

Importance of integration of the mixoplankton paradigm within Marine Framework Directives



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MixITiN

Project no. 766327

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Anna-Adriana Anschütz, Maira Maselli, Claudia Traboni,
Willem Stolte, Aditee Mitra, Kevin J Flynn

Acknowledgements

*Project **MixITiN** has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 766327. This document reflects only the author's view; the REA and the European Commission are not responsible for any use that may be made of the information it contains.*

How to cite this work

Anschütz AA, Maselli M, Traboni C, Stolte W, Mitra A, Flynn KJ (2024) Importance of integration of the mixoplankton paradigm within Marine Framework Directives.

Publisher: Zenodo, <https://doi.org/10.5281/zenodo.10684458>

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This report is an annex to MixITiN Network-wide training (secondments) MS5.

Project Coordinator

Aditee Mitra, School of Earth and Environmental Sciences, Cardiff University, Cardiff, CF10 3AT, United Kingdom. MitraA2@Cardiff.ac.uk

WP2 Lead

Kevin J Flynn, Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, UK

Secondment Supervisor

Willem Stolte, Deltares, Boussinesqweg 1, 2629 HV Delft, The Netherlands

Funding acquisition by AM; **Conceptualization & methodology** by WS, AM; **Supervision** by AM, KJF, WS; **Investigation** by AAA, MM, CT; **Writing, reviewing & editing** by AAA, MM, CT, WS, AM, KJF.

Importance of integration of the mixoplankton paradigm within Marine Framework Directives.

Anschütz, AA^{1,4}; Maselli, M²; Traboni, C^{3,4}; Stolte, W⁵; Mitra, A¹; Flynn, K.J.⁶

1 School of Earth and Ocean Sciences, Cardiff University, Park Place, Cardiff, Wales CF10 3AT, UK

2 Marine Biological Section, University of Copenhagen, Strandpromenaden 5,3000, Helsingør, DK

3 Institut de Ciències del Mar, CSIC, Passeig Marítim de la Barceloneta 37-49, 08003, Barcelona, Spain

4 Écologie des Systèmes Aquatiques, Université Libre de Bruxelles, Belgium

5 Deltares, Boussinesqweg 1, 2629 HV Delft, The Netherlands

6 Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, UK

Corresponding author: Willem Stolte; Willem.Stolte@deltares.nl

Acknowledgements

We thank colleagues within the MixITiN project (www.mixotroph.org) for discussions that have improved this work.

Author contributions: Anna-Adriana Anschütz, Maira Maselli and Claudia Traboni equally shared in literature analysis and drafting the paper under the supervision of Willem Stolte, Kevin Flynn and Aditee Mitra. All authors contributed to reworking the document to its final form.

Funding: This research was supported by EC MSCA-ITN 2019 funding to the project MixITiN (grant number 766327).

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Abstract

Marine plankton that are capable of photosynthesis and also predation (termed “mixoplankton”) comprise up to 50% of those organisms traditionally termed phytoplankton. Many harmful bloom species are mixoplanktonic. However, marine environmental management policies issued by governments, including the MSFD by the EU, have been designed assuming a strict dichotomy between autotrophic phytoplankton and heterotrophic plankton. Mixoplankton often differ greatly from these two categories in response to environmental pressures and have effects on the marine environment that we are only beginning to understand. While the management policies may conceptually provide scope for recognising mixoplankton, such action is rarely implemented. We suggest that the effectiveness of monitoring and management programs such as MSFD could benefit from explicit implementations regarding the role of mixoplankton. As an example, here we propose a revision of the MSFD explicitly including mixoplanktonic activity in management strategies, including descriptions of the management components that would benefit from including mixoplankton and suggests for appropriate methods.

Introduction

The importance of monitoring plankton production and biodiversity is globally recognized as it provides an indicator for food web productivity and the formation of harmful algae blooms (HABs). Such action is essential for maintaining and achieving good environmental status in aquatic systems (Gowen et al. 2011; Asa et al. 2015; López-Jurado et al. 2015; McQuatters-Gollop et al. 2015; Scherer et al. 2016; Bueno et al. 2017; Bedford et al. 2018; Lipsewers and Spilling 2018; Varkitzi et al. 2018; Batten et al. 2019) that in turn describes healthy productive ecosystems and relates to biomass and quality of food sources (European Commission 2019).

Traditionally, global ocean through to local coastal surveys have reported and classified planktonic organisms according to a simple dichotomic view of marine food web structure based on the photoautotrophic “phytoplankton”, their heterotrophic grazers, the “zooplankton”, together with other heterotrophs (Asa et al. 2015; Bresnan et al. 2015; Lima-Mendez et al. 2015; López-Jurado et al. 2015; McQuatters-Gollop et al. 2015; Scherer et al. 2016). The last decade has witnessed something of an overhaul in the way that science perceives the structure of the marine planktonic trophic structure. Many, if not on occasion most, of the protist “phytoplankton” and “proto(micro)zooplankton” are now recognised as being capable of both phototrophy and phago-heterotrophy simultaneously in the same single cell (Stoecker et al. 2009; Flynn et al. 2013; Mitra et al. 2016). These organisms are now collectively termed “mixoplankton” (Flynn et al. 2019). Of the chlorophyll-containing protist plankton, only the diatoms are exclusively not mixoplanktonic, and may thus be labelled “phytoplankton”. Cyanobacteria are also phytoplankton, being incapable of phagocytosis, though like diatoms they can be mixotrophic through the use of dissolved organic nutrients.

Although mixotrophy in the sense of using dissolved organic matter has long been recognized (Flynn and Butler 1986; Flynn et al. 2013, 2019), combined photo-autotrophic and phago-heterotrophic growth in mixoplankton redraws the conceptual framework of marine plankton ecology (Fig. 1.) that is used as the basis for ecosystem-based coastal zone management. In the first place, many mixoplankton are causative agents for harmful algal bloom events (HABs). We can now appreciate that a focus on inorganic nutrient supplies as a driver may not always explain the occurrence of such events. Secondly, some of the mixoplankton HABs, e.g. *Dinophysis* spp. are known to have very specific prey requirements (Park et al. 2006); understanding the trophic structure may improve early detection and better measures in cases where this is needed. Recently, more and more of such complex relations involving mixoplankton have become apparent (Pitta et al.

2001; McManus et al. 2004; Park et al. 2006; Calbet et al. 2012; Schoener and McManus 2012). While the importance of mixotrophy as a frequent strategic trait for many HAB species is recognised (Smayda 1997; Heisler et al. 2008; Anderson et al. 2012) concrete proposals on incorporating that knowledge into monitoring and management are still usually lacking. Only in a few specific instances does the role of mixotrophy come to the fore (e.g., *Alexandrium tamarense*, *Karenia brevis* and *Dinophysis spp.*; Erdner et al. 2008; Anderson 2009).

Recent revisions of potential indicators for the MSFD mentioned mixotrophy but do not discuss it an essential factor (Gowen et al. 2011; Caroppo et al. 2013; Jaschinski et al. 2015; López-Jurado et al. 2015; McQuatters-Gollop et al. 2015; Caruso et al. 2016; Bedford et al. 2018; Varkitzi et al. 2018). Papers that do mention the need to consider what we now term mixoplanktonic activity in coastal management (Davidson et al. 2014; Lehtinen et al. 2016), seldom distinguish between types of mixoplankton nor propose a concrete plan to implement changes in monitoring that recognise their activity. There are two indicators for the MSFD that could be interpreted to explicitly involve mixoplankton: the Phytoplankton Community Index PI(mp) (Tett et al. 2008; Whyte et al. 2017; Lei et al. 2018) used as an indicator for biodiversity, food web, eutrophication and seabed integrity (Lei et al. 2018) and the diatom/dinoflagellate index (Dia/Dino index) (Wasmund 2017) for eutrophication. Neither of these indices distinguish between mixoplanktonic modes of activity, within which the contributions of phototrophy and heterotrophy can vary significantly. In addition, the Dia/Dino index groups together autotrophic and mixoplanktonic dinoflagellates.

More profoundly, while the potential role of mixotrophic plankton growth (i.e., phototrophy + heterotrophy) is already covered in water directives by implicit mention of the importance of dissolved organics, mixoplanktonic activity differs significantly from osmotrophy-enabled mixotrophy. This is because the phagotrophy of mixoplankton requires the killing of competitor prey species and thus has scope to rapidly and radically recast the plankton community. In turn, while an osmotrophic-enabled mixotrophic activity may be accounted for by simple reference to the concentration of dissolved organics, mixoplanktonic activity requires monitoring and measurement of plankton community structure in more detail. Measuring inorganic and organic nutrients, together with chlorophyll for what is often a phytoplankton or diatom-centric analysis then appears to be an inadequate strategy. Indeed, we may speculate why the output of many models designed for the prediction of HAB events (Reguera et al. 2012; Moita et al. 2016) still greatly differs from observation (Moita et al. 2016; Flynn et al. 2018) is at least in part due to a failure to recognise the mixoplanktonic activity of the causative organisms.

Mixoplankton cannot be readily distinguished from “normal” phytoplankton by simple methods and monitoring them is therefore a challenge. Some monitoring programs distinguish

between general groups of plankton at different taxonomic level, such as dinoflagellates, cryptophytes and diatoms (Paerl et al. 2003; Beaugrand 2005; Baltic Marine Environment Protection Commission 2016; Abad et al. 2016). The continuous plankton recorder (CPR) survey identifies around 500 taxa of plankton (McQuatters-Gollop et al. 2009) while there are also monitoring programs which discriminate to the species level, for example, but not limited to, those that focus on harmful algal bloom (HAB) species. (e.g, USA (NOAA 2019), Ireland (Silke and Cusack 2012)). A lot of knowledge about biodiversity on a taxonomic level is gained by these classifications, but only little about the diversity in trophic modes.

Monitoring is the core of coastal management. At the moment no consistent monitoring for mixoplankton is implemented, and therefore, management cannot make use of the increasing knowledge on these types of plankton in achieving good ecosystem health. In the following, we aim to make visible how the mixoplankton paradigm (Fig. 1) can be connected to coastal management procedures. As an exemplar we make specific mention of regulations and management in the European Union (EU), however the concepts have universal application for marine and coastal water management.

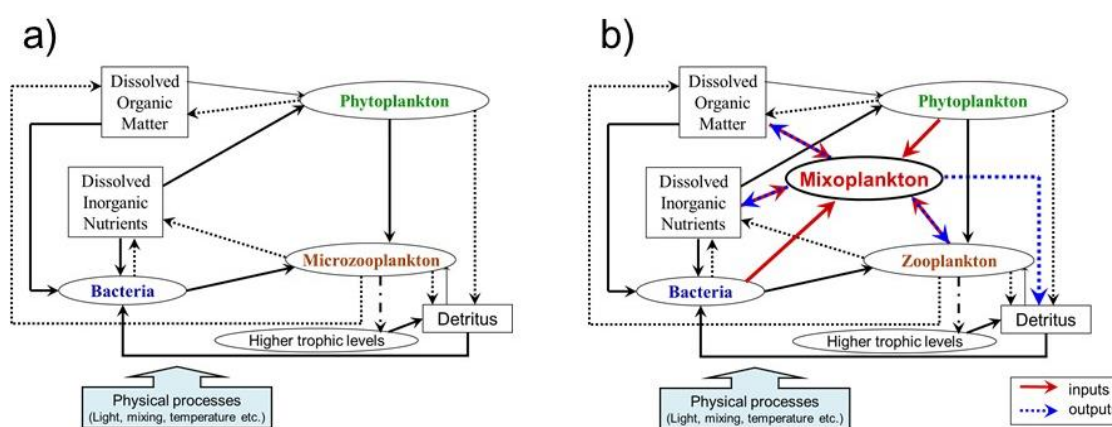


Figure 1 Schematic representation illustrating the difference between the traditional plankton food-web (a), and the restructured web (mixoplankton paradigm) that recognises the importance of mixoplanktonic activity (b).

