conditional mean number of nauplii released by an adult was the same for the three experimental treatments (see Electronic Supplement 2 for details). We repeated the entire analysis while omitting the outlier from the *S. marinoi* treatment (see Results) because the outlier could have undue influence on the results.

We considered the possibility that the number of nauplii that we found in the jars at the end of each experiment could differ among treatments if the rates of cannibalism by adults were also different among treatments. Thus, in addition to comparing the

number of nauplii in the jars, we also examined the percentage of treatment replicates in which adults were inferred to have participated in "mass release" (defined as 95% or more of adults in a jar with empty mantle cavities). Mass release could only be inferred, not verified, because it was impossible to determine whether adults were gravid at the beginning of the experiment without sacrificing them.

Predation rate of adults on newly released larvae under normal or turbid conditions

We tested the null hypothesis that turbidity would not affect the rate of cannibalism on newly-released barnacle larvae by exposing adults either to larvae and synthetic beads or to larvae alone. The experiment took place in New Hampshire on February 19, 2010. By this date, most (>75%) of the barnacles we sampled in the field had already released their larvae, so we assumed that the adults in the experiments also had released their larvae.

The experiment consisted of 5 jars with a control treatment (seawater filtered through 100 µm mesh) and 5 jars with an experimental treatment (filtered seawater with 40 µm Dynoseeds® added at 10^7 beads L^{-1}). Each 1-L jar contained one rock covered with barnacle adults. The number of adults in a jar was random, and not significantly different among treatments. We added at least 250 live, actively swimming nauplii to each jar, noting the exact number used. To obtain the nauplii, we scraped adults off pier pilings along the uppermost limit of the barnacle colonies. A few (<25%) of these adults still had viable eggs inside their mantle cavities. We collected the eggs from 10 individuals and placed them in seawater (pre-filtered through 100 µm mesh). The eggs

hatched within minutes, and nauplii swam to the surface. Using a glass pipette, we suctioned actively swimming larvae and added them to the experimental jars. We sealed the jars with lids, placed them in the plastic cages described above, and hung the cages off the pier so that the jars were submerged in seawater.

The experiment began at 14:00 and ended at 20:00. At the end of the experiment, we filtered water from each jar through 100 µm mesh and counted the number of nauplii that remained. We calculated the percentage of nauplii that survived in each jar without being consumed and performed a one-way ANOVA to detect any differences in the means for the two treatments.

RESULTS

Field observations of larval release

There were five major larval release events at the three sites. Two of the events coincided with higher chlorophyll levels, but all five coincided with higher turbidity levels. Two release events occurred in Massachusetts, one between December 12 and December 14, and the other between December 18 and December 22 (Fig. 2). On December 8, the increase in percent of empty adults might suggest that there was a release event, but that is unlikely because the next three samples had a lower percentage of empty adults. These barnacles reproduce once per year (Barnes 1963), so it is not possible for them to release larvae and become gravid again a few days later. Similarly, the decrease in percentage of empty adults on December 17 and 24 is likely due to

209 sample variability. The percentage of adult barnacles that were brooding viable larvae
210 generally increased until reaching a maximum on December 17. The two decreases in this
211 percentage coincided with the two larval release events.

212 Chlorophyll concentration fluctuated between approximately 0.6 and 3 μg L^{-1} in
213 Massachusetts (Fig. 2). A short-lived, modest increase in chlorophyll concentration
214 occurred during the second larval release event, but not during the first. Turbidity ranged
215 from approximately 0.8 to 6.5 Nephelometric Turbidity Units (NTU). NTUs measure the
216 amount of light scattered by particles. A high-turbidity event was ending when the
217 instrument was placed in the water, and another event followed it the next day. These two
218 events coincided with the first larval release event. A second high-turbidity event
219 coincided with the second larval release event. Water level relative to mean lower low
220 water fluctuated between -0.1 and 1.4 m. Water temperature declined steadily from 11° to
221 1.5° C.

222 In Rhode Island, major larval release events occurred between January 9 and
223 January 10 and between January 11 and January 13 (Fig. 3). During the first release, there
224 was an increase in turbidity, but no noticeable increase in chlorophyll. During the second
225 release, there was one high-chlorophyll event and two high-turbidity events. The
226 percentage of adult barnacles brooding viable larvae decreased during the larval release
227 events. Water level fluctuated between -0.5 and 1.7 m. Salinity and water temperature
228 fluctuated with a semi-diurnal period, so they were probably tidally influenced. Salinity
229 ranged from 30.4 to 31.8 psu. Water temperature ranged from 1.4° to 3.9° C. During the
230 evening of January 12, an extreme low tide caused the instruments to be briefly exposed

to air, so chlorophyll, turbidity, salinity, and water temperature data are missing for that period.

In New Hampshire, larval release occurred between February 12 and 15 (Fig. 4). The percentage of adult barnacles brooding viable larvae increased until it reached a maximum on January 29. The percentage remained high until the larval release event began on February 12. Chlorophyll values were generally low. Turbidity was generally higher near the beginning of the sampling period and then decreased, but there was an increase at high tide during the larval release period. Turbidity, salinity, and water temperature fluctuated semi-diurnally with the tides. Water level ranged from -0.7 to 3.5 m. Salinity ranged from 22.7 to 31.7 psu. Water temperature ranged from 0.7° to 4.2° C. In general, salinity and temperature increased and decreased as the tide flowed and ebbed, respectively.

Larval release in response to phytoplankton or turbidity

Larval release response was significantly stronger (Likelihood Ratio [LR] test, $p << 0.001$) in the phytoplankton and turbidity treatments than in the control treatments, even when the outlier in the *Skeletonema marinoi* treatment was removed (LR test, $p << 0.001$). The difference in larval release response between the phytoplankton and turbidity treatments was not significantly different (LR test, $p \approx 1$) (Fig. 5).

The statistical model estimates of π (the probability that an adult is gravid and receptive to a larval release cue) are shown as percentages in Table 2. The model estimates of θ (the unknown shape parameter of the negative binomial distribution), along

with the estimated mean number of nauplii released by each gravid adult, are shown in Table 3.

Twenty-seven percent of replicates for the control treatment had mass larval release. In contrast, forty-six percent and fifty-four percent of bead and diatom replicates, respectively, had mass release (Fig. 6).

Predation rate of adults on newly released larvae under normal or turbid conditions

The mean percentage of nauplii that escaped predation in the turbidity treatment (85.4) was significantly greater (ANOVA, $p = 0.015$) than in the control treatment (64.7) (Fig. 7)

DISCUSSION

Gravid *Semibalanus balanoides* barnacles brood their larvae until they perceive some cue from the environment that triggers naupliar release. The generally accepted hypothesis has been that barnacles release their larvae in response to phytoplankton blooms because high concentrations of phytoplankton provide abundant food for nauplii. In contrast, Gyory and Pineda (2011) proposed that high turbidity (which can be caused by phytoplankton blooms, sediments re-suspended by storms, or other sources) triggers the release of larvae, since a highly turbid environment may protect poorly-swimming, newly-released larvae from cannibalism and predation. Our field observations and experiments tested the predictions that (1) high phytoplankton concentrations or (2) high

turbidity levels trigger larval release in *S. balanoides*. We found that larval release in the field and in controlled experiments could be triggered by high-turbidity events in the absence of phytoplankton blooms.

