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A comparative analysis of *Alexandrium catenella/tamarense* blooms in Annaba Bay (Algeria) and Thau lagoon (France); phosphorus limitation as a trigger

Analyse comparative des efflorescences d'Alexandrium catenella/tamarense en baie d'Annaba (Algérie) et dans l'étang de Thau (France) : limitation en phosphore comme élément déclencheur

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Abstract:

Environmental conditions ultimately leading to blooms of the toxic dinoflagellate *Alexandrium catenella/tamarense* were investigated at two Mediterranean sites (Annaba Bay, Algeria and Thau lagoon, France). Three years were examined in details: 1992 (a pre-*Alexandrium* period), 2002 (a year with the first bloom in Annaba) and 2010 (a year with a major bloom in Annaba). Most conditions were similar, but ammonium concentrations were much higher in Annaba (up to 100 µM) than in Thau (up to 10 µM). First records of *A. catenella/tamarense* were in 1995 for Thau and 2002 for Annaba, and coincided with soluble reactive phosphorus (SRP) decreasing below a concentration of about 1 µM. No other environmental variable could be related to those blooms. Thus, it is likely that the large reductions in SRP at both sites led to phosphorus limitation of a certain number of phytoplankton species and favored the development of *A. catenella/tamarense*.

Résumé

Les conditions du milieu amenant à des efflorescences du dinoflagellé toxique *Alexandrium catenella/tamarense* ont été suivies sur deux sites méditerranéens (la baie d'Annaba, Algérie et l'étang de Thau, France). Trois années ont été examinées en détail : 1992 (période pre-*Alexandrium*), 2002 (première efflorescence à Annaba) et 2010 (efflorescence majeure à Annaba). La plupart des conditions étaient comparables, sauf pour l'ammonium, avec des concentrations bien plus fortes à Annaba (jusqu'à 100 µM) qu'à Thau (jusqu'à 10 µM). Les premières observations d'*A. catenella/tamarense* ont été réalisées en 1995 à Thau et 2002 à Annaba, et ont coïncidé avec le passage des concentrations en phosphore réactif dissous (PRD) sous la barre des 1 µM. Aucune autre variable du milieu n'a pu être reliée à l'apparition de ces efflorescences. Il est donc probable que les fortes diminutions en PRD aux deux sites aient entraîné une limitation par le phosphore d'un certain nombre d'espèces phytoplanctoniques et favorisé le développement d'*A. catenella/tamarense*.

Keywords : *Alexandrium catenella/tamarense* ; Blooms ; Algeria ; France ; Mediterranean

Mots clés *: Alexandrium catenella/tamarense* ; Efflorescences ; Algérie ; France ; Méditerranée

1. Introduction

The genus *Alexandrium* belongs to one of the most important genera of harmful algal blooms (HABs) producing Paralytic Shellfish Poisoning (PSP) toxins [1]. *Alexandrium* species have a worldwide distribution, being present in coastal zones, continental shelf waters, in temperate as well as tropical areas [2]. The species *A. catenella, A. tamarense* and *A. fundyense* are the most toxic of the genus *Alexandrium*. Together, these species make up the *A. tamarense* complex [1] and [3]. Such species are often observed around the Mediterranean region [2]. In the northern part, *A. catenella* has first been reported in the Balearic islands in 1983 [4], and later on the French [5], Spanish [6] and Italian [7] coasts. In the southern part, *Alexandrium* species have been reported in Morocco [8], [9], [10] and [11], Tunisia [12] and [13], and Algeria [14].

A. catenella blooms in inorganic nutrient rich [6] and [15] and nutrient poor [16] and [17] waters. This is from the viewpoint of inorganic nutrients. The *A. tamarense* complex has been classified as a « frontal zone taxon » or « mixing-drift » group [18]. and therefore characteristic of areas that are unstable and intermediate between high and low inorganic nutrients. Very little is known generally about dissolved organic nutrients at those sites. It is therefore difficult to find recurrent patterns of bloom development for this mixotrophic species in relation with trophic conditions. Comparative studies of HABs ecosystems have been recognized as a way to deal with such difficulties and could "reveal fundamental processes governing population development" [19].

