1 **Mixotrophic phytoplankton dynamics in a shallow Mediterranean water body: how to make a**
2 **virtue out of necessity**
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12 **Abstract**

13 Mixotrophy is a combination of photosynthesis and direct access to organic carbon sources, mainly

14 through osmotrophy or phagotrophy. This strategy is adopted by several, phylogenetically distinct,

15 phytoplankton groups and is commonly occurring in marine, brackish and freshwater ecosystems.

16 Traditionally, it has been put in relation to both scarcity of inorganic nutrients and poor light

17 conditions. However, we observed blooms of the mixotrophic, toxic haptophyte *Prymnesium*

18 *parvum* in different periods of the year, and under variable resources availability. The analysis of a

19 6.5-year data set of phytoplankton weekly records from a Sicilian shallow lake (Biviere di Gela,

20 south-eastern Sicily) allowed us to hypothesize that a depleted condition as regard inorganic

21 nutrients is not the main fuel to the growth of *Prymnesium parvum,* neither this is due to light

22 limitation. The results achieved show that an increased availability in suitable preys can stimulate

23 the growth of this phagotrophic photoautotroph. Contemporary, it was not found any clear

24 environmental patterns to explain species dominance and growth patterns as related to inorganic

25 nutrient availability. Moreover, it is shown that these organisms tend to monopolise resources when

26 these become available irrespectively of seasons, and under variable conditions as regard inorganic

27 nutrient availability.

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Introduction

The evolutionary pathways followed by protists in the last 2 billion years resulted in a legacy of several, often amazing, nutritional modes, which witness the complexity of the polyphyletic origins of these organisms (Zimmer, 2009; Simpson et al., 2017). By overturning this perspective, the wide spectrum of nutritional modes exhibited by protists has been certainly contributing to ensure their persistence and to improve their chance of survival (Yafremava et al., 2013).

Among these nutritional modes, the holophytic (photosynthetic) and holozoic (zoo-trophic, ingestive or phagotrophic) are the most popularly renowned since they are ascribed to "plants" and "animals", respectively.

Eukaryotic phytoplankton form an ecological group of photosynthetic protists adapted to live in suspension in the water column of aquatic ecosystems (Reynolds, 2006). Although, they show a nutritional mode superficially similar to that of plants, phytoplankton encompass a broader range of both phylogenetic diversity and life history strategies (Rengefors et al., 2017). This diversity is mirrored by their broad spectrum of nutritional modes, and several phytoplankton taxa can heterotrophically access energy and carbon.

One of the most important steps giving birth to photosynthetic eukaryotes, according to endosymbiont theory, was an error recurrently occurring in phagotrophy, which has played a central role in the evolution of photosynthetic eukaryotes (Raven, 1997). Actually, through a sequence of complex endosymbiotic events involving engulfment (i.e. ingestion lacking digestion) of unicellular photosynthetic prokaryotes and/or eukaryotes, the plastids, and other organelles, developed in algae (Keeling, 2013). Kleptoplasty is a quite common phenomenon among holozoic organisms and it supports the endosymbiotic origin of chloroplasts: several ciliate and dinoflagellate species photosynthesize by sequestering chloroplasts from their photosynthetic preys, and by maintaining these organelles in their cytoplasm for a variable amount of time (Johnson et al., 2016). This strategy, adopted not only by protists but also by some marine molluscs (Händeler et al., 2008),

probably represented the first step in the evolutionary path that has led to actual photosynthetic eukaryotes.

Since the recognition of the fundamental role exerted by the microbial loop in the functioning of aquatic ecosystems (cf. Azam et al., 1983), a net distinction between photoautotrophy and heterotrophy appeared insufficient to fully explain the pathway of carbon and nutrient flux in aquatic ecosystems; therefore, the importance of a further nutritional mode, mixotrophy, started receiving greater attention (Jones, 2000). Mixotrophy couples the photosynthetic and the heterotrophic nutritional modes, and forms a continuum in the way in which protists, but also bacteria (Eiler, 2006), can access different sources of energy and carbon (Jones, 1997; Stoecker, 1998). Recently, Mitra et al. (2016), proposed a functional grouping of planktonic protists in an eco-physiological context which includes among constitutive mixotrophs (those organisms bearing their own chloroplasts) also those ciliates able of kleptoplasty (non-constitutive mixotrophs).

Several phytoplanktonic organisms, especially found among those bearing flagella, exhibit a mixotrophic nutritional mode: they can therefore photosynthesize and contemporary, or alternatively, can derive energy from the uptake of dissolved organic compounds (osmotrophy) or from the ingestion of organic particles or other microorganisms (phagotrophy). In addition, depending on resource availability, some species must employ mixotrophy obligately and some others can do it facultatively (Ghyoot et al., 2017). Mixotrophy, along with species morphology (Naselli-Flores & Barone, 2011), and nitrogen fixation, represents an important trait related to resource acquisition by phytoplankton and therefore to their trophic niche (Litchman & Klausmeier, 2008). However, the existing literature reports contrasting information as regard the levels of nutrients and light which can make mixotrophs more competitive than phototrophs in natural environments. Both oligotrophic (e.g. Arenovski et al., 1995) and eutrophic conditions (e.g. Burkholder et al., 2008) were reported as favourable for mixotrophic phytoplankton. According to some authors, mixotrophy cannot allow phytoplankton to escape the need of photosynthesizing and

the availability of sufficient light remains a fundamental requisite for mixotrophic phytoplankton

(e.g. Caron et al., 1993). Nevertheless, there are papers suggesting that constitutive mixotrophy can

play a positive role when severe and prolonged light limitation occurs (e.g. Jones et al., 2009).

