ASCIDIAN NEWS*

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Thanks to all of you who sent in contributions and for letting me know how important AN continues to be. There are 102 new publications listed at the end of this newsletter. Please keep in touch and continue to send me contributions for the next issue! Keep safe, keep working, and good luck to everyone.

***Ascidian News is not part of the scientific literature and should not be cited as such.**

NEWS AND VIEWS

1. The 11th Intl. Conference on Marine Bioinvasions, scheduled for 16-20 May in Annapolis, Maryland has been postponed to 2023 due to Covid.

2. The next International Tunicata Meeting will be held on July 12-16, 2022 both online and onsite (Konan Univ., Kobe, Japan).

The meeting that had been scheduled in 2021 was postponed for one year. Hopefully, many researchers will be able to gather on-site. However, we are wondering whether visitors from abroad can meet in Japan in person. This is because visiting Japan is still severely restricted because of the Omicron variant. Currently our government is starting to reduce the restriction level, but it is not easy for us to predict what situation will be at the time of the meeting. This is the reason why we are planning to hold the meeting as a hybrid style.

It will be difficult to hold international meetings online because of time difference in the globe. We are going to manage recording presentations by zoom function and the movies will be provided for on-demand viewing. There are some issues we are not used to: security, server, budget and so on.

1. Every paper will be given in zoom meeting, irrespective that speakers are on-site or not. No poster sessions. Tentatively, 30 min (talk and discussion) for PI and 15 min for others (students and postdocs). This is still tentative, and could be modified depending on the number of talks registered. Zoom movies of talks will be recorded by organizers on time during the meeting days and will be uploaded for on-demand viewing as soon as we can. However, one or two-day lag will happen after the talk.

2. Because of shortage of time to organize this meeting, we will ask only a limited number of researchers to serve as the scientific advisory committee, compared to previous ITMs. We will not have to select presentations as talks or posters, nor have to assign talks to particular topics sessions (see below). One chair of each session will be selected from researchers based in Japan who can be on-site to secure session progress.

3. The international attendees have to give your talk in Japan-time daytime. Sorry for this, but we will do our best for maximizing your convenience in terms of your time differences when we assign your talk to a session, as we further explain below.

4. To increase comfortability of every speaker from different time zones, each session will be organized taking account of the speaker's time zone. Thus, a session will be a mixture of various topics that are irrespective of research fields. This is a big difference from the previous ITMs, but we hope this will help all attendants to contribute every topic on tunicates as in the previous onsite ITMs.

5. One can make questions using chat and comment functions even in on-demand viewing.

6. The registration fee will be less expensive, but not free. We need some amount of budget to administer the meeting online for professional assistant in security issues and web pages. We are thinking about proposing a discount fee for students. We ask that everyone, irrespective of speaker and audience, will register and pay the attending fee, avoiding only one member registering for whole lab. Otherwise, we will have serious trouble to estimate the budget income of the meeting. At the moment, we expect 150 attendees.

7. Researchers based in China will not be able to view talks on demand because of the blockage to access the Vimeo web site (https://vimeo.com/ondemand and https://vimeo.com/184141942) as Google and YouTube in China. You need to attend the meeting on time using Zoom. Sorry for this, but we do not have a good idea to avoid this problem. Please let us know if there is another nation having similar issue.

8. During the meeting, Takeo Horie and Alberto Stolfi will organize a technical session on single cell RNA-seq. The tentative title is "10X Genomics: Ascidian tips and how to use the available data". Round Table Discussion will also be planned although time difference is still problematic.
Scientific committee:
Kai Chen - Kunming University of Science and Technology
Bo Dong - Ocean University of China
Patrick Lemaire - CRBM - CNRS
Lucia Manni - University of Padova
Noa Shenkar - Tel-Aviv University

