



Distribution of the *Tripos* species (dinoflagellata) from Annaba Bay (Southwestern Mediterranean Sea)

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Abstract

The genus *Tripos* has been described as a key component in the World Ocean and in Mediterranean phytoplankton. Despite its high species diversity, the *Tripos* genus has also been considered to be the most important taxa, contributing significantly to the dinoflagellates and phytoplankton abundance. Though information on the phytoplankton of Algerian marine waters is still lacking, and the few available data are very limited to some local and sparse works. Here we study the seasonal distribution and composition of *Tripos* species regarding the environmental framework and assess changes in the environmental conditions that may affect the *Tripos* populations. The phytoplankton was monthly sampled during February 2010-January 2011 at two contrasted sites of Annaba Bay, Algeria. The phytoplankton biomass (measured as chlorophyll *a*) and the hydrological parameters (temperature, salinity and macronutrients) were jointly measured. The innermost area (site 1) which is directly impacted by riverine, industrial and household wastes input had high levels of nutrients and strong phytoplankton biomasses. The outer area (site 2), located at the entrance of the bay which is directly influenced by the open sea, namely the Modified Atlantic Water (MAW) current, showed very low concentrations for both nutrients and phytoplankton standing stock. In the present work we report for the first time 47 *Tripos* species; almost all of them can be considered as tropical and subtropical species. These types of species seem to be adapted to the warm waters, showing long horns that help maintaining the body buoyancy. The majority of the identified *Tripos* species showed affinities to the open waters, at site 2, where numerous of them can develop minor spring bloom. Only few species were associated to the eutrophicated area of the innermost bay (site 1).

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Introduction

Within the dinoflagellates phytoplanktonic taxa, the genus *Triplos* is a key component in the World Ocean and Mediterranean coastal waters (Gómez, 2003; Alina *et al.*, 2009). Despite its high species diversity, the genus *Triplos* significantly contributes in enhancing the occurrence and abundance of marine dinoflagellates (Gómez and Gorski, 2003). This dinoflagellate is considered to be the most important taxa, contributing to 42% the dinoflagellates abundance (Alina *et al.*, 2009). The genus has recently been reviewed in the work of Fernández-Gómez *et al.* (2013). It was suggested to replace *Triplos* instead of *Ceratium* or *Neoceratium* (Gómez *et al.*, 2010). The genus *Triplos* comprises 80 species, widely distributed. The species belonging to *Triplos* have the advantage to be easily sampled and preserved, and, their determination is based on simplest morphological criteria, compared to other marine phytoplankton taxa. Additionally, *Triplos* species have large size spectra (100-1000 μm), and their body shape with cellulosic ornamented theca plaques, facilitates their isolation and preservation.

As *Triplos* populations are widely distributed, the zooplankton grazing and nutrient availability have been determined as the main factor controlling their dynamics rather than the temperature and salinity variability. Worldwide, numerous works have been performed on the geographical distribution of *Triplos* genus (Sournia, 1986; Smayda, 1980; Balech, 1988; Heaney, 1988; Sun, 2006; Hikma, 2012; Alina Tunin, 2013). For the southwestern Mediterranean side, few are known about *Triplos* of this area (Halim, 1982; Gailhard, 2003; Alina Tunin, 2007, 2009; Elghrib, 2012).

In the Algerian marine waters, the Genus *Triplos* is still little known, despite some punctual and local studies. The first information about the phytoplankton composition from Annaba Bay was provided in the studies of Frehi (1995) and Ounissi and Frehi (1999). The study of Ounissi and Frehi (1999) only reported 5 *Triplos* species (previously called *Ceratium* species), which have been described as characteristic to neritic and well renewed waters.

More recently, Frehi *et al.* (2007) studied the harmful dinoflagellates from Annaba Bay and Hadjaji *et al.* (2014) described the harmful species of *Alexandrium catenella/tamarensis*. However, no study has been dedicated to the *Triplos* genus distribution in the region, despite its numerical and ecological importance in coastal water systems. The aim of this work is to better understanding the distribution and composition of *Triplos* populations regarding the environmental framework and to assess possible changes in the environmental conditions that may affect the *Triplos* populations.

