# SCOR Working Group Proposal for *Mixotrophy in the Oceans – Novel Experimental designs and Tools for a new trophic paradigm* (MixONET)


## 1. Abstract

Traditional and contemporary methods in Biological Oceanography assume a false plant/animal dichotomy. This dichotomy has been the bedrock of marine science, operationally separating organisms into phototrophic or phagotrophic compartments. We now know that most protist plankton at the base of the oceanic food-web can photosynthesise (plant-like-photo-autotrophy) **and** ingest food (animal-like-phago-heterotrophy) thus contributing to **both** primary and secondary production. While recent conceptual and quantitative models have been adapted to accommodate this synergistic merging of dichotomies, sampling and monitoring methods have not done so. Thus, a significant knowledge gap remains about the very foundation of the ocean food-web. We propose an international collaborative effort with a multidisciplinary team of experts in ecophysiology and molecular biology of mixoplankton, field sampling and monitoring technology, biological and physical oceanography to propose methods to determine contributions of mixoplankton to primary and secondary productions. We will approach this from the perspective of (1) **ocean biogeochemical cycling** especially under climate change by identifying methods for an accurate evaluation of mixoplanktonic activities in global oceans; descriptions of new networks of organisms are expected to emerge from our work; (2) **global food security** by establishing processes for assessing Mixoplanktonic-Harmful Algal Bloom dynamics, specifically associated with anthropogenic factors (e.g., eutrophication, climate warming, invasive species introductions) and impact on fishery and recreational industries. Through educational activities we will train the next generation, highlighting the importance of the mixoplankton paradigm in biological oceanography. MixONET will thus make a solid contribution to addressing priorities of the United Nations Decade of the Ocean.


## 2. Scientific Background and Rationale

### 2.1 The New Mixoplankton Paradigm

About half of Earth's carbon fixation and oxygen production are due to activities of microscopic marine plankton – the phytoplankton. Historically, methods have been developed to study the abundance and activity of these single-celled photosynthetic organisms (capable of growing with only light and inorganic compounds). However, we know now that many phytoplankton species are not exclusively phototrophic but also ingest other plankton – i.e., they are 'mixoplanktonic' (Flynn *et al*. 2019). Furthermore, about one-third of the protozooplankton (primary consumers) are also recognized as mixoplankton. Biogeography studies have shown the ubiquity of mixoplankton (Leles *et al*. 2017, 2019; Faure *et al*. 2019). Well-known phytoplankters that are actually mixoplankton include the coccolithophore *Emiliania huxleyi* (Avrahami and Frada 2020); the diverse bacterivorous phytoflagellates of the microbial carbon pump (Unrein *et al*. 2014); the toxin-producing *Alexandrium* and *Dinophysis* whose blooms (termed HABs) result in shellfish contamination and harvesting closures (Reguera *et al*. 2014); *Karlodinium* and raphidophytes that cause mass mortalities of farmed and wild fish. Various experiments have demonstrated that mixoplankton are major grazers of picoplankton responsible for >50% of the total bacterivory in oligotrophic systems (Unrein *et al*. 2007; Zubkov and Tarran 2008); this has important implications for carbon flux within the microbial food-web. The base of the oceanic food-webs is thus comprised of photosynthesizers that also eat and consumers that also photosynthesize, muddying the photo-autotroph/phago-heterotroph distinction that has dominated biological oceanography (Fig.1).

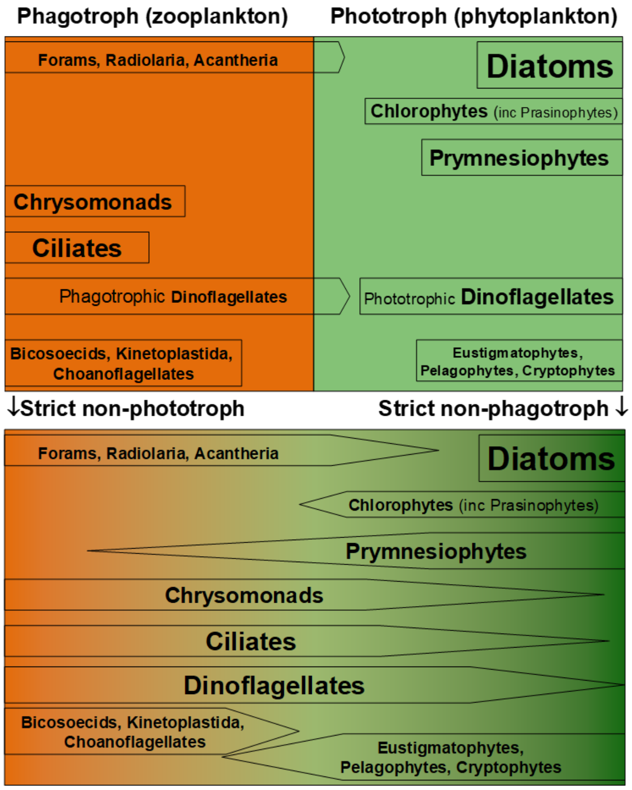


**Fig.1** The traditional dichotomy of protist plankton (top panel) is now replaced by a gradation (lower panel) from non-phototroph to the strict non-phagotrophy. In contrast to the classic misrepresentation shown in the upper panel, the potential for individual organisms to contribute both to primary and secondary production is now acknowledged. Flynn et al. (2013) J.Plankt.Res. 35: 3

