Limnol. Oceanogr., 58(3), 2013, 932–944

doi:10.4319/lo.2013.58.3.0932

An inducible antipredatory defense in haploid cells of the marine microalga *Emiliania huxleyi* (Prymnesiophyceae)

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Abstract

Microzooplankton are important consumers of phytoplankton primary production, but phytoplankton defenses against these predators are not well understood despite their expected importance. We tested for inducible defenses in the coccolithophore *Emiliania huxleyi*, an abundant and cosmopolitan bloom-forming species with a heteromorphic haploid–diploid life cycle, against the ciliate predator *Strombidinopsis acuminatum*. We hypothesized that the two life-cycle phases (calcifying diploid and motile noncalcifying haploid) of *E. huxleyi* are differently defended against predation. Using short-term (30 min) ingestion rate assays, we compared predation on *E. huxleyi* that had been previously exposed to predators for 24 h to predation on naïve (unexposed) *E. huxleyi*. Prey were considered to have a defense response when ingestion rates on naïve cells were higher than ingestion rates on predator-exposed cells. Haploid *E. huxleyi* had a strong defense response, with a 25–43% reduction in ingestion rate due to predator exposure, whereas diploid *E. huxleyi* had no defense response. Haploid *E. huxleyi* grown with reduced nutrient availability showed no defense response when initially offered to ciliates (although there was evidence of a delayed defense response). Additionally, the presence of defended haploid *E. huxleyi* did not reduce ciliate ingestion on another prey species (*Heterocapsa triquetra*). This defense system points to complex predator–prey interactions as key factors controlling the structure and function of marine planktonic communities.

Marine phytoplankton are important players in the global ecosystem, contributing up to 50% of global primary productivity and strongly influencing biogeochemical cycling (Falkowski et al. 1998; Field et al. 1998). Predation by microzooplankton, a group consisting of hetero- and mixotrophic protists and some metazoan larvae, is now understood to be one of the most important causes of mortality for phytoplankton. A worldwide estimated average of 67% of phytoplankton daily production is consumed by microzooplankton (Calbet and Landry 2004), suggesting strong selective pressure for adaptations that reduce mortality due to microzooplankton predation. Defenses against consumers, although well characterized and known to be common in terrestrial plants (Howe and Jander 2008), are not well understood in the phytoplankton despite their expected importance. However, several chemical, mechanical, and behavioral defenses against consumers have been proposed or demonstrated in the phytoplankton (reviewed by Wolfe 2000; Van Donk et al. 2011).

We investigated a defense system, induced by cues from the ciliate predator *Strombidinopsis acuminatum*, in the coccolithophore *Emiliania huxleyi*. *Emiliania huxleyi* is the most abundant living coccolithophorid and is one of the most abundant and ubiquitous species of eukaryotic phytoplankton in the ocean (Campbell et al. 1994). The production of calcium carbonate coccoliths by this species has large-scale consequences for the carbonate chemistry of ocean photic zones and the sequestration of carbon in sediments (Holligan et al. 1993; Milliman 1993). Elucidating predator–prey interactions is essential for our understanding of the ecology of this important species.

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Inducible defenses against consumers have long been well known in terrestrial plants, in which the production of noxious or toxic chemical compounds in response to insect wounding is common (Green and Ryan 1972; Howe and Jander 2008). More recently, there have been a few reports of examples of similar defenses in marine macroalgae that inhibit grazing by small crustaceans and gastropods (reviewed by Hay 1996; Amsler 2001). Within the plankton, an inducible defense system has been extensively investigated in the freshwater crustacean *Daphnia* (Stabell et al. 2003). Inducible defenses have also more recently been described in a few species of freshwater and marine phytoplankton, including the toxin-producing cyanobacterium *Microcystis* sp. (Jang et al. 2007), the colony-forming prymnesiophyte *Phaeocystis* sp. (Long et al. 2007), and the toxin-producing dinoflagellate *Alexandrium* sp. (Selander et al. 2006). Mounting evidence points to these types of specific predator–prey interactions as important processes controlling the structure and function of marine planktonic communities.

As a bloom-forming phytoplankter, *E. huxleyi* has long been suspected of being well defended against predators. Microzooplankton predation on *E. huxleyi* in nature can be low in comparison with predation on other phytoplankton, a possible factor contributing to the formation of blooms (Fileman et al. 2002; Olson and Strom 2002; but *see* Holligan et al. 1993). However, the reasons for weak control of *E. huxleyi* populations by predators remain unknown.

Like many other prymnesiophytes, *E. huxleyi* has a haploid–diploid life cycle with two morphologically different phases that can both undergo indefinite asexual reproduction (Green et al. 1996; Houdan et al. 2004). Haploid–diploidy is an important characteristic that

distinguishes the coccolithophores from other successful phytoplankton groups, including the diploid diatoms and the primarily haploid dinoflagellates (Houdan et al. 2004). In *E. huxleyi*, the diploid phase is nonmotile and produces distinctive calcium carbonate scales called coccoliths, whereas the haploid phase produces only noncalcified organic scales and possesses two flagella. The two phases of *E. huxleyi* likely occupy very different ecological niches, raising interesting questions about this species' interactions with predators (Frada et al. 2008; von Dassow et al. 2009; Rokitta et al. 2011).

We tested for the presence of an inducible defense mechanism in diploid and haploid *E. huxleyi*. We considered a prey species to have an inducible defense if a predator's ingestion rate on prey that were previously exposed to the predator were lower than baseline ingestion rates on "naïve" prey that had no exposure to the predator. Because costs associated with inducible defenses against herbivory have been demonstrated in many plant species (reviewed by Strauss et al. 2002), we hypothesized that *E. huxleyi* might require sufficient nutrients to mount an effective defense. Therefore we also investigated the influence of nutrient availability on *E. huxleyi* inducible defenses. Last, to test whether the defense was specific to the producing cell or whether it may have been a compound released in bulk into the medium, we investigated the effect of the presence of defended *E. huxleyi* on ciliate ingestion of another phytoplankton species.

Methods

Organisms and culture conditions—We used calcifying *E. huxleyi* strain CCMP3266 (synonyms RCC1216, TQ26-2N) and flagellated, noncalcifying strain CCMP3268 (synonyms RCC1217, TQ26-1N). Strain 3268 was isolated from 3266 when partial phase change occurred in 1999. Strains 3266 and 3268 have been confirmed to be stable as diploid and haploid, respectively, for many years (Houdan et al. 2004; von Dassow et al. 2009). We performed frequent checks of strain morphology using light microscopy and flow cytometry. Strain 3266 was consistently highly calcified, as indicated by high side-scatter values on live flow cytometer samples (Jacquet et al. 2002) and frequent microscopic observations. Calcification was never observed in strain 3268. Cultures of 3268 consistently appeared well mixed, indicating motility. Flagella and active motility were frequently observed in cells from this strain under microscopic observation. Strains 3266 and 3268 will hereafter be referred to as diploid and haploid, respectively.

Emiliania huxleyi cultures were maintained under 75 μmol photons s^{-1} m^{-2} irradiance on a 12 : 12 h light : dark cycle at 15°C, and transferred every 7–10 d into microwave-sterilized f/50 medium made by nutrient addition to 0.2 μm of filtered seawater (Keller et al. 1988). Cultures were used for experiments in mid- to late exponential phase.

We used aloricate ciliate *S. acuminatum* (Choreotrichida) isolates SPMC142 and SPMC153 as predators in this study. Single-cell isolations were obtained in July 2010 from Bellingham Bay, Washington (SPMC142), and August 2011 from Rosario Strait (SPMC153). We maintained *S. acuminatum* on a prey mixture consisting of *Heterocapsa triquetra* and smaller amounts of *Isochrysis galbana*, *Mantoniella squamata* (CCMP480), and *Rhodomonas* sp. (CCMP755). Ciliates were kept under approximately 5 μmol photons s^{-1} m^{-2} irradiance on a 12 : 12 h light : dark cycle at 15°C and were fed and transferred into ciliate medium (autoclaved filtered seawater enriched with ethylenediaminetetraacetic acid and trace metals; Gifford 1985) twice per week.

