

ASCIDIAN NEWS*

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A big thank-you to all who sent in contributions. There are 85 New Publications listed at the end of this issue. Please continue to send me articles, and your new papers, to be included in the June 2019 issue of AN. It's never too soon to plan ahead.

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

NEWS AND VIEWS

1. From **Stefano Tiozzo** (tiozzo@obs-vlfr.fr) and **Remi Dumollard** (dumollard@obs-vlfr.fr): The **10th Intl. Tunicata Meeting** will be held at the citadel of Saint Helme in Villefranche sur Mer (France), **8-12 July 2019**. The web site with all the information will be soon available, save the date! We are looking forward to seeing you here in the Riviera. A bientôt! Remi and Stefano
2. The **10th Intl. Conference on Marine Bioinvasions** was held in Puerto Madryn, Patagonia, Argentina, October 16-18. At the conference website (<http://www.marinebioinvasions.info/index>) the program and abstracts in pdf can be downloaded. Dr. Rosana Rocha presented one of the keynote talks: "Ascidians in the anthropocene - invasions waiting to happen". See below under Meetings Abstracts for all the ascidian abstracts; my thanks to Evangelina Schwindt for compiling them. The next (11th) meeting will be in Annapolis, Maryland, organized by Greg Ruiz, Smithsonian Invasions lab, date to be determined.
3. Conference proceedings of the May 2018 Invasive Sea Squirt Conference will be peer reviewed and published in a special issue of the REABIC journal Management of Biological Invasions. Submit your manuscripts to Stephan Bullard (bullard@hartford.edu) or Mary Carman (mcarman@whoi.edu) by **January 1, 2019**. They are Associate Editors for the journal and will be overseeing the review process for the manuscripts. Manuscripts should be prepared according to the author guidelines for the journal Management of Biological Invasions <http://www.reabic.net/journals/mbi/Submission.aspx>.
4. From **Bella Galil** (galil@post.tau.ac.il) and the others listed at the end of this contribution: **EVERY VOICE COUNTS!** We seek to document the key drivers and issues that may impact, over the next three decades, the magnitude, study, and management of marine bioinvasions through the collective voices of scientists, managers, conservationists, and other stakeholders around the world. Your answers to the questions in this survey will provide the key status, trend, and geospatial data to identify the priority issues of invasions in the sea in order to produce local, regional, national and global intensity maps of the pressures and risks we face.
The need for a fully international perspective – and for your participation in achieving this goal – on marine bioinvasions could not be more pressing. Climate change is altering and shifting spatial and habitat availability for marine species globally, coastal infrastructure (artificial habitat) amenable to

alien species colonization is rapidly increasing world-wide, global transport of marine life continues unabated through a growing diversity of vectors, and one of the world's greatest interoceanic corridors, the Arctic Ocean, is opening.

PLEASE USE THIS LINK: www.tinyurl.com/mbi2050 . Deadline for receiving completed questionnaires: March 29, 2019. We are deeply grateful for your participation. Bella Galil, Henn Ojaveer, Alejandro Bortolus, James Carlton, Joao Canning-Clode, Elizabeth Cottier-Cook, Vadim Panov, Gregory Ruiz, Evangelina Schwindt and Thomas Therriault.

5. Making waves by turning sea pests into fuel, fertilizer. Jannike Kihlberg Dagens Nyheter. <https://www.straitstimes.com/world/europe/making-waves-by-turning-sea-pests-into-fuel-fertiliser>

“SWEDEN • Large grey plastic drums bob up and down in rows on the waters of Hake Fjord, a bay in Sweden. They are the only visible signs that an offshore area east of Tjorn is a biogas cultivation site, and that it is harvest time. As the boat approaches the shore, sea squirts underwater can be seen. "They are basically mussels without shells - sessile filter feeders that eat phytoplankton. After about a year, they die and sink to the bottom, where they become food for other animals," said Mr Fredrik Noren, head of research and development at Marin Biogas. The company aims to extract biogas and eco-fertiliser from sea squirts, which are about to be harvested on a large scale for the first time. "It's actually the world's first sea squirt harvest," said Mr Noren, a marine biologist who also works on projects for the IVL Swedish Environmental Research Institute.



The idea of growing sea squirts has been around for years. In 2009, Mr Noren applied to patent a method for extracting biogas from them. The sea squirt is an invertebrate that grows rapidly, making it a good potential source of biogas. Each tonne of harvested sea squirts can produce biogas equivalent to 20 litres of petrol, along with fertiliser. Until now, their rapid growth rate made sea squirts a nuisance for mussel farmers. They like the same habitats as mussels and can invade mussel farms. "The cultivation we have here was originally an unsuccessful mussel farm that sea squirts took over. If our project is successful, it could enable mussel farmers to get something out of even unsuccessful cultivation," said Mr Noren. He pointed out that they were still at a trial stage, and said: "Proteins can be used to make food products, such as animal feed, and the cellulose is also appealing. We have many ideas in the pipeline."

6. **“Spinialo. This Greek dish maintains an old fisherman's tradition of preserving one's catch in bottles of seawater.** Kalymnos, located in Greece's Dodecanese group of islands in the southeastern Aegean Sea, is known for its diverse array of seafood dishes. These delights range from lobster tails to fried octopus ink sacs. One Kalymnos dish represents not only the island's unique cuisine, but also its rich seafaring tradition: *spinialo*, a dish of sea squirts (*fouskes*) marinated in a bottle of seawater. <https://www.atlasobscura.com/foods/spinialo-sea-squirt-fouskes>



Although they are cleverly disguised as rocks, sea squirts are actually primitive marine vertebrates. They feel like a hard sponge and attach themselves to rocks, shells, and even ships' hulls. When cut in half, they reveal soft, yellow-orange flesh, which has the texture of scrambled eggs. The flesh's strong, bitter flavor is reminiscent of iodine and is definitely an acquired taste.

