FEATURE ARTICLE

First successful culture of the marine dinoflagellate *Dinophysis acuminata*

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ABSTRACT: The dinoflagellate genus *Dinophysis* includes several species that cause diarrhetic shellfish poisoning, none of which have yet been established in culture. We report on the maintenance of *Dinophysis acuminata* cultures that were established in December 2005 and also on its feeding mechanism, and growth rates when fed the ciliate prey *Myrionecta rubra* with and without the addition of the cryptophyte *Teleaulax* sp. *D. acuminata* grew well (growth rate of 0.95 d^{-1}) in laboratory culture when supplied with the marine ciliate *M. rubra* as prey, reaching a maximum concentration of about 2400 cells ml^{-1} at the end of the feeding experiment. In contrast, *D. acuminata* did not show sustained growth in the absence of the ciliate or when provided the cryptophyte *Teleaulax* sp. as prey (*D. acuminata* used its peduncle to extract the cell contents of the prey organism, *M. rubra*). Based on the prey–predator interactions occurring among *D. acuminata*, *M. rubra,* and *Teleaulax* sp. in this study, establishment of permanent culture of the dinoflagellate *D. acuminata* may facilitate a better understanding of the ecophysiology, biology, and toxicology of *Dinophysis* species, as well as the evolution of dinoflagellate plastids.

KEY WORDS: *Dinophysis acuminata* · Cultivation · *Myrionecta rubra* · Diarrhetic shellfish poisoning · Feeding

INTRODUCTION

The marine dinoflagellate genus *Dinophysis* includes both phototrophic and heterotrophic species and is globally distributed in coastal and oceanic waters (Hallegraeff & Lucas 1988, Hallegraeff 1993).

Dinophysis acuminata feeding on the marine ciliate *Myrionecta rubra* by extracting its cytoplasm through a peduncle.
Photo: Myung Gil Park

Cell abundances of *Dinophysis* species are usually low (<100 cells l^{-1}), but at times they form seasonal blooms with a few thousand cells per liter in some areas of Europe and Japan (Dahl et al. 1996, Nishitani et al. 2005). *Dinophysis* species are of economic and public importance as they cause diarrhetic shellfish poisoning and have a significant effect on shellfish industries in many parts of the world (Boni et al. 1993, Dahl et al. 1996, Giacobbe et al. 2000) because of the threat to human health after consumption of contaminated shellfish (Hallegraeff 1993). Nonetheless, further detailed exploration of the ecophysiology, biology, and toxicology of the *Dinophysis* species has been hampered by an inability to culture them. Therefore, our current knowledge about *Dinophysis* species has been derived only from natural populations.

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Photosynthetic species of *Dinophysis* do not survive when cultured in various media that support growth of many other phytoplankton species (Sampayo 1993, Maestrini et al. 1995). Microscopic observations (Jacobson & Andersen 1994, Nishitani et al. 2002) show that photosynthetic species often contain food vacuoles, reflecting mixotrophy, indicating that feeding may be necessary for successful culture of *Dinophysis* species. However, despite the supply of potential prey organisms, including cryptophytes (Nishitani et al. 2003), all attempts to cultivate members of the genus *Dinophysis* have failed. While ultrastructural and molecular studies and pigment analyses all demonstrate that photosynthetic *Dinophysis* species contain plastids of cryptophyte origin (Schnepf & Elbrächter 1988, Lucas & Vesk 1990, Hewes et al. 1998, Takishita et al. 2002, Hackett et al. 2003, Janson & Granéli 2003, Janson 2004), the way in which they enter *Dinophysis* cells has not yet been confirmed. In the present study, we report on the establishment of *Dinophysis acuminata* in culture, its feeding mechanism, and its growth rate using the ciliate prey *Myrionecta rubra* with and without the addition of the cryptophyte *Teleaulax* sp.

