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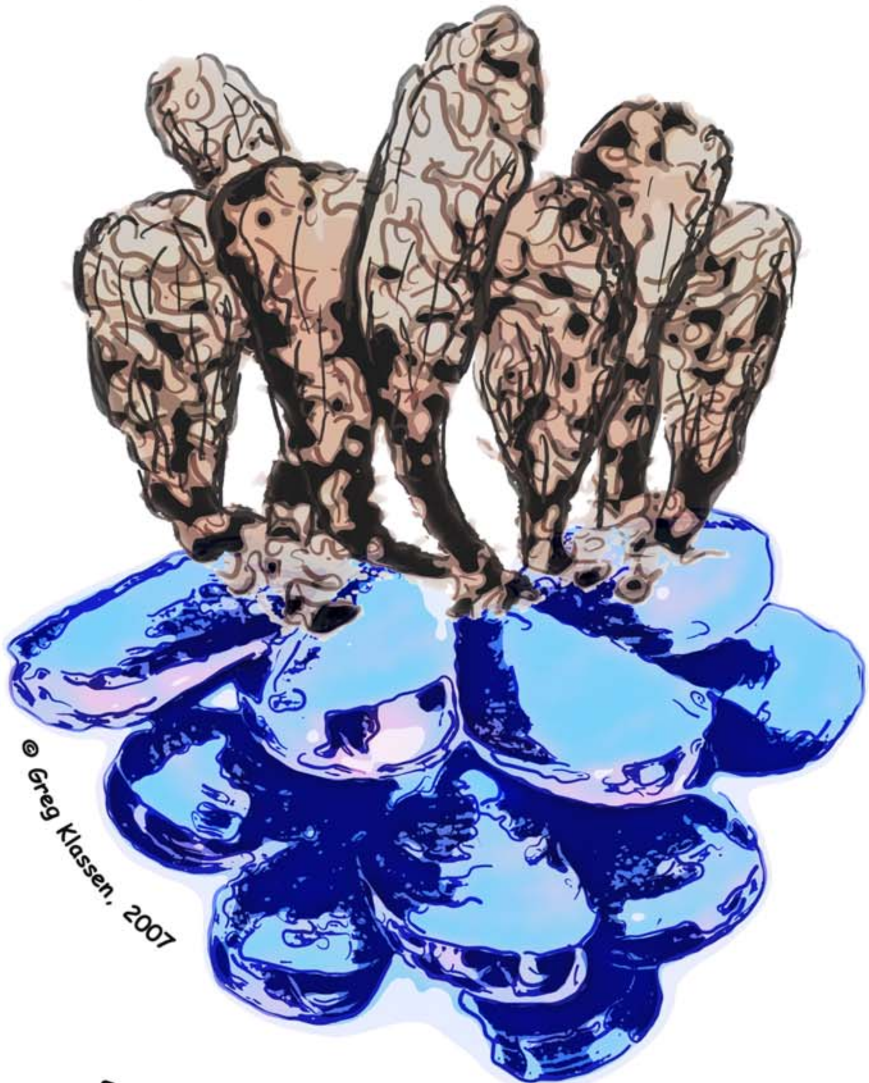

International Invasive Sea Squirt Conference

Brudenell, PEI,

Oct. 2-4, 2007

International Invasive Sea Squirt Conference-II-Canada

October 2-4, 2007

Rodd-Brudenell River Resort, Prince Edward Island

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Aquatic Invasions

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International Invasive Sea Squirt Conference-II-Canada
October 2-4, 2007

Program Schedule

Monday October 1

Registration 7-8 pm; posters up
Steering Committee meeting 7:30 pm

Tuesday October 2

7:00-8:00 Registration; posters up

8:00-8:30 **Welcome**: Allan Campbell, Minister of Fisheries and Aquaculture for PEI and Jim Jones, Regional Director General, Department of Fisheries and Oceans Canada-Gulf Region

8:30-9:00 Plenary speaker
History of the tunicate invasions in PEI, their impact on the cultured mussel industry and mitigation strategies employed since 1998
MacNair N. [1], Mills C. [1], Gillis B.[1], Smith M.[1], Landry T. [2], Locke A. [2], Smith A.[2], Davidson, J.[3], and Warris P. [4]
[1]Prince Edward Island Department of Fisheries and Aquaculture, Aquaculture Section, PO Box 2000, Charlottetown, PE, C1A 7N8; [2] Fisheries and Oceans Canada, P.O. Box 5030, Moncton, NB, E1C 9B6; [3] AVC 550 University Ave, Charlottetown, PE C1A 4P3; [4]PEI Aquaculture Alliance, 101 Longworth Ave, 2nd Floor, Charlottetown, PE C1A 5A9

9:30-12:00 **Boat Tour** with lunch, leader Neil MacNair

1:00-5:00 **Taxonomic Workshop**, leaders Gretchen Lambert and Charles Lambert

5:00-6:00 Break

6:00-8:00 **Poster Session** (abstracts listed alphabetically at end of program) and light refreshments

Wednesday October 3

7:30-8:25 Continental breakfast

8:25-8:30 **Welcome and announcements: Mary Carman**

"Biology, Systematics and Biogeography"; Session Head: Andrew Bagnall

8:30-9:00 Plenary speaker
The invasion process: Why it's so hard to predict the next sea squirt invasion
Carlton, James T.
Maritime Studies Program, Williams College – Mystic Seaport, Mystic CT 06355

9:00-9:20 **Adventures of a sea squirt sleuth: the remarkable story of *Didemnum* sp., a global ascidian invader**

Lambert, Gretchen[1] and Stefaniak, Lauren[2]
[1]*University of Washington Friday Harbor Laboratories, Friday Harbor, WA 98250. Mailing address: 12001 11th Ave. NW, Seattle, WA 98177;* [2]*Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Rd, Groton, CT 06340*

9:20-9:40 **Genetic conspecificity of worldwide populations of the colonial tunicate, *Didemnum* sp.**

Stefaniak, Lauren[1], Lambert, Gretchen [2], Gittenberger, Adriaan [3], Zhang, Huan [1], Lin, Senjie [1], and Whitlatch, Robert B.[1]
[1]*Department of Marine Sciences, University of Connecticut, Groton, CT;*
[2]*University of Washington Friday Harbor Laboratories, Friday Harbor, WA;*
[3]*National Museum of Natural History Naturalis, Leiden, The Netherlands*

9:40-10:00 **Seasonal water temperature cycles and the recruitment of larvae of the colonial ascidian *Didemnum* sp. in New England coastal and offshore waters**

Valentine, Page C.[1], Carman, Mary R.[2], Dijkstra, Jennifer[3], Blackwood, Dann S.[1], Westerman, Erica.[3], and Harris, Larry. G.[3]
[1]*U.S. Geological Survey, Woods Hole, MA 02543;* [2]*Woods Hole Oceanographic Institution, Woods Hole, MA 02543;* [3]*University of New Hampshire, Durham, NH 03826*

10:00-10:30 Coffee Break

10:30-10:50 **Global dispersion and phylogeography of *Styela plicata* (Leseuer, 1823) (Tunicata, Ascidiacea)**

Barros, R. C. and Rocha, R. M.
Universidade Federal do Paraná, Departamento de Zoologia. CP 19020, 81.531-980, Curitiba, Paraná, Brazil

10:50-11:10 ***Styela clava* – a new threat to the Mediterranean shellfish industry?**

Davis, Martin H. and Davis, Mary E.
Fawley Biofouling Services, 45, Megson Drive, Lee-on-the-Solent, Hampshire. PO13 8BA, UK

11:10-11:30 **Sea squirts in Brudenell estuary: Documenting the invasion**

Ramsay, Aaron[1], Davidson, Jeff[1], Landry, Thomas[2], and Arsenault, Garth[1]
[1]*Department of Health Management, Atlantic Veterinary College, Charlottetown, PE C1A 4P3;* [2]*Department of Fisheries and Oceans Canada, Moncton, NB E1C 9B6*

11:30-11:50 **Growth of the colonial ascidian *Didemnum* sp. under different environmental conditions (depth, salinity, coastal land use patterns)**
Bullard, Stephan G.[1] and Whitlatch, Robert B.[2]
[1]University of Hartford, Hillyer College, 200 Bloomfield Ave, West Hartford CT 06117 USA; [2]University of Connecticut, Department of Marine Sciences, 1080 Shennecossett Rd Groton CT 06340 USA

11:50-12:10 **Developing a study of the didemnid – mytilid epibiotic relationship**
Auker, Linda A.
Department of Zoology, University of New Hampshire, Durham, NH 03824

12:10-1:10 Lunch

"Ecology and Genetics"; Session Head: Peter Warris

1:10-1:40 Plenary speaker
Invasive ascidians: conservation challenges and ecological opportunities
Stachowicz, John J.
Department of Evolution and Ecology and Bodega Marine Lab, University of California, Davis 95616 USA

1:40-2:00 **Settlement and post-settlement interactions determine the distribution of invasive and native ascidians**
Rius, Marc[1], Marshall, Dustin J.[2], and Turon, Xavier[1]
[1]Departament de Biologia Animal, Universitat de Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain; [2]Centre for Marine Studies, The University of Queensland, 4072, Queensland, Australia

2:00-2:20 **A tale of two seas: ecological aspects of the ascidian community along the coast of Israel**
Shenkar, Noa and Loya, Yossi
Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel Aviv 69978, Israel

2:20-2:40 **Untangling temperature tolerance discrepancies between growth, abundance, and recruitment in *Botryllus schlosseri* and *Botrylloides violaceus***
Westerman, Erica L.[1], Whitlatch, R.B.[2], Dijkstra, J.[3], Harris, L.G.[3], and Bolker, J.A.[3]
[1] Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, [2]Department of Marine Sciences, University of Connecticut, Groton, CT 06340, [3]Zoology Department, University of New Hampshire, Durham, NH 03824

2:40-3:00 **Predicting the possible geographic distribution of the colonial ascidian *Didemnum* sp. on the Georges Bank fishing grounds (Gulf of Maine) based on water temperatures required for the development and release of larvae and on substrate availability**

Valentine, Page C.[1], Collie, Jeremy S.[2], and Reid, Robert N.[3]
[1]U.S. Geological Survey, Woods Hole, MA 02543; [2]University of Rhode Island, Narragansett, RI 02882; [3]National Marine Fisheries Service, Sandy Hook, NJ 07732

3:00-3:30 Coffee Break

3:30-3:50 **Potential impacts of predation on survival and growth of non-indigenous tunicate species in British Columbia, Canada**

Epelbaum, A.[1]*, Pearce, C.M.[1], and Therriault, T.W.[1]
*[1] Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9T 6N7; *NSERC Visiting Fellow*

3:50-4:10 **Invasive species alter the maintenance of biological diversity**

Dijkstra, Jennifer and Harris, Larry
Department of Zoology, University of New Hampshire, Spaulding Hall, 46 College Road Durham, New Hampshire 03824

4:10-4:30 **The invasive colonial ascidian *Didemnum* sp. on Georges Bank – Ecological effects and genetic identification**

Lengyel, Nicole L.[1], Collie, Jeremy S.[1], andValentine, Page C.[2]
[1]Graduate School of Oceanography, University of Rhode Island, South Ferry Road, Narragansett, RI 02882, USA; [2]U.S. Geological Survey, 384 Woods Hole Road, Woods Hole, MA, 02543, USA

4:30-6:00 Break

6:00-8:00 **Poster Session and Banquet Supper**

Thursday October 4

7:30-8:30 Continental breakfast

"Impacts"; Session Head: Jeff Davidson

8:30-9:00 Plenary speaker
Predicting the next one: or, trying to be the scientist who cried tunicate (but at the right place and time)

Locke, Andrea
Fisheries and Oceans Canada, P.O. Box 5030, Moncton, NB, E1C 9B6

9:00-9:20 **Extent of ecological interactions between the vase tunicate (*Ciona intestinalis)* and the farmed blue mussel (*Mytilus edulis)* in Nova Scotia, Canada**

Daigle, Rémi M. and Herbinger, C.M.
Department of Biology, Dalhousie University, Halifax, NS

9:20-9:40 ***Didemnum* sp. as an agent of change: Can this be detected regionally?**
Pederson, Judith
MIT Sea Grant College Program, 292 Main Street, E38-300, Cambridge, Massachusetts 02139

9:40-10:00 **Impacts of the invasive tunicate *Didemnum* sp. on scallop recruitment**
Morris, James[1], Carman, Mary R.[2], Hoagland, K. Elaine[3], Green-Beach, Emma[4], and Karney, Richard[4]
[1] *National Centers for Coastal Ocean Science, National Ocean Service, 101 Pivers Island Road, Beaufort, NC 28516;* [2] *Geology and Geophysics Dept., Woods Hole Oceanographic Institution, Woods Hole, MA 02543*; [3] *IM Systems Group, 3401 Bexhill Pl, Kensington, MD 20895 and National Centers for Coastal Ocean Science, National Ocean Service, NOAA, 1305 East-West Hwy, Silver Spring, MD 20910;* [4] *Martha's Vineyard Shellfish Group, Inc., Oak Bluffs, MA 02557*

10:00-10:30 Coffee Break

10:30-10:50 **Worldwide genetic structure of *Microcosmus squamiger*: contrasting genetic diversity patterns between introduced and native populations**
Rius, Marc[1], Pascual, Marta[2], and Turon, Xavier[1]
[1]*Departament de Biologia Animal, Universitat de Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain;* [2]*Departament de Genètica, Universitat de Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain*

10:50-11:10 **International DNA bank for ascidians**
Stewart-Clark, Sarah[1] and Davidson, Jeff[2]
[1] *Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave, Charlottetown, PEI, Canada, C1A 4P3;*
[2] *Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave, Charlottetown, PEI, Canada, C1A 4P3*

11:10-11:30 **Inter-specific ascidian overgrowth in New England coastal habitats**
Dijkstra, Jennifer[1] and Carman, Mary R.[2]
[1] *Department of Zoology, University of New Hampshire, Spaulding Hall, 46 College Road, Durham, New Hampshire 03824*; [2] *Geology and Geophysics Dept., Woods Hole Oceanographic Institution, Woods Hole, MA 02543*

11:30-1:00 Lunch

"Risk Assessment and Management"; Session Head: Art Smith

1:00-1:20 **Incorporating the New Zealand Tunicate Treatment Technology into a tunicate management strategy for Indian Point Marine Farms (Nova Scotia, Canada)**
Carver, Claire E. and Mallet, A.L.
Mallet Research Services, Dartmouth, Nova Scotia B2X 3H3

