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Parasitism as a biological control agent of dinoflagellate blooms in the California Current System

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ABSTRACT

Amoebophrya is a marine parasite recently found to infect and kill bloom-forming dinoflagellates in the California Current System (CCS). However, it is unknown whether parasitism by *Amoebophrya* can control dinoflagellate blooms in major eastern boundary upwelling systems, such as the CCS. We quantified the abundance of a common bloom-forming species *Akashiwo sanguinea* and prevalence of its parasite (i.e., % infected cells) in surface water samples collected weekly from August 2005 to December 2008 at the Santa Cruz Wharf (SCW), Monterey Bay, CA. Additionally, we measured physical and chemical properties at the SCW and examined regional patterns of wind forcing and sea surface temperature. Relative abundance of the net phytoplankton species was also analyzed to discern whether or not parasitism influences net phytoplankton community composition. Epidemic infection outbreaks (>20% parasite prevalence in the host species) may have contributed to the end or prevented the occurrence of *A. sanguinea* blooms, whereas low parasite prevalence was associated with short-term (≤2 weeks) *A. sanguinea* blooms. The complete absence of parasitism in 2007 was associated with an extreme *A. sanguinea* bloom. Anomalously strong upwelling conditions were detected in 2007, suggesting that *A. sanguinea* was able to outgrow *Amoebophrya* and 'escape' parasitism. We conclude that parasitism can strongly influence dinoflagellate bloom dynamics in upwelling systems. Moreover, *Amoebophrya* may indirectly influence net phytoplankton species composition, as species that dominated the net phytoplankton and developed algal blooms never appeared to be infected.

1. Introduction

Biological control processes, more specifically parasitism, may strongly influence the dynamics of dinoflagellate blooms. Slobodkin (1989) argued that, except for a highly specific infectious disease, there is no "magic bullet" to end algal blooms. Recently, it has been demonstrated that the marine parasitic dinoflagellate, *Amoebophrya*, which infects free-living dinoflagellates, can have high or moderate host specificity (Kim et al., 2008). Moreover, such a parasite is able to retard or prevent dinoflagellate blooms through epidemic infection outbreaks in estuarine systems (Nishitani et al., 1985; Coats et al., 1996; Chambouvet et al., 2009). Accordingly, Montagnes et al. (2008) showed through mathematical models that marine parasitism by *Amoebophrya* might have a greater impact on the demise of toxic dinoflagellate blooms than do microzooplankton grazers.

The ability of *Amoebophrya* to efficiently control dinoflagellate populations is likely the result of a faster growth rate and higher offspring production of the parasite than of the host. For example, an average intracellular development time of 2.16 days has been estimated for *Amoebophrya* infecting *Akashiwo sanguinea* populations from Chesapeake Bay (Coats and Park, 2002). As it kills and leaves the host in ~2 days, *Amoebophrya* can release up to hundreds of infective dinospores (Chambouvet et al., 2009), while healthy free-living dinoflagellates have a mean growth rate of 0.6 doublings per day (Tang, 1996).

Amoebophrya infections in bloom-forming dinoflagellate species from the California Current System (CCS) north of Baja California have only been recently observed (Mazzillo, in preparation). Our goal in the present study was to investigate whether or not *Amoebophrya* can regulate dinoflagellate blooms that occur in locations influenced by the CCS. Our observations were made in Monterey Bay, an open embayment in central California that is highly influenced by the upwelling dynamics of the CCS. We focused on a common dinoflagellate *A. sanguinea* (previously known as *Gymnodinium sanguineum* and *Gymnodinium splendens*), which often forms red tides in coastal locations influenced by major eastern boundary current systems (Trainer

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et al., 2010). Red tides are defined here as dinoflagellate blooms ($>10^4$ cells L^{-1}) that may discolor surface seawater. Although this particular species is not known to produce toxins, mass mortality of marine birds and increase in upper respiratory symptoms (i.e., sinus congestion) in humans have been documented during an *A. sanguinea* red tide in Monterey Bay (Jessup et al., 2009; C. O'Halloran, pers. comm.). Additionally, abalone mariculture farms are likely threatened by *A. sanguinea*, as this dinoflagellate can prey on abalone larvae (Botes et al., 2003). Thus *Amoebophrya* may help reduce negative effects of *A. sanguinea* blooms, when it controls the abundance of this common red tide former.

Physico-chemical processes in major coastal upwelling systems that directly influence red tides dynamics have been extensively studied (Bolin and Abbott, 1963; Kudela et al., 2005, 2010; Pitcher et al., 2010). For example, in Monterey Bay, physical processes that may influence bloom initiation are associated with the upwelling and downwelling circulation of the California Current (CC) and include development of vertical density stratification (which may be followed by the intrusion of CC warm offshore waters) and convergent frontal zones that may aggregate dinoflagellates (Ryan et al., 2005, 2010a). In addition, upwelling and downwelling circulation can spread and disperse red tides that are initiated in the Bay (Ryan et al., 2009). Thus, an additional goal was to evaluate the role of parasitism within the physico-chemical scenario in which *A. sanguinea* red tides occur. Upwelling circulation patterns that influence Monterey Bay were inferred from wind speed and direction and sea surface temperatures measured at 2 moorings located in the outer Bay region. Additionally, we measured sea surface temperature, salinity and inorganic nutrients (nitrate–nitrite and phosphate) at our long-term, nearshore study site, the Santa Cruz Wharf, where we monitored *A. sanguinea* abundance and infection levels.

