SCOR Working Group 165 MixONET

Mixotrophy in the Oceans – Novel Experimental designs and Tools for a new trophic paradigm

1. Brief summary with the main highlights

The overarching aim of MixONET is to propose methods to determine contributions of mixoplankton to primary and secondary productions. MixONET entered the third year in January 2024. This year the WG has focussed primarily on consolidating ongoing works and delivering to the different ToRs (as reported in Sections 3 and 4).

A fantastic achievement from this year has been the addition of, previously ignored, 81 protist species from the **Mixoplankton Database** (MDB) to the World Register of Marine Species (**WoRMS**). This task. overseen by **Stephanie Dekeyzer** (at WoRMS) in discussions with **Mitra**, included allocation of new AphiaIDs to these species new to WoRMS (e.g., *Collozoum ellipsoides*, *Karlodinium azanzae*, *Mesodinium coatsi*). Furthermore, the functional type descriptions of all species listed within the MDB were incorporated into WoRMS; these data can be found within the 'Attributes' tab for each species (e.g., *Karenia brevis*). These functional type classifications from the MDB have also been allocated to the mixoplankton species within the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae; these data are available within 'Sources' and 'Attributes' tabs in the IOC-UNESCO database (e.g., *Karlodinium armiger*).

Over the last MixONET year, various members have contributed to the research community and society through publications (details provided in **Section #3**), plenary lectures at international conferences (**Mitra** and **Santoferrara**), delivering in-person and online teaching modules to national and international audiences comprising UG-PG students as well as professionals (**Ciotti** and **Reguera**).

The MixONET team has also started planning a legacy course on mixotrophy for the Ocean Teacher Global Academy to be finalised in 2025.

2. Activities since previous report to SCOR

(1) WG meetings: this year all the MixONET WG meetings were held online (via Zoom) taking into account the differences in time zones. Between September and November 2023, monthly meetings were held (on 12 Sept, 10 Oct and 14 Nov 2023) to aid progress of the different deliverables. During these meetings various task-and-finish (T&F) groups were established to work on the different deliverables; all full and associate MixONET members were provided the opportunity to join these T&F groups. At the meeting in November 2023, it was decided that the T&F sub-groups needed dedicated and frequent meeting times. It was further decided that it was unnecessary to have monthly all member WG meetings, instead these would be held at 3-6 month intervals where the whole group would be provided summary updates from each of the T&F groups; following this all member Zoom meetings were held on 12 Mar and 11 Jun 2024.

(2) Task-and-Finish (T&F) group meetings: there are currently nine T&F subgroups working towards the different ToR deliverables (listed in #4 below). The leads of these groups arrange meetings as and when required (ca. at intervals of 2-8 weeks) to progress the required tasks. They also provide updates at the all member WG meetings.

(3) Invited presentations:

(a) Mitra gave **a plenary lecture** at the 4th International Conference of Community Ecology held in Trieste, Italy (20-22 September 2023). Results from the development and deployment of an acquired phototrophy simulator was presented at this conference; this work has been published as an OA article in the Special Issue within the journal of Community Ecology (Mitra 2024). Both the presentation and the paper acknowledged the MixONET SCOR WG.

(b) Santoferrara gave an **invited presentation** at the 7th International Zooplankton Production Symposium held in Hobart, Australia (17-22 March 2024). The talk included discussions on the use of the **Mixoplankton Database** (MDB) on protist datasets and acknowledged the MixONET SCOR WG.

(5) Reguera attended an Ad hoc International Workshop on Dinoflagellate Cysts hosted by the University of Vigo, Spain (18-21 June 2024). In a round table discussion chaired by Reguera, attention was drawn to the palynologists' dichotomy in the classification of cyst producing dinoflagellates into 'autotrophs' and 'heterotrophs'. The former are mixotrophs and the later are no longer considered microalgae, but protozooplankton; Reguera noted that the term 'mixotroph' appeared in only one title. In their models, palynologists assume that the number of heterotroph (e.g., *Protoperidinium*) cysts is proportional to the primary production of diatoms they feed upon in upwelling systems. Reguera introduced them to the updated functional groups described in the MixONET website and in the recent marine 'Mixoplankton Database', and commented on the oversimplification of their conceptual trophic chains. A summary of the meeting has been submitted to Harmful Algae News for publication in the upcoming issue #76.

3. Documents published since previous report to SCOR

Publications listed chronologically and within each year alphabetically. All these publications resulted directly from WG related activities and acknowledge SCOR WG MixONET. The MixONET WG members are indicated in **bold font.**

2023 publications:

Díaz PA, Figueroa RI (2023) Toxic algal bloom recurrence in the era of global change: Lessons from the Chilean Patagonian fjords. *Microorganisms* 11:1874. https://www.mdpi.com/2076-2607/11/8/1874.