1:20-1:40 **Management of sea squirts in New Zealand**
Bissmire, Sonya E. and Stratford, Peter J.
MAF Biosecurity New Zealand, Wellington, New Zealand

1:40-2:00 **Hull fouling and overland transport of boats on trailers as vectors of spread of clubbed tunicate (*Styela clava*)**
Darbyson, E.[1]*, Hanson, J. Mark[2], Locke, A[2], and Willison, J. H. M.[1]
*[1]Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1 and [2] Gulf Fisheries Centre, Fisheries and Oceans Canada, P.O. Box 5030, Moncton, New Brunswick, Canada, E1C 9B6; * Present address: Department of Biology, McGill University, Montreal, Quebec, Canada, H3A 1B1*

2:00-2:20 **The temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island**
Arsenault, Garth and Davidson, Jeff
Department of Health Management, Atlantic Veterinary College, Charlottetown, PE, C1A 4P3

2:20-2:40 **Rapid nationwide surveys for *Styela clava* in New Zealand: delimitation methods and detection probabilities**
Gust, Nick and Graeme, Inglis
National Institute of Water and Atmospheric Research, Christchurch, New Zealand

2:40-3:10 Coffee Break

3:10-3:30 **Market potential for *Styela clava*, a non-indigenous pest invading New England coastal waters**
Karney, Richard C.[1] and Rhee, Walter Y.[2]
[1]Martha's Vineyard Shellfish Group. Inc. Oak Bluffs, MA 02557; [2] Honolulu, HI

3:30-3:50 **Mussel processing plants a potential vector for invasive ascidians?**
Bourque, Daniel[1], Barkhouse, Carla[1], Landry, Thomas[1], Mills, Chris[2], LeBlanc, Angeline R.[1], Davidson, Jefferey[3], and Miron, Gilles[4]
[1]Fisheries and Oceans Canada, Moncton, N.B. E1C 9B6; [2]Department of Agriculture, Fisheries and Aquaculture, Charlottetown, P.E.I. C1A 7N3; [3]Atlantic Veterinary College, Charlottetown, P.E.I. C1A 4P3; [4]Département de biologie, Université de Moncton, Moncton, N.B. E1A 3E9

3:50-4:10 **Mitigation strategies for *Styela clava* fouling on mussel seed collectors**
Gill, Kim L.[1], Davidson, J.[1], Landry, T.[2], Stryhn, H.[1], and MacNair, N.[3]
[1]Atlantic Veterinary College, Department of Health Management, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island, Canada, C1A 4P3; [2]Department of Fisheries and Oceans, Oceans and Science Branch, Aquatic Health Division, Gulf Fisheries Centre, P.O. Box 5030, Moncton, New Brunswick, Canada, E1C 9B6; [3]Department of Fisheries, and Aquaculture, P.O. Box 2000, Charlottetown, Prince Edward Island, Canada, C1A 7N8

4:10-5:00 **Open Forum; Moderator: Art Smith**

Questions to be discussed:

1. What invasive tunicate species are present in your area and what impact have they had on aquaculture operations?

2. What management practices have been put in place in an attempt to control the further spread of tunicates and how successful have the practices been?

3. What (if any) mitigation techniques have been developed to control the fouling and how successful they have been?

Close of conference

Poster Session: Tuesday and Wednesday evenings

(in alphabetical order by first author's last name)

Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada

Bernier, Renée Y., Locke, A., and Hanson, J. Mark
Fisheries and Oceans Canada, Gulf Fisheries Centre, 343 Université avenue, P.O. Box 5030, Moncton, New-Brunswick, E1C 9B6, Canada

Discovery of inhibitors of tunicate larval settlement

Bunyajetpong, S.[1] and Kerr, R.[2]
[1]Biomedical Sciences, University of Prince Edward Island, Charlottetown, PE C1A4P3; [2]Department of Chemistry, University of Prince Edward Island, Charlottetown, PE C1A4P3

Monitoring the distribution of indigenous and non-indigenous ascidians and macroinvertebrates in harbours around Newfoundland

Callahan, A.G.[1], Deibel, D.[1], McKenzie, C.H.[2], and Sargent P.[1]
[1]Ocean Sciences Centre, Memorial University, St. John's, Newfoundland Labrador, Canada A1C 5S7; [2]Northwest Atlantic Fisheries Centre, Department of Fisheries and Oceans Canada, St. John's, Newfoundland Labrador, Canada A1C 5X1

Can the non-indigenous common periwinkle snail *Littorina littorea* be used as a biologic control for the invasive tunicate *Didemnum* sp.?

Carman, Mary R.[1], Allen, Hannah M.[2], Tyrrell, Megan C.[3], and Byers, James E.[4]
[1]*Geology and Geophysics Dept., Woods Hole Oceanographic Institution, Woods Hole, MA 02543*; [2] *Falmouth Academy, Falmouth, MA 02541*; [3] *Mashpee, MA 02649*; [4] *Department of Zoology, University of New Hampshire, Durham, NH 03824*

Tunicate faunas at two North Atlantic-New England islands: Martha's Vineyard, Massachusetts, and Block Island, Rhode Island

Carman, Mary R.[1], Hoagland, K. Elaine[2], Green-Beach, Emma[3], and Grunden, David W.[4]
[1] *Geology and Geophysics Dept., Woods Hole Oceanographic Institution*; [2] *IM Systems Group, 3401 Bexhill Pl, Kensington, MD 20895 and National Centers for Coastal Ocean Science, National Ocean Service, NOAA, 1305 East-West Hwy, Silver Spring, MD 20910*; [3] *Martha's Vineyard Shellfish Group, Inc., Oak Bluffs, MA 02557*; [4] *Town of Oak Bluffs Shellfish Department, Oak Bluffs, MA 02557*

Interviews from Prince Edward Island, Canada, on boater habits and the potential spread of the clubbed tunicate, *Styela clava*

Darbyson, E.[1], Locke, A.[2], Hanson, J.M.[2] and J.H.M. Willison[3]
[1]Dept. of Biology, Dalhousie University, Halifax, NS, Canada; [2] Fisheries and Oceans Canada, P.O. Box 5030, Moncton, NB, Canada, E4Z 3A7; [3] Environmental Studies Program, Dalhousie University, Halifax, NS, Canada

***Styela clava* Herdman, 1881 or 1882?**

Davis, Martin H. and Davis, Mary E.
Fawley Biofouling Services, 45, Megson Drive, Lee-on-the-Solent, Hampshire. PO13 8BA, UK

Invasive tunicates in Prince Edward Island estuarine systems: the use of colonization plates as a community-based monitoring tool

Desreux, Joe[1], Quijon, Pedro[1], and Davidson, Jeff[2]
[1]Department of Biology and [2]Department of Health Management-AVC, University of Prince Edward Island, Charlottetown, PE, C1A 4P3, Canada

Assessing eradication: development and application of a generalized, practical eradication framework

Paul Edwards[1,2] and Brian Leung[1,2]
[1] Department of Biology, McGill University, Montreal, QC H3A 1B1; [2] McGill School of Environment, Montreal, QC H3A 2A7

Effects of temperature and salinity on survival and growth of non-indigenous botryllid tunicates

Epelbaum, A.[1]*, Pearce, C.M.[1], and Therriault, T.W.[1]
[1] Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, BC V9T 6N7;
** NSERC Visiting Fellow*

Washington state tunicate species management plan

Eveningsong, O. and Pleus, A.
Washington Department of Fish and Wildlife, Aquatic Nuisance Species Unit, Olympia, WA

Localised patterns of connectivity for the invasive sea-squirt, *Styela clava*

Goldstien, Sharyn J., Schiel, David R., and Gemmell, Neil J.
School of Biological Sciences, University of Canterbury, New Zealand

Biotic and abiotic regulation of *Botrylloides violaceus* populations along the Strait of Juan de Fuca, Washington, U.S.A.

Grey, Erin K.
University of Chicago, Chicago, IL, U.S.A.

***Didemnum* sp. in North America: Can anything eat it? Will it do them any good?**

Guida, Vincent G., Candelmo, A., Wieczorek, D., Cleary, T.H., Williams, S., and Leimburg, E.
NOAA, NMFS, Northeast Fisheries Science Center, J.J. Howard Laboratory, Highlands, NJ

One ring to divide them all: mitochondrial genomics unveils two cryptic species in *Ciona intestinalis*

Iannelli, Fabio[1], Pesole, Graziano[2], Sordino, Paolo[3], and Gissi, Carmela[1]
[1]Dipartimento di Scienze Biomolecolari e Biotecnologie, Università di Milano, Italy; [2]Dipartimento di Biochimica e Biologia Molecolare "E. Quagliariello", Università di Bari, Italy; [3]Laboratory of Biochemistry and Molecular Biology, Stazione Zoologica "A. Dohrn", Naples, Italy

Non-indigenous ascidians in the Mediterranean Sea

Izquierdo, Andrés[1], Díaz-Valdés, M.[2] and Ramos-Esplá, A.A.[1]
[1]Centro de Investigación Marina de Santa Pola, Ayuntamiento de Santa Pola-Universidad de Alicante, 03130 Santa Pola (Alicante, Spain); [2]Departamento de Ciencias del Mar y Biología Aplicada, Universidad de Alicante, 03080 Alicante (Spain)

Hierarchical genetic structure of invasive tunicates

Lejeusne, Christophe, Cristescu, Melania, and MacIsaac, Hugh
Great Lakes Institute for Environmental Research (GLIER), 401 Sunset Avenue, Windsor, ON, Canada N9B 3P4

Secondary dispersal in the invasive ascidian *Styela clava* at the North Sea shore

Liebich, Viola and Reise, K.
Alfred Wegener Institute for Polar and Marine Research, Wadden Sea Station Sylt, Hafenstrasse 43, 25992 List, Germany

Environmental consequences of tunicate management practices in estuaries of Prince Edward Island

Locke, Andrea[1] and MacNair, N.G.[2]
[1]Fisheries and Oceans Canada, P.O. Box 5030, Moncton, NB, E1C 9B6; [2]PEI Department of Fisheries and Aquaculture, Charlottetown, PEI

Developing a rapid response plan for *Didemnum*: a model plan for Prince Edward Island

Locke, Andrea[1] and Smith, A.H.[2]
[1]Fisheries and Oceans Canada, P.O. Box 5030, Moncton, NB, E1C 9B6; [2]Fisheries and Oceans Canada, Charlottetown, PEI

Getting along with the tunicates? Co-occurring mobile invertebrates on blue mussel lines colonized by invasive tunicates in eastern Prince Edward Island

Lutz, Vanessa[1], Quijon, Pedro[1], and Davidson, Jeff[2]
[1]Department of Biology and [2]Department of Health Management-AVC, University of Prince Edward Island, 550 University Avenue, Charlottetown, PE, C1A 4P3, Canada

Potential impacts of an ascidian invader (*Didemnum* sp.) on seafloor communities in southern New England, USA

Mercer, J. and Whitlatch, R.B.
Department of Marine Sciences, University of Connecticut, Groton, CT 06340

The new European on-line journal "Aquatic Invasions": services for marine biodiversity related information systems

Panov, Vadim[1] and Gollasch, Stephan[2]
[1] St. Petersburg State University, Russia; [2] GoConsult, Hamburg, Germany

Tunicate nanocrystals as a novel nanomaterial for the preparation of ultra-strong composites, optical coatings, and cell culture platforms

Podsiadlo, P.[1]; Sui, L.[2]; Shim, B.[1]; Elkasabi, E.[1]; Burgardt, P.[1]; Lee, J.[6]; Miryala, A.[1]; Kusumaatmaja, W.[1]; Carman, M. R.[5]; Shtein, M.[2]; Kieffer, J.[2]; Lahann, J.[1,2,3]; and Kotov, N. A.[1,2,4]
[1]Department of Chemical Engineering, [2]Department of Materials Science and Engineering, [3]Program in Macromolecular Science and Engineering, [4]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, 48109-2136; [5]Department of Geology and Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543; [6]Department of Nanomedical Engineering, Pusan National University, Busan, South Korea

Introduced ascidians along the coast of South Africa: water temperature as a predictor of their geographical distribution

Rius, Marc[1], Griffiths, Charles[2], and Turon, Xavier[1]
[1]Departament de Biologia Animal, Universitat de Barcelona, Av. Diagonal 645, 08028, Barcelona, Spain; [2]Department of Zoology, University of Cape Town, Rondebosh, Cape Town 7700, South Africa

Invasive tunicates in oyster cultivation: potential to colonize the natural substrate

Rocha, R.M. and Baptista, M. S.
Universidade Federal do Paraná, Departamento de Zoologia. CP 19020, 81.531-980, Curitiba, Paraná, Brazil

Seasonal water temperature cycles and the recruitment of larvae of the colonial ascidian *Didemnum* sp. in New England coastal and offshore waters

Valentine, Page C.[1], Carman, Mary R.[2], Dijkstra, Jennifer[3], Blackwood, Dann S.[1], Westerman, Erica.[3], and Harris, Larry. G.[3]
[1]U.S. Geological Survey, Woods Hole, MA 02543; [2]Woods Hole Oceanographic Institution, Woods Hole, MA 02543; [3]University of New Hampshire, Durham, NH 03826

Predicting the possible geographic distribution of the colonial ascidian *Didemnum* sp. on the Georges Bank fishing grounds (Gulf of Maine) based on water temperatures required for the development and release of larvae and on substrate availability

Valentine, Page C.[1], Collie, Jeremy S.[2], and Reid, Robert N.[3]
[1]U.S. Geological Survey, Woods Hole, MA 02543; [2]University of Rhode Island, Narragansett, RI 02882; [3]National Marine Fisheries Service, Sandy Hook, NJ 07732

Development of genetic markers in the invasive tunicate *Ciona intestinalis*

Vercaemer, B.[1], Sephton, D.[1], Nicolas, J-M.[1], Howes, S.[1,2], and Herbinger, C.[2]
[1] Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS B2Y 4A2; [2] Department of Biology, Dalhousie University, 1355 Oxford Street, Halifax, NS B3H 4J1

Pilot general and targeted surveillance program for tunicates in Nova Scotia; April 2006 – August 2007

Vercaemer, B.[1] Sephton, D.[1], Nicolas, J-M. [1], Howes, S. [1,2], Locke, A. [3], Landry, T.[3], Bagnall, A. [4], and Mullen, J.[5]
[1] Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, NS B2Y 4A2; [2] Department of Biology, Dalhousie University, Halifax, NS; [3] Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, NB; [4] Aquaculture Division, Nova Scotia Department of Fisheries and Aquaculture, Halifax, NS; [5] Aquaculture Association of Nova Scotia, Halifax, NS

All Abstracts in Alphabetical Order

The temporal and spatial development of an infestation of *Styela clava* on mussel farms in Malpeque Bay, Prince Edward Island

Arsenault, Garth and Davidson, Jeff
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The first confirmed identification of the invasive solitary ascidian *Styela clava* (clubbed tunicate) in the mussel production area of Malpeque Bay occurred in September of 2002. Mussel farms in eastern Prince Edward Island waters had been challenged with infestations of this tunicate since 2000 causing both production and processing problems for the industry. A multi-year study was initiated in June of 2003 to document the spread of *S. clava* within the mussel producing areas of Malpeque Bay. The study design consisted of the establishment of a series of quadrant lines extending outwards from the initial area of discovery and subsequent deployment of PVC collector plates at predetermined distances. Collection plates were retrieved at the end of each growing season and *S. clava* specimens were quantified for growth and abundance. The mean recruitment levels for each set of collection plates increased from 5 animals in 2003 to 3444 animals in 2006. Additionally, the geographical spread within Malpeque Bay has extended by about 12 kilometers from the initial area of discovery.