Characteristics of mixoplankton of relevance to marine management

Before ca. 2013 (Flynn et al. 2013), and often still since, planktonic organisms were considered to be either exclusively phototrophic or heterotrophic while mixotrophy was mostly considered in the form of osmotrophy. Mixotrophy as a mode of nutrition combining both photo- and heterotrophy in the same organism, was considered as the exception. Furthermore, where

mixoplankton have been recognised they have traditionally been viewed as inferior to their pure photo- or heterotrophic counterparts, as “Jack-of-all-trades-and-master-of-none”. This view appears to be poorly substantiated, if not simply incorrect (Flynn et al. 2013, 2019). There is a growing body of scientific research (Fig. 2) that recognises the importance of mixotrophic activity in individual species (which includes HAB and EDAB species) and for ecology, especially in the temperate water during summer. However, the distinction between osmotrophy (uptake of dissolved organics) and phagotrophy (actual prey capture by functional mixoplankton) was seldom explicit. Thus, based on the current knowledge (Stoecker 1998; Flynn et al. 2013, 2019; Mitra et al. 2016), energy transfer, competition and selection in plankton food webs are influenced by mixoplankton in a way that is often not considered in current conceptual and simulation models (Fig. 1, (Mitra et al. 2014; Caron 2016)).

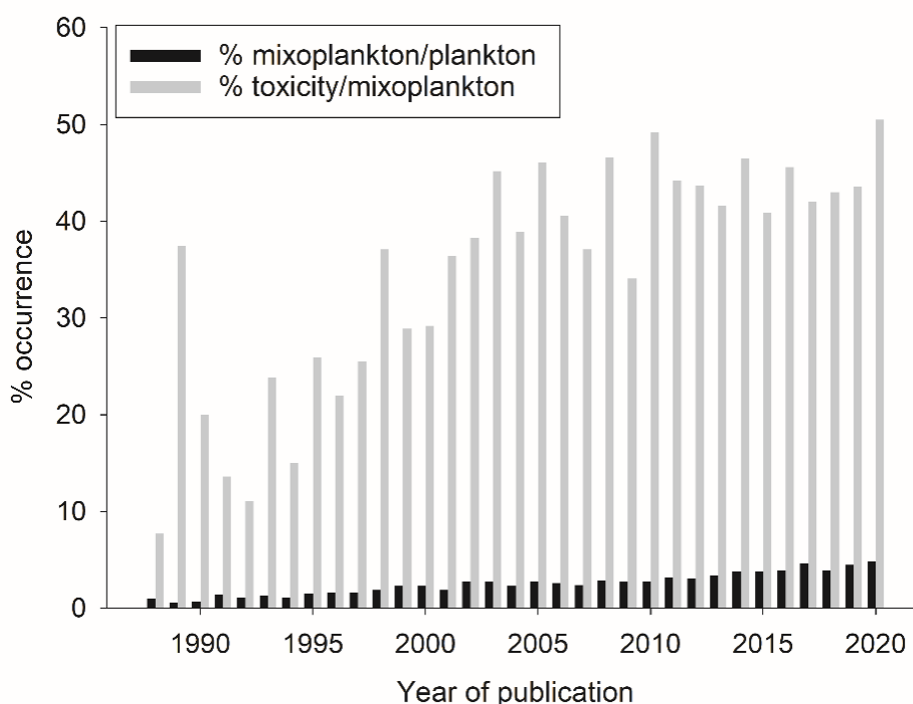


Figure 2 Proportion of scientific publications on plankton ecophysiology describing mixotrophic plankton over the years from 1988 until 2019. Black bars indicate the increasing acknowledgement of mixoplankton out of the overall plankton research. Grey bars reflect the trend of mixoplankton species involved in toxic outcomes. Data were obtained from Scopus datasets using the keywords “marine”, “plankton*”, “mixotroph*” and “toxic*”.

There are two major groups of mixoplankton termed “constitutive” and “non-constitutive”. Of those that acquire phototrophy from their prey (non-constitutive mixoplankton), some require a near-continuous supply of phototrophic prey from which they acquire their chloroplasts, while others only feed occasionally. The toxic genus *Dinophysis* is included in the last-mentioned group and thus its ecology is linked to that of several other specific species growing in the preceding weeks or months (Park et al. 2008; Nielsen et al. 2012; Hansen et al. 2013, 2016) and not simply upon the supply of light and nutrients as one may expect a regular phytoplankton to depend.

Constitutive mixoplankton, which are not dependent on specific prey for their chloroplasts, can often outcompete pure autotrophs in stratified waters, which become depleted in dissolved inorganic nutrients (Stoecker 1998; Burkholder et al. 2008) in summer. Consistent with such empirical observations, *in-silico* experiments conducted with appropriately constructed models have shown that mixoplankton also perform, and change their environment, in distinctly different ways from pure autotrophs and heterotrophs (Hammer and Pitchford 2005; Mitra et al. 2014; Ghyoot et al. 2017; Anschütz and Flynn 2020). Which type of plankton will succeed in an ecosystem will therefore affect this ecosystem further than in mere species composition, but may also change the conditions for higher trophic levels, the maximum biomass yield and/or the consecutive plankton bloom.

As further complications, traditional predator-prey allometric patterns (expecting prey to be smaller than the predator by ca. ten-fold) do not apply to many mixoplankton. On the contrary, many can feed on prey their own size or even bigger than themselves (Hansen et al. 1994; Calbet 2008). Because mixoplankton can feed on a wider range of prey than may be expected for their cell size (Tillmann 2003), they can take up a lot of nutrients by consumption of just one prey item at once (Tang 1995). At high cell abundances, mixoplankton can also gang-up on competitors and predators. For example, swarms of *Karlodinium* spp. have been observed in field samples in Denmark to kill and feed on copepods that are 100's of times larger than the mixoplankton themselves (Berge et al. 2012).

Different mixoplankton genera, and even species and strains, may be more or less affected by light attenuation (e.g. due to high sediment perturbation), as they can resort to predation as a source of energy (Skovgaard 1996; Stoecker 1998). That said, they often appear to have an obligate necessity for a small fraction of their C-budget to be derived from phototrophy. Prey availability, on the other hand, may be a limiting factor for some mixoplankton types and it can influence their population size and their toxicity (in the case of HAB species) (Gao et al. 2017; Hernández-Urcera et al. 2018). That the growth rate of a mixoplankton may be limited by the growth rate of its prey

(Riisgaard and Hansen 2009) requires a more holistic view of plankton ecology than just placing all “phytoplankton” in a few or often just one functional group “box” in a model. The complexity of the synergism between heterotrophy and phototrophy in mixoplankton also advises against implementing mixoplankton as the simple addition of the two trophic modes in one organism in such models. The corollary is that management policies must also not group such organisms together with what could be termed regular phytoplankton and zooplankton.

Many larger mixoplankton can exhibit vertical migration to optimise their growth, balancing needs for light, dissolved nutrients and prey, thus hydrodynamics (stratification and turbulence) may play a very different role in their ecology than for pure autotrophic phytoplankton (Sjöqvist and Lindholm 2011). It has long been argued that dinoflagellates undertake diel vertical migration to photosynthesise at the surface and acquire inorganic nutrients at the nutricline; it is quite possible that they also feed at the nutricline, where particles accumulate. In addition, experimental evidence highlights that mixoplankton decrease the variability in seston stoichiometry when compared to non-mixotrophic food webs (Moorthi et al. 2017) because they are stoichiometrically (N/P) more stable than autotrophs even under fluctuating inorganic nutrient ratios. They also promote zooplankton growth and reproduction as observed in marine calanoid copepods and freshwater daphnids (Ptacnik et al. 2004; Katechakis et al. 2005). Furthermore, mixotrophy has been suggested to enhance the transfer of biomass up the food chain, causing the sinking carbon flux to increase by ~35 % (Ward and Follows 2016). This showcases the importance of carefully considering mixoplankton when predicting nutrient fluxes and food web status in the marine environment for management purposes.

Impact of mixoplankton on ecosystem services

Many ecosystem services such as harvestable fish biomass, climate regulation and water quality are heavily impacted by plankton (Ryther 1969; Costanza et al. 1998; Hays et al. 2005) that we now define as mixoplankton (Leles et al. 2017, 2019). The most obvious impact is through the production of toxins during episodic HAB events. Indeed, the terminology, “HAB”, reflects the traditional microalgal-centric view of these organisms as photoautotrophs rather than the reality of the ecophysiology of most of the causative organisms as mixoplanktonic. While, indeed, some HAB species (certain dinoflagellates) have long been recognised as mixoplanktonic, the full ramifications of this mode of feeding and the implications for ecology and species proliferation have often been downplayed.

As an example, *Karlodinium veneficum* which feeds more when nutrient-stressed (Li et al. 2000) also has a higher toxin content when actively feeding (Adolf et al. 2009). Due to the observation of metazoan mortality in field samples, blooms of this toxic mixoplanktonic dinoflagellate would likely hamper the potential trophic transfer and secondary production by acting directly on copepod recruitment (Berge et al. 2012). In addition, the release of haemolytic karlotoxins could simultaneously cause an imbalance in food web structure due to downstream cascading effect at the level of fish populations affected by the ichthyotoxin (Place et al. 2012).

A mismatch between the utilisation of inorganic nutrients and the outbreak of mixoplankton growth due to phagotrophy (Jeong et al. 2010) hinders attempts to predict HAB events; management of HABs need to be reviewed taking in consideration different indicators, other than dissolved inorganic nutrients. Model predictions assuming a simple direct linkage between (phyto-) plankton growth and changes in nutrient discharge, or in N:P nutrient ratios, to test economic and ecological consequences of these measures may be inappropriate or even counterproductive. The benefit of decreasing nutrient run-off on the ecosystem needs to be balanced against the monetary investment required to minimise the effects of eutrophication, as seen for the Baltic Sea (Gren et al. 1997) and North Sea (Lancelot et al. 2011); the investment may be insufficient to avoid undesired blooms and restore the nutrient ratios at the impacted locations.

Over the last three decades (1987-2000 estimates), USA and European coastlines were financially impacted by HABs causing losses of several \$ million in different sectors (Hoagland and Scatista 2006) (Fig. 3). Europe was more impacted in the touristic sector in comparison to the USA, where fisheries and public health were more severely affected. In most reported cases, HAB events led to the closure of shellfisheries and, as a resulting 'halo effect', the price of shellfish sharply increased because of a drastic cut in seafood supply (Davidson et al. 2014). In Spain, Denmark and Norway, harmful bloom outbreaks led to the establishment of monitoring programs due to public health and industry loss (Anderson et al. 2001). Since the observed effects, in most of the countries at risk, regular toxin bioassays, nutrient and Chlorophyll *a* (Chl_{*a*}) analyses are routinely performed and "phytoplankton" monitoring is being conducted to aid in predictions of harmful events.

