Harmful Algae 30 (2013) 1–9

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Sexual life stages and temperature dependent morphological changes allow cryptic occurrence of the Florida red tide dinoflagellate *Karenia brevis*

Agneta Persson [a,*], Barry C. Smith [b], Steve Morton [c], Andrew Shuler [c], Gary H. Wikfors [b]

[a] Department of Biological and Environmental Sciences, Göteborg University, Box 461, SE-405 30 Göteborg, Sweden
[b] National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, Milford Laboratory, 212 Rogers Avenue, Milford, CT, USA
[c] NOAA/NOS/Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Road, Charleston, SC, USA

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 22 August 2013
Accepted 22 August 2013

Keywords:
Behavior
Bloom
Cryptic
Cyst
Dinoflagellate
Gamete
Karenia brevis
Mating
Morphology
Zygote

ABSTRACT

Karenia brevis, the Florida red tide dinoflagellate, has been studied extensively, but very little attention has been paid to its sexual life cycle. We found that the life cycle of *K. brevis* is heterothallic, most probably not resting cyst-producing, but with life stages of different morphology. The isogamous gametes were slightly smaller than vegetative cells and not as broad and flat. The late zygote was yellow-brown in appearance with a thicker wall and more rounded shape lacking carina. Pellicle cysts of these zygotes closely resembled the few earlier descriptions of "possible cysts" of the species. In addition, temperature-dependent, morphological changes and pellicle-cyst formation were observed. Cells placed in the cold (15 °C) formed spherical, thin-walled pellicle cysts that germinated into cells that were round in cross-section and longer than wide – so morphologically different from vegetative cells that they would not be correctly identified if encountered in field samples. Cells grown at 25 °C were wider and flatter than cells grown at 20 °C. Cells warmed from cold conditions became flat and wide within hours, returning to the typical shape. Also the morphological differences between sexual life stages were large enough to allow misidentification and cryptic occurrence of *K. brevis*. The cell shape of *K. brevis* was not fixed, but could vary from very wide and flat to elongate with rounded cross-section in the same culture of clonal cells and in the same cells within a short time (hours).

In addition to the culture studies, sediment samples from a *Karenia* "hot spot" area were concentrated, and the dinoflagellate cyst fraction was investigated for resting cysts. Cysts were not found, and *Karenia* cells did not germinate from slurry cultures of the concentrated cyst fraction.

1. Introduction

Karenia brevis is a well-known toxic dinoflagellate that causes severe harmful blooms in the Gulf of Mexico (reviewed in e.g. Steidinger, 2009; Vargo, 2009; Brand et al., 2012). Since the mid 1980s, blooms of *K. brevis* have occurred annually along the Florida coast, and semiannually along the coasts of Texas and Mexico, with cell densities reaching 10^5–10^7 per liter (Spear et al., 2009). Impacts include massive fish kills, marine mammal, sea turtle and sea bird mortalities, benthic community die-off, and public-health effects from shellfish contamination and inhalation of air-borne toxins (Pierce and Henry, 2008; Watkins et al., 2008; Landsberg et al., 2009). Brevetoxins, the class of chemical toxins produced by this species, can be transferred throughout the food web (Landsberg et al., 2009; Bricelj et al., 2012). Benthic planktivores in a number of taxa can serve as vectors transporting brevetoxins within the food web (Sotka et al., 2009). High concentrations of toxin may accumulate in the tissues of benthic, suspension-feeding invertebrates that may be transferred to higher-level consumers (Echevarria et al., 2012). This propagation of brevetoxin to higher trophic levels can lead to the death of animals in places and at times when there are no blooms of *K. brevis* (Brand et al., 2012). *K. brevis* is considered to be primarily autotrophic, but appears to be adapted for assimilating recycled nutrients as it is able to use organic compounds (Brand et al., 2012) and ingest microbes (Bronk et al., 2004) to supplement photosynthesis. It is present in low numbers (1–100 cells L^{-1}) year-round throughout the Gulf (Tester and Steidinger, 1997; Tomlinson et al., 2004). Blooms occur most often during the fall months at the peak of the wet season and land runoff in South Florida (Brand and Compton, 2007) when salinity fronts are generated along the coastline. *K. brevis* bloom initiation

* Corresponding author. Present address: Smedjebacksvägen 13, SE-771 90 Ludvika, Sweden. Tel.: +46 70 650 6227.
E-mail address: agnetapersson77@gmail.com (A. Persson).

1568-9883/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.hal.2013.08.004

has been obscure, but some evidence suggests that offshore, near-bottom populations may contribute to the more obvious coastal, near-surface HAB events often associated with fronts (Tomlinson et al., 2004; Sinclair and Kamykowski, 2008). Surface waters of the West Florida Shelf have low concentrations of nutrients, particularly nitrogen (Vargo, 2009). These low-nutrient concentrations do not reflect low nutrient inputs, however. The wide West Florida Shelf is rather shallow, and much of it is in the photic zone; therefore, most nutrients are rapidly taken up by benthic microalgae and the benthic plant community (macroalgae and seagrasses). Benthic chlorophyll on the West Florida Shelf is much higher than in the water column above (Walsh and Steidinger, 2001), and the area is famous for the plentiful and species-rich community of filter-feeding and deposit-feeding mollusks resulting in beaches covered with seashells (http://www.sanibelisland.com/shelling.html). Nutrient inputs in the region are very high and have, as also has the *K. brevis* biomass, increased significantly over the past 50 years from the large increase in human activities (e.g. Brand and Compton, 2007; Brand et al., 2012). Over time, the watersheds in Florida have changed from low-nutrient loading marshes and wetlands to high-loading urban and agricultural land uses. Wetlands have been drained, and the application of synthetic fertilizers is commonplace. Groundwater inputs to coastal waters have also increased dramatically in recent decades (Brand et al., 2012).

