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Can bivalve selective feeding shape phytoplankton community structure in the Ria Formosa coastal lagoon?

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Mestrado em Biologia Marinha

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Can bivalve selective feeding shape phytoplankton community structure in the Ria Formosa coastal lagoon?

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I declare to be the author of this work, which is original and unpublished. Authors and works consulted are properly cited in the text and included in the reference list.

María Nohales Coscollá

Dançarinos num universo silencioso,

seguindo a dança das correntes e das marés,

nas suas pequenas dimensões possuem a grandeza,

de tecer as teias da vida.

Pequenos na imensidão azul,

velas num mundo escuro,

são os guardiãs do equilíbrio,

guardiões de um ciclo eterno que alimenta e sustenta a respiração do planeta,

nossa casa azul

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I dedicate this Master’s thesis to my mother, the strongest and kindest woman I know. The woman with the biggest heart. How lucky I am to have such a beautiful person as my mum.

Abstract

Phytoplankton plays a vital role in aquatic ecosystems, driving primary production, carbon cycling and nutrient recycling. The impact of bivalve feeding on phytoplankton biomass and community composition has been extensively documented. Nevertheless, there is currently no published research examining the impact of bivalve grazing and its selectivity on natural phytoplankton assemblages within the Ria Formosa coastal lagoon, a region responsible for 90% of Portugal's bivalve mollusc production. The aim of this study is to evaluate the impact of grazing by *Mytilus galloprovincialis* on natural phytoplankton assemblages in Ria Formosa. In particular, the effects of grazing on phytoplankton biomass, abundance, community composition, and the mussel's selectivity for different phytoplankton functional groups. The abundance and composition of phytoplankton were assessed using chlorophyll *a* concentration and microscopy: epifluorescence for pico- and nanophytoplankton and inverted for microphytoplankton. Mussel feeding was assessed by clearance rate. The microcosm experiments revealed a significant reduction in phytoplankton abundance due to mussel grazing in March, with a consistent decline in diatom abundance across all experiments. However, no significant changes were observed in phytoplankton biomass or community structure. The estimated *per capita* clearance rates of *M. galloprovincialis* ranged from 0.82 to 4.36 L h^{-1} individual^{-1} based on total chlorophyll *a* concentration, 1.07 to 1.95 L h^{-1} individual^{-1} based on total phytoplankton abundance, and 0.41 to 23.63 L h^{-1} individual^{-1} 1 for specific phytoplankton groups. The results indicate that mussels selectively graze diatoms without significantly affecting overall phytoplankton biomass or community composition in Ria Formosa. Given the region's ecological and economic importance, understanding bivalve selective grazing is crucial for effective ecosystem management. Further studies should investigate the long-term impacts of this grazing on phytoplankton diversity and ecosystem resilience, particularly in light of changing conditions and growing aquaculture demands.

Key words: Phytoplankton, *Mytillus galloprovincialis*, clearance rate, selectivity, Ria Formosa

Resumo

O fitoplâncton apresenta um papel vital para o ciclo do carbono e o fluxo de energia em ambientes aquáticos, suportando as teias alimentares marinhas e contribuindo para o sequestro global de carbono. As comunidades fitoplanctónicas são compostas por grupos funcionais variando em tamanho e função ecológica. As dinâmicas de suas comunidades são influenciadas tanto por fatores que regulam o crescimento (e.g. luz e nutrientes) quanto por processos de remoção (e.g. predação e sedimentação). Em ecossistemas de águas rasas, os bivalves como *Mytilus galloprovincialis* desempenham um papel crucial na regulação da população fitoplanctónica através da alimentação seletiva. Estes bivalves filtradores demonstram plasticidade fisiológica na seleção de partículas, preferindo fitoplâncton de maior tamanho, como diatomáceas. Este comportamento de alimentação pode alterar significativamente a composição da comunidade fitoplanctónica, afetando a estrutura e o funcionamento das teias alimentares marinhas. A laguna costeira da Ria Formosa, em Portugal, é um sistema importante tanto no ponto ecológico quanto econômico, oferecendo um estudo de caso único para examinar os impactos dos bivalves sobre o fitoplâncton. Embora a biomassa e a dinâmica do fitoplâncton neste sistema já tenham sido amplamente estudadas, não há uma pesquisa profunda sobre o papel dos consumidores metazoários, em particular os bivalves, no controlo da abundância e composição das comunidades fitoplanctónicas. Este estudo teve como objetivo avaliar os efeitos da predação de *M. galloprovincialis* sobre as assembleias naturais de fitoplâncton na Ria Formosa. Os principais objetivos incluíram avaliar o impacto da herbivoria dos mexilhões na biomassa, abundância e composição da comunidade fitoplantoníca, bem como examinar os comportamentos de filtração seletiva do bilvalve em questão. Foi analisada a hipótese de que *M. galloprovincialis* teria preferência de consumir grupos de fitoplâncton de maior tamanho como por exemplo, diatomáceas, e que os mexilhões reduziriam significativamente a biomassa do fitoplâncton, alterando a estrutura e composição da comunidade. Para investigar o impacto da alimentação do mexilhão mediterrânico *M.galloprovincialis* nas comunidades de fitoplâncton, foram realizados três experimentos de microcosmos. Foram recolhidas amostras de água da Ria Formosa em três ocasiões (novembro de 2022, março e abril de 2023), juntamente com espécimes do mexilhão amostrados na região subtidal. Os mexilhões foram recolhidos durante a maré baixa, e aclimatados no laboratório por duas horas antes da experimentação.

Dois tratamentos foram estabelecidos: controlo (C), sem mexilhões, e tratamento com bivalves (B), ambos realizados em triplicata. A abundância e composição do fitoplâncton foram avaliadas por meio da concentração de clorofila *a* e de microscopia: epifluorescência para pico- e nanofitoplâncton, e invertida para microfitoplâncton. A alimentação dos mexilhões foi avaliada através da taxa de filtração. A concentração de clorofila *a* foi usada como um indicador da biomassa total de fitoplâncton, enquanto a abundância dos grupos foi estimada no início (t_0) e no final (t_{30}) de cada experimento. As métricas de fitoplâncton entre os tratamentos foram comparadas utilizando o teste de Kruskal-Wallis e o teste post-hoc de Dunn. As diferenças na estrutura das assembléias de fitoplanctónicas foram analisadas utilizando a PERMANOVA aplicada sobre a matrix de similaridade de Bray-Curtis, seguida de comparações parwise. A análise SIMPER foi utilizada para identificar os grupos de fitoplâncton que contribuíram para as dissimilaridades dos dados. Para a taxa de filtração dos mexilhões, o teste de Wilcoxon foi utilizado para avaliar as taxas de clearance da clorofila *a* e do fitoplâncton, enquanto o Kruskal-Wallis foi utilizado para testar as diferenças entre grupos de fitoplâncton. Os resultados demonstram que as condições iniciais de clorofila *a* apresentaram variação entre os experimentos, com concentrações entre 0,78 e 2,40 $\mu g\ L^{-1}$. A alta abundância de fitoplâncton não coincidiu com concentrações elevadas de clorofila *a*. Em novembro, foi detectada uma menor concentração de clorofila *a*, enquanto se observou uma alta abundância de células menores e de baixo conteúdo de carbono, como cianobactérias e picoeucariotos fitoplanctônicos. Os valores máximos de clorofila *a* foram registados em abril, coincidindo com elevadas abundâncias de criptófitas e diatomáceas. Inicialmente, dominaram os fitoplâncton de tamanho nano- e pico-. No entanto, em março e abril, outros grupos como criptófitas e diatomáceas tornaram-se mais prevalentes. O *M. galloprovincialis* não teve impacto significativo na biomassa total de fitoplâncton. No entanto, em março, os mexilhões causaram uma redução significativa de 32,37% na abundância de fitoplâncton ($p < 0{,}05$). Os mexilhões não alteraram significativamente a estrutura do agrupamento de fitoplâncton em novembro e março ($p > 0{,}05$), mas observaram-se mudanças significativas em abril ($p < 0{,}05$). Apesar dessas mudanças, o teste parwise não revelou diferenças significativas entre os tratamentos do presente estudo. Não foi detectada dissimilaridade significativa entre as condições iniciais (t_0) e o controlo em qualquer experimento. No final dos experimentos, as diatomáceas foram o único grupo de fitoplâncton que diminuiu consistentemente no tratamento com mexilhões, com reduções de 66,4% em novembro, 91,8% em março e 83,0% em abril. A abundância de diatomáceas e outros grupos, como cianobactérias e picofitoplâncton eucariota, manteve-se estável no tratamento de controlo. Em contraste, os nanoflagelados autotróficos diminuíram 90,00% em março e aumentaram 142,31% em abril no tratamento com mexilhões. As taxas de filtração *per capita* estimadas para *M. galloprovincialis* com base na concentração total de clorofila *a* (0,82-4,36 $L \cdot h^{-1}$ indivíduo^{-1}), na abundância total de fitoplâncton (1,07-1,95 $L \cdot h^{-1}$ indivíduo^{-1}) ou na abundância de grupos específicos (0,41-23,63 $L \cdot h^{-1}$ indivíduo^{-1}) ficaram dentro da faixa de valores relatados para esta espécie. As diatomáceas foram consistentemente filtradas a uma taxa média de 5,30 L ind^{-1} h^{-1}. As taxas de filtração para cianobactérias, picofitoplâncton eucariota e dinoflagelados foram muito baixas ou indetermináveis. Este achado apoia a hipótese inicial do estudo e está em consonância com a sugestão de Barbosa (2006) de que os filtradores bentônicos contribuem para a redução da proporção de diatomáceas na abundância total de fitoplâncton, apesar de suas altas taxas de crescimento na Ria Formosa. A preferência por este grupo fitoplanctônico já foi observada em outras espécies de bivalvos. Essa preferência pode ser atribuída ao tamanho das diatomáceas, à sua qualidade nutricional, à facilidade de digestão e à sua vulnerabilidade à predação bentônica, uma vez que tendem a afunda. As taxas de filtração que não puderam ser determinadas podem ter sido influenciadas pela falta de consideração do grazing dos bivalvos sobre o microzooplâncton, outro predador importante do fitoplâncton. Além disso, a ausência de nutrientes inorgânicos em ambos os tratamentos pode ter evitado crescimentos do fitoplâncton devido às excreções de amônia e fósforo dos mexilhões. Recomenda-se que estudos futuros considerem o grazing dos bivalvos em fitoplâncton e microzooplâncton, assim como as espécies de bivalvos dominantes, para uma compreensão mais abrangente do impacto dos bivalvos nas comunidades de plancton e para melhorar os modelos de fluxo de carbono, ciclo de nutrientes e transferência de energia na lagoa costeira da Ria Formosa.

Palavras-chave: Fitoplâncton, *Mytillus galloprovincialis*, taxa de depuração, seletividade, Ria Formosa

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List of Abbreviations

Chl *a*	-	Chlorophyll *a*
RF	-	Ria Formosa
t_0	-	Initial time
C	-	Control treatment
B	-	Bivalve treatment
Cyano	-	Cyanobacteria
Epp	-	Eukaryotic picophytoplankton
Crypto	-	Cryptophytes
O-anf	-	Other autotrophic nanoflagellates
Dia	-	Diatoms
Dinofl	-	Dinoflagellates

1. Introduction

1.1 Ecological relevance of phytoplankton and its drivers

Phytoplankton are microorganisms that are essential for energy and carbon cycling in aquatic environments, supporting marine food webs and helping to mitigate the greenhouse effect through carbon sequestration (Singh and Ahluwalia, 2013). Phytoplankton photosynthesis fixes up to 50 Gt of carbon per year, contributing almost half of global primary production (Field et al. 1998). The biogeochemical consequences and fate of this production are determined by the composition of the phytoplankton community, which can be organised into several functional groups (see Anderson, 2005). This classification often combines size classes, motility types (e.g., *coccoid* vs. *flagellate* phytoplankton) and higher taxonomic groups that have specific biogeochemical functions or requirements (Cloern & Dufford, 2005). Examples of these functional groups include diatoms, which require silicate for cell structure formation, nitrogen-fixing cyanobacteria, which use atmospheric nitrogen as a nutrient source, or coccolithophores, which produce calcium carbonate exoskeletons (Sommer, 2017). However, of all the traits that classify functional phytoplankton groups (see Litchman, 2008), cell size is considered a 'master trait' by Litchman et al. (2010) and Sommer et al. (2017), as this feature is significantly correlated with many other ecophysiological traits - such as growth rates, nutrient uptake, dissolved organic carbon (DOC) exudation, sinking rates and predator interactions - that shape the ecological niches of phytoplankton (Banse, 1976, Finkel, 2001, Litchman et al. 2007; Nasielli-Flores et al. 2007; Litchman et al. (2010); Sommer et al. 2017). For example, small cells such as picophytoplankton have lower sinking rates that follow Stokes' law (Smayda, 1970). They also acquire limiting nutrients more efficiently due to their higher surface to volume ratio and tend to have higher maximum growth rates (Banse, 1976). Phytoplankton communities span a wide range of sizes, from less than 1 µm to millimetre-sized cells and even centimetre-sized colonies, covering six orders of magnitude (Finkel et al. 2010).

The distribution, abundance, and community composition of phytoplankton communities is highly dynamic, exhibiting significant variability over time (D'Alelio et al. 2015), as well as within (Cloern and Dufford, 2005) and among marine ecosystems (Carstensen et al. 2015). The biomass and production dynamics of these phytoplanktonic communities are controlled by growth-regulating variables— such as light, inorganic nutrients, biochemical inhibitors, and other bioactive substances—; and by processes that regulate the removal rate —such as cell mortality by predation, sedimentation, and viral lysis— and/or processes that control biomass advection (losses and gains) —such as horizontal and vertical advection of actively growing cells (vegetative cells) or reproductive cells (spores)— (see Barbosa, 2006). However, studies of phytoplankton dynamics have tended to focus on resource availability, often underestimating the substantial influence of processes that affect removal rates, such as grazing by predators (Banse, 2007; Prowe et al. 2012, Banse, 2013). Neglecting predation control may result in a limited understanding and low predictability of the processes that influence the structure and functioning of marine pelagic food webs (e.g., Chenillat et al. 2021). Furthermore, of the existing studies on phytoplankton removal rates in coastal regions, few focus on specific groups of phytoplankton, with primary attention given to microzooplankton as grazers (Domingues et al. 2021; Santos, 2023).

In shallow aquatic environments such as estuaries, bays, lagoons and coastal waters, benthic suspension feeders, including bivalves, play a dual role in shaping phytoplankton communities (Lucas et al. 2016; Jiang et al. 2022). Regulatory functions of bivalves include reducing phytoplankton biomass through grazing activities, but interestingly they can also act as potential stimulators of phytoplankton *blooms* (Asmus and Asmus, 1991). This is due to their ability to contribute to nutrient enrichment in the environment through processes such as nutrient excretion, bioturbation and biodeposition of faeces and pseudofaeces (Prins et al. 1995; Sun et al. 2022). These interactions can have important consequences for the overall phytoplankton structure and dynamics in shallow marine ecosystems (Mo et al. 2023). Therefore, the study of the regulatory factors of phytoplankton, especially at the level of functional groups (Cloern and Dufford, 2005; Barbosa, 2006), is essential for the understanding of carbon fixation in the oceans and the reduction of atmospheric CO_2 levels (Chávez et al. 2011), improving predictive models of carbon fluxes in aquatic systems (Barbosa, 2006). It is also useful for assessing trophic status, predicting eutrophication and forecasting fishery yields in coastal waters (Nixon, 1982).

