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Toxic marine microalgae and noxious blooms in the Mediterranean Sea: A contribution to the Global HAB Status Report

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1	Toxic marine microalgae and noxious blooms in the
2	Mediterranean Sea: a contribution to the global HAB status
3	report
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25 ABSTRACT

26 We review the spatial distribution of toxic marine microalgal species and the impacts of all types of harmful algal events (Harmful Algal Blooms, HABs) in the 27 28 Mediterranean Sea (MS), including the Black Sea, the Sea of Marmara, coastal 29 lagoons and transitional waters, based on two databases compiled in the Ocean Biogeographic Information System (OBIS). Eighty-four potentially toxic species have 30 31 been detected in the MS (2,350 records), of which 16 described from these waters 32 between 1860 and 2014 and a few suspected to have been introduced. More than half 33 of these species (46) produce toxins that may affect human health, the remainders ichthyotoxic substances (28) or other types of toxins (10). Nevertheless, toxicity-34 35 related events are not frequent in the MS (308 records in 31 years), and mainly consist 36 of impacts on aquaculture, caused by the dinoflagellates Dinophysis and Alexandrium, 37 along with a few actual shellfish poisoning cases. Pseudo-nitzschia blooms are widespread, but domoic acid in shellfish rarely exceeds regulatory levels. Fish kills 38 39 are probably less sporadic than reported, representing a problem at a few places along the southern MS coasts and in the Ebro River Delta. Since the last decade of the 20th 40 41 century, blooms of the benthic dinoflagellates Ostreopsis cf. ovata have regularly occurred all along rocky shores of the MS, at times with human health problems 42 43 caused by toxic aerosol. New records of Gambierdiscus and Fukuyoa, until now 44 reported for the westernmost and easternmost MS coasts, raise concerns about the risk 45 of ciguatera, a syndrome so far known only for subtropical and tropical areas. Recent discoveries are the dinoflagellates Vulcanodinium rugosum, responsible for the 46 47 presence of pinnatoxins in French lagoons' shellfish, and the azaspiracid-producers Azadinium spp. Mucilages and discolorations have a major impact on tourism in 48 49 summer. Reports of toxic species and HABs have apparently increased in the MS over

50	the last half century, which is likely related to the increased awareness and monitoring
51	operations rather than to an actual increase of these phenomena. Indeed, while the
52	case of Ostreopsis appears as a sudden upsurge rather than a trend, no actual increase
53	of toxic or noxious events has so far emerged in intensively studied areas, such as the
54	French and Spanish coasts or the Adriatic Sea. Moreover, some cases of decrease are
55	reported, e.g., for Alexandrium minutum blooms disappearing from the Harbour of
56	Alexandria. Overall, main HAB risks derive from cases of massive development of
57	microalgal biomass and consequent impacts of reduced coastal water quality on
58	tourism, which represents the largest part of the marine economy along the MS coasts.
59	
60	Keywords: HABs; Mediterranean Sea; microalgae; toxicity; OBIS

62 **1. Introduction**

The Mediterranean Sea (MS, from the Latin *mare Mediterraneum* = the sea 63 surrounded by land) is an enclosed basin surrounded on the north by southern Europe 64 65 and Anatolia, on the south by North Africa and on the east by the Levant. It occupies an area of approximately 2,510,000 km² lying between latitudes 30° and 46° N. The 66 narrow and shallow Strait of Gibraltar to the West connects it with the Atlantic 67 68 Ocean, the Dardanelles to the East with the Black Sea through the Sea of Marmara and the Bosporus, while to the south-east the Suez Canal, opened in 1869 and recently 69 70 expanded, allows the exchange with the Red Sea. In spite of its geographic position 71 within the northern temperate latitudes, the quite shallow sill (170 m) at the Atlantic 72 boundary blocks the entrance of deep, cold oceanic waters and determines temperate-73 subtropical conditions in the whole area, with minimum temperatures rarely and only 74 at certain locations going below 12 °C. The size, location, and morphology of the MS are at the base of its complex physical 75 76 dynamics with a distinctive thermohaline circulation and permanent or semi-77 permanent sub-basin gyres. A marked oligotrophy, increasing along both the west-78 east and the north-south directions, characterizes the MS (Siokou-Frangou et al., 2010). However, along the Mediterranean coasts there are densely populated areas 79 80 while a number of large rivers with extended catchment basins flow in the MS (e.g., 81 the Po in the northern Adriatic, the Nile in Egypt, the Ebro in Spain, and the Rhone in France). This implies that meso- and eutrophic conditions, and at times pollution, can 82 83 affect various coastal areas (UNEP/MAP, 2012). 84 The MS has been the crossroad of various cultures since the very beginning of the human colonization and the development of ancient civilizations. Trading routes, 85 86 migrations, invasions and the struggle for power have shaped the dynamic history of

87 populations around the basin for millennia. The population grew from 281 million in 1970 to 419 million in 2000 and 472 million in 2010, and is predicted to reach 572 88 million by 2030. Coastal administrative entities make less than 12% of the surface 89 90 area of the Mediterranean countries, but host more than a third of the population of the whole region. Coastal population grew from about 100 million in 1980 to 150 91 92 million in 2005 and could reach 200 million by 2030 (UNEP/MAP, 2017). 93 The MS also represents a unique geographic landscape that generates wealth but 94 requires cooperation among the different countries to preserve the environment and 95 the biological resources. The conservative value of the economic assets of the MS has been estimated to be in the order of US\$ 5.6 trillion, generating an annual economic 96 97 value of US\$ 450 billion (Randone et al., 2017). A large fraction of the economic 98 value is represented by tourism and related activities; fisheries come as second but 99 >80% of the fish stock is presently threatened. Aquaculture in the MS has 100 considerably expanded over the last decades reaching about 1.3 million tons in 2009 101 with an estimated value of US\$ 3,700 million (Rosa et al., 2012). Most of the marine aquaculture production comes from the north Mediterranean countries, which are also 102 103 the most intensively monitored, but it is rapidly expanding also in Turkey and Egypt. In spite of the dramatic alteration of habitats, depletion of natural resources and 104 105 increased number of alien species, the MS is still characterized by high biodiversity in 106 most animal and algal groups and a considerable number of endemic species (Coll et 107 al., 2010). The rate at which climatic conditions (e.g., surface temperature, heat waves and sea 108

level) have changed in the MS over the last decades is higher than the global average

110 (Cramer et al., 2018). These changes, coupled with increased population size,

111 urbanization and changes in land use at many coastal places, may pose at serious risk

the quality of the environment, the quality and quantity of food and consequently the health and safety of the local populations (Cramer et al., 2018). Especially in view of the growing need to exploit marine resources, HABs and toxic species may represent an increasing risk for human health and economic activities.

Few are the papers reviewing the occurrence of harmful species and/or events at the 116 scale of the whole Mediterranean basin. Fifty years ago Jacques and Sournia (1978-117 1979) published a first account of the cases of water discoloration ('eaux rouges') and 118 the species involved. The overview included mainly dinoflagellate blooms, along with 119 120 a few cases of anoxia but with no evidence of toxic effects in humans or marine fauna 121 in those years when microalgal toxins were still almost unknown. In an overview of 122 nearly twenty years later, cases of PSP and DSP - mainly attributable to Alexandrium 123 *minutum* and *Dinophysis* spp., respectively – were reported from the northern coasts 124 of the basin, along with the records of various potentially toxic or ichthyotoxic dinoflagellates at different sites (Honsell et al., 1995). A subsequent overview of toxic 125 126 and harmful microalgae covering up to 2009 pointed at the sudden spreading of Ostreopsis cf. ovata blooms along the rocky Mediterranean shores (Zingone, 2010). 127 The present overview covers the MS distribution of marine, toxin-producing 128 microalgae, as included in the IOC-UNESCO Taxonomic Reference List of Harmful 129 130 Micro Algae (Moestrup et al., 2009) and the cases of toxin-related harmful events 131 (Sections 2.1 and 2.2), including direct impact on human health or natural resources 132 or indirect impact to aquaculture industry. In addition, we review non-toxic events that include high biomass harmful algal blooms (HB-HABs) causing seawater 133 134 discolorations, anoxia or any other damages to the environment or human activities (Section 2.3). Finally, we discuss the trends of HABs in the MS in general and 135 136 particularly in the Adriatic Sea, which is considered a HAB hotspot (Section 3). The