In this paper we present data on the phytoplankton of a natural, shallow lake in south-eastern

Sicily (Italy). The lake is largely dominated by constitutive mixotrophic phytoplankton and has

been regularly sampled since 2005. A 6.5-year dataset, constituted by weekly phytoplankton

samplings, was used to verify the hypothesis that the abundance of mixotrophic algae is not related

to the availability of inorganic nutrients, whether they are present in high or low concentrations.

Moreover, our aim is to show that these organisms tend to monopolise resources when these

become available irrespectively of seasons, and under variable environmental.

Materials and Methods

Study Site

The Biviere di Gela (BdG, 37°01'12" N, 14°20'28" E) is a natural coastal lake located in SE Sicily

(Italy) at 1.3 km from the Mediterranean Sea, 8 m a.s.l. The lake has no surface outflow and no

natural inflow and it is mainly fed by underground waters flowing from North to South. The lake

lies in a depression which intercepts the water table. The downgradient underground flow also

constitutes a barrier against seawater intrusion and avoid that the electrical conductivity of the lake

waters exceeds 2 mS cm^{-1}.

In 1988, the lake has been designated for the List of Wetlands of International Importance

and included in the ''Ramsar Act''. Nine years later, a Nature Reserve has been created by the

Sicilian Region to preserve the several rare and endangered vertebrate species (e.g., amphibians,

turtles, and birds) that inhabit the lake, and the lake itself which has an historical value, being

mentioned by Pliny the Elder in his *Naturalis Historia*. Nevertheless, the lake is surrounded by

greenhouses supporting intensive agriculture. These infrastructures compete with the lake for water

resources and exploit the same water table for their irrigation purposes.

As a result of overexploitation of groundwaters and climate change (Barone et al., 2010), the

lake risked to disappear at the end of the last century. This caused great concern, and an artificial

canal downstream of a dam reservoir (Lake Dirillo – 37°07'19" N, 14°41'54" E) was therefore

opened in 2004. Immediately after the BdG was quickly re-filled and the rich macrophyte belts

which characterised the littoral zone of the lake, and which were destroyed by the drop of lake level

and the concurrent increase in salinity, started recovering. Lake Dirillo is a eutrophic reservoir

receiving the untreated sewages from two urban centres (Vizzini – 6300 inhabitants, and Licodia

Eubea – 3100 inhabitants) located in its catchment. During the study period, inflows from Lake

Dirillo quite regularly reached the BdG through the canal, from 2011 to early spring 2013. This was

done to minimize the extent of water levels fluctuations in the BdG (also due to the overexploitation

of the water table caused by the irrigation needs in the nearby greenhouses) and to avoid the

vanishing of this Ramsar site. From spring 2013 to autumn 2016, also thanks to higher

precipitation, no significant water abstraction from Lake Dirillo was necessary to maintain the lake

levels. In November 2016, a quite rapid decrease in the lake level occurred and caused concern in

the Management of the Nature Reserve, which again moved waters from Lake Dirillo to refill the

Biviere di Gela.

Phytoplankton of Biviere di Gela has been regularly studied since 2005 and further

information on the lake is available in Barone et al. (2010), Naselli-Flores & Barone (2012),

Jeppesen et al. (2015) and Naselli-Flores et al. (2016).

Data collection and analysis

Phytoplankton data in the present study refers to weekly collections, from January, 2011 to May,

2017. Water samples for phytoplankton counting were collected at 10 am (± 30 min) in a station

located in the central part of the lake, approx. 20 cm below the surface, using a 100 ml dark-glass

bottle. These samples were preserved in Lugol's iodine solution immediately after collection.

Net samples were contemporary collected by towing vertically and horizontally a 40 μm

mesh size plankton net. Samples were immediately fixed in 4% buffered formaldehyde. However,

live samples were also taken for species identification. Taxa were identified at the lowest taxonomic

rank (species) in the majority of cases using the most up-to-date phycological literature.

A Zeiss Axiovert 100 microscope was used for cell counting which was performed in

accordance with the sedimentation method developed by Utermöhl; all the individuals (cells or

colonies) present in forty random fields at 400x magnification were counted.

The biovolume was computed approximating the shape of the cell or colony to simple

geometric shapes as shown in Hillebrand et al. (1999). To calculate the cell or colony volumes, 10

individuals of each recorded species were measured using a Zeiss AxioVision image analysis

system on the same microscope used for counting. The average value of each measurement was

used to calculate the average biovolume of species. Biovolumes were transformed in biomass

values assuming that phytoplankton has unit specific gravity (10^9 μm^3 = 1 mg). Wet weight biomass

of each population was calculated multiplying the recorded abundance of populations by the

average biomass estimates of their individuals.