Separate ciliate cultures were prepared for experiments. We fed these cultures only *H. triquetra* for 5–7 d before each experiment, with the last feeding at 48 h before each experiment. This prey species promotes good ciliate growth and is easily discriminated from *E. huxleyi* under epifluorescence microscopy (*see* below) due to its much larger size. Between 24 and 36 h before experiments, the majority of remaining prey was removed from *S. acuminatum* cultures by gentle reverse sieving through 20 μm mesh (Graham and Strom 2010). After reverse sieving, cultures were diluted with ciliate medium to the desired *S. acuminatum* concentration.

Emiliania huxleyi *characterization*—We performed gas chromatographic analysis of cellular (particulate) β-dimethylsulfoniopropionate ($DMSP_p$, a potential signal molecule) content of *E. huxleyi* culture samples with a Hewlett Packard 5890 chromatograph equipped with a flame photometric detector and a packed Supelco Chromosil 330 column. Triplicate samples were gravity filtered through glass fiber filters, quickly placed in gas-tight 16 mL serum vials with Teflon-lined septa containing 3 mL of 10 mol L^{-1} NaOH for alkaline hydrolysis of DMSP to dimethyl sulfide, and analyzed using the method of Wolfe et al. (2002).

Total particulate nitrogen and organic and inorganic carbon content of algal cultures was measured with a ThermoQuest Flash EA 1112 Series elemental analyzer using the method of Hedges and Stern (1984). We filtered culture samples onto precombusted 13 mm diameter type A/E glass fiber filters. Two sets of samples were taken for each culture, one of which was acid fumed to drive off inorganic carbon.

To obtain biovolume estimates of phytoplankton, we measured at least 30 live cell diameters using Image J 1.44 image analysis software. Biovolume was calculated assuming spherical cell shape for *E. huxleyi* and prolate spheroid cell shape for *H. triquetra*. For all experiments, we used equivalent prey biovolume concentrations (μm^3 mL^{-1}) of each algal prey type rather than equivalent cell or carbon concentrations. This better controlled for different predator encounter rates across a range of prey sizes as well as the different organic and inorganic carbon composition of diploid and haploid *E. huxleyi*.

Preliminary experiment—To estimate ciliate ingestion rates over a 24 h period, we added haploid *E. huxleyi* (10,700 cells mL^{-1}) to *S. acuminatum* at four different concentrations (0, 2, 6, 10, or 14 ciliates mL^{-1}) in triplicate bottles. These were incubated for 24 h on a plankton wheel

Table 1. Defense induction experiment I treatments. Treatments are listed as naïve (N) or predator-exposed (PE). Biovolumes are mean ± SEM.

Prey	Medium	Biovolume (μm^3 $cell^{-1}$)	Treatment	Test stage initial prey concentration (cells mL^{-1})	Replicates	Time points sampled
Diploid *Emiliania huxleyi*	f/50	218±15.2	N	6200	Four	All
			PE	6450	Four	All
	f/2	158±12.6	N	8700	Four	All
			PE	8850	Four	All
Haploid *Emiliania huxleyi*	f/50	45.0±3.16	N	31,200	Four	All
			PE	31,200	Four	All
	f/2	47.1±2.47	N	29,900	Four	All
			PE	29,800	Four	All
Heterocapsa triquetra	f/2	2355	Positive control	3390	One	30 min, 24 h
None	—	—	Unfed control	—	One	30 min, 24 h

under 1 μmol photons s^{-1} m^{-2} irradiance to minimize *E. huxleyi* growth. We sampled at the start of the experiment from one bottle and after 24 h from the remaining two bottles (effective $n = 2$), immediately adding a fluorescent bead standard to each sample and using the flow cytometer to obtain an estimate of *E. huxleyi* concentration (Collier and Palenik, 2003). Ingestion rates were calculated as in Frost (1972).

Vacuole count method of ingestion rate measurement—For the principal experiments of this study we used epifluorescence microscopy to determine ingestion rates by counting *E. huxleyi* visible inside *S. acuminatum* food vacuoles. After incubations with prey, we fixed 18 mL samples in vials preloaded with 0.6 mL alkaline Lugol's solution and added 0.5 mL of buffered 37% formalin, 2.5 mL 3% sodium thiosulfate, and 2 drops of 10 μg mL^{-1} 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid stain (procedure modified from Sherr and Sherr, 1993). After fixation, all samples were stored in the dark at 4°C for 3–5 h. We filtered the samples onto 5 μm Nucleopore polycarbonate filters, mounted them on slides with immersion oil, and froze them at −20°C until analysis.

The ingested prey inside the food vacuoles of the first 50 to 100 ciliates encountered (*see* experiment details) were counted under blue (450–490 nm wavelength) light excitation on a Leitz epifluorescence microscope. Only ciliates that were live at the time of fixation were included, as confirmed by the presence of intact, bright blue DAPI-stained nuclei when viewed under ultraviolet (340–380 nm wavelength) illumination. At time points when ingested prey were very numerous (occurring almost exclusively at 30 min time points in treatments with diploid *E. huxleyi* as prey, *see* below), we assigned all ciliates with more than 40 prey to a "40+" category. For calculations of ingestion rate, we used 40 cells $ciliate^{-1}$ as an estimate for this category, which resulted in slight underestimates of vacuole content.

Defense induction experiment I—To test for a defense mechanism in *E. huxleyi* that is induced in the presence of *S. acuminatum*, we compared *S. acuminatum* (SPMC142) ingestion rates on previously predator-exposed *E. huxleyi* to rates on unexposed naïve *E. huxleyi* using the vacuole count method of ingestion rate estimation. We also investigated whether the strength of the defense response depended on ploidy or nutrient availability. All treatments are summarized in Table 1.

Emiliania huxleyi cultures were transferred into their respective media (f/50 or f/2) and grown for 7 d before starting the experiment. All cultures were in late exponential growth phase at the start of the experiment. We sampled *E. huxleyi* stock cultures for biovolume measurements ($n = 30$ cells) and for $DMSP_p$ ($n = 3$ subsamples) and elemental ($n = 2$ subsamples) analyses on the day before the experiment. We used biovolume measurements to calculate saturating cell concentrations for each prey type, which we set to 1.4×10^6 μm^3 mL^{-1} (equivalent to 200 μg C L^{-1} of haploid *E. huxleyi*). Cell counts were done by microscope on samples preserved with 3% alkaline Lugol's solution.

We used a two-stage design for this experiment. In the "induction" stage, we exposed *E. huxleyi* to *S. acuminatum* (predator-exposed [PE] treatment) or an equivalent volume of ciliate medium (naïve [N] treatment) for 24 h. After 24 h, we removed *S. acuminatum* to obtain PE *E. huxleyi* cell suspensions. In the "test" stage, we added fresh *S. acuminatum* to PE and N *E. huxleyi* and compared ingestion rates on the two treatments. Within this basic design (e.g., of paired PE and N *E. huxleyi* treatments), we had four different *E. huxleyi* prey types: (1) diploid grown in f/50 medium; (2) diploid grown in f/2 medium; (3) haploid grown in f/50 medium; and (4) haploid grown in f/2 medium. We also included control treatments of *S. acuminatum* that were unfed or fed only *H. triquetra*.

