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Effects of microalgal exudates and intact cells on subtropical marine zooplankton

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Harmful algal blooms (HABs) affect coastal waters worldwide and very often lead to the disruption of seafood harvesting and commercial activities, because of potential hazards to human health associated with the consumption of contaminated mussels, crustaceans and fish. HAB events are frequently caused by outbreaks of toxin-producing dinoflagellates, which are subject to top-down control by zooplankton. The aim of this study was to analyze the effects of dinoflagellate exudates and intact cells on the survivorship and mobility of zooplankton taxa from a subtropical location (Ubatuba, Brazil). Lethal effects were observed in five out of six taxa investigated, three of which (copepod nauplii, tintinnids and gastropod larvae) when exposed to dinoflagellate exudates and two (rotifers and brachyuran zoeae) when exposed to intact cells. In addition, gastropod larvae displayed mobility impairment during exposure to dinoflagellate exudates. Only polychaete larvae were not apparently affected during the course of the experiments. Zooplankton responses usually varied according to the dinoflagellate species tested. For instance, exudates from *Alexandrium tamiyavanichii*, *Gonyaulax* sp. and *Gymnodinium* sp. decreased survivorship of planktonic copepod nauplii but did not affect bottom-dwelling harpacticoid nauplii, which were in turn killed by exudates from *Prorocentrum lima*, a epibenthic dinoflagellate. These results suggest that HAB events do not cause indiscriminate zooplankton mortality, but may instead generate community shifts and complex cascading effects through the pelagic and benthic food web. Species-specific monitoring of zooplankton responses to HABs is therefore an important step to understand the ecological implications of dinoflagellate outbreaks in coastal waters, and their impact on marine farming activities.

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KEYWORDS: dinoflagellates; exudates; HABs; zooplankton; survivorship

INTRODUCTION

The last decades have witnessed an apparent increase in the intensity, duration and geographic extent of harmful algal blooms (HABs) world-wide, which may be related to global climate change and increasing human perturbations on coastal environments, such as eutrophication, ballast water discharge and transport of shellfish stocks or other aquaculture products containing phytoplankton cysts (Hallegraeff, 2003). These events can cause severe economic, ecosystem and health impacts, particularly in regions lacking comprehensive monitoring and contingency programs (Hallegraeff, 2010).

The effects of toxic microalgal blooms on marine food webs through their consumption by zooplankton, especially copepods, are often studied *in situ* during bloom episodes (Kozlowsky-Suzuki *et al*., 2006), or in laboratory experiments comparing diets of toxic and non-toxic microalgae (Schultz and Kiørboe, 2009). The effects on zooplankton are diverse and highly species- and site-specific. Examples of sublethal effects following toxin ingestion include reduction in feeding, fertility or egg hatching rate or physiological incapacitation (Frangópulos *et al*., 2000).

More recently, studies have aimed at understanding the influences of toxic microalgal blooms on other pelagic groups in addition to copepods, such as protozoans and meroplanktonic larvae (Almeda *et al*., 2011). Direct exposure to intact cells or their exudates may have negative effects on zooplankton, such as reduction in filtration and ingestion rates (Thompson *et al*., 1994), behavioral alterations (Buskey and Stoecker, 1989; Bagøien *et al*., 1996), reduced survival rates (Hansen, 1989; Hansen *et al*., 1992; Ajuzie and Houvenaghel, 2000) and decreased reproduction and recruitment (Ajuzie, 2007).

The Brazilian coast encompasses the entire tropical and subtropical range of the Southwest Atlantic shoreline. Few reports exist on HABs along this extensive coastline because of the lack of continued, long-term phytoplankton monitoring programs (Odebrecht *et al*., 2002). Recently, a growing industry farming of the bivalve *Perna perna* (Linnaeus, 1758) has led to the establishment of water quality monitoring programs and health certification of open-ocean mussel farms in the subtropical coast of southern Brazil. HABs have received growing attention after the 2007 diarrheic shellfish poisoning event in Santa Catarina (southern Brazil), when at least 150 people were affected (Proença *et al*., 2007).

Annual mussel production on Santa Catarina coast, the major mussel farming area in Brazil and second largest in Latin America, increased from 190 to 15 635 tons from 1990 to 2010 (Santos *et al*., 2010). HAB research in Brazilian coastal waters is concentrated in that region (Odebrecht *et al*., 2002), where both toxic (e.g. *Dinophysis cf. acuminata*, Proença *et al*., 2007; *Dinophysis acuminata*, Souza *et al*., 2009) and non-toxic dinoflagellate blooms (e.g. *Alexandrium fraterculum*, Omachi *et al*., 2007) have been reported. In other Brazilian regions, the growing mariculture industries and the risks associated with seafood poisoning have also led to studies on the detection and monitoring of toxic microalgae (Nascimento *et al*., 2012).

Some reports describe mortality events of benthic fauna in the Southwest Atlantic coast associated with dinoflagellate occurrence: (i) mollusc mortality associated with *Gymnodinium* sp. (Machado, 1979), (ii) mortality of polychaetes, molluscs, cnidarians and echinoderms associated with *Gymnodinium cf. aureolum* (Rosa and Buselato, 1981), (iii) mortality of intertidal benthic invertebrates, mainly bivalves, associated with *Gymnodinium cf. aureolum*, *Dinophysis acuminata* and *Noctiluca scintillans* (Garcia *et al*., 1994), and (iv) bivalve mortality associated with *D. caudata*, *D. acuminata* and other dinoflagellates (Méndez, 1995). However, despite their importance in understanding the dynamics and impacts of regional HAB events, no studies are available on zooplankton responses to exposure to potentially toxic dinoflagellates.