1.2 The selective feeding behaviour of bivalves and its implications for the phytoplankton community structure

Filter-feeding bivalves are often dominant in the macrobenthos of coastal waters due to their high fecundity and potential for rapid growth (Rong et al. 2021). Bivalves form extensive populations of great economic, social, and ecological importance (Ward and Shumway, 2004; Rosa et al. 2018), providing a wide range of goods and services (see review by Smaal et al. 2019). From an ecological point of view, bivalve molluscs play a crucial role in the benthic-pelagic coupling (Ward and Shumway, 2004; Dame and Olenin, 2005). By biodepositing and excreting filtered pelagic material to the sediment surface, they effectively transfer energy from the pelagic to the benthic environment. This process not only increases deposition rates, but also triggers cascading effects such as sediment enrichment and enhanced microbial growth, which in turn provide vital resources for organisms living in the adjacent benthos (Naddafi et al. 2007). In addition, bivalves significantly influence the structure and dynamics of pelagic marine food webs by selectively consuming certain particles while rejecting others. This selective feeding behaviour ultimately affects the development, functioning and sustainability of coastal ecosystems (Lonsdale et al. 2009; Filgueria et al. 2012; Lucas et al. 2016). Bivalves demonstrate notable physiological plasticity in their feeding mechanisms, enabling them to adapt to alterations in seston composition and particle loading (Bayne, 2009; Pascoe et al. 2009). The physiological response is characterised by particle selectivity at the level of the ctenidia, which are located in the gills, and the labial palps. This ensures that only high-quality food is consumed, thereby maximising energy uptake (Bayne, 1993; Ward and Shumway, 2004). Furthermore, bivalves have the ability to reduce their clearance rates (CR) in unfavourable conditions. This is the rate at which water-borne particles are captured by their gills (Bayne, 2017). In extreme cases, filtration may cease completely by reducing the natural opening of the valves and retracting the mantle edges (Jørgensen et al. 1988). This can occur when there is an excess of food, which may result in an overload of the filtration and/or digestive systems (Riisgård, 2001a). The quality of food is typically evaluated based on the level of particulate organic matter or chlorophyll content (Ward and Shumway, 2004 and references therein). The sorting process in mussel feeding involves several distinct steps: (a) particle uptake in the ctenidia from the environment, (b) post-capture processing or pre-ingestive sorting of particles in the labial palps, leading to either their ingestion or the formation of pseudofaeces (material rejected before ingestion), and (c) differential absorption in the gut, known as post-ingestive sorting (Ward and Shumway, 2004; Rosa et al. 2018). At each of these stages, bivalves have the opportunity to select particles from the seawater based on both quantity and quality, given the large variability in suspended particulate matter (Ward and Shumway, 2004; Lucas et al. 2016; Safi and Hayden, 2010). It should be noted that selection efficiency will vary among bivalve species and, in some cases, between populations of the same species. This is due to differences in gill morphology and the relative sizes of the gills and labial palps (e.g., Meyhöfer, 1985). For instance, Rosa et al. (2015) demonstrated that the blue mussel *Mytilus edulis* has an enhanced capture efficiency (CE) for particles in the 4-10 µm size range, due to the characteristics of its pallial organs. The seston they feed on is composed of a variety of living and non-living components, including phytoplankton of different sizes and palatability, zooplankton, heterotrophic prokaryotes and detrital particulate organic matter (Vaughn and Hakenkamp, 2001; Ward and Shumway, 2004; Caroppo and Giordano, 2022). Seston selection is influenced by factors such as particle size, nutritional value - favouring organically enriched particles - and surface properties, with positively charged and hydrophilic particles being captured more efficiently than neutral or hydrophobic particles (Rosa et al. 2018). Phytoplankton is generally considered the main food source for filter-feeding bivalves among seston components, due to its high retention efficiency (Biandolino et al. 2008; Caroppo and Giordano, 2022). While there is evidence of a relationship between mussel growth and phytoplankton biomass (Dupuy et al. 2000; Trottet et al. 2008; Safi and Hayden, 2010; Jiang et al. 2016), the correlation between the two is variable (Asmus and Asmus, 1991; Ogilvie et al. 2000; Kimmerer and Thompson, 2014). This indicates that bivalve condition may also be influenced by factors other than biomass, such as phytoplankton size and species composition. It has been demonstrated that mussels have the ability to selectively feed on different microalgal species (Shumway et al. 1985), including algae of a similar size (Lesser et al. 1991; MacDonald and Ward, 1994; Shumway et al. 1997). This behaviour has the potential to affect the structure of the phytoplankton community (e.g. Froján et al. 2014; Richard et al. 2019; Gianasi et al. 2023). Despite this understanding, there is still a lack of research exploring the specific influence of bivalves in shaping phytoplankton composition. This is highlighted by studies such as those by Lucas et al. (2016) and Jiang et al. (2022), which indicate a need for further investigation in this area. The retention efficiencies of mussels indicate that small phytoplankton (< 5 µm) and very large phytoplankton (> 200 µm) are often unsuitable as food sources for bivalves (Rahman et al. 2020; Caroppo and Giordano, 2022). Therefore, mussels are unable to efficiently collect picophytoplankton (<2µm) (Kiorboe and Mohlenberg, 1981; Naddafi et al. 2007; Safi and Hayden, 2010; Rahman et al. 2020). Instead, mussels demonstrate a clear preference for consuming a significant portion of the large fraction of phytoplankton rather than consuming smaller picoplankton and bacteria (Trottet et al. 2008). Diatoms (Dupuy 2000b; Rosa et al. 2018; Jiang et al. 2022; Safi and Hayden, 2010; Zhang et al. 2023) and cryptophyes (Jiang et al. 2022; Naddafi et al. 2007) have been identified as the preferred phytoplankton food for filter-feeding mussels. A significant number of studies have been conducted to examine mussel filtration rates under controlled laboratory conditions, using pre-selected algal cultures (Shumway et al. 1985; Navarro et al. 1996; Vanderploeg et al. 2001; Pales Espinosa et al. 2016; Rosa et al. 2017) and prey items larger than 5 µm (see review by Rosa et al. 2018), not natural phytoplankton assemblages. While these experiments yield estimates of maximum clearance rates, it is generally expected that clearance rates in natural environments will be lower (Jacobs et al. 2015). It is therefore clear that there is a need for data on actual realised clearance rates under natural conditions and insights into the specific utilisation of natural plankton by mussels (Cranford et al. 2011; Trottet et al. 2008). Furthermore, the majority of the available information on the impact of bivalve grazers on phytoplankton has been gathered from studies documenting rapid temporal changes, particularly in the context of species introductions (Kimmerer and Thompson, 2014). It is notable that there is a lack of focus on native species such as the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819), an indigenous species to Ria Formosa (Portugal). This mussel is an optimal species for human consumption and aquaculture due to its rich nutrient profile (see Silvia et al. 2021).

1.3 The removal of phytoplankton by bivalve grazing in Ria Formosa

Ria Formosa is a shallow coastal lagoon system located in the Algarve region of southern Portugal. It plays an important role in supporting diverse marine life, as well as sustaining local livelihoods and contributing to national economic prosperity through fishing, aquaculture and tourism (see Barbosa, 2010 and references therein). Shellfish farming is a particularly significant contributor to the local economy, with Ria Formosa being Portugal's leading clam-producing site (Machas et al. 2003; Cravo et al. 2022). It accounts for approximately 90% of the country's bivalve mollusc production (Chícharo and Chícharo, 2001), which is ensured by the fundamental role of microbial plankton in this ecosystem. Microbial plankton is capable of using and significantly altering DOC and POC, thereby sustaining the larval and adult stages of this particular production (Barbosa, 2006). Previous studies of phytoplankton in this well-mixed meso-tidal system have addressed a number of key areas, including the spatial and temporal dynamics of chlorophyll *a* (Barbosa, 2010; Cravo et al. 2021; Rosa et al. 2022), the structure of phytoplankton assemblages (Morais et al. 2003; Loureiro et al. 2006; Barbosa, 2006; Jacob et al. 2020; Cravo et al. 2022). Furthermore, research has identified the occurrence of harmful algal blooms and the effects of physico-chemical variables on phytoplankton growth and composition as key areas of investigation. These include variables such as temperature, light, CO_2, nutrients and ultraviolet radiation. However, there is currently a lack of available information on phytoplankton to address the issue of microbial mortality due to grazing in RF coastal lagoons. The research has focused on microzooplankton herbivory (Barbosa, 2006; Sá, 2017; Domingues et al. 2021; Santos, 2023). However, there is currently no information available on metazoan grazers, including bivalve molluscs. Barbosa (2006) postulated that benthic filter feeders were responsible for the reduced average contribution of diatoms to total phytoplankton abundance, despite their high in situ growth rates in the interior of Ria Formosa. It is essential to assess the impact of mussel filtration on plankton communities in order to determine the carrying capacity of the lagoon system. If this capacity is exceeded, there is a risk of food shortages for cultured bivalves and environmental degradation (Trottet et al. 2008).

1.4 Aims and hypothesis

The aim of this study is to evaluate the effect of *M. galloprovincialis* spp. predation on natural phytoplankton assemblages in Ria Formosa.

The specific objectives of this study are:

(1) To evaluate the effects of *M. galloprovincialis* grazing on phytoplankton biomass, abundance, and assemblage composition.

(2) To evaluate *M. gallorpovincialis* grazing rates and its pre-ingestive selective feeding on phytoplankton.

Based on previous studies analysing bivalve selective grazing on phytoplankton, this study hypothesized that: (1) *M. galloprovincialis* would show a preference for larger phytoplankton groups such as diatoms and cryptophytes in respect to other phytoplankton groups (Dupuy et al. 2000b; Beninger and Decottignies, 2005; Naddafi et al. 2007; Trottet et al. 2008; Safi and Hayden, 2010; Rosa et al. 2018; Jiang et al. 2022); (2) Grazing by *Mytilus galloprovincialis* will significantly reduce phytoplankton biomass and alter community composition (Prins and Escaravage, 2005; Lucas et al. 2016; Marescaux et al. 2016).

The study yielded valuable insights into the phytoplankton dynamics in the Ria Formosa coastal lagoon due to bivalve grazing, offering a more comprehensive understanding of the relationships between bivalve grazing and the phytoplankton assemblage structure in coastal ecosystems.

2. Materials and methods

2.1 Study area

The Ria Formosa coastal lagoon (Figure 2.1) is a shallow (mean depth = 2 m) system, ~55 km long and ~6 km wide (see review by Barbosa, 2010), located in the southern region of Portugal (Algarve). It is partially enclosed by a system of barrier islands, which separate it from the Atlantic Ocean. However, it remains connected to the ocean through six inlets: two artificially relocated (Ancão and Fuseta inlets), two artificially opened and stabilised (Faro-Olhão and Tavira inlets) and two natural inlets (Armona and Lacém inlets) (Matias et al. 2008). The Ria Formosa has a Mediterranean climate, with mild winters, and hot, dry summers. Maximum summer temperatures reach 25°C, while minimum winter temperatures can fall below 9°C (see Aníbal, Esteves, and Rocha, 2011). Ria Formosa is subject to a semi-diurnal mesotidal regime, with a large fraction of the lagoon water volume (about 50-75%) exchanged with the ocean on a daily basis. This large tidal exchange, together with the reduced freshwater inflow, contributes to the euryhaline nature, with salinity levels typically similar to the adjacent coastal ocean waters (Newton and Mudge, 2003; Barbosa, 2010). In addition, the water column is well mixed due to its shallowness, with no significant thermal or haline stratification (Mudge et al. 2008). The coastal lagoon, with a wet surface area of 105 km^2 out of a total of 185 km^2 (Andrade et al. 2004), is a wetland area that is internationally recognized for its ecological value as a breeding and transit area for fish and birds, as well as supporting a wide range of human uses, including tourism, fishing, and shellfish farming (see review by Barbosa, 2010). For this reason, it has been a Natural Park since 1987, a Ramsar and Natura 2000 site, and is included in the Special Protection Area for Birds.

2.2 Experimental approach

Three short-term microcosm experiments were conducted using natural water samples from the Ria Formosa coastal lagoon to study how the feeding activity of the Mediterranean mussel (*Mytilus galloprovincialis*) affects phytoplankton assemblage groups and their structural composition. The experiments were carried out on 07 November 2022, 13 March 2023 and 26 April 2023.

2.2.1 Sampling Strategy

Water samples and *Mytilus galloprovincialis* specimens were collected at low tide from an inner site in the coastal lagoon of Ria Formosa (Figure 2.1, coordinates 37.03064°N, -7.593964°W). For each experiment, 21 litres of surface water were collected at a depth of approximately 50 cm using 6 L water jugs, with an additional 24 litres reserved for the acclimation system. Approximately 30 specimens of *M. galloprovincialis*, 50 to 60 mm in size, were collected by hand from the subtidal area by cutting the byssal threads with a knife. Both water samples and bivalves were transported to the laboratory in the dark within 30 minutes of collection.

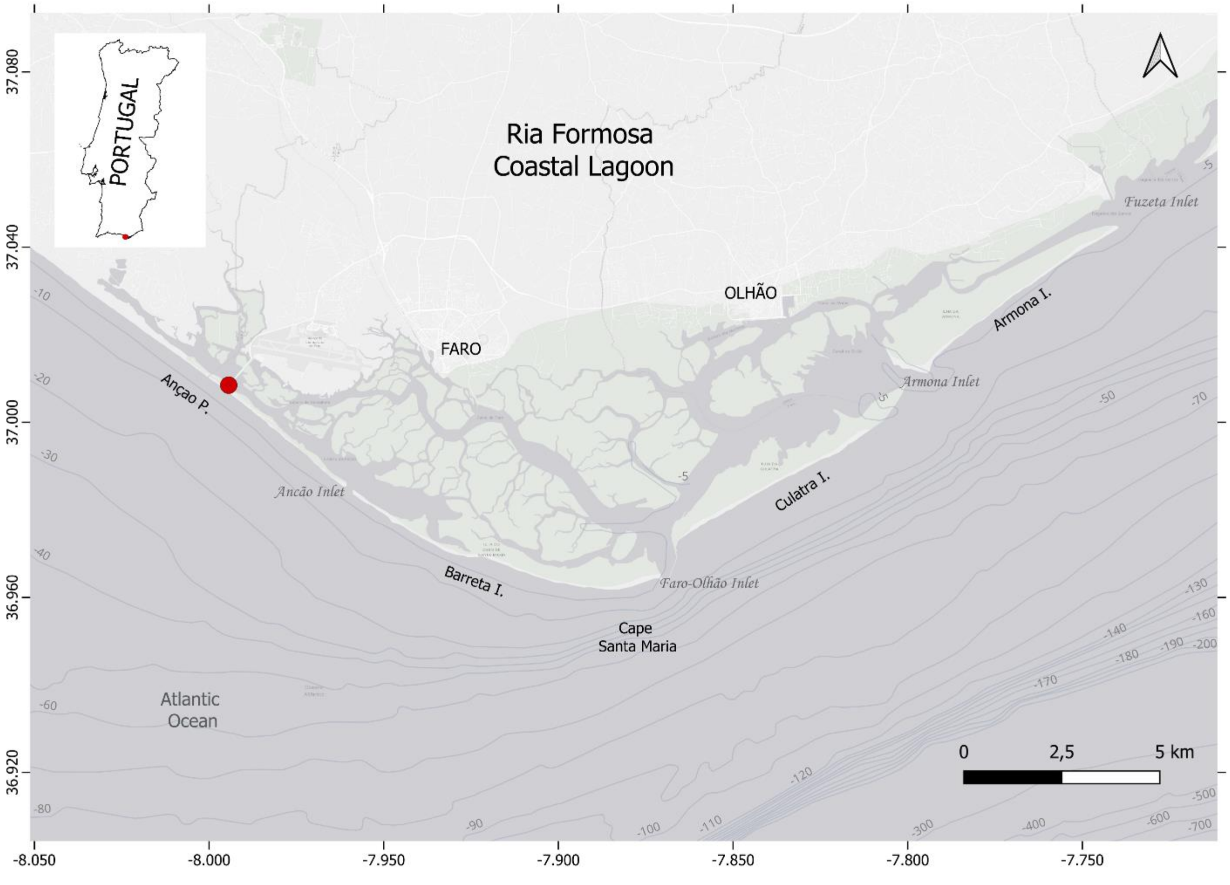

Figure 2.1: Location of the sampling station in the western subembayment of the Ria Formosa coastal lagoon. Sampling efforts included the collection of water samples along with *M. galloprovincialis* specimens at this designated station, denoted by the red dot.

2.2.2 Experimental design

Prior to the experiments, the mussels were acclimated in the laboratory for 2 hours to reduce stress from collection and transport. They were placed in a 50 L opaque bucket filled with 24 L of natural water from the Ria Formosa coastal lagoon, under ambient light conditions and aerated by air pumps (Figure 2.2, left). In contrast to the recommendation by Dupuy et al. (2000b), epibionts were not removed from the bivalves (see Dupuy et al. 2000b) to minimize further stress or damage on bivalves (Lacoste and Gaertner-Mazouni, 2014).