Like fried octopus ink sacs, spinialo was originally a fishermen's dish, eaten by the sponge-divers of Kalymnos who had to preserve their food to last for long sea voyages. Before modern refrigeration came to the island, fishermen would preserve sea-squirt flesh in empty wine bottles filled with seawater and a dash of olive oil. The flesh of sea urchins and fan mussels were commonly added, as well. The resulting mix was served with lemon and sometimes the double-baked bread known as barely rusk. Thanks to the salt in the seawater, a bottle of spinialo could last for months on end. To this day, authentic spinialo is still made in a bottle of seawater. Although it can be occasionally found in restaurants, fishermen still make it on their boats and sell it in fish markets, carrying on their time-honored culinary heritage. Spinialo can be found at some restaurants, tavernas, and fish markets in Kalymnos and, less frequently, in mainland Greece. Although spinialo can technically be made at home, the preservation process can be difficult to replicate accurately."

7. From **Shigeki Fujiwara** tatataa@kochi-u.ac.jp

I would like to announce that Professor Kaz Kawamura at Kochi University will officially retire next March. (He might continue research, but will not teach.) I am planning to give him a booklet containing warm-hearted messages from students and colleagues. Those who know Kaz, please give me a message to celebrate the start of Kaz's new life. Please send a message as a main text of e-mail or a pdf or docx file. If you happen to stay in Japan around 15th of March, how about joining us and drink with Kaz for a while? Best wishes, Shigeki Fujiwara, Dept. of Chemistry and Biotechnology, Kochi University, Kochi, Japan.

WORK IN PROGRESS

1. From **Gastón Alurralde** mitocondriarevelde@gmail.com We have recently established ourselves as a working team named Biología de Ambientes Acuáticos. The group comprises researchers and students from the Universidad Nacional de Córdoba, Argentina (ARG) and the Instituto de Diversidad y Ecología Animal, led by Dr. Marcos Tatián. We have launched several communication channels in the following social networks (the web page is still under construction):

Twitter: @AmbAcuaticosCBA, Facebook: <https://www.facebook.com/BioAmbAcuCba/>

Instagram: @biologia.amb.acuaticos

Institutional web page: <http://www.idea.conicet.unc.edu.ar/grupos/biologia-ambientes-acuaticos/>

2. From **Serina Lee**, Univ. of Singapore serina.lee@nus.edu.sg

Biodiversity in the tropics tends to be high but conversely abundance of the individual species low. In order to support marine research in the fields of toxicology, developmental biology, biomedical, physiology, biotechnology etc., St John Island National Marine Laboratory is currently looking at establishing a few tropical marine model organisms. In a perspective piece by Cook et al. (2016), the authors succinctly summarized the potential of non-conventional organisms and presented examples of nonstandard marine invertebrate species that have in recent years made important contributions to advancing knowledge in biological processes. One such example highlighted was ascidians. Over the years, the interest in using ascidians as model organisms has taken flight and progressively researchers are working on developing other species of ascidians for research studies. To date, there has been no establishment of tropical ascidian species as model organism for scientific studies although scattered work was done on a few species that were collected from the field. As such, we have identified tropical solitary ascidians as potential candidates.

Our preliminary efforts in culturing *Phallusia nigra* showed that we are able to yield reproductive individuals in 4 to 5 months. We are now working on optimizing loading density and feed quantity to accelerate growth rate. At the same time, we have just started cultures of 4 other species of solitary Phlebobranchs - *Phallusia philippinensis*, *Phallusia arabica*, *Ascidia gemmata* and *Ascidia sydneyensis* - to assess if our diet design can be applied across the different species to achieve similar growth rate. This would allow us to shortlist 1 or 2 faster growing species for long term in-house culture. (Reference: Cook CE, Chenevert J, Larsson TA, Arendt D, Houlston E, Lénárt P (2016) Old knowledge and new technologies allow rapid development of model organisms. Bement W, ed. Molecular Biology of the Cell, 27(6):882-887. doi:10.1091/mbc.E15-10-0682.)

10 week old *Phallusia philippinensis* in culture. Photo Serina Lee



ABSTRACTS FROM RECENT MEETINGS

1. 7th meeting of the European Society for Evolutionary Developmental Biology (EURO EVO DEVO CONFERENCE) 26th – 29th June 2018, National University Ireland, Galway, Ireland

An Evo-Devo approach to neurogenesis and neurodegeneration. Chiara Anselmi¹, Mark Kowarsky², Fabio Gasparini¹, Karla J. Palmeri^{3,4}, Katherine J. Ishizuka^{3,4}, Margherita Martini¹, Arianna Targonato¹, Ayelet Voskoboynik^{3,4}, Lucia Manni¹

1Department of Biology, Padova University, Padova, Italy; 2Dept. of Physics, Stanford Univ., CA, USA; 3Institute for Stem Cell Biol. and Regenerative Medicine, Stanford Univ. School of Medicine; 4Dept. of Biol., Stanford Univ. and Hopkins Marine Station, Pacific Grove, CA, USA.

In tunicates, the sister group of vertebrates, the larval central nervous system (CNS) develops from a typical chordate neural plate during embryogenesis. When larvae undergo metamorphosis, the embryonic CNS degenerates and a new brain (the cerebral ganglion of adult individuals) forms. In the colonial tunicate *Botryllus schlosseri*, there is the added feature that it reproduces asexually through weekly cycle in which neurogenesis occurs simultaneously to neuro-degeneration events. In this cycle, new buds regenerate from stem cells to replace the previous generation's zooids after they die through massive apoptosis. Therefore, brain regeneration takes place in the colony's developing buds as brain degeneration occurs in the resorbing adults. Taking advantage of this cycle, we studied brain differentiation and degeneration in buds and zooids combining: morphological data (ultrastructural and histological serial sections, 3D reconstructions, confocal microscopy), behavioral tests, and transcriptome analyses. We found that there is a progressive decrease in the number of neurons within the cerebral ganglion as the zooid ages. Accompanying CNS degeneration, we observed a decrease in zooid activity. Many of the genes involved in neurodegenerative human diseases are detected and differentially expressed in the ageing brain. Our results show that *B. schlosseri*, with its peculiar life cycle, is a powerful model in studying the evolution of neurogenesis and neurodegeneration processes, and exploring new strategies to deal with neurodegenerative diseases.

