NBP 23-03
Cruise Report

Photo Credit: Scott Crabbe, Australian Antarctic Division, Davis Station

Projects:

B-305-N	Halanych and Mahon	OPP-1916661 and OPP-1916665
B-014-N	Learman and Steen	OPP-2147045
B-010-N B-237-N	Gerken and Kocot	OPP-2138993 and DEB-1846174
B-252-N	Bik	OPP-2132641
O-271-N	Sarmiento	

The following is a brief report on the science that occurred on board the *RVIB Nathaniel B Palmer* from March 19 to May 6, 2023.

Overview:

The NBP 23-03 cruise funded by the USA National Science Foundation upon the *RVIB Nathaniel B Palmer* spanning March 19 to May 6, 2023. The expedition included 31 science participants from 8 different institutions/organizations to study the biodiversity and patterns of genomic structure in Antarctic organisms. The Halanych & Mahon effort focuses on using population genomics of Antarctic marine invertebrates. Gerken and Kocot's team explored the diversity of Antarctic meiofauna with an emphasis on cumaceans and meiofaunal mollusks. The Learman and Steen group's interests focused on the microbial diversity and function in sediments. The Bik group's focus was on meiofaunal nematode biodiversity. All of the projects involved sampling the benthos with various techniques (Blake trawl, Epibenthic sled, megacore, box core). These efforts were complemented by CTDs and benthic imaging (Yo-Yo Camera/video).

Our original plan was to head from New Zealand (Lyttleton) to East Antarctica and work our way west along the Antarctic continental shelf towards Prydz Bay and to include some sampling on the Kerguelen Plateau outside of the French and Australian EEZs. Due to a number of issues including COVID-19 delays (ship crew, not science team), ice coverage, weather, and the medical situation/emergency with ~10 science days remaining in our cruise, we were unable to sample into Prydz Bay or to sample the Kerguelen Plateau.

Two SOCCUM floats (Sarmiento, Talley, Becker, Matsumoto, et al.) were released on our southbound transit from Lyttleton to the Antarctic shelf and due to instructions from NSF, no floats were released on our northbound transit. SOCCUM floats also included outreach efforts organized by George Matsumoto at MBARI.

Notable NBP 23-03 cruise dates:

Arrival in Christchurch:	February 28, 2023
Embark NBP:	March 18, 2023
Depart for Antarctica:	March 19, 2023
Arrive at Station 1:	April 2, 2023
Science halted:	April 21, 2023
Depart Antarctica for Perth, Australia	April 26, 2023
Disembark:	XXXX

From our pre-cruise planning discussions and agreements with NSF, a total of 31 science days and 5 days for weather contingency were supposed to occur on NBP 23-03 with the following breakdown of time:

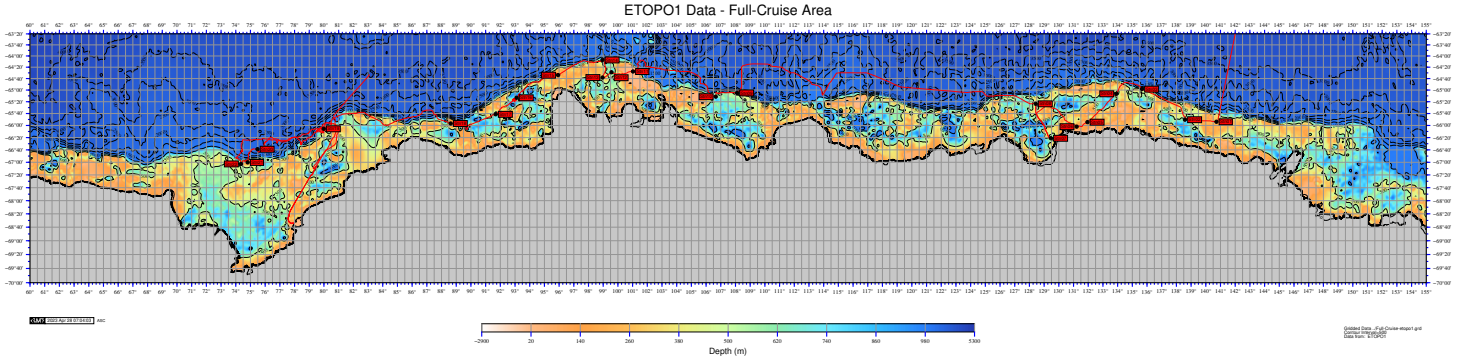
Halanych and Mahon (25 total days)
Bik, Gerken, Kocot, Learman and Steen (4 combined days in total)
Sarimento (2 total days)

With the delays of COVID on our trip south, the actual travel time it took us to get to our sampling region after leaving Lyttleton, New Zealand, and the sudden stoppage of science due to an onboard medical situation on April 21, 2023, our science time was cut to a total of

approximately 20 days which included 4-5 days lost due to weather. **<u>This resulted in an approximate 35% cut in total science time for the teams on NBP 23-03</u>**.

COVID Pandemic impact:
Several aspects of this cruise were impacted by the COVID pandemic. Due to a number of crew testing positive for COVID-19 while the science team was isolating in Christchurch, New Zealand, our departure for the cruise was delayed for 15 days (from March 4, 2023 to March 19, 2023). Given the time of year, this 2-week delay had a significant impact in that sea ice was rapidly forming in several regions along the Antarctic shelf meaning more time and effort to get through ice.

Cruise Track (available as Appendix 1 also):



Science Team Participants NBP 23-03

Mahon, Andrew R.	Central Michigan Univ.	Chief Scientist
Bik, Holly	Univ. of Georgia	PI
Gerken, Sarah	Univ. of Alaska Anchorage	PI
Halanych, Ken	UNC Wilmington	PI
Kocot, Kevin	Univ. of Alabama	PI
Howland, Katherine	Central Michigan Univ.	PI Representative and Graduate Student
Bergmeier, Franziska	Univ. of Alabama	Postdoctoral Fellow
Cobo Llovo, Maria Carmen	Univ. of Alabama	Postdoctoral Fellow
DeSantiago Perez, Alejandro	Univ. of Georgia	Graduate Student
Donnely, Kyle	UNC Wilmington	Graduate Student
Farris, William	Univ. of Alabama	Technician
Flaherty, Sophie	Central Michigan Univ.	Undergraduate Student
Gott, Madison	Central Michigan Univ.	Undergraduate Student
Grimes, Candace	UNC Wilmington	Postdoctoral Fellow
Halanych, Coral	Univ. of Washington	Undergraduate Student
Judge, Conor	Central Michigan Univ.	Undergraduate Student
Mancke, Harrison	UNC Wilmington	Graduate Student
Marcelino, Mirayana	Univ. of Georgia	Graduate Student
McLaughlin, Emily	Univ. of Alabama	Graduate Student
Noor, Nusrat	Auburn Museum of Natural History	Collaborator
Nygaard, Hannah	Central Michigan Univ.	Graduate Student

Olson, Chandler	Univ. of Alabama	Graduate Student
Pereira, Tiago	Univ. of Georgia	Postdoctoral Fellow
Perez, Jacob	Univ. of Tennessee	Graduate Student
Roberts, Nickellaus	Univ. of Alabama	Graduate Student
Schreiter, Samantha	UNC Wilmington	Graduate Student
Schutte, Virginia	Univ. of Georgia	Collaborator
Uzarski, Lindsay	Central Michigan Univ.	Undergraduate Student
Vandersommen, Victoria	Univ. of Alaska Anchorage	Graduate Student
Waits, Damien	UNC Wilmington	Technician
Zehnpfennig, Jessica	Central Michigan Univ.	Graduate Student

*ASC and ECO crew listed in Appendix 2

ASC and ECO support:
As with any scientific cruise, success is highly dependent on a number of factors including ship's crew and support staff. On NBP 23-03 the help, efficiency and effort by both ASC and ECO personnel were stellar. We were able to operate in a friendly professional manner with good communication between science, ASC, and ECO. From the science side, we were very appreciative of the support we received from the ASC and ECO crews. They helped make the cruise a resounding success.

Outreach Efforts:
The overall ability to complete outreach for all teams was hindered by a lack of internet connectivity during NBP 23-03, but we knew this would be the situation prior to the cruise. All groups participated in a daily blog that was posted to http://icyinverts.com. Additionally, the Bik group describes their outreach efforts while underway below. Post-cruise efforts will include K-12 visits and presentations, a significant increase in online efforts by all groups (e.g., Twitter), etc.

SOCCOM Outreach: The SOCCOM float group as an outreach effort organized by Dr. George Masumoto were K-12 classrooms adopt floats and track their progress. The NBP 23-03 crew aided these by decorating and deploying the floats. Images form these efforts were circulated to classes. They have also been featured on a flicker stream (www.flickr.com/gp/139764369@N07/W0X71Q) and presented in an American Geophysical Union (AGU) Town Hall meeting on BGC Argo floats.

Equipment and deployments
For NBP 23-03, each location visited was given a unique sequential "Station" number, and each overboard operation was given a sequential "Event" number. A complete list of this is given in Appendix 3 (attached as a separate file).

