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First Study of Dinoflagellates Phytoplankton of the Coastal Waters of Haouzia Bay (El Jadida, Doukkala Atlantic Coast, Morocco)

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ABSTRACT

A taxonomic study was carried out in July 2017 during a sampling campaign at a station in the Haouzia Bay of El Jadida, open on the Moroccan Atlantic coast and located two miles from the port of El Jadida. In this work, we presented for the first time an inventory of the dinoflagellates phytoplankton of Haouzia Bay, El Jadida. A specific richness of 57 dinoflagellate taxa was noted. Most taxa were illustrated by micrographs based on light microscope observations. The taxonomic list of dinoflagellates was used to identify specific species known to possess potential toxicity effects, including *Dinophysis* spp., *Alexandrium* spp., *Gymnodinium catenatum*, *Lingulodinium polyedra*, *Gonyaulax* spp., or harmful effects, including *Prorocentrum micans*, *Gyrodinium spirale*, *Scrippsiella trochoidea*, among others.

INTRODUCTION

Studies on the taxonomy of the dinoflagellates from the Doukkala coast have focused on the Sidi Moussa-Oualidia lagoon complex, located some sixty kilometers from El Jadida **(Bennouna, 1999**, **2008; Natij *et al*., 2014)**, or the mouth of the lagoon **(Akallal, 2001; Akallal *et al.*, 2006)**. However, the dinoflagellates from the Haouzia Bay coastline have never been reported before. Nevertheless, there has never been any data concerning the dinoflagellates present along the coast of Haouzia Bay (El Jadida). This bay was chosen because of the lack of phytoplankton data from the area. This approach represented the first study to examine the dinoflagellates component of the coastal waters of Haouzia (El Jadida).

Morphological identification of the dinoflagellates collected from the marine waters of Haouzia Bay enabled us to draw up a taxonomic list, the majority of which was illustrated.

MATERIALS AND METHODS

Study area

Haouzia Bay, situated along the Moroccan Atlantic coastline, spans from 33°15'00" to 33°21'40" North latitude and 8°18'00" to 8°30'00" West longitude with a north-east to a south-west orientation. The tidal patterns in this area follow a semi-diurnal cycle, characterized by two daily tidal cycles that vary depending on the day, months, and season **(Chaibi *et al.*, 2014)**. Haouzia coast boasts significant socio-economic diversity, with activities such as fishing and tourism thriving in the region. Moreover, the coast is renowned for its extensive and easily accessible wild mussel beds, which are harvested on a small-scale basis **(Merzouki *et al.*, 2009)**. Phytoplankton plays a crucial role in the local ecosystem, serving as a primary food source for both wild and farmed shellfish. Our phytoplanktonic sampling station, focusing on the dinoflagellates, is situated two miles from the port of El Jadida on the Haouzia coast (Fig. 1).

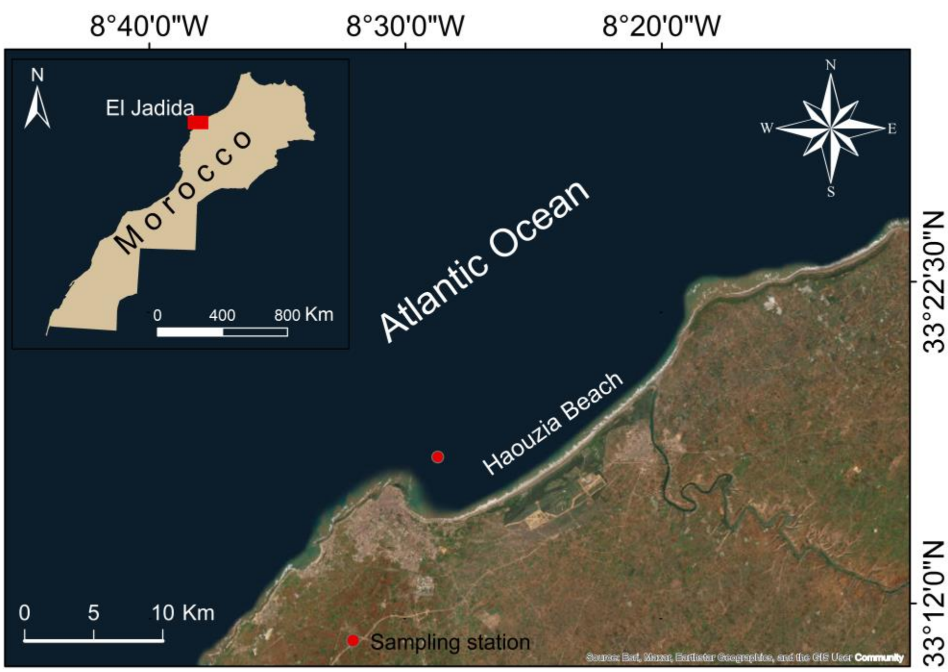

Fig. 1. Geographical location of the sampling station - Haouzia Bay (El Jadida)

Physicochemical data (temperature, conductivity, dissolved oxygen, and pH) were measured using a multi 340i multiparameter analyzer model WTW, coupled with an oximeter, a conductivity meter, and a pH meter. Dissolved oxygen concentration was checked using Winkler’s volumetric method **(Aminot & Chaussepied, 1983)**. The **Lorenzen (1967)** method was used to calculate chlorophyll (*a*) concentration.