For the induction stage, we added *S. acuminatum* (for 20 ciliates mL^{-1}) to polycarbonate bottles containing *E. huxleyi* (for 2X saturating concentrations) and the required amount of ciliate medium diluent to bring the volume to 600 mL. The corresponding bottles for the N treatment received only *E. huxleyi* and ciliate medium. Bottles were placed on a plankton wheel under 7 μmol photons s^{-1} m^{-2} irradiance for 24 h.

For the test stage, we collected the *E. huxleyi* cell suspension from each induction stage bottle, removing all *S. acuminatum* by gently reverse sieving through 20 μm mesh (N treatment bottles were also reverse sieved for

consistency). We ensured that no ciliates passed the mesh by inspecting all suspensions under a dissecting scope. We repeated *E. huxleyi* cell counts and corrected any increases in cell concentrations above 2X saturating levels with additions of ciliate medium. Note that in almost all treatments, including those with ciliates present, *E. huxleyi* concentrations showed a net increase of 3–27% over 24 h; a single treatment (PE f/2-grown diploid cells) showed a net decrease of 7%, which we did not amend.

We divided each *E. huxleyi* suspension into 75 mL aliquots in four replicate 150 mL polycarbonate bottles (for 1X saturating levels, Table 1) and added 75 mL of new (not previously *E. huxleyi*-exposed) *S. acuminatum* culture (for 20 ciliates mL^{-1}). We fixed samples for estimation of ingestion from each replicate bottle at 5, 10, and 30 min after *S. acuminatum* addition. The remaining volume in each bottle was used to fill 41 mL culture flasks, which were placed on the plankton wheel at 7 μmol photons s^{-1} m^{-2} irradiance and sampled again after 24 h. Only 30 min and 24 h samples were taken for *H. triquetra*-fed and unfed control treatments ($n = 1$).

For 5 and 10 min samples, we counted all *E. huxleyi* prey inside each of 100 ciliates. We counted prey inside only 50 ciliates for most 30 min and 24 h slides, after finding no significant difference between estimates of mean vacuole content on the basis of counts of 50 vs. 100 ciliates. For easy comparisons of the amount of prey ingested in each treatment, all vacuole content values are reported in units of prey biovolume ingested per ciliate as calculated from prey cell diameters measured at the start of the experiment (Table 1).

For unfed control samples, we counted all ingested prey of a similar size and shape to *E. huxleyi* (most likely previously ingested *I. galbana*). Experiment vacuole content values were not corrected for background prey in the unfed control because levels were very low (0.1 cells $ciliate^{-1}$, with only 7% of the population containing one or more *E. huxleyi*-like prey). We enumerated all *H. triquetra* ingested in the *H. triquetra*-fed control samples to obtain an ingestion rate on this prey at 30 min (vacuole content divided by 30 min) and vacuole content at 24 h. We corrected these values for background *H. triquetra* in the unfed control (a rate correction of minus 30%).

Ingestion rates were calculated as the slope of the linear regression of vacuole content per ciliate over time (0–30 min) for each replicate bottle separately. We used polynomial contrasts (trend analysis) to test for nonlinear relationships. When polynomial contrasts showed a significant quadratic component, we obtained ingestion rates over appropriate time intervals and calculated a weighted mean ingestion rate over the entire time period (0–30 min) for comparisons with treatments with linear relationships. We tested for effects of exposure (PE vs. N) and media type (f/2 vs. f/50) on ciliate ingestion rates during 30 min incubations with a two-factor analysis of variance (ANOVA). Haploid and diploid treatments were considered separately. Significant interactions were examined with tests for simple main effects. For 24 h time points, we did not calculate ingestion rates because digestion rates are unknown; we report only the mean vacuole content per ciliate. For the 24 h time point, we performed a priori contrasts using Student's *t*-tests to compare vacuole content between PE and N treatments for each medium type. All analyses were performed with Predictive Analytic Software Statistics (PASW; http://www.spss.com.hk/statistics/) 17.0.

Defense induction experiment II—We repeated the above experiment on haploid *E. huxleyi* grown in f/2 medium only (the prey type giving the largest induced defense response, *see* Results) with the newer *S. acuminatum* isolate (SPMC153) to confirm the results in defense induction experiment I and begin to explore the mechanism behind the defense. To achieve the latter, we expanded the design to test two other hypotheses: that *S. acuminatum* has reduced ingestion rates on a high-quality prey, *H. triquetra*, in the presence of PE haploid *E. huxleyi*; and that *S. acuminatum* has reduced ingestion rates on *H. triquetra* in the presence of a filtrate from PE haploid *E. huxleyi*. Testing these two hypotheses allowed us to discriminate between two types of signals (i.e., infochemicals) that could be involved in the defense: prey cell surface-associated signals, and signals released by prey into the environment. We assumed that a released infochemical would have the potential to influence ingestion on other prey cells (including those of other species) in the vicinity, whereas the effects of a surface-associated chemical would remain specific to the chemical-producing cell. To test these hypotheses, we mixed PE or N *E. huxleyi* cells (or *E. huxleyi*-derived filtrates) with *H. triquetra* and offered these mixtures to *S. acuminatum*. If ciliate ingestion rates on *H. triquetra* are reduced in the presence of PE *E. huxleyi* (compared with in the presence of N *E. huxleyi*), then *E. huxleyi* must release into the medium some compound that is broadly inhibitory to ciliate feeding. In contrast, if ingestion rates on *H. triquetra* are unchanged by the presence of PE vs. N *E. huxleyi*, the mechanism of defense must be some property of individual *E. huxleyi* cells.

The design of this experiment was the same as the first except for the following differences. During the induction stage, ciliates or medium and prey were combined in 4 liter polycarbonate bottles, which were too big to be placed on a plankton wheel. Instead, the bottles were mixed by inversion twice during the incubation period; because haploid *E. huxleyi* is motile the cultures likely remained well mixed. We took samples both before the induction stage (from *E. huxleyi* and *H. triquetra* stock cultures) and at the end of the induction stage (from PE and N *E. huxleyi*) for elemental analysis ($n = 4$ subsamples) and biovolume measurements ($n = 30$ cells).

For the test stage, we added new *S. acuminatum* to the following three paired prey treatments and two control treatments ($n = 5$): (1) PE or N haploid cells; (2) PE or N haploid cells + *H. triquetra*; (3) PE or N haploid cell filtrate + *H. triquetra*; (4) *H. triquetra* alone; and (5) ciliate medium (unfed control). Filtrates were prepared from PE and N treatments by sequentially filtering aliquots with a hand pump at < 5 mm Hg vacuum through 1.0 μm and 0.2 μm Nucleopore polycarbonate filters to remove all phytoplankton and bacteria. *Emiliania huxleyi* cell suspensions or

filtrates and ciliate medium diluent were mixed in 150 mL polycarbonate bottles for 1X saturating concentrations of each prey (33,300 cells mL^{-1} for 3268 and 621 cells mL^{-1} for *H. triquetra*) or an equivalent volume of filtrate. Each prey mixture was divided into five 60 mL polycarbonate bottles, to which we added the required volume of ciliate culture. We fixed samples for estimation of ingestion rate at a single time point, 20 min.

We counted all *E. huxleyi* and *H. triquetra* prey inside each of 50 ciliates and calculated ingestion rates for each prey type by dividing by the sampling time. All ingestion rates are presented in units of cells $ciliate^{-1}$ min^{-1}. We used vacuole content in the unfed control treatment to correct ingestion rate estimates on *E. huxleyi* and *H. triquetra* (a maximum rate correction of −8% and −6% for *E. huxleyi* and *H. triquetra*, respectively).