The cruise included the 101 deployments, including:

20 Blake trawls
21 CTDs
11 Epibethic sleds
24 Megacores
1 Box cores
23 Yo-Yo camera/video transects
1 Plankton tow

Blake trawls were conducted with a scope of 3:1 (wire-out:depth) with the tow lasting 10 mins once the desired wire-out length was achieved. Towing was completed at 1.0 knot.

The newly obtained Epibenthic sled (EBS) was used by the Gerken/Kocot group with much success. Its use is described in their report in detail, below in the Gerken/Kocot research summary.

The Yo-Yo camera/video transects were conducted for a 1 km linear transect once on the bottom with the ship moving at 0.5 knots. Due to wave swells and winds/ice, a few transects were cut short to protect the equipment. With the help of the ASC Electronic Technicians Alex Brett and Barry Bjork, we were able to utilize the live wire tow and visualize the bottom in real time along with capturing both still and video footage of the bottom during the transect. The addition of a live feed on the camera sled was a powerful new tool that allowed us to more rapidly assess the environment and served to engage the full science team in preparation for sampling.

For the CTD deployments, the science team opportunistically collected and filtered water collections from the surface, above and below the halocline, and at the bottom at each drop for further analyses of the environmental DNA contained in each region. Mahon (Chief Scientist/PI) was able to expand his collections from the NBP 20-10 cruise and has preliminary data analyzing the samples for macroinvertebrates, fish, birds, and mammals in the Peninsula-Northwestern Weddell region. He plans to expand these analyses with the samples collected in East Antarctica. The Bik group collected and filtered water samples for a colleague (Bradley Tolar at UNC Wilmington) who will perform eDNA analyses for microbes (focusing on Archaea).

Summary of scientific work:

B-305-N Halanych & Mahon – Population genomics of invertebrates
The overall objective of the Halanych & Mahon team on this cruise was to explore patterns of genetic structure among Antarctic marine invertebrates Specifically, we are testing the idea of whether a trans-Antarctic seaway has existed when the Western Antarctic Icesheet was reduced. This fits into a backdrop of previous efforts to examine endemism and circumpolar distributions in Antarctic marine invertebrates. This information will have direct implications for understanding past and future range shifts of organisms. To this end, the team employs an integrative approach that focus on the genetic signatures of historical gene flow or isolation. We are also assembling genomes for select invertebrate to search for signatures of selection versus genetic drift which differ under proposed speciation mechanisms at work in the Antarctic. Upon our return of samples to our respective universities, we will data collect genomic level data on variety of target organisms.

Although we were not able to sample as far south into the Prydz Bay region or on the Kerguelen Plateau, the collections we were able to make will allow us to expand our understanding of the biodiversity, phylogeography, and evolution of a number of groups of Antarctic benthic invertebrates. More specifically, we were able to expand our collections of numerous taxa with emphasis on sea spiders (Pycnogonida; Chelicerata), Echinoderms (urchins, brittle stars, sea stars, sea cucumbers, and crinoids), nemerteans, bryozoans, and crustaceans. Our new collections will allow us to sequence the entire genome of *Nymphon australe* (Nymphonidae) and *Ophionotus victoriae* (Ophiuoidea). As funds permit, we will include additional species (*Colossendeis megalonyx, Ammothea carolinensis, Sterechinus antarcticus*).

With new samples in hand, we will also be able to expand our whole genome scan work (RADSeq) for investigating population connectivity for multiple species from throughout the regions we have sampled in this and previous cruises. Our previous studies have looked at population connectivity in *Nymphon australe*, *Ophionotus victoriae* and *Astrotoma agassizi*, and this work will allow us to greatly expand our work for other species/groups including *Colossendeis* spp., *Ammothea* spp., *Pallenopsis* spp., *Parborlasia corrugatus*, *Cellarinella edita*, and *Sterechinus neumayeri*. Additionally, we will be able to map data in population studies (Single Nucleotide Polymorphisms; SNPs) to our new genomes, allowing us to look at portions of the genome under selection. This is a central tenant of our current proposal.

Blake Trawls:
We employed Blake trawls to collect organisms for genetic work to test the idea of whether a trans-Antarctic seaway has existed when the Western Antarctic Ice sheet was reduced. This information will have direct implications for understanding past and future range shifts of organisms. To this end, the team employs an integrative approach that focus on the genetic signatures of historical gene flow or isolation.

The focus of this cruise was to gain representative samples from Eastern Antarctica. Collections started at 141° E longitude and finished at 73° E along the continental shelf. Both Prydz Bay and the Kerguelen Plateau were targets for NBP 23-03, but collection in these regions was not

possible due to the various factors described above (other than 2 samples in Prydz Bay). Upon return of samples to our respective universities, we will data collect genomic level data on variety of target organisms.

Notably the continental shelf in Eastern Antarctica is much narrower than much of Western Antarctica and is even more limited when ice conditions are considered. Although the Blake trawl performed well, trawl payloads tended to be much smaller than observed in Western Antarctica with the same methods, a result consistent with camera based (Yo-Pro) observations. Additionally, the several of the target species of interest (e.g., brittle stars - *Ophionotus vicrtoriae, Astrotoma agassizii*; nemertean - *Parborlasia corrugatus*, urchin - *Sterechinus neumayeri*) were captured in low abundances and were spotty in terms of distribution.

Figure 1: Representative Blake trawl and on deck processing.

Most of the Blake trawl sampling was between 300-500 m deep. In general, sites that were on the shallower end tended to be very hard bottom and sediment depth was very limited even at slightly deeper (600-700m) sites. Notably, the 2-week delay in leaving port in New Zealand resulted in more sea ice, limiting the work to the edge or outer margins of the continental shelf. There are a few observations are worth mentioning: first, the outer shelf tended to have higher flow velocities (likely due in part to Eastwind Drift); second, the edge of the shelf tended to be well scoured and antidotally seemed to be an iceberg grounding line; and third, the continental slope was very steep (one might argue there was no slope, just continental shelf dropping into the depths greater then 1500m). This lack of a continental slope had an impact on sampling as we could not simply predict where to find deep benthos and more sedimented environments. Our original intention was to get to the more sheltered Prydz Bay or less scoured Kerguelen Plateau. The two Blake trawls in Pry Bay did prove different from the other sites in the Eastern Antarctic. For example, the sea star *Labidiaster annulatus* is common in the Antarctic Peninsula regions but, in Eastern Antarctic trawls, we only saw it in one Prydz Bay trawl (out of 20 total trawls) where it was abundant.

For the more holistic vantage point, brittle stars were the dominant fauna in Eastern Antarctica but not in the abundances seen in Western Antarctica. Specific sites were variably dominated by brittle stars, crinoids, pycnogonids, sea stars, or crustaceans. Although over all abundances were low, diversity in some cases was surprisingly high. This was true for annelids and crustaceans and one particular trawl yielded 29 species of Asteroidea (sea stars). Holothuroids, sea cucumbers, tended to be persistent throughout but their overall diversity was low. As for

crustaceans, we have a high diversity of amphipods, but low diversity of isopods including ones common to Eastern Antarctica (e.g. serolid isopods, *Glytoptonotus antarcticus* were rare). The urchin biodiversity was also notably different than the Western Antarctic. *Sterechinus neumayeri* and pencil urchins (*Ctenocidaris*) were less common, whereas the irregular urchins (e.g. *Abatus*) were more prevalent in sites sites with only modest a mounts of sediment (sediment depth 4-10cm). When we did see *Sterechinus*, they were overwhelmingly *S. antarcticus* and all were relatively small in size. Pycnogonids were also patchy in abundance (covered elsewhere in the report).

Sediments and Coring:
In addition to population genomics of individual species, we collected the top 0-2 cm of sediment from the megacore to assess diversity and community structure of meiofauna with genomic tools. This work is an extension of previous work using metabarcoding techniques which provided interesting results about the distribution of meiofauna species across 5500 km of the Western Antarctic. These new samples, from about 10 sites, will help us understand if some meiofaunal species also have a circumpolar distribution. The present sampling will help fill in major gaps in our sampling around the continent.

Environmental DNA analyses:
Because a portion of the Mahon lab's research focuses on using environmental DNA (eDNA; DNA that is shed from organisms into the water column that can be collected and analyzed), we used opportunistic sampling from CTDs performed at each station to collect and filter ~160 water samples from throughout our cruise track. These samples included, at most stations, collections at the surface and bottom of the water column and also from just above and just below the halocline. This will allow us to look at location and representation of eDNA in the Antarctic water column and how well it compares to benthic sampling performed during our cruise. We will process these samples to generate preliminary biodiversity data for additional studies in the future.

Underwater imaging for NBP 23-03
Prepared by Candace J. Grimes (Postdoctoral Fellow, UNC Wilmington) with help from ET Alex Brett

Participants: Kenneth M. Halanych, Andrew R. Mahon, Jessica Zehnpfennig, Madison Gott, Lindsay Uzarski, Coral Halanych, Conor Judge, Harrison Mancke, Will Farris, Damien Waits, Harrison Mancke, and Samantha Schreiter

The Yoyo camera device is a drop-camera system that is lowered to the seafloor and captures photos of the benthic community every 15s at from a height of approximately 2.5m for 1km at 0.5 knots. The general layout of the device consisted of 1 Nikon D3300, two GoPro Hero 10 Blacks, 2 SeaLite Spheres and 1 Multi-SeaLite (Figure 2). The two GoPros were included in order to attempt to create 3D models of the seafloor, but it seems as though we did not have sufficient lighting and tracking of the camera frame for success.