We observed five major larval release events at three sites along the northeastern coast of the United States. Two events coincided with increased chlorophyll concentrations, three did not, but all five events coincided with increased turbidity. Other authors have also noted that *Semibalanus balanoides* sometimes releases larvae in the field in the absence of phytoplankton blooms. Barnes (1962) identified 2 years (1950 and 1960) in which larval release in Millport, Scotland occurred in the absence of blooms. Another barnacle species, *Chamaesipho brunnea*, has been observed to release larvae under conditions when turbidity would be expected to be high. In New Zealand, they brood mature larvae during neap tides and calm weather, and release them during spring tides and stormy weather (Foster 1965 as cited in Luckens 1970).

In Massachusetts, the two larval release events coincided with an increase in turbidity. There was a small increase in chlorophyll during the second event, but not during the first. Since macroalgal interference with our instrument sensors required eliminating bad values from the data, it is possible that we failed to detect short-lived pulses in chlorophyll. This is unlikely, though, because we were able to detect short-lived pulses in turbidity after filtering the data, so we should have been able to do the same with chlorophyll. There is an increase in the percentage of adults with no embryos from 35% on December 18 to 51% on December 19 with seemingly no corresponding increase in chlorophyll or turbidity. The data filtering process may have obscured an increase in

one or both of these variables. In Rhode Island, there were increases in both turbidity and chlorophyll at the second larval release event, but not during the first.

In New Hampshire, there was an increase in turbidity at the time of larval release, but there was no major increase in chlorophyll. 92% of adults were brooding viable larvae during the highest turbidity event of the time series, on January 29. Why did the barnacles fail to release during the high-turbidity events at the end of January? We speculate that the extreme salinity fluctuations associated with the spring tide may have stressed the barnacles and caused them to close their opercular openings. Cawthorne and Davenport (1980) found that when gravid barnacles in the laboratory were exposed to large and rapid salinity fluctuations, they closed their opercular openings, halting larval release. Moreover, the peaks in turbidity in late January and early February occurred as the tide was ebbing, so a substantial portion of the adult population may have been out of the water and unable to release larvae. Finally, there is the possibility that another factor not taken into account here also affects larval release.

To examine the relationship between phytoplankton abundance and the timing of barnacle larval release, we used *in vivo* chlorophyll fluorescence to estimate chlorophyll-*a* concentrations, though this is known to be an imperfect method. The ratio of fluorescence to chlorophyll-*a* can vary depending on the species composition of the phytoplankton, the health of the cells, and the ambient light conditions (e.g., Loftus and Seliger 1975; Dandonneau and Neveaux 1997). In our data, we see decreases in fluorescence almost daily during the middle of the day. This is likely due to non-photochemical quenching. Non-photochemical quenching processes protect phytoplankton from photooxidative damage when light energy exceeds the capability of

322 the cell to utilize it (Müller et al. 2001). Quenching appears as a reduction in fluorescence
323 during periods of high light intensity. Thus, care must be taken in interpreting the data
324 from the brightest period of the day.

325 The results of laboratory and field experiments lend further support to the
326 hypothesis that turbidity triggers larval release. Starr et al. (1991) found that in the
327 laboratory, the larval release response is strongest when adult barnacles are fed
328 phytoplankton in concentrations 3-6 times greater than those found in typical blooms.
329 Barnacles in that study may not have been responding to the phytoplankton *per se*, but to
330 the mechanical stimulus from turbidity caused by high concentrations of phytoplankton
331 cells. This would explain why the barnacles did not respond to phytoplankton culture
332 filtrates, only to the presence of the cells themselves (Starr et al. 1991). The barnacles
333 also released when they were exposed to high concentrations of brine shrimp nauplii,
334 which are not a normal food item for them or their larvae in the field (Starr et al. 1991).

335 In the present study, we conducted field experiments to examine the larval release
336 response of gravid adults to *Skeletonema marinoi* diatoms and synthetic beads. The larval
337 release response was stronger when barnacles were exposed to the diatoms and beads
338 than when they were exposed to control conditions. The responses to diatoms and to
339 beads did not differ, suggesting that the barnacles respond to mechanical stimulation
340 from the particles, not to the identity of the particles.

341 Starr et al. (1991) suggested that particles in the water column might indicate that
342 a phytoplankton bloom is underway. Gyory and Pineda (2011) proposed that cannibalism
343 and predation may be an important source of mortality for newly-released larvae, and that
344 particles in the water column would signal turbid conditions that may provide a

345 temporary refuge for barnacle nauplii. *Semibalanus balanoides* will consume its own
346 nauplii in the laboratory (Crisp and Patel 1960), and the gut contents of other barnacle
347 species sometimes contain substantial numbers of conspecific larvae (Navarrete and
348 Wieters 2000). Because suspension-feeding barnacle adults tend to be found in high
349 abundance and high densities in the intertidal zone, larvae released into this environment
350 could be at risk for cannibalism. A highly turbid environment may reduce that risk by
351 temporarily swamping the filter-feeding appendages of adults with other particles. The
352 results of our predation experiments showed that *S. balanoides* adults consumed fewer
353 nauplii in turbid conditions than in control conditions, suggesting that mortality of larvae
354 is indeed lower when turbidity is high.

355 Our study provides a new explanation for the synchrony of larval release in the
356 barnacle *Semibalanus balanoides*. We show that high turbidity triggers release, whether
357 the source of turbidity is a phytoplankton bloom or not. However, it is possible that
358 phytoplankton blooms also play an important role in the timing of release. The timing of
359 reproduction in this species may have evolved so that larvae are developmentally ready to
360 be released by the onset of winter/spring phytoplankton blooms in order to maximize the
361 likelihood of a plentiful food supply, and the actual timing of larval release may have
362 been fine-tuned further by cannibalism and predation pressures. As seen in our data,
363 increases in phytoplankton abundance were often very brief, so the food limitation
364 hypothesis would imply that short-lived increases in food supply have a substantial
365 benefit on the growth or survival of larvae. Turbidity increases were also very brief, but
366 the potential benefit to larval survival (reduced risk of cannibalism) would only be
367 needed for a short period until nauplii dispersed away from the adult population. Other

368 crustaceans employ larval release strategies that reduce predation on newly-released
369 larvae (e.g., Morgan and Christy 1995). Releasing larvae during turbid conditions to
370 protect them from cannibalism or predation may be a strategy shared by other marine
371 organisms that release propagules into the water column.
372

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Figure 1. Sampling sites along the northeastern coast of the United States: 1) Woods Hole, Massachusetts; 2) Narragansett, Rhode Island; and 3) New Castle, New Hampshire. *Semibalanus balanoides* release larvae sequentially, in this order, at these three sites.

Table 1. Number of replicates for three treatments (*Skeletonema marinoi*, Beads, and Control) used in four experiments conducted in Rhode Island and New Hampshire.

