

**Bloom dynamics of the newly described toxic benthic dinoflagellate
Ostreopsis fattorussoi along the Lebanese coast (Eastern Mediterranean)**

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ABSTRACT:

This study involves monitoring both benthic and planktonic abundances of the toxic dinoflagellate *Ostreopsis fattorussoi* in 5 stations along the Lebanese coast (Eastern Mediterranean); it extended from June 2014 to July 2016 and aims to study the dynamics of its development. Results revealed its presence year round along the coast, presenting seasonal blooms, both epiphytic and planktonic. A major bloom (maximum of 880 694 cells/g FW and 18 000 cells/L) occurred early in the summer (June-July), followed by a minor one in the fall (October-November) ($\leq 50\ 000$ cell/g FW). Its development seemed influenced by a combination of multiple environmental parameters. Water temperature seemed to play a major role in its development, as it appeared to be a thermophilic species, with maximum abundances per site reached between 27°C and 30.5°C. Its development could be facilitated by some agitation resulting from winds. Results concerning the role of nutrients and other environmental parameters remain inconclusive.

Keywords: *Ostreopsis fattorussoi*, Benthic HABs, Dinoflagellates, microphytobenthos, Lebanese coast, Eastern Mediterranean.

1. INTRODUCTION:

The geographical distribution of the benthic dinoflagellate *Ostreopsis* spp. covers tropical and subtropical areas (Ballantine et al., 1988; Grzebyk et al., 1994; Parsons and Preskitt, 2007; Tindall and Morton, 1998) as well as temperate areas in New Zealand (Chang et al., 2000; Rhodes et al., 2000), Australia (Pearce et al., 2001), Japan (Taniyama et al., 2003) and the Mediterranean Sea (Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2011; Vila et al., 2001). Some studies suggest that it was possibly introduced from tropical to temperate areas via ship ballast water (Butrón et al. 2011), or as a result of the anthropogenic global climate change and warming of sea water (Maso and Garcés, 2006; Miraglia et al., 2009), whereas others suggest it could be indigenous to the Mediterranean Sea (Tognetto et al., 1995).

Two species, *Ostreopsis* cf. *siamensis* J. Schmidt and *Ostreopsis* cf. *ovata* Fukuyo, have been known for years in the North Western Mediterranean Sea; the latter in particular has formed blooms in the area (Mangialajo et al., 2011; Tognetto et al., 1995) and is the subject in most available literature. Its presence was first reported in the Mediterranean Sea in the bay of Villefranche-sur-mer (France) in 1972 (Taylor, 1979). Both species are known to produce palytoxin-like metabolites. Palytoxin (PTX) is among the most potent known naturally produced toxins of marine origin even at extremely low concentrations (Amzil et al., 2012; Moore and Scheuer, 1971) and has been linked to several human intoxications in tropical areas (Alcala et al., 1988).

Since the early 2000s, *Ostreopsis* spp. has emerged as a threat to public health in the Mediterranean Sea. Cases of human intoxication have been linked to this genus in the Western Mediterranean

Sea (Durando et al., 2007; Illoul et al., 2012; Kermarec et al., 2008;; Pfannkuchen et al., 2012; Vila et al., 2008 and 2016). In some cases, more than 200 individuals were affected in one bloom episode, e.g. Genoa in 2005 (Durando et al., 2007) and Algeria in 2009 (Illoul et al., 2012). The absence of reports of intoxication in the Eastern Mediterranean Sea could be due to undiagnosed cases or is simply a reflection of the lack of related studies in the area. The intoxication occurs either through direct contact with cells or through inhalation of aerosols (Ciminiello et al., 2006 and 2014; Sansoni et al., 2003; Tartaglione et al., 2015; Tichadou et al., 2010). The affected population is therefore not limited to swimmers and people involved in water activities, but also includes residents and people in the vicinity of the bloom area who are not in direct contact with the water. Reported symptoms in the Mediterranean Sea are mild and include irritation to the eyes, nose, skin and respiratory system, fever, while respiratory distress was reported on a few rare cases (Ciminiello et al., 2006; Simoni et al., 2003; Tichadou et al., 2010; Vila et al., 2016; Durando et al., 2007). In addition, PTX and PTX-like metabolites have been found in edible marine organisms from different trophic levels (Biré et al., 2013 & 2015). For these reasons, despite the fact that cases of intoxications through ingestion of seafood have not yet been reported in the Mediterranean Sea, they remain a potential risk. These toxins also represent a threat to the ecosystem itself, as they have been linked to a mass mortality (Illoul et al., 2012), decrease in population (Blanfune et al., 2012; Shears and Ross, 2009) and a negative impact on reproduction of some marine fauna (Guidi-Guilvard et al., 2012). As the occurrence of *Ostreopsis* blooms is accompanied by closing of beaches (Tichadou et al., 2010) and negative press, the consequences extend into the economy. An estimated “worst case scenario” could translate into losses of up to millions of euros in tourist areas such as the South East of France (Lemée et al., 2012) and this could be expected in other areas where the economy is based on fishing, aquaculture, ports and/or seasonal tourism attracted

by coastal activities. As a result, the genus *Ostreopsis* has been studied extensively in the Western Mediterranean Sea in the last two decades. The biotic and abiotic factors driving its development are still not fully understood. In the Eastern Mediterranean Sea, related studies remain scarce. In Lebanon, the genus *Ostreopsis* has been detected since 1979 (Abboud-Abi Saab, 1989). In a previous study, it was identified as *Ostreopsis* cf. *siamensis*, based on morphological characteristics (Abboud -Abi Saab et al., 2013); but in 2016, Accoroni et al., using molecular analysis, found mainly the new species named *Ostreopsis fattorussoi* Accoroni, Romagnoli & Totti and very few *Ostreopsis* cf. *ovata*. The presence of this last species was almost negligible (Casabianca S. and Penna A. pers. comm., in Accoroni et al., 2016). It was then likely, but not certain, that *Ostreopsis* cf. *siamensis* cited by Abboud-Abi Saab et al. (1989) was a misidentification. The geographic distribution of *O. fattorussoi* also includes Cyprus, and the same genotype has also been reported prior to its description in Greece, and outside the Eastern Mediterranean in Spain and in Puerto Rico (Penna et al., 2010,2014; David et al., 2013; Accoroni et al., 2016, Tartaglione et al., 2016). As for toxicity, three of the five strains of *O. fattorussoi* from Lebanon were found to be toxic, producing ovatoxins (OVTX): OVTX-a and structural isomers OVTX-d and -e, so far found only in *O. cf. ovata* (Accoroni et al., 2016). The same was found for other strains from Cyprus, whereas others produce three exclusive palytoxin-like compounds (OVTX-i, OVTX-j1, OVTX-j2, and OVTX-k; Tartaglione et al., 2016). It is therefore crucial to study the ecology of this newly described species. The present work is the first to study the ecology of *O. fattorussoi* in Lebanese coastal waters (Eastern Mediterranean) based on both epiphytic and planktonic abundances, through investigation of their temporal and spatial distribution along the coast and their relation to environmental factors. It extends from June 2014 to July 2016, thus covering 3 summer bloom periods in 5 sites along 172 km of the coastline.

2. MATERIALS AND METHODS

2.1. Study area and sampling frequency

Lebanon is situated in the East central part of the Levantine Basin (Eastern Mediterranean), with 220 km of coast, from Aarida in the north to Naqoura in the South. The continental shelf is very narrow 3 to 4 km (Pfannenstiel, 1960), with a mean depth of 20 m to 40 m (Goedicke, 1972). The bottoms are rough with wide rocky patches (Majdalani, 2004), potentially providing support for proliferation of benthic biota. The general circulation along the coast of Lebanon is dominant northwards during most of the year along with the general counterclockwise current gyre of the Eastern Mediterranean (Goedicke, 1972). This current progressively loses force as it approaches eastwards (Nakhlé, 2003). The tide is semidiurnal and is among the lowest in the Mediterranean with a maximum range of 15 to 20 cm (Abboud-Abi Saab, 1985). The current is locally modified by the configuration of the coastline and the topography of the narrow continental shelf. Water movement along the coast is also strongly related to surface currents and seasonal meteorological factors (Goedicke, 1972). As a result, the small scale hydrodynamism is dominated by local winds. All sampling sites were in exposed rocky shores where west winds can easily be associated to waves and then be a representative of local agitation. Preliminary sampling was conducted in May 2014 at eleven sites in order to survey most rocky beaches of the Lebanese Coast. It revealed the presence of *O. fattorussoi* in all eleven sites. The monitoring campaign that followed starting June 2014 was limited to five of the sites due to logistic constraints (such as site accessibility year round). The five sites were distributed from North to South and are thus representative of the entire coast. They are labeled as follows (North to South): TRI in Anfeh (close to Tripoli), BAT in Batroun, BYB in Byblos-Jbeil, BEY in Beirut and NAQ in Naquoura (Figure1, Table 1). Sampling

was conducted on weekly basis at BAT, and on monthly basis at the remaining sites until December 2015. Additional sampling was conducted at TRI and BAT in the spring and summer of 2016 (Table 1). Streams nearby BYB and TRI are seasonal as most of the water comes from rain and snow melting in the nearby mountains. They potentially bring in municipal wastewater and agricultural effluents rich with nutrients. In the presence of Northern winds, BAT can be affected by the discharges from a chemical plant located 3.9 Km its North (Abboud-Abi Saab, 1985). BYB and TRI are nearby beach resorts. NAQ and BEY are in proximity of small ports.