While photo-autotrophic organisms such as diatoms and cyanobacteria can also form blooms, most HAB events in the marine environment are in fact caused by mixoplankton (e.g., *Alexandrium* spp., *Phaeocystis* spp., *Dinophysis* spp., *Karenia* spp.), including in European countries (Blauw et al. 2006). In Table 1 a selected list of mixoplanktonic HAB events recorded in European marine waters is reported for informative purpose. Norway, in particular, was severely affected in the late '90s as massive salmon kills were reported upon blooms of (the now recognised mixoplanktonic) *Chrysochromulina* spp. and *Prymnesium* spp. for a total annual loss of US\$ 3M

(Anderson et al. 2001). In a recent Norwegian HAB event (May 2019), caused by *Chrysochromulina leadbeateri*, the estimated salmon loss accounted for \$ 69M, with over ten thousand dead fish and economic drop of fish market (www.fiskeridir.no/English, 2019). It is noteworthy that the biomass in the latest reported *Chrysochromulina* bloom is one order of magnitude higher than the one registered in 1995 (Table 1).

The link between eutrophication and HABs has been questioned (Davidson et al. 2014), in part because HAB species may be cryptic. At least some of those cryptic species actually require other (prey) species which themselves may not be cryptic; monitoring and preventional programmes of recognized mixotrophic HAB organisms like *Dinophysis* may well benefit from a specific revision to recognise the importance of the mixoplankton paradigm and thence of plankton community composition in general.

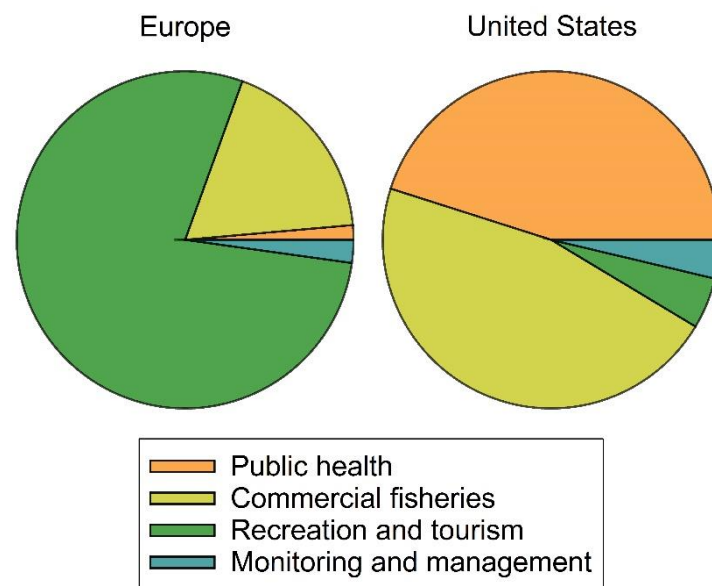


Figure 3. Averaged contribution of economic effects of harmful algal bloom events on several sectors in Europe and the United States between 1989 and 2000. Credit: Hoagland and Scatasta. 2006. Percentages calculated are relative to total annual losses of \$813 M for EU and \$82 M for the US (monetary estimates were converted into 2005 dollars). Different methodologies were applied in the computation of the presented economic loss, therefore quantitative comparisons should be taken with caution.

Table 1 A selected list of HAB events caused by mixoplankton species reported in different EU countries. Related causative species and cell concentrations, bivalve vectors and toxins analysed, impacts on economy and management decision are reported when available. Data are extracted from the Harmful Algae Information System (www.haedat.iode.org).

Year	Country	Mixoplankton Species	cells L ⁻¹	Shellfish vector	Toxin/syndrome	Impact
1994	Norway	<i>Prymnesium</i> spp.	2·10 ⁶			Threatened fish farms cleared the area.
1995	Norway	<i>Chrysochromulina</i> spp.	5·10 ⁶			Gill damage and osmoregulatory problems
1998	France	<i>Alexandrium tamarense</i>	90,000	<i>Ruditapes decussatus</i> ; <i>Mytilus</i> sp.	SXT/PSP	The lagoon was closed to harvesting shellfish
1989	The Netherlands	<i>Gyrodinium aureolum</i>	1,900	<i>Mytilus</i> sp.	DSP	Closure of mussel production
1999	Germany	<i>Prorocentrum triestinum</i>				High biomass
2001	Denmark	<i>Dinophysis acuminata</i>	1,300	<i>Mytilus edulis</i>	OA/DPS	Harvest was prohibited
2006	Italy	<i>Alexandrium pacificum</i>	2,240	<i>Mytilus galloprovincialis</i>	SXT/PSP	Water discoloration - Visual nuisance for locals
2010	Slovenia	<i>Dinophysis caudata</i>	330		DSP	Closure of shellfish harvesting
2014	Finland	<i>Alexandrium ostenfeldii</i>	6 ·10 ⁶		STX-GTX-GYM	Media alert to local inhabitants and vacationers
2014	Greece	<i>Karlodinium</i> spp.	3.3·10 ⁶			Ichthyotoxicity and visual nuisance for locals
2016	United Kingdom	<i>Dinophysis</i> spp.	90,274	<i>Mytilus edulis</i>	OA/DPS	Closures enforced at multiple sites around the site
2017	Norway	<i>Alexandrium tamarense</i>	3,760	<i>Mytilus edulis</i>	SXT/PSP	Harvesting was locally banned.
2018	Spain	<i>Dinophysis acuminata</i>	3,080	<i>Donax trunculus</i> ; <i>Mytilus galloprovincialis</i>	OA/DSP	Compromised shellfish industry
2019	Portugal	<i>Gymnodinium catenatum</i>	6,440		PSP	Production area interdicted to bivalve harvesting
2019	Norway	<i>Chrysochromulina leadbeateri</i>	1.5·10 ⁷			Gill damage and osmoregulatory problems; mass salmon mortality.

Anthropogenic pressures and environmental conditions promoting mixoplankton growth.

In general mixoplankton dominate in mature ecosystems (Mitra et al. 2014, Schneider et al in re-submission), that is in systems that contain a complex diverse ecology, typically in which most nutrients are bound as organics. In marine systems, the temperate spring bloom is an immature ecosystem, while the summer conditions typically support mature planktonic systems.

Anthropogenic drivers acting on mixoplankton growth and distribution, and the effects that organisms have on ecosystems and humans are shown in Figures 4 & 5. These drivers are (with reference to Fig. 5):

- **High nutrient inputs**, which cause eutrophication and light attenuation, leading to decreased water quality and anoxia due to high respiration (MSFD, 2008; European Parliament and Council of the European Union 2008). While eutrophication alone is not a trigger exclusive to mixoplankton HABs, mixoplankton have an advantage here as they can compensate for low light conditions and progressive nutrient limitation with other trophic modes (Anderson et al. 2002; Reynolds 2006; Glibert and Burkholder 2011; Mitra et al. 2016). In estuaries, with low light and very low dissolved inorganic to organic nitrogen ratios mixoplankton can outcompete autotrophs (Gobler et al. 2011). Hence, monitoring of nutrients per se would be helpful especially to predict the potential prey composition, in turn indicating the development but also the persistence of future mixoplanktonic bloom (e.g., *Teleaulax-Mesodinium-Dinophysis* complex; Gustafson et al. 2000; Park et al. 2006; Reguera et al. 2012).

The type of eutrophication and specifically the nutrient composition have also to be considered (Anderson et al. 2008). For example, elevated ammonium versus nitrate promotes growth and toxicity levels of some mixoplankton (Hattenrath-Lehmann et al. 2015); however, a distinction in monitoring between nitrogen sources (inorganic, organic, dissolved and for mixoplankton particulate) is not always made. Additionally, selective nutrient inputs from land-based activities cause imbalanced (non-Redfield) nutrient ratios, exacerbated by changes in water run-off patterns with precipitation and ice melts. This changes the silica:nitrogen:phosphorus (Si:N:P) supply ratio, promoting the growth of potential mixoplankton over its phototrophic counterpart (diatoms) which depend on silica to proliferate. Changes in mixoplankton N:P, and thus by inference nutrient input N:P, is also associated with increased toxicity (John and Flynn 2000; Granéli and Flynn 2006). In some cases, however, mixoplankton may also balance the effects of changes in light and nutrient supply on transfer efficiency of energy to higher trophic levels (Katechakis et al. 2005).

- **Aquaculture** that often provokes inorganic and organic eutrophication and light limitation. Mixoplankton may take advantage of these conditions for the reasons mentioned above and give rise to HABs and associated halo economic effects in this sector (Glibert et al. 2005).
- **Increasing temperature and stratification**, due to global and local warming (but also due to anthropogenic modifications of hydrodynamics on local scales), enhance communal respiration over gross primary production, decreasing the relative effectiveness of carbon sequestration from the atmosphere (Regaudie-De-Gioux and Duarte 2012). On an ecological level, this can cause a simultaneous shift from a diatom-based community to dinoflagellates (of which phototrophic forms are mixoplankton) (Klais et al. 2011; Gomes et al. 2018), and most likely also to other smaller mixoplanktonic flagellates that are usually not monitored.
- Compromising **seafloor integrity** by dredging or dumping works can lead to resuspension of resting stages of mixoplankton types that were formerly considered autotrophs into the water column, leading them to reappear and persist in seasons they are not expected in (Nehring 1996; Balkis et al. 2016). Some mixoplankton types such as *Dinophysis* require the presence of their specific prey for growth (Park et al. 2006), which is not considered as a risk factor for potential HAB organisms that are falsely labelled “phytoplankton”. Germination events from mixoplanktonic dinoflagellate cysts outnumber those from their heterotrophic counterparts throughout the year (Balkis et al. 2016). The HAB-forming mixoplankton (e.g., *Alexandrium tamarense*, *Protoceratium reticulatum*, *Heterocapsa triquetra*, *Gymnodinium catenatum*, *Scrippsiella trochoidea* and *Lyngulodinium polyedrum*) appear to be most abundant and persistent in sediments and showed the highest experimental germination success (Balkis et al. 2016). In some instances, resting stages of toxic species may contain a higher toxin load than the vegetative counterparts (Oshima et al. 1992). In addition, dredging and dumping works can cause resuspension of sedimented nutrients but also higher turbidity at the same time. These are conditions that mixoplankton could grow in but are unfavourable for autotrophs due to the lack of light (Anschütz and Flynn 2020). Dumping and dredging operations release nutrients buried in the seafloor and bring up fine sediments in suspension increasing light limitation. This could create more favourable conditions for mixoplankton species to thrive in at the expenses of pure autotrophic species (Flynn and Hansen 2013).
- **Non-indigenous species** which set ecosystems at risk of biodiversity loss and HAB formation (Hallegraeff and Gollasch 2006). Due to dual metabolism and flexible physiology, allochthonous mixoplankton can outcompete strict auto- and heterotrophs in some

instances. The spread of toxic *Gymnodium catenatum* (Hallegraeff and Gollasch 2006) and of “green *Noctiluca*” (Harrison et al. 2011) are examples.



Figure 4 Schematic of the effects of anthropogenic activities upon marine ecology and mixoplankton and their subsequent effects on human health and economy. Black = anthropogenic pressures, orange = bio/ecological effects, red/green = eco-human health and socioeconomic impacts. See also Fig. 5 for further description of linkages to mixoplankton.

