

Research Article

## Spatial and seasonal fluctuations of Ichthyoplankton assemblage in the Mediterranean coast of Morocco (Southwestern Alboran Sea)

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## Abstract

Ichthyoplankton represent the first life stages of fish. The study of ichthyoplankton is crucial to understanding marine ecosystems and plays an important role in the management and durability of fisheries resources. During March and October of 2019, two oceanographic ichthyoplankton surveys were conducted in the Mediterranean Sea of Morocco from Tanger to Saadia by studying the horizontal structure of the ichthyoplankton species assemblage and its relation to environmental parameters. The average surface water temperature was (15.8°C in spring and 16.4°C in autumn). The fish eggs and larvae were more abundant in March than in October (21268 eggs/10m<sup>2</sup> and 14084 larvae/10m<sup>2</sup> in spring and 10094 eggs/10m<sup>2</sup> and 13796 larvae/10m<sup>2</sup>). In both seasons, fish eggs from the families Sternoptychidae and Sparidae were dominant (10101 eggs/10m<sup>2</sup> and 7527 eggs/10m<sup>2</sup> in spring and 4422 eggs/10m<sup>2</sup> and 3928 eggs/10m<sup>2</sup> in fall, respectively). However, Myctophidae larvae were the most abundant in the study area, reaching 7601 larvae/10m<sup>2</sup> in spring and 11021 larvae/10m<sup>2</sup> in autumn. The environmental parameters: temperature, salinity and chlorophyll-a (surface) seem to directly influence the spatial distribution of ichtyoplancton. On the other hand, it seems that predation by jellyfish (*Pelagia noctiluca*)was a very important factor that added to the factors that influenced the distribution of the species of fish eggs and larvae. This work represents the first survey conducted in the southwestern Alboran Sea, which studies the horizontal structure of the ichthyoplankton species assemblage and its relation to environmental factors in the spring and autumn of 2019.

Keywords: Distribution, Environmental parameters, Ichthyoplankton, Marine ecosystems, Mediterranean Sea, Pelagia noctiluca

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## INTRODUCTION

The Moroccan Mediterranean coast is part of the Alboran Sea located in the southwestern basin of the Mediterranean Sea (Diouri et al., 2020). This region is characterized by primary productivity that increases seasonally (Idmoussi et al., 2021). These relatively the most productive areas provide suitable conditions for marine fish reproduction and habitat for the life cycle of small pelagic fish (Diouri et al., 2020; Idmoussi et al., 2021; Papaconstantinou et al., 2000; Agostini et al., 2002; Coombs et al., 2003 ; Lloretet al., 2004). Its hydrology is mainly conditioned by the Atlantic jet (AJ), which enters through the Gibraltar strait (Idmoussi et al., 2021, Bormans et al., 1989 and Lafuente et al., 1998). This jet contributes to the maintenance of two anticyclone gyres: the Western Alboran Gyre and the Eastern Alboran Gyre, conferring specific properties to this part of the Mediterranean Sea (Idmoussi et al., 2021, Arnone et al., 1990 and Vargas et al., 2002). Most fish pass through the pelagic stage, which lasts from weeks to months (Blabolilet al., 2023, Brothers et al., 1983 and Victoret al., 1986). Since the 20<sup>th</sup> century, understanding the population dynamics of marine fish's early life stages and the processes that influence their survival has been the interest of the fisheries oceanography. The new theoretical work identified that biodiversity, ecosystem functioning, and abiotic factors are interrelated (Gonzalez et al., 2020). Indeed, many scientists highlighted the influence of the spatial and temporal variations of the environmental parameters on the ichthyoplankton distribution (Lima et al., 2022; Badú et al., 2022; Randall et al.; 2019, Berraho et al.; 2005;Zarrad et al., 2003).

