Cruise Report and post-cruise sample processing
R/V Gulf Challenger “GC Mixo 23-03”, July 18-19, 2023
Judd Gregg Marine Research Complex, July 20-21, 2023
Report prepared by Ann Tarrant, Rebecca Gast, Phil Alatalo

Report available at:
Biological and Chemical Oceanography Data Management Office
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
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NSF project #OCE-2201365 (PIs: Gast, Tarrant) “Investigating Mixotrophic Algal Contribution to Copepod Diet and Reproduction”

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1. Acknowledgements

We are grateful for the support and expertise of Captain Bryan Soares, Mate Frannie Lux, and marine technician Sean Shellito. Nate Rennels facilitated access to the Judd Gregg Marine Research Complex. Jeff Runge, Shawn Shellito and Doug Vandemark shared sampling equipment and information regarding sampling locations and protocols. PJ Bernard assisted with cruise mobilization and demobilization and generally kept our spirits up. Carin Ashjian shared supplies for conducting egg production experiments.

2. Background

A primary topic of interest in the field of biological oceanography is the role of planktonic productivity in the global carbon cycle. Over the past 20+ years, the traditional food web of algal production, zooplanktonic consumers and higher trophic level predators has been undergoing revision with a stronger understanding of the contributions made within the microbial loop. Of particular interest has been mixotrophy, the blurring of trophic mode assignments within the

microbial eukaryotes. Recognized for over a century, the combination of phototrophy and heterotrophy by a single cell has gone from being considered a physiologically unfavorable oddity to a diverse and widespread adaptation. Empirical observations of the prevalence of mixotrophy in the world's oceans have led to a recent series of modeling efforts. These assessments suggest that the inclusion of mixotrophy can enhance primary production and trophic transfer efficiency, supporting the function of the biological carbon pump and larger organisms at higher trophic levels. Yet, there have been very few empirical studies to assess how mixotrophic organisms interact with and impact their zooplankton grazers. The goal of our proposed project is to help define mixotrophic contributions to higher trophic levels in marine pelagic food webs. We will accomplish this by experimentally testing whether they can support copepod reproduction under conditions when phototrophic food is of poor quality and by assessing mixotrophic contributions to the diet of two abundant copepod species within the Gulf of Maine.

3. Participants

Science: Becky Gast, Ann Tarrant, Phil Alatalo, Rodrigo Zuñiga, Cameron Johnson

Crew: Bryan Soares, Frannie Lux, Sean Shellito

4. Objectives and Station Plan

The overall goal of this cruise was to obtain a snapshot of the prevalence of mixotrophy within the Gulf of Maine and the potential contributions of mixotrophs to copepod diets. We proposed to accomplish this goal by sampling water and zooplankton from 3 stations. At each station (plan):

1. Water sampling (CTD)
    a. Collect profile as CTD descended.
    b. Collect water at 2 depths (nominally at the surface and deep chlorophyll maximum) for:
        i. incubations with labeled bacteria (to assay the potential for mixotrophy in the water column) - <100 micron seawater, 1.5L in bag plus bacteria to $1x10^5$ cells/ml, 500ml into dialysis bag in two of the incubation bags
        ii. amplicon sequencing (duplicates) – 400 ml <100 micron seawater onto 47 mm 0.2 micron Isopore filters. Stored frozen.
        iii. nutrients (duplicates) – 100ml whole seawater for particulate nutrients, 50 ml < 0.2 micron seawater for dissolved nutrients. Stored frozen.
        iv. chlorophyll a (duplicates) 50 ml whole seawater onto 25 mm GFF. Stored frozen.
    c. Collect additional water to maintain live animals and conduct experiments.