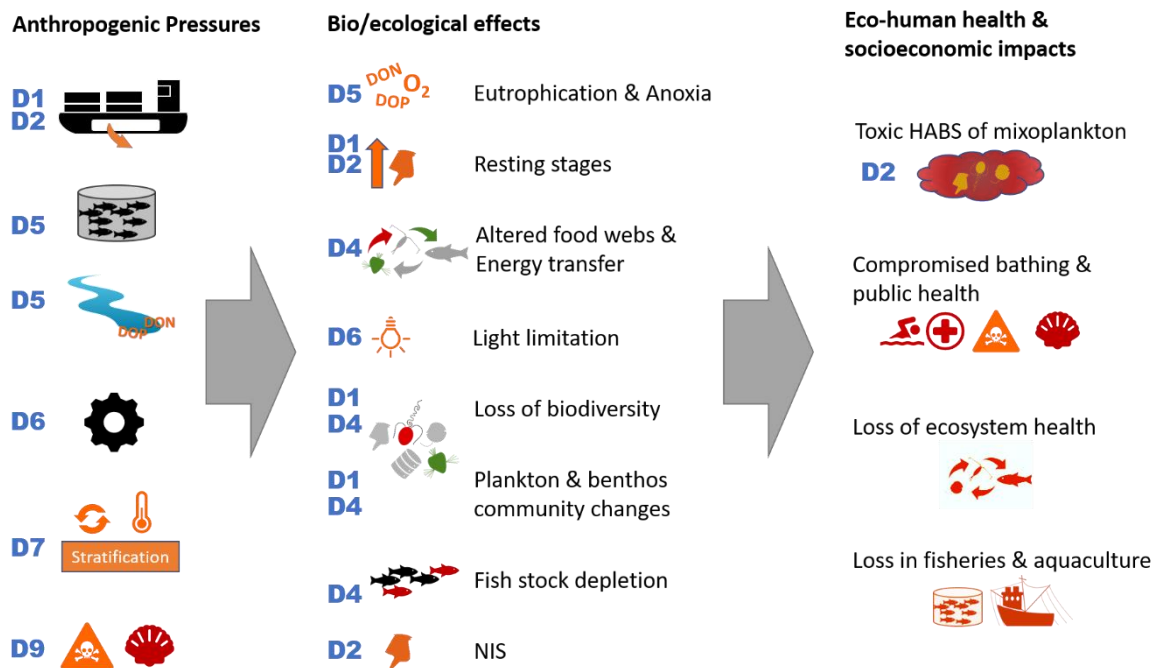


Figure 5 Linkage between anthropogenic pressures and mixoplankton, related bio-ecological effects and eventually eco-human health and socioeconomic impacts. The combination of the letter “D” and a number refers to the MSFD descriptor that applies to the respective point (see Fig. 6b). Icons match those in the schematic shown in Fig. 4; icons in black symbolise pressures, icons in orange the effects and icons in red and green possible impacts. The chart does not feature all possible relations of mixoplankton and their environment, only those considered most relevant for achieving Good Environmental Status. NIS - non-indigenous species.

The need to increase resolution in plankton monitoring

Currently, unicellular plankton communities are monitored by a variety of technical approaches including microscopy, high-performance liquid chromatography, Chl_a fluorescence, flow cytometry and molecular analyses for diversity and abundance on different scales and more recently the use of digestive vacuole staining as a sign of ingestion. None of these techniques can, however, discriminate between a non-constitutive mixoplankton that has just digested its prey from a pure heterotroph. Likewise, these methods do not allow to distinguish a constitutive mixoplankton (which has its own plastids) from a pure autotrophic organism. In fact, despite being good proxies to quantify the amount of fixed C at a specific location by the overall community, these tools do not inform us in detail on the plankton food web structure and energy flows.

Since there are no universal traits for the unequivocal distinction of mixoplankton, the latter ultimately end up being merged between the categories of phytoplankton (if containing Chl_a) or protozooplankton (if bearing digestive vacuoles). However, vacuole staining does not prove to be always efficient because a) if plastids or other prey components have been digested already, there is no remnant of previous feeding history; b) if the species does not feed the prey in its entirety (e.g., tube feeders), it is more difficult to detect intact confined structures; c) acidotropic staining can unselectively bind to acidic organelles in the cytoplasm other than food vacuoles, biasing our interpretation (Hansen 2011). Additionally, the use of fluorescently labelled algae (FLA) are known to suit in grazing experiments both in mono-diet and in mixed food suspension as a means to distinguish cells from one another (Martínez et al. 2014). Yet, the efficacy of fluorescent vital stain retention varies across prey and predator species and over time, pinpointing the importance of considering these aspects for the development of experimental protocols of mixotrophic grazing. Attempts to experimentally “isolate” mixoplanktonic grazing from that of pure heterotrophs have been made with the use of rotenone, but results seem to vary depending on the species and its growth phase, rendering the technique not reliable in all conditions (Ferreira and Calbet 2020).

Perhaps, the most accurate and controlled technical procedure for the estimation of nutrient flow and origin is the use of stable isotopes. Both carbon and nitrogen can be traced by tagging the nutrient source with an alternative isotopic form and infer whether mixoplankton acquired the essential nutrients from the prey or produced them via the photosynthetic pathway (Michener and Kaufman 2007). Yet, it has been seldom applied systematically because it requires *in vivo* measurement in comparison to Chl_a fluorescence which can be also inferred remotely via satellite data. Apart from technical challenges, in plankton monitoring there are still gaps when accounting

for relative contribution of plankton groups. In fact, most of the emphasis in the above-mentioned research is on dinoflagellates. There are other important mixoplankton taxa, including the similarly sized Raphidophytes (relatively large species and so easily monitored) but also the smaller mixotrophic flagellates such as *Prymnesium spp.* and *Chrysochromulina spp.*, which are common but often ignored until they cause a bloom event (Jones et al. 1993; Edvardsen et al. 1998). In addition, even if not toxic, high concentrations of the kleptoplastidic ciliate *Mesodinium rubrum* are the precursor to the proliferation of its predator, the toxic *Dinophysis spp.*, which has species-specific dietary and plastidial requirements (Reguera et al. 2012). Therefore, accurate annotation of these trophic linkages may help in forecast undesired HAB events.

Microscope counting and identification to the species level could at least identify the abundance of mixoplankton. There is a need for a comprehensive list of species that now, and in the future, are recognized as mixoplankton and preferably, also the type of mixoplankton (CM or NCM). Still, especially small flagellate species, microscopic identification may be difficult and will not be routinely done. Even though mixoplankton are ubiquitous in all marine ecosystems (Leles et al. 2017, 2019), they do not always form abundances that make them easy or appear important to sample (Burkholder et al. 2008). Neither is their physiology easy to measure (Anderson et al. 2017).

In conclusion, improving monitoring studies is difficult. The cost of improving monitoring techniques for general or specific mixoplankton species will therefore likely be balanced by the local need and potential risks of mixoplankton interference with good ecological status of marine waters.

Implication of the new knowledge about mixoplankton on specific aspects of the MSFD

The different stages of the MSFD are reviewed every six years. In the last review, plankton were recognized as important and are now increasingly integrated into directives (ICES 2015; OSPAR Commission 2017a). But very little consideration was given to mixoplankton. Our understanding of mixoplankton has developed greatly since the MSFD came into effect in 2008 (Marine Strategy Framework Directive 2008). Based on that, conceptual and methodological approaches to plankton ecology warrant some reconsiderations.

The inclusion of mixoplankton interactions in simulation models could eventually integrate the key acquired knowledge and may serve as a tool to help in forecasting future changes in the main ecosystem services related to plankton (harvestable fish biomass, climate regulation and water quality; see “Impact of mixoplankton on ecosystem services”). However, before that becomes possible, there is a need for more quantitative data on mixoplanktonic protists, but also qualitatively

different data to better characterize mixoplankton types (Mitra et al. 2016) and enhance models for prediction and their validation. This would be achieved through a targeted coordination of basic research and monitoring programs. This means that at the least monitoring programmes categorise plankton into functional types that explicitly include mixoplankton.

In the MSFD, eleven descriptors (Fig. 6b) define the criteria to assess the current status of the marine environment giving a robust and standardized qualitative description (European Environment Agency 2014). However, the methodological approach used to monitor such features does not always consider mixoplanktonic activity. In Table 2, we list the criteria in which the new knowledge about mixoplankton is to be integrated especially highlighting the methodological implication these would have on monitoring. (Morán et al. 2010; Guinder and Molinero 2013; Wilken et al. 2013; Boscolo-Galazzo et al. 2018)

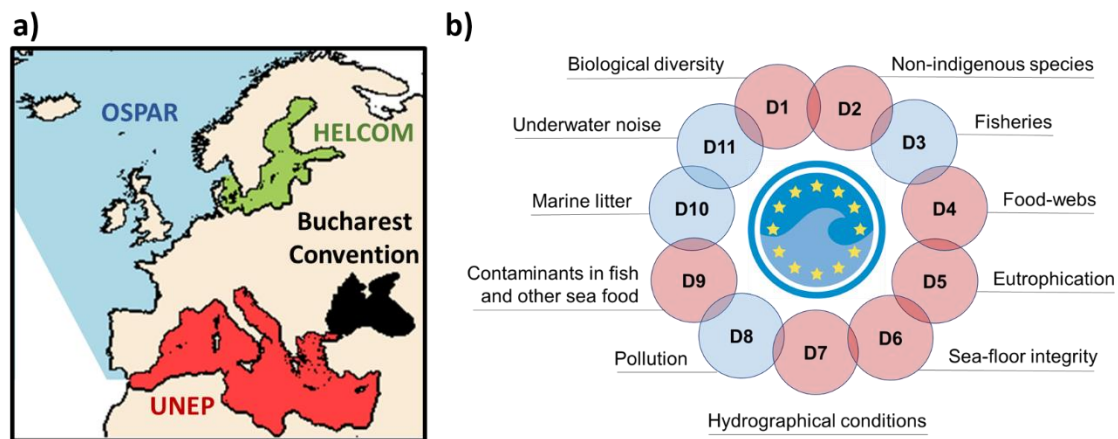


Figure 6 a) European sea regions identified within the Regional Sea Conventions (RSC), with four different commissions responsible for the implementation of the MSFD: Blue = OSPAR, Green = HELCOM, Red = UNEP (Mediterranean Sea), Black = Bucharest Convention. b) Schematic representation of descriptors used within the MSFD structure for the achievement of “good environmental status” (GES) by 2020. The descriptors highlighted in red are expected to interface with the mixoplankton paradigm and their implementation on a national level might thus warrant revision.

Table 2 MSFD descriptors that may benefit from revisions in light of the mixoplankton paradigm, the criteria that define these descriptors and the indicators currently in use to monitor them. The table also shows new relevant knowledge on mixoplankton, how it affects current monitoring and how it could be adapted.

Descriptors (D)	Criteria (and sub-criteria)	New knowledge impact	Implication on monitoring	Proposed adaptation
1 Biodiversity	6. Habitat condition (1) Condition of the typical species and communities (2) Relative abundance and/or biomass (3) Physical, hydrological and chemical conditions	Some pelagic habitats favour mixoplankton	Sampling method and frequency	-Collection of entire water samples -Assessment of molecular keys for identification -Definition of the sampling strategy considering changes in environmental conditions
	7. (1) Ecosystem structure	New knowledge on species feeding mode is important for interpretation	Identification of species and trophic mode	Identification of the ecological role of low and intermediate trophic groups
2 NIS	1.(1) Trends in abundance of NIS 2. Environmental impact of NIS 2.(1) Ratio between invasive NIS to native species	Some mixoplankton are NIS		Implementation of keys for taxonomical identification with putative trophic mode indication
	4 Food webs	3.(1) Abundance trends of functionally important selected groups/species		Mixoplankton change the energy transfer efficiency between trophic levels
5 Eutrophication	2. Direct effects of nutrient enrichment (1) Chlorophyll concentration (2) Water transparency	Enhanced chlorophyll-a and water discolouration may be due to mixoplankton	Estimation of mixoplankton biomass and primary production	In vivo measurement of photosynthetic activity at local scale
	(4) Shift in floristic species composition	New knowledge on species feeding mode is important for interpretation		
6 Sea-floor integrity	6.1.2 Extent of seabed affected	Resuspension of mixoplankton cysts; sedimented nutrients; increased turbidity may favour mixoplankton	Measurement of turbidity; Offshore dumping	Sampling for mixoplanktonic cysts, nutrient resuspension and turbidity
7 Hydrographical conditions	2. (2) Changes in habitats, in particular, the functions provided by it	Some pelagic habitats favour mixoplankton	Impact evaluation	Integration of mixoplankton into predictive models
9 Contaminants in fish and other seafood	1. Levels, number and frequency of contaminants (1) Levels of contaminants in seafood (2) Frequency of levels exceeded in seafood	Causative species for Shellfish poisoning is often mixoplankton	Identification of early indicators for the occurrence of HABs	Relate toxicity to biotic and abiotic factors (prey, nutrients)

Mixoplankton can alter the energy transfer efficiency between trophic levels, especially in mature ecosystem conditions. Mixoplankton and their relations should therefore be explicitly considered in food web analysis (D4).