Our working hypothesis is that cell exudates or intact cells of potentially harmful dinoflagellates cause either lethal or sub-lethal effects on small-sized zooplankton. The zooplankton tested (copepod nauplii, tintinnids, rotifers and larval stages of gastropods, polychaetes and brachyurans) are among the most common taxa in the micro- to mesozooplankton size range along the Brazilian shelf and in other coastal ecosystems worldwide; therefore, deleterious effects on them are likely to have extensive impacts on marine ecosystem functioning.

METHODS

Phytoplankton cultures

The toxic dinoflagellates *Alexandrium tamiyavanichii*, isolated from Porto Seguro, Brazil, and *Prorocentrum lima*, originated from Vigo, Spain, were obtained from UNIVALI (Itajaí, Brazil); *P. lima* is also present in

Brazilian waters (Nascimento *et al.*, 2012). Toxicity assessments of these two species were reported by Menezes *et al.* (Menezes *et al.*, 2010) (*A. tamiyavanichii*—strain A1PSA; seven toxins—STX predominated) and Bravo *et al.* (Bravo *et al.*, 2001) (*P. lima*—strain PL2V; dinophysis toxin—DTX1). *Gonyaulax* sp. and *Gymnodinium* sp. were isolated by us from the coastal water of Ubatuba and did not display toxicity in screenings for okadaic acid; dinophysis toxins 1 and 2; yessotoxins (Ytx) such as Ytx, 45-hyd-Ytx and 45-hyd-HomoYtx; azaspiracids 1, 2 and 3; pectenotoxins 1 and 2; gymnodimine; and spirolide (Proença, personal communication). However, the presence of other bioactive metabolites cannot be excluded. Filtered seawater was used as a control; in some experiments, we also included non-toxic microalgal species for comparison (Table I). All phytoplankton species were cultured in f/2 medium (with silicate for diatoms), a salinity of 35 psu, light intensity of 100 $\mu E^{-2}\ s^{-1}$, dark:light regime of 12:12 h and temperature of 23 ± 1°C.

Exudate experiments

Exudates were collected by filtering 50 mL of each microalgal culture through GF/F filters under the lowest possible pressure. Before filtration, the cell density of each culture was measured with a particle counter (Beckman Z2 Series Coulter Counter; Table I). Filtrates were used immediately or stored at 10°C for <1 h before experiments. Microzooplankton were collected off Ubatuba (São Paulo, Brazil) using a plankton net with 15- or 50-µm mesh size and a closed cod-end, with the exception of rotifers (*Brachionus plicatilis*), which were obtained from our own cultures, and crab zoeae (*Pachygrapsus transversus*), which were obtained from egg-laying females collected on a rocky beach shore in Ubatuba prior to the experiments. Zoeae were tested on the day of hatching (zoea I). Experiments were performed in 12- or 24-well plates depending on the zooplankton size. In earlier experiments (December 2010), exudates were directly added to the wells. In later experiments (January−February 2011), exudates were first filtered through a 0.2-µm filter. This step was added after apparent bacterial contamination was observed in some treatments during the initial experiments. The behavior and mobility of the animals was observed under a stereomicroscope at regular intervals (3−4 h). The cell plates were maintained at 21°C in a natural photoperiod scheme, and exposure to direct light was avoided.

Exudate incubations were performed in December 2010 and January−February 2011. The taxa studied were copepod nauplii (*Acartia lilljeborgii*, *Paracalanus* sp., *Longipedia* sp.), tintinnids (*Favella ehrenbergii*), rotifers (*Brachionus plicatilis*), gastropod larvae (unidentified veligers) and polychaete larvae (unidentified trochophores). Copepod nauplii were sorted from freshly collected samples and fed *Isochrysis galbana* until use (usually within 10 h). In the case of tintinnids, survival rates were calculated taking into account new individuals produced by asexual reproduction during incubations. Juvenile and female rotifers were sorted based by size, i.e. organisms of a size similar to that of newly hatched rotifers were selected as juveniles, and larger organisms carrying eggs were selected as females. Females were then further separated into amictic (resulting from parthenogenetic reproduction) and mictic (resulting from sexual reproduction) females, except in the early experiment, when they were combined. Gastropod larvae were sorted into two size classes: small veligers were

Table I: Potentially harmful algae and control treatments, and original site of strain isolation

