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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.

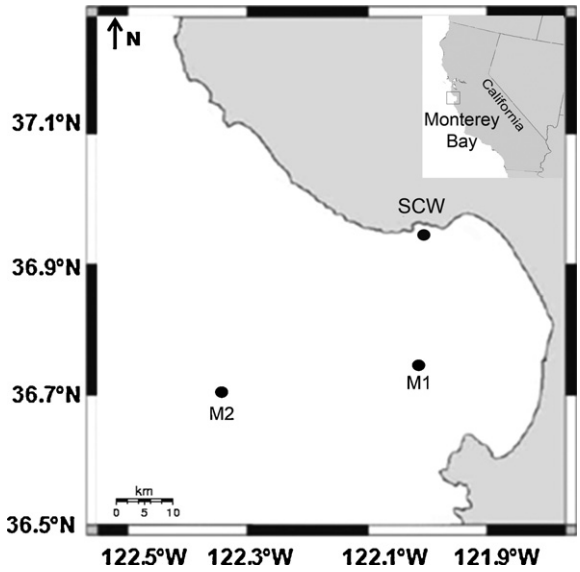

Fig. 1. Monterey Bay, CA, showing sampling sites, the Santa Cruz Wharf (SCW), and the moorings M1 and M2.

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).

Infection time of 1.42 days for *Amoebophrya* in Monterey Bay was calculated using average intracellular phase time of 2.16 days (Coats and Park, 2002), corrected for Monterey Bay average temperature from days where infection was detected (13.9 °C) and using a Q_{10} of 2.

2.4. Physicochemical analysis

Water temperature was measured with a digital thermometer upon sample collection. Samples for salinity and nutrient analysis were prepared by filtering 200 mL through a 25 mm Whatman GF/F filter. Salinity samples were tested using a portable salinometer (Guildline Portasal mod. 8410) shortly after collection. Nutrient samples were frozen at −20 °C and tested later for nitrate–nitrite and phosphate concentrations on a LaChat Instrument automated ion analyzer (8000 series) using standard methods (Diamond, 2003a,b).

2.5. Mooring observations

Two moorings, one at the mouth of Monterey Bay (M1) and another 23 km further offshore (M2) provided hourly data on sea surface temperature and surface wind, respectively, from 1995 to 2009 (Fig. 1). Surface wind direction and speed were measured hourly on M2 (36.7N, 122W) by a RM young model 05103 wind monitor. Sea surface temperatures (SSTs) from the same time period were obtained from M1 (36.7N, 122W) by temperature sensor at 3.5 m. SST anomalies were calculated by the difference between the average SST from 1995 to 2009 from the same time period that *A. sanguinea* abundance and parasite prevalence were monitored in 2005 (Aug–Dec), 2006 (Jan–Apr and Jun–Dec), 2007 (Sep–Dec) and 2008 (Aug–Dec) and the mean SST from the actual study periods of 2005–2008.

2.6. Satellite remote sensing

To place our Monterey Bay observations in a greater regional context during the biologically and physically anomalous 2007 study period, we examined satellite sea surface temperature (SST) data from the Advanced Very High Resolution Radiometer (AVHRR) satellite sensor. SST anomaly patterns were computed for the 2007 study period as the average SST at each pixel during the study minus the average for the same annual period averaged between 2004 and 2007. AVHRR processing methods are published (Ryan et al., 2010b).

2.7. Phytoplankton species composition

Live net tow material was examined in the laboratory using a dissecting scope (Olympus SZH Stereozoom) and magnified 64×. The net phytoplankton community (>20 μm) was characterized by identifying taxa to genus or species level and estimating the relative abundance contribution of each individual taxon to the total net phytoplankton taxa in the sample. Relative abundance categories used were: dominant (taxa contributing >45% of the total cell number within the net phytoplankton community), common (taxa contributing <45% but >10%) and present (taxa contributing <10% of the total net phytoplankton community). Taxa were identified to genus level to avoid misidentification of species, except for the case of *A. sanguinea*, which was an easily recognized species. Diatom presence in the net phytoplankton community was noted, but is not described here, as it was not directly relevant to the focus of this paper.

2.8. Statistical analysis

Systat was used for linear and multiple regressions and ANOVA analyses. Linear regression was used to evaluate whether *A. sanguinea* (i.e., host) density was correlated with *Amoebophrya* infections. A multiple linear regression model was used to determine whether *A. sanguinea* density was correlated with physicochemical variables (i.e., temperature, salinity, nitrate–nitrite and phosphate). Finally, ANOVA was used to evaluate whether sea surface temperatures differed among years when *Amoebophrya* was detected. A value below the detection limit was added to host densities and nitrate samples to avoid the use of zeros in models. For each of these analysis, nitrate–nitrite and phosphate were log $(x + 1)$ transformed, host densities were log transformed, and the % of infected *A. sanguinea* was forth root transformed to comply with linearity and normality assumptions of the respective tests.

3. Results

3.1. A. sanguinea red tides and Amoebophrya infections

Red tides of *A. sanguinea* ($>10^4$ cells L^{-1}) were frequently detected in surface water samples collected in the summer at the Santa Cruz Wharf during 2005–2007 (Fig. 2A). Additionally, red tides were detected in the fall of 2005 and 2007, and throughout all seasons during 2006. The highest density of *A. sanguinea* was observed in the fall of 2007 (i.e., $>10^6$ cells L^{-1}). During that time, *A. sanguinea* cell concentration remained above the red tide threshold for 8 consecutive weeks (17 Oct to 5 Dec). In 2008, *A. sanguinea* populations remained just below red tide threshold.

Parasite prevalence ranged between 0.5 and 10% with a maximum of 40%. Fig. 2B shows parasite prevalence from August 2005 to December 2008 after forth root transformation. Average parasite prevalence was lowest during red tides and vice versa (Fig. 3). The highest parasite induced daily mortality rate of 56% was calculated for 17 August 2005 and minimum rate of 0% throughout the fall of 2007.