2. Zooplankton sampling: one or more vertical tows using a dual rig.
    a. At WB7, preserve contents of one net in formalin for WBTS collaborators. Collect net contents onto a 53 μm filter and then transfer into a jar with formalin.

b. At each station, preserve contents of one net in formalin for community assessments by project personnel.
c. To collect live animals for experiments, the openings in the sides of the cod end should be covered with duct tape. Dilute contents of these tows into seawater in buckets. Store buckets in coolers with cold packs.
d. For genetic analyses, preserve the contents of a net (full contents or partial contents of the “live tow”) in ethanol. Ethanol samples are not considered quantitative.

After sampling was completed, conduct egg production experiments within the Judd Gregg Marine Lab. In brief, identify female *Calanus finmarchiucs* and *Centropages typicus* from each station and incubate them in egg production chambers overnight. After 24 hours, separately preserve female copepods and eggs.

5. Cruise Narrative

Some Logistics:

- We wore regular clothing (t-shirts, sweatshirts, lightweight pants or shorts) and were comfortable with the temperature during the cruise. We had various forms of rain gear (rain coat or full foul-weather gear). Steel-toed shoes strongly recommended.
- Phil borrowed a WHOI truck. We drove 3 vehicles (Becky’s car, Ann’s car, WHOI truck)
- We stayed at the Holiday Inn (300 Woodbury Ave, Portsmouth NH 03801), which was perfectly adequate. Rooms Tues through Thurs.
- Water Sampling:
    - We started with Station WB7 and did a CTD cast as planned. After the cast concluded, a problem was noted related to the CTD termination, and the CTD/rosette could no longer be used.
    - We collected the remainder of water from Station WB7 (for transporting zooplankton and for live animal experiments) using a Niskin bottle and messenger.
    - An incubation was started on board the ship. Water was transported back to Woods Hole for filtration.
- Zooplankton sampling:
    - As before: we used the WBTS net system: 200 µm mesh, 0.75 m diameter ring net in dual ring configuration. A weight is attached to the center of the dual ring, in between the two nets, to keep the nets parallel to the surface. The boat was stopped during the (vertical) tow, and it is assumed that there is no sampling on the way down as the wire is being let out. Retrieval rates generally started around 0.5 m/s (30 m/min) and increased to about 0.7 m/s near the top.
    - Due to the problem with the CTD (described above), zooplankton was only sampled from WB7.
- We worked out of the Judd Gregg Marine Lab (29 Wentworth Rd., New Castle, NH 03854). We needed to send car make/model/plate # to the lab manager (Nate Rennels: Nate.Rennels@unh.edu) ahead of time.

- The Ceratium bloom that was noted in April still continues.

Station Coordinates:

WB7: 42° 51.731’ N, 69° 51.719’ W

Timeline:

7/18/23

~12:30 Left Woods Hole

~15:30 Arrived at dock. Unloaded/loaded gear. Set up a little bit (not much) in the lab. Drove to hotel and checked in. I launched some HOBO loggers that we didn’t end up using.

~18:30 Left dock. We delayed leaving a little bit because of a passing storm (lightning and rain). Weather was overcast, air felt heavy, and temperature was in the high 70’s.

~20:45 (8:45 pm) Arrived WB7. Water was rough on the way out but a little calmer on station.

20:52 Start WB7 CTD cast 1 (full profile, sampling 11 m and 5 m, for Brdu experiments and metabarcoding). Surface Chl was high, but there was no deep chlorophyll max. Water very warm at the surface (~22°C but around 8°C by 50 m).

21:56 - 22:30 Niskin Casts (8) to 50 m for live animal transport and/or experiments). Water for transporting live animals was poured directly into 7-to-12-l beakers inside coolers with cold paks. Water for experiments was poured through a 53-um sieve and then into two round 12-L beverage coolers. This was relatively fast, especially because we could open the Niskin bottle directly into a bucket to empty it.

22:45 - 23:04 Zooplankton net tow #1 for formalin-preserved samples.

23:20 – 23:41 Zooplankton net tow #2 for ethanol-preserved and live samples. The cod end was taped for the live tow.