2.2. Environmental parameters:

Air temperature (T_{air}) was measured *in situ* using a mercury thermometer. Wind speed (WS) in m/s, and total precipitation in the week preceding sampling date (Rain) were obtained from the website windguru.cz. Wind speed was used as a proxy of water agitation at the sampling date. Maximum wind speeds reached in the previous week (WS_{max}) of sampling were also used in each sampling site in order to analyze influence of previous conditions on *Ostreopsis* development. Water temperature (T_{water}) was measured at each site using a mercury thermometer at 10 cm depth. Salinity was measured using the Beckman © RS7-C salinometer. Samples were also collected for the measurements of three nutrients: Nitrites (NO_2^-), Nitrates (NO_3^-) and Orthophosphates (P- PO_4). They were kept at -20°C until they were analyzed within a month. Nitrites (NO_2^-) concentrations were measured following the methodology described by (Bendschneider and Robinson, 1952) and Nitrates (NO_3^-) were obtained according to the methodology described by (Strickland and Parsons, 1968) with a slight modification by Grasshoff (1964). Orthophosphates (P- PO_4) concentrations were measured following the methodology described by (Murphy and Riley, 1962).

2.3. Biological parameters

Sampling

Ostreopsis fattorussoi being a tychoplanktonic species, both benthic (macroalgae) and planktonic (sea water) samples were collected, in triplicates at three sampling stations assigned 10 m apart on each site. Planktonic samples were collected first, at 20 cm depth (30 cm above the macroalgae) using plastic bottles of 250 ml, with minimal disturbance to the substrate in order to avoid the resuspension of epiphytic *O. fattorussoi* cells into the water column. For the benthic samples, 9.5 ± 6.0 g of macroalgae were collected along with some surrounding water at the same spot at 50 cm depth, using plastic bottles of 250 ml. Typically, the red macroalgae *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders was collected. The choice of macroalgae (Table 1) was based on dominance per site and availability on all sites, and was maintained for the most part throughout the sampling period to limit the influence of different substrata that has been reported by several authors (Totti et al., 2010; Vila et al. 2001). Lugol solution at 1% vol/vol was added to the samples for preservation and they were stored at 4°C.

Quantification of *O. fattorussoi*

For planktonic samples, after homogenization, subsamples of 50 ml were settled in a counting chamber for 24 hours, according to Utermöhl (1958). Counting was performed using an inverted microscope Wild M40. Cell abundances were expressed in number of cells per liter (cells/L). For benthic samples, the sample was shaken vigorously for 10 seconds in order to dislodge the cells from the macroalgae. The macroalgae was then separated from the water using a 200 µm mesh sieve, and rinsed twice with 100 ml of filtrated sea water, for the removal of any remaining epiphytic *O. fattorussoi* cells. The total water (sample + rinsing water) was homogenized using a Motoda box. This box is a splitting apparatus (often used for zooplankton samples) allowing the

separation of homogeneous subsamples (Motoda, 1959). One final 50 ml subsample was kept at 4°C. Counting was done using a 1ml Sedgwick-Rafter © counting chamber and an optic microscope (Motic BA 400). Cell abundance was expressed in number of cells of *O. fattorusoi* per gram of fresh weighed macroalgae cells/g FW. Identification of *Ostreopsis* cells at the species level was carried out in some samples through observation of thecal plates' arrangements using calcofluor staining and epifluorescence microscope (Figure 2), since *O. fattorusoi* does show some morphological features that lead unequivocally to its identification (Accoroni et al., 2016). At least two samples per site, selected from different bloom events during different seasons were observed for this purpose.

2.4. Statistical analysis:

All statistical analysis was applied using R programming language version 3.3.2 and its user interface R studio. Weekly data obtained in BAT were included in the graphs but excluded from statistical analysis as certain corresponding environmental data were not available; monthly data from this site were included in the analysis. As *O. fattorusoi* was sampled in triplicates, the site average was used in the dataset. The Shapiro test of normality was applied to each variable (benthic and planktonic *O. fattorusoi* abundances as well as all available environmental variables). Accordingly, the non-parametric spearman rank correlation test was applied to a matrix including all the above mentioned variables in order to determine their relationships. The same test was repeated per site. Kruskal–Wallis non-parametric test of the analysis of variance was applied to both planktonic and benthic abundances of *O. fattorusoi* in order to evaluate possible spatial variations between the sites; a Friedman non-parametric repeated measurement test was also applied at each site to determine temporal variability. A PCA (Principle Component Analysis) was

applied using environmental variables as active variables, benthic and planktonic abundances of *O. fattorussoi* as quantitative illustrative variables, and sites as qualitative illustrative variable. Analyses were based on Euclidean distance-transformed data.

3. RESULTS

3.1. Environmental parameters

Climatological parameters

Overall, measured air temperature (T_{air}) varied between a minimum of 14°C (BAT and BYB, Jan 2015) and a maximum of 36.3°C (NAQ, May 2015) (Table 2). It varied following a typical seasonal pattern in all 5 stations as the highest temperatures were recorded in the summer and lowest in winter (Figure 3(a)).

Wind speed ranged between a minimum of 0 m/s (Dec 2015, BAT) and a maximum of 13 m/s (Feb 2015, NAQ) (Figure 3(b)). Maximum Wind speed recorded in the previous week (WS_{max}) ranged between 3 m/s (Dec 2015, BEY) and 17 m/s (Jan 2015, BAT). WS_{max} was generally lower in the autumn months. WS_{max} was also highest in the most northern site TRI, (Figure 3(c)). Total precipitation during the previous week reached a maximum of 125 mm/week (NAQ, Jan 2015), followed by a max of 79.9 mm/week in BAT (Jan 2015) (Figure 3(d)). Summer was the dry season and maximum precipitation for each site was in January.

Hydrological parameters

Water temperature (T_{water}) ranged between a minimum of 17 °C (BAT, Mar 2015) and a maximum of 32°C (BAT, Aug 2014). In all 5 sites, T_{water} variation followed a typical seasonal pattern with the highest temperatures in the summer and lowest in winter (Figure 4(a)). In TRI the autumn of 2014 water temperature remained high throughout autumn. High water temperatures also stood

out at BAT in the summer of 2014 and spring of 2016. In each site, water temperature corresponding to the maximum of abundance of *O. fattorussoi* (T_{Cmax}) never exceeds the maximum temperature (T_{max}) reached on site (Figure 5 (a)). A gradient of T_{Cmax} vs. latitude was observed, as T_{Cmax} increases from south to north (Figure 5(b)). The average measured salinity was 38.6 ± 1.3 , ranging between a minimum of 34.5 (Jan 2015, BYB) and a maximum of 39.6 (Oct 2014, TRI). Salinity was higher in the most northern sites TRI and BAT as it rarely drops below 38.5; It was lower in NAQ (South) and BYB as it barely reaches 39.1 (Figure 4(b)).

Nutrients

Nitrites concentration ranged between a minimum of 0.008 $\mu\text{mol/L}$ (TRI, Jun 2015) and a maximum of 0.37 $\mu\text{mol/L}$ (NAQ, Aug 2014) (Table 2). They were generally highest in BEY, and lowest in TRI and NAQ (Figure 6 (a)). Nitrates concentration ranged between a minimum of 0.06 $\mu\text{mol/L}$ (TRI, Mar 2016) and a maximum of 25.12 $\mu\text{mol/L}$ (NAQ, Jan 2015) (Table 2); it was highest in NAQ and BYB and lowest in BAT and TRI (Figure 6(b)). Orthophosphates concentration ranged between a minimum of 0.01 $\mu\text{mol/L}$ (BEY, Sep 2014) and a maximum of 3.53 $\mu\text{mol/L}$ (NAQ, Aug 2015) (Table 2). They remained below 0.5 $\mu\text{mol/L}$ in all sites except for occasional peaks in the summer (Figure 6(c)).

