

Note

Viability of the Texas brown tide alga, *Aureoumbra lagunensis*, in fecal pellets of the copepod *Acartia tonsa*

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The Texas brown tide was the longest phytoplankton bloom ever documented, lasting without interruption from January 1990 through October 1997 in the Laguna Madre of south Texas (Buskey et al. 1996, Buskey et al. 1998, Buskey et al. 2001). The bloom was caused by a 4–5 µm diameter pelagophyte, *Aureoumbra lagunensis* (DeYoe et al. 1997). The reasons for the persistence of this bloom are largely unknown but it is believed that at least part of the answer is associated with the apparent inability of planktonic grazers in controlling the abundance of *A. lagunensis* (Buskey et al. 1997).

Before the onset of the brown tide, *Acartia tonsa* was the most abundant mesozooplankton in Laguna Madre, but its population, egg production and grazing rates declined during bloom years (Buskey & Stockwell 1993). *A. lagunensis* is a low quality food for *A. tonsa*, due to its small size and perhaps low nutritional value (Buskey & Hyatt 1995). However, *A. tonsa* ingests *A. lagunensis* at comparable rates to those for a similar sized species (Bersano 2000), and therefore avoidance or inability to ingest brown tide cells due to its small size was not the cause for the low egg production observed in *A. tonsa*.

It is possible that *A. lagunensis* is either of poor nutritional value to copepods, or that *A. lagunensis* cells are not completely digested during gut passage. Previous studies have revealed that some alga cells can still be viable after copepod gut passage (Porter 1973, Porter 1976, Adrian 1987, Epp & Lewis 1981, Gottfried 1988), suggesting that they pass through the copepod's gut undigested. This study explores the hypothesis that *A. lagunensis* cells resist digestion and are still viable in copepod fecal pellets. This would not only explain the poor nutrition of copepods in brown tide impacted areas, but might also help explain why grazers have so little impact on brown tide populations.

The objectives of this investigation were to look for the presence of undamaged brown tide cells in *A. tonsa* fecal pellets produced in the laboratory under diets composed of both wild populations and laboratory monocultures of *A. lagunensis*. Culturing *A. lagunensis* cells from fecal pellets

was also attempted to confirm the viability of cells passing through the copepod's gut.

Cultures of *A. lagunensis* used in the experiments were maintained at a salinity of 30 PSU, at 20°C with a light intensity of 120 µmol photons m⁻² s⁻¹ on a 12:12 h light:dark cycle in a 3.0 liter polycarbonate culture flask containing a modified f/2 media, consisting of NH₄Cl instead of NaNO₃ and K/20 levels of trace metals (Keller et al. 1987). All the experiments were run with cultures kept in exponential growth.

Copepods were collected in upper Laguna Madre (27°41'N, 97°13'W) Texas (USA) using a 0.3 m diameter plankton net (1.5 m in length) with 330 µm mesh. Zooplankton samples were screened through 5 mm mesh to remove macrodetritus and jellyfish, placed in a plastic bucket and immediately transported back to the laboratory. Samples of natural water containing wild populations of *A. lagunensis* were also collected for later use in the laboratory.

In the laboratory, three groups of about 30 *A. tonsa* females were sorted and transferred to 1-liter polycarbonate bottles containing natural water screened through a 100 µm mesh to remove large grazers. Replicate samples from the natural water were taken and preserved in formaldehyde (2%) for later determination of *A. lagunensis* cell density. Another three groups of about 30 females were transferred to 1-liter polycarbonate bottles containing monocultures of *A. lagunensis* (approximately 1 mg C liter⁻¹). Both sets of bottles were then placed on a plankton wheel within an environmental chamber under controlled temperature (20°C ± 0.5) and controlled light conditions (12:12 h light:dark cycle). After 24 hours the water of all the bottles was screened through a 153 µm mesh in order to retain copepods, which were immediately transferred to bottles containing fresh food as described before. After another 24 hours, the fecal pellets produced under the natural water diet were retained in a 20 µm mesh and preserved in a 2% formaldehyde solution for later testing by the antibody method developed for *A. lagunensis* by Lopez-Barreiro et al. (1998).

