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COMMENTARY: BECOMING "BOUNDARY PIONEERS": ROLES FOR ACADEMIC SCIENCE DEPARTMENTS IN UNDERSTANDING AND ADDRESSING INTERACTIONS BETWEEN SCIENCE AND RELIGION

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ABSTRACT

In this essay, I assert that academic scientists are ideally suited to address frequent and often contentious interactions between scientific and religious perspectives that occur on our campuses and well as among our colleagues and within our communities. I first define and provide specific examples of four historical approaches that characterize relationships between science and religion: (1) the *Warfare* or *Conflict* thesis, (2) the *Independence* approach, (3) the *Harmony* thesis, and (4) the *Complexity* model. Given that discussions about science and religion are often manifested in ongoing controversies surrounding biological evolution, I then summarize the origins of anti-evolution movements in the United States via the rise and persistence of Christian Fundamentalism. The essay concludes by comparing the religious beliefs of academic scientists to the general public and offering practical suggestions for serving as "boundary pioneers" between science and religion. [J PA Acad Sci 87(1): 3-9, 2013]

INTRODUCTION

Science and religion are two indisputably profound and durable cultural and historical forces. In Western society, for example, Judeo-Christian beliefs, practices, and theology continue to exert widespread influence as they have for two millennia whereas the rise of science and technology has accelerated during the post-Industrial Revolution era of the past three centuries. It should come as no surprise that science and religion have a complex history of interaction that includes frequent controversy and mutual suspicion, but also ongoing cooperation and accommodation.

In contemporary society, science-religion relationships are often characterized as hostile and divisive, in particular, with conflicts over the teaching of evolutionary biology in the United States. A casual observer could easily form the opinion that religious and scientific perspectives are inherently at odds based on judicial arguments, frequently proposed state legislation and school board policies, and the numerous institutions that discredit evolution and other generally accepted scientific theories to lay, often religious, audiences.

In this essay, I discuss some conflict-driven examples and clarify the roots of anti-evolution sentiments in the United States. I intend, however, to broaden the perspective by introducing more general scholarship describing the historical interactions between science and religion. I will restrict the examples to those from the United States given their particular relevance to *JPAS* readers. I also summarize contemporary research describing the religious beliefs of professional scientists and how those compare to the general population and selected subgroups of religious adherents. These studies lend insight into the varied perspectives of our colleagues, students, and communities.

Ultimately, I assert that science departments in colleges and universities are well positioned, and to a degree obligated, to help step beyond familiar oppositional descriptions or simplistic dichotomies. I provide specific and practical advice in the conclusion based on my own experiences and the recommendations of individuals and institutions dedicated to increasing understanding between scientific and religious perspectives.

OVERVIEW OF HISTORICAL INTERACTIONS BETWEEN SCIENCE AND RELIGION

Barbour (1997) and Principe (2006) describe four historical approaches to how science and religion interact: (1) the *Warfare* or *Conflict* thesis, (2) the *Independence* approach, (3) the *Harmony* thesis, and (4) the *Complexity* model.

The first approach is likely to be the most familiar to us, namely the *Conflict* or *Warfare* thesis that suggests that science and religion are philosophically and/or methodologically opposed and that progress in one field necessarily impedes the other. One advocate for this position was John William Draper (1811-1882), a chemistry Professor at Hampden-Sydney College in Virginia and the first President of the

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American Chemical Society. Draper accepted the notion from the Enlightenment philosopher August Comte that society was naturally progressing away from religion in favor of a society based more on reason. Draper explained his ideas in *A History of the Conflict Between Religion and Science* (1876) which was quite popular in its day.

Charles Hodge (1797-1878) was a contemporary of Draper who represented the other side of the *Warfare* model. Shortly after *On the Origin of Species* was published, Hodge wrote a widely read essay, *What is Darwinism?* (1874), that equated evolution with atheism and cautioned against adhering to strictly scientific perspectives. His views were quite influential given his national prominence at the Princeton Theological Seminary (PTS).

In modern society, Richard Dawkins might also be placed in this conflict category given his arguing for atheism and against religious belief in *The God Delusion* (2006) and via his eponymous foundation (richarddawkins.net). Dawkins is unsurprisingly a frequent target of anti-evolution groups such as Answers in Genesis (www.answersingenesis.org), the Institute for Creation Research (www.icr.org), and the Discovery Institute's Center for Science and Culture (www.discovery.org/csc/). Such organizations campaign against accepted evolutionary theory in favor of Young or Old Earth Creationism and the notion that empirical evidence suggests the existence of a supernatural, intelligent designer. These organizations frequently argue that acceptance of evolution or rejection of supernatural causation leads to materialism, atheism, and an erosion of morality. The Wedge Document (Discovery Institute, 1998) and The Young Earth (Morris, 1994) are particularly stark examples of their positions.

The *Independence* approach suggests that science and religion are simply two separate epistemological and methodological realms that should not, in Principe's (2006) words, have any "border transgressions." A highly respected voice for this position, particularly among scientists, was Stephen Jay Gould (1999) and the notion of Non-Overlapping Magisteria or NOMA that he described in *Rock of Ages: Science and Religion in the Fullness of Life*. Gould, like Dawkins, made immense contributions to biology and he was a thoughtful student of history. Using many of the same resources, however, he arrived at quite different conclusions about how the relationship between science and religion should be viewed.

Both Francis Collins (2006), current director of the National Institutes of Health, and Kenneth Miller (2007), cell biologist and public advocate for evolution, epitomize the *Harmony* thesis which suggests that common ground must be established when conflict is perceived between one's scientific and religious perspectives. Collins' and Miller's views are consistent with prominent scientists of the past such as Galileo, Newton, and Boyle who viewed scientific inquiry as a form of religious worship. Within theological circles, Saint Augustine (354-430), often cited as the most important Christian writer outside of the Bible, asserted that persons of faith must accommodate their theological beliefs to their understanding of the natural world (2002). He referred to the Book of Nature (science in modern parlance) as an *ancilla* or "handmaiden" to understanding the Book of Scripture. In post-Industrial Revolution times, approaches such as Paley's (1802) *natural theology* arose in order to reconcile the vast expansion of scientific knowledge with religious belief.

Finally, the *Complexity* thesis is most favored by historians since the actual interactions between science and religion cannot be precisely classified as in conflict, independent of one another, or in harmony. Rather, the historical and cultural contexts of the interactions must be taken into account. The Galileo affair, for example, was influenced more by the strained relationship between Galileo and Pope Urban VIII and political circumstances than outrage in the Catholic Church over heliocentrism and geokineticism. When geology became a formal discipline in 17th-century Europe, some used evidence from fossils and strata to disprove the Genesis account of creation whereas others saw evidence of a worldwide flood described by many ancient cultures. More broadly, historians generally agree that Christianity's emphasis on a monotheistic creator coupled with post-Reformation notions of individual interpretation of scripture established a necessary precedent for modern science, namely the belief in an ordered and intelligible universe that can be studied and understood (Barbour 1997).

The above summary should sufficiently demonstrate the diverse and complex interactions between science and religion interact. In the 21st century and, in particular, throughout the United States, their interactions and implications for science education at all levels cannot be understood outside of the ongoing controversy concerning evolution. As any experienced science instructor can attest, opposition to evolution is often motivated by gross misunderstandings about scientific inquiry in general as well as perceived conflicts between scientific and theological accounts of, for example, the origin and age of the universe, the mechanism of speciation, and the nature and purpose of human morality. To address these issues, it is essential to understand the historical roots of anti-evolution sentiments that remain so pervasive in contemporary American culture.

ORIGINS AND VESTIGES OF ANTI-EVOLUTION MOVEMENTS

Barbour (1997), Principe (2006), and Larson (2002) remind us that Darwin's ideas were rapidly and widely accepted in scientific circles. Natural selection based on variation in physical traits and population-level thinking helped biology develop from a largely descriptive field to one with an explanatory and predictive theoretical framework. Evolutionary theory has been compared to atomic theory in chemistry and the four fundamental forces in physics to the extent it is difficult to imagine what these scientific

disciplines would be in the absence of these foundational theories.

Immediate reactions from theologians and religious leaders were understandably mixed. Many opted to follow Augustine's doctrine of accommodation and asserted that natural selection was one mechanism through which a supernatural creator interacted with the physical world; an approach referred to as *theistic evolution*. Others, like Charles Hodge, argued that evolution via natural selection denied the existence of a world designed and guided by a supernatural creator and necessarily led to atheism and a strict materialist perspective.

Shortly after Darwin's death in 1882, however, social and religious controversies around evolution largely receded as scientists and theologians in Europe and the United States became convinced by the preponderance of empirical evidence independently gathered from a variety of scientific disciplines. Many Christians, for example, adopted a day-age interpretation of the six days in Genesis to account for geologic time.

Circumstances in the United States changed dramatically at the turn of the 20th century with the publication of a twelve-volume series called *The Fundamentals*. Authors included Charles Hodge and one of his colleagues at Princeton Theological Seminary, Benjamin Warfield who actually favored theologically accommodating evolution. These writings established the three complementary concepts behind the American Christian movement aptly named Fundamentalism: dispensational millenarianism, naïve literalism, and inerrancy. The first concept suggests that society currently exists in the sixth of seven ages or dispensations to be followed by the millennium, a period of reckoning by a supernatural creator. Apocalyptic, millenarian sects were common in the United States during this period and they based many of their beliefs and end-of-time predictions on the Book of Revelation in the New Testament.

To lend credence to their beliefs and predictions, Fundamentalists routinely asserted that the meanings of Christian scripture were neither dependent on the historical and cultural contexts within which they were written nor representative of multiple literary forms such as mythology, allegory, and metaphor. Fundamentalists held that the truths contained between the Bible's covers were evident in the surface appearance of the words and syntax. This Fundamentalist interpretation, called naïve or strict literalism, stands in contrast to more traditional exegesis (scriptural interpretation) where context, form, as well as one's personal circumstances are taken into account in order to derive meaning. Thus, from a Fundamentalist perspective, Adam and Eve were actual historical figures, the story of Noah in Genesis was an actual event rather than another example of ancient flood mythologies, and the Book of Revelation is a prediction of future events rather than a story written to give hope to the exiled tribes of Israel.

Biblical inerrancy is a necessary addition to Fundamentalist Christian theology. In order for naïvely literal interpretations and millenarian predictions to be unquestionably true, Christian scripture must be completely and historically accurate as written and internally consistent across books and Testaments.

It is essential to understand not only the tenets of Fundamentalism, but also the circumstances that sparked this significant historical movement in the United States. Early 20th century America experienced rapid economic and social changes. Industrialization and urbanization shifted our economy away from small-scale, rural agriculture. Immigration expanded the demographic profile beyond the dominant Anglo-Saxon Protestants. Science became more influential and professionalized during the same period that Protestant denominations continued to factionalize and, in many cases, reduce entry requirements for clergy. American seminaries were adopting European techniques of literary and historical criticism to interpret and analyze scripture. Public education was viewed by many as a form of government oppression. World War I and the rise of Germany and Russia lent credence to Christian apocalypticists of the time.

In this historical context, Christian Fundamentalism was largely a reaction to these circumstances, and evolution was included among their perceived threats to society. Early arguments by anti-evolution groups and vociferous individuals such as William Jennings Bryan, lead prosecuting attorney in the *Scopes* trial, are still used today by the contemporary organizations mentioned previously. Many simply misconstrue common ancestry and speciation through genetic variation and natural selection to falsely claim that evolution suggests that man descended directly from, for example, chimpanzees or that the mechanisms of evolution are strictly random events. Others assert that evolution is a worldview or a religion rather than a scientific theory built upon nearly two centuries of empirical evidence.

It is quite common to hear anti-evolutionists use the term *theory* dismissively to mean a guess or a hunch rather than an explanatory and predictive framework that guides scientific research. Some organizations go so far as to propose alternative explanations to scientific data that use some form of supernatural causation which is antithetical to contemporary science. Creationism, creation science, special creation for humans, and intelligent design are four common examples. Finally, the fact that unanswered questions or "gaps" remain in our understanding of evolutionary biology is interpreted by some to mean that it is flawed science.

I encourage readers to consult scholars such as Scott (2004) and Larson (1998) for more complete accounts of anti-evolution arguments. As *JPAS* readers are aware, anti-evolution groups rarely engage established scientific organizations. They tend to focus their efforts on religious organizations and schools, political entities such as state legislatures and school boards, and the judiciary. As

documented by the National Center for Science Education (NCSE, ncse.com) and previously cited resources, anti-evolution groups have been largely unsuccessful when their ideas are brought for public scrutiny. Although the *Scopes* decision was dismissed on a technicality upon appeal, banning the teaching of evolution in public schools was declared unconstitutional in the 1968 *Epperson v. Arkansas* trial. More recently, the 2005 *Kitzmiller vs. Dover Area School Board* case linked intelligent design to its creationist, and thus religious, precursors which had been previously deemed unconstitutional for inclusion in public science classrooms.

Thus, the anti-evolution movement, and to a degree Christian Fundamentalism in general, is defined not only by opposition to cultural circumstances, but also by retreat and retrenchment. While many of the arguments of, for example, The Discovery Institute (DI) remain unchanged, anti-evolution groups frequently alter their political and legal strategies. At the time of this article, the DI was lobbying for "academic freedom" legislation that has the potential to undermine the teaching of evolution in several states. This is a strategic change from their previous efforts during the *Kitzmiller* trial to have intelligent design taught as an alternative to evolution.

HOW CAN WE BE BOUNDARY PIONEERS?

Studies indicating that approximately half of the United States population does not accept the basic tenets of evolution (The Pew Forum on Religion & Public Life, 2009) might be less surprising given the persistence of anti-evolution efforts during the past century. There is also evidence that a *majority* of biology teachers simply do not teach evolution (Berkman and Plutzer, 2010, 2012) whereas a significant percentage teach some form of supernatural causation in spite of (or without the knowledge of) judicial history. When these data are coupled with survey data from The Pew Forum on Religion and Public Life (www.pewforum.org) indicating that many Americans retain Fundamentalist beliefs, we can expect that members of our communities and many of our students will be skeptical and antagonistic towards evolutionary theory.

Perhaps these conclusions are well understood by *JPAS* readers. Every science department eventually contends with students who question or are conflicted about accepted scientific evidence for the age of the earth and universe, biochemical origins of life, and natural selection. Experienced science instructors do not need survey data to convince them of this, but they may be surprised by ethnographic studies (Long, 2011) illustrating the anti-evolution efforts of many university students.