Identifying and comprehending the primary factors that impact the spatial distribution of marine fish are essential for evaluating the present distribution patterns of marine organisms (Diouri et al., 2021 ; Lasram et al., 2010). Seasonal variations of early life stages may drive changes in species distribution. During the early development of small pelagic species, environmental changes, such as temperature variations, ecosystem productivity and climatic forcing, impact survival rates and, thus, recruitment (Vargas et al., 2003; Santojanni et al., 2006; Palomera et al., 2007; Houde et al., 2008; Engelhard et al., 2014; Abdelouahab et al., 2016; Aldanondo et al., 2016). As per Cushing (1974), the main factor that affects the recruitment of fish is predation in their early life stages (Bailey et al., 1989), Jellyfish are often viewed as a threat to the survival of fish populations because they compete for food and directly prey on fish eggs and larvae (Brodeur et al., 2008, Mölleret al., 1980 and Purcell et al., 2001), especially Two species of fish are being referred to: the European anchovy (Engraulis encrasicolus) and the sardine (Sardina pilchardus)(Sabatés et al., 2000; Morote et al., 2010; Costalago *et al.*, 2012; Tilves *et al.*, 2016). This work represents the first study along the Moroccan Mediterranean coast related to the study of the composition of the ichthyoplankton assemblage to investigate the horizontal structure of the ichthyoplankton assemblage and its relationship with environmental factors in early spring and autumn. This objective is motivated by the particular hydrological status of this particular Mediterranean region as an Atlantic dynamic transition zone.

#### MATERIALS AND METHODS

## Collection and analysis of ichthyoplankton samples

Two oceanographic surveys were conducted in 2019, along the Moroccan Mediterranean coast from Tanger to Saadia, during the early spring and autumn seasons (March and October, respectively) on board the R/V Al Amir Moulay Abdellah. A total of 48 stations were sampled. The samples were collected between the coast and 500m isobaths (Fig.1). To collect plankton, and oblique hauls were conducted using a 147 $\mu$ m net with a 20 cm diameter. The hauls were carried out from a depth of 0 to 100 m and to the bottom in shallower waters. The volume of filtered water was measured using a flowmeter fixed in the net opening.

The ichthyoplankton samples were immediately treated with a 5% borax-buffered formalin solution for preservation. Later, all fish eggs/larvae were counted and identified to the lowest possible taxonomic level in the laboratory under a binocular loupe.

To normalize the number of egg and larval catches to a specific area of 10 square meters, the filtered depth and water volumewere taken into account. At each station, Sea Surface Temperature and Salinity were measured using a Neil Brown CTD (Conductivity-Temperature-Density) multisonde, the concentration of surface chlorophyll-a was measured by obtaining water samples through 1L-Niskin bottles, which were then analyzed using a 10-AU fluorometer (Idmoussi *et al.*, 2021).

#### Data processing

A standardized principal component analysis (PCA) was employed as the statistical treatment. To account for the differing nature and units of measurement of the hydrological parameters. Subsequently, an analysis of variance (ANOVA) was performed to test whether significant differences in abundance could be observed between different groups of stations. The density of ichthyoplankton was expressed per unit area according to the formula:

D = (N/Vf) \* H Eq. 1

Where N is the number of eggs or larvae counted, Vf is the volume filtered in  $m^3$  and H is the depth in meters reached by the net.

The existence of spatial and temporal variation in den-



Fig. 1. Study area where work was carried out in March and October 2019

sity was also tested by analysis of variance. The relationship between the characteristics of the environment and the presence of eggs and larvae of the different species was studied using a canonical correspondence analysis (CCA), the objective of which was to simultaneously process two tables of data (environmental variables and fauna variables) relating to the same surveys. The significance of the Canonical Correspondence Analysis (CCA) was assessed by performing a Monte-Carlo test. This test helps determine the stability and significance of the decomposition of the total inertia of the cross-tabulation by permuting only the rows of the environmental parameters table (Kazi *et al.*, 1995). This test showed a sign of the projected inertia at the 5% threshold for 1000 permutations.

#### **RESULTS AND DISCUSSION**

#### **Environmental conditions**

In spring, the mean sea surface temperature (SST) at 5m depth was  $15,8 \pm 0.38$  °C. A boundary was observed between the west and east of the Cape of Three Forks. Relatively colder water was found in the western part of the Cap Three Forks. However, (Fig.2a) illustrates that the proportion increases significantly as one moves from western to eastern regions. Regarding sea surface salinity (SSS) homogeneous distribution has been observed during this season with an average of  $36,5 \pm 0.15$  psu (Fig. 3a). The concentrations of chlorophyll-a surface at the surface level remained below  $0.5\mu g. I^{-1}$ ,

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compared to the east part, relatively higher concentrations of chlorophyll-a surface were recorded in the west part (Fig. 4a). In autumn, the mean SST was  $16.4 \pm 2.9$ °C. High SST (>20. 5°C) was observed near the coast, mainly in the east part of the Cape of Three Forks, the region of M'diq and Sidi Hssain (Fig. 2c). Contrary, low SST was detected in the west part and in the offshore area of the east part of the Cape of three Forks (<13.5° C). Concerning SSS, generally, an offshore-inshore decreasing gradient has been observed (Fig. 3c). Chlorophyll-a surface concentrations recorded in autumn were lower than those observed in spring. Towards the west, the coastal region between Fnid'q and Oued Laou was characterized by high concentrations (>0.7 µg.l<sup>-1</sup>) (Fig. 4c).

