

**FIELD OBSERVATION OF *ORNITHOCERCUS* SPP. (DINOPHYSIALES, DINOPHYTA):
REPRODUCTIVE STAGES AND PHASED CELL DIVISION (SOUTH ATLANTIC OCEAN,
BRAZIL).**Eveline Pinheiro de AQUINO¹Marcos HONORATO-DA-SILVA^{1,2}Fernando Antônio do Nascimento FEITOSA^{1,3}Maria Luise KOENING^{1,3}José Zanon de Oliveira PASSAVANTE^{1,3}

Recebido em: 11/01/2014

Aceito em: 20/10/2014

ABSTRACT

Studies of the marine dinoflagellates life cycle has been limited. Cell division is often initiated at specific times of the day (phased cell division). This study report the stages of cell division (megacytic zone and dorsal megacytic bridge) in *Ornithocercus* population from the South Atlantic Ocean (Archipelago of Fernando de Noronha) at different times of the day. The samples were collected every 3h, covering 24h, from a two fixed samplings stations, during the years 2010 and 2012. The plankton net (20µm

mesh size) was dragged vertically. The dividing cells of *Ornithocercus* was registered. Were identified *O. magnificus* Stein, *O. thumii* (Schmidt) Kofoid & Skogsberg, *O. quadratus* Schütt, and *O. steinii* Schütt, which the phased cell division occurred and presented at least one of the cell division stages. *O. quadratus* occurred at a wide range of sampling times, extending from before, during, and after dawn, to during and after sunset.

Key words: Phytoplankton, Dinoflagellate, Nictemeral variation.**RESUMO**

O ciclo de vida dos dinoflagelados oceânicos tem sido pouco estudado. A divisão é iniciada em determinados tempos do dia (divisão celular faseada). Este estudo relata os estágios de divisão (zona e ponte dorsal megacítica) para a população de *Ornithocercus*, no Oceano Atlântico Sul (Arquipélago de Fernando de Noronha), em diferentes tempos do dia. As amostras foram coletadas a cada 3h, durante 24h, em dois pontos fixos, nos anos 2010 e 2012. Foi feito o arrasto vertical da rede de plâncton (malha

de 20µm). As células em divisão de *Ornithocercus* foram registradas, em que ocorreram *O. magnificus* Stein, *O. thumii* (Schmidt) Kofoid & Skogsberg, *O. quadratus* Schütt e *O. steinii* Schütt, os quais apresentaram a divisão faseada e pelo menos um dos estágios de divisão celular. *O. quadratus* ocorreu no maior número de horários, desde antes, durante e depois do amanhecer, bem como durante e depois do pôr do sol.

Palavras chave: fitoplâncton, dinoflagelados, variação nictemeral.**INTRODUCTION**

Organisms of the order Dinophysiales thrive in ocean waters, and those in the waters of the Brazil Current have been well documented (BALECH, 1988; HARAGUCHI; ODEBRECHT, 2010; KOENING et al., 2009). These dinoflagellates are relatively diverse, showing a wide range of characteristics and physiological needs, as well as they show autotrophic, mixotrophic, and heterotrophic characteristics.

There has been limited research on the life cycle of marine dinoflagellates mainly because of the difficulty associated with its isolation and the need for highly specific growth

1. Universidade Federal de Pernambuco/ Programa de Pós-Graduação em Oceanografia/ Laboratório de Fitoplâncton
2. Colégio de Aplicação/ Universidade Federal de Pernambuco
Departamento de Oceanografia/ Universidade Federal de PernambucoContatos:
eveline_aquino@yahoo.com.br; marcos_honorato2003@yahoo.com.br; feitoc@bol.com.br;
koening@terra.com.br; passavante@gmail.com

conditions. Most life cycle studies have thus focused on continental and neritic species (WALKER, 1984). Vegetative division represents the main form of reproduction in dinoflagellates, resulting in two identical cells of the same initial size (REGUERA; GONZALEZ-GIL, 2001).