137 overview is based on information from more than 600 scientific publications and

technical reports collected in two curated databases in the Ocean Biogeographic

139 Information System OBIS (Zingone et al., submitted): the MS-HABMAP-OBIS

- 140 (<u>https://obis.org/</u>), gathering records of toxic species occurrence, and the Harmful
- 141 Events Database (HAEDAT, <u>http://haedat.iode.org/</u>), collecting information of either
- toxic or non-toxic events, i.e., cases of intoxications, closures of aquaculture plants,
- seawater discolorations and mucilages. The present review is a contribution to a first
- appraisal of the current knowledge of HAB occurrences across the world seas,
- namely, the Global HAB Status Report, (Hallegraeff et al., 2017; Zingone et al.,
- 146 2017). The requirement for such an assessment has emerged from the apparent
- 147 worldwide increase and spreading of HABs and their negative impacts contrasted by
- the lack of an overview founded on a robust basis of data.
- 149

150 2. HABs in the Mediterranean Sea: toxic species and harmful event distribution 151 2.1 Toxic species

Of the more than 140 potentially toxic species listed in the IOC-UNESCO taxonomic 152 153 reference list (Moestrup et al., 2009), 84 have been found in the MS so far: 17 diatoms, 54 dinoflagellates, 3 dictyochophytes, 6 haptophytes, and 4 raphidophytes 154 155 (Table 1, and some examples in Fig. 1). These records cover both species actually 156 found to produce toxins in the MS and species known to be toxic from other areas. 157 Given the known variability in toxin production among strains of the same species, non-tested local populations are only 'potentially toxic' in most cases, but for brevity 158 159 they will be referred to as 'toxic' in the context of this paper. Sixteen of the toxic species have actually been discovered and described from the MS (Table 2), the first 160 161 ones (Prorocentrum lima, Dinophysis caudata, D. sacculus and D. tripos) in the

second half of the 19th century and the most recent ones (*Vulcanodinium rugosum*,

163 Azadinium dexteroporum, Nitzschia bizertensis and Ostreopsis fattorussoi) in the

164 current decade. Some of the HAB species of the MS, such as *D. caudata* and

165 *Chattonella subsalsa*, are widely distributed worldwide while others, including the

recently described *N. bizertensis* and *O. fattorussoi*, so far seem to be restricted to

167 specific areas of the MS.

168 The discovery of potentially toxic species in the MS has undergone an evident escalation over the years (Fig. 2), from the first descriptions of more than a century 169 170 before the discovery of their toxicity to the rapid increase after the 1960s and the most recent findings. Information on their distribution has also markedly increased along 171 172 with the intensification of monitoring operations and studies on planktonic and 173 benthic microalgae (e.g., Zingone et al., 2006; Aligizaki et al., 2009; Pistocchi et al., 174 2012; Balkis and Taş, 2016; Fernández et al., 2019) and of their resting stages in the sediments (Bravo et al., 2006; Satta et al., 2013) or sediment traps (Montresor et al., 175 176 1998). Yet the actual range of most toxic species in the MS is far from being known. Indeed, the identification of some of the most represented genera in the MS, such as 177 Alexandrium, Karenia, Karlodinium and Pseudo-nitzschia, as well as of many other 178 flagellates, is quite problematic. In many cases the observation of live material or 179 180 methods more complex than light microscopy are needed. Cryptic diversity 181 discovered in many microalgal taxa over the last decades also concerns several harmful genera and species, which have undergone careful taxonomic investigations 182 more than other non-toxic taxa. This trend has led to the discovery of non-toxic taxa 183 184 morphologically similar to toxic ones, such as several species in the P. delicatissima and P. pseudodelicatissima species-complexes (Bates et al., 2018), the non-toxic A. 185 tamutum hardly distinguishable from A. minutum (Fig. 1A, Montresor et al., 2004), 186

and the non-toxic chain-forming *Gymnodinium impudicum* (as *Gyrodinium impudicum*, Fraga et al., 1991) which was misidentified as *Gymnodinium catenatum*in studies predating its discovery (e.g., Carrada et al., 1991). Recent studies coupling

detailed morphological investigations with the analysis of different molecular markers

and toxin production have attempted to clarify species identity within the

192 Alexandrium tamarense-species complex (John et al., 2014; Litaker et al., 2018). The

193 case of *Chattonella subsalsa* is interesting because, based on several molecular

194 markers, two different genotypes with different geographic distributions exist for the

species (Klöpper et al., 2013). All these taxonomic insights have invalidated many

196 previous identifications of presumed toxic taxa, as detailed in the following sections.

197 In recent years, information on the presence of toxic species is also gathered through

198 molecular identification of environmental DNA samples (e-DNA metabarcoding),

199 which may give relevant information on the presence and seasonality of cryptic or

rare species (Ruggiero et al., 2015; Dzhembekova et al., 2017; Grzebyk et al., 2017).

201 Nonetheless, new findings of species through molecular methods should always be

202 confirmed by morphological studies.

203 Some of the toxic species of the MS have been suspected to be non-indigenous

species (NIS), i.e., introduced outside their natural past or present distribution. The

205 main possible NIS in the MS are *Pseudo-nitzschia multistriata*, *Alexandrium*

206 pacificum and Ostreopsis cf. ovata. The first MS record of Pseudo-nitzschia

207 *multistriata*, a chain-forming diatom having a distinctive sigmoid shape (Fig. 1G),

was in 1992 in the Gulf of Naples, where phytoplankton have been intensively studied

since the beginning of the 1980s. The species has shown an increasing trend

afterwards in the same area (D'Alelio et al., 2010) and has subsequently been found in

211 Spanish (Quijano-Scheggia et al., 2008), Greek (Moschandreu and Nikolaidis, 2010),

212 Tunisian (Sahraoui et al., 2011) and Moroccan waters (Rijat Leblad et al., 2013) and in the Adriatic Sea (Pistocchi et al., 2012; Turk Dermastia et al., 2020). The chain-213 forming dinoflagellate Alexandrium pacificum (as A. catenella) was found for the first 214 215 time in low density in 1983 along the Spanish coast (Margalef and Estrada, 1987). In 216 the following years, A. pacificum formed blooms on the Spanish coast (Gomis et al., 1996; Vila et al., 2001) and in the Thau Lagoon (as A. tamarense/catenella, Abadie et 217 218 al., 1999; Lilly et al., 2002). Subsequently it was progressively found eastward along 219 the Italian (Lugliè et al., 2003, 2017; Satta et al., 2013), Algerian (Frehi et al., 2007) 220 and Tunisian coasts (Turki and Balti, 2007; Fertouna-Bellakhal et al., 2015), whereas it is still unrecorded in the rest of the MS. The benthic dinoflagellate Ostreopsis cf. 221 ovata showed a sudden emergence in the MS at the end of the last century (see section 222 223 2.2.4). A much higher genetic variability and several cryptic species characterize this 224 taxon along the Japanese coasts compared to the Mediterranean-Atlantic area (Sato et al., 2011; Penna et al., 2012) where genetic differences are seen only at the population 225 226 level with AFLP markers (Italiano et al., 2014). This situation suggests a relatively recent radiation of the species in the latter area and, given the lack of hydrographic 227 links between the two regions, a possible man-mediated transport, although it is 228 impossible to establish when this occurred (Sato et al., 2011). In lack of type material, 229 230 or material from the type locality, it has not been established which of the numerous 231 morphologically similar taxa corresponds to Ostreopsis ovata. Therefore these taxa should be referred to as O. cf. ovata (Penna et al., 2010; Sato et al., 2011). Benthic 232 Gambierdiscus and Fukuyoa species are also a novelty in the MS, and their 233 234 distribution, presently limited at the two ends of the basin, hints at a possible recent introduction from both the Atlantic and the Red Sea. 235

236

237 2.2 Toxic events

238 2.2.1 Diarrhetic Shellfish Poisoning (DSP)