The potential influence of *Mytilus galloprovincialis* filtration on the natural planktonic community was investigated by comparing changes in phytoplankton abundance and assemblage structure with and without mussels over a 30-minute period (Figure 2.2, right). For each experiment, three replicate 6-litre containers per treatment were prepared, each filled with 4 litres of natural, unfiltered RF water. Two experimental treatments were carried out in triplicate: control (C), in which plankton in natural water samples were allowed to develop without mussels; and water samples exposed to actively filtering mussels (B). For the bivalve treatment, two (in the first experiment) to three (in subsequent experiments) actively filtering bivalves of comparable size were used. No nutrient additions were applied to the experimental treatments (see Vanderploeg et al. 2001). Given the short duration of the experiment (30 min), any potential nutrient release by bivalves would be insufficient to stimulate phytoplankton growth in Ria Formosa, as previously demonstrated by Domingues et al. (2017) in nutrient enrichment experiments. No visible production of pseudofaeces was observed at the end of all experiments, in the experimental treatments with bivalves (B).

Chlorophyll *a* concentration and phytoplankton abundance and composition were evaluated, for each experimental treatment, at the beginning (t_0) and end of each experiment. As the same water sample was used for both treatments, the same t_0 value was applied to both. The experimental containers were gently homogenized prior to sampling to resuspend dense, non-motile cells that may have been deposited at the bottom, such as diatoms (see Anderson and Sweeney, 1978).

A preliminary experiment, undertaken on 7 November 2022, using *in vivo* chlorophyll *a* fluorescence measured with a 10-AU-005-CE fluorimeter (Turner Designs Instruments) on indicated a significant decrease in chlorophyll *a* concentration within the first hour, with reductions of 66,76% in the control (C) and 82,46% in the mussel treatment (B). Based on these results, the experiment duration was adjusted to 30-minute, as shorter periods could include variable bivalve activity until establishment of regular feeding (see Dupuy et al. 2000b).

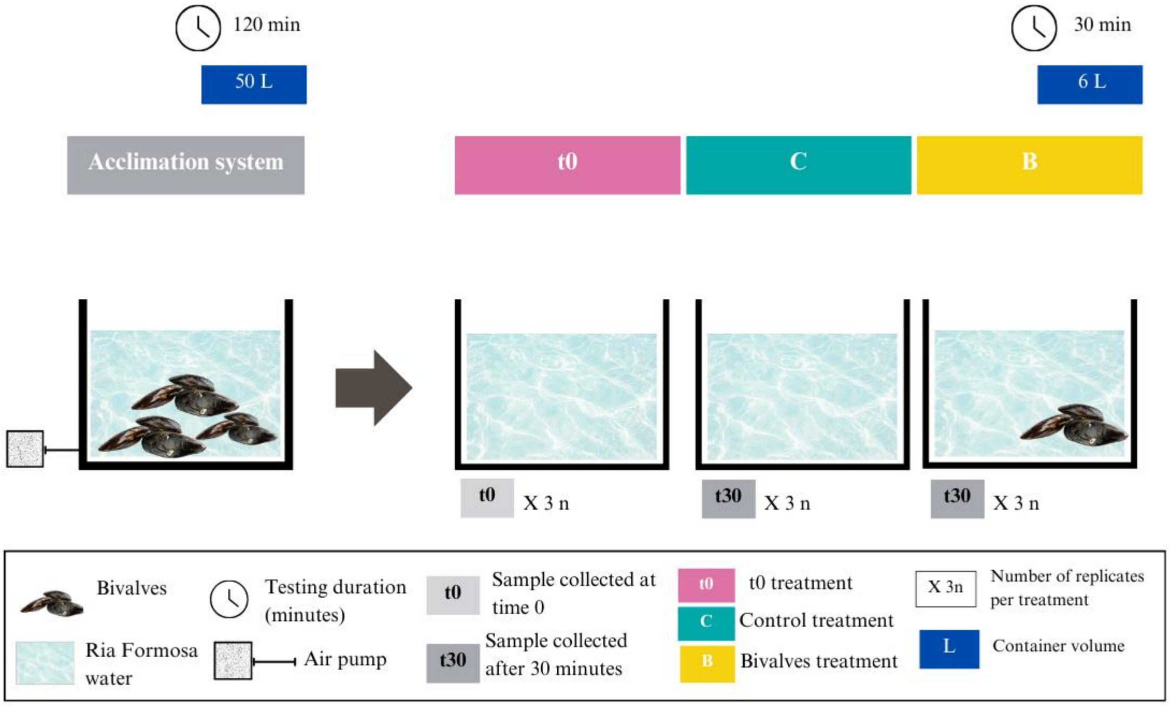

Figure 2.2: Schematic representation of the experimental design, including the acclimation stage (left) and the experimental stage (right). The experimental phase includes water sampling at the start (t_0) and after 30 minutes, for both the control treatment without mussels (C), and the experimental treatment with mussels, with 2-3 mussels per container (B). The same t_0 value was used for all treatments, carried out in triplicate.

2.3 Analysis of phytoplankton metrics

2.3.1 Chlorophyll *a* concentration

Chlorophyll *a* (Chl*a*) concentration was used as a proxy for total phytoplankton biomass (Cullen, 1982) at the start (t_0) and end (t_{30}) of each experiment. Chl*a* concentration was determined using the spectrophotometric method described by Parsons et al. (1984). One-L of Ria Formosa water sample was filtered through glass fibre filters (GF/F, pore diameter 0.7 μm) in triplicate, under low light conditions and low pressure (<100 mm Hg) to minimise cell damage or loss. After filtration, the filters were placed in test tubes, wrapped in aluminium foil, and stored in a freezer at -20ºC until further analysis.

Chl$a$ was extracted from the filters overnight at 4ºC using 90% acetone. After extraction, the filters were centrifuged (2000 rpm, 20 min), and the absorbance of the extract was then measured spectrophotometrically (Hitachi U-2000) at 750 and 665 nm, before and after acidification with 0.5 M HCl to correct for phaeopigments. The Chl$a$ concentration was calculated using the standard equation of Lorenzen (1967) (see equation 1), where $K = 2.43$ is the factor to equate the decrease in absorbance to the initial Chl$a$ concentration, E_{665}= absorbance at 665 nm before acidification, E_{750}= absorbance at 750 nm before acidification, E_{665a}= absorbance at 665 nm after acidification, E_{750a}= absorbance at 750 nm after acidification, v is the volume of acetone added (mL), V is the volume of sample filtered (L), and L is the path length of the spectrophotometer cuvette (cm).

$$Chla\ (\mu g\ L^{-1}) = \frac{11{,}4K[(E_{665} - E_{750}) - (E_{665a} - E_{750a})] \times v}{V \times L} \tag{1}$$

2.3.2 Abundance and composition of phytoplankton assemblages

Phytoplankton abundance and composition were evaluated using two microscopy techniques. Epifluorescence microscopy was used to determine the abundance of pico- (<2 μm) and nanophytoplankton (2-20 μm), and inverted microscopy was used for microphytoplankton (20-200 μm), following the methods of Haas (1982) and Utermöhl (1958), respectively. According to Barbosa (2006) and Domingues et al. (2008), the use of both techniques simultaneously is the most effective strategy for a comprehensive, detailed and quantitative analysis of the entire phytoplankton community.

Water samples for the assessment of pico- and nanophytoplankton abundance were collected and immediately fixed with glutaraldehyde 25% (final concentration 0.2%), stored in a dark glass bottle, and refrigerated at 4°C for no more than one week prior to slide preparation. Slides were prepared by staining with proflavine for 3 minutes and then vacuum filtering (pressure <100 mm Hg) onto a black polycarbonate membrane filter (Whatman, pore diameter = 0.4 µm). The membrane filter was placed on a glass slide with a drop of non-fluorescent immersion oil (Cargille type A) and immediately observed under an epifluorescence microscope (Leica DM LB) with blue and green light at 1250× magnification. Observations were made in the dark to increase contrast and minimise fluorescence loss. Specific groups of phytoplankton were distinguished under blue and green light using a graticule (New Porton G12) mounted on the eyepiece. Phytoplankton were aggregated into the following groups: cyanobacteria, eukaryotic picophytoplankton, cryptophytes and other plastidic nanoflagellates.

For the inverted microscopy, 330 mL samples were preserved immediately after collection with acidic Lugol's solution in a plastic bottle and stored in the dark. The preserved samples were then transferred to 50 mL sedimentation columns, where the phytoplankton cells were allowed to settle by gravity over a period of 24 hours. The sedimentation columns were sealed with a cover glass and kept in the dark during sedimentation. After settling, the samples were observed under an inverted microscope (Zeiss Axio Observer A1) at 400x magnification. Cells were identified as diatoms, plastidic dinoflagellates, and euglenophytes (flagellates). In addition, phytoplankton groups were analysed to the lowest possible taxonomic level, using Kraberg et al. (2010) and Tomas (1997) as a guide for taxonomic identification.

For both epifluorescence and inverted microscopy, a minimum of 50 visual fields and 400 cells were counted per sample. Assuming that the cells were randomly distributed, the counting precision was ±10% (Venrick, 1978). For each sample, the abundance of the different phytoplankton groups was estimated at the beginning (t_0) and end (t_{30}) using equation 2, where x is the total number of cells, A is the area (mm^2) of the polycarbonate filter / sedimentation chamber, d is the sample dilution factor due to the addition of fixatives, a is the area of the visual field (mm^2), n is the number of visual fields observed, and V is the sample volume filtered/settled (L). Total abundance of phytoplankton was calculated as the sum of all groups.

$$Abundance\ (cell.L^{-1})\frac{X \times A \times d}{a \times n \times V}$$

(2)

2.4 *Mytillus galloprovincilis* grazing on phytoplankton assemblages: analysis of clearance rates

To assess the selective grazing on phytoplankton by *M. galloprovincialis*, the clearance rate (F) was calculated. Clearance rate (F), defined as the volume of water cleared of particles per unit time (l h^{-1}) (Bayne and Widdows, 1978), was calculated using the equation 3 from Vanderploeg et al. (1995):

$$F = (V/nt)\ln\,(C_{wc}/Z_{wc})$$

(3)

where C_{wc} is the mean phytoplankton concentration in the water column at the end of the experiment in control containers, Z_{wc} is the phytoplankton concentration in the water column in experimental containers at the end of the experiment, *V* is the volume of water in the container during the experiment, *n* is the number of mussels, and *t* is the duration of the experiment (30 minutes).

All grazing rates were normalized per individual bivalve.

2.5 *Statistical analysis*

Basic assumptions for parametric analyses, including data normality and homogeneity of variance, were tested using the Shapiro-Wilk and Levene tests, respectively, and non-parametric methods were used when required. All statistical analyses were considered at a significance level of 0.05 and conducted using R version 4.1.1 (R Core Team, 2021) and specific R packages.

2. 5.1 Phytoplankton metrics and community structure

Differences in phytoplankton metrics between treatments were tested using the Kruskal-Wallis test, a one-way analysis of variance by ranks, followed by Dunn’s post-hoc test (Sokal and Rohlf, 1995). Kruskal-Wallis post-hoc test was computed using the R package ‘dunn.test’ (v1.3.5; test (Dinno, 2017). For November phytoplankton abundance, however, a Wilcoxon test was used to compare the control (C) and bivalve (B) treatments due to the absence of t0 replicates.

Differences in the structure of phytoplankton assemblages between treatments were tested using permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) based on a Bray-Curtis similarity matrix. Treatment (3 levels) was used as a fixed factor. Significant effects were tested using post-hoc pairwise comparisons with the PERMANOVA F-statistic and 999 permutations, using the R pairwise

Adonis function 'pairwise.adonis'. To assess the percentage contribution of each phytoplankton group to the average dissimilarity between factor levels, Similarity Percentage Analysis (SIMPER) was used (Clarke and Gorley, 2006), based on the R vegan function 'simper', allowing the identification of potentially discriminating phytoplankton groups. Multivariate analyses were computed using the following R packages: vegan (v2.6.4; Oksanen et al. 2022) and pairwiseAdonis (v0.4.1; Martinez Arbizu, 2017).

2.5.2. *Mytillus galloprovincilis* grazing on phytoplankton assemblages: analysis of clearance rates

A one-tailed Wilcoxon signed-rank test was used to test whether the clearance (F) rates of chlorophyll *a* and total phytoplankton were significantly different from zero. In addition, a one-way Kruskal-Wallis test followed by Dunn's post-hoc test (Sokal and Rohlf, 1995) was used to test whether there were significant differences between the clearance rates (F) of different phytoplankton groups within an experiment.

3. Results

3.1. Conditions at the beginning of the experiments

Initial conditions varied considerably among the experiments (Table 3.1). Initial chlorophyll *a* values ranged between 0.78 and 2.40 µg L^{-1}. The initial chlorophyll *a* concentration and phytoplankton abundance in the experimental water did not show a clear concordance, especially in November when chlorophyll *a* values were lowest and total phytoplankton abundance was highest compared to the rest of the experiments. This abundance is noteworthy as it is mainly composed of phytoplankton groups belonging to the pico-size category. The maximum chlorophyll *a* values were observed in April, which also coincided with the highest abundances of cryptophytes and diatoms (Figure 3.1 and Table 3.1).

Table 3.1: Mean (±SE) initial chlorophyll *a* concentration (C_0, µg L^{-1}), initial phytoplankton abundance (cell L^{-1}) and number of mussels per container.

Date	C_0 ($\mu g\ L^{-1}$)	Phytoplankton abundance (cells L^{-1})
November 2022	0,78 ± 0,61	$1{,}88x10^7$
March 2023	1,48 ± 0,42	$2{,}87x10^6$
April 2023	2,40 ± 0,89	$5{,}88x10^6$

Six phytoplankton groups were identified to estimate mussel feeding rates (Figure 3.1 and Table 3.2). Initially, the main contributors to phytoplankton abundance were nano- and pico-sized cells, in particular autotrophic nanoflagellates (excluding cryptophytes), eukaryotic picophytoplankton and cyanobacteria. However, with the onset of spring (March and April experiments), other nanoplanktonic groups, such as cryptophytes and diatoms, were more abundant. The presence of the centric diatoms *Thalassiosira* spp. and *Chaetoceros* spp., and the pennate diatom *Cylindrotheca* spp., seems to be more pronounced compared to other taxa evaluated (Table 3.2).

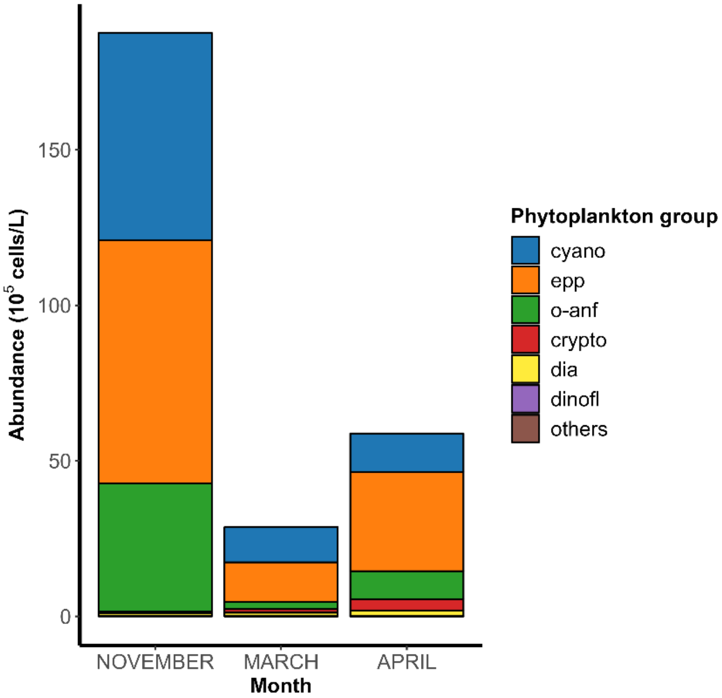

Figure 3.1: Initial abundance (x10^5 cell L^{-1}) of specific phytoplankton groups in water samples from the coastal lagoon of RF coastal lagoon. Abbreviations: Cyano -cyanobacteria; epp -eukaryotic picophytoplankton; anf autotrophic nanoflagellates; crypto -cryptophytes; dia -diatoms; dinofl -dinoflagellates; others.

Table 3.2: Abundance (x10^3 cells L^{-1}) of specific phytoplankton taxa in the initial water samples from the Ria Formosa coastal lagoon.