Ingestion rate data for *E. huxleyi* and *H. triquetra* were considered separately. For ingestion rates on *E. huxleyi*, we tested for effects of exposure (PE vs. N) and prey mixture (haploid cells alone vs. haploid cells mixed with *H. triquetra*) with a two-factor ANOVA. For ingestion rates on *H. triquetra*, we tested for effects of *E. huxleyi* exposure (PE vs. N) and prey mixture (*E. huxleyi* cell suspension present vs. *E. huxleyi* filtrate present) with a two-factor ANOVA. We used a Dunnett's *t*-test to compare ciliate ingestion rates on *H. triquetra* in mixture treatments to the control fed only *H. triquetra*.

Results

Preliminary experiment—We determined apparent ingestion rates of *S. acuminatum* on haploid *E. huxleyi* at four concentrations of *S. acuminatum*. *Emiliania huxleyi* cell disappearance during 24 h incubations with ciliates gave indirect evidence of an inducible defense. Although the apparent ingestion rate on haploid cells was substantial when only two ciliates mL^{-1} were present, ingestion rates were negative in treatments with 6, 10, and 14 ciliates mL^{-1} (Fig. 1). The method of calculating ingestion rates in Frost (1972) yields erroneously negative or low ingestion rates when net growth of prey is higher in the presence of predators than it is in the predator-free control. We subsequently found that *E. huxleyi* growth under the same conditions used in the preliminary experiment was indeed stimulated significantly by the presence of ciliate culture filtrates that included the bacteria size fraction (data not shown). However, this stimulation of *E. huxleyi* growth in the presence of ciliate culture material was only a small fraction (20%) of that required to account for the apparent lack of feeding at higher ciliate concentrations. This led us to hypothesize that *E. huxleyi* has a defense response that is induced by the presence of ciliates.

Defense induction experiment I—To test for an inducible defense, we compared ingestion rates of *S. acuminatum* on PE vs. N treatments of haploid and diploid *E. huxleyi* grown in two strengths of medium. Evidence of an induced defense was clearly present in experiments with haploid *E. huxleyi* grown in f/2 medium. Predator exposure of f/2-grown haploid cells resulted in a 43.0% ± 2.3% decrease in ingestion rates relative to N cells during 30 min incubations (8.5 ± 0.4 vs. 15.0 ± 1.4 μm^3 $ciliate^{-1}$ min^{-1}, respectively, mean ± SEM, $n = 4$; ANOVA, $F_{1,12} = 23.6$, $p < 0.001$; Fig. 2A). This difference was due in part to a consistently higher percentage of ciliates feeding in the N treatment. From 5 to 30 min, 10–30% more ciliates had ingested one or more cells when they were given N prey than when they were given PE prey (in all other treatments the percentage of the population feeding was similar for PE and N treatments; data not shown). The effect of predator exposure was still evident at 24 h, by which time ciliates had ingested a 45% lower biovolume of PE than N prey (*t*-test, $t = -2.95$, df = 6, $p = 0.026$).

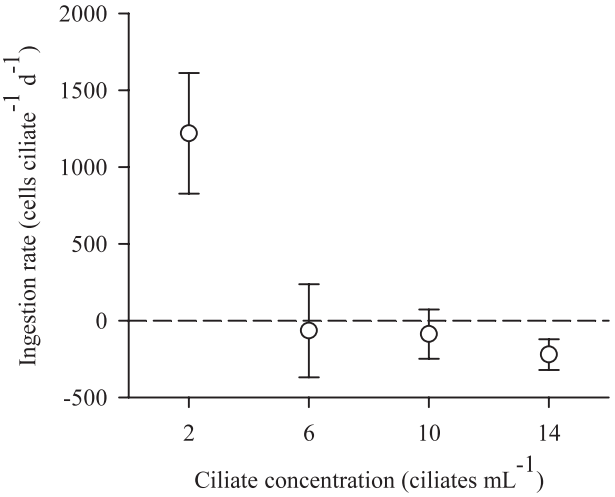

Fig. 1. Apparent *Strombidinopsis acuminatum* ingestion rates over 24 h during a preliminary experiment on haploid *Emiliania huxleyi* prey at four ciliate concentrations. Error bars display ± range, $n = 2$.

The defense response in haploid *E. huxleyi* was dependent on prey growth medium (significant interaction, ANOVA, $F_{1,12} = 16.9$, $p = 0.001$). No reduction in ingestion rate due to predator exposure was observed for f/50-grown haploid cells during 30 min incubations (10.3 ± 1.1 vs. 11.5 ± 0.6 μm^3 $ciliate^{-1}$ min^{-1} for N and PE prey, respectively; ANOVA, $F_{1,12} = 0.89$, $p = 0.36$; Fig. 2A). However, by 24 h, ciliates fed f/50-grown haploid *E. huxleyi* had ingested a 45% lower biovolume of PE than N prey (*t*-test, $t = -5.22$, df = 6, $p = 0.002$). This suggests that a defense response did eventually occur in this prey type, despite equal ingestion rates on PE and N prey observed within 30 min. Independent of the effects of predator exposure, ciliates fed f/50-grown haploid *E. huxleyi* also had an overall reduced ciliate vacuole content by 24 h compared with the 30 min time point; in contrast, ciliate vacuole content of f/2-grown haploids continued to increase slightly up to 24 h. Reduced availability of nitrogen for f/50-grown *E. huxleyi* is a likely reason for these observed differences: *E. huxleyi* had much higher particulate organic carbon to total particulate nitrogen (POC : TPN) ratios when grown in f/50 medium than in f/2 medium (Table 2).

In contrast to f/2-grown haploid *E. huxleyi*, there was little evidence for an induced defense in diploid *E. huxleyi*.

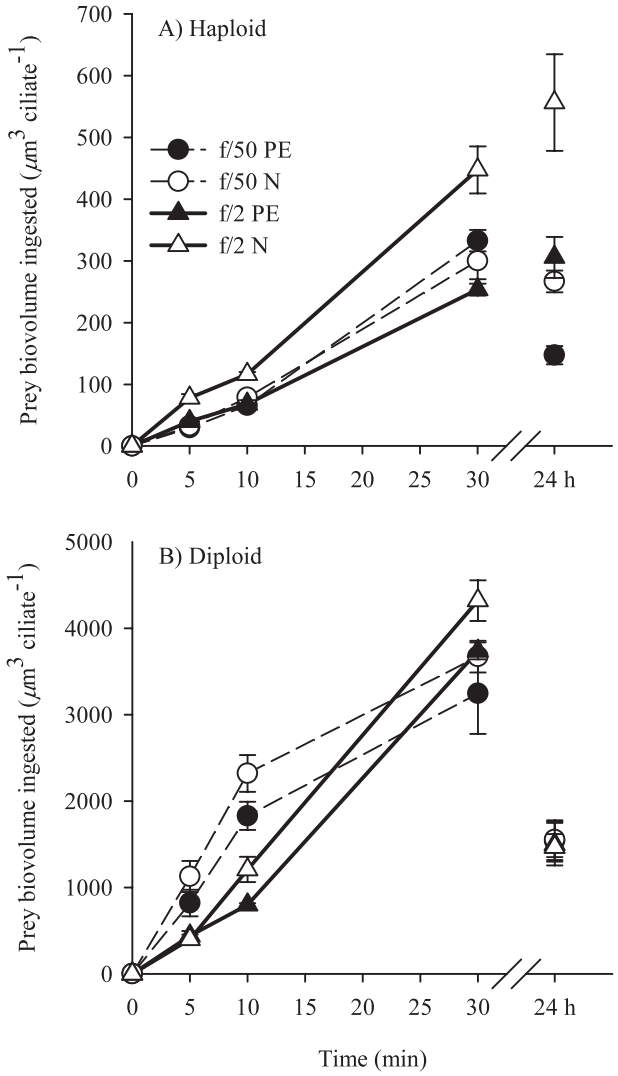

Fig. 2. *Strombidinopsis acuminatum* vacuole content of (A) haploid and (B) diploid *E. huxleyi* over time in defense induction experiment I. Open symbols represent naïve (N) treatments, black symbols represent predator-exposed (PE) treatments. Error bars, where visible, display ± SEM, $n = 4$.