In the spring of 2019 (March), the horizontal profiles of temperature and salinity surface recorded in the study area showed a large variation between the western and eastern parts, while in the autumn of 2019 (October), the values of temperature and salinity in the surface layers were homogeneous throughout the study area and thus presents higher values of temperature and salinity. Surface salinity was constantly between 36.5 and 36.6 psu during October, whereas it was about 36.2 psu during March. The small variations recorded would be related to local coastal processes or freshwater inflow to the outlets of major rivers, especially during the March period on the Chefchaouen coast (Zarrad *et al.*, 2004) and (Berraho *et al.*, 2016). On the other hand, the highest salinities were concen-



**Fig. 2**.Relationship between ichthyoplankton density distributions and surface water temperature in spring and autumn 2019 (SST: Sea Surface Temperature, a and b:SST in March, c and d: SST in October)

trated on the land, especially in the Al Hociema-Nador region. They would be due to contact with more saline Mediterranean surface waters. In October, values were similar throughout the littoral zone of the Alboransea, with values higher than those recorded during the March period (>36.5 psu).In October 2019, surface water temperatures ranged from 19°C to 22°C. The same trends were observed for March 2019, but with lower surface temperatures than October, below 16° C. According to Renault et al. (2012) during the winterspring phase, two gyral scale features can be observed: the Western Anticyclonic Gyre (WAG) in the western area, which is a familiar phenomenon, and the Central Cyclonic Gyre (CCG) is an unknown new structure not yet reported and it expands from the centerto the eastern parts of the Alboran Sea, the circulation system during the summer-autumn phase is characterized by a stable double anticyclonic gyre regime, wherein the Eastern Anticyclonic Gyre (EAG) forms in the eastern Alboran basin. In this scenario, the Central Cyclonic Gyre (CCG) is situated closer to the Western anticyclonic Gyre (WAG) and is narrower. Surface waters were cooler in March in the west between Sebta and Al-Hoceima with values <16°C and warmer east of Al-Hoceima with values >16°C according to Idmoussi et al., 2021 (Idmoussi et al., 2021). In contrast to the October period, the entire study area showed higher values above 21°C than those recorded during March. The highest values were recorded east of the Cap of Three Forks (>22°C).

Average chlorophyll-a levels were higher in March than in October 2019 along the Mediterranean coast. Indeed, the primary production of chlorophyll a was more abundant in March between Sebta and Al Hoceima, where the combination of two phenomena enhanced its development during this period, that is, upward water fluxes from the Spanish coast and residual fluxes from continental waters. This would have caused a significant enrichment in nutrient salts and, consequently, chlorophyll 'a' in the surface waters at these sites, and this was also confirmed by the study by Idmoussi et al. (2021) carried out in the spring of the year 2018 in the same study area. In October 2019, measurements of chlorophyll-a showed that concentrations were generally low along the Mediterranean coast <  $0.01(\mu g.l^{-1})$  except for Tetouan and Nador coasts which present values more or less high between 0.01 and 0.08 ( $\mu$ g.l<sup>-1</sup>). Indeed, despite the very pronounced presence of the gyre and upwelling of the Spanish coast in this period of early autumn 2019, only the coast of coasts of Tetouan and Nador would have benefited from the effect of the gyre that was well-marked in the form of a filament arriving in the bay.

#### Eggs/larvae fish community

In spring, a total of 21268 eggs/10m<sup>2</sup> of fish from 7 families and 14084 larvae/10m<sup>2</sup> of fish of 14 families were identified, while in autumn, 10094 eggs/10m<sup>2</sup> of fish and 13797 larvae/10m<sup>2</sup> of fish belonging to 6 and 12 families, respectively were identified.