Net growth rates were computed from weekly cell abundance data using the equation:

$$r = \ln(N_1)\text{-}\ln(N_0)/(t_1 - t_0) \qquad [\text{d}^{-1}]$$

where r is the net growth rate, and N_1 and N_0 are the number of phytoplankton cells in a unit volume

at time 1 (t_1) and time 0 (t_0), respectively.

In the same sampling station, water temperature was measured weekly, along vertical

profiles, using a YSI 556 MPS multiparametric probe. Water level data were supplied by the

Management Board of the Nature Reserve ''Biviere di Gela''.

155   Water samples for nutrients (Reactive Phosphorus – RP, and Dissolved Inorganic Nitrogen –

156   DIN) were collected sub-superficially, at monthly intervals. DIN refers to the sum of $N-NO_3$ and N-

157   NH_4. The chemical analyses were performed according to Tartari & Mosello (1997).

158   BOD_5 was measured as a surrogate of the degree of the organic pollution of the water

159   flowing from Lake Dirillo. The values were considered as proxies of bacterial abundance in the

160   waters. Samples for BOD_5 were taken every two weeks and additional samples were collected when

161   an evident flow in the artificial canal from Lake Dirillo occurred. The water was taken sub-

162   superficially and stored in 300 ml sterilized dark bottles. The analyses were performed in the lab of

163   the Nature Reserve, within a few minutes (15-30 minutes) from water collection. The analytical

164   method used (APAT, 2003), allows a direct determination of BOD_5 by measuring the concentration

165   of dissolved oxygen (Winkler method) before and after a five days incubation at 20 °C.

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167   **Results**

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169   Trends in selected environmental variables

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171   Lake depth ranged between 5.5 and 7.9 m. Seasonal water level fluctuations (lower depth in

172   summer and higher in winter) between 0.5 and 1.5 m occurred in the lake during the studied period

173   (Fig. 1). Water temperature ranged between 12.5 (±0.4) °C (February) and 29.5 (±0.6) °C (July-

174   August). Due to the high summer water temperatures, the water column occasionally showed a

175   weak stratification in July and/or August in spite of the shallowness of the lake. These occasional

176   thermoclines were punctual, and they were seldom recorded in two consecutive sampling dates.

177   The monthly dynamics of nutrients showed a marked and repetitive seasonal pattern with

178   highest concentrations in winter (RP above 15 µg L^{-1} and DIN above 200 µg L^{-1}) and lowest in

179   summer (RP below 10 µg L^{-1} and DIN below 200 µg L^{-1}). In general, DIN showed a decreasing

tendency from the beginning of the study period and until summer 2016. Later on, the values

increased again (Fig. 2). Conversely, RP values were slowly growing throughout the study period.

Oxygen contents in the superficial lake waters were generally close to, or slightly higher

than, 100% of saturation. BOD_5 values were generally around 2.0 mg L^{-1}. However, significantly

higher values (up to 6.9 mg L^{-1}) were sporadically recorded in the period 2011-2013 and from the

end of 2016 onward (Fig. 1). Values above 3 mg L^{-1} generally followed water inflows from Lake

Dirillo.

Phytoplankton structure and dynamics

Phytoplankton in the BdG was quite rich and diversified. A total number of 141 phytoplankton taxa

were identified in the lake during this study. These were ascribed to the following taxonomic

groups: cyanobacteria (8), haptophytes (3), cryptophytes (7), chlorophytes (91), bacillariophytes

(8), dinoflagellates (5), euglenophytes (12), xanthophytes (2), ochrophytes (3). "Picoplankton" and

"unidentified flagellates < 5 μm" were considered as monospecific taxa. Ochrophytes (two

unidentified species of *Mallomonas* and *Pedinella hexacostata* Vysotskij) appeared

idiosyncratically and punctually in a few winter samples but, when present, they were quite

abundant.

Phytoplankton total biomass varied between 2.5 and 25 mg L^{-1} in the period January 2011 -

April 2013 and was characterised by frequent peaks of the haptophyte *Prymnesium parvum* Carter

(Fig. 1). This species contributed up to 97% to total haptophyte biomass (other haptophytes present

in the lake were *Hymenomonas roseola* Stein and *Chrysochromulina parva* Lackey) and up to 80%

to total phytoplankton biomass in spring 2011. It dominated the phytoplankton assemblage from

mid-October 2011 to the beginning of June 2012 with percentage values ranging between 50% and

95%. Virtually absent in July and August 2012, the species again started dominating in the

beginning of November 2012 showing relative biomass values higher than 60% until mid-April

2013. Since then, *Prymnesium parvum* showed relatively low values of biomass until October 2016, when it started growing again and peaked at values above 35 mg L^{-1} in the last week of November. Later on, the species dominated the phytoplankton assemblage until mid-April 2017.

In the period May 2013 – September 2016, phytoplankton biomass ranged between 3.1 and 47 mg L^{-1} and was characterised by summer peaks of Cyanobacteria [mainly *Cylindrospermopsis raciborskii* (Wołoszyńska) Seenaya & Subba Raju and *Chrysosporum ovalisporum* (Forti) E.Zapomelová, O.Skácelová, P.Pumann, R.Kopp & E.Janecek]. Their dominance frequently lasted until mid-autumn with absolute biomass values peaking above 30 mg L^{-1} at the end of October, 2014, in mid-June 2015, and at the end of September 2016 (Fig. 3).