The water from the bottles containing monocultures of *A. lagunensis* was screened first through 153 µm mesh sieves in order to retain copepods, and then through 20 µm mesh sieves

to retain fecal pellets. Both sieves containing copepods (153 μm) and sieves containing fecal pellets (20 μm) were intensively, though gently, rinsed using a squeeze bottle filled with filtered, sterile sea water. Subsequently, they were washed at least 10 times in a series of five 1.5-liter dishes containing filtered-sterile seawater in order to avoid contamination by surface-attached algae from copepods and fecal pellets. As an additional precaution against contamination by external *A. lagunensis* cells, sieves containing copepods were placed in a beaker filled with filtered-sterile seawater and then left at room temperature for approximately 2–4 hours, in order to allow the copepods to produce fecal pellets. This procedure ensured that fecal pellets released under this condition, were never in contact with *A. lagunensis* cultures, further reducing the possibility of contamination by unwanted attached cells.

Fecal pellets produced under both procedures, were placed in different petri dishes and used for a series of laboratory tests. Most of them were transferred to both 50 ml culture vials (about 20 fecal pellets for each of two vials for each procedure) and cell well culture plates (5–10 fecal pellets for each of three 7 ml wells used for each procedure) containing filtered-sterile F/2 modified culture medium. Some of the fecal pellets (about 40% of the total) were broken using a fine pipette to ensure that cells situated in the core of the pellet would be released. Control samples of approximately 1 ml of seawater from the 1.5-liter dish used for the last wash of the sieves, were also taken and added to both 50 ml (three vials) culture flasks and cell wells culture plates (three compartments) containing filtered-sterile F/2 modified culture medium. All the culture vials were then placed in an incubator with controlled temperature (20°C) and light conditions (12:12 light:dark cycle). The 50 ml culture vials were untouched for the whole period of incubation (4–5 weeks), being opened only when discoloration of the water was noticed. The fecal pellets in the cell well plates were checked frequently under the inverted microscope for a period of 2–4 weeks. After the period of incubation, formaldehyde preserved samples were taken from all the vials for latter testing by the antibody method developed for *A. lagunensis* by Lopez-Barreiro et al. (1998).

The remaining fecal pellets left in the petri dishes were collected and divided into two groups. One group containing about 20 fecal pellets was placed in a 2 ml-Utermöhl's counting slide filled with culture medium, covered by a cover slip and kept in an incubator (20°C and 12:12 h light:dark cycle). Observations and photographs were taken during 3 consecutive days under an epifluorescence microscope using blue light excitation in order to check for possible natural chlorophyll fluorescence. Photographs were taken under magnifications of 40 \times and 100 \times (immersion oil).

The other set of fecal pellets (about 25) was used to read potential photosynthetic activity of *A. lagunensis* cells within the fecal pellets. Fecal pellets were transferred to individual drops of culture medium placed on glass slides. Readings of the photosynthetic activity were done on individual whole fecal pellets under a microscope (25 \times magnification) attached to a pulse-amplitude modulated (PAM) fluorometer (Villareal & Morton,

in press). This fluorometer measures the photochemical efficiency of electron transport occurring in photosystem-II during photosynthesis, and provides information about the physiological status of phytoplankton cells as the parameter $F_v:F_m$. The maximum potential quantum yield of PSII ($F_v:F_m$) has been used as an index of photosynthetic capacity, and as a proxy for the general health of the cell in both laboratory (Parkhill et al., 2001) and field studies (Babin et al., 1996). Variable fluorescence (F_v), is the difference between maximum and minimum fluorescence as measured with a series of modulated light flashes, and the ratio $F_v:F_m$ is the variable fluorescence normalized to the minimum fluorescence. In short, this parameter measures changes in the potential for electron flow in PSII. In the Heinz Walz system, maximum values are approximately 0.8, depending on the species.

The concentration of *A. lagunensis* in upper Laguna Madre was ca. 5×10^5 cells ml^{-1} one day before the copepod collection, based on counts of fluorescently labeled cells using the antibody method (Lopez-Barreiro et al., 1998).