Figure 2. Field surveys of the reproductive condition of adult *Semibalanus balanoides* barnacles in Massachusetts in relation to environmental variables. (a) Percentage of adult barnacles with no embryos, shown with standard error bars, suggests that there were two major larval release events, indicated by grey vertical shading. (b) Percentage of adult barnacles that were brooding viable larvae. (c) Chlorophyll concentration. (d) Turbidity. (e) Water level relative to mean lower low water. (f) Water temperature.

Figure 3. Field surveys of the reproductive condition of adult *Semibalanus balanoides* barnacles in Rhode Island in relation to environmental variables. (a) Percentage of adult barnacles with no embryos, shown with standard error bars, suggests that there were two major larval release events, indicated by grey vertical shading. (b) Percentage of adult barnacles that were brooding viable larvae. (c) Chlorophyll concentration. (d) Turbidity. (e) Water level relative to mean lower low water. (f) Salinity. (g) Water temperature.

Figure 4. Field surveys of the reproductive condition of adult *Semibalanus balanoides* barnacles in New Hampshire in relation to environmental variables. (a) Percentage of

adult barnacles with no embryos, shown with standard error bars, suggests that there was one major larval release event, indicated by grey vertical shading. (b) Percentage of adult barnacles that were brooding viable larvae. (c) Chlorophyll concentration. (d) Turbidity. (e) Water level relative to mean lower low water. (f) Salinity. (g) Water temperature. In (c), (d), (e), (f), and (g), the gray line indicates values when water level was below 1.5 m above MLLW, and the black line indicates values when it was above 1.5 m.

Figure 5. Pooled results of the Rhode Island and New Hampshire experiments in rank order. Each bar represents the number of nauplii released in each replicate jar at the end of experiments in which adult barnacles were exposed to *Skeletonema marinoi* diatoms, inert synthetic beads, or control treatment (plain filtered seawater). We rejected the null hypothesis that the larval release response was the same in all three treatments ($p << 0.001$). We cannot reject the null hypothesis that the larval release response was the same for the *S. marinoi* and bead treatments ($p \approx 1$).

Table 2. Probability, estimated by the statistical model, that an adult barnacle produced nauplii for each of the four experiments conducted in Rhode Island and New Hampshire.

Table 3. Estimates of θ (the unknown shape parameter of the negative binomial distribution) under the null hypothesis and under the three experimental treatments (including and excluding an outlier), and the estimated mean number of nauplii that each gravid barnacle adult released.

Figure 6. Pooled results of the Rhode Island and New Hampshire experiments in rank order. Each bar represents the percentage of adult barnacles that had not released larvae by the end of the experiment within a replicate jar. Replicate jars that had fewer than five percent of adults brooding larvae at the end of the experiment were considered to have undergone "mass release." Twenty-seven percent of replicates for the control treatment had mass release. In contrast, forty-six percent and fifty-four percent of bead and diatom replicates, respectively, had mass release.

Figure 7. Results of experiments in which adult barnacles were exposed to newly-released nauplii under high-turbidity (Experimental) or low-turbidity (Control) conditions. Predation rates by adult barnacles on nauplii were lower in high-turbidity than in low-turbidity conditions (ANOVA, $p = 0.015$). Triangles represent the means, boxes represent the median and standard error of the mean. Whiskers represent the minimum and maximum values. On average, 85.4% of nauplii in the turbidity treatment escaped predation, compared to 64.7% in the control treatment.

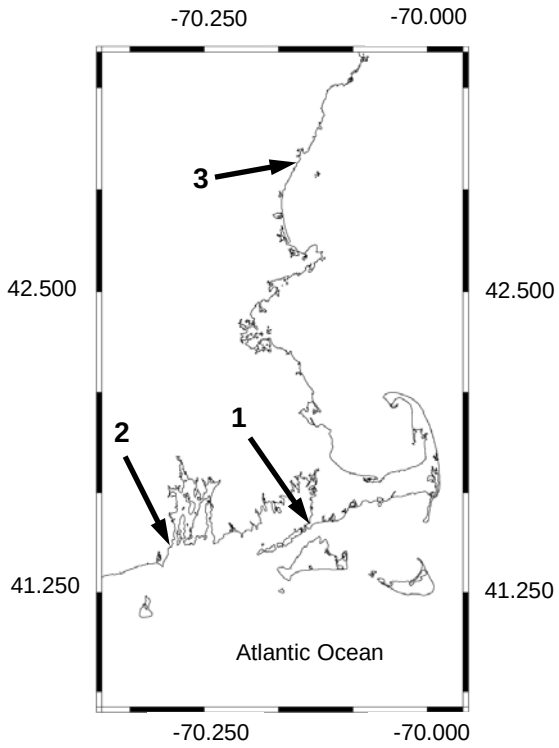
-70.250
-70.000
3
42.500
42.500
2
1
41.250
41.250
Atlantic Ocean
-70.250
-70.000

Figure 1

574

	Rhode Island	Rhode Island	New Hampshire	New Hampshire
	Jan 11 2010	Jan 13 2010	Jan 31 2010	Feb 02 2010
Skeletonema	4	3	5	5
Beads	4	3	5	5
Control	2	1	5	5

575

576 **Table 1**

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	Rhode Island	Rhode Island	New Hampshire	New Hampshire
	Jan 11 2010	Jan 13 2010	Jan 31 2010	Feb 02 2010
Probability under H_0	31.0%	3.2%	3.0%	4.1%
Probability under H_1	24.0%	4.5%	3.0%	4.7%
Probability under H_1 with outlier removed	18.0%	5.0%	3.0%	5.0%

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581 **Table 2**

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	Under H_0	Under H_1 Control	Under H_1 Beads	Under H_1 Diatoms
Estimate of θ	0.0018	0.038	0.005	0.001
Estimate of θ with outlier removed	0.006	0.042	0.0049	0.0051
Estimated mean number of nauplii released per gravid adult	165.67	22.81	203.08	195.08