When *Prymnesium parvum* and cyanobacteria biomass values were relatively lower, other phytoplankton taxa could grow up and reached relatively higher biomass percentages, although without showing a clear seasonal pattern. In particular, cryptophytes (*Cryptomonas* spp. and *Plagioselmis* sp.) were generally more abundant or sub-dominant in winter, whereas "chlorococcalean" chlorophytes and diatoms (*Cyclotella* spp. and *Diatoma tenuis* C.Agardh) tended to be more abundant in spring and/or in summer (the trends of these taxa are shown in the Electronic Supplementary Material). In these seasons, also euglenophytes and dinoflagellates were relatively more abundant, but never exceeded 7% of total biomass.

Mixotrophic (which included, in order of abundance, haptophytes, cryptophytes, dinoflagellates, euglenophytes and the ochrophyte *Pedinella hexacostata*) and phototrophic species were alternatively present in the lake on a seasonal basis (Fig. 4). In winter mixotrophs were relatively more abundant, whereas in summer autotrophs prevailed. This was particularly evident in the periods 2011-2013 and 2016-2017, when mixotrophic species formed the bulk of biomass in the BdG. A lower relative abundance of mixotrophs was recorded in 2014-2015, when cyanobacteria showed summer blooms, and the alternation with autotrophs was less marked in that period.

No significant relationships were found among the different phytoplankton taxonomic groups, nor between mixotrophs and phototrophs biomass values. Analogously, no significant

relationships were detected when correlating phytoplankton groups and environmental variables. However, when the positive values of *Prymnesium parvum* growth rates were correlated to the increase in water level which had occurred one week before, a significant correlation was clear (Fig. 5; n=89, r= 0.91, p<0.001). Figure 5 also shows the different seasonal contributions to the relationship: 16 points refer to winter growth, 8 to spring growth, 6 to autumn growth and 4 to summer growth. Although more frequent in winter, *Prymnesium parvum* also showed relatively high growth rates in all the seasons, including summer. All these points were related to an increase in depth higher than 10 cm, which corresponded to water inflows from Lake Dirillo. The remaining 55 points corresponded to "natural" water incomes and were related to growth rates lower than 0.05 d^{-1}.

Discussion

Environmental variability

Water level fluctuations in the BdG were not only due to the natural balance between inflows and outflows. The increase in water level also occurred "upon request" from Lake Dirillo to refill the water body and avoid the risks of excessive decrease in water level as already recorded in the past years (Barone et al. 2010). The period of inflows from Lake Dirillo were therefore monitored by the Nature Reserve Management Board and were easily discernible from inflows due to precipitation.

Biochemical Oxygen Demand (BOD) represents the quantity of oxygen consumed by microorganisms to fuel the aerobic decomposition of organic materials The BOD therefore has been considered a reliable proxy of bacteria density in water samples (e.g. Kagalou et al., 2002). In addition, Olutiola et al. (2010) found that it was also positively correlated to inorganic nitrogen concentrations, which constitutes an end-product of organic matter degradation. The results of the present study show that BOD5 in the BdG showed frequent peaks in the period 2011-2013 and at the end of 2016. In the same periods, DIN values higher than those recorded in 2014-2016 were measured in the BdG. These periods corresponded to water inflows from Lake Dirillo, which

receives untreated sewage from the municipalities located in its catchment. Although no data are available on the amounts of organic matter in Lake Dirillo, these repeated peaks make reasonable to hypothesize that Lake Dirillo represents a source i) of bacteria and/or ii) of organic pollution which triggers bacterial growth in the BdG.

Phytoplankton dynamics

Mixotrophy is considered an advantageous mode of nutrition when inorganic nutrients are limiting and/or the light regime is poor (Pållson & Granéli, 2004). Lake BdG, due to its Mediterranean location, is unlikely subjected to a poor light regime (Obrador & Pretus, 2008). Moreover, planktonic organisms entrained in the turbulent water mixing, have similar chances to access light when turbidity has an algal origin, especially when biomass is not dominated by buoyant cyanobacteria (Reynolds, 2006; Naselli-Flores, 2014). In addition, dissolved nutrients in BdG where generally above the limiting threshold, even though the biomass values reached by phytoplankton in the lake were well above those attained in other Sicilian lakes showing a comparable nutrients availability (Naselli-Flores & Barone, 1994; Naselli-Flores, 1999; 2000).

