OCB working group: Driving the direction of future mixotrophic research

Working Group Participants

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Scientific Summary

Plankton have traditionally been categorized using a false dichotomy of either heterotrophs (strict phagotrophs) or autotrophs (strict phototrophs). It is now accepted that a large number of species fall on a spectrum between the two trophic strategies, and are actually a type of plankton known as mixotrophs (Flynn et al. 2013; Mitra et al. 2014; Stoecker et al. 2017). There is general consensus that a wide range of research is needed on the topic of mixotrophy, but there is limited effort to attempt most of the necessary research. To date, the majority of mixotrophic research has focused on how grazing by individual constitutive mixotrophs - species that have their own photosynthetic machinery and supplement with ingestion - respond to changes in environmental conditions, such as light or nutrient availability (Li et al. 2000; McKie-Krisberg et al. 2015; Millette et al. 2016). This work has expanded our understanding of the different types of mixotrophs and created a growing list of mixotrophic species. However, now that it is clear that mixotrophs account for a substantial portion of the plankton community, future research should transition to studying mixotrophs as their own group, similar to how we study phyto- and zoo-plankton, in order to understand mixotrophs' role in the marine food-web and ecosystem (Millette et al. 2018).

The ability to conduct *in situ* research on mixotrophs has been hindered by the limitations of current popular methods for detecting mixotrophy in the field (Anderson et al. 2017; Wilken et al. 2019). The most common approach to studying mixotrophs is through the use of fluorescent microspheres, fluorescently labeled bacteria, or fluorescently labeled algae (Cynar and Sieburth 1986; Li et al. 1996; Sherr et al. 1987). The application of all these methods *in situ* is the same: estimate the abundance of active mixotrophs and measure their ingestion rates of fluorescently labeled material cell-by-cell (Domaizon et al. 2003; Sanders et al. 1989; Unrein et al. 2007). Other methods have been used to study mixotrophs, but their efficacy remains unclear. These methods include Brd-U labeled bacteria (Gast et al. 2018), ^{14}C (Adolf et al. 2006), lysotracker and flow cytometry (Anderson et al. 2017), qPCR (Gast et al. 2014), gene expression (Liu et al. 2016), stoichiometry (Moorthi et al. 2017), and imaging with cell-staining (Brownlee et al. 2016). Currently, there is a lack of consensus in the oceanography community on the appropriate use of fluorescent prey to study mixotrophs, and little direction for new method development. As a result, *the study of mixotrophs has not yet reached its full potential*.

Theoretical models and synthesis papers have attempted to fill the vacuum created by the lack of new and innovative *in situ* mixotrophy research. Food web and ecosystem modelling have demonstrated the substantial impact mixotrophy can have on ecosystem function when included in simulations. For example, global carbon transport and sequestration are altered when models allow mixotrophy as a trophic strategy in the plankton (Mitra et al. 2014, Ward and Follows 2016). Modelling efforts have helped observational and experimentally driven oceanographers develop testable hypotheses, and highlighted the data needed to ground-truth model outputs. The bottom line: *the scientific community is positioned to make major advancements in mixotrophy research, but first we must reach a consensus on how to best use the current available methods for mixotrophy*

research, collaboratively define new paths forward for methodological development, and put forth a proposed list of the most pressing mixotrophy research needs.

Objectives and Products

The ultimate goal of this working group is to *bring together experts in order to outline a clear set of top priority mixotrophy research needs and identify methodological gaps in achieving these needs*. There is widespread agreement among the oceanography community that mixotrophs are a high research priority, but currently there is a limited number of large-scale projects focused on mixotrophy. One of the reasons for the lack of necessary funded projects is prevailing distrust of current methods being used to study mixotrophs *in situ*. Our working group will consist of scientists who have studied the *in situ* mixotrophic community using a range of methods, modelers who are interested in incorporating mixotrophs into their models, and scientists who are interested in participating in future mixotrophic research. This will allow scientists with a diversity in research goals and expertise to provide input in the group's final products. We have outlined four specific objectives to achieve the working group's overall goal:

1. **Synthesize currently available data on *in situ* mixotrophic activity/presence and the methods used in peer-reviewed literature**. There have been a few, small scale studies that have studied *in situ* temporal and/or spatial variability in mixotrophs as a group (Table 1). These publications provide a starting point to begin synthesizing currently available *in situ* mixotrophy data, identifying major research gaps, and discussing the potential and shortcomings of the methods utilized to conduct mixotrophy research. All of the participants in the working group will be encouraged to contribute to a literature search, using the papers in Table 1 as a starting point. Interested participants will then work on a paper that synthesizes what the current published data says about the occurrence and activity of mixotrophs across environments. To date, there has only been one publication that has attempted to synthesize *in situ* mixotrophy data based on experimental measurements (Edwards 2019). Edward's synthesis compiled data for eleven studies and largely focused on spatial variability in mixotrophic abundance vs heterotrophs and autotrophs. Our planned synthesis will include more studies and emphasize the grazing activity of the mixotrophs.