Phytoplanktonic study

Phytoplankton material was first collected using a plankton net with a mesh size of 30µm. It was horizontally towed to obtain a sample of the surface layer. Vertical tows were then made using both the net of plankton and a Niskin bottle, two miles from the port of El Jadida (bounded by the cape of Mazagan). Samples were immediately transferred to jars for live observations for exploratory identification, while others were fixed with Lugol and/or formalin solutions and stored in 100ml bottles in the shade for microscopic analysis.

Two microscopes were used to analyze the counting and morphological identification of the samples. After decanting the samples, fixed with Lugol, onto a sedimentation tank, an inverted microscope, Olympus model CK 40, was used to observe and quantify the phytoplankton using the **Utermöhl (1958)** method. A second upright swift microscope was used to observe and determine taxa. Each microscope was equipped with an appropriate camera. Species were recognized based on books and articles on the taxonomy and classification of Dinophyceae **(Trégouboff & Rose, 1957; Sournia, 1986; Ricard, 1987; Fensome *et al*., 1993; Paulmier, 1994; Nézan & Piclet, 1996; Hasle *et al.*, 1997; Adl *et al*., 2012; Gómez, 2013)**. Numerous other authors and specialist websites were also required **(Guiry & Guiry, 2018)**.

The species list is accompanied by microphotographs illustrating several Lugol/Rhode-fixed specimens from observations under an inverted microscope at magnification (x40) or from fresh (unfixed) material examined under an upright light microscope at magnification (x100).

RESULTS AND DISCUSSION

Sampling was carried out two nautical miles from the port of El Jadida, at surface water level and at a depth of 10 meters. The main temperature between these two points was 23.22°C, with a heat intensity value UTC equivalent to 0.88°C. Conductivity was 53.4ms/ cm, and the average pH detected was 8.33. The average dissolved oxygen level was 8.92mg/ l, while the recorded chlorophyll (*a*) content was approximately 0.47µg/ l (Table 1).

Table 1. Physico-chemical readings two miles from the port at surface level and -10m depth

Station	GPS coordinates	Temperature °C	Conductivity ms/cm	pH	Chlorophyll (*a*) µg/l	Dissolved oxygen mg/l
Surface	33°16'01''N 8°27'30''W	24,9	53,2	8,32	0,67	9,24
-10 m		21,55	53,6	8,34	0,27	8,6

An assessment of the species richness of the dinoflagellates was carried out in Haouzia Bay (Moroccan Atlantic). The July 2017 taxonomic inventory revealed a significant number of the dinoflagellate taxa from this ecosystem. 57 dinoflagellates taxa were reported for the first time from the Bay of Haouzia (El Jadida), 54 of which were classified at species level and 3 at genus level. In total, these taxa are grouped into 25 genera, which include 15 families and two classes.