According to Walker (1984), the life cycle of Dinophysiales involves the following stages: During a rapid lateral expansion associated with cell division, a semi-meridional band called the megacytic zone is formed, which corresponds to an area developed at the dorsal region of the cell. While the cells are still attached and undergoing formation, the megacytic zone maintains the integrity of the mother cell walls during complete cytokinesis of the cell body, which culminates with the formation of a new cell wall. The megacytic zone then undergoes natural dissolution, leading to the separation of the daughter cells and subsequent development of fins and ribs. Thus, the dorsal region is the last area of attachment in some species. The daughter cells may remain attached during formation of the remaining fins and ribs. This occurs because of maximal extension of the megacytic zone, leading to the formation of a striated structure (PFIESTER; ANDERSON, 1987) known as the dorsal megacytic bridge. After full separation of daughter cells, remnants of this bridge can persist for an unknown but presumably short period (TAYLOR, 1973). Cell division in dinoflagellates is often initiated at specific times of the day, and division phases vary among species. This phenomenon is known as phased cell division.

Because of this synchronicity, population growth occurs during specific periods, thus, generating a step-by-step growth curve. Local environmental conditions also affect the reproductive cycle of these species. For example, in adequate conditions, marine photosynthetic organisms often undergo cell division during the early hours of the day, when exposure to sunlight is more intense. According to Walker (1984), maximum division rates for the dinoflagellate *Pyrodinium bahamense* Plate (Gonyaulacales) were observed during the first two hours after sunrise, whereas for laboratory-grown species of *Ceratium* (Gonyaulacales), reproduction occurs just before or soon after dawn.

In this context, this study aimed to report the stages of cell division (megacytic zone and dorsal megacytic bridge) in species of *Ornithocercus* (Dinophysiales) from the South Atlantic Ocean waters (Brazil) at different times of the day.

MATERIAL AND METHODS

The samples were collected from a two fixed samplings stations, during the years 2010 (year I; 32° 20' 10'' W and 3° 46' 49'' S) and 2012 (year II; 32° 27' 78'' W and 3° 54' 65'' S). The samples stations were situated about 6.35 km from the northwest (year I) and southeast (year II) coast of the Archipelago of Fernando de Noronha, South Atlantic Ocean, Brazil (Fig. 1). The sampling occurred aboard the oceanographic research vessel NHo Cruzeiro do Sul. The samples were collected every 3 h, covering 24 h, during the dry season (August 2010 e September 2012). The plankton net (20 µm mesh size) was dragged vertically for 5 min. Net dragging extended to a maximum depth of 130 m. The samples (n = 12) were stored in plastic containers (500 mL) and fixed in neutral formalin (THRONDSSEN, 1978). The aliquots were obtained of 0.5 mL for species identification using an optical microscope coupled to a camera at the Phytoplankton Laboratory of the Federal University of Pernambuco. Thus, the dividing cells of *Ornithocercus*, which were defined by the presence of the megacytic zone and the dorsal megacytic bridge were registered. For the taxonomical classification was used the system of Guiry and Guiry (2012).

