SABBATICAL REPORT – JASON M. GARVON, PH.D.

Executive Summary:
Throughout the academic year 2015/2016 and the summer of 2016 I became competent in techniques of DNA extraction and PCR analysis. I believe that I have achieved the goals of outcomes one and two from my proposal for sabbatical leave. After completion of last year's activities I am confident in my abilities to critically evaluate merits of research involving PCR and am comfortable incorporating the latest PCR related data into my teaching. In addition to completion of objectives I also completed and published a manuscript from existing data (June 2016, Journal of Wildlife Diseases), and an additional two were prepared to various stages to be submitted over the spring/summer 2017 with a third having data analyzed and ready to write up upon completion of the other two.
I also took the liberty of completing a few things I had always wanted to do but lacked time: I gave three invited talks: Bayliss Public Library on the Science of Evolution (July 2015), the Michigan Education Association regarding compensation trends in higher education (October 2015), and one to the EUP MDNR Citizens Advisory Council on the biology of Chronic Wasting Disease (April 2016).
Overall, I accomplished more than originally planned. I have returned to LSSU in the fall of 2016 an updated and current biologist which not only makes me a more effective instructor, but has prepared me for my new role and School of Biological Sciences Chair.

Summary of proposal justification
Introduction:
The fields of parasitology and wildlife, like most other areas of Biology, have evolved to the point where molecular techniques (DNA analysis) are a mainstay. A common procedure in DNA analysis is the use of Polymerase Chain Reaction (PCR). The process involving PCR is much like photocopying DNA, a single copy (or few copies) of DNA are obtained, isolated, and replicated to end up with millions of copies. This is necessary because often only small amounts of DNA can be originally obtained. Once the product has been amplified, analysis, such as determining the presence of specific species, or subspecies, within samples, identification of discrete populations, and establishing species relationships in the construction of phylogenetic (species) trees, can be completed. In the field of parasitology, this procedure has become so common that it is now being used for diagnosis of infections that would have traditionally been done through microscopy. For example, the 1996 edition (5^{th}) of Roberts and Janovy "Foundations of Parasitology" lists the preferred technique of diagnosing *Plasmodium falciparum* (which causes malaria) microscopically by examination of a blood smear slide. The 2009 edition (8^{th} edition) lists microscopy as a technique requiring great training and then lists DNA analysis as the preferred method of determining the exact species of *Plasmodium*, as there are four species capable of infecting humans.

For example, when some of my colleagues working on parasite of arctic wolverines discovered *Trichinella*, DNA analysis was used to discover that we actually had two unique species, *Trichinella native* and *Trichinella T6* (Reichard et al. 2008). This would not have been possible with microscopy alone, and illustrates how the field of Parasitology and Biology in general has modernized.

Proposed Outcomes:

Through learning the techniques of PCR analysis I will be continuing research already conducted by myself and other colleagues and previously published. In particular I will be addressing issues identified as weaknesses in previously published manuscripts. One area of focus will be on the populations of Blue-winged teal migrating through the southern US. Data from parasite communities suggests that there is a sub-population of Blue-winged teal that stay along the Atlantic coast and use primarily brackish environments. This was hypothesized in the 1950's but never truly tested. Reviewers of the published manuscript of helminth community dynamics in migratory populations of Blue-winged teal asked about genetic analysis to support evidence from parasite community analysis and there was none at the time. In addition, there are a few papers recently published regarding comparisons of community assessment using qPCR and traditional parasitology techniques, but none focus on both blood born parasites and helminth parasites together. Furthermore, by me simply becoming proficient in PCR I can once again be of value to my field and have a broader impact as a researcher and peer reviewer.

Second, as an instructor of BIOL 132 General Biology II-organisms I teach students about phylogenetic analysis and ways in which we decide when two organisms are the same species or different species. I know in theory, but not practice, how these decisions are made using DNA analysis. This issue is more pronounced in BIOL 420 – Evolutionary Analysis as we discuss how Biologists go about documenting evolution, which currently is heavily dependent upon PCR and DNA analysis. In my BIOL 422 – Parasitology class, I can introduce a lab to identify an infection using PCR. Finally, with proficiency in PCR and DNA analysis I can further help senior thesis students with projects in wildlife as they seek to examine differences in populations of presence of hybrids in populations. Currently we have one faculty member who can assist in such matters, but has a background in plants rather than wildlife.

Report on success of Sabbatical Leave during the 2015/2016 academic year.
Overall, my sabbatical was a great success. On a personal note, I left the spring of 2015 feeling burned out and out of date. Through the year I learned a new skill, created collaborative potential, completed several outreach projects I had wanted to do for some time, and returned in the fall of 2016 re-energized and refocused.