Fragile/cryptic mixoplankton (most notably bacterivorous nanoflagellates and NCM ciliates) are currently under-sampled. Thus, there is a need to increase the resolution in plankton monitoring. Collection of entire water samples (rather than net samples that damage fragile organisms) and the employment of molecular tools for identification may help to overcome this challenge. At the same time, keys for taxonomical identification could be implemented with putative trophic mode indication, which should be indicated together with presence/absence of the species (enhancing resolution on biodiversity assessment, D1).

On a regional scale, some good examples of indicators that enable a more thorough assessment of the planktonic community already exists, such as the ratio between the abundance of diatoms (pure phototrophs) and dinoflagellates (phototrophic forms being mixoplankton) adopted by EU commissions HELCOM and OSPAR (Fig. 6 a) as an indicator of GES.

For an understanding of nutrient inputs, direct eutrophication effects and indirect effects, it is important to understand the flow of energy and matter through plankton food webs. This requires knowledge of the contribution of mixoplankton to plankton production and consumption, so better measures to reduce eutrophication effects (D5) can be formulated.

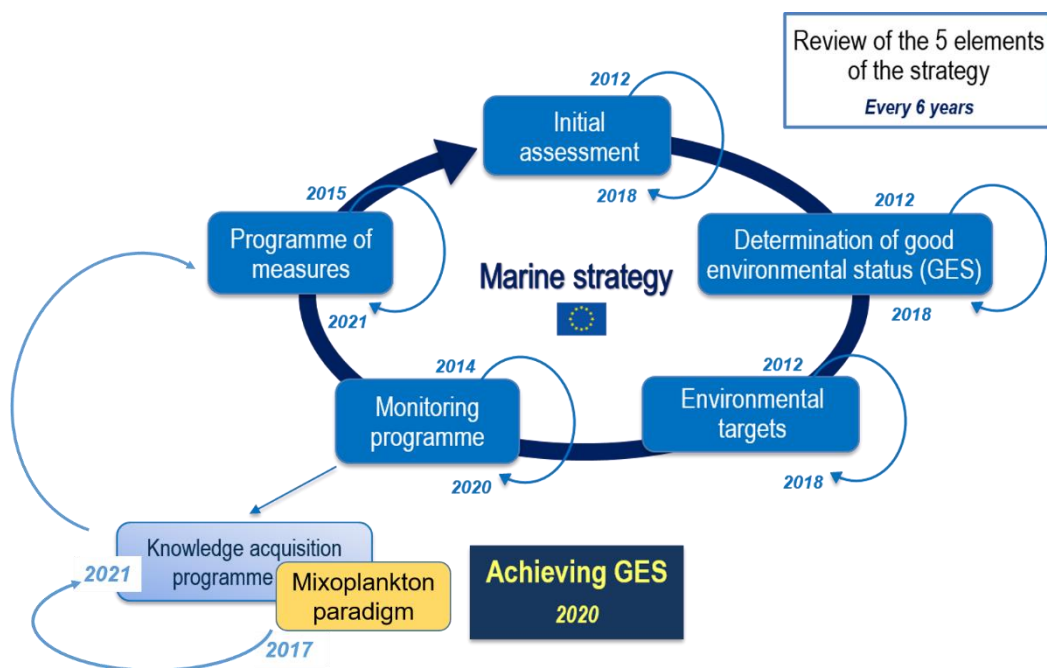
The increase in resolution on plankton monitoring towards the above-mentioned directions would be accompanied with the implementation of information about conditions that favour mixotrophy in pelagic habitats. This will enhance the evaluation of the impact of anthropogenic activities on the ecosystem (D7.2) and the identification of early indicators for occurrence of HABs (D9). Mixoplankton are, indeed, potentially favoured in conditions of stratification of the water column and their toxicity may be related to biotic and abiotic factors (prey, dissolved nutrients).

A more accurate description of the planktonic community assemblage would furnish the bases to build historical data on which to assess the likelihood of successful introduction of mixoplankton species by human activities into regions of species that are not indigenous (NIS, D2) which is supposed to be enhanced by their physiological plasticity. At the present, no protist species nor plankton are considered in the list of Invasive Species of Union Concern held by the EC related directive (European Union 2017). On the other hand, it is already recognized that the introduction of NIS can have a role in HAB events (Katsanevakis et al. 2014).

Proposed roadmap for including the mixoplankton paradigm within regional implementation plans under the MSFD

Above, we suggested integration of new knowledge on mixoplankton in descriptors 1, 2, 4, 5, 6, 7 and 9 of the MSFD (Figures. 6b, 5) The forms of change required to achieve this aim are methodical (i.e. sampling), analytical (species identification), but most importantly conceptual (and thence also model-based): mixoplankton need to be acknowledged as important plankton functional types within our understanding of marine ecosystems. The distinctive features of mixoplankton need to be considered in the initial assessment (Fig. 7a). It then follows that the characterisation of Good Environmental Status must include features affecting and developing in consequence of the activity of mixoplankton populations. Such appraisals will give rise to environmental targets and a monitoring programme of the marine environment that can better reflect the growth of organisms that we formally viewed as being phototrophs and now appreciate are mixoplankton.

The proposed changes need to be implemented on a regional level targeting those waters where mixoplankton contribute significantly to human pressures-related impacts. For example, water bodies that are troubled by recurring HABs caused by mixoplankton will more likely benefit from alterations in their programmes while other regions may have no demand for it. The challenge will be to identify simple improvements that make better use of the mixoplankton paradigm theories. This paper identifies the descriptors and criteria which should be considered to integrate this newly developed scientific knowledge (Fig. 7b).



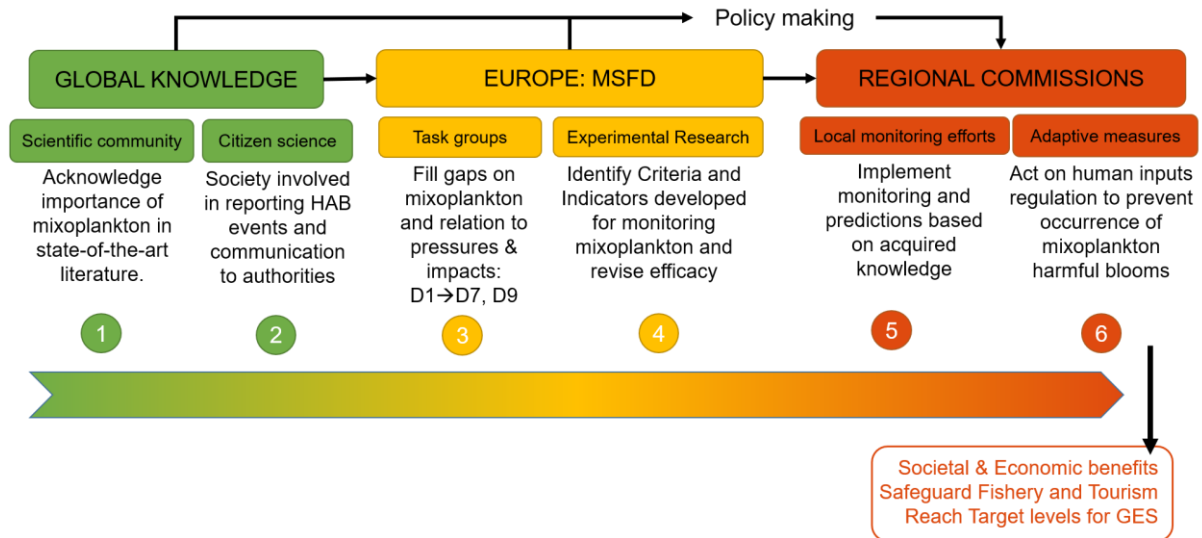


Figure 7. Roadmap highlighting the main steps towards the integration of mixoplankton paradigm (Fig.1) into mainstream ecological research within the MSFD to the overall aim of predicting and preventing nuisance and harmful mixoplanktonic blooms. Policymaking is crucial to allow the application of knowledge to local monitoring actions through regional implementation plans established according to local needs. Steps 4, 5 and 6 are those requiring significant monetary investments. The link between the two panels is to be found at the level of “Knowledge acquisition programme” within the 5-year “monitoring programme”. For monitoring strategies and policymaking purposes, refer to Table 2.

Discussion and conclusions

All management policies come under a periodic review when there is scope to introduce new approaches. Thresholds of quantifiable biological indicators of GES for European waters were set to be achieved by 2020 by process targeted over a decade earlier (MSFD 2008). There is considerable inertia in all management programmes but we argue that the mixoplankton paradigm (Flynn et al. 2019) represents such a fundamental change in our understanding of the ecophysiology and trophic dynamics of the plankton that are so responsible for characterising GES, that explicit inclusion of “mixoplankton” should occur in relevant elements of these processes as soon as possible. The Descriptors discussed above, using as an example the EU MSFD, contain features that pertain to mixoplankton and that may be weakened significantly by failing to explicitly include mixoplankton.

We make this argument because as we are confronted with climate change, we need to be more confident that we understand the functioning of a “normal” ecosystem, and that includes the role of mixoplankton. Unlike classical phytoplankton, mixoplankton can exploit other resources and bloom in unexpected scenarios. This requires a more holistic understanding of anthropogenic pressures that influence mixoplankton, and of the effects that mixoplankton have on the

environment. This may in relevant cases mean that monitoring plans would have to ensure a higher resolution to preserve ecosystem services and ensure GES in a timely fashion (Fig. 7).

We propose a series of actions that may lead to better assessment of environmental state (Tables 2). The most important of these is the need to improve the detection, classification of trophic mode and enumeration of a wider range of plankton species with a higher temporal resolution. This will require research efforts that will likely lead to the deployment of robust autonomous methodologies. Along with the usual monitoring of inorganic nutrients (ammonium, nitrate, nitrite, silicate, phosphate), particulate organic nitrogen should be included as a key indicator of trophic regime and possible nutritional base for mixotrophic protists. Integration of the increasing knowledge on mixoplankton in predictive models provides a better base for understanding complex causal relationships, leading to better programs of measures. However, many details of mixoplankton ecology are still unknown and basic research is required to supplement the century-long effort expended on studying what we can now see was only half of the ecophysiology of these planktonic protists.

Economic investments aimed at increasing our knowledge are paramount in order to gain technical expertise on mixoplankton ecophysiology, identify the environmental triggers or threshold of these for their episodic harmful blooms and enhance the predictive capabilities of models. Monetary and research efforts coupled with coordinated policymaking have the potential to safeguard future economic losses resulting from inaccurate predictions. Society could indeed benefit from such an investment if the reason behind specific measures and the impact that undesired mixoplankton blooms may have on the commercial, public health, tourism and societal sectors is acknowledged. Monitoring programmes would benefit from adapting to the newly proposed measures where the local needs require and providing results most coherently for comparative purposes Europe wide.