Materials and methods

Sampling sites

Annaba Bay is located between Cape Rosa (8° 15'E, 36° 68'N) and Cape de Garde (7°16'E, 36° 38'N), as shown in Fig. 1. On the land side, the bay receives riverine water discharge from Seybouse and Mafragh catchment's outlets and direct untreated industrial and domestic wastes. These inputs are heavily charged with nutrients and particulate materials (Ounissi and Frehi, 1999; Frehi *et al.*, 2007; Ounissi *et al.*, 2014). From the marine side, the bay is submitted to the open water intrusion through the Modified Atlantic Water current (MAW), which crosses eastward the north and central bay. The riverine plumes can extend several kilometers inside the bay by river flooding events and can then affect large part of the western sector (Ounissi *et al.*, 1998; Ounissi *et al.*, 2016). The MAW and its residual currents deployed however, all around the seasons and allow renewing a major part of the bay particularly during the mixing period, which extends from autumn to late spring.

However, by summertime, the southwestern sector of the bay is still suffering from confining conditions due to the establishment of sharp thermocline. Additionally, the westward winds affecting the area can reduce the external inflowing oceanic waters, and hence encourages the existence of so such confining state.

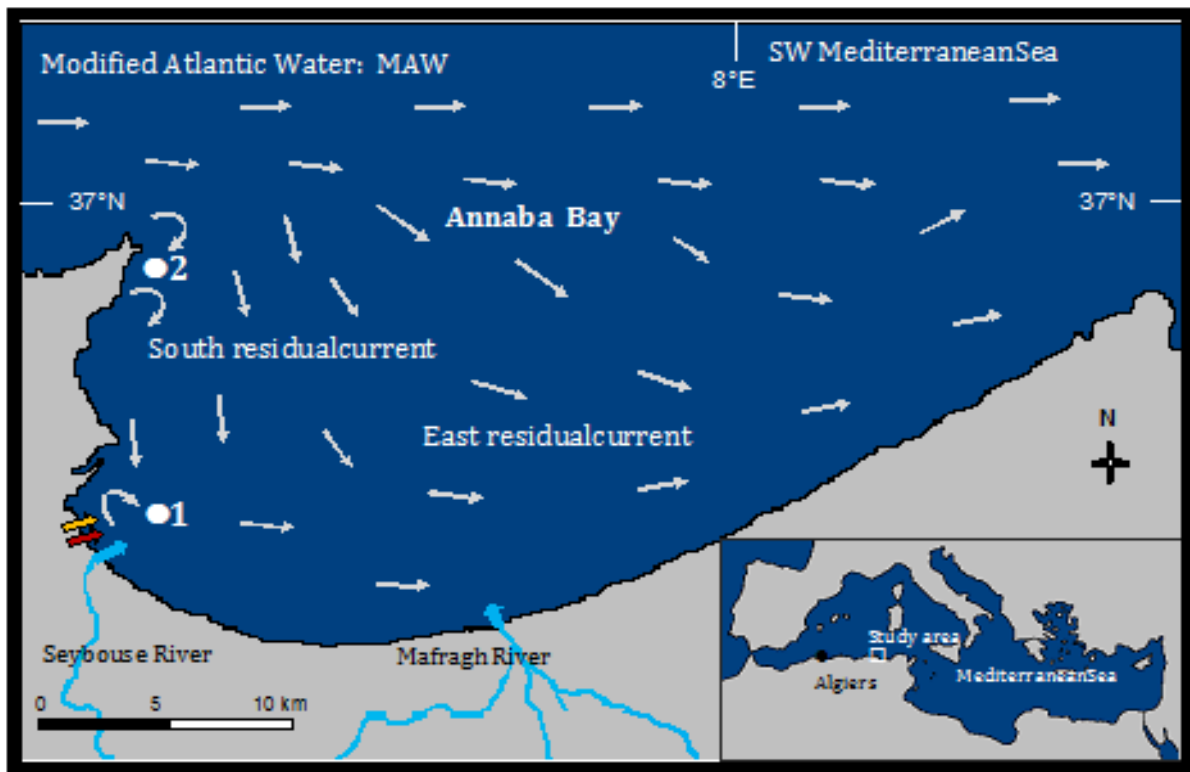


Fig. 1. Map showing the surface circulation affecting Annaba Bay (modified from LCHF, 1976; Ounissi *et al.*, 1998) and the location of the sampling sites 1 and 2. Riverine inputs (blue arrow), direct industrial waste input (red arrow) and direct household waste input (yellow arrow) are also represented.

