



Effects of barley straw (*Hordeum vulgare*) on freshwater and brackish phytoplankton and cyanobacteria

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Received 31 March 2003; revised and accepted 12 August 2003

Key words: phytoplankton, cyanobacteria, barley straw

Abstract

A short-term laboratory study was conducted to investigate the effect of barley straw in controlling several common phytoplankton and cyanobacterial species. Following a one-month incubation of barley straw in coarsely filtered fresh Potomac River and brackish Patuxent River waters, the growth of six autotrophic taxa was followed in culture. Barley straw slurry reduced the yield of three taxa (*Ankistrodesmus falcatus*, *Chlorella capsulata*, *Isochrysis* sp.) in comparison with cultures not receiving the slurry. Although no significant changes in growth were detected with three other taxa (*Cyclotella* sp., *Prorocentrum minimum*, freshwater *Pseudanabaena* sp.), some patterns indicated potential impacts of the barley straw. First, a higher addition of straw to *Cyclotella* sp. resulted in a lower biomass accumulation than in cultures receiving lower levels. Second, the bloom-forming dinoflagellate *Prorocentrum minimum* was apparently stimulated at low barley straw levels, perhaps suggesting conditions associated with the straw (metals-chelation, bacterial-produced nutrients) might stimulate dinoflagellate growth. Third, species shifts were observed in two of the cultures, with barley straw favoring shifts from *Isochrysis* to a *Cyclotella* sp. – *Thalassiosira* sp. mixture and shifts from *Pseudanabaena* to a *Pseudanabaena* – *Scenedesmus* mixture. These results provide new records for the susceptibility of freshwater and brackish phytoplankton taxa to barley straw exposure, including species-specific responses and shifts in species dominance in mixed assemblages.

Introduction

In an attempt to reduce the growth and accumulation of algae in some aquatic systems, several techniques have been explored over the last half century, including the addition of copper sulfate, aeration, manipulation of top piscivores, and in the last decade, the addition of barley straw to ponds and small reservoirs. The use of the last method is becoming increasingly frequent, particularly in the United Kingdom (Everall & Lees, 1996; Barrett et al., 1999), with firms providing bundled barley straw for algal control in fish ponds, canals, and small commercial systems. Exposures vary, but generally barley straw must age in

ambient waters prior to mass algal accumulation to be most effective.

Concentrations of barley straw limiting algal biomass vary across laboratory and small freshwater systems. Barrett et al. (1999) reported effective control of reservoir phytoplankton including diatoms and cyanobacteria, at dosages as low as 6 g m⁻³ (6 mg L⁻¹). Much higher levels, 440 g dry wt barley straw m⁻³, were required in a canal with a turnover time of 5.3 h (Welch et al., 1990). Similarly, barley straw applied to the Press Top Reservoir, UK, reduced the spring diatom bloom and caused a shift in dominance in spring and summer assemblages, severely reducing the summer *Aphanizomenon* – *Anabaena* bloom (Everall &

Lees, 1996). In the laboratory, *Microcystis aeruginosa* was inhibited at 2.57 g dry wt barley straw m^{-3} in one study (Newman & Barrett, 1993), a concentration much lower than most field applications; Butler (1998) & M.D. Ferrier, B.R. Butler, D.E. Terlizzi & R.V. Lacouture (pers. comm.) have found a similar inhibition in this taxon. Martin & Ridge (1999) reported this taxon to be the most sensitive of 22 species and strains tested, but with 70 g dry mass m^{-3} required for 50% reduction in yield in 4–8 d assays. Several taxa were insensitive to barley straw, or were even stimulated. A number of examples of stimulation were reported in a study by Butler (1998). Of 12 species examined, 8 species increased over a two-week period on exposure to barley straw extract.

Brackish waters also routinely experience algal 'blooms', but the application of barley straw to brackish or tidal waters has not been reported, although there are some unpublished observations suggesting highly variable, species-specific responses. In a laboratory study, barley straw enhanced growth of three estuarine dinoflagellate taxa *Prorocentrum minimum*, *P. micans*, and *Gyrodinium instriatum*, inhibited growth in *Karlodinium micrum* (*G. galatheanum*), *Akashiwo sanguinea* (*Gymnodinium sanguineum*), *Heterocapsa triquetra*, and *H. pygmaea*, and had no effect on *G. estuariale*, *G. uncatenum*, *Ceratium furca* and *Peridinium* sp. (Terlizzi et al., 2002).

