North American Black Fly Association
Eighth Annual Meeting

Program Agenda

February 4-5, 2010
Archbold Biological Station
Lake Placid, Florida

John P. Walz, President

Schedule of Presentations and Events

Thursday, February 4

6:30 AM – 8:00 AM	CONTINENTAL BREAKFAST IN DINING ROOM
9:00 AM – 9:25 AM	OPENING COMMENTS AND INTRODUCTION OF PARTICIPANTS **John Walz**, Metropolitan Mosquito Control District, St. Paul, MN
9:25 AM – 9:50 AM	AQUATIC HABITAT CHARACTERISTICS OF NEW ZEALAND BLACK FLY LARVAE **Doug Craig**, Department of Biology, University of Alberta, Edmonton, Canada
9:50 AM – 10:15 AM	RECENT ADVANCES IN UNDERSTANDING ORGANISM-FLOW INTERACTIONS FOR THE DISPERSAL OF *SIMULIUM TRIBULATUM* LARVAE **Jonathan Fingerut**[1], David Hart[2], James Thomson[3], Lindsay Schamel[1], Anthony Faugno[1], Michele Mestrinaro[4], and Piotr Habdas[4] [1]Saint Joseph's University, Department of Biology, Philadelphia, PA, [2]University of Maine, Senator George J. Mitchell Center, Orono, ME, [3]Monash University, Australian Centre for Biodiversity: Analysis, Policy and Management, School of Biological Sciences, Victoria, Australia, [4]Saint Joseph's University, Department of Physics, Philadelphia, PA
10:15 AM – 10:45 AM	**BREAK**
10:45 AM – 11:10 AM	GOOD SPECIES BEHAVING BADLY **Michael J. Kratochvil**[1], Ida M. Conflitti[2] and Gerald F. Shields[1] [1]Dept. of Natural Sciences, Carroll College, Helena, MT, and [2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada and Department of Natural History, Royal Ontario Museum, Toronto, Ontario, Canada
11:10 AM – 11:35 AM	SPECIATION AND SPECIES BOUNDARIES IN BLACK FLIES (DIPTERA: SIMULIIDAE) **Ida M. Conflitti**[1,2], Gerald F. Shields[3] and Douglas C. Currie[1,2] [1]Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada, [2]Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada [3]Department of Natural Sciences, Carroll College, Helena, MT, USA
11:35 AM – 12:00 PM	THE INFLUENCE OF SELECTED ANTIBIOTICS ON THE RESPONSE OF BLACK FLY (*SIMULIUM VITTATUM*) LARVAE TO INSECTICIDAL PROTEINS PRODUCED BY *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* **Joe Iburg**, Elmer Gray, Ray Noblet, and Roger Wyatt University of Georgia, Athens, GA
12:00 PM – 1:30 PM	**LUNCH**

Thursday, February 4 (continued)

1:30 PM – 1:55 PM	PRESENCE OF *WOLBACHIA* TRANSCRIPTS IN THE *SIMULIUM VITTATUM* TRANSCRIPTOME **Kristina Nowtzke** and Charles Brockhouse Marine and Aquatics Genetics Lab, Biology Department, Creighton University, Omaha, NE
1:55 PM – 2:00 PM	SUMMARY OF PROGRESS IN THE BLACK FLY GENOME PROJECT Charles Brockhouse **(presented by Kristina Nowtzke)** Marine and Aquatics Genetics Lab, Biology Department, Creighton University, Omaha, NE
2:00 PM – 2:25 PM	REPRODUCTIVE STATUS AND CONTINUITY OF TAXA OF THE *SIMULIUM ARCTICUM* COMPLEX AT THE CLEARWATER RIVER, MONTANA (2007-2009) **Gerald F. Shields**, B.A. Christiaens, M.L. Van Leuven, and A.L Hartman Department of Natural Sciences, Carroll College, Helena, Montana
2:25 PM – 2:50 PM	FACTORS EFFECTING *BTI* EFFICACY IN LARVAL BLACK FLIES **Elmer W. Gray**, Joe Iburg, Ray Noblet, and Roger D. Wyatt University of Georgia, Athens, GA
2:50 PM – 3:20 PM	**BREAK**
3:20 PM – 3:45 PM	THE NORTHERN BIODIVERSITY PROGRAM: ASSESSING THE RESPONSE OF INSECTS TO CLIMATE CHANGE IN CANADIAN'S NORTH **Douglas C. Currie**, Department of Natural History, Royal Ontario Museum and Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada
3:45 PM – 4:10 PM	HYBRIDIZATION OF *SIMULIUM SAXOSUM* AND *S. ARCTICUM S.S.* AT THE COEUR D'ALENE RIVER, IDAHO **Gerald F. Shields** and Michael J. Kratochvil Department of Natural Sciences, Carroll College, Helena, MT
4:15 PM – 4:30 PM	**GROUP PICTURE**
5:00 PM – 6:00 PM	**SOCIAL HOUR**
6:00 PM	**BBQ DINNER**