It is instructive, however, to consider the religious beliefs of professional and academic scientists in addition to our students' religious backgrounds. Ecklund's (2010) Religion Among Academic Scientists (RAAS) study began with surveys of 1,646 natural and social scientists from research-intensive universities such as Columbia, University of Chicago, and UCLA. Some of the more salient statistical conclusions for this essay are provided in Table 1.

Insofar as the RAAS sample is representative and compared to aforementioned studies, we can conclude that there are significant disparities between the religious beliefs of academic scientists, the U.S. population, and presumably many students in our classrooms. It is useful to keep in mind recent Gallup data (2012) indicating steady increases in the percentage in those who do not identify with any religious tradition; the so-called "nones." Twenty-seven percent of those between the ages of 18 and 29 placed themselves in this category.

Although nearly half of the RAAS participants self identified as part of a religious group, nearly two-thirds professed either an atheistic or agnostic position which is far greater than the U.S. population in general. Similar conclusions can be drawn from Larson and Witham's (1998) continuation of a 1914 survey of members of the National Academy of Science.

In the second phase, Ecklund (2010) conducted 275 interviews with RAAS participants from each sub-discipline to illuminate their beliefs and practices as well as their experiences and recommendations for approaching issues related to science and religion on campus. Many experienced an "anticonversion" at some point in their lives and concluded that religion was a societal detriment. This group

Table 1. Selected Survey Data from Religion Among Academic Scientists (RAAS) Study.

Summary of Survey Prompt	Percentage of Scientists in RAAS	Percent of U.S Population
Member of established religious organization	46	84
No belief in God	34	2
Do not know if there is a God and do not believe there is a way to know	30	4
No doubts about God's existence	9	63
Little truth can be found in any religion	26	4

often asserted that their universities should only include secular topics which Ecklund called a "No God on the Quad" approach. Questions from students and discussions with colleagues about religion and its interactions with science and public life were often suppressed by dismissal of religion as anti-intellectual or by invoking Constitutional principles of the separation of church and state. Such sentiments were quite common in the RAAS along with misconceptions that religious individuals, Christians in particular, are inherently anti-science and fundamentalist, and that the religion's influence on society can be lessened by simply ignoring it.

Similarly, those scientists that identified with a religious tradition were frequently hesitant to discuss their beliefs for fear that they would either not be viewed by their colleagues as serious scholars (a significant issue with regards to tenure and promotion) or that they might violate the Constitution. Simply avoiding confrontation was another frequent motivation for not discussing religious beliefs.

Part of Ecklund's (2010) motivation for the RAAS study was to identify "boundary pioneers" referring to academic scientists who were particularly adept at communicating their work to the general public and who overcome the *Warfare* thesis to generate thoughtful dialogue about the interactions of science and religion among their students, colleagues, faith communities, and the general public. Short-term approaches at crossing boundaries among the RAAS scientists included connecting environmentalism with Christian notions of dominion and stewardship and making NOMA-like distinctions between science and religion. Others developed undergraduate or graduate courses and seminars on science and religion specifically for science students.

Developing science-religion curricula is one important approach, but Ecklund (2010) suggests three broad areas for academic scientists to consider. This first might be particularly challenging for those that did not have a religious upbringing or, as mentioned previously, have had an "anticonversion" experience. Given the likely disparities between university science instructors and their students with regard to faith, it is essential to recognize the diversity within religious traditions and the varied reactions to evolution and science in general. While certain denominations such as the Missouri Synod branch of the Lutheran Church and the Southern Baptist Convention unequivocally oppose evolution (The Pew Forum, 2009; Lee, Tegmark, and Chita-Tegmark, 2013), the majority have official statements that either support evolution or state that there is no conflict between evolution, science, and their particular faith tradition. Pope John Paul II's 1996 encyclical and The Clergy Letters (The Clergy Letter Project, 2013), for example, are powerful statements in support of evolution that make eloquent distinctions and connections between science and spirituality. Simply recognizing the diversity is a good start.

Acknowledging the philosophical and methodological bounds of the natural and social sciences alongside their accomplishments is a second point to consider. Several biologists in the RAAS study indicated that this was particularly important in their field given the public scrutiny. For example, although sociobiology and neurotheological studies (Newberg and D'Aquili, 2001; Newberg, 2010) lend insight into ethics and spiritual experience, questions of ultimate purpose and origins lie beyond causal, empirical inquiry. Even those scientists who take an extreme view that science is the only valid form of knowledge (scientism) or that existence is confined to the physical world (materialism) could generate a useful dialog about the boundaries of their work.

The third and final step for academic "boundary pioneers" is to actively engage students, colleagues, and communities in discussions about how science and religion interact and to overcome any reticence that such endeavors are either unworthy or unnecessary. I assert that academic scientists are well positioned, but perhaps not fully prepared, for these activities in light of the religious disparities with the general public and many students.

Institutions of higher education often have the expertise and resources to address complex and potentially contentious issues. Partnerships between the sciences and departments of religious studies, sociology, philosophy, history, and anthropology may be required. Examples include Ecklund's Religion in Public Life Program (rplp.rice.edu) at Rice University, the Science for Ministry Institute at PTS (www.ptsem.edu/Offices/ConEd/SciMin/), and the Center for the Study of Science and Religion at Columbia University (cssr.ei.columbia.edu). The Templeton Foundation (www.templeton.org) often provides funding for such programs.

Colleges and universities can also work with K-12 schools, religious organizations, and political bodies to understand the complexities of science and religion and to respond to the frequent efforts of anti-evolution and indeed anti-science organizations. Ceding responsibility to any one of these groups alone will, at best, preserve the status quo. Since K-12 teachers and administrators are perhaps more beholden to elected officials and state-mandated curricula, they might be reluctant to assume leadership roles. Similarly, members of religious organizations may not wish to contradict official positions or the beliefs of clergy or community and family members.

PRACTICAL SUGGESTIONS AND PERSONAL ACCOUNT

By this point, I hope that I have offered insights into the viewpoints of both your students and colleagues. I conclude with some additional and practical suggestions for addressing interactions between science and religion on your campus and in your community. Instead of the literature review from previous sections, I offer a personal narrative of my involvement in this topic and reference additional resources where appropriate.

My interest in how science and religion interact began in 2005 as my science education students and I closely followed the *Kitzmiller* trial. Coincidentally, this was the same year that Ecklund (2010) conducted many of her interviews for the RAAS study. I read the Honorable Judge John E. Jones' (2005) ruling where he determined intelligent design to be inherently religious and thus in violation of the Constitution's Establishment Clause. I quickly realized how little I knew about the controversy surrounding evolution as well as the basics of evolutionary theory or the extensive scholarship in science and religion. As I read the references from this essay, I organized the first of five annual forums on science and religion at my university. In the first session for which I received a small internal grant, I organized a panel discussion centered on the *Kitzmiller* trial. The five panelists were the biology department chairperson from my university, Judge John E. Jones, two biology teachers from Dover Area High School (Robert Eshbach and Jennifer Miller), and a theologian from a local Lutheran Seminary (also the father of Robert Eshbach).

The theologian, Dr. Warren Eshbach, returned the next year to advocate for evolution from a religious viewpoint and he organized a panel discussion with seminary students with scientific backgrounds for the following year's forum. After researching more on the topic and interacting with concerned university colleagues, I prepared and gave a presentation entitled Beyond Evolution: A Brief Introduction to the Historical Interactions Between Science and Religion, and for the last forum I invited documentary filmmaker Israel Kacyvenski to screen *Wake Up Darkness* (www.wakeupdarkness.com) which chronicles his departure from a strict Fundamentalist upbringing.

Each forum was co-sponsored by our student chapter of the National Science Teachers Association (NSTA) and our university's ecumenical spiritual center. Currently, I work closely with our campus minister to organize these sessions and to facilitate campus dialogue. I have to admit that, although the forums are not costly, they are time consuming to arrange. To make this a more sustainable effort, I am considering applying for a Templeton Foundation grant from their Science in Dialogue funding category to invite more expert speakers to campus.

During the past two years, I have taught short courses on science and religion at my church as well as another in the region and I have given the aforementioned presentation to numerous audiences including the general public, university faculty, and our state NSTA chapter. After each forum, class, or presentation, I have received enthusiastic support and few antagonistic responses. In my experience, an historical approach that is not limited to evolution tends to, in the words of a local biology teacher, "bring the threat level down." I hope to expand the audiences to include a wider range of religious denominations, school and district administrators, and parent-teacher organizations.

In my science education methods class for pre-service teachers, I require my students to write a report comparing *Finding Darwin's God* (Miller, 2007) to the NOVA documentary about the *Kitzmiller* trial, *Judgment Day: Intelligent Design on Trial* (Public Broadcasting System, 2007). I provide them with the option of including their personal perspectives and nearly all do so. This report couples well with standard assignments such as writing lesson plans for teaching evolution using resources from the NSTA (2013), NCSE (2013), and the National Association of Biology Teachers (2013). Additionally, the instructor of our university's upper-division biology course in evolutionary theory includes some of these materials in the curriculum. I have encouraged other departments to do so as well, especially with pre-service teachers.

In the future, I hope to organize two groups that can regularly address issues of science and religion. As Ecklund (2010) notes, informal conversations between students and university faculty are particularly helpful. The challenge will be to identify colleagues who are willing to share their spiritual perspectives and how they reconcile their beliefs with their scientific work. Such groups have been formed at research-oriented institutions such as the Massachusetts Institute of Technology and they can be a regular feature of campus life that can be expanded to include local students, teachers, churches, and families.

It will also be useful to convene a group to support K-12 science teachers and to respond to legislative and school board actions that undermine evolution and other accepted scientific theories. The Louisiana Coalition for Science (www.lasciencecoalition.org) is a particularly good example. Advocacy groups such as these can include, for example, university and industrial scientists, teachers, school administrators, clergy and religious educators, attorneys, and interested community members.

Regardless of the degree of involvement and irrespective of one's personal viewpoints, I encourage academic scientists to become more aware of the history of science and religion and how their intersections influence the views of our students and colleagues. I assert that we are ideally suited for this task since willing, talented, and yet-to-be-discovered "boundary pioneers" may exist on your campus and colleagues from other departments and institutions can provide needed background and support.

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THE INVASIVE NEW ZEALAND MUD SNAIL (*POTAMOPYRGUS ANTIPODARUM*) NOT DETECTED IN WESTERN LAKES HURON AND ST. CLAIR[1]

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ABSTRACT

The New Zealand mud snail (*Potamopyrgus antipodarum*) is a world wide invasive species with established invasive populations in Australia, Europe, Japan, and North America. In the Laurentian Great Lakes, the snail has been found in each major water body except for Lakes Huron and St. Clair. Here we report data from samples taken from 78 sites in Lake St. Clair, the Mackinaw Straits, and two locations in western Lake Huron (Saginaw Bay and Thunder Bay) from time periods ranging from 1997 to 2009. *Potamopyrgus* was not found in the samples taken from any of the sites. Thus, there is no evidence from this study that the New Zealand mud snail has established populations in Lakes Huron and St. Clair. [J PA Acad Sci 87(1): 10-15, 2013]

INTRODUCTION

The Laurentian Great Lakes have become a hotspot for aquatic invaders (Ricciardi and MacIsaac 2000). Among the more recent invaders is the New Zealand mud snail, *Potamopyrgus antipodarum*. *P. antipodarum* exists in its native range in New Zealand in mixed populations of sexual and asexual individuals (Lively 1987). However in its invaded range, including Europe (Ponder 1988), Australia (Schrieber et al. 1998), Japan (Shamida and Urabe 2003), and North America (Bowler 1991; Zaranko et al. 1997), the populations are entirely asexual and are composed of a number of different clones.

Invasive *P. antipodarum* in North America is distributed into two broad populations. One population exists primarily in streams and rivers in the western United States and Canada (Proctor et al. 2007). The western US population is composed of three different clones with only one being widespread (Proctor et al. 2007; Dybdahl and Drown 2011). A second population exists in the Laurentian Great Lakes, where it was first discovered in Lake Ontario in 1991 (Zaranko et al. 1997). Since that time it has expanded its range within Lake Ontario (Levri et al. 2008) and within the Great Lakes into Lake Erie (Levri et al. 2007), Lake Superior (Grigorvich et al. 2003; Trebitz et al. 2010), and Lake Michigan (T. Nalepa, pers. comm.). The snail has also been discovered in flowing waters emptying into Lake Ontario (Levri and Jacoby 2008, Levri et al. in prep.). The number of clones in the Great Lakes is not known. The clone found in Lake Ontario is the same as one of the three clones found in Europe. Thus it appears that the snail was introduced via trans-Atlantic shipping.

Studies of *P. antipodarum* in rivers and streams in the Western US and in Australia have demonstrated that the snail can have a substantial ecological impact on native communities (reviewed in Proctor et al. 2007). The snail has been shown to alter the nitrogen and carbon cycles in streams (Hall et al. 2003), dominate secondary production (Hall et al. 2006), outcompete native grazers (Riley et al. 2008), and it is possible that they influence the distribution of other macroinvertebrates (Kerans et al. 2005) and negatively influence higher trophic levels (Vinson and Baker 2008; Bruce and Moffitt 2010). The impacts of the snail in the Great Lakes are not known, largely because most of the populations in the Great Lakes are found at depths where study of its ecology is difficult (Levri et al. 2008). In most locations in Lakes Ontario and Erie where the snail has been found it exists at depths between 15 to 40 m (Levri et al. 2007; Levri et al. 2008).

The purpose of this study was to determine if the snail exists in the two remaining large water bodies of the Laurentian Great Lakes where it has yet to be found, Lakes Huron and St. Clair.

METHODS

Benthic samples were taken from the Mackinaw Straits, Saginaw Bay, and Thunder Bay in Lake Huron (Figure 1; Appendix 1). Samples were also taken from uniformly distributed sites in Lake St. Clair (Figure 1; Appendix 1). Samples from Lake St. Clair, Saginaw Bay, and the Mackinaw Straits were taken by other researchers for other purposes.

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The samples from the Mackinaw Straits were taken in 1997 and repeated in 2003 by Don Schlosser and colleagues. Samples from Saginaw Bay were taken in 2000, 2001, 2002, 2003, and 2008 by Michael Thomas and colleagues, samples from Thunder Bay were taken in 2009, and the samples from Lake St. Clair were taken in 2007 by Don Schlosser and colleagues. Samples from all sites were collected using a Ponar dredge, except for the samples from Thunder Bay where a petite Ponar dredge was used. All organisms were preserved in 70% ethanol. All samples were examined using a dissecting microscope at 10x magnification.