3.2. Temporal and spatial variation in *O. fattorussoi* abundances

Observation of thecal plates' arrangements confirmed the exclusive presence of a single species: *Ostreopsis fattorussoi*. The species demonstrated a perennial presence in all the years of the study along the Lebanese coast, both in planktonic and benthic samples, although it was occasionally undetected in some of the sites during the winter (Figures 7 & 8). Lowest abundances were

recorded typically in winter months, and increases in abundance were noted starting early spring (Figure 8). The most important benthic blooms in terms of maximum abundances reaching more than 200 000 cells/g FW (i.e. major blooms) occurred almost exclusively during the summer. Less intense blooms with maximum abundance not exceeding 50 000 cells/g FW (i.e. minor blooms) occurred in autumn, with the exception of one autumn bloom at NAQ in 2014 which reached 228 257 cells/g FW. In 2014, important benthic summer blooms occurred at all of the sites (often more than 200 000 cells/g FW), whereas in 2015, such abundances were only recorded at TRI, BYB and NAQ. In 2016, data was only available for TRI and BAT; in both cases, summer blooms of 2016 (TRI and BAT) were minor in terms of abundance (< 50 000 cells/g FW). In addition, minor spring blooms were detected in BAT (April and May). Planktonic abundances generally followed the same pattern as the benthic ones. In addition, planktonic blooms were observed in BAT and BEY in the summer of 2015, where benthic blooms were not detected (Figure 7). Yearly maximum abundances were variable; in 2014, they reached 880 694 cells/g FW (BEY, June) and 9 000 cells/L (BAT, June & July), as opposed to 397 242 cells/g FW (BYB, July) and 18 065 cells/L (NAQ, June) in 2015, then 53 228 cells/g FW and 4 346 cells/L (BAT) in 2016. The ANOVA Friedman test showed no significant difference in cell abundances in either of the sites, neither in terms of seasons nor in terms of years ($p > 0.05$). Spearman rank correlation test showed a very strong significant correlation between benthic and planktonic *O. fattorussoi* ($r = 0.88$, $p < 0.01$) (Table 3).

In 2015, weekly sampling in BAT allowed the detection of two consecutive major blooms, during the second half of June then during the second half of July. In 2016, minor summer blooms occurred in both sampled sites, reaching their maximum abundances of 32 262 cells/g FW (BAT) and 45 398 cells/g FW (TRI) mid-June. Planktonic abundance followed the same pattern with a

relatively high maximum of 4 346 cells/L (BAT). Spring blooms were detected in BAT starting in April and reached a maximum benthic abundance of around 50 000 cells / g FW both in April and in May.

The Kruskal-Wallis test showed that no significant difference was noted between the 5 sites for both benthic (Kruskal-Wallis chi-squared = 104.04, p-value > 0.05) and planktonic abundances (Kruskal-Wallis chi-squared = 70.061, p > 0.05).

Relation between *O. fattorussoi* abundances and environmental parameters

Water temperature was strongly correlated with benthic abundances of *O. fattorussoi* ($r = 0.61$, $p < 0.01$) and moderately correlated with planktonic abundances ($r = 0.49$, $p < 0.01$) when considering all 5 sites; however, it varied between the sites from very strong in TRI ($r = 0.8$, $p < 0.05$) to non-significant in NAQ ($p > 0.05$) (Table 3). A weak positive correlation was noted between wind speed and planktonic abundances ($r = 0.26$, $p < 0.01$), but none with WS_{max} . Strong negative correlation with precipitation was noted only in BEY (Table 3). No significant correlation was noted with the measured nutrients, with the exception of a moderate negative correlation between nitrates and benthic abundances in BYB ($r = -0.53$, $p < 0.05$) and BEY ($r = -0.59$, $p < 0.05$) (Table 3). The first two principal components (PC) of the PCA analysis (Figure 9) amount to a cumulative percentage of the variance of 61.5%. The first PC (29.37%) is primarily a measure of T_{water} and Salinity, and is strongly but negatively correlated to Nitrates. The second PC (18.95%) mainly reflects wind speed, and is strongly correlated to WS_{max} . The PCA did not reveal a significant influence of a specific environmental parameter on *O. fattorussoi* abundances.

4. DISCUSSION

4.1. Temporal and spatial variation in *O. fattorussoi* abundances

During this study, bloom occurrences of *O. fattorussoi* were site and year specific, but a clear seasonal pattern was established. Typically, cells of *Ostreopsis fattorussoi* were barely detectable in winter. An increase in abundance was visible starting early spring, and blooms occurred in the summer with maximum abundances exceeding 200 000 cells / g FW occurred, followed by less intense autumn blooms in terms of maximum abundances as they remained at 50 000 cells / g FW or below. This succession of a summer bloom followed by a less intense autumn bloom has been reported in 2001 in the same area in a study that surveyed planktonic abundances only Abboud - Abi Saab et al., 2013) (Table 4); it has also been reported for *O. cf. ovata* in the North Western Mediterranean Sea (Mangialajo et al., 2011). In terms of timing of the blooms, summer blooms of *O. fattorussoi* detected in this study occur early compared to all other Mediterranean sites (Table 4). In addition, a couple of blooms were recorded as early as April and May, making them - along with one occurrence in the Aegean Sea (Aligizaki and Nikolaidis, 2006), the only spring blooms of *Ostreopsis* spp. reported in the Mediterranean Sea (Table 4). In terms of bloom intensity, maximum abundances reached during blooms of a species belonging to genus *Ostreopsis* in the Mediterranean Sea are variable. Both benthic and planktonic abundances recorded during this study remained considerably lower than those of Northern Mediterranean Sea, where the species *O. cf. ovata* is predominant, as they reach millions of cells/g FW in benthic samples, and exceed 10^5 cells/L in planktonic samples (Table 4). In this study however, maximum abundances remained at 18 000 Cells/L and below 400 000 cells / g FW, with only one exception ($880\,694 \times 10^5$ cells / g FW at BEY). It was worth indicating that as maximum abundances are year and site specific in NW Mediterranean sea, some sites presented lower maximum abundances than neighboring sites (Mangialajo et al., 2011), that were similar to those of *O. fattorussoi* in this study. In contrast, maximum *O. fattorussoi* abundances reached in this study exceed those reported in the Eastern

and Southern Mediterranean. Outside the Mediterranean Sea, maximum abundances are variable. Compared to this study, they were much higher in New Zealand (Shears and Ross, 2009), and much lower in Mexico (Okolodkov et al., 2007) and in the Sea of Japan (Selina et al., 2014). Nevertheless, comparing maximum benthic abundances of *Ostreopsis* species is always difficult, since biotic substrates preferences can heavily affect this parameter, even at low spatial scale (Cohu et al., 2011). Data from the more frequently sampled site (BAT) suggest that bloom duration is around one month, and bloom decline occurs within a week from reaching maximum abundances. Longer blooms such as the ones occurring in NAQ are not uncommon in the North western Mediterranean Sea (Mangialajo et al., 2011).

4.2. Influence of environmental factors on *O. fattorussoi* abundances

Temperature:

Our data suggests that water temperature plays a major role in the development of *O. fattorussoi*. We found a moderate and strong positive correlation between water temperature and planktonic and benthic cell abundances, respectively. Maximum abundances per site were reached at relatively high water temperature ($27^{\circ}\text{C} < T_{\text{Cmax}} < 30.5^{\circ}\text{C}$). In the Mediterranean Sea, several studies have linked optimum growth of *Ostreopsis* spp. to relatively high temperatures (Cohu et al., 2013; Mangialajo et al., 2008; Sansoni et al., 2003; Simoni et al., 2003;), whereas others did not find a correlation between temperatures and its abundances (Accoroni et al., 2011 & 2012; Cohu et al., 2011; Mangialajo et al., 2011; Totti et al., 2010; Vila et al., 2001). In the present study, *O. fattorussoi* was found year round, thus in all the measured range of temperature during the study (17°C to 32°). We might consider 18°C (threshold temperature associated with cell abundances of $\geq 1\ 000$ cell/g FW) triggers a swifter proliferation of available cells in their vegetative state. In the present study, maximum abundance was recorded as water temperature was still increasing,

whereas in Adriatic Sea, maximum abundances of *O. cf. ovata* were recorded as water temperature is decreasing (Accoroni et al., 2012 & 2015; Totti et al., 2010). In both cases, water temperature corresponding to the maximum of abundance of *O. fattorussoi* $T_{C_{max}}$ is lower than maximum temperature recorded T_{max} (in the present study in each of the 5 sites). The different $T_{C_{max}}$ per site could be due to the presence of different strains of *O. fattorussoi* on different sites; in fact, this is not unusual for different species, as several studies have showed species, clades or subclades to respond differently in terms of temperature (Guerrini et al., 2010; Pezolesi et al., 2012; Tawong et al., 2014).