DSP toxins in mollusks represent the most frequently reported cases of seafood 239 240 contamination in the MS. Eight toxic species of the genus *Dinophysis*, plus 241 Phalacroma rotundatum (Table 1), have been observed along the Mediterranean 242 coasts (Fig. 3A). Dinophysis caudata and D. sacculus (Fig. 1C), the most frequently 243 reported species, were both described from the MS more than one century ago (Kent 1881; Stein 1883), but risks for human health have first been recognised only in the 244 245 1980s in the Gulf of Lion (Belin et al., 1995). In the northern Adriatic Sea, DSP toxicity events have occurred on both the western and eastern side, often causing the 246 247 closure of shellfish farms (Sedmak and Fanuko, 1991; Boni et al., 1992, 1993; Della 248 Loggia et al., 1993; Orhanović et al., 1996; Bernardi Aubry et al., 2000; Francé and 249 Mozetič, 2006; Marasović et al., 2007; Ninčević-Gladan et al., 2008). In the period 1989-2018, such closures occurred regularly along the Slovenian coast (northern 250 251 Adriatic) with an exceptionally long period from May 2010 to March 2011 in which relatively high *Dinophysis* abundances were recorded (around 2,000 cells L^{-1} of *D*. 252 fortii, Francé et al., 2018). These high abundances, never recorded again, were related 253 254 to long-lasting low salinity and extremely high temperatures in June – July surface 255 waters (<30 °C) causing a marked water column stratification (Francé et al., 2018). High levels of okadaic acid (OA) and/or dinophysistoxin (DTX) in several instances 256 also led to halt shellfish harvesting along the French (Belin et al., 2020) and Spanish 257 coasts of the MS (García-Altares et al., 2016; Fernández et al., 2019). Recurrent toxic 258 259 Dinophysis blooms have been recorded in the Thermaikos Gulf (Greece, North Aegean Sea) since 2000, when they caused great economic losses (EU 5 million) to 260 aquaculture (Koukaras and Nikolaidis, 2004). More occasionally, high levels of DSP 261

toxins have been reported from the eastern Mediterranean (Orhanović et al., 1996;

263 Bazzoni et al., 2018) and Tunisian waters (Armi et al., 2012).

Nonetheless, there have been just a few cases of DSP diagnoses in humans, in the

- Adriatic (Boni et al., 1992) and Tyrrhenian Seas (Lugliè et al., 2011), and two major
- accidents. One occurred in 2000, when 200 people were hospitalized following the
- 267 above-mentioned Dinophysis bloom in the Thermaikos Gulf (Koukaras and
- Nikolaidis, 2004). The other happened in 2010 in Piemonte (north-western Italy), with
- 269 more than 150 people harmed by the consumption of toxic mussels from the northern
- 270 Adriatic Sea (Pistocchi et al., 2012).
- 271 Other DSP producers widely distributed in the MS are two benthic species of the
- 272 genus Prorocentrum (Fig 3A), P. lima (Fig. 1F) and P. rhathymum, but no toxicity

events have been related with their presence.

274

275 2.2.2. Paralytic Shellfish Poisoning (PSP)

276 PSP events in the MS are related to toxins produced by species of the genus

277 Alexandrium and by Gymnodinium catenatum. Of the six Alexandrium species known

to produce PSP toxins found in the MS, *A. minutum*, the type species of the genus

- 279 (Fig. 1A), and A. pacificum (as A. catenella in records before 2014) are the most
- commonly reported ones (Table 1, Fig. 3B). In some cases these species have reached

high densities (up to 10^7 cells·L⁻¹) causing seawater discolorations. *Alexandrium*

taylorii has also caused discolorations at several Spanish and Italian touristic places

283 (section 2.3.1, table S1) but no toxicity has ever been found in MS populations of this

species.

- 285 Reports of PSP events initially associated with A. tamarense (Boni et al., 1983;
- Honsell et al., 1992; Abadie et al., 1999), a species that should not produce saxitoxins

287 (John et al., 2014), were later reinterpreted and attributed to A. minutum (Pistocchi et al., 2012) or A. pacificum (Lilly et al., 2002). However, one strain of A. tamarense 288 from Sardinian coasts has recently been found to be toxic (Lugliè et al., 2017). Since 289 290 the first observations of massive natural fish mortalities in Egypt (Zaghloul and 291 Halim, 1992), A. minutum produced toxic blooms with consequent ban of both fishing 292 and shellfish harvesting in Morocco (Labib and Halim, 1995), Spain (Delgado et al., 293 1990; Forteza et al., 1998), France (Belin et al., 2020) and Italy (Honsell et al., 1996). After 2000, only a few cases of shellfish farm closures attributed to A. minutum have 294 295 been reported in northern Sardinia (Italy; Lugliè et al., 2011), Catalonia (Spain; Vila et al., 2005; Bravo et al., 2008; Sampedro, 2018) and southern France coasts (Belin et 296 297 al., 2020). Because of a very similar non-toxic species discovered in the MS, A. 298 tamutum, the identification of A. minutum can be problematic and should be 299 confirmed by molecular or toxin analyses. Alexandrium pacificum was responsible for toxic blooms along the Catalan coast (Bravo et al., 2008), in the Thau Lagoon 300 301 (Abadie et al., 1999), in Sardinia (Lugliè et al., 2011) and Sicily (Dell'Aversano et al., 302 2019), at times causing shellfish harvesting closures (Vila et al., 2001; Bravo et al., 2008). 303 Alexandrium and ersonii and A. ostenfeldii are much less frequently recorded and 304 305 possibly overlooked or misidentified in plankton studies. At times their presence has 306 been traced as resting stages (e.g., Montresor et al., 1998; Bravo et al., 2006; Satta et

al., 2013). Two other *Alexandrium* species recorded in the MS, *A. balechii* and *A.*

308 *pseudogonyaulax*, do not produce PSP toxins but are considered potentially

309 ichthyotoxic.

310 Gymnodinium catenatum was first reported in southern Spain in 1987 (Bravo et al.,

1989). The worst, and apparently unique, fatal case of human intoxication in the

312 whole Mediterranean was due to a bloom of this species that caused 4 deaths and the

hospitalization of 23 people in Morocco in 1994 (Tagmouti-Talha et al., 1996).

314 Shellfish harvesting ban due to high concentrations of *G. catenatum* have however

been frequent in Andalusia (Spain) during the last 3 decades (HAEDAT). Records of

this species in the central and eastern MS should be considered with caution because

317 of possible misidentification of *G. impudicum* (Gómez, 2003).

318

319 2.2.3 Amnesic Shellfish Poisoning (ASP)

320 Sixteen of the 26 *Pseudo-nitzschia* species known to produce domoic acid (DA) have

been found so far in the MS. Species-level identification is problematic in light

322 microscopy and often requires the use of electron microscopy and/or molecular

323 markers. It follows that in most publications only the genus is reported, or taxa are

324 clustered into two 'groups', only distinguishing the thin (*P. delicatissima*-group) and

325 the thicker morphotypes (*P. seriata*-group). In the last decades, potentially toxic

326 *Pseudo-nitzschia* species have been identified properly from several locations of the

327 MS (Fig. 3C) where the presence of the cold-water species *Pseudo-nitzschia seriata*,

328 often reported in old studies, has never been confirmed.

329 Seasonal blooms of *Pseudo-nitzschia* spp., at times including toxic ones, occur all

along Mediterranean coasts (Fig. S1), with abundances up to several million cells L^{-1}

331 (e.g., Caroppo et al., 2005; Cerino et al., 2005; Quiroga et al., 2006; Quijano-Scheggia

332 et al., 2008; Ljubešić et al., 2011; Marić et al., 2011; Cabrini et al., 2012; Ruggiero et

al., 2015; Taş and Lundholm, 2017; Totti et al., 2019a). Nevertheless, the detection of

334 DA has caused the closure of aquaculture plants only in a limited number of cases

335 (4% of toxicity events in HAEDAT) in southern Spain (HAEDAT) and France (Amzil

et al., 2001), whereas DA values below the regulatory limit have occasionally been

found in shellfish from the Adriatic Sea (Ciminiello et al., 2005; Ujević et al., 2010;

Arapov et al., 2016), Greece (Kaniou-Grigoriadou et al., 2005), and in 65% of 180

mussel samples from mid-Tyrrhenian waters (Rossi et al., 2016). In a few cases, the

340 presence of DA in bivalves was related to a specific taxon, i.e., *P. calliantha* along the

341 Croatian coast (Marić et al., 2011) and in the Gulf of Trieste (Honsell et al., 2008) and

342 *P. brasiliana* in the Bizerte Lagoon in Tunisia (Sahraoui et al., 2011).