Citation	**Environment**	**Focus**
Sanders et al. 1989	Lake Oglethorpe, USA (eutrophic)	Compares temporal and vertical variability in heterotrophic and mixotrophic abundance and bacterivory
Domaizon et al. 2003	Lake Annecy, FR (oligotrophic)	Compares temporal variability in heterotrophic and mixotrophic abundance and bacterivory
Unrein et al. 2007	Mediterranean Sea (oligotrophic)	Compares temporal variability in heterotrophic and mixotrophic abundance and bacterivory
Sanders and Gast 2012	Arctic Ocean (Canada Basin)	Compares spatial variability in heterotrophic and mixotrophic abundance and bacterivory
Gast et al. 2014	Ross Sea, Antarctica	Compares spatial and temporal variability in mixotrophic abundance and bacterivory
Princiotta and Sanders 2016	Lake Lacawac, USA (mesotrophic)	Compares temporal and vertical variability in heterotrophic and mixotrophic abundance and bacterivory

Gast et al. 2018	Ross Sea, Antarctica	Compares spatial and vertical variability in heterotrophic and mixotrophic abundance and bacterivory

Table 1: A select list of known peer-reviewed literature on *in situ* mixotrophic research. A more in-depth literature search will be completed over the course of the project.

2. **Outline the most pressing research questions related to mixotrophy.** The field of mixotrophy is replete with potential future research directions, and we will use this working group as an opportunity to produce a white paper of what the group identifies as the most important research questions, at this time. We will provide detail on how addressing each question will lay the foundation to address future important mixotrophy research topics. We will engage the larger OCB community early in the working group through a webinar as a way to encourage feedback from scientists with a wider range of expertise. Tentatively committed working group members consist of 50% early career scientists (Brownlee, Lambert, Leles, Luo, Millette, Moeller, Stamieszkin). We hope that by engaging so many early career scientists, the priorities laid out by this working group will serve as a framework for guiding research directions for years to come.
3. **Develop guidelines for the best application of current methods used to research mixotrophs in the environment, and identify potential new methods for advancing *in situ* studies.** Based on the research questions developed for the white paper, we will assess which methods used to study mixotrophs can be applied with confidence to address these questions. Common methods include fluorescent microspheres and fluorescently labeled bacteria, but there is a lack of confidence in the application of these methods. There is limited direct comparison of results from the different methods, but a meta-analysis could be possible to assess if current methods provide similar results, on the whole. We will develop "best practices" for each method and encourage future methodological intercomparison. Interested group participants will write a paper reviewing the pros and cons of common and uncommon current methods, and identifying promising new methods to be explored.
4. **Chair an OSM session on bridging the gap between modeling and environmental mixotrophy research.** The working group lead (Millette) will chair a session at the 2022 OSM in Honolulu, HI with two other interested participants in the group. The session will bring modelers and field/laboratory researchers together to enable discussion of how these two groups approach mixotroph studies and research gaps. By hosting a session at a large international meeting, we will cast a wide net to foster cross-disciplinary thought and discussion. As we finalize our proposed mixotroph research goals and methods, this session will provide the most up-to-date view of the wider scientific community's approach to mixotrophy.

<u>Participants</u>

The working group will consist of early, mid, and late career members who have a range of experience and interest in mixotrophs. We currently have 13 people who have tentatively committed to being a part of the working group. While we have only budgeted for 13 people to attend our two in-person working group meetings (see budget justification), certain portions of our discussions will be open to others via virtual participation. We will advertise these opportunities through the OCB newsletter and host a OCB webinar. Our current committed members include scientists who are considered experts on mixotrophs (Johnson, Leles, Sanders), have conducted *in situ* research on mixotrophs (Johnson, Gast, Millette, Sanders), and have or are currently attempting to model mixotrophs (Andersen, Dutkiewicz, Moeller).

Work Plan

Year 1 *(2021-2022)*: Starting in January, we will host zoom meetings every other month. At each zoom meeting, two group members or outside invitees will give a short talk (~15-20 minutes) on a similar mixotrophy topic, followed by a community discussion. These talks will be structured as overviews of larger ideas in the field of mixotrophic research, and not about a specific research project. In the first few months we will also host a webinar for the OCB community to introduce our working group and encourage wider participation in our zoom meetings. Simultaneously, the working group members will be conducting a literature search in order to identify all peer-reviewed publications that have published experimental data on *in situ* mixotrophy abundance and/or activity. All of the papers will be evenly distributed among the working group members, so that the key information for each study can be summarized. This will include: the study environment, how the methods were applied, the type of data that was collected, and the main findings. We will develop a template for everyone to follow to keep the paper summaries as uniform as possible. This literature search will provide an understanding of what *in situ* mixotrophy research has been done and the methods that were used. From here, the group will begin to discuss gaps in the research and the application of current methods. This will serve as the foundation for our proposed synthesis paper.