Undamaged cells of *A. lagunensis* were found in the fecal pellets of *A. tonsa*. Natural fluorescence of Texas brown tide cells within *A. tonsa* fecal pellets was observed for three consecutive days in fecal pellets kept in Utermöhl slides (Figs. 1a, 1b, 1c and 1d). Growth of *A. lagunensis* cells from *A. tonsa* fecal pellets occurred in all treatments (Fig. 2) and they tested positive for *A. lagunensis* when treated by the antibody method (Figs. 3a, 3b). No growth of *A. lagunensis* cells was observed for the controls. Fecal pellets produced under a diet composed of wild populations of *A. lagunensis* contained undamaged cells as well (Figs. 4a, 4b). Photosynthetic capacity ($F_v:F_m$) was observed to occur in 38% of a total of 24 fecal pellets of *A. tonsa* analyzed (Table 1). The mean value of ($F_v:F_m$) was 0.177 ± 0.087 , while the maximum and minimum were 0.373 and 0.122 respectively. *A. lagunensis* cells from laboratory cultures had a mean value of 0.403.

In the present study there were two important questions to be answered. The first one was if cells of *A. lagunensis* could be found undigested in the fecal pellets of *A. tonsa*, and the second was if they were still viable after gut passage. Cells of *A. lagunensis* were found in apparently good condition in *A. tonsa* fecal pellets (Figs. 1a, 1b). It was possible to see very clearly that some chloroplasts and cellular membranes were in perfect condition within the fecal pellets using epifluorescence microscopy (Fig. 1a, 1c). Natural chlorophyll fluorescence was observed for the same group of fecal pellets for at least three consecutive days, strongly suggesting that the cells inside the fecal pellets remained in good physiological condition (Figs. 1a, 1b, 1c).

The viability of *A. lagunensis* cells after passing through the digestive system of *A. tonsa* was confirmed by the results of the incubation experiments. *A. lagunensis* cells grown from fecal pellets (Figs. 2) were observed in all treatments. The results obtained from the trials using the PAM fluorometer also supported the concept that some cells inside the fecal pellets were physiologically healthy and therefore viable (Table 1). It is possible that the relatively low ($F_v:F_m$) values were due to