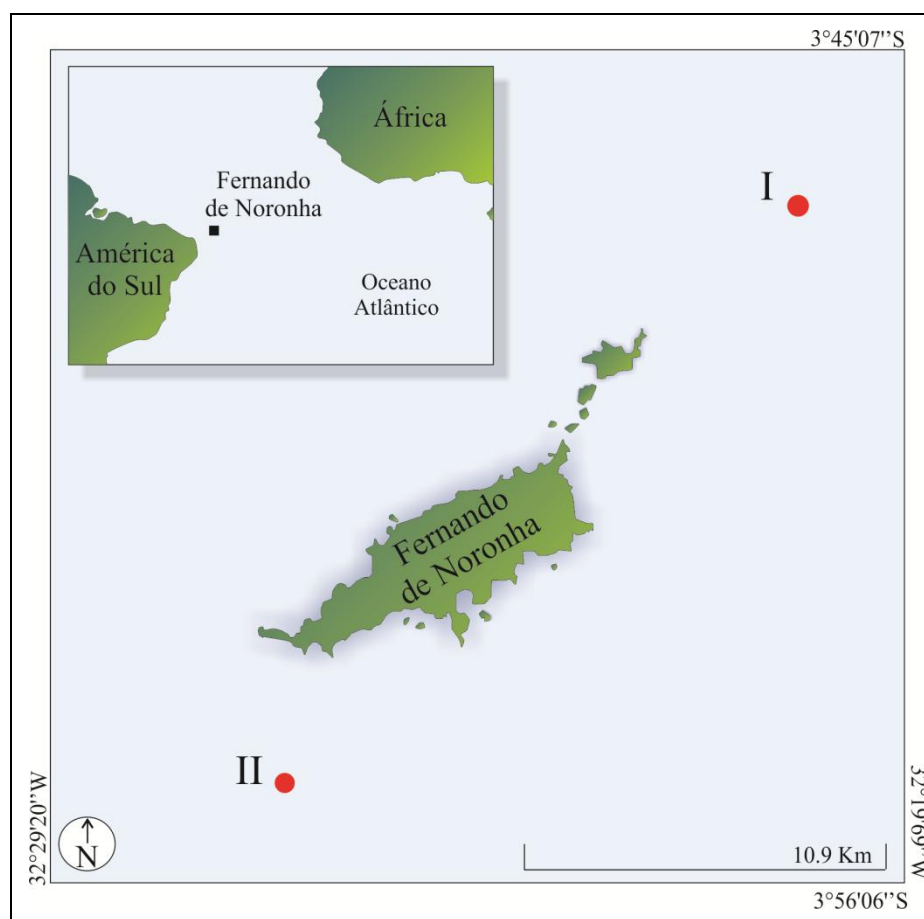


Figure 1 – Archipelago of Fernando de Noronha, South Atlantic Ocean, Brazil, where is located the sampling stations during the years I (northwest coast) and II (southeast coast).

RESULTS

Results from the both years, including vegetative and dividing cells, was identified the following species: *O. magnificus* (Fig. 2A and 2B), *O. thumii* (Schmidt) Kofoid & Skogsberg (Fig. 2C), *O. quadratus* (Fig. 2D and 2E), and *O. steinii* Schütt (Fig. 2F). During the year I, *O. magnificus* cells occurred in all times of this study, with highlight of your dominance during three hours before sunrise. Whereas, *O. quadratus*, occurred since dawn until three hours after sunrise. Thus, this species was absent before the sunset. Finally, *O. steinii* and *O. thumii* occurred before and after sunrise and sunset. During the year II, only *O. magnificus* and *O. quadratus* occurred. *O. quadratus* was observed during sunrise (where was dominant), and before and during sunset. On the other hand, *O. magnificus* was observed since before dawn until sunrise, and thus this species was not observed only three hours after sunrise.

All of these species presented at least one of the cell division stages (megacytic zone and/or dorsal megacytic bridge) in at least one sample. However, *O. magnificus* presented only the megacytic zone stage; were not observed the dorsal megacytic bridge in this species. Thus, two stages of cellular division were identified: the first consisted of the megacytic zone characterised by the presence of paired cells, which were attached by the dorsal margin (Fig. 2B); and the second included the dorsal megacytic bridge (Fig. 2C-2F), which displayed a strong horizontally striated pattern. The dorsal megacytic bridge occurred in two forms: as connected cells (Fig. 2C and 2D) or as remnants of a single individual (Fig. 2E and 2F). When connected, the cells were of the same size. The recently divided daughter cells was observed, commonly showed a left sulcal fin and incomplete anterior and posterior ribs, whereas the anterior and posterior cingulate fins were fully developed (Fig. 2C-2E).