I will stay in the following hotel during the meeting.
Kobe Tor Road Hotel Sanraku, English page is not available. 6500 yen per night. Walking distance to Kobe-Sannomiya station of Hankyu railway. It will take approximately 30 min to reach the meeting venue. You can book the hotel room at the following site (booking.com).
https://www.booking.com/hotel/jp/kobe-tor-road-sanraku.html?aid=356995&label=gog235jc-1FCAsodUIVa29iZS10b3Itcm9hZC1zYW5yYWt1SDNYA2h1iAEBmAEVuAEHyAEP2AEB6AEB-AEMiAIBqAIDuALswuKTBsACAdICJDhiNWNlODE4LTc2ZGYtNDAyNi1hMzgyLTc0NGNjMWZlYTEzMNgCBuACAQ&sid=312e577878338cc0a1770840e62c632c&dist=0&lang=en-us&room1=A%2CA&sb_price_type=total&soz=1&type=total&lang_click=other&cdl=ja&lang_changed=1

Dormitory in Konan Univ. is also available for limited number of attendants. If you want to stay there, please contact Takehiro Kusakabe (tgk@konan-u.ac.jp) as soon as possible.
Dormitory in Konan Univ. (the meeting venue). HIRAO Seminar House. 3,000 yen per night.
2-29-15, Suriyoshi-honmachi, Higashinada-ku, Kobe, Hyogo 658-0051, Japan
http://www.konan-isc.co.jp/kaikan2.html in Japanese

If you have any question and comment, do not hesitate to contact me. We hope you will enjoy the 11th ITM and are still hoping we can meet in person in Kobe. Thank you.
Best wishes, Takehito Kusakabe, Takahito Nishikata, Yasunori Sasakura, Hiroki Nishida
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Hiroki Nishida, Department of Biological Sciences, Graduate School of Science, Osaka University, Japan email: hnishida@bio.sci.osaka-u.ac.jp
http://www.bio.sci.osaka-u.ac.jp bio_web/lab_page/nishida/index.html

3. Eating sea squirts reverses signs of cognitive decline and aging.
https://www.studyfinds.org/sea-squirts-reverse-aging/

https://neurosciencenews.com/aging-sea-squirts-20570/
Click on the web links to read the articles!

4. Strange Deep-sea Creatures Found Inhabiting the Endurance Shipwreck
https://www.natureworldnews.com/articles/49842/20220310/strange-deep-sea-creatures-found-inhabiting-the-endurance-shipwreck.htm **The Discovery of Ernest Shackleton's Ship**

Almost 10,000 feet down in the Weddell Sea, the ship was discovered by the team based on Worsley's initial coordinates. This was just a few weeks after they began their mission in early February. Under the Antarctic Treaty, the wreck will be protected as a historic site and monument, ensuring that it will not be harmed during surveys and filming. This voyage was part of the trust's larger plan to convey the tale of Shackleton, his ship, and the members of his team to new and younger audiences. Shackleton died in 1922, making the ship's discovery 100 years later.

Can anyone identify this species?

4. We are very saddened to report the death of **Dr. Richard Cloney**, retired professor of the Dept. of Biology, Univ. of Washington, Seattle WA. His widow Rita Rae Cloney wrote that he died Feb. 6, just shy of his 92nd birthday. He had a stroke just after his 90th, a second one New Years's eve, followed by a third on Jan 28. Many tributes have been posted; here are a few (edited somewhat by me, your Ascidian News editor; my own tribute is below).

a) From David Perkel, Depts. of Biology and Otolaryngology: I write with the sad news of the passing, on Feb. 6, 2022, of Professor Emeritus of Zoology Richard Cloney at the age of 91. Richard grew up in Northern California, and earned a Bachelor's degree in Biology and a Master's Degree in Education from Humboldt State (then College, now University), followed by a Ph.D. in Zoology at the Univ. of Washington. After a postdoc in the UW Department of [then] Anatomy, he joined the Zoology faculty and rose through the ranks. He spent considerable time both in Seattle and at Friday Harbor Laboratories. He retired in the mid-1990s and pursued quite avidly a hobby of geology and mineralogy.

b) From Billie Swalla: Dept. of Biology, Univ. of WA: I just wanted to add a few remembrances of Professor Dick Cloney, who worked on ascidians and did beautiful E.M. ultrastructural work on them. He taught Invertebrate Embryology at the U.W. Friday Harbor Labs for many years, and stimulated many students around the world to choose working on invertebrates and their embryos and larvae for their thesis projects.