Nitrites, Nitrates and orthophosphate

In this study, taking into account the entire dataset, we found no correlation between *O. fattorussoi* abundances and any of the measured nutrients. Orthophosphate concentrations in BAT did not seem to be affected by the nearby chemical factory. We did however find a moderate negative correlation when the correlation test was performed on site basis, between nitrates and benthic abundances (in BEY and BYB). These results are inconsistent with a previous study in the same area (3 sites in common) that found a positive correlation of *O. cf. siamensis* abundances with Nitrites and Nitrates, and a negative correlation with orthophosphate (Abboud -Abi Saab et al., 2013). Results were also contradictory in the Mediterranean, and the link between nutrients and *Ostreopsis* spp. has yet to be established. Several studies found no evidence of a role of nutrients in the bloom dynamics of *Ostreopsis* spp. (Accoroni et al., 2011 & 2012; Vila et al., 2001). (Carnicer et al., 2015) found a negative correlation between *O. cf. ovata* and nitrates, whereas, despite the absence of correlation, (Cohu et al., 2013) has reported a positive association between Phosphate concentration, (rather than nitrogen or silicate), and *O. cf. ovata*. Accoroni et al. (2015) found that N/P ratio around the Redfield value was necessary for cell proliferation of *O. cf. ovata*. The lack of a statistically significant correlation

in these studies as well as the present one was not unexpected, as during the bloom cells were presumably consuming the nutrients (Accoroni et al., 2015). Ammonium was not analyzed in the present study, while this nutrient was found to be important *Ostreopsis* species growth in a recent study (Jauzein et al., 2017). This could have influenced the present interpretation. More generally, analysis of every supplementary parameter, including organic sources, could be useful for interpretation when studying the ecology of *Ostreopsis* species, since at least one species has been proved to be mixotrophic (*Ostreopsis* cf. *ovata*; Jauzein et al., 2017).

Cell Resuspension and Wind

In this study we found a strong positive correlation between benthic and planktonic abundances of *O. fattorussoi*, as confirmed by Vila et al. (2001) for *Ostreopsis* sp. also on samples of the macroalgae *Ellisolandia elongata*. A relatively good correlation between the two is also reported in the Mediterranean Sea for *O. cf. ovata* and *O. cf. siamensis* (Aligizaki and Nikolaidis, 2006; Mangialajo et al., 2008 & 2011). This could be due to re-suspension of epiphytic cells into the water column caused by waves (Mangialajo et al., 2011; Totti et al., 2010; Vila et al., 2001) as reflected by the positive correlation between planktonic abundances and wind speed, which in turn influences water agitation on a local level. Re-suspension is also brought on due to human activity causing movement of the substrata (Mangialajo et al., 2011), or due to daily cycle vertical migrations (Vila et al., 2008). Cell resuspension might partially explain the absence of a major benthic bloom at BAT and BEY in the summer of 2015, despite the occurrence of a planktonic one. Another explanation could be due to the heterogeneity of *Ostreopsis* sp. distribution on a small scale (within a few meters) (Jauzein et al., 2018), revealed in this study by high standard deviation of *O. fattorussoi* abundance between triplicate samples (Figures 7 and 8). In addition, there was a positive correlation between wind speed and benthic abundances of *O. fattorussoi* at

TRI; as water agitation at each site could be influenced by local winds, these results suggest that the species might prefer some agitation, as also observed for *Ostreopsis* spp. in other studies (Selina et al., 2014; Vila et al., 2001). The effects of water agitation on blooms of *Ostreopsis* spp. in the Mediterranean Sea might be variable, as it has been suggested that has a negative effect as higher abundances are found in sheltered or calm areas (Accoroni et al., 2012; Barone, 2007; Mangialajo et al., 2008; Totti et al., 2010), as wind and waves could disperse the bloom (Cohu et al., 2011; Accoroni and Totti, 2016). A single environmental parameter did not solely influence the development of *O. fattorusoi*, but it is a combination of several factors; the PCA did not highlight the influence of a specific environmental factor on its abundance. This has also been reported for *O. cf. ovata* (Accoroni et al., 2015; Mangialajo et al., 2008).

5. Conclusion

The present study found that *O. fattorusoi* is present year round in Lebanese coastal waters, with seasonal blooms along the coast. A major bloom occurred early in the summer (June-July), followed by a second minor one in the fall (October-November). Results showed that a combination of several environmental factors affect its development. It was revealed to be a thermophilic species, as the highest concentrations were reached between 27°C and 30.5°C depending on the site. A minimum of 18°C is suggested as a threshold temperature promoting proliferation of *O. fattorusoi*. Moreover, its development seemed slightly facilitated by some water agitation due to wind. Results concerning the role of nutrients and other environmental parameters still inconclusive. Major blooms coincide with the summer where beaches are most frequented and minor blooms coincide with autumn, a busy period for recreational fishing (Sharif Jemaa, personal communication, June 6th, 2016). It is therefore recommended to maintain a monitoring program throughout the year, focusing on benthic samples as they are more

conservative (Lemée et al., 2012). It should be intensified mid spring until the end summer in order to allow more thorough ecological studies of all phases of the bloom. It is also important to assess its toxins in the food chain, as it is customary for local inhabitants of the coastal zone to consume certain species of macroalgae and seafood including filtering sea urchins and shellfish (*Patella* sp.). Cases of intoxication, if any, would have gone undiagnosed due to the lack of awareness on the issue in public health officials and medical practitioners as well as the general public in Lebanon.

Declarations of interest:

none

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Table 1 - Description and geographical coordinates (in decimal degrees) of the five sampling sites

Site	Latitude	Longitude	Site description	Sampling end date	Sampling frequency	Dominant macroalgae
TRI	34.3675	35.736	Rocky	Jul 2016	Monthly*	<i>E. elongata</i>
BAT	34.2515	35.6568	Rocky	Jul 2016	Weekly	<i>E. elongata</i>
BYB	34.1144	35.6482	Sandy/ Rocky	Dec 2015	Monthly	<i>Variable</i>
BEY	33.902	35.4753	Rocky	Dec 2015	Monthly	<i>E. elongata</i>
NAQ	33.1284	35.1458	Rocky	Dec 2015	Monthly	<i>E. elongata</i>

Footnotes:

*: Sampling frequency was on weekly basis in 2016.

	All Sites Mean \pm Sd Min-Max	TRI Mean \pm Sd Min-Max	BAT Mean \pm Sd Min-Max	BYB Mean \pm Sd Min-Max	BEY Mean \pm Sd Min-Max	NAQ Mean \pm Sd Min-Max
OstreopI (Cells/L)	1097 \pm 2372 0 - 18065	791 \pm 1648 0 - 7480	897 \pm 1883 0 - 9207	327 \pm 718 0 - 2680	647 \pm 1022 0 - 4224	3400 \pm 4 0 - 180
Ostreob (Cells/g FW)	31887 \pm 87362 0 - 880694	31232 \pm 70706 0 - 317308	18886 \pm 43514 0 - 285704	32485 \pm 97036 0 - 397243	58058 \pm 191704 0 - 880694	60555 \pm 6 0 - 264
T_{water} (°C)	25.18 \pm 4.39 17 - 32	25.09 \pm 4.25 17.50 - 31.00	25.3 \pm 4.4 17 - 32	24.72 \pm 4.68 17.80 - 30 .50	25.22 \pm 4.42 18.50 - 31.50	24.99 \pm 4 17.50 - 3
T_{air} (°C)	25.3 \pm 4.8 14 - 36.3	24.9 \pm 5 14.5 - 32	25.2 \pm 4.5 14 - 33.5	25.2 \pm 5.9 14 - 35	25.7 \pm 4.0 17 - 31	25.7 \pm 5 17.5 - 3
Salinity	38.82 \pm 1.07 28.52 - 39.60	39.16 \pm 0.30 38.30 - 39.60	39.08 \pm 0.42 36.72 - 39.55	37.34 \pm 2.61 28.52 - 38.98	38.96 \pm 0.45 37.57 - 39.54	38.33 \pm 0 36.20 - 39
ws (m/s)	4.2 \pm 1.7 0 - 13	4.6 \pm 1.3 1.0 - 7.0	4.2 \pm 1.7 0 - 10	3.6 \pm 1.5 1.0 - 6.0	3.8 \pm 1.6 1.0 - 7.0	4.9 \pm 2 2 - 13
WS_{max} (m/s)	7.1 \pm 2.3 3 - 17	7.6 \pm 1.7 5 - 10	7.2 \pm 2.5 4 - 17	6.9 \pm 2.6 4 - 15.0	6.6 \pm 2.2 3.0 - 13.0	6.9 \pm 2 4 - 13
Rain_{WB} (m/s)	5 \pm 13.6 0 - 125.3	1.3 \pm 2.5 0 - 10.5	4.9 \pm 12.1 0 - 79.9	4.7 \pm 6.7 0 - 19.4	3.6 \pm 7.0 0 - 25	12.3 \pm 2 0 - 125
Nitrites (μmol/L)	0.11 \pm 0.08 0.01 - 0.37	0.06 \pm 0.04 0.01 - 0.14	0.09 \pm 0.06 0.01 - 0.25	0.13 \pm 0.08 0.02 - 0.27	0.16 \pm 0.11 0.05 - 0.37	0.09 \pm 0 0.03 - 0
Nitrates (μmol/L)	2.40 \pm 3.76 0.06 - 25.12	0.80 \pm 1.60 0.06 - 7.50	0.77 \pm 0.58 0.13 - 2.4	3.46 \pm 4.35 0.08 - 19.05	1.99 \pm 1.36 0.35 - 5.34	5.88 \pm 6 0.55 - 25
Orthohosphates (μmol/L)	0.19 \pm 0.42 0.01 - 3.54	0.24 \pm 0.33 0.01 - 1.27	0.22 \pm 0.33 0.01 - 1.33	0.18 \pm 0.38 0.01 - 1.62	0.09 \pm 0.06 0.01 - 0.27	0.22 \pm 0 0.01 - 3

