ASLO 2023 Scientific Session SS018

Mixoplankton: The New Paradigm Testing the Resilience of Our Science in the UN Ocean Decade

Organizers:
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Session description:
The past decade has seen the recognition of diverse plankton functional groups in marine ecology, beyond the classical phyto- and zoo- plankton. The new mixoplankton paradigm incorporates into the food web those protist plankton that engage in photo-phago-trophy to satisfy their nutritional needs. Mixotrophy, as the coupling of photo-osmo-mixotrophy, has been researched within phytoplankton, and especially associated with Harmful Algal Bloom (HAB) events, for decades. However, there are major differences between the photo-osmo-mixotrophic phytoplankton and the photo-phago-mixotrophic mixoplankton. Mixoplankton actively hunt, kill and eat thus removing competitors from the environment, acquiring nutrients, and directly impacting trophic dynamics. Further, we now know that various exemplar 'phytoplankton' and 'protozooplankton' species – such as the food web supporting *Tripos* sp. (*Ceratium*), the ecosystem disruptive bloom forming green *Noctiluca scintillans*, the HAB forming species within the *Alexandrium*, *Prorocentrum*, *Dinophysis* genera - are all in fact mixoplankton. The base of the marine food web is not as traditional science describes, raising profound questions about how we project climate change and allied anthropogenic events in reshaping the marine ecosystem and thence how we manage resources. Methods (field, laboratory, modelling) developed for phytoplankton and protozooplankton need to be adapted for mixoplankton science - where required, new in vivo as well as in silico methods need to be developed. Thus, environmental monitoring needs new methods to replace current sampling protocols which are known to selectively destroy groups of mixoplankton; ecosystem and management models need to properly reflect mixoplankton ecophysiology. To address such challenges, we recently formed the SCOR Working Group 165 – 'MixONET'. An overarching aspiration of MixONET is to extend the mixoplankton paradigm beyond blue-sky research to real-life applications such as environmental monitoring, management, policy making, and education. Changes in marine ecosystems with climate change, which will inevitably see changes in mixoplankton (including HAB) populations, will test the resilience of our science as much as of nature. In this session we invite people from all affected sectors to share their ideas and results on this challenging and timely topic.

LIST OF ABSTRACTS [**presenter*]

Mixotroph or Mixoplankton: what's in a name?

Aditee Mitra, Kevin Flynn, Urban Tillmann, John Raven*

Clarity in terminologies is vital in science. The term 'mixotroph' has a long history and is defined in Henderson's dictionary of biology as 'an organism using an inorganic compound as energy source and an organic compound as carbon source'. However, while the common exemplar 'mixotroph', the Venus flytrap, feeds for nutrients and not C, phytoplankton that consume dissolved free amino acids for C, energy and N via osmotrophy are often not viewed as mixotrophs even though by definition they are. In contrast, photo-phagotrophic protists are always referred to as mixotrophic, irrespective of why they feed. Ecologically, there is a critical distinction between protist plankton that are mixotrophic via osmotrophy versus via phagotrophy; the latter play a vital role in trophic dynamics through removal of prey/competitors. To provide a clear distinction between such organisms the term 'mixoplankton' was proposed, with a concise definition – 'protist plankton that are photo-phago-osmo-trophic'. Mixoplankton are thus all mixotrophs, but not all planktonic mixotrophs are mixoplankton. Importantly for science, 'mixoplankton' also sets these protists apart from the photo-osmo-mixotrophic 'phytoplankton' and heterotrophic 'zooplankton'. In moving away from a plant-animal dichotomy in marine ecology the term mixoplankton provides concise clarity. It provides a tool for us to redefine the core underpinning of marine science, to perform keyword searches, to differentiate database entries, model descriptions, and to reflect the biogeochemical and ecological consequences.