Mixotrophic phytoplankton largely dominated the phytoplankton of BdG during the periods characterised by water inflows from Lake Dirillo. Among mixotrophic species, *Prymnesium parvum* frequently bloomed during 2011-2013 and at the end of 2016. The species is well-known to produce a variety of toxins (Eikrem et al., 2017) endangering the aquatic fauna (both invertebrates and vertebrates). Moreover, it has been proven to exert an allelopathic effect addressed toward outcompeting other phytoplankton species (Granéli & Johansson, 2003). These metabolites can in some cases be used to directly kill potential preys, or, indirectly to promote bacteria growth through the decomposition of killed organisms. In addition, as shown by Croft et al. (2006), many different species of phytoplankton, including several haptophyte genera, cannot synthesize some vitamins and get them from bacteria by phagotrophy. *Prymnesium parvum* is actually able to support population growth through heterotrophy employing both osmotrophic and phagotrophic nutritional

284   modes (Roelke et al., 2016 and literature therein). In the studied lake, a significant positive

285   relationship was found between the instantaneous growth rates of *P. parvum* and the increment of

286   water level recorded in the lake one week before. These increments were due to water inflows from

287   Lake Dirillo, which caused an increase in the BOD_5 values measured in BdG, and occurred in any

288   season, when the intense use of water for irrigation was considered a menace for the water level

289   stability of the BdG. Accordingly, high growth rate of the haptophyte did not show a well-defined

290   seasonality, even though they were more frequent in winter. These repeated coincidences allow to

291   hypothesize that *Prymnesium parvum* benefited from an increased organic matter (and bacteria)

292   entering the lake through the inflows from Lake Dirillo. When the water inflows from Lake Dirillo

293   stopped (2014-2016), *P. parvum* biomass remained below 5 mg L^{-1}, and the species was virtually

294   absent for long periods. In the absence of *P. parvum*, also a decrease in inorganic nitrogen was

295   recorded. Filamentous nitrogen fixing cyanobacteria became very abundant, especially during

296   summer 2014, 2015 and 2016. This negative interaction between *P. parvum* and cyanobacteria had

297   been already observed immediately after the establishment of the water connection between Lake

298   Dirillo and the BdG (Barone et al, 2010). However, the dominance of the haptophyte was in that

299   case attributed to the decrease in conductivity caused by the inflows from Lake Dirillo (Jeppesen et

300   al., 2015). The present study offered an alternative explanation for *P. parvum* growth, which was

301   likely to be supported by the organic (and eventually bacteria) loads from Lake Dirillo.

302   Other supposed mixotrophic organisms in the phytoplankton of the studied lake belonged to

303   cryptomonads, euglenids and dinoflagellates.

304   Cryptomonads represented, according to their relative biomass, the second most important

305   group of mixotrophs in the lake. These organisms show ultrastructural details (a furrow-gullet

306   system surrounded by ejectosomes) suggesting an "attitude" to phagotrophy and several reports

307   indicate that these organisms may ingest bacteria (e.g. Tranvik et al., 1989). However, other more

308   recent studies refuse bacterivory (Hoef-Hemden & Archibald, 2017; Tanifuji & Onodera, 2017),

309   and a lack of consensus pervades the available literature. Although less abundant in summer, these

algae were persistent throughout all the seasons, as already observed in several Sicilian lakes (Barone & Naselli-Flores, 2003). No relationships were found among Cryptophytes biomass and growth rates, environmental variables and other phytoplankton groups in the studied lake.

Although belonging to a group of organisms largely considered as "heterotrophic", photoautotrophic euglenids have a feeding apparatus highly reduced and lost the predatory ability. Some species are probably osmotrophic but, in general, their predatory impact on prokaryotes and eukaryotes is negligible compared to that of heterotrophic euglenids (Leander et al., 2017).

Photosynthetic dinoflagellates are widely recognised as mixotrophic species, and are able to display peculiar phagotrophic strategies (Saldarriaga & Taylor, 2017). According to Reynolds et al. (2002), large thecate dinoflagellates are often associated to the coccalean cyanobaterium *Microcystis* spp. This is probably due to the reduced light availability caused by the colonies of *Microcystis* floating on the water surface, and to the possibility for these large mixotrophic dinoflagellates to ingest single cells of the cyanobacterium as an additional source of carbon.

However, both euglenids and dinoflagellates were never important in the lake, even though their representatives were present throughout the year. As for cryptophytes, no relationships were found with other phytoplankton groups and environmental variables.

As regard strictly-phototrophic phytoplankton (i.e. "cholorococcalean" green algae and diatoms) and cryptophytes, these were able to reach quite high biomass value any time there was a decrease in the relative biomass of haptophytes. This would suggest that these algae may benefit from the legacy of nutrients released in the water by haptophytes and deriving from their heterotrophic mode of nutrition.

In conclusion, it can be stressed that *Prymnesium parvum*, thanks to its mixotrophic behaviour and toxicity is able to monopolise resources and exert a negative effect on all the other biological components of the ecosystem. However, to do that, the species needs a continuous support in organic matter and bacteria. Lacking this "fuel", which positively influences its growth, the species is rapidly outcompeted by phototrophic organisms which can rapidly grow up thanks to

the legacy of nutrients left by *Prymnesium* itself. The alternation between mixotrophic and

phototrophic organisms is therefore a way to make a virtue out of necessity, and allow the entire

phytoplankton assemblage of the lake to quickly access both organic and inorganic nutrient

resources.

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Figure Captions

Fig. 1. Seasonal trends of depth, Biochemical Oxygen Demand (BOD5) values, and *Prymnesium parvum* biomass in the Biviere di Gela during the study period.

Fig. 2. Trends of inorganic nutrients (RP: reactive phosphorus; DIN: Dissolved Inorganic Nitrogen) in the Biviere di Gela during the study period. The dotted lines represent the best fitting polynomial line.