	11-Nov.-22	13-March-23	26-April-23
Picoplanktonic Cyanobacteria	6667.83	1130.47	1235.85
Eukariotic picophytoplankton	7817.46	1274.17	3190.21
Cryptophytes	57.48	114.96	364.05
Other autotrophic nanoflagellates	4109.91	229.93	900.54
Diatoms			
Centric diatoms	5.74	21.28	5.52
Chaetoceros spp.	4.30	0.72	21.8
Thalassiosira spp.	22.96	14.35	75.33
Dactyliosolen fragilissimus	0.00	0.00	2.15
Proboscia sp.	0.00	0.00	0.24
Rizosolenia spp.	5.02	0.96	0.00
Leptocylindrus spp.	6.46	0.00	3.35
Cerataulina pelagica	1.43	0.00	0.00
Odontella sp.	0.72	0.00	0.00
Pennate diatom	5.74	15.78	33.48
Cylindrotheca spp.	2.87	17.93	33.72
Pseudo-nitzchia spp.	6.46	4.3	0.72
Pleurosigma spp.	0.00	0.96	0.24
Navicula spp.	0.00	16.98	0.00
Asterionellopsis glacialis	0.00	18.65	0.00
Diploneis sp.	0.00	0.00	0.24
Licmophora spp.	0.00	1.91	0.72
Other diatoms	25.83	1.67	0.24
Plastidic Dinoflagellates			
Prorocentrum sp.	7.89	0.00	0.00
Other dinoflagellates	2.87	2.15	14.35

3.2. *Effects of bivalves on phytoplankton biomass, abundance, and community structure*

The presence of the bivalve *Mytilus galloprovincialis* had no significant effect on phytoplankton biomass as reflected by total chlorophyll *a* content (Figure 3.2). However, a significant 32.37% reduction in total phytoplankton abundance was observed in the mussel treatment group in March ($p < 0.05$).

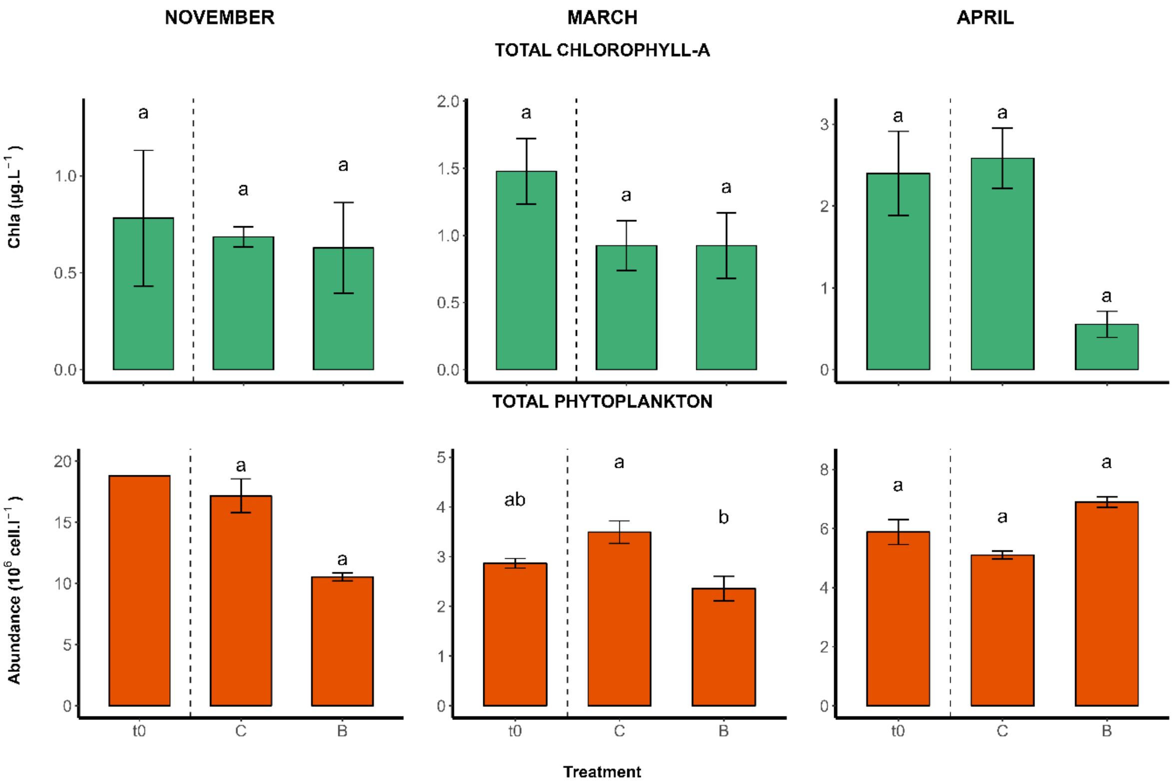

Figure 3.2: Chlorophyll *a* concentration (µg L^{-1}) and total phytoplankton abundance (10^6 cells L^{-1}) in the RF coastal lagoon at the beginning (t_0) and after 30 min for the control (C, no mussels) and experimental (B, 2-3 mussels) treatments. Data from November 2022, March 2023 and April 2023 are shown, with mean ± 1 SE for the phytoplankton metrics. Different letters (a, b) indicate significant differences within each experiment (Kruskal-Wallis, Bonferroni test, $p < 0.05$).

PERMANOVA analysis showed that *M. galloprovincialis* did not significantly affect the structure of the phytoplankton assemblage in the November and March experiments ($p > 0.05$). However, there was a significant change in phytoplankton structure in April ($p < 0.05$). Despite this overall significant effect in April, pairwise comparisons between specific treatments did not reveal statistically significant differences ($p > 0.05$, Figure 3.3, Table 3.3).

The SIMPER analysis ranked the phytoplankton groups according to their contribution to the overall dissimilarity of the phytoplankton assemblage structure between treatments and the initial composition. No statistical dissimilarity was observed in the phytoplankton assemblage structure between the initial conditions (t_0) and the control treatment after 30 min in any experiment. In November, the overall average dissimilarity in phytoplankton assemblage structure between the mussel treatment (B) and the other two conditions (t_0, C) was 31% and 24%, respectively (Table S1). The dissimilarity between baseline and final conditions with mussels (B) was mainly driven by eukaryotic picophytoplankton (17%) and cyanobacteria (12%), both of which were more abundant at baseline. Diatoms and dinoflagellates also contributed to the dissimilarity, but at low levels (<1%). For the final treatments -bivalve and control-, the dissimilarity was explained by autotrophic nanoflagellates (6%), cryptophytes and diatoms (<1%), all of which were more abundant in the control. In March, this dissimilarity in phytoplankton assemblage structure between the mussel treatment and the other two conditions (t_0, C) was 19% and 27%, respectively (Table S2). The dissimilarity between baseline and final conditions with mussels (B) was significantly driven by diatoms, which contributed 2% and were more abundant at baseline. For the final treatments, dissimilarity was attributed to autotrophic nanoflagellates (7%) and diatoms (2%), both of which were more abundant in the control. In April, the overall average dissimilarity in phytoplankton assemblage structure between the mussel treatment (B) and the other two conditions (t_0, C) was 17% and 19%, respectively (Table S3). The dissimilarity between baseline and final conditions with mussels (B) was driven by other autotrophic nanoflagellates (7%) and diatoms (<2%). The other autotrophic nanoflagellates were more abundant in the bivalve treatment. For the final treatments, dissimilarity was attributed to other autotrophic nanoflagellates and eukaryotic picophytoplankton, both contributing >5% to the total dissimilarity, with other autotrophic nanoflagellates showing greater abundance in the control treatment (C). Diatoms also contributed 1% to the dissimilarity.

Table 3.3 Summary of PERMANOVA (Permutational Multivariate Analysis of Variance) tests assessing differences in phytoplankton assemblage structure in the RF coastal lagoon, with treatment as a factor. The analysis includes data for t_0 and 30 min for control (C, no mussels) and experimental (B, 2-3 mussels) treatments. The table gives the interpretation of the PERMANOVA results for this factor. Partial R^2: partial coefficient of determination; Pseudo-F: pseudo-F ratio; P-value (perm): permutation P-value. An asterisk (*) indicates a p-value < 0.05.

Source of variation	**Partial R^2**	**Pseudo-F**	**P-value (perm)**	**Interpretation**
November 2022				
Treatment	0.68	8.36	0.10	No significant changes in phytoplankton composition
Residual	0.32			
March 2023				

Treatment	0.40	2.03	0.125	No significant changes in phytoplankton composition
Residual	0.60			
April 2023				
Treatment	0.71	7.48	0.019 *	Significant changes in phytoplankton composition
Residual	0.29			

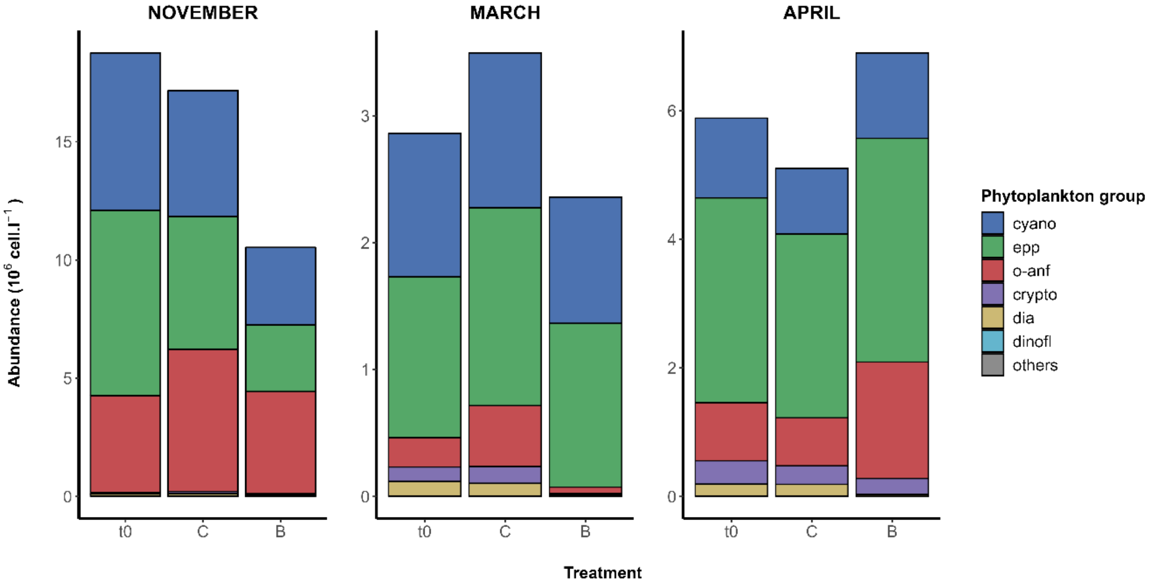

Figure 3.3: Abundance of specific phytoplankton groups (10^6 cells L^{-1}) in water samples from the Ria Formosa coastal lagoon at the start (t_0), and after 30 min at the control (C, no mussels) and experimental (B, 2-3 mussels) treatments. Abbreviations: Cyano - cyanobacteria; epp -eukaryotic picophytoplankton; o-anf -other autotrophic nanoflagellates; crypto -cryptophytes; dia -diatoms; dinofl -dinoflagellates; others.

At the end of the trials, diatoms were the only phytoplankton group to decrease significantly in the mussel treatment across all the experiments, with reductions of 66.4% in November, 91.8% in March and 83.0% in April (Figure 3.4). The abundance of diatoms remained unchanged in the control. Similarly, the abundance of other autotrophic nanoflagellates did not decrease in the control, but their abundance in the mussel treatment was 90.00% lower in March and 142.31% higher in April compared to the control. No significant changes were observed in the abundance of other phytoplankton groups, including cyanobacteria, eukaryotic picophytoplankton, cryptophytes and dinoflagellates, either compared to baseline values or between the mussel and control treatments at the end of the experiments (Figure 3.4).

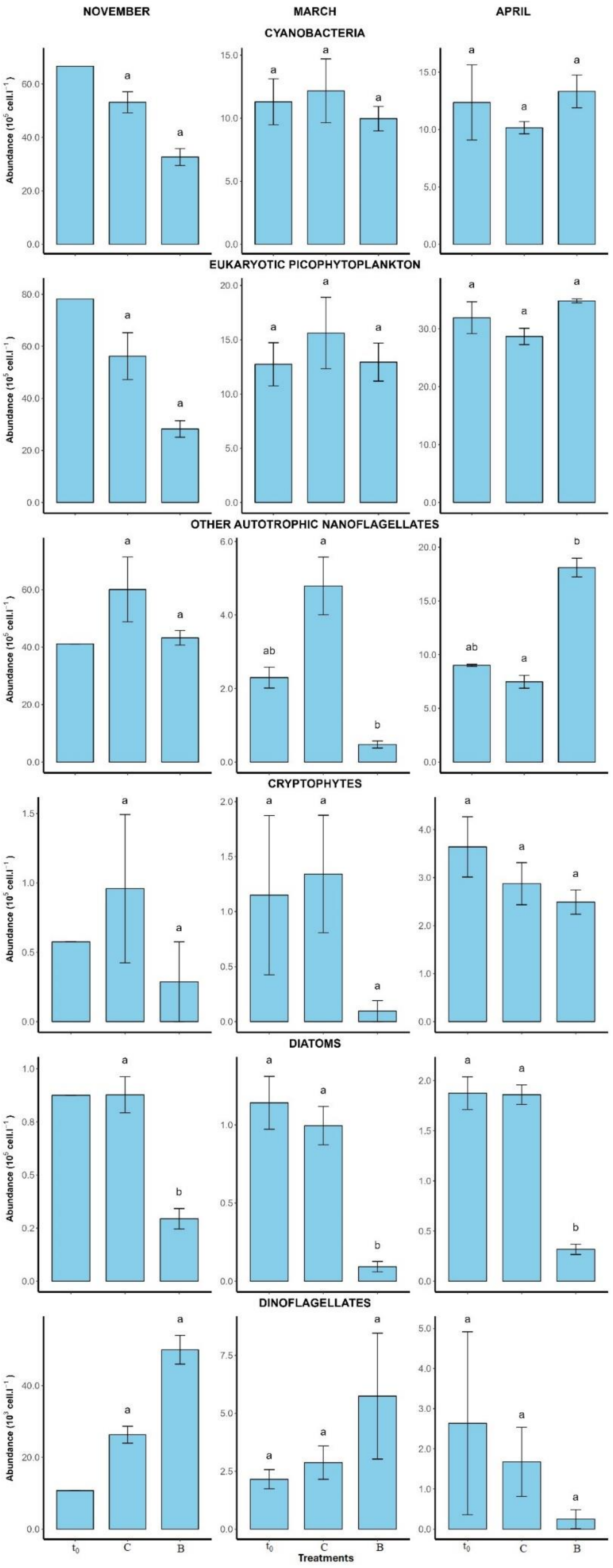
NOVEMBER
MARCH
APRIL
CYANOBACTERIA
EUKARYOTIC PICOPHYTOPLANKTON
OTHER AUTOTROPHIC NANOFLAGELLATES
CRYPTOPHYTES
DIATOMS
DINOFLAGELLATES
Abundance (10^5 cell.l^{-1})
Abundance (10^3 cell.l^{-1})
0.0
20.0
40.0
60.0
80.0
5.0
10.0
15.0
20.0
30.0
2.0
4.0
6.0
0.5
1.0
1.5
3.0
4.0
0.2
0.4
0.6
0.8
2.5
7.5
5.0
a
ab
b
t$_0$
C
B
Treatments

Figure 3.4. Abundance (cells L^{-1}) of specific phytoplankton groups in Ria Formosa coastal lagoon at the beginning (t_0) and after 30 min at the control (C, no mussels) and experimental (B, 2-3 mussels) treatments. Data from November 2022, March 2023 and April 2023 are shown, with mean and standard error. Phytoplankton groups labelled with different letters (a, b) indicate significant differences within an experiment (Kruskal-Wallis, Bonferroni test).

3.3. Grazing rates of M. galloprovincialis: analysis of clearance rates

Individual *M. galloprovincialis* clearance rates for chlorophyll *a* and total phytoplankton in natural RF water samples were not significantly different and showed closely comparable values (Table 3.4). The highest clearance rate for chlorophyll a occurred in April, coinciding with peak initial chlorophyll *a* level (Table 3.1), while intermediate and lowest rates were observed in November and March, respectively. The total phytoplankton clearance rate could not be determined in April due to higher phytoplankton abundance in the mussel treatment compared to the control at the end of the experiment (Figure 3.4). In November the F values for chlorophyll *a* were slightly higher than those for total phytoplankton abundance, whereas in March the clearance rate for phytoplankton appeared to be higher than that for chlorophyll *a*.