2. 64th Meeting of the Italian Society of Developmental and Cell Biology, 11th -13th June 2018 L'Aquila (Italy). Abstract published in the European Journal of Histochemistry ISSN 1121-760X 62/ supplement 1

Cyclical neurogenesis and neurodegeneration in the colonial tunicate *Botryllus schlosseri*.

Chiara Anselmi, Margherita Martini, Arianna Targonato, Fabio Gasparini, Lucia Manni. Dept. of Biology, Univ. of Padova, Italy.

In *Botryllus schlosseri*, a colonial tunicate, neurogenesis occurs simultaneously to neuro-degeneration. During its asexual weekly cycle, three different generations of zooid coexist: adult individuals, their buds, and budlets produced by the latter¹. At change of generation, or takeover, adult zooids regress and are substituted in filtration by their buds that become the new adults; in the meantime, budlets become buds and produce a new generation of budlets. This process is well synchronized and necessary for colony survival. While in developing budlets and buds brain development takes place, brain degeneration occurs in reabsorbing adults. In *B. schlosseri*, therefore, this neurodegenerative process is genetically controlled and not pathological. Taking advantage on this weekly zooid turnover, we studied brain differentiation and degeneration combining morphological data (ultrastructural and histological, 3D reconstructions, confocal microscopy) and behavioral tests. We found that, in adult filtering zooids, the brain neuron number is not constant. At the beginning of adult life, an initial increase of neuron number is recognizable; this is followed by a decrease, in which apoptosis is involved. Eventually, the brain is completely resorbed during the takeover. This trend of neuron number reflects the zooid behavior to respond to both oral siphon and oral tentacles stimulations. These two stimulations evoke two different behavioral answers (the direct response and crossed response), which are reflexes mediated by different nerve circuits. Experiments showed a greater sensibility to stimuli when the neuron number is maximum, followed by a decrease in zooid sensitivity during the last period of the adult life. Since *B. schlosseri* is a tunicate, the taxon considered the closest one to vertebrates², we propose this species as a useful model to provide new insights into compensatory mechanism protecting neurons from diseases associated

with neuron loss and aging. 1. Manni et al. PLOS ONE 2014, vol. 9(5); 2. Delsuc et al. Nature 2006, 439(7079):965-968.

3. Tenth Intl. Conference on Marine Bioinvasions, Puerto Madryn, Chubut, Patagonia, Argentina, October 16th-18th, 2018. Many thanks to Evangelina Schwindt (schwindtcnp@gmail.com) for assembling all the ascidian presentations as a packet for me. Here is a photo of most of the presenters, submitted by Rosana Rocha.



The Conference Abstract eBook can be downloaded here: <https://bit.ly/2Dijcp1>

KEYNOTE SPEAKER: ROSANA ROCHA

a. Ascidians in the anthropocene -invasions waiting to happen. Universidade Federal do Paraná, Departamento de Zoologia, Curitiba, PR, Brazil. rmrocha@ufpr.br

Only recently did we realize that the heretofore enigmatic disjunct distributions of many ascidian species were likely to be consequences of human activities, which may also explain why ports and marinas often have very similar assemblages even when separated by great distances. Also, only in this millenium did we note the huge impact of ascidian fouling in the bivalve aquaculture industry. Part of the reasons for the surprising influence of ascidians is because they do not have a typical “good invader” profile. They lack efficient osmoregulatory control that prevents them from surviving wide variation in salinity, typical of ports and marinas. Often they have delicate tunics that do not resist the force of drag on ship hulls. Finally, they face starvation due to the oligotrophic waters of the open ocean. On the other hand, they regenerate rapidly so small pieces can often recover from damage and become complete individuals or colonies. Also, fast growth rates and reproductive traits, such as hermaphroditism, allow rapid establishment once propagules arrive. Consequently, ascidians are becoming interesting and useful models for a broad spectrum of research that focuses on marine bioinvasion. The impressive data that has been gathered during the last 30 years demonstrate that 1) ascidians are less stenohaline than we thought, 2) tunics are often very resistant to detaching and tearing, 3) niches exist on ship hulls that promote transport without drag, and 4) construction in coastal areas offers new habitat and substrate that is now extensively occupied by ascidians. The aggregation of vast numbers of solitary ascidians and the smothering capacity of colonies result in a very high risk of impact by invasive ascidians in natural communities. While the scale of ascidian impact is not yet large, we know that it is simply a question of time. Are we prepared for the consequences of changing biodiversity?

b. Can anthropogenic forcing shape ascidian invasive potential in a warming ocean?

Clutton EA (1), Alurralde G (2), Repolho T (3). (1) Institute of Marine Sciences, University of Portsmouth, UK. (2) UNC, FCEyN, DDBE, Ecología Marina, Córdoba, Argentina. / (IDEA) CONICET, Córdoba, Argentina. (3) MARE - Marine and Environmental Sciences Centre, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Portugal. mitocondriarevelde@gmail.com

High alleged levels of pollutants in anthropogenically-modified marine environments may exert high pressure on maximum physiological responses of sessile organisms acting as powerful agents of selection. Thus, local adaptation or acclimation capacity play an important evolutionary role by enabling or conditioning species tolerance to stressful environmental conditions. Temperature modulates marine ectotherms physiology, influencing survival, abundance and distribution of sessile organisms. While native species could be susceptible to ocean warming, thermal tolerance might favour the spread of non-native species. We studied the effect of a simulated heat wave on the developmental performance of *Ciona intestinalis* early life stages (ELS), derived from populations of anthropogenically modified and undisturbed environments. The fertilization rate, embryo and larvae development, settlement and metamorphosis success, and juvenile heart beat rate were assessed as experimental endpoints. Excepting fertilization and heart beat rates, temperature affected all analysed endpoints. *Ciona intestinalis* ELS derived from undisturbed sites were the most negatively affected by increased temperature conditions. Oppositely, populations from anthropogenically modified sites showed an overall positive response to thermal stress. Interaction between temperature and population origin (i.e. anthropogenically modified or undisturbed), was statistically significant for embryo and larvae development, and metamorphosis. For anthropogenically modified sites, a higher proportion of larvae development and settlement and metamorphosis success were observed under increased temperature conditions. No differences were observed for heart beat rates between sampled populations and experimental temperature conditions. We propose that selection resulting from anthropogenic forcing could shape stress resilience of species in their native range and subsequently confer them advantageous traits for invasion.