He was very welcoming to me at the University of Washington when I arrived in 1999, and to my first student, Dr. Brad Davidson, who now is an Associate Professor at Swarthmore. Brad wanted to work on metamorphosis and Dick Cloney had written several wonderful reviews on the way that ascidian tadpole larvae retract their tails, after settling on a surface and then rotate their body axis. He served as an unofficial committee member and Brad did one of the first molecular analyses of what happens during ascidian metamorphosis for his Ph.D. work.

Whenever we had an ascidian scientist come to town, my lab would get together with Charlie and Gretchen Lambert and Dick and Rita Cloney and talk nothing but tunicates for an evening with the visitor. I think his paper that describes all of the different ways the ascidian tadpole tails are retracted is one of my favorites of his. I heard from several others with stories of Dick's dry sense of humor, kindness and willingness to help students and young faculty with research efforts. What a wonderful legacy for UW Biology!! Ninety one years is a good run, but I wanted to share what a great scientist and man that Dick Cloney was, and how much I will miss him.

c) From Bill Moody, Dept. of Biology, UW: I second Billie's nice comments. When I arrived in Seattle, with the plan of doing electrophysiology on ascidian embryos, it was Dick who introduced me to *Boltenia villosa*, with its orange-pigmented muscle lineage. I worked on those animals for a decade and he was always helpful. His suggestion of *Boltenia* put me on the track to record from developing muscle at all stages of larval development. One of those papers made it to Science, with a nice cover photo of a *Boltenia* gastrula. He also had a edgy sense of humor. He got a good laugh about one of my encounters with animal care people at UW, when they were debating whether non-vertebrate chordates should be regulated. They came to look at my seawater tanks and one of them saw the adult *Boltenia* (sessile filter feeders) and said "What in the world are those?" I simply replied that because they didn't move around, they were classified as plants. As a result, I became a botanist in the eyes of the regulators for about 10 years.

d) From Richard Strathmann, retired professor, Dept. of Biology, UW and former assoc. director of the UW Friday Harbor Labs: Co-teaching comparative invertebrate embryology with Richard Cloney was always fun and enlightening because of his knowledge of the animals and his ingenuity with methods. An example of his discoveries of diversity in structure, development, and function was the several quite different ways that ascidian tadpole larvae resorb their tails at metamorphosis. The tails are homologous and similar, and resorption serves the same function, but the mechanics and the ultrastructural basis for the mechanics have diverged remarkably. Most of us assume that when we have demonstrated a process in an organism, it is the same in related organisms that are similar in structure and function. It takes a person like Richard Cloney to show that evolution doesn't work that way.

There is so much more to remember with pleasure, like his invention of the simple way to attach tape labels to all those little dishes with embryos, partly submerged in aquaria for cooling: clothes pins. The method is still in use at FHL. And that from a person whose photomicroscopy was fine art.

e) From Dennis Willows, retired professor, Dept. of Biology, UW and former director of the UW Friday Harbor Labs for over 30 years: Dick and Rita Rae Cloney made many contributions to the scholarly life, and the fun at FHL in the first 2-3 decades I spent there. They were generous cheerleaders and collaborators with the "gang of five" (Art Martin, Helen Whiteley, Arthur Whiteley, Dixy Lee Ray, and Bob Fernald) who really launched the place after the doldrums of WWII. Anyone interested in those times, please take a look at https://tinyurl.com/DWillowsBook and send me your memories. it is 'in progress' and I will try to keep it updated and accessible.

