Limnology & Oceanography Research Exchange (LOREX) Application
I am writing to provide my very strong support for Antrelle Clark, my Ph.D. student, to receive a second LOREX fellowship to study in Israel. Antrelle is completing a NSF-NRT fellowship and will transition to her GRFP during 2024. Antrelle was awarded a LOREX fellowship to study in Israel during the summer of 2023, to work with Dr. Dror Angel at the University of Haifa. Antrelle is the best prepared, most energetic, independent and focused graduate student that I have had in my 30+ years at Auburn; she is also one of the best graduate TA’s we have had in our program. She will move to candidacy and so will be well-positioned to focus on her dissertation. Antrelle is keenly interested in understanding the relationships between organisms and the impact of climate change on host-microbial symbioses in marine and estuarine environments. We have previously discovered that Gulf of Mexico ctenophore *Mnemiopsis leidyi* bears small, lobose gymnamoebae (‘amoebae’), among other eukaryotic microbes attached to its comb plates, reported in two publications (*J. Euk Protozool.* 44:420; *Hydrobiol.* 251:495) and two MS theses (Connie Versteeg, Nova Southeastern; Kristian Smith, Auburn Univ.).

In the past year, Antrelle successfully cultured multiple amoebae morphotypes from Gulf of Mexico *Mnemiopsis* and found that ctenophores carry more diverse amoeba assemblages today than was observed previously. These exciting results provide support for our proposal that current ctenophore amoeba assemblages are sensitive to environmental conditions, resulting in different assemblages in different locations over different seasons, which we think is occurring in many places. We suspect that US Atlantic seaboard comb plate amoeba are migrating northward due to climate change. *M. leidyi* of the European seas bear gymnamoebae (unpublished observations) although different European seas contain ctenophores derived from different US coastal locations (*Mol. Ecol.* 19:2690; *MEPS* 485:25) and may therefore carry different amoebae. Dr. Angel, who is expert on Mediterranean ecology, is well positioned to help us understand the host/microbe dynamics. He has very generously agreed to host Antrelle in the coming year. In his lab in 2022, Antrelle cultured a single comb plate amoeba, thereby establishing that eastern Mediterranean *M. leidyi* do indeed carry them. Her amoebae were collected from very few animals, and so she needs additional field time to collect more material. She will bring back material for DNA extraction and will characterize her collections using 18S, COI and ITS3/4 sequence analyses after recently published methods (*Protists.* 161:102; *Amer. Micro.* 11:1). Her efforts will provide important new information about marine symbioses and their invasion dynamics and potential larger effects.

Antrelle’s work via the LOREX program will continue to expand our understanding of host-microbial invasion by one of the world’s most notoriously invasive marine animals. It will very likely open new doors for global collaboration on the microbial biology of invasive marine organisms. Antrelle will work hard to disseminate her results at regional, national and international meetings, and will use her insights in her teaching. The LOREX fellowship would greatly help her establish an international reputation. I hope that you agree. Thank you very much for making available this remarkable opportunity. Please contact me if you have any questions.

Sincerely,

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Dr. Adina Paytan, LOREX Principal Investigator
Association for the Science of Limnology and Oceanography
Re: Antrelle Clark, applicant for a LOREX IRES fellowship

2024

Dear Dr. Paytan,

Wednesday, January 3,
Letter of Support

Dear LOREX,

It is a pleasure to write another letter in support of Antrelle Clark’s application for support from the Limnology and Oceanography Research Exchange program. As you know, Antrelle came to Israel last summer as a LOREX intern to work on her doctoral research, on the effect of climate change on host-associate interactions in ctenophores. Although we have observed large populations of *Mnemiopsis leidyi* in Mediterranean Israeli coastal waters since 2009, the ctenophores were extremely rare last summer, despite our numerous searches for these. It is interesting to note that this “absence” was probably the result of regional climate change effects, which is Antrelle’s doctoral topic. Ultimately, through some concerted efforts, a few *M. leidyi* specimens were collected and Antrelle found amoebae in these which she proceeded to culture. Antrelle took these cultures back to her lab in Alabama for further characterization and is still working on these.

The advantage of returning to a “new” field site, as Antrelle is proposing to do, is that many of the novel research and logistical challenges that are discovered upon initial arrival are known and have been addressed. Following her experiences last summer, Antrelle knows how to plan her visit and her research so that she will be a lot more efficient in summer 2024. As before, I have discussed the research and current goals with both Antrelle and her advisors in an effort to streamline the work she plans to do. Although at the moment (January 2024), the sea is full of medusae and ctenophores, there is no guarantee they will be abundant in the summer. Nonetheless, based on our records over the past 12 years, chances are high that the ctenophores will be present when Antrelle plans to come and search for them.