Predator exposure did not result in significant decreases in ingestion rate relative to N cells for either f/2-grown (149 ± 7.9 vs. 128 ± 3.8 μm^3 ciliate^{-1} min^{-1} for N and PE prey, respectively) or f/50-grown (177 ± 11.8 vs. 146 ± 15.7 μm^3 ciliate^{-1} min^{-1} for N and PE prey, respectively) diploid *E. huxleyi* during 30 min incubations (ANOVA, $F_{1,12} = 3.39$, $p = 0.090$; Fig. 2B). Diploid *E. huxleyi* grown in f/2 medium were ingested at an overall higher rate than diploid *E. huxleyi* grown in f/50 medium (ANOVA, $F_{1,12} = 5.82$, $p = 0.033$). However, by 24 h, ciliate vacuoles in all treatments contained the same biovolume of prey in all cases at dramatically reduced levels compared with the 30 min time point.

Prey cell biovolume had a large effect on ciliate ingestion rates. If we consider only initial ingestion rates (from 0 to 10 min) when ingestion rates were not constant (i.e., for diploid cells grown in f/50), there was a strong and significant linear relationship between prey biovolume and ingestion rate on N prey ($p < 0.001$, $R^2 = 0.90$, $n = 16$; Fig. 3). This relationship disappeared when the weighted mean ingestion rate on f/50-grown diploid cells was used.

Because of the potentially large influence of prey biovolume on *S. acuminatum* ingestion rates, we investigated the possibility that predators in the induction stage ingested larger cells at a higher rate, thereby causing reduced ingestion rates on the smaller PE cells remaining during the test stage. Preserved samples taken for haploid cell counts before and after the induction stage showed that mean biovolume was not significantly different between PE and N treatments (*t*-tests, $t = -0.98$, df = 58, $p = 0.33$, and $t = 0.79$, df = 58, $p = 0.43$ for f/2- and f/50-grown, respectively). Although preserved samples of diploid treatments were too degraded for accurate biovolume measurements, flow cytometer forward scatter (a proxy for cell size) of live samples gave estimates indicating that PE f/2-grown diploid cells were 14% smaller compared with N cells (there were no differences for f/50-grown diploids). This suggests that more large cells were removed by ciliates during the induction stage, possibly causing an overestimate of the already nonsignificant f/2-grown diploid defense response.

Ciliates fed only *H. triquetra* in experiment I had an ingestion rate of 127 μm^3 ciliate^{-1} min^{-1} at 30 min, which was comparable with ingestion rates on f/2-grown diploid

Table 2. Defense induction experiment I prey characteristics. Chemical analyses and biovolume measurements were performed on stock cultures 24 h before the induction stage. Values are mean ± SEM (or range for carbon and nitrogen data). PIC, particulate inorganic carbon.

	Diploid *E. huxleyi*		Haploid *E. huxleyi*		*H. triquetra*
Medium	f/50	f/2	f/50	f/2	f/2
TPN (pg N cell^{-1})	0.66±0.10	0.91±0.13	0.61±0.09	1.4±0.25	—
POC (pg C cell^{-1})	7.2±0.18	5.7±0.01	7.1±0.25	7.8±0.60	1100*
PIC (pg C cell^{-1})	6.2±0.46	4.8±0.85	0.28±0.25	0.48±0.60	—
PIC : POC	0.86±0.08	0.83±0.15	0.04±0.04	0.06±0.08	—
POC : TPN	11±1.5	6.3±0.92	12±2.0	5.5±0.76	—
$DMSP_P$: POC (pg DMSP pg C^{-1})	0.12±0.002	0.13±0.009	0.11±0.002	0.14±<0.001	—
Biovolume (μm^3 cell^{-1})	218±15.2	158±12.6	45.0±3.16	47.1±2.47	2355*

* From Graham and Strom 2010.

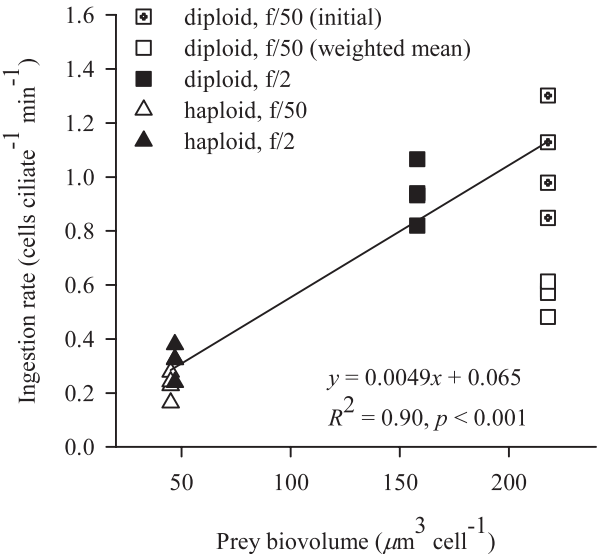

Fig. 3. Relationship of *S. acuminatum* ingestion rate and *E. huxleyi* prey biovolume observed in defense induction experiment I. Ingestion rates are presented from naïve treatments only. Ingestion rates were calculated over the 0–30 min interval for all treatments except f/50-grown diploid *E. huxleyi*, where the initial 0–10 min interval was used because of the nonlinear response. Ingestion rate strongly and significantly increased linearly with prey biovolume ($p < 0.001$, $R^2 = 0.90$, $n = 16$). The weighted mean for f/50-grown diploids is also included in the plot, but not in the regression (*see* text).

E. huxleyi. However, unlike in ciliates fed diploid *E. huxleyi*, the biovolume of *H. triquetra* ingested increased substantially from 3815 μm^3 ciliate^{-1} at 30 min to 11,445 μm^3 ciliate^{-1} at 24 h.

Defense induction experiment II—We repeated part of defense induction experiment I (f/2-grown haploid *E. huxleyi* treatment only) and additionally investigated the effect of predator-exposed haploid *E. huxleyi* cells and derived filtrates on ciliate ingestion of an alternate prey species, *H. triquetra*. Although this experiment used a new isolate of *S. acuminatum*, we again observed evidence for an induced defense response in f/2-grown haploid *E. huxleyi*. Previous predator exposure caused ciliates to reduce their ingestion rate on this prey (when offered alone) by 25% (ANOVA, $F_{1,16} = 38.9$, $p < 0.001$; Fig. 4). Ingestion rates were 0.52 ± 0.025 vs. 0.39 ± 0.014 cells ciliate^{-1} mL^{-1} for N and PE cells respectively (equivalent to 18.3 ± 0.88 vs. 13.6 ± 0.52 μm^3 ciliate^{-1} min^{-1}, mean ± SEM, $n = 5$). Contrary to defense induction experiment I, there was no difference in the percentage of ciliates having ingested one or more prey between N and PE treatments. When *H. triquetra* were mixed with *E. huxleyi*, the defense response was even stronger (a 46% reduction in ingestion rate on PE vs. N *E. huxleyi* cells).

Adding PE haploid *E. huxleyi* or filtrate to treatments fed *H. triquetra* did not reduce ciliate ingestion of *H. triquetra* relative to N treatments (ANOVA, $F_{1,16} = 0.81$, $p = 0.38$; Fig. 4), despite a reduced ingestion rate on PE *E. huxleyi* (Fig. 4). This supports our hypothesis that the defense response in haploid *E. huxleyi* was a property of individual cells rather than a generally inhibitory compound released into the medium. When *E. huxleyi*-derived filtrates were present, ingestion rates on *H. triquetra* were significantly higher overall in both PE and N treatments than when haploid cells were present (ANOVA, $F_{1,16} = 24.6$, $p < 0.001$). The addition of filtrate thus did cause an overall stimulation of ciliate feeding on *H. triquetra*, but the degree of stimulation was equivalent whether filtrates were from N or PE *E. huxleyi*. Ingestion rates on *H. triquetra* in the presence of filtrates from N haploid *E. huxleyi* were significantly higher in comparison with the control fed only *H. triquetra* (Dunnett's *t*-test, $p = 0.02$).