f) From Gretchen Lambert: I first met Richard Cloney when I was a beginning graduate student and spent the summer of 1964 at the UW Friday Harbor Labs taking classes (and where I met my future husband Charley). Richard and Rita Rae became good friends to us over so many years and I will miss him very much. Richard's research was primarily on the details, especially ultrastructurally, of metamorphosis of ascidian tadpoles. One of the many discoveries he made and published on was the function of the test cells, something a number of researchers had studied over many years but which were still a mystery. When tadpoles hatch, the test cells stick to and coat the outside of the tadpole. He realized that they prevent newly hatched tadpoles from sticking to the underside of the sea water surface film as they swim upward before they switch to downward swimming when ready to settle. Richard hated to get up early; he knew that the tadpoles would be swimming upward for a while after hatching so when he was ready to work on them (like about 10AM) he'd just put a light at one end of the aquarium, the tads swam there and he quickly pipetted them up. Besides his remarkable abilities in electron microscopy, one of his hobbies was photography, of people and landscapes, He preferred black and white, and for many years did his own developing and printing. As with everything else he did, the results were exceptional, especially his portraits of colleagues. He and Rita Rae loved to travel; one of their favorite destinations was Japan, where Richard had many colleagues. We all could go on and on about Richard, a unique and original person and researcher.

5. Skeleton Panda Sea Squirts. Check out this website https://totallythebomb.com/skeleton-panda-sea-squirts for the full article and more cute photos! (and unfortunately some incorrect statements about ascidians). *Ecteinascidia*?

Work in Progress

1. From Dr. Larry Dishaw, Univ. of South Florida, St. Petersburg, Fl (ldishaw@usf.edu)
In the past few years, our lab has become especially focused on better understanding mechanisms shaping transkingdom interactions in the gut and modeling it with *Ciona robusta*. Together with my colleague, Assunta Liberti, from the SZN in Naples, we just launched a special Research Topic issue for Frontiers in Immunology that is focused on the evolution of secreted immune effectors and their role in shaping the ecology of mucosal environments. It is open to submissions of all types from all model systems. We would appreciate spreading the word: https://www.frontiersin.org/research-topics/34987/evolution-of-secreted-immune-effectors-in-shaping-the-microbial-ecology-of-mucosal-environments .

2. From **Stefano Tiozzo,** Directeur de Recherche CNRS, Regeneration Team, Sorbonne Univ., CNRS,Laboratoire de Biologie du Développement de Villefranche-sur-mer, France (tiozzo@obs-vlfr.fr) : A reminder that in our servers we have public available transcriptomes of some species of Styelidae: http://octopus.obs-vlfr.fr/public/botryllus/blast_botryllus.php
Also from September 24th 2022 until March2023, the Museum of Modern and Contemporary Art of Nice (MAMAC - https://www.mamac-nice.org/en/) will host an exhibition of the artist Irene Kopelman (http://www.irenekopelman.com/) "Marine Models . Drawing regeneration", where *Botryllus schlosseri* and its regenerative capabilities will be the main subject.

3. From **Sébastien Darras**, Observatoire Océanologique, Banyuls-sur-mer, France. Our group DEEVA has sequenced the genomes and transcriptomes of 3 ascidian species: *Ascidia mentula*, *Molgula appendiculata* and *Clavelina lepadiformis*. People interested in using these datasets may contact Sébastien DARRAS (sebastien.darras@obs-banyuls.fr).

Meetings abstracts

1. Life Sciences Switzerland annual meeting, 21-22 April 2022, Zurich

In-lab breeding of *Botrylloides diegensis* requires a suitable marine microbiome. Simon Blanchoud (University of Fribourg). simon.blanchoud@unifr.ch

Tunicates are highly diverse marine invertebrate filter-feeders that are vertebrates' closest relatives. These organisms, despite a drastically different body plan during their adulthood, have a tissue complexity related to that of vertebrates. Ascidians, which compose most of the Tunicata, are benthic sessile hermaphrodites that reproduce sexually through a motile tadpole larval stage. Over half of the known ascidians species are able to reproduce asexually by budding, typically leading to the formation of colonies where animals, called zooids, are interconnected through an external vascular system. In addition, colonial ascidians are established models for important biological processes including allorecognition, immunobiology, aging, angiogenesis and whole-body regeneration. However, the current paucity in breeding infrastructures limits the study of these animals to coastal regions.

To promote a wider scientific spreading and popularity of colonial ascidians, we have developed a flexible recirculating husbandry setup for their long-term in-lab culture where specimens develop on hanging microscopy glass slides. Through more than 3 years of breeding of *Botrylloides diegensis*, a species of colonial ascidians, in recirculating artificial seawater over 600 km away from their natural habitat, we show that these animals can be proficiently bred in-land and suggest that our results can be extended to other species of colonial ascidians to promote research on these fascinating animals. We also show that a suitable marine microbiome is necessary for *Botrylloides*' development with colonies regressing within a few days in sterile artificial seawater. We investigate this dramatic interplay between environmental bacteria and colony's health by studying transcriptomics and microbiomics time-courses.