Friday, February 5

6:30 AM – 8:00 AM	CONTINENTAL BREAKFAST IN DINING ROOM
9:00 AM – 9:25 AM	NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES) UPDATE **John Walz** and Dave Clark, Metropolitan Mosquito Control District, St. Paul, MN

Friday, February 5 (continued)

9:25 AM – 9:50 AM	OVERVIEW OF THE TWIN FALLS COUNTY PEST ABATEMENT DISTRICT'S BLACK FLY CONTROL PROGRAM **Kirk Tubbs**, Twin Falls County Pest Abatement District, Twin Falls, ID
9:50 AM – 10:15 AM	MODELING THE RELATIONSHIP BETWEEN BLACK FLIES AND TRICHOMYCETES **JW McCreadie** and M Byrnes, University of South Alabama, Mobile, AL
10:15 AM – 10:45 AM	**BREAK**
10:45 AM – 11:10 AM	EXTANT AND EXTINCT SUBFOSSIL SIMULIIDS FROM THE COOK AND AUSTRAL ISLANDS: A BIT OF FINGER POINTING **Doug Craig**, Department of Biology, University of Alberta, Edmonton, Canada
11:10 AM – 11:40 AM	**GENERAL DISCUSSION, BUSINESS MEETING (DISCUSS 2011 MEETING LOCATION AND THE WEBSITE) AND PRESENTATION OF STUDENT AWARD**
12:00 PM – 1:30 PM	**LUNCH**
6:00 PM	**DINNER**

Saturday, February 6

6:30 AM – 8:00 AM	CONTINENTAL BREAKFAST IN DINING ROOM

Thank You

Thanks to Archbold Biological Station for allowing NABFA to host our meeting at their facility.

http://entweb.clemson.edu/biomia/nabfa/

Presentations Titles with Abstracts
8th Annual NABFA Meeting

February 4th and 5th 2010
Lake Placid, Florida

AQUATIC HABITAT CHARACTERISTICS OF NEW ZEALAND BLACK FLY LARVAE

Doug Craig, Department of Biology, University of Alberta, Edmonton, Canada

As part of the taxonomic revision of New Zealand *Austrosimulium*, characteristics of the running water habitats are being collected. This includes exact GPS position, photographic record, velocity, pH, conductivity, spot temperatures and other variables. All eventually to be available online through Landcare Research In., Auckland. Some 300 localities are now documented.

One particular example will be highlighted and that is the so-called 'beech gap' on the west coast of the South Island. Originally referring to the absence of *Nothofagus* (southern beech) it also is exemplified by reduction in aquatic organisms, including simuliid larvae. Often suggested as a result of detrimental brown water in the region, another possibility for the gap will be given. That is, the granite substrate common in the region, provides a further 'proximate stressor' to larvae through its rough surface.

RECENT ADVANCES IN UNDERSTANDING ORGANISM-FLOW INTERACTIONS FOR THE DISPERSAL OF *SIMULIUM TRIBULATUM* LARVAE

Jonathan Fingerut[1], David Hart[2], James Thomson[3], Lindsay Schamel[1], Anthony Faugno[1], Michele Mestrinaro[4], Piotr Habdas[4]

