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Multiannual *Chattonella subsalsa* Biecheler (Raphidophyceae) blooms in a Mediterranean lagoon (Santa Giusta Lagoon, Sardinia Island, Italy)

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ABSTRACT

Recurrent blooms of *Chattonella subsalsa* (Raphidophyceae) were associated with fish kills in Santa Giusta Lagoon (Mediterranean Sea). This study investigated the population dynamics of *C. subsalsa* and its relationship with environmental and meteorological conditions, using multiannual ecological data (1990–2016). In addition, for the first time, this study examined the presence of *C. subsalsa* cysts in lagoon sediments. The species was first detected in Santa Giusta Lagoon in July 1994. Bloom events coinciding with fish kills were recorded in 1994, 1998, 1999, and 2010. The timing and dynamics of *C. subsalsa* blooms and fish kills varied over the examined period. Presence of *C. subsalsa* was strongly influenced by temperature, especially in the early years of the series (1990–2002). Temperature control may have been lesser important in the more recent years, when higher temperature may have generated continuative suitable conditions for *C. subsalsa* affirmation, especially in July. Thus, the variations in the availability of food (via autotrophy and/or mixotrophy) could be one of the control keys on the proliferation of this species in the future in SG. Cysts of *C. subsalsa* were present in lagoon sediments at abundances ranging 200–2000 cysts g^{-1} wet sediment. This study is among a few that have examined *C. subsalsa* population dynamics and bloom events in the field over a long time period. Findings from this study contribute to a better understanding of *C. subsalsa* bloom development, by identifying environmental and meteorological variables that may promote blooms of this species in the Santa Giusta Lagoon.

1. Introduction

Red tide-causing Raphidophycean flagellates, belonging to the genus *Chattonella*, are responsible for harmful algal blooms as well as farm-raised and wild fish kills worldwide. In some cases, these fish kills have led to extensive ecological damage and economic loss (Imai and Yamaguchi, 2012; and references therein). The genus *Chattonella* includes *C. subsalsa*,*C. minima* Hara & Chihara, and a complex of other species: *C. marina* (Subrahmanyan, 1954) Hara & Chihara, *C. marina* var. *antiqua* (Hada) Demura & Kawachi, and *C. marina* var. *ovata* (Hara & Chihara) Demura & Kawachi, although Imai and Yamaguchi (2012) suggest that further taxonomic studies should be conducted before definitively accepting the *C. marina* complex. Recently, *C. subsalsa* strains from the western Adriatic Sea were found to be distinct from worldwide isolates, based on morphological and molecular characteristics (Klöpper et al., 2013). Additionally, a new species, related to *C. subsalsa*, was described from the Oman Sea (Attaran-Fariman and Bolch, 2014), indicating the need of further taxonomic studies.

Species belonging to the *Chattonella marina* complex are considered to be the most harmful of the genus and, therefore, have been the most thoroughly studied. Much information is available on their ecophysiology, life cycles, ecological strategies, bloom dynamics (Imai and Yamaguchi, 2012 and references therein), and sources and pathways of toxicity (Shimada et al., 1983; Khan et al., 1996; Marshall et al., 2003; Dorantes-Aranda et al., 2015 and references therein). In contrast, little information is available on the ecology (Handy et al., 2005; Zhang et al., 2006; Portune et al., 2009) or deleterious effects (Bridgers et al., 2004; Keppler et al., 2006; Pérez-Morales et al., 2014) of *Chattonella subsalsa*.

The distribution of *Chattonella* species in the Mediterranean seems to be geographically limited, and little is known about the ecology of the genus in this area. Among species of the *C. marina*

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complex, only *C. antiqua* has been reported from the coast of Egypt (Mikhail, 2007), whereas *C. subsalsa,* which represents the holotype of the genus, was first described by Biecheler (1936) in the Saline of Villeroy, near Séte, along the Mediterranean coast of France. Later, *C. subsalsa* was observed during bloom events in Algeri harbor (Holland and Enjument, 1956) and Barcelona harbor (Margalef, 1968). More recently, *C. subsalsa* was recorded in the Gulf of Naples, the Gulf of Salerno, Lago di Fusaro (Zingone et al., 2006), and the Adriatic Sea (Pistocchi et al., 2012; Klöpper et al., 2013).

Blooms of *Chattonella* have been associated with fish kills since the mid-1990s in Santa Giusta Lagoon (SG) (Sardinia, western Mediterranean Sea). The responsible species were initially classified as *C.* cf. *marina* (Sechi et al., 2001). Then, the presence of *C. subsalsa* in Santa Giusta Lagoon was assessed in 2003 (Bowers et al., 2006) and was confirmed over a multiannual series of blooms, by using molecular approaches (Stacca et al., 2016).

In this study, a multiannual series (1990–2016) of ecological data collected from SG was analyzed, including data on *Chattonella subsalsa* blooms associated with fish kills. The presence of viable *C. subsalsa* cysts in lagoon sediments was verified. The main objectives of this study were: 1) to provide new useful information on the population dynamics of *C. subsalsa* in a Mediterranean lagoon, 2) to characterize blooms coincident with fish kills, 3) to identify environmental factors driving the proliferation of the species, and 4) to identify resting cyst banks in the lagoon that provide a potential sediment repository of bloom inocula.

