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"Spread of potentially toxic benthic dinoflagellates in the Mediterranean Sea: a response to climate change?"

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Abstract

Records of toxic benthic dinoflagellates are increasing along the Mediterranean coasts, especially during the last decade. On the western coasts, *Ostreopsis ovata* blooms have been associated with human health problems due to palytoxin-like (p-PLT) toxin production, whereas, on the eastern Mediterranean (Greek) coasts, the presence of dense *Ostreopsis* spp. populations has resulted in shellfish contamination by similar (p-PLT) compounds. Furthermore, the recent detection of dinoflagellates once considered as tropical, such as the ciguatera causing genus *Gambierdiscus*, raises issues regarding changes in the biogeographical distribution of tropical benthic dinoflagellates. The possible relationship of climate and other relevant environmental changes to the increased records and intense blooms of temperate benthic dinoflagellates, and the detection in the Mediterranean of species previously considered tropical is discussed.

Introduction

Benthic dinoflagellates are characterized as a group of special interest since they comprise a high number of toxic and/or potentially toxic species (Yasumoto *et al.*, 1980b; Nakajima *et al.*, 1981). The biogeographical distribution of the majority of benthic dinoflagellate species was, until recently, limited mainly to tropical and subtropical areas, especially to coral reef ecosystems (Besada *et al.*, 1982; Faust, 1991; 1995; Faust *et al.*, 1996; Chinain *et al.*, 1999a; Chang *et al.*, 2000; Rhodes *et al.*, 2000; Pin *et al.*, 2001), while some others are known from temperate or even cold waters (Meunier, 1919; Hoppenrath and Leander, 2008).

Regarding their toxicity, it is worth pointing out that species of the genus *Ostreopsis* are capable of producing palytoxin analogs, with palytoxin being one of the most potent biologically synthesized marine toxins (Usami *et al.*, 1995; Ukena *et al.*, 2001; Taniyama *et al.*, 2003; Katikou, 2008); humans are affected with poisonous or even fatal consequences after consumption of fish contaminated with palytoxin and/or analogs, as in the case of clupeotoxism (Onuma *et al.*, 1999). Additionally, toxins produced by the benthic dinoflagellate genus *Gambierdiscus* (Yasumoto *et al.*, 1977; Bagnis *et al.*, 1980) are responsible for ciguatera, a disease that causes illness to more than 50,000 people each year after fish consumption, mainly in circumtropical areas (Glaziou and Legrand, 1994; Lewis, 2001).

Benthic dinoflagellates in the Mediterranean Sea before the year 2000

Prorocentrum lima was described from the Gulf of Sorrento in Italy (Ehrenberg, 1860) and *C. monotis* has been detected in the Mediterranean Sea since the early '60s (Halim, 1960a)

BOOK IN STOCK

following its initial description from North European waters (Meunier, 1919). On the contrary, the majority of the representatives of the toxigenic benthic dinoflagellate genera *Ostreopsis, Prorocentrum* and *Gambierdiscus* have been considered inhabitants mainly of tropical areas.

The first records of *Ostreopsis* species in the Mediterranean Sea go back to 1972 (Taylor in Zingone *et al.*, 2006) and 1979 (Taylor, 1979; Abboud-Abi Saab, 1989) on both the western and the eastern coasts. In the next two decades (1980-2000), records were relatively limited and concerned mainly the Italian coasts (for details see Table 1 *in* Zingone, this volume), while in some cases high cell densities were reported (Tognetto *et al.*, 1995). On the other hand, *Gambierdiscus* had never been detected, neither on the northern and more studied coasts nor in the southern and warmer areas of the Mediterranean; this absence was often used to discriminate benthic dinoflagellate assemblages of the Mediterranean Sea from similar ones in circumtropical areas (BENTOX-NET, 2007; Ismael and Halim, 2007).

Benthic dinoflagellates in the Mediterranean after the year 2000

During the last decade, records of *Ostreopsis* spp. have increased rapidly on both western (Vila *et al.*, 2001c; Masó *et al.*, 2003; Sansoni *et al.*, 2003; Turki, 2005; Turki *et al.*, 2006; Zingone *et al.*, 2006; Monti *et al.*, 2007; Mangialajo *et al.*, 2008) and eastern Mediterranean coasts (Aligizaki *et al.*, 2004; Aligizaki and Nikolaidis, 2006; Ismael and Halim, 2006; Aligizaki *et al.*, 2009a). Furthermore, *Ostreopsis* spp. (*O. ovata* & *O.* cf. *siamensis*) (Penna *et al.*, 2005) are not only detected in more geographical areas inside the Mediterranean Sea, but they also form intense blooms, reaching abundances comparable or even higher to those of tropical areas (Vila *et al.*, 2001c; Aligizaki and Nikolaidis, 2006; Mangialajo *et al.*, 2008). Recently, *C. monotis* was found proliferating in Tunisian waters (Armi *et al.*, 2009); in this latter case, the cell densities of *C. monotis* were quantified in water column samples, while high epiphytic abundances of this species have been recorded in Greek and Spanish waters (Vila *et al.*, 2001c; Aligizaki and Nikolaidis, 2006).