Treatments	Strain and site of isolation	Treatment abbreviation	Known toxicity	Concentration (cells L^{-1}) Exudate experiments	Concentration (mgC L^{-1}) Exudate experiments
Control treatments					
Dunaliella tertiolecta	Dun. Ter (Cabo Frio, Brazil)	DUN	No	1.0×10^8	2.35 ± 0.01
Isochrysis galbana	Iso.g.-Th1 (Tahiti)	ISO	No	2.0×10^7–2.0×10^{10}	16.07 ± 9.97
Tetraselmis gracilis	Tetra.g.-C1 (Cananéia, Brazil)	TET	No	2.3×10^6–2.2×10^9	50.91 ± 4.77
Diatom mixture	(Ubatuba, Brazil)	DMIX	No	5.9×10^7–4.6×10^8	1.67 ± 0.19
Filtered seawater	–	FSW	–		–
Potentially harmful treatments					
Alexandrium tamiyavanichii	(A1PSA—Porto Seguro, Brazil)	ALEX	Yes; Menezes *et al.* (Menezes *et al.*, 2010)	2.5×10^5–1.5×10^7	2.62 ± 2.59
Gonyaulax sp.	(Ubatuba, Brazil)	GO	Negative for several toxins; see text	1.1×10^5–1.2×10^7	1.64 ± 1.30
Gymnodinium sp.	(Ubatuba, Brazil)	GYM	Negative for several toxins; see text	1.1×10^6–2.0×10^7	3.11 ± 0.11
Prorocentrum lima	(PL2V—Vigo, Spain)	PRO	Yes; Bravo *et al.* (Bravo *et al.*, 2001)	4.4×10^5–8.8×10^6	1.42 ± 2.39

used in the exudate experiments; larger veligers were used in experiments with intact cells as described below. Polychaetes of the medium size were selected for the exudate experiments. Treatments with apparent bacterial contamination were excluded from the analysis. Whenever possible, the percent saturation of dissolved oxygen was measured using a membrane sensor at the end of each experiment, and oxygen percent saturation was always in the range of 70% or above. Incubation time was determined by two criteria: (i) the occurrence of high mortality (minimum of 60% death; usually 80−100%) in a potentially harmful treatment in contrast to a high survival rate in control treatments, and (ii) for cases in which no adverse effects were detected after 30 h, wells were monitored until the onset of apparent bacterial proliferation (typically 48−60 h) observed as a thin biofilm on the air−water interface. For copepod nauplii, tintinnids and gastropod larvae, experiments were performed in six-well plates, with 10 individuals per well and six replicates for each microalgal treatment. Polychaetes were individually incubated in 12-well plates. Different treatment combinations were applied depending on the availability of cultures in exponential growth phase.

Experiments with intact cells

Incubations were performed in December 2010 and February 2011. Rotifers, gastropod veligers and crab zoeae were exposed to intact phytoplankton cells under saturating concentrations (>1000 μg C L^{-1}) (Table I). Cell biovolumes (μm^{-3}) were measured by the particle counter, and cell carbon content was then calculated based on published volume-to-carbon conversion factors (Edler, 1979). Gastropod veligers and amictic egg-carrying rotifer females were individually incubated in 12-well plates. Ten replicates were performed for each treatment. For crab zoeae, experiments were performed in six-well plates with six replicates for each microalgal treatment. The plates were kept under daytime light levels throughout the experiment to allow for photosynthesis and oxygen production. Treatments with apparent bacterial contamination were discarded. Incubation periods and treatment combinations were determined according to the same criteria described for exudate experiments.

RESULTS

Exposure to microalgal exudates

Acartia lilljeborgii nauplii incubated in filtered seawater or exudates from diatom mixtures, *D. tertiolecta*, *I. galbana* and *Tetraselmis gracilis* showed relatively low mortality (between 2 and 23%) after 25 h. In comparison, the *P. lima* exudate caused slightly more rapid death (31%). The strongest harmful effects were found in exudates from *A. tamiyavanichii* and *Gonyaulax* sp., which caused 88−100% mortality within 29.6 h of exposure (Fig. 1A). For *Longipedia* nauplii, only the *P. lima* exudate caused higher mortality than filtered seawater and other treatments, and 70% of the nauplii died after 35 h (Fig. 1B). For paracalanid nauplii, exudates from *A. tamiyavanichii*, *Gonyaulax* sp. and *Gymnodinium* sp. resulted in reduced survivorship relative to filtered seawater and *T. gracilis* exudate (Fig. 1C). Paralytic effect was also observed in the *A. tamiyavanichii* exudate, in which the nauplii remained motionless with their antennules twisted up for several minutes prior to death. The tintinnid *F. ehrenbergii* was highly sensitive to exudates from *A. tamiyavanichii* and *Gonyaulax* sp., with its survival dropping to 10−20% within 25 h (Fig. 1D); in contrast, incubations in filtered seawater or *P. lima* exudate resulted in similarly high survival (∼75%).

The mobility of small gastropod veligers was reduced by 55% in filtered seawater after 35 h (Fig. 2A), although their survivorship was hardly affected (Fig. 2B). Exposure to exudates from *A. tamiyavanichii*, *Gonyaulax* sp. and *P. lima* strongly reduced their mobility and survivorship (Fig. 2A and B). Exudates from *A. tamiyavanichii* immobilized all individuals within a day, and exudates from *Gonyaulax* sp. and *Gymnodinium* sp. decreased the percentage of actively swimming veligers to 3 and 17%, respectively. Gastropod visceral retraction was also observed under exposure to *A. tamiyavanichii* and *Gonyaulax* sp. exudates. Only 34−48% of the veligers in these three treatments remained alive at the end of the experiment.