[1]Saint Joseph's University, Department of Biology, Philadelphia, PA 19131

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Black fly larvae often disperse when conditions are sub-optimal. Without the ability to swim, however, they should be at the mercy of ambient flow when trying to move beyond their current bed element. Even if they are delivered to a location with suitable conditions the reality of hydrodynamics means that the regions best suited for them, riffles, may also be the hardest to settle in due to drag, turbulence and advection. Moreover, the areas that are easiest to settle in, pools, may be fatal for them due to starvation and predation. This presents an interesting question as to how their populations are maintained in riffles, whether they can influence where they are dispersed to, and what exactly is the landscape, from their perspective, that they must contend with? Our recent studies indicate that the chances of a larva settling in their preferred fast-flow habitats may be improved both by actions taken on the part of the larva as well as the interaction between flow and the topography of the bed itself. Specifically, the use of silk threads produced before initiating dispersal may increase the chances of settling in riffles by increasing the effective size of the larvae and its ability to snag onto small topographic elements such as edges and ridges, while also increasing the chance of successfully transiting pool habitat by decreasing its fall velocity and increasing the chance of resuspension if on the bed. Further, the interaction of flow and individual bed elements leads to hydrodynamic heterogeneity at the sub-element scale providing regions on each piece of gravel or cobble where settlement may be facilitated. Together these findings provide some insight into the factors shaping the spatial distribution of larval populations in their stream environment.

GOOD SPECIES BEHAVING BADLY

Michael J. Kratochvil[1], Ida M. Conflitti[2] and Gerald F. Shields[1]
[1]Dept. of Natural Sciences, Carroll College, Helena, Montana, 59601 and [2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, M5S 3B2 and Department of Natural History, Royal Ontario Museum, Toronto, Ontario M5S 2C6

We used DNA sequences of cytochrome oxidase I and II mtDNA genes to derive phylogenic relationships of five siblings and eight cytotypes of the *Simulium arcticum* complex from chromosomally identified larvae and compared them to relationships derived from sequences of 12S, COII, *cyt b* and ITS-1 genes from three siblings and two cytotypes from chromosomally unidentified larvae from presumably "taxon pure" sites. Maximum parsimony and Bayesian analyses of both data sets indicate that taxa are not reciprocally monophyletic. That is, DNA sequences from different species are more closely related to each other than to those of conspecific alleles. Species-level non-monophyly can result from gene paralogy, imperfect taxonomy, inadequate phylogenetic signal, incomplete sorting of alleles and/or introgressive hybridization between taxa. We found no paralogous sequence patterns and since the two analyses mirror one another, we reject the cause of imperfect taxonomy. Although phylogenetic patterns likely reflect true species-level non-monophyly, we cannot confidently reject inadequate phylogenetic information as their cause. We conclude that incomplete lineage sorting and/or interspecific gene flow explain species-level non-monophyly. Finally, our data support Rothfel's chromosome-based model of speciation in which polymorphic sex-chromosomes are prerequisite for divergence and differentiating taxa undergo mating "trials' which promote the transition to coadapted chromosome systems. Patterns present in the *S. arcticum* phylogeny likely represent the initial stages of chromosome-based speciation.

SPECIATION AND SPECIES BOUNDARIES IN BLACK FLIES (DIPTERA: SIMULIIDAE)

Ida M. Conflitti[1,2], Gerald F. Shields[3], and Douglas C. Currie[1,2]
[1] Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada
[2] Department of Natural History, Royal Ontario Museum, Toronto, ON, Canada
[3] Department of Natural Sciences, Carroll College, Helena, MT, USA

Cytological analyses reveal that black fly morphospecies are often composites of chromosomally distinct populations. Whether these entities are considered reproductively isolated depends on their stage of chromosome evolution. Using the diverse *Simulium arcticum* species complex, containing nine 'cytospecies' and twenty one 'cytotypes', we aim to understand the molecular basis of chromosomally induced speciation. Specifically, we will test whether cytologically defined taxa reflect underlying genetic structure by estimating population differentiation, gene flow, and phylogenetic history with a suite of molecular markers. Preliminary results of such studies will be presented and future plans discussed.

THE INFLUENCE OF SELECTED ANTIBIOTICS ON THE RESPONSE OF BLACK FLY (*SIMULIUM VITTATUM*) LARVAE TO INSECTICIDAL PROTEINS PRODUCED BY *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS*

Joe Iburg, Elmer Gray, Ray Noblet, and Roger Wyatt
University of Georgia, Athens, GA

Bacillus thuringiensis subsp. *israelensis* (*Bti*) produces proteins that suppress populations of black flies and mosquitoes. Speculation has arisen that antibiotic contamination of waterways may mitigate the toxicity of these proteins. Experiments were conducted in which black fly larvae were exposed to enrofloxacin, tylosin, sulfamethoxazole, and trimethoprim followed by exposure to *Bti* ICPs. These antibiotics were selected based on their use in agricultural settings and documented anthropogenic contamination of rivers. Anthropogenic concentrations of a mixture of these antibiotics did not affect the response of the larvae to *Bti* ICPs. Subsequent experiments were conducted with antibiotic concentrations 10,000-80,000 times higher than typically found in contaminated rivers. Exposure of larvae to high levels of enrofloxacin (0.5 mg/L) had no effect upon the susceptibility of *Bti* ICPs, however, exposure to high levels of tylosin (8 mg/L) resulted in an increase in susceptibility of the larvae to *Bti* ICPs at 72 hrs of exposure. Exposure of black fly larvae to high concentrations

of a mixture of sulfamethoxazole and trimethoprim resulted in an increase in the efficacy of the larvicide after 48 and 72 hrs. These results suggest that impairment of the efficacy of *Bti* ICPs is not due to antibiotic contamination of the larval environment.