MATERIALS AND METHODS

Cultures. *Dinophysis acuminata* was established in culture by isolating single cells from seawater samples collected in Masan Bay, Korea (128° 34′ E, 35° 12′ N) on 20 December 2005. The *Dinophysis* culture was grown in 30 psu f/2-Si medium at 20°C under continuous light (60 μmol photons m^{-2} s^{-1}) with addition of the marine ciliate *Myrionecta rubra* as the prey species every 2 to 3 d. Cultures of *M. rubra* (strain MR-MAL01) were grown using the cryptophyte *Teleaulax* sp. (strain CR-MAL01) as prey, as described in detail by Yih et al. (2004). The cryptophyte culture was grown under the same conditions described above. All of the 3 cultures were non-axenic.

Feeding experiments. A dense culture of *Dinophysis acuminata* in exponential growth was split into 3 aliquots and diluted with fresh medium to prepare triplicate 300 ml bottles for each of 3 experimental treatments. Treatment bottles received either *Myrionecta rubra*, the cryptophyte *Teleaulax* sp., or a mixture of *M. rubra* and *Teleaulax* sp. as prey for *D. acuminata*. For controls, triplicate bottles were established for *D. acuminata* without prey, *M. rubra* alone, *Teleaulax* sp. alone, and a mixture of *M. rubra* and *Teleaulax* sp. Initial concentrations of *D. acuminata*, *M. rubra*, and *Teleaulax* sp. in experimental and control bottles were 100, 500, and 500 cells ml^{-1}, respectively. All treatments and controls were incubated at 20°C under continuous light (60 μmol photons m^{-2} s^{-1}) for 7 d. Daily subsamples were fixed

with acid Lugol's solution and cells were enumerated using a Sedgewick-Rafter chamber.

Microscopy. Live observations of the feeding process were made on a glass slide using an Olympus BX51 microscope at 400× magnification and recorded with a Sony Progressive 3CCD colour video camera attached to a digital imaging time-lapse recorder. Video sequences were frame grabbed and individual frames were exported in JPEG format. For observations of plastid density and autofluorescence in *Dinophysis* cells, light and epifluorescence micrographs of live cells were taken at 1000× magnification using a digital camera (PowerShot G5, Canon) coupled to the Olympus BX51 microscope equipped with differential interference contrast and fluorescence cube (U-MWB2, 450-480 nm excitation, 500 nm emission).

RESULTS AND DISCUSSION

Cultivation of *Dinophysis acuminata*

When grown in 30 psu f/2-Si medium at 20°C in continuous light (60 μmol photons m^{-2} s^{-1}) and supplied with the marine ciliate *Myrionecta rubra* as prey, *Dinophysis acuminata* reached densities greater than 1.1×10^4 cells ml^{-1}. No sustained growth was observed in the absence of the ciliate prey.

Growth and feeding of *Dinophysis acuminata*

Dinophysis acuminata grew well when offered *Myrionecta rubra* as prey (Fig. 1a), with cell numbers remaining constant during the first day and increasing exponentially at a growth rate of 0.95 d^{-1} (doubling time 17.5 h) over the next 3 d. After 4 d, initial *M. rubra* cells had declined by 97.5%, yet *D. acuminata* slowly continued to increase in numbers, reaching a maximum concentration of about 2400 cells ml^{-1} at the end of the experiment. In control bottles without the predators, *M. rubra* cell numbers increased exponentially with a growth rate of 0.61 d^{-1} by Day 5, and remained constant thereafter (Fig. 1b). When cryptophytes were offered as prey, *D. acuminata* cell numbers increased slightly to about 280 cells ml^{-1} (growth rate 0.31 d^{-1}) over the first 4 d and thereafter declined rapidly until the end of the experiment (Fig. 1c). The slight initial increase in *D. acuminata* cell numbers in the presence of *Teleaulax* sp. did not appear to reflect growth supported by predation or kleptoplastidy, or both, on cryptophyte cells since growth of *D. acuminata* occurred at a similar rate (0.32 d^{-1}) in control cultures without prey (Fig. 1e). When grown with both *M. rubra* and *Teleaulax* sp., *D. acuminata* cell numbers increased to about 2500 cells