Addressing the challenges of modelling ecosystem services in 3D under the mixoplankton paradigm

Molly James, Helen Powley, Jerry Blackford, Ricardo Torres, Aditee Mitra*

Mixoplankton, protists with the ability to obtain nutrition through photo-phago-osmo-trophy, are ubiquitous globally and important members of the marine plankton community. Over the last decade our knowledge and appreciation of these organisms in marine ecology has increased significantly. We now know that a large proportion of the harmful algal bloom forming species are mixoplanktonic with various species having a deleterious impact on aquaculture. While some recent studies have embraced the mixoplankton paradigm within hydrodynamic models and investigating the impact on biogeochemical cycling, the incorporation and application of the paradigm in 3D modelled systems specifically to understand the implications on ecosystem services (such as aquaculture) remains poorly explored. We have developed a 3D hydrodynamical-biogeochemical coupled model-framework to include mixoplankton, for a small bay region in the UK with aquaculture. Ocean hydrodynamics are described using the Finite-Volume Community Ocean Model (FVCOM). Protist plankton are represented through the Perfect Beast (PB) eco-physiological model and implemented within the European Regional Seas Ecosystem Model (ERSEM) to describe the lower-level trophic food-web (ERSEM-PB). In this session, we will discuss the methodological challenges in undertaking this work. We will focus on how functional diversity of marine protists can be incorporated within 3D coupled biogeochemical models to gain better understanding of the impact of environmental stressors on ecosystem services under the new paradigm in marine ecology.

Global distribution of marine mixoplankton based on morphological observations and metabarcoding

Luciana Santoferrara, Emile Faure, Suzana Gonçalves Leles, Michaela Larsson, Daniel Vaulot*

Studying mixoplankton species distributions is essential to support research on the ecological importance of this group in the ocean, and for applications like management of harmful algal blooms and food web modelling. Tracking mixoplankton distributions based on morphological identifications or DNA sequences is now facilitated by the recently launched Mixoplankton Database. Using this resource, we retrieved global distributional data from 1) the mostly microscope-based Ocean Biogeographic Information System database (OBIS), and 2) the metabarcoding Protist Ribosomal Reference database (metaPR2). Contrasting distribution patterns between databases emerged mainly due to differences in sampling coverage and methodological issues that bias the identification of certain mixoplankton groups. While OBIS provided a good spatial coverage and data for foraminiferan mixoplankton species that are absent in metaPR2, the opposite trend was observed for radiolarians. Other mixoplankton representatives, such as dinoflagellate and ciliate species, can be found in both databases but their distributions were more likely to be limited to coastal regions based on OBIS than on metaPR2. Given the recent expansion in the availability of metabarcoding data, we are now analyzing in more detail the metaPR2 dataset, which contains ~150 mixoplankton species in almost 5,000 samples from 59 studies worldwide. We expect to elucidate patterns related to latitude, coast-versus-ocean and vertical distributions.

Key parameters and procedures to improve monitoring and forecasting of rare specialized protist (*Dinophysis* species)

Beatriz Reguera, Patricio Díaz, Esther Velasco-Senovilla, Pilar Rial, Laura Escalera, Francisco Rodríguez, Manuel Ruiz-Villarreal, Enrique Nogueira, Aditee Mitra*

Species of the genus *Dinophysis*, producers of lipophilic toxins, are ubiquitous but their impact on the shellfish industry is variable depending on the toxic potential of local strains, and which resources are exploited. Site specific differences determine regional hot spots for diarrhetic shellfish poisoning (DSP) events. These events are caused by low biomass blooms (> 103 cells L^{-1}) of holoplanktonic species that most of the year occur close to detection level. Furthermore, *Dinophysis* is a highly specialized plastidic specialist non-constitutive mixoplankton (pSNCM), requiring ciliate prey to get kleptoplastids and light and nutrients for acquired phototrophy. Improved forecasting of Dinophysis blooms and their response to environmental changes is a formidable challenge for scientists and managers. To date, prediction has mainly been based on physical transport models applied to already established populations and short-term forecasts during well known settings promoting their advection or dispersal. Little has been done to unveil “the dark side of HAB dynamics”, i.e. biological interactions. Here we explore key biological parameters to estimate risks of bloom success. These include intraspecific (overwintering cells serving as inoculum; phases of population growth; small cell formation), and interspecific interactions (competition, ciliate prey availability). Some examples will illustrate large failures in expected events when the whole microplankton assemblage was not considered as well as biases due to inappropriate sampling procedures

Continuous automated imaging flow cytometry for early warning of the mixoplankton harmful algal bloom forming *Dinophysis ovum*