PRESENCE OF *WOLBACHIA* TRANSCRIPTS IN THE *SIMULIUM VITTATUM* TRANSCRIPTOME

Kristina Nowtzke and Charles Brockhouse
Marine and Aquatic Genetics Lab, Biology Department, Creighton University, Omaha, NE, 68178

Wolbachia are intracellular bacteria endosymbionts/parasites found in a wide variety of arthropods and nematodes. In many species, they cause significant population disruptions as part of their adaptations to spread through host populations. There has been great interest in them lately both as population control agents, and as vehicles for delivering novel genes for host species without a standard method of genetic transformation. Standard PCR assays for the presence of *Wolbachia* in *S. ochraceum* (Galapagos) and the colonized *S. vittatum* indicated the absence of the endobacterium (Brockhouse and Dobson, unpublished). The same negative result was found from a wide variety of other North American black fly species (Miranda Minhas, personal communication). Crainey and Post (pers. comm.), however, found a novel *Wolbachia* genome within the *S. squamosum* BAC library, indicating that this species carries the endsymbiont/parasite. The *Simulium* Genomics Project has sequenced a complete transcriptome of the colonized *S. vittatum*. To our surprise, several *Wolbachia* genes are included in the resulting database. Crainey and Post have provided PCR primers for taxonomically informative genes derived from the *S. squamosum Wolbachia*, to aid in correctly classifying the *S. vittatum* symbiont.

REPRODUCTIVE STATUS AND CONTINUITY OF TAXA OF THE *SIMULIUM ARCTICUM* COMPLEX AT THE CLEARWATER RIVER, MONTANA (2007-2009)

Gerald F. Shields, B. A. Christiaens, M. L. Van Leuven, and A. L. Hartman
Department of Natural Sciences, Carroll College, Helena, Montana 59601

We used conventional analysis of larval polytene chromosomes of a sibling, *S. arcticum* s. s. and a cytotype, *S. arcticum* IIL-22, to determine their reproductive status and to assess continuity of genotypes from 2007 to 2009. While other members of the *S. arcticum* complex were present in low numbers, *S. arcticum* s. s. and *S. arcticum* IIL-22 were abundant, present in remarkably similar frequencies from year-to-year, and in genetic equilibrium each year. *S. arcticum* IIL-22 is a cytotype new to science and occurs only at the Clearwater River. Two novel, Y-linked inversions, IIL-23 and IIL-24, are shared by the two taxa and may lead to derived Y chromosomes.

FACTORS EFFECTING *BTI* EFFICACY IN LARVAL BLACK FLIES

Elmer W. Gray, Joe Iburg, Ray Noblet, and Roger D. Wyatt

Water samples from two sites on the Susquehanna River were evaluated to determine the effectiveness of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) under operational conditions. Experiments demonstrated that the suspended solids present in the turbid sections of the Susquehanna River will significantly reduce *Bti* efficacy. Elemental analysis of the water from the turbid sites identified calcium, sodium, magnesium, silicon, potassium, boron, aluminum and iron as the most common elements. Filtration through a 0.2 µ Millipore filter demonstrated that the majority of the calcium, sodium and magnesium was dissolved in the river water. Experiments comparing the filtered river water and deionzied water indicate that water hardness does not reduce efficacy. Bioassays conducted with Kaolinite and Gibbsite demonstrated little effect on efficacy until turbidities of 40-50 NTU's were used. The clay Montmorillonite reduced efficacy at lower turbidities (~8.3 NTU's) and will continue to be evaluated. The clays, Kaolinite and Montmorillonite were mixed with the serial dilutions of *Bti* in an effort to evaluate the effect of *Bti* proteins binding to clay particles. No reduction in *Bti* efficacy was observed. Washed cells of the green alga *Scenedesmus quadricauda* significantly reduced *Bti* efficacy.