ml^{-1} over the first 4 d (growth rate of 0.91 d^{-1}), subsequently decreased slowly for 2 d, and then sharply declined to near zero values by the end of the experiment (Fig. 1f). The sharp decline in *D. acuminata* after 6 d was accompanied by a parallel decline in *M. rubra* prey. The

lack of ciliates, however, seems not to have been the primary cause for the decline in *D. acuminata* as this dinoflagellate is capable of surviving for many days in the absence of prey (Fig. 1e). A similar decline in *D. acuminata* was observed in bottles containing crypto-
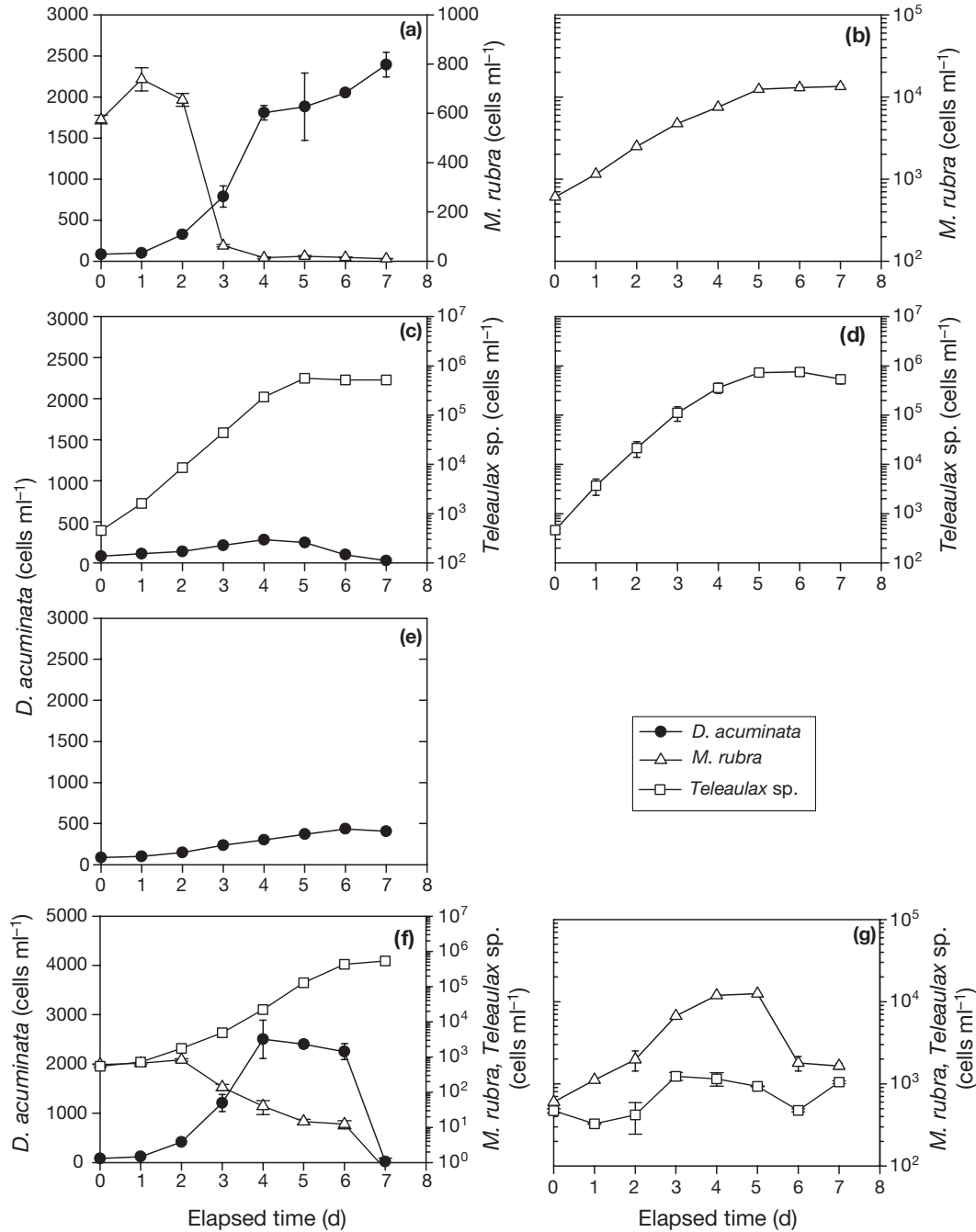