Lisa Campbell, James Fiorendino*

Mixoplankton are now recognized as an important functional group of plankton and include many harmful algal bloom (HAB) forming species. One promising method to identify these mixoplankton, which is not destructive and will allow enumeration, is the Imaging FlowCytobot (IFCB). IFCB combines flow cytometry and video technology to capture images of individual plankton cells. Continuous automated operation and data processing has produced a high-resolution time series of the microplankton community structure and has demonstrated the successful use of this instrument for HAB early warning. Sustained operation of this novel technology at the Texas Observatory for Algal Succession Time series (TOAST) in Port Aransas, TX since 2007 and Surfside Beach, TX since 2017 has provided observations of *Dinophysis ovum* and its prey, *Mesodinium rubrum*. In addition to detection and successful early warning of HAB events, time series data have been used in developing models to identify important factors in bloom initiation of *Dinophysis ovum*. Model results showed a strong effect of water temperature on *Dinophysis* bloom onset; blooms occurred as waters warmed and overlapped with the optimal temperature range for *Dinophysis* growth (18 – 24 °C). Allowing biomass of predator and prey to vary within the model showed a notable sensitivity of *Dinophysis* bloom intensity and duration to prey size.

Ecosystem size drives patterns and control mechanisms of mixotrophs success across tropical lakes: A large-scale assessment of the grand écart hypothesis

Mariana Costa, Leticia Quesado, Regina Nobre, Camila Cabral, Fabiola Dantas, Hugo Sarmento, André Amado, Vanessa Becker, Judit Padisák, Luciana Carneiro, Adriano Caliman*

Mixotrophy is a physiological trait that combines autotrophy and heterotrophy in one organism and contribute significantly to energy and matter transfer in aquatic ecosystems. However, there has been much uncertainty on how environmental factors affect mixoplankton success across freshwater ecosystems. The grand écart hypothesis is a framework that considers light and nutrients availability as the key components of mixotrophs' niche. It suggests that some properties of ecosystems lead to the segregation of nutrients and light, and the need to access both creates the environmental filtering for mixotrophs. Here, we rationalized that ecosystem size is a property of lake ecosystems that can determine opposing gradients of light and nutrients, as well as other key environmental factors that determine mixoplankton success. We hypothesized that lake ecosystem size can mediate the prevalence of patterns and control mechanisms predicted by the grand écart hypothesis on mixoplankton relative biomass (MRB). Using data from 98 tropical lakes spanning 36.000 km^2, we showed that lake size mediates the inverse relationship between light and nutrient availability. Bigger lakes have more light but low nutrients, and smaller lakes have more nutrients but shaded. Light availability predicts MRB in small lakes, and nutrients predict MRB in big lakes. MRB values were higher in small lakes, with secondary influence from zooplankton herbivory. Our results validate the grand écart hypothesis as a significant framework for predicting patterns and control mechanisms of mixoplankton. Highlights the significance of lake size as an ecosystem property that generates opposing light and nutrient gradients, further emphasizing its importance for understanding the regulation of freshwater aquatic ecosystems' biogeochemistry and functioning.

Modeling the effect of prey size on *Dinophysis* bloom dynamics

James M. Fiorendino, Daniel Roelke, Lisa Campbell*

Blooms of toxic *Dinophysis* threaten human health and coastal economies reliant on shellfish. Though a useful tool for studying bloom dynamics, models have lacked important biological data regarding *Dinophysis* physiology and ecology due to the difficulty of field and laboratory studies of mixotrophic *Dinophysis* species. Recent successes in culturing North American *Dinophysis* species, coupled with ongoing high-temporal-resolution monitoring of microplankton communities, have filled important gaps in data and expanded the potential for models of *Dinophysis* blooms. Over a decade of *Dinophysis* abundance, biomass, and environmental data (temperature, salinity) are available from the Texas Observatory for Algal Succession Timeseries where an Imaging FlowCytobot has operated since 2007. These data were used to parameterize and ground truth a new, mechanistic model of *Dinophysis* and prey population dynamics. The model incorporated previous hypothetical frameworks of mixotroph physiology and observed growth rates from extensive laboratory experiments. Not only did the model output illustrate the seasonal control of water temperature on bloom onset and duration, it also revealed an effect of prey size on bloom intensity and duration: large, less abundant prey may result in more intense, prolonged blooms than abundant, smaller prey. Considering the importance of mixoplankton to marine microbial dynamics, these results emphasize the need to accurately represent biomass in mixotrophic models of marine microbial systems.