Developing a study of the didemnid – mytilid epibiotic relationship

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The tunicate pest *Didemnum vexillum* is an epibiont on *Perna canaliculus*, the Greenshell™ mussel, an important New Zealand aquaculture species. The tunicate often covers the mussel so thoroughly that the valves are restricted from opening, likely preventing the mussel from feeding efficiently; however, the effects of this overgrowth on the mussel are not fully understood. As part of a developing project on *Didemnum* - mytilid epibiotic relationships, a pilot study was conducted in New Zealand in July and August, 2007, to investigate the effect of *D. vexillum* on mussel shell growth. *P. canaliculus* individuals, both clean and overgrown with *D. vexillum*, were collected from mussel farm lines in Ngaruru Bay in Queen Charlotte Sound. Approximately thirty mussels were placed into each of ten mussel socks: five socks were filled with overgrown mussels, and five more with clean mussels. These were suspended along a rope for two weeks below the water line in Port Nelson, an area already inoculated with *D. vexillum*. After this period, the mussels were removed from the water, with a select number from each sock measured for shell thickness index, lip thickness, tissue mass, and adductor muscle mass. With these preliminary results, long-term mussel growth studies and predation experiments will be designed for research on *Didemnum* sp. and *Mytilus edulis* in New England habitats.

Global dispersion and phylogeography of *Styela plicata* (Leseuer, 1823) (Tunicata, Ascidiacea)

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Styela plicata is a solitary ascidian found in some shallow water and protected environments of both tropical and warm temperate seas. Its origin is uncertain and, since its description, it was identified in several seas and oceans, with a large geographical distribution. Although historically qualified as a cosmopolitan species, in the last decades it has been considered an introduced or invasive species, in different regions of the world. In the present study, the genetic structure of *S. plicata* was investigated. The populations could not be morphologically differentiated in accordance to the site where they were collected: Brazil (Rio de Janeiro, Paraná and Santa Catarina), USA (Florida and California) and Japan. Eight haplotypes were found among the forty-three sequences obtained for the mitochondrial DNA fragment of the cytochrome *c* oxidase subunit 1 (CO1). The haplotype 8 presented an average of 3.3% of genetic divergence in relation to the others, what might represent speciation. Nevertheless, other evidences to support this hypothesis are necessary. Molecular data shows absence of geographic structure since the haplotypes are distributed in a random way among the geographic sites studied; besides that, populations are not isolated by distance, according to Mantel test. These results, associated with historical records, indicate that ship transport may be the main cause of *S. plicata* global spreading and that multiple introductions occurred in the localities studied. The North Hemisphere region was considered a potential area as a spreading center of the species under consideration. New data, however, coming from different populations and molecular markers, must be added to determine more accurately the phylogeographic relationships among the *S. plicata* populations.

Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada

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The spread of several non-indigenous tunicates along Prince Edward Island's coast has prompted further investigation into the vectors facilitating their dispersal. Anecdotal reports suggest tunicates occasionally occur on crabs and lobsters caught in commercial fisheries in these waters. A pilot project was conducted in November 2005 in tunicate-infested estuaries (St. Peter's Bay and Savage Harbour, PEI) and in the Northumberland Strait in August 2006 to determine the presence/absence of invasive tunicates on and under the carapaces of lobsters (*Homarus americanus*) and rock crabs (*Cancer irroratus*). No alien tunicates were found on rock crabs examined from either infested bay in November 2005. However, a small colony of *Botryllus schlosseri* was found attached to the ventral surface of one female lobster from St. Peter's Bay. Lobsters from the Northumberland Strait in August 2006 showed no signs of attached invasive tunicates. Small sea grapes (*Molgula* spp.) were found attached on the underside of most rock crabs and a few lobsters from both infested bays in November 2005 and on lobsters in the Northumberland Strait in August 2006.

Management of sea squirts in New Zealand

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New Zealand has discovered three confirmed exotic species of sea squirts in recent years. *Styela clava* was detected in September 2005, with historic records dating back to 2002. It has since been found to be well established over large areas of two of New Zealand's busiest ports and harbours: the Hauraki Gulf in the north and Lyttelton Harbour in the south. Very small infestations have been identified in Tutukaka Marina in Northland, as well as in Wellington and Nelson Harbours. MAF Biosecurity New Zealand initiated a major response to *Styela clava* and has learned many lessons about dealing with invasive sea squirts and marine biosecurity in general. Responses to sea squirts that have been detected post-*Styela* have been managed somewhat differently. The focus of MAF Biosecurity New Zealand's marine response programme is now on assisting marine users to manage their own biosecurity risks, on surveillance to enable early detection and on development of marine response tools to enable control of priority pests that are found early in the invasion cycle.

Mussel processing plants a potential vector for invasive ascidians?

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With the invasion of several problematic non-indigenous species of ascidians into Prince Edward Island coastal waters, the need to restrict further spread has become a top priority. Consequently, knowledge of potential vectors and associated risks are critical components of any management efforts. In Prince Edward Island, these invaders have predominantly affected the mussel aquaculture industry. These mussels are often transported to processing facilities situated in separate bodies of water for processing. The goal of the present study is to evaluate the risk of spread of invasive ascidians through processing facilities. Invasive ascidians could potentially be introduced via effluent waters in various forms such as whole live animals, colony fragments and early life stages. Risks associated to the processing stages, husbandry practices and environmental conditions within processing plants are identified and discussed. Potential mitigation strategies for high-risk scenarios are identified and evaluated. Strategies utilized include mechanical filtration, water parameter alteration (e.g. temperature, salinity, etc.) and sedimentation of effluent.

Growth of the colonial ascidian *Didemnum* sp. under different environmental conditions (depth, salinity, coastal land use patterns)

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The colonial ascidian *Didemnum* sp. is rapidly spreading on both coasts of North America. Although the species has the potential to alter community structure and impact human activities, relatively little is known about its biology. To help determine which coastal habitats may be most vulnerable to *Didemnum* sp. colonization and population expansion, we assessed the relative growth of the species under different environmental conditions (depth, salinity and coastal land use patterns). Pieces of *Didemnum* sp. colonies were deployed in plastic (Vexar) pouches at different coastal habitats located in southern New England, USA. After two weeks pouches were collected and the colonies were re-weighed to determine their change in biomass. The species grew significantly faster in shallower water (1.0 m compared to 4.0 m), in areas of higher salinity (26-30 ppt compared to 10-26 ppt) and in areas with relatively little coastal development (e.g., pristine areas compared to areas with significant coastal development). Survivorship of *Didemnum* sp. was significantly lower in lower salinity areas (< ~20ppt) suggesting that it may not be able to persist in these locations. Collectively, these results suggest that many coastal habitats may be at risk to invasion by *Didemnum* sp., particularly those associated with undeveloped coastlines (e.g., state and national parks).

Discovery of inhibitors of tunicate larval settlement

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Invasive fouling species, especially tunicates, are having a negative economic impact on the Prince Edward Island aquaculture industry. These tunicates foul on mussel socks and equipment and they compete for food with mussels. This makes a lot of work for farmers to harvest and causes economic impact to such industries. Using antifouling agents may be a sustainable solution for this problem. But organo-tin compounds such as butyltin compounds have been the most often used components in these agents. With their long half life, they cause a negative impact in marine ecosystems and have highly toxic effects in various aquatic species. In order to protect the environment and avoid accumulated toxins in mussel, finding new antifouling alternatives is of considerable value. This project aims to search for antifouling products from marine organisms in an environmentally sound manner. Recent data and strategies will be presented.

Monitoring the distribution of indigenous and non-indigenous ascidians and macroinvertebrates in harbours around Newfoundland

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Invasive, non-indigenous ascidians have been a significant biofouling problem for the aquaculture industry in Nova Scotia and Prince Edward Island since the mid-1990s. Given the high level of vessel traffic between Newfoundland and the Maritime Provinces, it may be a matter of when rather than if one or more of these species invade Newfoundland harbours. My thesis work is part of the first assessment of non-indigenous invertebrates in Newfoundland harbours. Field work has been conducted in the fall of 2006 and summer of 2007 in four ports (Port-aux-Basques, Corner Brook, Botwood and Argentia) to assess the abundance and biodiversity of macroinvertebrates on wharf pilings, including indigenous and non-indigenous ascidians. All four ports are visited regularly by a variety of ships sailing from locations in the southern Gulf of St. Lawrence. We are also exploring the use of sequence analysis of the cytochrome oxidase I gene (COI) of mtDNA to confirm taxonomic identity and to develop species-specific primers for early detection of larvae and juveniles of indigenous and non-indigenous ascidians. Quadrat samples, visual surveys and photographic records were taken at each harbour and when encountered ascidians were fixed for genetic analysis.

At this time, I am processing my field samples and am in the early stages of my genetic work. Early results have shown the presence of indigenous *Halocynthia pyriformis, Ascidia prunum, Molgula citrina, Boltenia echinata, Didemnum albidum*, *Molgula* spp. and the non-indigenous *Botryllus schlosseri* in high densities on the hull of vessels in three ports in Placentia Bay (Argentia, Long Harbour and Arnold’s Cove). I plan to present quantitative quadrat data from my samples, which are dominated by mussels. I am in the final stages of sequencing a portion of COI from the indigenous *Halocynthia pyriformis*, and will present data from this and the other species. As a result of the discovery of *B. schlosseri*, we are conducting a more intensive survey of several small harbours in Placentia Bay during 2007 to determine the spatial distribution of this potentially invasive species.

The invasion process: why it's so hard to predict the next sea squirt invasion

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The invasion process consists of a complex set of seven stages (entrainment, transport, discharge, survival, reproduction, establishment, and spread) with numerous embedded variables that are often unpredictable in time and space. In light of these variables, the likelihood of species becoming introduced would appear to be low (and often is for a given transport event), but this prediction is now balanced by the fact that marine organisms are transported at a larger number and faster rate than ever before, vastly increasing propagule pressure and the probability of survival. The breadth and depth of these variables (which now include global climate change), combined with the modern rate of global bioflow, insures that sea squirt invasions will continue. Detection programs should be in place for the spectacular introductions that will occur in the coming years.

Can the non-indigenous common periwinkle snail *Littorina littorea* be used as a biologic control for the invasive tunicate *Didemnum* sp.?

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Didemnum sp. is a non-indigenous tunicate that aggressively colonizes many types of substrates in New England's coastal and oceanic habitats. Most alarmingly, *Didemnum* sp. overgrows bivalves and is capable of completely encapsulating them, leading to diminished growth or death. Fouling by *Didemnum* sp. on aquaculture gear and product requires remediation. While manual methods of eradication are many, no biological control methods are currently implemented. Long recognized as an omnivore, the common periwinkle snail *Littorina littorea* is known to consume both plants and animals. Field observations indicate that *L. littorea* is consuming, scouring, or otherwise removing stressed *Didemnum* sp. from rocks in intertidal pools at Sandwich, Massachusetts, during all seasons. We examined the stable isotopic signatures and fecal pellets of *L. littorea* collected from *Didemnum* sp. in the field as well as *L. littorea* that had been maintained on stressed *Didemnum* sp. in the lab to determine if they were ingesting the tunicate tissue. We also examined changes in the abundance of *Didemnum* sp. maintained with and without *L. littorea*, and other potential predators in a laboratory setting. Preliminary experimental results indicate that neither *L. littorea* nor the other predators have strong potential as biological control for *Didemnum* sp. At this time, we recommend that other control methods that have a proven record of success be utilized by aquaculturists and others that are interested in controlling *Didemnum* sp.

Tunicate faunas at two North Atlantic-New England islands: Martha's Vineyard, Massachusetts and Block Island, Rhode Island

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A bay naturally abundant with scallops and a bay undergoing scallop restoration had similar tunicate faunas. We sampled natural and artificial substrata in the inner pond portion of the Great Salt Pond system of Block Island, Rhode Island, during late June 2007. We made similar observations in Lagoon Pond and adjacent portions of Vineyard Haven Harbor, Martha's Vineyard, Massachusetts, during July. These two marine pond regions are similar in that they are both enclosed regions of larger bays with natural scallop beds. However, Lagoon Pond has healthy scallop beds amidst native eelgrass *Zostera marina*, whereas Great Salt Pond has little eelgrass and is undergoing scallop restoration. From shore and with the use of small boats we examined natural surfaces: sand/pebble/cobble sediments, algae, and shellfish; and artificial surfaces: shellfish aquaculture equipment, buoys, floating platforms, fixed docks, and bridge pilings. Lagoon Pond and Great Salt Pond contained similar tunicate faunas, dominated by invasive species. We found the non-indigenous *Ascidiella aspersa, Botrylloides violaceus, Botryllus schlosseri, Didemnum* sp.*, Styela clava,* native *Aplidium constellatum* and *Molgula manhattensis,* and cryptogenic *Ciona instestinalis.* In both regions, most of the tunicate fouling was on hard artificial surfaces. Tunicate fouling did occur but at less biomass on natural benthic surfaces including marine plants and algae *Ulva lactuca, Sargassum filipendula, Fucus* spp., *Zostera marina,* and *Codium fragile* subsp. *tomentosoides.* Tunicates were absent on rocks, free-living scallops, the sedentary snail *Crepidula fornicata*, and open meadows of marine plants.