3.2. Oceanographic conditions during A. sanguinea red tides

At the Santa Cruz Wharf, sea surface temperature ranged from 10.9 to 18.1 °C and salinity from 30.63 to 33.56‰ during *A. sanguinea* red tides ($>10^4$ cells L^{-1}) in 2005–2007 (Fig. 4). Nitrate and phosphate concentrations ranged from 11.07 μM to undetectable and from 3.97 μM to 0.31 μM, respectively (Fig. 2C and D).

During the study periods of 2005–2008, mean surface winds were upwelling favorable (NW). Consistent with the exceptionally high abundances of *A. sanguinea* and the absence of parasites during the fall of 2007 (Fig. 2A and B), regional wind and SST patterns were unique during the 2007 study period. While average winds during study periods in 2005, 2006 and 2008 were very similar to the long-term (1995–2009) average for the same annual period as the studies, or weaker than average during the early 2006 study period, average winds during the 2007 study period were stronger than average by ~1 m/s (Fig. 5). The alongshore, equatorward wind direction (from the northwest) indicates anomalously strong upwelling during the 2007 study period. Accordingly, sea surface temperature (SST) anomalies calculated at M1 for the study periods indicated the coldest anomalies, ~1 °C, during the 2007 study period (Fig. 6). An anomaly map computed from satellite SST data revealed that during the 2007 study period cold anomaly extended throughout Monterey Bay and was part of a regional pattern detected along much of the central California coast (Fig. 7).

3.3. A. sanguinea abundance correlation with parasitism and/or enviromental variables

Linear regression results indicated significant and negative correlation between log of host density and parasite prevalence

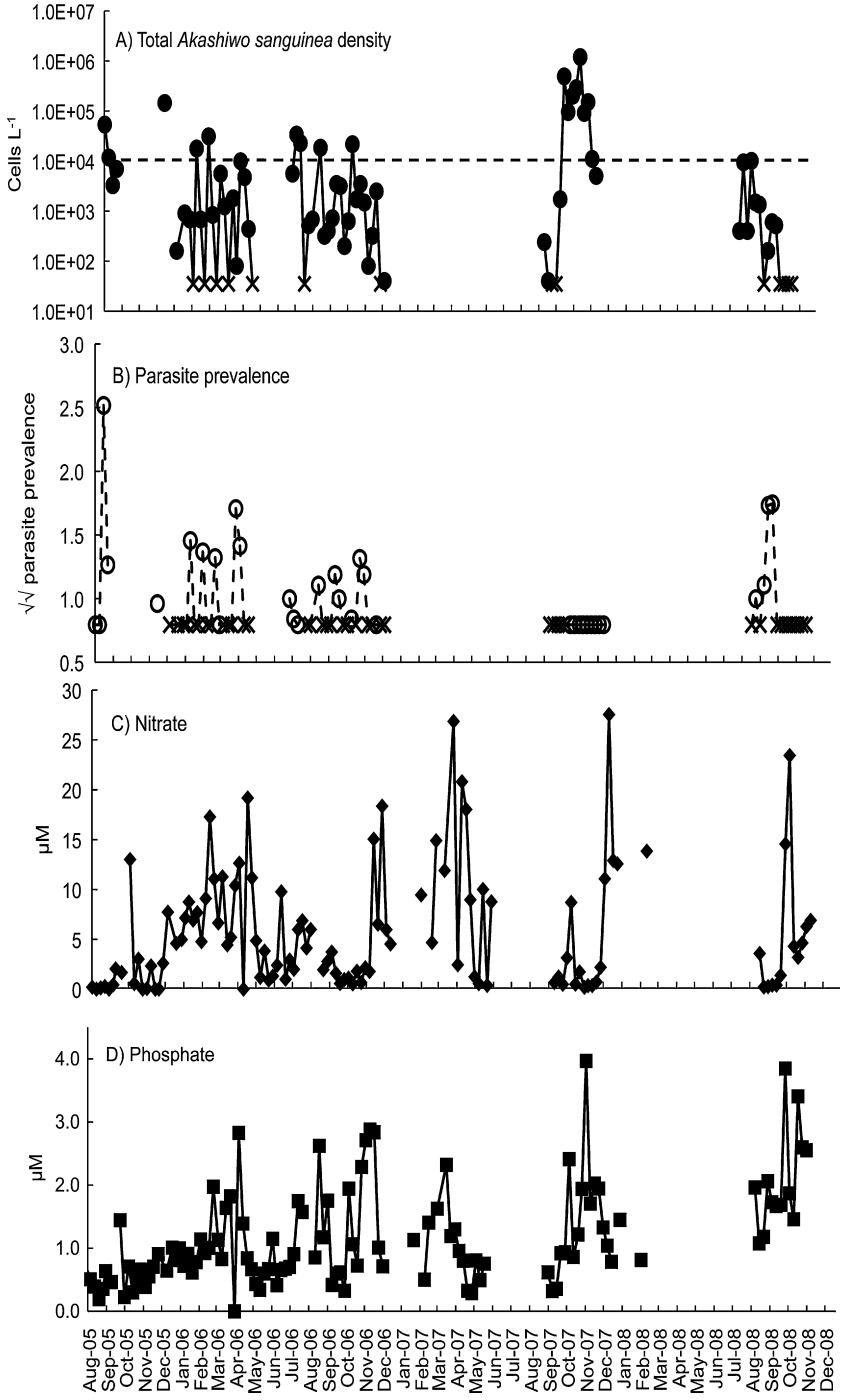

Fig. 2. *A. sanguinea* density (closed circles) (A), parasite prevalence (open circles) (B), nitrate (C), and phosphate (D) at the Santa Cruz Wharf from 08/2005 to 12/2008. Crosses indicate samples below limit of detection (LOD) of 40 cells L^{-1} on (A) and 0.5% parasite prevalence on (B). Dashed line on (A) indicates red tide threshold of $>10^4$ cells L^{-1}.