**This recognition where most oceanic primary producers cannot be analogized as miniature plants has led to a paradigm shift in trophic studies (Mitra *et al*. 2016), and there is thus a critical need to evaluate how new sampling and monitoring methods and global plankton databases can be optimized for this new perspective.**

Ecosystem models verify the importance of incorporating the mixoplankton paradigm into food-webs (Mitra *et al*. 2014, Leles *et al*. 2021). Combined photo+phago-trophic ability throughout the plankton size spectrum fundamentally changes the movement of nutrients and energy through the organism size classes. Therefore, models that ignore mixoplankton may provide erroneous predictions of plankton succession and potentially be unaware of allied deleterious implications for marine systems. For example, ignoring mixoplankton in biogeochemical cycling simulations leads to instability, with a decrease in C-fixation and DOC production, affecting the biological carbon pump (Mitra *et al*. 2014). Models have also shown seasonal variation in mixoplankton productivity and allied energy transfer to higher trophic levels (Leles *et al*. 2021).

During the current UN Decade of the Ocean, marine researchers are faced with the challenge of answering how climate change will impact biology, productivity and carbon sequestration, food-webs, and allied ecosystems services in global oceans. Mixoplankton are at the heart of all these processes, for example, dominating in various ecosystems (e.g., temperate summers) supporting juvenile fish growth. With increasing anthropogenic impacts, higher frequencies of harmful mixoplankton blooms are predicted with deleterious effects on biodiversity and ecosystem services such as fisheries production and coastal recreation (Glibert 2020, Griffith *et al*. 2020). In the Arabian Sea, spectacular blooms of mixoplanktonic dinoflagellate *Noctiluca* are leading to the collapse of the traditional phytoplankton-mesozooplankton link in the food-web, and the emergence of a new network of organisms operating at the higher trophic levels dominated by gelatinous-plankton, with severe food security and socio-economic hardships to a population of over 140 million (Goes and Gomes 2016).

**Our overarching aim is to update biological oceanography to quantitatively accommodate the mixoplankton paradigm, and to evaluate which emerging methods and technology will enable accurate assessment of mixoplankton abundance and activities. MixONET will thus integrate the mixoplankton paradigm with traditional and novel methods of plankton research to provide tools for predicting the response of the ocean's biological communities and element cycles in the face of ongoing climate change to better understand how humanity can maintain healthy sustainable oceans.**

### 2.2 The Challenge

There are no standard methods for monitoring mixoplankton. Microscope counts provide morphological identification of species but cannot reliably distinguish phagotrophic capability or trophic relationships (i.e., who eats whom). They thus cannot ascertain the separate contributions of mixoplankton to production and consumption.

Most developmental efforts directed at mixoplankton have been qualitative in nature with less emphasis on capturing quantitative information to support model validation and predictions. Thus, no consensus exists for how to estimate the contribution of mixoplankton to primary and secondary production rates, to biogeochemical cycling, to the microbial carbon pump, or how mixoplankton may react to climate change. In particular, we need:

- **simple standardized protocols** for routine incorporation into monitoring programmes so that plankton community composition in relation to global changes can be quantified;
- **rapid and accurate methods** for sampling, preserving samples and identifying species from the field to understand plankton succession and thus biogeochemical cycling;
- **to effectively catalogue mixoplankton** within existing diversity databases. Laboratory-based studies and models have indicated the impact of plankton nutritional mode on biogeochemical cycling. Some HAB species have inherent chloroplasts (e.g., *Karlodinium*, *Karenia*; constitutive mixoplankton) and will just grow faster with prey availability, but others acquiring chloroplasts from their prey (e.g., *Dinophysis*, green *Noctiluca*; non-constitutive mixoplankton) are more complicated as they depend on specific prey. Thus, accurate and routine methods for cataloguing mixoplankton need to be incorporated into existing diversity databases. We also need information on the prey items of the primary producers and also their predators to incorporate these into databases as well as climate and fisheries production models.
- Laboratory studies have shown that non-intrusive methods such as *in situ* flow cytometry and variable fluorescence could provide estimates of mixoplankton abundance and production. Thus, **extant and new methods need to be evaluated** and prioritized for monitoring and modelling.