In this study, we compare *A.catenella/tamarense* dynamics in two contrasted sites in the Mediterranean. The first one is the relatively eutrophic [14] Bay of Annaba (Algeria) and the second is the Thau lagoon (France) that has recently gone through a period of oligotrophication [20]. We try to relate those blooms to environmental factors in those two coastal sites on both sides of the Mediterranean Sea, and deduce possible causes of their recent development and recurring presence. We study 3 different years over an 18 year period, one being "pre-Alexandrium", one coinciding with the first *A. catenella* bloom in Annaba and the last one with a very large *A. catenella* bloom in Annaba. As the Thau lagoon blooms were connected with oligotrophication, we selected a period over which the same phenomenon appeared to take place in Annaba.

2. Material and methods

2.1. Study sites

The Bay of Annaba is located in the eastern part of Algeria (Fig. 1), between cape Rosa (36°38'N, 8°15'E) and Cape de Garde (36°38N, 7°16'E), with a maximal depth of 50 m. The main temporary rivers (oueds) are Oued Seybouse (second river in Algeria), Oued Boudjemâa carrying urban and industrial effluents, and Oued Kouba carrying sewage waters. *Alexandrium catenella* has bloomed periodically since 2002 in this bay [14].

The Thau lagoon is a shallow marine lagoon located on the French Mediterranean coast (43°24'N, 3°36'E) covering 75 km^2 (Fig. 2). The mean depth is about 4 m, with a maximum of 10 m. The lagoon is connected to the sea by 3 narrow channels. Three oyster farming areas are located along the northwestern shore. The lagoon represents 10% of French annual oyster production and is the main oyster production center on the Mediterranean. Since

1998, it has experienced recurrent blooms of *Alexandrium catenella* that periodically threaten economic activities [20].

2.2. Sampling

Sampling stations for Annaba Bay

Station 1 (36°54.073'N, 7°46.929'E) has a depth of 5 m and is sheltered from dominant (NW) winds, with a rocky/sandy bottom.

Station 2 (36°53.976'N, 7°47.111'E) is deeper (13 m) than station 1 and is located in front of the commercial harbor. It is subject to major land influence through input from Oued Seybouse and Oued Boudjemaa and is considered eutrophic relative to station 1. Bottom sediments are silty. The sampling frequency was twice a month.

Sampling stations for Thau lagoon

Station B (43°26.070'N, 3°39.920'E) is located at one of the deepest part of the lagoon (8 m) Station A5 (43°26.916'N, 3°40.300'E) is located in the Angle Creek and is shallower (2 m) and more sheltered than Station B. The sampling frequency varied from 1 to 8 samples per month for physical variables. For chemical and biological variables, sampling was carried out at least twice a month at station B.

2.3. Physical variables

In Annaba, surface temperature and salinity were measured with a WTW 191 multiparameter probe (precision 0.1°C for temperature and 0.05 for salinity).

For Thau, the Ifremer observation network provided records of surface water temperature and salinity (monthly means).

2.4. Nutrients

At both sites, samples for ammonium determination were immediately fixed and measured at the laboratory [21]. Samples for the other nutrients were kept in the cold and dark, then frozen at -20°C until analysis. Nitrate [22], nitrite [23] and soluble reactive phosphorus (SRP)[24] were measured after thawing at room temperature. Detection limits were 0.05, 0.01, 0.05 and 0.02 µM for nitrate, nitrite, ammonium and SRP respectively.

2.5. Biological variables

Chlorophyll

In Annaba, chlorophyll a was measured by spectrophotometry [25]. In Thau lagoon, chlorophyll a was estimated from 90% acetone extracts and fluorimetry [26] or spectrofluorometry [27].

Microphytoplankton.