2. Material and methods

2.1. Study area and sampling methods

Santa Giusta Lagoon (SG) is located on the western coast of Sardinia in the Mediterranean Sea (Fig. 1). The lagoon has an almost circular shape. Its surface area is about 8 km^2, and its mean depth is about 1 m. The lagoon is connected to the Mediterranean Sea through the 3-km-long Pesaria channel and to an industrial harbor by a man-made canal (Fig. 1). The morphology of SG is a result of substantial human modification (Satta et al., 2014). The lagoon is a research site of the "Marine Ecosystems of Sardinia" of the Italian Long-Term Ecological Research network (www.lteritalia.it). Moreover, SG is recognized as a Site of Community Interest for European Union (SCI ITB030037) and is designated by the Sardinian Government as a protected area for animals (INFS code: OR0211).

The main economic activity of the lagoon is fishing, which is undertaken by a cooperative of fishermen. Commercial catch consists mainly of mullet (*Mugil cephalus* Linnaeus, *Liza aurata* Risso, and *Liza ramada* Risso), sea bream (*Sparus aurata* Linnaeus), sea bass (*Dicentrarchus labrax* Linnaeus), and green crab (*Carcinus aestuarii* Nardo). Landings were substantial in the 1970s, (400–500 tons y^{-1}; Cannas et al., 1998). In the last several decades, landings began to decline due to recurrent dystrophic crises (Cannas et al., 1998).

Biweekly/monthly, rarely three times per month samplings, were conducted from February 1990 to September 2002 and from May 2011 to December 2016. Short time-scale samplings (every two days) were conducted from the 19th of July to the 4th of August in summer of 2010. During the sampling period several interruptions occurred. Information on the sampling timing and number of collected samples is reported in Supplementary Table S1.

Samples were collected in the morning/afternoon, between 10:00 a.m. and 1:00 p.m. from four sampling stations over the years of data collection (1, 2, 3, and 5; Fig. 1). This number was reduced to three stations (1, 3, and 5) from 1992 to 1994 and only one station (2) was sampled in 2002. In 2010, three more stations were sampled (4, 6 and 7; Fig. 1).

2.2. Phytoplankton and environmental parameters

Dissolved oxygen, salinity (Sal), and water temperature (Temp) were measured *in situ* using multiparameter probes (Idronaut and YSI 6600 v2). Samples for nutrient and phytoplankton analyses were collected at about 30 cm depth. Phytoplankton samples for cell abundance estimates were immediately fixed in 2% acid Lugol's solution (Throndsen, 1978a). Nonfixed samples were collected for

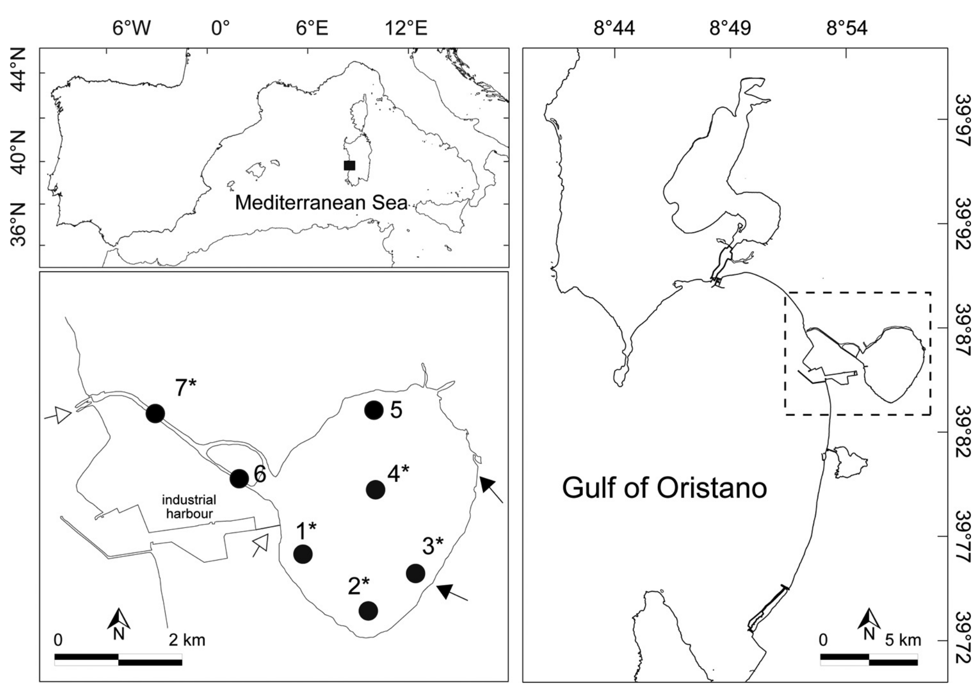

Fig. 1. Study area and location of sampling stations. Sediment sampling stations are marked with an asterisk. Black arrows: freshwater inputs; white arrows: inlets.