McManus G, Mitra A, Reguera B, Santoferrara L (2023) The Mixoplankton paradigm in plankton ecology. In *Harmful Algae News*. Editors: B Reguera, E Bresnan. 73: 15-18. https://doi.org/10.5281/zenodo.8406489

Mitra A, Leles SG (2023) A revised interpretation of marine primary productivity in the Indian Ocean: The role of mixoplankton. In: *Dynamics of Planktonic Primary Productivity in the Indian Ocean.* Editors: SC Tripathy, A Singh. Springer, Cham. https://doi.org/10.1007/978-3-031-34467-1_5

2024 publications:

Díaz PA, Álvarez G, Schwerter C, Baldrich ÁM, Pérez-Santos I, Díaz M, Araya M, Nieves MG, Rosales SA, Mancilla-Gutiérrez G, Arratia C (2024) Synchronic distribution of the dinoflagellate *Protoceratium reticulatum* and yessotoxins in a high stratified fjord system: Tidal or light modulation? *Harmful Algae* 135:102649. https://doi.org/10.1016/j.hal.2024.102649

Baldrich AM, **Díaz PA,** Rosales SA, Rodríguez-Villegas C, Álvarez G, Pérez-Santos I, Díaz M, Schwerter C, Araya M, **Reguera B** (2024) An unprecedented bloom of oceanic dinoflagellates (*Karenia* spp.) inside a fjord within a highly dynamic multifrontal ecosystem in Chilean Patagonia. *Toxins* 16: 77. https://doi.org/10.3390/toxins16020077

Mitra A (2024) Importance of dynamics of acquired phototrophy amongst mixoplankton; a unique example of essential nutrient transmission in community ecology. *Community Ecology* https://doi.org/10.1007/s42974-024-00202-9

Reguera B, García-Portela M, Velasco-Senovilla E, Rial L, Escalera P, **Díaz PA,** Rodríguez F (2024) *Dinophysis*, a highly specialized mixoplanktonic protist. *Frontiers in Protistology*. 1:1328026. https://doi.org/10.3389/frpro.2023.1328026

Rodríguez F, Escalera L, **Reguera B,** Nogueira E, Bode A, Ruiz-Villarreal M, Rossignoli AE, Ben-Gigirey B, Rey V, Fraga S (2024) Red tides in the Galician rías: historical overview, ecological impact, and future monitoring strategies. *Environmental Science: Processes & Impacts* 26: 16-34. https://doi.org/10.1039/D3EM00296A

Sixto M, Riobó P, Rodríguez F, **Díaz PA**, Figueroa RI (2024) Climate Change stressors, phosphate limitation, and high irradiation interact to increase *Alexandrium minutum* toxicity and modulate encystment rates. *Microorganisms* 12: 1480. https://doi.org/10.3390/microorganisms12071480

4. Progress toward achieving group's terms of reference (ToR).

ToR1 Biological Oceanography Databases And The Mixoplankton Paradigm

#1.1 The **Mixoplankton Database** (Mitra et al. 2023) has been applied to Indian Ocean protist plankton species. This work has been published as an OA book chapter (Mitra & Leles 2023; publisher: Springer).

#1.2 The **Mixoplankton Database** (MDB) highlighted the absence of 81 marine protist plankton species within the World Register of Marine Species (**WoRMS**) database. These species have now been added to WoRMS and new AphiaIDs allocated (e.g., *Collozoum ellipsoides*, *Karlodinium azanzae*, *Mesodinium coatsi*). Furthermore, the functional descriptions of all mixoplankton species within MDB were incorporated into WoRMS; these data can be found within the 'Attributes' tab for each species (e.g., *Karenia brevis*). These tasks were overseen by **Stephanie Dekeyzer** (at WoRMS) in discussions with **Mitra**. WoRMS is the most used public, global database for marine species.

#1.3 The functional type classifications from the MDB have also been allocated to the mixoplankton species within the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae; these data are available under 'Sources' and 'Attributes' tabs in the IOC-UNESCO database (e.g., for *Karlodinium armiger*).

#1.4 Work is ongoing to realign trophic strategies for the different microalgae in the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae database according to the MDB, making linkages to the PR2 database (**#1.5**, ToR1) and, also documenting various attributes and methodologies associated with study of HAB species (**#2.1**, ToR2).

#1.5 Santoferrara is working with the PR2 team to incorporate missing mixoplankton into the PR2 database of ribosomal genes and to revise the protist plankton functional traits g to the MDB.