Dinophyceae class

Order of **Prorocentrales**

Family Prorocentraceae

Prorocentrum Ehrenberg, 1834

P. arcuatum Issel

P. balticum (Lohmann) Loeblich III

P. donghaiense Lu

P. micans Ehrenberg

P. cf. *minimum* (Pavillard) Schiller

P. scutellum Schröder

P. sp.

Order of **Dinophysiales**

Family of Dinophysiaceae

Dinophysis Ehrenberg, 1839

D. acuminata Claparède & Lachmann

D. caudata Saville-Kent

D. fortii Pavillard

D. sacculus Stein

Phalacroma Stein, 1883

P. rotundatum (Claparéde & Lachmann) Kofoid & Michener

Order of **Gymnodiniales**

Family Brachidiniaceae

Karenia Hansen & Moestrup, 2000

K. mikimotoi (Miyake & Kominami ex Oda) Hansen & Moestrup

Familly of Gymnodiniaceae

Amphidinium Claperède & Lachmann, 1859

A. sp.

Gymnodinium Stein, 1878

G. catenatum Graham

Gyrodinium Kofoid & Swezy, 1921

G. britannicum Kofoid & Swezy

G. spirale (Bergh) Kofoid & Swezy

Order of **Gonyaulacales**

Family of Ceratiaceae

Tripos Bory de St.-Vincent, 1823

T. candelabrus (Ehrenberg) Gómez

T. furca (Ehrenberg) Gómez

T. furca var. *eugrammus* (Ehrenberg) Gómez

T. fusus (Ehrenberg) Gómez
T. macroceros (Ehrenberg) Gómez
T. massiliensis (Gourret) Gómez
T. muelleri (Cleve) Gómez
Family of Cladopyxidaceae
Micracanthodinium Deflandre, 1937
M. claytonii (Holmes) Dodge
Family of Goniodomataceae
Gambierdiscus Adachi & Fukuyo, 1979
G. cf. *excentricus* Fraga
Goniodoma Stein, 1883
G. cf. *sphaericum* Murray & Whitting
Family of Gonyaulacaceae
Gonyaulax Diesing, 1866
G. grindleyi Reineke
G. polygramma Stein
G. spinifera (Claparède & Lachmann) Diesing
G. turbynei Murray & Whitting
G. verior Sournia
G. scrippsae Kofoïde
Lingulodinium Wall, 1967
L. polyedra (Stein) Dodge
Family of Ostreopsidaceae
Alexandrium Halim, 1960
A. cf. *minutum* Halim)
Coolia Meunier, 1919
C. cf. *canariensis* Fraga
Ostreopsis Schmidt, 1901
O. ovata Fukuyo
O. cf. *siamensis* Schmidt
Family Pyrophacaceae
Pyrophacus Stein, 1883
P. horologium Stein
P. steinii (Schiller) Wall & Dale
Order of **Peridiniales**
Family of Diplopsalidaceae
Oblea Balech ex Loeblich & Loeblich III, 1966
O. rotunda (Lebour) Balech ex Sournia
Familly Heterocapsaceae
Heterocapsa Stein, 1883
H. circularisquama Horiguchi
H. psammophila Tamura, Iwataki & Horiguchi
H. triquetra (Ehrenberg) Stein

Family Protoperidiniaceae
Preperidinium Mangin, 1913
P. meunieri (Pavillard) Elbrächter
Protoperidinium Bergh, 1882
P. conicum (Gran) Balech
P. depressum (Bailey) Balech
P. diabolus (Cleve) Balech
P. divergens (Ehrenberg) Balech
P. leonis (Pavillard) Balech
P. pellucidum Bergh ex Loeblich & Loeblich
P. punctulatum (Paulsen) Balech
P. steinii (Jorgensen) Balech
Family Thoracosphaeraceae
Pentapharsodinium Indelicato & Loeblich III, 1986
P. dalei Indelicato & Loeblich III
Scrippsiella Balech ex Loeblich, 1965
S. trochoidea (Stein) Balech ex Loeblich
Order of **Oxyrrhinales**
Family Oxyrrhinaceae
Oxyrrhis Dujardin, 1841
O. sp.
Class of Noctilucophyceae
Order of **Noctilucales**
Family Noctilucaceae
Noctiluca Suriray, 1836
N. scintillans (Macartney) Kofoid & Swezy.

For the sake of convenience, we have counted around thirty species among all the taxa surveyed, to avoid an excessive number of species in low proportions. A species is considered dominant when it has the highest concentration within a given stand.

According to the quantitative analysis carried out, the dinophyceae stand was dominated by *Protoperidinium steinii*, represented by a density of 8060 cells/L, assessed as the most important taxon in the phytoplankton stand. *Prorocentrum scutellum* was detected at 7113 cells/L. Next, *Gyrodinium spirale* associated with *Gyrodinium britannicum* were counted at an average total of 5547 cells/L and *Heterocapsa circularisquama* was reported at 4107 cells/L.

The species with the lowest densities, among the assessed stand, were represented by *Dinophysis acuminata* (40 cells/L), *Dinophysis sacculus* (20 cells/L), *Tripos macroceros* and *T. massilensis* represented by a similar density (13 cells/L) and *D. caudata* (7 cells/L) (Fig. 2).