Incorporating the New Zealand Tunicate Treatment Technology into a tunicate management strategy for Indian Point Marine Farms (Nova Scotia, Canada).

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Although tunicate infestations are now widely recognized as a major biofouling issue for shellfish growers in many regions of the world, few mitigation strategies are available. One option, the New Zealand Tunicate Treatment Technology (NZTTT) was originally designed to eliminate the tunicate Ciona intestinalis from continuous mussel sleeves in New Zealand. The present study was aimed at adapting and evaluating the effectiveness of this system for use at Indian Point Marine Farms, a mussel operation located in Nova Scotia (Canada). Preliminary trials conducted in 2005 indicated that the NZTTT system could potentially eliminate 60-80% of the tunicates attached to the mussel sleeves, but certain equipment modifications were required to improve operational efficiency. Larger-scale trials undertaken in 2006 using the modified version of the system confirmed that 80-90% of the C. intestinalis on heavily-infested sleeves (400 tunicates/m) could be eliminated with minimal mussel losses. The costs associated with the set-up and operation of this system as well as its potential role in a tunicate management strategy will be discussed.

Extent of ecological interactions between the vase tunicate (*Ciona intestinalis)* and the farmed blue mussel (*Mytilus edulis)* in Nova Scotia, Canada

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The goal of this study was to quantify the ecological interactions between blue mussels (*Mytilus edulis*) and vase tunicates (*Ciona intestinalis*) at Indian Point Marine Farms Ltd., in Nova Scotia. The lack of benthic predators on mussel lines and the availability of food create an ideal habitat for mussels, but unfortunately, also for tunicates, who settle in large numbers directly on top of the mussel shells. In addition to the logistical problems caused by high tunicate densities (higher stress on mussel lines and harvesting equipment), tunicate fouling on mussel aquaculture sites has been anecdotally linked to decreased productivity; this decrease could be caused by competition for food or inhibition of the mussel's ability to feed. Recent developments in tunicate mitigation strategies have highlighted the need to quantify the impact of tunicate fouling on the productivity of mussel aquaculture in order to determine cost-effectiveness.

To quantify the extent of competition for food resources at varying temperatures, clearance rates for both species were calculated in feeding chambers using *Tetraselmis striata* (Chlorophyta) at cell concentrations of 20,000 cells ml^{-1}. Between 4-13°C, mussel clearance rates were at least 3 times higher when standardized to individual wet weights. This difference was even greater at the lowest temperatures, implying that mussels could theoretically consume a greater amount of the food resource than tunicates in this temperature range. At 16°C and 19°C, the mussel clearance rates fell to the same level as the tunicates. Clearance rates were also examined using different sized algal species (*Isochrysis* sp*., Chaetoceros muelleri, T. striata* and *Prymnesium* sp.) at 19°C. Mussels seemed to have slightly higher clearance rates with smaller particle sizes, while the reverse trend was seen with tunicates although there was a substantial overlap in particle size utilization.

To determine the effects of tunicate density on mussel productivity, mussel size and condition were quantified. This was done by deploying a series of mini-socks (1 m segments of mussel line) on a lease in 2005 and also through taking samples directly from an operational lease in 2006. Indices of size (length, weight, height, shell weight, and wet and dry tissue weights) and condition (meat yield and water content) were measured under varying tunicate density. Surprisingly, mussel size was found to increase with increasing tunicate density in December 2005, but this was likely due to potential mussel fall-off and a heavy second set of mussels particularly on the low tunicate density socks. In October and December 2006, the size of the mussel was found to decrease with increasing tunicate densities. This was most evident in October, when mussel shell length decreased by 1 mm for an increase in tunicate density of 1kg m^{-1}. Mussel condition followed a similar decrease; the largest effect was again seen in October, when meat yield was found to decrease 2% for an increase in tunicate density of 1kg m^{-1}. In addition, up to 50% mussel mortality was observed under heavy tunicate fouling (~2 kg m^{-1}).

In conclusion, mussels and tunicates seem to have the potential for substantial food resource competition, and the tunicates likely had a negative effect on the mussels when food supply was lowest. Since mussels feed proportionally faster in colder waters, it may prove advantageous to select leases that are subject to colder temperatures year round. Heavy tunicate fouling seems to be associated with higher mussel mortality, lower overall size and condition. This was most accentuated in October, when an increase in tunicate density of 1kg m^{-1} is estimated to be associated with the loss of up to \$1.86 m^{-1} of mussel line. Therefore, mitigation treatments should be conducted at some point before October, possibly in the spring or early summer, as the tunicates may not have influenced mussel size and condition yet.

Interviews from Prince Edward Island, Canada, on boater habits and the potential spread of the clubbed tunicate, *Styela clava*

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The role of several human-mediated vectors in the dispersal of the clubbed tunicate, *Styela clava*, was investigated using interviews with recreational and commercial boaters in Prince Edward Island. The species arrived in the southern Gulf of St. Lawrence within the past decade and is likely being spread locally by several natural and human-mediated vectors. Boaters were interviewed to determine how long their boat had been at the present location, the primary use of the boat, use of anchors, sounding equipment or fishing gear, whether any organisms were attached to these items when retrieved, and the fate of those organisms. Bilge water and hull scrapings collected from the vessels were found to contain 47 taxa and 71 taxa, respectively. Boater movements were also examined to predict future sites of spread. These patterns indicate that sites in northeastern Nova Scotia and the southern coast of Prince Edward Island are most at risk for the spread of clubbed tunicate by the boating vector.

Hull fouling and overland transport of boats on trailers as vectors of spread of clubbed tunicate (*Styela clava*).

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The invasive clubbed tunicate (*Styela clava*) was first identified in Georgetown harbor, Prince Edward Island (Canada), in 1998 and has since spread to other estuaries. Worldwide, the dispersal of this species is attributed to hull fouling and contamination of bivalve aquaculture products. We tested how clubbed tunicate settlement patterns differed among the most common boat hull surfaces and colors as well as the ability of these tunicates to survive extended atmospheric exposure similar to that of boats being transported on trailers during summer months. Untreated hulls made of fiberglass, painted wood, and bare aluminum were quickly colonized by larval tunicates with those painted black attracting significantly more colonists compared to white-painted hulls. Use of anti-fouling paint kept colonization to a minimum, even after 12 weeks in the water. Bare aluminum hull material attracted the highest numbers of tunicates, which is a problem because boats operating around bivalve aquaculture sites are mainly constructed of aluminum and can not be painted with anti-fouling paint according to industry codes of practice. Aluminum and fiberglass hull material that had one-year-old tunicates growing on them were exposed to open air for 48 h during September (average daytime temperature 29.7 C versus 8.5 C at night). Nearly all tunicates were alive after 8 hours with only 10 to 11 % mortality after 48 h. Based on a 48 h survival time, viable tunicates from boats removed from infested waters of PEI could be spread on boats transported on trailers to any waters within 1600-2000 km of PEI – a distance encompassing the entire Atlantic seaboard of Canada.

Styela clava – a new threat to the Mediterranean shellfish industry?

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The solitary ascidian *Styela clava* Herdman, 1882 has been recorded for the first time in the Mediterranean region. It was found in the Bassin de Thau, a lagoon that supports intensive oyster and mussel farming. Both *S. clava* and *Ascidielle aspersa* grow in abundance on the shellfish culturing ropes but both are perceived as a nuisance rather than an economic threat at present.

In Prince Edward Island, *S. clava* settles on the mussel ropes, competes with the mussels for food and costs the mussel industry millions of C$ per year in reduced mussel production. The recent discovery of *S. clava* in New Zealand caused considerable alarm. The green lipped mussel industry in New Zealand, worth millions of NZ$ per year, is now considered to be at risk.

There are many similarities between oyster and mussel farming in the Bassin de Thau and mussel farming in Prince Edward Island. Oyster farming is carried out with oyster "tables" rather than the traditional oyster "beds" found in northern Europe. Ropes are suspended from these tables through the water column; the oysters are fixed with cement to the ropes and allowed to grow until they reach a marketable size. Approximately 750 producers farm 2,750 oyster tables and harvest approximately 13,000 tonnes annually. In addition to oysters, approximately 3,000 tonnes of mussels are produced every year. Given the thriving commercial shellfishery, why is S. clava not considered a major threat? This paper explores the factors that act to mitigate the threat of S. clava on oyster production in the Bassin de Thau.

Styela clava Herdman, 1881 or 1882?

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Styela clava is variously referred to in the literature as *Styela clava* Herdman, *Styela clava* Herdman, 1881 and *Styela clava* Herdman, 1882. This is confusing and the authority should be clarified.

We believe that the International Invasive Sea Squirt Conference is the ideal forum in which to address this issue and achieve a consensus. To that end, we propose a paper designed to promote discussion of this problem. We will produce arguments for both authorities and conclude that the correct species name is *Styela clava* Herdman, 1882; other participants may wish to challenge this conclusion. By the close of the conference we hope to have a solution acceptable to all.

Invasive tunicates in Prince Edward Island estuarine systems: the use of colonization plates as a community-based monitoring tool

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The invasion of Prince Edward Island estuaries by solitary and colonial tunicates (*Styela clava, Ciona intestinalis, Botryllus schlosseri* and *Botrylloides violaceus*) has triggered the concern of coastal biologists and increased considerably the production costs for mussel growers. In order to detect the arrival of these tunicates to those areas not yet invaded, a role for community involvement has been discussed but not effectively assessed. In this study, we examined the use of a well know device (deployment of PVC plates) and its effectiveness as a community-based monitoring tool in the early detection of invasive tunicates. The 10x10cm plates provide artificial substrate for colonization by these organisms, and seem reliable and comparable to a large number of studies published in the region and elsewhere. Our study documents the results of an initial 2 month deployment (to be followed by a subsequent 2 month deployment) on a range of representative estuarine areas in eastern, southern, and northern Prince Edward Island. We discuss our results in relation to location (distance from habitats currently invaded by these tunicates), and their utility as a tool for spread monitoring and promotion of public awareness on invasive species.

Inter-specific ascidian overgrowth in New England coastal habitats

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The ability of colonial ascidian invaders to occupy secondary substrate and overgrow other sessile species, including other ascidian species, contributes to their dominance in intertidal and subtidal ecosystems. The purpose of this study was to assess overgrowth by ascidians through surveys and monthly examination of Plexiglas plates documenting community development. Overgrowth was documented at coastal sites in Massachusetts and Maine, using surfaces of floating docks, Plexiglas panels hung off docks, and natural surfaces in intertidal pools. The presence or absence of inter-specific ascidian overgrowth was recorded. The results indicate ascidian fauna varies between sites and habitats, from estuaries to marine environment, and intertidal pools, with some ascidian species predictably serving as secondary substrate for inter-specific ascidian overgrowth. The ability of specific ascidian species to overgrow other species also varied through space and time, contributing to their success in nearshore coastal zones.

Invasive species alter the maintenance of biological diversity

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The relationship between the introduction of non-indigenous species and the maintenance of diversity remains difficult to determine for most ecosystems. However, examining this relationship is critical to predict the effect of invasive species on abundance and species diversity patterns. In this study we examined the effects of invasive colonial ascidians on species abundance and diversity using short-term field studies. We then compared these results to long-term diversity patterns taken from two studies (1979 to 1982 and 2003 to 2006) whose dominant members have shifted from the blue mussel, *Mytilus edulis* (1979 to 1982) to the invasive colonial ascidians *Botrylloides violaceus* and *Didemnum* sp (2003 to 2006). Results from our short term studies revealed hard shelled and arborescent morphologies enhanced abundance and species diversity while colonial ascidians inhibited abundance and lowered species diversity. However overall diversity patterns between 1979 to 1982 and 2003 to 2006 showed species richness increased while abundance and diversity were similar. Further analysis showed diversity between 1979 and 1982 was maintained as secondary substrate provided by the hard shell of the blue mussel while diversity between 2003 and 2006 was maintained as primary substrate as a result of the life history characteristics of the dominant invasive colonial ascidians. These results will help to elucidate the effect of invasive species on the maintenance of diversity and the importance of the life-history characteristics of dominant species when examining the influence of invasive species on diversity.

Assessing eradication: development and application of a generalized, practical eradication framework

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Eradication is an eminent idea in invasion biology, but is rarely considered in marine systems. Unfortunately, without action, invasions can rapidly worsen causing damages, yet, managers are usually faced with incomplete data. Thus, there is a common dilemma -- act quickly or collect better information. Further, to date, eradication frameworks provide useful heuristics, but have not provided practical guidelines for management actions. We have built a quantitative model that offers not only criteria for eradication success, but also a method to estimate effort required, timing, and target area. This method allows a manager to target population dynamics research to only those required for management. We apply this framework to a new *Ciona intestinalis* invasion. *C. intestinalis* has recently established in Prince Edward Island, Canada, and is causing great damage to the mussel industry. We outline how, with the support of governing agencies, using a combination of vinegar treatment and manual removal, a team might be able to sweep an entire bay, leading to the eradication of *C. intestinalis* at a regional scale. If government and industry are interested in eradication, effort will need to be coordinated and will require multiple passes over the entire system. Based on laboratory studies, we estimate that each pass should occur within a time window of twenty or forty-five days, depending on the type of structure. Interestingly, the major uncertainty in this estimate is maturation time. Thus, additional research should focus on reducing this uncertainty to best formulate eradication strategy for PEI. Using a cost-benefit analysis and estimated treatment costs, this effort would be on the order of a few million dollars – far less than the cost of continued damages and control effort *ad infinitum*.

Potential impacts of predation on survival and growth of non-indigenous tunicate species in British Columbia, Canada.