#1.6 Work is ongoing to realign trophic strategies within the Long Island Sound phytoplankton monitoring database according to the MDB, making linkages to human activities.

ToR2 Repurposing Extant Methods

#2.1 A T&F group is collating methodologies used to study HAB species. This work is being undertaken in conjunction with task **#1.4** (ToR1).

#2.2 Díaz and **Reguera** have investigated how environmental stressors drive harmful blooms of mixoplanktonic microalgae through various field studies (see list of papers co-authored and/or led by Díaz and Reguera in 2023 and 2024).

#2.3 A T&F subgroup is analysing the sequence data available in the metaPR2 database under the mixoplankton paradigm.

ToR3 Development of New Methods

#3.1 Transfers of energy and nutrients from producers to consumers are fundamental to ecosystem structure and functioning. A highly specialised example is the transmission of acquired phototrophy between certain plankton. The MDB lists >250 species of marine plankton that exploit acquired phototrophy; the *Teleaulax-Mesodinium-Dinophysis* (TMD) trinity is the most studied complex. In the TMD-trinity, plastids and nuclear material produced by the cryptophyte *Teleaulax* are transferred during feeding to the ciliate, *Mesodinium* and these acquired plastids are subsequently transferred from *Mesodinium* to its predator, the dinoflagellate *Dinophysis*. These plastidic non-constitutive mixoplankton, *Mesodinium* and *Dinophysis*, are globally ubiquitous and ecologically important organisms. *Mesodinium* can form red-tide blooms, while *Dinophysis* spp. cause diarrhetic shellfish poisoning events and shellfisheries closures (see works by **Díaz** and **Reguera**). For the first time, the implications of the transmission dynamics of acquired phototrophy for the success of the TMD-trinity were explored under different nitrogen and phosphorus (N:P) nutrient ratios and loadings (eutrophic, mesotrophic, oligotrophic) using a newly developed model to describe acquired phototrophy for *Mesodinium* and *Dinophysis*. This work (Mitra 2024) highlights the need to enhance our understanding about how environmental stressors arising from anthropogenic activities (including climate change) will impact transference of acquired phototrophy between trophic levels and thence marine biodiversity and ecosystem services.

#3.2 Traditionally species belonging to the *Prorocentrum* genus have been considered to be phytoplankton engaging in primary production only. Recently, Tillmann et al. (2023) showed that the common and well-studied *Prorocentrum cordatum* and a newly identified species *P. pervagatum* can make mucus traps and deploy these to capture prey as well as remove competitors. This study, involving **Larsson** and **Mitra**, used a suite of methodologies including microscopy video observations, laboratory feeding experiments and system dynamics modelling to explore the ingestion rates and prey capture capabilities of these mixoplankton species.

#3.3 A PG Masters student, Josiah Gryzwacz, working under the supervision of **McManus**, used the novel Fluorescence Induction and Relaxation (FIRe) method to measure quantum efficiency in chloroplasts retained by a ciliate from its food. This work is in revision for a special section on mixoplankton in the *Journal of Plankton Research* for which McManus will be guest co-editor. The manuscript by Gryzwacz, Gorbunov & McManus is entitled Quantum efficiency of chloroplasts retained from food by the ciliate *Strombidium rassoulzadegani* and is currently under revision.

#3.4 An article reviewing the usefulness of utilizing the increase of *Mesodinium* populations as an early warning indicator for the advent of their potential predator, the HAB pSNCM *Dinophysis acuminata* has been authored and submitted by **Díaz**, **Reguera** et al. to the Journal of Plankton Research Special Issue.

#3.5 Laboratory experimental data have been integrated with the *Teleaulax-Mesodinium* food web simulator (developed in Mitra 2024) to study the contribution of phototrophy versus phagotrophy to carbon fixation by the globally ubiquitous species *Mesodinium*. This work has been undertaken by PhD students under the aegis of **Tong** and **Mitra**; manuscript currently in preparation.

ToR4 Ocean Literacy and Capacity Building

#4.1 A draft structure of the OTGA course, including 6 modules, has been developed. Six T&F subgroups associated with each of the modules have been established. Each of these groups are currently working on module outline structure and content.

#4.2 Ciotti has actioned the inclusion of the materials associated with the deliverables listed within www.mixotroph.org (home for MixONET) as reading material for the course CBM0140 - An introduction to marine plankton, offered for undergraduate programs on Biological Sciences of São Paulo University (Brazil).