Table 3.4: *M. galloprovincialis* clearance rates *per capita* on total chlorophyll *a* (F(Chl*a*)) and on total phytoplankton (F (Abund)) in different experiments. Values represent mean ± 1 SE. Values marked with an asterisk indicate results different from zero as defined by the Wilcoxon test.

Date	F (Chl*a*) $(L\ ind^{-1}\ h^{-1})$	F (Abund) $(L\ ind^{-1}\ h^{-1})$
11-november-22	2,31 ± 1,92	1,95 ± 0,20
13-march-23	0,82 ± 0,76	1,07 ± 0,51
26-april-23	4,36 ± 1,48	ud

^{ud} undeterminable

The mean clearance rate (F) of the Mediterranean mussel across different phytoplankton groups ranged from 0.41 to 23.63 L ind^{-1} h^{-1} (Figure 3.5). *M. galloprovincialis* filtered all phytoplankton groups at statistically similar rates, with none of the filtration rates significantly different from zero ($p > 0.05$). The highest filtration rates were recorded for cryptophytes, particularly in the first two trials, with a large standard error associated with these measurements. Diatoms were filtered in all the experiments, resulting in an average clearance rate of 5.30 L ind^{-1} h^{-1} across all trials. In contrast, the mussel clearance rates derived cyanobacteria (cyano) and eukaryotic picoflagellates (EPP) were lower (November and March) or undeterminable (April). Dinoflagellates were also filtered at lower rates, except in April when they were filtered at the highest rate with a large standard error associated with these measurements.

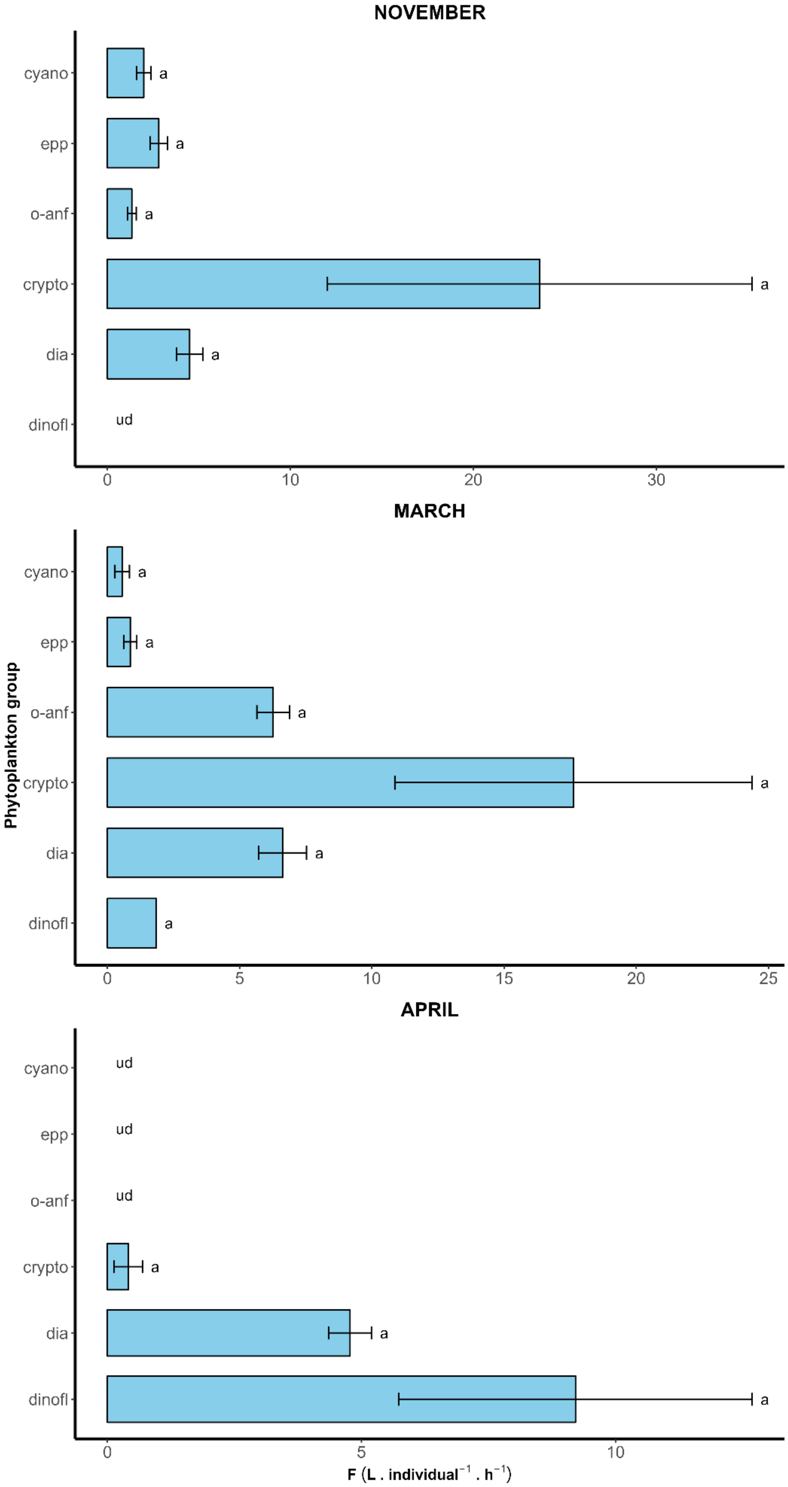

Figure 3.5: *M. galloprovincialis per capita* clearance rates (F) (L individual^{-1} h^{-1}) on different phytoplankton groups. Phytoplankton groups labelled with (a) indicate no significant differences from each other within an experiment (Kruskal-Wallis, Bonferroni test). Values represent mean ± 1 SE. An "ud" indicates that the corresponding values could not be determined.

4. Discussion

This study evaluated the clearance rate and selectivity of the Mediterranean bivalve *Mitillus galloprovincis* on phytoplankton, based on biomass, abundance and species composition in the Ria Formosa coastal lagoon. A significant decrease in total phytoplankton abundance due to bivalve feeding was detected only during March, but a consistent decrease in diatom abundance was observed in all experiments. However, no significant changes in total phytoplankton biomass, and structural composition were observed. This study represents the first assessment of clearance rates of *M. galloprovincis* based on different phytoplankton groups, using natural assemblages from Ria Formosa. Previous studies on bivalve grazing selectivity have mainly used pre-selected phytoplankton mixtures (Naddafi et al. 2007; Jiang et al. 2022) and prey items larger than 5 µm (see review by Rosa et al. 2018), not natural phytoplankton assemblages. Furthermore, much of the existing literature has focused on the impacts of bivalve grazers in the context of species introductions, with less emphasis on native species (Kimmerer and Thompson, 2014). Previous research by Sobral and Widdows (1997), addressing *Ruditapes decussatus* clearance rates in Ria Formosa, also used phytoplankton cultures.

4.1 Conditions at the beginning of the experiments

The chlorophyll *a* values at the beginning of the three experiments and temporal variability were consistent with those reported for the inner Ria Formosa coastal lagoon (Barbosa, 2006; 2010; Domingues, 2022; Domingues et al. 2017a; 2023). Higher chlorophyll *a* concentration in April (spring) and lower in November (autumn), followed the unimodal annual cycles typical of shallow estuaries and coastal lagoons, where phytoplankton biomass usually peaks in summer (Cebrián and Valiela, 1999). This pattern is usually associated with limitation on phytoplankton growth imposed by light availability and temperature, both in the Ria Formosa (Barbosa, 2006) and other temperate coastal ecosystems (Cebrián and Valiela, 1999).

Total phytoplankton abundance at the beginning of the three experiments was similar (November) or lower (March and April) than values reported by Domingues et al. (2017a, 2023), but similar to those reported by Santos (2023). Eukaryotic picophytoplankton (EPP) and picoplanktonic cyanobacteria were the dominant phytoplankton groups during all experiments, as reported in previous studies in the Ria Formosa coastal lagoon (Barbosa, 2006; Domingues et al. 2015, 2017a, 2017b, 2023) and other lagoon systems (Bec et al. 2005). The high surface area-to-volume ratio, which optimises light and nutrient absorption, along with efficient nutrient uptake via high-affinity enzymes, low sedimentation rates, and rapid growth, typically favour these picoplanktonic groups (Raven et al. 1998; 2005). Indeed, according to Barbosa (2006), EPP but also diatoms are the phytoplankton groups with the highest mean *in situ* growth rates in the inner Ria Formosa (0.044 ± 0.012 h^{-1} and 0.048 ± 0.010 h^{-1}, respectively).

During our study, high total phytoplankton abundance was not coincident with high chlorophyll *a* concentration. Lower chlorophyll *a* during the November experiment, was detected in tandem with high abundances of smaller cells with low carbon content, such as cyanobacteria and eukaryotic picophytoplankton (see Cermeño et al. 2005). In fact, despite its significant contribution to abundance, cyanobacteria and eukaryotic picophytoplankton represent a low contribution to phytoplankton biomass in the Ria Formosa lagoon (Barbosa, 2006). However, in relatively nutrient-poor waters, where total chlorophyll *a* is below 0.8–1 mg/m^3, picophytoplankton can account for up to 80% of the total chlorophyll *a* (Marañón et al. 2009).

4.2 Mussel clearance rates and effects of bivalve feeding on phytoplankton

Overall the estimated *per capita* clearance rates of *M. galloprovincialis* based on total chlorophyll *a* concentration (0.82-4.36 $L \cdot h^{-1}$ individual $^{-1}$), total phytoplankton abundance (1.07-1.95 $L \cdot h^{-1}$ individual $^{-1}$) or abundance or specific groups used (0.41-23.63 $L \cdot h^{-1}$ individual $^{-1}$) were within the range of values reported for this species (1.0 - 7.0 $L \cdot h^{-1}$ individual $^{-1}$, Navarro et al. 1996; and 0.2 - 2.5 $L \cdot h^{-1}$ individual $^{-1}$, Dennis, 1999). Mussels did not significantly reduce chlorophyll *a* concentration in respect to the control treatment, and the clearance rates based on chlorophyll *a* were not significantly different from zero. This lack of effect could be attributed to a chlorophyll *a* concentration below the activation threshold required for efficient bivalve filtration and metabolic benefit. Below this threshold, the energy cost of feeding exceeds the energy gained (see Widdows and Hawkins 1989). According to Filgueira et al. (2009), both low (< 2.08 µg L^{-1}) and high (> 26.91 µg L^{-1}) chlorophyll *a* concentrations can significantly reduce filtration rates of *M. galloprovincialis*. The initial chlorophyll *a* concentrations in this study were below the activation threshold, which may explain the lack of significant changes of bivalves on chlorophyll *a* concentrations. This finding challenges the second hypothesis of the study, which posited that grazing by *M. galloprovincialis* would significantly decrease phytoplankton biomass and alter community composition.

The clearance rates among different functional groups were found to be consistent, indicating that mussels were filtering the majority of plankton at similar rates. This finding is consistent with the results reported by Bastviken et al. (1998), who also observed comparable gross plankton clearance rates across various phytoplankton taxa in *Dreissena polymorpha*. However, considerable variation in net clearance rates was also observed, with differences of over fivefold among the various taxa. This discrepancy may be linked to the absence of pseudofaeces and/or faeces production, which makes it difficult to accurately assess the effective removal of phytoplankton and reflects the efficiency of the mussels' feeding (Bastviken et al. 1998). The absence of observable faeces or pseudofaeces in this study indicates that gross clearance measurements may not be a reliable indicator of phytoplankton metrics across different taxa. Furthermore, the lack of notable differences in clearance rates for various phytoplankton metrics may be also attributed to the presence of considerable standard errors in abundance estimates for specific groups, such as cryptophytes and dinoflagellates (Marescaux et al. 2016). Although the clearance rates among the various functional groups were comparable, the pronounced decline in the prevalence of diatoms and other plastidic nanoflagellates in March, in the presence of mussels, suggests that these bivalves are effectively filtering and feeding on them. This lends further support to the initial hypothesis of this study. Furthermore, the observed preference of bivalves for diatoms is in line with the hypothesis put forth by Barbosa (2006), which suggests that benthic filter feeders play a role in the observed decrease in the average contribution of diatoms to total phytoplankton abundance, despite their high *in situ* growth rates within the Ria Formosa. Similar trends were reported by Dupuy et al. (2000a), who found that *Crassostrea gigas* exhibited effective consumption of diatoms but demonstrated limited feeding on picophytoplankton. Lonsdale et al. (2009) additionally observed that the ribbed mussel (*Geukensia demissa*) exhibited greater efficiency in filtering diatoms than smaller organisms, such as bacteria. In Lake Taihu, Zhang et al. (2023) demonstrated that *Corbicula fluminea* exhibited significantly higher clearance rates for diatoms in comparison to cyanobacteria. Additionally, Naddafi et al. (2007) emphasised *Dreissena polymorpha's* selectivity for phytoplankton within the preferred size range of 7–50 µm. Diatoms are frequently selected as a food source due to their elevated concentrations of long-chain polyunsaturated fatty acids (PUFAs) (Vanderploeg et al. 1996; Brett and Müller-Navarra, 1997). Rouillon and Navarro (2003) posited that this preference is attributable to the rigidity of diatom frustules, which facilitates mechanical breakdown by the crystalline style, thereby enhancing their digestibility in comparison to more flexible phytoplankton, such as flagellates. Furthermore, Horgan and Millis (1997) highlighted that diatoms are more susceptible to predation due to their proclivity for sinking to the benthic zone, rendering them more vulnerable to benthic grazers.

4.3 Methodological limitations, and future challenges

The presence of undetermined F values (ud.) indicates instances where phytoplankton abundances were higher with bivalves than in control conditions. This observation is in line with the findings of Rahman et al. (2020), who reported that *M. galloprovincialis* exhibited negative filtration effects on small seston particles (< 5µm) during the spring season. Furthermore, Marescaux et al. (2016) documented similar undermined filtration values in *Dreissena* spp. and *Corbicula* spp., which were linked to chlorophyll *a* abundance. The increased abundance of certain phytoplankton groups may be attributed to bivalves consuming their natural predators, thus reducing top-down grazing pressure and allowing these phytoplankton to thrive. This phenomenon, known as a "trophic cascade", has been well-documented, with studies showing that the removal of a grazer can indirectly benefit its prey (e.g., Stein et al. 1995). A study by Calbet and Landry (2004) demonstrated that microzooplankton, the primary consumers of phytoplankton in marine systems, typically graze up to 67% of daily phytoplankton production. In diverse ecosystems, including the Ria Formosa lagoon, microzooplankton (100 µm) – mainly protistan grazers – consume 44% of daily phytoplankton production (Barbosa, 2006; Domingues et al. 2021). Bivalves, such as *Mya arenaria* and *Mercenaria mercenaria*, have been demonstrated to markedly reduce microzooplankton populations through predation of planktonic ciliates (Lonsdale et al. 2009). Similarly, observations in the Hudson River revealed a decrease in microzooplankton populations, including tintinnids and rotifers, due to predation by the zebra mussel *Dreissena polymorpha* (Pace et al. 1998). In a further study, Marroni et al. (2016) found that *Diplodon parallelopipedon* and *Corbicula fluminea* exhibited selective feeding on small zooplankton (rotifers and nauplii), avoiding medium-sized species like cladocerans and copepods. This selective predation resulted in a shift in the zooplankton community structure, with larger-sized species becoming more prevalent. Furthermore, Schmoker et al. (2013) demonstrated that microzooplankton consume a greater proportion of carbon than mesozooplankton, with microzooplankton consuming five times more carbon relative to primary production. Such shifts in zooplankton community structure can impact primary production, as phytoplankton are affected both directly by grazing and indirectly through changes in zooplankton composition. Given that bivalves impact both phytoplankton and zooplankton, they play a dual role, grazing on primary producers while also competing with and preying on secondary producers (Lonsdale et al. 2009).

