

# Abstracts

(in speaking order; as listed in program schedule)

## A remarkable case of non-invasion in British Columbia, Canada

Gretchen Lambert

Univ. of Washington Friday Harbor Laboratories, Friday Harbor WA 98177

Between July 21-August 11, 2017, a three-week comprehensive marine biodiversity survey was carried out at a small remote region of the central British Columbia coast at and near the Calvert Island Marine Station (Hakai Institute). There is no commercial shipping to this area and only a small amount of recreational-size boat traffic. The survey included daily sampling by the staff and a number of visiting taxonomists with specialties covering all the major groups of invertebrates. Many marine habitats were sampled: rocky and sand/gravel intertidal, eelgrass meadows, shallow and deeper subtidal by snorkel and scuba, plus artificial surfaces of settlement plates set out up to a year ago, and the sides and bottom of the large floating dock at the Institute. Many new species were recorded by all the taxonomists; in this limited remote area I identified 37 ascidian species, including 3 new species, which represents almost 1/3 of all the known North American species from Alaska to southern California. Remarkably, only one is a possible non-native, *Diplosoma listerianum*, and it was collected mostly on natural substrates including deeper areas sampled by scuba; one colony occurred on a settlement plate. There were no botryllids, no *Styela clava*, no *Didemnum vexillum*, though these are all common non-natives in other parts of BC and the entire U.S. west coast. Most of the species are the same as in northern California, Washington, and southern BC, with only a small overlap of a few of the known Alaska spp.

# Assessing Ascidian Use of Open Spaces throughout the Year

Judith Pederson and Carolina Bastidas

MIT Sea Grant College Program, Cambridge, MA

Climate change is altering the physical and biological disturbances in marine ecosystems. Along the Northeast coast of the U.S., ascidians, particularly non-native species often are dominant in the fouling communities. This study examined the settlement of fouling organisms on PVC plates throughout a year to assess the role of open space and early competition on established and newly placed plates. We compared patterns between two sites at different bioregions, in Buzzards Bay and Boston Harbor. These locations were chosen because pre-existing data from five rapid assessment surveys between 2000 and 2013, allowed comparisons between deployed plates with results from summer surveys of *in situ* communities. Settlement of native and non-native species were recorded over 12 months using plates removed at one, two, and three months after submersion. We assessed settlement of organisms and interaction among species and compared these data based on location and previous surveys. Non-native ascidians were present at both sites, often dominating plates, especially during the warmer months, with differences in which species became dominant.

# Impacts of Hurricane Sandy on recruit dynamics of *Botryllus schlosseri*

Paul Bologna & John Gaynor

Department of Biology, Montclair State University  
[bolognap@montclair.edu](mailto:bolognap@montclair.edu)

Hurricane Sandy had significant impacts to coastal regions of the western Mid-Atlantic. In particular, Barnegat Bay, NJ was especially impacted with an island breach, flooding, and infrastructure destruction. We used settling plates to evaluate spatial and temporal recruitment in the system in 2010, 2013, and 2014. Recruitment of *B. schlosseri* in 2010 indicated high settlement throughout the bay with peaks in June and July, but one site exhibited high recruitment into October. Following Hurricane Sandy, which destroyed large amounts of potential substrate, recruitment was limited with settlement essentially occurring only in June. By 2014, settlement patterns had begun to recover, albeit at lower rates and at fewer sites than 2010, but settlement was observed throughout the summer with similar peaks in June and July. The only other invasive tunicate to show recruitment on plates was *B. violaceus* in 2010. It was present at three sites and showed a settlement peak in July but continuous recruitment through to October. Our data demonstrate that the destruction of man-made structures which are important habitat for invasive tunicates by Hurricane Sandy reduced population recruitment in subsequent years. Future evaluation and monitoring can assess whether the impacts were short-term or substantially changed the community.

# Biotic exchange from the movement of 'static' maritime structures in an era of marine urbanization

Josephine C. Iacarella<sup>1</sup>, Ian C. Davidson<sup>2</sup>, Anya Dunham<sup>1</sup>

<sup>1</sup> Fisheries and Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Rd, Nanaimo, BC, V9T 6N7, Canada

<sup>2</sup> Smithsonian Environmental Research Centre, 647 Contees Wharf Road, Edgewater, Maryland 21037, USA

Coastal infrastructure provides extensive habitat for marine nonindigenous species (“NIS”), especially biofouling species. Ecosystem transplants can arise when floating artificial structures are relocated, typically under slow towing speeds following long periods of biofouling accumulation. These translocations of floating structures occur more often than acknowledged and represent a high-risk vector of marine NIS that is largely overlooked and unregulated. We characterized standing stock and movement patterns for oil platforms, derelict and obsolete vessels, barges, dry docks, floating lodges and homes, floating docks, aquaculture gear and buoys. Our review and synthesis of >10,000 geospatial locations of structures and NIS, movement case studies and tracked towing data for the NE Pacific coast reveal the growing NIS vector opportunities resulting from sprawling coastal infrastructure. As coastal development continues apace in the Anthropocene, this emerging management challenge will require strong frameworks and workable solutions for a global issue that currently lacks incentives to prevent species transfers and invasions.

# Understanding the Ecological Impacts of Invasive Tunicates and Their Response to Climate Change

<sup>1</sup>Yingqi Zhang, <sup>2</sup>Linda Deegan, and <sup>3</sup>Mary Carman

<sup>1</sup>Colgate University, Hamilton, NY 13346 and Marine Biological Laboratory, Woods Hole, MA 02543

<sup>2</sup>Woods Hole Research Center, Woods Hole, MA 02543

<sup>3</sup> Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Invasive colonial tunicates have become widely distributed in estuaries on Cape Cod over the past decades. Despite their ubiquity, little research exists on how they interact with other species in the food web. The goals of this study are to understand the ecology of invasive tunicates in comparison to native tunicate and blue mussels, and to explore the response of tunicates to future climate regime. Metabolic rates of two species of invasive tunicate (*Didemnum vexillum* and *Botrylloides violaceus*) as well as one species of native tunicate (*Aplidium glabrum*) were evaluated. To determine the impact of tunicate colonization on mussels, filtration rates of sixteen blue mussels (*Mytilus edulis*) were measured. Finally, the response of tunicates to the changing climate was tested using experimental manipulations of increased temperature and decreased pH. The result shows that *D. vexillum* and *B. violaceus* consumed oxygen at slightly faster rates than *Aplidium glabrum*. Fouling tunicates were strongly competing with blue mussels to filter feed, but were not inhibiting mussel's filtration rate. Finally, invasive tunicates might be more resilient to ocean warming and acidification, although this finding needs to be verified by further studies.