In Annaba, for the qualitative survey of phytoplankton, horizontal net tows were carried out at 50 cm below the surface, with a 20 µm mesh size net. During *Alexandrium* blooms and for the quantitative study, bucket sampling was used, and 40 liters were filtered on a 20 µm mesh size net. Samples were then resuspended in a 100 ml volume, then subsamples of 1 or 5 ml were used depending on the cell density. Samples were immediately fixed with buffered formaldehyde (5% final concentration). Identification was by electron microscopy in 2002 [14] and by optical microscopy in 2010, using morphological criteria. Both 2 cell and 4 cell chains were observed.

In Thau lagoon, at least one liter of sea water was taken with a sampling bottle. Additions of 0.2 ml to 0.4 ml Lugol's solution per 100 ml was carried out for quantitative analysis. A counting chamber was filled with 10 ml of fixed sample for sedimentation [28]. Phytoplankton cells greater than 10 µm equivalent cell diameter were counted by optical microscopy.

2.6. Statistical analyses

Variables were compared by Kruskal-Wallis one-way analysis of variance by ranks and Dunn’s multiple comparison tests.

3. Results

In 1992, *A. catenella/tamarense* had not yet been recorded either in Thau lagoon [5] or in Algeria [14].

3.1. *Alexandrium catenella/tamarense* blooms

Alexandrium catenella was first observed in Annaba in March 2002 and in Thau in July 1995. Temporal changes of cell densities during 2002 are shown for station 2 in Annaba and stations A5 and B at Thau (Fig. 3). Highest monthly mean cell densities (117000 cells L^{-1}) in Annaba were recorded in March with a secondary peak in December (200 cells L^{-1}). In Thau, monthly mean cell densities were generally higher at A5 (maximum of 66700 cells L^{-1} in May) than at station B (maximum of 5400 cells L^{-1} also in May), with a secondary peak in August (9600 cells L^{-1}).

In 2010, 2 stations were sampled in Annaba (Fig. 4) with higher cell densities at Station 2 (up to 681000 cells L^{-1} in May). At station 1, monthly mean cell densities never exceeded 73000 cells L^{-1} (also in May). For individual dates, a record peak of 1.38×10^6 cells L^{-1} was recorded in May 2010 at the surface of station 2. This was the highest cell density ever recorded so far in Annaba.

In Thau, during the same year, no bloom developed in the spring, but cell densities reached a maximum of 4400 cells L^{-1} in September at station A5 and 1500 cells L^{-1} also in September at station B.

In 2002, there appeared to be no synchrony in blooms between sites (Fig. 3), but in 2010, there was a bimodal distribution at both sites, with maxima in May-June and September-October (Fig. 4).

3.2. Environmental factors

Table 1 summarizes the ranges of physical and chemical variables at both sites. Sea surface temperatures were higher in Annaba than in Thau. The range of salinities was larger in Thau than in Annaba, with maximal values being greater in Thau lagoon. Maximal nitrate, ammonium and SRP concentrations were observed in Annaba, and were sometimes greater than in Thau by an order of magnitude, such as for ammonium. Concerning phytoplankton biomass, the range of chl a values was about the same.

Table 2 summarizes the changes in environmental parameters occurring over an 18 year period. The year 1992 can be considered as a “pre-Alexandrium” period for both sites. No significant changes in either annual mean SST, salinity (not shown) or ammonium occurred over this period at either site. In Annaba, the most spectacular changes were due to nitrate concentrations that decreased by a factor of 50 (significant difference with $p<0.001$) and specifically SRP concentrations that decreased by a factor of 250 (significant difference with $p<0.001$), from a mean annual value of 17.4 µM in 1992 to 0.07 µM in 2010. In Thau, the most dramatic decrease in SRP occurred between 1972 and 1992 (from about 10 to 1µM in summer and from 3 µM to undetectable levels in winter). Between 1992 and 2010, the decrease was significant ($p<0.05$).