(Fig. 8). Likewise, multiple linear regression results indicated that environmental variables (i.e., temperature, salinity, log nitrate and log phosphate) were significantly correlated with the log of *A. sanguinea* density (Table 1). Furthermore, higher R^2 and lower p values were obtained when *A. sanguinea* densities were significantly related to *Amoebophrya* parasitism but not to other variables (Table 1). An additional model that combined both biological and environmental variables, however, explained even more of the variability of *A. sanguinea* densities than the models that considered these variables separately (Table 1).

3.4. Parasitism influence in net phytoplankton species composition

In 83% of the days when *A. sanguinea* densities were above the red tide threshold ($>10^4$ cells L^{-1}) and low averaged infection rates were recorded, *A. sanguinea* remained the dominant species of net phytoplankton community and sometimes co-dominated with *Cochlodinium* sp. or *Ceratium* spp. (Figs. 9, 11 and 12). In the remaining 17% of these samples, *A. sanguinea* was a common species and no dominant species was observed (Figs. 9 and 12).

Conversely, on 75% of the days when *A. sanguinea* densities were below the red tide threshold and infections were detectable,

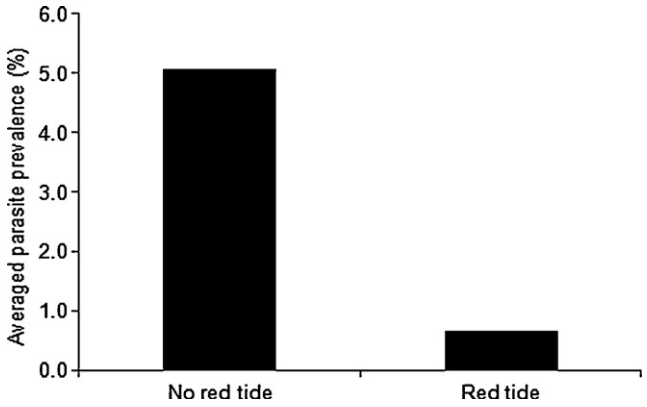

Fig. 3. Averaged parasite prevalence during days without a red tide ($<10^4$ cells L^{-1}) and days with red tide ($>10^4$ cells L^{-1}) at the SCW from study periods in 2005–2008.

the net phytoplankton community was dominated by species other than *A. sanguinea* (i.e., *Cochlodinium* sp., *Ceratium* spp. or diatoms) (Figs. 11 and 13) or no dominant species was detected (Figs. 9, 10 and 13). In the remaining 25%, *A. sanguinea* was dominant (Figs. 9 and 10) or co-dominant (Fig. 10).

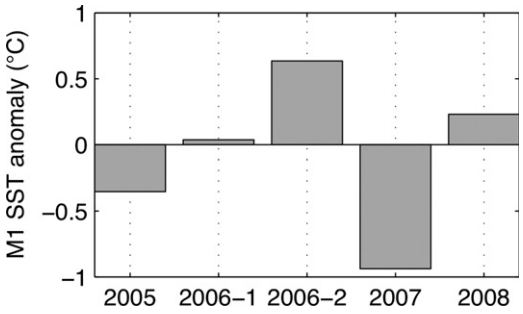

Fig. 6. Sea surface temperature (SST) anomaly at M1 (Fig. 1) during study periods in 2005, 2006-1 (Jan–April), 2006-2 (Jun–Dec), 2007 and 2008.

4. Discussion

The dinoflagellate *A. sanguinea* has been known to cause red tides along the coast of California since the early 1970s (Kiefer and Larsen, 1975; Fiedler, 1982). Although this species does not appear to produce toxins, it can negatively affect the marine fauna, human health and mariculture (Botes et al., 2003; Jessup et al., 2009; C. O'Halloran, pers. comm.). The goal of the present study was to

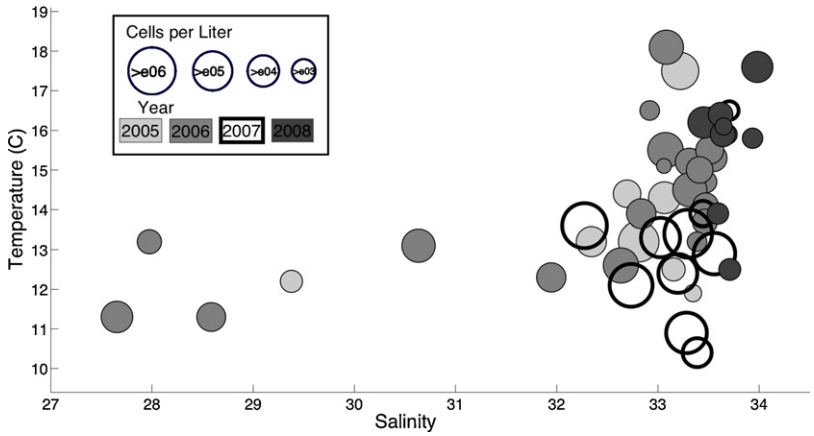

Fig. 4. Sea surface temperature and salinity relative to *A. sanguinea* abundance at the Santa Cruz Wharf surface waters on 2005–2008. Note that highest cell densities were observed in the coldest and saltiest waters during 2007.