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When introduced to new environments, non-indigenous species often are removed from predators, competitors, and pathogens or parasites that normally influence their population dynamics. In Canadian marine waters, including British Columbia (BC), four non-native tunicate species (*Styela clava*, *Botrylloides violaceus*, *Botryllus schlosseri*, and *Didemnum* sp.) may be experiencing this release thereby enhancing their invasion success. We conducted a series of laboratory experiments to identify potential predators and assess predation effects on survival and growth of these non-indigenous tunicate species. Predators which can potentially be used to control tunicate fouling on shellfish aquaculture gear (*e.g.* sea urchins and nudibranchs), as well as those which could control tunicate spread on natural substrates thereby limiting their ability to invade new habitats (*e.g.* various species of crabs and sea stars), were examined. Mottled sea stars (*Evasterias troschelii*), red rock crabs (*Cancer productus*), and green crabs (*Carcinus maenas*) (another invader within BC) were found to consume *S. clava*, while green sea urchins (*Strongylocentrotus droebachiensis*) grazed on all four tunicate species. In order to further investigate the potential of the 'successful' predator-prey combinations we initiated additional experiments to: (1) quantify specific predation rates on adult and juvenile tunicates and (2) assess feeding preferences of predators using multiple-choice experiments. Quantitative predation trials indicated that male red rock crabs may consume as many as 10 individuals of *S. clava* a day, with an average consumption rate of 6.7 individuals/day. Green sea urchins, on average, consumed 4.43 cm^2 of adult colonial tunicates and 31.5 individual juvenile colonies a day. These results indicate that using sea urchins as biological control organisms on shellfish aquaculture gear may significantly help to reduce tunicate fouling.

Effects of temperature and salinity on survival and growth of non-indigenous botryllid tunicates

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Currently, there are at least four species of non-indigenous tunicates in British Columbia, Canada that have global invasion patterns, including the solitary tunicate *Styela clava* and the colonial tunicates *Botrylloides violaceus*, *Botryllus schlosseri*, and *Didemnum* sp. Despite rather extensive invasion histories, the environmental tolerances that affect survival, reproduction, and growth remain relatively unknown for this group of organisms. Thus, it is difficult to identify areas susceptible to invasion, predict potential impacts of an invasion, or develop appropriate mitigation strategies. For marine organisms, including tunicates, temperature and salinity are two environmental variables known to affect distribution and growth, thus largely contributing to invasion success. Experimentally we examined the effects of temperature and salinity on survival and growth of juvenile colonial tunicates *B. schlosseri* and *B. violaceus*. Methods and techniques were developed for collecting and handling tunicates, obtaining larvae, and raising young and adult tunicates under laboratory conditions. While exploring acclimation rates for young colonies of *B. violaceus* to experimental salinities, the best survival and growth were attained when colonies were gradually introduced to experimental conditions by adjusting salinity by 2‰ every 24 hours. Also, we examined survival and growth of young colonies of *B. schlosseri* and *B. violaceus* across a range of temperatures and salinities in a replicated, orthogonal two-factorial design, using six levels of temperature (0, 5, 10, 15, 20, and 25ºC) and six levels of salinity (8, 14, 20, 26, 32, and 38‰) resulting in 36 treatment combinations. The experimental ranges of temperature and salinity were selected based on the maximum ranges these tunicates would likely experience in Canadian waters, ensuring direct usability of results on both Atlantic and Pacific coasts. Young colonies of *B. schlosseri* survived conditions between 10-25ºC and 14-38‰, while *B. violaceus* tolerated temperatures between 5-25ºC and salinities between 20-38‰. Maximal survival and growth for both species was observed at 20ºC and 26‰, followed by 20ºC and 32‰. Although both species showed growth variability within all treatments, both species possess very broad environmental tolerances that could enhance invasion success.

Washington state tunicate species management plan

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The Washington State Department of Fish & Wildlife is tasked with managing an invasion of *Styela clava*, *Ciona savigni*, and *Didemnum sp*. sea squirts in the waters of Puget Sound. Management actions to date have focused on containment of Styela clava in three infested marinas by removing specimens from the hulls of vessels and therefore eliminating what we believe is the primary vector of spread. We are now in the process of ramping up our management efforts by building our equipment infrastructure, having our current biologist certified as an agency diver, and adding a new dedicated biologist/diver to lead the development and implementation of a comprehensive tunicate management plan. We are also fortunate to have Gretchen and Charles Lambert, who live in Seattle, on contract to provide scientific expertise and review assistance in these efforts. New equipment includes two video drop cameras for conducting rapid surveys under docks, boats and other structures to provide a baseline assessment of current populations and dispersal. We have also purchased a share of a new SeaEye Falcon underwater ROV to assist in conducting surveys in open water situations and at depths too great for effective diver application. The management plan is being developed in consultation with other state agencies and public stakeholders to ensure that our actions are transparent and accountable. We are also actively seeking to coordinate and collaborate with international efforts to address the invasive sea squirt problem.

Mitigation strategies for *Styela clava* fouling on mussel seed collectors

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The blue mussel (*Mytilus edulis* Linnaeus) aquaculture industry in Prince Edward Island, Canada, depends on the success of wild mussel seed collection in the early summer months. However, the presence of the invasive clubbed tunicate (*Styela clava* Herdman) is negatively affecting mussel seed collection and has the potential to continue to do so. The effect of treatments on *S. clava*, and the impact on mussel seed collection were examined in this field trial. Two treatments were applied to mussel collectors; 5% acetic acid was sprayed on collectors and 4% hydrated lime was applied as an immersion to collectors. Treatments had a significant effect on mussel seed collection. Collectors treated with hydrated lime had a mean (±SE) mussel abundance of 104.6 ± 26.9 per 30 cm as compared to 38.7 ± 13.1 for acetic acid and 0 for the controls. Tunicate abundance showed a reverse trend; collectors treated with hydrated lime had 99.3 ± 22.7 tunicates per 30 cm in relation to 148.6 ± 26.3 for acetic acid and 489.8 ± 21.1 for the control collectors. In terms of the timing of treatment application, treatments of hydrated lime in early August and again in late August resulted in collectors free of tunicate fouling with the highest mussel abundances (253.4 ± 56.8) and greatest mussel lengths (19.51 ± 1.25 mm).

Localised patterns of connectivity for the invasive sea-squirt, *Styela clava*

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The Asian tunicate *Styela clava* is an unwanted invasive marine species that has spread throughout the northern hemisphere with detrimental effects on natural ecosystems and aquaculture industries. *S. clava* has recently established within two of New Zealand's main Harbours (Hauraki and Lyttelton) and has rapidly spread throughout the Hauraki Gulf and within the port of Lyttelton. Previous results from a Global phylogeographic study using the mitochondrial COI gene suggested limited vectoring of *S. clava* between Hauraki Gulf and Lyttelton populations. Here we explore this relationship further using 12 polymorphic microsatellite markers and a more extensive sampling regime within the Hauraki Gulf and Lyttelton areas. In the past year several new incursions have been observed in areas previously free of this tunicate. The microsatellite markers have also been used to more accurately assign a source population to these new incursions.

Biotic and abiotic regulation of *Botrylloides violaceus* populations along the Strait of Juan de Fuca, Washington, U.S.A.

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Large-scale surveys of fouling communities of the Strait of Juan de Fuca and Puget Sound revealed a significant negative correlation between species richness and the abundance of the non-native ascidian *Botrylloides violaceus* at the regional level, but no relationship at smaller spatial scales. Observational and experimental studies at 4 local sites were used to quantify the contribution of abiotic factors (temperature, salinity and productivity) and biotic factors (competition, predation) to this pattern. The relationship between space availability, diversity and predation in these communities was also investigated.

***Didemnum* sp. in North America: Can anything eat it? Will it do them any good?**

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The invasive colonial tunicate *Didemnum* sp. has come to dominate many hard bottom communities in New England, where it is displacing native organisms that serve as food for fishes. The success of *Didemnum* in dominating habitats depends on a combination of its abilities to colonize, to outcompete competitors, and to avoid disturbances like predation. Once established, its impacts on the fisheries ecosystem in New England and areas where it can potentially expand (Canadian Maritimes to Virginia, as well as on the west coasts of the U.S. and Canada) depend in part on the abilities of the ecosystem to adjust to the invasion. Regarding system impacts, two major questions arise; 1. What ability does the system have to integrate the invasive into the current trophic structure, i.e. can it provide nutritional value comparable to that which it is replacing? and 2. What is the ability of the system to moderate invasive monopolization of resources, i.e. what are the prospects for disturbance factors like predation that might reduce spatio-temporal dominance? To date, only a few consumers of *Didemnum* have been identified. In pursuit of these two related issues, we undertook two preliminary investigations: 1. Determination of the food value of *Didemnum* sp. colonies from a variety of locations in the northeastern U.S., and 2. Laboratory experiments in which the palatability of *Didemnum* sp. was tested with some likely predators. Total protein, lipid, and carbohydrates contents of 4%, 2%, and 1%, respectively, were calculated to impart a caloric content of 390-420 cal per gram wet weight for *Didemnum*: low compared with many other marine food organisms. Colonies from Georges Bank differed significantly from those from shoreline sites in having lower water content (66% vs. 83%), but higher ash content resulting from the incorporation of sand. All four predators tested experimentally (Asian shore crab, purple sea urchin, common spider crab, and cunner) consumed frozen *Didemnum* sp. colonies. The degree of consumption varied significantly between all predators: Asian shore crab (87%) > common spider crab (71%) > purple sea urchin (52%) > cunner (<10%). Taken together, these results suggest that *Didemnum* sp. degrades potential food value in habitats that it dominates, but that there is a potential for that dominance to be reduced spatially and/or temporally by one or more keystone predators, probably benthic invertebrates. Additional ecological investigations in the field and laboratory are recommended.

Rapid nationwide surveys for *Styela clava* in New Zealand: delimitation methods and detection probabilities

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Styela clava was first detected from New Zealand waters in October 2005. In late 2005 and 2006 a surveillance program determined the distribution and relative abundance of the introduced ascidian at over 30 high risk ports and marinas nationwide. At each survey location, we combined above-water searches with in-water surveys using SCUBA divers. *Styela clava* was detected at three of the surveyed locations, and estimates of its relative abundance were made visually using a semi-quantitative log-scale. Detection probabilities were calculated where *S. clava* was not found, to provide an indication of the relative confidence of detection among locations, search techniques and substrata. To calculate detection probabilities, an experiment was conducted to estimate the sensitivity of the search methods. Known numbers of *S. clava* mimics were deployed at twelve replicate sites under different conditions of water clarity. An independent team then searched the sites for the mimics using the standard survey protocols. Experimental results showed that search sensitivity for above-water searches was positively related to water clarity; however diver search sensitivity remained high irrespective of water clarity. At the locations where rapid surveys did not detect *S. clava*, we estimated the relative confidence that above-water surveys would have detected it if at least one individual was present. Above-water detection probabilities varied widely between locations (from 0.02 to 0.75), which often reflected the prevailing water clarity during searches, and its effect on search sensitivity. Above-water and in-water searches provided complementary approaches for rapidly determining the extent of the incursion at key locations nationwide.

One ring to divide them all: mitochondrial genomics unveils two cryptic species in *Ciona intestinalis*

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The circular mitochondrial genome (mtDNA) of metazoans represents a rich source of genetic markers for phylogenetic analyses at many taxonomic levels. Both sequences and genome-level features, such as gene arrangement, are used to resolve deep-level phylogenetic relationships, whereas single mitochondrial genes or regions are analysed in population genetic studies. We used a mitogenomic approach, based on the comparison of several sequence and higher order mitochondrial features, to unambiguously demonstrate the existence of two cryptic species in the ascidian *Ciona intestinalis*, a model chordate whose taxonomic status of a single species has been recently questioned. A comprehensive comparative analysis between the mtDNA of the two putative cryptic species revealed significant differences in gene order, size and number of non-coding regions, compositional features, and evolutionary rate of protein-coding genes. These mitochondrial features are clearly incompatible with intra-species variability, and strongly suggest the existence of the two cryptic species. Our results demonstrate that mitogenomics is useful in the resolution of cryptic species, as in the resolution of deep-level phylogenetic relationships. Furthermore, our approach allowed to set two PCR-based diagnostic tests for the discrimination of the cryptic species without recourse to morphological analyses, demonstrating that mtDNA represents an accessible and powerful tool to be used in routine analyses or high-throughput screening.

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Market potential for *Styela clava,* a non-indigenous pest invading New England coastal waters

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Styela clava is a solitary tunicate that has spread rapidly from its native waters in the Philippines and is now a dominant resident in marine habitats of much of the world. It was first reported on the east coast of the U.S. at Beverly, Massachusetts in 1970 and is now commonly found in marine environments throughout the state. The alien sea squirt is a pest to the shellfish industry. It ingests larval forms of economically important shellfish species and is responsible for nuisance fouling on aquaculture gear. In southern Korea, *Styela clava* is considered to be a seafood delicacy and has acquired a cultural distinction as an aphrodisiac. A consumer market already exists for the fouling tunicate in Korean markets in the U.S. that sell imported frozen *Styela* at a retail price of at least $8 per pound. It is suspected that local fresh tunicate product, if available, could sell for an even higher price. A market study to turn this alien pest into a profitable seafood product is proposed.

Adventures of a sea squirt sleuth: the remarkable story of *Didemnum* sp., a global ascidian invader

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The route to the identity of *Didemnum* sp. has been long and tortuous. The magnitude of its worldwide invasions has taken a number of years to be comprehended. During that time, it was identified as various species depending on its location—*D. carnulentum* in California, *D. lutarium* or *D. vestum* in New England, *D. lahillei* or *D. helgolandicum* in France and the Netherlands, *D. vexillum* in New Zealand, *D. pardum* in Japan. This talk summarizes a chronology of the steps in the development of our awareness and understanding of this species, lists the known invaded sites and the approximate minimum length of time it has been known in each area and will (hopefully!) unveil its true identity and origin, based on comparative morphology and genetics.

Hierarchical genetic structure of invasive tunicates

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The 3-year project *"Hierarchical genetic structure of invasive tunicates"*, supported by the Canadian Aquatic Invasive Species Network (CAISN) has started in August 2007 and has involved the analysis of hierarchical genetic structure of invasive tunicates at different spatial scales from subcolony/individual level to oceanic scale. A particular attention will be given to Canadian coasts where five invasive tunicates cause significant damages to ecosystems and human activities (clubbed tunicate *Styela clava*, violet tunicate *Botrylloides violaceous*, golden star tunicate *Botryllus schlosseri*, vase tunicate *Ciona intestinalis*, sea squirt *Didemnum* sp.).

The project focuses on i) characterizing spatial and temporal patterns of genetic structure during the establishment and subsequent spread of invasive tunicates; ii) identifing potential sources and vectors of invasions; and iii) addressing taxonomic problems related to color morphospecies.

