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Cover: Coronilla varia L. (Fabaceae)

Cover photo: David Garbary

**Back cover photos:** Mike Dadswell, Richard Brown, David Garbary, Peter Wells. Photo of Dr. Gareth Harding, winner of the Visionary Award of the Gulf of Maine Council on the Marine Environment, 2013.



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# **EDITORIAL**

# Supporting Science in Canada

The NSIS celebrated its 150<sup>th</sup> anniversary in 2012. In its optimism, the Society assumed that Canada will be looking forward to another 150 years of science - natural and social, basic and applied - serving Canadian society and humanity as a whole. With a series of special lectures, members of NSIS were able to reflect upon the contributions of the citizens of Nova Scotia and the Maritimes to this field of endeavor, some of which have been recorded in the pages of the PNSIS over the years.

Yet presently, some areas of science in Canada are facing very challenging times. The country is being governed nationally by a federal government that, at best, views science solely as a way to boost the natural resource industry and the economy, and at worst is seen by many citizens to be actively opposed to basic science and the acquisition of scientific (evidence-based) knowledge, unless it has immediate economic benefits. The latter view is supported by the very long list of recent cutbacks to environmental and living resource science and science capacity within the federal public service, starting in 2006 (Dupuis 2013, Nikiforuk 2013, Wells 2013). These include the closure of publically supported research laboratories and programs (e.g., the ELA or Experimental Lakes Area program), the unparalleled closure of government libraries and archives, the stance against climate change (in terms of national and international policy, and related science support), the unprecedented and highly embarrassing withdrawal from important international environmental conventions (Kyoto, Desertification), the elimination of important advisory groups (e.g., the National Roundtable on Environment and Economy) and positions (Office of the National Science Advisor), and the obvious dislike of evidence-based policy making (see Dupuis 2013, Hodson 2012, Wells 2013, www.eiui.ca, among many others). To its credit, the NSIS wrote a letter to the Prime Minister to express its concern about this state of affairs, and after many months received an unconvincing reply, to which it has replied (see NSIS website). Clearly, given these challenges, NSIS should continue to be a visible advocate for science in Canada, and join with regional groups such as "Save Our Science"

in St. Andrews, NB, to collectively, constructively and persistently voice our opinions.

The current situation (circa Dec. 2013) raises many important questions. These include: Do the cutbacks of some critical areas of science represent the wishes of Canadians? Do we, as scientists, collectively need to make a major new argument in favor of science for the public good? Do the general public and their politicians understand the roles and values of science in a modern industrialized democracy? If not, why not? How do scientific societies such as the NSIS engage the public in discussions about the direction of and support for Canadian science? How can societies such as ours add to an appreciation of science, along with the outstanding efforts of people and programs such as Quirks and Quarks (Bob MacDonald, CBC), other CBC programs (Bob Fournier, science commentator, CBC Radio 1, Halifax), the Nature of Things (David Suzuki), and NOVA (PBS). At present, we seem to be living (some say sleep walking) at a scientific crossroad; parts of our scientific enterprise are changing significantly in favor of selected industry and technology driven imperatives. Note the recent shift in the focus and operation of the prestigious National Research Council in Canada; it was once the pinnacle of the country's basic science and technology, and now it has been reorganized (some say reduced) to focus on supporting industry-based research. Answers to the above questions, and others that NSIS members undoubtedly can think of, are of paramount importance to the role of science in Canada's future.

Science has been an underpinning of the Canadian nation since Confederation. Unfortunately, it has had very few national champions, with some exceptions – e.g., Sir Sanford Fleming, John Polanyi, Michael Smith, David Suzuki. We have a host of productive and renowned research universities. To date, Canada has had 12 Nobel prize winners in diverse scientific fields (chemistry, physics, medicine), and many other awards as well, such as the Stockholm Water Prize to David Schindler (University of Alberta), and honourifics such as the Order of Canada. Major contributions have been made to many modern science fields, including original breakthroughs from pharmacology (e.g. the discovery of insulin) to marine science (e.g., the trophic biomass model), and contributions to the needs of industry from oil to forestry to mining. As a very large country with a relatively small (35.1 million, 2013) and widely distributed population, we have done reasonably well and with caveats continue to do so, according to the most recent evaluations (Council of Canadian Academies 2012, Science, Technology and Innovation Council 2013).

The question is - will our scientific contributions continue? We can always do better, in many areas, if we had strong leadership and support from various levels of government, and from our leading scientific societies and academies. This raises another set of questions: How do we as a country set our science priorities and keep advancing? How do we combat the current major cutbacks in certain core areas of Canadian environmental science - aquatic sciences and toxicology (Hodson 2012, Wells 2013), climate change and Arctic research (e.g. closure of PEARL or the Polar Environment Atmospheric Research Laboratory, cessation of the Northern Research Chairs program, withdrawing funding from the Kluane Lake Research laboratory in the Yukon, amongst others). How do we retain or even regain our leadership position in many fields with our science oriented neighbours to the south (i.e., the USA) and in Europe? How do we produce a science literate and empathetic population, with an interest in discovery and creativity? How do we vigorously promote the STEM topics (science, technology, engineering, mathematics) in our school systems? How do we emphasize the various rewards that careers in science bring? There are many questions and challenges, all urgent to the economy and overall health of our nation, its citizens, and its natural landscapes, ecosystems and wildlife. But we are capable of finding appropriate answers and opportunities to lead us back on the right road.

As a modern, resource-based nation, we need a balance between support for the basic sciences and support for the applied and industry directed sciences. The latter should strive for objectivity and not become mired in controversy, e.g., witness the current case in Nova Scotia with a university accepting an industry funded grant in coastal aquaculture. We also need to support small, one or two-investigator driven programs as well as large, elite, multi-investigator ones; the former has proven many times to lead to fundamental discoveries. New discoveries of a fundamental nature form the bedrock of eventual applied use, mostly not seen at the time, e.g., lasers, which are now ubiquitous, are used from grocery stores to ophthalmology; describing the structure of DNA revolutionized molecular biology and led to dynamic new fields such as genomics. Most of these discoveries are the work of inspired and energetic single investigators, not teams, though this does not apply to all fields (e.g., astronomy and space exploration) and may change in the future. We cannot easily predict where and when the next major breakthroughs will occur. For example, it is believed that we are entering the phase of the third life sciences revolution, with "the convergence of the life sciences with the physical sciences and engineering, the first two revolutions being the discovery of DNA and genomics (Kastner 2009). What will be discovered can only be imagined, and much will be completely unexpected and serendipitous. To facilitate basic discoveries and then to move them to the stages of technology and innovation, we need a science literate politic in Canada.

To be fair, the federal government in Canada in recent years has been supporting large programs and centres of excellence in specific topics, under its Canada Foundation for Innovation (CFI) granting process. This has led to many large programs, from understanding the human-computer interface to establishing the healthy oceans network. Recently, the government has announced investing in a new research station in Cambridge Bay, Nunuvat for science and technology research; critics say its location is too far south, and not as good an investment as at smaller more northerly sites. The current government likes big "flashy" pronouncements and studies that suggest instant applications to industrial applications, and has been reducing funding for smaller grant applications and facility support through the Canadian research councils (NSERC, SSHRC, CHRC). Though perhaps well intended, this benefits the few, not the majority of researchers. As well, the government has been eliminating many areas of public service research, especially in the environmental sciences, e.g., its marine ecotoxicology, climate change and polar science programs.

Two recent examples of how Canada is faring with its scientific enterprise should be mentioned again. These are described in 2013 reports of the Science, Technology and Innovation Council (an advisory council to the Government of Canada) and the Council of Canadian Academies (STIC 2013; CCA 2012). While we aspire to global leadership, there are "vitally important areas where our performance is lagging, where we must improve – in some cases significantly" (STIC 2013). Importantly, it was pointed out that "Canada's gross domestic expenditures on R&D (GERD) declined from their peak in 2008...... and Canada's rank in 2011 is 23<sup>rd</sup> of 41 economies" (STIC 2013). The CCA report is a critical read – while putting a shiny face on what the country excels in, "half of the S&T experts surveyed considered Canada to have lost ground in the past five years" and described a

#### **EDITORIAL**

decline in natural resources and environmental S&T (CCA 2012). NSIS members are encouraged to read these reports and make their own judgement as to how well we are doing in Canadian science and technology as a whole, and specific scientific fields important to the future of the Atlantic region.

On the value of science as a whole enterprise, there are general insightful comments in a recent book by Edward Wilson of Harvard University (Wilson 2013), regarding science and the future, and the classics of Peter Medawar (Medawar 1980) Carl Sagan (Sagan 1990) and Freeman Dyson (Dyson 2006) are worth revisiting in this context as history tends to repeat itself. Importantly, there is a need to continue to interest young people in all aspects of science, something that NSIS has long recognized and attends to annually through its writing program.

In conclusion, we need to support basic science in the region, utilize the information and knowledge that we already have, and encourage evidence-based public policy and decision-making on a lot of issues. The NSIS can continue to foster discussion of the many issues pertaining to the role of science in NS and the Maritimes, and in the larger Canadian fabric. The Society can encourage the young to pursue their dreams in areas of scientific and personal discovery. And it can defend Canadian science when it is unwittingly attacked and diminished. NSIS has many critical roles here, including holding workshops or round tables to discuss these issues. It is hoped that its members will ponder this editorial, offer their views, write letters to their MPs on issues vitally important to this region and the country, and continue to support the Society's programs.

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# EARLY SPRING FLOWERING IN NOVA SCOTIA: AN EXTREME SPRING IS REFLECTED IN ADVANCED FLOWERING

# NICHOLAS M. HILL<sup>1</sup>, DAVID J. GARBARY<sup>2\*</sup>

<sup>1</sup>Fern Hill Institute of Plant Conservation Bentley Rd., South Berwick, Nova Scotia

<sup>2</sup>Department of Biology, St. Francis Xavier University Antigonish, Nova Scotia, B2G 2W5

# ABSTRACT

Twenty species of herbaceous plants and four non-amentiferous shrubs were found in flower in March-April in Nova Scotia during the spring of 2012. Plants were observed primarily in Kings and Antigonish Counties, with several observations from Inverness County. The precocious flowering is attributed to an abnormally warm late winter and spring (February-March) in which climate normals for monthly average temperature were exceeded by a minimum of 1.2°C in February (Tracadie) to a maximum of 8.5°C in March (Kentville). Flowering was an average of 17 days earlier than herbarium records in the largest regional herbaria (ACAD, NSAC). Proportional contribution to the early flowering guild was greater for exotic species which featured weedy families not represented in the native group. These observations of spring climate conditions and flowering phenology are consistent with regional climate change associated with milder and earlier springs.

Key Words: climate change, Nova Scotia, phenology, spring flowering, exotic range expansion.

#### **INTRODUCTION**

Global climate change over the course of the 20<sup>th</sup> century is well established. It is generally recognized that mean air temperature has increased 0.5-0.6°C and that biological systems have responded accordingly (e.g., Houghton et al. 2001, McCarty 2001, Menzel et al. 2005, Walther et al. 2005, Core Writing Team IPCC 2007). Biological responses to global climate change have often been associated with changes in migration timing, distribution of organisms with regard to

<sup>\*</sup> Authors to whom correspondence should be addressed: fernhillns@gmail.com or dgarbary@gmail.com

elevation, changes in animal breeding times, and various aspects of plant phenology: timing of leaf bud burst, onset of leaf colour change and flowering phenology. Menzel (2003) argued that aspects of plant phenology provided an excellent proxy for temperature, and there is an extensive literature on advancement of flowering times in relation to climate change (e.g., Abu-Asab et al. 2001, Fitter and Fitter 2002, Gu et al. 2008, Houle 2007, Miller-Rushing & Primack 2008, Panchen et al. 2012).

Herbarium records of flowering phenology in Nova Scotia during the 20<sup>th</sup> century are concentrated between May and October, with a few stragglers in either spring or fall (Roland 1998). In Nova Scotia, responses of plants to changing temperature regimes have emphasized late fall flowering in which extensive flowering after November 1 has been documented. Thus, in the years following 2000 there have been two years when over 90 and 130 species flowered after November 1, and flowering continued into December and January in 2001 and 2006, respectively (Garbary and Taylor 2007, Garbary et al. 2012, Taylor and Garbary 2003). The highlight of these accounts was the occurrence of 20 species flowering in January, with the last day being January 21. The demonstration of advancement of spring flowering in Nova Scotia has been more problematic. Despite a well-documented increase in spring temperatures over the 20th century, Vasseur et al. (2001) were able to demonstrate limited advance in flowering that was associated with only a single cultivated shrub, Syringa vulgaris.

In this paper we demonstrate that temperatures for the late winter and spring of 2012 in Nova Scotia were considerably elevated relative to climate normals (Kings Co.) or for the previous nine years (Antigonish Co.). We then provide observations of flowering of 24 species during March and April, and compare the resulting phenology with the earliest flowering records in regional herbaria.

Given the record setting temperatures in mid March, we ask whether or not plants responded by coming into flower earlier than normal. We evaluate the resulting phenology in the context of potential impact of regional climate change.

# METHODS

The burst of abnormal temperatures in mid-March prompted us to examine the potential response of flowering in two areas of the province: Kings County in the Annapolis Valley (NH) and Antigonish County in northern Nova Scotia (DG). Habitats included agricultural fields and orchards, disturbed ground and building lots and natural forest communities. We avoided heat islands associated with urban developments such as garden plots immediately adjacent to buildings or at the base of walls. Records of flowering were compared with accounts in Roland (1998), records from herbaria at Acadia University (ACAD), Nova Scotia Agricultural College (NSAC) and St. Francis Xavier University Herbarium (STFX). An online database associated with the E.C.Smith herbarium (i.e., ACAD, http://herbarium.acadiau. ca) also includes records from Cape Breton University Herbarium. We avoided the catkin producing genera (i.e. *Alnus, Populus and Salix*) as they may be regarded as winter flowering, and many began flowering before our observations commenced. We also did not include trees (e.g., *Acer* spp.).

Climate data for Nova Scotia were obtained from the internet (http://www.climate.weatheroffice.gc.ca/Welcome\_e.html). Kentville (45°04'N, 64°29'W, Kings Co.) was used as the reference site for South Berwick (45°1.4'N, 64°42.6'W) where most of the observations were made by NMH. Tracadie (45°36'N, 61°40'W, Antigonish Co.) was used as the reference site for Antigonish town and environs (45°37.4 N, 61°59.5'W) where most of the observations were made by DJG. The Tracadie weather station has only been operating since 2003, and 'climate normals' were calculated based on data averaged from 2003-2011. Data for snowpack at the end of each month were only available for Kentville.

### RESULTS

#### Climate

As shown by the climate normals, February through April are not particularly conducive to plant growth and flowering in Nova Scotia (Table 1). There is typically extensive snowfall, and considerable snowpack remaining at the end of each month. While the data for Antigonish Co. are not available, the lower temperatures would suggest even greater snowpack than for the Annapolis Valley. The mild winter of 2012 was characterized by an 88% reduction in snowpack at the end of February and 100% reduction in March and April.

The relative absence of snow was reflected in the differences in temperature regimes between climate normals and 2012 (Table 1,

February-April 2012.						_
Climate normals	February	Kentville March	April	February	Tracadie March	April
Daily average (°C)	-5.2	-0.9	4.9	-5.0	-2.1	4.1
Daily maximum (°C)	-0.9	3.4	9.5	7.8	2.1	8.3
Daily minimum (°C)	-9.5	-5.2	0.4	-19.6	-14.2	-5.1
Degree days > 5°C	1.8	8.7	46.1	0.2	4.3	33.4
Snow on ground at						
month end (cm)	59	46	17.3	_	_	_
Weather 2012						
Daily average (°C)	-3.5	7.6	12.6	-3.8	5.2	10.4
Maximum (°C)	7.3	25.7	23.0	9.7	25.4	19.8
Minimum (°C)	-15.8	-11.4	-4.8	-18.6	-11.5	-6.1
Degree days $> 5^{\circ}C$	0	41.2	78.8	0.6	28.8	74.6
Snow on ground at						
month end (cm)	7	0	0	_	_	_

 Table 1
 Climate normals for Kentville (Kings Co., Annapolis Valley, 1971-2000), and Tracadie (Antigonish Co., 2003-2011), and weather regimes during February-April 2012.

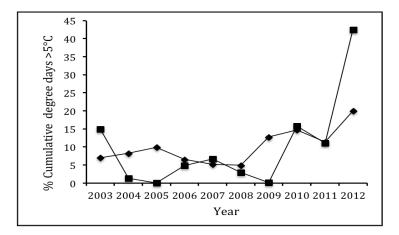


Fig 1 Percent contribution to decadal total of cumulative degree days >5°C for each of the individual years (2003 to 2012) for March (boxes) and April (diamonds).

Fig. 1). In both of the primary areas, climate normals showed daily averages below zero for February and March, and below 5°C for April (Table 1). The daily average temperature was 1.7°C and 1.2°C warmer for February relative to climate normals in the two areas, and these differences became more extreme in March and April. For Kentville, daily average temperatures were 8.5°C and 7.7°C warmer than normal for March and April, and the equivalent values for Tracadie were 7.3°C

and 6.3°C. Average daily maximum temperatures for climate normals did not exceed 10°C and extreme minima were -14.2°C and -5.1 for March and April, respectively. During spring, degree days >5°C are normally limited in Nova Scotia, with values of 4.3 and 8.7 (March) and 33.4 and 46.1 (April) for the two areas. Degree days >5°C are shown for Tracadie from 2003-12 (Fig 1), and the values for 2012 are extreme relative to previous years, with a 2.2 to 6.7 fold increase relative to means of the previous nine years, and a 30% to over 200% increase relative to previous maximum values.

#### **Plant Phenology**

Observations made of first flowering times for 24 plant species were compared with the first flowering records found among the collections in the E.C. Smith (ACAD) and Roland (NSAC) Herbaria (Table 2). On the one hand, since all of these records were based on opportunistic sampling across a wide geographic area (northern Cape Breton to the Annapolis Valley), we do not claim that the dates represent first flowering of the species. However, our observations are likely very close to first flowering in the following species: 1) Draba verna and Daphne mezerium from Antigonish where the sites were visited every few days until anthesis; 2) Senecio vulgaris from Antigonish where a single individual had only a few blossoms; and 3) Tussilago farfara where only a single site had a few flowers, when other populations were still in bud. On the other hand, many individuals of Caltha palustris were in flower in roadside wetlands around Cheticamp, suggesting that it had been in blossom for at least several days. In all but four species, first flowering was earlier in 2012 than for the herbarium specimens, a record spanning more than a century. Overall, flowering was  $17.5 \pm$ 17.4 days earlier than the earliest records in the herbaria. One of the species for which we did not claim an earlier record was for Taraxacum officinale for which the record comes from Sable Island with the habitat "west side of meteorological station" (ACAD #31833). We did not include observations of this species from equivalent heat islands in Antigonish where plants were in flower by April 1.

Five species were observed in both the Annapolis Valley and Antigonish Co. during March and April. In every case the Annapolis Valley observations were earlier, and the difference ranged from 2-20 days (mean of 10 d). At least four additional conspicuous species had not come into flower in Antigonish Co. that would have been documented if they had flowered. Table 2List of species collected and dates of collection in Annapolis Valley (Kings<br/>County) and Antigonish and Inverness Counties in 2012 with earliest previous<br/>collections based on records at E.C. Smith Herbarium (ACAD) and the A.E.<br/>Roland Herbarium (NSAC). Abbreviations: N- native, E- exotic, NO- not<br/>observed.

Species	Origin (native or exotic)	Collection date	2	Earliest Nova Scotia record in ACAD or NSAC	# days of advanced flowering
		Valley	Antigonish and Inverness C		
Amelanchier laevis Wieg.	N	Apr 25	NO	Apr 8, 1938	-17
Caltha palustris L.	Ν	Absent	April 27	May 24, 1999	27
Capsella bursa-			-	-	
pastoris (L.) Medikus	E	Mar 24	NO	Mar. 28, 1910	4
Cerastium vulgatum L.	E	NO	April 18	May 24, 1979	36
Claytonia caroliniana					
Michx.	Ν	NO	April 29	May 1, 1948	2
Corylus cornuta Marshall	N	Mar 25	NO	Apr 25, 1928	31
Daphne mezerium L.	E	Mar 25	April 8	Apr 10, 1920	16
Draba verna L.	E	Mar 17	April 6	Apr 14, 2002	28
Epigea repens L.	Ν	NO	April 15	Apr 20, 1928	5
Fragaria virginiana					
Duchesne	Ν	Apr 16	April 28	Apr 25, 1998	9
Glechoma hederaceae L.	E	NO	April 18	May 8, 1936	20
Hedyotis caerulea (L.)					
Hook.	Ν	Apr 20	NO	May 10, 1980	20
Lonicera canadensis Bart	r. N	Apr 20	April 29	May 1, 1997	2
<i>Myosotis scorpioides</i> L.	E	NO	April 29	May 14, 1980	15
Sanguinaria canadensis I	. N	Apr 18	April 20	May 17, 1952	29
Senecio vulgaris L.	E	Not	April 15	June 16, 2001	62
Stellaria media (L.)					
Cyrillo	Е	Mar 28	NO	Apr 20, 2002	23
Taraxacum officinale L.	Е	NO	April 26	Apr 25, 1953	-1
Tussilago farfara L.	Е	NO	March 20	Apr 25, 1995	36
Vinca minor L.	E	Apr 15	NO	May 20, 1949	35
Viola cucullata Ait.	Ν	Apr 30	NO	Apr 30, 1980	0
Viola macloskeyi Lloyd	Ν	Apr 30	NO	Apr 23, 1980	-7
Viola pubescens Ait.	Ν	Apr 30	NO	May 26, 1977	26
Viola tricolor L.	E	NO	April 26	May 16, 2002	20

# DISCUSSION

Atypical temperatures for one spring are not indicators of climate change, though they may be useful for indicating how communities may shift with the elevated temperatures of climate change. Spring of 2012 was exceptionally warm over much of North America, and March and April in Nova Scotia broke records. Temperatures in excess

of 25°C were recorded for several days in March and this atypical warmth followed a particularly mild winter with relatively little snowfall (Table 1). Subsequent spring weather in April was also above normal, although to a lesser extent than March.

How different members in a plant community respond to such a pulse of increased temperature may well reflect the inherent plasticity of different species. This in turn may be an indicator of performance over the next century when temperatures in temperate regions are projected to increase by a further 2°C.

Our scope was narrow: we set out to record the first species to flower in 2012 and to compare these flowering dates to historical data of flowering in local herbaria. It has been documented that flowering is occurring at earlier times of year in recent years than in the past, and Miller-Rushing and Primack (2008) have used historical data at Walden Pond, MA, to demonstrate this. Flowering onset in the temperate region is determined mainly by temperature (Miller-Rushing and Primack 2008); soil moisture levels and precipitation do not appear to be limiting factors in most years. Panchen et al. (2012) used a combination of herbarium records, photographs and naturalists' notes to document earlier flowering over the last century around Philadelphia. While we only formally consulted herbarium records, our informal observations over the last 20 years are congruent with the unusual phenology observed in 2012. Another botanical indicator of an early spring in Antigonish Co. included a harvest of Matteucia struthiopteris (L.) Todaro on April 29; this predates the earliest previous harvest from the last 25 years of May 10 (Dolna Garbary, personal communication). Several additional species of spring wildflowers showed expanded bud development at the end of April (Panax trifolius L., Erythronium americanum Ker-Gawler, Clintonia boreale (Aiton) Raf., Maianthemum canadense Desf.); however, we were unable to return to the site to observe anthesis of these species.