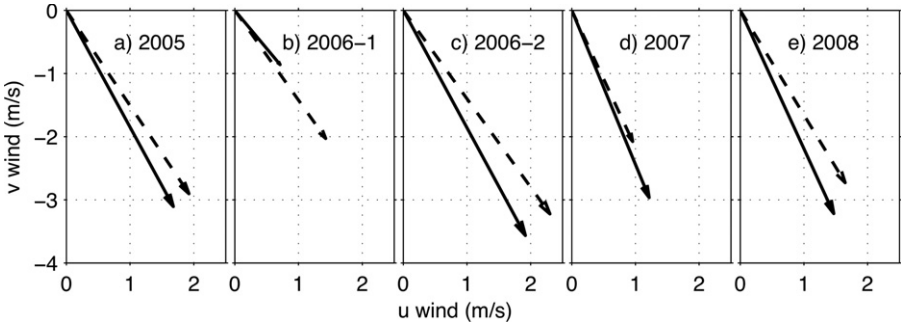

Fig. 5. Surface wind vector plots showing a comparison between mean wind direction and speed at M2 (Fig. 1) from 1995 to 2009 (dashed arrows) during study periods in 2005, 2006-1 (Jan–Apr), 2006-2 (Jun–Dec), 2007 and 2008, and average winds (black arrows) during the actual study periods.

Table 1
Results from linear and multiple regression models that used $\sqrt{\sqrt{}}$ parasite prevalence and/or environmental variables as predictors of log of *A. sanguinea* density variation.

Predicting variables of log of *A. sanguinea* density used in regression models	R^2	*df*	*p*	*N*	*F*
$\sqrt{\sqrt{}}$ Parasite prevalence	0.21	32	0.00	34	9.79
Environmental variables (temperature, salinity, log nitrate, log phosphate)	0.2	29	0.04	34	2.99
$\sqrt{\sqrt{}}$ Parasite prevalence and environmental variables	0.36	28	0.00	34	4.76

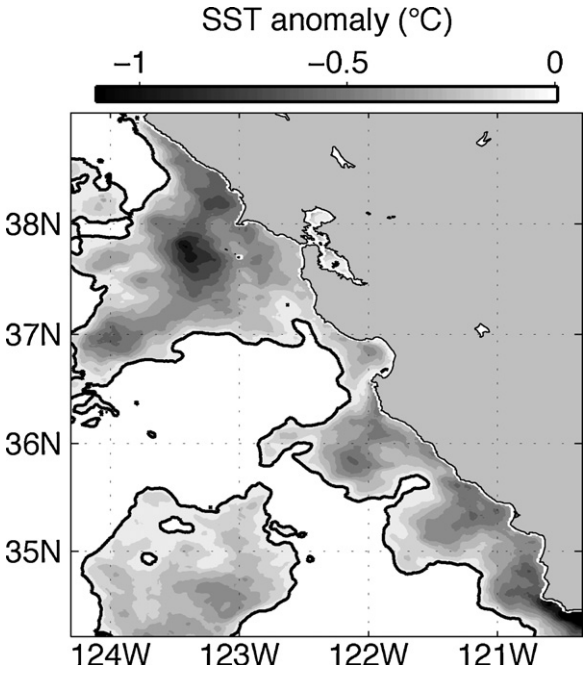

Fig. 7. Regional pattern of cold sea surface temperature (SST) anomalies during the 2007 study period.

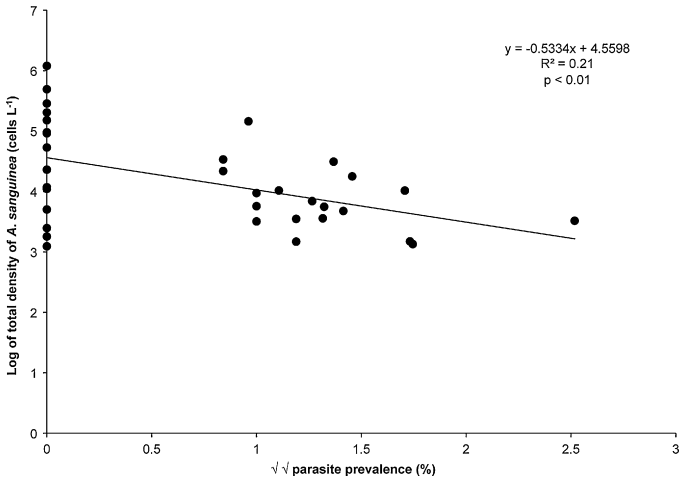

Fig. 8. Linear regression results (adjusted $R^2 = 0.21$, $df = 32$, $p < 0.01$, $N = 34$, $F = 9.79$) from log of host density (cells L^{-1}) versus parasite prevalence (4th root transformed) in surface water samples collected from study periods in 2005–2008 at the Santa Cruz Wharf. (Note: removal of highest parasite prevalence of 40% (i.e., 2.5% after 4th root transformation) changes results to adjusted $R^2 = 0.20$, $df = 31$, $p < 0.01$, $N = 33$, $F = 8.92$.)

determine whether or not the marine parasite *Amoebophrya* can regulate the abundance of *A. sanguinea* in Monterey Bay, CA as well as to understand the role of parasitism compared to that of environmental variables in controlling red tides in a coastal region strongly influenced by upwelling dynamics. An additional objective was to discern the consequences to the net phytoplankton community composition of parasitism of one of its dominant constituents, *A. sanguinea*.

4.1. Parasitism as a biological control of A. sanguinea population

Interestingly, highest *A. sanguinea* cell densities at the Santa Cruz Wharf (SCW) were observed in the absence of *Amoebophrya*