Mixoplanktonic events, and allied changes to ecosystem services, are not solely linked to the stimulation of purely phototrophic (plant-like, microalgal) activity. Rather, they depend on a host of factors that we currently poorly understand in a holistic fashion required for ecosystem management. Set against the backdrop of climate change, and indeed in changes in short term weather patterns, such enhancements to marine plankton monitoring and management cannot really come too soon.

Acknowledgements

References

- Abad D, Albaina A, Aguirre M, Laza-Martínez A, Uriarte I, Iriarte A, Villate F, Estonba A (2016) Is metabarcoding suitable for estuarine plankton monitoring? A comparative study with microscopy. *Mar Biol* 163:149. doi: 10.1007/s00227-016-2920-0
- Adolf JE, Bachvaroff TR, Place AR (2009) Environmental modulation of karlotoxin levels in strains of the cosmopolitan dinoflagellate, *Karlodinium veneficum* (Dinophyceae). *J Phycol* 45:176–192. doi: 10.1111/j.1529-8817.2008.00641.x
- Anderson DM (2009) Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean Coast Manag* 52:342–347. doi: 10.1016/j.ocecoaman.2009.04.006
- Anderson DM, Andersen P, Bricelj VM, Cullen JJ, Rensel JE (2001) Monitoring and management strategies for harmful algal blooms in coastal waters.
- Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, compositions, and consequences. *Estuaries* 25:704–726. doi: 10.1016/j.hal.2008.08.017
- Anderson DM, Burkholder JM, Cochlan WP, Glibert PM, Gobler CJ, Heil CA, Kudela RM, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. *Harmful Algae* 8:39–53. doi: 10.1016/j.hal.2008.08.017
- Anderson DM, Cembella A, Hallegraeff GM (2012) Progress in understanding harmful algal blooms (HABs): Paradigm shifts and new technologies for research, monitoring and management. *Ann Rev Mar Sci* 4:143–176. doi: 10.1146/annurev-marine-120308-081121
- Anderson R, Jürgens K, Hansen PJ (2017) Mixotrophic phytoflagellate bacterivory field measurements strongly biased by standard approaches: A case study. *Front Microbiol* 8:1–12. doi: 10.3389/fmicb.2017.01398
- Anschütz AA, Flynn KJ (2020) Niche separation between different functional types of mixoplankton: results from NPZ - style N - based model simulations. *Mar Biol* 167:1–21. doi: 10.1007/s00227-019-3612-3
- Asa J, Andonegi E, Can K, Danovaro R, Elliott M, Franco A, Garces E, Little S, Mazik K, Moncheva S,

- Papadopoulou N, Patrício J, Queir AM, Smith C, Stefanova K, Solaun O (2015) Marine biodiversity and ecosystem function relationships: The potential for practical monitoring applications. *Estuar Coast Shelf Sci* 161:46–64. doi: 10.1016/j.ecss.2015.04.008
- Balkis N, Balci M, Giannakourou A, Venetsanopoulou A, Mudie P (2016) Dinoflagellate resting cysts in recent marine sediments from the Gulf of Gemlik (Marmara Sea, Turkey) and seasonal harmful algal blooms. *Phycologia* 55:187–209. doi: 10.2216/15-93.1
- Baltic Marine Environment Protection Commission (2016) Pre-core indicator ‘Diatom-Dinoflagellate index’ – proposal to shift status to core indicator. In: Working Group on the State of the Environment and Nature Conservation (ed) Agenda Item: 4J6 – HELCOM indicators and assessments. Tallinn,
- Batten SD, Abu-Alhaija R, Chiba S, Edwards M, Graham G, Jyothibabu R, Kitchener JA, Koubbi P, McQuatters-Gollop A, Muxagata E, Ostle C, Richardson AJ, Robinson K V., Takahashi KT, Verheye HM, Wilson W (2019) A global Plankton diversity monitoring program. *Front Mar Sci* 6:1–14. doi: 10.3389/fmars.2019.00321
- Beaugrand G (2005) Monitoring pelagic ecosystems using plankton indicators. *ICES J Mar Sci* 62:333–338. doi: 10.1016/j.icesjms.2005.01.002
- Bedford J, Johns D, Greenstreet S, McQuatters-Gollop A (2018) Plankton as prevailing conditions: A surveillance role for plankton indicators within the Marine Strategy Framework Directive. *Mar Policy* 89:109–115. doi: 10.1016/j.marpol.2017.12.021
- Berge T, Poulsen LK, Moldrup M, Daugbjerg N, Juel Hansen P (2012) Marine microalgae attack and feed on metazoans. *ISME J*. doi: 10.1038/ismej.2012.29
- Blauw AN, Anderson P, Estrada M, Johansen M, Laanemets J, Peperzak L, Purdie D, Raine R, Vantera E (2006) The use of fuzzy logic for data analysis and modelling of European harmful algal blooms: Results of the HABES project. *African J Mar Sci* 28:365–369. doi: 10.2989/18142320609504179
- Boscolo-Galazzo F, Crichton KA, Barker S, Pearson PN (2018) Temperature dependency of metabolic rates in the upper ocean : A positive feedback to global climate change ? *Glob Planet Change* 170:201–212. doi: 10.1016/j.gloplacha.2018.08.017
- Bresnan E, Cook KB, Hughes SL, Hay SJ, Smith K, Walsham P, Webster L (2015) Seasonality of the plankton community at an east and west coast monitoring site in Scottish waters. *J Sea Res*

105:16–29. doi: 10.1016/j.seares.2015.06.009

Bueno M, Alberto SF, de Carvalho R, Costa TM, Ciotti ÁM, Christofolletti RA (2017) Plankton in waters adjacent to the Laje de Santos state marine conservation park, Brazil: Spatio-temporal distribution surveys. *Brazilian J Oceanogr* 65:564–575. doi: 10.1590/s1679-87592017129006504

Burkholder JAM, Glibert PM, Skelton HM (2008) Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8:77–93. doi: 10.1016/j.hal.2008.08.010

Calbet A (2008) The trophic roles of microzooplankton in marine systems. *ICES J Mar Sci* 65:325–331. doi: 10.1093/icesjms/fsn013

Calbet A, Martínez RA, Isari S, Zervoudaki S, Nejstgaard JC, Pitta P, Sazhin AF, Sousoni D, Gomes A, Berger SA, Tsagaraki TM, Ptacnik R (2012) Effects of light availability on mixotrophy and microzooplankton grazing in an oligotrophic plankton food web: Evidences from a mesocosm study in Eastern Mediterranean waters. *J Exp Mar Bio Ecol* 424–425:66–77. doi: 10.1016/j.jembe.2012.05.005

Caron DA (2016) Mixotrophy stirs up our understanding of marine food webs. *PNAS* 113:2806–2808. doi: 10.1073/pnas.1600718113

Caroppo C, Buttino I, Camatti E, Caruso G, De Angelis R, Facca C, Giovanardi F, Lazzara L, Mangoni O, MaGaletti E (2013) State of the art and perspectives on the use of planktonic communities as indicators of environmental status in relation to the EU Marine Strategy Framework Directive. *Biol Mar Mediterr* 20:65–73.

Caruso G, Azzaro M, Caroppo C, Decembrini F, Monticelli LS, Leonardi M, Maimone G, Zacccone R, La Ferla R (2016) Microbial community and its potential as descriptor of environmental status. *ICES J Mar Sci* 73:2174–2177. doi: 10.1093/icesjms/fsw101

Costanza R, D’Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O’Neill R V., Paruelo J, Raskin RG, Sutton P, van den Belt M (1998) The value of the world’s ecosystem services and natural capital. *Nature* 387:253–260. doi: 10.1038/387253a0

Council of the European Union Parliament (2008) Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Off J Eur Union* 164:19–40. doi: 10.1016/j.biocon.2008.10.006

- Davidson K, Gowen RJ, Harrison PJ, Fleming LE, Hoagland P, Moschonas G (2014) Anthropogenic nutrients and harmful algae in coastal waters. *J Environ Manage* 146:206–216. doi: 10.1016/j.jenvman.2014.07.002
- Edvardsen B, Paasche E, Edvardsen B, Paasche E (1998) Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*. Springer-Verlag, Heidelberg
- Erdner DL, Dyble J, Parsons ML, Stevens RC, Hubbard KA, Wrabel ML, Moore SK, Lefebvre KA, Anderson DM, Bienfang P, Bidigare RR, Parker MS, Moeller P, Brand LE, Trainer VL (2008) Centers for Oceans and Human Health: A unified approach to the challenge of harmful algal blooms. *Environ Heal A Glob Access Sci Source* 7:1–17. doi: 10.1186/1476-069X-7-S2-S2
- European Commission (2019) Legislation: the Marine Strategy Framework Directive. In: EU Coast. Mar. Policy. <https://ec.europa.eu/environment/marine/eu-coast-and-marine-policy/marine-strategy-framework-directive/>. Accessed 14 Nov 2019
- Ferreira GD, Calbet A (2020) Caveats on the use of rotenone to estimate mixotrophic grazing in the oceans. *Sci Rep* 10:1–11. doi: 10.1038/s41598-020-60764-2
- Flynn KJ, Butler I (1986) Nitrogen sources for the growth of marine microalgae : role of dissolved free amino acids. *Mar Ecol Prog Ser* 34:281–304. doi: 10.3354/meps034281
- Flynn KJ, Hansen PJ (2013) Cutting the canopy to defeat the “Selfish Gene”; Conflicting selection pressures for the integration of phototrophy in mixotrophic protists. *Protist* 164:1–13. doi: 10.1016/j.protis.2013.09.002
- Flynn KJ, Stoecker DK, Mitra A, Raven JA, Glibert PM, Hansen PJ, Granéli E, Burkholder JM (2013) Misuse of the phytoplankton-zooplankton dichotomy: The need to assign organisms as mixotrophs within plankton functional types. *J Plankton Res* 35:3–11. doi: 10.1093/plankt/fbs062
- Flynn KJ, McGillicuddy DJ, McGillicuddy Jr. DJ (2018) Modeling Marine Harmful Algal Blooms : Current Status and Future Prospects. Wiley Online Library
- Flynn KJ, Mitra A, Anestis K, Anschütz AA, Calbet A, Duarte Ferreira G, Gypens N, Hansen P, John U, Martin JL, Mansour JS, Maselli M, Medić N, Norlin A, Not F, Pitta P, Romano F, Saiz E, Schneider LK, Stolte W, Traboni C (2019) Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? *J Plankton Res* 00:in press. doi: 10.1093/plankt/fbz026
- Gao H, An X, Liu L, Zhang KK, Zheng D, Tong M (2017) Characterization of *Dinophysis acuminata* from