2. 50th Marine Benthic Ecology meeting 29 March-2 April 2022, Portsmouth, New Hampshire

https://www.bemsociety.org/schedule--program.html

a) Lopez-Guzman M. 1; López-Legentil S. 1; Hirose E. 2; Erwin P. 1.
Biogeography and Host--Specificity of Cyanobacterial Symbionts in Colonial Ascidians.
1University of North Carolina Wilmington, Wilmington, North Carolina, USA; 2University of the Ryukyus, Okinawa, Japan mal2227@uncw.edu

Ascidians, or seasquirts, are known to harbor bacterial symbionts in their tunics. In particular, the ascidian genus Lissoclinum can harbor abundant and diverse cyanobacterial associates. Here, we analyzed 32 samples of Lissoclinum collected from the Bahamas, Japan, Chile, and Spain in order to determine the presence and hostspecificity of symbiotic cyanobacteria. Ascidian hosts were identified using gross morphology and barcode sequencing of partial cytochrome C oxidase subunit I genes, while cyanobacterial symbionts were characterized using partial 16S rRNA and entire 16S23S rRNA internal transcribed spacer gene regions. We identified 8 species of Lissoclinum (L. aff. fragile, L. bistratum, L. midui, L. patella, L. perforatum, L. punctatum, L. timorense and L. verrilli) and found that both host species and geographic location played a role in structuring cyanobacterialascidian symbioses. Broad biogeographic trends included the dominance of Prochloron symbionts in Japan and the presence of a novel cyanobacterial lineage in the Bahamian Lissoclinum hosts. Within each geographic region, a high degree of hostspecificity was observed where similar symbionts were recovered from ascidian hosts across multiple collection locations. The results of this study add to the growing knowledge of cyanobacterialascidian symbiosis and symbiotic relationships as a whole.

b) Carman M.R., et al. **Distribution of tunicates (Ascidiacea) utilizing eelgrass as substrate in the Northwest Atlantic between New Jersey and Newfoundland.** mcarman@whoi.edu

Seagrass meadows are declining globally at an accelerating rate due to numerous interacting anthropogenic stressors. Invasive epiphytic tunicates have been recently recognized as an additional stress to seagrass meadows. Eelgrass (Zostera marina) is the dominant seagrass species in the Northwest Atlantic, where fouling communities are dominated by invasive tunicates. To investigate the extent of tunicate fouling on eelgrass, we surveyed 21 eelgrass meadows on the east coast of North America, ranging from New Jersey to Newfoundland. We found 8 tunicate species growing on eelgrass, of which 6 are invasive. Botrylloides violaceus and Botryllus schlosseri were most common, with B. schlosseri having the largest latitudinal range. Tunicate faunas attached to eelgrass were less diverse north of Gloucester, Massachusetts. At the highest latitude sampled, in Newfoundland, the solitary tunicate Ciona intestinalis was found on eelgrass, where it is a new invader. Other tunicate species found were the invasive species Ascidiella aspersa, Didemnum vexillum, Diplosoma listerianum, and native species Didemnum albidum, Molgula manhattensis. Tunicate coverage on eelgrass typically fell within the 125% range, with coverage up to >75100%. Eelgrass density typically ranged from 127 to 820 shoots/m2, and appeared unrelated to tunicate colonization.

c) Evans, J. 1, Erwin, P.1, Shenkar, N. 2, López-Legentil, S. 1.
1University of North Carolina Wilmington, Wilmington, NC 28403, USA; 2TelAviv University, Tel Aviv 69978, Israel jse5258@uncw.edu

Diversity and hostspecificity of microbial symbionts in ascidians from North Carolina harbors