RESULTS AND DISCUSSION

New Zealand mud snails were not found in any of the locations sampled during this study (Figure 1). The absence of New Zealand mud snails in the samples taken for this study does not necessarily indicate that the snail is not in Lakes Huron and St. Clair. The snail could be in regions of these lakes not sampled and/or exist at densities too low to detect. In Lake Erie, for example, some sites that were sampled were represented by only one snail (Levri et al. 2007). Thus it is very plausible that densities could be too low to detect using the procedures utilized in this study. The data reported here were collected over a relatively long time period (1997-2009). In areas where samples were taken some time ago, the snail may have invaded since. Especially in Lake Huron, the number of sites sampled was small in comparison to the size of the lake so it is very possible that locations where the snail exists were missed. In Lake Ontario studies have found that the snail is most commonly found at depths between 15 and 40 meters (Levri et al. 2008). Some of the sites sampled in Lake Huron and the Mackinaw Straits were within that depth range (Appendix 1). Lake St. Clair sites ranged from 2.6 to 6.0 m in depth (the lake itself ranges in depth from less than 1 m to about 8.0 m in the shipping channel [NOAA]), but some shallow locations in Lake Ontario and Lake Superior have been found to harbor New Zealand mud snails (Levri et al. 2008). Based on the previous findings in Lake Erie and Ontario, it seems like the most likely places to find the snail would be in the 15-40 m depth range relatively close to shore especially near ports where then snail may be more likely to

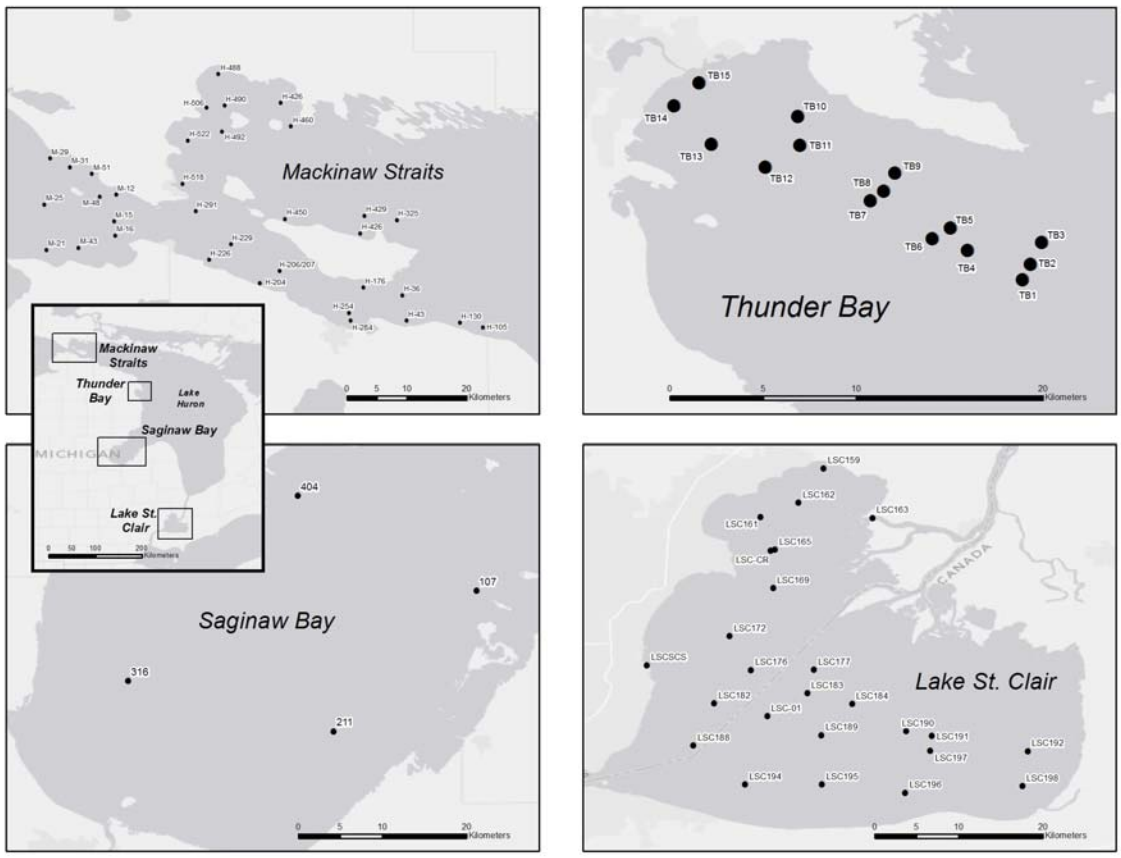

Figure 1. The sites sampled during the study of Lake Huron, Lake St. Clair and the Mackinaw Straits. Numbers in the figure correspond with sites in Appendix 1.

be initially introduced. Such locations include Thunder Bay, the deeper waters of Saginaw Bay, the northern mouth of the St. Clair River, and the deep water of the heavily-travelled Mackinaw Straits.

Since *P. antipodarum* has been found in all of the other Laurentian Great Lakes, we had expected to find it in Lake Huron and possibly in Lake St. Clair. Since the initial discovery of the snail in Lake Ontario in 1991, the snail was found in Lakes Erie, Michigan, and Superior within the next fifteen years. The mode of dispersal of the snail within the Great Lakes is unknown. It is possible that the presence of the snail in each of the individual lakes is due to separate introductions from Europe. It is also possible that the snail has dispersed from Lake Ontario to the other locations due to transfer of sediments via dredging, sediment on anchors from recreational users, or other means.

Negative data in invasive species research, and in science in general, is of limited value. However, the documentation of efforts of not finding an invasive is encouraged by the United States Federal Government (National Invasive Species Council 2003) and could be important for at least two reasons. First, by documenting locations where a species was not found, future research at the same locations can better determine the time of introduction if the species is later found. Second, the prediction of where invaders may potentially invade requires not only information of where invaders currently exist, but where they do not. Modeling approaches that use presence-only data rather than presence/absence data tend to be poorer predictors of the eventual range of an invasive species (Vaclavik and Meentemeyer 2009) and rare and endangered species (Engler et al. 2004). Unfortunately, pure absence data is rarely published.

Attempts to predict the future geographic range of the New Zealand mud snails utilizing genetic algorithm for rule-set production (GARP) models predict that parts of Lakes Huron and St. Clair should be suitable habitat for the New Zealand mud snail (Loo et al. 2007; Therriault, et al. 2010). One projection suggests that there is a high suitability of habitat for the snail in northern and southern Lake Huron, especially within twenty km of the shore, and in Lake St. Clair (Therriault, et al. 2010). Thus continued monitoring of these lakes should be a priority. Within the Laurentian Great Lakes themselves, it is not clear how *Potamopyrgus* is influencing the ecology of the lakes. However, in the streams and rivers of the western U.S. they appear to have a substantial effect (reviewed in Proctor et al. 2007 and Alonso and Castro-Diaz 2012). This impact seems to be correlated with density. Thus it is important that lotic locations adjacent to lakes with established populations should be monitored as densities of the snail higher than that found in the lakes may be possible if the snail migrates into or is introduced into rivers and streams.

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Appendix 1. Sites sampled in Lakes Huron, St. Clair, and the Mackinaw Straits

Location	Site	Depth (m)	Latitude	Longitude
Lake St. Clair	LSC-01		42 25.003	82 45.011
Lake St. Clair	LSC-CR		42 35.630	82 44.773
Lake St. Clair	LSC159	2.6	42 40.933	82 41.400
Lake St. Clair	LSC161	3.3	42 37.800	82 45.450
Lake St. Clair	LSC162	3.5	42 38.733	82 43.000
Lake St. Clair	LSC163		42 37.750	82 38.250
Lake St. Clair	LSC165	3.3	42 35.700	82 44.500
Lake St. Clair	LSC169		42 33.250	82 44.633

Location	Site	Depth (m)	Latitude	Longitude
Lake St. Clair	LSC172	5.2	42 30.167	82 47.417
Lake St. Clair	LSC176	5.5	42 27.983	82 46.050
Lake St. Clair	LSC177	5.9	42 28.000	82 42.000
Lake St. Clair	LSC182	5.9	42 25.833	82 48.417
Lake St. Clair	LSC183	6.1	42 26.500	82 42.433
Lake St. Clair	LSC184	5.9	42 25.800	82 39.550
Lake St. Clair	LSC188		42 23.133	82 49.750
Lake St. Clair	LSC189	5.8	42 23.800	82 41.533
Lake St. Clair	LSC190	6.0	42 24.067	82 36.083
Lake St. Clair	LSC191	6.0	42 23.767	82 34.433
Lake St. Clair	LSC192		42 22.767	82 28.283
Lake St. Clair	LSC194		42 20.633	82 46.417
Lake St. Clair	LSC195		42 20.633	82 41.500
Lake St. Clair	LSC196	5.0	42 20.100	82 36.150
Lake St. Clair	LSC197	5.7	42 22.804	82 34.556
Lake St. Clair	LSC198		42 20.517	82 28.617
Lake St. Clair	LSCSCS		42 28.292	82 52.737
Saginaw Bay	404	10.1	43 59.82	83 38.548
Saginaw Bay	316	7	43 47.928	83 49.445
Saginaw Bay	211	5.2	43 44.681	83 36.254
Saginaw Bay	107	4.3	43 53.707	83 27.092
Thunder Bay	TB1	26.0	44 57.627	83 15.860
Thunder Bay	TB2	25.0	44 58.074	83 15.625
Thunder Bay	TB3	21.9	44 58.697	83 15.298
Thunder Bay	TB4	19.1	44 58.466	83 17.459
Thunder Bay	TB5	19.6	44 59.125	83 17.953
Thunder Bay	TB6	17.8	44 58.820	83 18.468
Thunder Bay	TB7	14.3	44 59.916	83 20.272
Thunder Bay	TB8	13.4	45 00.185	83 19.881
Thunder Bay	TB9	13.0	45 00.715	83 19.546
Thunder Bay	TB10	9.8	45 02.356	83 22.361
Thunder Bay	TB11	9.4	45 01.519	83 22.302
Thunder Bay	TB12	9.1	45 00.890	83 23.307
Thunder Bay	TB13	4.9	45 01.542	83 24.872
Thunder Bay	TB14	4.9	45 02.656	83 25.948
Thunder Bay	TB15	4.9	45 03.322	83 25.231
Mackinaw Straits	M-12	4	45 51.3	84 48.7
Mackinaw Straits	M-15	68	45 48.9	84 48.9
Mackinaw Straits	M-16	26	45 47.6	84 48.8
Mackinaw Straits	M-21	9	45 46.3	84 55.0
Mackinaw Straits	M-25	28	45 50.4	84 55.2
Mackinaw Straits	M-29	9	45 54.6	84 54.7
Mackinaw Straits	M-31	9	45 53.8	84 52.9

Location	Site	Depth (m)	Latitude	Longitude
Mackinaw Straits	H-36	8	45 42.2	84 22.8
Mackinaw Straits	M-43	22	45 46.5	84 52.1
Mackinaw Straits	H-43	4	45 39.9	84 22.4
Mackinaw Straits	M-48	10	45 51.1	84 50.2
Mackinaw Straits	M-51	7	45 53.2	84 50.9
Mackinaw Straits	H-105	24	45 39.3	84 15.5
Mackinaw Straits	H-130	9	45 39.7	84 17.6
Mackinaw Straits	H-176	10	45 42.9	84 26.3
Mackinaw Straits	H-204	5	45 43.3	84 35.7
Mackinaw Straits	H-206/207	24	45 44.4	84 33.9
Mackinaw Straits	H-226	11	45 45.4	84 40.3
Mackinaw Straits	H-229	26	45 46.8	84 38.3
Mackinaw Straits	H-254	13	45 40.6	84 27.6
Mackinaw Straits	H-284	6	45 39.9	84 27.5
Mackinaw Straits	H-291	12	45 49.8	84 41.5
Mackinaw Straits	H-325	67	45 49.0	84 23.3
Mackinaw Straits	H-426	13	45 47.8	84 26.6
Mackinaw Straits	H-429	35	45 49.4	84 26.2
Mackinaw Straits	H-450	5	45 49.1	84 33.4
Mackinaw Straits	H-460	18	45 57.5	84 32.9
Mackinaw Straits	H-426	10	45 59.6	84 33.8
Mackinaw Straits	H-488	8	46 01.5	84 39.2
Mackinaw Straits	H-490	9	45 59.4	84 38.9
Mackinaw Straits	H-492	9	45 57.0	84 39.1
Mackinaw Straits	H-506	7	45 59.2	84 40.5
Mackinaw Straits	H-518	9	45 52.3	84 42.7
Mackinaw Straits	H-522	13	45 56.2	84 42.2

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THE STATUS OF WHITE OAK (*QUERCUS ALBA*) TREES AT THE GORDON NATURAL AREA, CHESTER COUNTY, PENNSYLVANIA[1]

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ABSTRACT

A field study was conducted in 2007 and 2008 to assess the population, distribution, size, and health status of white oak (*Quercus alba* L.) trees found in a forest at the Gordon Natural Area preserve in Chester County, Pennsylvania. A sweep survey was used to locate each oak, and then geographic coordinates, diameter at breast height (DBH), and crown vigor, were determined for each tree. Twenty-three trees were encountered in all, indicating a low density of < one tree per hectare, which was much lower than that of other co-occurring late successional species. Trees exhibited both clumped and linear distributions, likely resulting from acorn caching and past use of the species as a border around now reforested farmland. Trees were spread across most DBH size classes with a mean DBH = 57.4 cm. Most trees were relatively large and none occurred below 25 cm. Most trees were also healthy, though some were unhealthy. Overall, results suggest that white oak is an uncommon but widely dispersed species at the preserve, with a relatively healthy, but aging, population. Given these results, more studies of the species at the preserve are warranted, including new and periodic assessments of its recruitment status and canopy tree health, to better manage and ensure that white oak continues to have a presence in this forest and the region. [J PA Acad Sci 87(1): 16-19, 2013]

INTRODUCTION

White oak (*Quercus alba* L.) is a medium-large sized deciduous tree reaching up to 24 m in height (Harrison and Werner 1984). It is shade tolerant and late successional (DeWitt and Derbey Jr. 1955), and a foundation species that facilitates forest stability and provides food for many animals. It occurs throughout eastern U.S. forests in both lowland and upland communities (Rouse 1986, Chapman and Bessette 1990), but prefers more mesic habitats (Minckler 1965). While it has dominated many eastern forests throughout the Holocene (Abrams 2002), it declined after European colonization due to deforestation meant for farmland (Abrams 2003). It rebounded in the nineteenth century due to farm abandonment (Yahner 2000) only to decline again in the twentieth century due to poor recruitment (Abrams 2003). As a result, efforts to sustain the species have been made by ecologists and foresters. The goal of this study was to assist those efforts by assessing its density, distribution, size, and health status in one suburban southeastern Pennsylvania forest. A comparison of the species with past densities and to those of other hardwoods at the preserve and in other local forests was also a goal.