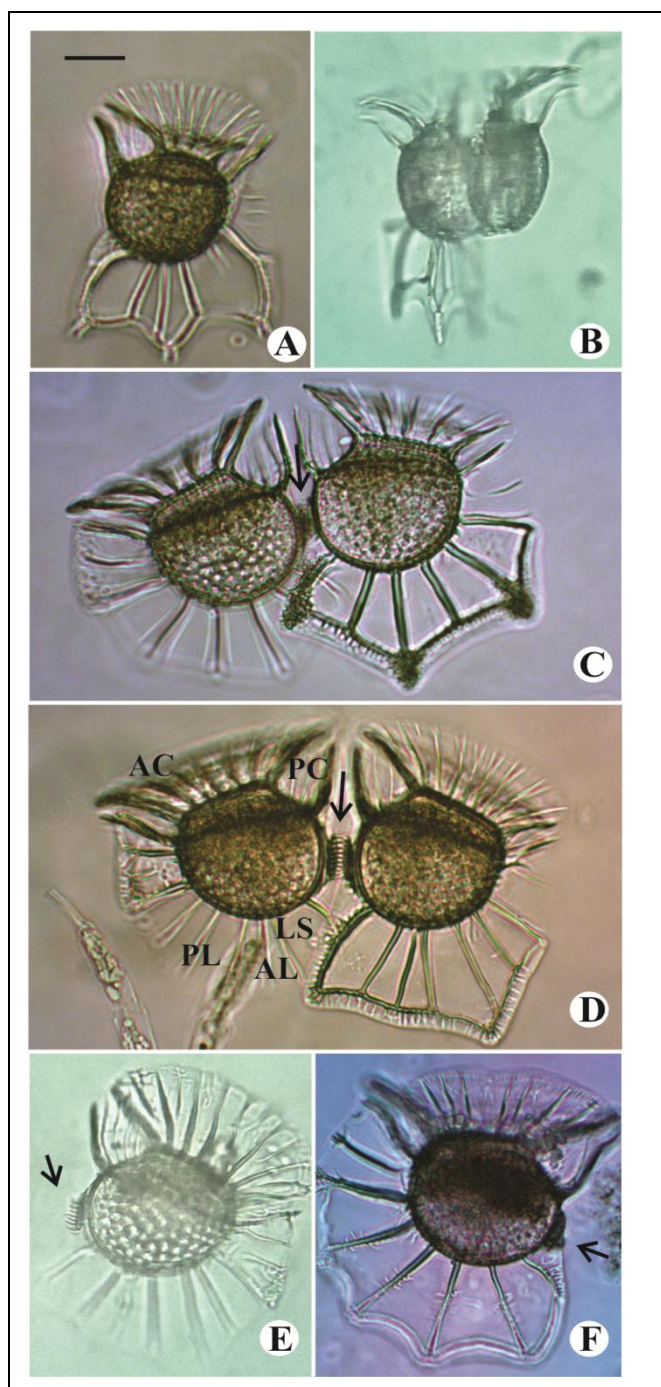


Figure 2 - **A)** Vegetative cell of *Ornithocercus magnificus* Stein; **B)** Dividing cell, at the megacytic zone stage; **C)** Two attached cells of *O. thumii* (Schmidt) Kofoid & Skogsberg; **D)** Two attached cells of *O. quadratus* Schütt; **E)** With incomplete lists and ribs; **F)** *O. steinii* Schütt; arrows show the megacytic dorsal bridge in attached cells or remnants of this bridge in an individual cell. Scale bar = 26 µm. Codes: LS = left sulcal fin; PC = posterior cingulate fin; AC = anterior cingulate fin; PL = posterior lobe; and AL = anterior lobe.

Based on the different sampling times, the phased cell division was observed in all four species of *Ornithocercus*. From the total of *Ornithocercus* cells observed, during the year I, cell division commenced three hours before dawn for *O. magnificus*, *O. thumii*, and *O. quadratus*. It occurred during sunrise only for *O. quadratus*. Whereas the reproductive stages of *O. steinii* and *O. quadratus* were observed up to three hours after sunrise. The latter also showed the presence of dividing cells during and up to three hours after sunset. Therefore, the results showed that dividing cells of *O. quadratus* occurred at a wide range of sampling times, extending from before, during, and after dawn, to during and after sunset. None of the species, during the year I, was present in samples taken either three hours before sunset or three hours after dawn and/or sunset. During the year II, the cell division were presents only to *O. quadratus*, during the sunrise and sunset times. These phases are summarised in Fig. 3.