# Color morphs of *Distaplia bermudensis* represent cryptic lineages with distinct microbiomes that remain structurally and functionally equivalent across different environments

Evans, J.S.<sup>1</sup>; Erwin, P.M.<sup>1</sup>; Sihaloho, H. F.<sup>1</sup>; Shenkar, N.<sup>2</sup>; López-Legentil, S.<sup>1</sup>

<sup>1</sup>Department of Biology & Marine Biology, and Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington NC 28409, United States of America

<sup>2</sup>School of Zoology, and The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies, Tel-Aviv University, Tel Aviv 69978, Israel

Harbor systems are notorious for their polluted conditions – reduced circulation, leaching and spills of toxic heavy metals and organic pollutants, and increased wastewater and runoff inputs all play a role. In addition, harbors also act as gateways for the passive introductions of non-native ascidian species. Once introduced, these ascidians can proliferate rapidly despite polluted harbor conditions, even spreading locally into adjacent natural reef systems. In North Carolina (southeastern USA), the colonial ascidian *Distaplia bermudensis* dominates coastal harbors, yet its origins and survival mechanisms in harbor habitats remain uncertain, complicated in part by the large variation in color morphologies exhibited by this species, and its global distribution. To investigate the genetic basis for color variation in *D. bermudensis* and potential microbiome contributions to host fitness, we characterized host genetic variation and symbiont communities in 23 distinct color morphs of *D. bermudensis*. Two distinct, well-supported lineages of *D. bermudensis* were revealed by analyses of both mitochondrial (cytochrome c oxidase) and ribosomal (18S rRNA) gene sequences. Next-generation sequencing of partial 16S rRNA genes revealed that each lineage harbored unique symbiont communities that differed significantly in both diversity and community structure between the lineages. To investigate the possible contribution of these host-specific microbial communities to the invasive potential of *D. bermudensis*, we compared microbiomes within one lineage collected from both natural reef and harbor environments. No significant variations in *D. bermudensis* microbial community diversity or structure across sites were detected. Moreover, metatranscriptomic analyses revealed that individuals from the harbor and reef possessed functionally equivalent microbiomes, including numerous genes associated with stress response and defense. Together, these results show that (1) *D. bermudensis* represents a species-complex comprised of at least two distinct genetic lineages, and that (2) each lineage of *D. bermudensis* forms host-specific and stable associations with its microbial symbionts that may improve fitness in high-stress environments and contribute to its successful establishment and spread.

# Prevalence of chimerism and colony fusion in *Didemnum vexillum*: a genomic approach

Xavier Turon<sup>1</sup>, Maria Casso<sup>1,2</sup>, Davide Tagliapietra<sup>3</sup>, Marta Pascual<sup>2</sup>

<sup>1</sup> Center for Advanced Studies of Blanes (CEAB, CSIC), Catalonia, Spain

<sup>2</sup> Dept. of Genetics, Microbiology and Statistics, and IRBio, University of Barcelona, Catalonia, Spain

<sup>3</sup> CNR - National Research Council of Italy, ISMAR - Marine Sciences Institute, Venice, Italy

Colony fusion and the formation of chimeric colonies is an important mechanism favouring colonisation of new substrates by modular organisms. In ascidians, colonial fusion is a process tightly regulated genetically in some groups and almost indiscriminate in others. The highly invasive ascidian *Didemnum vexillum* is said to gain a competitive edge through high inter-colony fusion rates, probably favoured by a reduced genetic diversity, in introduced populations. We wanted to test the prevalence of colony fusion in a well-established population of *D. vexillum* growing in oyster cultures in the Ebro Delta (NW Mediterranean). To this end, we sampled five different fragments from 9 large colonies. We examined through GBS (Genotyping-By-Sequencing) a genome-wide panel of 2,062 loci. WGA (Whole Genome Amplification) techniques were used to obtain enough DNA from a single zooid (only the thorax was used). We also cut some thoraces in half and processed the halves separately to calibrate the level of variability that could be expected from identical genotypes (technical noise). Our results showed that 3 out of 9 (33%) of the colonies were in fact chimeras, 2 of them made up of two genotypes and one comprising three genotypes. We then tested experimentally fusion rates of colonies in the Venice Lagoon (Italy) using cut surface assays. Five colonies were taken from separate zones of the Lagoon and cut into five pieces each. These fragments were paired in all combinations: five isogenic pairs from the same colony and 10 allogeneic pairs from different colonies. Pairs were placed in close proximity on microscope slides and monitored over one month. The experiment was repeated 3 times, totalling 15 different colonies. In about 50% of the pairs one or both of the fragments regressed and died. In the remaining pairs all of them showed fusion in the isogenic treatments, while only 29 % resulted in fusion in allogeneic contacts. We genotyped the Venice samples by GBS (obtaining a total of 1,837 loci) and found no difference in genetic distances between fused and non-fused colony pairs. We conclude that colony fusion occurs in Mediterranean populations of *D. vexillum* and is unlinked to genetic relatedness among fusing genotypes. Chimerism was only found in one third of the large colonies investigated, thus this factor may not be as important as previously thought to explain the high invasiveness of *D. vexillum* in these sites.

# How to make lemonade from invasive ascidians

Noa Shenkar<sup>1\*</sup>, Dror Avisar<sup>2</sup>, Aviv Ben-Tal<sup>1</sup>, Aviv Kaplan<sup>2</sup>, Zafir Kuplik<sup>1</sup>, Gal Navon<sup>1</sup>, Lion Novak<sup>1</sup>, Adi Torfstein<sup>3</sup>, Roni Tzafirri-Milo<sup>1</sup>, Gal Vered<sup>1</sup>

<sup>1</sup> School of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University and The Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies

<sup>2</sup> Water Research Center, School of Earth Sciences, Faculty of Exact Sciences  
Tel Aviv University

<sup>3</sup> Institute of Earth Sciences, Hebrew University, Jerusalem, and InterUniversity Institute for Marine Sciences, Eilat, Israel

Presenting author: Noa Shenkar [shenkarn@post.tau.ac.il](mailto:shenkarn@post.tau.ac.il)

The Mediterranean coastline has been undergoing dramatic changes in the past few decades. Although environmental and governmental agencies invest much effort in general monitoring and protection of this environment, there is a gap in our knowledge of the physiological impacts of current environmental stressors on the marine fauna, and of the possible potential of marine organisms as bio-indicators of environmental health. Sessile marine organisms such as ascidians may provide invaluable information regarding a wide scale of pollutants and stress factors over time, in contrast to common chemical assays. As highly efficient filter feeders, ascidians are well known for their ability to accumulate heavy metals, and to concentrate harmful compounds that are present in low or even only trace concentrations in the water column. The wide geographic distribution of invasive ascidians in both polluted and pristine habitats make them ideal candidates for bio-monitoring a wide variety of marine habitats, and investigating the pathological effects of a variety of stressors. The main objectives of the current study are to explore the potential use of the solitary invasive species *Phallusia nigra*, *Styela plicata*, *Microcosmus exesperatus*, and *Herdmania momus* as biological indicators for micro-plastic, phthalates, heavy-metals, and pharmaceuticals. By combining a suit of analytical methods including chemical, physiological, histological and proteomic analysis we aim to locate and describe 'hot-spots' of polluted zones along the Mediterranean and Red Sea coasts of Israel, and further understand the physiological effect of pollutants on benthic invertebrates.



# Proteomic profiling of ascidians from tropical and sub-tropical environments as a biomonitoring tool

Lion Novak\*<sup>1</sup>, Zafirir Kuplik<sup>1,2</sup>, Noa Shenkar<sup>1,2</sup>

<sup>1</sup> School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 6997801, Israel.

<sup>2</sup> Steinhardt Museum of Natural History, Israel National Center for Biodiversity Studies Tel Aviv University, Tel Aviv 6997801, Israel.