The timeline below outlines a month-by-month list of processes and outcomes. There is, however the more overarching accomplishments achieved throughout the year.

1. Collaboration with Algoma U – Throughout the process of attending meetings at Algoma University I was able to have several conversations around potential collaboration between Biology departments at both schools. While there may be some areas we can potentially work together the primary area they were interested in was a graduate program. This has been a sore subject with the LSSU faculty, and without additional faculty to help cover load we likely cannot

go any further.

2. Collaboration with the GLFC - Dr. Amanda Roe has indicated an interest to take on LSSU students for several smaller projects in her lab. The limiting factor is citizenship, so Canadian students are much more likely to obtain this opportunity. However, we did identify a few projects that can be run in the US. I believe a colleague of hers contacted the LSSU School of Engineering about another project he was interested in as a result of our meeting.
3. New projects from learning a new skill – From becoming competent in PCR I have spawned two new projects of interest. Dr. Kolomyjec and I are collaborating on the blue-winged teal population genetics project with a manuscript submission date set for February 15, 2016. In addition we have Piping plover feces frozen so that we can perform fecal DNA testing and PCR to identify common forage species. This will strengthen the manuscript in prep concerning the insect abundance on piping plover nest sites.
4. Confidence – Overall I gained a great deal of confidence in my own abilities through this process. As my main project took various turns I was forced to learn more than I set out to learn, and had to become more self-sufficient than I expected. Rather than tag along on an existing project to see the process, I had to jump in on my own project and make it work.
5. Preparedness for a new role – As a field-trained biologist with some lab skills I was limited more to the outdoor world. After completing this sabbatical I have more understanding of the other side of biology, the indoor world. As the chair I have found I can now understand both sides which makes me much more effective. I know what the outdoor side needs, and also the indoor side. As part of my role, I participated in a conference call with an Illumina representative. The Illumina platform is the latest/best in Next Generation Sequencing technology. Prior to my sabbatical I would have had no business in that room. After, I could contribute. This came from a failed part of my project, but because I had to research NGS I was prepared.

Timeline:

July 2015 –

I had always wanted to give a talk at the Bayliss Public Library summer lecture series and I took the opportunity to do so. I gave a talk entitled “The Science of Evolution” on Thursday July 7 at 7pm (See attached flyer). Attendance was around 20 people and it went pretty well. I did find out that some outlets did not promote the talk because of the topic, likewise there were no “non-believers” there to make a scene.

August 2015 –

This is where things got off track somewhat. I did meet with Dr. Pedro Antunes to discuss how to proceed and through that meeting realized that I needed to go back and do a bit of reading. While I thought that I had a grasp of the basics at the end of our first meeting I felt as if half of the time he was speaking a different language, and he was, that of molecular biology. I began with our current Freshmen level Biology book and went from there. It was really a nice refresher as so much has changed since I took the course, or really I never took the course as it is today. When I took Freshmen Biology it was

Botany and Zoology. Now it is cellular/molecular Biology, and Organismal Biology (Botany and Zoology, with Evolution and Ecology).

During this time, late August as classes were set to begin, I was notified by four of my senior research students of their desire to present their research at the Midwest Fish and Wildlife meeting in January, 2016. Abstracts were due by Oct 1 and all four had not completed data analysis. So I turned a bit of my attention to helping with data analysis and editing for the month of September.

September 2015 –
Much of the month was spent reading articles and tutorials on PCR to get a grasp of basic processes. As I was catching up with reading I only had one meeting with Dr. Antunes to discuss the potential project. He had none currently going on for me to tag along on through the process. As we discussed the idea of evaluating the population genetics of Blue-winged teal it was made apparent that this was an undertaking too large for my modest budget. We agreed for me to look at other possibilities within my data set. One idea was to look at microfilarid nematodes of blue-winged teal and variation in species by flyway. This was similar to my dissertation research on helminths of blue-winged teal between flyways, but requires PCR to identify the parasites to species. I began researching this topic.
During September I was approached by a colleague at Oklahoma State University about serving as an outside reviewer for grants issued by the National Center for Veterinary Parasitology. I had reviewed a few the year prior and found I did not have the time to evaluate those using PCR techniques. This year I had the time to read up on anything of question. Compensation was an issue and we agreed for me to be compensated in lab supplies. In this way I funded the research I did on my sabbatical.
Much of my month was spent assisting my research students with data analysis and writing up of their abstracts for the Midwest Fish and Wildlife Conference and in closing out a 2 year grant from the National Fish and Wildlife Foundation. The goal was to have everything spent by September 30 and I spent several days going back through financials and calculating unspent money from incomplete records. With Juliana Cox on maternity leave it made things difficult. In the end I did close out spending by the 1st of October and was confident that financials were in order. I began writing the end of grant reports due by December 30.