4. Discussion

Although nitrate decreased dramatically between 1992 and 2010 in Annaba, nitrogen limitation is unlikely because ammonium levels remained high (Table 2). The decrease in SRP was due to the discontinuation of phosphogypsum outputs from a local fertilizer plant in Annaba (M. Retima, pers. com.), and from implementation of sewage treatment plants in Thau [29]. Concerning *A. catenella* ability to use low SRP levels, laboratory studies show that the half -saturation constant for SRP uptake was variable in cultures, depending on the growth rate, but could reach values as low as 0.03 μM for strain ACT03 and 0.01 μM for strain TL01 [30]. This indicates that *A. catenella* could be a very strong competitor at the low SRP concentrations recently observed in Annaba Bay. An alternative to its periodic dominance is dissolved organic phosphorus use. Alkaline phosphatase activity was induced in *A. catenella* at SRP levels between 0.4 and 1 μM [30] that are intermediate values between those observed in 2002 and 2010 in Annaba Bay.

Thus it seems very likely that the appearance of blooms of *A. catenella* in Annaba Bay is due to SRP reaching very low levels where other members of the phytoplankton community cannot compete for the acquisition of this limiting resource.

This situation is similar to that experienced in Thau lagoon [20] where SRP reached undetectable winter levels in 1992-1993 and *A. catenella* was first reported in 1995.

A parallel can also be made with the Seto Inland Sea (Japan) where SRP decreased from 0.7 to 0.2 μM between 1978 and 1984 [31]. *A. catenella* vegetative cells were first reported in this area in 1982 [32], so that *A. catenella* probably started to bloom when SRP reached levels between 0.7 and 0.2 μM. In the Gulf of Olbia (Sardinia, Italy), the first A. catenella bloom was reported in 1999 [33] under conditions of SRP below 1 μM that were lower than observed in 1992-1993, a situation also similar to the results presented here.

5. Conclusions

This inter-site comparison between the Northern and Southern coasts of the Mediterranean has revealed that one of the causes of the development of *A. catenella/tamarense* is the onset of inorganic phosphorus limitation in both environments that allowed this organism to develop periodically and produce recurrent blooms. No other environmental variable could be related to the emergence of this organism. Such an explanation is an alternative to the general concept that eutrophication leads to harmful algal blooms and may require a revision of coastal water management policies.

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Table 1. General physical and chemical features at both sites. Range of values (monthly means) over several years (1992, 2002 and 2010 for Annaba ; 1992 to 2010 for Thau). S : salinity ; SRP : soluble reactive phosphorus ; SST : sea surface temperature ; U : undetectable

Site	SST (°C)	S	NO_3 (µM)	NH_4 (µM)	SRP (µM)	Chl a (µg/liter)
Annaba	14.1-28.6	35.3-38.1	U-65	U-110	U-43	0.8-32.8
Thau	5.0-25.6	29.5-39.2	U-20	U-10	U-10	0.3-37.4

Table 2. Changes in environmental factors at two coastal sites North and South of the Mediterranean for 3 selected years over an 18 year period.

Seasonal distributions are available at http://www.st.nmfs.noaa.gov/nauplius/media/time-series/site__mediterranean-thau-lagoon-phy/copepodite/index.html for Thau and in [34] and [14] for Annaba respectively in 1992 and 2002.