The present study was undertaken to explore and compare the impacts of barley straw on brackish taxa with some freshwater species, using both cultured and field assemblages collected from Chesapeake Bay tributaries. Results could indicate whether populations of unwanted members of the natural assemblages of the region might be controlled by the addition of readily available barley straw to ambient waters as well as expand community knowledge of taxa susceptible to barley straw control.

Materials and methods

The study was conducted at the Estuarine Research Center of the Academy of Natural Sciences laboratory in St. Leonard, MD, USA. Five phytoplankton cultures maintained at the laboratory were grown in filtered and autoclaved Patuxent River water approximating 11 psu. Culture conditions were 14:10 L:D cycles with cool-white fluorescent lighting and 19°C. Aliquots from exponential phase stock cultures of *Chlorella capsulata* (Provasoli-Guillard Col-

lection, CCMP245), *Isochrysis* sp. (T-iso, Provasoli-Guillard Collection, CCMP1324) and *Prorocentrum minimum* were transferred to Gelman AE-filtered brackish Patuxent River water enriched with f/2 nutrients (Guillard and Ryther 1962); each taxon was distributed to 9 tubes, with 0.04 L medium in each. Freshwater taxa, i.e., *Ankistrodesmus falcatus* and *Cyclotella* sp., were transferred to deionized water enriched with HYCO nutrients (Riedel & Sanders, 1996). A freshwater *Pseudanabaena*-dominated assemblage, obtained from field-deployed cubitainers (translucent, flexible containers) of upper Potomac River water enriched with f/2 nutrients, was transferred to filtered Potomac River water enriched with HYCO nutrients. All tubes were returned to the incubator for initial growth.

One month prior to setting up the culture tubes, 0.2 L of AE-filtered Patuxent River (12 psu) and tidal-fresh Potomac River water were decanted into small cubitainers containing 5 g diced barley straw. Foil-covered, loosely capped containers were returned to the incubator and shaken daily.

After an initial growth period of 4–7 d, *in vivo* fluorescence (IVF) of each tube was determined using a Turner Designs 10–005 field fluorometer. The aged barley straw slurry was then added to 6 of the 9 tubes for each taxon, yielding 3 tubes with no barley straw, 3 with low barley straw levels (312.5 mg barley straw L^{-1}), and 3 with high straw levels (1250 mg barley straw L^{-1}). IVF was again recorded. The IVF of algae-free filtered medium plus the low and high barley straw additions was also noted, and subtracted from IVF of each tube. All tubes were placed in the incubator and at day to two day intervals, the loosely capped tubes were gently inverted several times to mix tube contents. At 4–8 d intervals, IVF of each gently inverted tube was recorded.

Growth was rapid and consequently, dilution with medium and new additions of barley straw were necessary throughout the experiment. IVF was always determined prior to and after such dilutions, and final IVF recorded following corrections for dilution. Aliquots from tubes were also removed aperiodically, fixed with Lugol's iodine solution, and cell densities and composition determined by light microscopy at magnifications of 250–400 x.

Differences in cell yield were (with one exception) determined through an analysis of variance of IVF at the end of each taxon's growth period. IVF differences between the 3 barley straw levels for *Isochrysis* were determined at day 23, at a time just prior to a change

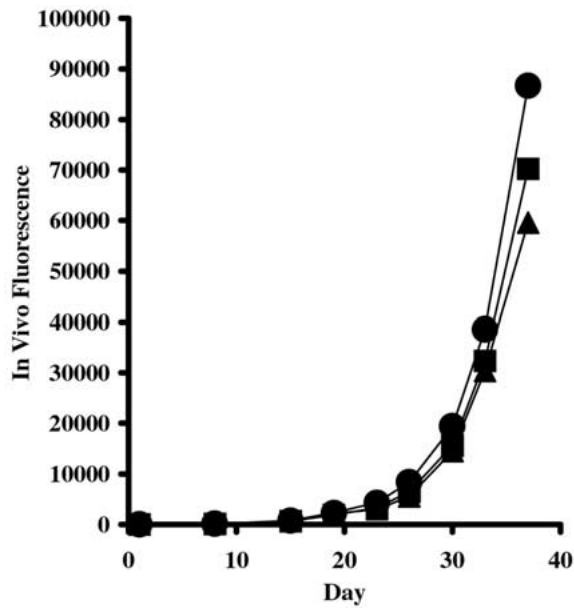


Figure 1. Effect of barley straw on growth of *Ankistrodesmus falcatus* ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹.