### 2.3 Why a SCOR Working Group now?

**MixONET** will convene pan-global cross-disciplinary experts to address challenges of incorporating mixoplankton into standard field and monitoring methods. The establishment of the mixoplankton paradigm was determined in such a manner several years ago when a Leverhulme Trust International Networking grant led to a series of high-impact publications in this area. We thus propose international brainstorming efforts among experts with knowledge of ecophysiology and molecular biology of mixoplankton, new sampling and observation technology, biogeochemical cycling and ecosystems services to find ways in which accurate estimates of mixoplankton primary and secondary productions can be made. No other single funding stream can enable such a discussion. Three of the five key challenges for the UN Decade of the Ocean are directly relevant to MixONET:

- *Understand and beat marine pollution* – many HAB species are mixoplanktonic and associated with eutrophication, fish-kills, and low oxygen events in coastal waters. Recognising these HAB species as mixoplankton, needing prey as well as light and inorganic nutrients, changes perceptions of what drives and controls these events. MixONET will propose quantitatively accurate and taxonomically precise methodologies for monitoring mixoplankton, including HAB species, in real-time mode at high frequency.

- *Protect and restore ecosystems and biodiversity* – mixoplankton dominate in high-diversity oceanic ecosystems but are also abundant in coastal areas impacted by anthropogenic nutrient loading and hypoxia, where HABs reduce diversity.
- *Sustainably feed the global population* – as both consumers and producers, mixoplankton play a central role in oceanic food-webs and are likely important food items for larval fish. On the other hand, some mixoplankton compete for food with, toxify, or even ingest the much larger mesozooplankton and thus may disrupt productive food chains. In areas like the Arabian Sea and the Gulfs of Oman and Thailand, they have led to new organism networks dominated by gelatinous-plankton, disturbing the classical food-web as well as industry and tourism.

## 3. Terms of Reference

**ToR1.** ***Biological oceanography databases and the mixoplankton paradigm***. Advocate for the realignment of existing plankton-facing databases in light of the mixoplankton paradigm. Identify connections between mixoplankton communities and essential ocean variables.

**ToR2.** ***Biological oceanography research methods under the mixoplankton paradigm***. Re-evaluate extant standard biological oceanographic research methods and practices for application under the mixoplankton paradigm. For example, conventional fixatives often destroy the delicate mixoplankton, while more gentle ones obscure the presence of chloroplasts. Also, pico- and nano-planktonic organisms are routinely counted using flow cytometry or epifluorescence microscopy; while standard protocols can discriminate between pigmented and colourless plankton, they are not geared for identification and quantification of mixoplankton.

**ToR3.** ***Development of new biological oceanography methods accounting for primary and secondary productions by mixoplankton***. Evaluate development of:

a. routine new methods and simple protocols that could be incorporated routinely in ongoing monitoring programmes to better quantify mixoplankton and interpret their activities;
b. new experimental and observing methods (including autonomous technologies) for quantifying and monitoring mixoplanktonic abundance and activity.

**ToR4.** ***Ocean literacy***. Development of multi-lingual training material for Early Career Researchers (ECRs), ecosystem managers, teachers and students, to enhance ocean literacy. The mixoplankton paradigm needs to be brought to the attention of students through to policy-makers. A Decision Support Tool (DST) will be developed to aid configuration of mixoplankton-centric experiments to determine contributions to primary versus secondary production by these organisms.

## 4. Working Plan

To address **ToR1**, we will collate a list of extant databases used in biological oceanography and climate modelling studies. Once compiled, we will align the plankton species within these databases to the functional group classifications according to the mixoplankton paradigm (Mitra *et al*. 2016, Flynn *et al*. 2019) through reference to the mixoplankton database (Mitra *et al*. in prep due to be completed 2021 summer; building from Leles *et al*. 2017, 2019, Faure *et al*. 2019). This methodology has been recently applied to the [IOC-UNESCO Taxonomic Reference List of Harmful Microalgae](), an IOC-HAB programme service used in monitoring centres worldwide, with a preliminary alignment of HAB species already undertaken (Mitra and Flynn 2021).

To address **ToR2**, we will collate details of standard biological oceanographic research methods and practices used for field monitoring and/or observation of the different protist species. These will be broadly divided into optical (e.g., microscopy, flow cytometry, video or holographic *in situ* instruments, satellite sensors, etc.) and genetic (e.g., DNA barcode markers, metagenomics, etc.) methods. We will then investigate to what extent these approaches may provide information on photo- &/or phago- trophic activities and conduct a re-evaluation and re-purposing of the methods for mixoplankton.

The findings from **ToRs 1** and **2** will inform **ToR3**. The activities of **ToR3** can be broadly divided into: (a) development of accessible methods which can be used for regular (low cost) monitoring, and, (b) development of methods which can be applied via autonomous technologies.

Planning of **ToR4** activities starts at the inception of the MixONET Working Group with activities planned throughout the funding period. Three face-to-face ECR-focussed training workshops are planned, one of which will be organised in conjunction with the International Congress of Protistology (ICOP) and the second and third ones in conjunction with MixONET WG meetings; 3 further virtual workshops are planned to enhance global accessibility. A Decision Support Tool (DST) will be developed and made available so that use of appropriate methods can be introduced into new and existing monitoring programmes efficiently. A dedicated section for MixONET will be assigned at www.mixotroph.org and links to all the educational and training material will be deposited here and also in an allied YouTube channel. Below, we list a summary of the activities; for further details please also see the Gantt chart in the appendix (Section 11).