My lab will be available for Antrelle’s research, as before and my team is looking forward to her visit. I am hoping that you will see fit to approve her application and help support her research.

Sincerely,

Prof. Dror Angel
Project Title: Spatial distribution and diversity of comb plate amoebae

Student Name and Email: Antrelle D. Clark, adc0064@auburn.edu

Current Institution: Auburn University
Program (MS, PhD, other) and Year in Program: Marine Biology Ph.D. and Graduate Certificate in Geographic Information Systems Science; third year
Advisor(s) Name: Anthony G. Moss and Moises A. Bernal
Thesis Title: Climatic impacts on the symbiotic association between ectosymbiotic gymnamoebae and *Mnemiopsis leidyi*

ASLO Member ID: 13183

Potential exchange site and collaborator: University of Haifa, Israel under Dr. Dror Angel, Ph.D. of the Department of Maritime Civilizations

Abstract (300 words maximum):

After discovering that the eastern Mediterranean Israeli coast populations of the invasive ctenophore, *Mnemiopsis leidyi*, bear ectosymbiotic amoebae, the main objective of this project is to continue my previously proposed project to determine whether the amoebae are displaying phenotypic plasticity based on location and environmental conditions. It is important to note that the amoebae may not be displaying phenotypic plasticity and could be of a different assemblage, since the invasive *M. leidyi* originated from the northern Gulf of Mexico (Reusch et al. 2010; Bolte, 2013). During this project, I intend to correlate environmental conditions with phenotypic plasticity of the coastal US (native) and Israeli (invasive) ctenophore ecto-amoebae populations. Ctenophores will be collected during the 8-week period from three sites along the Israeli Mediterranean coast via tow net and net scooping. During the time of collection, water properties and GPS coordinates will also be documented. Ctenophores will be scanned for the presence of amoebae, and cultured for growth, imaging, and phenotypic description. Samples and preservatives will be shipped to Auburn University where genotypic (molecular) analysis and descriptions will take place. Results stemming from this study will help us better understand the role symbionts play in their relationship with ctenophores, potentially shedding light on the reasoning behind the invasive invertebrates’ success in invading all of the European seas during the 1990-2012 period.

Keywords: symbiosis, invasive, climate, gymnamoebae, ctenophore
Project Description (3-page maximum):

Introduction

Research focused on the association of microbes with invertebrates has become one of the fastest growing attractions in the world as scientists have realized that the microbiome of an organism is critical to their well-being (Trench, 1993). *Vibrio fischeri*, for example, are responsible for illumination mechanisms found in the bobtail squid (Ruby and Asato, 1993). Also, zooxanthellae are known to be critical to the survival of corals as they provide nutritional support in exchange for protection for predators (Fitt, 2000). However, while invertebrates are able to tolerate such associations with microbes, some pose a threat to humans making the study of the invertebrate – microbe relationships not only critical to the well-being of the invertebrate, but also to the human population.

Rational and problem to be addressed. The Western Atlantic lobate ctenophore, Mnemiopsis leidyi has been documented to carry a number of microbes (i.e., viruses, bacteria, bacterioplankton) on its epithelium. However, very few scientists (from the Moss lab) have addressed the amoebae found on the comb plates, sparking a new topic of discussion. The Moss lab previously discovered that gymnamoebae specifically associate with the comb plate of the ctenophore (Moss et al., 2001). In recent times, initial observations from my studies have shown a difference in amoebae with respect to their morphology and motility. In other organisms, there has been a correlation between their phenotypic characteristics and surrounding environments. From this initial observation and knowing what could cause a difference in appearance, two main questions were developed:

1. Are the amoebae the same from previous studies? and if so,
2. Is there a correlation between amoebae morphological plasticity, location (spatial) and environmental conditions?

This research will be essential for understanding the impacts of climate change on symbiotic relationships as this particular study represents climate change in today’s time due to amoebae being sampled from different locations, and thus, being exposed to different conditions. The Mediterranean Sea is a good location for ctenophore – amoeba research as all European populations of the *M. leidyi* ctenophore originate from the Gulf of Mexico – eastern United States (Fuentes et al., 2010; Reusch et al., 2010; Bolte et al., 2013; Ghabooli et al., 2013).

Project Objectives

The overarching goal of this project is to determine whether exposure to different environmental conditions impact a gymnamoeba – ctenophore symbiosis. To aid in this goal, we specifically aim to [1] correlate salinity, temperature, pH and oxygen regimes with the geographic range of ctenophore amoebae, and [2] determine if there are morphological plasticity the amoebae display based on the conditions in the given environment.