Fig. 3. Trends of phytoplankton biomass in the Biviere di Gela during the study period. HAP: Haptophytes, CYA: Cyanobacteria.

Fig. 4. Relative contribution of mixotrophic and phototrophic phytoplankton to total biomass in the Biviere di Gela during the study period.

Fig. 5. Relationship between weekly *Prymnesium parvum* growth rates and the increment in depth recorded in the Biviere di Gela one week earlier. Water incomes from Lake Dirillo are seasonally differentiated by colours: red dots refer to winter growth, green dots refer to spring growth, yellow dots refer to autumn growth and dark blue refer to summer growth. Light blue dots correspond to "natural" water incomes and are related to growth rates lower than 0.05 d^{-1}.
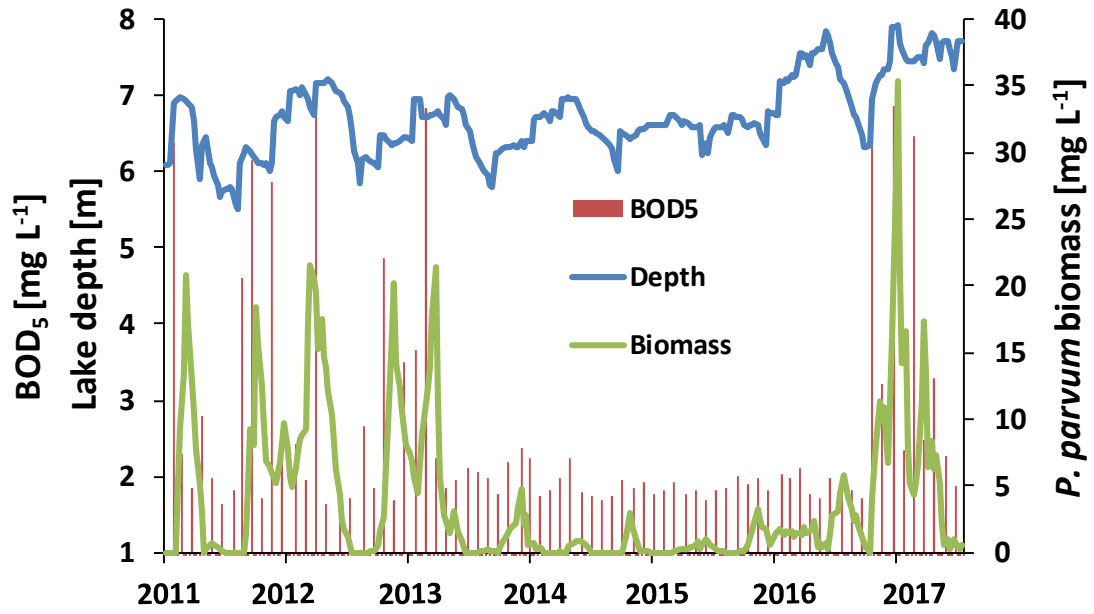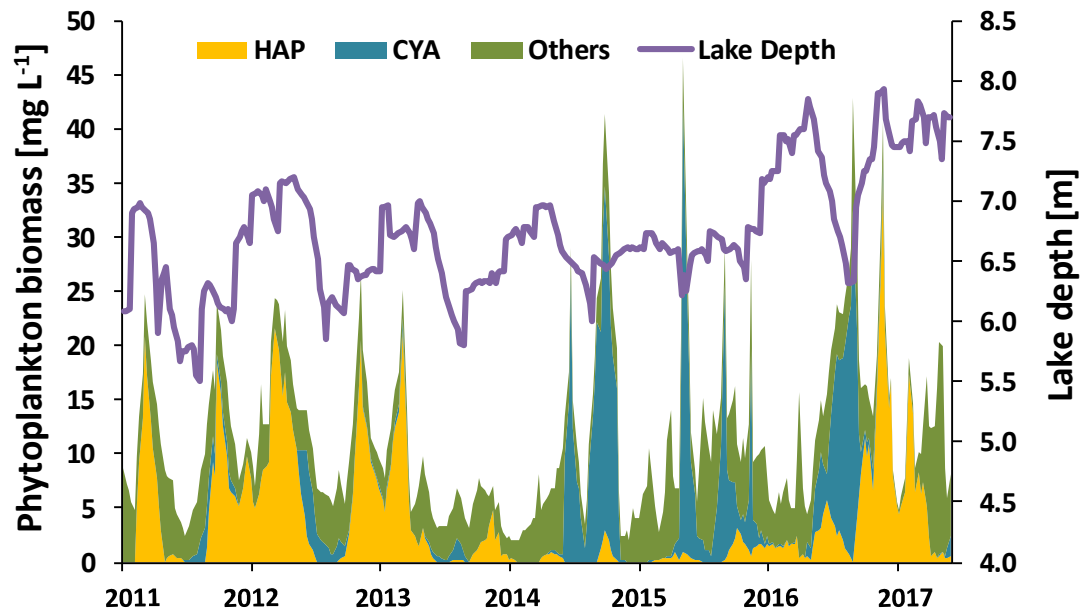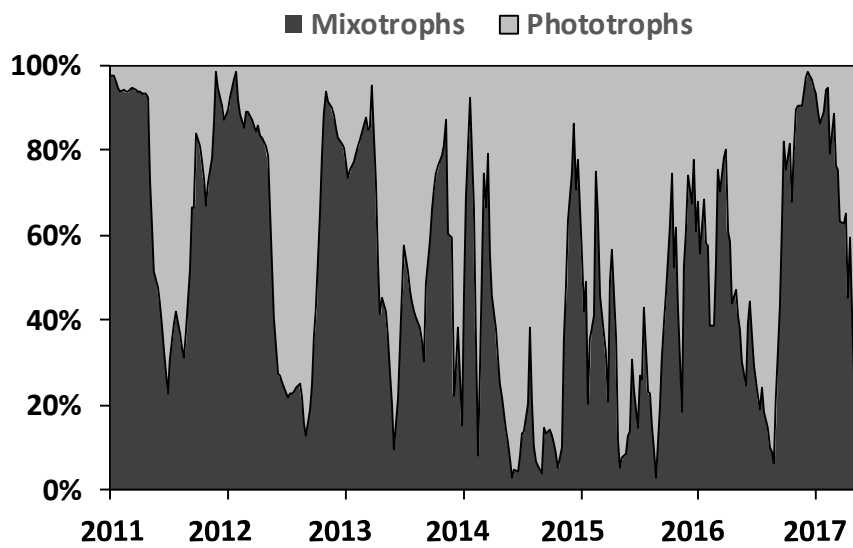
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484   Fig. 1