Recent weather extremes in late fall and early winter were associated with late fall flowering in 2000 when flowering terminated in mid December (Taylor and Garbary 2003) and in 2005-2006 when flowering extended until 21 January (Garbary et al. 2012, Garbary and Taylor 2007). While there is realistically a two-month gap between the dates of extreme late flowering in 2006 and the extreme early flowering in 2012, these records suggest that with a repeat of the late fall-early winter conditions of 2005-2006, accompanied by an early spring (i.e., as in 2012), we would anticipate flowering in 11 months of the year.

Vasseur et al. (2001) examined long-term records of spring phenology in selected plants in Nova Scotia using historical records between 1892 and 1923 and more recent observations from 1998 based on Nova Scotia Plantwatch. Only one species, *Syringa vulgaris*, showed significant flowering at an earlier date. Vasseur et al. (2001) used their analysis to point out the earlier flowering in the southern and western parts of Nova Scotia relative to the northern and eastern portions where there is a harsher climate zone (zone 5 versus 6). This change in climate zones is consistent with our observations on nine species. Regardless of the fact that the warm March weather was apparent in most of the province, flowering in Antigonish was about 10 days later.

Native and exotic taxa were equally represented among the 24 early flowering species. Since exotic species are a minority of the total flora, a weighted analysis was required. Exotics account for more than a third of the flora in Nova Scotia (Hill and Blaney 2010) but even this overestimates their real frequency, since many of these exotics are transitory and fail to become established in the regional flora. When a Chi-square analysis is performed using only the common native and exotic species, (i.e., subtracting the rare and transient species from both groups), exotic species are significantly (p<0.05) overrepresented in the early spring flowering guild. In addition to a greater proportional representation of exotics among the early flowering plants, their seasonal shift in flowering time, from the earliest records in herbaria, was significantly earlier than that of native plants (see Table 2). The average shift in flowering time was two weeks earlier for the group of exotic plants than the natives (2012 flowering time shifts= 24.5  $\pm$  16.7 versus 10.6  $\pm$ 15.7 days for exotics and natives respectively). Many of the exotic species are ruderals, plants adapted to frequently disturbed habitats through fast growth rate and early and often lethal reproduction (Grime 1979). The evolution of this set of ruderal traits is pronounced in several highly successful families which are also those best represented among the world's worst weeds (Huston 1994). Early flowering exotic representatives in Nova Scotia were with one exception all short-lived herbs representing three of the top weedy families (3 species of Asteraceae, 2 Brassicaceae, and 1 Lamiaceae) that contribute 10 or more species to the World's Worst Weeds (Holm et al. 1979, cited in Huston 1994). Although herbs were best represented among the early flowering native plants, this group shared only one family with the exotics (Violaceae) and it represented only one of the common weedy families (viz. the Rosaceae, 2 represented in native groups). Based on the above differences in their relative contribution to the early flowering records, their 2012 flowering shift, and their phylogenetic origins, these exotics are clearly the group poised to expand, given a fundamental change in spring climate.

Our observations provide the earliest flowering records of over 20 species based on herbarium records, and these records average 17 days earlier than the earliest of these specimens. While it is tempting to conclude that we have shown an advancement of 17 days in average flowering, such a conclusion is moderated by the fact that our records are based on haphazard observations in 2012 rather than systematic observations over many years. Furthermore, it is unlikely that previous herbarium specimens were collected to demonstrate earliest flowering times. Regardless, our observations provide a baseline in two areas of Nova Scotia for first flowering times for some conspicuous wildflowers. The congruence of record spring temperatures (Table 1, Fig. 1) along with the flowering phenology (Table 2) suggests that flowering was indeed advanced for these species.

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# Coronilla varia L. (Fabaceae): AN INVADER OF A COASTAL BARRIER BEACH IN NOVA SCOTIA, CANADA

# ANDREA FLYNN, ANTHONY G. MILLER and DAVID J. GARBARY<sup>\*</sup>

Department of Biology, St. Francis Xavier University, Antigonish, NS B2G 2W5 Canada

# ABSTRACT

*Coronilla varia* L. (crown vetch) is described as an invasive plant on a coastal sand dune system (Mahoneys Beach) in Nova Scotia facing the southern Gulf of St. Lawrence. This is the first time that *C. varia* has been shown as invasive in Atlantic Canada, and the first time it has been characterized as invasive on coastal sand dunes. Accordingly, colonies of *C. varia* forming more than 90% of the plant cover occupy 8% of the total dune system and account for 32% of the area in the vegetation zone where *C. varia* has become abundant. *C. varia* can achieve high percentage cover, with all of the major dune plants, i.e., *Ammophila breviligulata*, *Lathyrus japonicus*, *Toxicodendron radicans* and *Myrica pensylvanica*. Where *C. varia* achieves maximum abundance (i.e., >80% cover and ca. 200 shoots m<sup>2</sup>), it has seemingly replaced *A. breviligulata* and *L. japonicus*. We do not know when *C. varia* first colonized Mahoneys Beach, but conspicuous colonies were recorded there by 2005, and a colony was found on the adjacent dune system (Dunns Beach) for the first time in 2010.

Key Words: *Coronilla varia*, crown vetch, Fabaceae, invasive species, Nova Scotia, sand dunes

#### **INTRODUCTION**

Many recent studies have focused on the substantial threat that invasive alien plants pose to global biodiversity (e.g., Callaway & Aschehoug 2000, Lake & Leishman 2004, Pyšek et al. 2004, McClay & Faechner 2008, Rejmanek 2011). In Nova Scotia, roughly one third of the terrestrial vascular plants are alien species, most of these are found in highly disturbed sites, but only a handful may be considered invasive, i.e., cause significant displacement of native species in natural communities. A recent review by Hill and Blaney (2009) contained

<sup>\*</sup> Author to whom correspondence should be addressed: dgarbary@gmail.com

lists of both herbaceous and woody alien plants in Nova Scotia, and discussed some species that were likely to become significant threats (i.e., invasive) in native plant communities. Our interest here is the coastal sand dune ecosystem, which is under threat from anthropogenically derived disturbances and from sea-level rise (Beveridge 2012). Among these disturbances is the potential impact of invasive species. One woody plant suggested by Hill & Blaney (2009) as a potential invasive alien on coastal sand dunes was Rosa rugosa Thunb. Hill et al. (2010) later demonstrated its invasive properties on a series of sand dune systems on the north shore of mainland Nova Scotia and the west coast of Cape Breton Island. Here we provide the results of an investigation of Coronilla varia L. (crown vetch), on a coastal sand dune. Whereas C. varia establishes well in sandy soils (Gucker 2009), we found no account regarding its occurrence on sand dunes, and we describe it as an invasive species in this habitat for the first time. While Hill and Blaney (2009) did not consider C. varia as a potential invasive plant in Nova Scotia, it has become invasive elsewhere in North America in a wide variety of plant communities including roadsides, open fields, waste areas and gravel bars along streams (Harper-Lore 1996, Tu 2003, Symstad, 2004, Gucker, 2009, Losure et al. 2009).

Coronilla varia [also known as Sercurigera varia (L.) Lassen] is native to the Mediterranean region of Europe, southwest Asia, and northern Africa (Roland 1998), suggesting that it is adapted to arid environments such as the coastal sand dunes that are the focus here. The species was first introduced to North America in the 19th century and has subsequently spread throughout the continental United States (except Alaska and North Dakota) and all Canadian provinces (Thompson 1997, Gucker 2009, USDA 2009). Its main use in Nova Scotia has been for roadside erosion control (Roland & Smith 1969, Hill & Blaney 2009), and, like other Fabaceae, it is a nitrogen fixer (Cardina & Hartwig 1988, Madigan et al. 2010). The species is now classified as an invasive alien species in the USA (Harper-Lore 1996). However, Roland & Smith (1969) considered C. varia as "doubtfully hardy" in Nova Scotia, and it has only been classified as alien in Nova Scotia, not invasive (NatureServe 2011). Here we describe the ecology and natural history of C. varia on Mahoneys Beach, a coastal sand dune on the north shore of Nova Scotia facing the southern Gulf of St. Lawrence. While we do not know when C. varia colonized Mahoneys Beach, it was not recorded here or on any of the 45 Nova Scotian sand beach systems > 1 km long studied by Hales (1992), or on any of the 11 beaches surveyed on the west coast of Cape Breton Island by Beveridge (2012). *C. varia* was recorded by one of us (AGM, unpublished field notes) on Mahoneys Beach from 2005. By 2010 it had become abundant and we initiated the current study.

# MATERIALS AND METHODS

This study took place on Mahoneys Beach (now Mahoneys Island), an extensive sand dune system in St. Georges Bay, along the extreme southern portion of the Gulf of St. Lawrence of Nova Scotia (Fig 1).

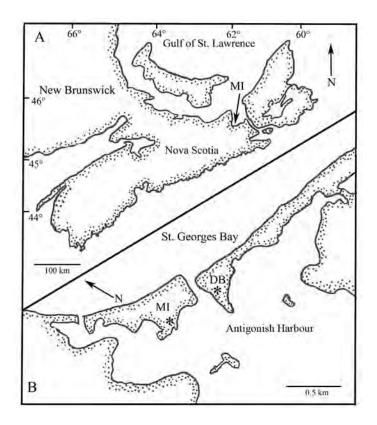


Fig 1 Map of Nova Scotia (A) indicating general location of Mahoneys Island (MI, arrow), and (B) enlargement of barrier beach system for Antigonish Harbour, showing Mahoneys Island (MI) and Dunns Beach (DB). Asterisks in B indicate outlier populations of *Coronilla varia*.

The sand dune is a 1.2 km long island, formed when it separated from the original 1.6 km spit on December 26, 2004 during a storm. Currently (2013), the island is relatively undisturbed by human activity and it has been a successful nesting site for the regionally rare shorebird *Charadrius melodus* (Ord) (i.e., Piping Plover). There is severe scarping visible on the seaward side of the island and the dune is retreating by an average of  $1.2 \text{ m y}^{-1}$ , with the western end recently retreating by over 5 m y<sup>-1</sup> (Garbary and Miller, unpublished observations). In addition to the impact of sea level rise and regional subsidence, armouring of a headland adjacent to the beach is starving the shore of sediment (Utting and Gallacher 2009).

A walking survey in the late summer of 2011 (September 1) was performed to locate all dense colonies of *Coronilla varia* on the island. Colonies were measured using a transect line to obtain dimensions for area calculation, and coordinates of each colony were recorded using a Garmin eTrex (Olathe, Kansas) GPS unit. Colonies were considered dense when cover of *C. varia* was at least 80%. In 2010, *C. varia* was first observed on Dunns Beach and a similar colony survey was conducted there in September 2012.

Irradiance was determined inside a dense stand of *C. varia* and on adjacent non-vegetated sand, using a HOBO Pendant Temperature/ Light Data Logger (UA-002-XX, Onset Computer Corporation, Pocasset, MA). While the HOBO records light in 'lumens', these data were converted to photosynthetically active radiation (PAR) based on the proportion of the overall spectrum between 400 nm and 700 nm sensed by the HOBO. The HOBOs were suspended 10 cm above ground level and data were collected from July 21 to August 31, 2011, at 10 min intervals. A LI-COR light meter (LI-250, Lincoln, NE) with a cosine corrected detector was used to determine photosynthetic photon flux density ( $\mu$ mol m<sup>-2</sup> sec<sup>-1</sup>) at ground level within quadrats (n = 5) where either *C. varia* or its primary associated species, i.e.,

 Table 1
 Ground level irradiance underneath five species following removal of the primary competitor within each quadrat (n = 5).

Species	Irradiance (µmol photons m <sup>-2</sup> sec <sup>-1</sup> )	
Coronilla varia	$565 \pm 69$	
Ammophila breviligulata	$561 \pm 109$	
Lathyrus maritimus	$432 \pm 136$	
Toxicodendron radicans	$419 \pm 87$	
Myrica pensylvanica	$217 \pm 88$	

Ammophila breviligulata Fern. (beach grass), Lathyrus maritimus (L.) Bigelow (beach pea), Myrica pensylvanica Mirbel (bayberry) and Toxicodendron radicans (L.) Kuntz (poison ivy), had been removed. Data were collected on a day with full sun between 10 am and 2 pm. The resulting data (Table 1) were used to infer whether or not light limitation has a role in competitive interactions between *C. varia* and the other species.

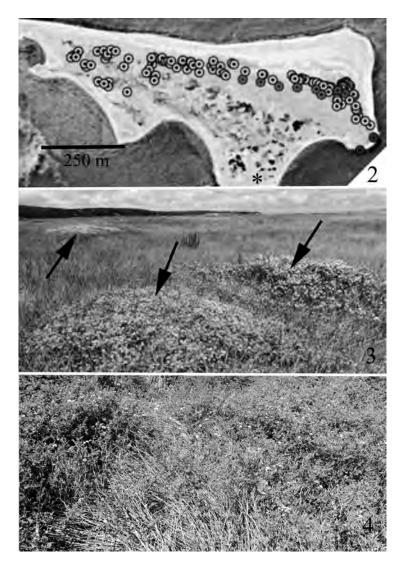
Soil pits were dug to 1.0 m depth within pure stands of each of the dune species, and the depth of root penetration into the sand dune by the various species was observed. Individual shoots were removed with intact roots and rhizomes for photography. Accordingly, differences in zonation would permit inferences regarding below-ground competition.

In the same year (2011) that we mapped colony distribution of Coro*nilla varia*, we determined cover and biomass values in a series of 24 plots where C. varia was associated with one of four abundant native plants on Mahoneys Beach, i.e., Ammophila breviligulata, Lathyrus maritimus, Toxicodendron radicans, and Myrica pensylvanica. In early June 2011, for each of the species associations, six experimental plots (replicates) were established (each 1 x 3 m) with three subplots (each 1 x 1 m), and percent cover of the major species was estimated visually in each subplot. In subplot 1, C. varia was removed by clipping at ground level, in subplot 2 (control) no harvesting occurred, and in subplot 3, the other species were harvested by clipping at ground level. For each subplot the percent cover of the major species were determined in early June, 2011. In late July the two harvested subplots were again evaluated for cover and biomass, and the control subplots were not harvested. These plots were established to experimentally evaluate competitive interactions between C. varia and each of the other species. Thus, we evaluated the following pairs of questions for each species combination: 1) when C. varia is removed, does Species 2 increase in cover and biomass relative to control plots, and 2) when Species 2 is removed, does C. varia increase in cover and biomass relative to control plots. These plots were to be re-evaluated in 2012; however, beach erosion in the winter of 2012, and overwash of the primary portion of the beach had removed most of the plots. For each species combination in the experiment, the Wilcoxon paired sample test (Zar 1999) was conducted in which the final values for cover and biomass in 2011 were evaluated against the values in the control subplots where no initial harvest was done.

# RESULTS

Field surveys revealed that colonies of *Coronilla varia* were abundant and easy to locate on Mahoneys Beach (Figs 2, 3, 4). The colonies were considered dominant (containing almost exclusively *C. varia* plants), when cover of *C. varia* was at least 80%. Small *C. varia* plants were also observed colonizing open sand, sometimes alongside native species, such as *Lathyrus maritimus*. The total area of Mahoneys Beach (island portion) in 2011 was estimated to be 342,144 m<sup>2</sup>. Of the total area of the island, the area of the zone inhabited by *C. varia* was 93,312 m<sup>2</sup>. Within the zone inhabited by *C. varia*, the total area of all the colonies of *C. varia* was 29,985 m<sup>2</sup>. Therefore, dense colonies of *C. varia* took up 32% of the zone that was inhabited by *C. varia*, and 9% of the entire island. In addition, it occurred with other species where it had lower cover.

There were 125 dense colonies present on Mahoneys Beach on the dune proper (Fig 2). In addition, a small population was found amongst populations of Rosa virginiana Mill. adjacent to a small salt marsh and grove of Populus tremuloides Michx. on the extreme landward side of the dune system. The average area of the Coronilla varia colonies was  $238 \pm 578$  m<sup>2</sup> (mean  $\pm$  s.d.). The large standard deviation resulted from a highly skewed size distribution (Fig 5) in which 88 out of 125 colonies were between 0 and 50 m<sup>2</sup>, and 5 colonies were more than 2,000 m<sup>2</sup>. There was also one very large, continuous colony of C. varia in the middle of the island that was approximately 330 m long and had an area of nearly 9,100 m<sup>2</sup>. This very large colony was flanked on the northwest and southeast ends by many smaller colonies. The southeast end of Mahoneys Island had more small clumps than the northwest end. On the southwest end, the spaces between colonies with native plant species also contained C. varia, but the latter tended to have lower cover values (25-75%). Therefore, C. varia had spread over almost the entire southeast end of the island. If cover values lower than 80%had been used to define a colony, then the entire southeast end would have been considered a single colony. These areas of lower cover value also occurred elsewhere. There were large areas on the northwest end of Mahoneys Island that only contained native or non-invasive alien species (i.e., no C. varia), unlike on the southeast end of the island where C. varia was certainly the most conspicuous plant species, and there was almost no area where only native species were present.



- Fig 2 Mahoneys Beach with 125 colonies of *Coronilla varia* indicated (round markers) and outlier population associated with *Rosa virginiana* and copse of *Populus tremuloides* (asterisk). Scale bar = 250 m.
- Fig 3 Portion of Mahoneys Island dune dominated by *Ammophila breviligulata* showing three clumps (arrows) of dense *Coronilla varia*, each about 5 m diameter.
- Fig 4 Portion of continuous sward of *Coronilla varia* with 100% cover showing dense aggregation of stiff, erect shoots; photograph shows about 5 m of width within a larger colony.

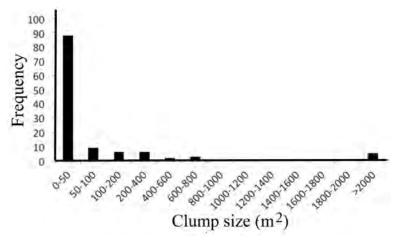


Fig 5 Size frequency distribution of Coronilla varia colonies on Mahoneys Island.

*Coronilla varia* achieved high biomass on Mahoneys Island, with biomass up to almost 700 g m<sup>2</sup> dry mass. The relationship between cover and biomass was highly variable, even in colonies where there was 100% cover (Table 2). Plants of *C. varia* have very robust stems that allow them to be self-supporting, and over 70% of the above ground biomass is in stems with about 20% in leaves and the balance in flowers and fruits.

While our initial plan for the experimental evaluation of competitive interactions between Coronilla varia and four other species was not fully realized (due to loss of plots from dune retreat), it did allow us to evaluate changes in growth of C. varia in the presence of other species. In these 1 x 1 m quadrats the cover of C. varia tended to increase when in the presence of the other species, i.e., from 13% to 32% during the four to six week experimental period (Tables 3 and 4). While none of these increases in growth were statistically significant at p < 0.1, when values for increase in cover for all four species were combined, C. varia showed a significant increase (Student's t test, p < 0.1). In the same quadrats, three of the four other species (all but L. japonicus) tended to decrease in cover; however, this decrease was only significant for A. breviligulata (Student's t test, p < 0.05). When the cover values for the four other species were combined, these species collectively showed a significant decline in the same quadrats where the *C*. *varia* had increased (Student's *t* test, p < 0.05).

	Erect shoots m <sup>2</sup>	Total Dry Mass (g m <sup>-2</sup> )	Stems (%)	Leaves (%)	Flowers and fruits (%)
Minimum	100	254	71.5	18.1	10.3
Maximum Mean ± s.d.	$292$ $199 \pm 66$	679 437 ±160	73.5 $70.3 \pm 3.8$	23.8 $22.4 \pm 4.9$	2.6 7.4 ± 4

 Table 2
 Annual, above-ground production of Coronilla varia on Mahoneys Island in early September 2011 from different colonies with 100% cover (n=10).

 Table 3
 Percent cover and biomass of Coronilla varia in naturally occurring quadrats with Species 2, i.e., Ammophila, Toxicodendron, Myrica and Lathyrus (mean ± s.d., n=6). Note Time 1 is mid June and Time 2 is late July 2011.

	Cover Time 1 (%)	)	Cover Time 2 (%)	Ti	Biomass ime 2 (g m	-2)
Coronilla with:	Coronilla	Species 2	Coronilla	Species 2	Coronilla	Species 2
Ammophila Toxicodendron Myrica Lathyrus	$30.0 \pm 9.5$ $12.5 \pm 7.6$	$\begin{array}{c} 40.0 \pm 20.7 \\ 42.5 \pm 11.7 \end{array}$	$\begin{array}{c} 24.2 \pm 10.7 \\ 34.2 \pm 16.3 \\ 20.8 \pm 13.2 \\ 30.0 \pm 13.2 \end{array}$	$29.2 \pm 7.4$ $40.0 \pm 21.7$	$\begin{array}{c} 103 \pm 31 \\ 62 \pm 49 \end{array}$	$217 \pm 75$ $378 \pm 266$

Table 4Increases in percent cover of Coronilla varia and other dune species after<br/>four weeks following removal in experimental plots with four different<br/>species. Note: control subplots were between and contiguous with clipped<br/>plots (Wilcoxon paired-sample test, n=6; NS, not significant at p < 0.1).</th>

Experiment (Species 2)	Response of Species 2 after removal of <i>Coronilla</i>	Response of <i>Coronilla</i> after removal of Species 2		
1. Ammophila	NS	NS		
2. Lathyrus	p < 0.1	p < 0.1		
3. Myrica	NS	NS		
4. Toxicodendron	p < 0.1	NS		

Average daily maximum irradiance within pure stands of *Coronilla* varia in mid-summer was  $246 \pm 417 \,\mu$ mol photons m<sup>-2</sup> sec<sup>-1</sup>. This was about 25% of the value from an adjacent non-vegetated area (935 ± 1120  $\mu$ mol photons m<sup>-2</sup> sec<sup>-1</sup>). The LI-COR irradiance measures taken from beneath stands of the various species on a single day showed species-specific variation in light transmission. In stands where *C*. varia had comparable cover to the other species, light penetration was similar for *C*. varia and Ammophila breviligulata (Table 1), reduced by one third under Lathyrus maritimus and Toxicodendron radicans,

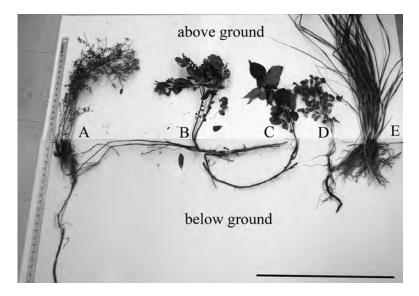


Fig 6 A comparison of root-rhizome vertical extents from A) Coronilla varia,
B) Myrica pensylvanica, C) Toxicodendron radicans, D), Lathyrus maritimus and E) Ammophila breviligulata. Scale bar = 50 cm.

and reduced by two thirds under canopies of *Myrica pensylvanica*. The latter is comparable to the irradiance under pure stands of *C. varia*.

