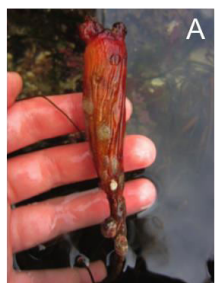


## Microbiome and stress: examining host-microbe responses in ascidians following acute low-salinity exposure

**Key words:** microbiome, ascidian, bioinformatics, salinity stress

**Background:** Marine bottom-dwelling species, such as tunicates<sup>1</sup>, sponges<sup>2</sup>, and corals<sup>3</sup>, are well-known for harboring a rich diversity of microbiota in the surrounding mucus layer, with evidence of symbiotic interactions<sup>4</sup> and horizontal gene transfer<sup>5</sup> between microbiome and host. Because of this tight relationship, when there is an environmental change, it is not clear whether physiological responses originate from the host, its microbiome, or the host responding to the dynamic microbiome. Tunicate ascidians are a unique model for studying host-microbiome interactions because they are our closest invertebrate relatives, providing insight into how the host innate immune system responds to its native microbiome. This will help us to better manage

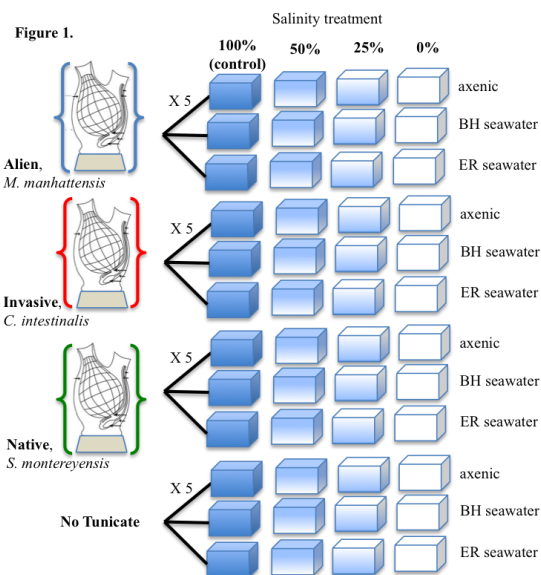


these cosmopolitan species. *Styela montereyensis* (A) is a solitary tunicate species native to the northern California coast. *Ciona intestinalis* (B) is a solitary tunicate and an invasive species of concern in the western US, implicated in fouling marine surfaces, threatening oyster fisheries, and spreading easily into ports via ship hulls and ballast water.<sup>6</sup> *Molgula manhattensis* is a tunicate species originating from the east coast of the U.S., now invasive on the west coast.<sup>7</sup> Previous studies have shown that microbial populations associated with native ascidian species are stable over time,<sup>8</sup> whereas non-native species have high variability in microbial composition.<sup>9</sup> There is evidence that the tunicate host is selecting for beneficial species.<sup>10</sup> However, the role of host-microbe interactions in the health of ascidians remains understudied.

**Goal:** Separate the physiological responses to osmotic stress between those specific to the host from those specific to the associated microbiome

**Methods:** Adult *C. intestinalis* and *S. montereyensis* will be collected from the same area within Bodega Harbor (BH), then transported to the Bodega Bay Marine Laboratory (BML) at the University of California Davis where the experiments will take place. To examine adaptability of alien species, *M. manhattensis* and water samples will be collected from the East River (ER) by Dr. Lionel Christiaen from New York University.

Samples of each species and vacuum-filtered surrounding water will be preserved immediately following collection in Trizol (nucleic acid preservative) to control for the effects of the *ex situ* experiment. Ascidians will be allowed to acclimate in tanks with flow-through seawater from where they were originally collected to assess viability. Tunicates will be randomly assigned to one salinity treatment (0, 25%, 50%, or 100%) using BH seawater, sterilized axenic Instant Ocean saltwater, or ER seawater (Figure 1, colored-brackets indicate surrounding microbial community in mucus layer). Acute salinity exposures will take place for 24 hrs, simulating the time period of a long storm event. Tunicates will immediately be preserved in Trizol following



exposure. For the three tunicate species plus control there will be four different salinities, three sources of water (48 different combinations), five replicates each, for a total of 240 samples.