October 2015 – “The beginning of the Big Change”
By October 2015, I had helped students submit 4 abstracts for a regional meeting, completed financials on a two year grant, and realized that I needed to get going on a manuscript. I had intended to draft one on our method of louse removal from birds, and one on the endohelminths of Wolverines from Nunavut, Canada. I found that the data I was sure was sitting in a binder concerning our trials of louse removal was incomplete, and that the nematodes from the Wolverine study needed for submission to a museum (required for publication) were improperly fixed (put in 95% ETOH rather than 70% with 8% glycerine as I had instructed) and not suitable for submission. At this point I realized that my entire work area needed a change. Nancy Kirkpatrick put her office (CRW 228) up for anyone who wanted to move. I decided that completely gutting my office and

moving was the only way to get things under control and accepted the new office. It would take me four months to move everything.
I contacted my dissertation advisor and we decided to try to publish some data about blood parasites from blue-winged teal. Ironically, this was part of the justification for my sabbatical. One journal editor (Journal of Parasitology) said the data was no good without the PCR analysis when we approached him about the topic prior to drafting the manuscript. We decided to try another journal (The Journal of Wildlife Diseases – an international journal) that said it could have a chance at publication and I began preparations of the manuscript. In the process I also considered performing the PCR part of the work with Dr. Antunes, but the sample size was too great. We would have to screen 184 samples for 4 parasites each and the chances of a methods publication (looking at microscopy versus PCR) were deemed to small to make the research worthwhile. In the process I went through my blue-winged teal genetic samples and discovered that I had about 130 samples that were not mine, and was missing 86 samples that were mine. When I contacted Dr. Gene Rhodes of the University of Georgia (formerly of Purdue when I sent the samples originally) he said that I had all of the blue-winged teal data he knew of, but that they had a freezer fail and had to move things around to keep them frozen. He agreed to look for my remaining samples (or have his grad students do it) but was not optimistic.
I spent a week reviewing 12 grant applications for the National Center for Veterinary Parasitology and earned $600 in lab supplies which were to be ordered by me when I was ready for my research. Reading the grant proposals was a nice break and a great chance for me to review what I had been reading about the PCR techniques. It felt very good to be confident that I knew exactly what people were proposing and if it was appropriate for the questions they wanted to answer. I did have to do some additional reading to understand some techniques and was happy to hear that the one proposal I had serious reservations about was met with the same criticism of those who commonly use the techniques.
Late in September I was contacted by the MEA and asked to present at the annual MEA Higher Ed conference in Lansing, MI on the 23rd. I attended the meeting and presented on “Trends in Higher Ed Compensation” along with Dr. David Jesuit from Central Michigan University. We were asked to present at the NEA national meeting in California in March of 2016. I believe that David did present, however I declined as I travelled two weeks in March/April. This presentation was a nice activity to put closure on my tenure as FA president, and allowed me to look forward to new roles at LSSU.

November 2015
During November I met with Dr. Antunes to discuss the microfilarid project. I had methods ready to discuss and began to assemble a list of materials I would need. I left our first meeting and began to investigate and learn GENBANK. This is where researchers publish their genetic sequences from research. Within the system you can look up sequences of organisms published by others, see primers used to get such sequences, and run searches on sequences to see how similar they are to prior published results. This process took a few weeks for me to learn and is one of those things that you simply have to do in order to learn. In our next meeting we uncovered that I would be looking at only 9 positive samples out of 102. This fact made it seem unlikely to yield

publishable results unless we could get into Next Generation Sequencing (NGS) using the Illumina platform. Needless to say, this spawned another two weeks of reading for me.

I continued to work on the blood parasite manuscript and had a draft to my co-authors for comment by the end of the month. I prepared and submitted slides for deposit in a museum (Sam Houston State University - parasite museum), which is part of the requirement for publication of the manuscript. I also helped my four presenting senior thesis students (only 1 of whom was going to the Midwest meeting in January) finalize their papers, posters, and presentations.

December 2015

I met briefly with Dr. Antunes in December as it is a short month for academics and he was finalizing fall grades. Also, at this point I realized that NGS (costing around $1,100 per sample) was not going to work for me. Also, the whole beauty of NGS is that you simply send out a sample and get back an immense amount of data. I intended to learn the basic PCR technique, which was the point of my taking a sabbatical. With the likelihood of publication not there, Dr. Antunes, who is a research chair and in the publish or perish world, was less receptive to the project. I had something I was interested in, publishable or not, wanted to pursue and had funding to look at. I spent some time reflecting on what to do and exploring options. One was to use LSSU resources and my colleague from Oklahoma State as my sounding board. I spent some time developing this avenue.