MATERIALS AND METHODS

We surveyed white oak in a forest at the Robert B. Gordon Natural Area (GNA), located on the West Chester University campus. The GNA is a 68 ha preserve containing early and late successional forest, serpentine grassland, and wetland habitats and is one of the largest open spaces in eastern Chester County. The forest is even-aged, 150-years old, and dominated by American beech (*Fagus grandifolia*), maple (*Acer*) and oak (*Quercus*) species, and tuliptree (*Liriodendron tulipifera*). From 2007-2008 white oaks were located in the forest using a sweep survey. Each tree was numbered, geolocated using a Trimble GPS Pathfinder Pro, and measured for size with a diameter at breast height (DBH) tape. The health of each tree was assessed using crown vigor (CV), which relied on observing the limbs of each tree to determine how many were healthy, damaged, or dead. A 0-3 scale was used to assign values of 0 (dead), 1 (unhealthy = many broken/dead limbs), 2 (healthy = few broken/dead limbs), and 3 (very healthy = no broken/dead limbs). Data were graphed and mapped to show size patterns and the distribution of all trees.

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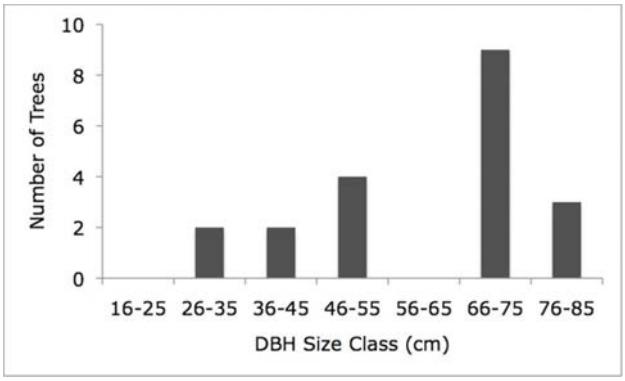

Figure 1. Map of the Gordon Natural Area showing the locations and distribution of white oak trees encountered in the study forest.

RESULTS AND DISCUSSION

Twenty-three trees were found, with a density of 0.58 per ha. Their distribution varied with some clumped in a northern part of the forest, but most growing in a sinuous line in western and southeastern parts (Fig. 1), indicating that most trees were non-randomly distributed. Sizes varied from 30-81 cm (Fig. 2), with mean DBH = 57.4 cm. Most trees were moderately large-large and several were assigned to one large 66-75 cm class, while others were spread across smaller classes. No trees were found in the smallest 16-25 cm class or in a moderately large 56-65 cm class. Crown vigor assessments found that most trees were healthy (15, CV = 2), but more trees were unhealthy (5, CV = 1) than very healthy (3, CV = 3). No discernable white oak snags or logs were found.

Based on results, white oak density was low as of study time, and has declined when compared to surveys made over the last century. Gordon (1941), for example, noted that the species was moderately abundant in the early twentieth century, which agrees with the forest's Red oak-mixed hardwood community classification type (Fike 1999) in which white oak can be co-dominant with red oak. Overlease (1973) found that the species was less abundant in 1970 than in previous decades, especially when compared to other oak species and tuliptree. However, it is important to note that their accounts were more qualitative, so it is hard to say whether the species was highly dense at the GNA during the last century, or that it was much more dense in comparison to our study. However, when compared to pre-colonial levels, when the species comprised up to 33% of trees in southeastern Pennsylvania forests (Black and Abrams 2001), and may have been co-dominant with red oak, our findings suggest that white oak density is now historically low, a trend that is not unique to the GNA since it was also found at low densities in other surrounding forests.

Recent contract surveys made in Chester County township forests, for example, found that white oak was sparse in each (J. Ebbert, pers. comm.). In addition, trail surveys made in 2013 at Ridley Creek and Edinburg State Parks, and at Valley Forge National Historic Park, in forests similar in area and composition to the GNA forest, found an average of 15 white oaks of similar sizes to those we found (G.D. Turner, pers. comm.). Also interesting was that in each township and trail survey, white oak was much less dense than other hardwoods. This is not surprising given that a comparative 1970-2003 GNA study found that American beech, red oak, and tuliptree were far more abundant than white oak (i.e., 35, 24, and 82 trees per ha, respectively, versus 0; Turner et al. 2007) and a comparison of surrounding Chester County forests in 1973 found that of eight sampled, white oak was relatively abundant in comparison to American beech, other oak species, and tuliptree in only one, was moderately so in two, and absent or sparse in five (Overlease 1973). Further, other timely regional studies report similar findings. Black and Abrams (2001), for example, found that white oak represented only 4% of dominant tree composition in Lancaster County forests while Abrams (2003) found that it represented just 1% in regional forests. Thus, it is reasonable to assume that white oak has not been abundant in area forests for some time, and that it is less common than other hardwoods.

While white oak recovered at the GNA following farm

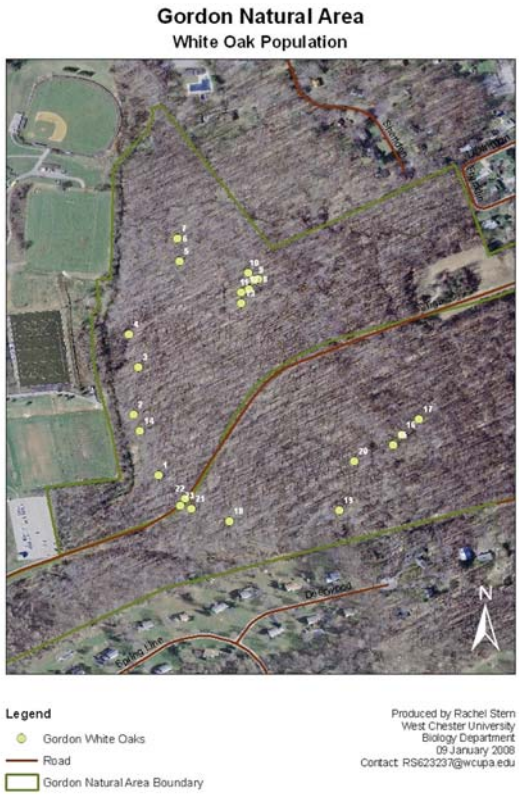

Figure 2. White oak frequencies per size class (cm) based on diameter at breast height (DBH).

abandonment (Overlease 1987), there is little evidence to suggest that it ever became abundant there, given that the forest is even-aged, 150-years old, and dominated by other hardwoods. It is possible that it was more abundant during that time in comparison to the time of this study, but declined more so than other hardwoods due to selective harvesting, given its high economic value (Abrams 2003). However, the lack of stumps or trees grown from root sprouts suggests that any harvesting, if it occurred, was minor. Further, given that chestnut blight (*Endothia parasitica*) decimated American chestnut (*Castanea dentata*) at the GNA (Overlease 1973), white oak should have increased in abundance, but there is no evidence that it did. Instead, other hardwoods were likely more abundant than white oak when the blight hit, and thus benefited accordingly.

We do know that white oak has declined at the GNA over the last century, regardless of its prior densities then, which raises the question of why it did. Many factors have been proposed, namely fire suppression and deer browsing, which have affected other hardwoods less (Abrams 2003). There has been no fire at the preserve for over 50 years, as there is no evidence of fire scars or charcoal, and only one minor fire has been reported since 1960 (Overlease 1973). White-tailed deer (*Odocoileus virginianus*) impacts were also likely, as their GNA population grew from a few in 1960 to 80 by 2012, which is far greater than is sustainable (G.D. Hertel, pers. comm.). Deer affect recruitment directly by consuming seedlings, and white oak seedling density declined from 1970-2003 (Turner et al. 2007), suggesting that deer were at least partly culpable since the decline coincided with their population growth. This trend was not unique to white oak, however, as other hardwoods experienced similar seedling declines during that time. The same seedling comparison, for example, found that only American beech and white ash seedlings were relatively dense in 2003, though both were lower than in 1970, while densities of the exotics Princess tree (*Paulownia tomentosa*) and tree of heaven (*Ailanthus altissima*) increased over that time (Turner et al. 2007). However, recent seedling surveys at the GNA have found few for any species, native or exotic, due to intense deer browsing (G.D. Hertel, pers. comm.). Only American beech, whose sprouts deer avoid, and white ash, which seeds prolifically, have shown any recruitment, though there are few saplings of either species. Thus, few species are replacing white oak, not even exotics, at the GNA.

Regardless of its status, white oak was present and distributed across the GNA forest as of study time. Some trees were clumped, likely a result of acorn caching, but most grew in a sinuous line through the forest, perhaps a legacy of use as field border trees. The species also ranged across size classes, suggesting some regeneration until recently. Interestingly, no trees were found in the 56-65 cm size class, suggesting that some factor(s), such as poor growth conditions or pests, hindered recruitment for many years during the last century. While most trees were healthy, more were unhealthy than very healthy, suggesting that its population may lose more individuals sooner than later. Other hardwoods, namely American beech, red maple, red oak, and shagbark hickory (*Carya ovata*) were healthier than white oak (C. Cummins and G. Turner, pers. comm.), though flowering dogwood (*Cornus florida*) and sugar maple (*Acer saccharum*) were less so due to disease and potential warming.

Overall, white oak was found to be a sparse but widely distributed and relatively healthy species at the GNA. Compared to other surrounding forests, this was typical. There were both younger and older trees based on a range of DBH sizes, but there were no young trees or saplings. Given this status, white oak could remain a minor component of forest composition at the GNA for many years, but only if recruitment improves and the current population stays healthy. Thus, new studies monitoring its recruitment and health status are needed, as are proactive plantings and deer exclusion. Such efforts could help sustain this important species in forest habitats at the GNA and across the surrounding region.

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SURVEY OF THE PREVALENCE AND DIVERSITY OF INTESTINAL PARASITES THROUGH SCAT ANALYSIS OF CANIDS AT LETTERKENNY ARMY DEPOT, FRANKLIN COUNTY, PENNSYLVANIA[1]

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ABSTRACT

The increased spread of *Echinococcus multilocularis* into novel areas has created a need for early detection and monitoring of parasites within wild canid populations. In order to survey the prevalence and relative intensity of helminthes in wild canid populations, coyote, red fox and gray fox scat samples were collected during February and March 2012 at Letterkenny Army Depot, in south central Pennsylvania, USA. Using standard fecal flotation, 13 different parasites were identified in 75 fecal samples, of which 40% of coyote (n=35) and 72.5% (n=40) of fox samples contained evidence of at least one parasite. This represents 8 new species now known to parasitize coyotes and foxes in Letterkenny Army Depot when compared with previous published research. Eleven of the 13 parasites identified were shared between coyotes and foxes. Fox samples had a higher prevalence of parasitism than did coyote samples; however, the relative intensity of parasitism was greater in coyote samples. While parasitism with *Taenia sp.*, *Capillaria sp.*, *Isospora sp.*, *Toxocara canis*, *Toxascaris leonina*, *Strongyloides stercoralis* and *Uncinaria stenocephala* is likely detrimental, we did not identify *Echinococcus sp.* or other zoonotic parasites. We recommend continued surveillance for parasites found within wild canids through standard fecal flotation techniques as well as molecular and specific DNA analyses. [J PA Acad Sci 87(1): 20-26, 2013]

INTRODUCTION

Coyotes (*Canis latrans*) began migrating eastward from the western half of North America around 1900 (Parker, 1995). Deforestation, conversion of land to agriculture and the reduction of the gray wolf (*Canis lupus*), mountain lion (*Felis concolor*), and grizzly bear (*Ursus arctos*) populations favored expansion into the east (Bekoff, 1978; Tomsa, 1995). Colonization of the Mid-Atlantic States: Delaware, Maryland, North Carolina, Pennsylvania, Virginia and West Virginia, occurred south from New York, northeast from Georgia and Tennessee, and east across the Ohio River and along Lake Erie. Coyotes were first reported in Pennsylvania in the late 1930s and early 1940s (Mastro, 2011; Parker 1995; Hayden, 2003). Coyotes are generalist predators that consume mammals, birds, insects, and vegetation. In winter, their diet shifts toward white-tailed deer (*Odocoileus virginianus*) (Steinmann et al., 2011). Coyotes at Letterkenny Army Depot (LEAD) are known to host fox lungworm (*Capillaria aerophila*), hookworms (*Ancylostoma sp.* and *Uncinaria stenocephala*) and roundworms (*Toxascaris leonina*) (Bixel, 1995) and a variety of other helminthes in other eastern locations (Foster et al., 2003; Gompper et al., 2003).

Ancestry of the red fox (*Vulpes vulpes*) in North America originated from natural range expansions from boreal and western montane sections of North America, not from translocation of European lineages, as was widely believed (Statham et al., 2012). Red foxes use habitats adjacent to streams, rivers and lakeshores which serve as natural boundaries between coyote territories. Their diet consists of small mammals, birds, eggs, invertebrates, frogs, snakes, vegetable matter, and carrion, although competitive exclusion by coyotes can cause foxes to consume prey items from higher trophic levels (Harrison et al., 1989; Lavin et al., 2003). Small mammals serve as intermediate hosts for parasites, and infect foxes upon ingestion (Macpherson, et al., 2000). Red foxes are known to be hosts to numerous species of roundworms, heartworms, tapeworms and flukes (Rankin 1946; Dibble et al., 1983; Merritt, 1987).

Unlike the red fox, the home range of the gray fox (*Urocyon cinereoargenteus*) extensively overlaps that of the coyote. They still maintain core areas that are not used by coyotes, but have not been displaced by the eastward expansion of the coyote (Chamberlain and Leopold, 2005). The gray fox is more closely associated with deciduous forests than either the red fox or the coyote. The gray fox prefers hardwood forests with rocky terrain and brushy cover, but has also been observed using meadows, grasslands and abandoned fields. Seventy-five percent of the gray fox diet consists of rabbits, mice, rats, and other wild mammals. Additional

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food items include passerine birds, eggs, invertebrates, frogs and carrion. During the late summer and autumn, insects, fruits, nuts, grasses and corn are a food staple. In winter, rabbits, small mammals and plants form the bulk of the diet (Hockman and Chapman, 1983; Merritt, 1987). The gray fox is considered to be dominant to the red fox due to its variable diet and ability to climb trees to escape predators (Bozarth et al., 2011). The gray fox is host to flukes, tapeworms, roundworms and spiny-headed worms (Buechner, 1944; Merritt, 1987; Davidson et al., 1992).

