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Research Article

First Report on the Occurrence and Dynamics of the Ectoparasitic Dinoflagellate *Amyloodinium ocellatum* in the Moroccan Atlantic Coast

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Abstract

Background and Objective: Dinoflagellates are commonly found in marine ecosystems and contain both photosynthetic and heterotrophic representatives. Among the latter, several species have been reported as parasites of various marine organisms. This study mainly focused on the proliferation of the ectoparasitic Dinoflagellate *Amyloodinium ocellatum* (*A. ocellatum*), documented for the first time in the Atlantic coast of Morocco. **Methodology:** As part of a monitoring program of harmful marine phytoplankton along Casablanca-El Jadida Atlantic axis, monthly sampling of phytoplankton and water was conducted from March, 2014 to November, 2015 to determine the temporal abundance and distribution of phytoplankton species in El Mrissa bay (33°31'48.5"N 7°48'55.3"W) 20 km South of Casablanca city. Particularly, taxonomic characteristics and environmental conditions encountered during the proliferation of *Amyloodinium ocellatum* are presented and discussed. **Results:** The taxonomic structure of phytoplankton populations showed a composition generally dominated by Diatoms (57%) and Dinoflagellates (38%) essentially represented by *Peridinales* and *Gymnodinales* species. Mass proliferations of *Amyloodinium ocellatum* has been detected during September, 2014 and October, 2015 with cellular densities of 1×10^6 and 2.5×10^4 cells L^{-1} , respectively. The main environmental factors leading to the ecological success of *Amyloodinium ocellatum* were relatively high temperature (21.2-25.8°C), high concentration of ammonium (10-10.4 mg L^{-1}) and orthophosphates deficiency (0.8-0.9 $\mu g L^{-1}$). **Conclusion:** This is the first record of the ectoparasitic Dinoflagellate *Amyloodinium ocellatum* in the Atlantic coast of Morocco and one of the rare instances in planktonic realm. The ecological importance of this species is particularly evident during epidemic outbreaks that cause mass mortality of host organisms. Parasitic Dinoflagellates are poorly investigated in Moroccan waters and accordingly further taxonomic and ecological investigations centering on parasitic Dinoflagellates are badly needed.

Key words: Marine phytoplankton, ectoparasitic Dinoflagellates, *Amyloodinium ocellatum*, Morocco

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Harmful algal blooms (HABs) are a global threat to living marine resources and human health¹. These phytoplanktonic events have an array of economic impacts, including large mortalities of marine organisms, toxic contamination of filter-feeding organisms such as bivalve shellfish that subsequently enter the market for distribution to consumers, economic hardships for fisheries, aquaculture and recreational and tourism-related industries and a compromised quality of life for people living or working along affected shorelines². HABs may be caused by the explosive growth of a single species that rapidly dominates the water column, but they may also be the result of highly toxic cells that do not accumulate in high numbers³.

Dinoflagellates are one of the major components of marine phytoplankton assemblages considered as food sources for fishery resources. They can be autotrophic, heterotrophic or mixotrophic, whereas photosynthetic species are globally important primary producers^{4,5}. When associated with harmful events, species identification of these organisms is critically important⁶. About 140 of the approximately 2000 known living Dinoflagellate species are parasites, most being parasites of invertebrates. Five genera have been reported as fish parasites: *Amyloodinium*, *Piscinoodinium*, *Crepidoodinium*, *Ichthyodinium* and *Oodinioides*. These parasitic forms can have a great impact on health and reproductive behavior of the infected hosts⁷ and therefore, understanding of taxonomy and ecology of the parasitic Dinoflagellates are of paramount importance. *Oodinium* and *Amyloodinium* species are the most common and

important Dinoflagellates parasitizing fish⁸. They cause serious morbidity and mortality in brackish and marine fish at aquaculture facilities worldwide, occurring on the gills, but sometimes on the external surface of the body causing a type of fish velvet disease⁹.

The main objective of this study was to describe taxonomic characteristics and environmental conditions encountered during blooms of *Amyloodinium ocellatum* reported for the first time in the Atlantic coast of Morocco.