The application of environmental proteomics is becoming an increasingly useful tool for biomonitoring of marine environments worldwide. Benthic filter-feeders are considered ideal candidates for this practice. While previous studies have used bivalves for this purpose, the current study investigated the potential of solitary ascidians to serve as bioindicators based on their proteomic profile. The solitary invasive species *Microcosmus exasperatus* was collected from five sites along the Mediterranean coast of Israel, while the native Red Sea species *Polycarpa mytiligera* was collected from four sites. Both species were collected from both artificial and natural substrates. Following tunic removal and digestion of the whole zooid, proteins were extracted, trypsinized, and sent for LC-MS analysis. A total of 431 proteins were identified in *Microcosmus exasperatus* and 126 proteins in *Polycarpa mytiligera*, of which 193 and 13 proteins, respectively, demonstrated significant differential expression. Principal component analysis revealed significant differences between the proteomes from northern and southern sites sampled along the Mediterranean coast, while in the Red Sea dissimilarity was identified between the southern sites and a unique northern site. Significant proteins were associated with various functional groups, mainly protein biosynthesis, response to stress and the cytoskeleton. These groups comprised more than 50% of the total proteins, some of which had previously been shown to be affected by environmental stressors, and thus could be used as biomarkers in our study species. Our study indicates that the application of proteomics to ascidians, and in particular invasive ascidians which can be easily collected, offers a reproducible method by which to monitor the health of marine environments.

## Ascidian Invasion Perspectives: IV. The Definition of "Invasive," and the Shifting Baseline of Biodiversity History, Revisited

James T. Carlton

Williams College - Mystic Seaport Maritime Studies Program, Mystic, Connecticut 06355 USA

As the science of bioinvasions advances, certain terms and concepts are worthy of continued re-assessment. The word "invasive" is widely used to refer to a perceived subset of introduced (non-indigenous, non-native, alien, exotic) species, but remains without an objective, quantitative scientific definition that would permit robust interspecific or inter-regional comparisons. As such, the use of "invasive" falls within Bachrach's (1972) definition of a "daily," but not a scientific, meaning. Although an attempt has been made to define "invasive" for terrestrial plants, it may be best to reserve the use of the word for subjective policy, management, and social questions, although even in those contexts it remains a challenge to argue that non-native species fall into one of two categories (invasive and non-invasive). For scientific purposes, a more robust, fine-grained framework should focus on the broad continuum of (versus a dualistic approach to) aspects of the characteristics of introduced species. Equally important is a deeper understanding of the extent to which shifting baselines influence our interpretation of the modern-day structure and function of marine communities, relative to the proportion of native and introduced species. There remains an appeal to interpreting species as native if they demonstrate a widespread distribution, were present in the earliest biological surveys, or both. Assuming that such taxa are not in fact cryptic genospecies, we still have a coarse-grained understanding of the depth and breadth of ascidian invasions that occurred for (at least) 300 years between the 1500s and 1700s. A major goal of ascidian ecology, biogeography and systematics is to ferret out what may be the 100 or more species (now considered native wherever they occur) that had achieved anthropogenically-driven intra-oceanic and inter-oceanic distributions before marine biological investigations commenced. Elucidating these historical invaders may add substantially to our knowledge of the traits of successful non-indigenous species. In the absence of a more thorough grasp of pre-1800s invasions, our understanding of historical community structure remains one of the fundamental challenges of historical biodiversity and thus evolutionary community ecology.

# Ocean Invader Tactics Revealed: The Role of Copper Pollution in the Presence and Invasiveness of Colonial Ascidians

Kristin Osborne<sup>1</sup> and Helen Poynton<sup>2</sup>

<sup>1</sup> Massachusetts Maritime Academy, 101 Academy Drive Buzzards Bay, MA 02125

<sup>2</sup> University of Massachusetts Boston, 100 Morrissey Boulevard, Boston MA 02532

Colonial ascidians are a group of prolific marine invaders found in urban biofouling communities. Despite often high levels of copper pollution in these areas, many species of ascidians thrive in these conditions, while many native species suffer. The role of pollution tolerance in establishment success is often overlooked, and therefore the strategies employed by invaders are poorly understood. To explore the underlying mechanisms responsible for invader success relative to native species, a series of tests were performed to assess toxicity thresholds, interspecific competition, and tolerance mechanisms for the invasive *Botrylloides violaceus* and the native *Aplidium glabrum*. Then, both species were exposed to sublethal copper concentrations in the presence and absence of interspecific competition to determine if an interaction between these two factors exists. Finally, a comparative transcriptomic approach was employed to identify physiological pathways involved in invader tolerance. Acute toxicity tests revealed that *B. violaceus* larvae are significantly more tolerant than *A. glabrum* larvae (settlement inhibition EC<sub>50</sub> values = 107.9 and 46.7 µg/L, respectively). Longer-term impacts to *A. glabrum* were achieved at environmentally relevant concentrations of copper, while *B. violaceus* values were over twice the concentration (approximately 8 and 20 µg/L, respectively). In terms of interspecific competition, *A. glabrum* was impacted, even at the lowest concentration tested (1 µg/L), while competition did not have a significant effect on *B. violaceus* at any concentration tested. Global gene expression changes indicate that *A. glabrum* and *B. violaceus* respond using two distinctly different mechanisms and *A. glabrum* expression is over an order of magnitude greater, suggesting a higher stress response. Overall, this research demonstrates that *B. violaceus* is consistently more tolerant than *A. glabrum* and that the invader efficiently utilizes molecular mechanisms to endure in copper-polluted environments.

# Sea squirts (Ascidiacea) dominate fouling communities and impact aquaculture and eelgrass in Edgartown, Massachusetts

Emily Reddington<sup>1</sup>, Mary Carman<sup>2</sup>, Ryan Smith<sup>3</sup>, and Kristin Gribble<sup>4</sup>

<sup>1</sup>Great Pond Foundation, Edgartown, MA <sup>2</sup>Woods Hole Oceanographic Institution, Woods Hole, MA,  
<sup>3</sup>Signature Oysters, Edgartown, MA <sup>4</sup>Marine Biological Laboratory, Woods Hole, MA

Both native and invasive sea squirt species inhabit Edgartown waters and dominate fouling communities. Edgartown is located on the southern coast of Martha's Vineyard, an island off Massachusetts in the Northwest Atlantic. Six ascidian species were observed on natural and artificial substrates during 2017 surveys. There were 6 species at aquaculture sites, including invasive, non-native species *Styela clava* and *Didemnum vexillum* (the most common) and *Botrylloides violaceus*, cryptogenic species *Ciona intestinalis*, and native species *Didemnum albidum* and *Molgula manhattensis*. Oyster cages, lines, rafts, and oysters were often fouled by tunicates, profoundly impacting oyster aquaculture farms in Katama Bay, an important area for commercial oyster shellfishery. Tunicate fouling increases the weight of gear, blocks water flow and food, probably weakens oyster shells, creates competition for nutrients, and requires additional work for shellfishers. Tunicates are not permitted on market ready product in Massachusetts. Management strategies must be employed in order to combat the effects of tunicate fouling on aquaculture operations. *Molgula manhattensis* was observed fouling nearby eelgrass beds in Edgartown Great Pond (EGP). The density of tunicate coverage on eelgrass blades varied between eelgrass beds in EGP and may have an association with water quality. Quadrat (25cm x 25cm) surveys in EGP in 2017 reveal that tunicate density on eelgrass ranged from 0-420 individuals/quadrat at Slough Cove to 0-25 individuals/quadrat at Mid-Beach. *Molgula* was also common on nearby artificial substrates. The density of tunicates on eelgrass in EGP in August is alarming as eelgrass reproduction takes place in late summer and the binding presence of tunicates inhibits the plants from naturally defoliating, probably blocks sunlight from reaching the blades thus inhibiting photosynthesis, and potentially reduces growth rates.

