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Note

Reconstructing the initiation of the Texas brown tide bloom of *Aureoumbra lagunensis* from archived samples using an immunofluorescence assay

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Harmful algal blooms in coastal marine environments have been increasing in frequency in recent years (Anderson 1989; Smayda 1989; Hallegraeff 1993) and this has been related to the increasing anthropogenic influences on coastal waters. To our knowledge, the longest lasting harmful algal bloom ever reported has been the "brown tide" bloom of the alga *Aureoumbra lagunensis* (Stockwell, DeYoe, Hargraves et Johnson) that has impacted the Laguna Madre of Texas, USA, from January 1990 through October 1997 (Buskey et al. 1996; Buskey et al. 1998). The Laguna Madre is a shallow, often hypersaline coastal lagoon (Armstrong 1987) that contains extensive seagrass beds (Quammen & Onuf 1993). The reduced water clarity associated with this persistent bloom reduced the distribution of seagrasses in deeper waters (Dunton 1994; Onuf 1996). The extended bloom also had negative impacts on other parts of the Laguna Madre ecosystem, including reductions in biomass and diversity of planktonic and benthic grazers on phytoplankton (Buskey & Stockwell 1993; Montagna et al. 1993; Buskey et al. 1997).

Algal blooms generally undergo several distinct phases in their population dynamics including initiation, growth, maintenance and dissipation (Steidinger et al. 1998). Many harmful algal blooms are not recognized until they are late in the growth phase and they have become abundant enough to cause visible harmful effects. As a result, there is very little information on the environmental conditions during the initiation of harmful algal blooms. In the case of the Texas 'brown tide' bloom, an ecosystem study had started in March 1989 in the region of the Laguna Madre where the brown tide began, resulting in nearly a year of data preceding the initiation of the bloom. This previously unknown phytoplankton species is morphologically indistinct and therefore impossible to recognize in phytoplankton samples at low concentrations using traditional microscopic examination of samples. Using archived zooplankton samples preserved in whole seawater from the site of collection, an immunofluorescence assay was used to identify *A. lagunensis* cells at pre-bloom concentrations, and to reconstruct the population dynamics of the bloom initiation. Understanding the precise timing of the *Aureoumbra lagunensis* bloom initiation is important because it has been hypothesized that a severe freeze in December 1989 may have stimulated this bloom through an extensive fish kill that released a large pulse of dissolved inorganic nitrogen into the Laguna Madre (Whitledge 1993; DeYoe & Suttle 1994).

Zooplankton samples were collected from March 1989 through December of 1990 at two locations in the upper Laguna Madre (Stns A, B) and two locations within Baffin Bay (Stns C, D, Fig. 1). Replicate samples were collected using a pair of 20-cm diameter, 153-μm mesh plankton nets mounted on a bongo frame. The nets were towed below the surface for 5 min. The contents of the cod end were passed through a course mesh (5 mm) to remove ctenophores and cnidarian medusae before being placed in the sample jar. The samples were placed in one liter wide mouth plastic jars, whole seawater and 50 ml of buffered formaldehyde were added to the jars to bring the volume to one liter. Upon return to the laboratory, the zooplankton samples were split into equal halves using a Folsom plankton splitter. One half was further subsampled for enumeration of zooplankton and the other half was stored at room temperature. In 1997, the archived samples were thoroughly mixed and a small subsample (20 ml) was passed through a 153-μm mesh sieve to remove mesozooplankton, and these subsamples were used for the immunofluorescence assay.

The immunofluorescence assay followed the protocol of Lopez-Barreiro et al. (1998). Between 100 and 400 μl of sample was added to a test tube containing 1 ml of 3% goat serum (Sigma Immuno Chemicals G-9023). The sample was gently agitated and incubated at room temperature for 30 min. The contents were poured into a filter funnel with a 5-μm porosity nitrocellulose backing filter under a 2.0-μm black polycarbonate filter. The tube was rinsed with 10 mM phosphate buffered

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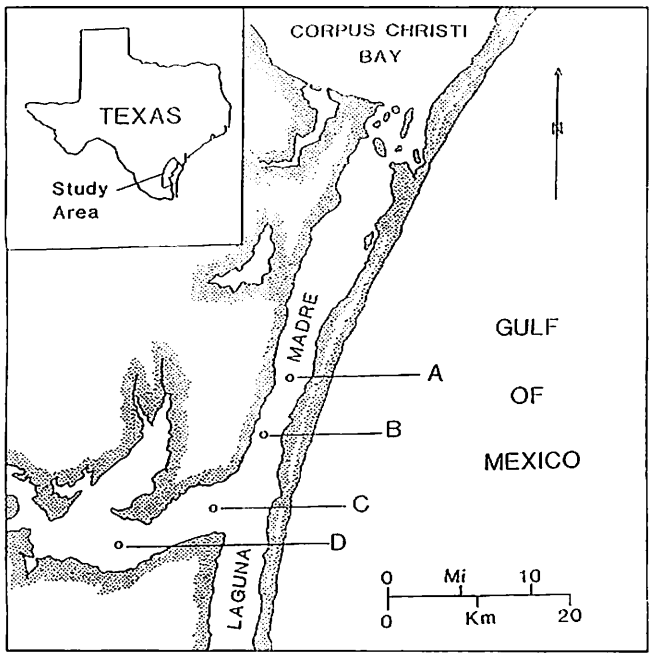

Fig. 1. Locations of sampling sites in the Laguna Madre, Texas, USA.