**Fig. 3.**Relationship between ichthyoplankton density distributions and surface water salinity in spring and autumn 2019 (SSS: Sea Surface Salinity, a and b: SSS in March, c and d: SSS in October)

In both seasons, fish eggs from the families Sternoptychidae and Sparidae were dominant with a percent abundance of 10101 eggs/10m<sup>2</sup> and 7527 eggs/10m<sup>2</sup> in spring and 4422 eggs/10m<sup>2</sup> and 3928 eggs/10m<sup>2</sup> in autumn, respectively. However, Myctophidae larvae were the most abundant in the study area, reaching 7601 larvae/10m<sup>2</sup> in spring and 11021 larvae/10m<sup>2</sup> in autumn. Concerning the family Sternoptychidae and Sparidae, there was a non-significant variation between seasons. Regarding the larvae, there was no variation in abundance between spring and autumn, except for some species that showed a significant variation, such as sardines and Sparidae. On the other hand, Clupeidae (Sardina pilchardus) and Engraulidae (Engraulis encrasicolus) showed a significant variation between seasons. Sternoptychidae eggs were present in more than half of the stations in spring, where the maximum egg density recorded was 1239 eggs/10m<sup>2</sup> in the M'dig area. However, in autumn, Sternoptychidae eggs were only found in a few stations in the western part, near the Mdi'g area and the Cala Iris area in the western part of Al Hoceima, also east of the Cap of The Three Forks (the maximum density did not exceed 1250 eggs/10m<sup>2</sup>). The majority of Sparidae eggs were found in the offshore area during March. The maximum density recorded was 7527 eggs/10m<sup>2</sup> and 3928 eggs/10m<sup>2</sup> in spring and autumn, respectively.In spring, the density of larvae of the family Myctophidae was 7601 larvae/10m<sup>2</sup>. The highest density was recorded in the east of M'diq with a value of 1999 larvae/10m<sup>2</sup>. However, in

autumn, the density was higher than in spring, with a 11021 larvae/10m<sup>2</sup> value. The larvae were more concentrated between Jebha and Al Hoceima and in the east of Nador, with values of 4521 larvae/10m<sup>2</sup> and 1152 larvae/10m<sup>2</sup>, respectively.

On the other hand, a significant difference between the seasons was observed for the Clupeidae and Engraulidae families. Indeed, in autumn, the egg density of *S. pilchardus* was 939 egg/10m<sup>2</sup> and 1234 egg/10m<sup>2</sup> in spring, however for the larvae, a value of 102 larvae/10m<sup>2</sup> in autumn and 1424 larvae/10m<sup>2</sup> in spring. Concerning *E. encrasicolus*, the lowest density values of eggs were recorded in autumn, while anchovy larvae were more concentrated in this autumn season with a value of 550 larvae/10m<sup>2</sup>.

# Relationship between the seasonal environmental parameters and the distribution of fish eggs and larvae

#### Eggs

During March, areas with high values of fish-egg density were concentrated in the east part and in some coastal stations in the western region of the study area where the SST registered was >15.5°C (Fig. 2a). Study area's sea surface salinity (SSS) gradually increased from March to October. In March, the waters with high fish-egg density were concentrated in areas where the SSS ranged between 36.2 and 36.7 psu (Fig. 3a), while in October, the high-density areas were found in waters with an SSS greater than 36.7 psu (Fig. 3c). The highest density of fish eggs in spring were recorded in areas where the values of chlorophyll-a surface were between  $0.01\mu$ g.l<sup>-1</sup> and  $0.35 \mu$ g.l<sup>-1</sup> (Fig. 4a), while in autumn, are recorded between  $0.01\mu$ g.l<sup>-1</sup> and  $0.07\mu$ g.l<sup>-1</sup> (Fig. 4c).

#### Larvae

In March, the fish-larvae density recorded in the western area was generally higher than in the Eastern area expected of some stations located in the offshore areas. During the study period, the density of fish larvae reached its highest level near Oued Laou, with a count of 2461 larvae per 10 square meters. Similarly, to the fish-egg density, larvae were concentrated in seawater areas of 16.1 - 16.7 °C (Fig. 2b), an SSS of 36.3 - 36.68 psu (Fig. 3b) and chlorophyll-a surface values between 0.02  $\mu$ g.l<sup>-1</sup> and 0.14  $\mu$ g.l<sup>-1</sup> (Fig. 4b). In autumn, larvae were more abundant than in March. Larvae density recorded varied between 29 and 5067 larvae.10m<sup>-2</sup>.