**Year 1**

**January 2022:** WG meeting to launch MixONET. This meeting will focus on addressing **ToR1** as follows - collate database list, identify subset of MixONET members to undertake alignment of species to mixoplankton functional group classification within respective databases and publish findings in appropriate open access avenues (**ToR1**, **D1**, Fig.2). At this meeting, a roadmap for MixONET ocean literacy activities will be drafted (**ToR4**).

**June 2022:** WG meeting to set out roadmap for ToR2. Convene a Workshop/Session at the ICOP in Seoul Korea: *Optical methods for mixoplankton identification.* Convene experts (including vendors) in fluorescence microscopy, flow cytometry, laser holographic *in situ* microscopy, video plankton recording, FlowCAM, flowcytobot, cytobuoy, and other optical technologies for a workshop on the pros and cons of different methods. A subset of Working Group members will produce a journal article summarizing the methods and prioritizing them for measurement of mixoplanktonic activities (**ToR2**, **D2**, Fig.2). The MixONET co-chairs will apply to ISOP for funding for this; McManus and Mitra have successfully applied for similar grants from ISOP in the past. The last grant funded by ISOP has led to the development of the Mixoplankton Database (Mitra *et al*. 2021).

**Year 2**

**February 2023**: WG meeting to compile findings from ToRs 1 and 2 to help plan **ToR3** and, also to discuss development of the DST (**ToR4**, **D4, Fig.2**).

**July 2023:** WG meeting and ECR training workshop at CEBIMar (Brazil). This meeting will specifically focus on: *Molecular methods to aid plankton monitoring under the mixoplankton paradigm* such as plastid 23S rDNA and *psbA* gene sequencing to identify kleptoplastidy and other mixoplanktonic-related behaviours. This will result in a summary paper on identification of 'omics methods that can be potentially useful for mixoplankton fieldwork and monitoring (**ToR2**, **D2** and **ToR3**, **D3**, Fig.2). For this meeting, we will seek additional funding from CNPq and FAPESP; we will explore capacity building options via the FAPESP-UKRI Memorandum of Uniderstanding.

**Year 3**

**February 2024**: WG meeting to initiate drafting of the MixONET legacy product – an open access book on biological oceanography methods under the mixoplankton paradigm (**ToRs1-4**; **D5**, Fig.2). This will bring together the outputs from ToRs 1-4 summarizing identification, genetics, isolation, cultivation, and *in situ* sampling for mixoplankton (cf. ICES Zooplankton Methodology Manual).

**August 2024:** WG meeting and training workshop to trial new methods (outputs from **ToR3**). Workshop will be held at the Institute of Marine Science in Burapha University (Thailand) and the Institute will cover costs of hosting, infrastructure and transportation from airport to location.

## 5. Deliverables

**MixONET** will generate a suite of open-source deliverables including data, knowledge and methods for applications in blue-sky research, ocean management and ocean literacy. All the MixONET ToRs, activities and deliverables have been designed to answer global questions through new developments which will advance science. In view of the global impact of the subject of this WG, MixONET brings together, for the first time, a multidisciplinary team with expertise from every field of marine research. The MixONET deliverables can be categorised into four broad outputs which will culminate into a legacy manual. All the members will engage in development and deployment of these deliverables (Fig.2).

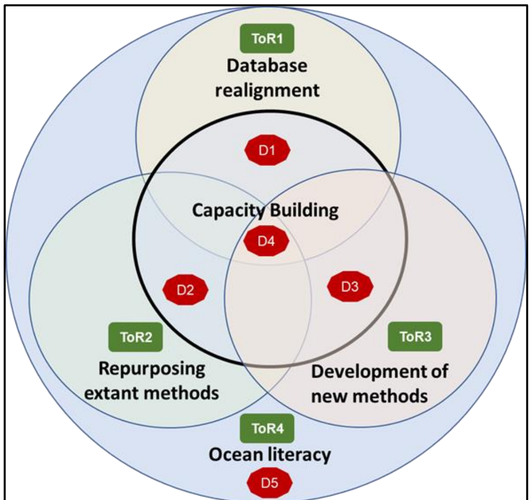


**Fig.2:** Inter-connectivity between ToRs and deliverables; see also, Gantt chart in Appendix.

**D1.** Peer-reviewed open access paper on reappraisal of historic global ocean databases and model outputs in light of the mixoplankton paradigm, including linkages to canonical ocean variables. **[ToR1]**

**D2.** Peer-reviewed open access paper on repurposing and realignment of extant biological oceanographic research methodologies and interpretations for application under the mixoplankton paradigm. **[ToR2]**

**D3.** Technical paper(s) on new method development(s). **[ToR3]**

**D4.** Decision Support Tool: an open access and free to end-user simulation model to help design and conduct laboratory or field experiments to determine mixoplanktonic activity. For example, this DST would help configure studies to determine contributions to mixoplankton growth from phototrophy versus phagotrophy. **[ToR4]**