Background

In the face of a changing climate, *Mnemiopsis leidyi* has become one of the most notorious marine invaders in the world (Reusch et al., 2010). The success behind the ctenophores’ ability to invade and establish itself in numerous water bodies have yet to be discovered; however, it has been suggested that their association with microbes may be key (Hammann et al., 2015).
While there are numerous microbes associated with the ctenophore (i.e., the ciliate *Trichodina ctenophorii* and *Pentapharodinium*-like dinoflagellate), my study focuses on their relationship with gymnamoebae (Moss et al., 2001; Smith, 2011; Versteeg, 2007). Many years ago, the Moss lab (Auburn University) discovered that *M. leidy* bears 'naked' (nontestate) gymnamoebae on the comb plates where they appear to play a role in keeping the comb plates free of other material; early work in the lab produced both a publication (Moss et al., 2001) and a MS thesis (Versteeg, 2007). While working on my study over the past 2 ½ years, we have still seen the presence of the ciliate and dinoflagellate; however, recent observations show that the amoebae appear to be different suggesting that the original amoebae are either no longer present or no longer of a single morphotype. The goal of this project is to figure out if varying locations (12 points along the southeastern and eastern United States coastline and 3 along the Israeli Mediterranean coastline) and thus, varying environmental conditions influence ecto-amoebae phenotypic plasticity.

**Approach/Work plan**

**Site Selection, Imaging, and Removal.** Three research sites will be selected along the Israeli Mediterranean coastline with all three sites being between the Ashkelon Marina (southern Israel) and the Haifa harbor (northern Israel). Sites will be selected based on nutrient loading and overall water quality; the local pH, salinity, coordinates, and sea surface temperature will be documented at the time of specimen collection. Based on previous experience from being a part of the third LOREX cohort, the best sampling technique in the Mediterranean Sea will be to travel by boat to open water and sample specimens via tow net, surface dipping, or snorkeling. Collected specimens will be transported back to the AMBER lab where the comb plates of the ctenophore(s) will be scanned using an inverted microscope for the presence of amoebae. Amoebae infested comb plates will be photographed using a camera that is attachable to the inverted microscope, as well as microscopes equipped with phase contrast, Hoffman modulation contrast, and differential interference contrast (DIC). Removal of comb plate amoebae will be completed using methods recently developed by me: removing 3-5 amoebae infested comb plate rows using sterilized microscopic dissecting scissors and placing them into a sterilized petri dish (standard size or greater) that is 75% filled with sterilized 26 o/oo seawater and containing a sterilized pearled barley grain. Amoebae are negative phototaxis so cultures will be placed in a dark room that is within their optimum growing temperature range (64-72℉).

**Growth, isolation, phenotypic description, and DNA preparation.** Primary cultures will be cleaned heavily using sterilized 26 o/oo seawater and fed via sterilized pearled barley grain. Heavy cleaning will be necessary to remove other microbes associated with the ctenophores' comb plates, which will free up the competition for food and provide more room for growth in the petri dish. Different morphological amoebae cells will be isolated using a sterilized pipette tip; the target amoeba cell will be lightly scrapped using a pipette tip, and the tip of the pipette tip will be cut off using sterilized scissors and placed into an individual petri dish. Cultures will be placed in their optimum growing conditions: ¾ filled with sterilized seawater, one pearled barley grain, a dark room, and a temperature between 64-72℉. Successful cultures will be imaged and recorded using the attachable camera, allowing for the phenotypic description (i.e., length, uroid structure, hyaline, internal structures, motility, shape) of the different amoeba cells to be completed. Some of the ctenophore comb plates will be removed and placed into a preservative for transport to Auburn University where ctenophore genomic analyses will be completed.

**Timeline.** During the first research exchange, my research was put to a halt for the first five weeks because the specimens were not blooming as expected because the sea-surface temperature was colder than normal during the early summer season. Therefore, instead of
partaking in the research exchange start of the blooming season (June-July), I would like to start my exchange at the start of peak blooming season (August-September). All three sites will be selected based on their environmental differences. Each site will be allotted a two-week period to complete the methodologies as listed above; the longest part of the methodology will be the waiting period as the cultures grow. Based on previous experience, week one will be dedicated to making sure we have everything we need to complete the targeted research and everything I may need in a general sense. Weeks 2 and 3 will be dedicated to site one, weeks 4 and 5 will be dedicated to site two, and weeks 6 and 7 will be dedicated to site three. Week 8 (and other free time) will be allocated as see fit: more time for cell growth, additional water sampling, additional imaging/recording of viable cells for descriptive analysis, etc.