We also investigated the influence that parasitism might have on net phytoplankton community composition. In a recent study in a marine coastal estuary, Chambouvet et al. (2009) found that when parasites exhibit high host specificity, the release of dinospores from one infected species may not suppress the bloom of other local dinoflagellates. Therefore, parasitism may shape phytoplankton community composition by selectively infecting species that might otherwise dominate the community. The specific questions being addressed in the present study are: (1) Does density variation of *A. sanguinea* populations from Monterey Bay, CA correlate with *Amoebophrya* infections and/or with physical and chemical variables? (2) Can *Amoebophrya* parasitism influence the net phytoplankton community composition?

2. Materials and methods

2.1. Water sample collection

Surface seawater samples were collected weekly from 3 August 2005 through 10 December 2008 at the Santa Cruz Wharf (SCW) (36.95N, 122.02W) (Fig. 1). Samples were collected by net tow (35 μm mesh) and surface bucket. Bucket samples provided subsamples for host and parasite enumeration, temperature, salinity and nutrient analyses.

2.2. Enumeration of A. sanguinea and Amoebophrya

Aliquots of 100 mL from bucket samples were preserved in 4% formalin final concentration for host and parasite enumeration. Enumeration of *A. sanguinea* (the host) and parasite prevalence (i.e., % of *A. sanguinea* infected by *Amoebophrya*) were done in samples where *A. sanguinea* was recorded as the dominant net phytoplankton species (see phytoplankton composition, below). To observe the progress of infections at the start and end of *A. sanguinea* blooms, samples collected one week after and before *A. sanguinea* were recorded as the dominant species of the net phytoplankton were also selected for host and parasite enumeration. As a result, 5 study periods (Aug–Dec 2005, Jan–Apr 2006, Jun–Dec 2006, Sep–Dec 2007, Sep–Dec 2008) were weekly monitored for parasite prevalence and host abundance.

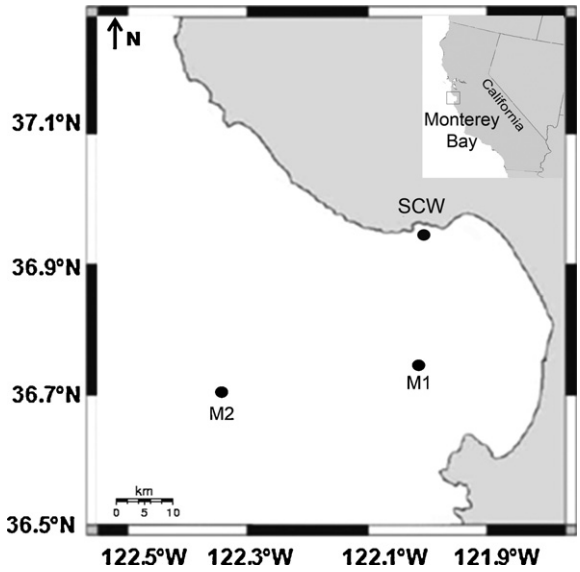

Fig. 1. Monterey Bay, CA, showing sampling sites, the Santa Cruz Wharf (SCW), and the moorings M1 and M2.

To detect *Amoebophrya* infections within hosts, subsamples of 25–75 mL were filtered on 5 μm polycarbonate black filters and DAPI (final concentration of 500 mg mL^{-1}) was added at 40 μl. DAPI stains allowed us to identify only the mature trophont stage of *Amoebophrya*, also know as the "beehive" stage and hereafter referred to as such. To estimate parasite prevalence, a minimum of 100–200 total *A. sanguinea* cells were counted. Thus, our limit of detection for parasite prevalence was between 1 and 0.5%. Enumeration of healthy and infected *A. sanguinea* cells was done on an epifluorescent compound microscope (Zeiss Axio Imager) using a 10× objective (40× was used when needed).

2.3. Parasite daily induced mortality

The percentage of a given host population killed per day by *Amoebophrya* was adapted from Coats and Bockstahler (1994) and estimated as:

$$\text{daily parasite induced mortality} = \frac{(\%\,\text{of infected host cells estimated with DAPI} \times 1.97)}{\text{infection time in days}}$$

We calculated a correction factor of 1.97 to account for host cells parasitized with early life history stages of *Amoebophrya* since DAPI stains allowed us to detect only the mature beehive life history stage. In contrast, quantitative protargol staining (QPS) allows the observation of initial stages of infection as well as the beehive stage. Thus one sample from each year where infections were previously detected with DAPI was also analyzed with quantitative protargol staining (QPS) as described in Montagnes and Lynn (1993) and Coats and Bockstahler (1994). The correction factor of 1.97 was then calculated as the averaged ratio between parasite prevalence detected with QPS and DAPI in 3 samples (17 Aug 2005, 29 Mar 2006 and 16 Sep 2008).