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 (200-300 words)

The overarching aim of MixONET is to integrate the mixoplankton paradigm with traditional and novel methods of plankton research. Results will provide tools for (i) predicting the response of the ocean's biological communities and element cycles in the face of ongoing climate change, and, (ii) better understanding about how humanity can maintain healthy sustainable oceans. MixONET entered the second year in January 2023. Major highlights include publication of the first ever comprehensive Mixoplankton Database (MDB) [ToR1] and delivery of a special session – *The New Paradigm Testing the Resilience of Our Science in the UN Ocean Decade* - at the ASLO Aquatic Sciences meeting 2023.

Santoferrara [USA] was awarded a grant from the "Exceptional" Call for SCOR Project and Working Groups Scholars to work with Unrein [Argentina]. Santoferrara's 12-day visit to Argentina included laboratory work at the INTECH institute and the UNNOBA University, both in the province Buenos Aires, a talk for the INTECH Waters research group, and knowledge exchange with graduate students. The goal of the project was to design and test a methodology to isolate and identify nano-mixoplankton, using a combination of feeding experiments, flow cytometry and DNA metabarcoding [ToRs2&3]. Several experiments were carried out between February and March 2023. The mixoplankton were successfully quantified, sorted and sequenced, obtaining promising results.

As part the ocean literacy and capacity building aspirations of MixONET [ToR4] Santoferrara delivered a 'Mixoplankton' module within the 2 week 'Marine Microbiology and Biogeochemistry' OTGA course in the medium of Spanish while Mitra [UK] hosted a POGO-SCOR funded Fellow, Sangeeta Naik [India] for 8 weeks from 30th January 2023. The POGO-SCOR Fellowship training programme was designed such that the capacity-building at Goa University could be implemented directly, both for research and for the upskilling of PG students, as a direct output of this effort.

2. Activities since previous report to SCOR (e.g., virtual or in-person meetings, email discussions, special sessions). Limit 1000 words

- All working group meetings (Sept 2022 – June 2023) have been held via Zoom this year.
- Following the meeting in Baiona (June 2022), various sub-groups were set up to address the tasks and deliverables associated with the different ToRs. These subgroups have been discussing progress via emails and via online meetings. As an example, various Zoom meetings have been held to identify items for the applications of the MDB with respect to the Biotoxin, IOC-UNESCO and metaPR2 databases. We also took the opportunity to discuss and progress various tasks through small-group in-person meetings at the ASLO Aquatic Sciences Meeting 2023.
- WG member Santoferrara [USA] applied for and received funds from the "Exceptional" Call for SCOR Project and Working Groups Scholars to work with WG member Unrein [Argentina]. Santoferrara traveled to Unrein's lab in Chascomús (Argentina) in March 2023 with the SCOR seed-corn funding, to perform experiments associated with ToRs 2&3. The 12-day visit included laboratory work at the INTECH institute and the UNNOBA University, both in the province Buenos Aires, Argentina. There was also a talk delivered to the INTECH Waters research group,

and knowledge exchange meetings with graduate students. A total of four experiments were performed. Flow cytometry cell sorting were carried out in collaboration with Romina Schiaffino from UNOBA, Junín (Argentina). DNA of sorted cells was extracted and sequenced. The mixoplankton were successfully quantified, sorted and sequenced, obtaining promising results. Funding to conduct further experiments is needed to validate the methodology. The full report of this activity is available here.

- The results obtained by Santoferrara and Unrein will be presented as a poster in the ISME-LAT Congress to be held in Buenos Aires, Argentina from August 7 to 10, 2023. Ref: Unrein, F., L.F. Santoferrara & R. Schiaffino. Testing a new method for quantifying mixoplankton in natural water samples. Buenos Aires (Argentina).
- WG co-chair Mitra [UK] hosted a POGO-SCOR funded Fellow, Sangeeta Naik [Goa University, India] for 8 weeks from 30th January 2023. The aim of the fellowship was to aid Goa University's aspiration towards capacity-building through implementation of system dynamics (simulation) modelling within their PG teaching modules. As the Fellow had no prior experience of modelling, the training programme focused on exercises from two open access books – 'Dynamic Ecology' and 'A simple N-based mixoplankton model'; both of these books have been successfully used for training senior school (16+ year old) pupils, undergraduate students and at international graduate training workshops. The training delivered over the 8 week period thus aimed to provide the Fellow (Naik) an appreciation of the challenges of simulation modelling of plankton both for research and for the upskilling of PG students at Goa University. Dr Naik's testimonial on her experiences as a POGO-SCOR Fellow at Cardiff University is available here.
- MixONET delivered a special session at the ASLO Aquatic Sciences 2023 meeting. The session entitled – *The New Paradigm Testing the Resilience of Our Science in the UN Ocean Decade* - comprised 12 oral presentations and 1 poster. The session focussed primarily on bridging the gap between blue-sky research and applied work. The contributions to our session were excellent. The session was very well attended and received congratulatory comments including from the current ASLO President Prof Patricia Glibert and ASLO President-elect Prof Susanne Menden-Deuer. Further details about this session can be obtained here.