343 Nitzschia bizertensis, described from the Bizerte Lagoon (Tunisia), is one of the two

344 *Nitzschia* species known to produce domoic acid. At least in one case, the presence of

this species was related to the detection of domoic acid in mussels (Bouchouicha-

346 Smida et al., 2014). Less clear is the toxicity and the distribution of the other benthic

- 347 species *Halamphora coffeaeformis*.
- 348

2.2.4. Ostreopsis and species responsible of Ciguatera Fish Poisoning (CFP) 349 The benthic dinoflagellate Ostreopsis cf. ovata produces ovatoxins, which are 350 351 palytoxins-like molecules that can intoxicate humans by inhalation or ingestion of contaminated seafood. The species was first detected in the MS in the plankton of 352 353 Villefranche-sur-Mer (France) after a strong mistral wind event in 1972 (Max Taylor, pers. comm.), when it was identified with the name of the only species known at that 354 355 time, O. siamensis. The presence of the species was then documented from the coasts 356 of Lebanon in 1980 (Abboud-Abi Saab, 1989) and central Italy in 1986 (Zingone in Tognetto et al., 1995). Around the 2000s, monitoring programs implemented 357 following a series of harmful events (see below) made it evident that Ostreopsis 358 359 species were growing all along the rocky shores of the northern MS (Fig. 4A) in summer/autumn, thriving as epiphyte on macroalgae or epibionthic on a number of 360 benthic substrata, with concentrations up to 10^6 cells g^{-1} fresh weight of macroalgal 361

362 thalli (Mangialajo et al., 2011). At lower concentrations Ostreopsis spp. were also found along the northern African coasts (Illoul et al., 2012; Ben Gharbia et al., 2019). 363 Of the three species so far identified in the MS, the most common and widespread is 364 365 O. cf. ovata, whereas O. cf. siamensis and O. fattorussoi have a much more restricted distribution (Fig. 1E). An interesting aspect of the annual dynamics of Ostreopsis 366 species is the rather repetitive patterns of summer and/or autumn peaks, with timing 367 368 that vary from place to place and is scarcely related to temperature or to other obvious environmental parameters (Zingone, 2010; Accoroni and Totti, 2016). 369 370 First problems caused by Ostreopsis in the MS were fish and invertebrate kills in 1998 along the coasts of Tuscany (northern Tyrrhenian Sea) (Sansoni et al., 2003; 371 372 Simoni et al., 2003). Some years later (2002) more than 200 people coming from the 373 beach of the city of Genoa (Ligurian Sea) were hospitalized with fever, red eyes and 374 wheeze (Ciminiello et al., 2006). The only known problems caused by benthic microalgae at that time were those related to ciguatera fish poisoning (CFP) in 375 376 subtropical areas, whereas cases of toxic aerosol were only known for planktonic 377 Karenia brevis blooms in the Gulf of Mexico. In those years, similar human health problems and dermatitis cases were reported from the Catalonia and Balearic Islands 378 (Vila et al., 2008), French (Cohu et al., 2013) and Algerian coasts (Illoul et al., 2012), 379 380 and are still reported nowadays at several MS places (e.g., Croatian coast, Ninčević 381 Gladan et al., 2019). Both the presence of toxins in the aerosol (Ciminiello et al., 2014) and toxicological data on the effects of inhalation exposure in mice (Poli et al., 382 2018) support a link between Ostreopsis toxins and the respiratory symptoms reported 383 384 during blooms. However, those health problems do not occur during all phases of a bloom (Vila et al., 2016) and are quite sporadic compared to the widespread and often 385 386 massive presence of the suspected causative species.

387	The presence of Ostreopsis toxins in marine animals used as food and their impacts
388	on the animal health are relevant for their sanitary implications, which are still
389	controversial (Tubaro et al., 2011). Apparently healthy organisms (e.g., mussels and
390	sea urchins) during Ostreopsis blooms can accumulate fairly large amount of toxins
391	(Aligizaki et al., 2008; E. Fattorusso & V. Soprano, pers. comm.), but macroscopic
392	damages have been reported for various benthic organisms in the MS (Sansoni et al.,
393	2003, Simoni et al., 2003; Accoroni and Totti, 2016) and elsewhere (Shears and Ross,
394	2009). In mussels, Ostreopsis can induce important and not completely reversible
395	ultrastructural damages (Carella et al., 2015) and immunological, histological and
396	oxidative responses (Gorbi et al., 2013) while in sea urchins Ostreopsis blooms affect
397	reproduction and offspring health (Migliaccio et al., 2016).
398	Four species of the dinoflagellate genus Gambierdiscus, which can produce CFP
399	toxins, have recently been found in the MS. Gambierdiscus australes, G. cf.
400	belizeanus, G. carolinianus, G. silvae and some unidentified Gambierdiscus spp.,
401	have been reported from the Balearic Islands (Tudó et al., 2018), Greece and Cyprus
402	(Aligizaki and Nikolaidis, 2008; Holland et al., 2013; Aligizaki et al., 2018; Tudó et
403	al., 2018), with the highest diversity in Crete. Fukuyoa paulensis also has been found
404	in the Balearic Islands (Laza-Martínez et al., 2016) and Cyprus (Tudó et al., 2018).
405	Yet CFP cases are not known in the MS countries with the exception of a suspected
406	case of ciguatoxins in rabbitfish (Siganus rivolutus) reported from Israeli coasts
407	(Bentur and Spanier, 2007).

408

409 2.2.5. Azaspiracid Shellfish Poisoning (AZP)

410 The toxins azaspiracids (AZAs), produced by a number of dinoflagellate species of

411 the genera *Azadinium* and *Amphidoma*, and the human syndrome they can cause,

412 AZP, have been discovered at the beginning of this century (James et al., 2002).

413 Subsequently AZAs have been reported in shellfish from numerous sites, including

the MS (Bacchiocchi et al., 2015). A new species described from the MS, A.

415 *dexteroporum* (Percopo et al., 2013, Fig. 1 B), produces a whole suite of AZAs that

416 can cause direct harm to molluscs (Rossi et al., 2017; Giuliani et al., 2019). Another

417 toxic Azadinium, A. poporum, has been found in Greek waters (Luo et al., 2018) but

418 no impacts related to AZAs have been reported so far.

419

420 2.2.6 *Ichthyotoxicity*

421 About half of the potentially toxic MS species produce a variety of toxins that differ 422 from those related to the syndromes mentioned in the previous sections. Of these, the 423 majority (28 species, Table 1) produce substances that have been associated with fish 424 and/or shellfish kills. With a few exceptions, species in this list are unarmoured dinoflagellates, e.g., Karenia and Karlodinium, and other flagellates belonging to the 425 426 prymnesiophytes, raphidophytes and dictyochophytes, which are all hardly 427 identifiable in fixed material under the light microscope, and hence are overlooked in 428 most monitoring and ecological investigations. The large majority of the information on the presence of these ichthyotoxic species (Fig. 4B) comes from fish mortality 429 430 events, mainly located near fish-farming plants, in which the identification of the 431 culprit became necessary. The few fish mortality events in the MS known before 1975 were related to HB-432 433 HABs of non-ichthyotoxic species causing anoxia in bottom waters (see section 2.3.1) 434 rather than to ichthyotoxic species (Jacques and Sournia, 1978-79). In the subsequent years, fish kills by ichthyotoxic species were reported sporadically from Catalan 435

436 coasts, Spain (Garcés et al., 1999), caused by *Karlodinium* spp., and Sardinia (Italy),

- 437 caused by *Chattonella subsalsa* (Stacca et al., 2016). Occasional fish mortality events
- 438 were related to *Prymnesium* spp., in the Ebro Delta (Spain, Comín and Ferrer, 1978)
- and in a Tuscany lagoon (Italy, Mattioli and Simoni, 1999), Karenia selliformis in the
- 440 Gulf of Gabes (Tunisia, Romdhane et al., 1998; Feki et al., 2013) and Karenia brevis
- 441 and Pseudochattonella cf. verruculosa in Greece (Ignatiades and Gotsis-Skretas,
- 442 2010). In other cases, fish kills occurred during blooms of species toxic to humans,
- like in Egypt in 1987 (Zaghloul and Halim, 1992; Labid and Halim, 1995) where
- 444 Alexandrium minutum was the culprit. No fish or shellfish kill accidents in the MS
- have ever been associated with blooms of two potentially ichthyotoxic Alexandrium
- 446 species, A. balechii and A. pseudogonyaulax.
- 447 Benthic cyanobacteria are poorly investigated in Mediterranean waters, but blooms of

448 filamentous cyanobacteria have been the cause of massive fish mortalities in

449 Alexandria waters (Egypt) during spring 2005 (Ismael, 2012).

450

451 *2.2.7 Other toxins*

452 The dinoflagellates Gonyaulax spinifera, Lingulodinium polyedra and Protoceratium

- 453 *reticulatum*, which are quite widespread in the MS (Fig. 4 B), produce yessotoxins
- 454 (YTX). These substances were initially associated to DSP because their presence
- 455 gives similar positive results in mouse bioassay, but they are not considered toxic to
- 456 humans (Tubaro et al., 2010). However, YTXs caused economic impacts in 2002,
- 457 2004 and 2007, when mussel harvesting was halted for a long time (average closure
- 458 153 days) in the north-western Adriatic Sea (Poletti et al., 2008).
- 459 Vulcanodinium rugosum produces pinnatoxins (Rhodes et al., 2010; Nézan and
- 460 Chomérat, 2011) a neurotoxin that has lethal effects on sea urchin larvae, oysters and

461 *Artemia*. Currently there are no problems related to this species, while toxic effects on462 humans are not known.