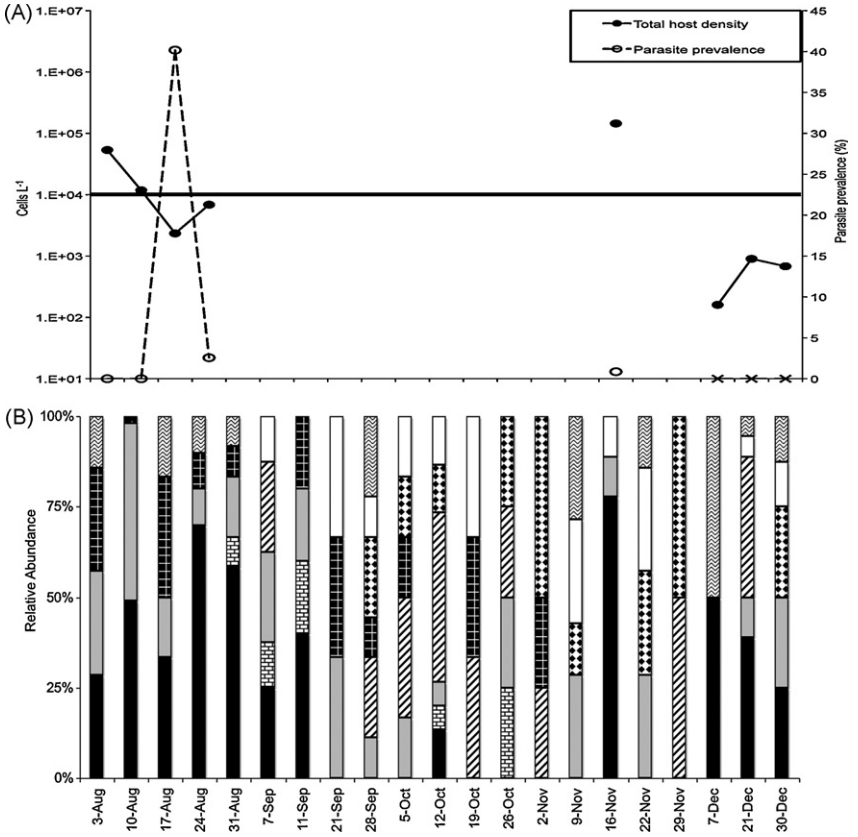

Fig. 9. (A) *A. sanguinea* total density (cells L^{-1}) (closed circles) and parasite prevalence (open circles) for 2005 study period. Crosses indicate samples below the detection limit. Line indicates red tide threshold. (B) Relative abundance patterns of dinoflagellates (by genus): *Akashiwo sanguinea* (■), *Alexandrium* (▩), *Ceratium* (▨), *Cochlodinium* (▨), *Dinophysis* (▩), *Gymnodinium* (▩), *Prorocentrum* (□), and diatoms (▨) (all genera combined) from August to December 2005 at the Santa Cruz Wharf.

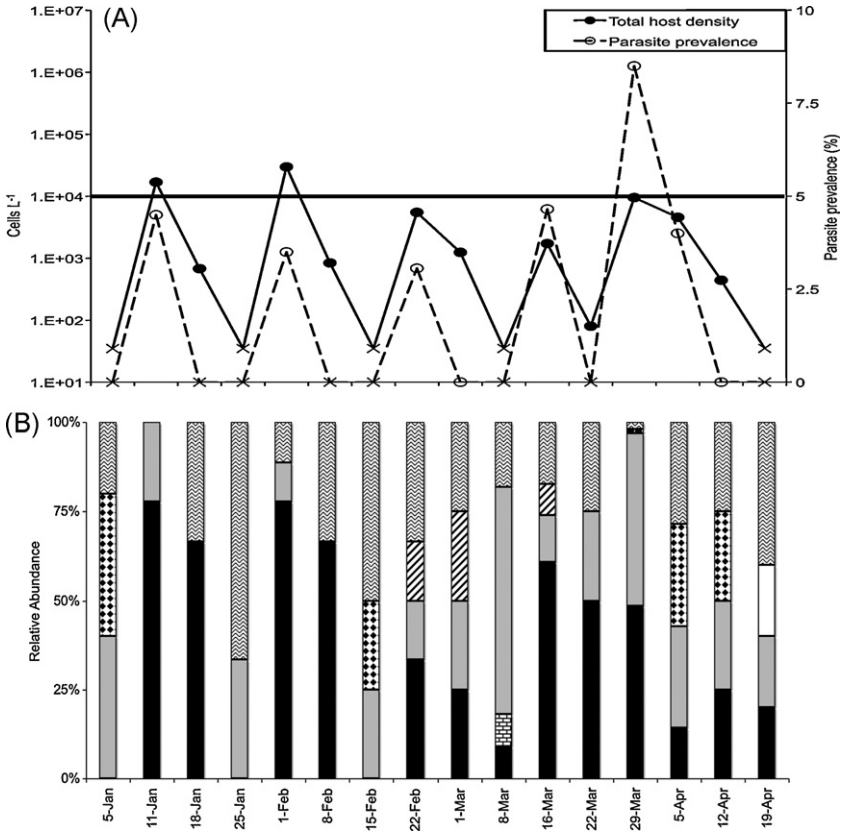

Fig. 10. (A) *A. sanguinea* total density (cells L^{-1}) (closed circles) and parasite prevalence (open circles) for 2006 study period (winter and spring). Crosses indicate samples below the detection limit. Line indicates red tide threshold. (B) Relative abundance patterns of dinoflagellates (by genus): *Akashiwo sanguinea* (■), *Alexandrium* (▩), *Ceratium* (▨), *Cochlodinium* (▨), *Dinophysis* (▩), *Gymnodinium* (▩), *Prorocentrum* (□), and diatoms (▨) (all genera combined) from January to April 2006 at the Santa Cruz Wharf.