Table 2 - Descriptive statistics (average \pm Standard deviation and Minimum-Maximum) of all variables in the 5 sites

Table 3 - Spearman rank correlation between *O. fattorussoi* abundances and environmental variables

	All sites		TRI		BAT		BYB		BEY	
	OstreoPI	OstreoB	OstreoPI	OstreoB	OstreoPI	OstreoB	OstreoPI	OstreoB	OstreoPI	OstreoB
OstreoPI (Cells/ L)	1**		1**		1**		1**		1**	
OstreoB (Cells / g FW)	0.88**	1**	0.92**	1**	0.82**	1**	0.71**	1**	0.89**	1**
T_{water} (°C)	0.49**	0.61**	0.8**	0.83**	0.43*	0.52**	0.38	0.52*	0.6**	0.8**
T_{air} (°C)	0.41**	0.47**	0.67**	0.66**	0.38	0.43*	0.36	0.43	0.51 *	0.67**
Salinity	0.25**	0.31**	0.52**	0.54**	-0.04	0.16	0.26	0.34	0.29	0.53*
WS (m / s)	0.26**	0.19	0.42*	0.39*	0.46*	0.21	0.19	0.32	0.3	0.27
WS_{max} (m / s)	-0.07	-0.12	-0.3	-0.35	0.27	0.22	0.05	0.21	-0.14	-0.17
Rain_{WB} (m / s)	-0.38**	-0.38**	-0.29	-0.37	-0.32	-0.27	-0.39	-0.24	-0.72**	-0.82**
Orthophosphates (μmol/L)	0.13	0.1	0.33	0.26	0.36	0.22	-0.08	0.12	0.08	0.08
Nitrites (μmol / L)	0.04	0.1	0.11	0.23	0.07	0.05	-0.12	-0.05	0.16	0.21
Nitrates (μmol / L)	-0.06	-0.05	0.03	0.16	-0.09	-0.08	-0.34	-0.59*	-0.55	-0.53*

Footnotes:

“OstreoB”: benthic *O. fattorussoi* abundances

“OstreoPI”: planktonic *O. fattorussoi* abundances

(* $p < 0.05$, and ** for $p < 0.01$)

Table 4 - Maximum benthic abundances (MaxB in cells/g FW) and maximum planktonic abundances (MaxPI in Cells/L) of *Ostreopsis* spp. reported in various areas in and outside the Mediterranean Sea.

Area	Species	Year	Timing	MaxB	MaxPI	R	
NW MS	Italy (Ligurian Sea)	<i>O. cf. ovata</i>	2006	Jul	2.5x10⁶	87x10³	M
	Spain	<i>O. cf. ovata</i> & <i>O.cf. siamensis</i>	2008	Oct	7.24x10⁶	6.44x10 ³	M
	Spain	<i>O. cf. ovata</i> & <i>O.cf. siamensis</i>	2009	Jul	1.0x10 ⁶	385x10³	M
	Monaco	<i>O. cf. ovata</i> ***	2007	Jul	1.56x10⁶	20.9x10³	M
	France	<i>O. cf. ovata</i> ***	2009	Jul	1.61x10⁶	12x10 ³	M
	France	<i>O. cf. ovata</i> ***	2008	Jul	5.1x10 ⁵	104x10³	M
	France	<i>O. cf. ovata</i>	2008	Jul	8.54x10⁶	7x10 ³	C
NA Sea	Italy (Conero Riviera)	<i>O. cf. ovata</i>	2010	Aug	1.2x10 ⁶	102x10³	A
	Italy (Conero Riviera)	<i>O. cf. ovata</i>	2011	Aug	1.9x10⁶	39x10 ³	A
	Italy (Conero Riviera)	<i>O. cf. ovata</i>	2012	Sep	1.9x10⁶	77.9x10 ³	A
	Croatia	<i>O. cf. ovata</i> **	2010	Oct	3.34x10⁵	42.6x10³	Pr
S MS	Tunisia	<i>O.cf. siamensis</i>	2001	Aug-Sep	3.6x10³	n.a.	T
	Tunisia	<i>O. cf. ovata</i>	NA	Jul	n.a.	84	B
	Tunisia	<i>O.cf. siamensis</i>	2008-09	Jul & Jan*	1 to 5	n.a.	M
	Algeria	<i>Ostreopsis spp.</i> **	2009	Jul	7.9x10⁴	5.9x10³	III
East MS	Egypt	<i>O. cf. ovata</i> & <i>Ostreopsis morph1.</i>	2005	Jul	9x10 ³	n.a.	Is
	Greece (Aegean Sea)	<i>O. cf. ovata</i> & <i>O.cf. siamensis</i>	2003	Sep	4.05x10⁵	16x10³	A
	Greece (Aegean Sea)	<i>O. cf. ovata</i>	2005	May	n.a.	0.6x10 ³	Sp
	Lebanon	<i>O.cf. siamensis</i>	2001	July	n.a.	10.5x10³	A
			2001	Sep	n.a.	7.7x10³	A
			2014	Jun	8.8x10⁵	1.7x10 ³	Pr
2015	Jun	2.64x10 ⁵	18.10³	Pr			
Non MS	New Zealand	<i>O. siamensis</i>	2004	Feb	1.41x10⁶	n.a.	SI
	Sea of Japan	<i>O. ovata</i> & <i>O. siamensis</i>	2010	Sep	52x10³	n.a.	Sc
	Mexico	<i>Ostreopsis heptagona</i>	2005	May-Jun	1x10³	n.a.	O

Footnotes: *: Sampling limited to these two months; **: Associated with toxic events; ***: Sporadic co-occurrence of *O. siamensis* is possible; Bold = maximum abundance; MS: Mediterranean Sea; Non MS:

Non Mediterranean Areas; NW: North West; NA: North Adriatic; S: South; E: East; MaxB: Maximum benthic abundances; MaxPl: maximum planktonic abundances; n.a.: Not available)

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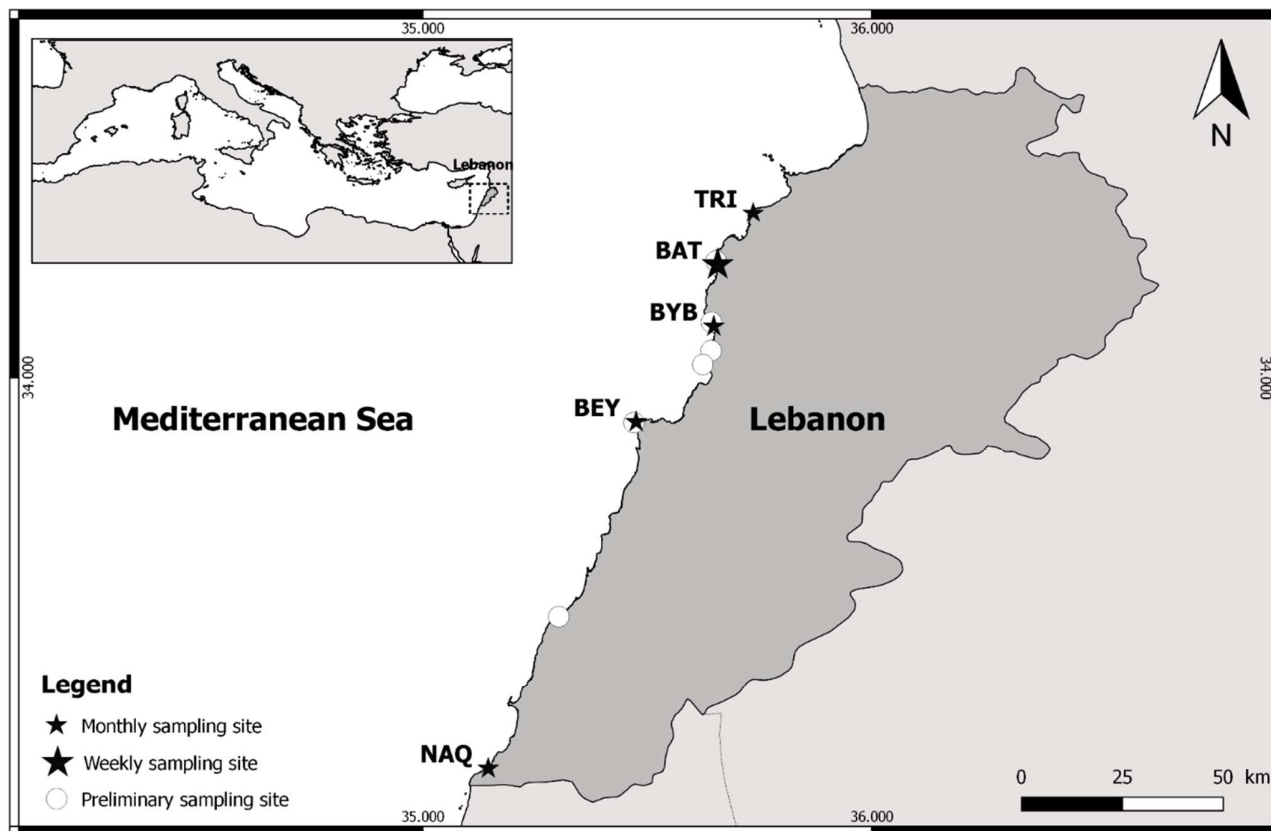