Some ascidian species are highly adaptable biological invaders, exhibiting remarkable success in crossing geographic borders. To examine the potential role of microbial symbionts in the success of these invasions, we determined the hostspecificity of microbial communities inhabiting 4 ascidian species commonly found off the North Carolina (NC) coast and compared them with seawater samples. Replicate samples (n=5) of two worldwide introduced species: Polyandrocarpa zorritensis and Clavelina oblonga (the latest considered native in NC), and two cryptogenic species (Distaplia bermudensis and Polyandrocarpa aff. maxima) were collected in January 2016 and their identity confirmed by genetic barcoding using partial cytochrome C oxidase subunit I genes. Microbial communities of ascidian hosts and ambient seawater were characterized by nextgeneration (Illumina) sequencing of 16S rRNA gene sequences. Ascidians hosted unique and diverse symbiont communities, consisting of 12,969 OTUs (at 97% sequenced identity) from 53 bacterial and 3 archaeal phyla. Permutational multivariate analyses revealed clear differentiation of ascidian symbionts compared to bacterioplankton in surrounding seawater and distinct microbial communities in each ascidian host species. These results suggest that the microbial symbionts in ascidians exhibit a high degree of hostspecificity, forming intimate associations with their hosts that may increase their fitness and invasive potential.

d) Villalobos, S. 1; Lambert, G. 2; Shenkar, N. 3; López-Legentil, S. 1. 1University of North Carolina Wilmington, Wilmington, NC 28403, USA; 2University of Washington Friday Harbor Labs, Friday Harbor, WA 98250, USA; 3Tel Aviv University, Tel Aviv Israel. smv7274@uncw.edu

Distribution and Population Dynamics of Key Ascidians in North Carolina Harbors and Marinas.

Ascidians have successfully invaded marinas and harbors around the world. In this study, we assessed the biodiversity and distribution of ascidians in 16 marinas of North Carolina. Ascidians were identified using morphological observations and barcode sequencing of a fragment of the mitochondrial gene cytochrome c oxidase I. Distribution patterns of native and introduced ascidians were analyzed using presenceabsence and relative abundance matrices in relation to sites and geographic distance. Finally, we monitored the dynamics of a wellestablished ascidian community in Wilmington, NC over 1.5 years using photo transects. For each of the five species present, we calculated percent coverage and relative abundance and then related those values to

temperature fluctuations using crosscorrelation analyses. We found 3 introduced, 5 cryptogenic, and 8 native ascidian species in the investigated harbors. Species distribution and relative abundance were unrelated to geographic location (Mantel test, p>0.14). The ascidian community in Wilmington consisted of 4 species that were present throughout the monitoring period and the solitary ascidian Ascidia interrupta, which was only present during warmer months. This is the first study describing the ascidian fauna in harbors and marinas along the North Carolina coast and providing insight into the population dynamics of key species.

e) Chase A.L., Dijkstra, J.A., Harris, L.G. **The influence of substrate material on ascidian larval settlement.**

Submerged manmade structures present novel habitat for marine organisms, and often host communities that differ from those on natural substrates. Although many factors are known to contribute to these differences, few studies have directly examined the influence of substrate material on organism settlement. We quantified larval substrate preferences of two species of ascidians, Ciona intestinalis (cryptogenic) and Botrylloides violaceus (nonnative), on commonly occurring natural (granite) and manmade (concrete, highdensity polyethylene, PVC) marine Low salinity compromises larval metamorphosis and growth in the colonial ascidian *Botryllus violaceus*.

f) Walter J. Lambert[1]; Jennifer A. Dijkstra[2]; Emily Clark[1]; Joanne Connolly[1]
Abiotic factors influence the success and distribution of many invasive marine invertebrates.
Poster
1 Framingham State College, Department of Biology, Framingham, MA 01701
2 School of Marine Science & Engineering, University of New Hampshire, Durham, NH 03824 USA
wlambert@framingham.edu