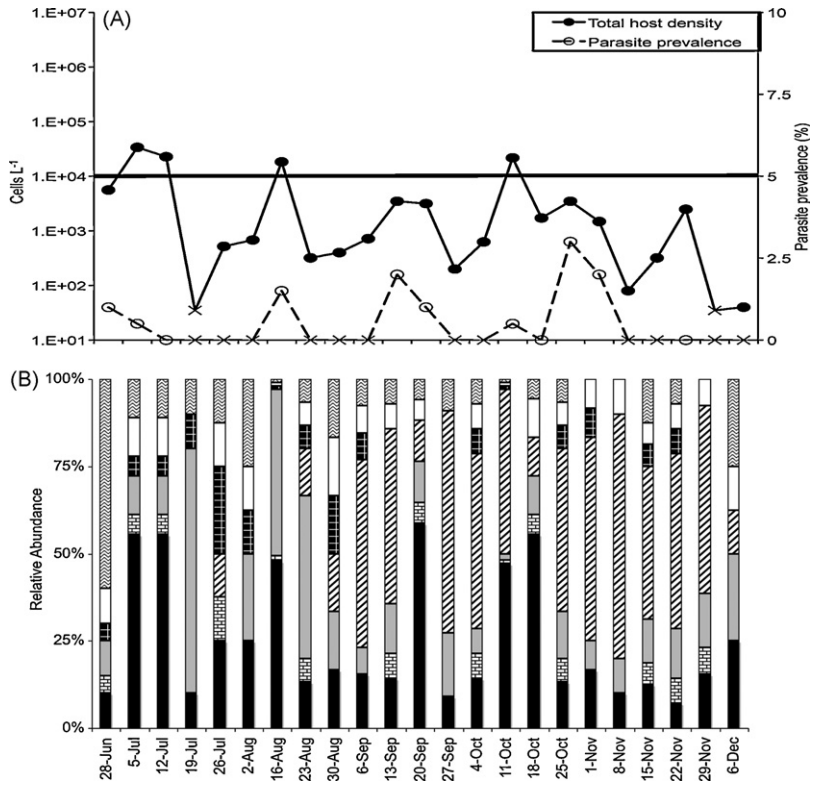

Fig. 11. (A) *A. sanguinea* total density (cells L^{-1}) (closed circles) and parasite prevalence (open circles) for 2006 study period (summer and fall). Crosses indicate samples below the detection limit. Line indicates red tide threshold. (B) Relative abundance patterns of dinoflagellates (by genus): *Akashiwo sanguinea* (■), *Alexandrium* (▩), *Ceratium* (▨), *Cochlodinium* (▨), *Dinophysis* (▩), *Gymnodinium* (▩), *Prorocentrum* (□), and diatoms (▨) (all genera combined) from June to December 2006 at the Santa Cruz Wharf.

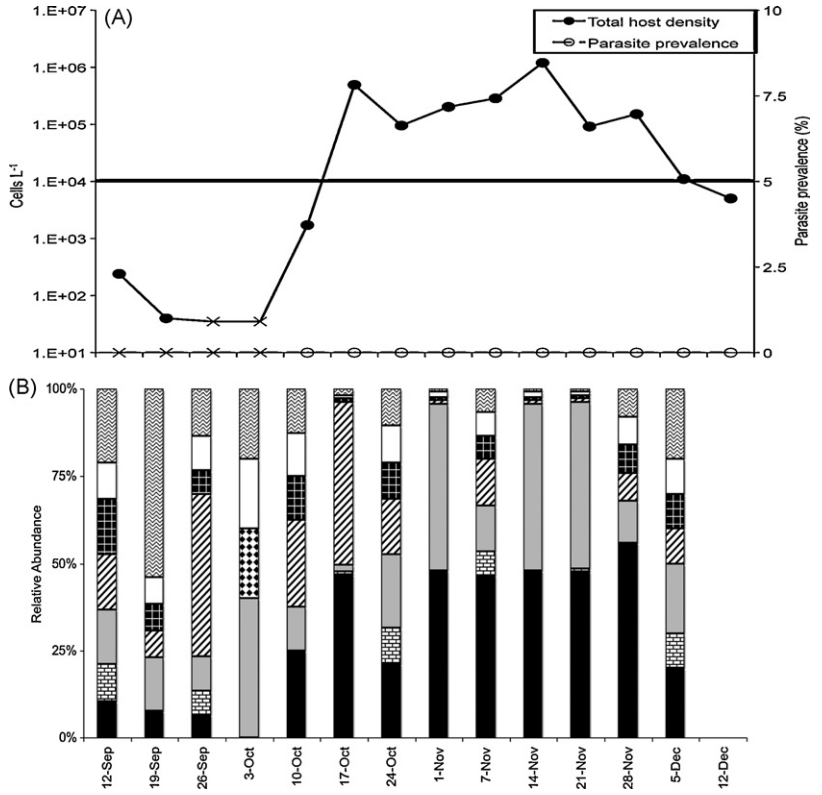

Fig. 12. (A) *A. sanguinea* total density (cells L^{-1}) (closed circles) and parasite prevalence (open circles) for 2007 study period. Crosses indicate samples below the detection limit. Line indicates red tide threshold. (B) Relative abundance patterns of dinoflagellates (by genus): *Akashiwo sanguinea* (■), *Alexandrium* (▩), *Ceratium* (▩), *Cochlodinium* (▨), *Dinophysis* (▩), *Gymnodinium* (▩), *Prorocentrum* (□), and diatoms (▩) (all genera combined) from September to December 2007 at the Santa Cruz Wharf.