c. Unexpected guests at the table: how a non-native ascidian uses food resources and its consequences on ecosystem functioning

Calcagno E (1), Giachetti C (2), Alurralde G (1), Battini N (2), Schwindt E (2), Tatián (1). (1) Univ. Nac. de Córdoba, FCEFN, Dpto. de Div. Biol. y Ecol, Ecología Marina/IDEA, CONICET, Córdoba, Argentina. (2) Grupo de Ecología en Ambientes Costeros (GEAC), IBIOMAR-CONICET, Argentina. calcagnoemilia@gmail.com

Harbors are prone areas for biological invasions since they provide diverse artificial structures that favor colonization by sessile marine organisms, like ascidians. These filter-feeding animals have a key role in pelagic-benthic coupling when reaching high densities: via the uptake of organic matter and the deposition of feces, ascidians fuel one of the major trophic pathways in marine ecosystems. In order to assess ascidian contribution in this pathway, we performed aquaria experiments using a non-native species (*Ascidiella aspersa*) and a cryptogenic one (*Asterocarpa humilis*) that flourish in a port area in Puerto Madryn city, Argentina. We evaluated the mass-specific biodeposition rate and the feces quality (C:N ratio) for both species, under two different diets (natural seston and natural seston enriched with phytoplankton). These parameters were related to ascidian densities, which were estimated after a year of deployment of artificial succession plates. Ascidians were the most abundant organisms of encrusting communities. *Ascidiella aspersa* predominated, reaching an annual average density of 233 individuals m⁻², that is up to five-folds the density reached by *A. humilis*. The trophic processes varied according to the species and the type of diet: biodeposition rates were similar under natural seston but higher in *A. aspersa* under natural seston plus phytoplankton. Feces quality of *A. aspersa* was higher than *A. humilis* after feeding with natural seston. Besides, the amount of carbon and nitrogen in feces is greater in *A. aspersa* than in *A. humilis* for both diets. Considering the densities observed and the estimated trophic processes, *A. aspersa* would have a major incidence in the flow of particles being able to alter the normal stoichiometry and the trophic pathways in the assessed ecosystem.

d. Hanging paradise: the role of benthic predators in colonization success of exotic ascidians

Giachetti C (1,2,3), Battini N (1,2,3), Castro K (1,2,4), Tatián M (5), Schwindt E (1,2). (1) GEAC, Argentina. (2) IBIOMAR-CONICET, Argentina. (3) FCEyN, UBA, Argentina. (4) CRUB, UNCOMA, Argentina. (5) IDEA-CONICET-UNC, Argentina. claragia88@gmail.com

Colonization success of marine exotic species in artificial structures depends on physical characteristics of these structures, and on environmental factors and/or ecological interactions among species. Predation plays a key role in tropical and subtropical areas with an apparent decrease at higher latitudes, establishing the hypothesis that tropical communities present a stronger biotic resistance to new exotic species. Among marine exotic species, ascidians appear to be the most successful sessile filter feeder in colonize new habitats as fouling communities on artificial structures. In this work, we (1) evaluate the effect of predation over colonization success of solitary ascidian community on different artificial structures, and (2) test the vulnerability of juveniles and adults of solitary exotic ascidian species in presence of different potential predators. We performed a field experiment in a cold-temperate port to evaluate the ascidian recruitment in fixed and hanging structures, in presence and absence of benthic and nektonic predators, and aquarium experiments under controlled conditions. We observed that benthic predators play a key role in the recruitment of exotic ascidians, which presented the highest cover in absence of these predators in both structures. Aquarium experiments showed that predation is higher over juveniles, while the effect on adults depends on the ascidian species. Sea urchins are the most successful predators, consuming both adults and juveniles. The results suggest that predation have a strong effect during the juvenile period with benthic species as the main predators. In this way, hanging structures might act as refuges from benthic predators for exotic ascidians in cold temperate regions.

e. Opportunistic epibiosis enables non indigenous invertebrates to insinuate themselves into space limited environments.

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Marine biofouling communities are often space-limited and characterised by intense overgrowth competition, often resulting in the mortality of the weakest competitor. Competitive hierarchies demonstrably influence larval settlement choice, with inferior competitors selecting settlement sites further away from superior competitors or not finding an adequate settlement sites at all. The ability of a non-indigenous species (NIS) to find adequate substrate to settle and grow has been shown to be an important function of success. Yet our understanding of the mechanisms that marine species use to insinuate themselves into extant, well connected communities remains elusive. Monthly observations of recruitment on PVC settlement plates over a year illustrated that NIS generally recruit in higher numbers and for longer periods than native species. However, the observed recruitment did not equate to species composition and dominance on plates that remained in-situ for three or six months, mostly due to the presence of the dominant native solitary ascidian *Molgula ficus*. Therefore, we examined changes in settlement preferences for settling on artificial or biogenic substrates by contrasting recruitment phenology with epibiotic settlement. We observed that in order to live and maintain a hold within space limited environments, NIS increasingly relied on epibiosis. More specifically, with the availability of space NIS tend to show no real preference with regards to settling on uncolonised space or as epibionts. However preference changes to settling as epibionts, when bare space became limited. In contrast native species that were recorded as epibionts generally showed a preference to settle on basibionts regardless of space availability.

f. Seasonal use of open spaces by non-native and native species.