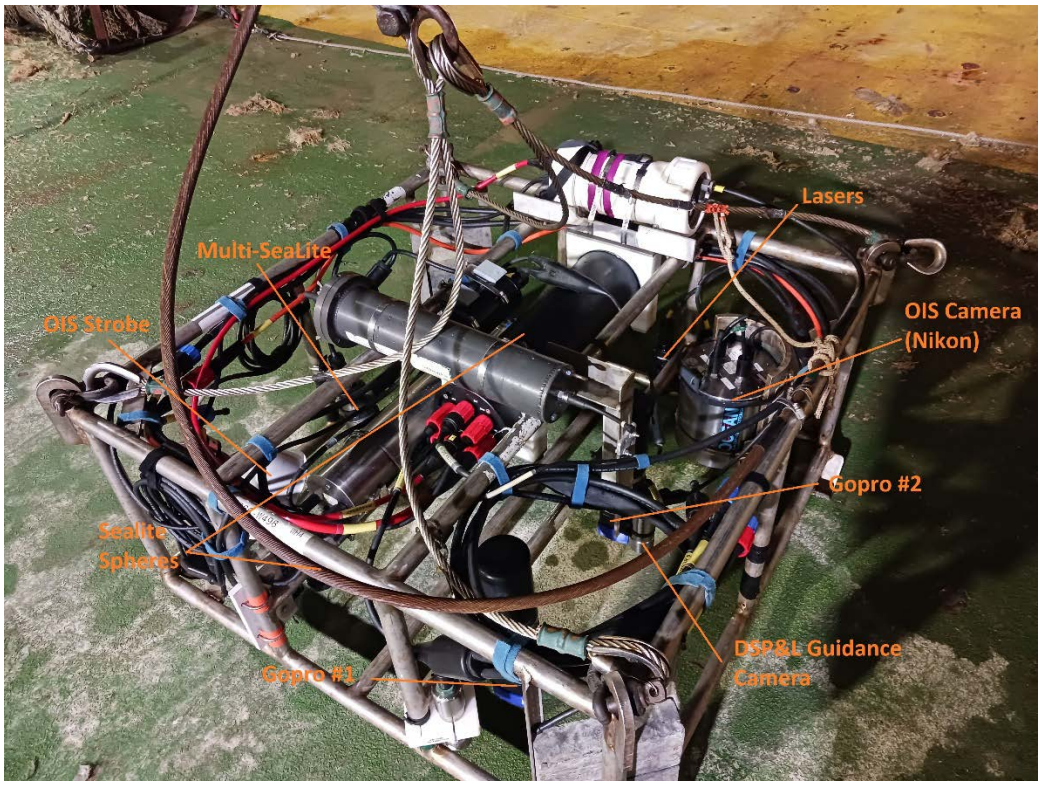


Figure 2 Annotated image of the Yoyo camera device.

The Yoyo camera was deployed at each station possible during the NBP 23-03 cruise, we began to count the dominant macrofauna taxa found at each station while in transit after the end of data collection with a “game” called, “Let’s name taxa!”. This game consisted of a group of 4 members of the scientific party and one leader (CJG) going through 50 randomly selected photos from each station. In order to compare with previously collected data, we are only able to use data collected from 10 of the 22 stations. This project came about because of the general lack of knowledge of the benthic communities in Eastern Antarctica and we had hoped to get through enough photos before we reach port to publish a description of the dominant taxa found there. We were able to record several of our target taxa in their natural environment (Figure 3 A-B) and

observe the abundance of sea cucumbers and phytodetritus after the summer algal bloom (Figure 4).

Figure 3. A. Photo from Station 7 showing two filter-feeding brittle stars, *Astrotoma agassizii*, and one crinoid. B. Photo from Station 21 with one fish, *Sterechinus* urchins, brittle stars, and sponges on rocky substrate.

Figure 4. Another photo from Station 7 showing an abundance of sea cucumbers which seemed to be the dominant taxa found and phytodetritus.

Sea spider parentage analyses
(J. Zehnpfennig, Graduate Student for A. Mahon, Central Michigan Univ.).

The analysis of parentage had provided fundamental insights into behavior, ecology and evolution and is considered a key facet of molecular ecology. Genetic data can be used to diagnose and infer natural parent-offspring relationships as well as provide information about sexual selection, conservation biology, population size, speciation, and natural selection. More recent advances in high throughput sequencing have highlighted the use of single nucleotide polymorphisms (SNPs) for analyses of parentage, relatedness and overall population structure. Furthermore, the use of SNPs in studies of parentage and relatedness have shown that SNPs perform as well, if not better than microsatellites.

Due to their small size, cryptic coloration and even with a purported circumpolar distribution for some species, pycnogonid (sea spider) reproductive habits and mating systems are vastly understudied. Furthermore, parental care is almost exclusively paternal in pycnogonids. In brooding families, the female sea spider will lay the eggs and transfers them to the male, who will then fertilize the egg and glue them to his ovigerous legs, then carry them around until they hatch. Observations on courtship and mating behaviors in sea spiders in nature are few and far between, and a majority of observations and studies that examined mating behaviors in pycnogonids have taken place in a laboratory setting. While, very valuable in learning courtship and copulation behaviors, studying sea spiders in laboratory conditions where there no predators, optimal conditions, unlimited food, and often a choice of multiple mates may not be representative of natural pycnogonid mating patterns. There has yet to be a study that examines natural mating systems within Antarctic pycnogonids.

Nymphon australe is reported to have a circumpolar distribution that extends to temperate waters in the southern hemisphere and is the most frequently captured pycnogonid in benthic trawls in the Southern Ocean. Collections from our previous cruises (NBP 12-10, LMG 13-12, and NBP 20-10) supported by the National Science Foundation (NSF ANT-1043670 and OPP-1916665 have collected *Nymphon australe* males carrying multiple egg clutches (up to six at a time) on their ovigerous legs. This project is aiming to use RAD-seq methods for SNP discovery from individual eggs taken from different clutches as well as the paternal parent to study parentage and relatedness and in specimen of *Nymphon australe,* from East Antarctica.

This cruise we observed the distribution of *Nymphon australe* to be more variable than previous cruises, with some trawls bringing up less than ten individuals, but other trawls bringing up over 500 individuals. It seemed that as the sampling sites moved west, we recovered more *Nymphon australe* specimen. There were egg-carrying males at every site we were able to find *Nymphon australe*. However, for the purpose of this study, we only chose males with two or more egg clutches. There were multiple sites where we only recovered egg-carrying males with a singular clutch. We also had sites where we recovered males carrying four or five egg clutches at a time, and males carrying two or more egg clutches that were in different developmental stages as well. There was also a site where the egg clutches on the same male were different colors, with two clutches being a pink color and the others being the characteristic yellow/off white color. Both observations indicate the male mated multiple times in the season, and that he may have mated with multiple females. We also saw the size of each individual clutch was variable, with some

males having small and large clutches. Males carrying more than one clutch were separated and the number of egg clutches per male was documented. Under a dissection microscope, the clutches were carefully dissected one at a time, with ten eggs from each clutch being flash frozen in liquid nitrogen for genetic analysis, and the dad as well as remaining clutches being preserved as well. The next steps will be to get back to our lab at CMU, perform the DNA extractions and downstream analysis on the individual eggs as well the paternal parents collected on the cruise.

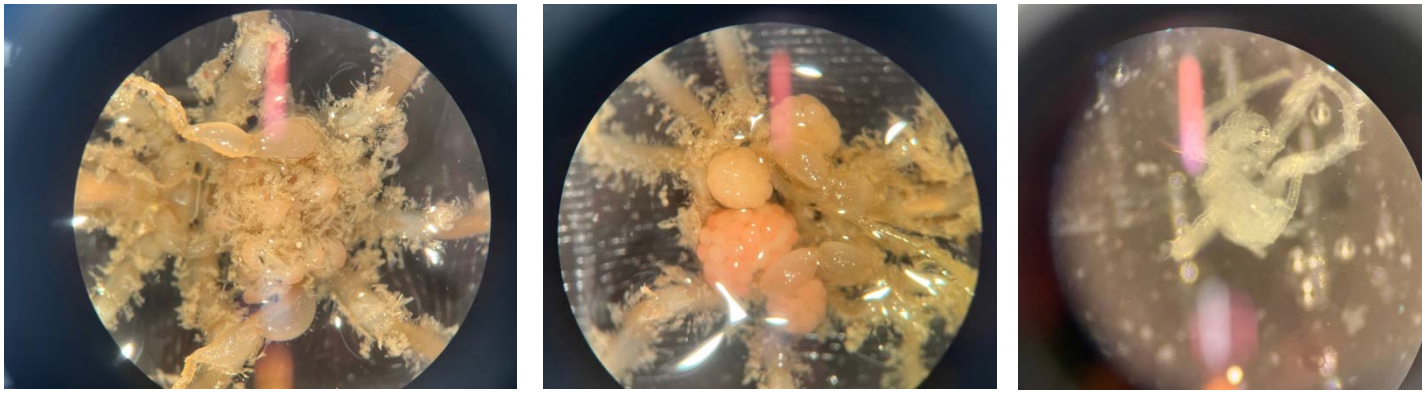


Figure 5. Photos of sea spiders, eggs, and developmental stages found on the NBP 23-03 cruise.