3. Documents published since previous report to SCOR (e.g., peer-reviewed journal articles, reports, Web pages) and should be limited to publications that resulted directly from WG activities and which acknowledge SCOR support

List of publications acknowledging SCOR support; MixONET WG members indicated using **bold** font:

(1) Endo H, Umezawa Y, Takeda S, **Suzuki K** (2023) Haptophyte communities along the Kuroshio current reveal their geographical sources and ecological traits. *Molecular Ecology* 32:110-123 https://doi.org/10.1111/mec.16734

(2) **Mitra A**, Caron DA, Faure E, Flynn KJ, Leles SG, Hansen PJ, **McManus GB**, Not F, **Gomes HdoR**, **Santoferrara LF**, Stoecker DK, Tillmann U (2023) The Mixoplankton Database (MDB): Diversity of photo-phago-trophic plankton in form, function, and distribution across the global ocean. *Journal of Eukaryotic Microbiology* 70:e12972. https://doi.org/10.1111/jeu.12972

(3) **Mitra A**, Flynn KJ (2023) Low rates of bacterivory enhances phototrophy and competitive advantage for mixoplankton growing in oligotrophic waters. *Scientific Reports* 13:6900 https://doi.org/10.1038/s41598-023-33962-x

(4) **Mitra A**, Flynn KJ, Stoecker DK, Raven JA (2023) Trait trade-offs in phagotrophic microalgae: the mixoplankton conundrum. *European Journal of Phycology* https://doi.org/10.1080/09670262.2023.2216259
(5) Tillmann U, **Mitra A**, Flynn KJ, **Larsson ME** (2023) Mucus-Trap-Assisted Feeding Is a Common Strategy of the Small Mixoplanktonic *Prorocentrum pervagatum* and *P. cordatum* (Prorocentrales, Dinophyceae). *Microorganisms* 11:1730 https://doi.org/10.3390/microorganisms11071730
(6) Yokoichi K, **Suzuki K**, Horiguchi T (2022) Comparative analyses of nutritional strategies among the species within the genus *Paragymnodinium* (Gymnodiniales, Dinophyceae). *Journal of Phycology* 58:490-501 https://doi.org/10.1111/jpy.13253

4. Progress toward achieving group's terms of reference. List each term of reference separately and describe progress on each one. Limit 1000 words

ToR1 Biological Oceanography Databases And The Mixoplankton Paradigm

• Mixoplankton Database (MDB) published (Mitra et al. 2023 in Journal of Eukaryotic Microbiology). The paper is open access and can be downloaded directly from the journal. This database has been applied to Indian Ocean species; this work (Mitra & Leles) is currently in press as an OA book chapter (publisher: Springer).

• MDB functional types have been incorporated into the PR2 database. This is the most used public, global database for annotation of protist plankton DNA sequences.

• Metabarcoding work from MDB was presented at ASLO2023 by Santoferrara. Further work on metaPR2 data is in process to investigate global distribution of diverse mixoplankton types in the global ocean.

• Following the publication of the MDB, a sub-group of WG members are working on the IOC-UNESCO HAB list realigning the trophic strategies for the different microalgae in this database, making linkages to the metaPR2 and biotoxin databases and, also documenting various attributes (e.g., life traits, distribution etc.). The aspiration is to develop a metadatabase providing: (i) information related to various marine monitoring and policy strategic directives (e.g., MSFD for Europe) and (ii) ocean indicators (e.g., EOVs, ECVs).

ToR2 Repurposing Extant Methods

• Work on manual including guides for setting up plankton laboratory has been started following in from discussions at our 2022 meeting in Baiona. At this meeting we discussed plans for technical documents that would serve to introduce new researchers to methods used in mixoplankton research and also assist early-career scientists in setting up a new laboratory for mixoplankton study. Co-chair McManus has created an outline for a methods manual that would be organized according to the relevant organisms (Rhizaria, Ciliates, Dinoflagellates, nanoflagellates, etc.), as represented by the expertise of various SCOR group members), with sections on field methods (sampling, preservation, quantification, etc. and lab methods (isolation, cultivation, etc.). Our goal is to complete the manual within the next year.

