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Evaluation of *Gambierdiscus* survival after exposure to ballast water

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ABSTRACT

The dinoflagellate *Gambierdiscus* was exposed to ballast water from a trans-oceanic vessel, and maintained at a variety of temperatures in the dark to determine if viability would be maintained. Logarithmically growing *Gambierdiscus* inocula were admixed (1:6, vol:vol) with ballast water, maintained in the dark at 22.6 °C, 24.6 °C, 26.8 °C and 29.0 °C and assessed for numerical abundance over six days. Calculated growth rates from the biomass time series showed no indication that ballast water negatively impacted *Gambierdiscus* viability; accompanying microscopic inspections supported this conclusion. Filtration of large volumes of collected ballast water failed to show the presence of any *Gambierdiscus* cells contained therein. Recovery and microscopic examination of the experimental inocula after 10 weeks in the dark, failed to show cyst development at any temperature regime. This examination of ballast water showed no evidence of cytotoxicity to *Gambierdiscus* spp.

1. Introduction

The need to better understand the vectors, pathways, and probabilities associated with the transport of invasive species (e.g., algae, mollusks, crustaceans, tunicates) is recognized to be crucial because of economic and ecological impacts in a number of coastal regions (Carlton, 1985; McCarthy and Crowder, 2000; Bailey et al., 2003; Levings et al., 2004). The ballast water in trans-oceanic shipping vessels has been implicated as a mode of transfer for invasive species, including the dinoflagellate, *Gambierdiscus* spp., the causative organism of ciguatera fish poisoning.

Ciguatera, a circumtropical malady caused by the ingestion of toxic reef fish, is the most prevalent form of natural marine seafood poisoning in the world (Lehane and Lewis, 2000; Bienfang et al., 2008; Dickey and Plakas, 2010). *Gambierdiscus* is a predominantly epiphytic genus that grows on marine macroalgae and produces a powerful suite of polyether compounds called ciguatoxins (Yasumoto et al., 1977; Holmes, 1998; Chinain et al., 2010; Roeder et al., 2010). These ciguatoxins enter the coral reef food web when herbivorous fish graze on macroalgae in the littoral zone, and inadvertently ingest the *Gambierdiscus* spp. and the ciguatoxins contained within. Ciguatera incidences are well known for the Pacific, Indian, and Atlantic oceans, as well as the Caribbean and Mediterranean Seas (Lewis, 1986; Lehane and Lewis, 2000; Bienfang et al., 2008; Dickey and Plakas, 2010); recent discoveries suggest an even more expansive geographic scale (Lu and Hodgkiss, 2004; Aligizaki and Nikolaidis, 2008; Fraga et al., 2010). Ciguatera outbreaks are temporally and spatially sporadic; the underlying trigger(s) for such outbreaks remain enigmatic. Hypothesized causes have included reef disturbances (Ruff, 1989; Bagnis, 1994), altered anthropogenic input, warming surface water temperature, transport of *Gambierdiscus* from one locality to another in ships' ballast water, and others.

This work addressed the issue of *Gambierdiscus* transport and survival via ship ballast water. Like many other dinoflagellates, *Gambierdiscus* spp. have a reputation for being difficult to maintain in culture in vitro due to sensitivities to agitation, abrupt changes in temperature, salinity, light, and high silicate and metal levels, particularly copper (Guillard and Keller, 1984; Steidinger and Baden, 1984; Durand-Clement, 1986; Bomber et al., 1988). These attributes created interest to examine empirically if *Gambierdiscus* spp. would survive exposure to a ship's ballast water. Following our contact, Matson Inc. offered to supply these experiments with ballast water from a vessel immediate after docking. The M/V Maunalei is four years old, carries a total ballast volume of 9652 metric tonnes in fourteen separate, lined tanks. Per requirements for docking in U.S. ports, vessels are required to purge/refill ballast tanks with deep sea water, defined as taken beyond 200 miles from land in ≥2000 m of

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depth. At sea, ballast tanks are periodically purged, refilled, and transferred to stabilize vessels in transit. The objectives of this work were to examine the ballast water for the presence of *Gambierdiscus* spp., to determine if the growth response of *Gambierdiscus* following exposure to ballast water reflected toxicity to the cells, and to subsequently examine for evidence of cyst formation by *Gambierdiscus* following prolonged exposure to ballast water.

2. Materials and methods

Ballast water was collected from the Matson M/V Maunalei into an acid-washed, deionized-water rinsed 20L polycarbonate container for transport to the lab; prior to sampling, the ballast water used in these had a residence time of ~4 days in the ballast tanks. After mixing, 3–5 L subsamples were passed (gravity pressure) over 35 μm mesh Nytex to examine the >35 μm particulates for the presence of *Gambierdiscus* spp. Subsamples were also taken into double acid-washed bottles for metals analyses, and the time series experiments. Metals samples were collected following trace element clean protocols (Spencer et al., 1995), and concentrations were determined by FIA-ICP-MS (De Carlo and Resing, 1998).

Gambierdiscus cultures (presumably *G. carpenteri*) to be used as inoculum were grown in modified K medium (Keller and Guillard, 1985), in which TRIS, copper, and silica were excluded (Morton and Norris, 1990), and maintained in a Thermo-Precision Model 818 incubator set on a 12:12 L:D cycle, at 25 °C, at 50 μE m^{-2} s^{-1}. Light, pH, and salinity were measured with Biospherical Instruments, Inc. Model QSL-100 irradiance meter, an Extech Instruments Model PH220, and an Autosal Model 8400A unit, respectively.