Polychaete bioluminescence
(H. Mancke, graduate student in the Halanych lab)

Polynoidae is a family within the suborder Aphroditiformia, commonly known as scale worms. These worms are relatively common in the Antarctic benthos and have relatively understudied visual systems. I sought out to collect as many worms as I could to understand what opsins, light-sensitive proteins involved in phototransduction, they are expressing in light-poor areas. The worms I managed to collect were *Eunoe opaline, Laetmonice cf producta, Harmothoe fuligineum,* and a handful of *Eunoe sp* and *Harmothoe sp.* These worms were placed in Big Antarctica, one of the walk-in freezers kept at 1°C., in total darkness between five and six hours. At the end of this darkness treatment, the worms were prepared under a red-light head lamp for decapitation. We plan to use the heads transcripts to identify the active opsins expressed, with additional body tissue samples to sequence and identify the worms formally, beyond the loose taxonomic identification.

It was during these trials that I noticed a majority of the polynoid worms bioluminesced, and the light they emitted was blue as opposed to the reported green in other members of Polynoidae. Bioluminescence can be used for a variety of tasks; predator evasion, mating, signaling to conspecifics, etc. The worms I worked with seemed to do one of two behaviors; they would separate from their torso just a few segments below the pharynx. The head would curl up and stay dark while the body would wiggle and flash very brightly. Secondly, some worms would release glowing elytra and attempt to flee. These behaviors seem to indicate predator evasion, but without observing them in a more natural setting, it's not definitive. Investigating both the opsins

expressed and the presence of bioluminescence could shed insight into the ecology, behavior, and sensory of evolution of this group.

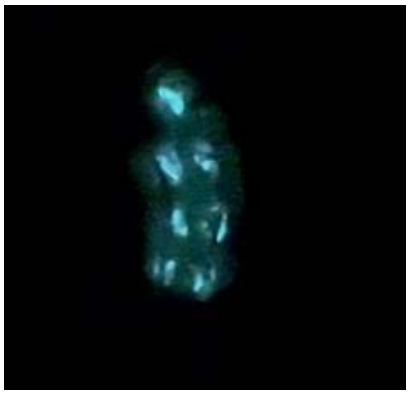



Figure 6. Organisms used in the polychaete sight and bioluminescence studies.

Echinoid Spawning
(S. Schreiter, graduate student for B-305)

I was brought on by Ken Halanych to spawn sea urchins, specifically the target species *Sterechinus neumayeri* and collect sperm for transcriptome analysis. Since most sea urchins that came up in the trawl were not alive, I dissected individuals to collect the gonads. During the NBP 23-03 cruise, I collected gonads from *S. neumayri, S. antarcticus, Abatus phillipi,* and *Urechinus* cf. *naresianus, Pourtalesia hispida, Ctenocidaris* species, and *Notocidaris* species. Most individuals that we collected were female, but we still found males. We collected both eggs and sperm, though sperm was our objective. The sperm was flash frozen using liquid nitrogen and stored at -80°C directly after. Eggs were put on ice and stored at -80°C. A few gonad samples were stored in ethanol. I also found *U.* cf. *naresianus* females that were actively brooding at the time of collection. The brooding females had about 10-20 juveniles in their marsupia (brooding pouches), which was on the internal aboral side of their test. A few juveniles were collected and stored in ethanol or formalin.

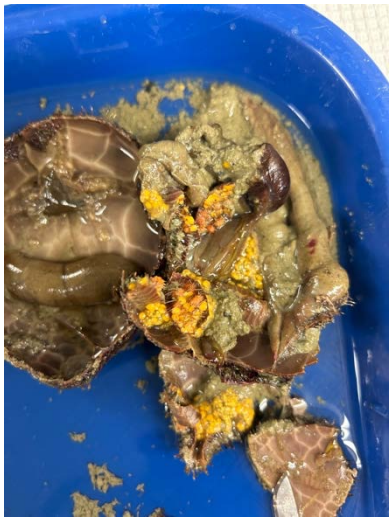



Figure 7. Urchins from the NBP 23-03 and dissections for spawning experiments.

Learman and Steen (B-014-N):
The overall goal of the Learman and Steen project was to obtain benthic sediment samples from the Eastern continental shelf in Antarctica to examine how microbial communities are degrading complex organic matter. This project will utilize enzyme function (extracellular enzymes), geochemistry, and gene potential (metagenomics) and function (metatranscriptomics) to elucidate how microbes in benthic sediments degrade complex organic matter, a process with implication to both benthic and pelagic organisms. To satisfy this goal we will use tools such as metagenomics and metatranscriptomics coupled with geochemical data to relate gene potential to function. We were able to successfully sample 10 stations. Of these 10 stations, we were able to obtain porewater from a drilled core liner at 9 stations. The porewater was used to collect geochemical data that will provide additional data such as dissolved inorganic carbon, dissolved phase total organic carbon, sulfate, and sulfide. Another goal of ours was to get sediment depths up to 15 cm. We were able to obtain depth samples between 6-15 cm at 8 out of the 10 stations. The cores were sectioned into 3 cm intervals and half of each of those intervals were flash frozen in liquid nitrogen to be shipped back home for DNA/ RNA extraction. While the other half of each 3 cm section was used for additional testing such as obtaining cell counts, porosity, testing for methane, and microcosm experiments that were set up and executed while on board the RVIB.

The two aims that were listed in the NSF proposal by CO-PI Learman and Steen were:
A1): Differentiate metagenomic potential and metatranscriptomic expression of sediment microbial communities to degrade complex organic matter across a transect that spans the entire continental shelf of Antarctica: Eastern Antarctica, Prydz Bay, Ross Sea, Bransfield Strait, and Weddell Sea.
A2): Compare how metagenome potentials compare to rates of microbially-catalyzed organic matter transformation using extracellular enzyme assays and metabolomics.

The samples collected will allow us to satisfy all of A1 with the exception of Prydz Bay, as we were not able to collect samples in that area. The benthic sediment samples that were collected from this cruise possessed different visual characteristics. Visually, (color, grain size, smell) we were able to collect a diverse set of samples on this cruise. Samples collected from depths ranging 235 -715 m. Additionally, these samples will also be used for nutrient and isotope analysis.

Figure 8. Megacore collections for the Learman/Steen group on NBP 23-03.

To satisfy the objective in A2, small-scale experiments were set up using the oxic top layer of our sediment cores with the addition of filtered seawater and selected metabolites. These include isotopically-labelled glucose, starch, N-acetyl-β-D-glucosamine, and algal cells. To study the catalysis rates of these substrates by extracellular enzymes, separate incubations were set up

using fluorogenic substrates including: MUB-α-D-glucopyranisole, MUB-β-D-cellobioside, MUB-N-acetyl-β-D-glucosamine, and leucine-AMC. These incubations were subsampled periodically and frozen for later analysis on enzyme rates and down-stream products.

These data collected from this cruise will be used to compare data collected from the 2020 cruise (in prep) comparing areas with relatively high and relatively low organic matter to examine how gene potential relates to gene function. Together these data will provide a comprehensive view of how microbial communities degrade complex organic matter by showing the correlation of gene potential and expression with enzymatic activity.

Gerken and Kocot (B-010-N and B-237-N)

Field Testing of the Aquatic BioTechnology Epibenthic Sled

NBP 23-03 was the inaugural cruise for the newly acquired USAP Aquatic BioTechnology epibenthic sled (EBS). This instrument (see Figures 9-11) is designed to be towed while sliding over the surface of non-rocky sediments to collect small organisms living on the surface of the sea bottom or in the first few centimeters of the sediment. The instrument consists of two stainless steel cases with attached plankton nets mounted on aluminum runners. The bottom case (epi) generally recovers more heavier specimens (e.g., shelled molluscs) while the top case (supra) generally recovers more lighter material.

This new instrument has several advantages over the EBS used previously by USAP. It is lighter in weight and has numerous bridle attachment points meaning the instrument is less likely to dig into the bottom and fill with sediment rather than collecting material kicked up into the water column by the wire. The stacked nets are an improvement over the previous EBS, which only had a single net. Also, the cases are designed to open only when in contact with the bottom, which depresses a lever on the underside of the instrument, such that benthic invertebrates rather than plankton are collected. The instrument can also be equipped with an underwater light and GoPro housing rated to 250 meters depth.