Pederson J, Bastidas C. MIT Sea Grant College Program, USA. jpederso@mit.edu
Climate change is altering physical and biological conditions in marine ecosystems in ways that have impacts on both native and non-native species. Along the Northeast coast of the U.S., non-native species especially ascidians 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 monthly available open space and early competition on established and newly placed plates. We compared patterns between two sites at different bioregions and slightly different physical conditions, in the Virginian (Buzzards Bay) and Acadian (Boston Harbor) Provinces. These locations were chosen because pre-existing data from five rapid assessment surveys between 2000 and 2013, and allowed comparisons between deployed plates with results from summer surveys of in situ communities in terms of species present. Settlement of native (~90-110) and non-native species (14) were recorded over 12 months using plates removed throughout the years 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. During the coldest months, plates were basically bare or covered with turf algae, except for two ascidians, *Didemnum vexillum* and *Botrylloides violaceus* that were present as diminished colonies in Buzzards Bay. Differences between the two sites were most marked by the presence of two non-native ascidians, *D. vexillum* more prevalent at Buzzards Bay and the *Asciidiella aspersa* more prevalent at Boston Harbor. Data on the timing of arrival and settlement of non-native species will be shared with marina operators and managers responsible for prevention and control.

g. When introduced equals invasive: Normative use of “invasive” with ascidians. Pereyra PJ, Ocampo Reinaldo M. CIMAS - CONICET - UNCOMA, Argentina. pereyranis@gmail.com
Since early 2000, there was ample debate on what is and how to define an invasive species. With several definitions coexisting and being used indistinctly, there was highlighted the possible use of the “invasive” in a normative way, meaning that instead of describing an ecological behavior related with invasiveness (i.e. spread, effects), the species are labeled as invasive for other reasons (e.g. habit, citation practices). To test this hypothesis, we have used as working example introduced ascidians worldwide. We also describe the type of research performed with introduced ascidians, with a focus in all ascidians vs those described in the literature as model species, to synthesize the research made with ascidians so far, and identified possible research gaps. A specific search in Web of Science was performed, and articles suitable for analysis were selected. Each article was classified according to the type of environment, species under study, type of effects and spread that ascidians are linked to. Most of the 184 articles analyzed did not consider any type of dispersal or effects as study subject (82 and 71%, respectively). Most research was conducted in laboratory conditions (41%) or man-made environments (32%) or indicating few escapes to natural environments. Almost half of the articles (47%) were made with the six model ascidians. These results indicate that the normative use is widely used regarding introduced ascidians. Spread and Effects, necessary conditions to consider a species as invasive, are notoriously understudied. Most research was not conducted in natural environments and over few species, weakening the perception of introduced ascidians as a conservation problem. This distinction allows us to discuss two separate aspects of the same phenomena: are some species intrinsically problematic for conservation (i.e. invasive) or is the movement of non-native species (i.e. biological invasion) the conservation problem?

h. Facilitation between two introduced species in Patagonia: a mechanistical approach. Pereyra PJ, Saad J, Gastaldi M, de la Barra P, González RC, Narvarte M. CONICET, Argentina. pereyranis@gmail.com

Positive interactions are increasingly studied in marine environments. We were recently able to document a novel interaction between two introduced species, the clubbed tunicate *Styela clava* and the macroalga “wakame” *Undaria pinnatifida*, in which the presence and abundance of *U. pinnatifida* was favored by the occurrence of *S. clava*. We inquired if this was due to a biological effect (i.e. presence of *S. clava* vs. an inanimate, erect substrate), or if it was due to a greater added complexity, either in total area available for settlement or in height (i.e. increasing access to light). To test this, we

made mimics of synthetic rubber that copied adult (10 ± 3 cm) and juvenile (3 ± 1 cm) individuals of *S. clava*, and arranged them in four different groups of structural complexity, from higher to lower complexity: (a) 5 adult mimics, (b) 5 juvenile mimics, (c) 1 adult mimic and (d) 1 juvenile mimic. We deployed them ($n = 20$ for each treatment) in an inner channel of San Antonio Bay below mature *U. pinnatifida* in November 2017 to promote spores settlement. In March 2018, we collected *S. clava* individuals with similar size to the adult mimics and attached them to tiles ($n = 20$) to test for biological effects. In May 2018 we retrieved all the experimental units. We found that more *U. pinnatifida* settled on alive *S. clava* than on mimics, showing that *S. clava* may offer additional benefits than only more area for settlement. We also found a larger number of *U. pinnatifida* per area in adult than in juvenile mimics, but the density of mimics did not affect the response, showing that *U. pinnatifida* takes more advantage at settling on adult than on juveniles mimics, possibly related with greater access to light.

i. Going tropical- genetic analysis of a Red Sea population of *Ciona intestinalis* type A reveals unique introduction scenarios. Chen Y (1), Shenkar N (2), Zhan A (1). (1) Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, China. (2) School of Zoology, Tel-Aviv University, Israel. shenkarn@post.tau.ac.il

The solitary ascidian *Ciona intestinalis* Type-A (*C. robusta*) is among the most damaging of invasive fouling species in the world. It is mostly known from cold-water or temperate environments, where it is able to form dense aggregations. However, in 2015 several individuals were found fouling the bottom of few floating docks in Eilat marina, Israeli Red Sea coast. While numerous cases of introduction are known from marine environments in temperate and sub-tropical latitudes, only a few introduced species have been reported from tropical regions, and none have been previously reported in Eilat. In order to elucidate the potential source of this unique tropical population we conducted a genetic analysis based on cytochrome c oxidase subunit 3-NADH dehydrogenase subunit 1 region (COX3-ND1) of 22 individuals. The Red Sea sequences were compared to over 300 sequences from 19 populations around the world, revealing 3 new haplotypes to the Red Sea. The Red Sea population genetic diversity was lower than the American populations but higher than that in European populations. Thus, based on polymorphism of this mtDNA fragment, we did not see a significant genetic bottleneck, suggesting of several introduction events. Previous studies showed that the optimal temperature for sustainable populations of Mediterranean *Ciona intestinalis* type A is 14–23.4 °C. The occurrence of a reproductive population in a Red Sea marina, with SST fluctuating between 19 and 27 °C and salinity of 40–41 ‰, indicates that this species possesses a wider environmental tolerance than previously assumed. The distinctive population genetic pattern in Red Sea may result from rapid adaptation to local conditions.

j. Distribution of *Ciona robusta* Hoshino & Tokioka, 1967 and *Rhodosoma turcicum* (Savigny, 1816) along Brazilian coast. Barboza D, Skinner L. UERJ - DCIEN/ PPGOCn, Brazil. lskinner@uerj.br