463

464 **2.3. Non-toxic events**

Independent from toxin production, all microalgae may exert a negative impact when 465 they reach a high biomass producing seawater discolouration, mucilages or anoxia in 466 467 bottom waters (Zingone and Enevoldsen, 2000). Although several microalgal species are frequently associated with these HB-HABs, as detailed in the next sections, the 468 469 number of species that may cause harm with no specific toxin production is in theory 470 unlimited, and can vary from place to place. For this reason it is not possible to define 471 a global or regional list of non-toxic harmful microalgae. In addition to HB-HAB-472 formers, some non-toxic species, mainly diatoms, may cause mechanical harm to 473 invertebrates' gills (Bell, 1961), but no information on such events is available for the MS. In case of fish or invertebrate kills, at time it is hard to discern whether the cause 474 475 has been anoxia, toxic substances or mechanical damages. In many cases, species known to produce toxins may produce non-toxic HB-HABs, which have no impact on 476 477 human or marine fauna health but important consequences for tourism. For all these reasons, the boundaries between events described in the previous and next sections 478 479 cannot always be well defined.

480

481 2.3.1 Discolorations

482 In the MS, discolouration or anoxia have frequently been caused by unarmoured

dinoflagellates either toxic (e.g., *Margalefidinium polykrikoides*) or non-toxic (e.g.,

484 *Noctiluca scintillans*), but also by numerous armored dinoflagellates, diatoms,

485 prasinophytes, prymnesiophytes and raphidophytes (Table S1). Change of seawater

color caused by HB-HABs (Fig. 5) have been noticed since the first half of the XX
century in both lagoons and coastal sites, where they were given several names (*purga de mar, punti verdi*) before the one of *red tides* gained popularity. The oldest records
include discolorations caused by *Chattonella subsalsa* in 1956 in the Algiers harbor
(Hollande and Enjumet, 1957), *Alexandrium minutum* in 1957 in the Alexandria
harbor (Halim, 1960) and *Prorocentrum cordatum* in the Gulf of Naples in September
1962 (Yamazi, 1964).

Different dinoflagellates (e.g., Alexandrium spp., Noctiluca scintillans, Karlodinium 493 494 spp.), raphidophytes (Chattonella subsalsa and Fibrocapsa japonica, Fig. 1D) and chlorophytes (Tetraselmis wettsteinii and Pyramimonas spp.) occasionally produced 495 discoloration (Table S1, Fig. 5), which in some cases were also associated with fish 496 497 kills and/or massive death of marine invertebrates caused by anoxic conditions (e.g., 498 Arzul, 1994; Halim and Labib, 1996; Garcés et al., 1999). A couple of such cases of fish mortality events attributed to anoxia were already reported in the review by 499 Jacques and Sournia (1978-79): in Ismir Bay (Nümann, 1955, in Jacques and Sournia, 500 1978-79) and in the Adriatic Sea (Piccinetti and Manfrin, 1969; Froglia, 1970), during 501 blooms of Gymnodinium sp. and Protoperidinium depressum, respectively. 502 Discolorations were particularly frequent in the northern Adriatic Sea in summer in 503 the 1970-'80s, when dinoflagellate blooms (e.g., Lingulodinium polyedra, 504 505 Alexandrium mediterraneum and Lepidodinium chlorophorum) turned the sea into various colours (Boni, 1983, Table S1), at times extending offshore as in the case of 506 507 N. scintillans in 1980 (Fonda Umani et al., 2004) and L. chlorophorum in 1984 (Artegiani et al., 1985). Some summer blooms were caused by diatoms (e.g., 508 Skeletonema marinoi and Chaetoceros spp.), particularly after intense freshwater 509

510 inputs (Boni, 1983; Regione Emilia Romagna, 1982-2018). Over the last decades

511 blooms of *F. japonica* (Fig 1D) became common in late summer (Cucchiari et al.,

512 2008) in shallow coastal waters where they lasted up to 20–40 days. Along the eastern

513 Adriatic coast, 'red tides' were limited to eutrophicated semi-enclosed bays

514 (Marasović et al., 1991) or to unusual phenomena such as bloom of the silicoflagellate

515 Octactis (formerly Distephanus) speculum in summer 1983 in bottom waters in the

516 Gulf of Trieste, causing anoxia (Fanuko, 1989). An increasing number of

517 discolorations have been observed over two decades in the Golden Horn Estuary of

the Sea of Marmara (Taş et al., 2016). An unusual bloom of the coccolithophore

519 Holococcolithophora sphaeroidea (as Calyptrosphaera sphaeroidea) caused a white-

520 green-turquoise discoloration in a vast area off the Tarragona harbor (Spain, Cros et

al., 2002). The most recent event has been a long-lasting bloom of *Margalefidinium*

522 cf. *polykrikoides* that produced a yellow brownish discoloration in a touristic area of

the Ionian Sea (Italy) in July-August 2018, recurring in the same place in summer

524 2019 (Roselli et al., 2020).

525 In summer, discolorations can be a serious problem along Mediterranean beaches

where they have an impact on tourism and recreational use of the sea. This is the case

527 of the recurrent *Alexandrium taylorii* blooms along the Sicilian and Sardinian coasts

528 (Italy) and in the Balearic Islands (Spain) (e.g., Basterretxea et al., 2005; Giacobbe et

signal, 2007; Satta et al., 2010; Sampedro, 2018).

530

531 *2.3.2 Mucilages*

532 In the MS, a number of cases of mucilaginous aggregate formation related to

533 microalgal growth have been described, the most conspicuous of which occurred in

the northern Adriatic Sea in the 1990s. Mucilaginous macroaggregates represent the

535 last stage of aggregation of organic matter, mainly refractory polysaccharides derived

536 from phytoplankton exudates (Myklestad, 1995) and/or from bacterial capsular material (Stoderegger and Herndl, 1998) whose hydrolysis cannot be sustained by 537 phosphorous-limited bacteria (Danovaro et al., 2005). Whereas marine snow 538 539 (aggregates of 0.5-1 cm diameter) is common in all the oceans (Simon et al., 2002), 540 the mucilage event in the northern Adriatic Sea was unique in that those aggregates covered hundred square kilometres of both coastal and offshore areas. The formation 541 542 of larger aggregates was favored by the strong stratification of the water column and reduced circulation that retained freshwater in the northern Adriatic basin (Russo et 543 544 al., 2005). The direct responsible of the phenomenon were often thought to be the most abundant phytoplankton species in the aggregates, such as Cylindrotheca 545 closterium (Revelante and Gilmartin, 1991) and Gonyaulax fragilis (Pompei et al., 546 547 2003), both capable to produce large amounts of refractory polysaccharides (Pistocchi 548 et al., 2005; Urbani et al., 2005). In fact, phytoplankton communities associated with mucilage aggregates largely vary, depending on sampling area and period (Totti et al., 549 550 2005, and references therein), while the aggregates represent a self-sustained microcosm hosting a rich microorganism community (Simon et al., 2002). 551 552 Pelagic mucilages have been reported at several other Mediterranean sites, such as the Greek (Gotsis-Skretas, 1995; Nikolaidis et al., 2008) and Catalan coasts (Sampedro et 553 554 al., 2007) where Gonyaulax fragilis was thought to be involved in their production, 555 and the Sea of Marmara (Turkey) where Cylindrotheca closterium, Skeletonema costatum and Gonyaulax fragilis were indicated as the most abundant species 556 (Tüfekçi et al., 2010). In the Tyrrhenian Sea, extensive pelagic aggregates were 557 558 observed in 1991, 2000 and 2012 (Fig. 5 A, Calvo et al., 1991; Innamorati et al., 1993; Escalera et al., 2018). Foam accumulated massively along the Catalan coast in 559

March 2006 during a *Phaeocystis* sp. bloom, an event that was related to anomalous
hydrographic winter conditions (Arin et al., 2014).