The invasive colonial ascidian *Botryllus violaceus* is a common inhabitant in fouling and shallow benthic communities in the Gulf of Maine. Although capable of tolerating a range of salinities, low salinities (<10 ppt) seem to be physiologically limiting. We exposed tadpole larvae to salinities (030ppt). Tadpoles metamorphose in salinities >16 ppt; percent metamorphosis of larvae was not significantly different at salinities >20 ppt. We then tested whether larval exposure to low salinity impacts the growth of the resulting colonies when allowed to grow in full strength seawater. Larvae were metamorphosed larvae in four salinities (18 ppt, 20 ppt, 25 ppt, and 30 ppt). Colonies were transferred to 30 ppt seawater and colony growth was monitored for 9 weeks postmetamorphosis by counting the number of zooids per colony and measuring the area occupied by the colonies. Colonies that resulted from larvae that metamorphosed in lower salinities (18 ppt, 20 ppt and 25 ppt) had fewer zooids and covered less area than colonies metamorphosed at 30 ppt. A brief period of salinity stress during the larval stage has compromised growth of the adult colony materials in laboratory trials. Larvae exhibited speciesspecific settlement preferences, but generally settled more often than expected by chance on concrete and HDPE. Variation in settlement between materials may reflect preferences for rougher substrates, or may result from the influence of leached chemicals on ascidian settlement and metamorphosis. These findings indicate that experimental plate material may influence larval behavior and may help us understand how substrate features can contribute to differences in settlement in the field.

g) Surprenant M, Jarvis J, López-Legentil S. **Ascidian diversity and relative abundance in North Carolina seagrass meadows**. LopezLegentils@uncw.edu

Ascidians are sessile marine invertebrates found all over the world in a variety of natural and artificial habitats. However, ascidian distribution and diversity has not yet been recorded in North Carolina (NC) natural habitats. The objective of this study was to provide the first catalog of ascidian diversity and abundance in seagrass meadows. Eight sites along the NC coast were surveyed. At each site, 20 random quadrats were deployed and all ascidian species present were counted and

either identified in situ or collected for morphological and genetic identification. Seagrass percent cover, biomass and shoot density were also quantified. Ascidians were found in six sites and four species were recorded: Molgula manhattensis, Styela plicata, Didemnum lutarium and Bostrichobranchus sp. Ascidian abundance changed significantly across sites (p=0.020) and with overall seagrass biomass (p=0.04) however, there was no significant relationship between ascidian abundance and seagrass percent cover (p=0.276). Colonial ascidians were generally attached to hard substrate while solitary species were more often found attached to seagrass rhizomes and blades. Temperate ascidians are known to have seasonal cycles, thus further research should include monthly surveys to assess ascidian diversity and abundance over time.

h) López-Legentil S, Palanisamy SK, Smith KF, McCormack GP, Erwin PM. **Biogeographic patterns in the microbiomes of introduced ascidians.** LopezLegentils@uncw.edu

Ascidians are among the marine taxa with the most introduced species. Recent studies have shown that microbial symbionts associated with their tunic may aid some species in their successful introduction worldwide. Here, we sequenced a fragment of the 16S ribosomal RNA gene to characterize symbiont diversity and host-specificity in the solitary species Syela clava and Ascidiella aspersa, and the colonial species Didemnum vexillum. Samples were collected from introduced populations in several marinas and mussel facilities around Ireland, and a marina in New Zealand. Two additional colonial species Botrylloides violaceus and Didemnum sp. were collected in Ireland, and ambient seawater was sampled from both countries for comparison. Data revealed a strong effect of host species and location on prokaryote symbiont composition, consistent with recent ascidian microbiome literature. However, a location effect did not manifest in alpha diversity metrics (e.g., the same ascidian species at different locations exhibited similar diversity) but was evident in beta diversity metrics (greater intra-specific differences across locations than within locations). Location effects were stronger than species effects only for the solitary species (i.e., A. aspersa from New Zealand was more similar to S. clava from New Zealand than to A. aspersa from Ireland). D. vexillum and A. aspersa hosted high abundance of prokaryotic symbionts previously found in other ascidian species, while S. clava symbiotic community was more closely related to bacteria common in the marine environment. Further studies should aim to unravel host-microbe coevolutionary patterns and the microbial role in facilitating host establishment in different habitats.

i) Hutchings B, Stiles E, López-Legentil S. **Swept away by the storm: Impact of Hurricane Florence on ascidian communities in North Carolina harbors and marinas.**