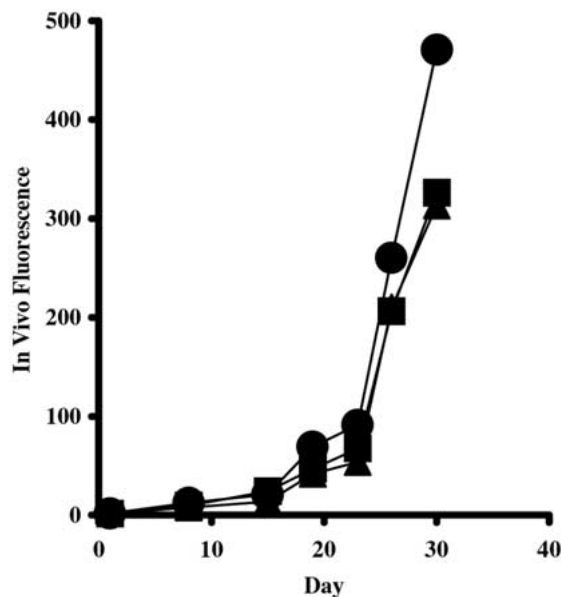


Figure 2. Effect of barley straw on growth of *Chlorella capsulata*. ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹.

in the dominant species to a *Cyclotella-Thalassiosira* mixture.

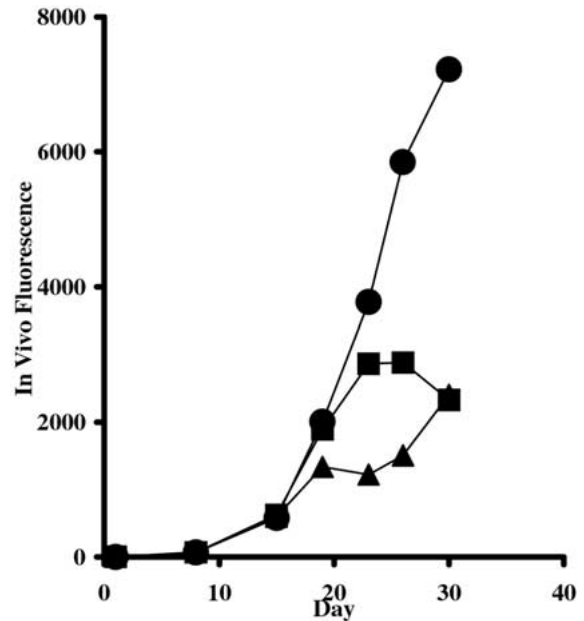


Figure 3. Effect of barley straw on growth of *Isochrysis* sp.: ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹. A mixture of *Cyclotella* sp. and *Thalassiosira* sp. became dominant by day 23.

Results

The growth of three taxa (Figures 1–3) was reduced in the presence of aged barley straw slurry (Table 1). *Ankistrodesmus falcatus*, *Chlorella capsulata*, and *Isochrysis* sp. IVF was higher with no addition of straw, and lower at 312.5 and 1250 mg L⁻¹, respectively. In the case of *A. falcatus*, there was a greater impact as the concentration of barley straw increased (Figure 1), while in *C. capsulata*, the two barley straw concentrations resulted in similar depressed yield in IVF (Figure 2). Figure 3 indicates similar results for *Isochrysis* sp. with the two levels, demonstrating a significant impact of the straw. However, barley straw depressed *Isochrysis* and either stimulated or opened a niche for a mix of two other taxa, another *Cyclotella* sp. and a small *Thalassiosira* sp., and the new taxa began to increase after day 23 (Figure 4). Therefore, differences in *Isochrysis* IVF were examined at day 23, and a pattern similar to that for *Ankistrodesmus* was observed: highest growth with no straw and decreasing production with increasing straw concentration.