The ratio of total particulate carbon to TPN (TPC : TPN) in this experiment was the same for PE and N treatments (Table 3). We also confirmed that ciliates did not alter the size distribution of haploid *E. huxleyi* during the induction stage (*t*-test, $t = 0.46$, df = 58, $p = 0.64$).

Discussion

Our data demonstrate a predator defense system in *E. huxleyi* that is induced in the presence of the ciliate *S. acuminatum*. This inducible defense specifically benefitted *E. huxleyi* without simultaneously inhibiting predation on another prey species (*H. triquetra*) when both were offered in mixture. The capacity for induction of this defense strongly depended on *E. huxleyi* life-cycle phase and nutrient availability. This defense system, found here to be present only in the haploid phase under nutrient-replete conditions, is evidence of the importance of the heteromorphic haploid–diploid life cycle in the ecology of this abundant and biogeochemically significant phytoplankton species.

Evidence for inducible defense—In both defense induction experiments, ingestion rates on f/2-grown haploid *E. huxleyi* were reduced when they had been previously exposed to predators, a result that meets our definition of an induced defense response. In addition to an induced defense response, there is another possible explanation for these results. If some determinant of ciliate ingestion (i.e., cell size) was variable in the prey population, ciliates may have ingested some prey cells faster than others during the induction stage, resulting in lower ingestion rates on the remaining, less-ingestible PE prey during the test stage.

Prey characteristics that could be determinants of ciliate ingestion rate and variable in culture include prey size, nutritional quality, and motility. Ingestion rates of planktonic ciliates are known to be sensitive to prey size (Jonsson 1986). However, changes in mean prey size during the induction stage occurred only in treatments with f/2-grown diploid *E. huxleyi* (further weakening the case for a defense response in this strain). Microzooplankton are also known to feed at higher rates on more nutritious prey (*see* below). However, if anything, prey nutritional quality should have increased in the presence of nutrients remineralized by ciliates or associated bacteria in the PE treatment. The C : N ratio of f/2-grown haploid cells was similar for PE and N prey after the induction stage of

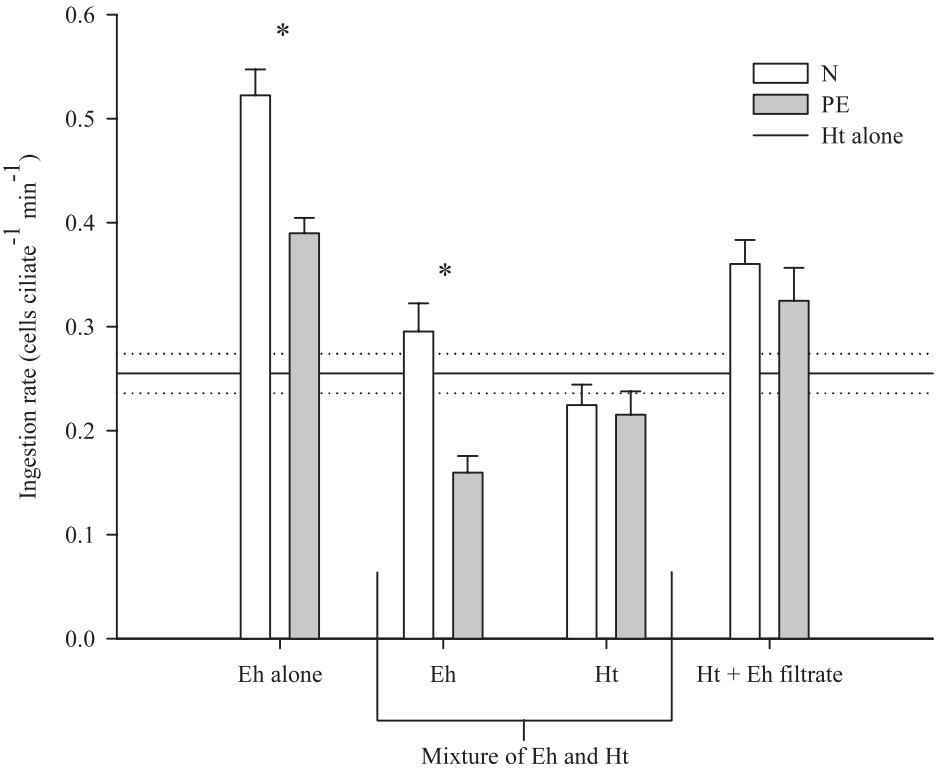

Fig. 4. *Strombidinopsis acuminatum* ingestion rates on haploid *Emiliana huxleyi* (Eh) and *Heterocapsa triquetra* (Ht) in defense induction experiment II. Ingestion rates are presented for *E. huxleyi* and *H. triquetra* separately in the mixed prey treatment indicated by the bracket. Ingestion rate on *H. triquetra* is presented in the treatment with *E. huxleyi* cell-derived filtrates. Horizontal line indicates mean ingestion rate when *S. acuminatum* was fed *H. triquetra* alone (dotted lines above and below indicate ± SEM). Error bars display ± SEM, $n = 5$; asterisks indicate significant differences between N and PE bars.

experiment II, suggesting no effect of ciliates or associated bacteria on nutritional quality over 24 h. Prey motility could also be an important factor in ciliate feeding. However, we saw no discernible differences in PE vs. N haploid *E. huxleyi* motility in qualitative microscope observations. Because swimming speeds of nanoflagellates (1–100 μm s^{-1}, lower end likely for haploid *E. huxleyi*) are orders of magnitude lower than swimming speeds of large ciliates such as *S. acuminatum*, (2000–4000 μm s^{-1}) (Crawford 1992), changes in haploid *E. huxleyi* swimming speed are not likely to affect feeding behavior (Wolfe 2000).

The potential effects of ciliate feeding during the induction stage on relevant (but unmeasured) characteristics of haploid cells remains an important caveat for our interpretation of these data as evidence of an inducible defense. Further experiments exposing *E. huxleyi* to predator feeding cues alone, without the presence of the predator itself, would be required to definitively test this

Table 3. Defense induction experiment II prey characteristics. Chemical analyses and biovolume measurements were performed on f/2-grown haploid *E. huxleyi* stock cultures before the induction stage and for both PE and N treatments after the induction stage. All measurements for *H. triquetra* were done on stock cultures before the induction stage. Note that we did not acidify samples for elemental analysis for this experiment because particulate inorganic carbon (PIC) values for this strain were small in defense induction experiment I; therefore TPC values (sum of PIC and POC) are presented. Values are mean ± SEM except for the "Diameter" of *H. triquetra*, which is oblong in shape; the "Diameter" data for that species are given as minor axis length × major axis length.

	Haploid *E. huxleyi*			*H. triquetra*
	Preinduction stage	PE	N	Preinduction stage
TPC (pg C cell^{-1})	6.11±0.07	6.85±0.05	6.11±0.05	328±1.97
TPN (pg N cell^{-1})	1.18±0.07	1.03±0.06	0.92±0.04	69.1±1.57
TPC : TPN	5.21±0.32	6.73±0.37	6.70±0.29	4.75±0.11
Diameter (μm)	4.3±0.09	3.97±0.09	4.03±0.09	14.5×20.4
Biovolume (μm^3 cell^{-1})	42.2±2.72	34.2±2.37	35.8±2.61	2265±100

hypothesis. However, we argue that any effects of predation during the induction stage should have been small because feeding was negligible (i.e., not enough to offset *E. huxleyi* growth in low light over 24 h). Finally, if ciliate feeding during the induction stage altered prey characteristics, we would expect to see similar test-stage results in all prey treatments (particularly between f/50-grown and f/2-grown haploid treatments), which did not occur.