562 Massive mucilage events have also concerned the benthic environment. Ostreopsis cf.

563 *ovata* during intense blooms forms a network-shaped mucilaginous biofilm that can

- harm benthic invertebrates (Schiaparelli et al., 2007). In the Tyrrhenian and Ligurian
- 565 Seas (western MS), benthic mucilages have occurred since 1991 (Sartoni and Sonni,

1991), and have been attributed to the massive growth of several macro- and

567 microalgae such as the filamentous brown alga Acinetospora crinita and the colonial

568 pelagophytes Nematochrysopsis marina and Chrysonephos lewisii (Giuliani et al.,

569 2005; Schiaparelli et al., 2007). The allochthonous pelagophyte *Chrysophaeum*

570 *taylorii*, recorded in the western MS since 2005, in recent years was involved in the

571 formation of dense layers of mucous covering macroalgae, gorgonians and the

572 surrounding rocks (Lugliè et al., 2008; Caronni et al., 2015).

573

574 **3. Trends in the Mediterranean HABs**

575 *3.1 General trends*

576 The MS has undergone profound changes over the last centuries. Human action has mainly been visible along the coasts of the basin, which have become increasingly 577 578 populated and deeply modified by coastal and riverine engineering and deforestation 579 which, along with cultural eutrophication, are all potential drivers of deep changes in phytoplankton communities (Garcés and Camp, 2012). Natural and/or man-induced 580 meteorological and climatic variations superimpose to these changes often with an 581 582 amplifying effect. The most striking characteristic of the MS HABs over the last 50 yrs, which approximately correspond to the time since when they have been studied 583 584 more intensively, is the remarkable increase of the toxic species list, from a few taxa

585 to the more than 80 of the present review (Fig. 2). Over the same period, the records of these species across the MS have also remarkably increased (Fig. 6). This trend is 586 parallel to that of the increased list of toxic species and of their records worldwide, 587 588 which is an obvious result of the intensification of the taxonomic and toxin studies on 589 marine microalgae (Zingone et al., 2017). The increase of the records of actual HAB events from the less than 30 cases listed by Jacques and Sournia (1978-1979) and 590 591 Honsell et al. (1995) to the several hundred cases of halted aquaculture operations, seawater discoloration and minor human health accidents presently recorded in 592 593 HAEDAT is also impressive (Fig. 7). Damages to aquaculture caused by ASP and PSP toxins in mussels have been limited over the last 30 years while DSP cases have 594 595 represented about 75% of the harmful events, with an increase between the decade 596 1987-1997 and the two following ones (Fig. 7). This trend should however be 597 interpreted with caution because it has been paralleled by a remarkable growth of the coastal MS population (section 1), much more intensive use of marine resources, and 598 599 consequent raise of the level of attention to the integrity and safety of marine 600 resources. 601 In fact, toxic blooms as well as mucilage events and discolorations in the MS have generally shown an unpredictable interannual periodicity, like in the case of the 602 603 conspicuous blooms of Noctiluca scintillans in the Adriatic Sea (Fonda Umani et al., 604 2004), Moroccan (Tahri Joutei et al., 2003), Catalan (Lopez and Arte, 1971) and French coasts (M.-O. Soyer in Jacques and Sournia, 1978-1979). There are cases of 605 decreases, e.g., the blooms of Alexandrium pacificum occurring on the Catalan coast 606 607 from 1996 to 1998 (Vila et al., 2001) but rarely recorded afterwards (Sampedro, 2018). Blooms of A. minutum were recurrent in Egyptian waters but not recorded any 608 609 longer after 1994 (Ismael and Halim, 2001), while their frequency doubled from 2000

611 rubrum hosting cryptophyte chloroplasts were not recorded in the MS (Jacques and Sournia, 1978-1979) until their occurrence in both the Adriatic (Sorokin and 612 613 Ravagnan, 1999) and Tyrrhenian Seas (Siano et al., 2006), and afterwards have only been observed in 2017 in the North Aegean Sea (Genitsaris et al., 2019). 614 615 In the case of Ostreopsis cf. ovata, rather than an increase the phenomenon in the MS 616 has shown a sudden upsurge around the 2000, followed by an expansion of the known 617 range for the species in the next years and a relative stability in the following decade. 618 Indeed Ostreopsis cf. ovata provides the most evident case of range expansion and increased impact over time in the MS. Although benthic microalgae have received 619 scarce attention until the late 20th century, it is unlikely that the species might have 620 621 been abundant but undetected before. The apparent sudden range expansion and 622 impact of Ostreopsis cf. ovata is in line with an increasing trend of species of the same genus in New Zealand and some other temperate areas around the world 623 624 (Parsons et al., 2012). On the other hand, no clear increase of the impact or of species abundance has been reported since the 2000 outburst, while the above-mentioned 625 626 range expansion has coincided with a dramatic increase in monitoring programs and research projects focused on benthic microalgae. Initially, the sudden relevance of the 627 628 phenomenon was associated with an increase of temperature in the MS, based on the 629 belief that all Ostreopsis species were of tropical origin. In fact, Ostreopsis cf. ovata 630 and its close relatives are widely distributed in temperate areas, also matching the apparent preference of the species for moderately high rather than very high 631 632 temperature (Mangialajo et al., 2011; Scalco et al., 2012). Overall, the trend observed for this species in the MS, with an outburst followed by a stabilizing trend, recalls that 633 634 of an invasive species rather than that of a species favored by a temperature increase.

to 2012 along the Catalan coast (Sampedro, 2018). Blooms of the ciliate Mesodinium

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636	3.2 HAB trends in the Adriatic Sea, a case study
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The Adriatic Sea (AS) represents a unique system for its semi-enclosed morphology, shallow depth and oligotrophic nature in most parts but with eutrophic characteristics along the north-western coasts driven by inputs from the Po River and other rivers (Mozetič et al., 2010; Cozzi and Giani, 2011). The AS is considered one of the

hotspots of MS HABs (Garcés and Camp, 2012), in terms of both occurrence and

642 impacts. However, compared to the great variety of potentially toxic species (Mozetič

643 et al., 2019), toxicity cases are limited, and the most common toxins found above the

regulatory limits in the Adriatic shellfish to date are DSP toxins (okadaic acid group)

and other lipophilic toxins (yessotoxins and pectenotoxins).

646 Because of the early development of marine-related activities, there is a wealth of

647 information from the area dating back to the last century, which allows some insights

on possible HAB trends. Phytoplankton in certain areas of the AS (e.g., Gulf of

649 Trieste, Gulf of Venice, Senigallia-Susak transect, Kaštela Bay) have been

extensively studied for decades (Ninčević-Gladan et al., 2010; Bernardi Aubry et al.,

651 2012; Marić et al., 2012; Mozetič et al., 2012; Cerino et al., 2019; Totti et al., 2019a),

highlighting a number of changes, such as trends or regime shifts in main

653 phytoplankton groups (Mozetič et al., 2010; Totti et al., 2019a) and in bloom forming

654 species (Cabrini et al., 2012). However, no trends specifically related to toxic species

655 is evident from these long-term studies, neither in terms of increased frequency nor of

- abundance. In fact, most studies on HAB species are snapshots of isolated toxic
- episodes (Pistocchi et al., 2012, and references therein). Similar conclusions can be

drawn also from toxicity events: aquaculture operations have been halted frequently

over the last 20 years (section 2.2.1), but without any significant trend for DSP events.