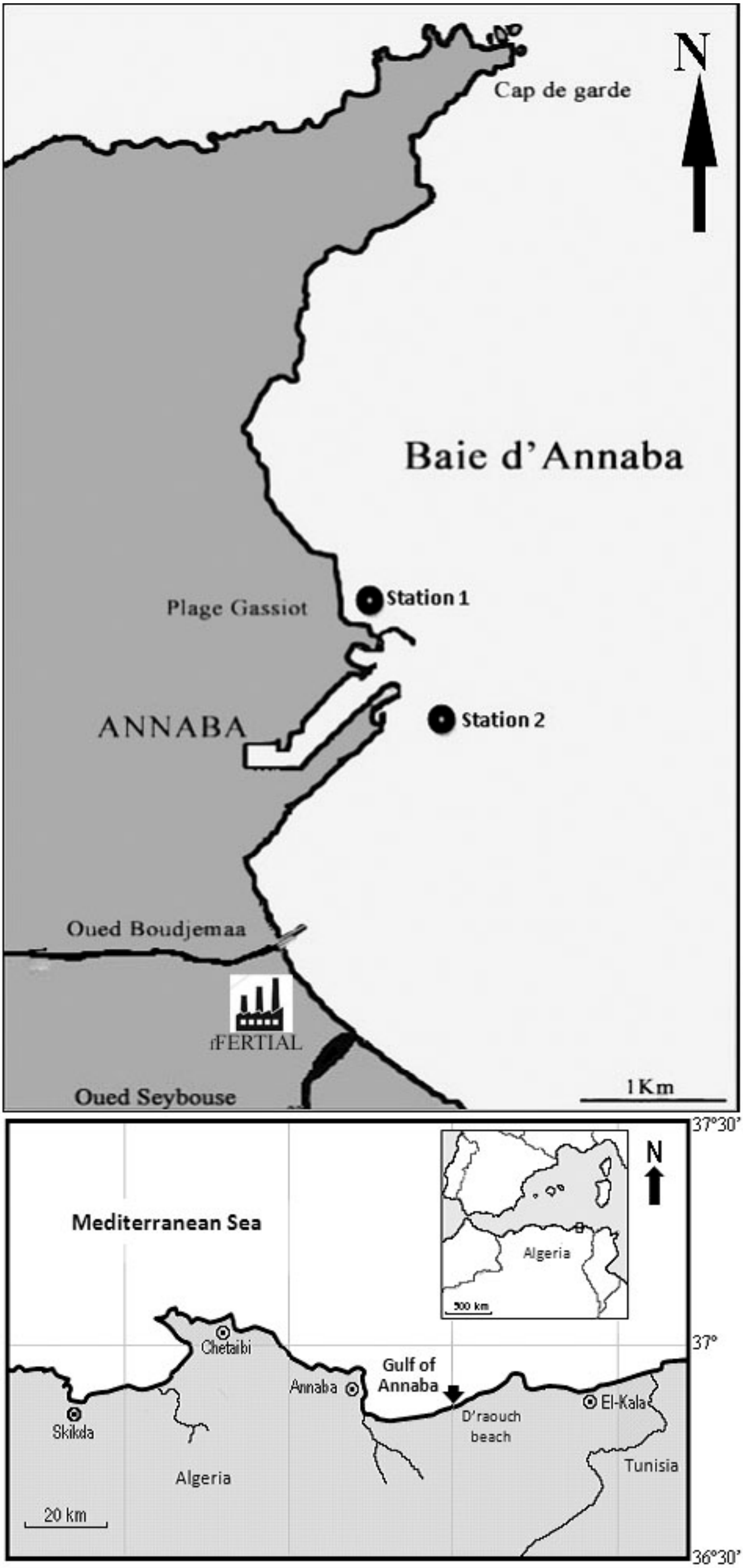
NA: not available; P: phosphorus; SD: standard deviation; SST: sea surface temperature.

Year	SST			nitrate			ammonium			soluble reactive P			Chlorophyll a		
Annaba	(°C)			(µM)			(µM)			(µM)			(µg/liter)		
	Mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
1992	21.6	4.7	8	25.8	20.5	8	49.9	37.7	8	17.4	14	8	10.3	9.5	6
2002	19.5	4.1	12	1.9	3.0	12	11.6	15.3	12	1.2	1.8	12	9.0	11.3	11
2010	20.7	4.8	11	0.5	0.9	12	23.6	24.3	11	0.07	0.13	11	2.4	1.4	11
Thau															
1992	18.4	7.1	12	0.5	0.5	11	1.4	3.1	10	1.2	1.2	10	1.5	0.6	9
2002	15.9	5.6	12	0.2	0.3	3	1.1	0.7	10	0.5	0.2	10	3.0	1.5	12
2010	15.1	6.9	12	0.2	0.1	3	0.7	0.6	3	0.2	0.1	3	2.2	1.1	11

Figure legends

1. Station positions in Annaba Bay, Algeria.
2. Station positions in Thau lagoon, France.
3. Changes in monthly mean *Alexandrium catenella/tamarense* cell densities during 2002. Diamonds : Thau station A5 ; squares : Thau station B ; triangles : Annaba station 2. Detection limits: 100 cells L^{-1}.