I spent the rest of the time in December working on the blood parasite manuscript, getting museum accession numbers from those specimens I had submitted, and finishing the reporting from my NFWF grant.

January 2016

I submitted the Blood parasite manuscript on January 12, 2016.

After a bit of soul searching, and a review of my proposed outcomes of the sabbatical, I decided to go ahead and do the research I was interested in on my own. I spent some time exploring the possibility of performing ELISA analysis on microfilarids to get a second look at my samples. I figured that this would possibly allow me to increase my positive sample size. The ELISA is a common SNAP test used by Veterinarians to screen for heartworm (also a microfilarid nematode). After long consult with my colleague from OSU we decided against it, as the commercially available tests (that I have in the refrigerator from prior studies) are made specifically for dog antigens. I then focused on performing my PCR research on my own. Dr. Stephen Kolomyjec (my sabbatical replacement) offered to give me suggestions along the way, as did Dr. Barbara Evans and Jesse Wesolek. I began in earnest my research on PCR identification of Microfilaria from blue-winged teal. I dove into the methods I would use, asked questions from my resources, worked more in GENBANK and sorted my samples.

For some time mid-month I worked with my three students (Michelle Kane, Gislaine Peters, Jacob Northius) who had not graduated and were presenting at the Midwest Fish and Wildlife Conference. We got their posters done and printed without incident, along with an edited version of Thomas Flanagan's poster (he graduated in December, 2015 - See attached posters). I attended the meeting from January 23-27. During this time I met

with Tom Flanagan and we decided to attempt to publish his senior thesis data as a methods paper. He was not working and was going to begin manuscript prep.

February 2016
I received the letter from WDA editor on the 22nd accepting the manuscript after revisions and rebuttal. I spent a few days immediately after working of revisions and more importantly the rebuttal letter.
I ordered my lab supplies to begin the month and was ready for lab work to begin. While I waited for supplies I finished (mostly) moving offices and in the process found data sheets from the louse study. I used the time waiting for supplies to analyze data from the louse study in program 'r'. I had always wanted to learn 'r' and this was a good chance. As I was ready to begin writing that manuscript I had the reply from the WDA and began my rebuttal. By the time the manuscript was re-submitted my supplies were in and I finally began lab work. It was very slow and cumbersome at first.
I was invited to join, and did join, the EUP ISD Math and Science Advisory team. My primary focus on this committee is making the science fair a larger part of the local curricula and a more successful activity overall.

March 2016
The manuscript "Blood parasites from blue-winged teal (*Anas discors*) using two flyways" was accepted on March 11. In the process of having page charges taken care of by my dissertation advisor we discussed the possibility of blue-winged teal population genetics. Discussions with Dr. Kolomyjec yielded a different approach than Dr. Antunes considered. We thus, began discussions of a summer contract to perform genetic analysis of blue-winged teal populations.

I spent the first three weeks of March in the lab and successfully isolated DNA from my 14 year old frozen samples. This was a good first step. Next I had to isolate and amplify the DNA from the microfilarids. What I learned is that isolating animal DNA (Microfilarid) from within animal DNA (blue-winged teal) is a bit tricky and that isolating bacterial DNA from animal DNA is much easier. I did succeed in isolating microfilarid DNA but it was not enough to perform sequencing on. So the process was repeated as I attempted to optimize my PCR reaction to yield the best result. This is where I left my research prior to travelling the last weeks of March/April.

April 2016
I returned to begin optimization trials again. By this time I was out of DNA from the original extractions and had to begin again. This was a bit easier the second time around. I left April with the technique down and feeling confident in my ability to perform and understand PCR.
As April began, I realized that we did not yet have a contract for the summer Piping Plover research. This was the first year under a new grant and I had heard nothing from the agency. Thus began a rush on paperwork to complete prior to May.
I prepared and gave a talk about the biology and ecology of Chronic Wasting Disease to the Eastern Upper Peninsula Citizens Advisory Council of the MDNR on April 21.

Because the manuscript with Tom Flanagan is about insects I made an appointment to meet with Dr. Amanda Roe from the Great Lakes Forestry Centre (A.K.A. = The Bug Lab). We met to discuss appropriate journals and whether or not our method of identifying insects with GoPro cameras is of value. The meeting was very positive. Because of this meeting I was asked to present our data and method as a Plenary speaker at the Ontario Entomology Society meeting in October, 2016.

May 2016
With the piping plover season in full swing all other work took a back seat. I was elected as School of Biological Sciences Chair at the end of April and spent some time meeting with Nancy Kirkpatrick about my impending duties.

June-August 2016
I taught BIOL 122 during the second summer session and worked with Dr. Kolomyjec to get the blue-winged teal population genetics study off the ground.