We conclude that the presence of an inducible defense is the most likely explanation for reduced ingestion rates observed on PE f/2-grown haploid *E. huxleyi*. The defense was activated within 24 h upon exposure to cues from feeding *S. acuminatum*. The density of ciliates required for defense induction was 20 ciliates mL^{-1} (in the defense induction experiments), or potentially as low as 6 ciliates mL^{-1} (preliminary experiment, Fig 1). These are comparable with observed ciliate densities in upwelling systems: 1.2–7.7 aloricate ciliates mL^{-1} off the Oregon coast (Fessenden and Cowles 1994), 7–88 total ciliates mL^{-1} in Puget Sound, Washington (Paul 2010), 10–45 total ciliates mL^{-1} during an *E. huxleyi* bloom in the Bering Sea, Alaska (Olson and Strom 2002), and 10–50 *Strombidinopsis* spp. mL^{-1} during an *E. huxleyi* bloom off the coast of Devon, United Kingdom (Fileman et al. 2002). The ciliate predator responded to defended haploid *E. huxleyi* with 25% (experiment II) to 43% (experiment I) reductions in ingestion rates within 30 min; reduced ingestion persisted for at least 24 h. In defense induction experiment II, predators ingested defended prey at an even lower rate (46% rate reduction) when *H. triquetra* was available as prey. This suggests that ciliates were more sensitive to *E. huxleyi* defenses when an alternative and high-quality prey species was available. Finally, the defense specifically benefitted haploid *E. huxleyi*, without a corresponding reduction in ciliate ingestion rates on an alternate prey species (*H. triquetra*) when offered in mixture (defense induction experiment II).

Mechanism of defense—The mechanism of the defense in haploid *E. huxleyi* remains unknown. A morphological defense system has been postulated in the related colony-forming species *Phaeocystis globosa* and *P. antarctica* (Prymnesiophyceae). Waterborne chemical cues from predators can alter the proportion of cells in colonies (Long et al. 2007) as well as the size of colonies (Tang et al. 2008), which is suggested to create a size-mismatch problem for predators. The defense response in *Phaeocystis* sp. is relatively slow, with morphological changes in *Phaeocystis* sp. observed only after 3–5 d of predator cue exposure. We observed no obvious morphological changes in *E. huxleyi* after predator exposure. Combined with the rapid ($\leq$ 24 h) activation of a response to predators under conditions with minimal growth, this suggests that the *E. huxleyi* defense may have a chemical basis.

Defensive chemicals include both predator deterrents and toxins. For maximum effectiveness, predators must respond to defensive chemicals produced by phytoplankton by avoiding ingestion of the producer cell and, if available, switching to a more palatable prey species. Infochemicals important for mediating microzooplankton predation may exist on the prey cell surface or be released into the boundary layer or ''phycosphere'' immediately surrounding the cell, creating a near-cell concentration gradient to which predators can respond directionally (Wolfe 2000; Pohnert et al. 2007). In contrast, the community-wide release of a toxic or inhibitory compound is less likely to be a successful defense mechanism evolutionarily. Unless the prey population consists of many genetically related cells that benefit from the community-wide protection (resulting in high inclusive fitness for toxic/inhibitory compound-producing cells), this system is vulnerable to invasion by cheater cells that produce no toxic/inhibitory compounds but still receive the benefit (Thornton 2002; Pohnert et al. 2007). This is unlikely because phytoplankton populations (including *E. huxleyi* blooms) are often genetically diverse (reviewed by Medlin et al. 2000; Iglesias-Rodriguez et al. 2006). In addition, any net benefit to a group of genetically related cells would be reduced if competitors also benefitted (Lewis 1986). A similar argument applies to toxic phytoplankton cells that harm the predator after ingestion; in this case, prey cells receive no benefit for potentially costly toxin production unless predators can detect some signal of prey toxicity and avoid ingesting them (Wolfe 2000; Pohnert et al. 2007).

We propose that the production of a predator-deterrent infochemical (either on the cell surface or released into the phycosphere) is the most plausible mechanism for the defense response observed in haploid *E. huxleyi*. In defense induction experiment II, the presence of PE haploid cells and associated medium did not inhibit ciliate ingestion of *H. triquetra*. Indeed, somewhat surprisingly, the presence of both PE and N filtrates slightly stimulated ingestion of *H. triquetra.* (We speculate that the lysis of *E. huxleyi* during filtration caused the release of compounds that stimulated *S. acuminatum* feeding.) Taken together, our findings point to a defense mechanism that is a property of individual *E. huxleyi* cells, rather than an inhibitory compound released generally into the environment.

Similar inducible chemical defense systems have been clearly demonstrated in only a few phytoplankton species. One of the most widely studied systems is the production of paralytic shellfish toxins (PST) by the dinoflagellate *Alexandrium minutum*. Waterborne cues from copepods can induce *A. minutum* to increase intracellular PST content within 3 d. Predator-exposed *A. minutum* cells with higher PST content are ingested by copepods at a $\sim$ 15% lower rate than naïve cells when offered in a prey mixture that includes a nontoxic dinoflagellate species. In agreement with our findings, the presence of PE vs. N *A. minutum* had no effect on ingestion of a nontoxic dinoflagellate, suggesting that copepods are capable of perceiving and responding to some indicator of *A. minutum* toxicity associated with individual cells (Selander et al. 2006).

Microzooplankton predators, even the more generalist suspension-feeding ciliates, can be highly selective in choosing prey (Christaki et al. 1998; reviewed by Montagnes et al. 2008). There is strong evidence that protists (including the marine predatory dinoflagellate *Oxyrrhis*

marina) have specialized receptors (e.g., lectins) that bind to molecules such as carbohydrate residues extending from the prey cell surface or to small dissolved molecules in the immediate vicinity of the prey cell, allowing the identification of suitable prey (Ricci et al. 1996; reviewed by Roberts et al. 2011). Such prey selection mechanisms could be exploited by phytoplankton that evolve to produce signals mimicking unsuitable prey, thereby acquiring an effective defense system. Specific molecules involved in such signaling pathways remain largely unidentified, however. One candidate is DMSP; when DMSP is added in bulk to the medium, feeding is inhibited in several microzooplankton species (Strom et al. 2003). The release of DMSP into the phycosphere could theoretically act as an effective directional signal deterring potential predators. However, another study showed that microscale pulses of DMSP actually act as attractants to some microzooplankton (Seymour et al. 2010).

If the key signal involved in inducible defense is present on the cell surface rather than released into the phycosphere, the lack of a defense response in diploid *E. huxleyi* could be related to the presence of coccoliths obstructing predator access to important cell-surface molecules. The defense response observed in haploid *E. huxleyi* could well be expressed by the diploid phase, but masking by coccoliths may reduce its efficacy.

Finally, an interesting aspect of this study was the observation that reduced ingestion rates on PE haploid cells in experiment I were due in part to a smaller percentage of ciliates that were feeding, further supporting the idea that ingestion of defended *E. huxleyi* is not necessary for infochemical detection by *S. acuminatum*. This may especially be the case when ciliate food vacuoles contain more prey at the start of the experiment, as they did in experiment I. Ciliates in experiment II had a slightly different feeding history, resulting in lower initial vacuole content of the maintenance prey *H. triquetra*. In this experiment, we did not observe a lower percentage of ciliates feeding on PE than N *E. huxleyi*, suggesting that hungrier ciliates were less discriminating in their choice to feed or not feed.