#4.3 Over the last year, **Reguera** delivered various online teaching courses (**#4.3.1** & **#4.3.2**) on the topic of mixotrophy and mixoplankton making reference to works associated with the MixONET ToRs, that have been and are currently being undertaken.

#4.3.1 *International Course on Taxonomy and Ecology of HAB-forming Microalgae* (7, 14, 21 and 28 October 2023) organised by Fitoland-Perú, a Latin American platform organizer of training courses online. Groups of 20 postgraduate students and professionals attended these modules in order to upgrade their knowledge base and skills. Reguera delivered the following modules:

- Marine Phytoplankton. Generalities and classification criteria. Morphological, physiological and life cycle traits.
- Taxonomy of toxigenic species of *Dinophysis*. Intraspecific variability related to feeding behaviour, cell-cycle phases and life-cycle stages.
- Sampling strategies to study rare and/or patchily distributed protists
- Practical guide for the unambiguous identification of the causative agents of shellfish poisoning events.

#4.3.2 *Postgraduate Hybrid course on Phytoplankton, Algal Blooms and Phycotoxins* (15-26 Jan 2024) organised by the Academic Unit of Mazatlán, National Autonomous University of Mexico (UNAM). Reguera delivered the following modules:

- Morphological and toxinological characterization and functional traits of toxic species of the genus *Dinophysis*. Monitoring Implications.
- Cultivation of microalgae of the genus *Dinophysis*.

5. WG activities planned for the coming year.

Below, is a list of tasks we aspire to undertake between now and end of 2025:

#5.1 Harmful Algal Bloom (HAB) species under the mixoplankton paradigm (Tasks #1.4 & #2.1, Section 4): creating a database of known HAB species including information pertinent to ecosystem functioning. The article will also collate methodologies used to study HAB species. This work stemmed from the initial efforts to assign the revised functional group classifications to the species recorded within the

IOC-UNESCO Taxonomic Reference List of Harmful Microalgae. This IOC-UNESCO database has 168 species. Two T&F groups have been established to complete these deliverables.

#5.2 Protist plankton genetic database under the mixoplankton paradigm (Task #1.5, Section 4): MixONET WG members and members from the PR2 team are updating and revising the protist plankton functional traits within the PR2 reference database of ribosomal genes according to the mixoplankton paradigm with reference to the MDB.

#5.3 Long Island Sound Database under the mixoplankton paradigm (Task #1.6, Section 4): the different species within this 8-year phytoplankton monitoring dataset (US National Estuaries Program) is being aligned to the MDB functional type (FT) descriptors. The aim is to investigate whether human activities (e.g., sewage and fertilizer runoff) leading to hypoxia result in interannual differences in the appearance of FTs.

#5.4 Global distribution of protist plankton functional groups in the metaPR2 metabarcode database (Task #2.3, Section 4). A T&F MixONET subgroup is collaborating with various MDB authors and metaPR2 team to analyse the metaPR2 data. A manuscript is under development.

#5.5 Development and delivery of OTGA course on mixotrophy under the mixoplankton paradigm (Task #4.1, Section 4): The aim is to develop and deliver a full course on 'mixotrophy in marine ecology under the mixoplankton paradigm'. The aspiration is to develop this course in the medium of English, if feasible possible, at least one other language (e.g., Spanish, Chinese) by taking advantage of the language skills of the WG members. We have drafted a plan for this course and set up six T&F subgroups to work on the six modules. The T&F groups are currently working on content and outline for each module. The plan is to develop the basic skeleton of the course via online meetings and then spend a 'retreat' week working on finalising content for this course. MixONET has various members who have experience in UG and PG teaching. We are also fortunate to have two WG members who have been involved in delivering OTGA courses in the past. Santoferrara has been associated with development of a module on mixoplankton within a microbial ecology OTGA course which was developed and delivered in Spanish. Reguera has decades of experience in developing and delivering courses on Harmful Algal Blooms taxonomy, monitoring and management in the framework of the IOC-IEO Science and Communication Centre on Harmful Algae in Vigo and other IAEA and FAO initiatives. As most of the MixONET WG members involved in the delivery of this OTGA course are on academic contracts, the in-person meeting is scheduled to be held in March 20205 (outside of academic term time).

6. Is the group having difficulties expected in achieving terms of reference or meeting original time schedule?

No

7. Any special comments or requests to SCOR.

In March 2024 we requested an extension of at least 12 months till end of 2025 so that we can deliver the tasks (**#5.1-#5.5**) listed in Section 5 (above). We are very grateful to have received this extension.

Additional information can be submitted and may be posted at the SCOR Annual Meeting webpage at the discretion of the SCOR Executive Committee Reporter for the WG and the SCOR Secretariat.