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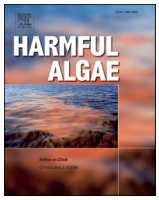

Abiotic and biotic factors controlling sexual reproduction in populations of *Pseudo-nitzschia pungens* (Bacillariophyceae)

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ABSTRACT

Pseudo-nitzschia pungens is a widely distributed marine pennate diatom. Hybrid zones, regions in which two different genotypes may interbreed, are important areas for speciation and ecology, and have been reported across the globe for this species. However, sexual reproduction between differing clades in the natural environment is yet to be observed and is difficult to predict. Here we carried out experiments using two mono-clonal cultures of *P. pungens* from different genotypes to measure the frequency and timing of sexual reproduction across varying biotic (growth phases and cell activity potential) and abiotic conditions (nutrients, light, turbulence). We found the mating rates and number of zygotes gradually decreased from exponential to late stationary growth phases. The maximum zygote abundance observed was 1,390 cells mL^{-1} and the maximum mating rate was 7.1%, both which occurred during the exponential growth phase. Conversely, only 9 cells mL^{-1} and a maximum mating rate of 0.1% was observed during the late stationary phase. We also found the higher the relative potential cell activity (rPCA) in parent cells, as determined by the concentration of chlorophyll a per cell and the ratio of colony formation during parent cultivations, revealed higher mating rates. Furthermore, sexual events were reduced under nutrient enrichment conditions, and mating pairs and zygotes were not formed under aphotic (dark) or shaking culture conditions (150 rpm). In order to understand the sexual reproduction of *Pseudo-nitzschia* in the natural environment, our results highlight that it is most likely the combination of both biotic (growth phase, Chl. *a* content) and abiotic factors (nutrients, light, turbulence) that will determine the successful union of intraspecific populations of *P. pungens* in any given region.

1. Introduction

Diatoms are the most abundant and diverse siliceous micro-organisms in the world (Finkel et al., 2005). They contribute as much as 20% of the primary production on Earth and up to 40% of marine primary production (Field et al., 1998). Whilst asexual reproduction through rapid cell division contributes to this production via photosynthesis, sexual reproduction is a more critical life cycle strategy both from an ecological and evolutionary perspective (Brawley and Johnson, 1992; Jewson, 1992; Poulíčková et al., 2019). A diatom cell carries out vegetative cell division resulting in a decreasing of cell size, until cell size recovery occurs by sexual reproduction and auxosporulation (Chepurnov et al., 2005; Davidovich and Bates, 1998; Mann, 1993). Apart from size recovery of the cell, sexual reproduction also allows the maintenance of genetic diversity via genetic recombination (Amato et al., 2005; Kim et al., 2020). The diatom genus *Pseudo-nitzschia* are diploid (2n) heterothallic organisms (Quijano-Scheggia et al., 2009), meaning they require another sexual type (e.g. male or female) for sexual reproduction. In this case, the sexual events between genetically distinct populations may provide significant insights for investigating

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