In the case of cultures started as *Cyclotella* sp. (Figure 5), assessing the impact of straw by comparisons with and without the straw proved impossible, as two of the three control tubes perished within the first week. As a result, there were only sufficient replicates

Table 1. Barley straw-induced differences in cyanobacteria or phytoplankton

Taxon	Day	Barley straw effect on IVF	df	F-statistic	p	Other effects
<i>Ankistrodesmus falcatus</i>	37	decrease	2	31.000	0.0007	–
<i>Chlorella capsulata</i>	31	decrease	2	6.752	0.029	–
<i>Isochrysis</i> sp.	23	decrease	2	93.629	0.00002	shift to <i>Cyclotella-Thalassiosira</i>
<i>Cyclotella</i> sp.	31	no detectable effect*	1	4.494	0.101	decrease at high straw
<i>Prorocentrum minimum</i>	31	no detectable effect	2	1.987	0.218	stimulation by straw?
<i>Pseudanabaena</i> sp.	31	no detectable effect	2	1.714	0.258	shift to <i>Scenedesmus abundans</i>

* Growth was observed in only 1 of 3 control replicates so no comparison could be made between the control cultures and low or high barley straw levels.

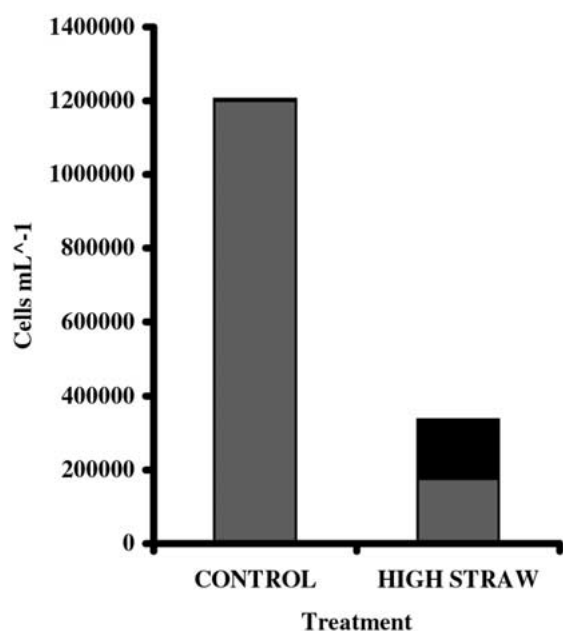


Figure 4. Shift in composition over 23 d from assemblage overwhelmingly dominated by *Isochrysis* (gray bar) to assemblage with the co-dominants *Cyclotella-Thalassiosira* (black bar) after exposure to high levels of barley straw extract (1250 mg barley straw L⁻¹).

to compare yields with the two different concentrations of barley straw slurry. The single control with *Cyclotella* sp. yielded similar IVF values as the tubes with the lower level of barley straw.

Changes in IVF were not detected using the three barley straw slurry levels for the *Pseudanabaena*-dominated assemblage tubes (Figure 6). However, microscopy indicated a shift in species in the barley straw replicates, from *Pseudanabaena* to a *Scenedesmus* (*S. abundans*)–*Pseudanabaena* mixture (Table 1, Figure 7). Tubes without straw were characterized by filaments of *Pseudanabaena* or amorphous ag-