4. Changes in monthly mean *Alexandrium catenella/tamarense* cell densities during 2010. Diamonds : Thau station A5 ; Squares : Thau station B ; triangles : Annaba station 2 ; circles : Annaba station 1. Detection limits: 100 cells L^{-1}.



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ANNABA
Station 2
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Oued Seybouse
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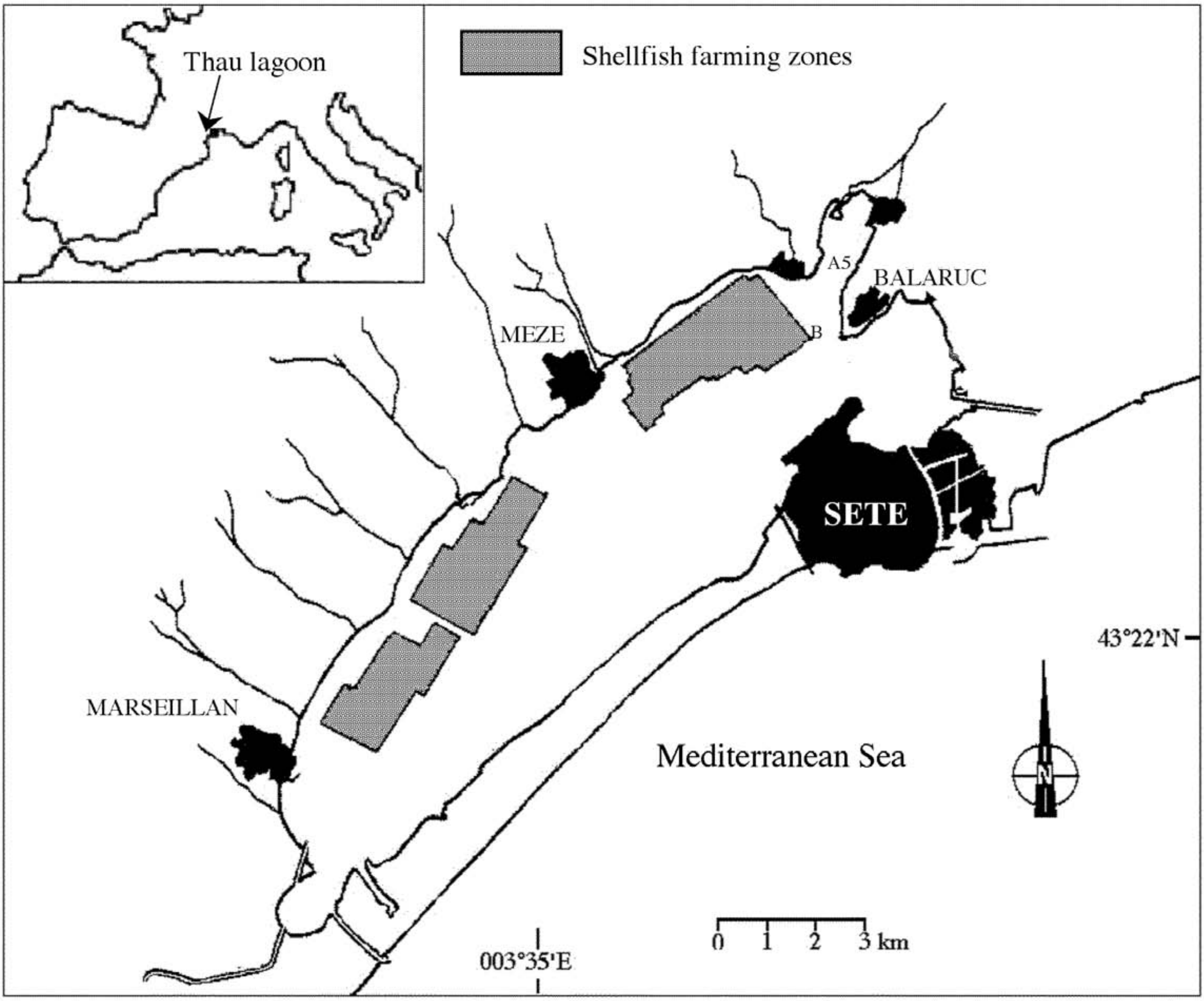
Mediterranean Sea
N
Algeria
500 km
37°
Chetaibi
Gulf of Annaba
Annaba
Skikda
D'raouch beach
El-Kala
Algeria
Tunisia
20 km
36°30'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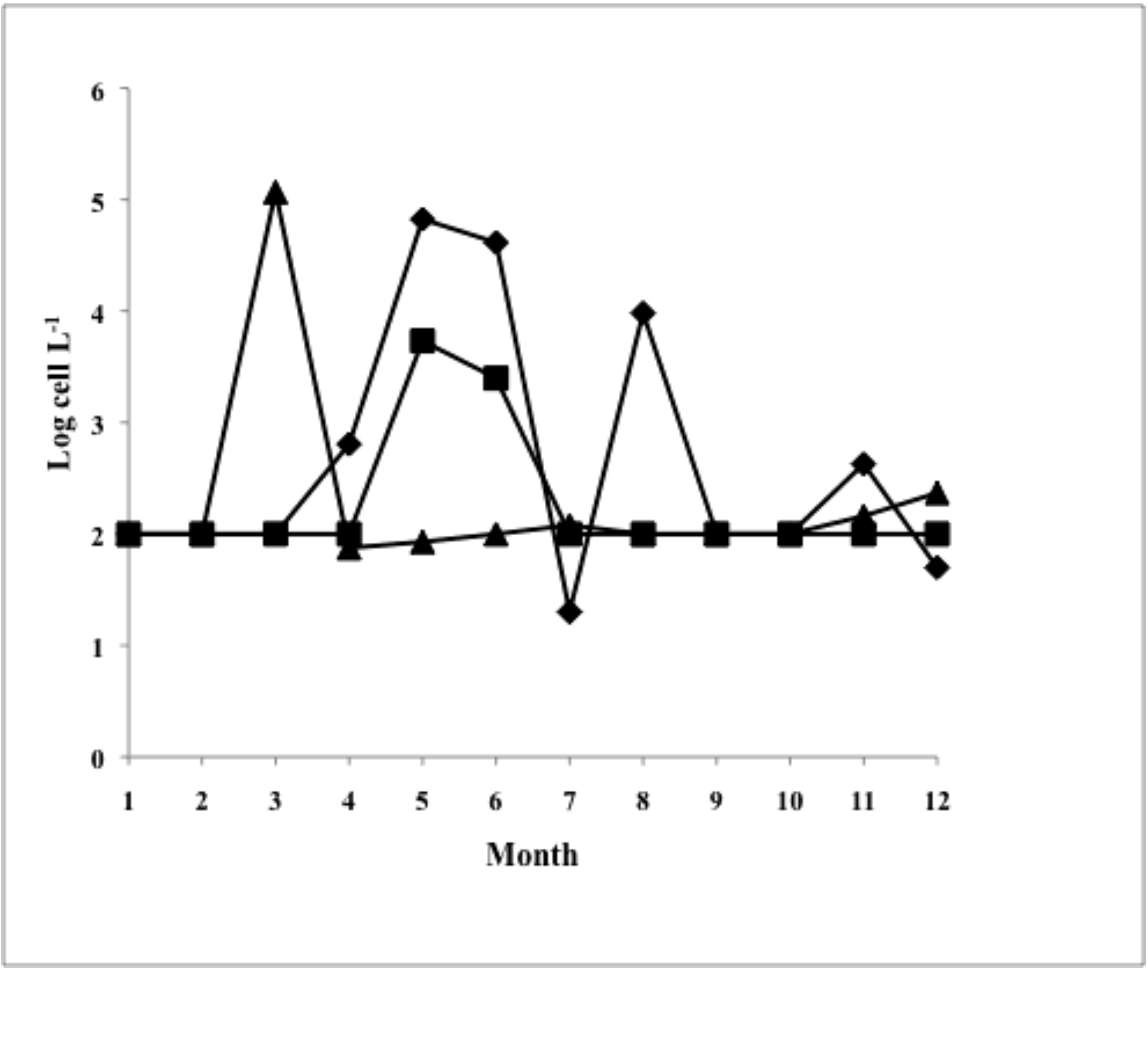

