Deep-Sea Research II 57 (2010) 248–255

Contents lists available at ScienceDirect

Deep-Sea Research II

journal homepage: www.elsevier.com/locate/dsr2

A massive and simultaneous sex event of two *Pseudo-nitzschia* species

Diana Sarno*, Adriana Zingone, Marina Montresor

Stazione Zoologica Anton Dohrn, Napoli, Italy

ARTICLE INFO

Available online 20 September 2009

Keywords:
Life cycle
Diatoms
Pseudo-nitzschia
Pseudo-nitzschia cf. *delicatissima*
Pseudo-nitzschia cf. *calliantha*
Sexual reproduction
Mediterranean Sea
Tyrrhenian Sea
Gulf of Naples

ABSTRACT

Sex is an obligatory event in the life cycle of many diatoms, being the means of restoration of maximum cell size that counteracts the progressive miniaturization induced by asexual divisions. Nonetheless, sexual stages and evidence for sexual reproduction in diatoms have rarely been directly reported from the marine environment. On September 20, 2006, different stages of the sexual cycle of two species, *Pseudo-nitzschia* cf. *delicatissima* and *P.* cf. *calliantha*, were detected in surface waters of the Gulf of Naples, at the Long Term Ecological Research Station MareChiara. The sampling date corresponded with a seasonal peak of abundance of the two species, which reached maximum concentrations of 9.1×10^6 and 7.0×10^5 cells L^{-1}, respectively. Gametangia, auxospores of different size, and initial cells within and outside the perizonium were observed. Up to 9.2% of *P.* cf. *delicatissima* and 14.3% of *P.* cf. *calliantha* cells were undergoing sexual reproduction. The sexual event was mainly concentrated in surface waters and no sexual stages were observed a week before or after the date. The low percentage of sexual stages and the short duration of such events are probably the reasons for the scarcity of sexual stage observations in nature.

1. Introduction

Pennate diatom species of the genus *Pseudo-nitzschia* are abundant and widespread in coastal and oceanic waters all over the world (Hasle, 2002). Over the last 20 years they have become the subject of numerous studies, following the discovery that some species produce the toxin domoic acid, the cause of the Amnesic Shellfish Poisoning (ASP) syndrome in humans (Mos, 2001) and of mass mortality in marine mammals and birds (Sierra-Beltrán et al., 1997; Lefebvre et al., 1999; Scholin et al., 2000).

Due to the fact that diatom cells are surrounded by a rigid frustule composed of two slightly unequal halves (epi- and hypotheca), at each vegetative division the two daughter cells inherit maternal epivalves of different sizes and, as a consequence, one of the cells is smaller than the other. In some diatoms the deposition of cingular bands can compensate for the size reduction process (Crawford, 1980; Hildebrand et al., 2007), whereas in others cell enlargement can occur through the formation of an auxospore-like structure that is produced asexually (e.g. von Stosch, 1965; Gallagher, 1983; Sato et al., 2007). However, in many cases, and especially in pennate diatoms, the progressive reduction in the population cell size that accompanies vegetative divisions is interrupted by the sexual phase, in which maximum cell size is restored (Chepurnov et al., 2004). In the genus *Pseudo-nitzschia*, the asexual phase is the dominant form, but sexual stages have been observed in laboratory investigations. All the *Pseudo-nitzschia* species studied so far are heterothallic (Davidovich and Bates, 1998; Amato et al., 2005; Chepurnov et al., 2005; D'Alelio et al., 2009), as sexual reproduction requires a mixture of strains of two different mating types, with the exception of *Pseudo-nitzschia brasiliana*, which is homothallic (Quijano-Scheggia et al., 2009). The mode of sexual reproduction is similar in all species of the genus. Haploid, non-motile gametes are produced following meiosis in gametangial cells, which pair together allowing migration of two active gametes towards the passive ones. Gamete conjugation gives rise to two auxospores that are initially spherical but quickly elongate while they are still attached to the gametangial frustule. Elongation is made possible by the fact that the auxospore lacks rigid siliceous valves and is surrounded by a soft covering, the perizonium. Within the auxospore, large-sized initial cells are produced, which resume the asexual phase. As maximum size restoration in all *Pseudo-nitzschia* species studied so far seems to depend upon the onset of the sexual phase, this should be a regular event in nature. Nonetheless, despite the high level of attention that has been paid to these species over recent decades, *Pseudo-nitzschia* auxospores have never been reported from the natural environment. This lack of reports mirrors the very few findings of sexual events so far known for all marine diatoms in nature (e.g. Waite and Harrison, 1992; Crawford, 1995; Potapova and Snoeijs, 1997; Assmy et al., 2006; Koester et al., 2007).