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585 **Table 3**

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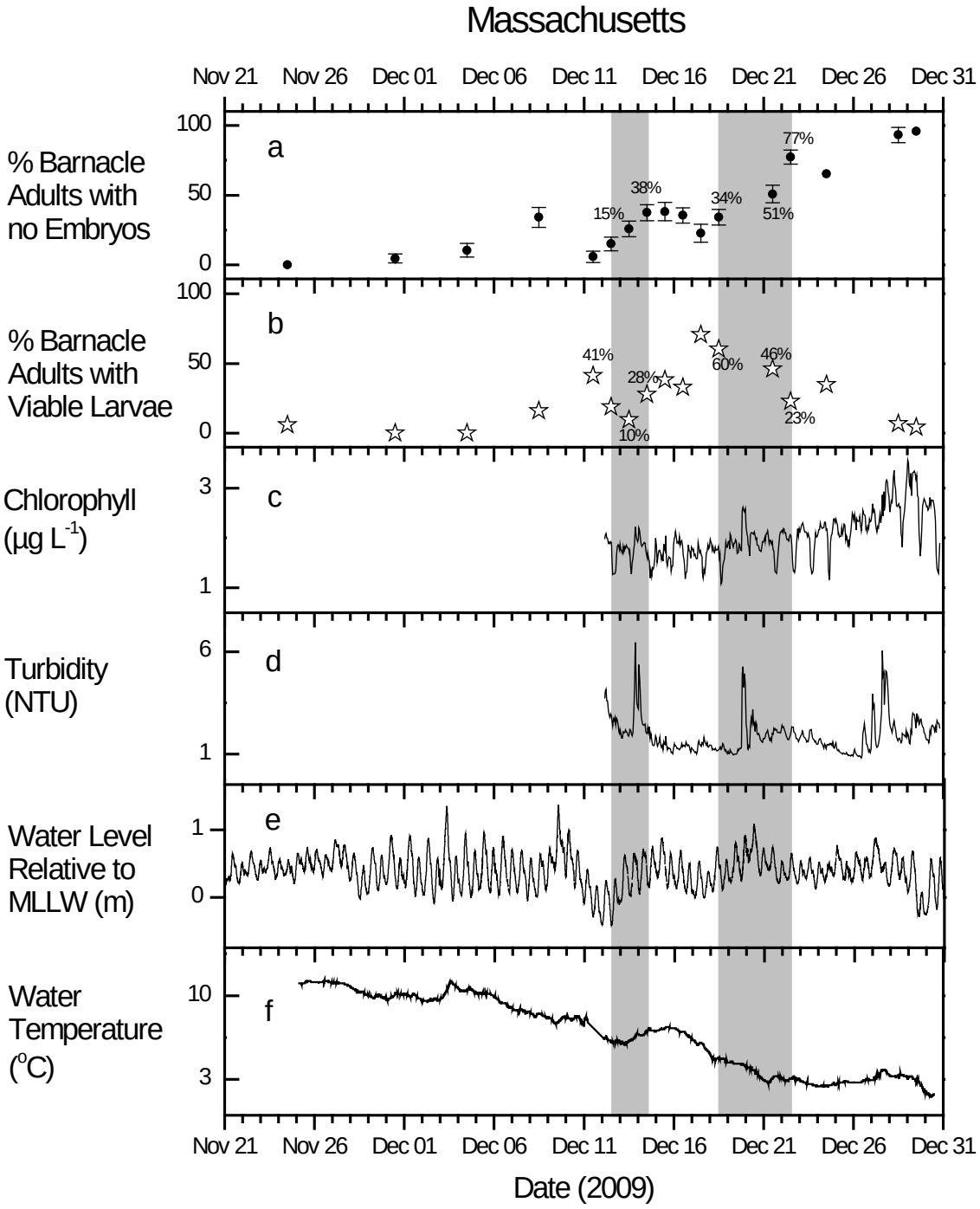

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589 **Figure 2**
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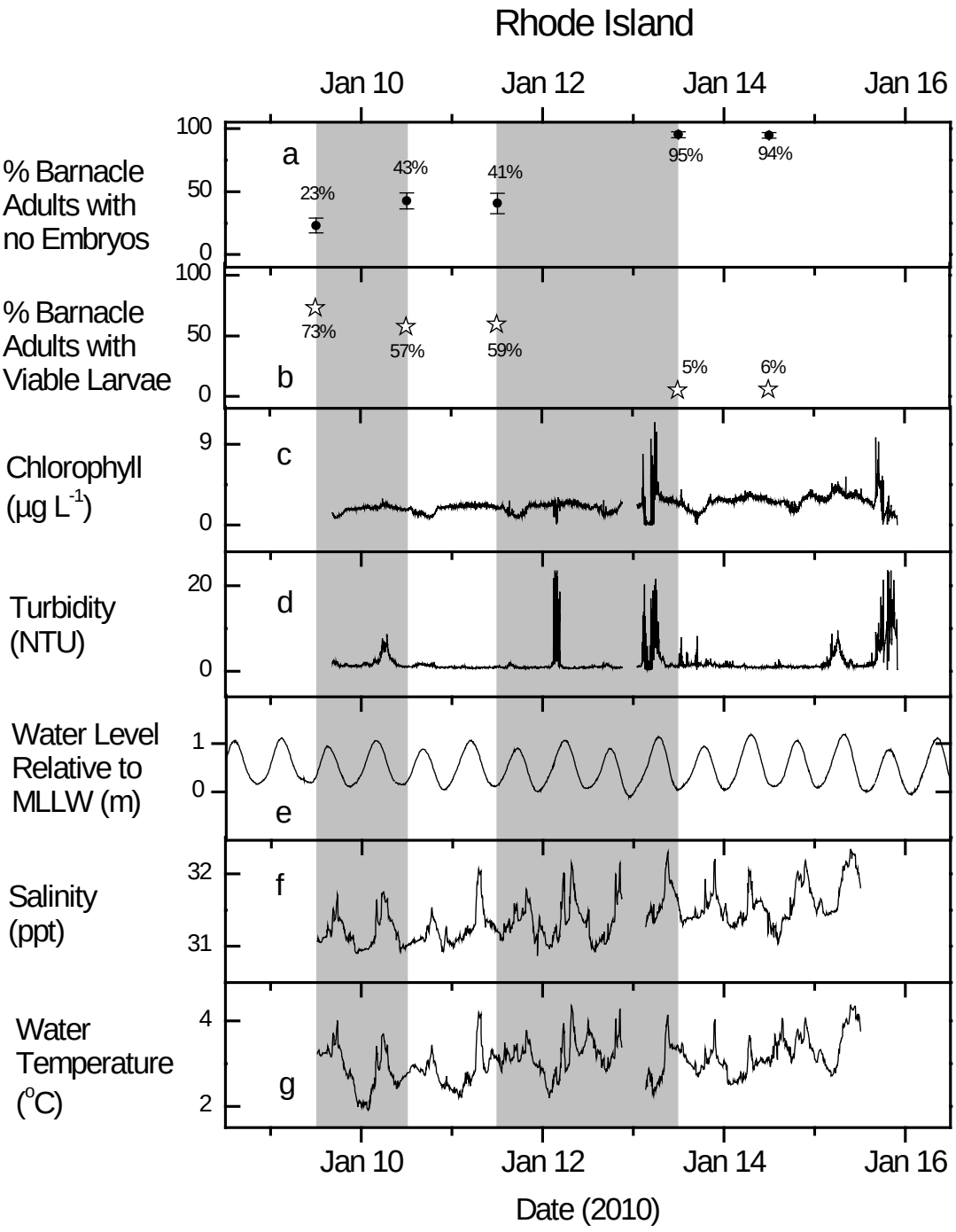