660 Nevertheless, some changes in phytoplankton community structure of the AS have involved a number of HAB species, such as Pseudo-nitzschia multistriata, an 661 allochthonous species (section 2.1) that became a regular component of the autumn 662 663 phytoplankton communities of the NW AS (Totti et al., 2019a). In the Gulf of Trieste, previously rare *Dinophysis tripos* have become a regular member of the autumn 664 phytoplankton assemblages since 2010, along with higher temperatures recorded in 665 666 this decade (Francé et al., 2018), whereas further south D. sacculus has replaced D. 667 *caudata* as one of the indicator species of spring phytoplankton communities (Totti et 668 al., 2019a). 669 HB-HABs caused by dinoflagellates, occurring in summer and often associated with 670 water discoloration and bottom anoxia, were a major problem in the AS until the end 671 of the 1980s (see section 2.3.1). At the time, because of the heavy impact on the local 672 economy, the Italian government adopted countermeasures to reduce P content in 673 detergents and improve the urban wastewater treatment plants, leading to a strong 674 reduction of P load in coastal waters. Since the end of the 1980s, summer dinoflagellate blooms became a rarer phenomenon, their decline coinciding with the 675 676 years of large mucilaginous macroaggregate appearance. Mucilages in the AS (see section 2.3.2) were known since the beginning of 1700, 677 678 when they were named 'mare sporco'. In more recent years, massive episodes have

occurred in the years 1988 to 1991 and 1997 to 2004, typically in summer (Giani et

al., 2005), while a spatial and temporal reduction occurred in subsequent years. An

anomalous occurrence in autumn-winter was reported in 2006-2007, probably in

relation to a water temperature increase (Danovaro et al., 2009). The mucilage

appearance, and the concurrent disappearance of summer water discolorations have

both been associated with the decrease of inorganic and organic P (Degobbis et al.,

2005), but also to hydrographic changes related to large-scale climatic changes around
the end of the '80s, which could have driven a regime-shift affecting not only the AS
but also other European Seas (Conversi et al., 2010).

In the last decade (2008-2018), HB-HABs of both diatoms and dinoflagellates

occurred without a regular temporal pattern, reflecting the meteorological events that

690 nowadays tend to be more intense and unhampered by a regular seasonal rhythm

691 (Totti et al., 2019a, b). Blooms of *Fibrocapsa japonica* that were common at the end

of the 1990s seem to be rarer since 2012 (Regione Emilia-Romagna, 1982-2018), and

693 mucilage events occurred shortly in 2014 and in 2018 (Regione Emilia-Romagna,

694 1982-2018).

As a whole, HABs in the AS show unpredictable time variability that is partly related

to the irregularity and intensity of meteorological events in the last decades.

697 Prolonged periods of drought (Cozzi et al., 2019) with oligotrophic conditions

698 (Mozetič et al., 2010) alternate with nutrient pulses from continental water runoff that

can drive the occurrence of anomalous intense blooms at any time of the year (Totti etal., 2019a).

701

702 **4. Conclusions**

A deep knowledge on the spatial and temporal distribution of harmful species and the blooms that they produce is an indispensable goal towards a safe use of marine resources and an informed management and planning of the coastal zone. In the MS this goal is even more crucial considering the importance of the economy deriving from the use of the sea for tourism and recreational use, fishery and aquaculture. The information about HABs has grown remarkably over the last 50 years since the first review (Jacques and Sournia, 1978-1979) all over the MS areas. However, the marked

710 west-east and north-south gradients in the knowledge of HABs and HAB species 711 distribution persist, with long traits of coast with scarce or no information available. Overall, the MS hosts a high number of potentially toxic species, many of which have 712 713 a wide distribution across its coastal waters. Yet the cases of intoxication are 714 extremely rare, while the impact on aquaculture appears to be limited to a few hot 715 spots in the northern Adriatic, Spain and France coasts. A variety of toxins have 716 actually been detected in several instances in microalgae strains from the MS, while seafood toxicity, when detected, has commonly remained below the safety limits. The 717 718 typical oligotrophic offshore Mediterranean waters that influence most coastal areas 719 and the enhanced alongshore circulation in many places may play a role in keeping 720 toxic algae at levels rarely exceeding critical density thresholds, thus preventing their 721 excessive accumulation in seafood. On the other hand, quite effective monitoring 722 operations have accompanied the development of aquaculture over the last decades, 723 thus reducing the possibility of accidents to a minimum level. 724 In terms of microalgal toxins, the only major concern seems to reside in the large 725 amount of palytoxin-like substances that every summer accumulate along the rocky 726 Mediterranean shores because of Ostreopsis blooms. Although sea urchins and wild mussels inhabiting those environments at time accumulate those toxins to 727 728 considerable levels, no cases of seafood intoxication have occurred so far. 729 Contaminated herbivorous fishes represent a problem in areas where they ingest 730 macroalgal substrates colonized by toxic microalgae, i.e. in the ciguatera areas, but 731 species capable of this transfer link may be missing in the MS trophic webs, or toxins 732 are neutralized in the transfer. Nonetheless, the guard level must be kept high because sudden changes might occur, e.g., due to penetration of benthic herbivorous fish in the 733 734 MS and consequent novelties in the local food webs.

735 Overall, the present overview demonstrates a relatively low risk deriving from toxic 736 blooms and a higher risk from high biomass blooms affecting the aesthetic qualities of coastal areas devoted to tourism in the MS. No clear trends in occurrence nor 737 738 expansions emerge for either toxic or HB-HABs. While EU regulation and national initiatives have promoted actions addressing seawater quality and aiming at a good 739 740 environmental status (GES), human densities along the coasts is predicted to keep on 741 increasing in the next decades. Therefore, a larger use of marine resources in the 742 future, in the MS like in other coastal areas of the world, will probably lead to an 743 increased impact of the risks posed by HABs even in absence of any trends in their abundance and frequency (Zingone and Wyatt, 2005). In addition, predicted changes 744 745 in climate and consequent modifications in hydrographical features may drive local 746 variations in microbial populations both in the plankton and in the benthos. Continued monitoring and further studies on HAB patterns and trends are therefore mandatory 747 goals to be able to predict their evolution and protect human health and wellbeing in 748 749 the MS.

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- 1487 **Figure captions**
- 1488 Figure 1: Examples of toxic species from the Mediterranean Sea. A) Alexandrium
- 1489 minutum stained with calcofluor. B) Azadinium dexteroporum. C) Dinophysis
- 1490 sacculus. D) Fibrocapsa japonica. E) Ostreopsis fattorussoi stained with Calcofluor
- 1491 (courtesy of S. Accoroni). F) Prorocentrum lima. G) Pseudo-nitzschia multistriata.
- 1492 Scale bars in A and B: 5 μ m; in C, D, E, F and G: 20 μ m.
- Figure 2: Cumulative numbers of known toxic species in the Mediterranean Sea indifferent years.
- 1495 **Figure 3:** Geographic range of potentially toxic species in the Mediterranean Sea.
- 1496 Distribution of species known to produce toxins related to: A) Diarrhetic Shellfish
- 1497 Poisoning (DSP), *Dinophysis* spp. and the benthic species *Prorocentrum lima* and P.

1498 rhathymum. B) Paralytic Shellfish Poisoning (PSP), Alexandrium spp. and

1499 Gymnodinium catenatum. C) Amnesic Shellfish Poisoning (ASP), Pseudo-nitzschia

1500 spp. and Nitzschia bizertensis. For the genera Dinophysis, Pseudo-nitzschia and

- 1501 *Alexandrium*, which include both toxic and non-toxic species, the maps represent only
- toxic species and, in case of cryptic or problematic species, only the records validated
- 1503 by electron microscopy, molecular methods and/or toxin production.
- **Figure 4:** Geographic range of potentially toxic species in the Mediterranean Sea. A)

1505 Ostreopsis spp. (mostly O. cf. ovata) and species related to the Ciguatera Fish

1506 Poisoning (CFP). B) Species producing ichthyotoxins (Alexandrium

1507 pseudogonyaulax, Karenia spp., Karlodinium spp., Chattonella spp., Vicicitus

- 1508 globosus, Prymnesium spp., etc.) and other toxins. The latter include mainly a few
- 1509 widespread dinoflagellate species that produce yessotoxins (Lingulodinium polyedra,

1510 Gonyaulax spinifera and Protoceratium reticulatum), but also other dinoflagellates

1511 producing azaspiracids (*Azadinum* spp.), pinnatoxins (*Vulcanodinium rugosum*) and

1512 other toxins with poorly known effects (e.g., Prorocentrum spp., Margalefidinium

1513 *polykrikoides*). See Table 1 for a complete list.

1514 **Figure 5:** A) Mat of *Oscillatoria acutissima* in the Eastern Harbour of Alexandria

1515 (Egypt). B) Bloom of Noctiluca scintillans in Thermaikos Gulf (Thessaloniki,

1516 Greece). C) Discoloration caused by Euglena viridis in the Golden Horn Estuary (Sea

- 1517 of Marmara, Turkey). D) Shellfish mortality in Ras El-Bar (Egypt) in 2011 due to the
- 1518 proliferation of *N. scintillans* and consequent oxygen depletion. E) Pelagic mucilages
- 1519 in the Gulf of Naples (Italy).