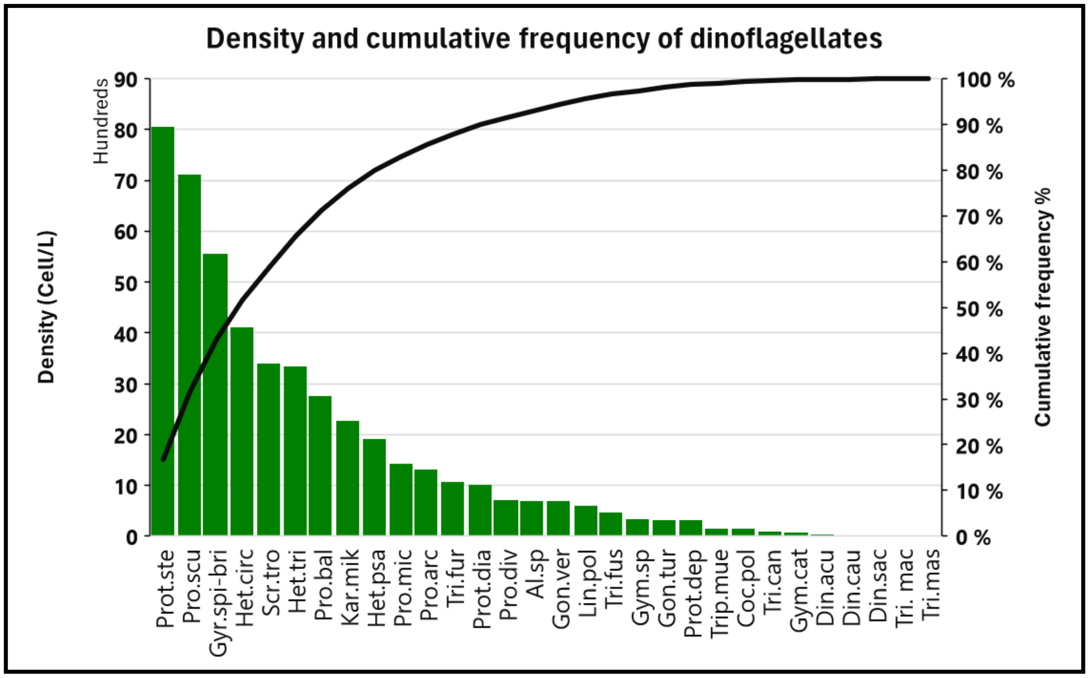

Fig. 2. Diagram of density (Cells/L) and cumulative frequency (%) of dinoflagellate taxa - July 2017

The Pareto plot combines a bar graph showing all taxa in a descending order of abundance with a cumulative curve showing which taxa dominate across all stands. Of the thirty taxa counted, four species (*Protoperidinium steinii*, *Prorocentrum scutellum*, *Gyrodinium spirale* combined with *G. britannicum* and *Heterocapsa circularisquama*) can be considered dominant, accounting for 55.07% of the cumulative frequency (CF) of the total number of cells counted. The remaining portion was distributed among the 25 other taxa included in this taxonomic assessment.

Protoperidinium steinii had a relative frequency (fi) of 17.88%, followed by *Prorocentrum scutellum* with 15.78%, *Gyrodinium spirale* and *G. britannicum*, together they had a relative frequency of 12.30%, while *Heterocapsa circularisquama* had a relative frequency of 9.11%. The range of relative frequencies between 6 and 9% included *Scrippsiella trochoidea* (7.56%), *Heterocapsa triquetra* (7.39%), *Prorocentrum balticum*, and *P.* cf. *minimum* (6.11%). In contrast, the range of values between 0.01 and 6% encompassed the relative frequencies of the twenty-one other taxa identified in the count.

The upwelling zones are very productive. The upwelling phenomena observed along the Moroccan coasts lead to substantial primary production (**Franchimont, 1998 and 2001; Penaud, 2009; Elghrib *et al*., 2012)**. The study area experiences seasonal upwelling effects during the warmer months. Throughout the sampling campaign, a diverse array of species was identified.

The presence of certain potentially toxic or harmful taxa has been noted. Their appearance is a natural phenomenon that can sometimes be increased by a significant enrichment of the environment in nutritive elements, but without harmful consequences. Nevertheless, under certain conditions, their blooms can have harmful effects through their impact on marine species and/or human health, which seem to have increased in frequency and geographic extent **(Hallegraeff *et al*., 2003)**.

The species *Alexandrium minimum, Gonyaulax* spp. and *Gymnodinium catenatum*, are known to be potentially associated with the production of paralytic toxins through the consumption of PSP-type seafood **(Amzil & Motteau, 2000; Herzi, 2013)**. *Alexandrium minutum* is one of the species that has already led to toxic episodes in the Atlantic **(Lassus *et al*., 2016)**.

The *Dinophysis* complex contains several species, including *D. acuminata*, *D. caudata*, *D. fortii* and *D. sacculus*, all of which can produce diarrhoeal toxins by eating seafood of the DSP (Diarrheic Shellfish Poisoning) type **(Néz & Rocher, 2003; Suzuk *et al.*, 2009)**. *Dinophysis* never proliferates in high concentrations, but the toxins it produces are capable of contaminating bivalves at a rate of around one hundred cells per liter. *Dinophysis acuminata* is considered an "indicator" species in relation to *D. fortii*. On certain occasions, the appearances of *D. acuminata* preceded that of *Dinophysis fortii* **(Lassus, 1984)**. Four species of *Dinophysis* were identified in the water off the coast of Haouzia coast. *D. fortii* was found with a lower frequency (fi = 0.003%) compared to *D. acuminata* (fi = 0.09%). Due its very low frequency (fi), the latter was not included among the taxa selected in Fig. (2).

Other species found in the Haouzia littoral could have detrimental effects if they proliferate. *Karenia mikimotoi*, which typically produces hemolytic toxins affecting marine flora and fauna, has been linked to marine organism mortality **(Nézan *et al.*, 1997; Cadour *et al*., 1997)**. Additionally, other harmful species have been observed, including *Gyrodinium spirale, Prorocentrum micans* and *Lingulodinium polyedra* (formerly *Gonyaulax polyedra*), which produce toxins such as homoyessotoxin, saxitoxin, responsible for shellfish contamination **(Charbonnier, 2006)**, as well as paralytic toxins. *Lingulodinium polyedra* has a wide distribution, and an occurrence of stained water caused by it was documented along the Moroccan Atlantic coast in July 1999. It was observed in the Kenitra region and spread as far south as Safi, affecting areas under health surveillance **(Bennouna *et al.*, 2002)**. This species was also identified on the Haouzia coast during the current study.