Fig. 1. *Dinophysis acuminata*. Characteristics of batch culture growth. (a) Changes in cell numbers of *D. acuminata* and *Myrionecta rubra* in cultures supplied with the ciliate *M. rubra* as prey. (b) Growth of *M. rubra* in cultures without the predator *D. acuminata*. (c) Changes in cell numbers of *D. acuminata* and *Teleaulax* sp. in cultures with the cryptophyte *Teleaulax* sp. provided as prey. (d) With only cryptophytes. (e) With only *D. acuminata*. (f) Growth of *D. acuminata* in cultures with a mixture of ciliate and cryptophytes provided as prey. (g) Growth of ciliate and cryptophytes in cultures without predator. Data were shown as meanvalues (±1 SE) of 3 replicate cultures

phytes as potential prey (Fig. 1c, f), which suggests that inhibition of *D. acuminata* growth was due to nutrient competition or allelopathy from *Teleaulax* sp. Another plausible explanation for the sharp decline in *D. acumi-nata* could be that the 3 species may differ in their pH limits for growth (Hansen 2002, Pedersen & Hansen 2003, Hansen & Fenchel 2006). The cryptophyte *Teleaulax* sp. may have a higher pH limit for growth compared with *M. rubra* (Hansen & Fenchel 2006) or *D. acuminata* and, thus, these 2 species may reach their pH limits for growth before *Teleaulax* sp.