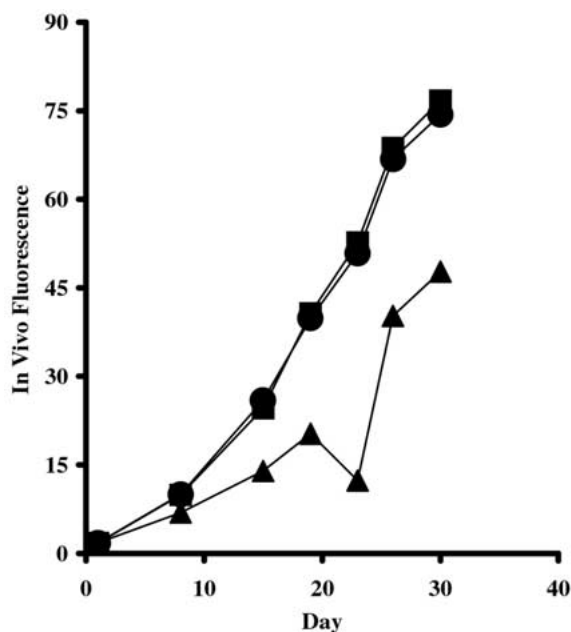


Figure 5. Effect of barley straw on growth of a second *Cyclotella* sp.. ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹.

gregates of this cyanobacterium. Although the few samples enumerated indicated potential inhibition of *Pseudanabaena* by straw, the number of preserved samples was insufficient to assess whether this effect was significant. *S. abundans* density also appeared to be higher in samples with straw compared with the control, but low sample number again prevented a clear-cut assessment.

Barley straw had no detectable impact ($p = 0.055$) on the bloom-forming dinoflagellate *Prorocentrum minimum* (Figure 8). However, barley straw at low levels may possibly enhance growth of *P. minimum*, judging by the higher IVF levels of cultures receiving the straw slurry.

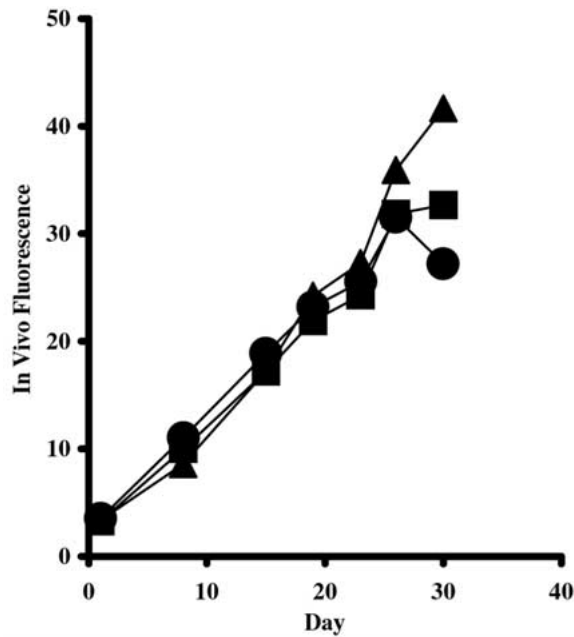


Figure 6. Effect of barley straw on growth in an *Pseudanabaena*-dominated assemblage from the tidal fresh Potomac River. ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹. By the end of the experiment, the *Pseudanabaena*-dominated assemblage had shifted to a mixture of *Scenedesmus abundans*-*Pseudanabaena* in tubes enriched with barley straw extract.

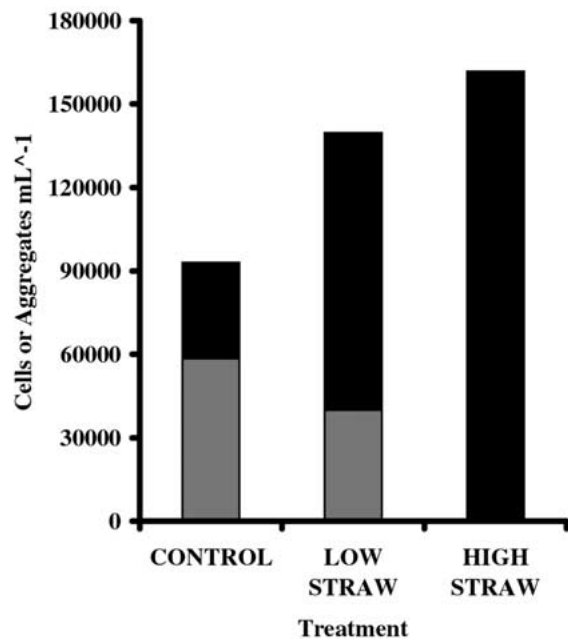


Figure 7. Increasing *Scenedesmus abundans* (black bar) over 29-d exposure to barley straw extracts from an initial Potomac River assemblage dominated by *Pseudoanabaena* (gray bar). Control, low and high straw are equivalent to 0, 312.5, and 1250 mg barley straw L⁻¹, respectively.