Haploid–diploidy and defense—There were important differences in how *S. acuminatum* interacted with the two phases of *E. huxleyi*. Overall, ingestion rates were higher on diploid *E. huxleyi.* This was true on a biovolume basis (Fig. 2) as well as a per cell basis (Fig. 3) and was possibly due to an increased capacity to capture or handle the larger diploid cells. However, ciliates appeared to drastically reduce or cease ingestion of diploid cells sometime after 30 min, resulting in reduced vacuole prey content by 24 h in all treatments (Fig. 2). This decrease in vacuole content did not occur for ciliates fed f/2-grown haploid *E. huxleyi* or *H. triquetra*; in these treatments, vacuole content continued to increase at least up to 24 h (although only slightly for f/2-grown haploids). Diploid *E. huxleyi* (and f/50-grown haploids) may be poor-quality prey, causing ciliates to stop or slow their feeding to conserve resources. Alternately, the coccoliths produced by diploid *E. huxleyi* may be indigestible, causing a slowing of feeding once food vacuoles are filled with particulate inorganic carbon. Low nutritional quality of the diploid phase may protect it from predators, reducing the need for an inducible defense as observed in the haploid phase, and promoting bloom formation.

The different capacity for inducible defense in the two life-cycle phases of *E. huxleyi* suggests an important role for the haploid–diploid life cycle in this species. Several hypotheses exist regarding the selective pressures involved in the maintenance of a haploid–diploid life cycle, the best supported of which are the challenges of a variable or seasonally changing environment. The existence of separate spatial or temporal niches can select for multiple morphotypes in a single species, and it has been proposed that one of the simpler mechanisms by which this could occur is via development of morphologically different life-cycle phases (Valero et al. 1992; Mable and Otto 1998).

Several examples exist supporting the variable environment hypothesis for the role of haploid–diploidy in the macroalgae, some of which involve herbivore defenses. In the macroalgae, haploid–diploidy involves the alternation of generations of multicellular sporophytes (diploid) and multicellular gametophytes (haploid). In the genus *Iridaea* (Rhodophyceae), the two life-cycle phases are separated spatially and seasonally, and results of herbivory experiments show that diploid sporophytes are more resistant to grazing (Hannach and Santelices 1985). Similarly, Vergés et al. (2008) found that in *Asparagopsis armata* (Rhodophyceae), diploid sporophytes had higher chemical defense metabolite concentrations and were correspondingly less preferred by grazers than were male haploid gametophytes.

The two phases of *E. huxleyi* are differently adapted to a variety of environmental and biological factors, and transcriptome analyses have shown extensive differences in gene expression. Haploid *E. huxleyi* have a more streamlined metabolism and express genes related to vitamin synthesis and the use of ammonium as a nitrogen source; these are apparently suppressed in the diploid phase (Rokitta et al. 2011). In addition, haploid *E. huxleyi* have $\sim$ 20% lower transcriptome richness than diploids (von Dassow et al. 2009). Haploid *E. huxleyi* are also well defended against a virus that readily attacks diploid cells (Jacquet et al. 2002; Frada et al. 2008). However, haploid *E. huxleyi* begin to show photoinhibition above $\sim$ 400 μmol m^{-2} s^{-1} irradiance, whereas the diploid phase tolerates high irradiance levels (up to 1000 μmol m^{-2} s^{-1}) with no photoinhibition. This may allow diploid *E. huxleyi* to maintain high growth rates and form blooms during conditions with light levels damaging to the haploid phase as well as many other phytoplankton species (Houdan et al. 2005; Loebl et al. 2010).

Recent evidence points to the importance of the haploid phase in the ecology of *E. huxleyi*, which has been shown to exist at low concentrations concurrent with diploid populations (Campbell et al. 1994; Frada et al. 2012). Meiosis by diploids to produce haploids apparently occurs near the end of diploid bloom periods (Frada et al. 2012), supporting the hypothesis that haploid *E. huxleyi* are uniquely adapted to persist during periods unfavorable to the diploid phase by having a streamlined metabolism and

possessing effective defenses against predators and viruses. If haploid *E. huxleyi* act as gametes in addition to reproducing asexually (as has been observed in the haploid phases of two other species of coccolithophore, Houdan et al. 2004), sexual recombination could be another important role for this cell type. Indeed, an antipredatory defense would improve the chances of haploid cells, as gametes, surviving to locate a suitable partner for sexual fusion. Further research is necessary to explore the role of the haploid phase and its inducible defense for *E. huxleyi* sexual reproduction and bloom dynamics.

Role of nutrients—The medium in which *E. huxleyi* was grown (f/2 vs. f/50) had a large effect on *S. acuminatum* ingestion rates, both inherently and in combination with predator exposure. Considering the response to N cells shows the effect of nutrient status alone. Ciliates initially ingested f/50-grown diploids at a higher rate than f/2-grown diploids, possibly a response to the larger size of the former. However, after 10 min, ingestion on the larger f/50-grown diploid cells was reduced to a rate lower than found on f/2-grown diploids, possibly via some mechanism of prey quality identification (Fig. 2). In the case of haploid *E. huxleyi*, ingestion rates were higher on N cells grown in f/2 than on N cells grown in f/50, as expected, without the complicating factor of differing cell sizes. In contrast to diploid *E. huxleyi*, where vacuole content dropped drastically, or haploid *E. huxleyi*, where vacuole content increased or decreased slightly, by 24 h ciliates fed the high-quality prey *H. triquetra* contained 3X as much prey as at 30 min. This occurred despite initial ($<$ 30 min) ingestion rates on *H. triquetra* being comparable with those for diploid *E. huxleyi*. *S. acuminatum* likely has multiple strategies for selecting and ingesting prey that interact in complex ways. Our observations that low prey nutritional quality can lead to reduced predation rates are in good agreement with previous work (John and Davidson 2001; Strom and Bright 2009).

We observed a large defense response in haploid *E. huxleyi* under nutrient-replete conditions, with a substantially delayed defense response (evident only at 24 h) when haploids were grown in f/50 medium. This reduced or delayed capacity for defense in f/50-grown haploid cells suggests that the defense response is costly and that cells lacking sufficient nitrogen (and possibly other nutrients) are unable to spare the resources required for fast activation. The costs associated with induced defenses in terrestrial plants can be high (Strauss et al. 2002). Our findings are similar to those of Selander et al. (2008), who found that predator-induced increases in *A. minutum* PST content did not occur under nitrogen limitation. Rotifers also require higher prey densities to maintain positive growth after morphological defenses are induced by the presence of a predator (Aránguiz-Acuña et al. 2011).

Although *E. huxleyi* grown in f/50 had reduced nitrogen availability (as evidenced by high C : N ratios), other nutrients required for defense induction might also have been in short supply. Clearly, the effect of nutrient availability on defense in haploid *E. huxleyi* is significant, with implications for community ecology under conditions of variable nutrient supply in the ocean. Identifying the mechanism of defense, associated cellular machinery, and specific nutrient requirements is necessary to further our understanding of this inducible defense and its consequences for natural communities and *E. huxleyi* bloom formation.

Acknowledgments

We thank Stephen Sulkin, Benjamin Miner, and Brady Olson for contributing invaluable input regarding experimental design, statistical analyses, and manuscript revision. Gordon Wolfe gave advice on methods development and experimental design. Kelley Bright and Kerri Fredrickson provided extensive culturing, flow cytometry, and microscopy support. Tristen Wuori assisted with sample processing and methods development. We also thank two anonymous reviewers for their comments, which greatly improved this manuscript.

This research was supported by grants 0604296 and 1021189 from the National Science Foundation, as well as grants from the Graduate School, Shannon Point Marine Center, and the Biology Department at Western Washington University.

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Associate editor: David A. Caron

Received: 07 August 2012
Accepted: 16 January 2013
Amended: 24 January 2013