Feeding process of *Dinophysis acuminata*

Microscopic observations of live cells using our established cultures revealed that *Dinophysis acu-minata* uses a peduncle to extract the cell contents of the ciliate *Myrionecta rubra* (Fig. 2). While pe-duncle feeding has been reported for the heterotro-phic species *D. rotundata* and *D. hastata* (Hansen 1991), the feeding mechanism used by photosyn-thetic or mixotrophic species of *Dinophysis* has not been previously observed. However, ultrastructure
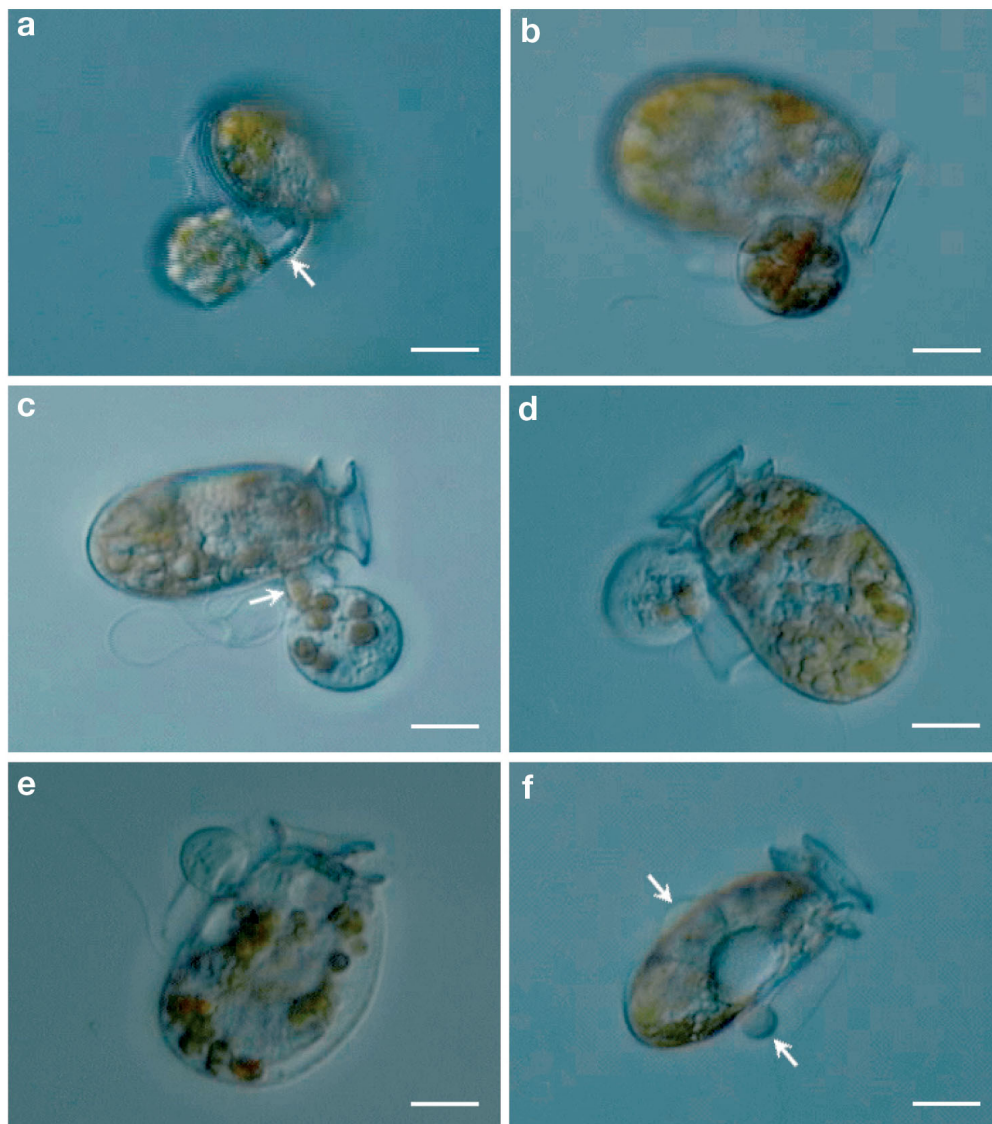

Fig. 2. *Dinophysis acuminata*. Light micrographs of live cells feeding on the ciliate *Myrionecta rubra*. (a) Peduncle (arrow) of the dinoflagellate *D. acuminata* during swimming with a captured *M. rubra* cell suspended. (b) *D. acuminata* with a recently captured *M. rubra* prey organism. Note that all cilia of the ciliate were detached from the body. (c) *D. acuminata* feeding on *M. rubra* by extracting its cytoplasm through a peduncle. The ciliate prey has only 6 chloroplasts left and 1 chloroplast is passing through the peduncle (arrow) into the dinoflagellate. (d,e) *D. acuminata* that has acquired most of the chloroplasts of the prey. (f) *D. acuminata* with balloon-like spheres (arrows) near its surface after feeding. Scale bars = 10 µm. Video available as supplementary material at: www.int-res.com/articles/suppl/a045p101_video