Ascidians are marine invertebrates commonly surveyed to assess invasion processes. However, no studies to date have examined the impact of hurricanes on ascidian community composition and resilience in harbors and marinas. Here, we revisited eighteen previously surveyed harbors in North Carolina (NC) one year after Hurricane Florence's landfall in 2018. The distribution and community structure of native and introduced ascidians at each location were analyzed using presence-absence and relative abundance data for each species in relation to latitudinal position and distance between harbors. A loss of three native and one cryptogenic species was observed post-hurricane, with the introduction of four previously unreported species in NC. Changes in ascidian communities were overall non-significant. Additionally, ascidian community changes over time at Seapath Yacht Club (Wrightsville Beach) were monitored before and after Hurricane Florence using monthly photographic transects. Average abundances for all ascidian species except for Styela plicata were significantly lower after Florence. This study illustrates the impact of hurricanes on ascidian communities, and provides numerical evidence that introduced species recover faster than native species.

Thesis Abstracts

1. Ascidian abundance and diversity in North Carolina seagrass meadows. Mina Surprenant, Univ. of N. Carolina Wilmington. MS thesis, advisor Susanna López-Legentil LopezLegentils@uncw.edu

Ascidians are sessile marine invertebrates found all over the world in a variety of natural and artificial habitats. However, ascidian distribution and diversity has not yet been recorded in North Carolina (NC) natural habitats. The objective of this study was to provide the first catalog of ascidian diversity and abundance in NC seagrass meadows. Eight sites along the NC coast were surveyed. At each site, 20 random quadrats were deployed, and all ascidian species present were counted and either identified in situ or collected for morphological and genetic identification. Seagrass percent cover, biomass and shoot density were also quantified. Ascidians were found in six sites and four species were recorded: Molgula manhattensis, Styela plicata, Didemnum lutarium and Bostrichobranchus sp. Ascidian abundance changed significantly across sites ($p=0.020$) and with overall seagrass biomass ($p=0.040$) however, there was no significant relationship between ascidian abundance and seagrass percent cover ($p=0.276$). Colonial ascidians were generally attached to hard substrate while solitary species were more often found attached to seagrass rhizomes and blades. Temperate ascidians are known to have seasonal cycles, thus further research should include monthly surveys to assess ascidian diversity and abundance over time.

2. Genetic and microbial community analyses in two physiological states and color morphs of the colonial ascidian *Polyclinum constellatum*. Sammy Morrison, Univ. of N. Carolina Wilmington. MS thesis, advisors Susanna López-Legentil (LopezLegentils@uncw.edu) and Patrick Erwin (erwinp@uncw.edu) .

Genetic analyses allow the differentiation of conspecific organisms and cryptic species that otherwise would not be distinguishable. Polyclinum constellatum is a colonial ascidian that has several color variants: red, green, brown, purple, and sometimes multiple colors within one colony. In addition, the species is known to enter a resting period where the zooids stop filtering. In this study, 61 colonies collected from Puerto Rico were sequenced using a fragment of the nuclear 18S rRNA gene and the mitochondrial cytochrome oxidase subunit I gene to conduct phylogenetic analyses and determine whether different color morphs corresponded to different genetic lineages. A partial fragment of the 16S rRNA gene was also sequenced from 9 green and 9 red colonies (10 filtering and 8 resting) to characterize the microbiome of two color morphs and physiological states. Phylogenetic analyses did not reveal genetic differences among color morphs. Likewise, there were no significant differences in microbial structure between the green and red color morphs. However, the physiological state of the host (resting or filtering), did yield a marginally significant difference ($p = 0.05$) on microbial community composition. A set of 10 OTUs drove community dissimilarity, all of which were members of the phylum Proteobacteria (classes Alphaproteobacteria and Gammaproteobacteria). These results indicate that the color variants observed in P. constellatum are due to phenotypic plasticity rather than genetic variation. The higher abundance of some microbes in a resting state over an actively filtering state suggests a putative role in nutrient retention and recycling while the ascidian is not actively acquiring nutrition from its external environment, though the presence of a microbial core maintains that microbial communities are host-specific.

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