No discernible harmful effects were observed in exposure of *B. plicatilis* females and juveniles to dinoflagellate exudates, and the survival rates remained high (≥90%) and comparable with those in filtered seawater (Fig. 3A and B). Survivorship of polychaete larvae in dinoflagellate exudates decreased to 75−85% after 27 h but remained stable afterward (Fig. 3C); no mobility impairment was found.

Exposure to intact cells

Large gastropod veligers did not show ill effects when exposed to dinoflagellates when compared with filtered seawater or other microalgae (Fig. 4A). However, *A. tamiyavanichii* and *Gymnodinium* sp. had a negative effect on *B. plicatilis* amictic females (Fig. 4B), reducing their survival to 0 and 17%, respectively, relative to non-dinoflagellate microalgae. In the same experiment, mortality in filtered seawater was similar to the dinoflagellate *Gonyaulax* treatment, indicating a starvation

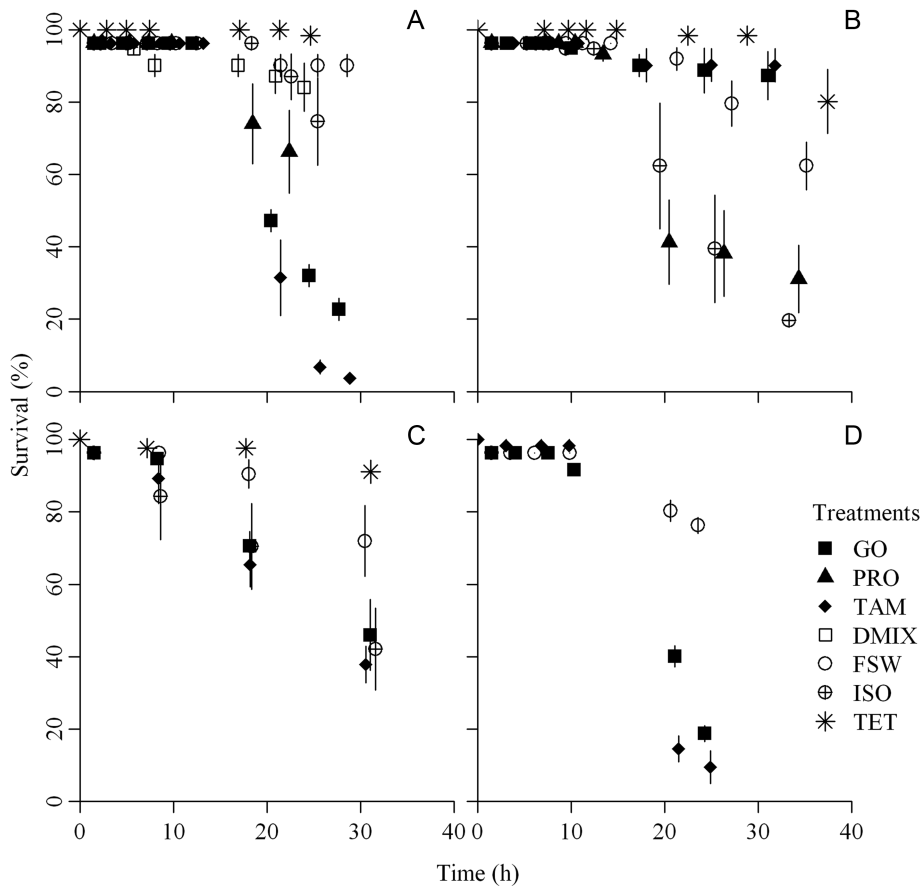

Fig. 1. Exudate experiments with lethal effects. *Acartia lilljeborgii* (**A**), *Longipedia* (**B**), Paracalanid nauplii (**C**) and tintinnid ciliates (*Favella ehrenbergii*) (**D**) survival as a function of exposure time to different treatments of cell exudates. Vertical bars: standard error considering six replicates. Treatment abbreviations as in Table I.

effect. Crab zoeae were not affected by *Gonyaulax* sp., but they all died in the *A. tamiyavanichii* treatment after 23 h (Fig. 4C). Prior to death, we observed changes in larval swimming behavior including erratic movements and reduced mobility.

DISCUSSION

Zooplankton can be affected by HAB toxins when ingested, but even for species that do not prey on HAB species (due to size mismatch or active avoidance) exposure to HAB exudates may cause negative impacts, as summarized in Table II.

Exudates tested in our experiments were collected from dense dinoflagellate cultures, (e.g. 2.5×10^5 to 1.7×10^7 cells L^{-1} for *A. tamiyavanichii*), the lower concentrations being close to average bloom-level concentrations reported in Brazilian coastal waters (*A. tamarense*, 2×10^5 cells L^{-1}, Odebrecht et al., 1997; *A. fraterculum*, 7.0×10^4 to 8.8×10^5 cells L^{-1}, Omachi *et al.*, 2007). Toxin-producing microalgae often aggregate in thin layers (Sullivan *et al.*, 2010), where cell concentrations can reach as high as 10^5–10^7 cells L^{-1} (Rines *et al.*, 2010). Therefore, our experiments are a realistic representation of field conditions as experienced by local zooplankton.

In exudate experiments, copepod nauplii, tintinnids and small veliger larvae suffered substantially higher mortality in dinoflagellate exudates than in filtered seawater or other microalgal exudates, clearly indicating a toxic effect in addition to starvation (Figs 1 and 2).