**D5.** Open access book: *'How to' Guide to Mixoplankton for Biological Oceanography*. This will include realigned as well as new methods and, also, discuss methods such as isolation, cultivation, measurement of phototrophy and ingestion, etc. **[ToRs 1-4]**

## 6. Capacity Building

The global oceans together form the single largest ecosystem on our planet, and ocean life is very much dependent on global anthropogenic activities. We now know that primary and secondary production occur simultaneously and synergistically in mixoplankton. The mixoplankton paradigm thus brings to the forefront the importance of ocean-dwelling organisms which have hitherto been incorrectly labelled as either primary producers or primary consumers. Changes in the environment (e.g., pollution, climate change, ocean acidification) can result in the "good" primary producing mixoplankton being replaced by "bad" mixoplankton (e.g., mixo-HABs), impacting ocean health, biodiversity, food security, recreational activities, property prices etc. It is thus important that we build global capacity for enhancing our understanding of mixoplankton. Over a decade ago, the Leverhulme Trust funded an international network which led to a global effort investigating and reporting the importance of mixotrophic protists. Over the last few years, the European Marie Skłodowska-Curie Actions have funded an Innovative Training Network MixITiN; outputs from this network have shown the importance of synergistic photo+phagotrophic activities in mixoplanktonic species. It is, therefore, now time to build a global team centred on oceanography to implement our understanding of mixoplanktonic activities, impacting primary and secondary production, to global ocean observation and monitoring.

MixONET will address this global capacity-building effort through a range of different activities as listed below.

- All working group meetings except those associated with training workshops in conjunction with international conferences will be held *in silico*. This will enable all full and associate members to participate in the planning and deployment of activities and, also will decrease the carbon footprint of the group.
- One WG meeting, including training workshops for ECRs, are planned to be held in conjunction with an international conference (ICOP Seoul 2022); the organisation, timing and application for funding of this will be guided by Prof McManus (ISOP committee).
- Two other in-person WG meetings are planned to be held in CEBIMar (Brazil) and Burapha University (Thailand) and will include workshops for researchers and stakeholders to enhance regional participation.
- Upon completion of **ToR1** (Yr1), virtual training workshops on data management and data compilation methodologies under the mixoplankton paradigm will be organised in Yrs 2 and 3.
- **ToRs 1 and 2** will directly develop current best-practice manuals for field monitoring providing step-by-step guidance. Potential publishers of these open access manuals include Xenodo, UNESCO Technical Papers in Marine Science, the UNESCO Monographs on Oceanographic Methodology, the IOC Manuals and Guides series etc.
- Benchtop development of new instruments and methods is beyond the scope of this WG. Rather, within **ToR3**, we anticipate an important role of MixONET in evaluating new and emerging technologies and judging which of these are most suitable for development and deployment in the light of the mixoplankton paradigm. This includes ocean observing systems with concomitant *in situ* sensors such as laser holographic or video imaging, optical particle counting, fluorescence induction and relaxation, PAM fluorometry, flowcytobot and cytobuoy, as well as developing molecular techniques such as meta-barcoding, -genomics, and -transcriptomics.
- As part of **ToR4** we will specifically develop educational material targeted for next generation to enhance ocean literacy (these would include a DST, videos, books). Various members of MixONET have extensive experience of public and media engagement. One example would be videos that explain mixoplanktonic activities and ocean food-webs for K-12 education (e.g., https://youtu.be/TjWio2OzXRI). Based on the composition of our WG, we have already identified various languages that we could provide voiceovers for - Arabic, Bengali, Croatian, Hindi, Japanese, Mandarin, Portuguese, Spanish, Swahili, Thai.
- SCOR Visiting Scholarships and POGO-SCOR Visiting Scholarships: Prof McManus has hosted a successful POGO-SCOR Fellow (Dr Luciana Santoferrara) in the past and will support further applications. Dr Mitra has received requests from post-doctoral level researchers (Brazil and Cuba) wanting to apply for these schemes; Cardiff University will be supporting these applications in the

next rounds. There are various other programmes (e.g., NERC UK -FAPESP Brazil, NSF-NERC, GCRF etc.) which the members will explore to further develop the network.

- We will develop various online courses for undergraduate and graduate students. For example, we will develop "crash courses" (short episode of 8-10 min classes) given by the MixONET members in their respective laboratories demonstrating instruments, sampling etc. In addition, we will make available graduate level lectures. For example, Prof McManus has taught the UConn graduate Biological Oceanography course for many years and will make his plankton lectures (recorded during the pandemic) available on the web. This material covers both traditional and mixoplankton paradigmatic views of the ocean food-web.
- The MixONET activities are well aligned with the UN Sustainable Development Goals. The activities would significantly improve the advancement of SDG 14 (Conserve and sustainably use the oceans, seas and marine resources for sustainable development) and consequently other SDGs related to society, environment and innovation. Through MixONET, we will aim to create educational materials that can be used for ocean literacy and provide guidelines towards development of a more sustainable society.