Karenia brevis is an athecate dinoflagellate that is both pleomorphic and polymorphic; natural populations often appear with multiple shapes and sizes (Steidinger and Williams, 1964; Brand et al., 2012). Wilson wrote in 1967, "The absence of a cell wall permits considerable variation in the size and shape of *Gymnodinium breve*; probably no two individuals are alike" and "many forms would not be recognized as *G. breve* in field samples." Morphological variations in field samples were documented microscopically by Dragovich (1968). More recently, photographs taken by the automated camera FlowCytobot (Texas Observatory for Algal Succession time series: http://gcoos.tamu.edu/products/phytoplankton/Image_Archives.html), and by FlowCAM (Jena Campbell's web site at https://sites.google.com/site/jenacampbell23/research/research-projects) illustrate the same phenomenon. When studying the several hundred photos taken from field samples, it is very difficult to find two cells that are exactly alike, or to sort them into distinct morphological groups.

Sexual conjugation in *Karenia brevis* was observed early (Wilson, 1967). In 1982, Walker showed that sexual behavior, with mating and zygote formation, occurred in cultures. Gametes and hypnozygotes were observed in low numbers in stock cultures of non-clonal strains. Sexual stages were induced by subjecting the cultures to nitrogen deficiency or by crossing six separate, haploid strains. Also a few "possible" resting cysts were seen in culture and one with a similar morphology in a field sample (Walker, 1982). In the early research on *K. brevis*, a dormant, resting-cyst stage often was hypothesized to exist (e.g. Wilson, 1967; Steidinger and Ingle, 1972; Walker, 1982), because the disappearance and appearance of the blooms had no clear, alternative explanation. Further, the behavior of *K. brevis* blooms was similar to those of dinoflagellates with a known sexual life cycle that includes a dormant, resting cyst stage. The uncertainty regarding the resting cysts remained, and despite the intense interest and number of studies performed on *K. brevis*, no further studies or descriptions of cysts were published until 2010. At this time, a report of "possible *K. brevis* cysts" appeared in a resting-cyst survey from the Florida west coast (Kang, 2010). Dinoflagellate cyst studies on the coast of Florida have been performed using routine paleontological methods for preparation of cyst samples that include hot treatment with strong acid that destroys unfossilizable cyst species (e.g. Cremer et al., 2007; Vásquez-Bedoya et al., 2008). Kang found the "possible *K. brevis* cysts" similar in appearance to the cysts described by Walker without using acid treatment. She suggested they were cysts, or forming cysts, but added that they should be germinated to confirm the identity.

Previous, detailed studies of the sexual life cycle of *Alexandrium fundyense* and *Scrippsiella lachrymosa* revealed that it can be possible to recognize the differences in behavior between the different sexual life stages (Smith and Persson, 2004, 2005; Persson et al., 2008, 2012, 2013; Persson and Smith, 2013). Our experience with this approach led us to investigate the sexual life cycle of *Karenia brevis*, especially the question regarding the existence of resting cysts. In addition, a cyst-concentrating device for the collection of undisturbed dinoflagellate resting cysts from large volumes of sediment in which they occur in low concentrations (Persson and Smith, 2009; Smith et al., 2009) allowed us to investigate the possible presence of dinoflagellate cysts, both fossilizable and unfossilizable, in sediment samples from coastal locations in Florida where *K. brevis* blooms recur. Cyst concentration, in combination with a slurry culture technique (Persson, 2001, 2002), was expected to improve chances of encountering and recognizing a cyst-producing species in sediments, especially in samples collected from a "hot-spot" bloom area for the species in question.

2. Materials and methods

2.1. Culture experiments

2.1.1. Encystment experiment comparing three different temperatures

All culture experiments were performed at NOAA/NOS/Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC, USA, between 25 April and 6 May 2011. Four different, reliably growing, clonal cultures of *Karenia brevis* were selected from the culture collection; NOAA-1, C2, JAX C5 and C5. We suspected heterothally (as indicated by Walker, 1982, and sexual stages have not been observed in clonal cultures) and could not know which clones could be compatible; therefore, all four clones were mixed together in the experiment (Table 1).

Stock cultures of *Karenia brevis* in the culture collection were grown at 25 °C, 65 μE m^{-2} s^{-1}, in light room 2 (Table 2), in autoclaved Gulf stream water (from the running seawater system in the Florida Institute of Technology, Vero Beach Laboratory) with L1 nutrients sterile-filtered before addition. In this experiment, we used L1 medium for growth, and L1 without nitrogen addition as "encystment medium" (nitrogen deficiency is known to induce gamete formation in dinoflagellates and is often used in encystment experiments; Persson et al., 2008 and references therein). One liter of each medium was prepared, and cells were added aseptically to each container. The containers were mixed gently but thoroughly, and the contents were poured aseptically into 50-mL tissue-culture flasks and placed in the different conditions described in Table 2. Cell-count sub-samples from original cultures were preserved with Lugol's Iodine and counted in a Sedgewick–Rafter chamber using a light microscope.

Table 1
The combination of strains used in L1 (growth) and L1-N (encystment medium).

Strain	Origin	Cell density (cells ml^{-1})	Volume added (ml)	Cells added to 1 L
NOAA-1	Sarasota, FL	21,500	12	258,000
C2	Sarasota, FL	9214	13	119,782
JAX C5	Jacksonville, FL	29,000	10	290,000
C5	Sarasota, FL	13,200	13	171,600