THE NORTHERN BIODIVERSITY PROGRAM: ASSESSING THE RESPONSE OF INSECTS TO CLIMATE CHANGE IN CANADIAN'S NORTH

Douglas C. Currie
Department of Natural History, Royal Ontario Museum and Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, ON, Canada

The Arctic is among the most fragile ecosystems on Earth; it is also under immense environmental pressure as the effects of global warming are felt most acutely at northern latitudes. With their diversity and potential for rapid population growth, arthropods can serve as barometers of environmental change. The Biological Survey of Canada's Northern Biodiversity Program (NBP) aims to document changes in Canada's arthropod fauna by capitalizing on the vast but underused collections derived from the half century old Northern Insect Survey (NIS) - an unprecedented initiative that sampled diversity at 72 arctic, subarctic and northern boreal localities at a time when climate change was not yet a global concern. The overarching research objective is to understand how northern biodiversity has and is adapting to a changing environment. Arthropod biodiversity will be sampled from three major ecoclimatic zones in northern Canada by strategically revisiting sites that were part of Canada's 1947-1962 NIS. The specific objectives are: (1) to test how and to what degree the structure of arthropod communities change between the Boreal, Subarctic, and Arctic Ecoclimatic zones, (2) to assess how northern arthropod communities have adapted to recent (50-60 yr.) changes to their environment, (3) to use cutting-edge molecular genetic techniques to aid species identification and to help elucidate longer-term (i.e., phylogeographic) patterns, and (4) to provide a sound foundation from which to launch future studies. Plans for the 2010 field season will be discussed, with special reference to biting flies.

HYBRIDIZATION OF *SIMULIUM SAXOSUM* AND *S. ARCTICUM S. S.* AT THE COEUR D'ALENE RIVER, IDAHO

Gerald F. Shields and Michael J. Kratochvil
Department of Natural Sciences, Carroll College, Helena, Montana 59601

Preliminary analyses of larval polytene chromosomes in 2004-05 indicated the presence of hybrids between *S. saxosum* (IIL-2) and *S. arcticum* s. s. (IIL-3) at the Coeur d'Alene River, Idaho. More extensive analyses of larvae from the Coeur d'Alene site in 2009 indicated the presence of parental and hybrid types in roughly equal frequencies suggesting that the hybrids may have been there for at least five years. Analyses of larvae from nearby (S. Fork of the Coeur d'Alene, Spokane River and Latah Creek) and distal (Cle Elum River and St. Regis River) sites indicate that the large majority of hybrids are restricted to the Coeur d'Alene. The Coeur d'Alene River site is at the eastern end of the distribution of *S. saxosum* and the western end of that of *S. arcticum* s. s. Initial hybridization may have been influenced by the catastrophic flooding of northern Idaho and eastern Washington by waters from Glacial Lake Missoula flowed west 12,000 years ago. Thus, present-day allopatric species form hybrids at a narrow range of overlap. It is possible that IIL-2/IIL-3 types could, in time, give rise to a new diploid lineage.

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM (NPDES) UPDATE

John Walz and Dave Clark
Metropolitan Mosquito Control District, St. Paul, MN

No abstract submitted

OVERVIEW OF THE TWIN FALLS COUNTY PEST ABATEMENT DISTRICT'S BLACK FLY CONTROL PROGRAM

Kirk Tubbs, Twin Falls County Pest Abatement District, Twin Falls, ID

No abstract submitted

MODELING THE RELATIONSHIP BETWEEN BLACK FLIES AND TRICHOMYCETES

JW McCreadie and M Byrnes
University of South Alabama, Mobile, AL

No abstract submitted

EXTANT AND EXTINCT SUBFOSSIL SIMULIIDS FROM THE COOK AND AUSTRAL ISLANDS: A BIT OF FINGER POINTING

Doug Craig, Department of Biology, University of Alberta, Edmonton, Canada

Simuliids of the subgenus *Inseliellum* are widespread across Polynesia and the Cook Islands. One lineage with an unusual displaced chromosome centromere is found on the Marquesas, Austral and Cook Islands. Recently subfossil (ca 2000 ybp) specimens of larval head capsules have been recovered in large numbers from swamps on both Cook and Austral islands where simuliids are known to not be extant - for some that is not known. One Austral island, Raivavae, has a new extinct species, others are of known species. Major questions arise as to why simuliids are now extirpated from some islands and that includes the question of how small can an island be to maintain simuliids? Another problem is that ages of possible colonization are troublingly-some low.