Mortality following exposure to *P. lima* exudates, as well as naupliar inactivation during exposure to *Alexandrium* exudates, both observed in our study, were reported by Ajuzie and Houvenaghel (Ajuzie and Houvenaghel, 2000) for *Artemia salina* nauplii, and by Bagøien *et al.* (Bagøien *et al.*, 1996) for *Euterpina acutifrons* nauplii (Table II). This is in contrast with observations made in adult copepods, which usually reveal exudate sensitivity only after a long exposure time (e.g. 5 days, *A. minutum*, Bagøien *et al.*, 1996).

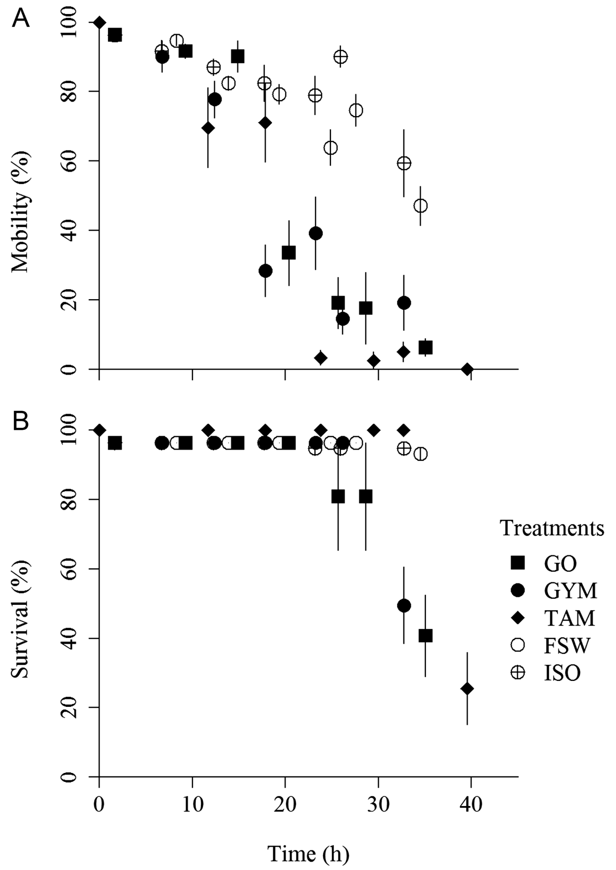

Fig. 2. Exudate experiments leading to sublethal and subsequent lethal effects. Gastropod larvae mobility (**A**, percentage of swimming organisms) and survival (**B**, percentage of live organisms) as a function of exposure time to different treatments with cell exudates. Vertical bars: standard error considering six replicates. Treatment abbreviations as in Table I.

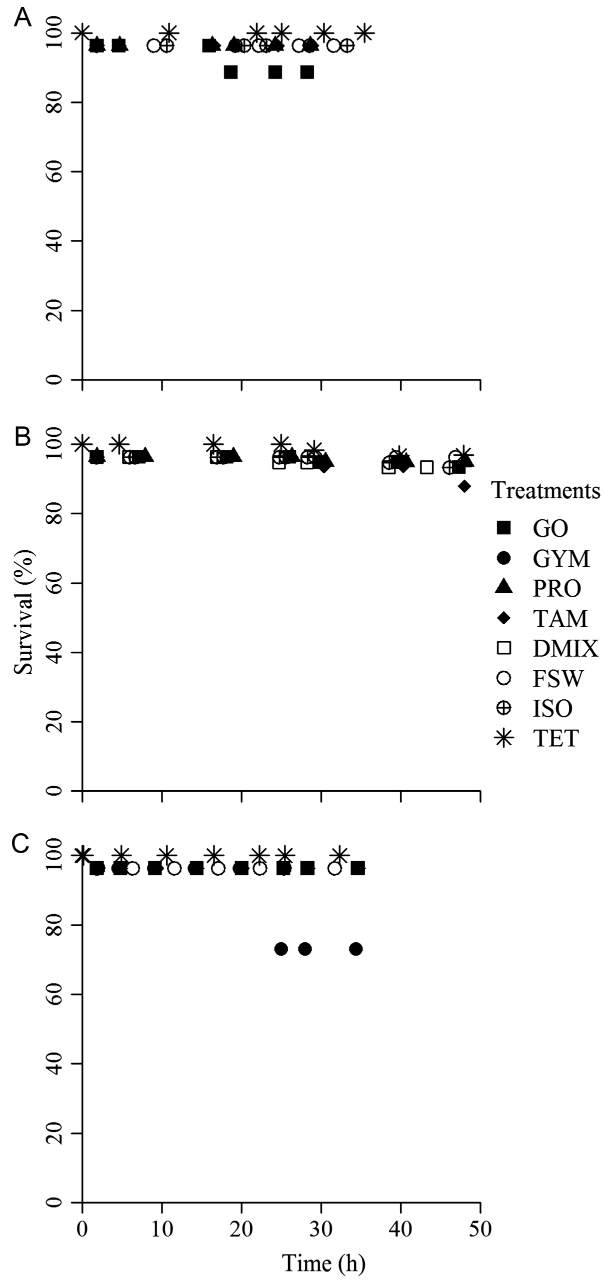

Fig. 3. Exudate experiments with the absence of effects. Rotifer (*Brachionus plicatilis*) mictic and amictic females (**A**) and juveniles (**B**), and polychaete larvae (**C**) survival as a function of exposure time to different treatments with cell exudates. Treatment abbreviations as in Table I.

