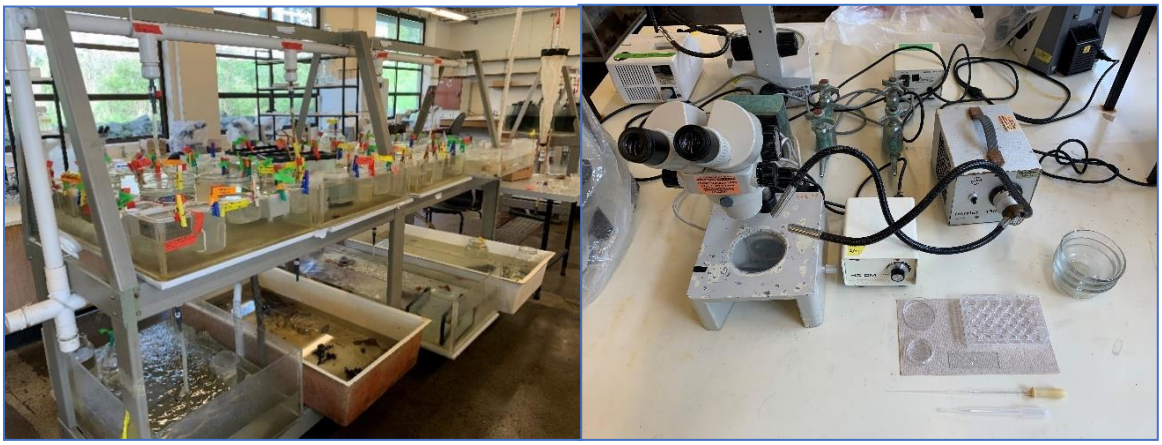
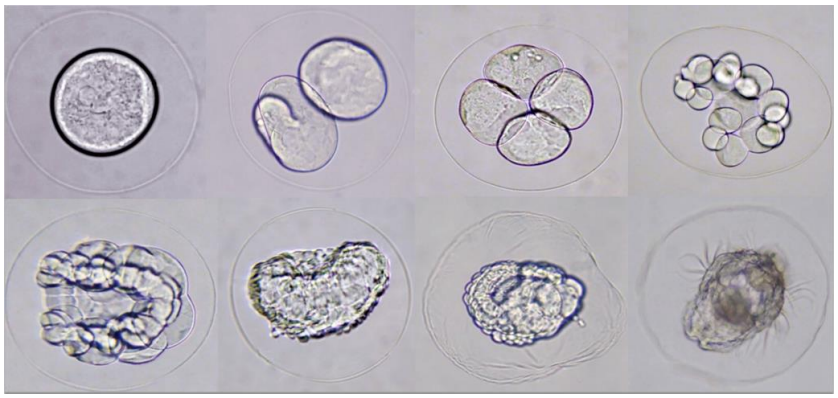
Relazione Attività Ricerca Tirocinio e Tesi: Biodiversity through the Lens of Single-cell Genomics.

Vincenzo Petrillo M74000113 – Corso di Laurea Magistrale in "Biology and Ecology of The Marine Enviroment and Sustainable Use of Marine Resources"

With the aim of forming students and guiding them turning into scientists, Research Apprenticeships are run since decades at Friday Harbor Laboratories UW. I've been able to attend "The Marine Biodiversity Through The Lens of Single-Cell Genomics", a full-time apprenticeship, thanks to Professor Leonid Moroz (University of Florida) and its collaboration with Professor Anna Di Cosmo (University of Naples Federico II). Throughout the course I've learnt concepts, skills and techniques for research at the intersection of marine biodiversity and functional genomics, integrating perfectly for the activities I'm conducting in Di Cosmo's lab at the Department of Biology in Naples as part of my Master's Degree work of Thesis. During my stay at FHL (March 27th – June 10th) I was engaged almost 24/7 with the course activities, I went through a phase of getting acquainted with the study of marine invertebrates in a practical way followed by a phase of setting and working on a personal project. I've been sharing the course with other 15 students and contributed to some of their personal and team projects.

On the first two weeks the main focus of the whole group was on producing material to contribute to a book chapter on Ctenophores that Prof. Moroz was working on. Our team's task was to document all developmental stages of these animal's embryos and report all the methods. I've collected the animals (*Pleurobrachia bachei*, *Bolinopsis infundibulum*, *Euplokamis sp*.) made them spawn in jars, collected the embryos and analysed them on the dissection microscope and validate the stage using slides and compound microscope. We met regularly as a team to discuss and elaborate the data.

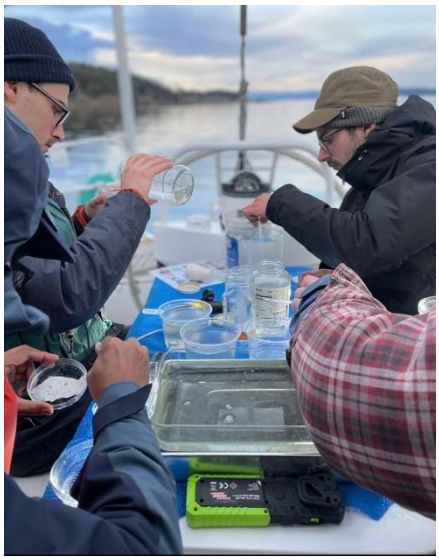




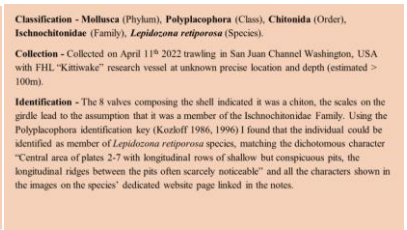
Euplokamis sp. develop*m*ental stages from 1-cell stage to pre-hatch larvae