MATERIALS AND METHODS

Sampling site, water and phytoplankton analysis: The study was conducted in El Mrissa site located at 20 km South of Casablanca city on the Atlantic coast of Morocco (Fig. 1; 33°31'48.5"N 7°48'55.3"W). According to preliminary results, this site is considered as potential habitat for the proliferation of algal toxic species due to a limited water movement and stagnant environment. For quantitative and qualitative analyses of phytoplankton, cell counts and composition, water samples were collected monthly during the high tide-times in 2 L capacity plastic containers and preserved using 4% formalin solution and/or Lugol's iodine solution and stored in the dark until analyzed. Identification and cell counts of phytoplankton were made under a photonic microscope (Leica DM 1000) and an inverted microscope (Nikon Eclipse TS100) using settling chambers following the Utermöhl method. Micrographs were captured with SI-5000 digital camera and processed with DsCap 3.91 software (EJC Associates). Environmental variables were measured *in situ* using a thermometer, salinometer and pH meter for water

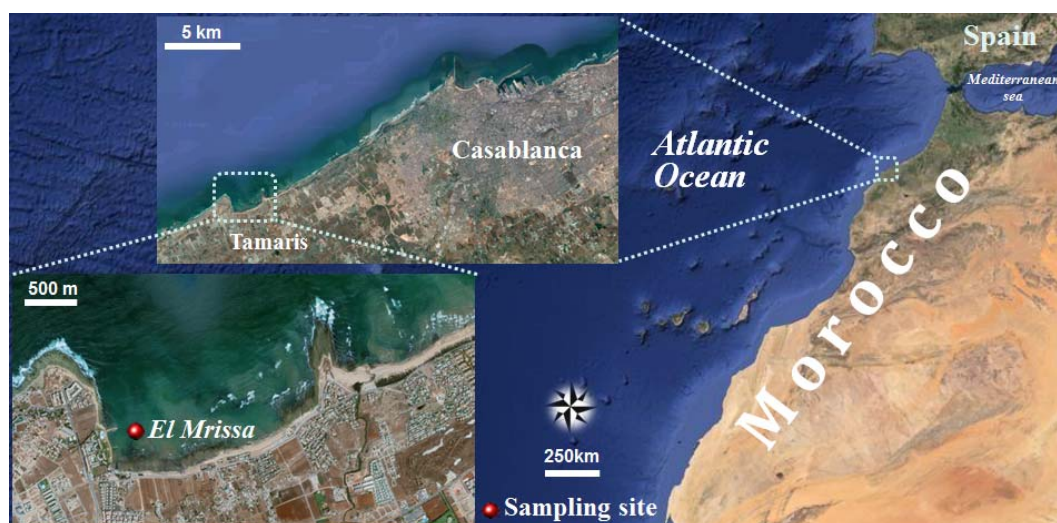


Fig. 1: Sampling site of El Mrissa on the Atlantic coast of Morocco

temperature (°C), salinity (psu) and pH, respectively. For nutrients analysis, water samples were analyzed in the laboratory within 24 h according to standard methods NF T90-023 for orthophosphates and NF T90-015 for ammonium as described in AFNOR¹⁰.

Statistical analysis: Water and phytoplankton analysis were performed by duplicate. Microsoft Excel (Windows 10) was used to process experimental data.

RESULTS AND DISCUSSION

General phytoplankton composition: A total number of 87 phytoplankton taxa were identified in the studied coastal area. *Bacillariophyceae* (57%), mainly represented by *Chaetoceros* and *Navicula* species, predominated in El Mrissa site, followed by *Dinophyceae* (38%) represented by *Peridinales* and *Gymnodinales* species. Other minor groups (Cyanophyceae, Euglenophyceae, Dictyophyceae...) were represented by a smaller number of taxa corresponding to 5% of the total phytoplankton diversity. Regarding potentially toxic species, a total of 15 taxa were identified most of them were Dinoflagellates, except for the diatom *Pseudo-nitzschia australis*. Seasonal quantitative changes in groups of phytoplankton (Fig. 2) showed a sub-continuous dominance of Diatoms and Dinoflagellates. Diatoms increased their share in the density from winter to summer while Dinoflagellates particularly dominate phytoplankton during autumnal periods.