- the Yellow Sea, China, and its response to different temperatures and *Mesodinium* prey. *Oceanol Hydrobiol Stud* 46:439–450. doi: 10.1515/ohs-2017-0043
- Ghyoot C, Lancelot C, Flynn KJ, Mitra A, Gypens N (2017) Introducing mixotrophy into a biogeochemical model describing an eutrophied coastal ecosystem: The Southern North Sea. *Prog Oceanogr* 157:1–11. doi: 10.1016/j.pocean.2017.08.002
- Glibert PM, Burkholder JAM (2011) Harmful algal blooms and eutrophication: “strategies” for nutrient uptake and growth outside the Redfield comfort zone. *Chinese J Oceanol Limnol* 29:724–738. doi: 10.1007/s00343-011-0502-z
- Glibert PM, Seitzinger S, Heil CA, Burkholder JM, Parrow MW, Codispoti LA, Kelly V (2005) The role of eutrophication in the global proliferation of harmful algal blooms. *Oceanography* 18:198–209. doi: 10.5670/oceanog.2005.54
- Gobler CJ, Berry DL, Dyhrman ST, Wilhelm SW, Salamov A, Lobanov A V., Zhang Y, Collier JL, Wurch LL, Kustka AB, Dill BD, Shah M, VerBerkmoes NC, Kuo A, Terry A, Pangilinan J, Lindquist EA, Lucas S, Paulsen IT, Hattenrath-Lehmann TK, Talmage SC, Walker EA, Koch F, Burson AM, Marcoval MA, Tang YZ, LeClerc GR, Coyne KJ, Berg GM, Bertrand EM, Saito MA, Gladyshev VN, Grigoriev I V. (2011) Niche of harmful alga *Aureococcus anophagefferens* revealed through ecogenomics. *Proc Natl Acad Sci U S A* 108:4352–4357. doi: 10.1073/pnas.1016106108
- Gomes H do R, McKee K, Mile A, Thandapu S, Al-Hashmi K, Jiang X, Goes JI (2018) Influence of light availability and prey type on the growth and photo-physiological rates of the mixotroph *Noctiluca scintillans*. *Front Mar Sci* 5:1–12. doi: 10.3389/fmars.2018.00374
- Gowen RJ, Mcquatters-gollop A, Tett P, Best M, Bresnan E, Castellani C, Cook K, Forster RM, Scherer C, Mckinney A (2011) The Development of UK Pelagic (Plankton) Indicators and Targets for the MSFD. Belfast
- Granéli E, Flynn KJ (2006) Chemical And Physical Factors Influencing Toxin Production. In: Granéli E, Turner JT (eds) *Ecology of Harmful Algae, Ecological*. Springer-Verlag, Berlin, pp 229–241
- Gren IM, Elofsson K, Jannke P (1997) Cost-effective nutrient reductions to the Baltic Sea. *Environ Resour Econ* 10:341–362. doi: 10.1023/A:1026497515871
- Guinder VA, Molinero JC (2013) Climate Change Effects on Marine Phytoplankton Climate Change Effects on Marine Phytoplankton. doi: 10.1201/b16334-4
- Gustafson DE, Stoecker DK, Johnson MD, Van Heukelem WF, Sneider K (2000) Cryptophyte algae are

- robbed of their organelles by the marine ciliate *Mesodinium rubrum*. *Nature* 405:1049–1052. doi: 10.1177/0956247816647344
- Hallegraeff GM, Gollasch S (2006) Anthropogenic introduction of microalgae. In: Granéli E, Turner J (eds) *Ecology of Harmful Algae*. Springer-Verlag, Berlin, pp 279–390
- Hammer AC, Pitchford JW (2005) The role of mixotrophy in plankton bloom dynamics, and the consequences for productivity. *ICES J Mar Sci* 62:833–840. doi: 10.1016/j.icesjms.2005.03.001
- Hansen B, Bjornsen PK, Hansen PJ (1994) The size ratio between planktonic predators and their prey. *Limnol Oceanogr* 39:395–403. doi: 10.4319/lo.1994.39.2.0395
- Hansen PJ (2011) The Role of Photosynthesis and Food Uptake for the Growth of Marine Mixotrophic Dinoflagellates. *J Eukaryot Microbiol* 58:203–214. doi: 10.1111/j.1550-7408.2011.00537.x
- Hansen PJ, Nielsen LT, Johnson M, Berge T, Flynn KJ (2013) Acquired phototrophy in *Mesodinium* and *Dinophysis* - A review of cellular organization, prey selectivity, nutrient uptake and bioenergetics. *Harmful Algae* 28:126–139. doi: 10.1016/j.hal.2013.06.004
- Hansen PJ, Ojamäe K, Berge T, Trampe EC, Nielsen LT, Lips I, Kühl M (2016) Photoregulation in a kleptochloroplastidic dinoflagellate, *Dinophysis acuta*. *Front Microbiol*. doi: 10.3389/fmicb.2016.00785
- Harrison PJ, Furuya K, Glibert PM, Xu J, Liu HB, Yin K, Lee JHW, Anderson DM, Gowen R, Al-Azri AR, Ho AYT (2011) Geographical distribution of red and green *Noctiluca scintillans*. *Chinese J Oceanol Limnol* 29:807–831. doi: 10.1007/s00343-011-0510-z
- Hattenrath-Lehmann TK, Marcoval MA, Middlesdorf H, Golecki JA, Wang Z, Haynes B, Morton SL, Gobler CJ (2015) Nitrogenous nutrients promote the growth and toxicity of *Dinophysis acuminata* during estuarine bloom events. *PLoS One* 10:e0124148. doi: 10.1371/journal.pone.0124148
- Harmful Algae Event Database (2020) - <http://haedat.iode.org/eventSearch.php?searchtext%5BregionID%5D=4>
- Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. *Trends Ecol Evol* 20:337–344. doi: 10.1016/j.tree.2005.03.004
- HELCOM (2018a), Seasonal succession of dominating phytoplankton groups -

<http://www.helcom.fi/baltic-sea-trends/indicators/seasonal-succession-of-dominating-phytoplankton-groups>

HELCOM (2018b), Diatom-dinoflagellate index - <http://www.helcom.fi/baltic-sea-trends/indicators/Diatom-dinoflagellate-index>

Hernández-Urcera J, Rial P, García-Portela M, Lourés P, Kilcoyne J, Rodríguez F, Fernández-Villamarín A, Reguera B (2018) Notes on the Cultivation of Two Mixotrophic *Dinophysis* Species and Their Ciliate Prey *Mesodinium rubrum*. *Toxins* (Basel) 10:505. doi: 10.3390/toxins10120505

Hoagland P, Scatasta S (2006) The Economic Effects of Harmful Algal Blooms. *Ecol Stud Vol 189* 189:391–402. doi: 10.1007/BF02804908

ICES (2015) EU request to ICES on clarification of the advice on MSFD reviews of descriptors D3, 4, 6, and 11. ICES Tech Serv Northeast Atl Ecoregion 1–31.

Jaschinski S, Flöder S, Petenati T, Göbel J (2015) Effects of nitrogen concentration on the taxonomic and functional structure of phytoplankton communities in the Western Baltic Sea and implications for the European water framework directive. *Hydrobiologia* 745:201–210. doi: 10.1007/s10750-014-2109-9

Jeong HJ, du Yoo Y, Kim JS, Seong KA, Kang NS, Kim TH (2010) Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. *Ocean Sci J* 45:65–91. doi: 10.1007/s12601-010-0007-2

John EH, Flynn KJ (2000) Growth dynamics and toxicity of *Alexandrium fundyense* (dinophyceae): The effect of changing n:p supply ratios on internal toxin and nutrient levels. *Eur J Phycol* 35:11–23. doi: 10.1080/09670260010001735581

Jones HLJ, Leadbeater BSC, Green JC (1993) Mixotrophy in marine species of *Chrysochromulina* (Prymnesiophyceae): Ingestion and digestion of a small green flagellate. *J mar biol Ass U* 73:283–296. doi: 10.1017/S0025315400032859

Katechakis A, Haseneder T, Kling R, Stibor H (2005) Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light : Nutrient hypothesis might not hold for mixotrophs. *Limnol Oceanogr* 50:1290–1299. doi: 10.4319/lo.2005.50.4.1290

Klais R, Tamminen T, Kremp A, Spilling K, Olli K (2011) Decadal-scale changes of Dinoflagellates and Diatoms in the Anomalous Baltic Sea spring bloom. *PLoS One*. doi: 10.1371/journal.pone.0021567

- Lancelot C, Thieu V, Polard A, Garnier J, Billen G, Hecq W, Gypens N (2011) Cost assessment and ecological effectiveness of nutrient reduction options for mitigating *Phaeocystis* colony blooms in the Southern North Sea: An integrated modeling approach. *Sci Total Environ* 409:2179–2191. doi: 10.1016/j.scitotenv.2011.02.023
- Lehtinen S, Suikkanen S, Hällfors H, Kauppila P, Lehtiniemi M, Tuimala J, Uusitalo L, Kuosa H (2016) Approach for supporting food web assessments with multi-decadal phytoplankton community analyses-case baltic sea. *Front Mar Sci* 3:1–14. doi: 10.3389/fmars.2016.00220
- Lei Y, Whyte C, Davidson K, Tett P, Yin K (2018) A change in phytoplankton community index with water quality improvement in Tolo Harbour, Hong Kong. *Mar Pollut Bull* 127:823–830. doi: 10.1016/j.marpolbul.2017.10.005
- Leles SG, Mitra A, Flynn KJ, Stoecker DK, Hansen PJ, Calbet A, McManus GB, Sanders RW, Caron DA, Not F, Hallegraeff GM, Pitta P, Raven JA, Johnson MD, Glibert PM, Våge S (2017) Oceanic protists with different forms of acquired phototrophy display contrasting biogeographies and abundance. *Proc R Soc B Biol Sci* 284:20170664. doi: 10.1098/rspb.2017.0664
- Leles SG, Mitra A, Flynn KJ, Tillmann U, Stoecker D, Jeong HJ, Burkholder JA, Hansen PJ, Caron DA, Glibert PM, Hallegraeff G, Raven JA, Sanders RW, Zubkov M (2019) Sampling bias misrepresents the biogeographical significance of constitutive mixotrophs across global oceans. *Glob Ecol Biogeogr* 28:418–428. doi: 10.1111/geb.12853
- Li A, Stoecker KD, Coats DW, Stoecker DK, Coats DW (2000) Mixotrophy in *Gyrodinium galatheanum* (Dinophyceae): grazing responses to light intensity and inorganic nutrients. *J Phycol* 36:33–45. doi: 10.1046/j.1529-8817.2000.98076.x
- Lima-Mendez AG, Faust K, Henry N, Colin S, Carcillo F, Chaffron S, Cesar J, Roux S, Vincent F, Bittner L, Darzi Y, Wang J, Audic S, Berline L, Cabello AM, Cornejo-castillo FM, Ovidio F, De L, Ferrera I, Guidi L, Pesant S, Royo-lonch M, Salazar G, Sebastian M, Souffreau C, Dimier C, Searson S, Kandels-lewis S, Oceans T, Gorsky G, Not F, Ogata H, Speich S, Wincker P, Bontempi G, Acinas SG, Sunagawa S, Bork P, Sullivan MB, Bowler C (2015) Top - down determinants of community structure in the global plankton interactome Affiliations : Tara Oceans coordinators and affiliations are listed below . These authors contributed equally to this work.
- Lipsewers T, Spilling K (2018) Microzooplankton, the missing link in finnish plankton monitoring programs. *Boreal Environ Res* 23:127–137.
- López-Jurado JL, Balbín R, Alemany F, Amengual B, Aparicio-González A, Fernández De Puellas ML,