* Corresponding author.
E-mail address: diana.sarno@szn.it (D. Sarno).

0967-0645/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dsr2.2009.09.012

In September 2006, a considerable number of *Pseudo-nitzschia* auxospores formed by two different species were observed in natural samples collected at the long-term monitoring station MareChiara (LTER-MC) in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea) during the course of a bloom of several *Pseudo-nitzschia* species. We provide a report of this exceptional event, which represents the first such description for the genus to our knowledge, and includes detailed observations of the different stages observed in natural samples.

2. Methods

The station MareChiara (40°48.5′N, 14°15′E) located 2 miles offshore in the Gulf of Naples (Tyrrhenian Sea, Mediterranean Sea) was sampled weekly as part of the Long Term Ecological Research Program MareChiara (LTER-MC). Salinity, temperature and oxygen data were collected using an SBE 911plus CTD profiler (SeaBird, Bellevue, Washington, USA) with sensors and an SCUFA fluorometer. Data were processed using SeaSave Data Processing software. The CTD was connected to the automatic Carousel sampler equipped with 12 L Niskin bottles used to collect the water samples. Samples for nutrient analyses were collected in high-density polyethylene vials directly from the Niskin bottles and immediately stored at −20 °C until analysis in the laboratory. Phytoplankton samples, also collected using the Niskin bottles from 0.5 (indicated as 0 m or surface in the following), 10, 20, 30, 40 and 50 m, were fixed with neutralised formaldehyde at a final concentration of 0.8%. Depending on the richness of the samples, between 1 and 50 ml were analysed with an inverted light microscope (ZEISS Axiovert) at 400× magnification (Utermöhl, 1958). Cells falling within two transects representing ca. 1/30 of the whole chamber bottom were identified and enumerated. Species of the genus *Pseudo-nitzschia* were enumerated in the 0, 10, 20, 30, 40 and 50 m samples. All species present were enumerated in the 0 m samples. Carbon content was calculated from the mean cell biovolumes as outlined in Ribera d'Alcalà et al. (2004) using the conversion formulae proposed in Menden-Deuer and Lessard (2000).

Measurements of vegetative cells and of different stages of the life cycle of *P.* cf. *calliantha* and *P.* cf. *delicatissima* were taken with the inverted light microscope in the 0 and 10 m samples collected on September 20, 2006, and in the 0 m sample of September 12 and 26 at 400× using an ocular micrometer, with a resolution of 2.5 μm. At least 200 specimens of each of the two species were measured in each sample. Pictures of specimens and different sexual stages were taken with a Zeiss Axiocam digital system mounted on the Zeiss Axiovert microscope. TEM observations were performed with an LEO 912AB transmission electron microscope on clean frustules prepared as illustrated in Orsini et al. (2002). Fixed samples, not subjected to cleaning, were dehydrated in an ethanol series, critical point dried using CO_2 as intermediary fluid, sputter-coated with platinum and observed with a JEOL JSM-6700F scanning electron microscope.