Getting all kinds of live marine invertebrates for further experiments and genome sequencing is something everyone's been up during the whole spring quarter. We had regular field trips (2-3 times a week depending on the tide) to False Bay and Lime Kiln Point State Park sites collecting from intertidal environments, and going out on FHL research vessel Kittiwake at least 3 times a month, using both trolling net and dredging to get organisms from deeper waters. The nets would be pulled on board using a winch and unloaded on a table for sorting.

Around the end of the first month I also participated to a sailboat research trip lasted 4 days, with only 3 other course mates, Prof. Moroz and Captain Peter. While we were sailing north through the San Juan Islands archipelago we sampled planktonic organisms simple conical tow-net hauled by hand (150µm for zooplankton and 90µm when needed to retain phytoplankton too). We analyzed the fresh samples with a on-board lab set (microscope, containers to dilute the samples, jars and Eppendorf tubes to store the organisms). We got plenty of Chaetognaths and Larvaceans (important animals for evolutionary biology) and fixed them with ethanol for molecular and imaging analysis. During those days Peter, the captain, taught us everything about the sailboat and sailing, we were asked to do anything needed on deck, so we were learning by doing and had many duties.



Halfway of the course we had more than 300 different species of invertebrates in the lab's tanks and thousands of individuals. The species of interest for genome sequencing had to be validated, so each student used the dichotomous keys, the available literature and sometimes help from experts to properly ID the sampled organism, documenting everything on a ppt. For the sequencing 3 replicates per species were required, the sampling material was properly prepared in sterile condition and stored, then sent to University of Florida for the actual sequencing procedure.

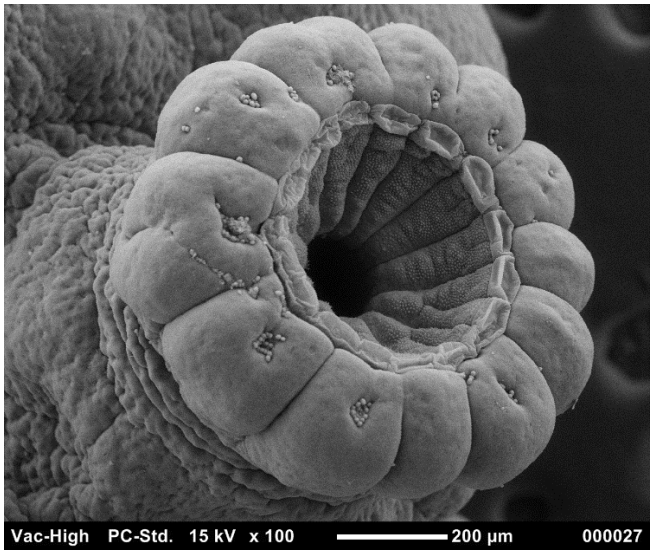


Dr. Eric Edsinger arrived from Salk Institute to assist the students during their personal projects. He also introduced us to GIGANTIC, an unpublished pipeline for phylogenetic analysis, and taught us how to run it. The project I've been focusing during the rest of the quarter is the assembly and differential expression analysis of the *Octopus bimaculoides* transcriptome. It allows to tell what

and how genes are expressed in different conditions, cells and tissues. The latter it's our case, we have 95 different Octopus tissues raw data to analyze and compare.

Reason why I chose to do that is because I recognized that this type of analysis could be useful for Di Cosmo's research and for my future as a researcher. The project is still on its way.

Dylan, the IT person set up a machine at FHL that I can access remotely from Italy. While working on this project I've experienced using major databases for biological information (NCBI SRA, Uniprot) and parsing sequences from them. I went to the process of learning how to use the existing literature to produce valid transcriptome from raw reads . Skills I'm growing include also python and R programming, and command line use to install and use bioinformatic software. I'm also going to use the computer resource to perform phylogenetic analysis for Dr. Eric Edsinger and Prof. Leonid Moroz. I've also participated to Yelena Fita's project on behavior and bioluminescence in *Bolinopsis*, and attended tissue preservation and imaging experiments including SEM and Confocal Microscopy.



SEM image of Octopus' Sucker.

These months I've spent abroad for my thesis work were certainly the most intense of my life. I've been in a complete different reality and even if Professors and Administration were there, I had to rely on myself, I had to face up my own issues and people that were hindering my learning and growth process. Despite my proficiency in English language in was not trivial to communicate with my peers, and during my time at Friday Harbor I've been improving my communication and team work skills significantly. In the end the whole experience was worth it and I'm satisfied with the outcome and much more opportunities are now open for me. I am infinitely thankful for anyone that helped me throughout this adventure, among them: Professor Anna Di Cosmo, Professor Leonid Moroz, Dr. Eric Edsinger, Professor Gianluca Polese, Mason Wiley. I would also like to express my great appreciation to University of Naples Federico II, especially to Ufficio Relazioni Internazionali staff for making all this possible.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