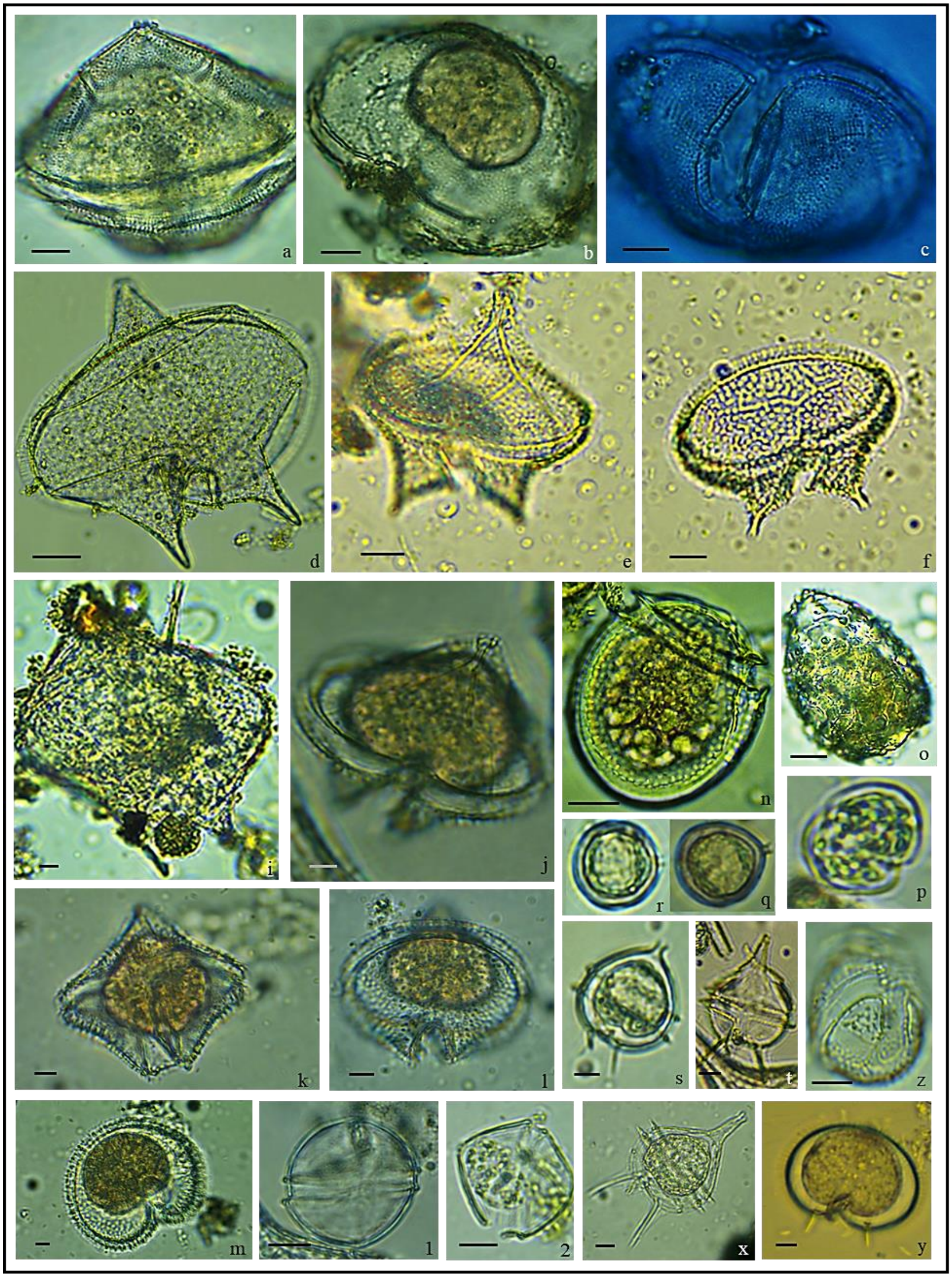

Plate 1. Dinoflagellates *in vivo* from Haouzia Bay (El Jadida) (G x100) -Jully 2017 showing: *Protoperidinium punctulatum* (a-c); *Protoperidinium sp.* (d); *Protoperidinium divergens* (e-f); *Protoperidinium depressum* (i); *Protoperidinium conicum* (j, k, l & m); *Phalacroma rotundatum* (n); *Ostreopsis ovata* (o); cf. *Karinia mikimotoi* (p); *Protoperidinium minutum* (q-r); *Protoperidinium steinii* (s); *Protoperidinium diabolus* (t-y); *Gonyaulax scrippsae* (z); *Oblea rotunda* (1); *Scrippsiella trochoidea* (2). All scale bars = 10µm