Canids can serve as a definitive host for tapeworm, *Echinococcus multilocularis*, while meadow voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus*) common to LEAD (Stewart et al., 2008) serve as the primary intermediate hosts. This is especially troubling because *E. multilocularis* causes cystic and alveolar echinococcosis or hydatid disease in humans (CDC, 2012). *Echinococcus multilocularis* eggs have become adapted to colder climates and can survive at temperatures of -50◦C (Macpherson, et al., 2000). *Echinococcus multilocularis* has been found in coyote, red fox, meadow voles, and deer mice in ten continuous states across the north-central United States: North Dakota, South Dakota, Iowa, Minnesota, Montana, Wyoming, Nebraska, Illinois, Wisconsin, Indiana, and Ohio (Storandt et al., 2002).

Previous research examining internal parasites at LEAD excluded foxes and tapeworms were absent in the sixteen coyote scats sampled (Bixel, 1995). Also, no research has been done comparing the internal parasites of coyotes with that of foxes in south-central Pennsylvania. The goal of this study was to survey the prevalence and relative intensity of helminthes in canid populations at LEAD using standard fecal flotation. The inclusion of red fox and gray fox fecal samples served to provide a more complete inventory and assessment of internal helminthes present and will provide data for future management decisions at LEAD. A secondary goal was to determine if the zoonotic tapeworm *E. multilocularis*, is present at LEAD, since its range is expanding from midwestern regions (Storandt et al., 2002)

MATERIALS AND METHODS

LEAD is a circa 7,000 ha military installation located in south-central Pennsylvania along the Kittatinny Ridge of the southern Blue Mountains (39° 58'N, 77° 42'W). This ridge has been identified by the Mammal Technical Committee of the Pennsylvania Biological Survey as an area of mammalian diversity importance (Pennsylvania Biological Survey, 2006). The Kittatinny Ridge runs from south-central Pennsylvania, and extends northeast approximately 300 km. The majority of the terrestrial habitat on LEAD consists of open fields and second- or third growth forest. Of the 7,000 ha on LEAD, approximately 35 percent is forested and 52 percent is open fields, one percent is water, and the remaining 12 percent is mostly developed with scattered vegetation. This area is actively managed by LEAD personnel and no feral dogs are tolerated within this area.

Fresh canid feces were collected every four days between February 7th and March 20th 2012, from 38.62 km of paved roads. Each sample was placed in an individual freezer bag labeled with the date and location and analyzed within 24 hours. The diameter of each sample was measured in two locations along the scat and average diameters 18mm or greater were designated coyote, while average diameters less than 18mm were designated as fox (Bixel, 1995; Danner and Dodd, 1982). Samples were processed using Fecal Diagnostic Kits (Revival Animal Health, Orange City, IA) and Feca-Med (VEDCO, Inc., St. Joseph, MO), standardized sodium nitrate solution with a specific gravity of 1.25-1.30. A simple flotation technique was used with one deviation, samples stood for 30 minutes before examination of the coverslip (Dryden et al., 2005). Ova and oocysts were identified by morphologic characteristics and size using a standard micrometer (Sloss, 1994; Foreyt, 2001; Bowman, 2009; Butterworth and Berverley-Burton, 1980): and were counted for each sample (Table 1). The prevalence of parasitism is the percentage of samples examined, which were positive for a given parasite or parasites. The relative intensity of parasitism is the mean number of parasite ova or oocysts per slide prepared from all positive samples.

RESULTS

Thirteen parasites were identified in 75 fecal samples (Table 2) in this study. Of these, six genera and four species of Isospora not previously reported at LEAD were detected including: *Taenia sp.*, *Capillaria plica*, *Capillaria putorii*, *Toxocara canis*, *Strongyloides stercoralia*, *I. canis*, *I. burrowsi*, *I. ohioensis*, *I. bigimina* and the earthworm parasite *Monocystis lumbrici*. Forty percent of coyote (n=35) and 72.5% (n=40) of fox fecal samples contained evidence of at least one parasite. Eleven of the 13 parasites identified were common to coyotes and foxes. Fox samples demonstrated a higher prevalence of parasitism than did coyote samples; however, the relative intensity of parasitism was greater in coyote samples. *Toxascaris leonina* and *Isospora bigimina* were present only in fox samples (Table 2).

Ova of seven nematode species were identified including: *Capillaria aerophila*, *Capillaria plica*, *Capillaria putorii*, *Toxocara canis*, *Toxascaris leonina*, *Strongyloindes stercoralia*, and *Uncinaria stenocephala*. *Capillaria aerophila* prevalence in foxes was double that of coyotes (28% and 14% respectively). Prevalence of *Monocystis lumbrici* oocysts, a protozoan parasite of earthworms and intermediate host for *Capillaria sp.* was nearly equal among coyotes (26%) and foxes (28%). *Capillaria plica* was observed in 17% of coyote and 18% of fox samples. *Capillaria putorii* was observed in three percent of coyote

Table 1. Published dimensions of parasite ova or oocysts collected from coyotes collected during this study. These measurements were used, in part, to positively identify each parasite.

	Length x Width (μm)	Reference	Length x Width (μm)	Reference
Cestodes				
Taenia sp.	38 x 32	Foreyt, 1997		
Nematodes				
Capillaria aerophila	70 x 35	Foreyt, 1997	64-83 x 26-38	Butterworth, 1980
Capillaria plica	58-71 x 25x31	Butterworth, 1980		
Capillaria putorii	53-64 x 20-28	Butterworth, 1980		
Toxocara canis	80 x 75	Foreyt, 1997		
Toxascaris leonina	80 x 70	Foreyt, 1997		
Strongyloides stercoralia	55 x 30	Foreyt, 1997		
Uncinaria stenocephala	75 x 45	Foreyt, 1997	Avg >70 μm long	Bowman, 2009
Protozoans				
Isospora canis	36 x 30	Foreyt, 1997	32-42 x 27-33	Bowman, 2009
Isospora burrowsi	17-22 x 16-19	Bowman, 2009		
Isospora ohioensis	24 x 21	Foreyt, 1997	19-27 x 18-23	Bowman, 2009
Isospora bigimina	13 x 10	Foreyt, 1997		
Monocystis lumbrici				

and eight percent of fox species samples. *Toxocara canis* was present in three percent of coyote and five percent of fox samples. Foxes (20%) had a higher prevalence of *Taenia sp.* than coyotes (11%), but coyote samples contained a greater mean relative intensity 16 to 3.5 respectively. *Toxascaris leonina* was only detected in one fox sample out of 40 examined and was not detected in any of the 35 coyote samples tested. The prevalence of the hookworm, *Uncinaria stenocephala* was ten percent in fox samples and 14% of coyote samples. *Strongyloides stercoralis*, an intestinal threadworm of canids and humans, was more prevalent in foxes than coyotes 10% and 3% respectively. Four species of *Isospora* were identified, *I. canis*, *I. burrowsi*, *I. ohioensis* and *I. bigimina*. Measurements were not taken on two *Isospora* ova, which were classified as *Isospora sp.* (Table 2).

Of the 75 fecal samples tested, nine percent of coyote and 28% of fox contained one species, nine percent of coyote and 18% of fox contained two species, 11% of coyote and 13% of fox contained three species, six percent of coyote and three percent of fox contained four species, three percent of coyote and ten percent of fox contained five species and three percent of coyote and no fox samples contained six species. No sample contained more than six parasite species (Figure 1) which is greater than previous research in which no sample contained more than two parasite species (Bixel, 1995).

DISCUSSION

Both foxes and coyotes share common parasites at LEAD, although foxes had higher parasite richness (13) than coyotes (11). Previous research on coyotes at LEAD identified five endoparasite species from 16 scats in summer. The increase in parasite richness may be due to increased sample size, varied diets between winter and summer months, or variable sympatric relationships between the coyote, red fox and gray fox as these will change through time. Of the five parasites identified by Bixel (1995); *Capillaria aerophila*, *Uncinaria stenocephala* and *Isospora sp.* were observed in both fox and coyote scat in this study, while *Toxascaris leonina* was only found in foxes, and *Ancylostoma caninum* was not detected in foxes or coyotes. The lack of detection of the hookworm *A. caninum* at LEAD in this study and the very low prevalence of detection in Bixel's (1995) study where a single scat (out of 16 sampled) contained ova, is potentially reassuring as *A. caninum* can cause severe human pathology (Prociv and Croese, 1996).

Prevalence of the lungworm *C. aerophila* in coyote samples (14%) is lower than previous research at LEAD where 38% of coyote feces contained *C. aerophila* ova (Bixel, 1995) but near the average prevalence of 12.4% from three sites in New York (Gompper et al., 2003). Presence of *C. aerophila* from fox scat in this study (28%) is similar to the prevalence

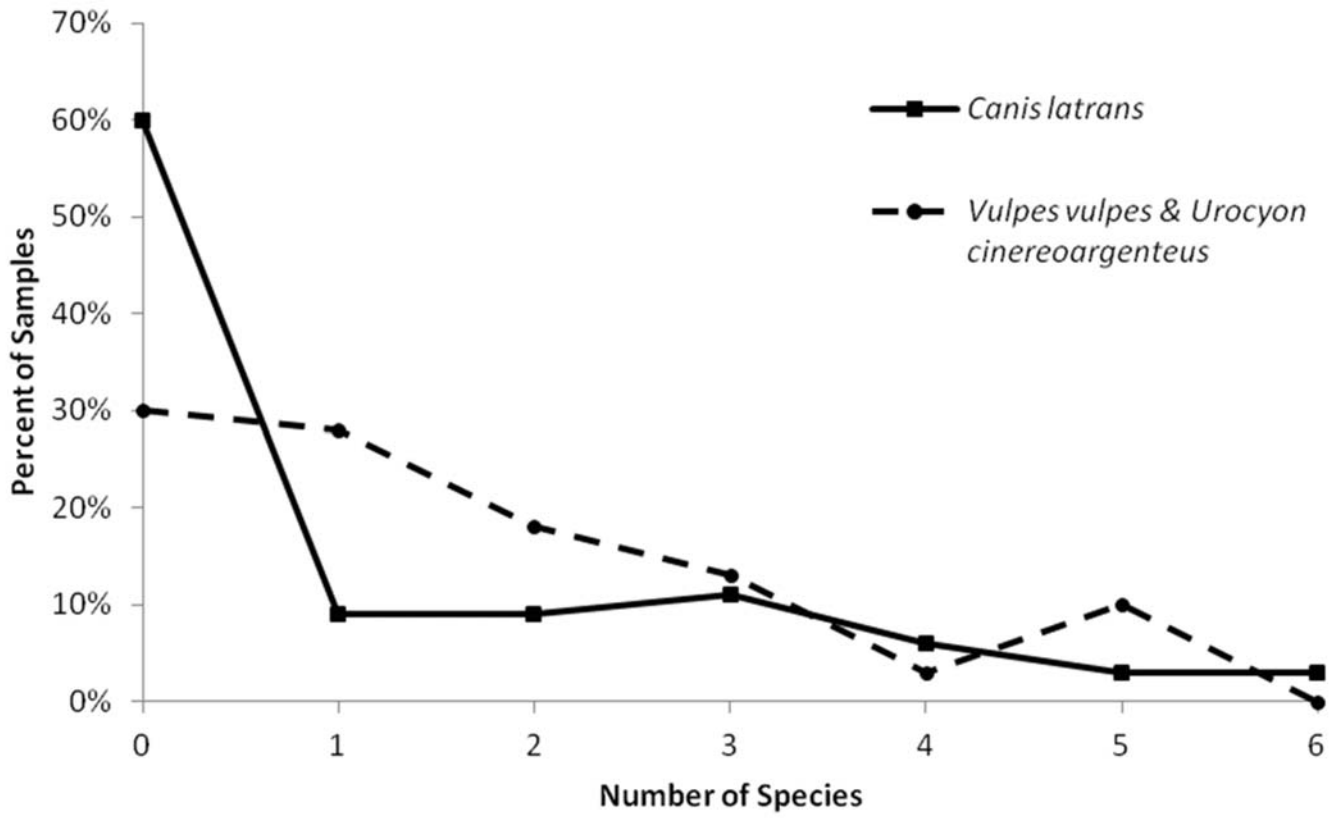

Figure 1. Percentage of coyote and fox samples infected with different number of intestinal parasite species at Letterkenny Army Depot during February and March 2012. It was not possible to determine if fox scat samples were from red (*Vulpes vulpes*) or grey (*Urocyon cinereoargenteus*).

observed by Bixel (1995) indicating that prevalence should be determined from all component hosts within a community. Infection of *Capillaria aerophila* can be much higher in wild fox populations as reported at 74.1% in red foxes from Denmark (Saeed et al. 2006). Common parasite prevalence between fox and coyote samples observed in this study demonstrate that some fecal ingestion occurs between canid species at LEAD. This and other intestinal parasite species with a direct lifecycle may be shared among and between canids due to their coprophagic tendency to remove feces from their territory (Livingston et al., 2005). Foxes with high *C. aerophila* worm burdens experience wheezing, coughing, weakness, poor growth, failure to shed properly and death due to bronchopneumonia (Bowman, 2009) so the high prevalence of this helminth may have management implications.

Capillaria plica (the dog bladder worm) was not detected by Bixel (1995) but was observed in 17% of coyote and 18% of fox samples in our study indicating contamination of samples with urine (Sloss et al., 1994). *Capillaria plica* infections occur from the incidental consumption of earthworms (Bowman, 2009 and Macpherson et al., 2000). The high prevalence of the Gregarine *Monocystis lumbrici* (26% for coyotes, and 28% for foxes) indicates that earthworms are likely a part of their diet since *M. lumbrici* is only a parasite in the seminal vesicles of *Lumbricus terrestris* and related earthworms (Roberts and Janovy, 2009) and is commonly found in scavaging animals whose diet includes earthworms (Anderson 2008).

Capillaria putorii, detected in one coyote and three fox scats in this study, is a parasite of the small intestine of hedgehogs, raccoons and various mustelids suggesting additional evidence of prey species (Bowman, 2009). *C. putorii* was not previously reported at LEAD but was detected in one of the 145 scats examined in NY by Gompper and coworkers.