When growing with four other sand dune species (Table 3), *Coronilla varia* showed a range in biomass from  $62 \pm 49$  g m<sup>-2</sup> of dry mass when growing with *Myrica pensylvanica*, to  $127 \pm 98$  g m<sup>-2</sup> when growing with *Lathyrus maritimus*. While these extremes are not statistically significantly different (p > 0.1), they do illustrate a trend for *C. varia* to move into the spaces occupied by other species. Furthermore, this must be a transitory stage from successful colonization to virtual elimination of the other dune species. This is particularly evident in the large portions of Mahoneys Island previously occupied by *Ammophila breviligulata* and *L. maritimus*, where *C. varia* is the dominant (i.e. > 85% cover) and can achieve biomasses of over 400 g m<sup>-2</sup> (Table 2).

Depth of root penetration varied among the different species on the sand dune (Fig 6). All of the native dune species (*Myrica pensylvanica, Toxicodendron radicans, Lathyrus maritimus and Ammophila breviligulata*) were mostly shallow rooted, with roots and rhizomes extending down to 20-30 cm, with only a few thin roots penetrating deeper. In a monospecific clump of *Coronilla varia*, roots extended beyond the final pit depth of 100 cm.

# DISCUSSION

*Coronilla varia* possesses characteristics of typical invasive species (Huenneke & Thomson 1995, Maron & Connors 1996, Symstad 2004; Walck et al. 1999). It often out-competes native species and forms large single-species stands (Harper-Lore 1996, Tu 2003, USDA 2006). C. varia suppresses the growth of other plants, and experiments by Ang et al. (1994, 1995) showed that it could suppress even the invasive Canada thistle. Baskin et al. (2000) showed that the number of reproductive structures increased on the endangered plant Solidago shortii Torrey & Gray when C. varia was removed from experimental plots, again demonstrating that C. varia suppresses the development of surrounding plants. This is consistent with our experimental plots where cover of Lathyrus maritimus and Toxicodendron radicans increased following removal of C. varia (Table 3). C. varia altered nutrient cycles in a sand prairie by increasing soil nitrogen availability (Symstad 2004). Altering nutrient cycles could encourage invasion by other invasive species that had previously been suppressed in nutrient poor and xeric environments (Symstad 2004), such as coastal sand dunes. C. varia can also regenerate from above-ground fragments, making it difficult to eradicate from an area once it is established (Losure et al. 2009).

As *Coronilla varia* interacts with and outcompetes other species, it proceeds from having sparse cover where there is little shading of the competitor species to where there is 100% cover and other species are excluded. In the quadrats where *C. varia* occurred with other species, it is intriguing that *C. varia* had the greatest light transmission (Table 1). From this we deduce that it is not competition for light that is allowing *C. varia* to dominate over the other species. Given that *C. varia* is becoming dominant in the vegetation zone normally occupied by *Ammophila breviligulata* and *Lathyrus maritimus*, it appears that *C. varia* is more tolerant of being shaded than either of the other species. In an agricultural ecosystem, *C. varia* survives and grows well under corn with shading in conditions ranging from 62-92 % of full sunlight, although shade treatments reduced nodulation by up to 50% (Cardina & Hartwig 1988). In an extensive review, Gucker (2009) lists seemingly contradictory reports of a wide range of shade tolerance by *C. varia*.

Losure et al. (2009) found no evidence that *Coronilla varia* builds up a large seed bank. This is to be expected given the low rates of sexual reproduction in clonal species (Erikkson 1989) such as *C. varia*. The root system can grow in sand, is multi-branched, and able to spread quickly by rhizomes (Tu 2003). Rhizomes can be up to 3 m long, and an individual plant is able to spread and cover 21 - 30m<sup>2</sup> in four years (USDA 2006). Our observations on below-ground roots and rhizomes, show that C. varia penetrates much deeper than other dune species. Thus C. varia has access to moister substrata, and can 'harvest' nutrients over the entire soil profile, not just the upper 50 cm where the root-rhizomes systems of the other species are found. In addition, C. varia can tolerate a range of conditions: drought to heavy precipitation, and warm to cold temperatures (NBII and ISSG 2005), though it grows best with high levels of precipitation (Tipping 2001). Our observations on Mahoneys Beach show that C. varia is tolerant of high salinity and beach overwash. Elias & Chadwick (1979) found that C. varia had low growth rates at establishment compared to other legume species. Regardless, the fact that C. varia easily regenerates from small vegetative fragments (Losure et al. 2009) may be the basis of its spread on Mahoneys Island and its subsequent spread to Dunns Beach.

Dune systems where invasive plant species have been present for a long time are less likely to return to the original vegetation, due to large seed banks of invasive species that are built up over time. Therefore, when the invasive species are removed, there is a higher reinvasion potential (Marchante et al. 2008, 2011). Although *Coronilla varia* has not been proven to build up a large seed bank, it does form monospecific stands that spread by robust rhizomes and perpetuate over time, and it can regenerate from above-ground fragments (Losure et al. 2009). Therefore, areas invaded by *C. varia*, in which this invasive plant has already colonized and lowered biodiversity, could become a self-sustaining system that is less resilient than normal, high biodiversity conditions.

Hill & Blaney (2009) described *Coronilla varia* from the Maritime Provinces as a nitrogen-fixing, alien species being used for roadside stabilization in the region. However, they observed that no alien, herbaceous legumes appeared to be a threat to native ecosystems in this area. This observation can now be questioned by our findings.

*Rosa rugosa* Thunb. is a prime example of an invasive alien species that is well known throughout Europe, as well as in Nova Scotia (Bruun 2005, Weidema 2006, Isermann 2008, Hill et al. 2010). Hill et al. (2010) identified *R. rugosa* as invasive to Nova Scotia sand dunes in the southern Gulf of St. Lawrence where it occupied up to 8% of the dune area; it has not yet colonized any of the beach systems in St. Georges Bay. Roads, tracks, and house plantings can act as conduits

for invasion of coastal sand dunes by this invasive shrub (Jørgensen and Kollmann 2009). Recently, R. rugosa was found to be invasive on Nova Scotia sand dunes, and its presence on the dunes was indeed associated with roads and nearby house plantings (Hill et al. 2010). The fact that C. varia is commonly used for roadside stabilization, and that prior to 2004 there was a track leading to the portion of Mahoneys Beach that is connected to the mainland, presents a possible mechanism for how C. varia was introduced to Mahoneys Beach. Pleasant Valley Nurseries (Henry Steghs, pers. comm.), in Antigonish, confirmed that C. varia has been sold and planted along roadsides and beachfront properties in an attempt to minimize coastal erosion near Mahoneys Beach. However, since C. varia seems to be more dominant and better established at the southeast end of the island, further from roads and housing, perhaps a marine dispersal method might be a better explanation, as for R. rugosa on Brier Island in the Bay of Fundy (Garbary, unpublished observations) and coastal Norway (Fremstad 1997). Animals could provide another vector, as seeds of C. varia can germinate from feces of white-tailed deer (Myers et al. 2004), and deer migrate over to the island portion of Mahoneys Beach for short periods.

The presence of nitrogen-fixing bacteria in root nodules allows the plant to grow in nutrient-deficient substrates, such as those found on sand dunes. Rodriguez-Echeverria et al. (2009) studied the invasive ability of Acacia longifolia (Andr.) Willd. They found that its associations with soil microorganisms and ability to nodulate profusely allowed it to fix nitrogen, and that this enhanced its invasive success. More recently, Rascher et al. (2012) demonstrated significant nitrogen inputs of A. longifolia in 3.5-fold the physical area occupied by the plant. The presence of native nitrogen-fixing species on Californian coastal prairies contributed to the success of invasive species because of the elevated nitrogen levels that they provided (Maron and Connors 1996). Thus, the presence of native nitrogen-fixing species (Myrica pensylvanica and Lathyrus maritimus in particular) may have aided in the establishment of Coronilla varia on Mahoneys Beach. The species was then able to persist due to its own nitrogen-fixing abilities and then outcompete other species based on its deeper roots that permit greater nutrient and water absorption over a larger soil depth.

*Coronilla varia* is well established on Mahoneys Beach. Approximately 9% of the island is now covered by 121 monospecific clumps of *C. varia*, with some being over 2000 m<sup>2</sup> (the largest clump covering an impressive 9100 m<sup>2</sup>). These large monospecific colonies of C. varia confirm its invasive qualities as defined by Harper-Lore (1996) and USDA (2006). The large colonies and extensive rhizome system of C. varia suggest that it is using a guerrilla strategy (Harper 1977; Lovett-Doust & Lovett-Doust 1982) to spread and increase the size of existing colonies. Herbaceous perennials, such as C. varia, with long distance clonal spreading (guerilla techniques), show the greatest relative increase in cover among moderately to highly disturbed habitats (Fahrig et al. 1994). C. varia also has the potential for prolific seeding (Tu 2003), but this does not occur in the first year of growth, and this can be severely limited by the abundance of major pollinators (e.g., honevbees, Gucker 2009). There was no conspicuous evidence of seedling establishment within stands of C. varia on Mahoneys Island. Mahoneys Beach can be considered a moderately to highly disturbed habitat because it is subject to storm surges (like the one in 2004 that breached the dune system and created the channel that now separates the island portion of the beach from the part connected to the mainland), and physical evidence of disturbance was observed (severe scarping on the seaward side of the dune).

Density and species richness of native species were compromised in areas invaded by *C. varia*. Pure colonies of *Coronilla varia* on Mahoneys Beach had shoot densities averaging 200 shoots m<sup>-2</sup> (Table 2) and excluded native species. Similarly Burkitt & Wootton (2011) found increasing density of the invasive plant *Carex kobomugi* Ohwi (a coastal dune sedge) increased the negative effects on native species. The exclusion of native species could be detrimental to sand dune ecology in the long term. *Ammophila breviligulata* and the nitrogen-fixers, *Myrica pensylvanica* and *Lathyrus maritimus*, are three of the native species that were examined in this study, and they are important for sand trapping and providing nitrogen in low nutrient soils, respectively.

Although it seems logical to associate high disturbance levels on sand dunes with invasion potential, the correlation between the two is unclear (Hill et al. 2010), especially on a relatively undisturbed system such as Mahoneys Beach. It is also possible that *C. varia* may prove beneficial to sand dunes by moderating erosion from sea level rise that is having major impacts on sand beaches in the southern Gulf of St. Lawrence (Garbary et al. in preparation). This raises the question of whether species diversity and preservation of native species is more important than the possible benefits of *C. varia* for preventing erosion of sand dune systems. Whether *C. varia* is detrimental, or not, to long term sustainability of sand dune plant communities remains to be

established; however, the obvious invasion and competitive strength of this species is a concern for the diversity and function of Mahoneys Beach and other sand dunes that have yet to be invaded.

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# A SCANNING ELECTRON MICROSCOPE ANALYSIS OF MORPHOGENESIS OF EMBRYOS AND JUVENILES OF THE DIRECT DEVELOPING ISOPOD, *Cyathura polita* (Stimpson, 1855)

# SARA C. MERCER, MICHAEL J. DADSWELL\* and GLENYS D. GIBSON

Department of Biology, Acadia University Wolfville, NS, Canada B4P 2R6

Isopods are a species rich, morphologically diverse group characterised by direct development of young within a marsupium. Collectively, these traits make isopods excellent models for understanding the changes to morphogenesis that occur in the evolution of direct development, but the overall understanding of this process is limited by a lack of comparative data. We investigated morphogenesis in Cyathura polita, an isopod common in estuaries along the Atlantic coast of North America, from gastrulation to release of young as benthic juveniles, using Scanning Electron Microscopy. We found that early development of *C. polita* from gastrulation to hatching from the extraembryonic membranes was generally similar to that described by earlier work using sectioned material, but found that development through the manca stage to free living juveniles differed from previous studies. For instance, the embryonic dorsal organ differed from that described generally for isopods as the apical surface is covered by a sieve plate and the typically reported microvilli were not observed. Differences in timing were also noted: for example, segments appeared throughout the pereon and pleon well before the post-naupliar appendages, and not coincident with them as described previously for isopods. Limb development occurred simultaneously except that percopod 7 did not form until the post-manca stages, as is typical for this group, suggesting there is non sequential (or suppression of) teloblast activity in postnaupliar development. Also, some broods contained young of different developmental stages (i.e., early embryos and late manca), indicating that spawning events are repeated and overlap, which is perhaps adaptive in the shortened reproductive season characteristic of our study population that is at the northern limit of the known range for this species. Mean brood size was  $53.2 \pm 18.9$  young (range 4-108), the largest reported for Anthuridae (a family of isopod crustaceans).

Key words: scanning electron microscopy, marsupium, manca, embryonic dorsal organ, direct development, crustaceans.

Author to whom correspondence should be addressed: mdadswell@eastlink.ca

# INTRODUCTION

Marine isopods are a large, diverse group (~4500 spp; Brusca 1997), which, as in other peracaridan crustaceans, are characterised by direct development of maternally brooded young (Anderson 1973). Reproductive biology is well known for a few species and presents some fascinating examples of mating behaviour, mate guarding, and both protandrous and protogynic sex change (reviewed by Johnson et al. 2001). Unfortunately, the understanding of isopod development is limited to a relatively few, but thorough, descriptions of embryonic development (McMurrich 1895, Strömberg 1965, 1967, 1971, 1972) and several descriptions of young following release from the marsupium (Elizalde and Sorbe 1993, Araujo et al. 2004). Detailed comparative data of morphogenesis throughout ontogeny is lacking despite the importance of these data to overall understanding of isopod diversity. Description of external morphogenesis in embryos and juveniles of Cyathura polita (Stimpson, 1855) (Anthuridae) will provide a more complete understanding of ontogeny for an isopod that is relatively well known in terms of early embryology (Strömberg 1972), physiology (Kelley and Burbanck 1976) and reproductive biology (Frankenberg and Burbanck 1963, Burbanck and Burbanck 1974, Burbanck et al. 1979).

Most isopods have large, centrolecithal eggs (>300 mm) that are released as zygotes into a ventral marsupium composed of overlapping oostegites (Strömberg 1965, 1971, Johnson et al. 2001). Cleavage is superficial and results in a thin blastoderm and a thickened, ventral germ band. As the germ band elongates, head bands give rise to 3 naupliar segments and associated antennae and mandibles. The metanaupliar region forms as teloblasts sequentially divide to generate the maxillulary and maxillary segments, followed by the segments of the pereon (thorax) and pleon (abdomen) (Strömberg 1967). Embryos hatch from the extraembryonic membranes as manca that continue to develop within the marsupium. Manca become morphologically similar to free living juveniles but lack legs on pereonite 7 (Strömberg 1967, Johnson et al. 2001). After the manca are released from the marsupium, they develop into adults through a series of molts as pereopods 7 are formed. Descriptions of isopod reproductive biology are provided in the comprehensive reviews of Anderson (1973) and Johnson et al. (2001).

The single previous study of embryology in the Anthuridae is Strömberg's (1972) analysis of internal morphogenesis of C.polita, and was based on sectioned material to describe cleavage, cell arrangement

and organ formation in early embryos. *Cyathura polita* is a benthic isopod that occurs in estuaries from the Gulf of Mexico to the Bay of Fundy (Burbanck et al. 1979). Adults are interstitial in coarse sand of intertidal and shallowly subtidal areas and feed mainly on detritus and algae in the sediment (Burbanck 1959, Burbanck et al. 1979). Most *C. polita* are protogynic hermaphrodites, however, a few mature directly as males (Burbanck and Burbanck 1974). Life-history traits vary with latitude: northern populations, as described here, have longer life cycles, reproduce later in the summer, contain larger females and have higher individual fecundity than do southern populations (Mercer et al. 2007). Strömberg's (1972) description of embryos ended at hatching from the extraembryonic membranes, and later development of young within the maternal brood pouch was not described.

Our objective was to examine external morphogenesis in embryo and juvenile *C. polita* using Scanning Electron Microscopy (SEM) to build a more complete understanding of the development of this relatively well known species. Our study builds upon Strömberg's (1972) description of *C. polita* embryos by focusing on external structures and extending the period of ontogeny in question to include the entire brooded period until release and then growth as benthic juveniles.

# **METHODS**

#### **Collection and Preparation for Scanning Electron Microscopy**

*Cyathura polita* were collected from the lower Saint John River Estuary, New Brunswick, Canada, the northern-most population known for this species (Mercer et al. 2007). Isopods were collected from four stations: Bell Point, Westfield Beach, Summerville and Millidgeville (approximately 45°20'N; 66°20'W). Samples were obtained bi-weekly from May 29 to August 27, 2004, by collecting surface sediments from shallow sub-tidal areas with water depths of 20 to 150 cm. Sediment was collected with a shovel and sieved on 1.0 mm or 0.50 mm mesh. Gravid females were fixed in formalin for 3 to 4 hours on the day of collection and stored in 70% ethanol until examination. During examination, young were removed from the marsupia, measured, and stage of development determined.

Most specimens examined with scanning electron microscopy were collected from Millidgeville on July 27, 2004. This included twelve females with gravid marsupia and eleven juveniles. The day following collection, young were removed from 10 of the marsupia. Intact adults and isolated young were anaesthetized in 3.5% magnesium chloride, fixed in 2.5% glutaraldehyde (1 hour) and post-fixed in 1% osmium tetraoxide (4 hours), both in 0.1M cacodylate in seawater. Additional young were removed from the marsupia of a few females that were fixed in formalin (above) and prepared for SEM by post fixing in 1% osmium tetraoxide. Specimens were dehydrated in ethanol and criticalpoint dried using a Bio-Rad E3000 critical point drier in liquid CO<sub>2</sub>. As embryos were placed on stubs, they were gently rolled on carbon tape to remove the chorion and vitelline membrane. Samples were coated with gold palladium using a Technics Hummer II sputter coater and viewed with a JEOL 5600LV Scanning Electron Microscope at 10kV. Digital images were adjusted for size and brightness in Corel Photo Paint 11, and arranged in plates using Corel Draw 11.

# Identification of adults and classification of young

Females were identified by the presence of oostegites forming a ventral marsupium. Males were identified by the presence of an appendix masculina on pleopod 2. Post-manca individuals that did not demonstrate either of these sexual dimorphisms were considered to be juveniles or non-reproductive adults.

Young were categorized into six stages of development: Class 1) embryos that were oval in shape and housed within two extra embryonic membranes; Class 2) embryos that had naupliar appendages (antenna 1, antenna 2, mandible); Class 3) embryos with post-naupliar appendages (other mouthparts, pereopods, pleopods); Class 4) early manca that had hatched from the embryonic membranes but were still housed within the marsupium; Class 5) late manca, also housed within the marsupium but with an arrangement of appendages similar to adults although pereopods 7 were absent; and Class 6) free-living juveniles with pereopods 7 but lacking sexually dimorphic traits.

#### RESULTS

#### Female anatomy and brood structure

Mature females examined were  $13.8 \pm 2.14$  mm long (mean  $\pm$  S.D., n=163). The percon consisted of seven elongate perconites, each with a pair of percopods (Fig 1A). In both sexes, percopod 1 is modified into large gnathopods and is used for feeding and mating. Percopods

2-7 are ambulatory and are arranged such that P2-P3 are anteriorly directed and P4-P7 are posteriorly directed. Pleopod 1 is operculate and covers pleopods 2-5. Gravid females had a well-developed marsupium that consisted of 4 pairs of oostegites arising from the coxa of pereonites 3-6 (Fig 1A,B). The leaf-like oostegites loosely overlapped on the ventral surface of the body to form a single pouch that housed the young (Fig 1C). Brood size was  $53.2 \pm 18.9$  (mean  $\pm$  S.D.) with a range of 4-102 young per brood (n=72 females).

## Development

# Embryos

Class 1 embryos were slightly oval-shaped, flattened on the ventral side, and covered by two extraembryonic membranes including an outer chorion and an inner vitelline membrane (Fig 2A, B). Class 1 embryos observed were blastulae and had a single-layered cellular blastoderm that covered a large central yolk mass (Fig 2B). Class 1 embryos were  $450 \pm 60 \,\mu$ m in length (x ± standard deviation; n=956). We were unable to measure the size of mature oocytes or zygotes (not collected) but the size of Class 1 embryos measured here were similar to the egg size given for the 8-cell stage of development (0.40 X 0.45mm; Stromberg 1972).

Class 2 embryos were  $1.05 \pm 0.15$  mm in length (n=630). The body regions, now well defined, were similar in size and the cephalon had two naupliar appendages forming the buds of antennae 1 and 2 (Fig 3A). The two dorsolateral embryonic organs, located on either side of the anterior pereon, were approximately 70  $\mu$  m in length and consisted of an ectodermal rosette around a central pore (Fig 3A, B). Early Class 2 embryos lacked external evidence of segmentation while later Class 2 embryos showed initial differentiation of pereonites and pleonites as paired whorls along the dorsal midline (Fig 3A, C).

Class 3 embryos were on average similar in size to Class 2 embryos  $(1.12 \pm 0.19 \text{ mm}; n=605)$ , but showed considerable variation in length among embryos (from 0.69 to 2.21 mm) with most growth occurring in the pereon (Fig 4A, B). After the chorion was removed, buds of the postnaupliar appendages were visible including the remaining mouth parts, pereopods 1-6, pleopods 1-5, and uropods. All appendages, held closely to the body, were positioned posteriorly and toward the ventral midline (Fig 4B). The antennae were segmented (antenna 1 had 4 segments, antenna 2 had 6 segments) but lacked setae or spines (Fig 4C). The buds of the mouthparts also lacked setae but the basic

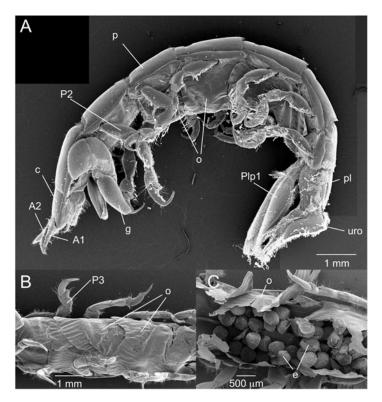


Fig 1 Scanning electron micrographs of female *Cyathura polita*. A. Composite image of a female with intact marsupium on ventral surface. B. Ventral view of marsupium, composed of overlapping oostegites. C. Marsupium with oostegites removed to show the brood of Class 1 embryos. A1=first antenna, A2= second antenna, c=cephalon, e=embryos, g=gnathopod, p=pereonite, P2=pereopod 2 (1<sup>st</sup> pair of walking legs), P3=pereopod 3, pl=pleon, Plp1=pleopod 1, o=oostegites, uro=uropod.

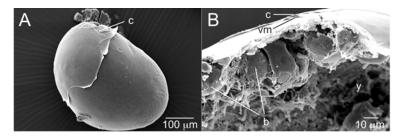


Fig 2 Scanning electron micrographs of Class 1 embryos of *Cyathura polita*. A. Ventral view of embryo with extra embryonic membranes partially removed. B. Fractured embryo showing the two extra embryonic membranes and the cellular blastoderm. Note that most of the central yolk mass is removed. b=blastoderm,c=chorion,vm=vitelline membrane,y=yolk.