Another potential factor contributing to the observed increase in phytoplankton in the mussel treatment, relative to the control, is the excretion of nutrients by bivalves. These organisms release dissolved inorganic nutrients, primarily ammonium and phosphate, which are rapidly taken up by phytoplankton (Carlsson et al. 2012; Cranford et al. 2012). Similar effects have been observed in other studies. In bivalve culture areas, for example, Lu et al. (2015) observed an increase in 2–5 µm autotrophic nanoflagellates resulting from nutrient excretion by *Chlamys farreri* and *Crassostrea gigas* in enclosure experiments. Dupuy (2000a) additionally documented the accelerated development of nanoflagellates measuring <5µm in an oyster farming pond in comparison to the control group. Such nutrient ‘fertilization’ has the potential to stimulate primary production in taxa that were initially limited by nutrients (Asmus and Asmus, 1991; Dame, 2013). Although nutrient enrichment by mussels is a plausible explanation, the 30-minute incubation period is likely to have been insufficient to allow for the detection of significant instantaneous phytoplankton growth. Indeed, as Barbosa (2006) observed, average *in situ* phytoplankton instantaneous growth rates in the Ria Formosa (0.040 ± 0.008 h^{-1}) are associated with average cell doubling times of 22.5 hours, making a 30-minute period too short for substantial increases. As proposed by Bastviken et al. (1998), this brief period is insufficient to discern significant alterations in relative abundance. Future studies, however, should include the addition of dissolved inorganic nutrients in excess to all treatments (bivalve and control), as proposed by see Vanderploeg et al. (2001).

Further studies should investigate additional grazing rates to gain a more comprehensive and detailed understanding of the selectivity of *M. galloprovincialis* within the phytoplankton community. Additionally, it is crucial to gather data on the feeding behaviour of other dominant species and measure ingestion rates not only on phytoplankton but also on microzooplankton, in order to gain a deeper insight into phytoplankton dynamics in the Ria Formosa coastal lagoon.

5. Conclusion

In order to gain insight into the dynamics of aquatic ecosystems, it is vital to quantify the removal rates of phytoplankton through grazing by benthic filter-feeders. This study, in line with others in the field, demonstrates that bivalve grazing is a key selective process with a significant impact on phytoplankton dynamics. Gaining an understanding of the processes that regulate phytoplankton in natural assemblages provides valuable insights into the fate of these microorganisms within the food web and their interactions in the Ria Formosa coastal lagoon. The high clearance rates on diatoms indicate their importance for the growth and maintenance of *M. galloprovincialis* populations in the lagoon. To the best of our knowledge, this is the first study to examine mussel grazing on natural phytoplankton biomass, abundance, and assemblage composition in the Ria Formosa coastal lagoon. Further research should assess the impact of bivalve grazing on not only phytoplankton but also microzooplankton, as well as measure ingestion rates by other dominant bivalve species. This would provide a more comprehensive understanding of the overall impact of bivalves on natural plankton communities and improve models of carbon flow, nutrient cycling, and energy transfer within the Ria Formosa coastal lagoon.

References

Anderson, L. W., & Sweeney, B. M. (1978). Role of inorganic ions in controlling sedimentation rate of a marine centric diatom *Ditylum brightwelli*. *Journal of Phycology, 14*(2), 204-214. https://doi.org/10.1111/j.1529-8817.1978.tb02450.x

Anderson, M. J. (2001). Permutation tests for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences, 58*(3), 626-639. https://doi.org/10.1139/f01-040

Anderson, T. R. (2005). Plankton functional type modelling: Running before we can walk? *Journal of Plankton Research, 27*, 1073–1081. https://doi.org/10.1093/plankt/fbi076

Andrade, C., Freitas, M. D. C., Moreno, J., & Craveiro, S. C. (2004). Stratigraphical evidence of Late Holocene barrier breaching and extreme storms in lagoonal sediments of Ria Formosa, Algarve, Portugal. *Marine Geology*, 210(1-4), 339-362. https://doi.org/10.1016/j.margeo.2004.05.016

Asmus, R. M., & Asmus, H. (1991). Mussel beds: Limiting or promoting phytoplankton? *Journal of Experimental Marine Biology and Ecology, 148*(2), 215-232. https://doi.org/10.1016/0022-0981(91)90083-9

Banse, K. (1976). Rates of growth, respiration and photosynthesis of unicellular algae as related to cell size: A review. *Journal of Phycology, 12*(2), 135-140. https://doi.org/10.1111/j.1529-8817.1976.tb00490.x

Banse, K. (2007). Do we live in a largely top-down regulated world? *Journal of Biosciences, 32*(4), 791–796. https://doi.org/10.1007/s12038-007-0080-6

Banse, K. (2013). Reflections about chance in my career, and on the top-down regulated world. *Annual Review of Marine Science, 5*, 1–19. https://doi.org/10.1146/annurev-marine-121211-172359

Barbosa, A. B. (2006). *Estrutura e dinâmica da teia alimentar microbiana na Ria Formosa* [Doctoral dissertation, University of Algarve]. University of Algarve.

Barbosa, A.B. (2010). Seasonal and interannual variability of planktonic microbes in a mesotidal coastal lagoon (Ria Formosa, SE Portugal): Impact of climatic changes and local-human influences. In H. Paerl & M. Kennish (Eds.), *Coastal Lagoons: Critical Habitats of Environmental Change* (pp. 335-366). CRC Press, Taylor & Francis Group. ISBN 978-1-4200-8830-4.

Bastviken, D. T., Caraco, N. F., & Cole, J. J. (1998). Experimental measurements of zebra mussel (*Dreissena polymorpha*) impacts on phytoplankton community composition. *Freshwater Biology*, 39(2), 375-386. https://doi.org/10.1046/j.1365-2427.1998.00283.x

Bayne, B. L. (2009). Carbon and nitrogen relationships in the feeding and growth of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology, 374*(1), 19-30. https://doi.org/10.1016/j.jembe.2009.04.003

Bayne, B. L. (2017). Feeding. In Developments in aquaculture and fisheries science (Vol. 41, pp. 209–329). Elsevier. https://doi.org/10.1016/B978-0-12-803472-9.00005-4

Bayne, B. L., & Widdows, J. (1978). The physiological ecology of two populations of *Mytilus edulis* L. *Oecologia, 37*(2), 137–162. https://doi.org/10.1007/BF00344987

Bayne, B. L., Iglesias, J. I. P., Hawkins, A. J. S., Navarro, E., Heral, M., & Deslous-Paoli, J. M. (1993). Feeding behaviour of the mussel, *Mytilus edulis*: Responses to variations in quantity and organic content of the seston. *Journal of the Marine Biological Association of the United Kingdom*, 73(4), 813-829. https://doi.org/10.1017/S0025315400034743

Bec, B., Husseini-Ratrema, J., Collos, Y., Souchu, P., & Vaquer, A. (2005). Phytoplankton seasonal dynamics in a Mediterranean coastal lagoon: Emphasis on the picoeukaryote community. *Journal of Plankton Research, 27*(9), 881–894. https://doi.org/10.1093/plankt/fbi061

Beninger, P. G., & Decottignies, P. (2005). What makes diatoms attractive for suspensivores? The organic casing and associated organic molecules of *Coscinodiscus perforatus* are quality cues for the bivalve *Pecten maximus*. *Journal of Plankton Research*, *27*(1), 11–17. https://doi.org/10.1093/plankt/fbh086

Biandolino, F., Prato, E., & Caroppo, C. (2008). Preliminary investigation on the phytoplankton contribution to the mussel diet on the basis of fatty acids analysis. *Journal of the Marine Biological Association of the United Kingdom, 88*(7), 1009–1017. https://doi.org/10.1017/S0025315408001598

Brett, M., & Müller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology, 38*(3), 483-499. https://doi.org/10.1046/j.1365-2427.1997.00220.x

Brito, A. C., Quental, T., Coutinho, T. P., Branco, M. A. C., Falcão, M., Newton, A., Icely, J., & Moita, T. (2012). Phytoplankton dynamics in southern Portuguese coastal lagoons during a discontinuous period of 40 years: An overview. *Estuarine, Coastal and Shelf Science, 110*, 147–156. https://doi.org/10.1016/j.ecss.2012.04.014

Calbet, A., & Landry, M. R. (2004). Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography, 49*(1), 51–57. https://doi.org/10.4319/lo.2004.49.1.0051

Carlsson, M. S., Engström, P., Lindahl, O., Ljungqvist, L., Petersen, J. K., Svanberg, L., & Holmer, M. (2012). Effects of mussel farms on the benthic nitrogen cycle on the Swedish west coast. *Aquaculture Environment Interactions, 2*(2), 177–191. https://doi.org/10.3354/aei00039

Caroppo, C., & Giordano, L. (2022). New insights on phytoplankton communities as a tool for the sustainable management of the mussel culture in a Mediterranean coastal site.

Journal of Marine Science and Engineering, 10(4), 462. https://doi.org/10.3390/jmse10040462

Carstensen, J., Klais, R., & Cloern, J. E. (2015). Phytoplankton blooms in estuarine and coastal waters: Seasonal patterns and key species. *Estuarine, Coastal and Shelf Science, 162*, 98–109. https://doi.org/10.1016/j.ecss.2015.05.005

Cebrián, J., & Valiela, I. (1999). Seasonal patterns in phytoplankton biomass in coastal ecosystems. *Journal of Plankton Research, 21*(3), 429-444. https://doi.org/10.1093/plankt/21.3.429

Cermeño, P., Marañón, E., Rodríguez, J., & Fernández, E. (2005). Large-sized phytoplankton sustain higher carbon-specific photosynthesis than smaller cells in a coastal eutrophic ecosystem. *Marine Ecology Progress Series*, *297*, 51-60. https://doi.org/10.3354/meps297051

Chavez, F. P., Messié, M., & Pennington, J. T. (2011). Marine primary production in relation to climate variability and change. *Annual Review of Marine Science, 3*(1), 227-260. https://doi.org/10.1146/annurev.marine.010908.163917

Chícharo, L. M., & Chícharo, M. (2001). Effects of environmental conditions on planktonic abundances, benthic recruitment and growth rates of the bivalve mollusc *Ruditapes decussatus* in a Portuguese coastal lagoon. *Fisheries Research, 53*(3), 235-250. https://doi.org/10.1016/S0165-7836(00)00290-3

Clarke, K.R.; Gorley, R.N. *PRIMER v6: User Manual/Tutorial*; PRIMER-E, Ltd.: Plymouth, UK, 2006

Cloern, J. E., & Dufford, R. (2005). Phytoplankton community ecology: Principles applied in San Francisco Bay. *Marine Ecology Progress Series, 285*, 11–28. https://doi.org/10.3354/meps285011

Cranford, P. J., Kamermans, P., Krause, G., Mazurié, J., Buck, B. H., Dolmer, P., Fraser, D., Van Nieuwenhove, K., O'Beirn, F. X., Sanchez-Mata, A., Thorarinsdóttir, G. G., & Strand, Ø. (2012). An ecosystem-based approach and management framework for the integrated evaluation of bivalve aquaculture impacts. *Aquaculture Environment Interactions, 2*(3), 193–213. https://doi.org/10.3354/aei00040

Cranford, P. J., Strohmeier, T., Filgueira, R., & Strand, Ø. (2016). Potential methodological influences on the determination of particle retention efficiency by suspension feeders: *Mytilus edulis* and *Ciona intestinalis*. *Aquatic Biology, 25*, 61-73. https://doi.org/10.3354/ab00660

Cranford, P. J., Ward, J. E., & Shumway, S. E. (2011). Bivalve filter feeding: Variability and limits of the aquaculture biofilter. In *Shellfish aquaculture and the environment* (pp. 81-124). Wiley-Blackwell.

Cravo, A., Barbosa, A. B., Correia, C., Matos, A., Caetano, S., Lima, M. J., & Jacob, J. (2022). Unravelling the effects of treated wastewater discharges on the water quality in a coastal lagoon system (Ria Formosa, South Portugal): Relevance of hydrodynamic conditions. *Marine Pollution Bulletin, 174*, 113296. https://doi.org/10.1016/j.marpolbul.2021.113296

Cravo, A., Cardeira, S., Pereira, C., Rosa, M., Alcântara, P., Madureira, M., Rita, F., Correia, C., Rosa, A., & Jacob, J. (2019). Nutrients and chlorophyll-a exchanges through an inlet of the Ria Formosa Lagoon, SW Iberia during the productive season: Unravelling the role of the driving forces. *Journal of Sea Research, 144*, 133-141. https://doi.org/10.1016/j.seares.2018.12.001

Cullen, J. J. (1982). The deep chlorophyll maximum: Comparing vertical profiles of chlorophyll a. *Canadian Journal of Fisheries and Aquatic Sciences, 39*(5), 791–803. https://doi.org/10.1139/f82-108

D'Alelio, D., Mazzocchi, M. G., Montresor, M., Sarno, D., Zingone, A., Di Capua, I., et al. (2015). The green-blue swing: Plasticity of plankton food-webs in response to coastal oceanographic dynamics. *Marine Ecology, 36*, 1155–1170. https://doi.org/10.1111/maec.12211

Dame, R. F. (Ed.). (2013). *Bivalve filter feeders: In estuarine and coastal ecosystem processes* (Vol. 33). Springer Science & Business Media. https://doi.org/10.1007/978-3-642-78353-1

Dame, R. F., & Olenin, S. (Eds.). (2005). *The comparative roles of suspension-feeders in ecosystems: Proceedings of the NATO Advanced Research Workshop on the Comparative Roles of Suspension-Feeders in Ecosystems, Nida, Lithuania, 4–9 October 2003*. Springer Netherlands.

Dinno, A. (2017). *dunn.test: Dunn's Test of Multiple Comparisons Using Rank Sums* (Version 1.3.5) [R package]. CRAN. https://CRAN.R-project.org/package=dunn.test (accessed on 10 July 2024)

Domingues, R. B. (2022). Seasonal and spatial variability of phytoplankton primary production in a shallow temperate coastal lagoon (Ria Formosa, Portugal). *Plants, 11*(24), 3511. https://doi.org/10.3390/plants11243511

Domingues, R. B., & Lima, M. J. (2023). Unusual red tide of the dinoflagellate *Lingulodinium polyedra* during an upwelling event off the Algarve coast (SW Iberia). *Regional Studies in Marine Science, 63*, 102998. https://doi.org/10.1016/j.rsma.2023.102998

Domingues, R. B., Barreto, M., Brotas, V., Guerra, C. C., Galvão, H. M., & Barbosa, A. B. (2021). Short-term effects of winter warming and acidification on phytoplankton growth and mortality: More losers than winners in a temperate coastal lagoon. *Hydrobiologia, 848*, 4763–4785. https://doi.org/10.1007/s10750-021-04672-0

Domingues, R. B., Guerra, C. C., Barbosa, A. B., & Galvão, H. M. (2015). Are nutrients and light limiting summer phytoplankton in a temperate coastal lagoon? *Aquatic Ecology, 49*, 127–146. https://doi.org/10.1007/s10452-015-9512-9

Domingues, R. B., Guerra, C. C., Barbosa, A. B., & Galvão, H. M. (2017a). Will nutrient and light limitation prevent eutrophication in an anthropogenically-impacted coastal lagoon? *Continental Shelf Research, 141*, 11–25. https://doi.org/10.1016/j.csr.2017.05.003

Domingues, R. B., Guerra, C. C., Barbosa, A. B., Brotas, V., & Galvão, H. M. (2014). Effects of ultraviolet radiation and CO_2 increase on winter phytoplankton assemblages in a temperate coastal lagoon. *Journal of Plankton Research, 36*(3), 672-684. https://doi.org/10.1093/plankt/fbu015

Domingues, R. B., Guerra, C. C., Galvão, H. M., Brotas, V., & Barbosa, A. B. (2017b). Short-term interactive effects of ultraviolet radiation, carbon dioxide, and nutrient enrichment on phytoplankton in a shallow coastal lagoon. *Aquatic Ecology, 51*, 91–105. https://doi.org/10.1007/s10452-016-9601-4

Domingues, R. B., Nogueira, P., & Barbosa, A. B. (2023). Co-Limitation of Phytoplankton by N and P in a Shallow Coastal Lagoon (Ria Formosa): Implications for Eutrophication Evaluation. *Estuaries and Coasts*, *46*(6), 1557-1572. https://doi.org/10.1007/s12237-023-01230-w

Domingues, R.B., Barbosa, A., Galvão, H., (2008). Constraints on the use of phytoplankton as a biological quality element within the Water Framework Directive in Portuguese waters. *Marine Pollution Bulletin* 56 (8), 1389–1395. https://doi.org/10.1016/j.marpolbul.2008.05.006

Dupuy, C., Pastoureaud, A., Ryckaert, M., Sauriau, P. G., & Montanié, H. (2000a). Impact of the oyster *Crassostrea gigas* on a microbial community in Atlantic coastal ponds near La Rochelle. *Aquatic Microbial Ecology, 22*(3), 227-242. https://doi.org/10.3354/ame022227

Dupuy, C., Vaquer, A., Lam-Hoai, T., Rougier, C., Mazouni, N., Lautier, J., Collos, Y., & Le Gall, S. (2000b). Feeding rate of the oyster *Crassostrea gigas* in a natural planktonic community of the Mediterranean Thau Lagoon. *Marine Ecology Progress Series, 205*, 171-184. https://doi.org/10.3354/meps205171

Field, C. B., Behrenfeld, M. J., Randerson, J. T., & Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*, 281(5374), 237-240. https://doi.org/10.1126/science.281.5374.237

Filgueira, R., Fernández-Reiriz, M. J., & Labarta, U. (2009). Clearance rate of the mussel *Mytilus galloprovincialis.* I. Response to extreme chlorophyll ranges Tasa de aclaramiento del mejillón Mytilus galloprovincialis. I. Respuesta a intervalos extremos de clorofila. *Ciencias Marinas*, *35*(4), 405-417. https://doi.org/10.7773/cm.v35i4.1645

Filgueira, R., Labarta, U., & Fernández-Reiriz, M. J. (2008). Effect of condition index on allometric relationships of clearance rate in *Mytilus galloprovincialis* Lamarck, 1819. *Revista de Biología Marina y Oceanografía, 43*(2), 391-398.