1520 Figure 6: Distribution of potentially toxic species, mucilages and discolorations in the

1521 Mediterranean Sea. A) Distribution of species known to be toxic and harmful events

- until 1995 as reported in Jacques and Sournia (1978-1979) and Honsell et al. (1995).
- 1523 B) Distribution of potentially toxic species (excluding *Ostreopsis* and CFP species)
- and harmful events updated to the present status of knowledge. The position of the
- 1525 circles in several cases has been slightly modified to reduce overlapping.
- **Figure 7**. Harmful events related to microalgae in the Mediterranean Sea (n=501)
- 1527 based on records in the Harmful Algae Event Database HAEDAT
- 1528 (<u>http://haedat.iode.org/).</u> High density phytoplankton blooms with no impacts were
- 1529 not considered. A) Relative abundance of different types of nuisance with details of
- 1530 seafood toxicity. B) Interannual variations of ASP, DSP and PSP toxicity events.


















Table 1: Potentially toxic species in the Mediterranean Sea and associated types of syndromes or impacts (see Moestrup et al., 2009 and Lassus et al., 2016 for details). ASP, Amnesic Shellfish Poisoning; AZP, Azaspiracid Shellfish Poisoning; DSP, Diarrhetic Shellfish Poisoning; PSP, Paralytic Shellfish Poisoning; CFP, Ciguatera Fish Poisoning. 'Other toxins' include unknown toxins or toxins with poorly known effects.

Bacillariophyceae	
Halamphora coffeaeformis	ASP
Nitzschia bizertensis	ASP
Pseudo-nitzschia australis	ASP
Pseudo-nitzschia brasiliana	ASP
Pseudo-nitzschia caciantha	ASP
Pseudo-nitzschia calliantha	ASP
Pseudo-nitzschia cuspidata	ASP
Pseudo-nitzschia delicatissima	ASP
Pseudo-nitzschia fraudulenta	ASP
Pseudo-nitzschia galaxiae	ASP
Pseudo-nitzschia hasleana	ASP
Pseudo-nitzschia multiseries	ASP
Pseudo-nitzschia multistriata	ASP
Pseudo-nitzschia pseudodelicatissima	ASP
Pseudo-nitzschia pungens ⁽¹⁾	ASP
Pseudo-nitzschia subfraudulenta	ASP
Pseudo-nitzschia subpacifica	ASP
Dictvochophyceae	
Pseudochattonella farcimen	Ichthyotoxicity
Pseudochattonella verruculosa	Ichthyotoxicity
Vicicitus globosus	Ichthvotoxicity
0	
Dinophyceae	
Alexandrium andersonii	PSP
Alexandrium balechii	Ichthyotoxicity
Alexandrium minutum	PSP
Alexandrium ostenfeldii	PSP
Alexandrium pacificum ⁽²⁾	PSP
Alexandrium pseudogonyaulax	Ichthyotoxicity
Alexandrium tamarense ⁽²⁾	PSP
Alexandrium taylorii	PSP
Amphidinium carterae	Ichthyotoxicity
Amphidinium klebsii	Ichthyotoxicity
Azadinium dexteroporum	AZP
Azadinium poporum	AZP
Dinophysis acuminata	DSP
Dinophysis acuta	DSP
Dinophysis caudata	DSP
Dinophysis fortii	DSP
Dinophysis infundibulum	DSP
Dinophysis ovum	DSP
Dinophysis sacculus	DSP
Dinophysis tripos	DSP

Entreno a nautonoia	CED		
Fukuyoa paulensis	CFP		
Gambieraiscus australes	CFP		
Gambieraiscus beitzeanus	CFP		
Gambierdiscus carolinianus	CFP		
Gambierdiscus silvae	CFP		
Gonyaulax spinifera	Other toxins		
Gymnodinium catenatum	PSP		
Karenia bicuneiformis	Ichthyotoxicity		
Karenia brevis	Ichthyotoxicity		
Karenia cristata	Ichthyotoxicity		
Karenia longicanalis	Ichthyotoxicity		
Karenia papilionacea	Ichthyotoxicity		
Karenia selliformis	Ichthyotoxicity		
Karlodinium armiger	Ichthyotoxicity		
Karlodinium corsicum	Ichthyotoxicity		
Karlodinium veneficum	Ichthyotoxicity		
Lingulodinium polvedra	Other toxins		
Margalefidinium polvkrikoides	Ichthvotoxicity		
Ostreopsis fattorussoi	Airborne disease		
Ostreopsis cf. ovata	Airborne disease		
Ostreopsis cf. siamensis	Airborne disease		
Pfiesteria niscicida	Ichthyotoxicity		
Phalacroma mitra	DSP		
Phalacroma rotundatum	DSP		
Polykrikos hartmannii	Other toying		
Provocentrum borbonicum	Other toxins		
Provocentrum cordatum	Other toxing		
Provocentrum corduium	Other toxing		
Provocentrum emarginatum			
Prorocentrum tima	DSP Otherstering?		
Prorocentrum mexicanum	Other toxins?		
Prorocentrum rnatnymum	DSP Other terring		
Protoceratium reticulatum	Other toxins		
Vulcanodinium rugosum	Other toxins		
Charles a human dia a la a dha atani	Labthractoriaita		
Chrysochromulina leaabeateri	ICIUIIYOUOXICITY		
Phaeocystis globosa	Other toxins		
Frymnesium caiathiferum	Ichthyotoxicity		
Prymnesium faveolatum	Ichthyotoxicity		
Prymnesium parvum	Ichthyotoxicity		
Prymnesium polylepis	Ichthyotoxicity		
Danhidanhyaaaa			
Chattonella marina ⁽³⁾	Ichthyotovicity		
Chattonella subsalsa	Ichuryotoxicity		
Unanonena subsaisa	Ichthyotoxicity		
Fibroganga izraniaz	Ichthyotoxicity		
тыносарѕа јаропіса	rentityotoxicity		

⁽¹⁾ Including *P. pungens* var. *aveirensis* ⁽²⁾ *A. pacificum* (group IV) and *A. tamarense* (group III), following the ribotype group designation in John et al. (2014) and Litaker et al. (2018)

(3) Including Chattonella marina var. antiqua

Table 2: Potentially toxic species described from the Mediterranean Sea

Species name	Described in	Described as	Type locality
Alexandrium minutum Halim	Halim (1960)		Harbour of Alexandria, Egypt
Alexandrium pseudogonyaulax (Biecheler)	Biecheler (1952)	Goniodoma	Thau Lagoon, Gulf of Lion, France
Horiguchi ex K.Yuki & Y.Fukuyo		pseudogonyaulax	
Azadinium dexteroporum Percopo &	Percopo et al. (2013)		Gulf of Naples, Italy
Zingone			
Chattonella subsalsa Biecheler*	Biecheler (1936)		Saltern of Villeroy, Sète, France
Dinophysis caudata Kent	Kent (1881)		Nearby Fano, Marche Region, Italy
Dinophysis fortii Pavill.	Pavillard (1923)		Thau Lagoon and/or Sète harbour,
			France
Dinophysis infundibulum J.Schiller	Schiller (1928)		Southern Adriatic Sea
Dinophysis sacculus F.Stein	Stein (1883)		Kvarner Gulf, Croatia
Dinophysis tripos Gourret	Gourret (1883)		South of Ratonneau, Gulf of
			Marseille, France
Karlodinium armiger Bergholtz, Daugbjerg	Bergholtz et al. (2006)		Alfacs Bay, Catalonia, Spain
& Moestrup			
Karlodinium corsicum (Paulmier, Berland,	Paulmier et al. (1995)	Gymnodinium	Diana Lagoon, Corse, France
Billard & Nézan) Siano & Zingone		corsicum	
Nitzschia bizertensis Bouchouicha-Smida,	Bouchouicha-Smida et		Bizerte Lagoon, Tunisia
Lundholm, Hlaili & Mabrouk	al. (2014)		
Ostreopsis fattorussoi Accoroni, Romagnoli	Accoroni et al. (2016)		Batroun, Lebanon
& Totti			
Prorocentrum lima (Ehrenb.) F.Stein	Ehrenberg (1860)	Crytoptomonas lima	Sorrento, Gulf of Naples, Italy
Prymnesium faveolatum Fresnel	Fresnel et al. (2001)		Beach of Roquebrun, Cap Martin,
			France
Vulcanodinium rugosum Nézan &	Nézan and Chomérat		Ingril Lagoon, France
Chomérat**	(2011)		

*A second, distinct genotype also discovered in Mediterranean waters (Klöpper et al., 2013).

** First report in Rhodes et al. (2010) from New Zealand.