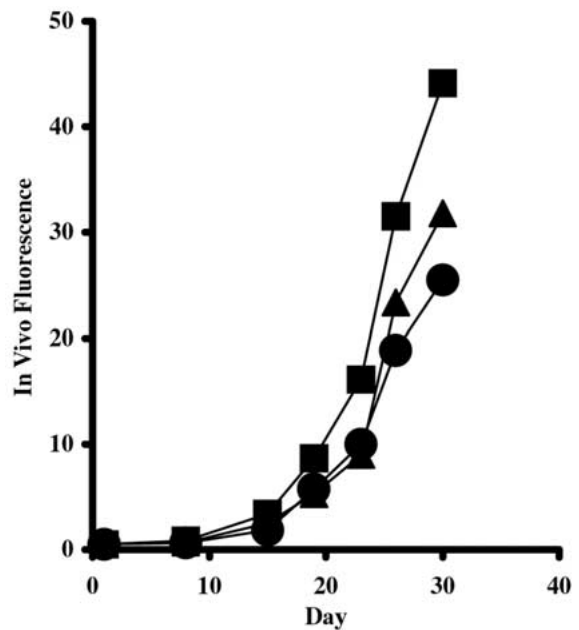


Figure 8. Effect of barley straw on growth of *Prorocentrum minimum*. ● no straw; ■ 312.5 mg L⁻¹; ▲ 1250 mg L⁻¹.

Discussion

The present study provides further evidence to support the use of barley in controlling algal growth in freshwater and brackish systems. In particular, it helps to emphasize the practical value of using such a readily affordable and easily available substrate in estuarine waters. Three taxa showed an obvious reduction in biomass when exposed to an aged barley straw slurry (312.5–1250 mg L⁻¹) in comparison to the controls. These included one freshwater (*Ankistrodesmus falcatus*) and two brackish taxa (*Chlorella capsulata*, *Isochrysis* sp.). There were also strong indications that growth of two other taxa was reduced. The presence of straw depressed growth of (freshwater) *Pseudanabaena* sp., it being replaced by *Scenedesmus abundans*. One of two freshwater strains of *Cyclotella* in the study showed the least growth at the highest level of barley straw. Thus, five of six initially dominant taxa examined were adversely affected by the addition of aged barley straw.

The effectiveness of barley straw for controlling algae and cyanobacteria, however, is most clear, when considered for individual species, as impacts are highly species-specific and not phyla-specific. For example, the reductions in *Pseudanabaena* (Figure 7) cannot be extrapolated to general control of all cy-

anobacteria with barley straw, as other investigators have reported stimulatory as well as inhibitory responses for particular species and strains. Susceptible populations of field *Aphanizomenon flos-aquae* were found in the Press Top reservoir in 1994 following treatment with 50 mg L⁻¹ barley straw; the straw eliminated the cyanobacterium even though it had occurred as a summer dominant during the previous three years, when no straw treatment had been used (Everall & Lees, 1996). This parallels declines in summer cyanobacteria noted for a reservoir near Aberdeen, Scotland following barley straw treatment (Barrett et al., 1999). In laboratory studies, M.D. Ferrier, B.R. Butler, D.E. Terlizzi & R.V. Lacouture (pers. comm.) noted reduced growth in *Microcystis aeruginosa* when exposed to 7.2 g extract L⁻¹ for 2 weeks, similar to responses noted for a cultured strain (Martin & Ridge, 1999) and field populations (Newman & Barrett, 1993). Martin & Ridge (1999) also noted susceptibility in *Oscillatoria redekei*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae*, and *Synechococcus*. However, growth of *O. animalis* (Martin & Ridge, 1999) and *Oscillatoria lutea* var. *contorta* and was enhanced, while another strain of *Anabaena flos-aquae* showed no response to the additions (latter two observations, pers. comm from M.D. Ferrier, B.R. Butler, D.E. Terlizzi & R.V. Lacouture). Hence, barley straw impacts must be explored at the individual species level.