Copepod nauplii died in a surprisingly short period of time in our experiments. While Bagøien *et al.* (Bagøien *et al.*, 1996) found a maximum inactivity rate of 52% in *Euterpina acutifrons* after 3 days of incubation, we observed inactivity percentages varying from 70 to 100% for other copepod species after 30 h exposure to dinoflagellate exudates (Fig. 4C). Our data give support to the concept that although toxin exudation may not represent an effective defense mechanism against adult copepods (Barreiro *et al.*, 2007), the inhibition of naupliar development by exposure to toxic exudates is an important survival strategy for dinoflagellates via suppression of future predator populations (Turner and Tester, 1997). This includes the well-known effects on embryonic development leading to impaired copepod hatching success (Frangópulos *et al.*, 2000).

The effects of *Alexandrium* exudates on the tintinnid ciliate *F. ehrenbergii* were previously studied by Hansen (Hansen, 1989), Hansen *et al.* (Hansen *et al.*, 1992) and Fulco (Fulco, 2007) (Table II), who observed a recurring behavioral response: reversal of ciliary movement and continuous swimming backwards with subsequent death. Using the same experimental design, our experiments indicated a reduction in survival after exposure to exudates, with no apparent change in swimming behavior

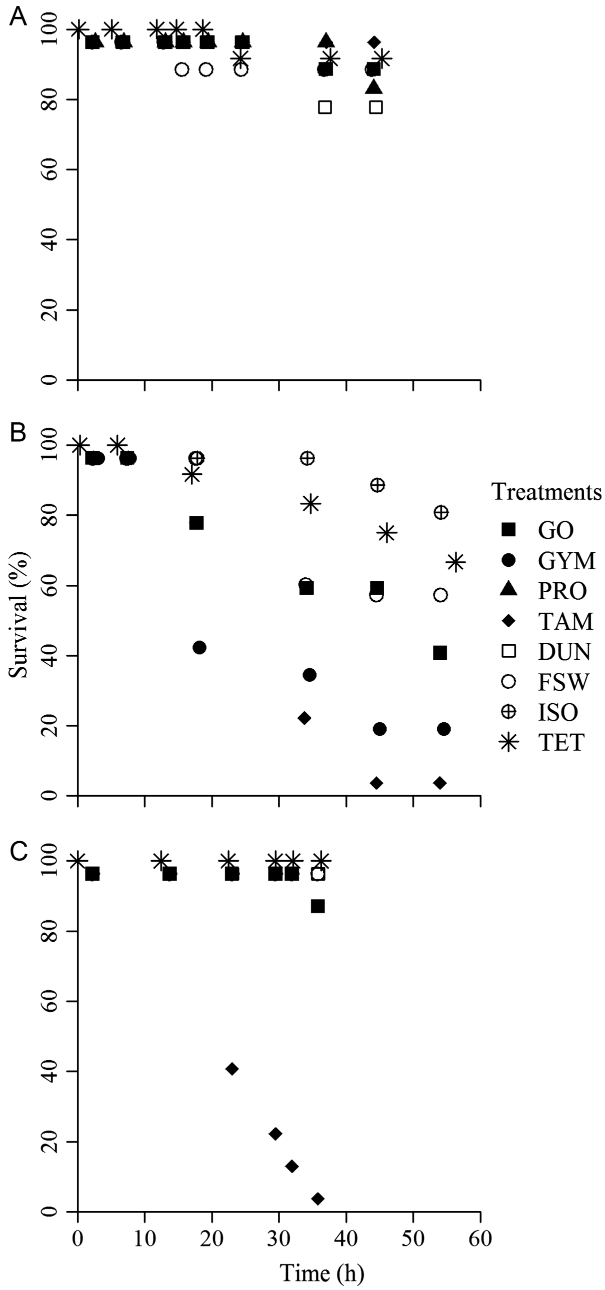

Fig. 4. Experiments with intact cells. Gastropod larvae (large veligers) survival (**A**), and rotifer (*B. plicatilis*) amitic females (**B**) and brachyuran zoeae (**C**) survival in function of exposure time to different treatments with phytoplankton intact cells. Treatment abbreviations as in Table I.

apart from progressive lethargy. This may be linked to a variable toxicity in the *Alexandrium* species and strains investigated. Although effects on tintinnids are reportedly acute under experimental conditions, these are not apparent in field studies, since *Favella* sp. may quickly excrete *A. tamarense* toxins even after 24 h of interaction with the prey (Kamiyama and Suzuki, 2006).

Although large veliger larvae appeared to be resistant to ingestion of dinoflagellates or possibly able to reject them (Fig. 4—intact cells experiments), small (and younger) veligers were not (Fig. 2—exudate experiments). Hence, if bloom events coincide with a recruitment phase, the gastropod population could still be severely impacted. Our observations on abnormal gastropod behavior after exposure to dinoflagellate cells, the so-called "morbidity" behavior (visceral retraction), were coincident with the findings of Juhl *et al.* (Juhl *et al.*, 2008) (Table II).