3. Results

3.1. Morphology

The two species undergoing sexual reproduction were identified as members of the *P. delicatissima* and *P. pseudodelicatissima* species-complexes (Fig. 1), respectively, based on their narrow valves (1.3–1.7 and 1.8–2.0 μm, respectively) and the shape of the cell ends in girdle view, which were rounded to truncated in the former species (Fig. 1B) and pointed and elongated in the latter (Fig. 1C) (Lundholm et al., 2003, 2006; Amato et al., 2007). *Pseudo-nitzschia pseudodelicatissima*-like cells were slightly more silicified compared to *P. delicatissima*-like cells. The cell length range was 40–92.5 μm in the *P. delicatissima*-like morphotype and 50–112.5 μm in the *P. pseudodelicatissima*-like morphotype, but the size distribution pattern was very distinct between the two species (Fig. 2).

Upon TEM examination, *P. delicatissima*-like specimens showed 22–26 fibulae and 38–42 striae in 10 μm. Each stria was biseriate, with 9–12 poroids in 1 μm (Fig. 1D). Based on the ultrastructural features of the frustule, these specimens could not be attributed unequivocally to a single species, and will therefore be referred to as *P.* cf. *delicatissima*. Specimens that, in light microscopy, resembled *P. pseudodelicatissima* in TEM had valves with 20–22 fibulae and 38–40 striae in 10 μm. Each stria was ornamented with square poroids (4–5 poroids in 1 μm) split into 4–9 sectors (Fig. 1E) as in *P. calliantha*. In most cells a central sector was present in more than 50% of the poroids. However, some specimens with a lower number of sectors per poroid and a lower percentage of poroids with a central sector were also found, which are typical features of *P. mannii* (Amato and Montresor, 2008). Given the possibility that both species were present in the samples examined, the *P. pseudodelicatissima*-like material is hereafter referred to as *Pseudo-nitzschia* cf. *calliantha.*

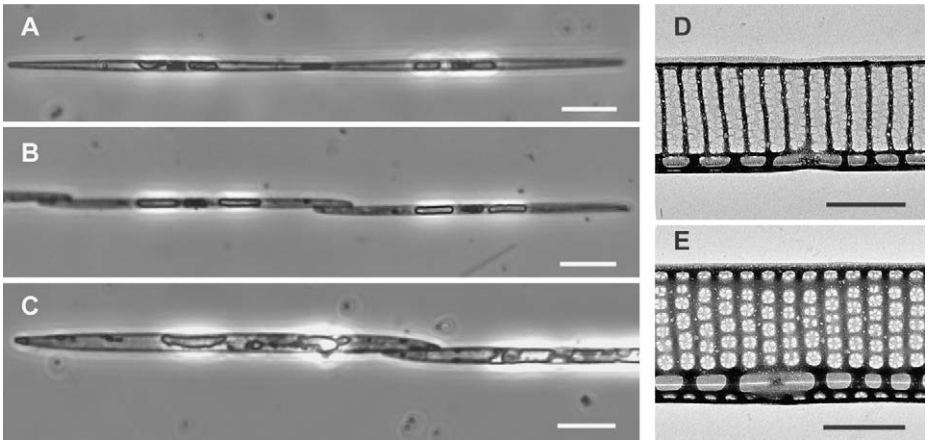

Fig. 1. *Pseudo-nitzschia* cf. *delicatissima* and *Pseudo-nitzschia* cf. *calliantha*, natural samples from the LTER-MC station, late summer 2006. Light (A–C) and TEM (D–E) micrographs of vegetative cells. (A) Two-celled chain of *Pseudo-nitzschia* cf. *delicatissima* in valve view. Scale bar: 10 μm. (B) Chain of *Pseudo-nitzschia* cf. *delicatissima* in girdle view. Scale bar: 10 μm. (C) Chain of *Pseudo-nitzschia* cf. *calliantha* in girdle view. Scale bar: 10 μm. (D) Detail of the central part of the valve of *Pseudo-nitzschia* cf. *delicatissima*. Scale bar: 1 μm. (E) Detail of the central part of the valve of *Pseudo-nitzschia* cf. *calliantha*. Scale bar: 1 μm.