The brackish water dinoflagellate, *Prorocentrum minimum*, was apparently stimulated by the presence of aged barley straw in the medium (Figure 8). Stimulation in *P. minimum* was also observed in other barley straw experiments (Terlizzi et al., 2002), with two other estuarine dinoflagellates *Gyrodinium striatum* and *P. micans* similarly enhanced. Further examples of stimulation include *Nitzschia filiformis* var. *conferta* (Martin & Ridge, 1999), and 8 freshwater taxa (*Ulothrix fimbriata*, *Scenedesmus quadricauda*, *Selenastrum capricornutum*, *Spirogyra* sp., *Chlorella vulgaris*, *Oscillatoria lutea* var. *contorta*, *Anabaena flos-aquae*, *Navicula* sp.) (Butler, 1998).

For *P. minimum*, a mixotroph, decomposing barley straw might provide needed micronutrients and/or other food particles for supplementing its autotrophic nutrition resulting in enhanced growth compared with photoautotrophic growth. Glibert et al. (2001) have argued that blooms of this dinoflagellate in a tributary of Chesapeake Bay are a response to increasing organic nutrients, specifically urea, due to its higher specific uptake rates for this substrate than for nitrate. The

response observed in the present study might complement those of Glibert et al., with dissolved organic matter derived from barley straw acting as growth substrates. Additional experiments should be undertaken to identify barley straw impacts on nuisance taxa that feed as mixotrophs (like *P. minimum*) or strict heterotrophs (e.g. *Pfiesteria* spp., *Dinophysis* spp.).

Scenedesmus abundans also appeared to increase in response to extract exposure (Figure 7) although only single samples were examined preventing definitive elucidation of straw-induced stimulation. This response is similar to stimulation noted for *S. subspicatus* exposed to 4 g dry mass barley straw L⁻¹ (Martin & Ridge, 1999) and slight increases in *S. quadricauda*, relative to control, when exposed to aged barley straw extract (7.2 g L⁻¹) for 2 weeks (Ferrier et al., unpubl. data). Although an extensive list of *Scenedesmus* species has not been assayed yet, the present results coupled with earlier observations suggest that this genus may be less susceptible to barley straw than others.

Detection of a barley straw effect on the phytoplankton used in the present study required 3–5 week exposures to pre-aged barley straw, and appear to be longer periods than Barrett et al. (1999) noted for impacts in the Aberdeen, Scotland, reservoir of their study. Terlizzi et al. (2002) employed 2 week incubations, while Martin & Ridge (1999) detected 50% reductions in algal production for their species in 4–8 d assays. The differences likely reflect a number of conditions unique to each experiment, including the decomposition process used in each study. Sufficient decomposition and accumulation of breakdown products must occur before an effect might be detected (Gibson et al., 1990). This was readily apparent in our investigations where dried barley straw was added to diluted water samples from three eutrophic systems in the Chesapeake Bay watershed, one freshwater cyanobacteria-dominant system and two estuarine systems, dominated by flagellates and dinoflagellates, respectively. In no case was there any impact from the barley straw, even at the elevated water temperatures (>24 °C) of Chesapeake Bay in summer, and the lack of impact from the added dry straw was attributed to insufficient time for decomposition and accumulation of inhibitory by-products. The elevated temperatures permitted rapid algal growth to levels too high to be impacted by the slowly accumulating straw decomposition products. This conclusion is partially supported by the results obtained with the

Pseudanabaena-dominant assemblage experiments as well as those with individual cultures.

The results of this study plus those reported previously suggest that the use of barley straw to control algae in fresh and brackish waters is a practical approach to increasingly frequent overgrowths observed in many eutrophic systems globally. Although species-specific in its impact, wider application and field experimentation should be considered, with field managers encouraged to look for reductions in weeks to months, rather than immediate, post-application collapses in some nuisance species.

Acknowledgements

The authors express their gratitude to A.M. Hartsig for providing the initial cultures. She and F. Acker also assisted in identifying species and their efforts are much appreciated. Our thanks also go to RV. Lacouture for permitting use of the Turner Designs fluorometer and to M. Bundy for use of culture tubes and incubator.

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