## 7. Working Group Composition

The primary aim of MixONET WG is to identify methodologies to integrate a new paradigm in marine ecology within extant research methods; this mixoplankton-centric paradigm overthrows over 100 years of understanding of biological oceanography. Thus, the team is composed of pan-global multi-disciplinary experts covering global oceans from the Atlantic to Indian and Pacific Oceans. Indeed, the MixONET team includes members from all the continents (except Antarctica). An important aspect of advancing science is integrating knowledge and experience with new recruits and ideas; the WG comprises members from a range of different career stages.

**Table1: Full Members; * indicates Early Career Researchers**

| Name | Gender | Place of work | Expertise relevant to proposal |
|---|---|---|---|
| **Aditee Mitra (Co-Chair)** | F | Cardiff University, Wales, **UK** | Ecosystem modelling, mixoplankton paradigm, project management, public & media engagement |
| **George McManus (Co-Chair)** | M | University of Connecticut **USA** | Plankton ecophysiology – field, laboratory & ’omics |
| **Anukul Buranapratheprat** | M | Burapha University, **Thailand** | Big data, autonomous vehicle, hydrodynamics modelling, physical oceanography |
| **Helga do Rosario Gomes** | F | Lamont-Doherty Earth Observatory, **USA** | mixoplankton physiology, eutrophication, hypoxia, field ecology & oceanography |
| **Robinson Mugo* [PhD: 2011]** | M | SERVIR E&SA/RCMRD, **Kenya** | Satellite oceanography, GIS, fisheries, food security |
| **Kunnatholickal Balakrishnan Padmakumar* [PhD: 2011]** | M | Cochin University of Science and Technology (CUSAT), **India** | Microalgal taxonomy, HAB dynamics study, biological oceanography, higher trophic levels, socioeconomics |
| **Beatriz Reguera** | F | Instituto Español de Oceanografia, | HABs autoecology, population dynamics, mixotrophic cultures |

| | | | |
|---|---|---|---|
| | | Vigo, **Spain** | |
| **Tina Šilović*<br>[PhD: 2012]** | F | CytoBuoy b.v.<br>**The Netherlands** | Field monitoring and development of new applications |
| **Mengmeng Tong** | F | Zhejiang University, **China** | Nutritional dynamics of mixotrophs, allelopathy, marine biotoxins, coastal monitoring, food security |
| **Fernando Unrein** | M | Instituto Tecnológico de Chascomús (UNSAM-CONICET), **Argentina** | Flow cytometry, bacterivory, mixoplankton and phytoplankton eco-physiology, microbial food-web |

**Table2: Associate Members; * indicates Early Career Researchers**

| Name | Gender | Place of work | Expertise relevant to proposal |
|---|---|---|---|
| **Ahmed Al-Alawi*<br>[MSc: 2020]** | M | Marine Science and Fisheries Centre, **Oman** | Phytoplankton blooms, fisheries, aquaculture |
| **Áurea Maria Ciotti** | F | University of São Paulo, **Brazil** | Phototrophy field method, chlorophyll fluorescence |
| **Patricio A. Diaz** | M | Centro i~mar, Los Lagos University, Puerto Montt, **Chile** | Higher trophic Levels, aquaculture, mariculture, public health, socioeconomics |
| **Amany Ismael** | F | Alexandria University, **Egypt** | Phytoplankton succession, Harmful Algae in North Africa (HANA), eutrophication, ballast water introductions |
| **Hae Jin Jeong** | M | Seoul National University, **Republic of Korea** | Phototrophy, phagotrophy, mixotrophy, vital rates, field sampling |
| **Michaela Larsson*<br>[PhD: 2019]** | F | Department of Water and Environmental Regulation (DWER) &<br>University of Technology Sydney, **Australia** | Microalgal taxonomy, mixoplankton physiology and ecology; field monitoring techniques |
| **Maite Maldonado** | F | University of British Columbia, **Canada** | Oceanography, microbial processes, carbon cycle Geotraces, Biogeoscapes |
| **Nikola Medić*<br>[PhD student; 2018- ]** | M | Copenhagen University, **Denmark** | European Climate Pact Ambassador, public & media engagement (Croatian TV, radio & printed matter), mixoplankton ambassador |
| **Tim Smyth** | M | Plymouth Marine Laboratory, England, **UK** | Physical oceanography, remote sensing, ocean colour |
| **Koji Suzuki** | M | Hokkaido University, **Japan** | Oceanic and sea-Ice protists, biogeography, diversity, ecophysiology |

## 8. Working Group Contributions

1. **Aditee Mitra** has been at the forefront of incorporating mixotrophy into plankton ecosystem models. She is one of the main drivers of the mixoplankton paradigm with broad experience in public and media engagement and project management.

2. **George McManus**, a biological oceanographer, brings his years of expertise in field and laboratory methods, including genomics and transcriptomics, to study mixotrophic ciliates. With his experience as an academic, Prof McManus brings a range of pedagogic and project management skillsets.

3. **Anukul Buranapratheprat**, a physical oceanographer, uses hydrodynamic modelling to study phytoplankton succession, including rising mixotrophic blooms in coastal seas under eutrophication. Dr Burapratheprat will also help with the organisation and logistics of the final workshop to be held in Burapha University in Thailand.