During the cruise, twelve deployments of the EBS were performed, eleven of them with gear reaching the bottom. All deployments of the instrument during NBP 23-03 were performed using 500 micron nets and codends. Working closely with the MTs, especially Matt Louis, we tested several approaches to determine the optimal towing strategy (Table 1). The approaches we tried included first laying out wire on the bottom to a set length and then hauling in the sled while the ship was not underway, and second laying out wire to a set ratio (from 1.4-2 :1) and then towing for 5-10 minutes before hauling in the sled while the ship was underway. Although one deployment was recalled due to wind, another essentially failed when the instrument tangled on the wire and two yielded suboptimal samples (one of the nets was clogged with mud), all eleven deployments that reached the bottom yielded samples and overall PIs Gerken and Kocot are ecstatic about this instrument and the samples of small macrofauna that it provided.

The video capability of the EBS could only be tested on one deployment as most were performed significantly deeper than the 250 m depth range of the housing. This video provided valuable insight into the performance of the instrument. In this case, the instrument was towed with the

ship underway rather than ‘hauled in’ with the ship at a full stop. The video showed that the instrument was being pulled too quickly resulting in it picking up too much sediment and larger mobile organisms (crinoids).

Overall, PIs Kocot and Gerken collected 1418 lots of specimens totaling over 5000 specimens using this instrument not including bulk-fixed material that could not be live sorted during the cruise. Notable finds included two different species of monoplacophorans in relatively high numbers. Monoplacophorans are very rare molluscs that are typically encountered in low numbers (1-2 specimens per cruise).

Concerns that arose during the cruise included the rear corner welds on both sides (at the tip of the tear drop at the rear) cracked free about halfway through the cruise. MT Matt Louis reinforced the cracked welds with bolts and was exploring other options including welding in structural supports, but nothing further was done during the cruise, as appropriate materials were not available. Structural reinforcement in the form of a triangular block in each corner is planned for the next port call in Punta Arenas. There was wear on the upper canvas of the supra (upper) net, due to rubbing on the metal box of the net mouth. Matt Louis reinforced the canvas material where wear was evident, but clearly this is an area to be monitored regularly for wear on all the nets during use, and reinforcement as needed. Also, by the last few deployments, the nets were showing some wear, acquiring pinholes that were reinforced to prevent the nets splitting. Thus, net inspection after each deployment, regular net replacement and several back up nets onboard are likely to be necessary when the gear is utilized in the future.

Table 1. Summary of EBS deployments during NBP 23-03.

Station #	Event #	Notes
1	5	Deployed at 0.5 knots. Paid out 500m after on bottom. Retrieval started when full stop requested. Both nets were clean with good samples in codends.
2	12	Deployed at 0.5 knots. Paid out 250m after on bottom. Retrieval started when full stop requested. Both nets were clean with good samples in codends.
4	21	Deployed at 0.5 knots. Paid out 250m after on bottom. Retrieval started when full stop requested. Both nets were clean with good samples in codends.
7	35	Deployed at 0.5 knots. Paid out 500m after on bottom. Retrieval started when full stop requested. Both nets were clean with good samples in codends.
11	54	Deployed at 0.5 knots. Paid out 250 m after on bottom. Underway at 0.5 knot due to ice during recovery. The nets were somewhat clogged as a large benthic nemertean (Parborlasia) was caught which exudes large quantities of thick slime. However, the sample was excellent, including monoplacophorans.

12	61	Deployed at all stop. Paid out 250 m after on bottom, waited until full stop achieved before recovery began. Instrument came up tangled with virtually no sample.
15	71	Deployed at 0.5 knots. Paid out 500 m. Did not come to all stop for retrieval, was underway at 0.5-1 knot due to ice during retrieval. Camera deployed, camera and light worked well. Video showed sled was moving too fast and digging into bottom. Supra net was clean with sample in codend, epi net was full of mud for half length of the net.
NA	NA	Deployment aborted shortly after equipment entered water due to increasing wind and ice conditions.
16	78	Deployed at 0.5 knots. Paid out 250 m after on bottom. Retrieval started after all stop requested. Supra net came up 1/3 full of mud, epi net was clean with sample in codend.
18	86	Deployed at 0.5 knots. Towed at 2:1 wire scope for 5 min for 1 knot. Supra net was very clean with sample in codend, epi net was filled with mud to about 35 cm above the codend, as the codend was corked up by a large sea pig.
21	95	Deployed at 0.5 knots. Wire ratio was 1.4:1, towed for 5 minutes before retrieval. Total deployment was about 1 hour, when all other deployments were about 2 hours. Both nets were clean with good but small sample in codends.
22	97	Deployed at 0.5 knots. Wire ratio about 1.5:1, 10 minute tow. Towed at 1 knot for 2 minutes then dropped to 0.5 knots. Both nets were clean with good sample in codends.

Figure 9. Aquatic BioTechnology Epibenthic Sledge (EBS).

Figure 10. Sunrise deployment of the EBS by MTs Matt Louis and Hila Shooter.

Figure 11. Successful sled deployment, note the clean nets and the codend going into the bucket. Photo by Heather Jackson.

Cumacean biodiversity:
NSF-OPP 2138993 Collaborative Research: ANT LIA. Cumacean-Omics to Measure Mode of Adaptation to Antarctica (COMMAA), lead PI S. Gerken, in collaboration with K. Kocot.

My team consisted of myself and MS student V. Vandersommen, working in conjunction with PI Kocot and his team (Grad students N. Roberts, E. McLaughlin, C. Olson, and post-docs C. Cobo Llovo and F. Bergmeier).

Goals of our participation in this cruise were collection of fresh material suitable for both morphology and -omics, and photodocumentation of living individuals, including pigmentation patterns and pigmented eyelobes.

Overall, 20 events produced cumaceans, ranging from 1 to more than 500 per event, totaling over 3000 individuals, although this is an underestimate as there were several lots that had so many individuals that number of specimens was estimated. The highest numbers of individuals and highest diversity were produced by the new Epibenthic Sled (EBS), while 1-few individuals per event were produced by Blake trawl, Boxcore or Megacore. In comparison with NBP 20-10, the new epibenthic sled was spectacularly successful, with two casts with diversity of greater than 20 species, and three casts with very high density of *Vaunthompsonia* (several hundred specimens per cast).

Diversity included 37 morphospecies identified, with 8-10 additional species that will require further work to identify to morphospecies. There were multiple undescribed species encountered, including new species of *Leucon*, *Schizocuma*, *Campylaspis,* and *Atlantocuma*. There were several species of Leuconidae with distinctly opaque eyelobes, likely new species of *Ommatoleucon*, a genus currently only reported from Australian waters. An advantage of taking

photographs of living specimens at sea is that features such as opaque eyelobes, eyelobe and carapace pigmentation can be recorded, which is especially important for features that may become very difficult to observe on preserved specimens, in which coloration typically fades quickly. As can be seen in the figure (Figure 12), cumaceans can be quite colorful. The coloration may be dramatic, as in the orange *Cyclaspis* and *Platytyphlops*, or more subtle, such as in the *Hemilamprops* and *Vaunthompsonia*. The eyelobe is typically opaque white with red ommatidia, as can be seen in the figure on *Hemilamprops, Cyclaspis,* several *Campylaspis, Diastylopsis,* and *Vaunthompsonia*. The new Nikon microscopes increased our ability to identify specimens at sea, enhancing our work and making sorting and identification even more productive.

The lifestages encountered on this cruise included many manca (very early juveniles) of some species, indicating recent reproduction by those species. In other species, adult males and brooding or pre-ovigerous females were encountered, indicating current reproduction. A notable finding was a few females were captured mid-molt, from pre-ovigerous to brooding, meaning they were captured essentially during mating. Cumaceans are typically sexually dimorphic, with most of the difference observed in the terminal molt of the adult males, and adult males are typically not present unless reproduction is occurring. This suggests that Antarctic cumaceans may reproduce towards the end of phytoplankton productivity (suggested in several areas by significant amounts of flocculent algal detritus observed in megacores), presumably to produce an overwintering generation of juveniles, as is known in the life histories of some boreal species.

Due to the excellent living samples procured by the new EBS, specimens of all morphospecies were preserved cryogenically and in RNAlater for future transcriptomic and genomic work. Additional specimens of all species were preserved in 95% ethanol with 5% glycerol for morphology and sequencing. In additional, we tested the potential for returning cryogenically preserved specimens to 95% ethanol and 5% glycerol for future morphological work, which was very successful.

Outreach activities included four blog posts (2 each by PI Gerken and V. Vandersommen), participation in an outreach panel by V. Vandersommen, and collection of video interviews for future use.