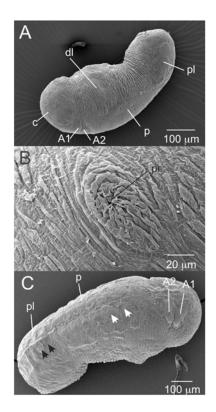


Fig 3 Scanning electron micrographs of Class 2 embryos of *Cyathura polita*. A. Lateral view of an early Class 2 embryo showing body regions but lacking defined segments. B. Dorsolateral organ. C. Dorsal view of a more advanced Class 2 embryo, showing early segmentation in the thorax (white arrows) and abdomen (black arrows). A1=antenna 1,A2=antenna 2, c=cephalon; dl=dorsolateral organ, p=pereon, pl=pleon, pr=pore.

segmentation pattern of their adult morphology was complete: the mandibles consisted of a stout peduncle, a palp with 2 articles and an incisor; maxilla 1 with single rudiments of both outer and inner lamella; maxilla 2 was uniarticulate and maxilliped with 4 articles (Fig 4C).

The percon of Class 3 embryos was composed of seven elongate segments. The six anterior perconites were equal in size, and each had a pair of uniramus percopods that were also similar in size (6 segments). All were oriented with the dactyl pointing posterior. Perconite 7 was small and lacked legs (Fig 4D, E). The buds of all pleopods and uropods were biramus and approximately the same size (Fig 4E). The embryonic dorsal organ was visible medially on perconite 1 as a

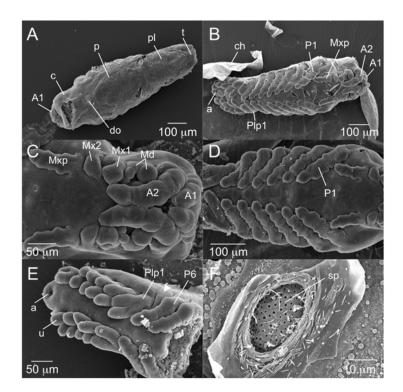


Fig 4 Scanning electron micrographs of Class 3 embryos of *Cyathura polita*.
A. Dorsal view of embryo with the chorion intact. B. Ventral view of embryo partially removed from the extraembryonic membranes. C-D. Ventral view of the head and thorax, respectively. Note that small setae are visible at the tips of the pereopods. E. Ventral view of posterior thorax and abdomen. Note that pereonite 7 (indicated by the arrow) lacks legs at this stage of development. Exopods and endopods are present on the pleopods. F. Dorsal organ. a=anal pore, A1=antenna 1, A2= antenna 2, c=cephalon,ch=chorion,do=dorsal organ,Md=mandibles,Mx1=maxilla a, Mx2=maxilla 2, Mxp=maxilliped, p=pereon, P1=pereopod 1, P6=pereopod 6, pl=pleon, Plp1=pleopod 1, sp=sieve plate, t=telson.

central sieve-like plate, surrounded by the chorion (Fig 4A, F). The dorsolateral organs were no longer visible at this stage.

#### Manca

Manca were hatched from the extraembryonic membranes but were still sheltered within the female marsupium. Two size classes were observed. Class 4 young (early manca) had body proportions that were similar to the adults but still had rounded, well separated segments and a smooth exoskeleton with small tufts of setae (Fig 5A, B). Average length was 1.8mm. The appendages had obtained the adult positions: the first antennae extended anteriorly; the mandible and maxilliped curved anteriorly over the mouth; pereopods 1-3 had rotated so that the dactyls were oriented anteriorly while pereopods 4-6 retained their original configuration; and pleopod 1 extended posteriorly to begin to enclose the other pleopods in the gill chamber (Fig 5B-G). The head appendages had the number of segments typical of adults: antenna 1 had 5 segments; antenna 2 had 6 segments and a broad peduncle; mandibles had a palp with 3 segments and apical setae; maxilla 1 and 2 were both smooth; and maxillipeds had 3 segments (Fig 5E). The gnathopods were much larger than the other pereopods. The pleonites

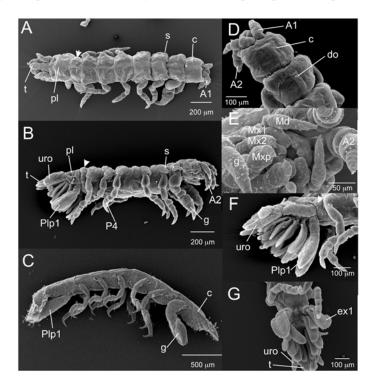


Fig 5 Scanning electron micrographs of Class 4 (early) and Class 5 (late) manca of *Cyathura polita* that were removed from the marsupium. A, B. Early manca, dorsal and lateral view. C. Late-stage manca. D, E. Dorsal and ventral aspects of the cephalon of an early manca. F, G. Lateral and ventral aspects of the pleon of an early manca. Arrow indicates pereonite 7 that lacks appendages at this stage. A1=antenna 1, A2=antenna 2, c=cephalon, do=dorsal organ, g=gnathopod, ex1=exopod of pleopod 1, Md=mandible, Mx1=maxilla 1,Mx2=maxilla 2,Mxp=maxilliped,P4=pereopod 4, pl=pleon, Plp1=pleopod 1, t=telson, uro=uropod. were short and fused dorsally, but small lateral divisions were visible between pleonites 1-5 with a fairly deep groove between pleonites 5-6 (Fig 5A,F). The pleopods had an exopod that was shorter, broader and with more setae than the corresponding endopod (Fig 5F,G). The uropods had migrated to their adult position and appeared to be fused with the telson (Fig 5F). The dorsal embryonic organ was visible on the dorsal midline of pereonite 1 as a small rosette of ectoderm; the central sieve plate, found in earlier stages, was not observed (Fig 5D).

Class 5 mancas were much larger (approximately 2.5 mm in length) and exhibited considerable differentiation of the exoskeleton indicating that perhaps at least one molt occurred between classes 4 and 5 (Fig 5C). Class 5 manca had a pointed rostrum, anterolateral corners of the cephalon and smoothly articulated pereonites. The pereopods were considerable modified, with very large gnathopods with a large propodus, and extensive bands of setae on the posterior side of the anterior pereopods, and the anterior side of the posterior pereopods. Pereopod 7 was absent. The lateral divisions between the pleonites were no longer visible. The exopods of pleopod 1 were similar to those of adults in terms of relative size and development of the setae (Fig 5B,C).

# Free-living juveniles

Class 6 or free-living juveniles, collected from the sediments, had the same overall morphology as adults but generally had fewer setae and lacked sexually dimorphic traits. Newly released juveniles (age 0) were first observed on July 31, 2004 and had a mean length of 2.70  $\pm$  0.24 mm (Mercer et al. 2007) close to the length of Class 5 mancas and suggesting that Class 5 manca were released as juveniles without a molt occurring (i.e., no percopod 7).

The juveniles collected for our SEM study were taken on July 24, 2004, had a mean length of  $10.82 \pm 2.50$  mm, were age 1 juveniles, and pereopod 7 at this stage was similar in size and orientation to the other posterior walking legs (Fig 6A). The antennae were covered in setae, and the peduncle of antenna 2 had the broad, sculpted morphology typical of adults. The juvenile mouthparts consisted of: mandibles with 3 palp articles, broad peduncle and flat, sculpted incisor, and the maxillipeds with 3 elongate, bristly articles that curved flat over the mouth and covered maxilla 1 and 2 (Fig 6B, C). The gnathopod of the juvenile was much larger in comparison to the walking legs and had both propodal and carpal teeth (Fig 6A).

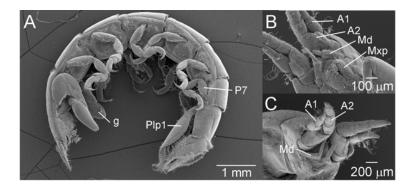


Fig 6 Scanning electron micrographs of free-living juveniles of *Cyathura polita*, collected from sediments. A. Lateral view of juvenile. B, C. Mouthparts of juvenile and adult, respectively. A1=antenna 1, A2=antenna 2, Plp1=exopodite of pleopod 1, g=gnathopod, Md=mandible, P7=pereopod 7.

# Reproductive Activity and Intra-Brood Synchrony in Development

Gravid females were found from July 1- August 17 and females brooding early embryos (Class 1) were collected throughout that period (Fig. 7). All offspring per brood were at the same stage of development for most broods (n=60 females; Fig 7). The marsupia of a few females (n=14) contained young of different but sequential developmental classes (i.e., Class 2 and 3 embryos). Females with young at sequential stages of development were found slightly later in the summer (July 17- August 17) and were collected from all 4 study sites. A few females (n=4) contained broods with young at non-sequential stages of development. In all 4 broods, the majority of young per marsupium were at an advanced stage and a few (<10%) were at an earlier stage. In 3 broods, most young were manca while a few were Class 1 (2 broods) or Class 2 (1 brood), while in the fourth brood, most young were Class 3 and a few were Class 1.All 4 females were collected later in the reproductive season (July 31).

# DISCUSSION

The marine peracaridan crustaceans are a large and diverse group, yet our understanding of their development rests on a few, well-studied species and incomplete descriptions of some additional taxa (Anderson 1973, Johnson et al. 2001). Our objective was to build upon a well

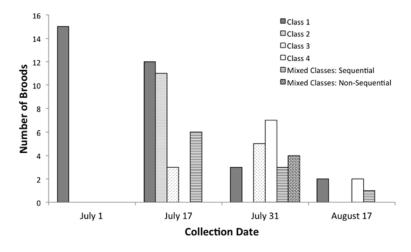


Fig 7 Stage of development of embryos brooded by female *Cyathura polita* collected throughout the study period (n=73 females).

established literature on *Cyathura polita* by investigating external morphogenesis, thus focusing on gaps in the existing knowledge that addresses their early embryology (Strömberg 1972), physiology (Kelley and Burbanck 1976) and life history (Frankenberg and Burbanck 1963, Burbanck and Burbanck 1974, Kruczynski and Subrahmanyam 1978, Burbanck et al. 1979, Mercer et al. 2007). Our study of morphogenesis throughout most of the life cycle is, to our knowledge, the first such description for the Isopoda using SEM. SEM revealed differences from earlier studies in timing of segmentation, new structural information on the embryonic dorsal organ, and suggests that several key modifications of adult morphology are acquired during the molt to the first manca stage.

Overall, our results support earlier descriptions of isopod embryology based largely on sectioned material (Strömberg 1965, 1967, 1971, 1972). Differences in timing of segmentation and appendage formation were noted. Strömberg (1965) describes the development of the naupliar appendages in isopods as arising almost simultaneously from the naupliar ecto-mesodermal headbands, with the mandibles arising slightly before the buds of the first and second antennae. We found the buds of the antennae appeared first, but the mandibular buds, once present, grew faster and soon outstripped the antennae in size. We also found external evidence that segmentation of the pereon and pleon began earlier in development than previously reported, as segments were observed at the same time as the naupliar appendages were forming while Strömberg (1972) observed segmentation did not occur until the metanaupliar region was forming. In the present study, the segments developed before the limb buds were externally visible and not sequentially with them, as in previous reports (Strömberg 1972). These differences likely reflect differences in method of observation. For example, the small antennae buds are much easier to observe with SEM than in section, and differentiation of the surface of the ectoderm (SEM) may provide earlier indication of segmentation than may be observed by internal cell differentiation (section).

Our study also revealed new information on the structure of the embryonic dorsal organ. With few exceptions (epicarids, Charmantier and Charmantier-Daures 2001), peracaridan embryos typically have three embryonic dorsal organs: a pair of dorsolateral organs that extend from the yolk mass to the space between the chorion and the vitelline membrane; and, a single dorsal organ that develops later and extends through both extraembryonic membranes to the marsupial fluid (Strömberg 1971, Johnson et al. 2001). These dorsal organs are lost at hatching and are not equivalent to the dorsal organ typical of adults (Martin and Laverack 1992). Earlier reports of the dorsal organ (Strömberg 1965, 1967, 1972) illustrate an apical surface covered with microvilli (observed in section), while our observations (SEM) reveal a distinct sieve plate covering the organ and surrounded by a rosette of extraembryonic membrane. The sieve plate would likely appear as apical folds in section. The actual function of the dorsal organ, as vet undetermined, has been suggested to include volk absorption, cell degradation, secretion of the larval cuticle, molting, and osmoregulation (Strömberg 1965, Johnson et al. 2001, Charmantier and Charmantier-Daures 2001). We also noted minor differences in timing including: the onset of the dorsolateral organ occurred earlier (here, before segmentation of the thorax) and the loss of the dorsal organ occurred much later (here, well after hatching), than was found in the analysis of the embryos observed by Strömberg (1972).

The morphological diversity of the telson has made it an important character in phylogenetic analysis (Brusca and Wilson 1991). The Anthuridae are characterised by uropods that extend dorsally over the pleotelson (a unique synapomorphy), while the presence of a true pleotelson (i.e., pleonite 6 fused to the telson) has been much discussed for this clade (Brusca and Wilson 1991). We found that the uropods originate as do pleopods 1-5 in size and early orientation and that differential growth to produce the adult arrangement occurs in the molt to the first manca stage (Class 4 young). In early manca (Class 4), the division between pleonite 6 (with uropods) and pleonite 5 is distinct, while the articulation between pleonite 6 and the telson was not externally visible. This supports Brusca and Wilson's (1991) conclusion that pleonite 6 and the telson are fused in the Anthuridae, and also, that fusion may occur in the molt to the first manca stage. The first manca is also the stage of development where the walking legs acquire their adult orientation (except for leg 7), and differential growth of the gnathopods is most evident.

Lack of intra-brood synchrony in development was found in approximately 20% of the marsupia observed, although cyathurans were generally thought to have synchronous development (Strömberg 1972). Most of these females carried embryos of sequential developmental classes suggesting young were in the process of hatching or molting at the time of collection; similar observations are reported for C. carinata (Bamber 1985). We also found a few gravid females (4 of 78 in total) that were brooding young of non sequential developmental stages. All of these females were collected late in the reproductive season and the majority of young in these broods were manca. This suggests that perhaps a few females underwent a second, smaller reproductive event shortly before the offspring from the previous event had been released, or, that late season broods contained a few embryos that were less viable or at least slower to develop than their siblings. Both conditions may be a consequence of the short reproductive season associated with the northern location of the Saint John population. Cyathurans are known to spawn several times per reproductive season (Marques et al. 1994) in populations where the reproductive season is long (e.g., 5 months for C. polita populations in Florida; Kruczynski and Subrahmanyam 1978). Cyathura polita in the Saint John population have a short reproductive season (2 months) but early broods can be found throughout that time suggesting repeated spawning may occur (Mercer et al. 2007). The presence of several developmental stages within a single marsupium was also observed in a parasitic entoniscid isopod by Müller (1862; in Strömberg 1971).

Marine isopods are typically thought to undergo 3 molts inside the marsupium (Johnson et al. 2001). These molts include: the first, or hatching molt, that involves loss of the extraembryonic membranes by naupliar intermolts with three pairs of naupliar appendages and poorly developed thoracic appendages; the second molt in which the embryonic cuticle is shed by early manca with well developed pereopods

and pleopods (excepting percopods 7); and the larval molt into a more advanced manca, occurring just prior to release from the marsupium (Johnson et al. 2001). We did not observe a typical hatching molt in *C. polita*. Instead, the loss of extraembryonic membranes (chorion and fertilization envelope) and embryonic cuticle appeared to occur at the same time and young hatched from the extraembryonic membranes after they had well developed abdominal and thoracic appendages (i.e., the first manca stage). The larval molt, from first to second manca stage, appeared to be more typical and was characterised by changes in size, shape and differentiation of the exoskeleton.

These, plus other, observations suggest that *C. polita* undergo 5 phases of development during their life cycle. The first 2 phases occur within the marsupium and include the development of embryos (organization of the body regions and appendages) and manca (young with a juvenile-like morphology but lacking the 7<sup>th</sup> pair of pereopods). The length of the brood period has been estimated as 30-45d (Mercer et al. 2007). Manca are released from the marsupium and as free living juveniles undergo at least one additional molt to become subreproductive adults; i. e., those with the 7<sup>th</sup> pair of pereopods. *Cyathura polita* are protogynic hermaphrodites (Burbanck and Burbanck 1974, Mercer et al. 2007) and as they mature, they undergo at least two additional molts: the first producing mature females with oostegites, and the second producing males with the appendix masculina. Mercer et al. (2007) estimated the life cycle takes 3 years in the Saint John River population.

This study of external morphogenesis supports earlier work on isopod embryology based on sectioned material and additionally describes the development of manca and free-living juveniles. Comparative analysis is difficult as so few developmental studies exist, but this preliminary work suggests that direct development in isopods includes shifts in the timing of the development of naupliar appendages relative to segmentation; the non-sequential development of appendages (i.e., the late appearance of the 7<sup>th</sup> pair of pereopods) suggesting temporary suppression of teloblast activity part way along the anterior-posterior axis; and the acquisition by most pericaridans of an additional juvenile stage (i.e., manca) in a life cycle that has lost a free-living larval stage. Our understanding of isopod development remains in its infancy as we lack sufficient comparative studies, undertaken within a phylogenetic framework, to both understand isopod diversity and also to more fully appreciate the origins of direct development in this group. Acknowledgements This project was supported by NSERC (Natural Sciences and Engineering Research Council of Canada) funding to G. Gibson. Images were taken at the Acadia Centre for Microstructural Analysis. We thank Martin Thiel and two anonymous reviewers for their helpful comments on the manuscript. The authors thank D. MacDonald and G. Cheeseman for their help with SEM, Dr. T. Avery for reviewing earlier versions of the manuscript, and R. Mercer and C. Mercer for assistance with field work.

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# THE FISHERY FOR SPECKLED TROUT, Salvelinus fontinalis, OVER A 28 YEAR PERIOD IN THE TANGIER GRAND LAKE WILDERNESS AREA, NOVA SCOTIA

# JOHN L. MACMILLAN<sup>\*</sup>, REGINALD J. MADDEN, TAMARA WILSON and MEGAN KENNY

Inland Fisheries Division, Nova Scotia Department of Fisheries and Aquaculture, Box 700, Pictou, Nova Scotia B0K 1H0

# ABSTRACT

The Tangier Grand Lake Wilderness Area (16,000 ha) is located about 100km east of Halifax, Nova Scotia, and supports a popular fishery for speckled trout. The purpose of this study was to assess the status of the trout fishery and address concerns related to over-exploitation. Angler check points were occupied during 1979 and 2007 on an access road to sample the catch of anglers during the May-June period of heavy angler activity. In 1979, a total of 1380 interviewed anglers spent 6889 hours to catch 1852 trout. In comparison, a total of 178 interviewed anglers spent 1363 hours to catch 593 trout during 2007. The differences associated with sample size between survey years reflect sub-sampling in 2007 rather than a change in angler activity. The majority of anglers retained less than three trout and of the total trout caught, anglers released 19% in 1979 and 50% in 2007. Catch per hour, size, age, and growth rate of trout were similar between surveys. Results indicated that there was little change in this fishery between the 28 years separating the two creel surveys.

# **INTRODUCTION**

Several factors have influenced the Nova Scotia sport fishery over the past 30 years. The number of licensed anglers in Nova Scotia has declined by approximately thirty five percent since the 1970s. Coincidentally, as one would expect, angler catches have also declined in many areas; however, the decline in the total speckled trout, *Salvelinus fontinalis*, catch has occurred at a greater rate than the decline in the number of anglers in the province. Introduction of invasive species, habitat loss and over-exploitation can all influence trout populations

<sup>\*</sup> Author to whom correspondence should be addressed: macmiljl@gov.ns.ca

and angler catches (MacMillan and Madden, 2007, NSDFA 2005). Regional differences in habitat condition may also influence the health of trout populations. For example, the Tangier Grand Lake Wilderness Area (TGLWA) is located in eastern Nova Scotia where freshwaters tend to be nutrient poor, acidic and sensitive to warming, whereas the geology in the Northern Mainland and Cape Breton provides cooler water in summer and in many areas there is a natural buffering capacity against the impacts of acidity that benefits speckled trout production (MacMillan et al. 2008). Introduction of invasive smallmouth bass, Micropterus dolomieu, and chain pickerel, Esox niger, have occurred to a much lesser extent in waters of the Northern and Eastern Nova Scotia (McNeill, 1995). Generally, speckled trout are considered to be poor competitors to most freshwater fish in Nova Scotia and this is especially true in marginal habitats (Hayes and Livingstone, 1955, Alexander 1975, Alexander et al. 1986). Quinn et al. (1994) demonstrated that trout production was related to the presence of other fish species in lakes in Ontario. The compounding influences of exploitation and habitat losses on trout has often been cited as allowing other populations of fish species to increase and eventually dominate the habitat of lakes and streams (Smith 1938, 1940, Browne and Rasmussen 2009, Munro and MacMillan 2012). Nova Scotia has made regulatory changes to reduce exploitation on the speckled trout resource including a reduction in the daily bag limit from 15 to 5, as well as a change from a retention season to a release season during September (Inglis 1995).

Many sport fisheries are influenced by over-exploitation (Post et al. 2002). Anglers, environmental organizations and fishery managers have become more interested in changing regulations to improve fisheries and conserve stocks in light of these trends. Further, some studies suggest that exploitation could influence genotypes for aggression and growth rates on targeted populations (Biro and Post 2008). Curry et al. (2003) estimated total fishing mortality at 44% on a speckled trout population in Meach Lake, Ontario, and recommended that fishing mortality should not exceed that level in other lakes.

Recently, eastern NS anglers at the public Recreational Fishing Advisory Council meetings provided input concerning the poor status of trout fisheries and the need for new regulations. Interest was expressed in designating the entire TGLWA a Special Trout Management Area where regulations could be implemented in an attempt to reduce harvest and improve the sport fishery for speckled trout. Sabean (1980) and Heggelin (2008) suggested that angling pressure could be influencing the status of trout populations in lakes of the TGLWA. Sabean (1980) reported that Egg Lake was potentially overfished. While studying 12 lakes within the TGLWA, Heggelin (2008) demonstrated that the presence of larger trout was correlated with less angling activity. Conversely, Halfyard et al. (2008) suggested that exploitation was not an important factor regulating trout populations in three interior TGLWA lakes.

In response to concerns expressed by anglers, the Inland Fisheries Division agreed to conduct an angler creel survey in the same region that was studied 28 years prior. The objective of this study was to assess the degree of change that may have occurred in the TGLWA fishery and whether there was a need for new regulations to reduce harvest.

# METHODS AND STUDY AREA

The results from an extensive creel survey in the Mooseland Region, Halifax County, carried out in 1979 (Sabean 1980) provided baseline data for the fishery in TGLWA. The southern portion of the Mooseland to Murchyville road borders the TGLWA which includes 16,000 hectares that have been designated under the Nova Scotia Protected Areas Program.