• Collation of different mixoplankton vital rates is ongoing with an aim to list different methodologies used to measure these in field as well as laboratory experiments.

ToR3 Development of New Methods

• Funded through the SCOR Exceptional funding call, this year Santoferrara and Unrein worked on the development of a new methodology for the routine study of mixoplankton. The method is based on the combination of ingestion of fluorescent preys and flow cytometry cells sorting that would allow the

quantification and identification of mixoplanktonic nanoflagellates. Several experiments were carried out in Argentina between February and March 2023. The mixoplankton were successfully quantified, sorted and sequenced, obtaining promising results. Further experimentation for method validation is required; Santoferrara and Unrein are exploring funding opportunities. Further details are available here.

• Preliminary experiments have been undertaken for deriving photosynthesis parameters using the Walz PhytoPAM II. Further experiments for new method development to be undertaken in final quarter of 2023. This work is being undertaken in Cardiff University under the aegis of co-chair Mitra in collaboration with WG member Tong.

ToR4 Ocean Literacy

• WG member Santoferrara [USA] prepared and delivered a 'Mixoplankton' module within a 2 week 'Marine Microbiology and Biogeochemistry' training course hosted by the Ocean Teacher Global Academy (OTGA); the course is in the medium of Spanish.

• WG member Gomes [USA] reported that their team is currently in the process of constructing a decision support tool - *Decision and Information System for the Coastal waters of Oman* (DISCO) - for detection of *Noctiluca* blooms in Oman. DISCO will provide real-time forecasts of atmospheric and sea state conditions using outputs from an atmosphere/ocean/biogeochemical coupled model tailored for the coastal waters of Oman, and provide real-time forecasts of outbreaks of blooms of the mixoplankton *Noctiluca scintillans* based on a fusion of model outputs and satellite ocean colour data.

• *Capacity building*: co-chair Mitra [UK] hosted a POGO-SCOR funded Fellow, Sangeeta Naik, from Goa University (India) for 8 weeks from 30th January 2023. The Fellowship training programme was designed such that the capacity-building at Goa University could be implemented directly as an output of this effort. The training delivered thus aimed to provide Dr Sangeeta Naik with an appreciation of the challenges of simulation modelling of plankton both for research and for the upskilling of PG students at Goa University. Accordingly, Dr Naik was provided training using two open access books - 'Dynamic Ecology' and 'A simple N-based mixoplankton model'. Both these books have been used for training undergraduate students in the UK as well as at international graduate training workshops. Dr Naik's work plan during the Fellowship included various modelling exercises from the Dynamic Ecology book (Flynn 2018) and also step-by-step training on replicating the phytoplankton sub-module within the simple N-based mixoplankton food-web model book (Flynn & Mitra 2021). In summary, the Fellowship training focussing on exercises from these two open access books should aid Goa University's aspiration towards capacity-building through implementation of system dynamics (simulation) modelling within the PG teaching modules in Goa University. Dr Naik's testimonial is available here.

• *Outreach*: WG member Gomes [USA] supported various summer high school interns at the Lamont-Doherty Earth Observatory Columbia Climate School. Some highlights from these include New York High Schooler, Leysha Steves, who researched the 'Ingestion of plastics by the mixoplankton *Noctiluca scintillans*', received the Stockholm Junior Water Prize and represented New York State at the National Junior Water Prize in Colorado. Another intern, Leonah Steves, working on 'The effects of hypoxia and low pH on the mixoplankton *Noctiluca scintillans*' received the Association for Women Geoscientists Award, the Creative Approach to Research Award and the 3rd place at the Regeneron Westchester Science & Engineering Fair, NY.

5. WG activities planned for the coming year. Limit 500 words

- Regular monthly meetings have been time-tabled (second Tuesday of every month for 45 min) to help progress the different ToRs and deliverables. All WG Members have been sent a Zoom calendar invite for these meetings; the first meeting is scheduled to be held on 12th September 2023.

- Further sub-group meetings to be scheduled by each subgroup chair as required for each task and deliverable.
- If possible, the aspiration is to hold an in-person meeting coupled with a capacity-building workshop in 2024.
- Various WG members are keen to work on a free OA course on 'Mixoplankton and Mixotrophy' for OTGA. As a first step, contact will be made with OTGA to ascertain whether there would be interest in such a course.
- The WG also has an aspiration to develop manuals/guides on protocols for sampling rare/delicate protists, laboratory experiments to determine vital rates.

6. Is the group having difficulties expected in achieving terms of reference or meeting original time schedule? If so, why, and what is being done to address the difficulties Limit 200 words

Not applicable

7. Any special comments or requests to SCOR. Limit 100 words.

None at present.

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.