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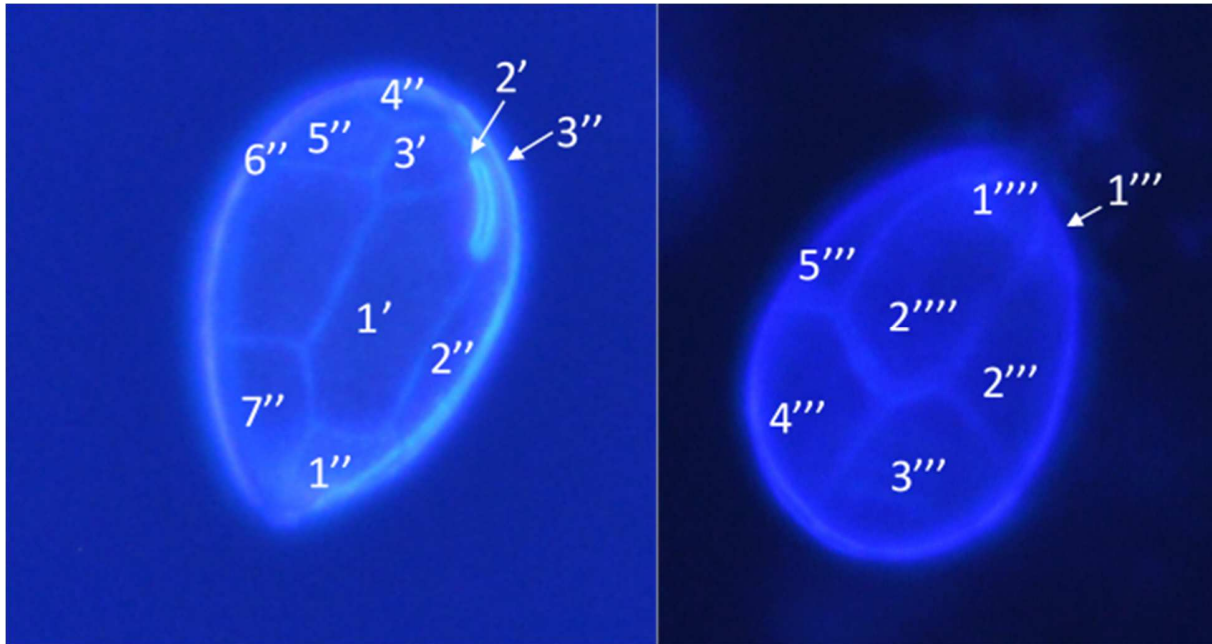


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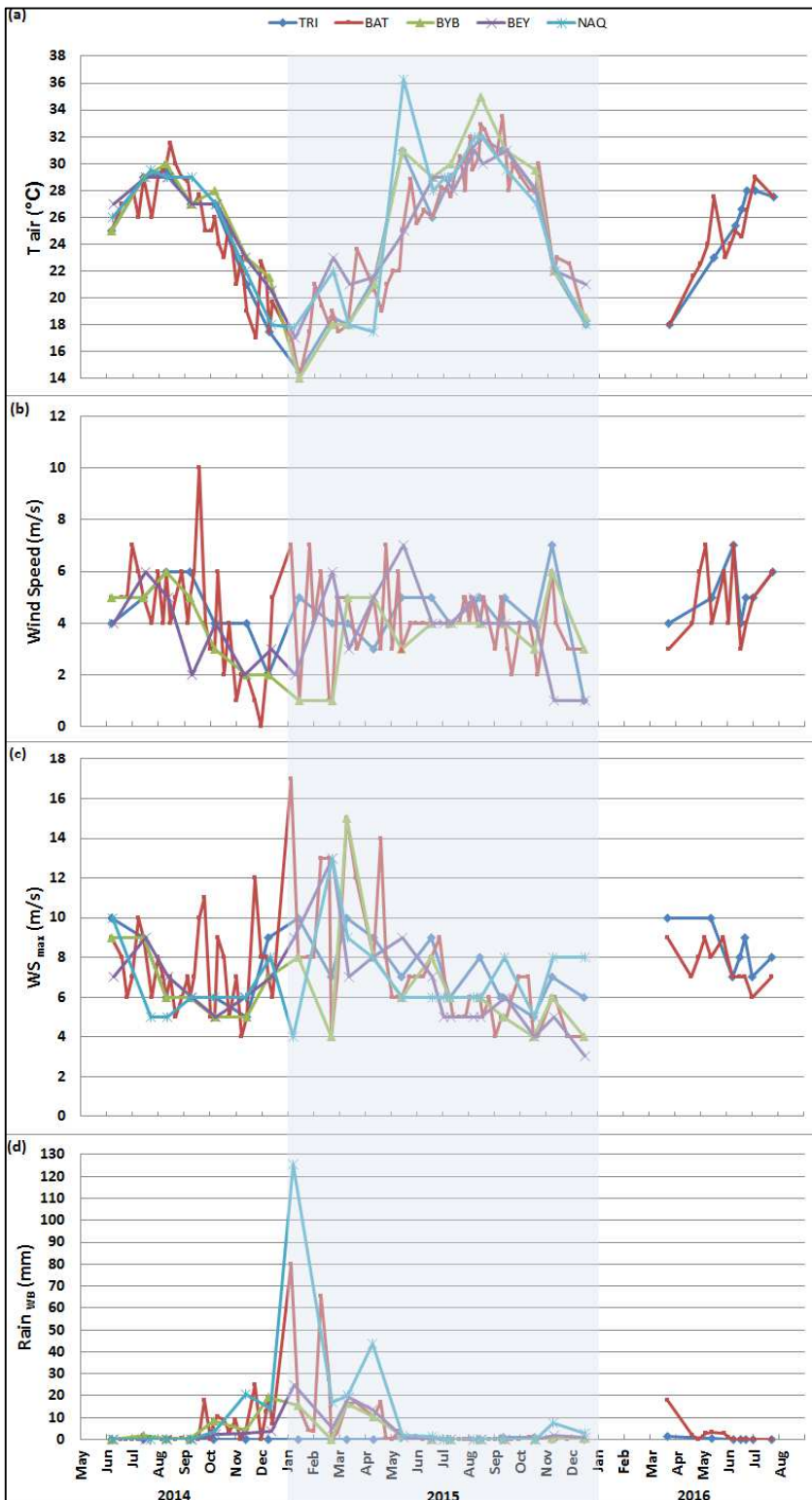


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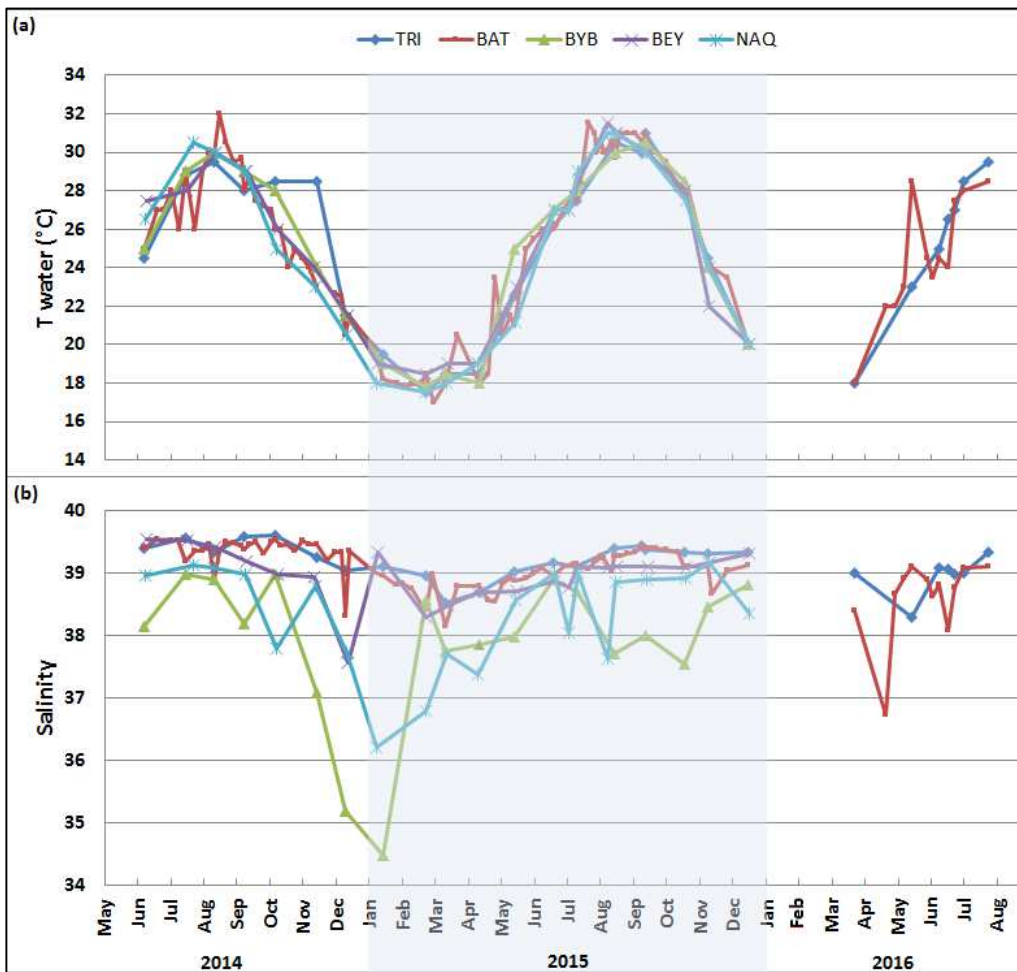