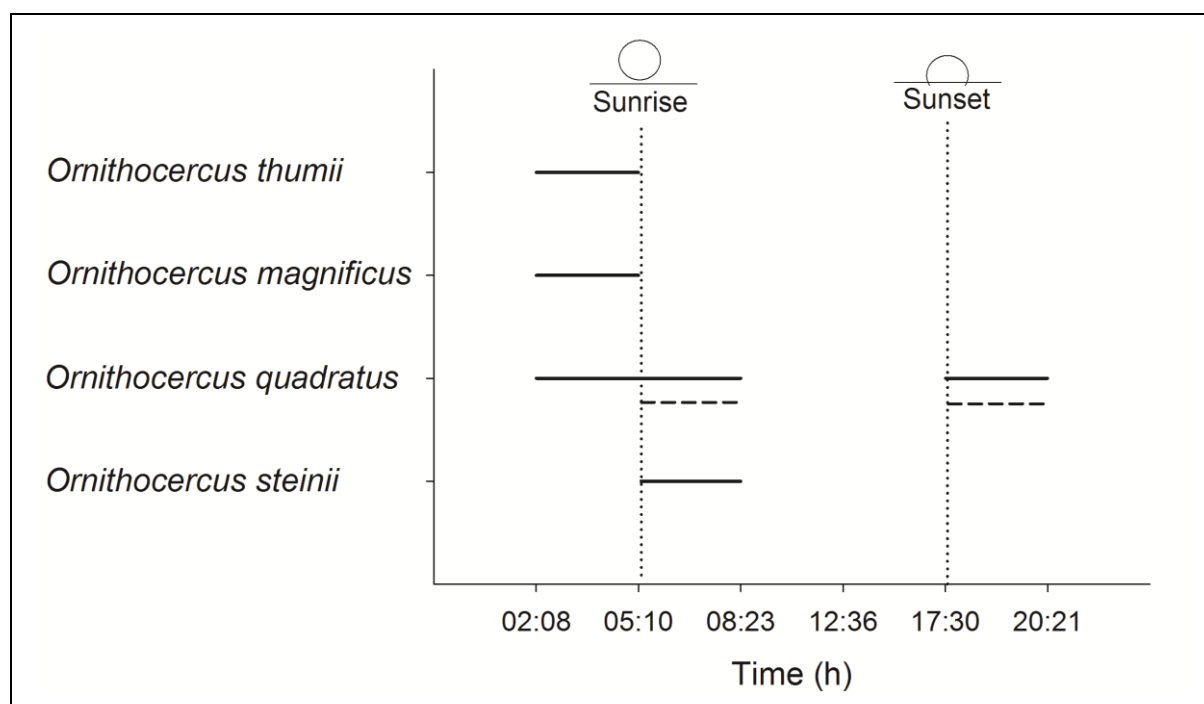


Figure 3 – Times (h) at which *Ornithocercus* species were undergoing cell division, during the years I (continuous line) and II (dotted line).

DISCUSSION

In general, among the dinoflagellates, the life cycle of *Dinophysis* spp. (Dinophysiales) has been broadly researched to generate information on the stages of cell division and the mechanism of phased cell division. Additionally, this group has been extensively studied for the following reasons: it includes coastal organisms that are highly accessible and whose growth mechanisms are already known, and some of the species produce toxins that are harmful to humans and the environment, thus further justifying research efforts on its life cycle.