**Fig. 4.** Relationship between ichthyoplankton density distributions and surface water chlorophyll-a in spring and autumn 2019 (Chl-a: Chlorophyll-a, a and b: Chl a in March, c and d: Chl-a in October)



**Fig. 5.** Inter-species relationship of ichthyoplankton eggs (principal component analysis PCA) in spring and autumn 2019. Abbreviations:O: Eggs, O not identified: Eggs species not identified, O Total: Total eggs, Ichthyoplankton eggs families identified in spring (7 families):Engrau: Engraulidae, Clup: Clupeidae, Bothi: Bothidae, Solei: Solidae, Spari: Sparidae, Scomb: Scombridae, Sternopty: Sternoptychidae. Ichthyoplankton eggs familiesidentified in autumn (6 families): Sternopty: Sternoptychidae, Engrau: Engraulidae, Spari: Sparidae, Clup: Clupeidae, Bothi: Bothidae.

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**Fig. 6.**Inter-species relationship of ichthyoplankton larvae (principal component analysis PCA), in spring and autumn 2019. Abbreviations: L: Larvae, L not identified: Larvae species not identified, L Total: Total Larvae, Ichthyoplankton Larvae families identified in spring (14 families): Mycto: Myctophidae, Gobi: Gobiidae, Engrau: Engraulidae, Gadid: Gadidae, Spari: Sparidae, Par: Paralepididae, Gonosto: Gonostomatidae, Bothi: Bothidae, Clu: Clupeidae, Caran: Carangidae, Stomi: Stomidae, Solei: Soleidae, Call: Callionymidae, Loti: Lotidae. Ichthyoplankton Larvae families identified in autumn (12 families): Mycto: Myctophidae, Gobi: Gobiidae, Engrau: Engraulidae, Spari: Sparidae, Par: Paralepididae, Gobi: Gobiidae, Engrau: Engraulidae, Gadi: Gadidae, Spari: Sparidae, Par: Paralepididae, Gobi: Gobiidae, Engrau: Engraulidae, Gadi: Gadidae, Spari: Sparidae, Par: Paralepididae, Gobi: Gobiidae, Engrau: Engraulidae, Gadi: Gadidae, Spari: Sparidae, Par: Paralepididae, Gobi: Gobiidae, Clu: Clupeidae, Caran: Carangidae, Stomi: Stomidae, Bothi: Bothidae, Clu: Clupeidae, Caran: Stomidae, Solei: Soleidae, Bothi: Bothidae, Clu: Clupeidae, Caran: Carangidae, Stomi: Stomidae, Solei: Solidae

High fish-larvae density was observed in inshore areas in the western part and some stations in the offshore areas in the eastern part (>350 larvae.10m<sup>-2</sup>). The area with low SST (Fig. 2d), high SSS (Fig. 3d) (<14°C and >38 Psu) and chlorophyll a, surface values not exceeding 0.01µg.l<sup>-1</sup> (Fig. 4d) were characterized by a high larvae density this season.

Correlation analysis proved a strong negative significant relationship between the abundance of ichthyoplankton larvae and eggs collected and the high sea surface water temperature recorded in October 2019 (P < 0.05). In March, a strong positive correlation was found between chlorophyll-a and the abundance of larvae and eggs. In October, the ichthyoplanktonic range decreased, coinciding with the distribution of salinity (≥36.5 psu.) due to stronger currents driven by the flow of Atlantic surface water masses to the Alboran Sea through the Strait of Gibraltar and the lack of freshwater input during this period. The highest values of ichthyoplankton were recorded in March, a period coinciding with the coldest temperatures in the study area, concluding that the peak of ichthyoplankton was related to the cold seasons. These results agree with those found by Arkhipov et al. (2019) off the northern part of the Moroccan coast.

Based on the principal component analysis, ichthyoplankton eggs and larvae show statistically significant differences between the fall and spring periods. Indeed, these differences would be due to multiple factors influencing the ichthyoplankton community in this area. The distribution of eggs in fall and spring is presented in Fig. 5 and 6.