***Amyloodinium ocellatum* description and dynamics:** Cells with morphological features corresponding to *Amyloodinium ocellatum*^{8,11-13} were, for the first time observed in survey samples early autumn 2014. This species falls within the Phylum Dinoflagellida, Class Blastodiniophyceae, Order Blastodinales, Family Oodiniaceae. The most frequently encountered specimens were those corresponding to the reproductive stage called tomons (Fig. 3a,b). They correspond to an opaque round to oval cysts range from 25-50 µm in length and 20-45 µm in diameter. The parasitic stage of *A. ocellatum* known as trophont is the feeding stage. Detached trophonts were occasionally observed (Fig. 3c,d) with single cells colorless, pear-shaped in the young stages and vary from 50-120 µm in maximum length with a fine transparent membrane, dense, finely granular. To date, only one species of the genus *Amyloodinium* has been identified, *A. ocellatum*. Recent genetic studies suggest that *A. ocellatum* isolates from diverse geographic origins and from different fish species are all the same species although some morphological studies by others have suggested that multiple species might exist^{8,14}. The Dinoflagellate described in this paper shows obvious similarity with the species *A. ocellatum* and some common characteristics with members of allied genera, such as *Oodinium*, *Crepidoodinium* and *Piscinoodinium*. However, *A. ocellatum* is clearly distinguishable from species of *Crepidoodinium* and *Piscinoodinium* which are characterized by the possession of chloroplasts and the lack of thecal plates. *Amyloodinium* can also be distinguished from *Oodinium* by the possession of

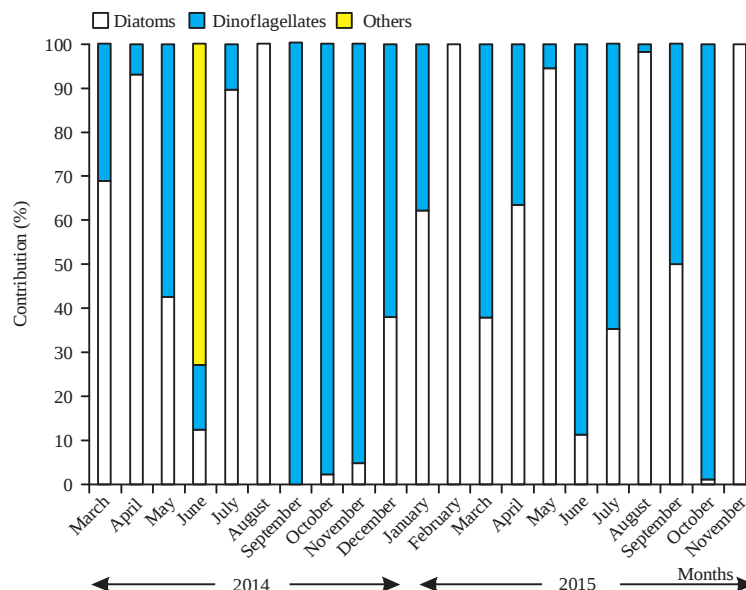


Fig. 2: Relative contribution of phytoplankton groups: Diatoms, Dinoflagellates and others (Cyanophyceae, Euglenophyceae, Dictyophyceae) to the total abundance in El Mrissa site from March, 2014 to November, 2015