The plankton communities undergo then these contrasted influences and evolve accordingly to their relative dominance. Considered like-hydrological strategic points, the two sampling sites have then been located according to these contrasted hydrological impacts (terrestrial and marine open water influences). Site 1 is located at the innermost bay, which is largely affected by terrestrial inputs, while site 2 is located at the entrance of the bay where the MAW current dominated the area (Fig. 1). This is a purposive sampling that can offer substantial amount of information relative to the sampling effort (Ounissi *et al.*, 2016; Scherrer 1984). The intentional placement of the sampling sites may be responsible for correlations between plankton populations and environmental conditions, but data issued from purposive sampling can reveal interesting findings if used in environmental diagnosis and to find trends along spatial gradient of the variables (Ounissi *et al.*, 2016; Scherrer, 1984).

Environmental framework

Both hydrological and biological parameters were monthly monitored at the two studied sites. Surface water temperature and salinity were measured using the multiparameter probe wtw Multi 197i. Water samples were determined for the macronutrients (ammonium: NH_4 ; nitrite: NO_2 and phosphate: PO_4) following the manual of Aminot and Chaussepied (1983). The phytoplankton biomass, measured as chlorophyll *a*, was determined according to the Lorenzen's (1967) method.

Phytoplankton sampling and determination

Phytoplankton samples were collected from the near bottom to the surface using a standard WP2 net (mesh size $63 \mu\text{m}$). The net was gently rinsed, and the samples were transferred into plastic bottles and fixed by the addition of borax-buffered formaldehyde to a final concentration of 4%. The samples for *Tripes* genus taxonomic composition were sub-sampled and three sub-samples were examined following the *Uthermhol's* method.

The genus *Triplos* has been identified to the species, infra-species, forms and varieties level. All of the other phytoplanktonic taxa were not considered.

The species of *Triplos* genus were determined based on numerous phytoplankton manuals and publications: Jörgensen (1911, 1920); Paulsen (1905); Schiller (1937); Taylor (1976); Sournia (1965, 1967, and 1986); Balech (1988); Tomas (1997); Nezan *et al.* (1997). The inverted microscope Wild M20 has been used for the observation and photographs of *Triplos* cells.

The nomenclature of *Triplos* species used here is that of Gómez (2013). More details on the *Triplos* species checklist and nomenclature of this new genus can be found in the works of Gómez *et al.* 2010, Gómez (2010; 2012 and 2013).

Results

Environmental framework

Table 1 provides the monthly variations of the various hydrological parameters and phytoplankton biomasses recorded at the two studied sites during an annual cycle. The two sites experienced large thermal range resulting in the feeble resilience of these shallower waters against the atmospheric influence.

Table 1. Hydrological parameters and chlorophyll *a* biomass variations at the two studied sites of Annaba Bay recorded during February 2010-January 2011. Mean values ± SD (standard deviation) are given for each parameter as well as the monthly precipitation data (pp in mm) over the bay with the annual wealth (543.3 mm). Precipitation data are from Annaba weather station.

Period	PP	Temperature		Salinity		NH ₄		NO ₂		PO ₄		Chlorophylla	
	(mm)	(°C)		(PSS)		(µmol/L)		(µmol/L)		(µmol/L)		(µg/L)	
	Bay	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
February 2010	53.5	16	15.6	36.2	36.5	75.8	0.0	0.45	0.0	0.72	0.0	0.72	0.18
March	60	14.5	15.8	36.1	36.7	288	0.0	0.88	0.04	2.20	0.04	1.46	1.26
April	32.4	20.5	16.8	36.6	36.8	365	0.0	1.42	0	0.45	0.01	3.02	1.88
May	40.6	22.2	20	35.7	36.7	7.7	0.0	0.26	0	0.24	0	0.65	0.36
June	19.3	26.2	22.6	36.6	36.8	15.6	0.2	0.23	0	0.10	0	4.14	0.46
July	0.0	28.7	26.9	36.6	37.1	10.9	0.0	0.65	0	6.65	0	8.65	0.18
August	1.1	30	26.8	37.1	37.5	78.6	0.0	0.60	0	7.60	0	2.88	0.65
September	27	27.3	24.8	35.4	37.2	54.6	0.04	0.36	0	2.65	0	1.26	0.46
October	99	24.4	22.9	36.7	36.6	25.6	0.0	0.36	0	1.33	0	2.65	0.46
November	120.7	20.2	18	35	36.7	6.6	0.9	0.26	0.05	0.26	0.03	0.88	0.26
December	40.7	17.5	16	36.4	36.6	48.2	0.0	0.65	0.12	3.60	0.01	0.46	1.14
January 2011	49.0	18	14.9	36.5	36.6	54.6	0.2	0.33	0.08	1.36	0.01	0.65	0.56
Mean ± SD	543.3	22.13	20.09	36.24	36.82	93.15	0.11	0.54	0.024	2.26	0.01	2.29	0.65
		± 5.17	± 4.52	± 0.60	± 0.30	± 119	± 0.26	± 0.34	± 0.04	± 2.52	± 0.01	± 2.33	± 0.52