Previous research at LEAD did not detect any T*oxocara canis*. This study found a slight increase in the prevalence of *T. canis* infection with 3% of foxes and 5% of coyote samples showing signs of infection. This is slightly higher than the 1.4% of coyote samples from New York (Gompper et al., 2003; Bixel, 1995). The increase at LEAD may be due to increased sample size from sixteen samples in 1995, to seventy-five in the present study as well as the inclusion of fox scat or differences in dietary patterns between summer and winter. Globally, infection rates in foxes can be up to

Table 2. Number and prevalence (%) of coyotes (*Canis latrans*) and foxes (*Vulpes vulpes* and *Urocyon cinereoargenteus*) infected with cestodes, nematodes and parasitic protozoa as detected through fecal floatation of scat collected during February and March 2012 at Letterkenny Army Depot, Chambersburg, PA. Relative intensities were calculated based on the total number of ova/oocysts.

	Canis latrans					***Vulpes vulpes* & *Urocyon cinereoargenteus***				
		Prevalence (%)	Intensity				Prevalence (%)	Intensity		
	# of samples	% all coyote samples n=35	Mean	SD	Range	# of samples	% all fox samples n=40	Mean	SD	Range
Cestodes										
Taenia sp.	4	11	16	20.3	1-45	8	20	3.5	4.8	1-15
Nematodes										
Capillaria aerophila	5	14	2	1.0	1-3	11	28	2.4	2.1	1-7
Capillaria plica	6	17	6	7.4	1-20+	7	18	4.7	7.3	1-21+
Capillaria putorii	1	3	20	NA	NA	3	8	2	1.7	1-4
Toxocara canis	1	3	1	NA	NA	2	5	1	NA	NA
Toxascaris leonina	0	0	0	NA	NA	1	3	1	NA	NA
Strongyloides stercoralis	1	3	4	NA	NA	4	10	1.25	0.5	1-2
Uncinaria stenocephala	5	14	2	0.9	1-3	4	10	1.75	0.5	1-2
Protozoans										
Isospora canis	3	9	2	2.3	1-5	3	8	5	3.6	1-8
Isospora burrowsi	4	11	18	22.2	1-48	4	10	1.25	0.5	1-2
Isospora ohioensis	2	6	3	2.1	1-4	2	5	10.5	13.4	1-20+
Isospora bigimina	0	0	0	NA	NA	3	8	4.3	4.9	1-10
Isospora ssp.	1	3	1	NA	NA	1	3	1	NA	NA
*Monocystis lumbrici**	9	26				11	28			

NA Standard deviation and range incalculable because n=1 or 0.

* Presence/Absence only

80% (Macpherson et al., 2000). Humans become infected through oral ingestion of infective eggs from contaminated soil, unwashed hands, unwashed raw vegetables or consumption of undercooked organ and muscle tissue (Macpherson et al., 2000).

The detection of *Toxascaris leonina* in only one fox sample, and no coyote samples is consistent with low prevalence reported by Bixel (1995) who detected two ova in one coyote fecal sample (0.6%). Similarly 1.4% of coyote samples in New York tested positive for *T. leonina* (Gompper et al., 2003; Bixel, 1995). *Toxascaris leonina* is considered to be less pathogenic than *T. canis* causing pot-belly and diarrhea in heavy infestations.

Strongyloides stercoralis, an intestinal threadworm of canids and humans, was detected in 3% of coyote and 10% of fox samples (Table 2). The parasite, typically found in the southern United States, was not detected in New York in 2003 or at LEAD in 1995 (Gompper et al., 2003; Bixel, 1995). The increase in *Strongyloides stercoralis* may be due to the lack of cold temperatures; the winter of 2011/2012 was one of the top five warmest on record in Pennsylvania (Dolce, 2012). *Strongyloides stercoralis* usually infects humans through penetration of the skin at the feet or lower legs. The parasite can be fatal to immunocompromised individuals and diagnosis can be complicated by intermittent larval shedding. Infectious larvae in stools maintained at room temperature for 24-96 hours have been known to develop into free-living adult life stages (Macphearson, 2000).

The prevalence of the hookworm, *Uncinaria stenocephala* was consistent with previous research at LEAD being present in ten percent of fox fecal samples and 14% of coyote fecal samples (Bixel 1995). Prevalence estimates from three sites in New York ranged from 1.5% to 26.1% (Gompper et al., 2003). *Uncinaria stenocephala* can develop at temperatures

below 15°C when outside of a host and infection can cause mild diarrhea and anemia in young canids with high worm burdens (Macpherson et al., 2000).

Taeniid ova were detected in 20% of fox and 11% of coyote samples. This is consistent with research in New York which reported *Taenia sp.* in 11% of coyote samples (Gompper et al., 2003). Coyotes experienced a greater mean relative intensity than foxes, 16 to 3.5 respectively, which was likely due to one individual having a large number of ova (SD 20.3). No *Taenia sp.* were reported in coyotes at LEAD in 1995 (Bixel, 1995). This increase may be due to the coprophagic tendency to remove feces from their territory or infected coyotes relocating into the area (Livingston et al., 2005). *Taenia* ova cannot be identified to species based on morphology alone, however, red foxes, gray foxes and coyotes from Ohio and Indiana carried *T. crassiceps* and *T. pisiformis* and coyotes in Florida carried *T. pisiformis* (Davidson et al., 1992 and Foster et al., 2003).

Coccidiosis, from *Isospora* infection, causes chronic diarrhea. The prevalence for *I. canis* (9% coyote and 8% fox samples) reported in our study was higher than previous research in New York (0.7% coyote), but comparable for *I. ohioensis*. *Isospora burrowsi* and *I. bigimina* were not previously identified in New York (Gompper et al., 2003), but were detected in 11% of coyotes and 10% of fox samples. Bixel, reported a 31% relative frequency of *Isospora sp.* at LEAD (Bixel, 1995). Our findings are comparable to those of Bixel, (1995), when *Isospora* species are grouped (29% of coyote and 34% of fox samples).

The number of fecal samples containing no evidence of internal parasites was higher in this study (coyotes 60% and foxes 27.5%) than previous research on coyotes in New York (44%) and LEAD (31%) (Gompper et al., 2003 and Bixel, 1995). This percentage may have been affected by the nine fecal samples collected the first two weeks of February. These samples consisted almost exclusively of white-tailed deer hair; no parasite ova or oocysts were observed in these samples. Alternatively, our choice of supersaturated solution (sodium nitrate) and/or method to isolate oocysts and eggs may have decreased our isolation ability since the specific gravity is 1.25-1.30 (Dryden et al., 2005). Centrifugation of the sample may have increased our ability to detect more ova; however, each sample was set aside for a time period (30 minutes) greater than was tested by Dryden and coworkers. Another possible explanation may be the intestinal parasite community within LEAD resides mostly within foxes and recent coyote invaders are not as important for maintaining these parasites.

Hunters annually harvest approximately 140 white-tailed deer per square kilometer at LEAD. Deer are field-dressed and the offal is left behind for scavengers. Our survey failed to detect *E. multilocularis* and no hydatid cysts have been found in the hundreds of deer tissues analyzed annually. Therefore, the eastward spread of *E. multilocularis* has most likely not reached south-central Pennsylvania. There remains a need to continue monitoring for the presence of *E. multilocularis* since alveolar echinococcosis is one of the most lethal helminthic infections of humans and its presence has been confirmed in Ohio (Storandt et al., 2002). We also recommend determining population sizes for canine species at LEAD to serve as a baseline for evaluating the effects of coyote migration into the region and the impact of parasite prevalence on each population.

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ANALYSIS OF GROUND WATER DRAWDOWNS USING FIELD MONITORING, COMPUTER MODELING AND THEORETICAL TECHNIQUES AT MONROE MARKETPLACE, HUMMELS WHARF, PA[1]

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ABSTRACT

A newly built shopping center called Monroe Marketplace Plaza, located in Hummels Wharf, Pennsylvania, raised concern among local residents after they heard about the high rate of two pumping wells placed in the vicinity of their homes. The study herein was conducted by monitoring 4 domestic wells from March 2008 through January 2010, theoretically by using Neuman and Witherspoon equation, and lastly by groundwater modeling. The results showed that both Neuman and Witherspoon equation and groundwater modeling results are in agreement with the observed data when a hydraulic conductivity of $9.26x10^{-7}$ ft/sec ($2.78x10^{-7}$ m/sec) was used. The results indicate that the initial drawdown of 19 ft (6 m) observed during the monitoring period in well 4 was caused by a nearby well that was pumping at high rate. The location of both pumping wells has arrested the cone of depression to the center of the plaza without major effect on the well 1, 2, and 3. The groundwater level gradually increased due to high specific yield of the aquifer and has since readjusted to new hydrological condition, fluctuating only to recharge effects. Overall, all 3 methods approximate similar results or complement each other and for a practical approach makes it highly unlikely that the New Marketplace's water consumption will affect residential water supply. [J PA Acad Sci 87(1): 27-33, 2013]

INTRODUCTION

Often in rural areas, commercial development causes concern with regard to ways in which allocation of water use may affect those already living in the area. Residents in the areas surrounding Monroe Marketplace in Hummels Wharf, PA were concerned about the impact of a commercial development on their water supply (figure 1). Monroe Marketplace is a newly built 750,000 ft^2 (69,677 m^3) sized shopping center. Prior to its construction, this project raised concern among the residents especially after they learned that two high-rate pumping wells will be placed in the center of shopping plaza. This study has focused on evaluating the impact of these pumping wells on groundwater level in surrounding areas. To address this problem, four domestic wells were selected near the shopping plaza and monitored from March 2008 through January 2010.

The geology and hydrology of the area was investigated and a groundwater model was constructed to conceptualize the general water table of the area. Neuman and Witherspoon's theoretical equation was found to fit the scenario best, and replicate very well the observed data from monitoring. Neuman and Witherspoon equation was also used to extrapolate the impact of pumping on the drawdown both in short and long-term periods. The results of this study added important knowledge to our understanding of the groundwater table behavior in general, but limited literature is available on the behavior of the aquifer at this particular area.

The first objective was to determine the groundwater level at various locations surrounding the study area to build a

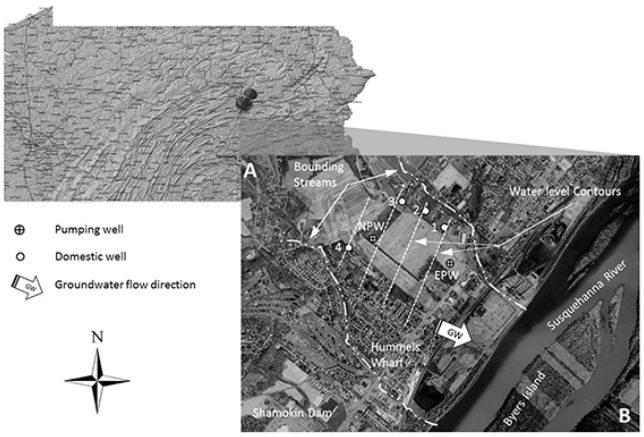

Figure 1. Geographical location of Monroe marketplace, the four domestic wells and the groundwater contour lines. AB represents the cross section line shown in Figure 2.

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piezometric map and to determine the existing groundwater flow direction. A geological study was also carried out and a stratigraphic cross-section of the aquifer was constructed to understand the aquifer properties (figure 2). Water level was regularly monitored to determine if the pumping wells were having any effect on the groundwater table. The measured water level was also used to validate the Groundwater Model built with MODFLOW software. This was created using a conceptual modeling approach using GMS 7.0 software. The model was constructed to replicate the hydrogeological conditions of Monroe Marketplace site. After defining the boundary and the initial conditions, MODFLOW was run with several Hydraulic Conductivity (*K*) Values until reaching the hydrological conditions matching the monitoring measurements. Hydraulic Conductivity values were selected from a previous study by Spotts, Stevens and McCoy (SSM) in 2009 that ranged from 0.03 to 13 ft/day. The underlying aquifer at this site was mostly Keyser and Tonoloway Formations (DSkt) (Socolow 1980). These formations are composed mostly of gray, mud-cracked limestone with dark gray shale interbeds. *K* value was determined by comparing observed water levels and contour line provided by groundwater modeling as different *K* values were used. Assuming all conditions that make Darcy's law valid, a theoretical expression by Neuman and Witherspoon (1969) was used and revealed a good match between the calculated drawdown and the observed water level and provide an explanation of why the drawdown was higher at the beginning of the monitoring.

Overtime the response of the water table, especially in the area close to the pumping well, was observed to follow the theoretical early drawdown patterns described by Neuman and Witherspoon equation. It was also determined that the overall effect of the pumping may had an effect on the area and was strong during the construction and the landscape irrigation of the plaza, yet after this period the drawdown was observed to decreases reaching a steady condition and shows no major adverse impacts on the water table. This conclusion obviously assumed average local climate without significant dryness taken in consideration.