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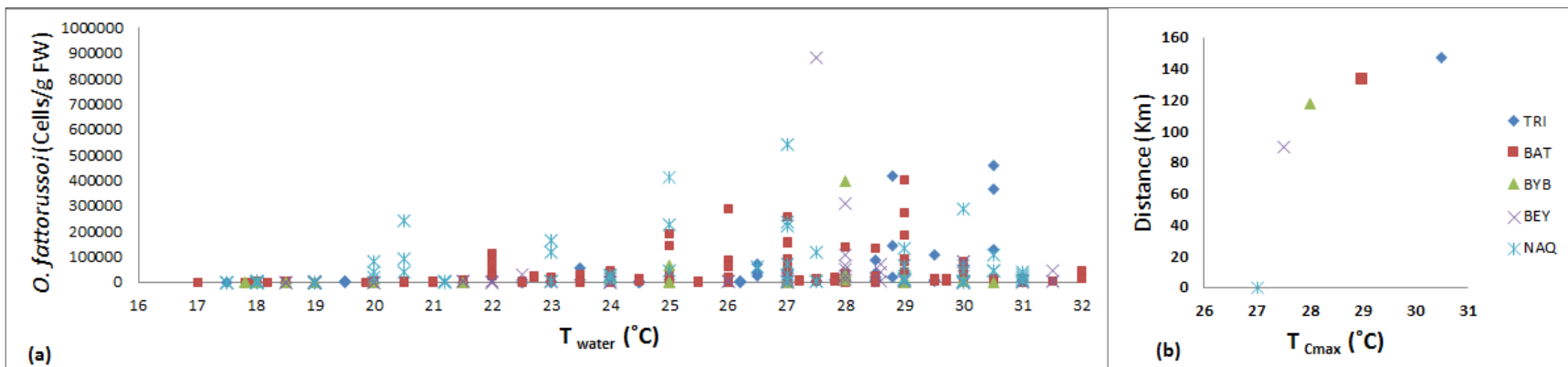


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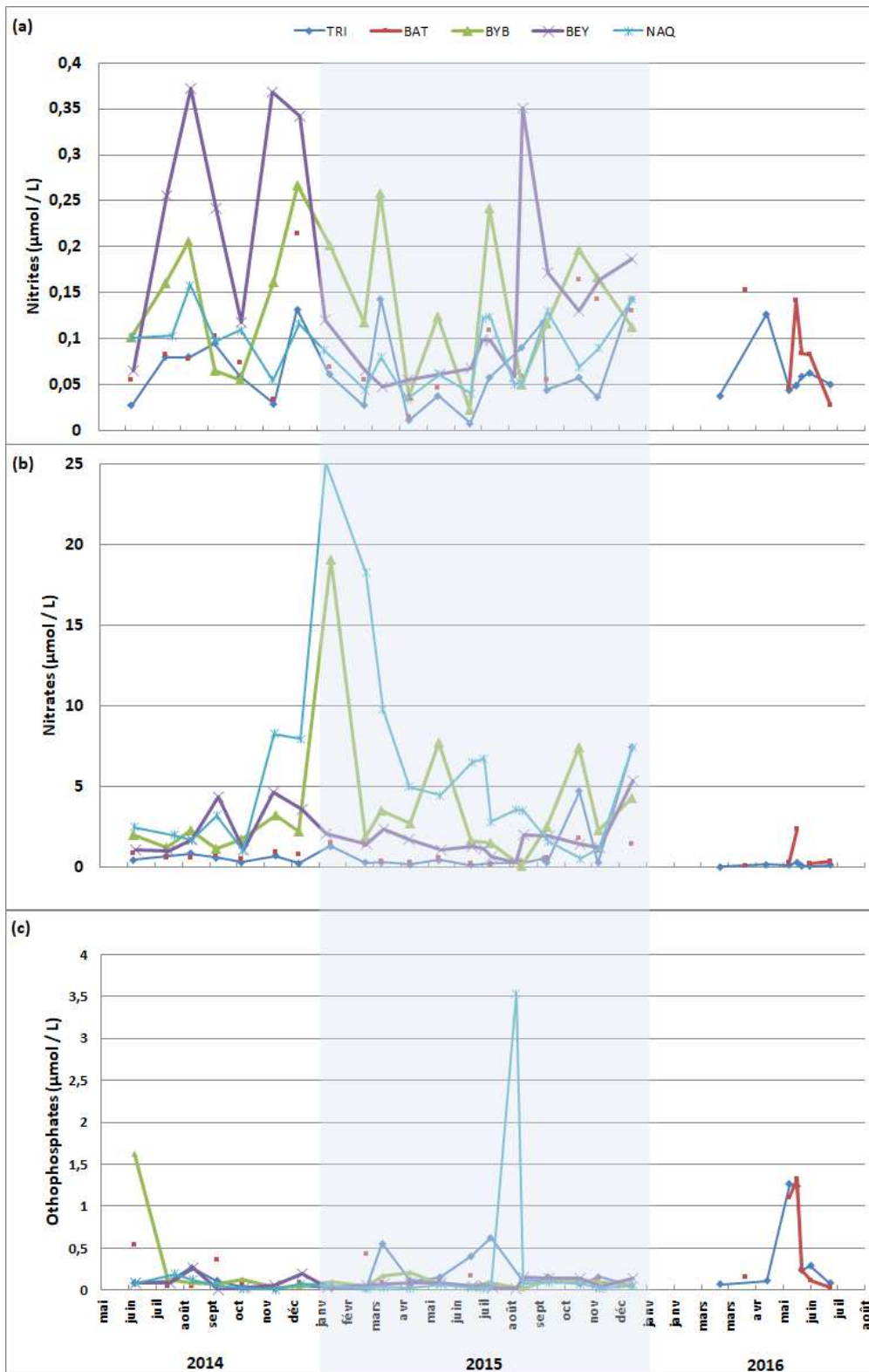


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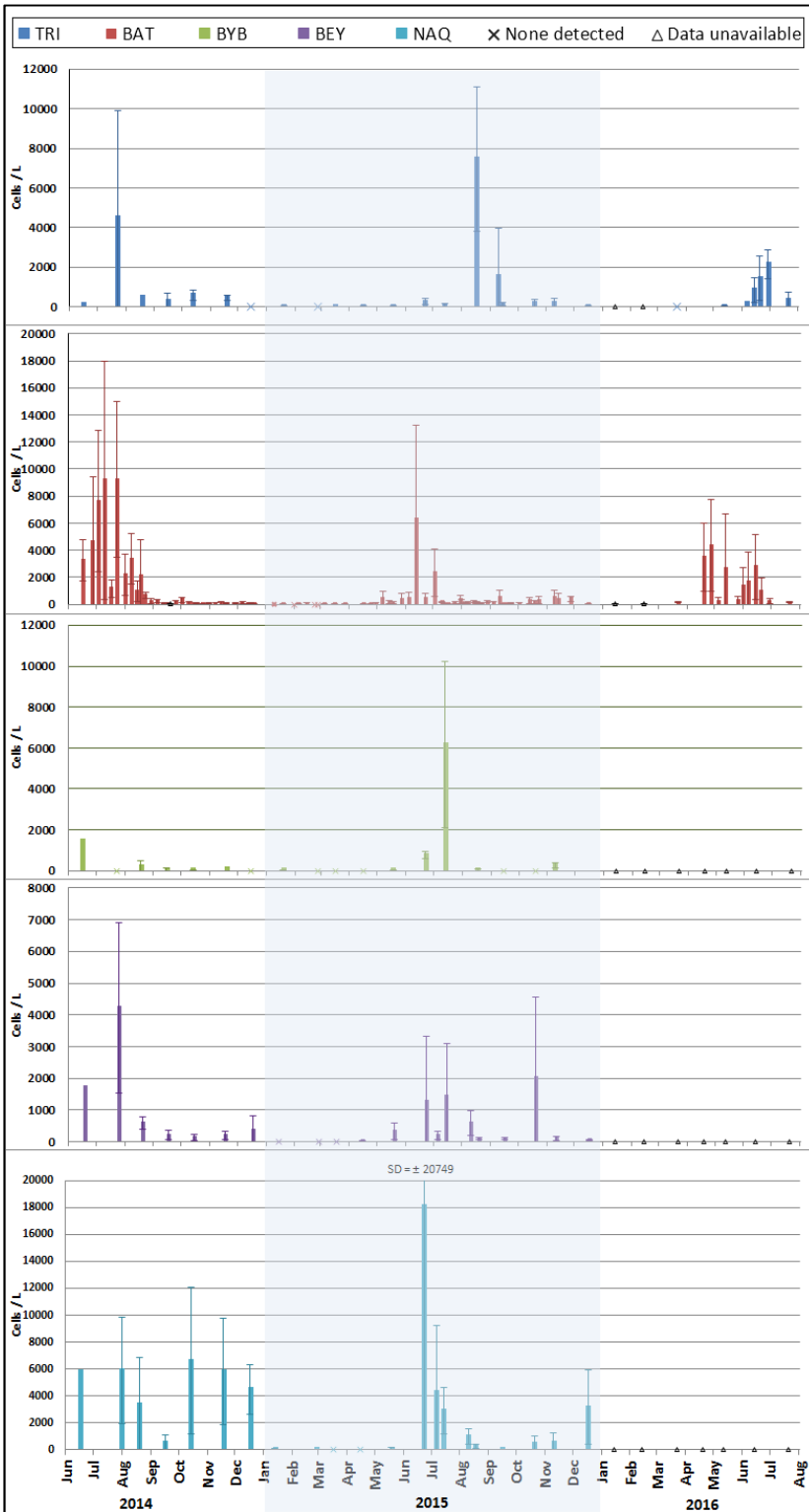