- García-Martínez MC, Gazá M, Jansá J, Morillas-Kieffer A, Moyá F, Santiago R, Serra M, Vargas-Yáñez M (2015) The RADMED monitoring programme as a tool for MSFD implementation: Towards an ecosystem-based approach. *Ocean Sci* 11:897–908. doi: 10.5194/os-11-897-2015
- Martínez RA, Isari S, Calbet A (2014) Use of live, fluorescently-labeled algae for measuring microzooplankton grazing in natural communities. *J Exp Mar Bio Ecol* 457:59–70. doi: 10.1016/j.jembe.2014.03.007
- McManus GB, Zhang H, Lin S (2004) Marine planktonic ciliates that prey on macroalgae and enslave their chloroplasts. *Limnol Oceanogr* 49:308–313. doi: 10.4319/lo.2004.49.1.0308
- McQuatters-Gollop A, Gilbert AJ, Mee LD, Vermaat JE, Artioli Y, Humborg C, Wulff F (2009) How well do ecosystem indicators communicate the effects of anthropogenic eutrophication? *Estuar Coast Shelf Sci* 82:583–596. doi: 10.1016/j.ecss.2009.02.017
- McQuatters-Gollop A, Edwards M, Helaouët P, Johns DG, Owens NJP, Raitos DE, Schroeder D, Skinner J, Stern RF (2015) The Continuous Plankton Recorder survey: How can long-term phytoplankton datasets contribute to the assessment of Good Environmental Status? *Estuar Coast Shelf Sci* 162:88–97. doi: 10.1016/j.ecss.2015.05.010
- Michener RH, Kaufman L (2007) Stable isotope ratios as tracers in marine food webs: An update. In: Michener R, Lajtha K (eds) *Stable Isotopes in Ecology and Environmental Science*, 2nd edn. Blackwell Publishing, pp 238–282
- Mitra A, Flynn KJ, Burkholder JM, Berge T, Calbet A, Raven JA, Granéli E, Glibert PM, Hansen PJ, Stoecker DK, Thingstad F, Tillmann U, Våge S, Wilken S, Zubkov M V., Våge S, Wilken S, Zubkov M V. (2014) The role of mixotrophic protists in the biological carbon pump. *Biogeosciences* 11:995–1005. doi: 10.5194/bg-11-995-2014
- Mitra A, Flynn KJ, Tillmann U, Raven JA, Caron D, Stoecker DK, Not F, Hansen PJ, Hallegraeff G, Sanders R, Wilken S, Mcmanus G, Johnson M, Pitta P, Våge S, Berge T, Calbet A, Thingstad F, Jin H, Burkholder JA, Glibert PM, Granéli E, Lundgren V, Jeong HJ, Burkholder JA, Glibert PM, Granéli E, Lundgren V, Jin H, Burkholder JA, Glibert PM, Granéli E, Lundgren V, Jeong HJ, Burkholder JA, Glibert PM, Granéli E, Lundgren V (2016) Defining Planktonic Protist Functional Groups on Mechanisms for Energy and Nutrient Acquisition : Incorporation of Diverse Mixotrophic Strategies. *Protist* 167:106–120. doi: 10.1016/j.protis.2016.01.003
- Moita MT, Pazos Y, Rocha C, Nolasco R, Oliveira PB (2016) Toward predicting *Dinophysis* blooms off NW Iberia: A decade of events. *Harmful Algae* 53:17–32. doi: 10.1016/J.HAL.2015.12.002

- Moorthi SD, Ptacnik R, Sanders RW, Fischer R, Busch M, Hillebrand H (2017) The functional role of planktonic mixotrophs in altering seston stoichiometry. *Aquat Microb Ecol* 79:235–245. doi: 10.3354/ame01832
- Morán XAG, López-Urrutia Á, Calvo-Díaz A, LI WKW (2010) Increasing importance of small phytoplankton in a warmer ocean. *Glob Chang Biol* 10:1137–1144. doi: 10.1111/j.1365-2486.2009.01960.x
- Nehring S (1996) Recruitment of Planktonic dinoflagellates: importance of benthic resting stages and resuspension events. *Int Rev der Gesamten Hydrobiol* 81:513–527. doi: 10.1002/iroh.19960810404
- Nielsen LT, Krock B, Hansen PJ (2012) Effects of light and food availability on toxin production, growth and photosynthesis in *Dinophysis acuminata*. *Mar Ecol Prog Ser* 471:37–50. doi: 10.3354/meps10027
- NOAA (2019) Harmful Algal Blooms. In: Natl. Ocean Serv. <https://oceanservice.noaa.gov/hazards/hab/gulf-mexico.html>. Accessed 12 Feb 2020
- Oshima Y, Bolch CJ, Hallegraeff GM (1992) Toxin Composition of resting cysts of *Alexandrium tamarense* (Dinophyceae). *Toxicon* 30:1539–1544.
- OSPAR Commission (2017) Changes in Phytoplankton and Zooplankton Communities. OSPAR Assess. Portal
- OSPAR (2017b), MSFD Intermediate assessment “Changes in Phytoplankton and Zooplankton Communities” - <https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/habitats/changes-phytoplankton-and-zooplankton-communities/>
- OSPAR (2017c), Pilot assessment of production of phytoplankton - <https://oap.ospar.org/en/ospar-assessments/intermediate-assessment-2017/biodiversity-status/fish-and-food-webs/phytoplankton-production/>
- Paerl HW, Valdes LM, Pinckney JL, Piehler MF, Dyble J, Moisander PH (2003) Phytoplankton Photopigments as Indicators of Estuarine and Coastal Eutrophication. *Bioscience* 53:953. doi: 10.1641/0006-3568(2003)053[0953:ppaioe]2.0.co;2
- Park MG, Kim S, Kim HS, Myung G, Kang YG, Yih W, Yi GK, Yih W (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquat Microb Ecol* 45:101–106. doi: 10.3354/ame045101

- Park MG, Park JS, Kim M, Yih W (2008) Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *J Phycol* 44:1154–1163. doi: 10.1111/j.1529-8817.2008.00579.x
- Pitta P, Giannakourou A, Christaki U (2001) Planktonic ciliates in the oligotrophic Mediterranean Sea: Longitudinal trends of standing stocks, distributions and analysis of food vacuole contents. *Aquat Microb Ecol* 24:297–311. doi: 10.3354/ame024297
- Place AR, Bowers HA, Bachvaroff TR, Adolf JE, Deeds JR, Sheng J (2012) *Karlodinium veneficum*-The little dinoflagellate with a big bite. *Harmful Algae*. doi: 10.1016/j.hal.2011.10.021
- Ptacnik R, Sommer U, Hansen T, Martens V (2004) Effects of microzooplankton and mixotrophy in an experimental planktonic food web. *Limnol Oceanogr* 49:1435–1445. doi: 10.4319/lo.2004.49.4_part_2.1435
- Regaudie-De-Gioux A, Duarte CM (2012) Temperature dependence of planktonic metabolism in the ocean. *Global Biogeochem Cycles*. doi: 10.1029/2010GB003907
- Reguera B, Velo-Suárez L, Raine R, Park MG (2012) Harmful *Dinophysis* species: A review. *Harmful Algae* 14:87–106. doi: 10.1016/j.hal.2011.10.016
- Reynolds CS (2006) Pelagic Ecology Based in part on the article “Pelagic ecology” by Colin Reynolds, which appeared in the Encyclopedia of Environmetrics. *Encycl Environmetrics*. doi: 10.1002/9780470057339.vap008.pub2
- Riisgaard K, Hansen PJ (2009) Role of food uptake for photosynthesis, growth and survival of the mixotrophic dinoflagellate *Dinophysis acuminata*. *Mar Ecol Prog Ser* 381:51–62. doi: 10.3354/meps07953
- Ryther JH (1969) Photosynthesis and fish production in the sea. The production of organic matter and its conversion to higher forms of life vary throughout the world ocean. *Science* (80-) 166:72–76.
- Scherer C, Gowen RJ, Tett P (2016) Assessing the state of the pelagic habitat: A case study of plankton and its environment in the Western Irish Sea. *Front Mar Sci*. doi: 10.3389/fmars.2016.00236
- Schneider LK, Fynn KJ, Herman PMJ, Troost TA, Stolte W (in re-submission) Exploring the trophic spectrum: placing mixoplankton into marine plankton communities of the Southern North Sea
- Schoener DM, McManus GB (2012) Plastid retention, use, and replacement in a kleptoplastidic

- ciliate. *Aquat Microb Ecol* 67:177–187. doi: 10.3354/ame01601
- Silke J, Cusack C (2012) Irish National Phytoplankton Monitoring (Sites 41–45). *ICES Coop Res Rep* 313:104–113.
- Sjöqvist CO, Lindholm TJ (2011) Natural co-occurrence of *Dinophysis acuminata* (Dinoflagellata) and *Mesodinium rubrum* (Ciliophora) in Thin Layers in a Coastal Inlet. *J Eukaryot Microbiol* 58:365–372. doi: 10.1111/j.1550-7408.2011.00559.x
- Skovgaard A (1996) Mixotrophy in *Fragilidium subglobosum* (Dinophyceae): growth and grazing responses as functions of light intensity. *Mar Ecol Prog Ser* 143:247–253.
- Smayda TJ (1997) Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol Oceanogr* 42:1137–1153.
- Stoecker DK (1998) Conceptual models of mixotrophy in planktonic protists and some ecological and evolutionary implications. *Eur J Protistol* 34:281–290. doi: 10.1016/S0932-4739(98)80055-2
- Stoecker DK, Johnson MD, De Vargas C, Not F (2009) Acquired phototrophy in aquatic protists. *Aquat Microb Ecol* 57:279–310. doi: 10.3354/ame01340
- Tang EPY (1995) The allometry of algal growth rates. *J Plankton Res* 17:1325–1335.
- Tett P, Carreira C, Mills DK, Van Leeuwen S, Foden J, Bresnan E, Gowen RJ (2008) Use of a Phytoplankton Community Index to assess the health of coastal waters. *ICES J Mar Sci* 65:1475–1482. doi: 10.1093/icesjms/fsn161
- The Norwegian Directorate of Fisheries (2019), 11 600 tonn død laks i nord - <https://www.fiskeridir.no/Akvakultur/Nyheter/2019/0519/11-600-tonn-doed-laks-i-nord>
- Tillmann U (2003) Kill and eat your predator: A winning strategy of the planktonic flagellate *Prymnesium parvum*. *Aquat Microb Ecol* 32:73–84. doi: 10.3354/ame032073
- Varkitzi I, Francé J, Basset A, Cozzoli F, Stanca E, Zervoudaki S, Giannakourou A, Assimakopoulou G, Venetsanopoulou A, Mozetič P, Tinta T, Skejic S, Vidjak O, Cadiou JF, Pagou K (2018) Pelagic habitats in the Mediterranean Sea: A review of Good Environmental Status (GES) determination for plankton components and identification of gaps and priority needs to improve coherence for the MSFD implementation. *Ecol Indic* 95:203–218. doi: 10.1016/j.ecolind.2018.07.036
- Ward BA, Follows MJ (2016) Marine mixotrophy increases trophic transfer efficiency, mean organism

size, and vertical carbon flux. *Proc Natl Acad Sci* 113:2958–2963. doi:
10.1073/pnas.1517118113

Wasmund N (2017) The diatom/dinoflagellate index as an indicator of ecosystem changes in the Baltic Sea. 2. Historical data for use in determination of good environmental status. *Front Mar Sci* 4:1–12. doi: 10.3389/fmars.2017.00153

Whyte C, Davidson K, Gilpin L, Mitchell E, Moschonas G, McNeill S, Tett P (2017) Tracking changes to a microplankton community in a North Atlantic sea loch using the microplankton index PI(mp). *ICES J Mar Sci* 74:311–325. doi: 10.1093/icesjms/fsw125

Wilken S, Huisman J, Naus-Wiezer S, Van Donk E (2013) Mixotrophic organisms become more heterotrophic with rising temperature. *Ecol Lett* 16:225–233. doi: 10.1111/ele.12033