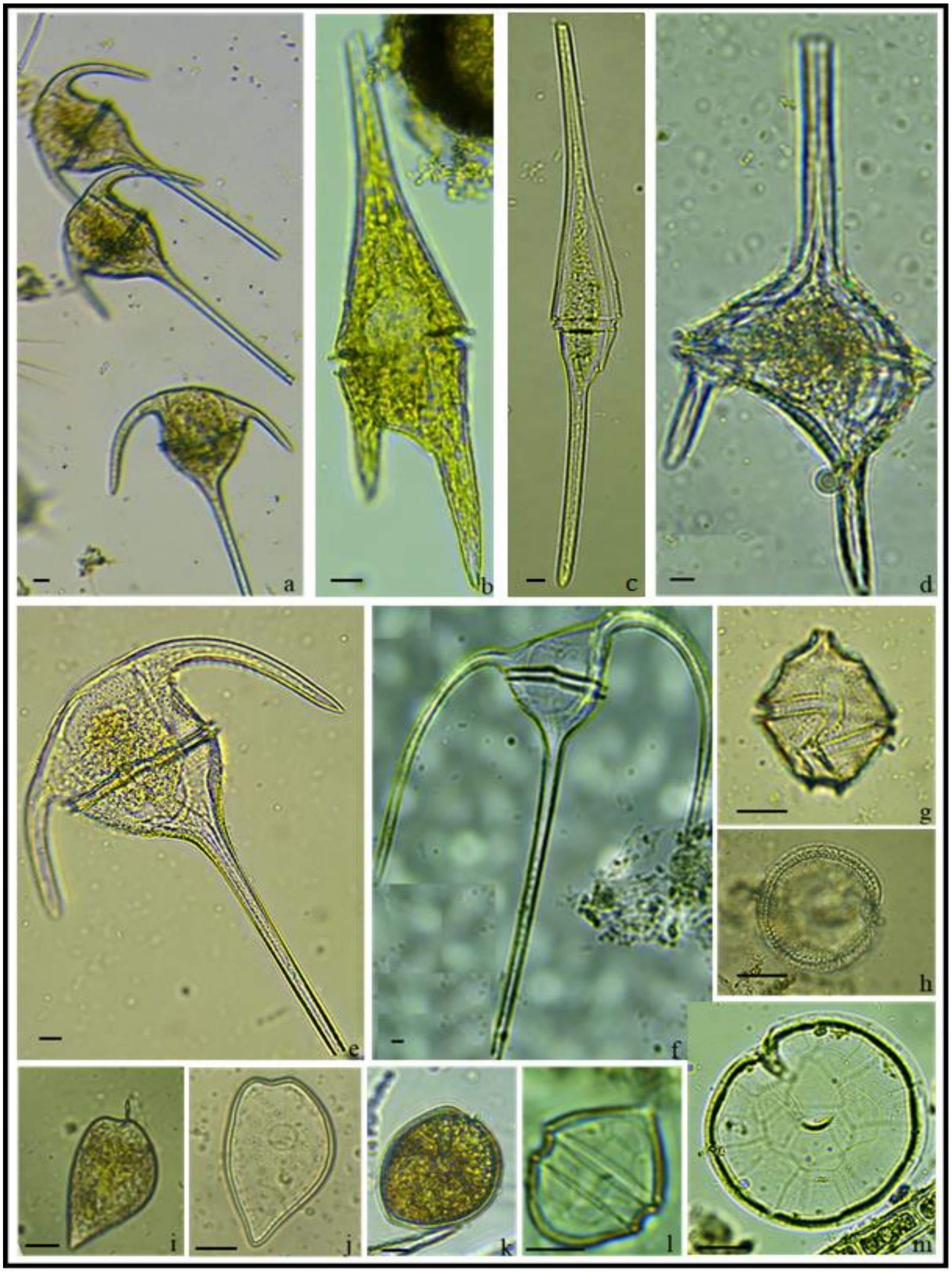

Plate 2. Dinoflagellates *in vivo* from Haouzia Bay (Gx 100)-July 2017 showing: *Tripos muelleri* (a & e); *T. furca* (b); *T. fusus* (c); *T. candelabrum* (d); *T. macroceros* (f); *Gonyaulax spinifera* (g-h); *Prorocentrum micans* cell and empty thequa (i-j); *P. scutellum* (k); *Pentapharsodinium dalei* (l); *Pyrophacus horologium* with plates and sulcus (m). All scale bars = 10µm