Coolia monotis strains from the Mediterranean Sea have been shown not to produce toxins (Penna *et al.*, 2005; Aligizaki, 2008; Aligizaki *et al.*, 2009a), unlike strains from Australia that have been found to produce cooliatoxin (Holmes *et al.*, 1995). On the other hand, Mediterranean *Ostreopsis* strains are capable of producing palytoxin analogs (Ciminiello *et al.*, 2006; Riobó *et al.*, 2006; Aligizaki *et al.*, 2008b; Ciminiello *et al.*, 2008; Riobó *et al.*, 2008; Ledreux *et al.*, 2009), as has been shown for *Ostreopsis* spp. from other areas (Usami *et al.*, 1995; Rhodes *et al.*, 2000; Taniyama *et al.*, 2003; Lenoir *et al.*, 2004).

However, *Ostreopsis* toxicity is expressed differently in the Mediterranean compared to circumtropical areas, where palytoxin originating from *Ostreopsis* cells has been associated with human poisonings or even death after fish consumption (Onuma *et al.*, 1999). In Greek waters, bivalve mollusc (mussels, clams, hairy mussels) tissues have been found contaminated with putative palytoxin (Aligizaki *et al.*, 2008b), but without any human poisoning after shellfish consumption. This fact can be attributed to the restriction of fisheries and commercial activities implemented by the appropriate authorities based on the results of the Greek Monitoring Program on Marine Biotoxins (National Reference Laboratory on Marine Biotoxins, Laboratory Unit for Toxic Marine Microalgae-AUTH). In contrast, on western Mediterranean coasts, and especially along Italian coasts, *Ostreopsis ovata* blooms have been associated with health problems (respiration, skin and eye irritations, fever) in swimmers (Brescianini *et al.*, 2006; Ciminiello *et al.*, 2006) resulting in bans on swimming and closure of several kilometres of coastline. Both expressions of *Ostreopsis* toxicity in the Mediterranean have direct consequences not only for human health but also for socioeconomic activities, since they afflict fisheries, aquaculture and tourism.

Apart from the obvious increase of *Ostreopsis* spp. records in the Mediterranean Sea, another important issue is the detection of species formerly considered as tropical. Species of the ciguatera causing *Gambierdiscus*, as well *Prorocentrum borbonicum, P. levis* and *Sinophysis canaliculata*, have very recently been recorded in this area, and specifically in Greek coastal waters (Aligizaki and Nikolaidis, 2008; Aligizaki *et al.*, 2008a; Aligizaki *et al.*, 2009b).

Based on these findings, the first record of *Gambierdiscus* sp. in the Mediterranean goes back to 2003 in Crete (Figure 1), indicating a northward expansion of *Gambierdiscus* "entering" the Mediterranean basin (Aligizaki and Nikolaidis, 2008). Prior to this record, the northernmost one concerned the Canary Islands in the NE Atlantic Ocean (Fraga *et al.*, 2004; Aligizaki *et al.*, 2008a). More recently (February 2009), *Gambierdiscus* was recorded in Saronicos Gulf, Salamina Island (Figure 1), at a latitude of about 38 °N extending the northern geographical boundaries of *Gambierdiscus* distribution even further within the Mediterranean basin (Aligizaki *et al.*, 2009a).