**Transport to home institution and further analysis.** Following the 8-week period of working with Dr. Dror Angel at the University of Haifa, viable cultures and prepared preservations will be transported to Auburn University for amoebae identification via molecular descriptive analysis (i.e., DNA sequencing). The extracting of amoebae DNA will be completed using the ZYMO Quick-DNA Fungal/Bacterial Prep Kit that has been known to be successful in extracting DNA from amoeba cells. The output from the DNA extraction will be tested for nucleic acid count and overall DNA quality using the nanodrop located in the Department of Biological Sciences at Auburn University. Successful DNA extractions will be subjected to PCR for DNA proliferation, and tested using an agarose gel electrophoresis before being sent off for sequencing. The details in the molecular methodologies are as described in Nassonova et al. (2010) and Zurita-Artaloitia et al. (2023).

**Dissemination of results**

Results from participating in this collaborative research with Dr. Dror Angel of the Applied Marine Biology and Ecology Research (AMBER) lab at the University of Haifa, Israel will be presented as a School of Marine Sciences seminar that focuses on the correlation between climate change and the loss of biodiversity via interspecific (symbiotic) relationships. Additionally, results from this research will be presented at the Auburn University NRT Water Climate Symposium and the ASLO 2025 Aquatic Sciences Meeting (Charlotte, North Carolina).
Project Justification (1-page maximum):

Importance of Research

Studying the ctenophore–amoeba symbiosis is important because symbiotic relationships are the key contributor to biodiversity. Biodiversity is the vital component that drives the natural processes that occur on Earth (i.e., nutrient cycling, weather patterns) and thus, supports all life forms. However, climate change (driven by human influences) is causing the dissociation of numerous symbiotic relationships, resulting in biodiversity loss. For example, recent studies have shown that the relationship between zooxanthellae and corals have shifted towards parasitic when exposed to a warming ocean, leading to coral bleaching and ultimately death (Baker et al. 2018). The coral-zooxanthellae and squid-Vibrio associations have become standardized models for understanding the climatic impacts on symbiotic relationships; and while researchers are tackling questions associated with those models, it is important to understand that there are numerous poorly understood models (such as the ctenophore–amoeba model) that need attention due to the economic and ecological threats they pose.

Importance of the University of Haifa research site

The Mediterranean Sea region has been noted as one of the most susceptible regions in the world in regard to climate change and the impacts of it as a result of human activity. The changes within this region continue to pose a threat to biodiversity and the abundance of the native species, allowing invasive species, such as the lobate ctenophore Mnemiopsis leidyi, to overtake Mediterranean waters. As previously stated by Dr. Dror Angel during my first LOREX exchange, the Mediterranean Sea is often infested with M. leidyi throughout the year and their location relative to the Mediterranean Sea makes the University of Haifa (Israel) a great host institution for my research. Dr. Angel has focused his career on medusae and ctenophores (Jasper et al., 2018; Shiganova et al., 2019) where he tackles different aspects concerning them. Before my first LOREX exchange, he had not performed any research on their symbionts; however, since then he has become familiar with their importance and contribution to the invertebrates. Dr. Angel and I hope to use my second LOREX exchange to continue bridging the gap between our individual research focuses to expand our knowledge on ctenophore invasion success, making the University of Haifa the right place to conduct my research.

Contribution to thesis and future career goals

While some microbial-invertebrate host symbioses have been previously examined (i.e., corals-zooxanthellae and bobtail squid-Vibrio), no such study has been conducted on ctenophores protist epibionts. With the support of a LOREX grant, my dissertation work at Auburn University will help provide an improved understanding of the dynamics of a microbe/invertebrate interaction. This project may reveal insight on how such microbe-host interactions could change under conditions of stress that mimic those anticipated in a changing coastal environment. To date, I have been awarded an NSF Graduate Research Fellowship and an NSF Climate Resilience Traineeship, providing me with the opportunity to make my mark in the science world on national level. Additionally, as a member of the third LOREX cohort, I have been able to step foot into the collaborative and international research world where I have acquired many networking opportunities and skills. With a second LOREX grant, I will be able to hone in on said acquired skills and gain others; components that will be helpful when applying to work for the Environmental Protection Agency as a Freshwater/Marine Biologist with a Geographic Information System (GIS) science background to continue my work on correlating climate change, symbiotic relationships, and biodiversity.
References (1-page maximum):