Marine bioinvasion is a worldwide problem and since the last decades, the number of scientific papers dealing with this theme have increased. Commonly, non-indigenous species (NIS) that became invasive could impact biodiversity by the reduction of populations, local extinction of species, and damage to economic activities. Prevention and persistence of non-indigenous species are dependent of environmental factors like temperature, or by biological factors like predation and competition. In Brazil, two species recognized as invasive in many parts of the world were recorded in recent years: *Ciona robusta* and *Rhodosoma turcicum*. The present study was conducted between January 2013 and May 2018 in four bays of Rio de Janeiro state: Ilha Grande, Sepetiba, Guanabara and Cabo Frio / Arraial do Cabo. Three sampling strategies were used: experimental structures composed of granite plates (21 x 12 cm) inside protected cages to prevent predation by fishes; black polyethylene plates (15x15 cm) mounted like a sandwich; and active collection by scuba diving, searching under boulders and other cryptic and predation protected habitats. These different methods

allow us to collect many individuals of *C. robusta* and *R. turcicum* both on artificial and on natural substrates. Confronting our data about *C. intestinalis* and *R. turcicum* with those from literature, we could relate it to regional characteristics of water temperature and ship traffic. *Ciona robusta* and *R. turcicum* were recorded for the first time at Ilha Grande Bay, including natural substrates. They were found at four sites, but only in one site both species were recorded together. Our previous samples and the literature indicate that *C. intestinalis* for Guanabara Bay (RJ), Sepetiba Bay (RJ) and Arraial do Cabo (RJ) and probably from São Sebastião (SP) correspond to *C. robusta*. *Rhodosoma turcicum* was recorded for Salvador (BA), São Gonçalo do Amarante (CE), Arraial do Cabo (RJ) and Ilha Grande Bay (RJ).

k. Ten years of non-indigenous species survey along southwestern Tropical Atlantic: the rise of vectors and environment. Skinner LF (1,2), Barboza D (1,2), Oliveira Marins F (1). 1: UERJ - DCIEN, Brazil. (2) PPGOCn, Brazil. lskinner@uerj.br

Surveys of non-indigenous species (NIS) are essential to understand the invasive potential of many marine species. Early detection of NIS and environmental monitoring of habitats are one of the most important issues to establish the invasive potential, the environmental impacts and the spread of NIS. Since 2008, we are surveying the occurrence of NIS, some of them described as invasive on other world oceans. Surveys were performed using predator exclusion cages, sandwich like plates and active search by scuba diving. We surveyed, from south to north, Ilha Grande Bay, Sepetiba bay, Guanabara bay and Arraial do Cabo/ Cabo Frio peninsula. During these 10 years, we detected and expand studies on many recently introduced species like the sponge *Paraleucilla magna*, the polychaeta *Spirobranchus giganteus*, the moluscan *Eualetes tulipa*, and the ascidians *Ciona robusta* and *Rhodosoma turcicum*. We identify, based on the distribution, abundance and range expansion of species the main factors influencing their invasive potential. Among these species, all of them except *P. magna* were not directly related to oil industry. The second factor that emerges from all data are the influence of water temperature limiting the occurrence of *C. robusta* and the role of predation controlling the establishment of species. Palatable species like the ascidians *C. robusta* and *R. turcicum* are still controlled by predators. The only detected species that is expanding its density is *E. tulipa*. *Rhodosoma turcicum* is the species with the highest range expansion since its detection, in 2009. Continuously survey is essential to follow increases on density and range expansion. However, the absence of the politics and funding to this survey limit the early detection and monitoring programs. Since marine activities like harbours, marinas, oil industry, aquaculture and urban development among others are expanding, new habitats is being created favouring species spread and increase in density.

I. Contrasting systematic, biogeography and historical data to define ascidian invasions in the SW Atlantic (Argentine Sea). Tatián M (1), Alurralde G (2), Calcagno E (2), Correa N (3), Giachetti C (4), Gimenez D (1), Lager C (2), Maggioni T (2), Meloni M (5), Scarabino F (6), Schwindt E (4), Sylvester F (7), Taverna A (2). (1) UNC, FCEyN, DDBE, Ecología Marina, Córdoba, Argentina, (2) IDEA-CONICET, Córdoba, Argentina. (3) SHN y Escuela de Ciencias del Mar, INUN Facultad de la defensa, UNDEF, Ministerio de Defensa, Argentina. (4) Grupo de Ecología en Ambientes Costeros (GEAC), IBIOMAR-CONICET, Argentina. (5) IEGEBA (CONICET-UBA), FCEyN, UBA, Argentina. (6) MNHN y Centro Universitario Regional del Este – CURE, Universidad de la República, Uruguay. (7) IEBI, FCEyN, UNS - CONICET, Argentina. marcostatian@gmail.com

The Southwestern Atlantic (SWA) is one of the poorest known regions in terms of biodiversity, in particular in the case of ascidians. This diverse group of animals has long time been considered in bioinvasion studies because of its association with negative ecological as well as economic impacts. Accurate species identification is crucial to detect and clarify invasion processes. Our aim is to permanently enhance the knowledge on species richness along the shelf and continental slope in order to upgrade the following ascidian statuses for the assessed area: native; exotic; cryptogenic

(those whose status is still doubtful). We performed field samplings, including coastal and oceanic areas, and evaluated ship fouling communities. We also studied old museum collections and compiled data from specific taxonomic and global literature. The general survey of species reveals at the moment a hundred ascidian species, being 14 of them, species whose status is considered exotic or cryptogenic. From these, two species: *Ascidiella aspersa* and *Styela clava*, have been stated to reach ecological impact at the SWA but, their distribution is still limited. The distribution of *A. aspersa* is probably restricted by low temperatures, while *S. clava* is a recent introduction. The use of morphological and genetic tools to clarify species identities is the first step in the evaluation of invasion processes. Besides, the study of these processes also helps to better understand the native biodiversity of this wide and not completely known area.

m. The ascidian *Asterocarpa humilis* (Heller, 1878) in the South Western Atlantic: a native or introduced species? Taverna A (1), de Aranzamendi MC (1), Maggioni T (1), Alurralde G (1), Turon X (2), Tatián M (1). (1) IDEA-CONICET-UNC, Córdoba, Argentina. (2) Centro de Estudios Avanzados de Blanes (CEAB-CSIC), Blanes, Spain. anabelataverna@gmail.com