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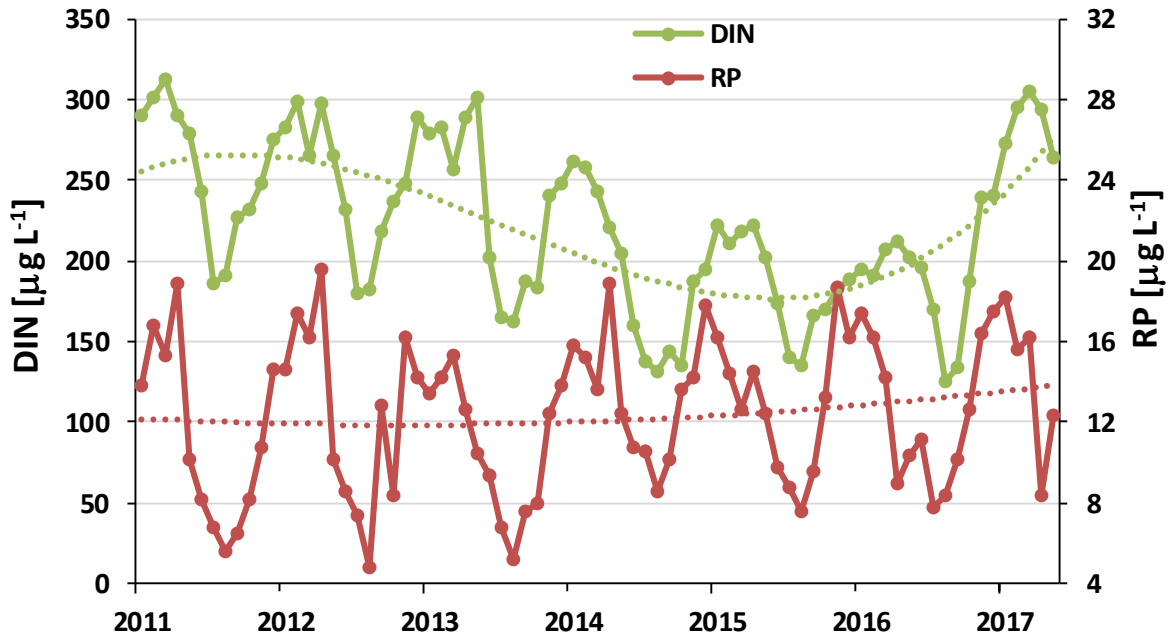
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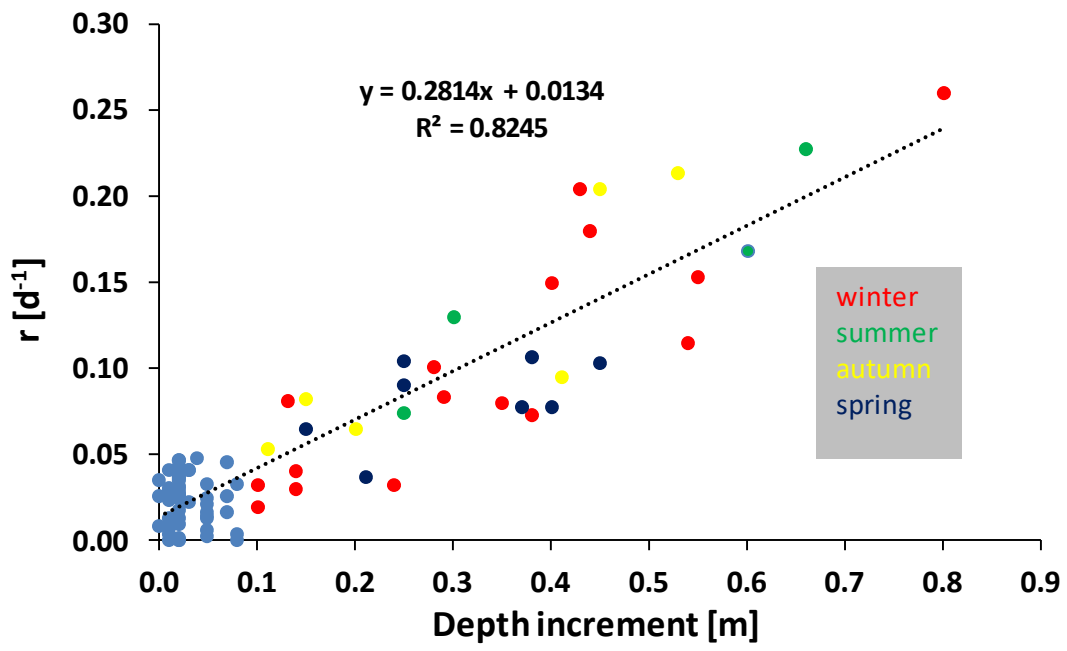
487   Fig. 2