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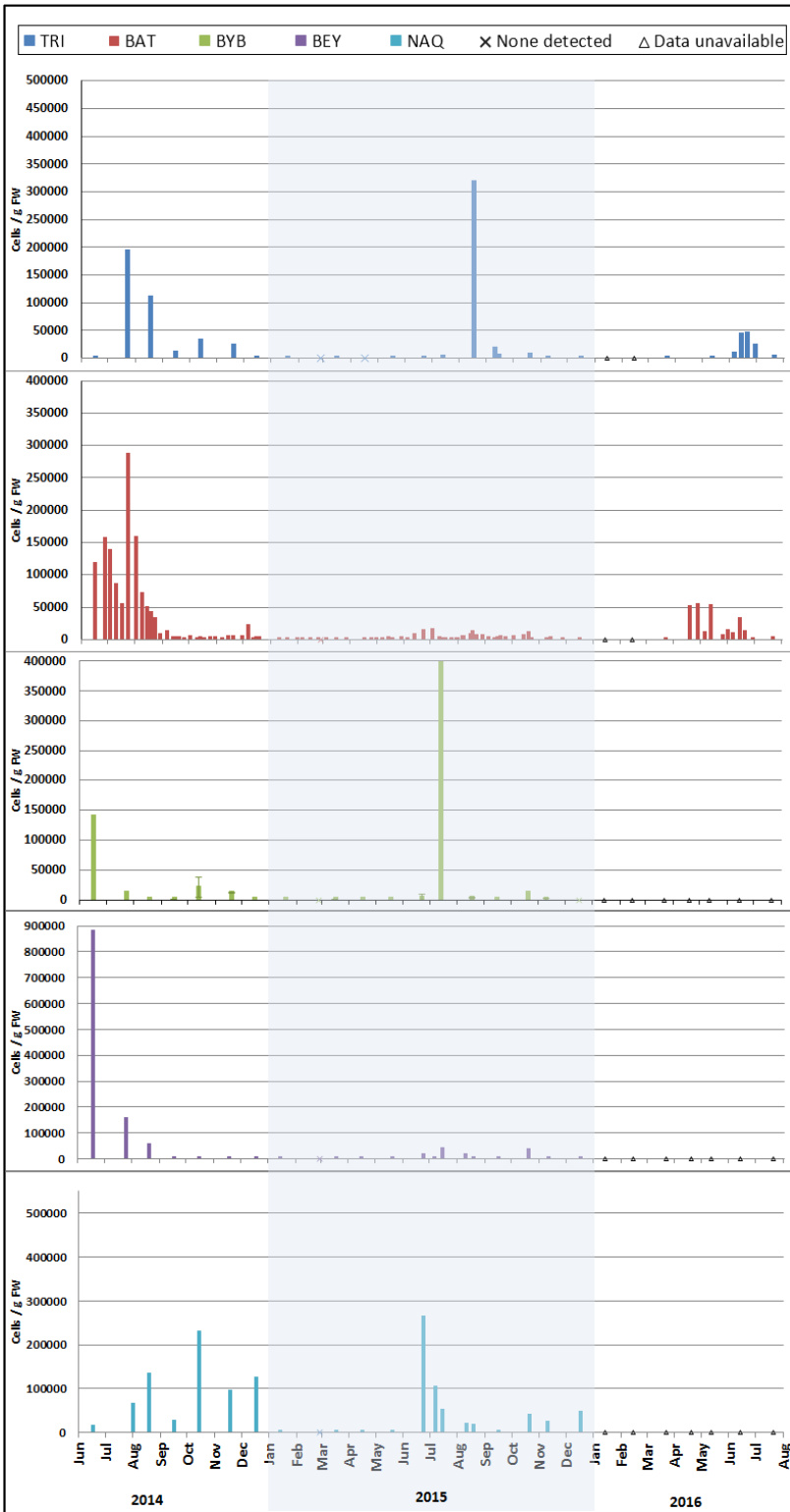


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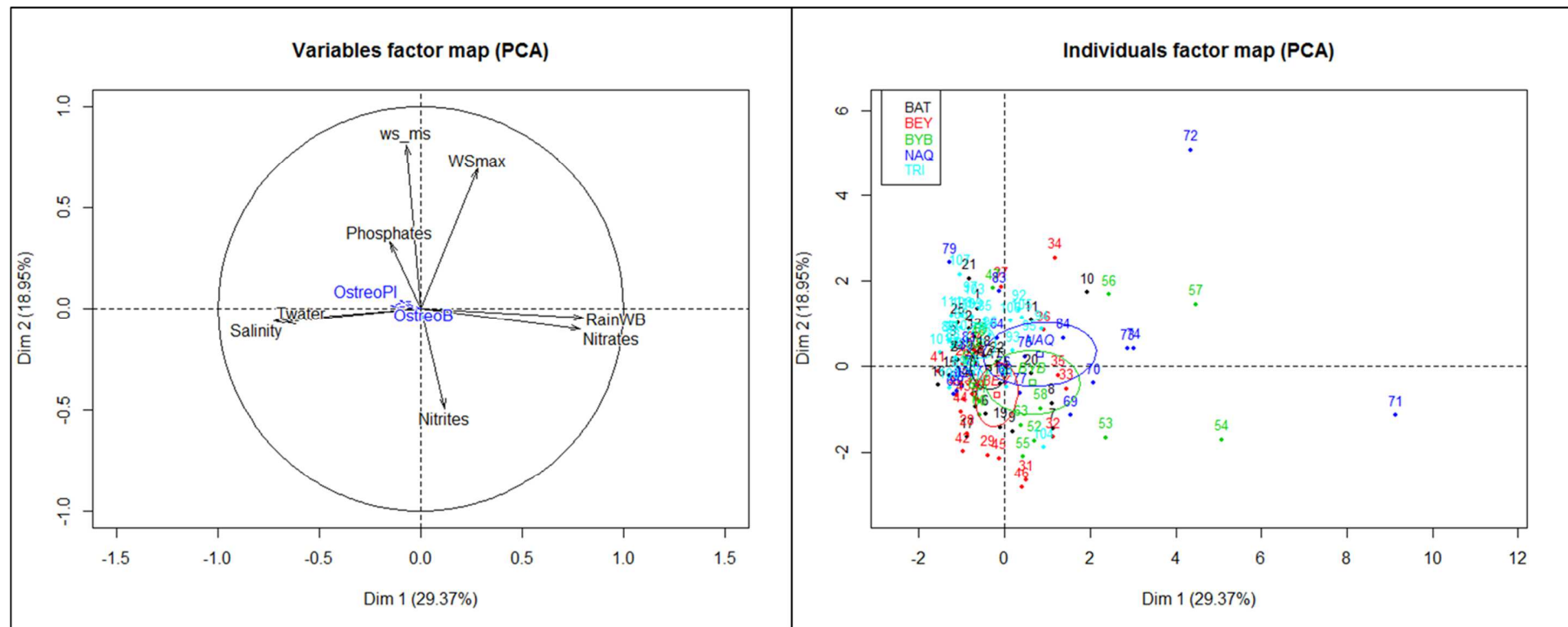


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