The surface water temperature at the two coastal sites largely varied according to the season but was more pronounced (14.5-30 C°) in the shallower water of site 1. Surface waters of site 1 were colder in winter and warmer in summer compared to site 2. The surface water salinity had slightly varied even for site 1, which is directly subject to freshwater input mainly from Seybouse River discharge. The study period was exceptionally dry so that the derived freshwater (directly or indirectly through riverine input) input

could be limited. As can be seen in Table 1, the surface water salinity at site 1 sensibly followed the precipitation yield and decreased with the precipitation increase. As site 2 is far from the freshwater discharge source, its surface salinity is still varying in very small range (36.6-37.5). Regarding the relative constancy of the water salinity over the whole bay, which contrasted the strong water thermal variations, the plankton communities would rather be submitted to seasonal water temperature changes.

The two sites also largely differ from both their water fertility (nutrients levels) and productivity (Chlorophyll *a* levels: Chla). Site 1 showed unusually

strong levels of ammonium (NH₄) that can be compared to those of domestic sewer or of some highly impacted rivers.

Table 2. Checklist of *Triplos* species recorded at two coastal sites of Annaba Bay during the period February 2010-January 2011.

	Site 1												Site 2											
	F.10	M	A	M	J	J	A	S	O	N	D	j-11	F.10	M	A	M	J	J	A	S	O	N	D	j-11
<i>Triplos arietinus</i> var. <i>arietinus</i>	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+
<i>T. arietinus</i> var. <i>gracilentus</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>T. belone</i>	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
<i>T. candelabrus</i> var. <i>candelabrus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. candelabrus</i> var. <i>depressus</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
<i>T. carriensis</i> var. <i>carriensis</i>	-	-	+	+	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>T. carriensis</i> var. <i>volans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>T. concilians</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	+
<i>T. contortus</i> var. <i>contortus</i>	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	-	+	-	+	+	+
<i>T. contrarius</i>	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
<i>T. declinatus</i> var. <i>normale</i>	-	-	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-	+	+
<i>T. euarquatus</i>	+	+	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
<i>T. extensus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. falcatus</i>	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	+	-	-	-	-
<i>T. furca</i> var. <i>eugrammus</i>	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. furca</i> var. <i>furca</i>	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-
<i>T. fusus</i> var. <i>fusus</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. fusus</i> var. <i>seta</i>	+	+	-	+	+	+	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+
<i>T. geniculatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>T. gibberus</i> var. <i>dispar</i>	-	-	-	-	+	-	-	-	-	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+
<i>T. gravidus</i>	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+
<i>T. hexacanthus</i> var. <i>hexacanthus</i>	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+
<i>T. horridus</i> var. <i>molle</i>	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
<i>T. horridus</i> var. <i>horridus</i>	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+
<i>T. inflatus</i>	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. karstenii</i>	-	-	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. longirostrus</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+
<i>T. longipes</i>	-	+	-	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
<i>T. lunula</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
<i>T. macroceros</i> var. <i>gralicus</i>	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. macroceros</i> var. <i>macroceros</i>	-	-	+	-	-	-	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>T. massiliensis</i> var. <i>massiliensis</i>	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. massiliensis</i> var. <i>protuberans</i>	-	-	-	-	-	+	-	+	+	+	-	+	-	+	+	+	-	-	-	-	-	-	-	+
<i>T. massiliensis</i> var. <i>armatus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>T. pentagonus</i> var. <i>robustus</i>	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>T. pentagonus</i> var. <i>tenerus</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+

Numerically, the *Triplos* populations peaked during spring-beginning summer (Fig. 2) and were more abundant at the neritic open site. The maximum density was recorded during June 2010 with about 2000 Cell L⁻¹ at site 2 and 1500 Cell L⁻¹.