Left station ~23:50 Due to CTD failure, we returned directly to the dock.

7/19/23

02:30 Gulf Challenger arrived at the dock. We unloaded all our gear, with the help of PJ. Becky and PJ dropped Cam off at the hotel, and then they brought the water incubations and nutrient samples back to Woods Hole. Phil, Ann, and Rodrigo went to the lab to process zooplankton samples.

7/19/23 (cont) Setting up egg production experiments (picking of target species)

For each station, the goal was to incubate 30 individual *C. finmarchius* females and 3 groups of 10 *C. typicus* females. Unfortunately, we were only able to sample WB7.

Within this sample, *C. typicus* was much more abundant than we had observed back in April. A mix of stages were present, along with both males and females. Anecdotally, it seemed like many of the females may have been newly molted. We collected 4 replicates of 10 females for group egg-production experiments. Different from last time, we had Phil set up all these experiments from a pool of animals set aside by Phil, Ann and Rodrigo (this was to confirm ID and ensure that the correct number of animals were in each incubation).

Calanus was also relatively abundant, but C5 copepodites were much more common than females. In much of the sample, the *Calanus* C5's appeared relatively sluggish, and we wondered if they had entered a diapause-like state. Later on in our sorting, we found some animals (from a different bucket) that seemed more active, so we wondered if the sluggishness may have been an artefact of insufficient dilution in the bucket. We set up 32 individual egg production experiments.

We also sorted ~30 *C. finmarchicus* C5 copepodites that were transported live to WHOI for a side project.

As previously, from a sub-sample of each station catch, copepods were pipetted from under a stereoscope into a 9-well plate to confirm species and stage identification. *C. typicus* females were pooled in group egg-collectors (200µ m mesh) suspended in beakers filled with approximately 800ml of natural seawater (filtered to 53 µm). Egg collectors were marked with color-coded cryolabels. *C. finmarchicus* females were next individually placed in 40-ml of natural seawater in specially designed egg-collecting chambers, outfitted with 200 µm mesh. All females were placed in a dark incubator (door covered with a garbage bag) at 8°C for 24-h incubation.

03:30 In the lab and setting up.
04:00 Actively picking copepods
04:38 Had set up the first four *Calanus* incubations and 1 *Centropages* incubation (10 females)
05:04 Second *Centropages* incubation set up
05:40 Finished setting up all 4x10 *Centropages* incubations
07:17 Done with setting up incubations and cleaning up.

7/20/23 Processing egg production experiments

Sample processing was timed to enable ~24 hour egg production incubations. The next morning, adult female egg-collecting chambers were processed.

For *C. finmarchicus*, the egg chamber was unscrewed from the incubation lid and the copepod removed with forceps. The copepod was examined briefly under a stereomicroscope to confirm species, staging and condition (live/dead) and transferred to a labeled (same cryolabel from egg chamber) 1.5-m microcentrifuge tube to which a 5% formalin solution was added. Contents of the incubation chamber were then rinsed into a Pyrex dish. Under the stereoscope, the contents

were swirled and visualized. The condition of the female (live, dead) was noted. No eggs were observed in any of the *Calanus* experiments. 6 of the 32 animals were dead, and some others were in poor condition. We wondered if the lack of egg production was a real ecological phenomenon or an artefact related to stress (overcrowding and/or warming) during transport.

For *C. typicus*, groups of adults were washed into a preservation jar, counted and preserved with formalin (5%). The egg-collection water was examined under a stereo microscope by Phil. He did not see any eggs within the samples, and the contents were discarded. Animals were initially sluggish upon observation (e.g., TW7-1, see Table 5). We attributed this to the water being too warm in the dish being used for visualization (which was later corrected). The temperature during the incubation was fine (8°C). Egg production was very low.

~05:00 Left hotel for lab.

06:00 Actively processing samples

06:30 About halfway through *Calanus samples* (Ann had done 1-11, Rodrigo had also done some).