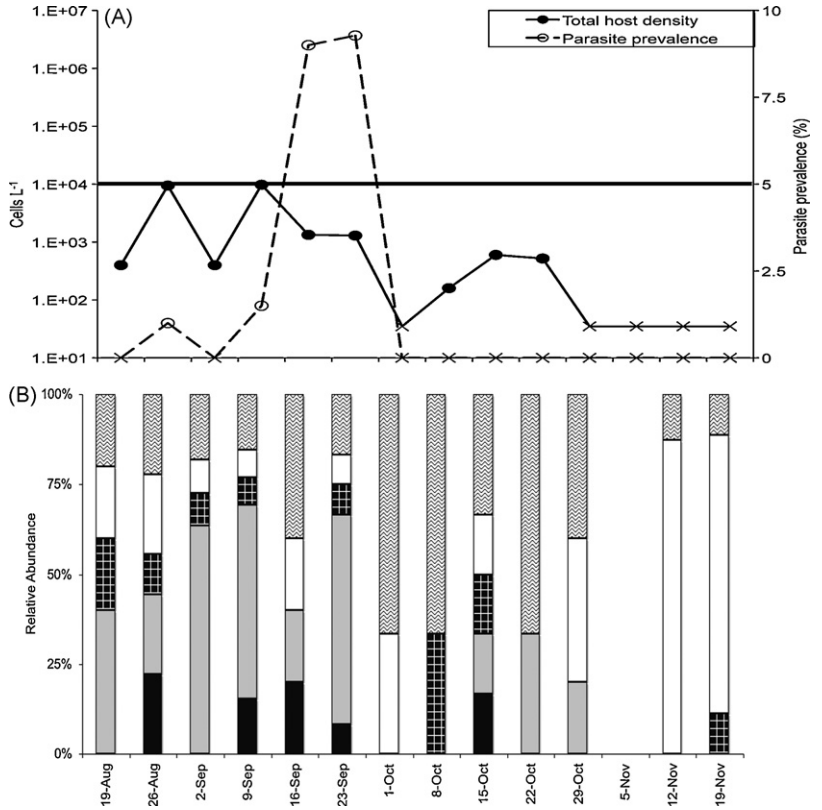

Fig. 13. (A) *A. sanguinea* total density (cells L^{-1}) (closed circles) and parasite prevalence (open circles) for 2008 study period. Crosses indicate samples below the detection limit. Line indicates red tide threshold. (B) Relative abundance patterns of dinoflagellates (by genus): *Akashiwo sanguinea* (■), *Ceratium* (▩), *Dinophysis* (▩), *Prorocentrum* (□), and diatoms (▩) (all genera combined) from August to November 2008 at the Santa Cruz Wharf.

infections, in the fall of 2007 (Fig. 1D). Early and late stages of infection were also not observed in a net samples from 2007 that were examined using quantitative protargol staining (QPS). Moreover, infections were not detected in water samples collected at the offshore Monterey Bay station M1 or in water samples collected along an inshore transect located on the northeastern shelf of the Bay during the 2007 study period (data not shown), suggesting that the 2007 red tide occurred as *A. sanguinea* was released from parasitism. Such a red tide caused harm in a previously undocumented manner. Specifically, organic matter from the senescing *A. sanguinea* bloom coated feathers of birds, fouling the insulating function of their feather and causing morbidity and mortality via hypothermia (Jessup et al., 2009).

The factors responsible for the absence of *Amoebophrya* infections in the 2007 fall bloom are unknown. Mechanisms that may lead the host to escape parasitism and, consequently, develop red tides may include (I) a low encounter rate of dinospore and host due to low host density or spatial segregation (Coats and Bockstahler, 1994); (II) ciliate and other microzooplankton grazing pressure on dinospores (Johansson and Coats, 2002); or (III) an environmental change that negatively affects parasite fitness and at the same time leads to a faster growth rate of the host. Alternative (I) may be rejected in this case, as high host densities were recorded in 2007, the Santa Cruz Wharf site is shallow (~9 m) and relatively well mixed making spatial segregation of host and dinospores unlikely. Alternative II could not be evaluated, as we did not recorded zooplankton biomass. On the other hand, anomalous environmental conditions were observed during the 2007 red tide and may have influenced host growth rate and/or parasite fitness.

During the 2007 red tide, the lowest sea surface temperatures (SSTs) along with the highest salinity values were recorded relative to temperature and salinity levels during other *A. sanguinea* bloom events in this study (Fig. 4). A comparison with mean climatological data from 14 years (1995–2009) indicated that during the 2007 red tide period upwelling favorable winds were stronger than average by 1 m/s and anomalously cold SST were detected throughout Monterey Bay (Figs. 5–7). Accordingly, climatic conditions of the California Current System (CCS) reflected a strong and persistent La Niña marked by low SST and above normal upwelling volumes in 2007 (McClatchie et al., 2007). Such upwelling conditions could have increased the growth rate of *A. sanguinea* by increasing the supply of nutrients. Likewise, Cloern et al. (2007) found that the shift to La Niña cold conditions, which implied increased upwelling intensity along with strengthened southerly flows transporting subarctic waters, was linked to an increase in the occurrence of red tides (including those caused by *A. sanguinea*) in San Francisco Bay.

While the cold nutrient rich upwelled waters may have "fueled" the bloom, it is also possible that an unknown mechanism may have decreased the growth rate of the parasite relative to the host growth rate. For example, the low SST could have altered *Amoebophrya* dinospore physiology. Accordingly, ANOVA results showed that mean SST measured at the SCW significantly differed among the years when *Amoebophrya* was detected (i.e., 2005–2008) ($p < 0.001$, $F_{3,30} = 7.65$). Pairwise comparison analysis further indicated that SST in 2007 was significantly lower than SST in 2006 ($p < 0.05$) and 2008 ($p < 0.01$), and lower than in 2005 ($p = 0.05$). Therefore, low SST could have signalized the presence of a habitat that reduced *Amoebophrya* infection ability.

An alternative hypothesis for the absence of infections in 2007 is that the observed bloom could have been caused by a strain of *A. sanguinea* resistant to *Amoebophrya* infections. Although different strains of *A. sanguinea* have not been identified in Monterey Bay, 6 different strains of this species have been identified in US waters (data from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton) and several strains of the same dinoflagellate species is common coastal embayments (Martinez et al., 2006; Touzet et al., 2007).

Moreover, *Amoebophrya*, which as originally described as one species (i.e., *Amoebophrya ceratii*), is actually a complex of several species as shown by studies using 18S rRNA gene sequences of *Amoebophrya* that infect different dinoflagellate species (Cachon, 1964; Jason et al., 2000; Gunderson et al., 2002; Salomon et al., 2003; Kim et al., 2008) and some of these strains can be host specific while others are not (Coats and Park, 2002; Kim, 2006; Chambouvet et al., 2009; Kim et al., 2008). *Amoebophrya* infections have been detected in 6 dinoflagellate species from Monterey Bay and simultaneously in at least 2 dinoflagellate species (Mazzillo, in preparation). The latter observation suggests lack of host specificity (assuming that the same species of *Amoebophrya* were detected). However, preliminary cross-infection laboratory experiments using dinospores of *Amoebophrya* ex. *Prorocentrum micans* into an *Alexandrium catenella* culture isolated from Monterey Bay were unsuccessful, and indicated some degree of host specificity – at least for the host-parasite system tested (FM, pers. obs.). Thus, whether different strains of *A. sanguinea* are present in Monterey Bay and whether the 2007 bloom was caused by a strain resistant to infections of *Amoebophrya* strains present in Monterey Bay are questions that have not yet been answered.