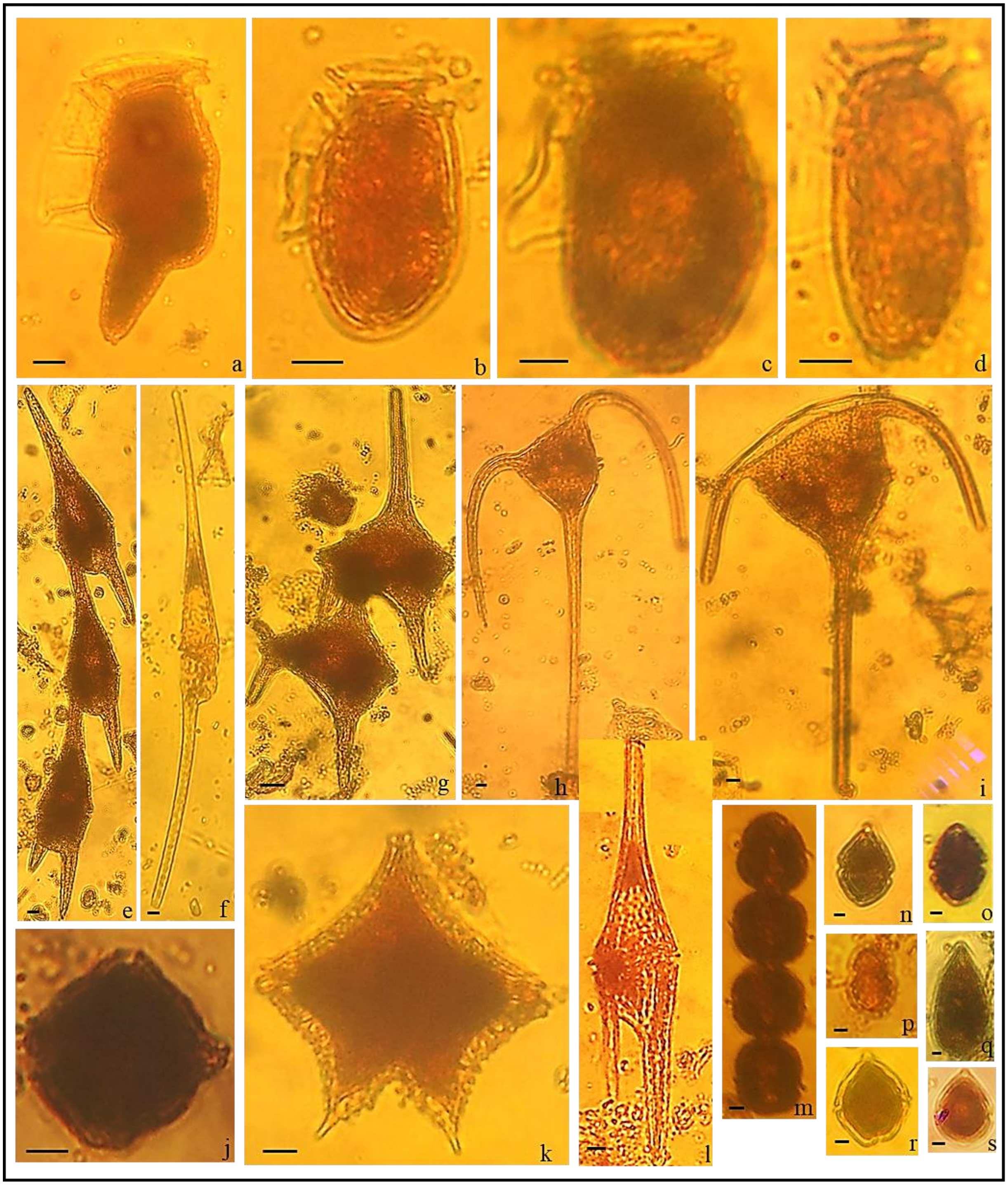

Plate 3. Dinoflagellates from Haouzia Bay (El Jadida) fixed with Lugol / Rhode (G. x40) -July 2017 showing: *Dinophysis caudata* (a); *Dinophysis acuminata* (b); *Dinophysis fortii* (c); *Dinophysis sacculus* (d); *Tripos furca* var. *eugrammum* (e); *Tripos fusus* (f); *Tripos candelabrus* (g); *Tripos macroceros* (h); *Tripos muelleri* (i); *Lingulodinium polyedra* (j); *Protoperidinium divergens* (k); *Tripos furca* (l); cf. *Gymnodinium catenatum* (m); *Scrippsiella trochoidea* (n); *Heterocapsa circularisquama* (o); *Heterocapsa psammophila* (p); *Ostreopsis* cf. *siamensis* (q); *Gonyaulax polygramma* (r); *Scrippsiella trichoidea* (s). All scale bars = 10µm