[Acknowledging mixoplankton within dilution grazing experiments]

Guilherme Ferreira, Filomena Romano, Nikola Medić, Paraskevi Pitta, Per Juel Hansen, Kevin Flynn, Aditee Mitra, Albert Calbet*

The dilution grazing technique established by Landry and Hassett in 1982 has become the most widely used methodology to measure planktonic herbivory in situ. This technique has, however, been questioned a few times, as some studies report positive regression slopes, i.e., potential "negative grazing rates". The reasons behind positive slopes are neither straightforward nor easy to discern, as these can be caused by several factors. Particularly, mixoplankton, single-celled organisms that combine phototrophy and phagotrophy, were not originally considered in the formulation of the theory behind the dilution technique. As a result, the technique is, currently, blind to photo-phago-mixotrophy, as mixoplankton behave simultaneously as predator and prey. Hence, there is a need to reappraise the technique to accommodate the mixoplankton paradigm. Our study explored the dynamics of the dilution experiment in a controlled environment, with known mixtures of phyto-, protozoo-, and mixo-plankton, operated under different light regimes and species combinations. Our results demonstrate the poor nature of chlorophyll as a proxy for phytoplankton biomass, as well as the importance of considering diel feeding rhythms in the experimental design. We hypothesize that in silico approaches may help disentangle the contribution of mixoplankton to the community grazing of a given system. Indeed, we hope that our results can inform modelling investigations in the future.

Mixotrophic growth of the ichthyotoxic Golden algae, *Prymnesium parvum*, under different sources of phosphorus

Clémence Boucher, Julie Andre, Damien Réveillon, Thomas Lacour, Per Juel Hansen, Francis Mairet*

Laboratory experiments were conducted with the mixotrophic and ichthyotoxic haptophyte *Prymnesium parvum* (strain CCAP 946/6) to test the effects of phosphorus (P) sufficiency and deficiency on its growth, phagotrophic and cytotoxic activities. P-deficient *P. parvum* cultures were grown without or with addition of P in the form of inorganic P (nutrients) and/or living algal prey (i.e. the cryptophyte *Teleaulax amphioxeia*). The ingestion rate of *P. parvum* and prey mortality rate were calculated based on the phycoerythrin (PE) fluorescence signal. Growth rates of *P. parvum* were higher in the treatments with live prey compared to inorganic P and maximal when both sources of P were added ($0.79 \pm 0.07\ d^{-1}$). In addition, the mortality rate of *T. amphioxeia* induced by toxic compounds was $0.2 \pm 0.02\ d^{-1}$ in the P-deficient treatment, while no mortality was observed in P-sufficiency. This experiment also revealed the mortality due to cell lysis in many cases exceeded that of prey ingestion. Therefore, additional experiments were conducted with lysed prey cells. In these experiments, when grown with prey debris, the mean growth rate of *P. parvum* was similar to strict autotrophy (0.30 ± 0.1 vs. $0.38 \pm 0.03\ d^{-1}$) meaning that *P. parvum* is able to grow on lysed prey cells, but not as fast as with live prey. These results provide the first quantitative evidence that ingestion of organic P in the form of living prey and/or debris of prey plays an important role in *P. parvum* growth and may explain its efficiency in forming blooms.

Mucus trap assisted mixoplanktonic activity in *Prorocentrum*

Urban Tillmann, Kevin Flynn, Michaela Larsson, Aditee Mitra*

Prorocentrum comprises a diverse group of bloom forming dinophytes with a worldwide distribution. They are photosynthetic, but mixotrophic phagotrophy has also described. Recently, the small *P. cf. balticum* was shown to use a remarkable feeding strategy by crafting globular mucus traps to capture and immobilize potential prey. Here we present video observations and experimental data showing that two additional related species, the recently described *P. pervagatum* and the cosmopolitan bloom forming *P. cordatum*, also produce large (120 µm) mucus traps enabling their mixoplanktonic activity. Both species fed on *Teleaulax* cells entangled in these traps. Through openings in the mucus traps, *Prorocentrum* cells create water movement eddying particles from outside into the trap, or cells are more passively entangled upon contact with the trap´s outside. Trapped prey cells were immobile but remained alive. Uptake of prey trapped in the mucus was via a peduncle which always extruded from the apical area of the *Prorocentrum* cell. Phagotrophy of *P. pervagatum* supported faster growth compared to unfed controls, and time series quantification of food vacuoles revealed ingestion rates of about 12 *Teleaulax* prey cells per day. This study demonstrates that the large size and immobilization properties of mucus traps successfully increase the availability of prey for small Prorocentrum species, whose peduncle feeding mode impedes consumption of actively moving prey, and that this strategy is common among certain clades of small planktonic *Prorocentrum* species.