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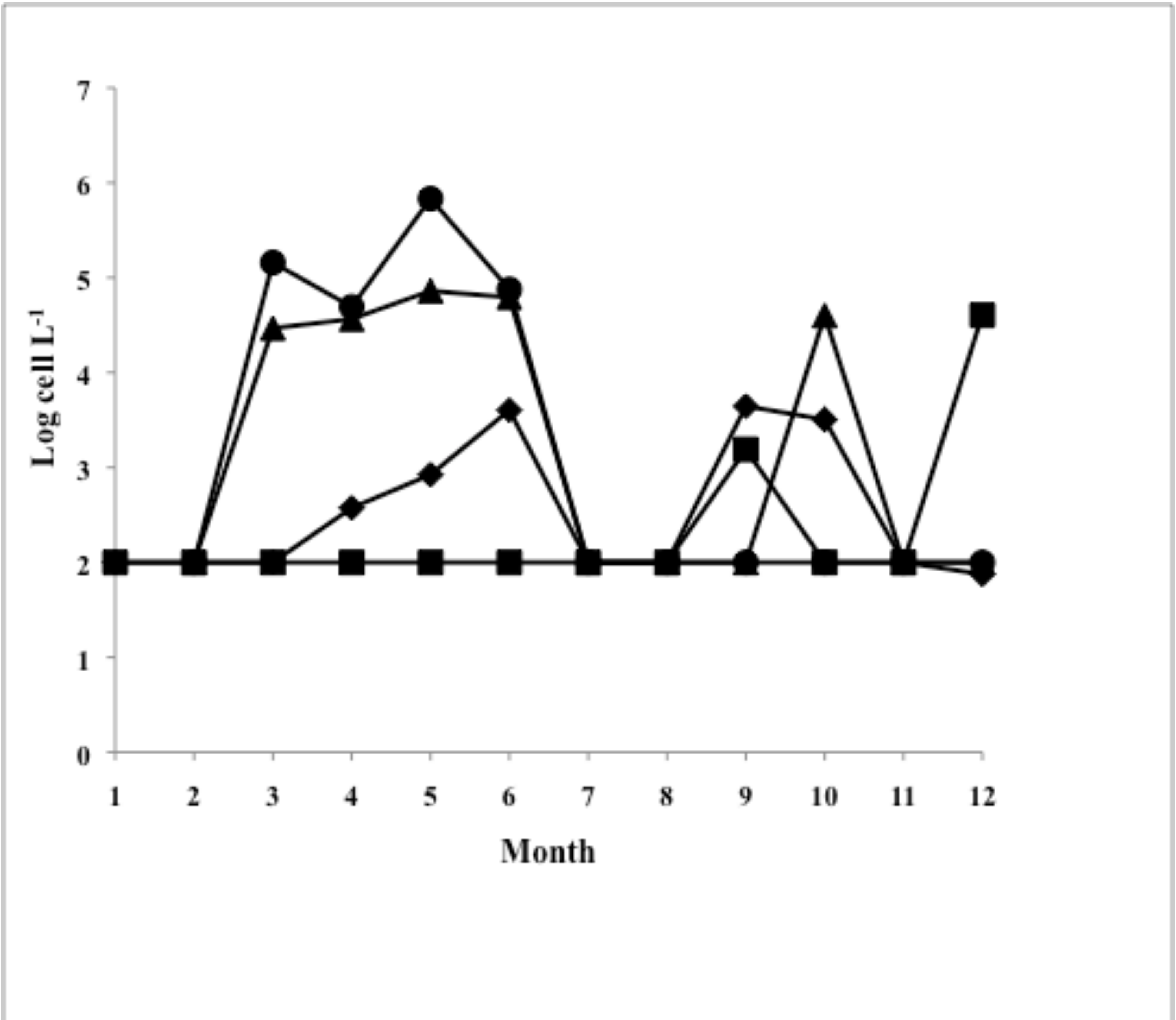

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