In the fall of 2021, we will host a two and half day, in person meeting with all the working group members at the Virginia Institute of Marine Science. The main goal of the meeting will be to come up with a draft of our white paper on priority mixotrophic research objectives and begin discussing which current methods can be applied to address each objective. At this point, the synthesis paper should have made substantial progress and, as a group, we will come up with the methods review paper outline. Members can be on both papers, but any members not working on the synthesis paper will be encouraged to take a large role in the methods review paper.

Year 2 *(2022-2023)*: In February 2022, Millette and two other working group members will host a session at the Ocean Sciences Meeting related to this group's objectives. At this point our white paper should have gone through some revisions and we use this opportunity to see what scientists outside the working group and OCB are thinking about mixotrophy. In June 2022, we will host a two-day meeting at Woods Hole Oceanographic Institution, right before the 2022 OCB meeting. The goals for the meeting will be to finalize the white paper and provide group feedback on the synthesis and method's review paper. By this time, there should be full drafts of both papers, so as a group we will be able to agree on the main takeaways of both papers. At the OCB 2022 meeting, we will then present the main products from our working group. Through the rest of the year we will continue to host meetings every other month with 20 minute seminars and ensure that our papers are submitted by the end of 2022.

Relevance to OCB

Convening a working group to define a path forward for mixotrophy research behooves the OCB and greater scientific communities. It is apparent that mixotrophy is not an oddity, but rather the norm at the base of the marine food web (Flynn et al. 2013). Theoretical modeling has shown that a global plankton food-web composed of 100% mixotrophs may enhance carbon trophic transfer and carbon sequestration by shifting carbon to larger-sized particles (Ward and Follows 2016). Mixotrophs may provide an efficient pathway for carbon to move from bacterial production to higher trophic levels when the traditional nutrient-phytoplankton-zooplankton food web is not present (Mitra et al. 2014). Therefore, through guiding future mixotrophy research forward, this working group will advance the OCB research priority, *"marine organism-mediated carbon cycling and export via the biological pump"*.

This working group will also address the OCB research priority, "*marine organism response to environmental change*". It has been hypothesized that as the ocean warms, plankton communities will become more heterotrophic (Lopez-Urrutia et al. 2006). Laboratory studies have shown that mixotrophs utilize heterotrophic strategies more under warmer conditions (Wilken et al. 2013), and mixotrophic dinoflagellates have increased in abundance in the subarctic Northeastern Atlantic over the last few decades, coincident with warming sea surface temperatures (Barton et al. 2016). Given that mixotrophs likely have a substantial impact on global carbon cycling, and that their activity may increase as global temperatures warm, observational and experimental research should begin as soon as possible to track changes in mixotroph distribution and abundance. The sooner we are able to collect *in situ* data addressing the distribution of mixotrophs and the use of mixotrophy as a trophic strategy, the better our understanding of their response to contemporary climate change will be. Thus, by advancing current methods and developing new ones to study mixotrophy in the marine environment, this working group will be pressing the field forward at a critical time.

The active members of OCB include a cross section of modelers, experimental researchers and observationalists, who study topics related to biogeochemical cycling, with a particular focus on the role of plankton, from viruses to large zooplankton. In recent OCB summer meetings, there has been a clear growing interest in mixotrophy among those in attendance. OCB is therefore the ideal group from whose expertise this working group will draw, driving forward our understanding of plankton trophic strategies and their impacts on marine biogeochemical cycles and trophic dynamics.

Budget and Justification

We are requesting $29,097 to cover the cost of hosting two in person meetings, one at the Virginia Institute of Marine Sciences in fall 2021 and one at the Woods Hole Oceanographic Institution in summer 2022. A majority of the funds will be used to cover the cost of hosting these meetings, travel, food, hotel rooms, etc. All but one of the 13 group participants are domestic and the international participant (Anderson) will only be attending the 2022 in-person meeting at WHOI. We have confirmed that there will be no costs associated with reserving a meeting room at VIMS and WHOI. Additional costs include the publication costs of two papers associated with the outcomes of the working group.

Budget Item	**Amount**	**Justification**
Food & Beverage	$3826	Estimated @ $55/day/person and $71/day/person per diem for Gloucester Point, VA (VIMS) and Woods Hole, MA (WHOI), respectively: 12 people for three days in 2021 and 13 people for two days in 2022.
Vehicle Travel	$1296	Estimated @ $0.54/mile for up to 300 miles per person, both ways: 1 person for fall 2021 and 3 people for summer 2020
Air Travel	$10350	Estimated @ $550 domestic and $1000 international: 10 domestic for fall 2021 and 7 domestic and 1 international for summer 2022
Hotel Rooms	$9625	Estimated @ $175/room/night: 3 nights for fall 2021 for 11 people (Millette is local and will not need a hotel) and 2 nights for summer 2022 for 11 people (Gast and Johnson are local and will not need a hotel).
Publication costs	$4000	Cover the publication of two open access papers

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