For the time series trials, 1200 mL of ballast water was admixed with 200 mL *Gambierdiscus* inoculum, and 40 mL of K media, distributed into 40 mL polycarbonate test tubes, and placed into the temperature-control unit in darkness to stimulate exposure in ballast tanks. 36 tubes containing the ballast water-*Gambierdiscus* mixture were placed in a temperature-controlled aluminum block array in darkness at 22.6 °C, 24.6 °C, 26.8 °C and 29.0 °C. At 0, 3, 6, and 9 days, one set of triplicate tubes from each temperature were removed for measurement of numerical abundance, microscopic examination, and returned to light to assess growth performance. Numerical abundance was determined using a Coulter Instruments Model Z1 electronic particle counter equipped with a 200 μm orifice, and set to count 1000 μL volume. Five replicate counts were taken on each sample; the relative standard deviation about the means of individual samplings ranged from 3% to 42%. Specific growth rates (day^{-1}) were calculated from slopes regressions of ln(cell density) versus time. Microscopic observations were done using an Olympus Model SZX-12 dissecting microscope.

3. Results and discussion

Consistent with its oceanic origin, the ballast water showed pH = 8.00, S = 33.20 psu, and low metal levels (Cu = 0.7 ppb, Zn = 4 ppb, and Mn = n.d.). We suspect that Mn was lost due to adsorption; an instrument problem precluded measurement of the concentrations of other metals. Microscopic examination of the >35 μm fractions from the 3 to 5 L ballast water samples failed to show any *Gambierdiscus* cells.

The time series of *Gambierdiscus* cell numbers in ballast water (Fig. 1) shows increases in cell density at all temperatures. Over the six-day exposure, the calculated growth rates ranged from 0.12 to 0.23 day^{-1}; the growth rates at the higher three temperatures were roughly half that observed at 22.6 °C. After 6 days of exposure to ballast water in the dark, the cell numbers showed about 1.5 doublings at 22.6 °C; cultures at the three temperatures $T \geq 24.6$ °C showed about 1 doubling. The progressive increase in cell numbers over time suggests the absence of pernicious effects of the ballast water to the cells. Microscopic examination of cultures exposed to ballast water for three days revealed no abnormalities in appearance, e.g., motility, normal coloration, and uniform distribution of chloroplasts. The *Gambierdiscus* inocula used in the above trials were also maintained under a 12:12 light:dark (50 μE m^{-2} s^{-1}) regime to confirm viability for this trial, and give a reference for growth rate of this isolate. The time series for cultures maintained in light in either K/5 media or enriched ballast water showed estimated growth rates of 0.21–0.22 day^{-1}(Fig. 2). The similarity of these two growth rate estimates to one another, and to the growth rate (0.23 day^{-1}) for T = 22.6 °C lends support to the

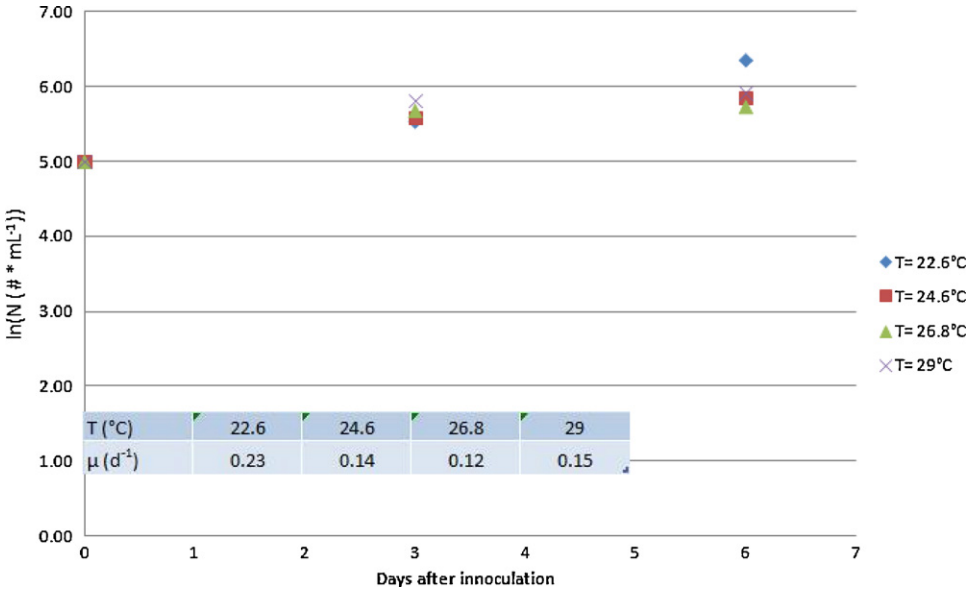

Fig. 1. Time series of *Gambierdiscus* biomass exposed to ballast water in the dark at various temperatures. Specific growth rates (μm) were calculated from regressions of ln(cells/mL) versus time (days). Mean cell densities were computed from five replicate measurements using an electronic particle counter; the relative standard deviation in all measures of cell density ranged from 3% to 42%.