The majority of the forest in the region is dominated by spruce, pine, fir, and occasionally hemlock. Red maple and yellow birch occur in areas of richer, deeper soils. This landscape was formed approximately 10,000 years ago by a receding glacier that scoured out numerous depressions which are now occupied by about 100 lakes and numerous streams. The geological makeup of this region includes mainly shallow soil cover over granite and greywacke and supports relatively unproductive waters that tend to be acidic (Davis and Brown 1996). Heggelin (2008) reported mean pH values ranged from 4.6 to 5.3 from twelve lakes in the TGLWA. Fish species that are considered to be competitors of speckled trout in some lakes within the TGLWA and surrounding region include yellow perch, *Perca flavecsens*, white sucker, *Catostomus commersoni*, brown bullhead, *Ameiurus nebulosus*, and American eel, *Anquilla rostrata* (Alexander et al. 1986).

The 2007 creel survey followed the methods used during 1979 (Sabean 1980). In 1979, anglers were interviewed as they exited a 30 km gated road that runs from Murchyville to Mooseland. The 2007

survey was shorter as the sampling period was from 6 May to 10 June compared to 1 May to 30 June in the 1979 survey. Sampling days during the recent survey were mainly on weekends and holidays. During the 1979 creel survey, angler check points were set up at both Murchyville and Mooseland Road exits whereas in 2007, only the Murchyville exit was sampled regularly (Fig 1). In 2007, a sign was placed beside the exit providing notice to anglers of the voluntary creel survey. While the majority of anglers provided information to creel clerks, some refused to participate.

On a number of occasions during the 2007 survey, a roving creel survey was conducted in order to gather information from additional anglers within TGLWA. On the 21 May, officers from Fisheries and Oceans, Nova Scotia Department of Natural Resources and Nova Scotia Department of Environment and Labour were involved in a cooperative enforcement patrol and angler survey. Angler surveys were conducted at check points on the Fish River Bridge as well as the Mooseland exit. Cool temperatures during the 21 May enforcement check may have resulted in relatively few anglers available for interviews. Creel data were sampled from anglers who collectively reported fishing at twenty-

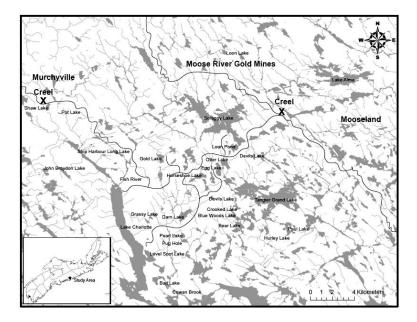


Fig 1 Mooseland to Murchyville creel survey sites and the lakes that were targeted by anglers in 1979 and 2007.

five locations; 22 lakes, Cowan Brook, Butler Lake still water, and Fish River. Data from Pug Hole Lake and Pearl Lake were combined as a number of anglers reported fishing from both during one angling trip.

Creel data included size of the catch, time spent angling, residence of the angler, site fished, gear type, species caught, and the collection of fish scales for aging. Catch per unit effort (CPUE) was presented in terms of trout caught per hour of angling. Trout harvested by anglers were measured for fork length (FL, mm). Commonly, anglers cleaned their catch prior to participating in the creel survey. Cleaned trout were measured and if heads were removed, the presence or absence of pectoral fins was recorded. If the pectoral fins were removed, an additional 35% of the length was added to approximate FL. If the pectoral fins were present, an additional 30% was added. These additions to length were incorporated into the study so that estimations of FL would remain consistent with the 1979 study. The proportions of large trout (FL > 30 cm) in the catch were compared with the 1979 data set (z-test).

Scales were sampled from the area on the body between the lateral line and dorsal fin. Scales were aged by two readers. If no agreement was reached, a third reader was used and a consensus for each age was reached. Mean length at age was calculated for most of the trout populations sampled.

Site specific comparisons of FL and CPUE (retained and total) were made for eight of the surveyed sites; Egg Lake, Fish River, Grassy Lake, Lake Charlotte, Level Spot Lake, combined Pearl & Pug Hole Lakes, Ship Harbour Long Lake, and Loon Lake (Fig 1). Data sets from Loon Lake, Egg Lake, and Level Spot Lake were supplemented by additional angling and gill netting by staff of the Inland Fisheries Division. Catch rates from the 1979 survey were only based on retained trout whereas catch rates for released trout, retained, and total trout caught were available from individual records in the 2007 survey. Total trout CPUE was estimated for 1979 sites based on the average release rate of 19% reported (Sabean 1980). The total (retained and released) trout CPUE was calculated for 2007. Differences between surveys for mean size of the catch, CPUE, and length at age of speckled trout were compared using t-tests (P<0.05).

# RESULTS

The mean distance travelled by anglers to Murchyville was 58 km (28 SD). The majority (60%) of the anglers interviewed during the 2007 Mooseland to Murchyville creel survey resided in the communities of Dartmouth/Halifax/Sackville, Enfield and Truro. The distance from Murchyville was 42 km from Enfield and approximately 60 km from Dartmouth/Halifax/Sackville and Truro. One angler interviewed was from Moncton, New Brunswick, an estimated 221 km from Murchyville. The remainder was from small communities, most of which was located within a 60km radius of the creel survey check point at Murchyville. In 2007, a total of 178 anglers interviewed spent 1363 hours and 284 days to catch 593 trout. In comparison, a total of 1380 anglers interviewed spent 6889 hours and 2085 days to catch 1852 trout in 1979. As expected, the number of angler hours found during weekends in 2007 was less compared to the 1979 survey.

Most angling hours were spent fishing on Fish River, Ship Harbour Long Lake, Paul Lake, Gold Lake, Bait Lake, Bear Lake, and Dam Lake. The number of angling hours recorded on each site ranged from four hours on Blue Woods Lake to 194 hours on Fish River. The mean release rate during 2007 by lake was  $0.38 (\pm 0.31,SD)$  when all sites were included and the average overall release rate based on the total retained trout compared to the total released was 0.50 of the total catch. The average release rate reported for 1979 was 0.194 (Sabean 1980), indicating that anglers are releasing a much greater proportion of their catch than in the past. During 2007, the total (retained and released) number of trout caught per hour of angling per site was variable and the overall mean CPUE per lake was  $0.64 (\pm 0.69, SD)$  and ranged from 0 to 2.8 trout. The mean number of trout caught per individual angler was 4.0 trout ( $\pm 4.2$ , SD) and ranged from 0-17 caught per angler per site (Table 1).

Creel statistics were compared for eight sites between the 1979 and 2007 surveys. During 2007, mean FL of retained trout was 25 cm ( $\pm$ 3.3, SD) and ranged from 20 - 31 cm compared to 24.9 cm ( $\pm$ 1.9, SD) and a range of 22 - 28 cm in 1979. During 2007, the number of trout caught per hour was 0.62 ( $\pm$ 0.6, SD) and ranged from 0.04 to 1.56. During 1979 the CPUE was 0.50 ( $\pm$ 0.2, SD) and ranged from 0.20 to 0.78 (Table 2). No significant difference was detected between mean FL and CPUE between surveys (t-test, P>0.05). The increased variability in the 2007 data was expected, given the smaller sample size.

1 Catch statistics 1	6 May - 10 Turi 2007
Table	

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Name	Latitude Longitude	Anglers	Hours	Trout retained	Trout released	Released as prop of catch	Catch per hour	Catch per angler
Bait Lake	44° 49' 62° 57'	17	136	25	5	0.1	0.2	1.6
Bear Lake		8	80	31	18	0.4	0.6	6.1
Blue Woods Lake		1	4	1	10	0.9	2.8	11.0
Butler Lake stillwater	53'	0	4	33	0	0.0	0.8	1.5
Cowan Brook		4	8	7	0	0.0	0.9	1.8
Crooked Lake	52'	5	17	10	10	0.5	1.2	4.0
Dam Lake	52' 62°	3	60	14	20	0.6	0.6	11.3
Devils Lake	52'	4	32	7	1	0.1	0.3	2.0
Egg Lake	54'	2	24	3	0	0.0	0.1	1.5
Fish River	53' 62°	35	194	23	40	0.6	0.3	1.8
Gold Lake	55'	6	140	20	18	0.5	0.3	4.2
Grassy Lakes	52' 62°	8	34	34	19	0.4	1.6	9.9
Horseshoe Lake	52'	2	16	0	0	ı	0	0
Hurley Lake		2	9	10	3	0.2	2.2	6.5
John Brayden Lake	54'	4	16	0	0	ı	0	0
Lake Charlotte	50' 62°	10	47	18	23	0.6	0.0	4.1
Level Spot Lake	50'	9	38	10	38	0.8	1.3	8.0
Loon Lake	57' 63°	7	23	0	1	1.0	0.0	0.1
Otter Lake	44° 51' 62° 55'	3	18	~	1	0.1	0.5	3.0
Paul Lake		5	150	25	09	0.7	0.6	17.0
Pug Hole / Pearl Lakes	55'	11	58	18	16	0.5	0.6	3.1
Pot Lake	44° 57' 63° 03'	2	8	1	0	0.0	0.1	0.5

# THE FISHERY FOR SPECKLED TROUT

Name	Latitude Longitude	Anglers	Hours	Trout retained	Trout released	Released as prop of catch
Scraggy Lake	44° 56' 62° 52'	4	32	1	0	0.0
Shaws Lake	44° 58' 63° 07'	6	38	7	4	0.4
Ship Harbour Long Lake	44° 55' 63° 02'	15	180	15	18	0.5
Total or mean (SD)		178	1363	291	302	0.38 (0.31)

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Table 2

				2007							1979		
Lake name	Trout N	FL	Angler hours	Kept N	Released N	CPUE kept	total	Trout N	FL	Angler hours	Kept N	CPUE kept	total *
Egg Lake**	6	31	24	с	0	0.13	0.13	31	28	231	40	0.17	0.20
Fish River	21	23	194	23	40	0.12	0.33	238	24	813	421	0.52	0.62
Grassy Lakes	19	26	34	34	19	1.00	1.56	156	25	388	220	0.57	0.68
Lake Charlotte	5	24	47	18	23	0.38	0.87	48	26	353	67	0.19	0.23
Level Spot Lake**	7	20	38	10	38	0.26	1.26	99	24	171	99	0.39	0.47
Pug Hole / Pearl Lakes	17	25	58	18	16	0.31	0.59	76	23	285	186	0.65	0.78
Ship Harbour Long Lake	6	27	180	15	18	0.08	0.18	54	22	179	78	0.44	0.53
Loon Pond **	7	27	23	0	1	ı	0.04	100	28	255	113	0.44	0.53
Total or Mean (SD)	87	25 (3.3)	575	121	154	0.33 (0.3)	0.62(0.6)	790	24.9 (1.9)	2675	1191	0.42 (0.2)	0.50 (0.2)

Trout angled by NSDAF staff was included in 2007 Trout N and FL columns: Egg Lake (6), Level Spot Lake (3), and Loon Lake (7) UE = trout catch per hour of angling Total CPUE estimated from addition of 19.4% to CPUE of kept fish in 1979 \* Totai \*

per angler

per hour Catch

Catch

4.0 (4.2)

0.64 (0.69)

 $1.2 \\ 2.2 \\ 2.2$ 0.3

 $0.0 \\ 0.3 \\ 0.2 \\ 0.2$ 

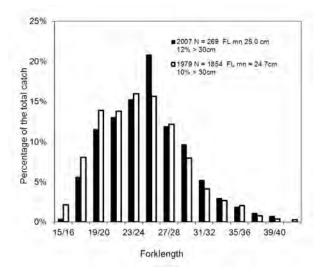


Fig 2 Fork length (cm) frequency distributions of the speckled trout catch from anglers interviewed during the Mooseland to Murchyville creel surveys in 1979 and 2007.

Change in catch size structure, and particularly in the proportion of larger fish, may reveal disturbances to the population, affecting mortality. The relative proportion of large trout (>30cm FL) in the catch was 0.10 in 1979 and 0.12 in 2007 (Fig 2). The data indicate that the mean FL of speckled trout caught by anglers and the CPUE were similar between surveys and no significant difference was detected in the proportion of large trout (FL>30cm) captured between the two surveys (z-test, P = 0.367).

In 2007, mean FL at age from eighteen lakes and Fish River was 22.4 cm ( $\pm$ 2.1,SD) for 2+y trout (18.8 - 25.7 cm), 27.7 cm ( $\pm$ 2.7,SD) for 3+y trout (21.6 - 33.5 cm), and was 34.9 cm ( $\pm$ 3.0,SD) for 4+y trout (31.5 - 38.9 cm). In 1979, mean FL at age was available from Scraggy Lake, Pug Hole/Pearl Lake, Fish River, and Lake Charlotte. During 1979, mean FL was 21.5 cm ( $\pm$ 1.7,SD) for 2+y trout (20.4 - 22.7 cm), 27.8 cm ( $\pm$ 2.4,SD) for 3+y trout (24.8 - 30.4 cm), and was 33.1 cm ( $\pm$ 2.4,SD) for 4+y trout (30 - 36.1 cm) (Table 3). No significant differences were detected for mean FL of trout at ages 2+, 3+, and 4+ y between the two surveys (t-test, P>0.05).

The age structure of the catch indicated that the majority of the trout caught by anglers were from the 2+ and 3+ age classes. Few 4+y trout were detected and 1+y and 5+y trout were absent from the samples

Year years	Name	1	Age 2+ y	ears		Age 3+	years		Age 4+	
ycars		Ν	FL	SD	Ν	FL	SD	N	FL	SD
2007	Bait Lake	7	22.1	2.4	17	27.2	2.7			
	Bear Lake	3	25.7	0.8	18	29.8	2.1			
	Blue Woods Lake				1	33.5				
	Cowan Brook	6	21.9	1.6	1	26.5	-	1	38.9	-
	Crooked Lake	2	25.7	3.1	8	29.2	4.2	3	36.4	2.4
	Dam Lake	10	21.0	1.7	2	21.6	0.5			
	Devils Lake	3	24.2	0.8	3	28.5	2.5			
	Egg Lake	13	19.5	3.6	7	31.0	3.9	1	32.4	-
	Fish River	19	21.0	2.2	9	25.0	1.7			
	Gold Lake	8	22.1	1.3	3	24.1	2.8			
	Grassy Lake	7	22.9	1.5	11	27.4	3.7			
	Hurley Lake				9	29.2	2.2	1	31.5	
	Lake Charlotte	4	20.9	1.9				1	35.4	
	Level Spot Lake	9	18.8	1.9						
	Loon Lake	17	20.2	1.7	5	28.6	1.2			
	Otter Lake	4	23.0	2.0	4	27.5	2.1			
	Pug Hole/	11	24.3	1.1	7	26.2	2.0			
	Pearl Lakes									
	Scraggy Lake				1	29.7				
	Shaws Lake	1	23.0	-	7	26.1	1.4			
	Ship Harbour	3	24.6	1.8	6	28.2	3.9			
	Long Lake									
	Summary	127	22.4	2.1	119	27.7	2.7	7	34.9	
1979	Scraggy Lake	17	22.7	1.7	83	29.3	3.5	17	36.1	3.7
	Fish River	27	20.4	2.2	22	24.8	2.4	1	30.0	
	Pug Hole/ Pearl Lakes	2	20.5	3.5	12	26.5	1.2			
	Lake Charlotte	6	22.4	2.1	6	30.4				
	Summary	52	21.5	1.7	123	27.8	2.4	18	33.1	2.4

Table 3Mean length at age of the trout catch from eighteen lakes and two stream<br/>systems from anglers interviewed during the Mooseland to Murchyville<br/>creel survey, 1979 and 2007.

from 1979 and 2007 (Fig 3). Age structure information was available for comparison among three sites surveyed in 1979 and included Pug Hole/Pearl Lake, Lake Charlotte, and Fish River. When these age structure data were pooled the proportion in each year class was 0.46 for 2+y, 0.53 for 3+y, and 0.01 for 4+y trout in 1979, and was 0.67 for 2+y, 0.31 for 3+y and 0.02 for 4+y in 2007. A significant difference was detected in the proportion of 2+ and 3+y trout captured between surveys (z-Test,P<0.05), but the age structure data were not significantly different when 1979 data from PugHole/Pearl Lake, Lake Charlotte, and Fish River were compared to the total age structure data from 2007 (z-test, P>0.05). The age structure of trout caught from Scraggy Lake was also available for comparison from the 1979 survey, however, the data were excluded as only two anglers with one trout were surveyed

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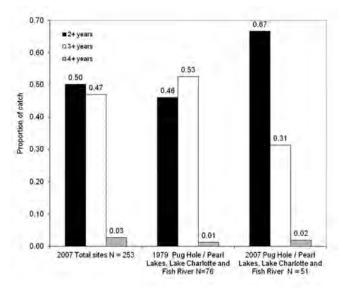


Fig 3 Age structure (y) of the speckled trout catch from anglers interviewed during the Mooseland to Murchyville creel survey, 1979 and 2007.

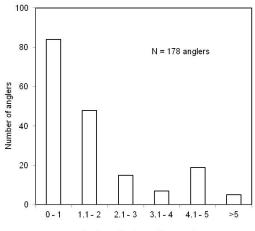
from this site in 2007. Scraggy Lake was located relatively close to the Mooseland exit and limited time was spent sampling in this location during 2007.

While angling, the most popular gear type used was bait or a combination of bait with lure or fly. The proportion of the CPUE using bait or combination of bait alone or bait with lure or fly remained relatively unchanged between the two surveys; 0.87 in 1979 and 0.88 in 2007. Angler effort using fly only was 0.09 in 1979 and 0.11 in 2007. The greatest number of trout caught per hour was accomplished angling with a fly or a combination of fly and bait, shown in both surveys (Table 4).

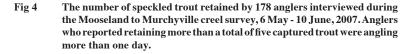
During 2007, the average number of trout retained by anglers was determined from 178 anglers in 62 angling parties as 1.7 trout and ranged from zero to 6.5 trout. The majority of the parties interviewed (82%) retained less than three trout per angler (Fig 4). Three percent of the parties retained more trout than five per angler but they were fishing for more than one day. These anglers did not exceed the possession limit of five trout per angler as they reported consuming some of the trout caught while on their multi-day fishing trip. The five trout possession limit was reached by nine percent of the anglers interviewed.

Gear type used by anglers as a percentage of the total number of angling hours and mean trout catch per hour during the Mooseland to Muchyville

creel su	rvey in 1979 and 2007	, Nova Sco	otia.	
	1979		2007	
	Percentage of effort	CPUE	Percentage of effort	CPUE
Bait	68%	0.46	26%	0.38
Bait / Fly	11%	0.53	35%	0.88
Bait / Lure	5%	0.35	26%	0.57
Bait / Lure / Fly	4%	0.23	0.2%	0.33
Fly	9%	0.50	11%	1.02
Lure	4%	0.31	1%	0.21



Number of trout caught per angler



# DISCUSSION

Creel survey data that related to angler abundance, angler catch rate, size of the catch and mean FL at age were all similar between the surveys. The sample size of the 2007 survey was smaller than that of the 1979 survey; however, it still represented a sizable angler effort and catch from the study area.

Although the percent of large trout caught between the two surveys did not reflect a change, the overall age structure was not available from the 1979 survey. Age structure information from PugHole/

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Table 4

Pearl Lake, Lake Charlotte, and Fish River did indicate a shift in the catch toward more two-year-old trout during 2007. This may represent a trend related to the impact of habitat change and exploitation, however, annual variability among year classes of speckled trout can be large (Platts and Nelson 1988).

Similar to the 1979 survey, daily bag limit was considered to have little direct impact on exploitation. The majority of anglers retained two trout per angler instead of the legal possession limit of five trout per angler. Angler effort and accessibility can apparently be more important than angling regulations on trout fisheries (Post et al. 2003). Since the total number of anglers in Nova Scotia has decreased by about 35% from a high of 85k in the late 1970s to about 55k in the 2000s (NSDFA 2000), direct comparison of angler activity in the Mooseland to Murchyville area was difficult to assess. Compared to the results of the 1979 survey, fewer overall angler hours were detected during weekends, but since only one of the two exits were sampled, this difference was anticipated and overall this region continues to be viewed as a popular destination for anglers. The recent wilderness designation of the TGLWA has reduced the ease of angler access into many of the inner lakes as vehicle traffic, mainly with all terrain vehicles, has been restricted. Remote regions of Wilderness Areas may receive less pressure than in the past because of these regulations. Restrictions to land use, in terms of forestry and other development associated with angling and hunting camps, may further reduce the potential for heavy exploitation to occur on some lakes.

On average, anglers interviewed during the 1979 creel survey released about 19% of their catch compared to an average release rate of 50% during the 2007 survey. This finding is in agreement with results from surveys conducted every five years which indicate that trout anglers released 26% of their catch in 1980 compared to 61% of their catch in 2000 in Nova Scotia (NSDFA 1995, NSDFA 2000). This change in angler behavior has probably reduced the impact of angling activity on exploited trout populations.

Anglers have often expressed a concern about high grading of catches and its impact on trout populations. Angling in general may select for faster growing, more aggressive individuals in fish populations (Biro and Post 2008). It has been suggested that high grading may result in slower growth and smaller individual size in targeted sport fish populations. A similar, mean FL at age found between the two surveys that were 28 y apart suggests that the growth rate of trout in the TGLWA has not changed significantly. Population density in trout populations is often related to habitat condition and may override selective impacts associated with exploitation in many lakes. Our findings are in agreement with Halfyard et al. (2008) who reported that the levels of harvest on three TGLWA lakes were less than fish yield estimates and low population density was correlated with size and condition of trout. Similarly, Heggelin (2008) demonstrated positive correlations among twelve TGLWA lakes between angler activity indices and catch rates, as well as inverse relationships between catch rates and the size of the catch. Accessible, productive trout habitats potentially receive more directed effort from anglers; however, the size of the catch may be smaller compared to populations at lower densities.

Catch rates can be highly influenced by weather and activity of the fish. Many anglers plan their fishing trips to correspond to the mayfly hatch in lakes. During this time, water temperatures are close to ideal for trout activity and feeding (10-16°C) (Power 1980). As a result, catch rates can be very high and may not be representative of trout population density in a lake. Limited production of trout combined with their ease of capture under some circumstances remains a concern; however, angling is only one of many factors that may influence a trout fishery. Although a change in the release rate of trout was detected and a change in angler effort was probable, the data suggests that the level of exploitation has not caused a decline in this fishery. The perspectives of anglers are often diverse and research efforts should be undertaken prior to implementing regulatory changes that may impact opportunities. More study is required to further assess populations of trout as well as their competitors in lakes to better understand the role of competition, exploitation, and habitat limitations.

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## FIRST VERIFIED RECORD FOR SHORTNOSE STURGEON, Acipenser brevirostrum LeSueur, 1818, IN MINAS BASIN, BAY OF FUNDY, NOVA SCOTIA, CANADA

### MICHAEL J. DADSWELL\*, GEORGE NAU and MICHAEL J.W. STOKESBURY

Department of Biology, Acadia University Wolfville, NS Canada B4P 2R6

A shortnose sturgeon was caught in fisherman Wayne Linkletter's intertidal fish weir in Minas Basin near Economy, Nova Scotia, on June 29, 2013. It was an adult, 73.7 cm fork length and weighed ~4.5 kg. Fishers in Minas Basin relate that they have captured shortnose sturgeons in their weirs in the past decade (1 or 2 fish/y) but this is the first sighting verified by photographic evidence. In Canada shortnose sturgeons were previously known only from the Saint John River and Harbour. The new record extends the coastal range of the species by approximately 165 km and is a new addition to the fish fauna of Nova Scotia.