Not all zooplankton groups tested were adversely affected by the exudates. For example, rotifers (both female and juvenile) and polychaetes appeared to be more resistant to any released toxins and did not exhibit any ill effects from exposure to the exudates (Fig. 3). On the other hand, exposure to intact cells led to high mortality in rotifers and crab zoeae (Fig. 2).

Sensitivity of decapod zoeae to a potentially harmful *Alexandrium* species was also described by Hinz *et al.* (Hinz *et al.*, 2001), Perez and Sulkin (Perez and Sulkin, 2005), Garcia *et al.* (Garcia *et al.*, 2011) (Table II) and Sulkin *et al.* (Sulkin *et al.*, 2003). Although crab and dinoflagellate species tested in this study were different, locomotion perturbations, concomitant with a reduction in the rates of oxygen consumption, are consistent with the results of Sulkin *et al.* (Sulkin *et al.*, 2003). For polychaetes, our results are coincident with those of Wilson (Wilson, 1981) who tested the dinoflagellate genera *Amphidinium*, *Gymnodinium*, *Prorocentrum* and *Scrippsiella*, and did not find apparent effects of either exudates or intact cells on the larvae (Table II).

Acartia nauplii and *Paracalanus* nauplii were all strongly affected by exudates from *A. tamiyavanichii* and *Gonyaulax* sp., but not *P. lima*. Conversely, *Longipedia* was strongly affected by the *P. lima* exudate but not the others. These results suggest that *A. tamiyavanichii* and *Gonyaulax* sp. exudates contained different toxic substances compared with *P. lima* exudates, as reported in the literature (Table III). These differences in toxicity also reflect the different life cycle strategies of the organisms: while *P. lima* is present in the water column, it has also a tendency to attach to macroalgae (Foden *et al.*, 2005), and *Longipedia* as a benthic harpacticoid copepod tends to reside near the bottom. The close spatial association between *P. lima* and *Longipedia* in the environment may have led to the evolution of *P. lima* toxicity more specific to *Longipedia*. Likewise, the more planktonic forms of *A. tamiyavanichii* and *Gonyaulax* sp. may have facilitated the evolution of their specific toxic effects to grazers in the upper water column.

The variable responses among the different zooplankton groups to the different dinoflagellates as

Table II: Review of the effects of intact cells and exudates of dinoflagellate genera on zooplankton, including only taxa covered by this study

Zooplankton taxa	Dinoflagellate taxa	Laboratory strain	Zooplankton Species	Intact cells	Exudates	Author (year)
Tintinnina	Toxic *A. tamarense*	Ply173a	*Favella ehrenbergii*	Adv.	Adv.	Hansen (Hansen, 1989)
		LF1		Adv.	Adv.	
		LF2		Adv.	Adv.	
		LF3		Adv.	Adv.	
		LF4		Adv.	Adv.	
		Wh7		Adv.	None	
	Toxic *A. ostenfeldii*	LF37	*Favella taraikaensis*	Adv.	Adv.	Hansen *et al.* (Hansen *et al.*, 1992)
		LF38		Adv.	Adv.	
	Toxic *A. tamarense*	AT2B	*Eutintinnus* sp.	None	None	Fulco (Fulco, 2007)
			Favella taraikaensis	Adv.	None	
	Toxic *A. tamiyavanichii*	–	*Favella ehrenbergii*	No data	Adv.	This study
	Non-toxic *Gonyaulax* sp.	–		No data	Adv.	
Gastropoda (larvae)	Toxic *A. minutum*	18-1T	*Nassarius* sp.	Adv.	None	Juhl *et al.* (Juhl *et al.*, 2008)
	Non-toxic *A. minutum*	18-1NT		Adv.	None	
	Toxic *A. tamiyavanichii*	–	–	None	Adv.	This study
	Non-toxic *Gonyaulax* sp.	–		None	Adv.	
	Non-toxic *Gymnodinium* sp.	–		None	Adv.	
Rotifera	Toxic *A. minutum*	–	*Brachionus plicatilis*	None	No data	Wang *et al.* (Wang *et al.*, 2005)
	Toxic *A. minutum*	–		None	No data	
	Non-toxic *A. tamarense*	AT-6		None	No data	
	Toxic *A. tamarense*	ATHK#		Adv.	No data	
	Toxic *A. tamarense*	AT5-1#		Adv.	No data	
	Toxic *A. tamarense*	AT5-3#		Adv.	No data	
	Toxic *A. tamarense*	ATCI02#		Adv.	No data	
	Toxic *A. tamarense*	ATCI03#		Adv.	No data	
	Toxic *Alexandrium* sp. 1	–		Adv.	No data	
	Toxic *Alexandrium* sp. 2	–		Adv.	No data	
	Toxic *Alexandrium* sp.	–		Adv.	None	This study
	Non-toxic *Gonyaulax* sp.	–		Adv.	None	
	Non-toxic *Gymnodinium* sp.	–		Adv.	None	
Brachyura (zoea I)	Toxic *A. fundyense*	1719 (Af)	*Cancer magister*	Adv.	Adv.	Hinz *et al.* (Hinz *et al.*, 2001)
			Glebocarcinus oregonensis	Adv.	Adv.	
	Toxic *A. tamarense*	115 (A5)	*Cancer magistes*	Adv.	None	
			Hemigrapsus oregonensis	Adv.	None	
			Rhinolithodes wosnessenskii	Adv.	None	
	Non-toxic *A. tamarense*	118 (A8)	*Glebocarcinus oregonensis*	Adv.	None	
			Cancer magistes	Adv.	No data	
			Hemigrapsus oregonensis	Adv.	No data	
			Rhinolithodes wosnessenskii	Adv.	No data	
	Non-toxic *G. spinifera*	409 (Gs)	*Cancer magister*	None	No data	Perez and Sulkin (Perez and Sulkin, 2005)
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Non toxic *A. catenella*	1911 (Ac)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Toxic *A. fundyense*	1719 (Af)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Non-toxic *A. tamarense*	115 (A5)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	