has revealed the presence of microtubular ribbons inside *D. acuminata* and *D. norvegica* (Jaconson & Andersen 1994) that are presumably used during feeding. The precapture behaviour of *D. acuminata* differs from that of other marine thecate dinoflagellates (i.e. searching type) (Jacobson & Anderson 1986, Hansen & Calado 1999) but is similar to that of the heterotrophic dinoflagellates *D. rotundata* and *D. hastata* (i.e. trapping type) (Hansen 1991, Hansen & Calado 1999). The ciliate *M. rubra*, which has pronounced jumping behaviour, is captured by the dinoglagellate upon mechanical contact. After making physical contact, *D. acuminata* pierces *M. rubra* with a peduncle. Once trapped, the ciliate temporarily swims for about 1 min, towing the attached *D. acuminata*. However, the ciliate soon becomes immobile and the dinoflagellate then swims freely around towing the attached ciliate. At this time, *D. acuminata* starts to gradually consume the ciliate. During the early stage of feeding (i.e. capturing of prey and swimming), most cilia are shed from the body of *M. rubra* (Fig. 2a,b). During feeding, *D. acuminata* extracts the contents of the prey using the peduncle that extends from the flagellar pore. During the last stage of the feeding process, which lasts for about 1 to 2 h, the *D. acuminata* cell is filled with vacuoles containing ciliate cytoplasm (Fig. 2d,e). In addition, plastids were frequently noticed within the cytoplasm of *D. acuminata* (Fig. 3a,b). Epifluorescence microscopy revealed that *D. acuminata* emitted bright yellow–orange fluorescence under blue light excitation (Fig. 3c), typical of cryptophycean phycobilin (phycoerythrin). After the feeding event, *D. acuminata* was frequently observed with balloon-like spheres of varying size distributed close to the cell surface (Fig. 2f).

Establishment of *Dinophysis acuminata* in culture and its implications

To our knowledge, this is the first report on extended cultivation of a species belonging to the genus *Dinophysis*. Despite considerable effort since the early work of Barker approximately 70 yr ago (Barker 1935), all attempts to cultivate *Dinophysis* species have failed. This has posed a major obstacle to detailed study of the ecophysiology, life history, toxicology, and evolution of the plastids in members of this genus. Dinoflagellates possess 5 different types of plastids and have acquired and lost them many times during their evolution (Schnepf & Elbrächter 1999). While *Dinophysis* species are now known to possess cryptophyte-type plastids, the route by which the plastid enters *Dinophysis* cells remains unknown.

Like *Dinophysis acuminata*, the planktonic ciliate *Myrionecta rubra* contains plastids of cryptophyte origin. The origin of the plastids has been proposed to be via kleptoplastidy following ingestion of the cryptophyte (Gustafson et al. 2000, Yih et al. 2004). Recently, however, Hansen & Fenchel (2006) have argued that the plastids of *M. rubra* are not kleptoplastids. They postulated, using morphological and experimental evidence, that *M. rubra* does not acquire chloroplasts from its cryptophyte prey; rather it feeds on cryptophytes in order to gain an essential growth factor for continuous growth. Similarly, *D. acuminata* may get its plastids as kleptoplastidy from ingesting *M. rubra*. If so, the plastids would be secondary kleptoplastids if Gustafson et al. (2000) and Yih et al. (2004) are correct about the origin of *M. rubra* plastids. If, however, Hansen & Fenchel (2006) are correct, then the plastids of *D. acuminata* would be primary kleptoplastids. Alternatively, *D. acuminata* may have its own plastids

Fig. 3. *Dinophysis acuminata*. Typical light and epifluorescence micrographs of well fed cells using the ciliate prey *Myrionecta rubra*: (a) surface focus; (b) central focus; (c) epifluorescence of the same cell. Scale bars = 10 µm

and simply eat *M. rubra* to acquire some essential growth factor, as Hansen & Fenchel (2006) argued for *M. rubra*.

The establishment of *Dinophysis acuminata* cultures promises to improve our knowledge of the evolution of the dinoflagellate plastids and the more complicated interactions among the 3 organisms *D. acuminata, M. rubra* and *Teleaulax* sp. within marine planktonic food webs. Cultivation of *Dinophysis* in this study solves a major bottleneck in this research and our findings will allow other laboratories around the world to expand research efforts on this cosmopolitan species.

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