Styela clava, a solitary ascidian and *Botrylloides violaceous* a colonial form are the two main species investigated in the project.

The invasive colonial ascidian *Didemnum* sp. on Georges Bank: ecological effects and genetic identification.

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The invasive colonial tunicate *Didemnum* sp. was first noted in an area of gravel habitat on Georges Bank in 2002. Since its discovery, research has focused on investigating the spread of the tunicate invasion, on evaluating the potential impacts on the benthic community, and on identifying it at the species level and determining its region of origin. Analysis of video and photo transects taken with a USGS SeaBed Observation and Sampling System (SEABOSS) in the infested areas has shown that the tunicate continued to spread annually from 2002-2006 and now is present in an area of 230 sq km in two adjacent gravel areas, one open and one closed to fishing. At some sites in the area closed to fishing, *Didemnum* sp. covers nearly 75 percent of the seabed.

An Analysis of Similarity (ANOSIM) test on the abundances of 120 species collected by Naturalist dredge from 1994 to 2006 indicated that *Didemnum sp*. has had a significant impact on the species composition of the benthic community. In particular, the abundance of two polychaete species, *Nereis zonata* and *Harmothoe extenuata*, increased significantly in areas infested by the tunicate compared to areas not infested. The polychaetes live beneath the tunicate mats, and the increased abundance of these prey species suggests they are avoiding predation by fish.

In order to determine the species and origin of the tunicate, 18S rDNA sequences were obtained for 16 Georges Bank samples and one sample of an unidentified species of *Didemnum* from the west coast of New Zealand, not *Didemnum vexillum*. A multiple sequence alignment (MSA) revealed 4 distinct nucleotide sequences among the 17 samples. Sequences 1, 2, 3 were from Georges Bank samples while sequence 4 was from New Zealand. A diversity/similarity matrix indicated that sequences 1 and 2 were highly similar to each other with a divergence of 3.7 percent. By contrast, sequences 3 and 4 were less closely related with a divergence of 8.2 percent. When the two pairs of sequences (1 and 2 vs. 3 and 4) were compared, they had an average divergence of 13.78 percent, suggesting the presence of at least two different species. The samples belonging to sequences 1 and 2 from Georges Bank were identified as *Didemnum albidum*, a species native to the east coast of North America. On the basis of physical characteristics, samples of sequence 3 (Georges Bank) and 4 (New Zealand) are reported to be separate species of *Didemnum*. Research is needed both to identify meaningful levels of genetic divergence in tunicates and to further clarify the taxonomy of *Didemnum* sp.

Secondary dispersal in the invasive ascidian *Styela clava* at the North Sea shore

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The NW-Pacific ascidian *Styela clava* has become established in European coastal waters with a range from Denmark to Portugal. At several sites around low tide level near the island of Sylt in the German Wadden Sea, *Styela clava* is gaining dominance, particularly on mussel beds. Abundances range up to 200 m^{-2}. Seasonal development was followed at one site in 2007 and increments in length of 2 cm per month were recorded during summer. Substrates were noted as well as epiphytes and epizoans on *Styela clava* itself. Unattached bunches of up to 200 individuals were found drifting across tidal flats. Buoyancy had lifted these off the bottom, usually together with their basibionts, i.e. stacks of *Crepidula fornicata*, oysters or mussels. This secondary dispersal may be significant in the spread of this ascidian across the tidal zone.

Predicting the next one: or, trying to be the scientist who cried tunicate (but at the right place and time)

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Of the 41 tunicate species that were reported by Hayes and Sliwa (2003) to have invaded somewhere in the world, four currently occur in Atlantic Canada. Does this mean that we should prepare for the imminent arrival of the other 37 species? Probably not, given the uncertainties of transport and the challenge of surviving the Atlantic Canadian winter climate. Refining this long list so as to know which species to watch for is an important step in the development of early detection and rapid response strategies, which in turn are essential components of any management program for invasive species. A "short list" of potential tunicate invaders will be developed, based on those species present in regions climatically similar to Atlantic Canada, and from where there is an existing vector to our waters. Environmental matching using the Global Ballast Water Programme's ports dataset will be used to compare source regions for invasive tunicates to Canadian waters. For the regions likely to pose a risk of invasions, shipping vectors will be examined using the Canadian Ballast Water Dataset.

Environmental consequences of tunicate management practices in estuaries of Prince Edward Island

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Since about the year 2000, invasive tunicates (*Styela clava, Botrylloides violaceus, Ciona intestinalis* and to a lesser extent *Botryllus schlosseri*) have become major pests on blue mussel (*Mytilus edulis*) aquaculture sites in Prince Edward Island estuaries. The location of these sites in warm, sheltered, nutrient-rich, soft-bottom estuaries with very limited natural hard substrate has resulted in a strong attraction for tunicates to settle on the aquaculture structures as preferred substrates. The sometimes large biomass of tunicates is problematic for handling and harvesting of the mussels, so growers in heavily infested estuaries sometimes use weak acetic acid (household vinegar) or calcium oxide (quicklime) solutions that kill tunicates but have imperceptible effects on the mussels. The extent to which these solutions may affect other biota in the estuaries has recently been questioned. We report here our mass-balance calculations to determine the rate of neutralization of hydrogen ion imbalances that may result from use of acetic acid or the alkaline quicklime, and *in situ* experiments measuring pH, eelgrass biomass, benthic and water column biota in the vicinity of treatment sites.

Developing a rapid response plan for *Didemnum*: a model plan for Prince Edward Island.

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"Rapid Response" is the capacity to quickly respond to an invasion in cases where prevention (of the invasion) fails. Normally, eradication is the primary goal. Other management options include containment of the population, suppression to keep its numbers below an economic or ecological threshold or slow its spread, or living with the effects of the species. The Rapid Response Plan outlines the decision-making process to select a management option and the tools to carry it out.

Locke et al. developed a draft National Framework for Rapid Response to Aquatic Invasive Species which will be formally reviewed in 2008 by Fisheries and Oceans Canada (DFO). The Rapid Response Framework is a "cookbook" of pre-invasion planning and post-invasion action steps. Despite the "draft" status of the Framework, it has now been used in several provinces as an aid to identifying actions to be taken after the discovery of invasive marine species, although in those cases it was too late for the pre-invasion planning stage.

The Prince Edward Island Aquatic Invasive Species Coordinating Committee identified the non-indigenous form of *Didemnum*, present in New England waters but not yet reported from Atlantic Canada, as a taxon for which a Rapid Response Plan should be developed. In March 2007, a workshop was held in Charlottetown, PEI, to start the development of a Rapid Response Plan for *Didemnum* in PEI. This is the first such plan which will be developed proactively by DFO before the detection of an invasion, and serves as the first field-test of the proposed National Framework. This poster reports on the outcomes of that workshop.

If *Didemnum* should arrive in PEI, this Rapid Response Plan will assist DFO and provincial and industrial partners in responding to the arrival of *Didemnum* in an organized and timely fashion. This can also serve as the base of a Generic Rapid Response Plan relevant to other invasive species in PEI, and those portions of the Plan not dependent on specific details of PEI's infrastructure would be applicable to other regions of Canada.

Getting along with the tunicates? Co-occurring mobile invertebrates on blue mussel lines colonized by invasive tunicates in eastern Prince Edward Island

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Blue mussel socks not only provide artificial substrate for invasive and native tunicates (such as *Ciona intestinalis* and *Mogula* sp, respectively). They also provide a habitat for mobile invertebrates such as polychaetes that eventually interact or respond to tunicate colonization and abundance. We explored patterns of abundance and diversity on these assemblages and their potential interactions with tunicate numbers in relation to four variables: mussel stocking density (90 versus 250 versus 500 mussels per 0.3 m length of mussel sock), season of mussel sock deployment (fall versus spring), location within the Montague-Brudenell estuarine system (three long-lines), and sampling period along the growing season (June, August and October). Our study documents one of the most representative epifaunal assemblages associated to this and other estuarine systems elsewhere and their variation in relation to the drastic changes in tunicate densities as the growing season progresses. We then discuss the implications of our results for the role of coastal invasions on the diversity of native invertebrates.

History of the tunicate invasions in PEI, their impact on the cultured mussel industry and mitigation strategies employed since 1998

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Since1997 four new tunicate species; *Styela clava* (the clubbed tunicate), *Botrylloides violaceus* (the violet tunicate), *Botryllus schlosseri* (the golden star tunicate) and *Ciona intestinalis* (the vase tunicate) have invaded various PEI river systems. The greatest impact of the tunicate invasion has been on blue mussel (*Mytilus edulis)* aquaculture operations as a result of tunicate fouling on mussels and aquaculture gear.

A cooperative approach between the mussel industry, federal and provincial governments and researchers at the Atlantic Veterinary College, has been utilized to understand the basic biology of these invaders in relation to the PEI environmental conditions and to develop strategies for reduction and control of the tunicate fouling.

Strategies utilized include studies to determine effective mitigation and control methods, and restrictions on shellfish transfers. Communication strategies have also been implemented targeting water users to assist in controlling the spread of tunicates in PEI waters. The work in these areas is ongoing. Despite the efforts to date, the tunicate species continue to spread and seriously impact the mussel industry.

Potential impacts of an ascidian invader (*Didemnum* sp.) on seafloor communities in southern New England, USA

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Unlike the other recent ascidian invaders in southern New England, *Didemnum* sp. has the ability to form extensive mats on deep water cobble-gravel substrates similar to those previously documented in Georges Bank, Gulf of Maine. It is well recognized that such habitat-modifiers have the potential of greatly modifying associated species abundance and diversity and well as food web structure, nutrient cycling and ecosystem functioning. We conducted an *in situ* sampling survey comparing infaunal and epifauna abundance and species diversity associated with areas inside and outside *Didemnum* sp. mats at a site located in eastern Long Island Sound at a depth of about 25 m. A two-way analysis of variance showed that the total number of individuals from all taxonomic groups collected in benthic core samples did not vary significantly with the presence or absence of *Didemnum* sp. mats or the sampling date; however, when the data from all sampling dates were combined, a paired t-test indicated that there were significantly more individuals found within mats ($p = 0.042$). Significant differences in individual species abundances were also observed. Paired t-tests indicated that nine species had significantly greater abundances inside of mats than outside of the mats and one species had significantly greater abundance outside mats than inside mats. Of the 82 benthic species sampled, 38 were found exclusively either inside or outside mats; 19 of these were found only outside of mats and 19 found only within the mats. These species comprised only 5.14% of the total number of organisms observed in samples. The majority of these location-specific taxonomic groups were rare and were represented by four or less individuals or were found in two or less samples. An epibenthic polychaete, *Lepidonotus squamatus*, and an infaunal polychaete, *Eusyllis lamelligera*, were the only location-exclusive organisms found in high abundances in a number of samples and were only found within *Didemnum* sp. mats. Multivariate statistical analysis indicated the benthic community composition was significantly different between samples taken inside *Didemnum* sp. mats and outside mats.

Impacts of the invasive tunicate *Didemnum* sp. on scallop recruitment

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The invasive colonial tunicate *Didemnum* sp. has become widespread in New England waters. *Didemnum* sp. has colonized many kilometers of shell-gravel bottom of Georges Bank, including commercial scallop grounds. *Didemnum* sp. colonies are also fouling coastal shellfish aquaculture gear and shellfish, affecting shellfish growth rates and increasing maintenance costs. We hypothesized that *Didemnum* sp. will continue to spread and may impact shellfish larval settlement and survival through physical (filter feeding) or chemical interactions. We have conducted a replicated experiment to investigate bay scallop larval interactions with *Didemnum* sp. We will report the results of a replicated experiment under shellfish nursery conditions to investigate bay scallop larval interactions with *Didemnum* sp.

The new European on-line journal "Aquatic Invasions": services for marine biodiversity related information systems

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Aquatic Invasions is a rapid on-line journal with a focus on (a) biological invasions in European inland and coastal waters and (b) potential donor areas of aquatic invasive species for Europe (ISSN: 1818-5487, **http://www.aquaticinvasions.ru**). The journal provides the opportunity of timely publication of first records of biological invaders for the consideration in risk assessments and early warning systems. Also, the journal provides the opportunity to publish relevant technical reports and other accounts not publishable in regular scientific journals. Conference proceedings may be published as Special Issues.

Aquatic Invasions is an important part of the developing Pan-European and regional early warning systems on aquatic invasive species, with an important protection service of author rights on primary geo-referenced records on introduced species. In 2006, more than 50 research articles and short communications in 4 regular issues of the first volume of *Aquatic Invasions* included geo-referenced information on range expansions and European first records of highly invasive species, such as Conrad's false mussel *Mytilopsis leucophaeata*, Wedge clam *Rangia cuneata,* grapsid crab *Percnon gibbesi*, Chinese mitten crab *Eriocheir sinensis*, ctenophore *Mnemiopsis leidyi* and round goby *Neogobius melanostomus*. Further a European distribution update of the Asian sea squirt *Styela clava* was published.

Start-up funding for *Aquatic Invasions* is provided by the European Commission Sixth Framework Programme for Research and Technological Development Integrated Project ALARM (GOCE-CT-2003-506675), with general networking support from the EC FP6 Strategic Targeted Research Project DAISIE (SSPI-CT-2003-511202). Each manuscript submitted to *Aquatic Invasions* are reviewed by at least two independent experts. Accounts on inland invaders may be submitted to Vadim Panov (**rbic@zin.ru**) and for coastal invaders please approach Stephan Gollasch (**sgollasch@aol.com**).

Didemnum sp. as an agent of change: Can this be detected regionally?

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Didemnum sp. has been the focus of much research over the past several years. Although *Didemnum* sp. appears to cover large areas of hard substrate and grow over many species, its impact locally and regionally is undocumented. Of particular concern is the presence of *Didemnum* in Georges Bank where it covers at least 150 sq km of gravel bed. One question of interest is what the impact of *Didemnum* on scallops and groundfish is and can this be documented. Do we have the tools to document its presence throughout its habitat range? Can we effectively mitigate its impact, limit its spread, and reduce new introductions? This presentation outlines approaches to identify the spatial extent of *Didemnum* in nearshore and offshore areas as the first step in identifying impacts. Results of diver education programs and proposed regional approaches to detection and prediction will be discussed.