This high abundance was mainly supported by the coexistence of several species (*T. exetnsus*; *T. fusus*; *T. macroceros* and *T. massiliensis*; *T. candelabrum*; *T. furca*) for site 2 (data not represented). In contrast the species *T. contortus* and *T. karsteni* were more represented at site 1. Overall, only 12 species (*T. candelabrus*; *T. contortus*; *T. extensus*; *T. furca*; *T. fusus*; *T. karsteni*; *T.*

macroceros; *T. massiliensis*; *T. muelleri*; *T. contortus*; *T. karsteni*; *T. candelabrum*) constituted the essential and key components of the of *Triplos* genus abundance over Annaba Bay.

Discussion

Information on the phytoplankton of Algerian marine waters is still scare and sparse, and not only does the few available data are very limited in space and time but are also restricted to some local and isolated works on a specific taxa. These works usually focused on harmful dinoflagellates species (Illoul *et al.*, 2005; 2008; 2012) and blooming and toxic dinophycea species (Frehi *et al.*, 2007).

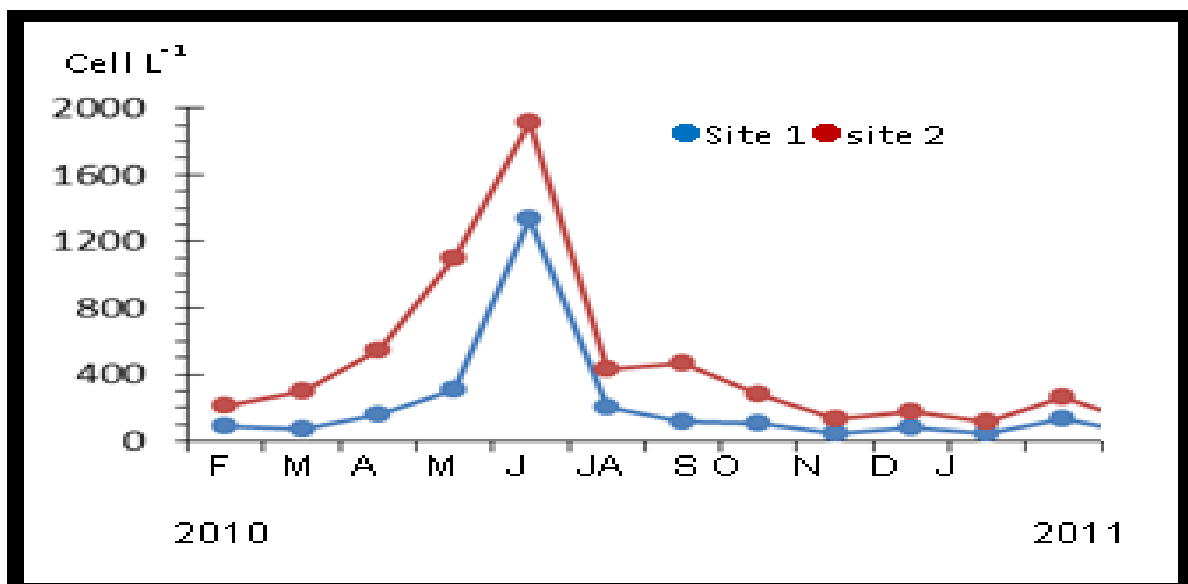


Fig. 2. Variations in *Triplos* species density at site 1 and site 2 for the period Februaury 2010-January 2011.

The only available picture we have on the phytoplankton as a whole is rather limited to the July MEDIPROD cruise data of 1990 (Raimbault, 1990). Data of MEDIPROD concerned however the Mediterranean oceanic waters and cannot be validly compared to the most Algerian studies that were restricted to coastal waters.