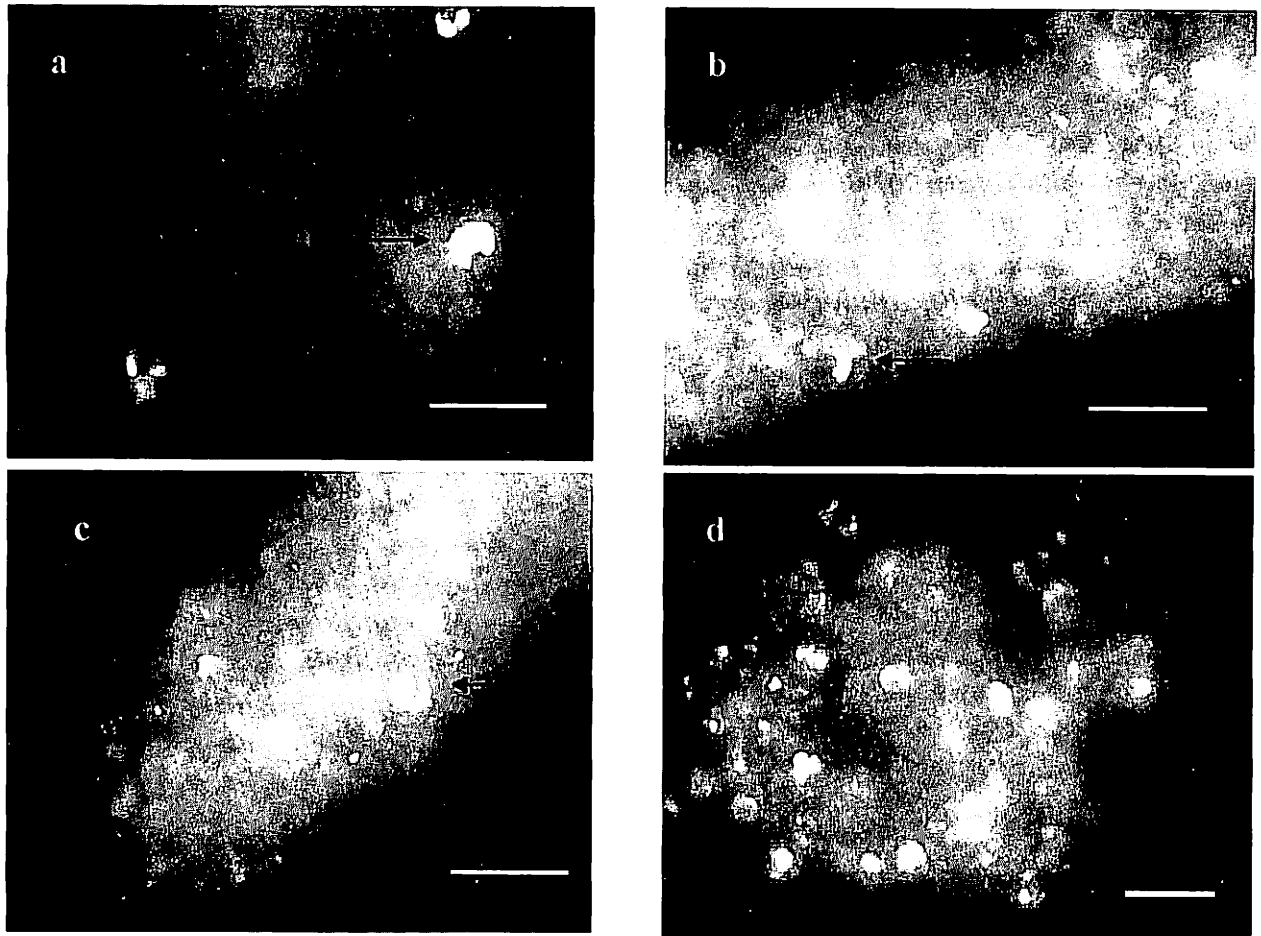


Fig. 1. Natural chlorophyll fluorescence of *Aureoumbra lagunensis* cells within *Acartia tonsa* fecal pellets observed under blue light excitation with an epifluorescence microscope. Arrows indicate cells of *A. lagunensis*. Scale bars = 10 μm .

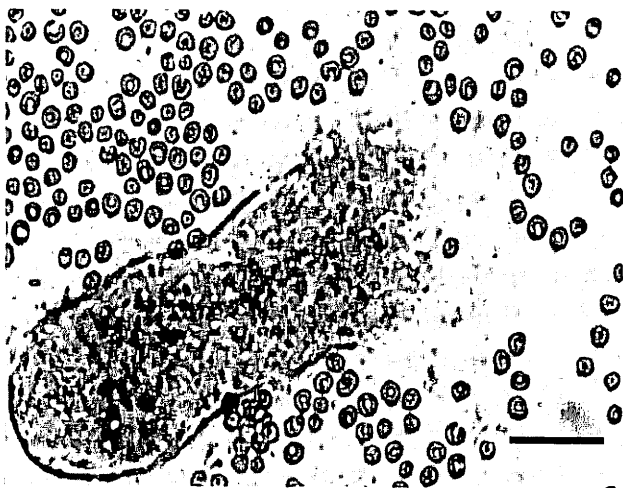


Fig. 2. Cells of *Aureoumbra lagunensis* cultivated from *Acartia tonsa* fecal pellets. Scale bar = 20 μm .

inactive chlorophyll associated with dead cells within the fecal pellets. This material would fluoresce and increase F_m without a measurable F_v .

The next question that emerges is why are some *A. lagunensis*

Table 1. Readings of photosynthetic activity obtained by a pulse-amplitude modulated (PAM) fluorometer in fecal pellets of *Acartia tonsa* fed cultures of *Aureoumbra lagunensis*.

PAM Readings ($F_v : F_m$)	
Max	0.373
Min	0.122
Mean*	0.177
SD	0.078

* The number of fecal pellets read was 24. Photosynthetic activity was recorded in 38% of the fecal pellets observed. The values of the yield for cultures used to feed the copepods in the laboratory were 0.403 on average.

sis cells not digested? This study does not address this question, but results from other studies may provide some insight. Gottfried (1988) reported that the major factor limiting survival after gut passage was cell size, and species having maximum dimension greater than 14 μm usually did not survive. However, the small size of *A. lagunensis* (3–5 μm) alone would not prevent the cells from being attacked by digestive enzymes within the copepod gut.