#### INTRODUCTION

The shortnose sturgeon, *Acipenser brevirostrum* LeSueur, 1818, is one of two anadromous sturgeon species that occur on the Atlantic coast of North America (Vladykov and Greeley 1963). It is a smallbodied species (for sturgeon) which seldom grows longer than 120 cm fork length (FL; Dadswell 1979). The Atlantic sturgeon, *Acipenser oxyrinchus* Mitchill,1814, the other anadromous species on the Atlantic coast, is known to grow to 459 cm FL and weigh up to 365 kg (Scott and Scott 1988).

Shortnose sturgeon is an estuarine species that usually occupies the low salinity regions of its natal estuary (Dadswell et al. 1984a) but is also known to make limited coastal migrations (Fernandes et al. 2010, Peterson and Farrae 2011). Shortnose sturgeon was first identified from the Saint John River estuary in 1957 (Leim and Scott 1966) and this locality remains the only known spawning population in Canada (Usvyatsov et al. 2013). It was listed as a 'species of concern' by the

<sup>\*</sup> Author to whom correspondence should be addressed: mike.dadswell@acadiau.ca

Canadian Species at Risk Act (SARA) in 2012. Shortnose sturgeon also occurs in at least 28 watersheds along the east coast of the USA (Dadswell et al. 1984a) but has been listed as 'endangered' by the USA Endangered Species Act since 1973. Recently, perhaps because of 40 years of protection, studies have shown that there are some large estuarine populations (10,000+ adults) throughout its range (Dadswell 1979, Hastings et al. 1987, Bain et al. 2007). Possibly because of the increased populations in natal estuaries, more sturgeon may be entering coastal waters to forage. Our studies (Dadswell et al. 1984b) and fishers' observations suggest the presence of shortnose sturgeon in Minas Basin is a recent occurrence. Because of its limited range in Canada and its listing by SARA, we believe it is important to provide the details of its verified presence in Minas Basin, Bay of Fundy and its addition to the fish fauna of Nova Scotia.

#### THE OCCURRENCE

The shortnose sturgeon was captured on June 29, 2013 in Wayne Linkletter's intertidal fish weir which is situated in Minas Basin near Carr's Brook, Economy, Nova Scotia (N45.38742; W063.96145). Intertidal weirs are a V-shaped construction of stakes, mesh and brush with each wing approximately 800 m long (Gordon 1993). There is a fish trap with a pool at the lower intertidal zone of the V where the catch accumulates at low tide. The weirs of Minas Basin capture numerous commercial fish species including Atlantic sturgeon, Atlantic herring, gaspereaux, American shad, winter flounder, Atlantic mackerel and striped bass (Dadswell et al. 1984b). Atlantic sturgeon catches occur from early May until late July each year. Catches vary annually from 10's to 100's of Atlantic sturgeon with about 2-10 captured on most low tides during the run period. Sturgeon captured in marine waters are required by the Canadian Department of Fisheries and Oceans to be released alive in which case all are handled manually by the fisher to allow them freedom as soon as the weir is fished. Our Acadia University crew was working with Mr. Linkletter tagging Atlantic sturgeon for an ongoing population study (McLean et al. 2013). In this way the shortnose sturgeon was identified during a midday tide (low water 12:33 pm). For comparison, there were also two Atlantic sturgeon in the weir on that tide.

The shortnose sturgeon was an adult, 73.7 cm FL and weighed approximately 4.5 kg (Weight-Length relationship; Dadswell 1979). Photographs of all the critical taxonomic characteristics were taken with a mobile telephone camera (head, top and bottom, body; iPhone-4) before the sturgeon was tagged with a yellow, FLOY FT-1 dart tag (Acadia #4057) under the anterior of the dorsal fin and released alive.

Shortnose sturgeon are easily recognized from Atlantic sturgeon because of their short, rounded snout (Fig 1, 2) and very slippery, smooth skin (Gorham and McAllister 1974). Atlantic sturgeon of a similar length (60-100 cm FL) has an elongated snout (Fig 1) and are very rough to the touch because of small, sharp dermal ossifications.

Taxonomic characteristics of the shortnose sturgeon easily discernible from our photographs are the wide mouth which exceeds 62% of the interorbital width (in our specimen 66%; Vladykov and Greeley 1963) and a large, triangular lower pectoral girdle (Fig 2). The lower

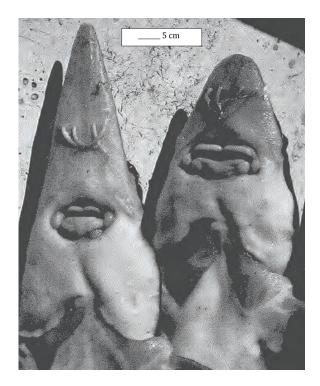


Fig 1 Ventral view of an Atlantic sturgeon (left) and a shortnose sturgeon (right); note short, round snout, wide mouth and large, triangular pectoral girdle of the shortnose sturgeon (after Dadswell et al. 1984a).



Fig 2 Ventral view of shortnose sturgeon captured in Minas Basin on June 29, 2013. Note the wide mouth (66% of interorbital width),rounded lateral protuberances of the lower lip, and wide, triangular pectoral girdle.

lip in the shortnose has rounded protubences at its lateral edges; in the Atlantic these are rectangular (Fig 1). In sturgeons the cleithrum of the pectoral girdle is fused to the clavicle (Vladykov and Greeley 1963) and the clavicle shows as a large flat, bony plate on the ventral side of the fish on each side of the body just behind the opercula (Hilton et al. 2011). In the shortnose sturgeon this plate is wide and triangular in shape, in an Atlantic sturgeon it is narrow and curves laterally on its inner side along the *linea alba* (Fig 1). Shortnose sturgeon have large nares that are set close to the eye (Fig 3). Atlantic sturgeon have smaller, less obvious nares.

#### DISTRIBUTION OF SHORTNOSE STURGEON

Shortnose sturgeon populations are known from the Saint John River estuary, New Brunswick south along the Atlantic coast of the USA to the Saint John's River, Florida (Dadswell et al. 1984a). Although shortnose sturgeon largely remain in the lower salinity reaches of their natal estuary, they often make coastal movements between watersheds, particularly during the warm water period of the year. In the southern



Fig 3 Lateral view of the head of the shortnose sturgeon captured in Minas Basin on June 29, 2013. Note the short, rounded snout and the large nares set close to the eye.

part of its range there is persistent movement between the Ogeechee and Altamaha rivers in Georgia, a distance of about 80 km (Peterson and Farrae 2011). In Maine, 83% of shortnose sturgeons tagged with acoustic tags in the Penobscot River were recovered by acoustic receivers in the Kennebec River, after moving an average distance of 120 km (Fernandes et al. 2010).

Minas Basin is warm during summer (18-20 C) and has lower salinity than the open Bay of Fundy (24-30; Bousfield and Liem 1959). The Basin has an abundance of intertidal shellfish (*Mya sp., Macoma sp.*), which are the preferred food of adult shortnose sturgeon (Dadswell et al. 1984a). The distance between the mouth of the Saint John River and Economy is about 165 km, easily within the coastal migration distances known for this species. In addition, various weir fishers have told us they had seen shortnose sturgeon in their weirs in recent summers. We are thankful that we were able to verify their observations and extend the known Atlantic coast range of the shortnose sturgeon. Acknowledgements We thank Wayne Linkletter, Tony Lewis and Darren Porter for allowing access to their weirs. The Atlantic sturgeon study, which made this work possible, is funded by the Ocean Tracking Network through the National Science and Engineering Research Council of Canada. MJWS is funded by the Canada Research Chairs Program. We thank John Gilhen for improvements made to the manuscript.

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### **Student Award-Winning Paper**

## AGE-RELATED CHANGES IN MOTOR ABILITY AND MOTOR LEARNING IN TRIPLE TRANSGENIC (3×TG-AD) AND CONTROL (B6129SF1/J) MICE ON THE ACCELERATING ROTAROD

### JONATHAN J. OORE, LEANNE M. FRASER and RICHARD E. BROWN\*

Department of Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4R2

#### ABSTRACT

Mouse models of Alzheimer's disease (AD) show both cognitive and neuromotor impairments. We measured motor ability and motor learning of male and female triple transgenic ( $3\times$ Tg-AD) and control (B6129SF1/J) mice on the accelerating Rotarod in a cross-sectional design at 2, 6, 9, 12, and 15 months of age. At all ages except for 2 months, transgenic mice performed better and had a steeper motor learning curve than controls. Female mice showed better motor performance than males, while males had a steeper learning curve than females. Age did not have a significant main effect on Rotarod performance of  $3\times$ Tg-AD mice peaked at 6 months of age and decreased as age increased. The performance of controls was below that of  $3\times$ Tg-AD mice at each age. Behavioural differences seen in the  $3\times$ Tg-AD mice may help us to understand the development of neuromotor dysfunction in AD.

*Keywords*: Alzheimer's disease, Alzheimer models, Amyloid beta, motor ability, motor learning, Rotarod, tau, transgenic, 3×Tg-AD mice

### **INTRODUCTION**

Alzheimer's disease (AD) is the most common form of dementia in humans (Alzheimer's Association 2011). Currently, over 26 million individuals have AD worldwide, and this number is expected to triple by 2050 (Brookmeyer et al. 2007). In Canada, AD accounts for more than 50% of dementia diagnoses, and there are over 100 thousand

<sup>\*</sup> Author to whom correspondence should be addressed: rebrown@dal.ca

new cases of dementia per year, which is predicted to double by 2030 (Alzheimer Society of Canada 2010). In the same time frame, Nova Scotia's 3,200 new cases of AD per year are projected to double, and more than 2% of Nova Scotia's population will suffer from dementia (Smetanin et al. 2009). Alzheimer's is a neurodegenerative disease, leading to an early death (Mayeux 2010). After onset, those with AD experience short-term memory loss and, as the disease advances, they develop symptoms of confusion, long-term memory loss, and impairments in language and motor function (Castellani et al. 2010, Budson & Solomon 2012).

Two principal markers of AD have been identified in the brain: extracellular deposits of amyloid beta (A $\beta$ ) protein and intracellular neurofibrillary tangles of tau proteins (Hardy & Allsop 1991, Huang & Mucke 2012). However, the link between A $\beta$  and tau proteins in the development of AD is not understood. Immunizations which reduce A $\beta$ accumulation had no affect on AD symptom development; therefore, there must be other factors at work (Holmes et al. 2008, Robakis 2011). At present, there is no cure for AD and few treatments have had success. To study AD, it is useful to turn to simple animal models. While no model accurately represents AD pathology as a whole, the changes in A $\beta$  neuropathology with age have been modeled in mice (Gilley et al. 2011). The present study investigates the triple transgenic mouse model of AD (3×Tg-AD), which has become an important tool in the development of pharmacological and behavioural therapy against AD (García-Mesa et al. 2011).

The 3×Tg-AD mouse model was created by microinjection of AP-P<sub>Swe</sub> and tau<sub>P301L</sub> mutant transgenes into a germline of PS1<sub>M146V</sub> knockin mouse embryos (Oddo et al. 2003a). The model hinges on the amyloid precursor protein (APP<sub>Swe</sub>), which leads to the production of A $\beta$  in the brain, and the tau<sub>P301L</sub> gene, leading to hyperphosphorylation of tau proteins developing into neurofibrillary tangles. In addition, the presenilin 1 mouse gene (PS1<sub>M146V</sub>) interacts with APP<sub>Swe</sub>, accelerating A $\beta$ accumulation in the brain (Oddo et al. 2003a, b). The 3×Tg-AD mice show an increase in the number of A $\beta$  plaques from three to twelve months of age, and in neurofibrillary tangles from six months of age (Oddo et al. 2008). Sex differences have been found in the development of A $\beta$  pathology in 3×Tg-AD mice. Female 3×Tg-AD mice show increased numbers of A $\beta$  plaques and more behavioural deficits than males. This difference has been attributed to the organizational effects of neonatal sex hormones (Carroll et al. 2010). Increased  $\beta$ -secretase levels seen in female mice are associated with increased A $\beta$  production and decreased neprilysin levels. Both of these changes are thought to lead to a decrease in the degeneration of A $\beta$  (Hirata-Fukae et al. 2008). The presence of these pathologies, however, cannot predict age-related changes in behaviour in 3×Tg-AD mice (Knight et al. 2013).

A number of studies have reported differences in motor skills between transgenic Alzheimer model mice and their wildtype controls. However, the results of such studies appear contradictory. Deficits in motor performance increase with age in both the  $APP_{751}/PS1_{KI}$  (double transgenic) and 5×FAD mouse models of Alzheimer's disease, and are attributed to axonopathy due to increased AB deposits (Jawhar et al. 2012). In contrast, compared to controls a tau<sub>INPL3</sub> mouse model showed increased Rotarod performance as well as better performance in the balance beam and coat hanger tests (motor performance) compared to controls and this was attributed to the presence of mutant tau protein (Morgan et al. 2008). Finally, the 3×Tg-AD mice were found to outperform controls on the Rotarod test and swam faster than controls in the Morris Water Maze (Filali et al. 2012, Stover 2012). Other studies use mice with similar  $(tau_{P301S})$  or the same  $(tau_{P301L})$  tau mutation as that in the 3×Tg-AD model and have found motor deficits (Scattoni et al. 2010, Xu et al. 2010). The differences in results in motor performance may be due to inherent differences in background strains of the transgenic mice (Brooks et al. 2004, McFadyen et al. 2003).

The accelerating Rotarod assesses motor ability and motor learning of rodents (LeMarec & Lalonde 1997, Hamm et al. 1994, Bohlen et al. 2009). Mice are placed onto a rotating rod and the latency to fall (the time from the beginning of the rod's rotation to when a mouse falls) is recorded. The Rotarod is considered a valid measure of motor ability and learning over short-term (trials) and long-term (days) test durations (Buitrago et al. 2004). This test is powerful because it can be used to detect motor behaviour abnormalities in genetically altered, brain damaged, or drug-treated mice (Rustay et al. 2003; Shiotsuki et al. 2010). However, differences in body weight can confound the measurements of mice tested on the Rotarod (Brown et al. 2002). Mice with lower body weights often outperform heavier mice and, since female mice weigh less than males (Stover 2012), it is important to control for body weight in the Rotarod test. The present experiment used the accelerating Rotarod to investigate age related changes in motor ability and motor learning of male and female  $3\times$ Tg-AD and control (B6129SF1/J) mice from 2 to 15 months of age, in a cross-sectional design. It was hypothesized that (1) the latency to fall for all mice would increase over trials as they learned to stay on the Rotarod (Hamm et al. 1994) and (2) mice with higher body weights would have shorter latencies to fall than those with lower body weights (Brown et al. 2002). Increases in A $\beta$  pathology in female  $3\times$ Tg-AD mice suggest that (3) males would outperform females (Carroll et al. 2010). Based on recent literature (Stover 2012, Morgan et al. 2008), it is hypothesized that (4) transgenic mice will have a longer latency to fall than controls.

#### METHODS

#### **Subjects**

Control (B6129SF1/J) and 3×Tg-AD mice were purchased from Jackson Laboratories (Bar Harbor, Maine) and bred at Dalhousie University. This experiment was approved by the Dalhousie University Committee on Laboratory Animals. Mice were weaned at 21 days of age and housed in same-sex groups of two to four in clear plastic cages measuring  $28 \times 18.75 \times 12.5$  cm containing woodchip bedding. *Ad libitum* access to Purina rodent chow and water was provided for all mice. Each cage included a PVC enrichment tube (4 cm diameter × 7 cm length). The colony room was kept at  $22\pm 2$  °C, with a reversed light-dark cycle (lights off from 09:30-21:30). Table 1 shows the distribution of mice tested by genotype, sex, and age. Table 2 gives details of mean body weights of the mice.

#### Apparatus

The accelerating Rotarod (AccuScan Instruments, Columbus, Ohio), consisted of an acrylic, horizontally-grooved rod (30 mm diameter), rotating about its long axis (Fig 1). The rod was divided into four 11 cm compartments by Plexiglas dividers (30 cm diameter), so that four mice could be tested simultaneously. Below each rod segment was a holding compartment connected to a timer that automatically recorded the latency of each mouse to fall. The Rotarod accelerated from 0.0 to 48.0 rpm over 360 seconds. The testing room ( $1.12 \times 2.60$  m) was illuminated by a 60W red light bulb. The experiment followed

Age	3×Tg-AD	Control	Total
2-month	10F, 8M	8F, 8M	34
6-month	5F, 7M	5F, 6M	23
9-month	9F, 4M	8F, 10M	31
12-month	8F, 5M	10F, 12M	35
15-month	10F, 3M	10F, 11M	34
Total	69	88	157

 Table 1
 Number of female (F) and male (M) 3×Tg-AD and wildtype control mice tested at each age.

 Table 2
 Mean body weights (g) of mice by age, genotype, and sex, abbreviated.

Age	3xTg-AD		Control	
	Female	Male	Female	Male
2-month old	18.9	24.2	21.0	26.5
6-month old	27.4	30.8	24.9	32.3
9-month old	33.3	33.1	29.8	33.4
12-month old	36.3	32.9	29.3	38.9
15-month old	38.0	30.8	30.3	40.5

a between-subjects design (genotype  $\times$  sex  $\times$  age), with experimenters blind to age and genotype.

#### Procedure

Mice were tested on the Rotarod for six trials per day for five consecutive days during the dark phase of the light-dark cycle. Mice were weighed daily before being tested. A trial began by placing each mouse onto the stationary rod, with its head facing opposite to the direction of rotation. The Rotarod was switched on and the latency to fall (sec) was displayed on a digital panel and recorded for each mouse. Mice were left in the compartments below the Rotarod until all mice had fallen. The next trial began 60 seconds after the fourth mouse fell. After six trials, the mice were returned to their home cages. If any mouse failed to fall for the entire 360-second trial, it was taken from the rod and placed in its holding compartment, and the latency to fall was recorded as 360 seconds. After each group of mice was tested, the Rotarod was cleaned with a damp paper towel and a drop of dishwashing liquid to remove residual odours.



Fig 1 The Rotarod apparatus, showing four mice being tested, the circular dividers between compartments, the holding compartments, and timers for recording latency to fall.

#### **Data Analysis**

Mean latencies to fall for each mouse were calculated for each test day using Microsoft Excel 2011. An "Improvement score" *Pay 5 Performance–Day 1 Performance* 

 $\left(\frac{Day 5 Performance-Day 1 Performance}{Day 1 Performance} * 100\right)$  was also computed for each mouse. All statistical analyses were conducted using SPSS 21.0. Correlations were performed between weight and performance on day 5. Repeated measures ANOVAs were performed with mean fall latencies for each day as the within-subjects variable, and genotype, sex, and age as between-subjects variables. Mauchly's (1940) test was performed, and the assumption of sphericity was found to be violated for the model. A second analysis of variance was conducted using the Greenhouse-Geisser model (Girden 1992), and the significance of the F-values computed using this model did not differ from those calculated using the unmodified ANOVA. Therefore the results of the unmodified ANOVA without corrected degrees of freedom are reported. The improvement scores were assessed using a factorial, genotype by sex by age, ANOVA. Post-hoc t-tests were used to examine differences between groups on each test day. Data was considered significant for p<0.05.

#### RESULTS

No significant correlation was found between body weight and Rotarod performance on day 5 (r=-0.151; df=155; p=0.058), thus weight was not used as a covariate in subsequent analyses. Performance for all mice increased significantly over the five days of testing (F(4,548)=261.47, p<0.001) (Fig 2A-F). A main effect was found for genotype (F(1,137)=60.54, p<0.001) as transgenic mice outperformed controls in latency to fall. There was also a main effect of sex (F(1,137)=4.49, p<0.05) as females outperformed males. No significant main effect was found for age. There was a significant genotype by day interaction (F(4,548)=26.89, p<0.001) (Fig 2F) as latency to fall increased more rapidly for transgenic mice than control mice over days. No significant sex by day or age by day interactions were found. The interaction between genotype and age was significant (F(4,137)=3.64, p<0.01). As shown in Figure 2 (A-E), the mean latency to fall of transgenic mice peaked at 6 months of age and decreased as age increased, with the 15 month old group having the lowest latencies. The mean latencies were lower in controls than in transgenic mice at each age, with peaks occurring among controls at 2 and 9 months of age. There was also a significant genotype by age by day interaction (F(16,548)=2.41, p<0.01). This three-way interaction reflects the finding that the genotype by day interaction was not significant at 2 months of age (F(4,128)=0.928, p=0.45) but was significant at all other ages: at 6 months (p<0.001); at 9 months (p<0.001); at 12 months (p<0.001); and at 15 months (p<0.01) (Fig 2A-E). Post-hoc t-tests indicate the significant differences between genotypes on each test day at each age (Fig 2A-E). Improvement scores were significantly higher in transgenic mice than in controls (F(1,137)=15.58, p<0.001) (Fig 3A); and higher in males than females (F(1,137)=8.08, p=0.005)(Fig 3B). Improvement scores were not significantly affected by age nor were any interactions found.

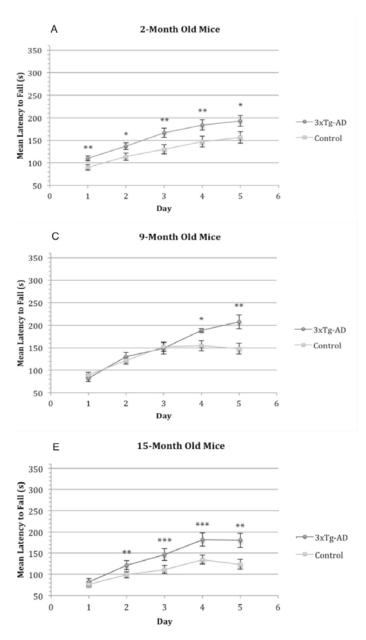
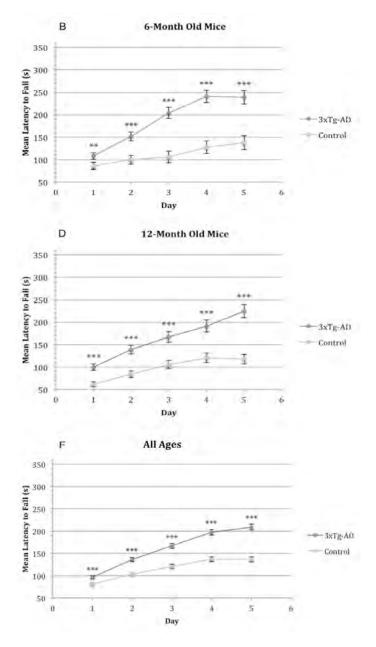


Fig 2 Mean (±SEM) latency (s) to fall over the 5 days of testing for 3×Tg-AD and control mice at (A) 2 months of age; (B) 6 months of age; (C) 9 months of age; (D) 12 months of age; (E) 15 months of age; and (F) at all ages. Asterisks indicate the level of significance between means for 3×Tg-AD and control mice for each day, at each age (\*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001).