## Tunicates (Ascidiacea) utilizing eelgrass as substrate in the Northwest Atlantic

Mary R. Carman

Biology Department, Woods Hole Oceanographic Institution

The number of reports of non-native tunicates (ascidians) invading seagrass habitat and using eelgrass, kelp and other marine vegetation as substrate in the Northwest Atlantic has been increasing over time. Seagrass meadows are ecologically important habitats that are declining globally at an accelerating rate due to natural and anthropogenic stressors. Their decline is a serious concern as this habitat provides many ecosystem services. Eelgrass (*Zostera marina*) is the dominant seagrass species in the Northwest Atlantic. Recently, tunicates have posed a threat and another form of stress for eelgrass beds. Colonization of eelgrass leaves by a density of non-native tunicates and native tunicates can negatively impact the eelgrass itself by reducing eelgrass growth and reproduction, while colonization of eelgrass by non-native tunicates can negatively impacts eelgrass ecosystems. For example, by growing where algae would otherwise grow for grazers and creating a secondary substrate that it uninhabitable by juvenile bay scallops and other sessile invertebrates. In 2013, we documented the distribution and diversity of tunicates on eelgrass in a Northwest Atlantic latitudinal study, from New Jersey to Newfoundland. Eight species of tunicates were found to be colonizing eelgrass, of which 5 are considered non-native (*Asciella dispersa*, *Botrylloides violaceus*, *Didemnum vexillum*, and *Diplosoma listerianum*), 2 native (*Didemnum albidum* and *Molgula manhattensis*), 1 cryptogenic (in the US) and non-native (in Canada) (*Ciona intestinalis*), and 1 site-specific cryptogenic (*Botryllus schlosseri*). *Botrylloides violaceus* and *Botryllus schlosseri* were the most common, with *B. schlosseri* having the largest latitudinal range. *Ciona intestinalis* was only found on eelgrass at the highest latitude sampled, in Newfoundland, where it is a new invader. Percent tunicate cover on eelgrass tended to fall within the 1–25 range, with occasional coverage up to 75–100. It appears that predation and leaf turnover rates keep tunicates in low abundance (1-25% coverage) but if there is a lack of predators or plants become encapsulated by excessive tunicate growth, then the tunicate density is considered extreme (75-100% coverage). The number of tunicate species that colonize eelgrass may be increasing, and eelgrass may be facilitating tunicate range expansion both by providing substrate in areas where the seabed is inhospitable to tunicates and by serving as a potential dispersal mechanism (i.e. rafting of tunicates on floating plant debris). In 2017, we again documented the ascidian fauna on eelgrass in the Northwest Atlantic. When attached to eelgrass, tunicates, both solitary and colonial, native and non-native species, can pose a potential threat to overgrow and weaken seagrass shoots and reduce the sustainability of seagrass meadows.

<https://www.youtube.com/watch?v=imaJW6DZSEs>

# Crab predation on eelgrass fouling epiphytes: sponge *Halichondria panicea*, colonial tunicate *Botryllus schlosseri*, and solitary tunicate *Molgula manhattensis*

David W. Grunden<sup>1</sup> and Mary R. Carman<sup>2</sup>

<sup>1</sup>Oak Bluffs Shellfish Department, Oak Bluffs, MA

<sup>2</sup>Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA

Eelgrass (*Zostera marina*) has been exposed to a wide range of stressors and consequently, abundance of this species has been declining. The proliferation of fouling species in the coastal zone can represent additional stress on eelgrass. The sponge *Halichondria panicea*, colonial tunicate *Botryllus schlosseri*, and solitary tunicate *Molgula manhattensis* commonly utilize eelgrass as substrate at Martha's Vineyard, Massachusetts. While sponge and tunicate abundance can vary widely, at high densities they can be an additional source of stress to eelgrass. We examined green crabs *Carcinus maenas* and spider crabs *Libinia dubia* in the lab as potential predators of sponges and tunicates; they may be able to provide some biocontrol over these biologic eelgrass stressors. Individual *Libinia* and *Carcinus* were offered similar amounts of *Halichondria*, *Botryllus*, and *Molgula* attached to *Zostera*. Consumption was assessed after 1 hour and after 24 hours. *Carcinus* did not display any predation, while predation by *Libinia* occurred within the first hour of our trials. After 24 hours, *Halichondria* specimens were 100% consumed; *Botryllus* specimens were 40% wholly consumed, 40% partially consumed, and 20% not consumed; and *Molgula* specimens were 60% completely consumed, 20% partially consumed, and 20% not consumed. A high density of *Molgula* often occurs on eelgrass in the marine ponds of Martha's Vineyard. We sought to determine a *Molgula* consumption rate for *Libinia* and found that a single *Libinia* in a controlled setting can consume up to 30 *Molgula* in 24 hours. Most often the spider crab would grasp the solitary tunicate with its claws, puncture and tear open the tunic (exterior layer) using both claws, and treat the tunic like a sack, use one claw to hold the sack and the other claw to selectively feed on internal tissues, leaving the tunic unconsumed. In some instances, the crabs consumed the entire tunicate including the tunic. In total, 14% of the *Molgula* specimens were consumed whole, 66% were partially consumed (not consuming the tunic), and 20% were not consumed. Our results were determined in a lab setting and further work should be done to confirm that similar results will be found in the field. Unconsumed tissue may have the ability to regrow and spread to other types of substrate. We experimentally placed fragments of *Halichondria* and *Botryllus* attached to eelgrass in plastic containers in the field and found that after one week, *Halichondria* reproduced on average 2.1 cm of new growth and *Botryllus* reproduced on average 1.4 cm of new growth on the containers. *Halichondria*, *Botryllus*, *Molgula*, *Carcinus*, and *Libinia* also live elsewhere in Northwest Atlantic eelgrass habitats. Our results imply that these eelgrass ecosystems could be experiencing similar fouling, crab predation, and new growth from partially consumed fouling species.

# Can invasive tunicates be useful for fish aquaculture? The replacement of menhaden fish meal by the tunicate *Styela plicata* in the diets of juvenile black sea bass *Centropristis striata*

Avery J. Delmaine\*, Wade O. Watanabe, Md Shah Alam

University of North Carolina Wilmington  
Center for Marine Science, Aquaculture Program  
601 South College Rd, Wilmington, NC 28403-5927, USA  
\*current email address [adelmaine@gmail.com](mailto:adelmaine@gmail.com)

The global pervasiveness of invasive tunicates and their persistent fouling of estuarine substrates make them potentially useful and abundant new candidate protein sources for use in fish diets. The aquaculture sector now produces more seafood for human consumption than wild capture fisheries do, but faces problems. The production of fish meal, which the industry has historically depended on as the primary protein ingredient in feeds, has plateaued while aquaculture production grows quickly. Alternate sources of protein such as soy, poultry byproduct, and even maggot meals are increasingly important, but nutrition experiments are necessary to test their viability. In the present study, the invasive tunicate *Styela plicata* was used in the diets of juvenile black sea bass *Centropristis striata* to determine the effects of different levels of substitution of fishmeal protein with tunicate protein on fish survival, growth performance, feed utilization, and whole body composition under controlled laboratory conditions.