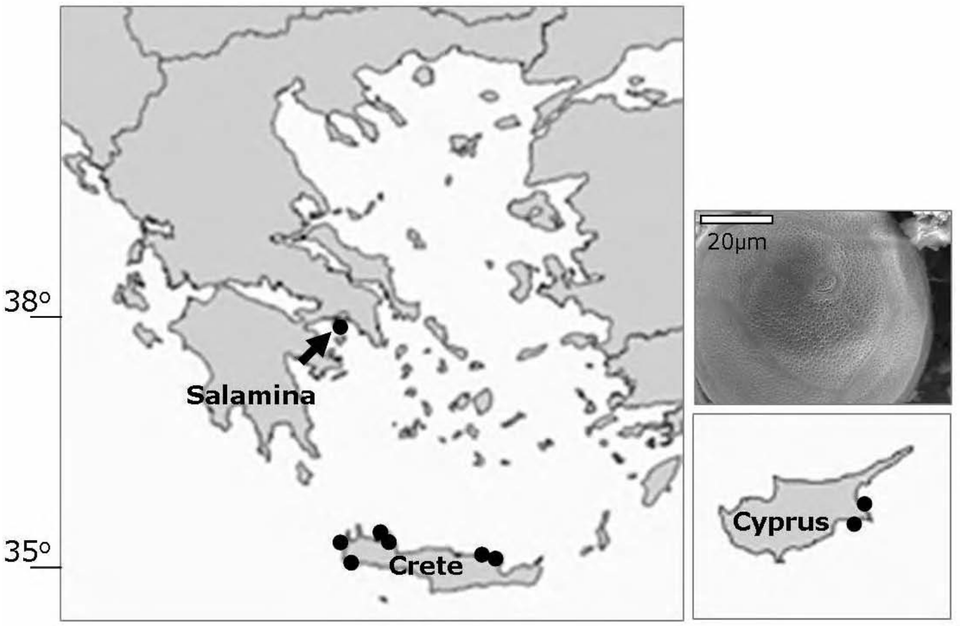

Figure 1. SEM micrograph and distribution of *Gambierdiscus* (KC81ALCCAUTH) in Greece and Cyprus. Arrow indicates the current northernmost record for the Mediterranean Sea.

Regarding the taxonomic identity of *Gambierdiscus* from the Mediterranean, it is possible that more than one species is present; in this context, several strains have been isolated, and morphological and molecular analyses are in progress, in view of recent new species descriptions and reinvestigation of the type species *G. toxicus* (Tester *et al.*, 2006; Litaker *et al.*, 2009). It is worth noting that preliminary toxicity analyses on two *Gambierdiscus* strains isolated from Greek waters indicated low toxicity compared to other strains from Malaysia and the Canary Islands based on mouse bioassay and cytotoxicity tests (Aligizaki *et al.*, 2009a; Caillaud *et al.*, 2009).

Is climate change responsible for benthic dinoflagellates expansion in the Mediterranean Sea?

The increased number of potentially toxic benthic dinoflagellates in the Mediterranean could reflect the apparent global increase of harmful algal blooms (Hallegraeff, 1993). Scientific interest in the impacts of climate variability on harmful algal blooms, and on marine ecosystems in general, is increasing (Navarra *et al.*, 2007; Moore *et al.*, 2008; Paerl and Huisman, 2008; Rabalais *et al.*, 2009; Shears and Ross, 2009; Valdes *et al.*, 2009). Regarding benthic dinoflagellates and their impact on human health, most surveys have focused on the link between climate change and ciguatera caused by *Gambierdiscus*-toxins (Hales *et al.*, 1999; Hales *et al.*, 2000; Llewellyn, 2010). Most authors suggest the possible spread of ciguatera to formerly non-endemic areas due to geographical expansion of its causative agent (*Gambierdiscus* spp.).

The fact that *Gambierdiscus* detection in the Canary Islands (Fraga *et al.*, 2004) was followed by ciguatera incidents in the same area (Pérez-Arellano *et al.*, 2005), indicates the potential hazard for

BOOK IN STOCK

future occurrence of ciguatera in the Mediterranean, since *Gambierdiscus* has been a Mediterranean inhabitant for at least six years (Aligizaki and Nikolaidis, 2008). But against this possibility is the fact that the first Mediterranean *Gambierdiscus* strains examined have shown low toxicity (Aligizaki *et al.*, 2009a; Caillaud *et al.*, 2009).

It is worth noting that suspected cases of ciguatera have been reported in the Mediterranean (Israel coast) in 1971-1972 (Herzberg, 1973) and 1988 (Spanier *et al.*, 1989), but they have not been associated with *Gambierdiscus* species. However, the latter report was questioned by Chevaldonné (1990), who suggested the possibility of misinterpretation of this case with "*Caulerpa* poisoning". Recently, two cases of another type of ichthyosarcotoxism, ichthyoallyeinotoxism, were described and distinguished from ciguatera poisoning based on the typical clinical signs and symptoms (De Haro and Pommier, 2006). The causative agents of ichthyoallyeinotoxism are still unknown, although representatives of the family Caulerpaceae have been suspected (Chevaldonné, 1990). Furthermore, the presence of ciguatoxin-like substances in edible fish of the eastern Mediterranean has been suggested (Bentur and Spanier, 2007). However, the aforementioned authors point out the necessity for additional analyses in order to determine whether these substances are ciguatoxins or related polyethers.