Finkel, Z. V. (2001). Light absorption and size scaling of light-limited metabolism in marine diatoms. *Limnology and Oceanography, 46*(1), 86-94. https://doi.org/10.4319/lo.2001.46.1.0086

Finkel, Z. V., Beardall, J., Flynn, K. J., Quigg, A., Rees, T. A. V., & Raven, J. A. (2010). Phytoplankton in a changing world: Cell size and elemental stoichiometry. Journal of Plankton Research, 32(1), 119–137. https://doi.org/10.1093/plankt/fbp098

Froján, M., Arbones, B., Zúñiga, D., Castro, C. G., & Figueiras, F. G. (2014). Microbial plankton community in the Ría de Vigo (NW Iberian upwelling system): Impact of the culture of *Mytilus galloprovincialis*. *Marine Ecology Progress Series, 498,* 43-54. https://doi.org/10.3354/meps10612

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography, 17*(6), 805–815. https://doi.org/10.4319/lo.1972.17.6.0805

Gianasi, B. L., McKindsey, C. W., Tremblay, R., Comeau, L. A., & Drolet, D. (2023). Plankton depletion by mussel grazing negatively impacts the fitness of lobster larvae. *Aquaculture, 574,* 739659. https://doi.org/10.1016/j.aquaculture.2023.739659

Haas L. W. (1982) Improved epifluorescence microscopy for observing planktonic microorganisms. *Annales del'lnstitut Oceanographique,* Paris, 58, 261-266.

Horgan, M. J., & Mills, E. L. (1997). Clearance rates and filtering activity of zebra mussel (Dreissena polymorpha): Implications for freshwater lakes. *Canadian Journal of Fisheries and Aquatic Sciences, 54*(2), 249–255. https://doi.org/10.1139/f96-276

Jacobs, P., Troost, K., Riegman, R., & Van der Meer, J. (2015). Length- and weight-dependent clearance rates of juvenile mussels (*Mytilus edulis*) on various planktonic prey items. *Helgoland Marine Research, 69,* 101-112. https://doi.org/10.1007/s10152-014-0419-y

Jiang, T., Chen, F., Yu, Z., Lu, L., & Wang, Z. (2016). Size-dependent depletion and community disturbance of phytoplankton under intensive oyster mariculture based on HPLC pigment analysis in Daya Bay, South China Sea. *Environmental Pollution, 219,* 804-814. https://doi.org/10.1016/j.envpol.2016.07.058

Jiang, T., Pan, H., Steeves, L., Jiang, Z., Filgueira, R., Strand, Ø., Strohmeier, T., & Cranford, P. J. (2022). Effect of *Mytilus coruscus* selective filtration on phytoplankton assemblages. *Frontiers in Marine Science, 9,* 1070737. https://doi.org/10.3389/fmars.2022.1070737

Jørgensen, C. B. (1996). Bivalve filter feeding revisited. *Marine Ecology Progress Series*, *142*, 287-302. https://doi.org/10.3354/meps142287

Jørgensen, C. B., Famme, P., Kristensen, H. S., Larsen, P. S., Møhlenberg, F., & Riisgård, H. U. (1986). The bivalve pump. *Marine Ecology Progress Series*, *34*(1/2), 69–77. http://www.jstor.org/stable/24824953

Jørgensen, C. B., Famme, P., Kristensen, H. S., Larsen, P. S., Møhlenberg, F., & Riisgård, H. U. (1986). The bivalve pump. Marine Ecology Progress Series, 34(1/2), 69–77. https://doi.org/10.3354/meps034069

Kimmerer, W. J., & Thompson, J. K. (2014). Phytoplankton growth balanced by clam and zooplankton grazing and net transport into the low-salinity zone of the San Francisco Estuary. *Estuaries and Coasts, 37*(5), 1202–1218. https://doi.org/10.1007/s12237-013-9753-6

Kiorboe, T., & Mohlenberg, F. (1981). Particle selection in suspension-feeding bivalves. *Mar. Ecol. Prog. Ser*, *5*(3), 291-296.

Kraberg, A., Baumann, M., & Dürselen, C.-D. (2010). *Coastal Phytoplankton: Photo Guide for Northern European Seas*. Dr. Friedrich Pfeil. ISBN 9783899371130.

Lacoste, E., & Gaertner-Mazouni, N. (2015). Biofouling impact on production and ecosystem functioning: a review for bivalve aquaculture. *Reviews in Aquaculture*, *7*(3), 187-196. https://doi.org/10.1111/raq.12063

Lesser, M. P., Shumway, S. E., Cucci, T., Barter, J., & Edwards, J. (1991). Size-specific selection of phytoplankton by juvenile filter-feeding bivalves: Comparison of the sea scallop *Placopecten megellanicus* (Gmelin, 1791) with *Mya arenaria* Linnaeus, 1758 and *Mytilus edulis* Linnaeus, 1758. In *An International Compendium of Scallop Biology and Culture* (pp. 341–346). The World Aquaculture Society.

Litchman, E., & Klausmeier, C. A. (2008). Trait-based community ecology of phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 615-639. https://doi.org/10.1146/annurev.ecolsys.39.110707.173549

Litchman, E., de Tezanos Pinto, P., Edwards, K. F., Klausmeier, C. A., Kremer, C. T., & Thomas, M. K. (2015). Global biogeochemical impacts of phytoplankton: A trait-based perspective. *Journal of Ecology, 103*(6), 1384-1396. https://doi.org/10.1111/1365-2745.12438

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters, 10*(12), 1170-1181. https://doi.org/10.1111/j.1461-0248.2007.01117.x

Litchman, E., Pinto, P. D. T., Klausmeier, C. A., Thomas, M. K., & Yoshiyama, K. (2010). Linking traits to species diversity and community structure in phytoplankton. *Hydrobiologia, 653*, 15–28. https://doi.org/10.1007/s10750-010-0326-3

Lonsdale, D. J., Cerrato, R. M., Holland, R., Mass, A., Holt, L., Schaffner, R. A., Pan, J., & Caron, D. A. (2009). Influence of suspension-feeding bivalves on the pelagic food webs of shallow, coastal embayments. *Aquatic Biology, 6*(263-279). https://doi.org/10.3354/ab00130

Lorenzen, C. J. (1967). Determination of chlorophyll and pheo-pigments: Spectrophotometric equations. *Limnology and Oceanography, 12*(2), 343-346. https://doi.org/10.4319/lo.1967.12.2.0343

Loureiro, S., Newton, A., & Icely, J. (2006). Boundary conditions for the European Water Framework Directive in the Ria Formosa lagoon, Portugal (physico-chemical and phytoplankton quality elements). *Estuarine, Coastal and Shelf Science, 67*(3), 382-398. https://doi.org/10.1016/j.ecss.2005.11.029

Lu, J., Huang, L., Luo, Y., Xiao, T., Jiang, Z., & Wu, L. (2015). Effects of freshwater input and mariculture (bivalves and macroalgae) on spatial distribution of nanoflagellates in Sungo Bay, China. *Aquaculture Environment Interactions, 6*, 191–203. https://doi.org/10.3354/aei00124

Lucas, L. V., Cloern, J. E., Thompson, J. K., Stacey, M. T., & Koseff, J. R. (2016). Bivalve grazing can shape phytoplankton communities. *Frontiers in Marine Science, 3,* 14. https://doi.org/10.3389/fmars.2016.00014

MacDonald, B. A., & Ward, J. E. (1994). Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus* (Mollusca: Bivalvia). *Marine Ecology Progress Series, 108*, 251–251.

Machás, R., Santos, R., & Peterson, B. (2003). Tracing the flow of organic matter from primary producers to filter feeders in Ria Formosa lagoon, Southern Portugal. Estuaries, 26(4), 846–856. https://doi.org/10.1007/BF02803343

Marañón, E., Steele, J. H., Thorpe, S. A., & Turekian, K. K. (2009). Phytoplankton size structure. In *Elements of physical oceanography: A derivative of the Encyclopedia of ocean sciences* (pp. 85–92). Academic Press. https://doi.org/10.1016/B978-012374473-9.00661-5

Marescaux, J., Falisse, E., Lorquet, J., Van Doninck, K., & Descy, J. P. (2016). Assessing filtration rates of exotic bivalves: Dependence on algae concentration and seasonal factors. *Hydrobiologia, 777*(1), 67–78. https://doi.org/10.1007/s10750-016-2764-0

Marroni, S., Mazzeo, N., Pacheco, J. P., Clemente, J., & Iglesias, C. (2016). Interactions between bivalves and zooplankton: competition or intraguild predation? Implications for biomanipulation in subtropical shallow lakes. *Marine and Freshwater Research*, *68*(6), 1036-1043. https://doi.org/10.1071/MF15454

Martinez Arbizu, P. (2017). *pairwiseAdonis: Pairwise Multilevel Comparison using Adonis* (Version 0.4.1) [R package]. CRAN. https://CRAN.R-project.org/package=pairwiseAdonis (accessed on 30 July 2024)

Matias, A., Ferreira, Ó., Vila-Concejo, A., Garcia, T., & Dias, J. A. (2008). Classification of washover dynamics in barrier islands. *Geomorphology*, 97(3-4), 655-674. https://doi.org/10.1016/j.geomorph.2007.09.010

Meyhöfer, E. (1985). Comparative pumping rates in suspension-feeding bivalves. *Marine Biology, 85*(2), 137–142. https://doi.org/10.1007/BF00397432

Mo, Y., Li, A., Luo, Z., Jia, C., Ou, R., Qiu, J., Chang, X., Huang, H., & Wang, L. (2023). Inapparent decrease in phytoplankton biomass accompanied by significant size composition succession in the shellfish aquaculture farm in the western Taiwan strait. Frontiers in Marine Science, 10. https://doi.org/10.3389/fmars.2023.1204508

Morais, P., Chícharo, M. A., & Barbosa, A. (2003). Phytoplankton dynamics in a coastal saline lake (SE-Portugal). *Acta Oecologica, 24*, S87-S96. https://doi.org/10.1016/S1146-609X(03)00008-0

Mudge, S. M., Icely, J. D., & Newton, A. (2008). Residence times in a hypersaline lagoon: using salinity as a tracer. *Estuarine, Coastal and Shelf Science*, 77(2), 278-284. https://doi.org/10.1016/j.ecss.2007.09.032

Naddafi, R., Pettersson, K., & Eklöv, P. (2007). The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. *Freshwater Biology, 52*(5), 823-842. https://doi.org/10.1111/j.1365-2427.2007.01732.x

Naselli-Flores, L., & Padisák, J. (2023). Ecosystem services provided by marine and freshwater phytoplankton. *Hydrobiologia, 850*, 2691–2706. https://doi.org/10.1007/s10750-022-04795-y

Naselli-Flores, L., Padisák, J., & Albay, M. (2007). Shape and size in phytoplankton ecology: Do they matter? Hydrobiologia, 578(1), 157–161. https://doi.org/10.1007/s10750-006-2815-z

Navarro, E., Iglesias, J. I. P., Camacho, A. P., & Labarta, U. (1996). The effect of diets of phytoplankton and suspended bottom material on feeding and absorption of raft mussels (*Mytilus galloprovincialis* Lmk). *Journal of Experimental Marine Biology and Ecology, 198*(2), 175–189. https://doi.org/10.1016/0022-0981(95)00210-3

Newton, A., & Mudge, S. M. (2003). Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal. *Estuarine, Coastal and Shelf Science,* 57(1-2), 73-85. https://doi.org/10.1016/S0272-7714(02)00332-3

Nixon, S. W. (1982). Nutrient dynamics, primary production, and fisheries yields of lagoons. *Oceanologica Acta, Special Issue*. https://archimer.ifremer.fr/doc/00246/35748/

Ogilvie, S. C., Ross, A. H., & Schiel, D. R. (2000). Phytoplankton biomass associated with mussel farms in Beatrix Bay, New Zealand. *Aquaculture, 181*(1-2), 71–80. https://doi.org/10.1016/S0044-8486(99)00219-7

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H. B. A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M. O., Lahti, L., McGlinn, D., Ouellette, M.-H., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C. J. F., & Weedon, J. (2022). *vegan: Community Ecology Package* (Version

2.6-4) [R package]. CRAN. https://CRAN.R-project.org/package=vegan (accessed on 10 July 2024)

Pace, M. L., Findlay, S. E., & Fischer, D. (1998). Effects of an invasive bivalve on the zooplankton community of the Hudson River. *Freshwater Biology, 39*(1), 103–116. https://doi.org/10.1046/j.1365-2427.1998.00266.x

Pales Espinosa, E., Cerrato, R. M., Wikfors, G. H., & others. (2016). Modeling food choice in the two suspension-feeding bivalves, *Crassostrea virginica* and *Mytilus edulis*. *Marine Biology, 163*(40), 1-10. https://doi.org/10.1007/s00227-016-2815-0

Parsons, T.R., Maita, Y., Lalli, C.M. (Eds.), (1984). A Manual of Chemical & Biological Methods for Seawater Analysis, 1st ed. *Elsevier*. https://doi.org/10.1016/C2009-007774-5

Pascoe, P. L., Parry, H. E., & Hawkins, A. J. S. (2009). Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish. *Aquatic Biology, 6*(2), 181–190. https://doi.org/10.3354/ab00123

Petersen, J. K., Holmer, M., Termansen, M., & Hasler, B. (2019). Nutrient extraction through bivalves. In *Goods and services of marine bivalves* (pp. 179–208). Springer Nature. https://doi.org/10.1007/978-3-319-96776-9

Prins, T., & Escaravage, V. (2005). Can bivalve suspension-feeders affect pelagic food web structure? In R. F. Dame & S. Olenin (Eds.), *The comparative roles of suspension-feeders in ecosystems* (Vol. 47, pp. 35-46). NATO Science Series IV: Earth and Environmental Series. Springer. https://doi.org/10.1007/1-4020-3030-4_3

Prowe, A. E. F., Pahlow, M., Dutkiewicz, S., Follows, M., & Oschlies, A. (2012). Top-down control of marine phytoplankton diversity in a global ecosystem model. *Progress in Oceanography, 101*, 1–13. https://doi.org/10.1016/j.pocean.2011.11.016

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Rahman, M. A., Henderson, S., Miller-Ezzy, P. A., Li, X. X., & Qin, J. G. (2020). Analysis of the seasonal impact of three marine bivalves on seston particles in the water column. *Journal of Experimental Marine Biology and Ecology, 522,* 151251. https://doi.org/10.1016/j.jembe.2019.151251

Raven, J. A., Finkel, Z. V., & Irwin, A. J. (2005). Picophytoplankton: Bottom-up and top-down controls on ecology and evolution. *Vie et Milieu/Life & Environment, 55*(4), 209-215.