Taxonomic problems when dealing with morphologically similar marine taxa can hinder the correct assignment of the status of a species as native or introduced. This is the case of *Asterocarpa humilis* (Heller, 1878) considered native for the Southern Hemisphere. The species was originally described for New Zealand and later cited for Tasmania and South Africa. However, for Chile *A. humilis* was considered introduced, as well as for the Northern Hemisphere in Great Britain and France. For the SW Atlantic, another species with a similar morphology, *Cnemidocarpa robinsoni* Hartmeyer, 1918 has been reported as a cryptogenic species. The aim of this work was to define the entity and status of these species: *Asterocarpa humilis* and *Cnemidocarpa robinsoni* through molecular analyses. We sequenced a partial fragment of COI gene of seven samples of *C. robinsoni* collected at the Argentine Sea. A median joining haplotype network was constructed including the seven new sequences and four sequences of *A. humilis* from Chile, New Zealand and France mined from published databases. Four haplotypes with eight variable sites were obtained. The haplotype network shows a more frequent haplotype present in South America and France, suggesting that the Argentine specimens described as *C. robinsoni* and those from Chile and France as *A. humilis*, correspond to the same taxonomic entity. Phylogenetic trees reconstructed by Bayesian Inference, Maximum Likelihood and Maximum Parsimony, grouped *A. humilis* and *C. robinsoni* into one well-supported monophyletic clade. The scarce genetic difference among individuals from distant populations proves the existence of a unique specific identity. Thus, the name *Asterocarpa humilis* takes precedence over *Cnemidocarpa robinsoni*; the latter must be considered a junior synonym of the former. The presence of this species in natural habitats in addition to its register in old collections (1920) in the SW Atlantic, suggests that the species is native for this area.

n. A brand new non-native species of *Didemnum* genus (Tunicata: Ascidiacea: Aplousobranchia) in Southeastern Brazilian coast. Castelo Branco LP (1), Vieira Granthom-Costa (2), Coutinho R (1,2) (1) Programa de Pós-Graduação em Biotecnologia Marinha - IEAPM/UFF, Brazil. (2) Departamento de Biotecnologia Marinha - Instituto de Estudos do Mar Almirante Paulo Moreira – IEAPM, Brazil. lucas.castelo20@gmail.com

Non-native species are major threats to the marine environment. During a field work in harbor area in the municipality of Arraial do Cabo, Rio de Janeiro (22°57'58"S-42°01'40"W), was found a colonial ascidian of the genus *Didemnum* unreported in previous studies. We conducted this study during november and december/2017 in order was to assess the invasive potential this brand new record considering spatial distribution, colonization ability, reproductive effort and predation this species named here as *Didemnum* sp. RJAC. Our results showed *Didemnum* sp. RJAC is confined to the Anjos Inlet occurring in both sides of the beach in natural and artificial substrates covering 69.10 m²/200m² in harbor area (iron cables, pillars) and rocky shores, covering 14m²/200m². We observed

that *Didemnum* sp. RJAC colonies were reproductively active during all year study where we found full larvae in all analyzed colonies mainly in (Autumn). During this study, we found lowest densities in January/2017 with 2 larvae/cm² and highest densities with 127 larvae/cm² in May/2017. The colonization ability of *Didemnum* sp. RJAC was assessed experimentally in two seasons during 2017 showing a significant difference between them (Student's t-test, T=4.45, df=278, p=0.01) and also both sides -upper and lower of the plates tests -(Student's t-test, T=5.35, df=278, p=0.00). Besides, predation pressure results based on experimental test showed that *Didemnum* sp. RJAC colonies were not bite in two different periods of the day. It was also observed that *Didemnum* sp. RJAC overgrows other native and non-native benthic species in region and may induce to death by suffocation or tissue necrosis. As conclusion, *Didemnum* sp. RJAC exhibit characteristics of invasive species, such as rapid growth, high fecundity and regeneration of colonies from fragments. We recommend the continuous monitoring of the population dynamics of this species where it already occurs in order to control and prevent to bioinvasion events.

o. Non-indigenous marine biofoulers in an upwelling region from Southwestern Atlantic Ocean. Vieira Granthom-Costa L (1), Messano L (1), Spotorno-Oliveira P (1,2), Altvater L (1), Kassuga A (1), Arruda Gonçalves JE (1), Coutinho R (1). (1) IEAPM, Brazil. (2) FURG, Brazil. lu.granthom@gmail.com

Bioinvasion is considered a threat to biodiversity and it has been modified benthic marine communities worldwide. Brazilian coastline is the longest in the Southwest Atlantic comprising three biogeographical provinces, in which the south is one of the most speciose marine ecoregion. According to Brazilian government environmental agencies, 32 non-indigenous (NIS) fouling species (detected, established and invasive) were recorded. Recent surveys added 24 fouling species to the list, totaling 56 NIS on natural and artificial marine substrates in the Brazilian Coast. Arraial do Cabo region (23° S-42° W) comprises a multiple-use Marine Protected Area located in the main upwelling system of Southwestern Atlantic. The local geomorphology consists in an inner embayment, with tropical environmental characteristics and an area exposed directly to the coastal upwelling, with subtropical characteristics. Furthermore, Arraial do Cabo holds a small commercial harbor that occasionally supports domestic and offshore vessels, that may act as a vector of NIS. In this study, we conducted a survey based on the literature in order to assess the NIS recorded to Arraial do Cabo. As a result, we listed 31 NIS recorded only in the inner embayment. Ten species are new recent records to the region – the green algae *Caulerpa brachypus*, the sponge *Darwinella cf. oxeata*, the serpulid *Branchioma luctuosum*, the gastropod *Eualetes tullipa* and six ascidians species *Didemnum* sp RJAC, *Ciona robusta*, *Ascidia curvata*, *Cnemidocarpa irene*, *Styela canopus* and *Rhodossoma turcicum*. We concluded that the harbor area is a receptor of NIS due to high number of species found inside the bay and may act as a donor to surrounding areas. Our data suggest that the inner area is open to introduction events, expansion and establishment of NIS. Although in the outside area the low water temperature may create a barrier to their spread, it is susceptible to the establishment of NIS with subtropical affinities.