The experimental system consisted of eighteen 75-L aquaria supported by a recirculating system in a controlled-environment laboratory. Tunicates collected from a local shellfish hatchery and marina were freeze dried, ground into meal, and analyzed for proximate, amino acid, and fatty acid compositions. A control fishmeal-based diet (45% crude protein, 13% crude lipid) was formulated to contain 30% (total diet) menhaden fishmeal. Five other isoproteinic and isolipidic diets were formulated to contain 5, 10, 15, 20, and 25% tunicate meal, which replaced 8.3, 16.7, 25.0, 33.3, and 41.6% of fishmeal protein respectively. Fish (7.1 g mean initial wt.) were stocked at a density of 15 fish per tank ( $N = 3$  per treatment) and fed the test diets daily to satiation for 55 days. Temperature (23 °C), salinity (32), pH (7-8) and dissolved oxygen (7 mg/L) were held constant. Fish survival, growth performance, and feed utilization were monitored. Proximate and amino analyses of diets and fish tissue were conducted, and fatty analyses were conducted on fish tissue. Tunicate meal was analyzed for the metals Cd, Co, Cr, Cu, Hg, Ni, Pb, V, and Zn. Treatment means were compared by one-way ANOVA.

At the termination of the study, black sea bass showed no significant differences in any survival, growth, or feed utilization metrics or in whole body proximate composition (protein, fat, moisture and ash). Tunicates contained all essential amino acids, and diets were similar in amino acid concentrations. Lipid, and therefore all fatty acid concentrations were relatively low in the tunicate meal; dietary fatty acids mostly came from other sources. Fatty acid concentrations were largely similar in whole fish bodies. Metal concentrations of tunicate meal were all within legal levels for diet formulation. Overall, the results suggest that juvenile black sea bass accept and grow well on diets replacing up to, and possibly more than, 41.6% fishmeal protein with tunicate meal protein without adverse effects on survival, growth, or feed utilization. Maximum replacement level of fishmeal protein with tunicate meal protein requires further study in black sea bass and in other fish species. Arsenic and lead concentrations in tunicates were high in other studies, and could be problematic for widespread tunicate use in aquafeeds. Questions are raised for future study about tunicate population density and abundance as well as scaling harvest.

# Management Strategies for Invasive Tunicates on Prince Edward Island Mussel Farms

John DP Davidson<sup>1</sup>, Luc A Comeau<sup>1</sup>, Thomas Landry<sup>2</sup>, Jeff Davidson<sup>2</sup>

<sup>1</sup> Department of Fisheries and Oceans Canada, Gulf Fisheries Centre, 343 University Avenue, P.O. Box 5030, Moncton, New Brunswick, Canada E1C 9B6

<sup>2</sup> Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave. Charlottetown, PEI. C1A 4P3

Culturing of the blue mussel, *Mytilus edulis* (Linnaeus, 1758) on Prince Edward Island (PEI), Canada, continues to be challenged by invasive tunicates; *Ciona intestinalis* (Linnaeus, 1767) (Vase tunicate), *Styela clava* (Herdman, 1881) (Clubbed tunicate), *Botryllus schlosseri* (Pallas, 1766) (Golden Star tunicate) and *Botrylloides violaceus* (Oka, 1927) (Violet tunicate). These invasive tunicates have established populations in numerous bays and estuaries and, depending on the species of tunicate, are impacting the mussel aquaculture industry. In addition to causing reductions in mussel productivity by competing for food and space with cultured mussels, removal of the tunicates from the mussel socks increases production costs and crop loss. *C. intestinalis* is particularly difficult to control and is considered the single main threat to the industry on PEI. High pressure water is currently the most common treatment against *C. intestinalis*. A considerable amount of research has been conducted on mitigating the effects of invasive tunicates on the mussel industry focusing on; tunicate ecology, adaptive management practices, mitigation strategies, and developing dynamic models for treatment options. Mussel growers have adapted to this challenge, through on farm experimentation with mitigation strategies. This presentation will concentrate on the on-farm management of tunicates resulting from research and growers experience.



# Exploratory baseline surveys for tunicates at Nantucket and Muskeget Islands off southern Massachusetts in the Northwest Atlantic

Dann S. Blackwood<sup>1</sup> and Mary R. Carman<sup>2</sup>

<sup>1</sup>US Geological Survey, Woods Hole, MA

<sup>2</sup>Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA

Tunicates (Ascidiacea) are common benthic marine invertebrates that are widespread in both the intertidal and subtidal zones in New England. Some areas of New England have been poorly surveyed for tunicates, and the fauna of Nantucket and Muskeget Islands had not been previously identified in published documentation. We conducted snorkeling surveys to a water depth of 3 m at Muskeget in 2016 and at Nantucket in 2016 and 2017 to identify species and to determine the biodiversity of the tunicate population. Muskeget is a small, uninhabited sand island less than 1 km<sup>2</sup> in area that lies 8.8 km west of Nantucket. There is little hard substrate for attachment of tunicates in the sandy nearshore area except for the extensive eelgrass beds that occur there. In July 2016, we found no tunicates at Muskeget. By contrast, Nantucket is a large island which is heavily populated in the summer. We found 8 species of tunicates at Nantucket attached to anthropogenic substrates including boat hulls, floating dock sides, and ropes hanging off docks, and on natural substrates including bivalves. The species identified included 5 non-native species (*Asciella aspersa*, *Botrylloides violaceus*, *Diplosoma listerianum*, *Didemnum vexillum*, and *Styela clava*), 2 native species (*Aplidium glabrum* and *Molgula manhattensis*), and 1 site-specific cryptogenic species (*Botryllus schlosseri*). When present, tunicate abundance or percent coverage commonly ranged between 50 to 75%. The Nantucket faunal composition, abundance, and substrates utilized are similar to tunicate populations living elsewhere in New England, including at the neighboring island of Martha's Vineyard which lies 21 km to the west and is heavily populated in the summer. Vectors for the distribution of these species to Nantucket were likely boat and ship traffic, as the island is an international hub for recreational boating. We wanted to determine if tunicates had spread to eelgrass on Nantucket, as it has on Martha's Vineyard, and therefore conducted a baseline survey of the eelgrass meadows at Jackson Point, the nearest point to Martha's Vineyard. In July 2017, the eelgrass meadow appeared healthy, both in density and canopy height, and no epiphytic tunicates were present. However, the presence of tunicates on the nearby town landing dock confirmed that tunicates are in the area. The coverage of tunicates (*B. schlosseri*, *B. violaceus*, and *M. manhattensis*) on the dock ranged between 75 and 100%. Although these species were a potentially large source of propagules in the summer reproductive period, larvae had not yet settled onto the nearby eelgrass beds, the nearest of which were within 1 m of the dock. Tunicates were absent at Muskeget Island, where eelgrass occurs, absent in the eelgrass beds at Jackson Point on Nantucket, and present on artificial substrates on Nantucket. Our surveys may provide useful baseline data for further research on the spread of tunicates into island environments.