Photoacclimation of Cryptophyte plastids *Plagioselmis prolonga* in their hosts *Mesodinium rubrum* and *Dinophysis acuminata*

Sarah Garric, Morgane Ratin, Dominique Marie, Foulon Valentin, Ian Probert, Francisco Rodriguez, Christophe Six*

Several lineages of cryptophyte microalgae are the prey of ciliates that steal and use their nuclei, mitochondria and plastids, thereby becoming photosynthetic. These ciliates can in turn be victims of kleptoplasty by species of the dinoflagellate *Dinophysis*. The objective of this study is to understand whether plastid photoacclimation capacities are altered in the cellular environment of the hosts. Using the triple culture system *Plagioselmis prolonga* - *Mesodinium rubrum* - *Dinophysis acuminata*, we set up an experiment in which the three organisms were shifted from high to low irradiances, in order to induce the synthesis of photosynthetic complexes, such as phycoerythrin. Our results show that photoacclimation to lower irradiance is possible in the three organisms but that it is much constrained by the availability and the photoacclimation level of preys. Confocal microscopy measurements showed that it does not rely on an increased ingestion rate of plastids. Interestingly, green light utilization by photosystem II was optimal in the cryptophyte but always lower in the ciliate and in the dinoflagellate stolen plastids, suggesting a modification of phycoerythrin light absorption efficiency. We hypothesize that phycoerythrin synthesis, which requires nuclear and plastidial genes, is altered in the hosts. Overall, our study suggests that light harvesting is less efficient in the translocated plastids and that competitiveness of the ciliate and the dinoflagellate for light is highly dependent on the photoacclimation level of their preys.

The combined effect of pH and dissolved inorganic carbon concentrations on the plastidic ciliate *Mesodinium rubrum* and its cryptophyte prey

Per Juel Hansen, Christine Eriksen, Melanie Walli, Dedmer van de Waal, Nico Helmsing, Emma Dahl, Helle Sørensen*

Ocean acidification is caused by rising atmospheric CO2 levels and involves a lowering of pH combined with increased concentrations of CO2 and dissolved in organic carbon in ocean waters. Many studies investigated the consequences of these combined changes on marine phytoplankton, yet only few attempted to separate the effects of decreased pH and increased CO2. Moreover, studies typically target photoautotrophic phytoplankton, while little is known of plastidic protists that depend on the ingestion of plastids from their prey. Therefore, we studied the separate and interactive effects of pH and DIC levels on the plastidic ciliate *Mesodinium rubrum*, which is renowned for forming red tides in coastal waters worldwide. Also, we tested the effects on their prey, which typically are cryptophytes belonging to the *Teleaulax*/ *Plagioslemis*/ *Geminigera* species complex. These cryptophytes not only serve as food for the ciliate, but also as a supplier of chloroplasts and prey nuclei. We exposed *M. rubrum* and the two cryptophyte species, *T. acuta*, *T. amphioxeia* to different pH (6.8 – 8) and DIC levels (~ 6.5 – 26 mg C L^{-1}) and assessed their growth and photosynthetic rates, and cellular chlorophyll a and elemental contents. Our findings did not show significant effects across the ranges in pH and/or DIC, except for *M. rubrum*, for which growth was negatively affected at pH 6.8. It thus seems that *M. rubrum* is resilient to changes in pH and DIC, and its blooms may not be strongly impacted by the changes in ocean carbonate chemistry projected for the end of the 21st century.

Laboratory studies with the model ciliate mixoplankter *Strombidium rassoulzadegani*

George McManus, Josiah Grzywacz, Maxim Gorbunov*

Only a few ciliate mixoplankters, among them *Laboea strobila*, *Mesodinium rubrum*, and several members of the oligotrich genus *Strombidium* have been cultivated for any length of time, so we do not know a great deal about their physiology and that of their retained chloroplasts. In recent years, we have isolated an oligotrich ciliate, *Strombidium rassoulzadegani* from multiple locations and cultivated it for up to 7 years. It can retain and use plastids from chlorophytes and cryptophytes, but appears to grow wholly heterotrophically on dinoflagellates. Using the FIRe (fluorescence induction and relaxation) instrument, we measured quantum efficiency in chloroplasts over time within the ciliate. Freshly-incorporated chloroplasts have an efficiency of about half that of the parent alga and decline by another half over three days within the ciliate. In this presentation, we review the role of captured chloroplasts in supporting ciliate growth.

Highlights from the ASLO 2023 SS018 Mixoplankton session