591
592
593 **Figure 3**
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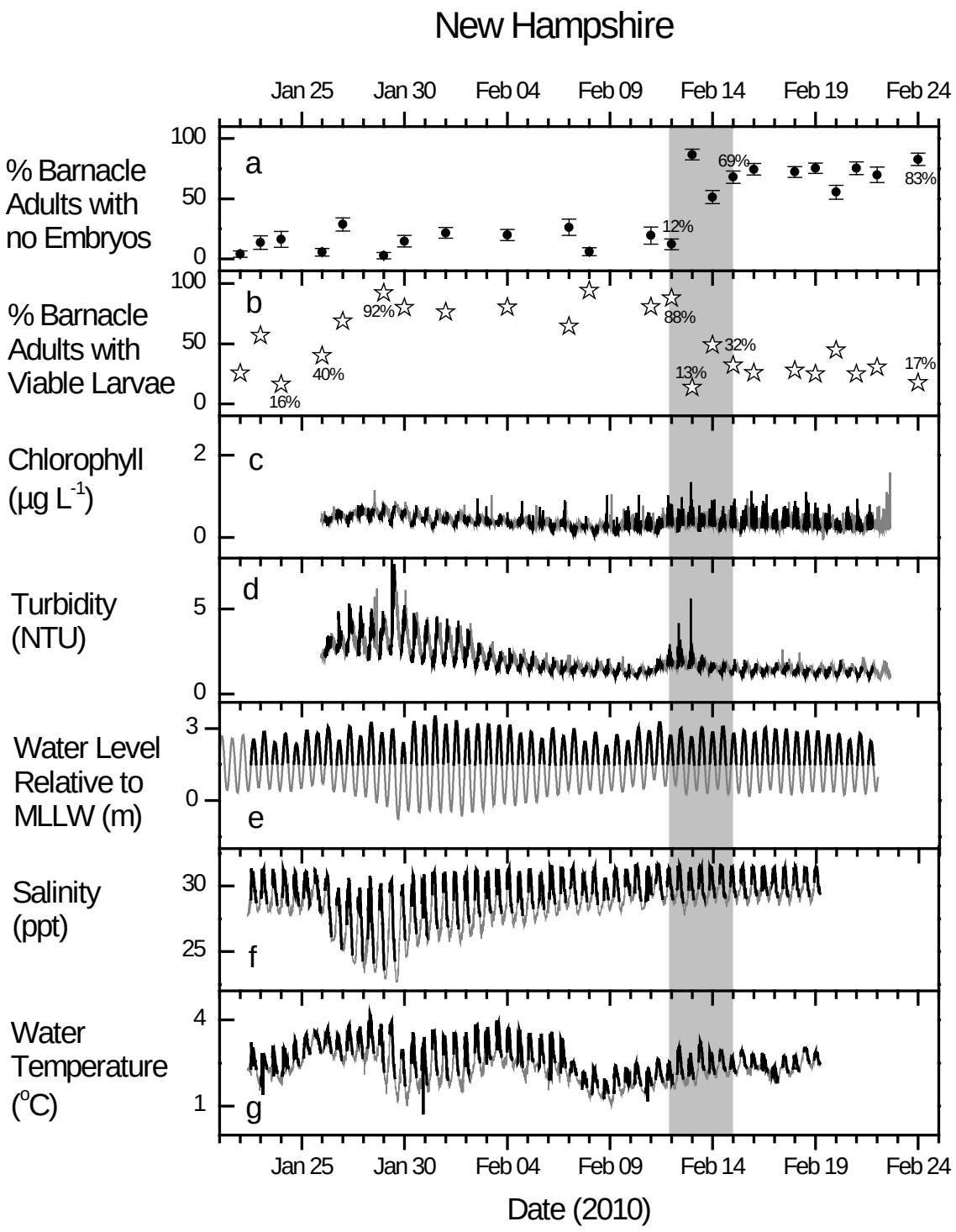

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598 **Figure 4**

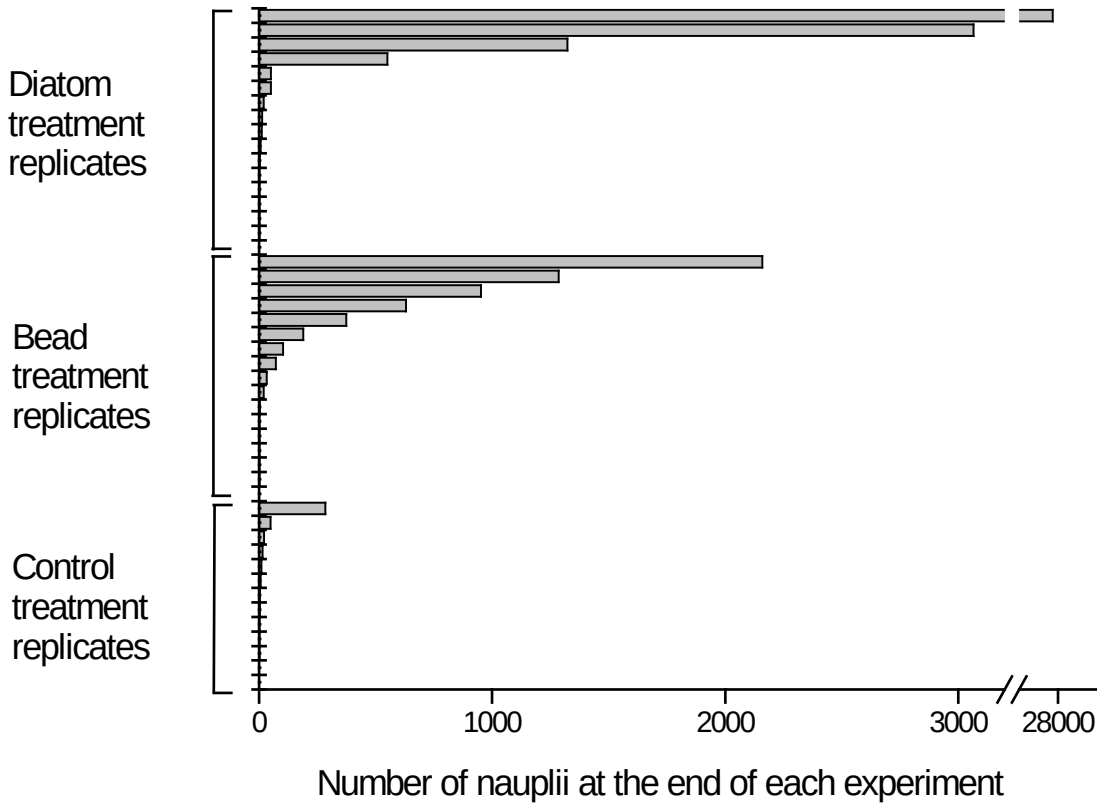

Diatom
treatment
replicates
Bead
treatment
replicates
Control
treatment
replicates
0
1000
2000
3000
28000
Number of nauplii at the end of each experiment