4. 11th International Vanadium Symposium. 5-9 November 2018. Montevideo, Uruguay. Genetic Mechanism of Vanadium Accumulation and Possible Function of Vanadium in Underwater Adhesion in Ascidians. Tatsuya Ueki, Marine Biol. Laboratory, Graduate School of Science, Hiroshima Univ., Hiroshima, Japan ueki@hiroshima-u.ac.jp

Ascidians are marine animals that belong to the same phylogenetic group (Phylum Chordata) as human beings do. One of the three suborders in ascidians can accumulate a high level of vanadium ions in blood cells. *Ascidia gemmata* has been reported to accumulate the highest levels of vanadium at 350 mM, which is 10⁷-fold higher than the vanadium concentration in seawater. Vanadium ions are absorbed from natural seawater in a +5 state, reduced to a +4 state through the branchial sac, intestine, and blood plasma and are stored in a +3 state in vanadocytes.

In order to identify comprehensively vanadium-related genes expressed in blood cells, we have done a transcriptome analysis on blood cells of a vanadium-rich ascidian *Ascidia sydneiensis samea*. We gathered huge data on nucleotide sequences and gene expression in blood cells. Most notable one is the sixth Vanabin, vanadium-binding protein, identified in this study. We are currently studying its biochemical properties.

Irrespective of vanadium accumulation, most ascidian species settle on underwater substrates during a short free-swimming tadpole larval period. We have recently published untrastructural, elemental and masspectroscopic observation of the adhesion sites². Based on the finding that levels of vanadium and iron in adhesion site are higher than those in non-adhesive sites in all four ascidians species examined, we postulated that vanadium and iron are involved in the adhesion in all ascidians. We performed comparative proteomic study on adhesion and non-adhesion sites and found several proteins that are solely expressed in the adhesion site. One of the proteins is von Willebrand factor which is known to be expressed in the blood cells of *Ciona intestinalis* and is also found in the transcriptome analysis in this study. We are currently working on this protein to examine whether this protein has a glue activity and has a metal binding activity.

5. Intl. Meeting on Marine Research. Peniche, Portugal (July 5-6).

Developmental performance of ascidian populations to an extreme warming event.

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Coastal environments are increasingly experiencing an upsurge of anomalously high seawater temperature events, with unpredictable effects over marine biota. Marine heat wave episodes have been identified as a major climate change driver, which can prompt, to a more or less extent, detrimental outcomes to marine species. As a result of an increase in the frequency and timespan of short-term acute warming events, invasive species proliferation is expected to intensify, thus resulting in a loss of native biodiversity, within invaded ecosystems. Under this perspective, we investigated the effect of an acute temperature increase (simulating a marine heat wave), on the developmental performance of *Ciona intestinalis* early life stages (ELS), derived from populations of anthropogenically-modified and undisturbed environments. Fertilisation rate, embryo and larvae development, settlement and metamorphosis success, and juvenile heart beat rate were assessed. With the exception for fertilization rate and heart beat rates, temperature had a significant overall effect over analysed endpoints. *Ciona intestinalis* ELS, derived from undisturbed environments, were the most negatively affected by increased temperature conditions. Opposingly, anthropogenically impacted populations showed an overall consistency in their positive response to thermal stress. Interaction between temperature and population origin (i.e. anthropogenically impacted or undisturbed environments), was statistically significant for three of the ELS analysed endpoints, namely: embryo and larvae development, and metamorphosis. A higher proportion of larvae development and settlement success was observed under increased temperature conditions. Additionally, a higher metamorphosis success rate was observed in individuals exposed to increased temperature conditions, derived from populations from anthropogenically impacted locations. Considering heart beat rates, no differences were observed between sampled populations and experimental temperature conditions. Our findings suggest that future marine heat wave events, together with stress resilience attained by *C. intestinalis* populations as a result of anthropogenic forcing exposure, could provide favourable breeding conditions towards their environmental proliferation, with unpredictable effects within invaded ecosystems.

6. X Jornadas Nacionales de Ciencias del Mar, XVIII Coloquio Nacional de Oceanografía. "Universidad, ciencia y sociedad: estrategias de hoy para sostener el mañana". 30 de julio-3 de Agosto 2018, Buenos Aires, Argentina.

Éxito en la fertilización y desarrollo embrionario en dos especies de ascidias.

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Las ascidias (Chordata, Tunicata) son animales hermafroditas con fertilización externa o interna. Al comienzo de su desarrollo, las larvas, a través de estructuras especializadas, encuentran un sitio adecuado para dar lugar al proceso de metamorfosis. Algunas especies presentan ventajas reproductivas en el adulto y comportamentales en la larva las cuales posibilitan su dispersión natural. Asimismo, pueden colonizar áreas distantes a través del transporte humano. El objetivo de este trabajo fue comparar el éxito en la fertilización y en el posterior desarrollo embrionario de dos especies de ascidias recolectadas en el intermareal de Playas Doradas: *Paramolgula gregaria* y *Ciona* sp. Esta última especie es exótica e invasora en el área. Mediante disecciones se extrajeron óvulos y espermatozoides de individuos de cada especie. Se hidrataron los óvulos, y se procedió a fertilizarlos luego de comprobar la movilidad de los espermatozoides. Se realizó un seguimiento y cuantificación de los diferentes estadios de desarrollo del cigoto de cada una de las especies, mediante la observación al microscopio óptico. Con los datos obtenidos, se estimó el éxito de la fertilización como la proporción de cigotos que llegan a larva. Asimismo, se determinó el número de estadios entre el cigoto y larva en cada especie. Se obtuvieron 8 estadios embrionarios de *Ciona* sp. y 10 estadios en *P. gregaria*, especie cuyo desarrollo concluyó con una larva urodela. Se observó un menor tiempo de desarrollo del cigoto en *Ciona* sp. que en *Paramolgula gregaria*, aunque la proporción de cigotos que llegaron al estadio de larva móvil fue mayor en esta última especie, cuyo desarrollo embrionario se estudia por primera vez. Los datos recopilados constituyen una información valiosa para estudios de manejo y prevención de invasiones biológicas.

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