SITE DESCRIPTION AND BACKGROUND

The study area is located within the Susquehanna River Valley lowland section of the linear ridges and valleys of central Pennsylvania. The study site is located in Monroe Township which lies within the Lower Penn's Creek watershed in Snyder County. The study area includes the new Marketplace Plaza and the surrounding residential areas bounded by two small streams oriented NW-SE and are perpendicular to Susquehanna River (figure 1). The two streams were selected to mark the boundaries of the study site. The topography of the study area varies from gentle slopes at about 538 ft above sea level in the northwest to the Susquehanna River floodplain laying at an average elevation of 407 ft. The central Ridge-and-Valley region is

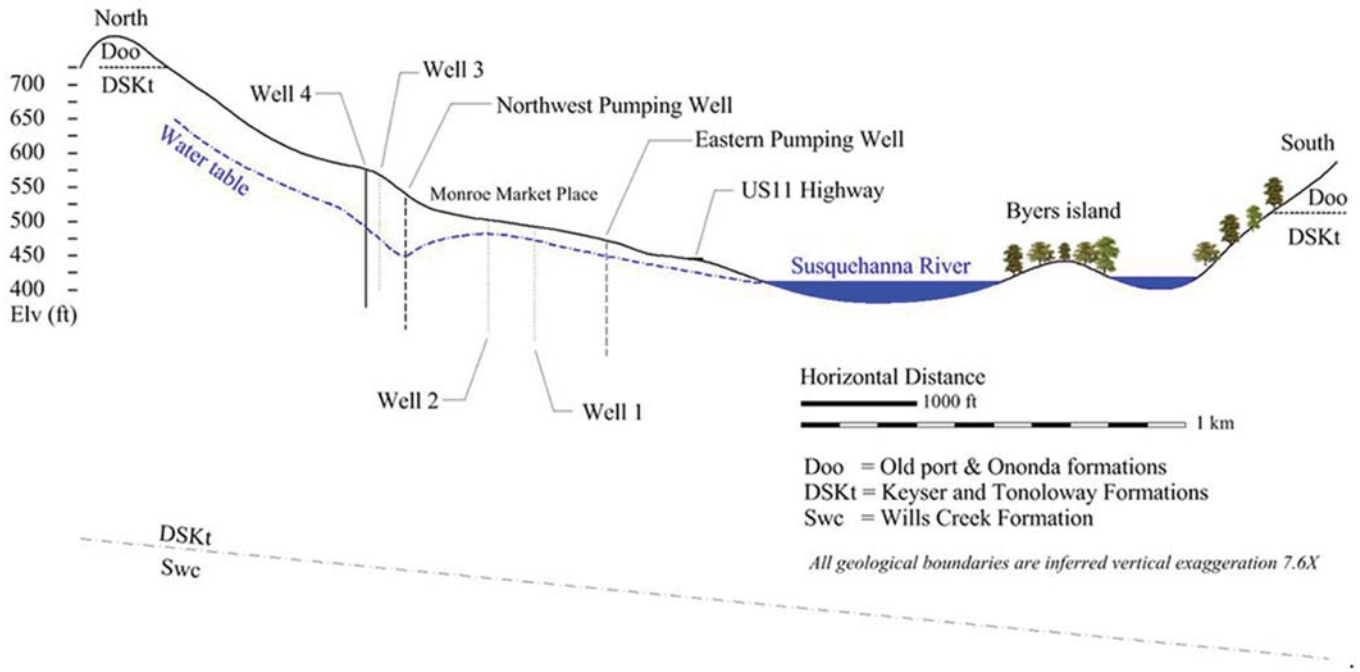

Figure 2. Cross-section showing the local geology (AB in Figure 1), wells and graphical representation of the cone of depression near well 4. Wells 1, 2 and 3 are projected of the cross section line.

characterized by an average annual precipitation of 40 inches and an average snowfall of 40 inches per year (Yarnal, 1989). To determine the groundwater flow direction, repeated tests were performed with water levels measured at different times. Groundwater contour lines and the hydraulic gradient were determined by interpolating the hydraulic head values obtained from 5 sets of randomly selected measurements. The hydraulic gradient and the direction of groundwater flow was calculated using the four-point method of plane analytic geometry as described by Vacher (1989) and Fetter (2001) (figure 1). These parameters were determined by placing the four monitoring wells on a known scale map and well pairs water level differences. Table 1 shows the geographical coordinates of the four domestic wells used to determine the hydraulic gradient and flow direction. The hydraulic gradient was found to be in average about 0.025 with a flow direction toward the south-east (figure 1).

GEOLOGICAL CHARACTERIZATION

Monroe Marketplace is located within the Susquehanna Lowland sections of the linear ridges and valleys physiographic province of central Pennsylvania. A geologic cross-section of the study area (figure 2) along AB line in figure 1 was completed based on a field survey, measurements of strike and dip performed on outcrops, and a local geological map. Most of the geologic formations found in lowlands consist of relatively soft shales, limestone, and siltstones. According to the Pennsylvania Department of Environmental Resources geologic map, the primary geological formations near the Monroe Marketplace Tonoloway Formation, Onondaga Formation, and Old Port Formation.

Socolow (1980), describes the Wills Creek Formation (Swc) as a Silurian formation comprised of multicolored gray, grayish red, yellowish and greenish-gray, interbedded calcareous shale, siltstone, sandstone and shaly limestone and dolomite. The thickness of this formation is estimated to range from 250 to 500 feet (Laughrey, 1999). Because it was also reported that this formation has a low permeability it was used as an appropriate no-flow boundary in the groundwater modeling study.

The Tonoloway Formation (DSKt) is situated above the Wills Creek Formation. It consists of medium-gray laminated, mud-cracked limestone containing some medium-dark olive-gray shale and siltstone interbeds (Socolow 1980). This formation is from the upper-Silurian period (Laughrey, 1999). The lower contact of the Keyser with the Tonoloway is distinct and conformable; however, they are usually classified geologically together as the Keyser and Tonoloway Formations and referred to in the literature as DSkt (Socolow, 1980).

The Keyser Formation consists of carbonates deposited from the Late Silurian into Early Devonian time. Socolow (1980) describes the Keyser as being made up of medium-gray, crystalline to nodular, fossiliferous limestone. The upper part of the Keyser Formation is made of thin-bedded limestone and dark-gray chert nodules. The remaining portion of the formation is thin to very thick bedded. The thickness of the Keyser ranges from 75 to 202 feet (22 to 62 m) (Laughrey, 1999). The Devonian-age Onondaga and Old Port Formations are also found symbolized under Doo (figure 2). The Onondaga Formation is comprised of medium-gray calcareous shale with marine fossils along with argillaceous limestone at the top referred to as the Needmore Formation within its Selinsgrove Member. The Old Port Formation is composed of fine to very coarse grained, light-gray sandstone (Socolow, 1980). However, these formations are found only at the high elevations of nearby ridges to the Monroe Marketplace and have no impact on the flow of water within the aquifer

MATERIALS AND METHODS

Groundwater monitoring

Groundwater level was monitored using four wells over a period of 534 days using a water level meter. The wells were spread within the limit of the study area and are supposed to cover all variation of the water table. The water level measurements were performed at short time interval at the beginning of the study and gradually on weekly to biweekly basis as water level started to stabilize. In addition to direct assessment of the water level, these measurements were also used to evaluate the appropriateness of the theoretical approach used in this study and the effectiveness of the modeling simulation.

Hydrological characterization

Based on the average precipitation, land use, geomorphology and the underlying geologic formations, the mean annual groundwater-recharge estimates of the study area is about 14.01 to 16 inches with an approximate average error of 2.01 to 3.00 inches (Reese and Risser, 2010). Most hydraulic properties of this aquifer were taken from a recent project funded by PADEP and completed by the consulting firm SSM in 2009. The stated values in SSM report were found for the same aquifer not far from the study area. The Hydraulic Conductivity (K) ranges between 0.03 and 13 ft/day and the Transmissivity (T) ranges between 10 and 1511 ft^2/day. Using Neuman equation for the drawdown caused by the pumping wells, the best fit value for transmissivity was determined to be 4 ft^2/day, which is less than the smallest value found by SSM. Furthermore, the aquifer exhibits vertical anisotropy with a ratio of horizontal to vertical hydraulic conductivity of 1/1.5.

Based on the hydrogeological conditions of this site, Neuman and Witherspoon equation was applied to study the impact of pumping on the drawdown. In an unconfined aquifer, the flow of groundwater toward a pumping well can be described by the following equation (Neuman and Witherspoon, 1969):

$$K_r \frac{\partial^2 h}{\partial r^2} + \frac{K_r \partial h}{r \partial r} + Kv \frac{\partial^2 h}{\partial z^2} = S_s \frac{\partial h}{\partial t} \qquad (1)$$

where
h: Saturated thickness of the aquifer (L)
r: Radial distance from the pumping well (L)
z: Elevation above the base of the aquifer (L)
S_s: Specific Storage (1/L)
K_r: radial Hydraulic Conductivity (L/T)
K_v: Vertical Hydraulic Conductivity (L/T)
t: Time (T)

Radial flow in unconfined aquifers is typically modeled based on a series of equations depending on specific conditions e.g., (Boulton and Streltsova 1975; Boulton 1954, 1955, 1963, 1973; Boulton and Pontin 1971; Streltsova 1972, 1973; Dagan 1967; Moench 1995; Neuman 1972, 1974, 1975; Gambolati 1976). The appropriate equation in these solutions can be difficult when compared to a qualitative description of how the water-table responds to pumping.

Neuman (1972, 1974, 1975, and 1987) refined a solution to equation (1) by making several assumptions in addition to the basic approximations made about the hydraulic conditions of the aquifer to better estimate drawdown in response to pumping. These are: 1) the aquifer is unconfined, 2) the vadose zone has no influence on the drawdown, 3) water initially pumped comes from the instantaneous release of water from elastic storage, 4) eventually water comes from storage due to gravity drainage of interconnected pores, 5) the drawdown is negligible compared to the saturated aquifer thickness, 6) the specific yield is at least 10 times the elastic storativity, 7) the aquifer may be – but does not have to be – anisotropic with the radial hydraulic conductivity different than the vertical hydraulic conductivity (Fetter 2001). With these assumptions Neuman's solution is:

$$h_o - h = \frac{Q}{4\pi T} W(u_A, u_B, \Gamma) \qquad (2)$$

where $(h_o\text{-}h)$ represents the drawdown, Q is the pumping rate and $W(u_A,u_B,\Gamma)$ is the well function for the water-table aquifer. Solutions of $W(u_A,u_B,\Gamma)$ are tabulated and can be found in Neuman (1975).

There are three phases of time-drawdown of the water table due to pumping well. During the first phase, the pressure in the annular region surrounding the well will drop. During this initial drop the aquifer will contribute a small volume of water due to the expansion of water and also the compaction of the aquifer matrix. During this period the drawdown is determined by the elastic storativity of the aquifer. Flow is mostly horizontal during this period because the water is being derived from the entire aquifer thickness (Fetter 2001). The initial phase for the Neuman solution for early drawdown data is shown as follow:

$$h_o - h = \frac{Q}{4\pi T} (u_A, \Gamma) \qquad (3)$$

with $u_A = r^2/4Tt$ (for early drawdown data), $\Gamma = (r^2 K_v)/(b^2 K_h)$

where r is the radial distance from the pumping well, S is the storativity, t is the time, K is the hydraulic conductivity along the vertical direction (K_v) and along the horizontal direction (K_h) and b is the initial saturated thickness of the aquifer.

As the drawdown continue over a longer time, the elastic storage coefficient approaches zero, the first stage of drawdown also approaches zero. As the specific yield approaches zero the length of time for the first stage increases (Gambolati, 1976). The later phase of drawdown data is used in equation 4:

$$u_B = \frac{r^2 S_y}{4\pi T} \qquad (4)$$

(for later drawdown data), $\Gamma = (r^2 K_v)/(b^2 K_h)$

where S_y is the specific yield

The groundwater level showed a high drawdown in all wells at the beginning of monitoring and a maximum drawdown of 19 feet was observed in well 4 (figure 3). The drawdown values using the Neuman and Witherspoon equation (equation 3) for an early drawdown was calculated over a period of 400 days. The same drawdown was also obtained after 200 days with a continuous pumping rate of 3,800 GPD. The rate of 38,000 GPD was announced by the Pennsylvania Real Estate Investment Trust (PREIT) and was published in the Daily Item of January 30, 2008. For this reason we rearrange our data to start from the 200th day as shown in figure 3 and figure 4a. Another reason was because the monitoring started toward the end of the construction of the plaza during the period of the highest drawdown. Monroe Market Place opened prior to the commencement of monitoring, which shows that the drawdown was most probably caused by the use of water for landscaping and gardening. Well 1, 2 and 3 show smaller fluctuations of water level, most probably due to their locations outside the caption zone caused by the eastern pumping well (figure 5 and 6). In addition, well 1 and 2 are located at a lower elevation near the Susquehanna River. The water table near major rivers are usually shallower compared to areas of

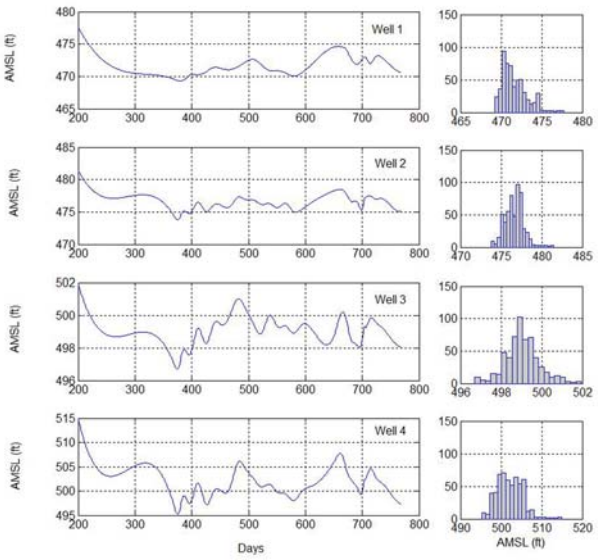

Figure 3. Water level monitoring of the four residential wells and their relative groundwater level frequency of fluctuations with respect to the main sea level since monitoring started.

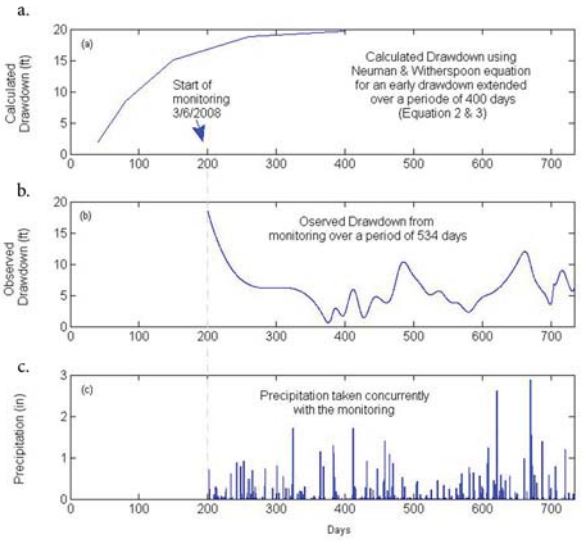

Figure 4. Well 4 calculated and observed drawdown with relation to precipitation.

higher altitude. The fluctuation of drawdown in this case becomes larger as the distance between the well and the river increases. The fluctuations of groundwater level with respect to the main sea level can also be seen in each well corresponding histogram (figure 3). Groundwater level in well 4 was significantly lower than the rest of the wells with a drawdown of 19 feet at the beginning of monitoring. Because well 4 has shown the worst scenario, it was selected to be examined by the theoretical approaches and the groundwater modeling. Aquifer characteristic parameters used in the theoretical evaluation are summarized in table 2. Equation 3 was applied to well 4 and lead to practical results.