Continued

Table II: Continued

Zooplankton taxa	Dinoflagellate taxa	Laboratory strain	Zooplankton Species	Intact cells	Exudates	Author (year)
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Toxic *A. tamarense*	118 (A8)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Toxic G. *catenatum*	1937 (G37)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Non-toxic G. *catenatum*	1940 (G40)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Toxic *A. andersoniii*	1718 (Aa)	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	Adv.	No data	
			Hemigrapsus nudus	Adv.	No data	
	Toxic *A. fundyense*	1719 (Af) CCMP	*Cancer magister*	None	No data	
			Cancer oregonensis	None	No data	
			Cancer productos	None	No data	
			Cancer gacilis	None	No data	
			Hemigrapsus oregonensis	None	No data	
			Hemigrapsus nudus	None	No data	
	Toxic *A. andersonii*	1718 (Aa)	*Glebocarcinus oregonensis*	Adv.	No data	Garcia *et al.* (Garcia *et al.*, 2011)
			Cancer magistes	Adv.	No data	
			Hemigrapsus oregonensis	Adv.	No data	
	Toxic *A. fundyense*	1719 (Af)	*Glebocarcinus oregonensis*	Adv.	No data	
			Cancer magistes	Adv.	No data	
			Hemigrapsus oregonensis	Adv.	No data	
	Toxic *Alexandrium* sp.	–	*Pachygrapsus transversus*	Adv.	No data	This study
	Non-toxic *Gonyaulax* sp.	–		None	No data	
Copepoda	*Gymnodinium simplex*	–	*Calanus pacificos*	None	No data	Huntley *et al.* (Huntley *et al.*, 1987)
	Toxic *A. minutum*	A12V	*Euterpina acutifron*s	Adv.	Adv.	Bagøien *et al.* (Bagøien *et al.*, 1996)
	Toxic *G. catenatum*	GC7B		Adv.	No data	
	Toxic *A. minutum*	A11V		Adv.	No data	
Copepoda	Toxic *A. tamiyavanichii*	–	*Acartia lilljeborgii*	No data	Adv.	This study
			Longipedia sp.	No data	None	
			Paracalanus sp.	No data	Adv.	
	Non-toxic *Gonyaulax* sp.	–	*Acartia lilljeborgii*	No data	Adv.	
			Longipedia sp.	No data	None	
			Paracalanus sp.	No data	Adv.	
	Toxic *Prorocentrum lima*	–	*Acartia lilljeborgii*	No data	None	
			Longipedia sp.	No data	Adv.	
			Paracalanus sp.	No data	Adv.	

Adv., adverse effect (lethal or sublethal effects). None: no effect. Dashes represent information not available.

Table III: Toxin characteristics of dinoflagelate taxa analyzed in this study

Dinoflagelate specie (strain)	Toxins	References
Alexandrium tamiyavanichii (A1PSA)	STX (3.4 pg cell^{-1}67.06%) neoSTX (0.39 pg cell^{-1}) GTX4 (1.6139 pg cell^{-1}) GTX3 (low concentrations) dcGTX2 (low concentrations) dcGTX3 (low concentrations)	Menezes *et al.* (Menezes *et al.*, 2010)
Gymnodinium sp.	PSP toxins	Hallegraeff (Hallegraeff, 2004)
Gonyaulax sp.	Yessotoxins	Rhodes *et al.* (Rhodes *et al.*, 2006)
Prorocentrum lima (PL2V)	OA (8.75 pg cell^{-1}) OA ester (8.11 pg cell^{-1}) DTX (3.02 pg cell^{-1})	Bravo *et al.* (Bravo *et al.*, 2001)

observed in our study highlight the non-uniform effects HAB events may have on coastal ecosystems. Depending on the species, HAB may not necessarily decimate all zooplankton, but rather shift the community composition to the more resistant species. For example, bloom events of *A. tamiyavanichii*, *Gonyaulax* sp. or *Gymnodinium* sp. would favor *Longipedia* and polychaetes, whereas a *P. lima* bloom would affect mostly the harpacticoid but have relatively little effects on the other zooplankton species. Changes in zooplankton species composition could then have secondary effects on the remainder of the food web. These taxon-specific responses also underscore the need for species-specific monitoring, assay and mitigation of HAB events. Although HAB events have been treated as infrequent or absent in most areas of the Brazilian coast (Odebrecht *et al.*, 2002), a large number of bloom events have been recorded in the past 10 years, leading to the suspension of mussel harvest and commercialization in affected areas (Souza *et al.*, 2009). In this context, information about ecological interactions between HAB species, zooplankton and other food-web components is of major importance for the adequate management of the growing mussel farming activity in Brazil.

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