Our data suggest that *Amoebophrya* epidemic infection outbreaks could have been responsible for the end or prevention of *A. sanguinea* red tides. Epidemic infection outbreaks are considered when >20% of the host is parasitized and thus most of the host population is killed due to infections (Coats et al., 1996). An epidemic outbreak in the summer of 2005 was estimated to remove 56% of the host population per day and thus could have been responsible for the short-lived red tide (Fig. 9). Another possible epidemic infection outbreak was observed in September 2008. Although 9.0% and 9.3% parasite prevalence was recorded in September 2008, our methodology allowed us to detect only the *Amoebophrya* beehive stage (i.e., the mature trophont). This life-history stage corresponds to less than half of *Amoebophrya* total generation time (see calculation of correction factor of 1.97 on methods) and thus the actual parasite prevalence could have been ~20% in September 2008. In this case, the calculated parasite induced mortality rate indicated that 13% of the host was being killed daily. Thus, high levels of parasitism may have been responsible for the demise and prevention of *A. sanguinea* red tides in Monterey Bay.

4.2. Parasitism as another piece of the red tide dynamics puzzle

Red tides dynamics are likely to be influenced by the synergism among environmental and biological processes. Although a significant negative correlation was observed between infections and host abundance, parasitism may have been one of several factors preventing the occurrence of red tides in Monterey Bay. Additionally, results of our multiple linear regression model that included both parasitism and environmental variables as predictors of *A. sanguinea* density variability were the most predictive, as opposed to models that used parasitism and environmental variables separately to predict changes in *A. sanguinea* density (Table 1). Similarly, an inverse correlation between parasite prevalence and host abundance was reported in an *Alexandrium* (=*Gonyaulax*) *catenella* population from Puget Sound, Washington, where the decline of an *A. catenella* bloom was associated with high infection rates as well as decreased host growth rate and depleted nutrients (Nishitani et al., 1985).

Other biological interactions that potentially influence red tide dynamics, such as grazing, also should be considered. Regarding grazers of *A. sanguinea*, Fiedler (1982) observed that zooplankton

avoided grazing *A. sanguinea* during a red tide. Likewise, experimental studies showed that some heterotrophic dinoflagellates have difficulty capturing/ingesting *A. sanguinea* (Jeong and Latz, 1994; Jeong et al., 1999). Additionally, the production of anti-grazing substances may diminish grazer control (Smayda, 2008). To date *A. sanguinea* anti-grazing substances have not been reported in the literature, but surfactant-like proteins were detected in the surface foam generated at the end of the 2007 bloom period (Jessup et al., 2009). Whether or not surfactants produced during the 2007 bloom acted as an anti-grazing substance and aided in the bloom development is unknown.

4.3. Parasitism influence in net phytoplankton species composition

Amoebophrya parasitism may indirectly affect net phytoplankton community species composition by keeping species that are susceptible to infection at low densities and allowing species that are resistant to infections to reach bloom levels ($>10^4$ cells L^{-1}). In most of the samples where *A. sanguinea* was parasitized, another species dominated the net phytoplankton community. These included 2 dinoflagellate species not known to be parasitized by *Amoebophrya* (i.e., *Ceratium divaricatum*, *Cochlodinium* sp.) in Monterey Bay or elsewhere (Park et al., 2004; Mazzillo, in preparation), and species of the diatom genus *Pseudo-nitzschia*, which cannot be infected by *Amoebophrya*. Accordingly, the other dinoflagellate species detected throughout the study never dominated the community and are susceptible to *Amoebophrya* infections (Mazzillo, in preparation).

It has been suggested that if parasitism specificity is high, the efficiency of natural biological control is reduced for exotic (enemy release hypothesis) (Kean and Crawley, 2002) or for rare species that become abundant due to environmental change (such as coastal eutrophication or climate change) (Chambouvet et al., 2009). The latter may be the case for the observed *Cochlodinium* bloom in 2006. *Cochlodinium* was regarded as a rare species in Monterey Bay prior to 2004 (Curtis et al., 2008). This dinoflagellate is adapted to low-nutrient environments, but also is capable of responding to eutrophication (Kudela et al., 2008). Moreover, *Cochlodinium* has similar ecophysiological characteristics to those of *A. sanguinea* (Smayda, 2002). Thus, it is possible that during the fall of 2006, while *Amoebophrya* controlled *A. sanguinea* population, *Cochlodinium* was able to exploit an ''open niche'' and dominated the phytoplankton assemblage.

5. Conclusions

Fatal parasitic infections by *Amoebophrya* can contribute to the dynamics of red tides that occur in coastal locations influenced by upwelling, as exemplified by red tides of *A. sanguinea* in Monterey Bay, CA. While *Amoebophrya* epidemic infection outbreaks may end or prevent the occurrence of red tides, strong and persitstent upwelling events may cause the host to grow at a faster rate than the parasite, which then escapes parasitism and, consequently develops extreme red tides. These findings suggest that red tides dynamics can be controled both by environmental (bottom up) and biological (top down) processes, and that the relative importance of each of these processes may temporaly vary. Additionally, throughout this study, only dinoflagellate species that were free of *Amoebophrya* infections (i.e., *Cochlodinium* sp., *C. divaricatum*) developed red tides in the Monterey Bay, further suggesting *Amoebophyra*'s significant role in indirectly contributing to the net phytoplankton species compositon.

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