Previous studies have identified the megacytic zone and the dorsal megacytic bridge as reproductive stages of *Dinophysis* (REGUERA; GONZALEZ-GIL, 2001; REGUERA et al., 2003; REGUERA; GONZALEZ-GIL, 2007; AISSAOUI et al., 2013). Likewise, based on our findings from the South Atlantic Ocean, these stages are also present in the genus *Ornithocercus*, whose dorsal megacytic bridge showed a heavily striated pattern. Remnants of this bridge can still be found in recently divided cells of *Ornithocercus* spp., even after dissolution of the megacytic zone and the separation of cells (TAYLOR, 1973). However, this structure can be easily destroyed by sample manipulation, especially during dragging of the net or because of formalin fixation (AISSAOUI et al., 2013). Thus, given that our results are based on field observations, laboratory experiments should be performed to confirm these results and generate further detailed information that is specifically aimed at assessing the time required for the formation of the dorsal megacytic bridge and how long the bridge structure lasts in *Ornithocercus* spp. It was also observed that during this stage of cell division, the recently formed daughter cell showed incomplete ribs and fins. This is a morphological characteristic routinely used to establish the end of a reproductive stage, which is important in studies focused on estimating cell division rates (REGUERA; GONZALEZ-GIL, 2001; HERNANDEZ-ROSAS et al., 2007).

Dinophysis spp. commonly shows phased cell division (REGUERA et al., 2003). This is also the case for the genus *Ornithocercus*, based on our findings in the presence of dividing cells throughout the day (before, during, or after dawn or sunset) in the four species. In a study of *Dinophysis acuminata* Claparède & Lachmann in the Mediterranean Sea, Aissaoui et al. (2013) showed that cell division reached a maximum soon before or after sunrise, which indicates that the first rays of sunlight activated cell division. On the other hand, these same authors observed that for other species of this genus, cell division reached its maximum at several hours after dawn, suggesting that these organisms require higher intensity sunlight.

Other species achieve its maximum cell division in the dark, thus suggesting the need for low intensity light to activate cell division (FARREL et al., 2013).

In this context, our observations have demonstrated that *O. steinii* requires high light intensity, whereas *O. magnificus* and *O. thumii* uses the first rays of sunlight. Regarding *O. quadratus* presented dividing cells at various sampling times, including both periods of light and dark, it can be explained by the need for sunlight and availability of prey, based on the observations in a previous study that *D. acuminata* requires a combination of low intensity light (REGUERA et al., 2003) and access to prey (KIM et al., 2008) for its long-term survival. As a mixotrophic species (HARAGUCHI; ODEBRECHT, 2010), *D. acuminata* can achieve high growth rates in the absence of light or in the presence of low intensity light. In this context, this combination could be applied to *O. quadratus* because this organism is also mixotrophic.

Cell division rates are subjected to seasonal and spatial (e.g. at different depths of the water column) changes and vary considerably among dinoflagellate species. Aissaoui et al. (2013) observed higher rates in different species of *Dinophysis* during the fall and summer months, with proliferation occurring between 13°C and 30°C and within a salinity range of 33 to 40, which is suggestive of the broad adaptation of these species to environmental changes throughout the year. Given that our study involved only one season (the dry season), additional research is necessary to determine the potential effects of environmental changes on the reproductive cycle of *Ornithocercus* spp. during the different seasonal climate periods of the region (rainy and dry). In addition, this approach can be utilised in assessing the effect of environmental conditions on dinoflagellate growth. Similarly, vertical spatial variation has been regarded as one of the factors that affect population growth rates, with higher rates found at narrower depths (VELO-SUAREZ et al., 2009; FARREL et al., 2013). However, as our study did not include sampling at different depths (vertical dragging of the net extended to a maximum depth of 130 m), we cannot describe the effects of vertical spatial variation on *Ornithocercus* spp. at different depths.

ACKNOWLEDGEMENTS

The authors wish to thank Professor Denise R. Tenebaum for providing scientific literature on the reproduction of dinoflagellates. We would also like to thank the Brazilian Ministry of Science, Technology, and Information (MCTI), the Brazilian Navy, and the Federal University of Pernambuco (UFPE) for the financial and logistic support; the Institute Chico Mendes de Conservação da Biodiversidade (ICMBio) for conceding the license required for conducting samples collections.