The phytoplankton of Annaba Bay is little known, especially because of small species (<20 μm) require specific equipment that we could not have. However, for the large dinoflagellate species some effort has been done on (Frehi *et al.*, 2007). This study is a logical continuation of the work of Frehi *et al.* (2007) and is focussed on the distribution of the large *Triplos*

genus (100-1000 μm), newly revised by Gómez (2013). Although their large body size, the *Triplos* species identification are still very difficult because of the huge varieties and forms of the species, in addition to the numerous nomenclature confusions (Gómez *et al.*, 2010; Gómez, 2012). For instance, according to Gómez (2013) the *Triplos muelleri* species alone has several tens of forms, varieties and subspecies and *Triplos fusus* and *Triplos furca* have numerous forms and varieties. However, as reported by Gómez (2003), the Mediterranean *Triplos* species have not so such morphological varieties and forms. In the Mediterranean Sea Gómez (2003) reported 57 *Triplos* species (previously named *Ceratium* species) from which 36 species inhabited the Algerian basin.

In the present study we report for the first time 47 species of *Triplos* including their respective various forms and varieties. Although the large seasonal variation in surface water temperature (annual thermal magnitude: 14 C°), the *Triplos* species inhabiting Annaba Bay seem to have limited forms and size variability. This might mean that other environmental factors could be responsible for *Triplos* species composition. The external water intrusion (the modified Atlantic water: MAW) can bring additional neritic and oceanic species as has previously been reported in the works of Ounissi and Frehi (1999), Ounissi *et al.* (1998) and Ounissi *et al.* (2016) for the plankton of Annaba Bay. Species can have however specific affinities to nutrient enrichment level (Smayda, 1980), such as *T. massiliensis*; *T. fusus* and *T. extensus*; *T. muelleri*, which have been reported to be characteristics for eutrophicated and warm waters (Le Corre and Treguer, 1976; Havskum *et al.*, 2005; Sullivan and Switt, 2003). For instance, *T. horridus* and *T. geniculatus* are found to be rather linked to open and cold waters.

On the whole, almost all of the identified taxa of Annaba Bay can be considered as tropical and subtropical species (e.g. Sournia, 1967; Tunin *et al.*, 2007). A number of species collected in Annaba Bay are known to be adapted and linked to warm waters. Similarly, Gómez and Gorski (2003) reported that the *Triplos* diversity increases during spring in the coastal waters of Villefranche (NW Mediterranean). These types of species showed long horns, that help maintaining the body buoyancy (Tunin *et al.*, 2007), such as *Triplos candelabrus*.

Among the 47 identified *Triplos* taxa only 8 species of them were common over the entire bay (*Triplos candelabrus*; *T. concilians*; *T. contortus*; *T. contrarius*; *T. carriensis*; *T. karsteni*; *T. inflatus* and; *T. horridus*). Moreover, *T. longirostris* and *T. pentagonus var. tenerus* were confined to the open area in contrast to *T. longipes*, which is linked to the eutrophicated area.

Numerous species always occurred at the well-renewed and oligotrophic waters of the entrance of the bay.

They preferably appeared during winter when the MAW current allow renewing the shallow water of the bay. However, about third the identified *Triplos* taxa were rare and only collected during the cold and mixing period. Overall, the *Triplos* species were more diversified and abundant at the neritic water of site 2, compared to those of the eutrophicated area of the innermost bay.

We have presented here our preliminary findings about *Triplos* species distribution and composition in a coastal area regarding the environmental framework. Future complementary works should address an exhaustive checklist of the *Triplos* of Annaba Bay, which is considered to be a relevant indicator for water temperature and climate changes (Alina *et al.*, 2009).

Conclusions

This study provides the preliminary findings about the *Triplos* species distribution and composition in Annaba Bay coastal area regarding the environmental variables.

The innermost area (site 1) showed strong levels of NH₄, PO₄ and phytoplankton biomasses and seems to function as eutrophicated area. By contrast, surface water of site 2, which is rather impacted by external oceanic waters, was impoverished in nutrients and phytoplankton biomass, and experienced core oligotrophic conditions. These highly spatially contrasted areas will significantly have clear impact on the actual phytoplankton distribution and composition.

In the present study we report for the first time 47 species of *Triplos*; almost all of them can be considered as tropical and subtropical species. To be adapted to warm waters, these types of species showed long horns that help maintaining the body buoyancy.

Nearly third the *Triplos* identified species were found to be rare in the samples and have frequently been collected during the cold and mixing period.

The highly common species are *Triplos massiliensis*; *T. trichoceros*; *T. furca*; *T. fusus*; *T. extensus*; *T. muelleri*; *T. macroceros*. Numerous species were highly frequent at the entrance of the bay, characterized by its renewed and oligotrophic waters. The abundant species are those collected from the innermost bay mostly during the warm period.

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