Figure 5

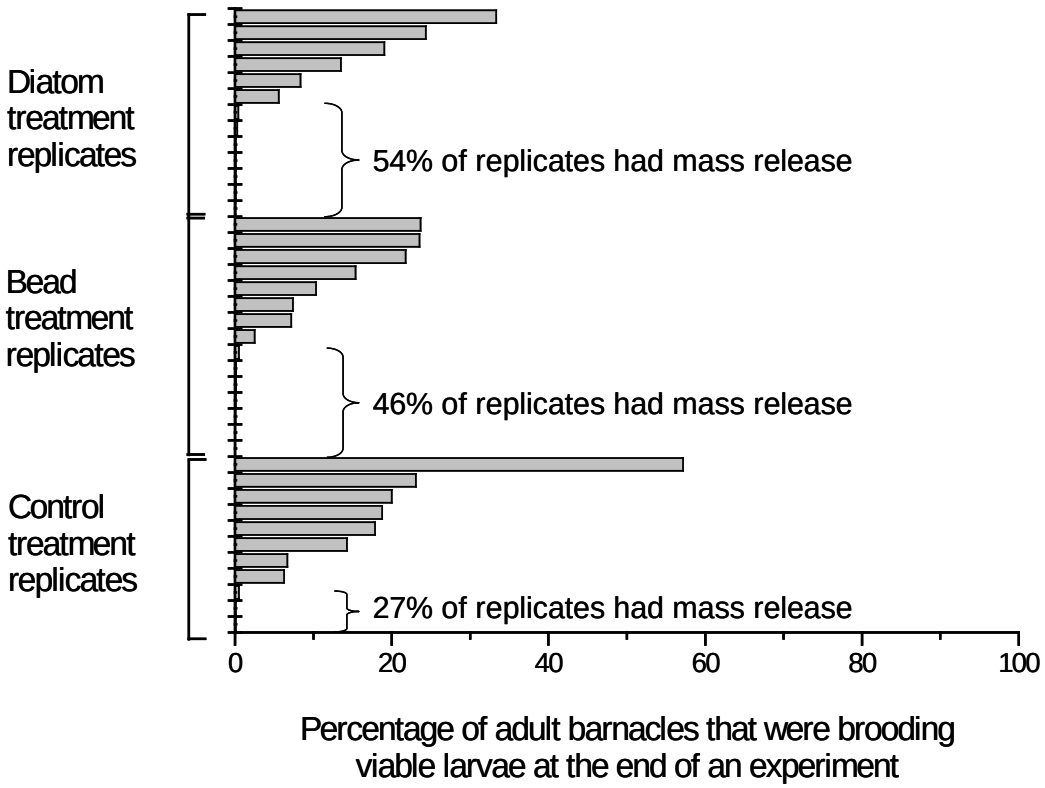
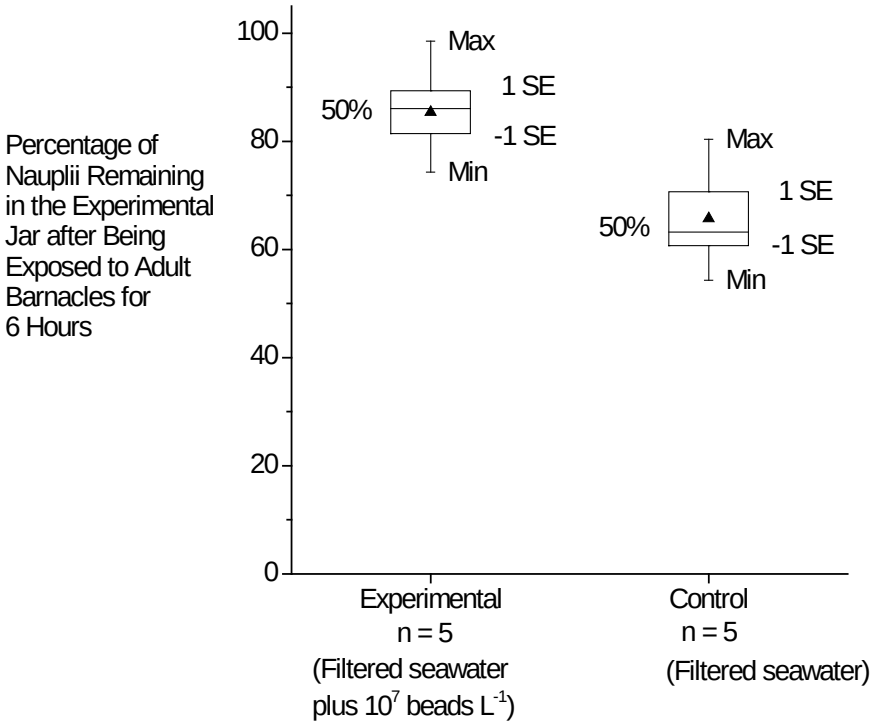

Diatom
treatment
replicates
54% of replicates had mass release
Bead
treatment
replicates
46% of replicates had mass release
Control
treatment
replicates
27% of replicates had mass release
0
20
40
60
80
100
Percentage of adult barnacles that were brooding
viable larvae at the end of an experiment

Figure 6



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614 **Figure 7**
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Electronic Supplement 1
Data filtering method for the Massachusetts site

The chlorophyll and turbidity raw data in Little Harbor, Massachusetts exhibited some unrealistically high values, probably caused by a piece of macroalga that wrapped itself around the instrument. Since the instrument sampled at a high frequency and not all of the data seemed to be contaminated, we devised an ad-hoc method for filtering out bad data. It proceeded in three steps:

1. The instrument sampled once per second for 5 seconds every 5 minutes, so we computed the median for each of the 5-second sampling bursts. This eliminated bad data in situations when only some of the values in the sampling burst were contaminated (Fig 1b, 2b).
2. To remove bad data in instances when the entire sampling burst was contaminated, we divided the sampling period into 2-hour bins, and we filtered the data in each bin as follows: we calculated the mean and standard deviation of the values in the 2-hour bin. If the standard deviation of the mean was equal to or greater than half of the mean, then we eliminated the highest 1/3 of values from the 2-hour bin (Fig 1c, 2c).
3. Finally, we calculated the median values for each 1-hour bin, and those are the values that we used in our analyses (Fig 1d, 2d, Fig 3).