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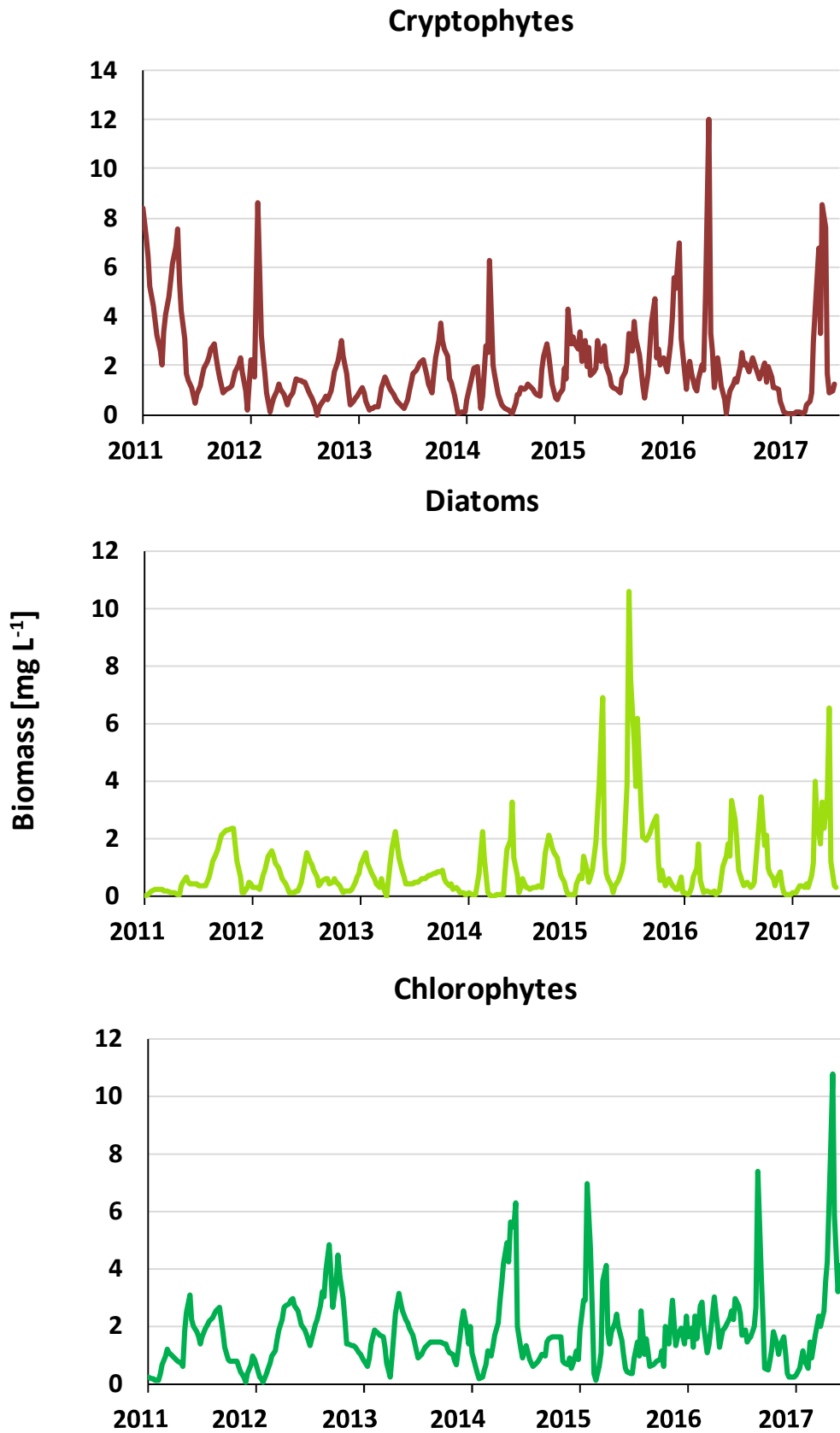492

493   Fig. 4
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499   Fig. 5

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Electronic Supplementary Material

502   Biomass trends of the main phytoplankton groups in the Biviere di Gela during the study period.

Cryptophytes

Diatoms

Chlorophytes

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