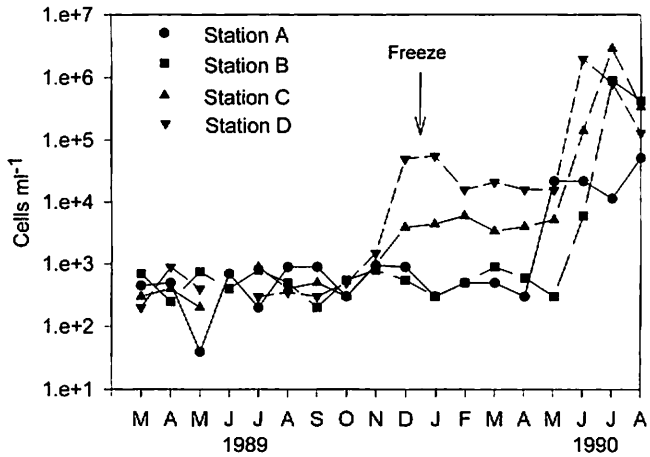

Fig. 2. Abundance of *Aureoumbra lagunensis* cells detected in archived zooplankton samples from March 1989 through August 1990 using the immunofluorescence assay. "Freeze" indicates a severe freeze on 23 December 1989 (see text).

saline (PBS), pH 7.4 (Sigma P-3813) and the rinse was also added to the filtration funnel. The sample was filtered to <2 ml volume without letting the filter dry, and rinsed two more times with 10-ml PBS. One ml of antiserum BTD-22 (R-5310 and R-5313, Charles River Pharmservices) at a concentration of 1 : 1000 (antibody : PBS) was added to the filtration funnel and incubated for 30 min. The filter was then rinsed three times with 10-ml PBS, 1 ml of secondary antiserum (Anti-Rabbit IgG FITC Conjugate; Sigma F-0382) was added and incubated for 20 min. The filter was again rinsed three times with 10-ml PBS, and the black polycarbonate filter was mounted on a glass slide, with one drop of glycerol-PBS (9 : 1) and topped with a cover slip. Slides were immediately frozen and stored in the dark until enumerated with an Olympus IMT-2 or BHS epifluorescence microscope at 1000× magnification using blue excitation. Antibody specificity was confirmed against 22 cultures in a double-blind test. At high titers (1 : 100), the antibody reacted with other Pelagophytes. At the dilution used (1 : 1000), only *A. lagunensis* reacted with the antibody. Details may be found in Lopez-Barreiro et al. (1998).

Since *A. lagunensis* had not been described as a species prior to this bloom (DeYoe et al. 1997), and since similar brown tides had not been previously reported in Laguna Madre, there was speculation that this might be an introduced species. Our data do not support this. Before the initiation of the bloom late in 1989, *A. lagunensis* cell densities ranged between 40 and 1000 cells ml^{-1} (Fig. 2). These are similar to cell densities found in other Texas bays under non-bloom conditions using the immunofluorescence assay (Lopez-Barreiro, 1998). Based on our findings and those of Lopez-Barreiro (1998), *A. lagunensis* appears to be normally present at low densities in coastal bays throughout the western Gulf of Mexico, and can be considered part of the "hidden flora" because of its small size and indistinct morphology.

During the bloom period, cell concentrations generally ranged from 1.6×10^4 to 2.9×10^6 cells ml^{-1} based on the immunofluorescence assay (Fig. 2). Cell counts made with a hemocytometer on Lugol's iodine preserved samples from these same stations (Buskey & Stockwell 1993; Buskey et al. 1996) are typically 2× higher on average. This difference may be due in part to overestimates of *A. lagunensis* cells densities in Lugol's preserved samples, since this species cannot be positively identified based on morphological characteristics alone and other similarly sized cells may have been included in counts. However, we cannot exclude the possibility that a significant fraction of *A. lagunensis* cells were lysed during the 8 year formalin storage, but we note that the counts using the antibody were the same order of magnitude as the hemocytometer counts made during the bloom study. In addition, Lopez-Barreiro (1998) found 30% higher cells counts during laboratory growth studies of *A. lagunensis* using transmitted light than using the immunofluorescence assay, providing evidence for some cell loss during processing for the immunofluorescence method.

The initiation of the bloom has been linked to a severe freeze on 23 December 1989, with the resulting fish kill releasing a large pulse of nutrients which stimulated bloom initiation (Whitledge 1993; DeYoe & Suttle 1994). However, samples from stations in the upper reaches of Baffin Bay preserved in Lugols iodine indicated that a bloom of cells similar in size to *A. lagunensis* began as much as 3 months prior to the freeze (Buskey et al. 1997). Unfortunately, these cells could not be positively identified since *A. lagunensis* is morphologically indistinct based on standard light microscopy, and the immunofluorescence technique does not work on Lugol's iodine preserved samples (Lopez-Barreiro 1998). In the present study, samples collected on 19 December 1989 in Baffin Bay (Stns C, D, Fig. 1) demonstrated the presence of *A. lagunensis* at con-

centrations in excess of 50,000 cells ml^{-1} (Fig. 2), confirming that the brown tide alga had already begun to bloom prior to the severe freeze, and therefore other factors must have also contributed to its initiation. Severe freezes under hypersaline conditions and their associated fish kills have occurred numerous times in the Laguna Madre without initiating major algal blooms (e.g. Gunter & Hildebrand 1951), suggesting that the severe freeze of 1989 may have been coincident with, rather than the cause of, the bloom. The collapse of grazer populations during extreme hypersaline conditions in the months prior to the initiation of the bloom (Buskey et al. 1997; Buskey et al. 1998) may have been an important factor in the initiation of the bloom, while the large pulse of nutrients released by the decomposition of the fish kill may have served to intensify the growth phase of the existing bloom.

The immunofluorescence assay was found to work well on formaldehyde preserved samples stored at room temperature for over 8 years. This raises the possibility of testing the hypothesis that harmful algal blooms have increased in frequency in recent years by developing immunofluorescent assays for target species, and testing archived samples held in marine science laboratories throughout the world.

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