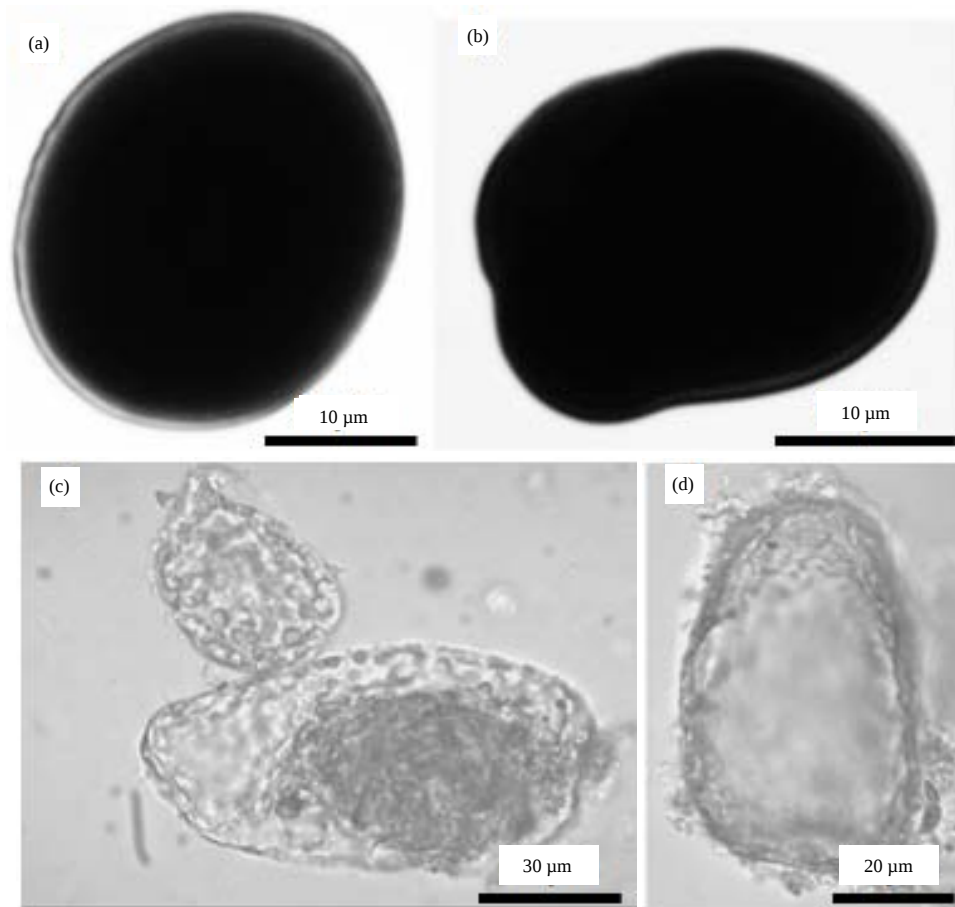


Fig. 3(a-d): Photomicrographs of specimens ascribed to *Amyloodinium ocellatum* with cell stages (a, b) Tomont and (c, d) Trophont

rhizoid- and root-like processes for attachment and by the production of starch grains⁷.

A. ocellatum is one of the most important pathogenic parasites affecting the culture of marine and brackish water fish¹⁵. The parasite produces a powdery or velvety appearance on infected fish and the resulting disease is commonly referred to amyloodiniosis or marine velvet disease. *A. ocellatum* has a broad host and geographic range causing fish mortalities in tropical and temperate environments. It is one of a very few organisms that can infect both teleosts and elasmobranchs¹⁶. According to Aaen *et al.*¹⁷, *A. ocellatum* have a classical direct life cycle without intermediate host. The trophont lives on the skin of its fish host and develops a network of rhizomes that pierce the epidermis and take up nutrition directly from the host. Once mature, this parasitic form detaches from the network of rhizomes and falls through the water column onto the substrate to become the tomont. This is an encysted stage that subdivides internally to form up to 256 numbers of infective motile dinospores^{18,19}.

Temporal distribution showed that *A. ocellatum* revealed a strong seasonality and was mainly present in samples taken during the autumn months (Fig. 4). Outside this period, its contribution to total phytoplankton was generally insignificant, never exceeding 1% and when considered within the Dinoflagellate assemblage, its abundance accounted for no more than 1.7%. During autumnal periods, contribution of *A. ocellatum* to total Dinoflagellates was higher (50-100%) with maximal abundances reached 1×10^6 cells L^{-1} in September, 2014 and 2.5×10^4 cells L^{-1} in October, 2015. These proliferations coincided with periods of relatively high water temperatures ranged from 21.2-25.8°C (Table 1). Salinity (35.5-35.6 psu) and pH (8.1-8.2) recorded during this Dinophycean event were normal when compared to values outside the peak period. Regarding analyzed nutrients, the studied species seems to be supported by higher ammonium concentrations (10-10.41 mg L^{-1}) and an opposite pattern in regard to orthophosphates (0.8-0.9 $\mu g L^{-1}$). In general, many of the physical, chemical and biotic variables act synergistically