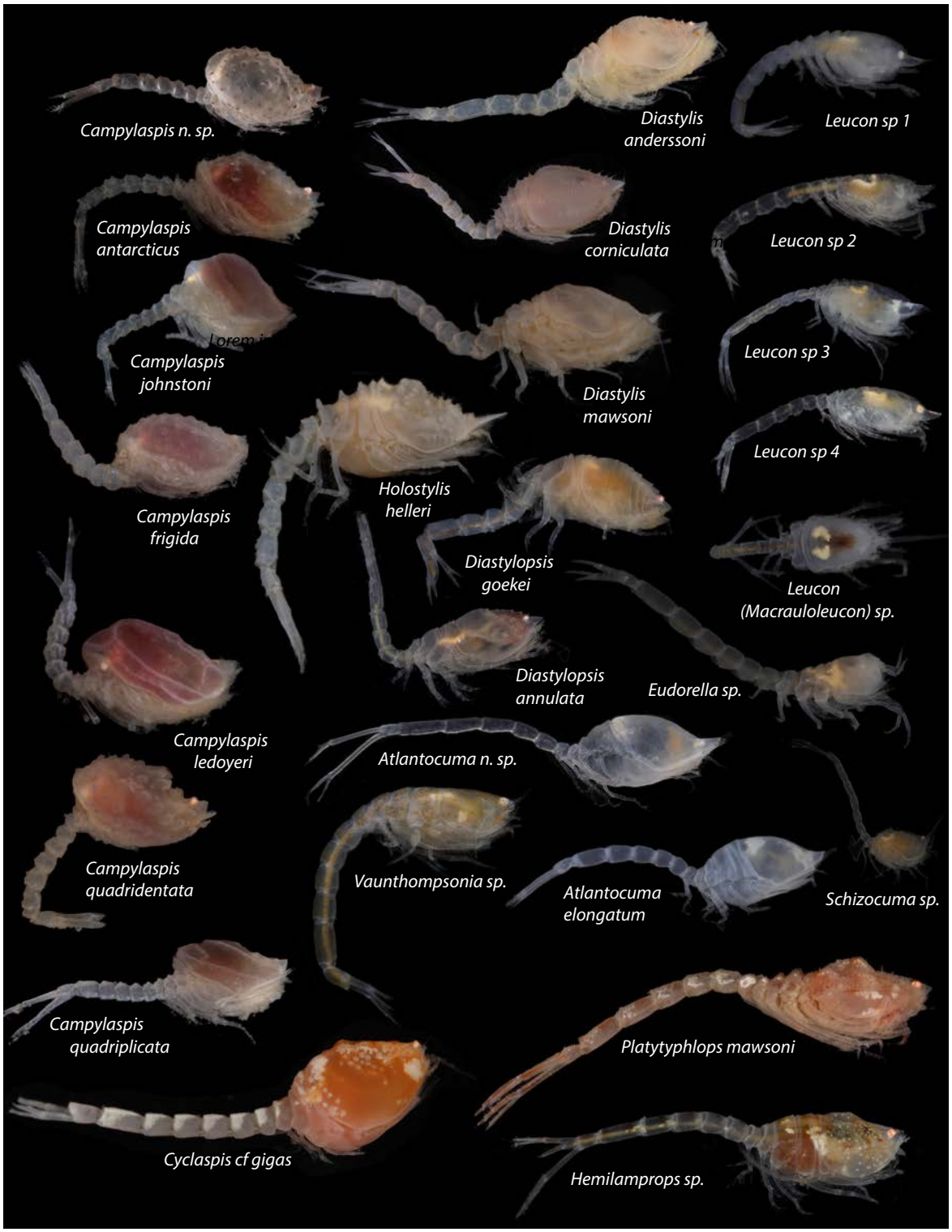


Figure 12. Cumacean biodiversity on the NBP 23-03 cruise. Photo credits for the figure are Sarah Gerken, Victoria Vandersommen, Emily McLaughlin

PI Kocot and his team of six (including himself) were funded through NSF DEB 1846174 ("CAREER: Revolutionizing Biodiversity and Systematics Research on Aplacophora (Mollusca) and Training the Next Generation of Invertebrate Systematists"). The overarching goal of this research is to advance knowledge on the biodiversity and systematics of aplacophoran molluscs while training the next generation of invertebrate systematists. Aplacophora is a group of shell-less, worm shaped molluscs comprised of two sub-clades, Solenogastres and Caudofoveata. Several species are ecologically important and Aplacophora plus Polyplacophora (chitons) make up the sister taxon of all other molluscs. The number of scientists studying aplacophorans is small with just a handful of expert researchers worldwide. However, Kocot's project (soon to be in its last year) is providing training opportunities for three Ph.D. students and two postdoctoral researchers. Collectively, this team is advancing understanding of this fascinating but poorly-understood group.

The objective of the Kocot team during NBP 23-03 was to collect aplacophoran mollusc material with emphasis on preservation of specimens for molecular work (in addition to morphological work) to support systematic research on this group including higher-level phylogenetics and an updated monograph on Antarctic Solenogastres. The latter builds on a 1978 monograph by Lutfried von Salvini-Plawen that served as our primary reference to Antarctic Solenogastres and has been translated from German into English by Ph.D. student Emily McLaughlin, who is leading the Antarctic monography work, with help from German postdoctoral researcher Dr. Franziska Bergmeier. Based on Salvini-Plawen (1978) and a handful of subsequent publications on Antarctic Solenogastres, Antarctica appears to have the most diverse aplacophoran fauna in the world with 73 described species. Eighteen of the 28 aplacophoran families and all traditionally recognized orders inhabit Antarctic waters. For comparison, the aplacophoran faunas of Spain and Norway are the best-known in the world, but just ~40 solenogaster species representing ten families have been described from Spanish waters and Todt (2013) stated "my own count of Norwegian solenogaster species currently has reached a number of more than 40 (about one-third of these are new to science)." These numbers may seem to suggest that the Antarctic Peninsula is not exceptionally more diverse than these more accessible regions. However, the majority of solenogaster species described from waters off Spain and Norway are small-bodied (<0.5 cm) whereas most described Antarctic solenogasters are relatively large-bodied animals (>1 cm), which are more likely to be collected in trawls, unlikely to be overlooked during sample processing, and are more easily studied than small animals. During NBP 23-03, solenogaster samples were obtained via a new epibenthic sledge (EBS; described above), Blake trawl, and box corer. Specifically, during the roughly three weeks of sampling, 266 lots of Solenogastres were collected, totaling nearly 500 specimens. These specimens were collected from 11 different stations and 25 different sampling events. The vast majority of these (208/266) were small-bodied animals collected using the EBS, unlike our sampling during NBP 20-10 where we primarily obtained large-bodied animals from the Blake trawl and relied on sieving mud collecting by this trawl to opportunistically obtain a small number of small-bodied animals. Samples from the EBS yielded an exceptionally high diversity and abundance of Solenogastres, further confirming the Antarctic as a hot spot for the biodiversity and richness of this taxon. The new EBS used during this cruise consistently yielded large numbers of small specimens while relatively few larger animals were collected by trawling

during this cruise (~15), probably due to the paucity of corals and hydroids (primary prey items of Solenogastres) at the sites sampled. Based on preliminary morphological examination, we estimate that between 50-70 different species were collected. Thanks to the large numbers of specimens collected by the EBS, most species were represented by several specimens and a few were found in high numbers.

EBS samples were live sorted to the extent possible on board using the USAP stereomicroscopes. Notably, two new Nikon Nikon SMZ 1270 stereomicroscopes with View 4K digital cameras were very beneficial to our sorting and the digital cameras hooked up to computer monitors were useful in teaching students to recognize certain organisms or characters while on board. Virtually every specimen was imaged using the Kocot lab Canon EOS Mark IV digital camera with a 100 mm or 65 mm macro lens and/or the ship's Leica/Wild M3C Stereomicroscope and Canon EOS 60D digital camera. For the latter, we connected a tethered speedlight and were able to take very high quality stereomicroscope photos – USAP may be interested in investing in such a setup (ca. USD $600) for other researchers in the future. A smaller number of specimens were also selected as subjects for videos and time lapse videos to capture behavior (see separate report by Chandler Olson and Nick Roberts). Overall, we were very pleased with the quality of live images and videos we were able to take of animals on board despite the challenges of photography on a moving ship. Sclerites from most morphospecies were imaged on board using the ship's Nikon E800 compound microscope. For most species, specimens were preserved intact for morphological work in 95% ethanol, 4% formalin, and 2.5% glutaraldehyde. Additional specimens of most species were dissected with tissue samples preserved in 95% ethanol, RNAlater, and frozen. For very large specimens, tissue samples were taken for molecular work while the anterior and posterior ends were retained intact for morphological work. Most specimens could only be identified to the order or family level on board as further examination of sclerites using a compound or scanning electron microscope and, in many cases, histology will be needed to identify specimens more specifically. However, a few species were easily recognized. These include the extremely large *Neomenia megatrapezata*, the uniquely 'warty' *Sandalomenia pappiligera*, the narrow and keeled *Sandalomenia carinata*, the bizarre sponge-inhabiting *Apodomenia enigmatica*, the abundant species *Dorymenia tricarinata*, and several species of *Entonomenia* (see Figure 13). Because some EBS samples were very large, these were divided with a fraction of the sample bulk-fixed in cold ethanol for future sorting.