#### DISCUSSION

The purpose of this study was to characterize age-related changes in the motor ability and motor learning of male and female 3×Tg-AD mice on the accelerating Rotarod. As hypothesized, (1) the latency to fall for mice significantly increased over the five days of testing at all ages. This indicated that all of the mice tested showed motor learning on the accelerating Rotarod. We hypothesized that (2) lighter mice would outperform heavier mice (Brown et al. 2002), but the results did not indicate any influence of body weight. Increases in A $\beta$  pathology of female 3×Tg-AD mice led to the hypothesis that (3) males would outperform females on the Rotarod (Carroll et al. 2010). This hypothesis was not supported; females had significantly longer latencies than males. This could be in part because female mice tend to have lower body weights than males (Stover 2012), and lower body weights are associated with longer latencies (Brown et al. 2002). However, the improvement scores were significantly higher for males than for females (Fig 3B). Thus, females performed better than males, but males learned more quickly, possibly due to less accumulation of A $\beta$  pathology (Hirata-Fukae et al. 2008). While no main effect was found for age, there was a significant genotype by age interaction. A notable shift occurred at 6 months of age (Fig 2B), where the learning trajectory becomes much steeper in transgenic mice than in controls. This coincides with detectable pathologies in 3×Tg-AD mice (Oddo et al. 2003b). Perhaps unknown neural changes within 3×Tg-AD mice promote motor learning while leading to cognitive and memory impairments (Morgan et al. 2008).

Recent studies on Rotarod performance for  $3\times$ Tg-AD mice (Filali et al. 2012, Morgan et al. 2008, Stover 2012) led to the hypothesis that (4) transgenic mice would outperform controls in motor ability and motor learning. Our results supported this hypothesis: transgenic mice had a longer latency to fall and exhibited a greater increase in performance over the five-day test period than controls (Fig 2F). As expected, the improvement score was also significantly higher in transgenic mice than in controls (Fig 3A). Morgan et al. (2008) attributed increases in Rotarod performance to the tau protein. In their study, the Rotarod was the only task that they found to be sensitive enough to detect improvements in transgenic mice with only A $\beta$  pathology over controls. However, Morgan et al. (2008) did not suggest any explanation for the effect of mutant tau. Filali et al. (2012) also noted a significant increase

in Rotarod performance in  $3\times$ Tg-AD mice and stated that this result was paradoxical. Similarly, they did not explain their finding. Indeed, this is paradoxical since many tau models display worse performance on the Rotarod than controls (Scattoni et al. 2010, Xu et al. 2010). Harada et al. (1994) found tau proteins to be integral in the organization and stabilization of axonal microtubules in mice. Both microtubules and A $\beta$ , when functioning correctly, are involved in cellular transport (Harada et al. 1994, Igbavboa et al. 2009). Despite that these are both mutant forms, which lead to the overproduction and accumulation of A $\beta$ and tau protein, perhaps together they may promote an ideal transport gradient resulting in the motor improvements seen in  $3\times$ Tg-AD mice. While this is conjecture, it may be useful in the future to examine the motor control processes regulated by tau and A $\beta$  proteins.

Mice may need an initial adjustment period to the Rotarod, regardless of genotype or sex. One limitation of a cross-sectional study is that this adjustment period is seen at all ages. In the future, it would be useful

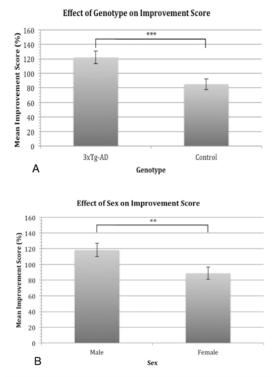


Fig 3 Mean (±SEM) improvement score (%) showing differences between (A) genotype and (B) sex. (\*=p<0.05, \*\*=p<0.01, \*\*\*=p<0.001).

to conduct more experiments longitudinally to look at motor behaviour in mice with extensive experience. Interestingly, Stover (2012) investigated how different starting ages progress longitudinally on the Rotarod (i.e. testing mice for the first time at 9-months and retesting the same mice at older ages) and found the same differences between  $3\times$ Tg-AD mice and controls as were found in this study (unpublished results, personal communication, 2013). Another limitation was that the mice were only tested until 15 months of age, though neuropathology in  $3\times$ Tg-AD mice exists at older ages (Oddo et al. 2003b). The 15 month  $3\times$ Tg-AD mice may not have enough neural loss to detect differences from younger ages. From 18 to 24 months of age, cognitive deficits increase, learning decreases, and the abundance of neuropathologies increase in  $3\times$ Tg-AD mice (Billings et al. 2007). Testing older mice, and testing more often throughout the life span, could increase the range of results and better reveal the trajectory of age effects.

There are differences in motor behaviour and motor learning in mice used as background strains for transgenic models (Brooks et al. 2004, McFadyen et al. 2003). The 3×Tg-AD background strain (B6129SF1/J) was used as a control. This strain is considered an appropriate control for the 3×Tg-AD mice. However, the choice of a control is crucial. Even sub-strains display varying behaviour (Stover 2012). Testing multiple sub-strains of the controls against 3×Tg-AD mice could aid in future comparisons. To characterize 3×Tg-AD mice, it would be useful to look at other motor behaviours that occur during Rotarod trials such as gait, foot slips, or Rotarod strategies, such as thigmotaxis, backward walking, and rearing against the dividers. Such observations could help to develop better motor profiles of 3×Tg-AD and control mice. Gait differences between mice (stride length and front paw stride synchrony) have been detected on the Rotarod (Stroombants et al. 2012). Buitrago et al. (2004) suggest that gait differences resulted in varying levels of improvement on the Rotarod. Looking at behavioural strategies or gait may help explain the superior performance seen in 3×Tg-AD mice, which may be linked to specific components of motor behaviour.

In summary, the results of this study suggest that the neuro-genetic mechanisms underlying motor behaviour with the 3×Tg-AD model may provide a link between motor ability and learning impairment in Alzheimer's disease, and provide some insight into the mechanism underlying the relationship between motor learning, sex and age. Gaining a better understanding of motor differences in relation to the

age- and sex-related development of brain pathologies will help to focus future research on this important issue.

Acknowledgements It is with great pleasure that I thank Dr. D. Gilin Oore for her tremendous support with the results and data analysis. Thank you to T. O'Leary for helping to formulate the original proposal for this project. Thank you to my good friend, B. Proudfoot for his tech-savvy creation of the significance indicators in the figures. And thanks to K. McParland, D.M. Oore, and A. Silburt, who each helped with revisions. Finally, I would like to thank my dear S.J. Yufe, whose total support throughout the entire project, and its subsequent redrafts, was a *joy* to have.

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## **NSIS COUNCIL**

### (Reports from the Annual General Meeting, May 2013)

### AGENDA

### **152<sup>ND</sup> ANNUAL GENERAL MEETING**

### 5 pm - 6 May 2013

### The Great Hall, University Club Dalhousie University, Halifax, Ns

- 1. Minutes of 151st AGM, 2012 meeting
- 2. President's Annual Report (Michelle Paon)
- 3. Treasurer's Annual Report (Angelica Silva)
- 4. Editor's Annual Report (Peter Wells)
- 5. Librarian's Annual Report (Michelle Paon)
- 6. Student Science Writing Competition (Hank Bird)
- 7. Hall of Fame Review (Richard Singer and John Rutherford)
- 8 Nominations for Positions on NSIS Council
- 9. Other Business
- 10. Adjournment

# REPORTS FROM THE NSIS COUNCIL NOVA SCOTIAN INSTITUTE OF SCIENCE PRESIDENT'S REPORT: 2012 TO 2013

The Nova Scotian Institute of Science is a long-standing organization that promotes science in Nova Scotia and supports the dissemination of peer-reviewed scientific research. When issues arise that affect the conduct and quality of science in the province, the Institute takes as its responsibility that it should express its views. Over the past year, NSIS Council has witnessed with trepidation a number of major changes which have occurred with regard to federally-funded science in Canada. Significant cuts have been applied to scientific research programs, and modifications have been made to the Fisheries Act and environmental assessment legislation via the successful passage of Bill C-38. In addition, federal scientists have been restricted in speaking to the media about their research results. At the time of this writing, plans are also underway to consolidate the federal Department of Fisheries and Oceans libraries, decreasing their number from eleven sites to two primary locations and two specialized collections.

On June 14, 2012, acting on a motion passed at its 2012 Annual General Meeting, NSIS Council sent a letter to Prime Minister Stephen Harper objecting to portions of Bill C-38. The letter expressed concerns about the proposed changes to the federal Fisheries Act and to environmental assessment legislation. In addition, NSIS noted its concerns about the limits being placed on federally employed scientists to communicate the results of their research. Copies of the letter were sent to the federal Minister of the Environment and the Minister of Fisheries and Oceans, the leaders of the Opposition, Liberal and Green Parties, Opposition critics for the Environment and Fisheries, Speaker of the Senate Noel Kinsella, all Nova Scotia Members of Parliament, and all NSIS members. Copies were also sent to *The Globe and Mail* and *The Chronicle-Herald*, the latter of which printed the letter on June 21<sup>st</sup>.

NSIS received email responses from Opposition Leader Thomas Mulcair, and three Nova Scotia Members of Parliament. Among the latter was Mr. Robert Chisholm (Opposition Fisheries Critic), who met with the NSIS President and Past President on July 25th. The discussion centred around the responses of NSIS members to the coming changes set out in Bill C-38.

In early September, we received a letter from the Prime Minister's Office, assuring NSIS that its concerns had been noted and thanking the Institute for its letter. Subsequently, in January 2013, we received a letter from federal Minister of Fisheries and Oceans Keith Ashfield, again thanking us for our communications and assuring the Institute that the new legislation would adequately protect the commercial, recreational and Aboriginal fisheries. However, he did not address the issue of federal scientists being prevented from speaking with the media about their research. NSIS Council agreed that the response had left a number of questions unanswered, and at the time of this writing, Council is preparing a letter to Minister Ashfield.

NSIS has a long history of engaging scientists to speak to the public about their research within the context of the NSIS monthly lecture series. The 2012-2013 lecture programme was organized by Michelle Paon, John Rutherford, and Angelica Silva. A variety of engaging topics were presented, attracting on average about 65 attendees per event. In fact, the January lecture drew a capacity crowd of 114!

The 2012-2013 lecture programme consisted of the following speakers and topics:

#### Monday, 1 October 2012 7:30

NS Museum of Natural History Dr. Tamara Franz-Odendaal: Department of Biology, Mount Saint Vincent University NSERC Atlantic Chair, Women in Science & Engineering GIRLS and Science: Why it's Important and How You Can Support Them

Monday, 5 November 2012 7:30

NS Museum of Natural History

## Dr. Arunika Gunawardena: Department of Biology,

### **Dalhousie University**

Dying to Live: The Role of Programmed Cell Death in Plant Development

Monday, 3 December 2012 7:30

NS Museum of Natural History Dr. Eric Mills: Professor Emeritus of History of Science, Department of Oceanography, Dalhousie University, and Inglis Professor, University of King's College Canadian Marine Science from Titanic to BIO

### Monday, 7 January 2013 7:30

NS Museum of Natural History

Dr. Doug Wallace: Department of Biology, Dalhousie University Canada Excellence Research Chair in Ocean Science and Technology

Sea Change in the Ocean: What do We Know? and How do We Know It?

#### Monday, 4 February 2013 7:30

NS Museum of Natural History Dr. Luigi Gallo: Department of Astronomy and Physics, Saint Mary's University Seeing the Universe in X-rays

### Monday, 4 March 2013 7:30

NS Museum of Natural History Dr. Jeff Dahn, FRSC: Department of Physics and Atmospheric Science, Dalhousie University NSERC/3M Canada Industrial Research Chair / Canada Research Chair How Good Can a Battery Be?

### Monday, 1 April 2013 7:30

NS Museum of Natural History Dr. Danny Silver: Director of the Jodrey School of Computer Science, Acadia University Getting a Machine to <del>Fly</del> Learn: Extending Man's Reach Beyond his Grasp

### Monday, 6 May, 2013 7:30

Great Hall, University Club, Dalhousie University **Dr. Sean Myles: Faculty of Agriculture, Dalhousie University** *Sex-Deprived Fruit: How a Lack of Breeding Threatens our Food's Future and How Genomics Can Help Fix the Problem* 

In addition to the Institute's regular lectures, a special joint lecture was organized to celebrate the NSIS 150<sup>th</sup> anniversary. On November 21<sup>st</sup>, **Dr. Suzanne Zeller** delivered a very engaging presentation entitled:

"The Natural History of a Sustainable Institution: The Nova Scotian Institute of (Natural) Science since 1862". To prepare for the lecture, Dr. Zeller had conducted research at the Nova Scotia Archives and had read the content of all volumes of the *Proceedings of the NSIS* – no small feat! This lecture was co-sponsored by NSIS, the Royal Nova Scotia Historical Society, the Dalhousie University School of Information Management, the History of Science and Technology Programme at the University of King's College, and the Situating Science Strategic Knowledge Cluster - Atlantic Node.

The Institute was also one of several co-sponsors of a public lecture presented by **Dr. Evelyn Fox Keller**, Professor Emerita of History and Philosophy of Science at the Massachusetts Institute of Technology. Organized by Situating Science, the lecture was entitled "Paradigm Shifts and Revolutions in Contemporary Biology". It took place on October 30<sup>th</sup> at Alumni Hall, University of King's College and was attended by over 250 people, including many scientists, philosophy professors, students, and members of the public.

This year, the Institute also connected with fans of classical music. On October 6 and 7, 2012, to celebrate the NSIS 150<sup>th</sup> anniversary and to explore the links between science and music, Symphony Nova Scotia performed Haydn's *Creation* oratorio. About 600 attendees enjoyed the Saturday evening concert, while in the range of 300 attended the Sunday afternoon performance. We were privileged to have the opportunity to work with the symphony on some of the plans for these events. NSIS recruited six scientists as speakers for the pre-concert panel discussions, facilitated by Adrian Hoffman. Panel speakers included: Helen Lau, Michael Steinitz and Cameron Ells; and Jordan Kyriakidis, Sageev Oore and Brad Peters. These events provided NSIS with increased exposure to the public and an opportunity to work closely with an established community partner.

In addition to participating in its own anniversary activities, NSIS was invited to take part in the 50<sup>th</sup> anniversary celebrations of the Bedford Institute of Oceanography. On behalf of the NSIS, I attended the *Symposium on Oceans and Climate Change* held in Dartmouth on October 24, 2012. During this enlightening day-long event, highly-regarded marine scientists from Germany, Denmark and Canada spoke on topics such as: changes in fisheries species, ocean water chemistry, coastal impacts, and marine policy issues.

In the fall, as NSIS 150<sup>th</sup> anniversary events drew to a close, Council decided to address in a more formal way the preservation of its archival records. In November 2012, Dalhousie University Archivist Michael Moosberger was invited to Council to speak in general about the importance of preserving institutional records. Then in December, the president and past president met with Provincial Archivist Lois Yorke to talk about the NSIS records held by the Nova Scotia Archives. They were acquired at some time in the past, but it has been many decades since new material has been added. These meetings have proven very useful to Council, which continues to deliberate on this matter.

With regard to our publications, a new issue of the *Proceedings* was published in December 2012 (volume 47, issue 1). In keeping with the new format, this issue's cover sported a full-colour aerial view of Lake Rossignol, the setting for a 2006 BioBlitz, whose results are described in the issue. Editor Peter Wells, Associate Editor David Richardson and their editorial review board are to be commended for their tireless work in producing the journal and for their efforts in networking with the scientific community to solicit new manuscripts as potential journal content. In addition, Gail LeBlanc is warmly thanked for her production and layout work on each issue. NSIS acknowledges the financial support of the Nova Scotia Department of Communities, Culture and Heritage, which funds part of the publishing costs of the journal.

During the spring, the Institute made progress towards its plans to transition to an electronic format for the *Proceedings*. After discussions with the Dalhousie University Libraries, NSIS has signed a Memorandum of Understanding with the university to publish the online version of the *Proceedings* on the Open Journal Systems (OJS) platform. The Libraries will host the journal, providing the server space and the electronic platform, while NSIS will load the journal content on the platform. It is hoped that the online *Proceedings* will provide greater exposure for the research published in the journal. With the assistance of the Dalhousie Libraries, the online platform will be prepared for use during the summer of 2013.

The Student Science Writing Competition was organized and overseen by Henry (Hank) Bird, whom we thank for his capable work in coordinating this initiative. NSIS also acknowledges the Situating Science Strategic Knowledge Cluster – Atlantic Node, which provided financial support for the competition. Our congratulations to the winners, two of whom were present at the April meeting of the NSIS. Prizes and/or certificates were awarded to the following: In the undergraduate category, the winner was **Jonathan Oore** (Dalhousie University) for a paper entitled, "Age-related Changes in Motor Ability and Motor Learning in Triple Transgenic (3xTg-AD) and Control (B6129SFiI/J) Mice on the Accelerating Rotarod".

An undergraduate Honourable Mention was awarded to **Laura Perry** (Cape Breton University) for a paper entitled, "The Effects of Climate Change on Several Seabird Species on the Coastal Regions of North America".

In the graduate category, Honourable Mentions were awarded to:

**Lynn Cornish** (St. Francis Xavier University) for a paper entitled "Managing Molecules in Health and Nutrition - A Literature Review".

**Jordi Segers** (Saint Mary's University) for a paper entitled "Summer Distribution and Movement Patterns of Short Distance Migratory Bats in Relation to a Wind Farm in Nova Scotia".

In addition to sponsoring the writing competition, NSIS continued to provide financial support to the ten Nova Scotia regional science fairs in the form of a donation of \$150.00 in prize money to each. A number of the science fair coordinators have already sent letters to Council, expressing their thanks for this year's contribution. Also to be noted, NSIS support of one fair, that of the Conseil Scolaire Acadien Provincial (CSAP), was mentioned in an April 19<sup>th</sup> news article in *Le Courrier*, Nova Scotia's French language weekly newspaper.

NSIS also funded several other initiatives, including the graduate student poster competition at the annual conference of the Fishermen & Scientists Research Society held in Truro in February 2013, and the RobotsEast Atlantic Championship for school children held in Halifax in May 2013.

News of the NSIS initiatives is regularly posted to the NSIS website, an important means of communication, which is now being transferred to the open source WordPress software platform. The adoption of this software is expected to make it easier for the NSIS webmaster to update the site with text, photos, and news items. Thanks to webmaster Suzuette Soomai for her diligence in pursuing this initiative. The work is being completed by Mike Reid, the creator of the *Environmental Information: Use and Influence* website and a graduate of the Dalhousie Marine Affairs program. As of this writing, work is progressing on the site and is expected to be complete by the summer of 2013, with a projected fall launch.

At the conclusion of such a successful year, it is with regret that I report the passing of several long-standing NSIS members during this

period. They include member Dr. Alan C. Grant; Past President Dr. Donald D. Betts; and Past President, Past Editor and Life Member Dr. Alan Taylor. The Institute recognizes with gratitude their contributions to NSIS and extends condolences to the family of each scientist.

In closing, I would like to thank all members of Council for their commitment to the Institute, their support for its initiatives and the time which they so generously give to the Institute in order to help it to achieve its goals. Several councillors will be retiring from Council this spring: First, let me acknowledge the contributions of John Rutherford, who will be leaving Council after having served on the Executive for the past three years. John led the Institute as its President during the very successful 150<sup>th</sup> anniversary celebrations and served on numerous planning committees in the lead-up to these events. We thank John for his conscientious leadership and sound advice. Another experienced member, past treasurer Elaine McCulloch, will also be leaving Council. Our thanks to Elaine for having provided an additional year's service, assisting our new treasurer to learn the ropes.

I also thank David Christianson, who since 2007, represented the Nova Scotia Museum on Council and who has recently retired as Manager of Collections from the Museum of Natural History. As the liaison with the museum, David provided a calm and dependable presence on Council and facilitated our use of the lecture venue. As you know, most NSIS talks are presented at the museum's auditorium on Summer Street, which we are fortunate to be able to use for a very reasonable rate due to our longstanding relationship with the museum. This relationship continues as we welcome Mr. Jeff Gray, the Museum's new representative on Council, who has already attended several of our meetings.

This year, it has been my great pleasure to serve as President and work with Council on numerous projects and initiatives, as well as to respond to member queries and to discuss suggestions via email and in person. I look forward to our continued engagement with NSIS members and the public in the coming years. Interest in science is certainly alive and well in our province!

Respectfully submitted, Michelle Paon May 6, 2013

## **TREASURER'S REPORT**

## NOVA SCOTIAN INSTITUTE OF SCIENCE

## March 31, 2013

ASSETS: Bank Account (BMO, March 30, 2013) Investments	10,642.53 44,106.61	
TOTAL ASSETS		54,749.14
LIABILITIES AND NET WORTH Accounts Payable		
NET WORTH TOTAL LIABILITIES AND NET WORT	H	54,749.14
<b>INVESTMENTS:</b> Renaissance High Interest Savings Account Manulife Bank Investment Certificate @2.45 % due Jan 31, 2018	t	2,806.61 21,000.00
National Bank of Canada Certificate @2.9% due May 13, 2013		10,300.00
Montreal Trust Co. Cert @3.25% due July 15, 2014	10,000.00	
TOTAL INVESTMENTS (AS OF MARCH 28, 2013)	5	\$ 44,106.61

### Finances

The net worth of NSIS is **\$54,749.14** as of March 31, 2013. For this 2012-2013 period, NSIS received a total of \$1,870.13 from ACCESS copyright for publication royalties. The province of Nova Scotia contributed \$1,000 for each 2011-2012 and 2012-2013 (2,000.00), and both contributions were received during 2012-2013. The Situation in Science group contributed \$250 to the NSIS Writing Competition.

Total Expenditures for contributions to special lectures, writing competition contest and others were \$ 3,450.00. These included \$750.00 for undergraduate and graduate students winners of 2012 writing competition, nine Regional Science Fairs for a total of \$ 1,050 (1 -2012; 6-2013; 4 did not cashed cheques before March 31, 2013), one best graduate poster at the 20<sup>th</sup> Fishermen and Scientist Research Society for \$150.00 and contribution to Robots East in the amount of \$500. Special lectures contributions were made to Sable Island Round Table; School of Information Management and Situation Cluster for a total of \$ 1,000.00.

### Membership

The NSIS has 98 individual members and include 2 students, 8 Life members and 20 Institutional Members. Dues from Individual members amounted to \$ 2,890.00. The NSIS also received \$ 346.25 from 20 institutional members, and \$1,982 from sales of NSIS proceedings and other Flora, Birds special publications.