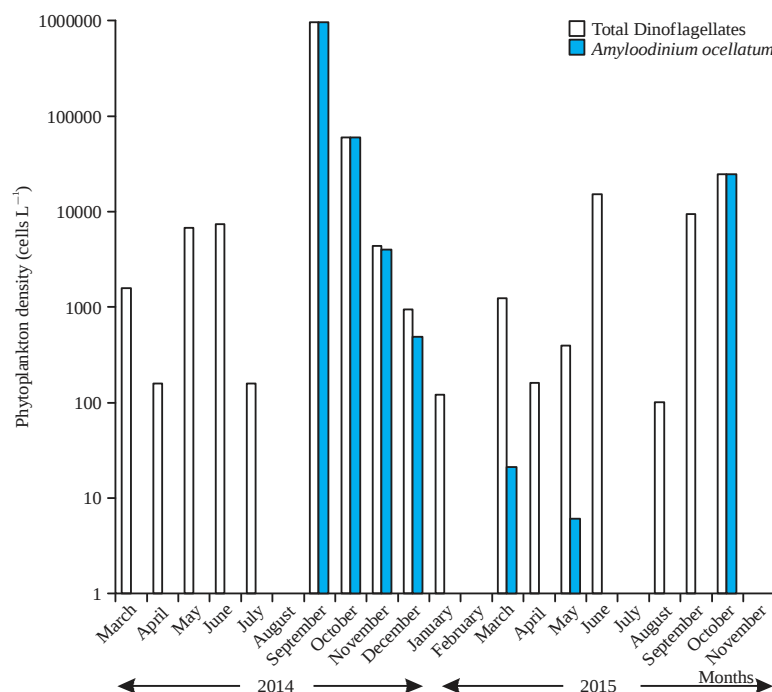


Fig. 4: Temporal total Dinoflagellates and *Amyloodinium ocellatum* dynamics in El Mrissa site from March, 2014 to November, 2015

Table 1: Some physico-chemical parameters of surface waters of El Mrissa site during and outside *Amyloodinium ocellatum* bloom periods

Parameters	Bloom period	Outside bloom
Temperature (°C)	21.2-25.8	20.9±4.9
Salinity (psu)	35.5-35.6	35.0±2.8
pH	8.1-8.2	8.3±0.3
Orthophosphate (µg L ⁻¹)	0.8-0.9	2.9±2.2
Ammonium (mg L ⁻¹)	10.0-10.4	8.3±2.1

on phytoplankton populations and consequently, it is not easy to elucidate the interactions between Dinoflagellate-dominated blooms, like *A. ocellatum* and environmental parameters. To date, all the most of the reports on *A. ocellatum* come from tropical or subtropical waters but it has become evident that this fish ectoparasitic also occurs in the colder parts of the Mediterranean region and in the European Atlantic coasts¹². In this study, some similarities regarding the effect of temperature were found by Paperna²⁰, who observed that temperatures from 18-30°C are favorable for *A. ocellatum* to mont division and that sporulation at 23-27°C reproduction was most efficient. As previous studies, Kuperman and Matey²¹ demonstrated high tolerance to both elevated water temperature and salinity of this parasite which successfully reproduces in temperatures up to 40°C and salinities up to 46 psu. In terms of nutrients, El Mrissa site seems to be favorable to massive development of phytoplankton since it is a relatively confined area receiving

wastewater and housing an intense fishing activity which probably increase water eutrophication. The obtained results show that *A. ocellatum* proliferates under phosphorus limitation and high ammonium concentrations. In a recent work, Seoud *et al.*¹³ demonstrated that however there is a weak significant correlation between ammonia levels and *A. ocellatum* infestation in different marine farms in Egypt which is not in agreement with Kuperman and Matey²¹, who reported that the frequently high levels of ammonia in the Salton Sea (California, USA) may promote *A. ocellatum* and fish infection. In general, the view that algal proliferations occur in response to enhanced nutrient loading has recently been reviewed by Gowen *et al.*²², who found that the evidence relating HABs to anthropogenic nutrient enrichment was often equivocal. Moreover, there is substantial and growing evidence that the traditional use of dissolved inorganic nutrients concentrations or their molar ratios cannot be used as an indicator for the probable bloom occurrence for Dinoflagellate HAB species that are mixotrophs/heterotrophs²³, like *A. ocellatum*.

CONCLUSION

In conclusion, the occurrence of *A. ocellatum* was recorded for the first time in the North African Atlantic coast.

Although there are no associated fish epizootic events, the presence of such ichthyotoxic species is alarming even though in low concentrations. In fact, the regular monitoring of toxin-producing microalgae and studies on factors leading to their ecological success should be expanded to ectoparasitic Dinoflagellates. In addition, further molecular, ecophysiological and toxicological investigations are required to clarify the real potential risk of this microalga on Moroccan coastal waters.

SIGNIFICANCE STATEMENT

This study discovers, for the first time in the Atlantic coast of Morocco, the occurrence, morphological description and dynamics of *Amyloodinium ocellatum*. Despite that phytoplankton was mostly dominated by diatoms from winter to summer, *A. ocellatum* displayed temporal dynamics with highest autumnal dominance (50-100%) and cell abundances (up to 1×10^6 cells L^{-1}). Proliferations of this species appeared associated with relatively high water temperature and ammonium concentrations. Although there is no ichthyotoxic incident reported in the studied coastal region, the initiation of regular monitoring of *A. ocellatum* in the water column and among susceptible animal hosts is highly recommended to model the life cycle dynamics and to clarify the potential risks of this ectoparasite Dinoflagellate along the Atlantic and Mediterranean Moroccan coasts.

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