## Ascidian diversity in Ireland with a focus on invasive species

Satheesh Kumar Palanisamy<sup>1</sup>, Olivier P. Thomas<sup>2</sup>, Grace P. McCormack<sup>1</sup>

<sup>1</sup>*School of Natural Science, Ryan Institute, Department of Zoology, National University of Ireland, Galway, Ireland*

<sup>2</sup>*School of Chemistry, Ryan Institute, Marine Biodiscovery Group, National University of Ireland, Galway, Ireland*

\* Presenting Author: [sathees.palanisamy@nuigalway.ie](mailto:sathees.palanisamy@nuigalway.ie)

Regular monitoring surveys are an important tool for detecting new arrivals of invasive species, for documenting patterns of invasion, and the impact of bio-invasion on the environment and human health. While invasive ascidians have been recorded in the Republic of Ireland, causing problems for the shellfish industry, the diversity and distribution of ascidians in Ireland are poorly known. This study aimed to report the abundance and distribution of ascidians in Ireland, and determine their taxonomy and phylogenetic relationships using mitochondrial cytochrome oxidase 1 sequence data. A total of 30 ascidian species have been collected to date, from 14 study sites in Irish waters (including yachting marinas, shellfish farm, salt lake, intertidal sites, depth range 1 to 20 m) between October 2016 and March 2018. The species identified are distributed across nine families and 19 genera, and include seven alien species. Here, *Didemnum candidum* (Savigny, 1816) belonging to family Didemnidae of order Aploubranchia and *Perophora japonica* (Oka, 1927) belonging to family Perphoridae of order Phlebobranchia are newly discovered invasive species in the west and the south. *Asciella aspera*, *Ciona intestinalis* and *Botryllus schlosseri* were the most abundant species throughout the year at all study stations. The exotic species *Botrylloides violaceus* and *Didemnum vexillum* are abundant in yachting marinas at Cork and Dublin during summer months, and both species were completely absent during winter. We have recorded potential expansion of invasive ascidian *A. aspersa* in six stations, and extensive biofouling of *A. aspersa*, *C. intestinalis* along with other ascidians on a blue mussel (*Mytilus edulis*) shellfish farm at Killary harbour, greatly reducing mussel production. Molecular data has been generated from nine species to date from 8 genera using a mt CO1 gene fragment. All genera were recovered monophyletic except for *Didemnum* as *D. candidum*, grouped with members of the Order Phlebobranchia rather than other *Didemnum* species. Chemically mediated interactions between invasive ascidians and shellfish will be investigated to detect defense metabolites and provide novel information about the biochemical pathway involved in the chemical defense.

Funding: This research work support by Irish Research Council (GOIPD/2016/100) is greatly acknowledged.

# Effects of Air Exposure on Markers of Oxidative Damage in an Invasive Tunicate (*Styela plicata*) and a Native Shellfish (*Crassostrea virginica*)

Peyton A. Thomas, S.T. Kinsey, S. Lopez-Legentil, A. Wilbur

University of North Carolina-Wilmington, Dept of Biology and Marine Biology, Center for Marine Science

The invasive solitary tunicate *Styela plicata* is distributed in coastal ecosystems around the world and it is known for its detrimental impacts on aquaculture operations. North Carolina has a growing shellfish aquaculture industry that provides economic benefits to the state and serves to rebuild natural Eastern oyster (*Crassostrea virginica*) reef populations. There is a need for a cost-effective method for the removal of the invasive tunicate from oyster aquaculture cages, where fouling by tunicates limits water flow and leads to competition for food. The aim of this research was to evaluate air exposure as a method to induce oxidative stress in tunicates that may limit their growth on oyster cages without significantly dampening oyster viability. Preliminary experiments found that 48 h air exposure under lab conditions led to 100% mortality of tunicates, whereas 24 h exposure had no mortality, so experiments were carried out at  $\leq 24$  h. Seventy individual tunicates and seventy individual oysters were collected from the docks of UNCW's Center for Marine Science. Tunicate size ranged from 19-80 mm while oysters ranged in size from 28-57 mm (approximate age of 6-9 months). Animals were acclimated to lab conditions for three weeks with daily feedings of algae provided by the UNCW Shellfish Research Hatchery and filtered seawater changes every other day. Ten individuals from each species were randomly placed in one of the following seven groups: control (continuous water immersion), 6, 12, or 24-h air exposure, or 6, 12, or 24-h air exposure followed by 6 h of water immersion. Individuals were placed in their specific treatment conditions in 5-minute intervals to ensure equal exposure periods when accounting for the time required for dissection. Tunicates were dissected to obtain branchial tissue while oysters were dissected to obtain gill tissue. Tissues were examined for oxidative damage using immunoblotting procedures for ubiquitin (a marker of protein degradation), protein carbonylation (a marker of protein oxidative damage), and 4-HNE (a marker of lipid oxidative damage). In general, tunicates had greater increases in oxidative damage markers than did oysters, suggesting that air exposure may lead to physiological stress that limits tunicate fouling of oyster cages. There was also evidence of a negative correlation between tunicate length and each marker of oxidative damage, suggesting that size of the individual plays a role in the physiological stress it endures. This interaction could mean that frequent air exposures will prevent larval and juvenile *S. plicata* from recruiting to oyster cages. Oyster height exhibited no significant relationship with any of the markers tested in this study. The results of this study will be used to guide field experiments from June through October 2018 to determine whether the lab results translate *in situ* and whether an air exposure protocol can be used for both seed oysters (5-mm) and 1-inch oysters in regions containing this particular invasive tunicate.

# Two lineages of *Polyandrocarpa zorritensis* co-exist in the US southeastern Atlantic

Jamie Webb<sup>1</sup>, Bailey Counts<sup>1</sup>, Marie Nydam<sup>2</sup>, Susanna López-legendil<sup>1,\*</sup>

<sup>1</sup>*Department of Biology & Marine Biology, and Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington, NC 28409, USA.*

*\*lopezlegendils@uncw.edu*

<sup>2</sup>*Division of Science and Mathematics, Centre College, 600 W Walnut St, Danville KY 40422, USA*

The ascidian *Polyandrocarpa zorritensis* (Van Name, 1931) is a colonial stolidobranch first described from Peru. Since then, the species has been recorded in many locations around the world, including both the Caribbean and Mediterranean Seas, the eastern and western USA coasts, Panama and India. Here we sequenced a fragment of the mitochondrial gene Cytochrome c Oxidase subunit I (COI) to determine the genetic diversity of this species in southeastern USA. Samples were collected in March 2017 from Sunset Cay (South Carolina, n = 5), Fernandina Beach (Florida, n = 16) and Smyrna Beach (Florida, n = 17). Additional samples (n = 3) were collected from Brazil by R.M. Rocha in May and September 2016. Two tree construction methods (Neighbor-joining and Maximum likelihood) were built using Mega v5 and additional *P. zorritensis* sequences retrieved from GenBank: 2 from southern India, 1 from northeastern Spain, and 5 from North Carolina (southeastern coast of the USA). A sample identified as *P. anguinea* was collected in August 2017 from Key Biscayne Florida to serve as an outgroup together with a sequence for *P. misakiensis* retrieved from GenBank. Phylogenetic analyses revealed that two lineages of *P. zorritensis* co-exist in southeastern USA (100% bootstrap support). The first clade (Clade I) contained the two sequences from India as well as a sequence from North Carolina and 2 of the sequences obtained herein: 1 from a colony collected in Fernandina Beach and one from Smyrna Beach. The second clade (Clade II) grouped all the remaining sequences obtained here for Florida, South Carolina and Brazil (n = 39), as well as GenBank sequences from North Carolina and Spain. No morphological differences were observed between individuals grouped in either clade. Our results indicate that two distinct lineages of *P. zorritensis* have successfully colonized different regions of the globe, living in sympatry in the southeastern US.