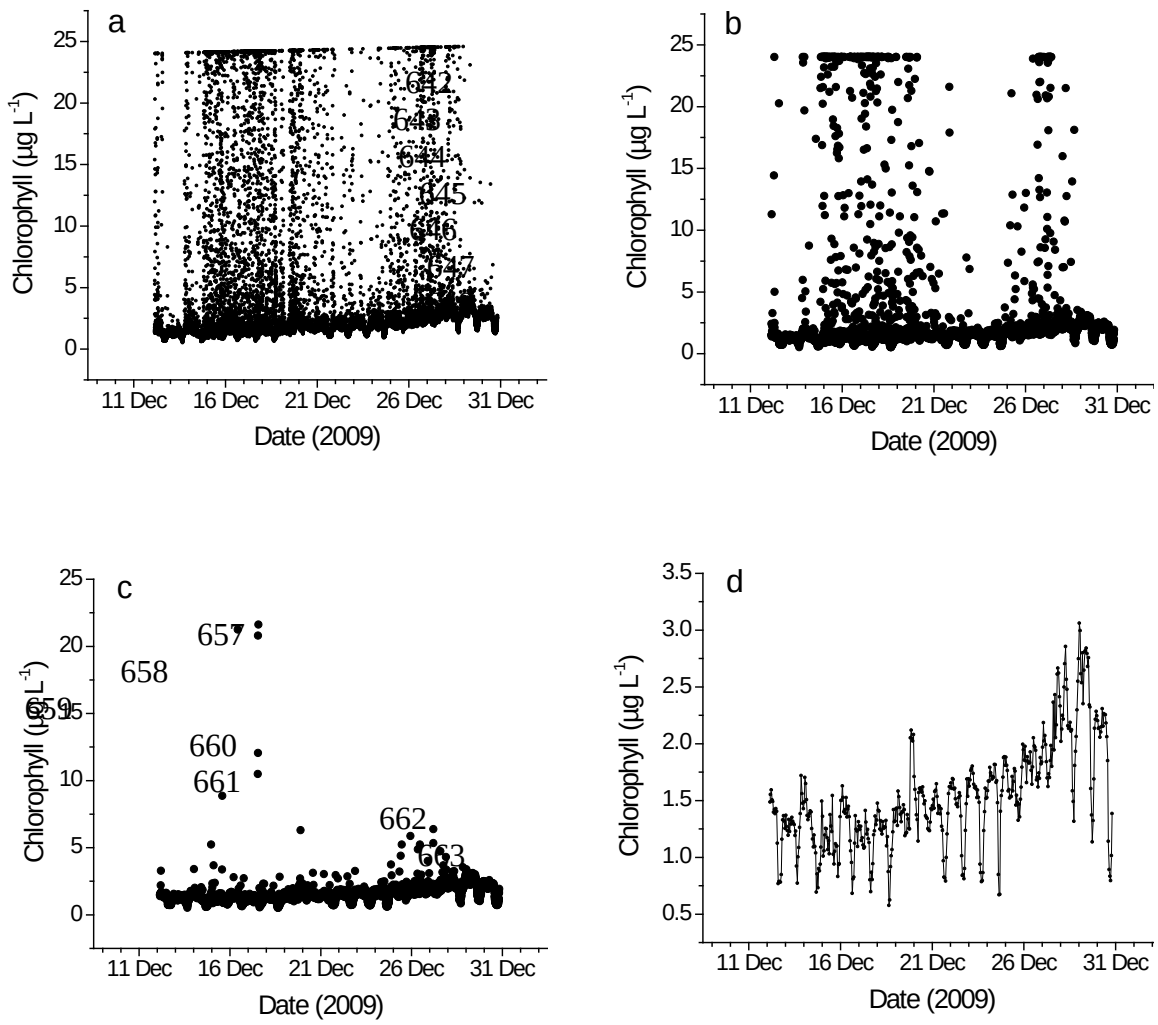

Figure 1. The process of data filtration for chlorophyll measurements from Little Harbor, Massachusetts. (a) Raw chlorophyll data (b) Median values for each 5-second sampling burst (c) Results of filtering data in 2-hour bins. (d) Median values for each 1-hour bin. Note that the y-axis scale differs from the other three plots.

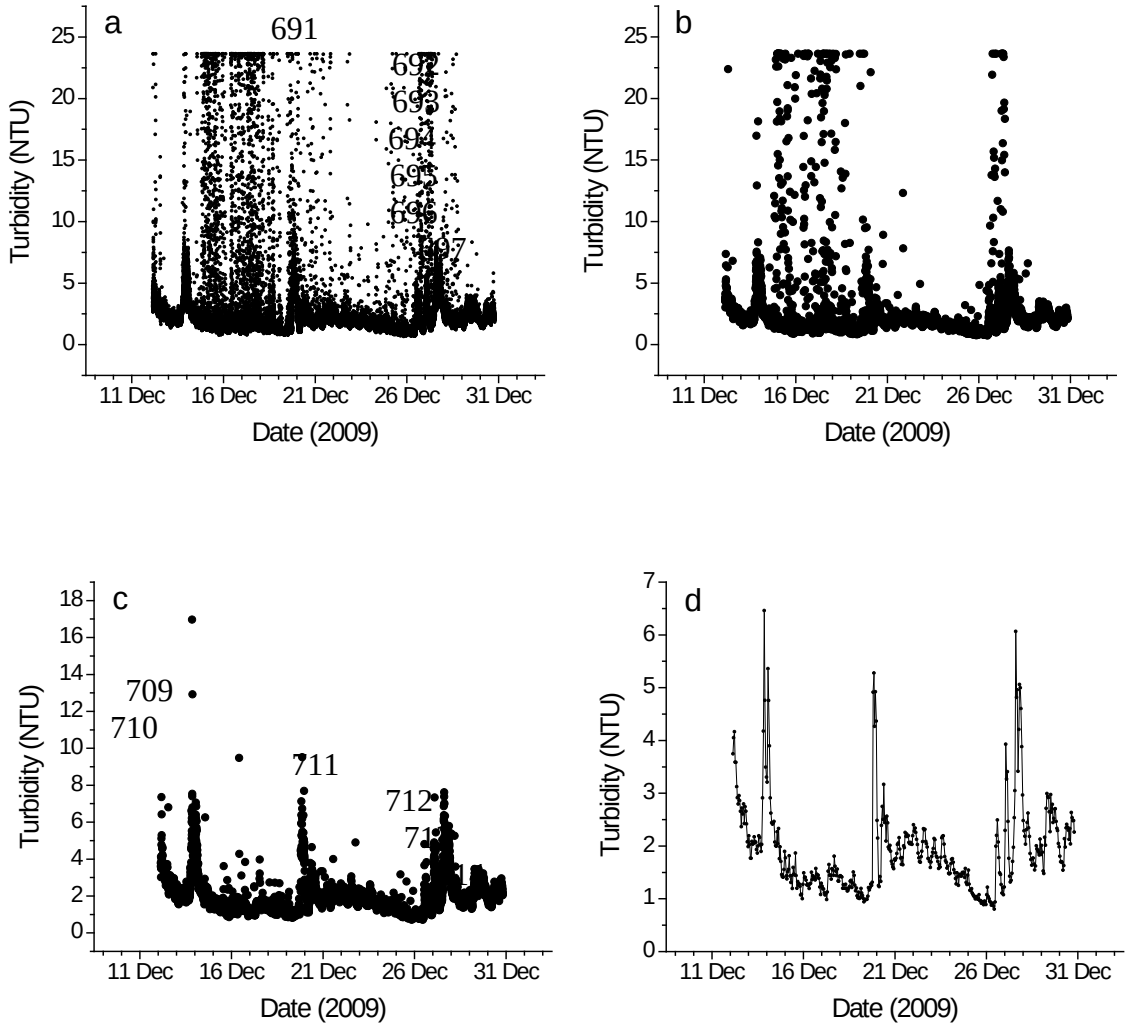

Figure 2. The process of data filtration for turbidity measurements from Little Harbor, Massachusetts. (a) Raw turbidity data (b) Median values for each 5-second sampling burst (c) Results of filtering data in 2-hour bins. (d) Median values for each 1-hour bin. Note that the y-axis scale differs among plots.