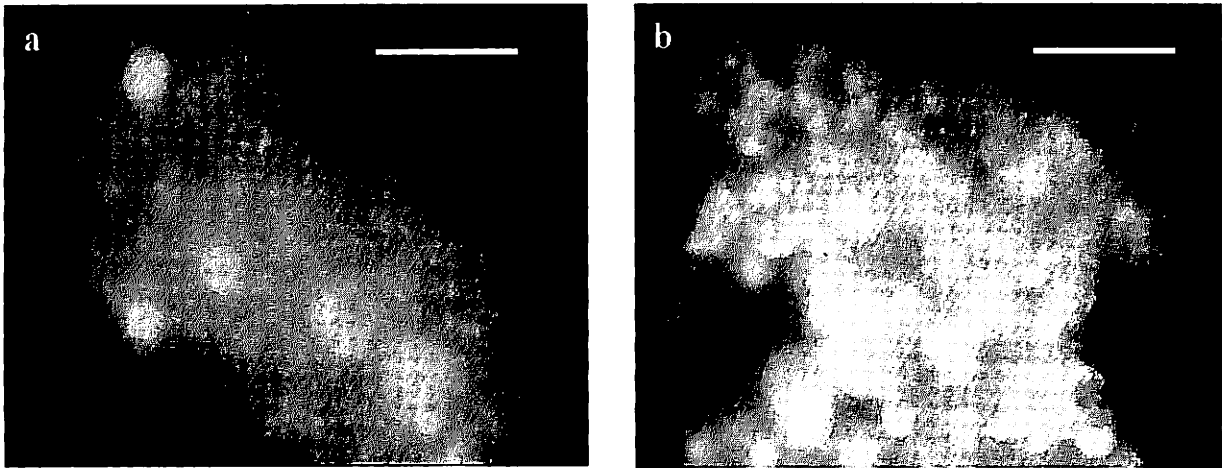


Fig. 3. *Acartia tonsa* fecal pellet produced under a diet of cultured *Aureoumbra lagunensis*. Fecal pellets were treated by the antibody method and examined under blue light excitation with an epifluorescence microscope. The bright spots are *Aureoumbra lagunensis* cells. Scale bar = 10 μ m.

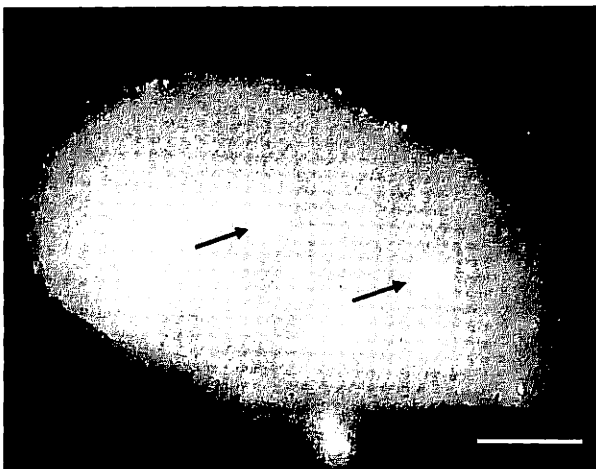


Fig. 4. *Acartia tonsa* fecal pellet produced under a diet of natural phytoplankton assemblage from Laguna Madre. Fecal pellets were treated by the antibody method and examined under blue light excitation with an epifluorescence microscope. The bright spots are *Aureoumbra lagunensis* cells. Scale bar = 10 μ m.

The Texas brown tide algae is one of the many phytoplankton species known to exude an extracellular matrix probably composed of polysaccharides (Stockwell et al. 1993, DeYoe et al. 1997, Liu & Buskey 2000a). This external polymeric substance (EPS), has been shown to reduce grazing on *A. lagunensis* by protozoa (Liu & Buskey 2000b). Porter (1973) suggested that polysaccharide sheaths act as molecular sieves and protect phytoplankton from digestion. The extracellular polysaccharide layer observed in *A. lagunensis*, might work in a similar way. Although not directly measured in this study, cultures of *A. lagunensis* grown at 30 PSU typically possess ca. 50 pg xanthan equivalent per cell of EPS (Liu & Buskey 2000a). In future studies, the possibility that EPS could also inhibit enzymatic activity through pH buffering within the copepod's gut, should be addressed. As a final comment, the

ability of *A. lagunensis* to resist digestion, could represent an important evolutionary adaptation against grazers. By reducing the number of copepod grazers through malnutrition and/or by causing them to prey more intensely on microzooplankton (Buskey et al., in press), the low digestibility of the Texas brown tide algae could reduce populations of both mesozooplankton and microzooplankton grazers, contributing to the persistence of the bloom in Laguna Madre. In addition, *A. lagunensis* cells may benefit from gut passage through uptake of nutrients released in the zooplankton's digestive system (Porter 1976, Epp & Lewis 1981).

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