# Analysis of the spatial distribution, and recruitment of native and non-native tunicate species on *Zostera marina* in New Jersey

Sarah Hoffman & Paul Bologna

Marine Biology and Coastal Sciences, Montclair State University, Montclair, New Jersey

Many tunicate species have invaded the Western North Atlantic coast in the last century. These tunicates can have negative impacts on important industries like shellfish aquaculture, but also foul numerous natural and anthropogenic surfaces leading to displacement of native communities. During the summer of 2017, we assessed the spatial distribution and recruitment of tunicate species in *Zostera marina* (Eelgrass) beds in Barnegat Bay, New Jersey. Samples were collected in June, July, and August using 0.25m<sup>2</sup> quadrats and assessed for tunicate presence, as well as percent coverage of tunicates. *Botrylloides violaceus* was the most commonly identified species and was found at Barnegat Inlet and Ham Island. *Botrylloides schlosseri*, *Didemnum vexillum*, and *Asciidiella aspersa* were also identified, but were less abundant. Tunicate coverage increased substantially at Barnegat Inlet from June to August, but decreased at Ham Island during this period. *Didemnum vexillum* was only found in Ham Island, while *Asciidiella aspersa* was only found in Barnegat Inlet. There was a positive regression found between *Z. marina* biomass and tunicate biomass. This suggests that when there is more seagrass biomass there is more spatial area for colonization and growth. There is one outlier that shows the potential negative effects of tunicate growth on seagrass. This outlier is evidence of the fact that tunicates can overgrow and kill seagrass. These results suggest that fouling invasive tunicates could have a negative impact on eelgrass communities by smothering blades and reducing plant viability.

# Rapid coastal survey of the distribution of invasive ascidians in Coastal Lake Faro, Messina- Italy

Satheesh Kumar Palanisamy\*, Andrea Cosentino, Salvatore Giacobbe

Department of Chemical, Biological, Pharmacological and Environmental Sciences,  
University of Messina, Messina-98166, Italy

Presenting Author: [sathees.palanisamy@nuigalway.ie](mailto:sathees.palanisamy@nuigalway.ie)

Introduction of non-indigenous species, NIS, notably affects the Mediterranean Sea, due to very high anthropogenic pressure. Estuarine and brackish environments are favourable habitat to early settlement of NIS, and notably contribute to their further spreading. The lake Faro, due to the crucial location in the central Mediterranean, in the Strait of Messina area, probably plays a relevant role in hosting and disseminating NIS, as testified by several recent records. Mussel farming also play a remarkable role in the Lake Faro in terms of anthropogenic disturbance and NIS introduction. A rapid survey, carried out by snorkelling in such basin, between July 2013 to December 2014 (depth range from 1 to 2 m) allowed to recognize sixteen ascidia species, belonging to 7 families and 11 genera. Three NIS have been recognized, *Styela plicata*, *Botrylloides violaceus* and *B. diegensis*. The solitary *S. plicata* is cited as potentially invasive and widely introduced in western Mediterranean (Pineda et al., 2013). The colonial *Botrylloides violaceus*, rarely reported from Mediterranean, is known as highly invasive in western Atlantic. All these three species might be arrived in the Strait of Messina by ballast water and ship hull, both important vector for the dispersal of marine invasive ascidian species. Once entered in the Lake Faro they rapidly colonized all hard substrates, successfully competing with the native species. Especially *S. plicata* dominated benthic communities, in association with native and naturalized mussels. Field observations confirmed that *S. Plicata* has the potential to alter marine communities and affect economically important activities, as observed for shellfish aquaculture in Lake Faro. Regular port surveys throughout the Messina Harbour and coastal brackish basins are recommended to provide an effective early warning system for exotic species in Mediterranean waters.

**Keywords;** Ascidians, Biodiversity, Distribution, Ecology, Faro Lake, Invasive species.

**Funding:** This research work support by University of Messina - Non- European Doctoral Research Fellowship 2013-2015 is greatly acknowledged.

# No introduced ascidians detected in reef and mangrove habitats of the Bahamas

Bailey Keegan Counts<sup>1</sup>; Xavier Turon<sup>2</sup>; Susanna López-Legentil<sup>1</sup>

<sup>1</sup>*Department of Biology & Marine Biology, and Center for Marine Science, University of North Carolina Wilmington, Wilmington NC 28409, United States of America*

<sup>2</sup>*Department of Marine Ecology, Centre for Advanced Studies of Blanes (CEAB-CSIC), Blanes (Girona), Spain*

The class Ascidiacea contains over 3,500 described species, eighty of which have been reported as globally introduced. Despite increased efforts to identify ascidian species around the globe, some regions within the Caribbean Sea remain under-sampled. In particular, the Bahamas were last surveyed over 60 years ago by Van Name (1945), resulting in the description of only eight species. In this study we aimed to identify common ascidian species in the Bahamas and to determine the prevalence of native and nonnative species in the area. Two sampling events took place in May 2008 and July 2010 at twelve reef and mangrove localities within five islands of the Bahamas. Species were identified based on their morphology and barcoded by sequencing a fragment of the mitochondrial gene Cytochrome Oxidase subunit I. Twenty-two ascidian species were identified: ten Stolidobranchia (*Pyura longispina*, *P. vannamei*, *Pyura* sp. 1, *Pyura* sp. 2, *Polycarpa spongiabilis*, *Eusynstyela floridana*, *E. tinctoria*, *Symplegma viride*, *S. stolonica*, and *Botrylloides* sp.), two Phlebobranchia (*Ecteinascidia conklini* and *E. turbinata*), and ten Aplousobranchia species (*Eudistoma hepaticum*, *E. obscuratum*, *E. amanitum*., *Aplidium* sp. 1, *Aplidium* sp. 2, *Diplosoma listerianum*, *Didemnum conchyliatum*, *Trididemnum savignii*, and *Trididemnum* sp. 1). All the species found are considered native to the area, except for *T. savignii* and *D. listerianum*, which were considered cryptogenic. Further monitoring efforts should include entry-points such as harbors and marinas to assess whether nonnative species are present there and their potential to spread to the surrounding reefs.