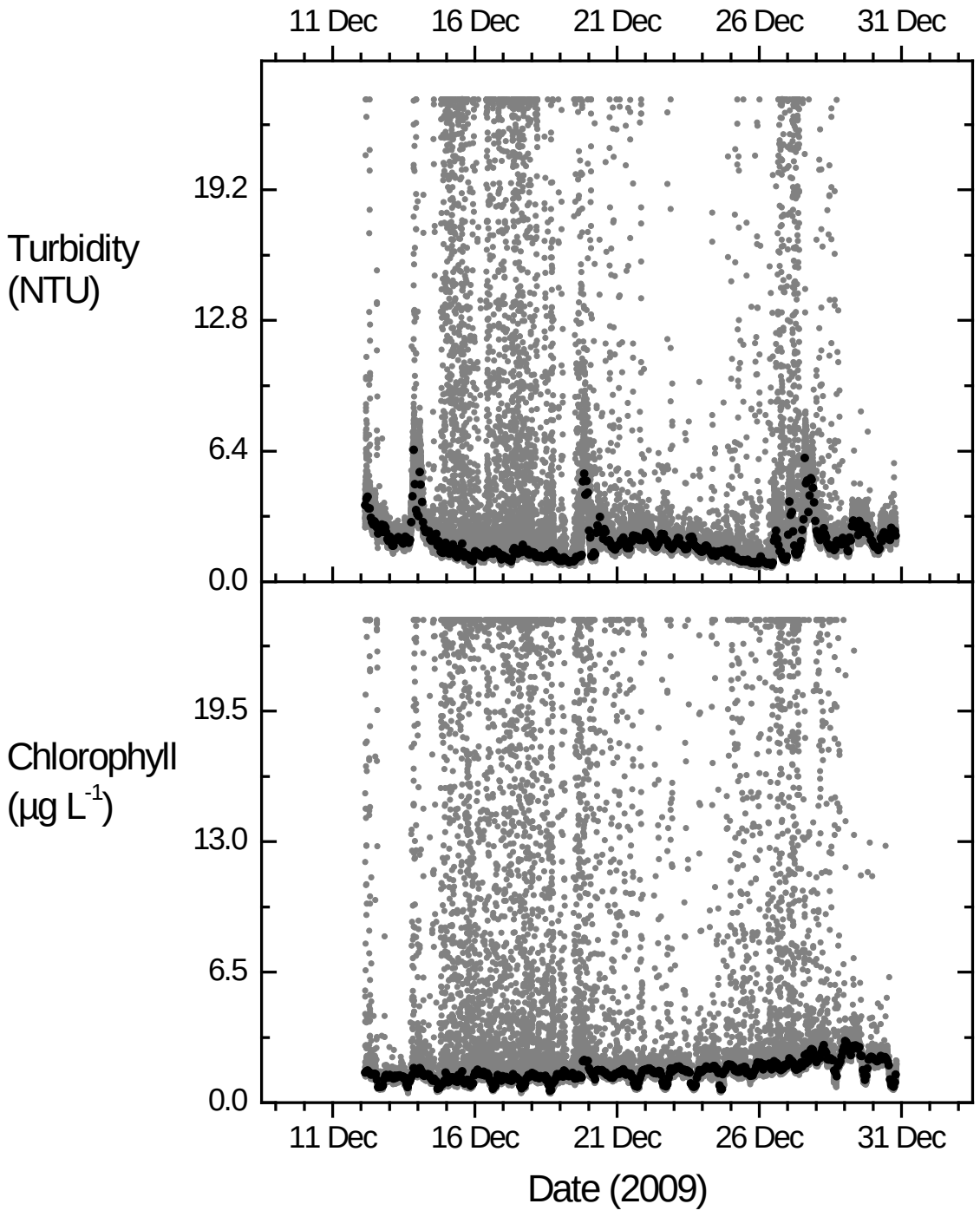

Figure 3. Raw turbidity and chlorophyll data are shown in gray dots. The black dots represent the data that have been processed via the filtering method described above.

Electronic Supplement 2

Statistical model for testing the hypothesis that there is a difference in the larval release response of adult barnacles when exposed to one of three treatments

To begin with, consider a single experimental jar. Let *m* be the known number of barnacle adults and let *y* be the observed number of nauplii in the jar at the end of the experiment. An adult is not necessarily capable of producing nauplii, either because it had released the larvae prior to the start of the experiment, or because it does not respond to the experimental treatment. Let π be the unknown probability that an adult is gravid and receptive to the larval release cue being tested. We allow π to be different for each of the four experiments we conducted.

Under the model, the unknown number *N* of adults capable of producing nauplii has a binomial distribution with probability mass function given by:

$$p(n) = \binom{m}{n} \pi^n (1-\pi)^{m-n} \qquad (1)$$

where *n* is the number of adults that release larvae.

Conditional on its being gravid and receptive to the larval release cue, we assumed that the number *x* of nauplii produced by a single adult follows a geometric distribution with probability mass function:

$$p(x) = \theta(1-\theta)^x \qquad x = 0, 1, 2, \ldots \qquad (2)$$

with unknown parameter θ ($0 < \theta < 1$). The geometric distribution is commonly used as a model for count data with a long upper tail. The mean and variance of x are $(1 - \theta) / \theta$ and $(1 - \theta) / \theta^2$, respectively.

The total number y of nauplii observed inside a jar at the end of an experiment represents the sum of a random number N of independent and identically distributed geometric counts. The probability mass function of y is given by:

$$p(y) = \sum_{n=0}^{m} p(y \mid n)\, p(n) \qquad (3)$$

where $p(y|n)$ is the conditional probability mass function of y given $N = n$, which can be shown to be negative binomial with scale parameter n and shape parameter θ. The negative binomial probabilities required for the calculation of (3) were approximated by the method of Best & Gipps (1974).

The analysis proceeded using the basic model outlined above, allowing π to vary among the 4 experiments and with interest centering on testing the null hypothesis H_0 that the geometric parameter p is the same for the three treatments (control, synthetic beads, and *Skeletonema marinoi* diatoms) against the alternative hypothesis H_1 that it is not. We used the likelihood ratio (LR) test, which involved fitting the model under both H_0 and H_1. The LR test statistic is given by:

$$\Lambda = 2\,[\log L_1 - \log L_0] \qquad (4)$$

where L_1 is the maximized likelihood value under H_1 and L_0 is the maximized likelihood value under H_0. Under H_0, Λ has an approximate chi-squared distribution with degrees of freedom given by the difference in the number of parameters under H_1 and H_0. In this case, there are 7 parameters under H_1 (one geometric parameter for each treatment and one binomial probability for each of the four experiments), and 5 under H_0 (one common geometric parameter and one binomial probability for each treatment). Thus, there are two degrees of freedom.

We repeated the entire analysis but omitted the outlier from the *Skeletonema* treatment, as this has undue influence on the results. We also used the LR test to test the null hypothesis that the geometric parameter is the same for the bead treatment and the *Skeletonema* treatment.

Summary of variables involved in the statistical analysis of experimental data:

m	number of adult barnacles in a jar
n	number of adults that release larvae
π	unknown probability that an adult is gravid and receptive to a larval release cue
N	the unknown number of adults that are gravid and receptive to a larval release cue
x	number of nauplii produced by a single adult
θ	unknown shape parameter of the negative binomial distribution
y	total number of nauplii inside a jar at the end of an experiment
Λ	test statistic of the likelihood ratio test

Literature Cited

Best DJ, Gipps PG (1974) An improved gamma approximation to the negative binomial. Technometrics 16: 621-624