REFERENCES

- AISSAOUI, A.; ARMI, Z.; TURKI, S.; HASSINE, O. K. B. Seasonal dynamic and in situ division rates of the dominant *Dinophysis* species in Punic harbors of Carthage (Gulf of Tunis, South Mediterranean). **Environmental Monitoring Assessment**, v.185, n.11, p.9361-84, 2013.
- BALECH, E. **Los dinoflagelados del Atlántico Sudoccidental**. 1ª Edição. Inst. Español Oceanogr., 1988. 310 p.
- FARRELL H.; VELO-SUAREZ L.; REGUERA, B.; RAINE, R. Phased cell division, specific division rates and other biological observations of *Dinophysis* populations in sub-surface layers off the south coast of Ireland. **Deep Sea Research II**, v.1, 2013.
- HARAGUCHI, L.; ODEBRECHT, C. Dinophysiales (Dinophyceae) no extremo Sul do Brasil (inverno de 2005, verão de 2007). **Biota Neotropica**, v. 10, n.3, p.101-114, 2010.
- HERNÁNDEZ-ROSAS, A.; MEAVE, M. E.; CASTILLO, M. E.; ZAMUDIO-RESENDIZ; CASTILLO-RIVERA, M. Morfometría y distribución de especies del género *Ornithocercus* (Dinophysiales: Dinophyta) del Pacífico Mexicano. **Hidrobiologica**, v. 17, p.257-272, 2007.
- KIM, S.; KANG, Y. G.; KIM, H. S.; YIH, W.; COATS, D. W.; PARK, M. G. Growth and grazing responses of the mixotrophic dinoflagellate *Dinophysis acuminata* as functions of light intensity and prey concentration. **Aquatic Microbial Ecology**, v. 51, p.301-310, 2008.

- KOENING, M. L.; WANDERLEY, B. E.; MACEDO, S. J. Microphytoplankton structure from the neritic and oceanic regions of Pernambuco State - Brazil. **Brazilian Journal of Biology**, v. 69, n.4, p.1037-46, 2009.
- PFIESTER L. & ANDERSON D.M. **Dinoflagellate reproduction**. In: TAYLOR, F. J. R. (Ed.). Biology of dinoflagellates. Oxford: Blackwell Scientific Publications, 1987. p. 611 - 648.
- REGUERA, B.; GONZÁLEZ-GIL, S. Small cell and intermediate cell formation in *Dinophysis* (Dinophyceae, Dinophysiales). **Journal of Phycology**, v. 37, p.318-333, 2001.
- REGUERA, B.; GARCÉS, E.; BRAVO, I.; PAZOS, Y.; RAMILO, I. In situ division rates of several species of *Dinophysis* estimated by a postmitotic index. Marine Ecology Progress Series, v. 249, p.117-131, 2003.
- REGUERA, B.; GONZALEZ-GIL, S. *Dinophysis diegensis* a life history stage of *Dinophysis caudata* (Dinophyceae, Dinophysiales). **Journal of Phycology**, v. 43, p. 1083-1093, 2007.
- TAYLOR, F. J. R. Topography of cell division in the structurally complex dinoflagellate genus *Ornithocercus*. **Journal of Phycology**, v. 9, p. 1-10, 1973.
- THRONDSSEN, J. **Preservation and storage**. In: Sournia, A. (Ed.). Phytoplankton Manual. New York: UNESCO, 1978. p. 69 - 74.
- VELO-SUÁREZ, L.; REGUERA, B.; GARCÉS, E.; WYATT, T. Vertical distribution of division rates in coastal dinoflagellate *Dinophysis* spp. populations: implications for modelling. **Marine Ecology Progress Series**, v. 385, p. 87-96, 2009.
- WALKER, L. M. **Life histories, dispersal and survival in marine, planktonic dinoflagellates**. In: STEIDINGER, K. A.; WALKER, L. M. (Ed.). Marine Plankton Life Cycle Strategies. Florida: CRC press, 1984. p. 19 - 34.