Raven, J.A., (1998). The twelfth Tansley Lecture. Small is beautiful: the picophytoplankton. Funct. Ecol. 12, 503–513. https://doi.org/10.1046/j.1365_2435.1998.00233.x

Reynolds, C. S. (2012). Environmental requirements and habitat preferences of phytoplankton: Chance and certainty in species selection. *Botanica Marina*, 55(1), 1-17. https://doi.org/10.1515/bot.2011.121

Richard, M., Bec, B., Vanhuysse, C., Mas, S., Parin, D., Chantalat, C., Le Gall, P., Fiandrino, A., Lagarde, F., Mortreux, S., Ouisse, V., Rolland, J. L., Degut, A., Hatey, E., Fortune, M., Roque d'Orbcastel, E., Messiaen, G., Munaron, D., Callier, M., Oheix, J., & Mostajir, B. (2019). Changes in planktonic microbial components in interaction with juvenile oysters during a mortality episode in the Thau Lagoon (France). *Aquaculture, 503*, 231–241. https://doi.org/10.1016/j.aquaculture.2018.12.082

Riisgård, H. U. (2001). Comment: Physiological regulation *versus* autonomous filtration in filter-feeding bivalves: Starting points for progress. *Ophelia*, *54*(3), 193–209. https://doi.org/10.1080/00785236.2001.10409465

Rosa, A., Cravo, A., Jacob, J., & Correia, C. (2022). Water quality of a southwest Iberian coastal lagoon: Spatial and temporal variability. *Continental Shelf Research, 245*, 104804. https://doi.org/10.1016/j.csr.2022.104804

Rosa, M., Ward, J. E., & Shumway, S. E. (2018). Selective capture and ingestion of particles by suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research*, *37*(4), 727-746. https://doi.org/10.2983/035.037.0405

Rosa, M., Ward, J. E., Holohan, B. A., Shumway, S. E., & Wikfors, G. H. (2017). Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs. *Journal of Experimental Marine Biology and Ecology, 486,* 59-68. https://doi.org/10.1016/j.jembe.2016.09.007

Rosa, M., Ward, J. E., Ouvrard, M., Holohan, B. A., Espinosa, E. P., Shumway, S. E., & Allam, B. (2015). Examining the physiological plasticity of particle capture by the blue mussel, *Mytilus edulis* (L.): Confounding factors and potential artifacts with studies utilizing natural seston. *Journal of Experimental Marine Biology and Ecology, 473*, 207–217. https://doi.org/10.1016/j.jembe.2015.09.005

Rouillon, G., & Navarro, E. (2003). Differential utilization of species of phytoplankton by the mussel *Mytilus edulis*. *Acta Oecologica, 24*, S299–S305. https://doi.org/10.1016/S1146-609X(03)00029-8

Sá, B. A. dos S. de S. e. (2017). *Trophic dynamics between phytoplankton and microzooplankton in the Ria Formosa coastal lagoon system* [Master's thesis, Universidade do Algarve]. Faro, Portugal.

Safi, K. A., & Hayden, B. (2010). Differential grazing on natural planktonic populations by the mussel *Perna canaliculus*. *Aquatic Biology, 11,* 113-125. https://doi.org/10.3354/ab00297

Santos, Y. D. (2023). *Growth and mortality of microbial plankton in the Ria Formosa coastal lagoon* [Master's thesis, Universidade do Algarve]. Faro, Portugal.

Schmoker, C., Hernández-León, S., & Calbet, A. (2013). Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps, and future directions. *Journal of Plankton Research*, 35(4), 691–706. https://doi.org/10.1093/plankt/fbt023

Shumway, S. E., Cucci, T. L., Lesser, M. P., Bourne, N., & Bunting, B. (1997). Particle clearance and selection in three species of juvenile scallops. *Aquaculture International*, *5*, 89-99. https://doi.org/10.1007/BF02764790

Shumway, S. E., Cucci, T. L., Newell, R. C., & Yentsch, C. M. (1985). Particle selection, ingestion, and absorption in filter-feeding bivalves. *Journal of Experimental Marine Biology and Ecology, 91*(1-2), 77–92. https://doi.org/10.1016/0022-0981(85)90222-9

Shumway, S. E., Cucci, T. L., Newell, R. C., Selvin, R. R. L., Guillard, R., & Yentsch, C. M. (1985). Flow cytometry: A new method for characterization of differential ingestion, digestion, and egestion by suspension feeders. *Marine Ecology Progress Series, 24*, 201–204. https://doi.org/10.3354/meps024201

Silva, D. C., Neto, J. M., Nunes, C., Gonçalves, F. J., Coimbra, M. A., Marques, J. C., & Gonçalves, A. M. (2021). Assessment of seasonal and spatial variations in the nutritional content of six edible marine bivalve species by the response of a set of integrated biomarkers. *Ecological Indicators*, 124, 107378. https://doi.org/10.1016/j.ecolind.2021.107378

Singh, U. B., & Ahluwalia, A. S. (2013). Microalgae: A promising tool for carbon sequestration. Mitigation and Adaptation Strategies for Global Change, 18(1), 73–95. https://doi.org/10.1007/s11027-012-9393-3

Smaal, A. C., Ferreira, J. G., Grant, J., Petersen, J. K., & Strand, Ø. (Eds.). (2019). *Goods and services of marine bivalves*. Springer Nature. https://doi.org/10.1007/978-3-319-96776-9

Smayda, T. J. (1970). The suspension and sinking of phytoplankton in the sea. *Oceanography and Marine Biology Annual Review, 8*, 353-414.

Sobral, P., & Widdows, J. (1997). Influence of hypoxia and anoxia on the physiological responses of the clam Ruditapes decussatus from southern Portugal. *Marine Biology*, *127*, 455-461. https://doi.org/10.1007/s002270050033

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practice of statistics in biological research* (3rd ed.). W. H. Freeman and Company, New York, NY

Sommer, U., Charalampous, E., Genitsaris, S., & Moustaka-Gouni, M. (2017). Benefits, costs and taxonomic distribution of marine phytoplankton body size. *Journal of Plankton Research, 39*(3), 494–508. https://doi.org/10.1093/plankt/fbw071

Stein, R. A., DeVries, D. R., & Dettmers, J. M. (1995). Food-web regulation by a planktivore: Exploring the generality of the trophic cascade hypothesis. *Canadian Journal of Fisheries and Aquatic Sciences, 52*(11), 2518-2526. https://doi.org/10.1139/f95-842

Strohmeier, T., Strand, Ø., Alunno-Bruscia, M., Duinker, A., & Cranford, P. J. (2012). Variability in particle retention efficiency by the mussel Mytilus edulis. *Journal of Experimental Marine Biology and Ecology*, *412*, 96-102. https://doi.org/10.1016/j.jembe.2011.11.006

Thompson, R., & Bayne, B. L. (1972). Active metabolism associated with feeding in the mussel *Mytilus edulis* L. *Journal of Experimental Marine Biology and Ecology*, 9(1), 111-124. https://doi.org/10.1016/0022-0981(72)90011-1

Tomas, C. R. (1997). *Identifying marine phytoplankton*. Academic Press.

Trottet, A., Roy, S., Tamigneaux, E., Lovejoy, C., & Tremblay, R. (2008). Impact of suspended mussels (*Mytilus edulis* L.) on plankton communities in a Magdalen Islands lagoon (Québec, Canada): A mesocosm approach. *Journal of Experimental Marine Biology and Ecology, 365*(2), 103-115. https://doi.org/10.1016/j.jembe.2008.08.001

Trottet, A., Roy, S., Tamigneaux, E., Lovejoy, C., & Tremblay, R. (2008). Influence of suspended mussel farming on planktonic communities in Grande-Entrée Lagoon, Magdalen Islands (Québec, Canada). *Aquaculture, 276*(1-4), 91-102. https://doi.org/10.1016/j.aquaculture.2008.01.038

Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton-Methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. *Internationale Vereinigung Für Theoretische Und Angewandte Limnologie: Mitteilungen*, *9*(1), 1–38. https://doi.org/10.1080/05384680.1958.11904091

Vanderploeg, H. A., Liebig, J. R., & Gluck, A. A. (1996). Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): The importance of size and polyunsaturated fatty acid content. *Journal of Great Lakes Research, 22*(1), 36-45. https://doi.org/10.1016/S0380-1330(96)70932-2

Vanderploeg, H. A., Liebig, J. R., & Nalepa, T. F. (1995). From pico plankton to microplankton: Temperature-driven filtration by the unionid bivalve *Lampsilis radiata siliquoidea* in Lake St. Clair. *Canadian Journal of Fisheries and Aquatic Sciences, 52*(1), 63–74. https://doi.org/10.1139/f95-006

Vanderploeg, H. A., Liebig, J. R., Carmichael, W. W., Agy, M. A., Johengen, T. H., Fahnenstiel, G. L., & Nalepa, T. F. (2001). Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences, 58*(6), 1208-1221. https://doi.org/10.1139/f01-066

Venrick, E. L. (1978). How many cells to count? In A. Sournia (Ed.), *Phytoplankton manual* UNESCO, Paris, 167–180.

Ward, J. E., & Shumway, S. E. (2004). Separating the grain from the chaff: Particle selection in suspension- and deposit-feeding bivalves. *Journal of Experimental Marine Biology and Ecology*, 300(1-2), 83-130. https://doi.org/10.1016/j.jembe.2004.03.002

Ward, J. E., & Shumway, S. E. (2004). Separating the grain from the chaff: Particle selection in suspension- and deposit-feeding bivalves. Journal of Experimental Marine Biology and Ecology, 300(1–2), 83–130. https://doi.org/10.1016/j.jembe.2004.03.002

Widdows, J., & Hawkins, A. J. S. (1989). Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. *Physiological Zoology, 62*(3), 764-784 https://doi.org/10.1086/physzool.62.3.30157926

Yu, N., & Culver, D. A. (1999). Estimating the effective clearance rate and refiltration by zebra mussels, *Dreissena polymorpha*, in a stratified reservoir. *Freshwater Biology, 41*(3), 481–492. https://doi.org/10.1046/j.1365-2427.1999.00393.x

Zhang, Y., Cheng, L., Li, W., Han, Y., Gu, J., Li, K., & Jeppesen, E. (2023). Growth and clearance rate of *Corbicula fluminea* in relation to fine sediment resuspension in eutrophic shallow lakes. *Hydrobiologia*, 850, 1045–1058. https://doi.org/10.1007/s10750-022-05105-2

Appendix

Supplementary Table S1 –Summary of SIMPER (Similarity Percentage) analysis results for November, comparing dissimilarity in phytoplankton assemblage structure at t_0 and after 30 min for control (C, no mussels) and experimental (B, 2-3 mussels) treatments. For each phytoplankton group, the mean abundance in each treatment (log (cells L^{-1} + 1)) and its percentage contribution to the total dissimilarity between treatments (Contrib.) are presented. The relative cumulative contribution (Relative Cumulative Contrib.) shows the cumulative percentage contribution of each group to the total dissimilarity. An asterisk (*) indicates a p-value < 0.05.

SIMPER overall average dissimilarity t0-C= 15,39%

	Abundance t0	Abundance C	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,89	6,75	6,30	41,00	0,956
o-anf	6,61	6,78	5,13	74,30	0,255
cyano	6,82	6,73	3,73	98,50	0,987
crypto	4,76	4,98	0,16	99,50	0,696
dinofl	4,03	4,42	0,04	99,80	0,981
dia	4,94	4,94	0,03	100,00	0,989

SIMPER overall average dissimilarity t0-B= 30,59 %

	Abundance t0	Abundance B	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,89	6,45	17,07	55,80	0,001 *
cyano	6,82	6,51	11,64	93,90	0,001 *
o-anf	6,61	6,64	1,38	98,40	0,933
dia	4,94	4,47	0,20	99,00	0,016 *
crypto	4,76	4,46	0,16	99,60	0,709
dinofl	4,03	4,70	0,13	100,00	0,001 *

SIMPER overall average dissimilarity C-B= 24,10 %

	Abundance C	Abundance B	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,75	6,45	9,88	41,00	0,234
cyano	6,73	6,51	7,55	72,30	0,121
o-anf	6,78	6,64	6,06	97,50	0,029 *
crypto	4,98	4,46	0,31	98,80	0,041 *
dia	4,94	4,47	0,21	99,60	0,008 *
dinofl	4,42	4,70	0,09	100,00	0,117

Supplementary Table S2 – –Summary of SIMPER (Similarity Percentage) analysis results for March, comparing dissimilarity in phytoplankton assemblage structure at t_0 and after 30 min for control (C, no mussels) and experimental (B, 2-3 mussels) treatments. For each phytoplankton group, the mean abundance in each treatment (log (cells $L^{-1} + 1$)) and its percentage contribution to the total dissimilarity between treatments (Contrib.) are presented. The relative cumulative contribution (Relative Cumulative Contrib.) shows the cumulative percentage contribution of each group to the total dissimilarity. An asterisk (*) indicates a p-value < 0.05.

SIMPER overall average dissimilarity t0-C= 20,43%

	Abundance t0	Abundance C	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,11	6,19	8,33	40,80	0,378
cyano	6,05	6,08	6,10	70,60	0,459
o_anf	5,36	5,68	3,83	89,40	0,613
crypto	5,06	5,13	1,72	97,80	0,671
dia	5,06	5,00	0,43	99,90	0,995
dinofl	3,33	3,46	0,02	100,00	0,963

SIMPER overall average dissimilarity t0-B= 18,68 %

	Abundance t0	Abundance B	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,11	6,11	6,03	32,30	0,947
cyano	6,05	6,00	5,10	59,60	0,857
o_anf	5,36	4,68	3,51	78,40	0,708
dia	5,06	3,97	2,00	89,10	0,002 *
crypto	5,06	3,98	1,96	99,60	0,385
dinofl	3,33	3,76	0,08	100,00	0,08

SIMPER overall average dissimilarity C-B= 27,07 %

	Abundance C	Abundance B	Contrib. (%)	Relative Cummulative contrib. (%)	p
epp	6,19	6,11	8,89	32,80	0,225
o_anf	5,68	4,68	7,30	59,80	0,001 *
cyano	6,08	6,00	7,09	86,00	0,198
crypto	5,13	3,98	2,18	94,10	0,223
dia	5,00	3,97	1,54	99,80	0,044 *
dinofl	3,46	3,76	0,07	100,00	0,41

Supplementary Table S3 –Summary of SIMPER (Similarity Percentage) analysis results for April, comparing dissimilarity in phytoplankton assemblage structure at t0 and after 30 min for control (C, no mussels) and experimental (B, 2-3 mussels) treatments. For each phytoplankton group, the mean abundance in each treatment (log (cells L-¹ + 1)) and its percentage contribution to the total dissimilarity between treatments (Contrib.) are presented. The relative cumulative contribution (Relative Cumulative Contrib.) shows the cumulative percentage contribution of each group to the total dissimilarity. An asterisk (*) indicates a p-value < 0.05.

SIMPER overall average dissimilarity t0-C= 9,75%

	Abundance t0	**Abundance C**	**Contrib. (%)**	**Relative Cummulative contrib. (%)**	**p**
epp	6,50	6,46	3,60	37,00	0,604
cyano	6,09	6,01	3,56	73,40	0,377
o-anf	5,95	5,87	1,38	87,60	0,987
crypto	5,56	5,46	0,98	97,60	0,211
dia	5,27	5,27	0,21	99,70	0,996
dinofl	3,42	3,22	0,03	100,00	0,176

SIMPER overall average dissimilarity t0-B= 16,50 %

	Abundance t0	**Abundance B**	**Contrib. (%)**	**Relative Cummulative contrib. (%)**	**p**
o-anf	5,95	6,26	7,12	43,20	0,016 *
epp	6,50	6,54	3,67	65,40	0,524
cyano	6,09	6,12	3,59	87,20	0,355
dia	5,27	4,50	1,22	94,50	0,010 *
crypto	5,56	5,40	0,89	99,90	0,413
dinofl	3,42	2,40	0,02	100,00	0,665

SIMPER overall average dissimilarity C-B= 18,52 %

	Abundance C	**Abundance B**	**Contrib. (%)**	**Relative Cummulative contrib. (%)**	**p**
o-anf	5,87	6,26	8,83	47,70	0,001 *
epp	6,46	6,54	5,14	75,40	0,044 *
cyano	6,01	6,12	2,72	90,10	0,745
dia	5,27	4,50	1,29	97,10	0,007 *
crypto	5,46	5,40	0,53	99,90	0,862
dinofl	3,22	2,40	0,01	100,00	0,724