The detection of tropical representatives of *Gambierdiscus, Prorocentrum* and *Sinophysis* in the Mediterranean may represent biological signs of climate change in this area; this accords with discussion of "tropicalization" of the Mediterranean (Bianchi, 2007). In this context, the possibility of geographical expansion of tropical microalgae, such as *Gambierdiscus*, due to climate change was somehow "foreseen" (Fraga, 2007).

However, climate change is not a new issue; the climate has always been changing on scales of million of years and shorter times. But what is new during the last decades is the anthropologically induced component of global warming. Regardless of the mode of climate change, temperature range alterations may have an impact on microalgae, since each species/taxon has a preferred temperature range in which it can survive or even bloom. In this context, it can be suggested that the gradual increase in sea surface temperature in the Mediterranean (Salat and Pascual, 2002) facilitated the acclimation and settlement of such tropical species in this area, as in the case of other marine organisms (e.g. mollusca, fish, macroalgae) (Occhipinti-Ambrogi, 2007).

Furthermore, taking into account that *Ostreopsis* species had already been recorded in the Mediterranean Sea since the 1970s (Taylor, 1979), but did not form enhanced blooms until the last decade, it can be proposed that even a small-scale shift in the surface temperature may have increased prevalence of the optimal growth conditions for these species.

Additionally, temperature alterations might trigger a series of changes in parameters, such as stratification, upwelling, freshwater run-off and cloud cover, which affect microalgal communities (Dale *et al.*, 2006). Diatoms can be disadvantaged due to silicate limitation that results from limited rainfall and freshwater run-off, while flagellates (e.g. dinoflagellates, raphidophytes) are favoured in stratified conditions against diatoms due to their vertical migration ability (Anderson and Stolzenbach, 1985; Levandowsky and Kaneta, 1987). In the same context, it has been shown experimentally, with all the restrictions that laboratory conditions can have, that climate change in the North Sea will increase the risk of harmful dinoflagellate and raphidophyte blooms (Peperzak, 2003).

However, the role of the climate change focuses mainly on the prevalence or otherwise of conditions that permit the organisms to survive and establish viable populations; environmental changes are not responsible for organisms' transport itself. Thus, when it comes to tropical taxa in the Mediterranean, it is tempting to try to understand how they were at first transferred into this area.

Microalgae are known to be transported in several ways (e.g. ships' ballast waters, aquaculture products, etc) mainly in the form of resting cysts (Hallegraeff *et al.*, 1988; Hallegraeff and Gollasch, 2006). Information on cyst formation in benthic dinoflagellates is limited (Pearce *et al.*, 2001; Aligizaki and Nikolaidis, 2006), but this does not exclude the fact that they may form cysts. Nevertheless, benthic dinoflagellates are able to "travel" attached to floating material, e.g.

BOOK IN STOCK

macroalgae or even plastic debris (Bomber *et al.*, 1988; Masó *et al.*, 2003). Thus, in the case of *Gambierdiscus*, it may have invaded the Mediterranean along with "alien" macroalgae, which are increasing in Mediterranean waters (Galil, 2000; Tsiamis *et al.*, 2008).

However, *Gambierdiscus* most probably invaded the Mediterranean at a specific, though unknown, time; this hypothesis is based on the fact that research on benthic dinoflagellates was underway some years before its finding, not only in the northern Mediterranean countries (e.g. Spain, Italy, France), but also in the warmer southern areas (e.g. Egypt, Tunis, Lebanon), and *Gambierdiscus* cells are usually noticeable due to their large size and characteristic shape. Furthermore, the limitations that usually arise when examining "microalgal invasions" due to taxonomic ambiguities do not apply in the case of *Gambierdiscus* since no taxon assigned to this genus had previously been observed in the Mediterranean.

Concluding considerations

It is apparent that records of benthic dinoflagellates in the Mediterranean have increased in recent years, in number of species, abundance, and geographical distribution. This situation is most probably related to climate change, but many issues have to be examined to come to accurate conclusions. It cannot be ruled out that the increasing records of benthic dinoflagellates in recent years reflect intense research in this field, especially in temperate areas (Shears and Ross, 2009).

Regardless of how and why, potentially toxic and/or toxic benthic dinoflagellates nowadays constitute a significant component of benthic communities in the Mediterranean. This fact requires further studies and collaboration among Mediterranean countries in order to understand the environmental changes that are occurring and to protect human health and human activities from the potential hazards of benthic dinoflagellate toxicity.