# Microbiome shifts across the native and invasive range of the ascidian *Clavelina oblonga*

Goddard-Dwyer, M.<sup>1,2</sup>; López-Legentil, S.<sup>1</sup>; Erwin, P.M.<sup>1</sup>

<sup>1</sup>*Department of Biology & Marine Biology, and Center for Marine Science, University of North Carolina Wilmington, 5600 Marvin K. Moss Lane, Wilmington NC 28409, United States of America*

<sup>2</sup>*University of Southampton, European Way, Southampton SO14 3ZH, United Kingdom*

Ascidians are prolific invaders of non-native environments, which may cause negative ecological and economic impacts in native ecosystems. Ascidians possess a range of well-studied features that contribute to their successful spread into new habitats, but the role of their symbiotic microbial communities in invasion success is relatively unknown. To investigate this concept, we utilized next-generation sequencing of partial 16S rRNA genes to provide the first comprehensive description of the microbiome in the colonial ascidian *Clavelina oblonga*, and examined differences in the composition, diversity, and structure of symbiont communities in the host's native (North Carolina and Florida, USA) and invasive (Brazil, Italy and Spain) ranges. To identify host haplotypes, we sequenced a fragment of the mitochondrial gene cytochrome c oxidase subunit I. *C. oblonga* harbored a diverse microbiome consisting of 42 bacterial phyla (dominated by Proteobacteria and Bacteroidetes; 81.7% of the community) and 3 archaeal phyla. Colonies in the invasive range hosted significantly less diverse symbiont communities compared to the native range (Inverse Simpson and Shannon diversity,  $P < 0.05$ ). In addition, differences in microbiome structure were detected across colonies in the native and invasive range, averaging 74% dissimilarity between the two ranges. The main drivers of these community-level differences consisted of novel bacteria representing symbiont lineages with putative roles in nitrogen cycling and heavy metal resistance. In particular, putative nitrate reducers exhibited elevated abundances in colonies from the invasive range. Variability in symbiont composition was also observed among sites within each range (PERMANOVA,  $P < 0.05$ ). Together, these data suggest that *C. oblonga* hosts a dynamic microbiome resulting from (1) reductions in symbiont diversity due to founder effects in host populations, and (2) environmental selection of symbiont taxa in response to new habitats within a range. Further investigation is required to document the mechanisms behind these changes and to determine how changes in microbiome structure relate to holobiont function and the successful establishment of *C. oblonga* worldwide.



# Invasive Ascidian Research – A recap of 13 years of the International Invasive Sea Squirt Conference

S.G. Bullard<sup>1</sup>

<sup>1</sup> University of Hartford, Hillyer College, 200 Bloomfield Avenue, West Hartford, CT 06117, USA

Invasive ascidian research has evolved since the first International Invasive Sea Squirt Conference in 2005. At the time of the first meeting, researchers were just coming to terms with the global scope of the invasive ascidian problem. Indeed, the 2005 meeting was largely held so diverse workers from around the world could come together to share their experiences with invasive ascidians, gauge the scale of the problem, and develop research strategies to deal with the issue. Research efforts have continued over the last 13 years, but the focus has somewhat shifted. In 2005, much of the work was aimed on describing the problem posed by invasive ascidians, assessing the current distribution of invasive ascidians, and addressing the ecological impacts of ascidians on invaded communities. Recent research has focused on the biology of specific invaders or invasion events and about how to deal with the global challenge of invasive ascidians. This talk will summarize the changing field of invasive ascidian research from 2005-2018.

# DNA BARCODING REVEALS CRYPTIC DIVERSITY IN THE ASCIDIAN *SYMPLEGMA RUBRA* FROM SOUTHEASTERN BRAZIL

L. V. Granthom Costa<sup>1</sup>, C. R. Guerra<sup>1</sup>, V. Padula<sup>1</sup>, E. M. Hajdu<sup>2</sup>, G. M. Dias<sup>3</sup>

<sup>1</sup>Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM), Departamento de Biotecnologia, Divisão de Biodiversidade e Genética, Arraial do Cabo, RJ; <sup>2</sup>Museu Nacional/UFRJ, Departamento de Invertebrados; Rio de Janeiro, RJ; <sup>3</sup>Universidade Federal do ABC (UFABC), Centro de Ciências Naturais e Humanas, São Bernardo do Campo, SP.

Cryptic species are two or more species morphologically very similar or indistinguishable that can be identified through genetic data and other evidence. The information provided by an informative gene in species level, generally the mitochondrial COI gene, is a powerful tool to reveal cryptic lineages. *Symplegma rubra* is a colonial ascidian described from Guadeloupe in the Caribbean Sea. It has a characteristic transparent tunic, varying in color from orange to bright red. In Brazil, *S. rubra* has been recorded from northeastern to southern coasts, in both natural and artificial substrates. Further records include the Pacific coast of Panama, where it was listed as an exotic species, and few records from the western Indian Ocean. As part of a project to evaluate the connectivity of some ascidian populations in southeastern Brazil, we collected and sequenced (COI gene) colonies of *S. rubra* from different regions along the coast of Rio de Janeiro state. The single COI sequence of *S. rubra* available in GenBank and sequences of other *Symplegma* species were downloaded to be included in the analyses. We then conducted both phylogenetic (maximum likelihood) and species delimitation (ABGD) analyses. For both analyses, the COI sequences of *S. rubra* resulted in two distinct groups, retrieved as distinct species in the ABGD analysis. Maximum uncorrected *p*-distance intra-group was of 0.36% and minimum uncorrected *p*-distance inter-groups was of 4.3%. This barcoding gap is larger than the divergence presented between some close related, distinct ascidian species, indicating that cryptic species apparently occur under the name *Symplegma rubra*. This hypothesis will be tested with a wider sampling, the inclusion of a nuclear gene and with a comparative morphological study.

Funding: Faperj/APQ1E-26/ 110.735/2013

# INVASIVE POTENTIAL OF *Didemnum* sp RJAC (TUNICATA: ASCIDIACEA) IN ARRAIAL DO CABO, RJ.

Lucas Paes Castelo Branco<sup>1</sup>; Luciana V. Granthom Costa<sup>2</sup>; Ricardo Coutinho<sup>1 2</sup>

*1*Programa de Pós-Graduação em Biotecnologia Marinha - IEAPM/UFF. *2* Departamento de Biotecnologia Marinha - Instituto de Estudos do Mar Almirante Paulo Moreira – IEAPM; [lucas.castelo20@gmail.com](mailto:lucas.castelo20@gmail.com)

In July 2016, it was detected an unidentified colonial ascidian species from *Didemnum* genus during a fieldwork in the harbor area in Arraial do Cabo, state of Rio de Janeiro. This species named here as *Didemnum* sp RJAC was living in the moorings and overgrowing the subtidal assemblage and its presence was considered a significant threat to this region. Our aim was to assess the invasiveness potential of this species in order to understand *Didemnum* sp RJAC distribution and/or its subsequently spread, colonization and succession pattern. We examined the adjacencies of harbor area – Anjos Beach and two sites on Forno Inlet (Forno and Fortaleza) - visiting each site (December/2016) with SCUBA dive using zigzag pattern. Besides, experimental plates were submerged in order to assess the colonizing ability and successional pattern of *Didemnum* sp RJAC. Twenty set of black polypropylene plates and each set (15x15cm) presents two faces (upper and shading) (from February to July 2017) were placed randomly between 2-4 meters depth. During four months, in every 15 days sampling using photoquadrats methods. CPCE software was used to quantify the percentage cover on the plates. Our results demonstrated that *Didemnum* sp RJAC colonies were recorded only in the breakwater boulders confined in the harbor area and covered 12 m<sup>2</sup> in 200 m<sup>2</sup> only between 2-4 meters depths. After four months, *Didemnum* sp RJAC showed a fast capacity of colonization on the plates with an increase in coverage over time from march (10,5%) to july (32,5%). Recruits of *Didemnum* sp RJAC preferred to attach on the shading face with 54% of percent cover. Our preliminary results, demonstrated that *Didemnum* sp RJAC showed characteristics of invasive species due to this combination of characteristics. Therefore, we recommend monitoring the population dynamics of this species as a contribution to a successful management of invasive species.

**Financial support:** FAPERJ, CNPq