Tunicate nanocrystals as a novel nanomaterial for the preparation of ultra-strong composites, optical coatings, and cell culture platforms

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The tunic of tunicates is rich in nearly perfect crystalline and high aspect-ratio cellulose nanocrystals (NCs). These NCs can be relatively easy to extract and purify in the form of a few nanometers in diameter and several microns-long nanowires. Similar NCs can also be extracted from other cellulosic organisms, e.g. wood, cotton, or bacteria. However, those crystallites have smaller dimensions, with the lengths of only 200 – 400 nm, which may be prohibitive for some of the applications. The perfect crystalline structure of NCs, which originates from perfect, parallel alignment of the cellulose chains, imparts them with exceptional mechanical properties: ultimate tensile strength is estimated at ~10 - 15 GPa, bending strength ≈ 10 GPa, and Young's modulus, $E \approx 150$ GPa. For comparison, strongest steel alloys have tensile strength up to 2 GPa and E ~200 GPa. They also possess liquid crystal properties, which in the case of cotton NCs, have been explored for the preparation of a security paper.

Given their natural and renewable origins, NCs are emerging as a new class of reinforcing nanomaterial for the preparation of high performance nanostructured composites. Here, we present preparation of thin film composites from cotton (100-300 nm long) and/or tunicate (>1 μm long) NCs with different polyelectrolytes using a bottom-up fabrication process called: the layer-by-layer (LBL) electrostatic assembly. We present our results from the evaluation of mechanical properties of the thin films both with standard stretching technique and Brillouin light scattering. LBL assembly of the cotton NCs with poly (diallyldimethylammonium chloride) (PDDA), results in nanocomposites with ultimate tensile strength, σUTS ≈ 40 MPa and Young's modulus, E ≈ 2 GPa (as high as 10 GPa from Brillouin light scattering). Post-assembly thermal-treatment increases σUTS to ≈ 130 MPa without a change in E.

We also show that LBL assemblies of tunicate NCs possess strong antireflection (AR) properties having an origin in a novel highly porous architecture reminiscent of a "flattened matchsticks pile", created by randomly oriented and overlapping NCs. This first example of LBL layers of tunicate NCs can be seen as an exemplary structure for any rigid axial nanocolloids. Given the refractive index match, AR properties are expected to be a common property. Similarly, the films exhibit exceptional mechanical properties with E reaching as high as 30 GPa. We have also found that the same network structure of tunicate NCs serve as an excellent platform for mammalian cells adhesion, guidance, and proliferation.

Sea squirts in Brudenell estuary: Documenting the invasion

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Over the past decade, four exotic tunicates (*Styela clava*, *Ciona intestinalis*, *Botrylloides violaceus* and *Botryllus* schlosseri) have been reported in the Brudenell estuary in Prince Edward Island (PEI), Canada. *Styela clava*, was the first exotic tunicate to arrive in 1997, rapidly establishing, spreading, invading, and eventually becoming nuisance in several estuaries of PEI. In the Brudenell estuary, *S. clava* remained the only exotic nuisance tunicate until 2003. In the fall of 2004, the vase tunicate *C. intestinalis*, was the second exotic tunicate reported in that system in low abundance, followed by the two colonial species, *B. schlosseri* and *B. violaceus*, reported in the spring of 2005. The abundance of *C. intestinalis* rapidly increased post-introduction, eventually replacing *S. clava* as the foremost nuisance species on mussel farms in the estuary. To date, *C. intestinalis* continues to colonize this estuary and neighbouring waters at epidemic proportions, resulting in the continuing drop of *S. clava* abundance. The abundance at the end of 2006 of *C. intestinalis* is estimated at $5/cm^2$, which is similar to *S. clava* abundance at its height in 2003. The 2006 abundance of *S. clava* is estimated to have fallen to near $0/cm^2$. The dominance of *C. intestinalis*, as a fouling organism on mussel farms, is considered a serious threat to this aquaculture industry, mainly due to its unmanageable weight. In the spring of 2007 observations from divers and mussel growers indicated there was a mass winter mortality of *C. intestinalis*; mussel socks once smothered by the tunicate were now clear of fouling. Continued monitoring of the re-development of *C. intestinalis* in this estuary is ongoing to determine if this mass winter mortality is an isolated event or whether a pattern of boom and bust emerges on a predictable time scale.

Invasive tunicates in oyster cultivation: potential to colonize the natural substrate

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Shellfish cultivation is rapidly becoming more common in Brazil, especially in the state of Santa Catarina. A problem with shellfish cultivation is that many encrusting invertebrates and algae can grow on the mussels or oysters, tunicates being one of the most important groups. To better understand the dynamics and potential impacts of non-native encrusting tunicates, we identified the encrusting species present at one oyster farm and we tested their colonizing ability on artificial (plastic) and natural (granite) substrate, with and without (as in oyster cultivation) predation. The study took place at the Fazenda Marinha Atlântico Sul in Florianópolis, in the southern Brazilian state of Santa Catarina. We used as substrates granite plates that were placed within the "lanterns" that are used in cultivation. Lanterns comprise 6 horizontal plastic discs within a nylon fishnet. In each lantern we placed three granite plates (11x23x1 cm), two within the lantern underneath two discs, and one external on the bottom disc. On 30 May 2006 we submerged 16 lanterns. We analyzed colonization of the granite plates (internal and external) and three adjacent areas of the plastic discs of the same dimensions (11x23 cm). Four lanterns were collected every three months and their encrusting communities were identified. Tunicates were classified as native, cryptogenic or introduced. Fourteen species were found: *Trididemnum orbiculatum* and *Polycarpa spongiabilis* were native, *Diplosoma listerianum*, *Didemnum perlucidum*, *Lissoclinum fragile*, *Distaplia bermudensis*, *Clavelina oblonga*, *Botrylloides nigrum*, *B. giganteum*, *Styela canopus*, *Symplegma rubra* and *Microcosmus exasperatus* were cryptogenic, *Ascidia sydneiensis* and *Styela plicata* were introduced. *Styela plicata* is also an invasive species that causes major economic costs to shellfish cultivation. It was clear that oyster cultivation favored establishment of exotic species, since only two species were from the region. Substrate was of little apparent importance: only *A. sydneiensis* was more frequent on plastic samples than granite in September, the remainder of species and substrates showed similar trends. Species were equally abundant on both substrates, suggesting a strong colonization potential. Internal substrates were always more covered by all species but three (*D. listerianum*, *S. canopus* and *S. rubra*) which indicates the important role of predation on controlling the ability of exotic species to become established in new environments and to dominate natural communities.

Worldwide genetic structure of *Microcosmus squamiger*: contrasting genetic diversity patterns between introduced and native populations

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The solitary ascidian *Microcosmus squamiger* is considered to be native from Australia and to have spread around the world through transoceanic vessels. We studied the genetic structure of 12 *M. squamiger* populations, including from its supposed native range as well as presumably introduced populations from the Indian, Pacific and Atlantic oceans, and the Mediterranean sea. We aimed to determine the phylogeographic relationships between the populations and to track the spread of this species from its presumed native range. We amplified 574 bp of the mitochondrial COI gene in 258 individuals and found a total of 52 haplotypes. The frequency of each group of haplotypes in each population showed important differences between the localities of western Australia and the remaining ones (eastern Australia and introduced populations). Likewise, a neighbour-joining tree included the localities of eastern Australia and the introduced populations in a single group, separated from the western Australia populations, a result also supported by genetic differentiation matrices (based on F_{ST}) and AMOVA analyses. We found no significant correlation between genetic differentiation and geographic distance, which suggests that the dispersal of the species was not only due to natural processes, but also to passive transportation (presumably through ship transport). Accordingly, a Nested Clade Analysis showed that worldwide *M. squamiger* populations have a restricted gene flow or dispersal but with some long distance colonisation events. However, when the analysis was restricted to the Australian populations, we found a contiguous range expansion between them. We conclude that *M. squamiger* is native to Australia and has expanded its range of distribution through shipping to other continents, especially from the harbours of the more populated East Australia. The high level of haplotype diversity of the introduced populations suggests that multiple introductions have shaped the present day distribution of the species.

Introduced ascidians along the coast of South Africa: water temperature as a predictor of their geographical distribution

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The extensive coastline of South African has been one of the world's main shipping routes since the first European sailors rounded the Cape Agulhas in the late fifteenth century. From previous ascidian taxonomic studies, nine introduced ascidian species and 8 cryptogenic species have been recorded at various locations along the South African coast.
Here we present the results of a survey undertaken to establish the current distribution of these introduced and cryptogenic species along the South African coast. We focused our sampling on 10 sites where it was most likely to find introduced ascidians (including all main harbours, two small recreational marinas and an oyster farm). Most species distributions were clearly separated by the different biogeographical regions, especially splitting east and west of the Cape, where the Agulhas (warm) and the Benguela (cold) currents dominate respectively. However, some species (e.g. *Diplosoma listerianum*) have a broader distribution and occur widely along the South African coast. In order to test how temperature affects the embryonic development of introduced ascidians, we chose two species, *Ciona intestinalis* for the west coast and *Microcosmus squamiger* for the east coast, which are both dominant species at their respective locations. *C. intestinalis* embryos only developed in a temperature range between 15-20ºC while the embryos of *M. squamiger* developed between 20-25ºC. The results indicate that water temperature is a good indicator to determine where potentially invasive ascidian species can be introduced.

Settlement and post-settlement interactions determine the distribution of invasive and native ascidians

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Invasive species can have a range of impacts on native species and while lethal effects are the most commonly studied, the non-lethal effects remain poorly understood. Furthermore, studies have generally been restricted to the interactions between native and invasive species during their adult stage. Non-lethal effects occurring during the initial stages of development in marine organisms may disrupt the fertility, population dynamic and dispersal of native broadcast spawners in the same way as pollination can be disruptive in terrestrial systems. Marine invasive species are often competitively dominant, and as a result the larvae of native species might reject settlement sites adjacent to invasive species. We examined the effects of a marine invasive species (*Styela plicata*) on an Australian native species (*Microcosmus squamiger*) from fertilisation to larval settlement and post-metamorphic performance. We found no significant effects of the presence of *S. plicata* sperm on the fertilisation of *M. squamiger*. We subsequently found that the presence of one species' recruits had a negative effect on the settlement of the other species, while the presence of homospecific recruits did not affect the settlement of the larvae. Finally, we examined the post-metamorphic performance of both species in the presence and absence of the other species in the field. The presence of *S. plicata* had a strong negative effect on the subsequent survival of *M. squamiger* while the presence of *M. squamiger* had no effect on the subsequent survival of *S. plicata*. Our results suggest that marine invasive species have the potential to dramatically change the population dynamics of native species through interactions occurring during the early phases of the life history.

A tale of two seas: ecological aspects of the ascidian community along the coast of Israel

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Human activities have and are continuing to shape the distribution of a number of species in the marine environment. This is true when taking into account changes in water quality and is particularly true for species traversing environments that have been connected via human activity. One example of human activities connecting bodies of water is the connection of the Eastern Mediterranean with the coral reef environment of the Red Sea via the Suez Canal. This proximity between the seas provides fertile ground for comparative studies of distribution and migration of marine fauna. The ascidian fauna of both the Gulf of Eilat (Red Sea), and the Mediterranean coast of Israel has been poorly studied and many questions have been left open concerning the validity of some species, the geographical distribution of others, and the arrival of non-indigenous species. In this study we investigated two populations of the solitary ascidian *Herdmania momus*; one from the Red Sea and the other from the Mediterranean. In order to understand parameters that may have lead to the migration of this ascidian from the Red Sea to the Mediterranean, we conducted a comparative study of the ecology of this species in the two environments. In the Mediterranean coast, *H. momus* exclusively inhabit artificial substrates, and are common at greater depths than in the Red Sea coast. *H. momus* individuals from the Mediterranean site were significantly larger than individuals from the Red Sea site. Gonad indices and oocyte diameter measurements indicate that in the Mediterranean *H. momus* have a short reproductive season, while in the Red Sea they reproduce year-round. These differences are attributed to different temperature ranges, food availability, water currents and wave exposure. Future results from molecular analysis will contribute to the understanding of arrival and dispersal modes. Other anthropogenic activity such as those that cause changes to water quality may also induce changes in the natural fauna. In the coral reefs of Eilat, the colonial ascidian *Botryllus eilatensis* has been observed overgrowing dead coral skeletons and rapidly colonizing artificial substrates. In order to study overgrowth dynamics and the relationship between coral morph, coral size, orientation and ascidian overgrowth, monthly monitoring of 50 tagged coral colonies interacting with the colonial ascidian were conducted. Results show that the rate of ascidians overgrowth on corals is influenced by coral morph and orientation combined with a seasonal appearance. Interestingly this seasonality is closely linked to the vertical mixing phenomenon that occurs in Eilat every winter which results in high nutrient levels in the water column. Increased anthropogenic activity, particularly the eutrophication occurring along the Israeli coasts, creates favorable conditions for filter-feeding organisms such as ascidians, providing them with an advantage in competition for space with reef-building corals and other organisms. It is therefore imperative to continue monitoring the ascidian populations and further study ecological aspects of this group.

Invasive ascidians: conservation challenges and ecological opportunities

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While invasions by exotic sea squirts undoubtedly pose numerous important challenges to both conservation and human enterprise, they also offer a number of important opportunities to gain critical insight into the functioning of ecological systems. They provide unanticipated (though often uncontrolled) experiments across large spatial and temporal scales that enable researchers to observe ecological and evolutionary processes in real time that are often difficult to study with native species, and to provide data that would often be deemed unethical to collect otherwise. This talk will touch on a number of basic ecological insights provided from the recent study of exotic sea squirts and discuss the implications of these insights for both our general understanding of invasion biology and the impacts of exotic species on native ecosystems.

I will discuss what the study of exotic sea squirts has taught us about the consequences of changing biodiversity for the invasibility and functioning of marine ecosystems. Both short-term experiments and longer-term surveys provide evidence that increasing community diversity reduces the susceptibility of these communities to future invasion. However, this is at odds with the common-sense observation that invasion into native communities has not slowed and if anything has increased in recent years. In addition to the well-demonstrated negative effects of diversity on invasion, there are also facilitative effects as well, mediated by the influence of habitat forming species that boost both native and exotic diversity. Both positive and negative effects are mediated through the effects of resident species on the availability of space, often the limiting resource in these systems. The balance of positive and negative effects appears to be context-dependent, and broad-scale surveys are beginning to elucidate the conditions under which each occur. However, native and exotic fouling organisms often differ in the degree to which they facilitate other natives vs. exotics, sometimes in surprising ways that appear to contradict the idea of ìinvasional meltdownî. For example, in central California, the non-native bryozoan Watersipora subtorquata actually: (1) increases total diversity of both sessile and mobile fouling organisms, and (2) disproportionately facilitates native epibionts relative to other native habitat forming species. Implications of these sorts of studies for understanding both the process of community assembly and the impacts of invasions on native communities will be discussed.