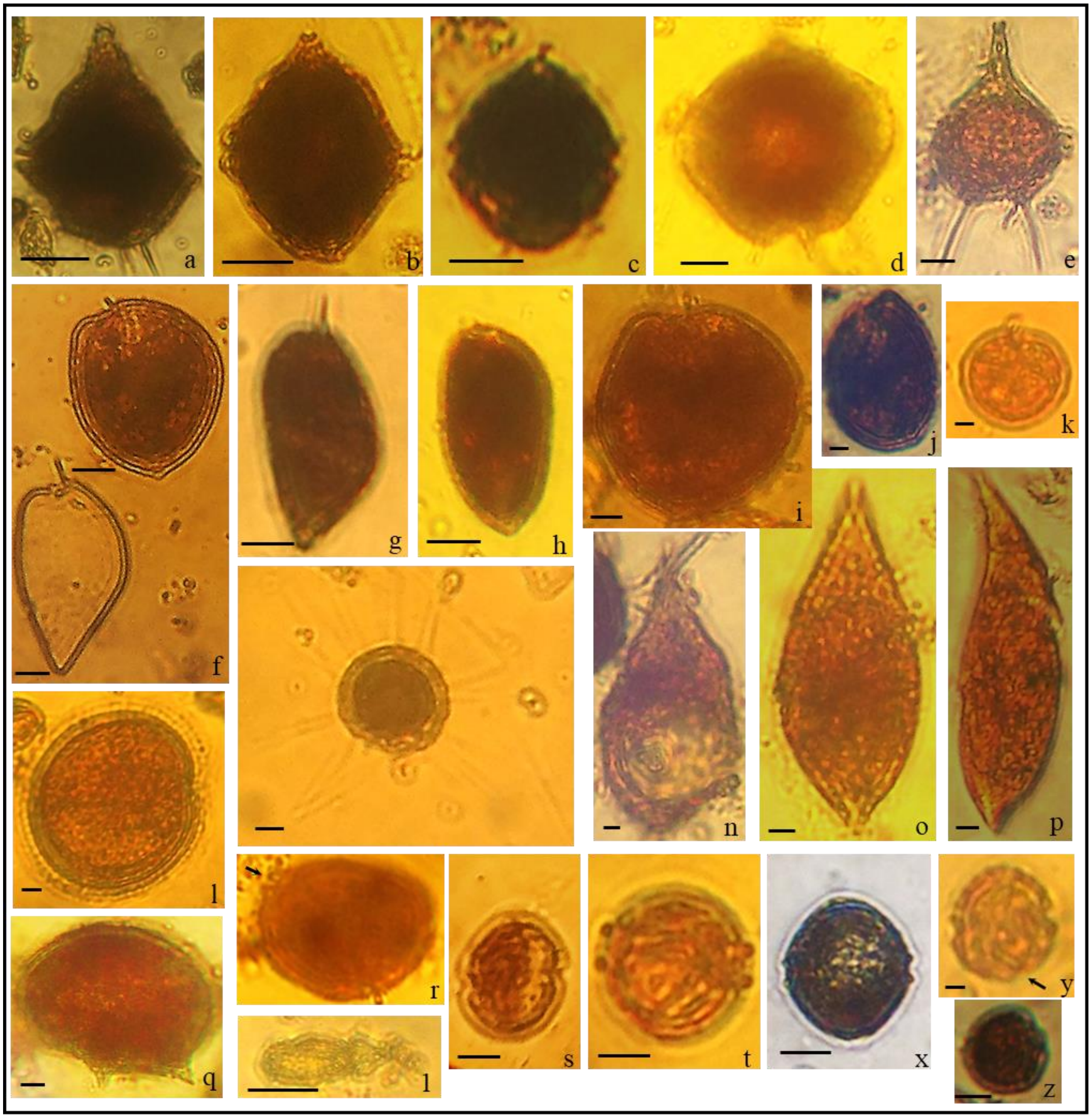

Plate 4. Dinoflagellates from Haouzia Bay (El Jadida) fixed with Lugol/Rhode (G. x40)- July 2017 showing: *Gonyaulax verior* (a); *Lingulodinium polyedra* (b); *G.* cf. *turbynei* (c); *Protoperidinium pellucidum* (d); *Protoperidinium diabolus* (e); *Prorocentrum scutellum* (up) et *P. micans* thèque vide (bellow) (f); *P. arcuatum* (g); *P. donghaiense* (h); *P. scutellum* (i); *Ostreopsis* sp. (j); *P. balticum* (k); *Preperidinium meunieri* (l); *Micracanthodinium claytonii* in antapical view (m); *Gyrodinium spirale* (n-o); *G. britannicum* (p); *Protoperidinium divergens* (q); cf. *Coolia canariensis* (r); *Gyrodinium* cf. *lebouriae* (s); *Oblea rotunda* (t); *Gonyaulax grindleyi*/*minitum* (x); *Gymnodinium* sp. (y); *Gambierdiscus* cf. *excentricus* (z); unidentified Cell / *Amphidinium* (1). All scale bars = 10µm

CONCLUSION

This study, conducted at the onset of the dry season (July 2017) in Haouzia Bay on the El Jadida coast, revealed notable diversity in dinoflagellates, which are considered a significant group alongside diatoms. However, the observed taxonomic richness cannot be attributed solely to the abiotic factors mentioned. The abundance of the dinoflagellates in these coastal waters appears to be a response to the seasonal upwelling dynamics in the region.

The presence of potentially toxic or harmful dinoflagellates in the study area poses a risk if consumed by mollusks that can accumulate toxins from these organisms. It is important to note that the toxicity or harmfulness of dinoflagellates can vary depending on environmental conditions and organism concentrations. Therefore, careful monitoring and assessment of these species in their specific context are essential.

Given that the study area serves as a transit zone for the artisanal fishing fleet, ongoing monitoring is recommended to observe interactions between toxic microalgae, other biological components, and abiotic factors. During the study period, however, concentrations of toxic or harmful dinoflagellates did not exceed the alert threshold.

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