In addition, maybe the presence of jellyfish during this same period (October 2019) is another parameter that adds to explain the low abundance in October compared to March (Aouititen et al., 2021) and M'Diq Beach has experienced successive outbreaks of Pelagia noctiluca since 2011, during the summer and autumn season, which is also known as the season of flowering and beaching of jellyfish. These same results have been confirmed by (Canepa et al., 2014 and Mghili et al., 2020). This species of jellyfish was abundant on the Alboran coast (the Mediterranean), mainly in summer, on the continental shelf region where high abundances of Ichthyoplankton were found (Sabatés et al., 2004). According to Bailey et al.(1989), the primary factor influencing fish recruitment is predation in their early life stages, and a number of fish larval species have been impacted by predation from various types of jellyfish. They also compete with other zooplanktivorous species, including fish larvae (Purcell et al., 2001). Furthermore, as reported in reference, these jellyfish species directly impacted fish stocks as they feed on fish larvae and eggs (Purcellet al., 1985).

During the autumn, the (PCA) analysis reveals the presence of two well-identified groups of ichthyoplankton eggs. Steronodphychidae and Clupidae formed the first group. The second group is formed by the Bothidae, Soleidae, Engraulidae and Sparidae. Maybe this distribution was explained by the fact that the first group was important prey for jellyfish, Sternoptychidae and Clupidae are significant food sources for jellyfish, in the northeastern Atlantic Ocean, deep-water Sternoptychidae were found to be an important food source for pelagic cnidarians such as jellyfish (Wiebe., *et al.*2003). Sardines, a type of Clupidae, were also identified as important prey for several species of scyphomedusae in the same region (Brotz *et al.*, 2012). Similarly, in the Black Sea, both Sternoptychidae and Clupidae were observed to be among the most abundant prey for several species of jellyfish. These findings suggest that these fish families play a crucial role in the food chain of jellyfish populations in different marine ecosystems (Mavruk*et al.*, 2016 and Mavruk *et al.*, 2020).

In contrast, according to Hinrichsen et al. (2022) and Alheit et al. (2005), species in the second group have no direct relationship with Medusae, which are known to feed on small zooplanktonic organisms that are important food sources for the larvae of Bothidae, Soleidae, Engraulidae, Sparidae. Thus, changes in the distribution and abundance of medusae may indirectly affect the feeding behavior and survival of Bothidae, Soleidae, Engraulidae, and Sparidae larvae. For spring, the distribution of egg abundance does not follow any gradient. Also, we observed the appearance of eggs of a new family, namely Scombridae. This is probably favored by the absence of Medusae, which compete with Scombridae on the same prey (Graham et al., 2003). Concerning the larvae, the distribution of their abundance does not know a significant difference between autumn and spring because of their mobility all along the water column. Nevertheless, during the spring, there is an appearance of larvae of Lotidae and Callionymidae, which compete with jellyfish on the same prey (Aioiet al., 1981 and Ohman et al., 2012)

## Conclusion

Analysis of the distribution of ichthyoplankton has shown that these species are good indicators of spatial variations in salinity and temperature controlled by the hydrodynamic characteristics of the system. In the spring, the southwestern Alboran Sea represents a favorable environment for the reproduction of different species of fish eggs and larvae due to the hydrological characteristics of the area, which provides ichthyoplankton species with a favorable environment for their development. Contrary to the autumnal period which recorded lower values in the density of ichtyoplankton than March, coinciding with temperature values higher than those recorded in the spring as a result of the stability of the area in this period which makes the environment favorable for the proliferation of jellyfish which previous studies have indicated that the Moroccan Mediterranean Sea represents an important area of proliferation and reproduction of Pelagia noctiluca, probably the predation factor by this specie seems to have negative effects on the density and distribution during the autumn period in the study area, the continuous presence of this specie in the Moroccan Mediterranean Sea makes it necessary to set up effective strategies to prevent and minimize their impact on economic activities. A monitoring network should be set up along the Moroccan Mediterranean sea to observe and study the temporal and spatial distribution of larvae, fish, and P. noctiluca. This monitoring should concern their abundances, their fluctuations, and the dominant hydrodynamic conditions to predict the conditions favouring their abundance in the Moroccan Mediterranean Sea.

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## **Conflict of interest**

The authors declare that they have no conflict of interest.

## **Ethics approval**

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Hassan II University of Casablanca (2018-321).

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