07:10 Finished processing samples and cleaning up.

BrdU ingestion experiments

Incubation bags were kept in the dark in coolers with frozen plastic ice after setup and transported to Woods Hole laboratory after the cruise (~6 am on 7/19) where they were placed in incubators at ambient light conditions (surface vs DCM) on a 12/12 hr light dark cycle. DCM samples were at 8C while surface samples were at 15C.

Experiments were incubated for ~57 hours and sampled at 9:30 am on July 21, 2023. All of the water from each dialysis bag was collected onto individual 0.2 micron 47mm Isopore filters. The experimental bags were divided into 750 ml aliquots and collected onto duplicate 0.2 micron 47mm Isopore filters. All filters were stored at -20C until processing.

Table 1: GoM Station Metadata

Site/Station	WB7
Date	17 July 2023
CTD cast #'s	01
Latitude	42 51.716 N
Longitude	69 51.334 W
Time	20:52
Cast type	01 full depth
Salinity	30.0 surface, dropping to 32 by 20 m
Temperature (°C)	20.6° surface, dropping to 10.1° by 20 m
Fluoro (mg/m^3)	Peak of 16.8 at 6-7 m
OXY (uM/kg)	256 surface, peak of 277 at 11 m
Depth (m)	259

Table 2: CTD Cast Log

Station	Cast	Date	Start	Max depth	Sampling
WB7	01	7/18/2023	20:52	250	8, 11 m: amplicon; mixotrophy ingestion; chl a; nutrients

Table 3: Zooplankton Tow Log

Station	Date	Tow	Time Start	Time End	Lat (N)	Long (W)	Max Depth (m)	Net A Start	Net A End	Net A Use	Net B Start	Net B End	Net B Use
WB7	07/18/23	1	20:45	23:04	42 51.716	69 51.334	250	46231	46948	Formalin (Mixo project)	55437	56254	Formalin (WBTS)
WB7	07/18/23	2	23:20	23:41			250	46948	47759	Live animals	56254	57110	Ethanol

Table 4: *Calanus finmarchicus* observations (Notes on female condition only, no eggs produced). Note that specimen numbers are not unique; they repeat each cruise.

Animal	Condition/Notes
FWB7-1	Had parasite. Was in water but looks dead.
FWB7-2	Alive but barely
FWB7-3	Live
FWB7-4	Live
FWB7-5	Different parasite. Alive
FWB7-6	Dead, good condition (recently dead?)
FWB7-7	Live
FWB7-8	Live
FWB7-9	Dead, dark
FWB7-10	Alive, swimming
FWB7-11	Live
FWB7-12	Live
FWB7-13	Dead/unresponsive; good condition
FWB7-14	Alive, responds to touch
FWB7-15	Alive but barely
FWB7-16	Alive but barely
FWB7-17	Alive and lively
FWB7-18	Alive, responds to touch
FWB7-19	Alive, responds to touch
FWB7-20	Alive and lively
FWB7-21	Alive, responds to touch
FWB7-22	Alive, twitching
FWB7-23	Barely alive
FWB7-24	Alive and lively
FWB7-25	Live
FWB7-26	Live
FWB7-27	Live
FWB7-28	Live
FWB7-29	Dead, dark
FWB7-30	Live
FWB7-31	Live
FWB7-32	Live

Table 5: *Centropages typicus* egg production observations Goal was 10 females per chamber. All animals female unless noted

Chamber	Copepods	Egg production/Notes
TWB7-1	10 total (?) 1 dead, 1 CV; not “perky”	No eggs, preserved anyway
TWB7-2	11 total, 1 CV (not moving but looks good)	2 eggs
TWB7-3	Sieve had hole. ~7 females observed, preserved	1 egg, preserved in Lugols
TWB7-4	~5 females observed	1 egg

Figure 1: Profiles of water column properties from WB7 (CTD cast 1)