Genetic conspecificity of worldwide populations of the colonial tunicate, *Didemnum* sp.

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A colonial tunicate of the genus *Didemnum* has recently been found in many temperate coastal regions throughout the world, as well as large areas of the Georges Bank in the NW Atlantic. It continues to spread rapidly and compete aggressively with other native, hard substrate species (e.g., mussels, barnacles, bryozoans, other ascidians). In addition, it can form dense mats of cobble-gravel substrates and influence the abundance and species composition of benthic epifauna and infauna. This invasive species, referred to in recent publications as *Didemnum* sp. A, has been misidentified as five previously described species native to the regions where *Didemnum* sp. has been discovered and described as two new species based solely on morphological characteristics. However, there are relatively few diagnostic characters and a great deal of variability in the relevant characters, making the task of identification very difficult. Adding to the confusion has been the widespread and often disjunct distribution of the species. While the presence of these organisms has caused severe detrimental economic and ecological impacts, the taxonomic identification remains problematic. Here we present molecular data from both mitochondrial and nuclear genes which strongly indicate that *Didemnum* sp. is a single species, possibly resident to the western Pacific Ocean, that has become established globally.

International DNA bank for ascidians

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Molecular studies are currently making significant contributions to the detection and management of aquatic invasive species, including invasive tunicates. To fully harness the potential of molecular studies in aquatic invasive tunicates, DNA samples from ascidian species on an international level must be readily accessible. Since tunicates have spread on a global level, it is imperative that samples be available on a global level. It is our proposal through CAISN, to develop and maintain a DNA bank of aquatic invasive tunicates in our lab at the Atlantic Veterinary College at the University of Prince Edward Island. We invite international researchers to send tissue samples to us of tunicates in their area. We will extract DNA, list them in an online database, maintain the collection frozen at -80, and send samples out to any researcher who requires samples for their research. We believe that this global access to DNA, will increase the efficiency and scope of molecular studies on invasive tunicates as well as to allow for collaborations and networking amongst researchers.

Seasonal water temperature cycles and the recruitment of larvae of the colonial ascidian *Didemnum* sp. in New England coastal and offshore waters

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The colonial ascidian *Didemnum* sp. occurs in many regions of the world. It aggressively overgrows hard substrates and many sessile species and has become a nuisance to the aquaculture industry. Its extensive mat-like colonies hypothetically could alter benthic communities. The species can survive in water temperatures ranging from <-1 to >24 °C. In order to predict the geographic spread of *Didemnum* sp. and to aid in developing strategies for managing it, we attempted to determine the relationship between water temperature and the larval recruitment that is necessary for successful colonization of an area.

Experiments using settlement plates in shallow New England coastal marine waters of up to 5 m depth showed that first larval recruits appeared at water temperatures of 19-20 °C in late June 2007 at Woods Hole, MA in the southern part of the region. Farther north, first recruits appeared at the same water temperatures but in late July 2006 at the Damariscotta River, ME.

Didemnum sp. has been observed over 6 years in two adjacent areas of gravel habitat in water depths of 45-65 m on the northern part of the Georges Bank fishing grounds. The areas are characterized by maximum temperatures in the August-October period that reach only 15-17 °C. It is not possible to monitor larval recruitment on Georges Bank, but fully-developed larvae have been observed in colony tissues. We assume larval recruitment is responsible for the presence of dense aggregations of colonies and the species' rapid spread in the two affected areas, which extend 14 and 30 km in their longest dimensions and encompass 230 sq km. Although tidal currents are strong in the region, the species is absent at sites only 4 to 6 km to the north that have identical gravel substrates and lie in depths of 45-55 m beneath cool water on the shelf edge, where temperatures are highly variable and generally do not exceed 12-14 °C.

The shallow coastal and deep offshore sites studied here are characterized by different annual temperature ranges and rates of warming. In winter, most shallow New England coastal sites decline to temperatures of 0 °C and colder. Georges Bank areas lie in deeper water and are no colder than 4-5 °C, which we suspect may also be typical of conditions in New England deep coastal areas. Our observations suggest that the development and release of *Didemnum* sp. larvae is governed by response to local temperature trends, which differ between shallow and deep water areas. The failure over 6 years of *Didemnum* sp. on Georges Bank to expand a relatively short distance from "warm" areas to nearby "cool" areas suggests that colonies or larvae of the species likely will not successfully colonize offshore or coastal areas where water temperatures do not exceed 15 °C.

Predicting the possible geographic distribution of the colonial ascidian *Didemnum* sp. on the Georges Bank fishing grounds (Gulf of Maine) based on water temperatures required for the development and release of larvae and on substrate availability

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Didemnum sp., an aggressive invasive colonial ascidian, has been observed over the past 6 years on gravel habitats of the Georges Bank fishing grounds. We have conducted long-term studies of the benthic gravel communities for the past decade in two affected areas and in four unaffected areas on the northern part of the bank. *Didemnum* sp. became obvious in one area in 2002, and as of July 2007 two large elongated areas in U.S. waters have been colonized that total 230 sq km. These areas are 14 and 30 km in length, respectively. One other area surveyed in U.S. waters on the bank and three on the Canadian part of the bank are unaffected. In all locations, the substrate is predominately pebble gravel, and water depths range from 45 to 65 m in the affected areas to 45 to 85 m in the unaffected areas. All areas are characterized by strong tidal currents and a water column rich in nutrients. *Didemnum* sp. can be spread between widely-separated areas by the transport of colony fragments, but colonization requires the release of larvae, which are reported to live only a matter of hours.

The principal difference between affected and unaffected areas is the seasonal maximum water temperature which occurs in the August-October period. We have relied on temperature records collected over the past 20 years by NOAA Fisheries and supplemented by our own data. In all study areas, the seasonal temperature minimum is 4-5 ^{o}C and occurs in March and April. The two areas affected by *Didemnum* sp. reach maximum temperatures of 15 to 17 ^{o}C, and the presence of larvae in colony tissues and the high density of colonies are strong indications that larval recruitment has occurred there. Unaffected areas all lie nearer to the shelf edge where water temperatures are appreciably colder. In a region of strong tidal and storm currents, the unaffected area in the U.S. lies only 4 to 6 km north of the two affected areas and reaches temperatures of just 12-14 ^{o}C. These observations suggest that *Didemnum* sp. larvae will not recruit in Georges Bank waters that do not reach temperatures greater than 15 ^{o}C. The three areas in Canada reach temperatures of only 10-12 ^{o}C. Thus, we suggest that the four unaffected areas we have studied will not be colonized by the species.

To identify areas on the bank susceptible to colonization also requires knowledge of the distribution of seabed types. Earlier observations have shown that *Didemnum* sp. depends on hard substrates and is susceptible to smothering by mobile sand and by muddy sediment. On the northern part of Georges Bank, seabed habitats include: gravel pavements; mobile sands moved daily by strong tidal currents; and mixtures of sand and gravel where cobble and boulder surfaces lie above moving sand. In 2006, *Didemnum* sp. was reported to occur west of Little Georges Shoal on the western part of the bank. We found that water temperatures there reach 16-18 ^{o}C at depths of 40 to 65 m, and in July 2007 we confirmed the species' presence on a substrate of mixed sand and pebble gravel. We suggest that Cultivator Shoals on the northwestern part of the bank, where hard substrates have been reported and where temperatures reach 17-18 ^{o}C at depths of 22 to 37 m, is an area favorable for colonization by *Didemnum* sp., initially by fragments of colonies transported from other areas followed by spreading of locally-produced larvae.

Development of genetic markers in the invasive tunicate *Ciona intestinalis*

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In 2005, the annual landings of cultured mussels in Nova Scotia were estimated at 2.3 metric tons, with a value of over $3,000,000 (Nova Scotia Department of Fisheries and Aquaculture). During the past decade however, fouling of mussel lines by the cryptogenic tunicate *Ciona intestinalis* has created a significant financial and technical burden for a number of Nova Scotia growers.

Several studies have demonstrated that the distribution of *C. intestinalis* along the coast of Nova Scotia is extremely patchy. It is not known if separate *Ciona* infestations are genetically related. The goal of the present work is to assess the origin of the *Ciona* infestation and document its colonization along the coast of Nova Scotia. To this end, we undertook the development of genetic markers specific to this species which would enable us to track populations, families and even individual organisms.

Ascidians such as *C. intestinalis* have been extensively studied as a model for ancestral chordates. Vertebrate gene families are typically found in simplified form in *Ciona*, suggesting that ascidians contain the basic ancestral complement of genes involved in cell signalling and development. A draft of the *C. intestinalis* genome was generated by the DOE Joint Genome Institute using a whole genome shotgun strategy (*Science* 13 December 2002: Vol. 298. no. 5601, pp. 2157– 2167). Using the database generated by JGI, we identified regions of the *Ciona* genome containing repetitive patterns of nucleotides also known as microsatellites. A microsatellite usually consists of repeats of two to four bases in length, and the number of repeats frequently differs between different members of a species. More than 100 potential microsatellite loci were tested. Surprisingly, this led to the development and characterization of only sixteen microsatellite loci for *C. intestinalis*. These included nine perfect tetranucleotide and seven perfect dinucleotide repeat regions. These loci exhibited a low rate of polymorphism with the number of alleles per locus ranging from 2 to 6.

Confirmation of Mendelian inheritance and segregation is ongoing for all sixteen loci, as well as the transferability of the-loci to animals belonging to distinct populations of *Ciona* from California, Japan and Europe. The development of these new polymorphic microsatellite markers may facilitate future linkage mapping efforts.

Pilot general and targeted surveillance program for tunicates in Nova Scotia; April 2006 – August 2007

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In the past decade, the shellfish culture industry in Atlantic Canada has been impacted by invasions of fouling tunicates. Five species of tunicates are of concern in the region: *Ciona intestinalis* (vase tunicate), *Botrylloides violaceus* (violet tunicate), *Botryllus schlosseri* (golden star tunicate), *Styela clava* (clubbed tunicate) and *Didemnum* sp,). A general and targeted surveillance program, with the involvement of community groups, was initiated by the Department of Fisheries and Oceans in the spring of 2006. Collection plates were deployed at 34 geo-referenced general monitoring sites along the coast of mainland Nova Scotia and Cape Breton. Sites were selected based on the presence of the following risk factors for tunicate arrival or establishment: shellfish/mussel processing, mussel/shellfish aquaculture in area, port with international traffic, marina with US traffic, fishing harbour, high risk ports with herring or US caught lobster processing. Additional sites were established and targeted surveillance was conducted, in association with the Fishermen's and Scientist's Research Society, in 3 "hot-spots" or areas of "high-risk".

Collectors were deployed in late May and collected in mid August (first set) or late October (full season) or deployed in mid August and collected in late October (second set). Physical measurements (temperature, salinity, oxygen, conductivity) were made at each deployment and collection, and surfaces surrounding collectors were examined for the presence of tunicates. In an effort to promote community based monitoring, a Tunicates Brochure was produced and circulated to members of the general public, lease holders and fishermen. A 1-800 tunicates reporting line was also established.

In 2006, *Ciona intestinalis* was found at 13 of 34 general monitoring sites, and in the 3 targeted areas. New documented locations for *C. intestinalis* were Sydney and Meteghan. Violet tunicate was found on 7 of 34 general monitoring sites, and in the Halifax-St. Margaret's Bay and Shelburne-Port La Tour targeted areas. Golden star tunicate was the most wide-spread species; present at 18 of 34 general sites and 14 additional sites in all 3 targeted areas. Tunicates were found in waters with 20.3 – 33.1 ppt salinity, and at oxygen saturations of 54.2 -93.8%. Tunicates were present on sites with every "risk-factor" selected; from aquaculture leases, to private marinas, to public fishing wharves. *Styela clava* and *Didemnum* sp. were not observed during 2006.

The project is continuing in 2007, with 24 general sites, and 15 targeted coastal sites from Mahone Bay to Digby, the area where *Didemnum* sp. may first appear. Identification Posters have been hung at about 100 locations, and brochures circulated to promote community based monitoring and reporting of new tunicate infestations.

Untangling temperature tolerance discrepancies between growth, abundance, and recruitment in *Botryllus schlosseri* and *Botrylloides violaceus*

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Increased awareness of climate change and invasive species has resulted in a surge of studies on how climate change may impact the invasibility of communities. One common method of conducting these experiments is to compare the temperature tolerances of native or naturalized species to those of highly invasive species through laboratory and field studies. Two animals that have received attention in fouling communities recently are the ascidians *Botryllus schlosseri* (a naturalized species in New England since the 1800's) and *Botrylloides violaceus* (an invasive species in New England waters since the 1980's). These two species present a unique problem for ecologists, as temperature tolerance comparisons of different life history traits have shown conflicting results. For example, *Botryllus schlosseri* recruitment and peak abundance occur earlier in the summer than that of *Botrylloides violaceus*, but asexual reproduction rates suggest *Botrylloides violaceus* is more cold tolerant than *Botryllus schlosseri*. We examined the life histories of these two species to identify a characteristic that could explain these discrepancies and found one outstanding feature: the brooding period in *Botrylloides violaceus* lasts at least six times longer than that of *Botryllus schlosseri*. To assess if this elongated brooding period could account for the observed variations in temperature tolerances of the two species, the relationship between recruitment and temperature was reanalyzed using estimated fertilization times 2001-2006 recruitment data collected at the University of Connecticut Avery Point Campus and Groton Long Point Marina, CT as well as 2006 recruitment data from Hawthorne Cove Marina, the University of New Hampshire Coastal Marine Laboratory, and the University of Maine Darling Marine Center. *Botrylloides violaceus* colonies fertilized their eggs both earlier in the year and at colder temperatures than *Botryllus schlosseri* for all sites. The observed delay in recruitment and peak abundances were a result of the elongated brooding period, not a reduced cold tolerance in *Botrylloides violaceus*. Therefore an analysis of recruitment data without taking brooding period into account may not be fully representative of reproductive environmental requirements and tolerances for species with relatively long brooding periods.