Respectfully submitted to the NSIS AGM Angelica Silva Treasurer May 6<sup>th</sup>, 2013

### **EDITOR'S REPORT**

# NSIS ANNUAL GENERAL MEETING May 6th, 2013

Volume 47 (Part1), 2012, of the PNSIS (Proceedings of the Nova Scotian Institute of Science) was published in December 2012. It continued the new journal format; this includes a colored cover with a theme-related photograph, and new typeface and spacing, giving the journal a completely new look.

Throughout 2012, the Editorial Board across the Province worked hard to attract papers and move them through the review process. The reviewing process is working well. The Journal is starting to attract more papers and ones on a wide range of scientific topics. The Board is thanked for its work and enthusiasm running the Proceedings.

To date in 2013, several contributed papers are completed or are in the review process for PNSIS Volume 47(2) 2013. An editorial is planned and a list of prospective papers and editorials is guiding future work. We are seeking ideas for a Special Issue for Volume 48(1). In discussions with Dalhousie University, progress has been made to make the Proceedings available in both print and electronic formats (on the website, one year after publication). All previous volumes will soon be available on the NSIS website. Eventually, with the concurrence of members, the Proceedings will only be available as e-copies.

Sales of the special issue on *The Birds of Brier Island* (PNSIS Vol. 46, Part 1, 2011) continue steadily. It is hoped that members will continue to advertise this outstanding volume, and help in its sales, especially during the busy tourist season. This will encourage support being given to future special issues.

Papers of original science, review papers, commentaries and editorials are requested from NSIS members and from other societies and individuals in the Province and beyond. Supported by the website, the Proceedings are the visible, written voice piece for the NSIS. Members and others are encouraged to keep contributing to it, as it provides a scholarly outlet for science related to the Maritimes and Atlantic Region.

Submitted by the Editors Peter G. Wells, Editor PNSIS (Dalhousie University, Halifax, NS)

David H.S. Richardson, Associate Editor, PNSIS (Saint Mary's University, Halifax, NS).

## LIBRARIAN'S REPORT 2012/2013

## Prepared for AGM May 6, 2013

As the new NSIS Librarian, it has been a pleasure to respond to requests related to the *Proceedings of the Nova Scotian Institute of Science* and to oversee the exchange journal collection during the past year.

# **Digitization of the** *Proceedings of the Nova Scotian Institute of Science*

During the summer of 2012, the Killam Library's graduate student interns continued to digitize the *Proceedings*, depositing volume 35 (1985) and volume 36 (1986) into DalSpace. We expect subsequent volumes of the *Proceedings* to be processed and deposited into DalSpace during the summer of 2013.

#### **Institutional Members and Exchange Partners**

There are currently 20 institutional members (four less than last year). Renewal notices were sent to institutional members on January 23, 2013 alerting them to the increase in membership fees to \$30.00 per year. By the end of March, we had received 13 institutional membership renewals for a total of \$346.25. One library cancelled its institutional membership: the NRC Institute for Marine Biosciences in Halifax is closing its library collection.

There are 89 NSIS exchange partners. During the 2012-13 year, we identified another partner from which we had received no publications since 1998, and thus removed its name from the list. An additional three partners cancelled their own exchanges. The Library of Congress also cancelled, but we moved this partner to complimentary status. The number of exchange partners has diminished by five since last year. [Note: You may recall that last year, a number of exchange partners were removed from the list because they had not sent publications to NSIS for two or more years. Through a typographical error, that number was reported as 75, but in fact, it was 85.]

Volume 47, part 1 of the *Proceedings* was published in December 2012. The issue was mailed to all institutional members in February 2013. So as to save on overseas postage, volume 47 (parts 1 and 2) will be sent to exchange partner institutions upon publication of volume 47(2).

## Sales of the Proceedings of the Nova Scotian Institute of Science

During 2012/2013, sales of volumes of the *Proceedings*, including the new issue (volume 47, part 1), generated \$1982.00 in revenue. Please see Appendix A (attached) for details. Of note, during the summer, 19 copies of the *Flora of Nova Scotia* were sold, and during the year, 40 copies of the *Birds of Brier Island* were sold, many by author Eric Mills, who has distributed copies to a retail outlet in the Westport area of Nova Scotia.

## **Access Copyright**

The past NSIS Librarian submitted the required forms to Access Copyright for the 2012 repertoire payment to publishers. NSIS subsequently received a payment of \$1870.13. As you may have noticed, this was a much larger sum than we normally receive; it included a one-time payment of \$906.96 designated as the K-12 Tariff Special Dividend for Publishers. Following a September 2012 decision by the Supreme Court of Canada, Access Copyright agreed to release the majority of royalties collected under the 2005-2009 K-12 Tariff. Publishers (such as NSIS) that had registered with Access Copyright prior to December 31, 2009 were eligible for this payment.

## **NSIS Exchange Journal Collection**

In December 2012, due to budget constraints, the Killam Library made the decision that it will no longer bind print journal issues. This measure will affect all journals received by the library including the journals which are sent to NSIS by exchange societies. Individual journal issues will be fitted with security tattle-tape, placed on the shelves and monitored for usage. If journal issues show signs of wear, selective binding will be conducted.

Last April, Sharon Longard initiated a project to inventory the older volumes of the NSIS exchange journals which are housed in the basement of the Killam Library. The next phase of the project is to identify freely available online copies of the journals. This is an ongoing project which will continue as staffing permits. NSIS exchange partners continue to send their new publications to NSIS from around the world. These items are processed and added to the collection. On behalf of NSIS, I would like to thank Carol Richardson and the Serials Department staff in the Killam Library who process the exchange journals and make them shelf-ready for both NSIS members and Killam Library patrons.

Respectfully submitted, Michelle Paon NSIS Librarian May 6, 2013

Date	Volume/ Issue of	# Sold	Price (\$)	Amount Received
	Proceedings of the Nova Scotian Institute of Science			(\$)
May 16, 2012	Birds of Brier Island	10	250.00	250.00
June 7, 2012	Birds of Brier Island	1	25.00 + 9.00 S/H	US34.00
June 7, 2012	v. 42 pt. 1 & 2	2	22.00 + 15.00 =37.00	37.00
June 2012	Flora of Nova Scotia (v.26, pt. 2 & 4)	19	665.00	665.00
August 17, 2012	Birds of Brier Island	14	350.00	350.00
September 26, 2012	v. 19, pt. 1	2	10.00	10.00
September 26, 2012	v. 23 pt. 1	2	10.00	10.00
October 2, 2012	Birds of Brier Island	5	125.00	125.00
October 9, 2012	Birds of Brier Island	2	50.00	50.00
October 15, 2012	Birds of Brier Island	1	25.00	25.00
December 3, 2012	Birds of Brier Island	1	25.00	25.00
December 3, 2012	Birds of Brier Island	2	50.00	50.00
December 11, 2012	Birds of Brier Island	1	25.00	25.00
December 13, 2012	Birds of Brier Island	1	25.00 + 5.00 S/H	30.00
January 7, 2013	Birds of Brier Island	1	25.00	25.00
January 10, 2013	v.47, pt. 1	1	10.00	10.00
January 11, 2013	v.47 pt. 1	1	10.00	10.00
January 17, 2013	v.42, pt. 1, 2002	1	22.00 + 5.00 S/H	27.00
January 22, 2013	v.42, pt. 1, 2002	2	44.00	44.00
January 22, 2013	v.47, pt. 1, 2012	2	20.00	20.00
February 15, 2013	v.47, pt.1, 2012	9	90.00 + 45.00 S/H	H 135.00
March 6, 2013	Birds of Brier Island	1	25.00	25.00
Total		81	\$1,982.00	\$1,982.00

#### Appendix A

Respectfully submitted, Michelle Paon NSIS Librarian May 6, 2013

## STUDENT SCIENCE WRITING COMPETITION 2013

## **Final Report**

This year's competition received the generous support of \$250 from the Situating Science Cluster of Halifax.

There were 23 students who expressed interest in submitting entries, and 10 actually did so. (That's a typical ratio.) The entries were from Acadia, Cape Breton Univ., Dalhousie, King's, Mount St. Vincent, St. F.X., and Saint Mary's. There were 6 undergraduate submissions and 4 submissions by graduate students.

Robert Cook, Stuart Grossert, David Richardson, John Rutherford and Peter Wells graciously and diligently assisted with the judging. The results were as follows:

## **Undergraduate Winner**

Jonathan Oore, Dal., Psychology

"Age-related Changes in Motor Ability and Motor Learning in Triple Transgenic (3xTg-AD) and Control (B6129SF1/J) Mice on the Accelerating Rotarod"

## **Undergraduate Hon. Mention**

Laura Perry, CBU, Biology, "The Effects of Climate Change on Several Seabird Species on the Coastal Regions of North America"

## **Graduate Honourable Mention**

**Lynn Cornish,** St. F.X., Biology, "Managing Molecules in Health and Nutrition; A Literature Review"

## **Graduate Honourable Mention**

**Jordi Segers,** S.M.U., Biology, "Summer Distribution and Movement Patterns of Short Distance Migratory Bats in Relation to a Wind Farm in Nova Scotia".

Hank Bird SSWC Coordinator

## REPORT OF THE COMMITTEE TO REVIEW THE CRITERIA AND PROCESS USED FOR MEMBERSHIP IN THE NOVA SCOTIAN INSTITUTE OF SCIENCE HALL OF FAME

Submitted by: Richard Singer and John Rutherford - May 6, 2013

## Introduction

At present, there appear to be three major criteria employed to evaluate a nominee for membership in the Nova Scotian Institute of Science Hall of Fame:

- 1. The nominee has made significant lifetime contributions to some field of science;
- 2. The nominee is Nova Scotian;
- 3. The nominee is deceased.

In addition, a nominee need not have pursued his or her scientific career in Nova Scotia.

There is no formal definition of what constitutes "significant contributions," and no limitation on the field of science to which such contributions have been made, except that "science" in this context appears to refer to the natural, rather than the social, sciences.

Nominations for inclusion in the Hall of Fame are made by members of Council, with the nominating Councillor(s) justifying the case for inclusion to Council.

Additions to the Hall of Fame appear to be made on an ad hoc basis.

## Discussion

In October, 2012, a suggestion was received by NSIS that nominations to its Hall of Fame be opened to the public, and that the criterion limiting eligibility to deceased individuals be dropped. Council discussed the implications of adopting these changes, and then referred the matter to a committee to consider the issue and present recommendations. We agreed to serve as this committee.

We began by considering the criteria that other organizations use to recognize, in one fashion or another, meritorious individuals. NSIS President Michelle Paon kindly supplied us with a list of relevant groups. These institutions included the following:

- Science Atlantic Outstanding Members Hall of Fame
- The Canadian Science and Technology Museum Hall of Fame
- The Nova Scotia Forestry Hall of Fame
- The Atlantic Agricultural Hall of Fame
- The Discovery Centre's Discovery Awards for Science and Technology
- Doctors Nova Scotia Awards for Contributions to the Medical Profession

We found that the criteria used by organizations on this list, although interesting, are not directly relevant to our situation. In some cases, recognition is conferred for service to a particular group. In others, recognition is based on achievement in a narrowly defined branch of science. Finally, some organizations use awards as a way of calling attention to excellence.

Other organizations' practices did, however, provide a basis for reflection on the nature and purpose of our Hall of Fame.

- A. Since the NSIS is interesting in promoting science in general, those inducted into the Hall of Fame have come from across the scientific spectrum.
- B. While being named to the Hall of Fame does honour an individual's contributions to science, it is not construed to be an 'award' in the personal sense.
- C. The NSIS Hall of Fame does, however, serve as an archive of Nova Scotians of note who have achieved recognition for contributions to their areas of science. In this way it is a historical record, and a reminder of the achievements of scientists linked to this province.

The NSIS, as a "generalist" organization, lacks the organizational capacity to make expert judgements on any specific field of science, other than those areas represented by members of Council. This situation is both a weakness and a strength. On the one hand, we may be, or be seen to be, susceptible to considering only those individuals whose accomplishments are in fields with which Councillors are familiar. On the other, and to offset this possible bias, we tend to consider Nova Scotians who have achieved prominence in the public eye or have been recognized by other organizations of note, making their inclusion in our Hall of Fame appropriate to its function as an archive. The recent

induction of Nobel laureate Dr. Willard Boyle is an example of the latter situation.

The current criterion that only deceased individuals are eligible for the Hall of Fame ensures that in comparing the accomplishments of one individual to another we are considering the totality of their contributions to science. This limitation may "level the field" a bit, making judgements somewhat more equitable and decisions a bit easier. Moreover, if we were to begin including living scientists, two "classes" of inductee would be created, one class inducted on the basis of a lifetime's accomplishment in science, and a second class whose careers are still in progress.

We discussed at some length the possibility of opening Hall of Fame nominations to the public. There are benefits to doing so. Wider consultation could identify meritorious individuals who might otherwise be overlooked, inasmuch as Councillors cannot be conversant with all areas of science. Broader consultation might also bring the NSIS to wider public attention, thus increasing recognition of the Institute and its activities. However, we were unable to identify reasonable means to limit the numbers of nominations that might be entertained at any one time; the administrative burden of identifying worthy inductees from a list compiled from public suggestions might soon become onerous. We considered "rotating" nominations from the various areas of science from year to year, but realized that this situation might lead to many nominations in fields of particular current interest in one year, and few (or none) in less prominent areas in a subsequent year. Moreover, there are, potentially, negative consequences to involving the public in nominating Hall of Fame candidates. Such a practice could lead to the process being viewed as a competition, which today it is not, and might result in calls that the NSIS justify its decisions to those whose nominees were not chosen. Indeed, to the extent that the process could become contentious, the outreach mission of the NSIS in promoting science and informing the public might be compromised.

#### Recommendations

- That the current practice of nominating only deceased Nova Scotian scientists for Hall of Fame membership be continued. Including living individuals is not consistent with the intended purpose of the Hall of Fame.
- 2. That nominations for induction continue to be generated by members of Council, with the following provisos:

- a. That criteria for membership in the Hall of Fame be reviewed on a regular basis (*e.g.* every two or three years) by Council, or by a committee struck by Council, with an eye to determining whether new names should be added;
- b. That Council, or a committee struck by Council, be empowered to consult selectively with third parties (*e.g.* local historians of science, other organizations whose input is deemed to be useful and appropriate) of *its own choosing* to aid in its deliberations.
- 3. That, as a means of drawing public attention to scientists of note who are still actively engaged in research, the NSIS maintain on its website a list of the speakers who have contributed to the public lecture series over the past five (or ten?) years. Such a list would include the affiliations of each contributor, the title of the talk presented, and a brief description of the topic covered. Where possible, photographs of the speakers would be included.

## NOMINATIONS TO POSITIONS ON NSIS COUNCIL 2013-2014

## Officers

President:	Tom Rand
Vice-President:	Patrick Ryall*
Past President:	Michelle Paon
Secretary:	Linda Marks§
Treasurer:	Angelica Silva
Editor:	Peter Wells
Librarian:	Michelle Paon
Webmaster:	Suzette Soomai
Publicity:	Vacant

## Councillors

Henry (Hank) Bird Leigh Ann Bishop Robert Boudreau Kevin Hewitt Ron MacKay Rick Singer John Young (Writing Competition Coordinator) (Science Teacher's Representative) (Mentorship)

## **Student Representative**

Allison Chua

## Observers

Jeff Gray<sup>1</sup> Vacant (Nova Scotia Museum) (Discovery Centre)

- \* New member on Council
- § Nominated to continue as Secretary
- <sup>1</sup> Appointed April, 2013

# **NSIS (OTHER BUSINESS)**

# CHANGES TO THE PUBLICATION OF THE PNSIS OCTOBER 2013

The NSIS website has migrated to a new platform. Please change your bookmarks to reflect the new URL:

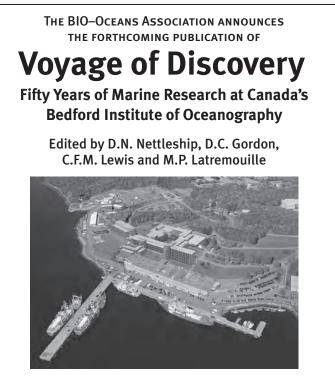
## http://nsis.chebucto.org

On the website's menu bar, under the "Publications" header, one can access the full-text of the Proceedings of the NSIS online from v.1 (1863) - v.41 (2001). The web address for these volumes is:

## http://dalspace.library.dal.ca/handle/10222/11192

In 2014, the full-text of v.42 (2004) to v.47.1 (2012) of the Proceedings will be available free online. In addition, the Institute plans to publish new issues of the Proceedings online in a collaboration with staff at the Dalhousie University Libraries. Each new electronic issue will be available only to NSIS members for the six months following publication. After that period, anyone accessing "Publications" on the NSIS website will be able to view the issue free of charge.

Michelle Paon Past President, and NSIS Librarian, Nova Scotian Institute of Science Email: mpaon@dal.ca



A commemorative volume in celebration of the 50th Anniversary of BIO: 1962 - 2012

VOYAGE OF DISCOVERY summarizes BIO research results on the oceanography of Arctic and Eastern Canada. In a series of 49 papers by past and present research staff from all oceanographic disciplines at BIO, the history of Canadian oceanography before BIO and a broad section cross section of the Institute's work spanning five decades is featured with particular emphasis on contributions to Canadian and global understanding and management of the marine environment and resources. Divided into 12 sections with a Preface and Epilogue, Voyage of Discovery is the most extensive overview of the history and scientific accomplishments of the Bedford Institute of Oceanography under one cover: Historical Roots, Arctic Studies, Ocean Life, Ocean Circulation and Chemistry, Hydrography and Seabed Mapping, Geological Oceanography, Fisheries-Ecosystems-Aquaculture, Marine Contamination, Technology and Instrument Development, Energy Developments, BIO and the Law of the Sea, and The BIO Experience. This book's well-written and illustrated accounts will appeal to a broad readership from professional oceanographers and environmental managers and decision makers to marine science students and lay persons interested in the Arctic and Atlantic oceans, and their present status and future welfare.

Voyage of Discovery
Hardcover, text on premium 8.5 x 11 inch glossy 100 wt Sterling paper with sewn binding.
ca. 432 pages. Price: \$35.00 Cdn plus shipping ISBN 978-0-993644-0-6
Forthcoming BIO-OA Publication: mid-April 2014
To reserve one or more copies, complete order form given below with details and mail to:
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## **RECOMMANDATIONS AUX AUTEURS**

Les auteurs peuvent soumettre leur manuscrit en anglais ou en français et doivent l'envoyer au rédacteur en chef par courriel (nsis@dal.ca et oceans2@ns.sympatico. ca). Le titre du manuscrit doit être suivi des noms de tous les auteurs, leurs adresses respectives et leurs adresses de courriel. Un résumé doit suivre qui comptera au plus 200 mots. Si approprié, il doit y avoir des sections tel que l'introduction, les méthodes, les résultats, la discussion, les conclusions et les références bibliographiques. L'orthographe doit suivre Le Grand Robert, et il est recommandé de se servir du Système international d'unités. Des frais de 25,00\$ par page sont présentement imposés, à moins que tous les auteurs sont membres du Nova Scotian Institute of Science. (Les frais d'adhésion pour membres réguliers sont 25,00\$ par an, et pour étudiants sont 10,00\$ par an.) Des tableaux, des illustrations et des photos en noir et blanc peuvent être inclus et seront reproduits sans frais supplémentaires. Au format copie papier du journal, les coûts de reproduction en couleurs seront aux frais des auteurs, et seront environ 500\$ par planche qui peut être une seule photo ou un collage. Chaque tableau ou illustration doit porter un titre et une légende auto-explicative.

Veuillez consulter des exemplaires du Journal pour vérifier le format du manuscrit. Chaque page doit être numérotée. Les références bibliographiques doivent être en ordre alphabétique et doivent montrer le nom complet de la revue, et si approprié, les numéros des revues, comme les exemples suivants:

Nielsen, K.J., & France, D.F. (1995). The influence of adult conspecifics and shore level on recruitment of the ribbed mussel *Geukensia demissa* (Dillwyn). *Journal of Experimental Marine Biology and Ecology* 188 (1):89-98.

Cushing, D. & Walsh, J. (1976). The Ecology of the Seas. W. B. Saunders Company, Toronto.

Lee, G.F. (1975). Role of hydrous metal oxides in the transport of heavy metals in the environment. In: Krenkel, P.A. (ed.), Heavy Metals in the Aquatic Environment. Pergamon Press, Oxford, pp. 137-147.

Communication personnelle: Smith A.J. (2001, pers. comm.) in text.

Document sur un site web: Auteur (l'année de publication) titre, URL et la date de consultation.

Les auteurs sont responsables pour la revue des épreuves en placard dans les plus brefs délais. Une reproduction électronique de l'article en format PDF sera fournie gratuitement aux auteurs. Comme un des avantages d'adhésion, les membres du NSIS reçoivent chaque numéro du journal au format copie papier. Des exemplaires des numéros spéciaux de la revue sont en vente chez NSIS aux frais établis par NSIS.

## NOVA SCOTIAN INSTITUTE OF SCIENCE COUNCIL 2013 - 2014

President:	Tom Rand	Treasurer:	Angelica Silva
Vice-President:	Patrick Ryall*	Editor:	Peter G. Wells
Past President:	Michelle Paon	Librarian:	Michelle Paon
Secretary:	Linda Marks	Webmaster:	Suzuette Soomai
		Publicity:	Vacant

Councillors: Henry (Hank) Bird (Writing Competition Coordinator), Leigh Ann Bishop (Sci. Teacher's Rep), Robert Boudreau (Mentorship), Kevin Hewitt, Ron MacKay, Rick Singer, John Young

#### Student Representative: Allison Chua

Observers: Jeff Gray (Nova Scotia Museum)

## **INSTRUCTIONS TO AUTHORS**

Papers may be submitted in either English or French and should be sent electronically to the Editor at (nsis@dal.ca and oceans2@ns.sympatico.ca). The title should be followed by names, addresses and e-mails of all authors. An abstract of up to 200 words should follow. As appropriate, sections devoted to introduction, methods, results, discussion, conclusions and references should be included. Canadian spelling and SI units should be used wherever possible. There is currently a page charge of \$25 per page but this will not be levied if all authors are NSIS members (Membership costs \$25 per year for regular members and \$10 year for students.) Tables, figures and black and white photographs may be included and will be published without an extra charge. Publication of colour figures in any hard copy of the journal will incur a charge that must be borne by the author and will likely be in the range of \$500 per plate which may be a single photo or a collage. **All tables and illustrations should have a title and a self-explanatory legend, sent in separate files at 300 dpi or higher.** 

Refer to more recent issues of the Journal for general layout of a paper. Pages should be numbered. **References should be in alphabetical order – name first, initials after, and give the full title of the journal and issue numbers where appropriate, thus:** 

Nielsen, K.J., & France, D.F. (1995). The influence of adult conspecifics and shore level on recruitment of the ribbed mussel *Geukensia demissa* (Dillwyn). *Journal of Experimental Marine Biology and Ecology* 188 (1):89-98.

Cushing, D. & Walsh, J. (1976). The Ecology of the Seas. W. B. Saunders Company, Toronto.

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Personal Communication: Smith A.J. (2001, pers. comm.) in text.

Website Citation: Author (year) title, URL and date accessed.

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