In addition to the large volume and diversity of specimens collected, we were able to make several interesting biological observations that are expected to result in additional publications beyond the scope of the originally proposed work. Three species of the order Pholidoskepia were observed to brood fully developed juveniles up to one third of their body length in their reproductive tract. While brooding of young in the mantle cavity has been reported in the order Cavibelonia, this is the first record of this in Pholidoskepia and the first record of juveniles so large compared to their mother to our knowledge. Further, we documented the first known parasites of Solenogastres. Two species belonging to the family Proneomeniidae were observed to contain nematode parasites. Tissue of these parasites was preserved for molecular and the head end of one worm was preserved in glutaraldehyde. We aim to collaborate with our nematologist colleagues to identify these worms. We were also able to collect two specimens of the strange, highly reduced (parasitic?) solenogaster *Apodomenia enigmatica*, which we

described in 2019. This material will be used for histology to improve our understanding of the anatomy of this species as the radula and some other characters could not be discerned in the type material.

Although our focus was on Aplacophora, we were opportunistic and also collected specimens of diverse invertebrate taxa for other ongoing and planned research projects and the Alabama Museum of Natural History Invertebrate Zoology collection. In total, we collected 1,418 lots of samples (including the cumaceans reported on by PI Gerken). Notable 'by-catch' included exactly 50 specimens (including a few dead shells) representing two species of the rarely collected molluscan class Monoplacophora (see separate report by Dr. Franziska Bergmeier) – a source of great excitement for our team! We also collected three specimens representing two new species of Xenoturbellida, a phylum currently consisting of only six species.
Despite delays to the start of the cruise and the early termination of science, we consider the cruise a great success. Aside from a desire for more science time, the main issue we faced during the cruise was the overwhelming amount of work it took to live sort our samples, image specimens, and process them as appropriate for both molecular and morphological work. These activities are important for our work as data on living animals and material preserved for genomics is needed. Therefore, we found our team of six individuals to be inadequate to conduct the necessary work and, in many cases, we raced to finish processing a sample just in time for the next EBS deployment. This hindered our ability to spend adequate time taking high quality photos, making observations of living animals, and taking much-needed breaks. Instead of keeping track of how many hours were worked by our team, I began recording the number of hours that the lab was *not* actively processing samples after science began until it was terminated – just over 72 hours (mostly during a single long transit and meal times). In the future, our team would be much more efficient with at least two additional members to help process samples.

We expect the work performed during this cruise to have immediate impact on our research and on the research programs of our colleagues. During the cruise, specimen collection data were entered into an Arctos (museum database management system) bulkloader spreadsheet and both collection data and photos will be made publicly available as soon as the cruise concludes. These data will be available not only via Arctos (http://arctos.museum.database/almnh_inv), but also through the Global Biodiversity information Facility (GBIF) and iDigBio.
Prepared by: Kevin Kocot

Figure 13. Examples of Solenogastres collected during NBP 23-03. **A.** Pruvotinidae sp. indet. **B.** Pholidoskepia sp. indet. containing embryos. **C.** Cavibelonia sp. indet. with orange material in the gut. **D.** *Micromenia* sp. **E.** Amphimeniidae sp. indet. **F.** *Neomenia megatrapezata*. **G.** *Entonomenia* (*carinata*?). **H.** *Apodomenia enigmatica*. Scale bar under A is 1 mm and corresponds to A-D. All other scale bars are 1 cm.

Invertebrate Filming and Behavioral Observation:
(Chandler Olson and Nickellaus Roberts, Graduate students for K. Kocot)

Prior to the NBP23-03 cruise, an experimental aquarium setup was prototyped and developed by Nickellaus Roberts to record small Antarctic invertebrates as close to their natural behavior as possible. This system is unique in that its design and accompanying equipment were developed and obtained for recording invertebrate taxa often overshadowed by other large charismatic relatives. The flowing seawater system in the Hydro Lab of the NBP was used to provide cool and clean fresh seawater to specimens. Prior to fixation, high-quality video, pictures, and observations of behavior were recorded in and sediment from the sites sampled. Specimens recorded in this way were sampled using both the Epi Benthic Sledge for small fragile invertebrates and the Blake Trawl for larger charismatic fauna. Observations captured using this experimental setup include, unrecorded sea spider (Chelicerata, Pycnogonida) feeding and burrowing behavior, as well as burrowing and locomotory behavior of Antarctic Solenogastres (Mollusca, Aplacophora). Over 11 diverse and morphologically disparate invertebrate phyla were capture using this setup. A short Nature Documentary, filmed and edited by Chandler Olson will be distributed digitally profiling the multitude of specimens captured using this experimental setup on the Seahorse and Co. YouTube Channel

Figure 14. Representative photos from the camera setup by Olsen and Roberts.

New insights into Antarctic Monoplacophoran Diversity (Mollusca)
(Franziska S. Bergmeier, Postdoctoral fellow for K. Kocot)

Monoplacophorans are enigmatic molluscs: on first glance resembling limpet-like gastropods, they actually constitute their own class. Findings of these rare molluscan "living fossils" are often limited to few individuals or empty shells. So far, three (out of a global total of currently 30 nominal species) have been reported and described from the Weddell Sea and Lazarev Sea.

While live sorting sediments collected with the new epibenthic sledge, we found overall 47 living monoplacophorans and additionally 3 empty shells belonging to two different morphospecies during two different sampling events (Ev. 54, and Ev. 95). Based on external morphology of the soft body and shell characters (e. g. shape and position of the apex, number of gills, postoral tentacles) both species most likely constitute new species within the genera *Laevipilina* McLean, 1979 and *Micropilina* Warén, 1989. Both species are very small, ranging between 1 – 1.5 mm (*Laevipilina* sp.), or less than 1 mm (*Micropilina* sp.). Like their congener *Micropilina arntzi* Warén and Hain, 1992, the newly discovered *Micropilina* sp. is a brooding species with juvenile monoplacophorans present in the pallial groove of adult individuals. Individuals of both species exhibit a characteristic reddish-brown coloration along their mantle and foot margins, indicating the presence of symbiotic bacteria.

We have preserved specimens of both species for future molecular work as well as histological and ultrastructural investigations. Finding the elusive Monoplacophora in such high abundance was unexpected and one of our personal highlights of the NBP 23-03. We are looking forward to shedding more light on the diversity of monoplacophorans in Antarctica.

Bik Lab (B-252-N)

Our NSF OPP Antarctic project is addressing three key science aims:

- **Aim 1:** To determine if molecular data supports high biodiversity and endemism of benthic meiofauna in Antarctic benthic ecosystems.
- **Aim 2**: To determine the proportion of marine nematode species that have a deep-sea versus shallow-water evolutionary origin on the Antarctic shelf, and assess patterns of cryptic speciation in the Southern Ocean.
- **Aim 3:** To determine the most important drivers of the host-associated microbiome in Antarctic marine nematodes.

Linking NBP23-03 sampling with Science Aims: Our sampling goals were hindered by the lack of soft-bottomed habitat appropriate for sediment coring on our East Antarctic cruise transect, and in addition the medical emergency and operational changes caused early cessation of science activities and prevented our team from obtaining material from a critical deep-sea sampling site that was integral for achieving Aim 2. Nevertheless, we obtained sufficient material to begin work on Aim 1 (eDNA metabarcoding) and Aim 3 (characterization of nematode microbiomes) with the NBP23-03 samples. Work on Aim 2 will be contingent on the availability of public

sequence data, donated sediments from collaborators, and/or future opportunistic deep-sea samples from the Southern Ocean.

Sediment Core Sampling. The Bik Lab was able to obtain a robust set of sediment cores at 10 out of 22 stations on NBP23-03; at these 10 stations we were able to collect multiple cores from the same megacore deployment for eDNA, phylogenetic/taxonomy, and nematode microbiome science aims. Additional small amounts of sediment were obtained at 3 additional stations, where the megacorer was only partially triggered or there were other issues preventing successful deployment for the Bik Lab. At the remaining 9 stations we were unable to obtain any sediment samples at all due to pervasive hard bottomed habitats, weather/ice conditions, or constraints on wire time. Overall the Bik Lab was extremely surprised by the lack of suitable sites for megacore deployment on the East Antarctic continental shelf. Many shelf stations appeared to be recently scoured by icebergs or potentially have year-round ice conditions that prevent typical sedimentation rates that one would expect on the continental shelf (e.g. which are seasonally driven by phytoplankton blooms during the summer in Antarctica). Water currents and steep/shallow bathymetry may also play a role in preventing higher sedimentation – we noticed that the most successful sites for coring had "bowl" type bathymetry (allowing sediments and flocculent material to easily accumulate) and/or were deeper than average (~700m where iceberg scour becomes less of an issue). Generally the cores we obtained were either 25cm+ deep with flocculent material on top of fine clay particles (**Figure 15A**) or were much shallower (<10cm) with a grainy/sandy consistency (**Figure 15B**).