4. **Helga do Rosario Gomes,** a biological oceanographer with expertise in mixoplankton ecophysiology, studies the advent of disruptive blooms from climate change and anoxia. Dr do Rosario Gomes brings a wealth of knowledge in emergent marine networks due to climate change.

5. **Robinson Mugo** uses satellite-derived products (temperature, ocean colour, currents) to model migration and ecological interactions of apex level predators such as tuna. His key expertise is GIS applications and spatial analyses.

6. **Kunnatholickal Balakrishnan Padmakumar** is a biological oceanographer with expertise in marine phytoplankton taxonomy and ecology, especially harmful Algal blooms. He is one of the few algal taxonomists in India and therefore, will be a key player in introducing the mixoplankton paradigm into Indian marine research and education.

7. **Beatriz Reguera** is a phytoplankton ecologist focussing on mixotrophic toxin producing HABs and impact on shellfisheries and thence aquaculture, ocean health and biodiversity. Dr Reguera has extensive experience in international UN (IOC, IAEA) training/cooperation programmes as well as engagement with various stakeholders including policy-makers.

8. **Tina Šilović** is an aquatic microbiologist with a long-term expertise in microbial oceanography and flow cytometry. She is currently employed as application scientist in Cytobuoy b.v. and is involved in research and development as well as marketing and communications.

9. **Mengmeng Tong**, a marine phytoplankton ecologist; studies nutritional dynamics of plankton, especially mixoplanktonic organisms (e.g., genera *Dinophysis*, *Prorocentrum*, *Karenia*, *Karlodinium*, and *Mesodinium)*.

10. **Fernando Unrein** is an ecologist with experience in trophic interactions within the planktonic food-webs. He specialises in the use of flow cytometry in aquatic microbial ecology; flow cytometry is widely used in field and laboratory-work of plankton (from fempto to nano- sizes).

For further details please see Table 3.

**Table3: Multidisciplinary expertise and synergistic contributions of the WG Full Members to the different MixoNET deliverables; see also Table1 and Fig.2**

| Full Members | Expertise | | | | | | | | | | | | | | | Deliverables | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mixoplankton | physical oceanography | chemical oceanography | biological oceanogrpahy | HABs | fisheries & aquaculture | socioeconomics | ecophysiology | ’omics | laboratory | field | autonomous & remote | modelling | public & media engagement | project management | **D1: database** | **D2: Repurposing methods** | **D3: new methods** | **D4: DST** | **D5: Legacy** |
| Mitra | ● | | | ● | ● | | | ● | | ● | | | ● | ● | ● | ● | ● | ● | ● | ● |
| McManus | ● | | | ● | | | | ● | ● | ● | ● | | | ● | ● | ● | ● | ● | ● | ● |
| Buranaprathеprat | | ● | ● | | ● | | | | | | ● | ● | ● | ● | | | ● | ● | | ● |
| do Rosario Gomes | ● | | ● | ● | | ● | ● | ● | | ● | ● | | | ● | ● | ● | ● | ● | ● | ● |
| Mugo | | ● | | | | ● | | | | | ● | ● | ● | ● | | | ● | ● | | ● |
| Padmakumar | ● | | | ● | ● | ● | ● | ● | | ● | ● | | | | | | ● | ● | | ● |
| Reguera | ● | | | ● | ● | ● | | ● | ● | ● | ● | | | ● | ● | ● | ● | ● | ● | ● |
| Šilović | | ● | ● | ● | | | | | | | ● | ● | | | | | ● | ● | | ● |
| Tong | ● | | , | ● | ● | | ● | ● | | ● | ● | | | ● | | ● | ● | ● | ● | ● |
| Unrein | ● | | | ● | | | | ● | | ● | ● | | | ● | | | ● | ● | | ● |

## 9. Relationship to other international programmes and SCOR Working Groups

MixONET integrates research questions and methods in biological oceanography with widespread implications related to ongoing international programmes and SCOR WGs.

### 9.1 International Programmes

**GlobalHAB**: this IOC-SCOR programme (2014-), successor of GEOHAB, aims to improve understanding and prediction of HABs in aquatic ecosystems, and management and mitigation of their impacts; e.g., **GlobalHAB symposium** on automated *in situ* observations of plankton (Sweden, 2022). Hae Jin Jeong is a member of the GlobalHAB Scientific Steering Committee (SSC).

**PICES S-HAB:** is an intergovernmental organization that aims to advance scientific knowledge about oceanic processes and impacts of climate change and anthropogenic inputs. Members of S-HAB reports on annual HAB events (HAEDAT) and species mapping (HAB-MAP), develop guidelines for quantifying changes in composition, magnitude and frequency of HAB events in PICES Nations related to expected and measured trends of climate change. Mengmeng Tong is a member of PICES S-HAB.