**Hypothesis 1:** *Osmotic stress in the tunicate host affects microbial composition differently in native vs. non-native ascidians.*

**Aim 1: Determine salinity tolerance threshold of native, non-native, and alien ascidians with gene expression profiling using transcriptome sequencing**

**Aim 2: Characterize the microbiome of native, non-native, and alien ascidians in response to low salinity stress using shotgun metagenomics sequencing** To identify microbial species and their transcripts present in tunicate hosts, genomic DNA and total RNA will be extracted from the outer mucus layer, tunicate tissue and vacuum-filtered seawater from each sample then sequenced on an Illumina HiSeq at the UC Davis Genome Center core sequencing facility.

Following genome and transcriptome annotation, gene expression profiles for salinity treatments vs. controls will be compared across host and microbial taxa. UC Davis and XSEDE high performance computing (HPC) resources will be used for the bioinformatics analyses using methods developed by Dr. C. Titus Brown's Lab for Data Intensive Biology at UC Davis.<sup>11</sup>

**Hypothesis 2:** *Host defense immunity in native tunicates will be more discriminating and have more of an effect on microbial composition than in non-native tunicates.*

**Aim 3: Measure holobiont response to salinity stress following knock-out of immune response genes** There are nine transcripts known to be associated with adult tunicate innate immune-related functions.<sup>12</sup> These transcripts will be knocked down with morpholinos,<sup>13</sup> suppressing expression in larvae from all three species. When adults grow to maturity, they will be exposed for 24 hrs to either full-strength (control) or the threshold low salinity determined in Aim 1. Metagenomics and metatranscriptomics will be analyzed compared to controls and results from Aims 1-2 to determine the role of the host immune function in microbial composition and role of microbial composition on the health of the host tunicate.

**Broader Impacts:** This study fills knowledge gaps identified by the U.S. Fish and Wildlife Service, Western Regional Panel (WRP) on Aquatic Nuisance Species in 2014 for understanding functional genomics of invasive tunicates for the purpose of developing species-specific biomarkers for monitoring.<sup>6</sup> Results from this research could be presented to the WRP annual meeting, in view of assessing threats to novel areas such as Alaska where invasions are not yet as severe but are of high concern. I will present results from this research to the Davis Senior High School Environmental Club to encourage STEM learning, stakeholders of the Gulf of Farallones National Marine Sanctuary, the Tomales Bay and Point Reyes National Seashore where oyster fisheries are economically important to the community, and the San Francisco Naval Shipyard to increase awareness of invasive species and hull-fouling organisms.

Data analysis pipelines developed will be presented in Software Carpentry and bioinformatics inquiry-based training workshops that are regularly held by Dr. C. Titus Brown at UC Davis and across the US. I plan to blog about this research and teach programming skills to future generations of women and underrepresented minorities through community organizations such as Software Carpentry and Girls Who Code.

**References** <sup>1</sup>Tianero et al. 2015 doi:10.1038/ismej.2014.152; <sup>2</sup>Hentschel et al. 2012 doi:10.1038/nrmicro2839; <sup>3</sup>Ainsworth et al. 2015. doi:10.1038/ismej.2015.39; <sup>4</sup>Fan et al. 2012 doi: 10.1073/pnas.1203287109; <sup>5</sup>Kojima and Hirose 2012 Biol Bull. Feb;222(1):63-73; <sup>6</sup>US FWS. 2014. WRP Invasive Tunicate Workshop Report; <sup>7</sup>Fofonoff et al. 2003. Cal-NEMO System:TSN=159557; <sup>8</sup>López-Legentil et al. 2015. doi:10.3389/fmicb.2015.01022; <sup>9</sup>Erwin et al. 2013 doi: 10.1007/s13199-012-0204-0; <sup>10</sup>Donia et al. 2011 PNAS. 108(51): E1423–E1432; <sup>11</sup>Howe et al. 2014 doi:10.1073/pnas.1402564111; <sup>12</sup>Davidson and Swalla. 2002 *Development*. 129:4739-4751; <sup>13</sup>Sataue et al. 2001. doi: 10.1002/gene.1040