Figure 15A: 25cm+ Muddy Cores (Station 2)

Figure 15B: ~10cm Sandy Cores (Station 17)

Summary of Bik Lab Sediment Cores collected on NBP23-03:

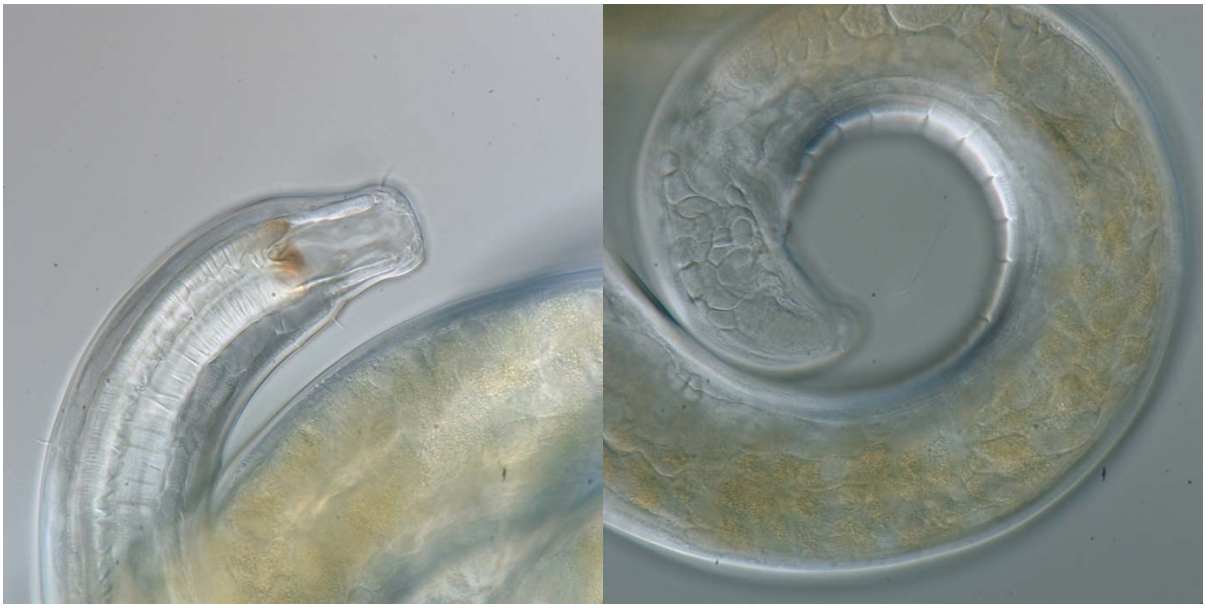
Science Aim	Preservation Method	No. of Stations	Total No. Cores Sampled	Notes
Sediment Analysis*	Frozen -80°C	10	10	For granulometry, TOC, etc.
eDNA metabarcoding	Frozen -80°C	11	52	Three replicate Falcon tube subsamples taken per core
Nematode Microbiomes*	Frozen -80°C	13	76	Parallel sampling from eDNA cores
Molecular Phylogenetics*	DESS (4°C)	11	34	Preservative enables morphological IDs + DNA barcoding
Nematode Taxonomy*	Formalin (room temp)	11	34	Samples for formal species descriptions + SEM

All samples represent top 0-10cm of megacore; Asterisk denotes cores that were vertically fractionated at 2cm intervals where possible. Some cores were split and used for multiple science aims (e.g. one core may be used for eDNA Falcon tube subsampling as well as subsampling for microbiome/phylogenetics/taxonomy aims).

Additional opportunistic samples of individually picked nematodes. The Bik Lab was additionally able to collect opportunistic samples of individual nematode specimens that were set aside by the Kocot Lab as they picked through meiofauna samples collected via the Epibenthic Sledge and Blake Trawl. We were able to sort these nematode specimens into morphospecies and capture some preliminary images using onboard microscopy systems, and when possible, preserve specimens in a way that will enable integrated molecular-morphological work when we return to UGA (using DESS, RNAlater, Repli-G buffer, and Formalin preservatives). Our most exciting find was a novel "toothless" nematode putatively belonging to the predatory Oncholaimidae family, a group not previously known to contain species without teeth. A summary of the most abundant nematodes collected is as follows:

Nematode Taxa (Genus/Family)	Station	Sampling Equipment	Number of Specimens	Preservatives Used
Halalaimus sp.	1	EBS	13	DESS, RNAlater, Repli-G, Formalin
Thoracostomopsidae sp.	1	EBS	3	DESS, RNAlater, Formalin
Halalaimus sp.	4	EBS	24	DESS, RNAlater, Repli-G, Formalin
Microlaimida sp1.	4	EBS	2	RNAlater, Formalin
Thoracostoma sp.	4	EBS	2	RNAlater, Formalin
Nemanema sp.	4	EBS	2	DESS
Anoplostoma sp.	9	Box Core	4	DESS, RNAlater, Formalin

Adoncholaimus sp.	11	EBS	2	RNAlater, Formalin
Sabatieria sp.	11	EBS	2	RNAlater, Formalin
"Toothless" Oncholaimidae sp. ***(pictured in below photos)***	11, 14	EBS, Blake	2	RNAlater, Formalin

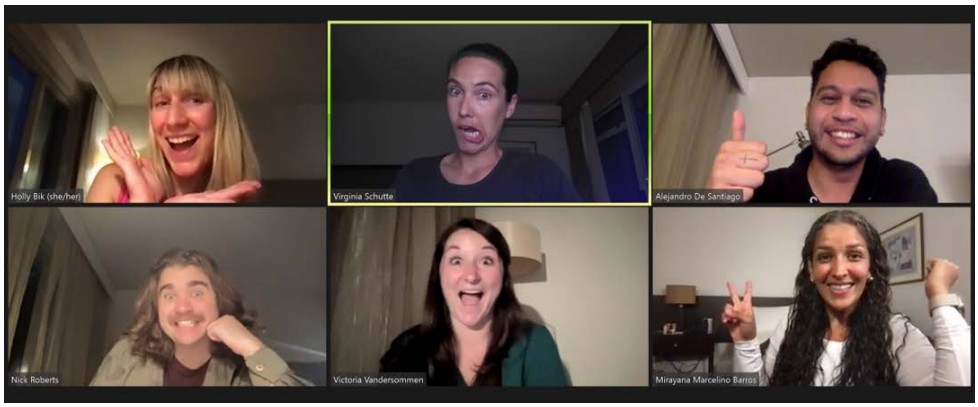


Filtered eDNA samples for collaborator Bradley Tolar (UNCW). We were also able to collect opportunistic water samples from CTD bottles at 21 stations for collaborator Bradley Tolar at the University of North Carolina, Wilmington, who plans to use these samples for generating preliminary -Omics datasets of Archaea communities in East Antarctic waters. At each station we aimed to collect 3 replicate 1 Liter water samples at each of four depths: 1) Surface Water, 2) Above the Halocline, 3) Below the Halocline, and 4) Bottom Water. Each 1L water sample was filtered across a 0.22μm Sterivex filter using the Mahon Lab's peristaltic pumping system designed for eDNA sample collection. In total, we were able to collect 244 eDNA samples on Sterivex filters, which will be shipped to UNCW along with the Halanych Lab's samples.

Outreach and Science Communication. The Bik lab's cruise budget included dedicated outreach funding for Science Media Specialist Dr. Virginia Schutte to join the science party of NBP23-03. Together, Drs. Bik and Schutte held multiple events at the beginning of the expedition, conducted real-time outreach throughout the expedition, and plan to publish a number of post-expedition products beginning summer 2023. The Bik lab's outreach philosophy centers heavily on experimentation, and outreach products include a diverse portfolio of materials targeting a variety of audiences. We ran a reddit AMA (Ask Me Anything) on March 12 through PI Bik's reddit account that connected audiences with all grantee teams and several staff members living and working on the ship. This Q&A was seen by millions of people and earned more than 7,000 engagements. Impact highlights include comments like "*It seems like you are all so passionate about what you're doing and are exactly where you're meant to be."* On March 14, we held an NSF Live presentation and Q&A attended by 300+ people over Zoom. This event featured a panel of 5 grantees from 3 teams discussing their work and life as scientists, and a ship tour given by ship's staff who answered questions alongside grantees during the Q&A. Including a tour in the NSF Live was a new format for this kind of event, and multiple attendees specifically named the ship tour as the best part of the Live. On March 20 we conducted two interviews once onboard the ship; one recorded over Zoom and another livestreamed to YouTube and Facebook. While we currently don't have the internet to pull typical metrics of success for the recorded interview, the livestream reached 500+ people and earned 150+ engagements.

An example photo shared to WhatsApp.

Finally, throughout the cruise we carried out real-time outreach via the WhatsApp messaging platform, which uses such little data that we could post nearly daily even when we only had access to an extremely slow internet connection. The mini blog reached an estimated 1,000+ people daily by the end of the expedition, and we plan to collect further post-cruise qualitative analytics to better characterize this micro-blogging audience on WhatsApp. Other future products include YouTube partnerships and photo features, which will be produced after the cruise using footage, photos, and interviews that Dr. Schutte collected during NBP23-03



The panel of scientists who participated in the NSF Live.

Appendix 1: Cruise Track map. attached as separate file (big.Full-Cruise.etopo1.pdf)

Appendix 2: NBP 23-03 roster including ASC and ECO crew

Appendix 3: Gear deployments for NBP 23-03 science teams. This is included as a separate file (NBP23-03_Event_log_FINAL.pdf)