**IOC REGIONAL HAB Networks** are working groups operating under the auspices of the IOC Intergovernmental Panel on Harmful Algal Blooms (IPHAB, 1992) to identify research and education priorities on HAB related issues, promote training and mutual assistance, compile reports of HAB events and establish databases at a regional level. Beatriz Reguera is a founding member of IPHAB.

**WESTPAC** is the IOC Sub-Commission for the Western Pacific established in 1989 by IOC-UNESCO to pursue the IOC mission in the Western Pacific and adjacent seas. WESTPAC-HAB is the Regional HAB Network for the WESTPAC Region. Their working group 'Rapid Detection Technology for Harmful Algal Blooms (RDT-HAB)’ approved in April 2021 aims to explore, adapt and apply rapid detection technology for HABs in the region. Mengmeng Tong is a member of WESTPAC-HAB.

**HANA**: is the IOC Regional HAB Network for Harmful Algae in North Africa established in 2007. Amany Ismael is the current HANA Chair and in charge of the group website.

We will explore synergies and complementarities between MixoNET and the activities of all these groups.

**9.2 SCOR WGs**

**WG149 COBS:** this WG has closed and is transitioning to an infrastructural project. Áurea Maria Ciotti, a full member of COBS, will help plan the next-steps to progress the findings from MixONET on completion of the ToRs.

**WG156 Active Chlorophyll fluorescence for autonomous measurements of global marine primary productivity:** this WG is working to standardize Chla measurements in primary producers; One of the aims of MixONET is to repurpose established methodologies such as these for mixoplankton productivity. We hope that we can learn from each other through knowledge exchange; Dr Ciotti is a member of WG156.

**WG157 MetaZooGene:** chaired by Ann Bucklin, is working to standardize methods for DNA-based identification of mesozooplankton. In a similar vein, we hope to evaluate how mixoplankton can be quantified in global genetic surveys. Currently, there are no universally accepted genetic markers for mixotrophy, but we hope our Group can facilitate their development through workshops and community discussion (cf. Leles *et al*. 2017).

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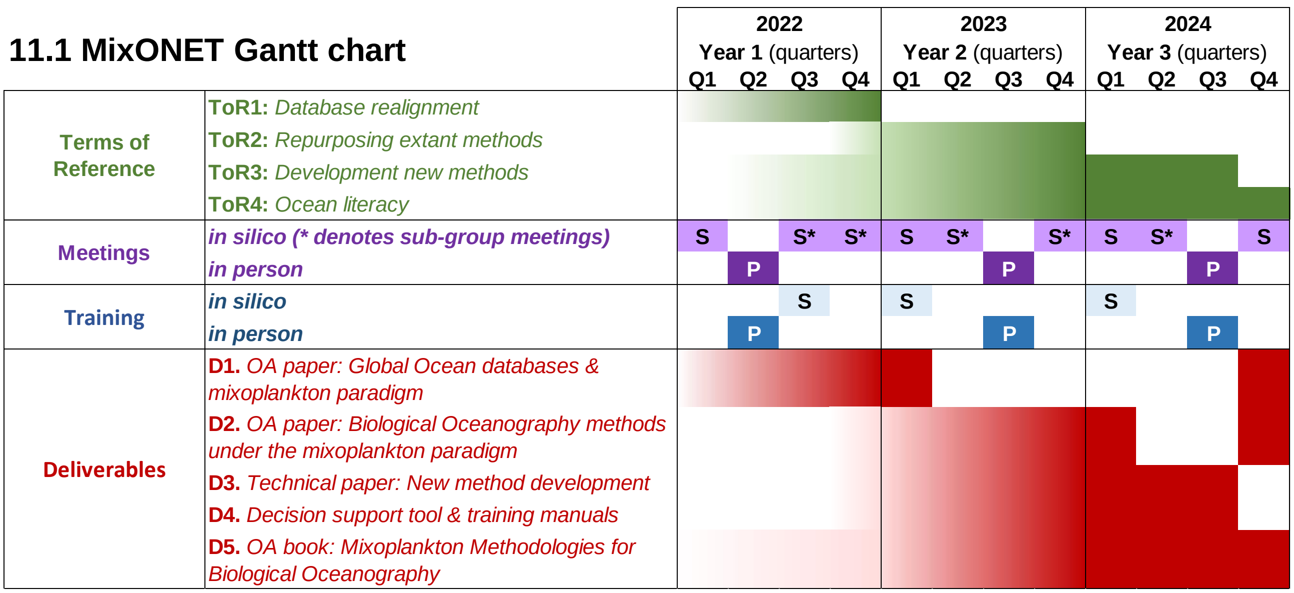
## 11. Appendix

### 11.1 MixONET Gantt chart




### 11.2 List of 5 key publications for each full member

**<u>Aditee Mitra</u> (Co-Chair), Cardiff University UK**

Leles SG, Bruggeman J, Polimene L, Blackford J, Flynn KJ, **Mitra A**. 2021 Differences in physiology explain succession of mixoplankton functional types and affect carbon fluxes in temperate seas. *Prog Oceanogr* **190**:102481 https://doi.org/10.1016/j.pocean.2020.102481

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