After this initial drawdown, the water table started to rise as shown by the monitoring data (figure 4b). The drawdown

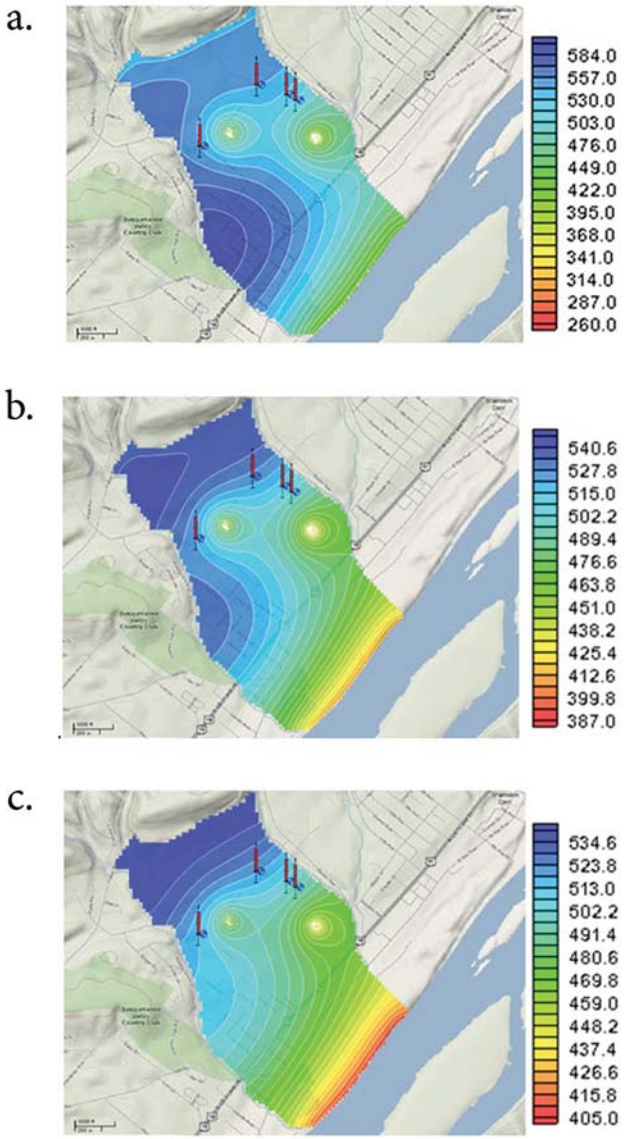

Figure 5. Water table scenarios for different hydraulic conductivity values. (a) K = 0.02 ft/day (0.003 m/day) caused Well 4 to have a head =580.951 ft (177.07 m) above MSL which is significantly higher than the observed head. (b) K = 0.05 ft/day (0.015 m/day) caused Well 4 to have a head =514.330 ft (156.767 m) above MSL which is significantly slightly higher than the observed head. (c) K = 1 ft/day (0.305 m/day) caused Well 4 to have a head =496.867 ft (154.445 m) above MSL which is significantly slightly lower than the observed head.

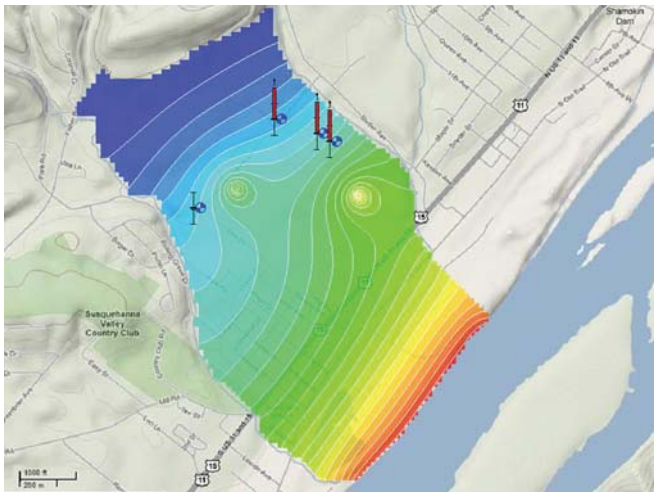

Figure 6. Contours of groundwater heads based on the best-fit hydraulic conductivity value K of 0.08 ft/day matching observed values.

in figure 4b shows a decrease of 14 feet bringing the water level back to a higher elevation. At this point, the fluctuations of drawdown were mainly affected by precipitation rather than pumping as shown by figure 4b and 4c.

Groundwater modeling

To create a groundwater model, a topographic map image of Monroe Marketplace was imported into MODFLOW and then georeferenced to have accurate distances between the wells and consistent with their corresponding orientation. The boundary conditions were represented by a ridge on the north of the study area, two small streams on the east and west sides and the Susquehanna River on the south with a specific head of 409 ft above the sea level (figure 1 and 2). The location of the two pumping wells used in Monroe Marketplace with a total rate of 38,000 GPD was added to the model coverage.

Residential wells were used as monitoring wells due to their negligible average daily pumping rate compared to the pumping wells and implemented into the model coverage. Water level was measured in early afternoon when water is at its low demand to estimate a good average level. figure 3 shows the drawdown trend in the four wells during the monitoring time. These values were also used to assess the efficiency of the groundwater model by comparing the modeling simulation to the observed head values.

Due to the fractured nature of the Keyser and Tonoloway formation, the hydraulic conductivity of this formation is difficult to estimate (Geyer and Wilshusen 1982). The fractures distribution, this formation is considered heterogeneous especially at small scale yet given the fact that the size of the site was relatively large; the assumption of homogeneity was made. To determine the accurate value of the hydraulic conductivity, several MODFLOW simulations were examined with *K* value ranging from 0.1 to 15 ft/day while comparing computed to the measured water level in the wells. Hydraulic conductivity values ranging between 0.08 to 0.1 ft/day have provided the best fit contour lines matching the observed heads. figure 5 shows the three different trials performed based on three different increasing *K* values. Because well 4 has shown the worst drop of water level during the monitoring period, it became obvious that any further drawdown in the area will most probably have an effect on this particular well first. Well 4 was specifically tested for several time periods within this range of *K* values and all led to similar results with the observed heads. As a result *K*= 0.08 ft/day was selected as the representative value for the aquifer's hydraulic conductivity. This value matches all fluctuation of well 4 (figure 6) and provides a good estimate of the other three wells as well.

RESULTS AND DISCUSSION

All wells in the study area have experienced a drawdown of different levels during the monitoring period right after the construction of Monroe Marketplace during the summer of 2008. The drawdown in well 4 at the beginning of the monitoring period was found to be equal to 19 feet. Using the early drawdown Neuman equation (equation 3), this decline must have started in about 200 days prior to the beginning of monitoring (figure 4a). The drawdown was calculated based on an average pumping rate of 38,000 GPD. Beyond this initial decrease, all four wells have stabilized and only fluctuated after rainstorm events (figure 3 and 4). Both calculated and observed drawdowns were found to be in good agreement. The drawdown decline at the end of construction of the plaza was most probably caused by the decrease in pumping as landscaping irrigation was stopped or significantly reduced. The 18 months monitoring have provided useful data matching Neuman theoretical equation results which was also found consistent with the groundwater model simulation.

The value of 0.08 ft/day (0.024 m/day) appears to be the best estimate for hydraulic conductivity (figure 6) in the study area. This value was obtained based on the matching of observed water water level in the residential wells specifically well 4 and their corresponding values obtained from groundwater modeling. Well 4 showed a significant drawdown most probably due to its elevated location. It lays directly up-gradient of the northern pumping well (NPW) and along the groundwater flow path that is within the capture zone of pumping well. In addition, the NPW supplies water to the adjacent water tower. The eastern pumping well (EPW) has no major effect on well 1, 2 and 3 due to their locations outside the cone of depression area. The location of EPW and the level of groundwater transmissivity have

restricted the cone of depression to the center of the plaza without major effect on these wells. Overall, all three methods have led to acquiescent results and makes it highly unlikely that the Marketplace's water usage will drop in the residetial wells.

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ANALYSIS OF THE IMPACT OF ACID MINE DRAINAGE ON BACTERIAL POPULATIONS IN THE UPPER TIOGA WATERSHED[1]

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ABSTRACT

The Upper Tioga Watershed (UTW) in northern Pennsylvania is exposed to acid mine drainage (AMD), resulting in decreased population sizes and diversity of macrobiota. Few studies thus far, however, have assessed the impact of AMD on the microbial communities in streams receiving AMD. Using taxonomic (terminal restriction fragment length polymorphisms (T-RFLP)) and metabolic (Biolog EcoPlates) analyses, bacterial biodiversity and community structure in AMD-impacted and non-impacted sites of the UTW were compared. The results indicate that bacterial communities in sediments of streams receiving AMD differ from those at a non-impacted site and are less diverse. Analysis of T-RFLP patterns and metabolic patterns from Biolog EcoPlates revealed two main clusters of community similarity among the sites. The pattern suggests that the bacterial communities may be more resistant to negative effects of AMD than macroscopic organisms. One AMD-impacted site is dominated by one taxonomic group, putatively identified as Beijerinckiaceae. [J PA Acad Sci 87(1): 34-41, 2013]

INTRODUCTION

Acid Mine Drainage (AMD) is the result of the flooding of abandoned mine shafts with ground water. The water inside the mine shaft reacts with iron pyrite (FeS_2) and other iron compounds that were exposed during the mining process. Acid results according to the chemical reaction $FeS_2 + 14Fe_3^+ + 8H_2O \rightarrow 15Fe_2^+ + 2SO_4^{2-} + 16H^+$ (Bond et al. 2000). The acidic water then flows from the mine shaft to nearby streams. In addition, water discharged from these abandoned mines frequently contains other contaminants such as high metal concentrations (iron, manganese, and aluminum in particular), elevated sulfate levels, and increased suspended solids (U.S. Environmental Protection Agency, 1997). In the mid-Atlantic region of the U.S. (DE, MD, PA, and WV), approximately 4,500 stream miles are degraded by AMD (http://www.Epa.gov/region3/acidification/index.Htm).

The Tioga River Watershed encompasses approximately 400 square miles within Tioga and Bradford Counties in North Central Pennsylvania (Orr 2003). Coal was discovered within the watershed in 1792 and mining began shortly after in 1812 (Orr 2003). Coal production peaked around the turn of the 20th century, and mining ceased in the Upper Tioga Watershed (UTW) in 1990 (Orr 2003). It has been determined by the Susquehanna River Basin Commission (SRBC) that due to the abandoned mine operations in the area, the UTW has been impacted by AMD (Orr 2003). The pH of several of the sampling sites tested by the SRBC falls below 4.5 (Orr 2003), easily below a tolerable pH for many organisms.

The poor biological health of the UTW is also indicated by the composition of the macrobiotic communities. Studies in the watershed have shown that downstream of mine outflows the macrobiotic communities are severely impaired due to AMD (Hughey 1993). As a result, no fish or benthic macroinvertebrates are found in Fall Brook, Morris Run, Coal Creek, Bear Creek, Fellows Creek, or McIntosh Hollow which are all downstream from mine outflows and are tributaries to the Tioga River (Moase et al. 1999). The meiobenthic fauna in the AMD-receiving streams also reflect their impacted state. Stress-tolerant species such as *Eunotia*, which have been shown to predominate in acid-impacted streams (Warner 1971) are common at these sites (J. Kirby, personal communication).

Although the role of prokaryotic communities associated with the formation of AMD has been extensively studied (Baker and Banfield 2003; Bond et al. 2000; Riesenfeld et al. 2004), few studies have been performed on the impact of AMD on the microbial communities in natural streams receiving mine discharge. Most of the existing studies rely solely on culture-based methods of assessing the microbial communities present (Leduc et al. 2002). One study using molecular methods, however, determined that the microbial populations within wetland communities constructed to treat AMD was dominated by two different species, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Nicomrat et al. 2006).

The observed effects on the microbial communities

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in constructed wetlands as well as the impact of AMD on macrobiota suggest that the diversity and size of the bacterial populations within AMD impacted streams will be greatly reduced compared to non-impacted streams. Such an impact could be measured by a variety of different methods such as bacterial enumeration as well as taxonomic and metabolic analyses. Terminal restriction fragment length polymorphism (T-RFLP) for instance, has been used in many studies to assess the diversity of the bacterial populations and to quickly compare the community structure and diversity in a wide variety of environments (Schütte et al. 2008). A method which has been used to complement taxonomic analyses is metabolic characterization of the microbial community using Biolog EcoPlatesTM (Gomez et al. 2004; Hitzl et al. 1997; Viti & Giovannetti 2005). These methods in combination can be used to evaluate the impact of AMD on the biodiversity and community structure of bacteria within stream sediments.

MATERIALS AND METHODS

Site selections and sediment sampling

Sediment from three different sites within the UTW (County Bridge, Fall Brook, and DFB 099) was sampled four times within the period of one year: April 2006, September 2006, January 2007, and April 2007. A fourth sampling site (Coal Creek) was added for the January and April 2007 dates. Each sample collection site was considered to be highly impacted or not impacted by AMD as indicated by the Susquehanna River Basin Commission (SRBC) from a water quality analysis in 2000-2001 in the Upper Tioga Watershed (Gannett Flemming Inc. 2003; Orr, 2003). Environmental conditions (pH and temperature) were recorded at the time of each sample collection (Table 1). All samples were collected in duplicate in separate sterile Mason jars using sterile trowels. Each jar was filled half with water and half with sediment from the site. Sediment samples were stored at 4°C and processed within 24 hours of the initial collection.

County Bridge (CB), the control site, is located upstream from AMD discharges and was therefore considered non-impacted. DFB099 (099) and Coal Creek (CC) are both located within 100 m of a mine shaft outflows and are considered highly impacted sites. The average pH at 099 is 3.1 and CC has an average pH of 2.5. The SRBC determined CC to be the second largest contributor to AMD in the Upper Tioga Watershed and 099 has been determined to be the third largest contributor, accounting for 5.5% of the acidity in the impacted portion of the Tioga River (Gannett Flemming Inc. 2003) Fall Brook (FB), our fourth sampling site, is located down stream from 099 and, although considered highly impacted by the SRBC (Gannett Flemming Inc. 2003), has a more moderate pH (average 4.1).

Metabolic Analysis

Sediment for the Biolog EcoPlatesTM (Biolog, Hayward, CA) was prepared in the same manner as mentioned above, with the addition of a 12 hour incubation to remove potential carbon sources from the sediment (Hitzl et al. 1997). An aliquot (100 µL) of the sediment suspension in 0.85% NaCl (settled for 30 minutes following shaking) was inoculated in each well. The plates were incubated for 3 days at room temperature (20°C). Absorbance was measured at 590 nm using an HTS 700 Bio Assay Reader (Perkin-Elmer, Norton, OH).

DNA extractions from sediment

DNA was extracted from 1 gram of each sediment sample in triplicate using an UltraCleanTM Soil DNA Isolation Kit by MoBio Laboratories, INC. (Carlsbad, CA).

Table 1. Site Conditions. Temperature and pH were recorded during each sample collection using a Hanna HI 991300 electronic pH probe. N/A – not applicable.

		Apr. 06		Sept. 06		Jan. 07		Apr. 07	
	Coordinates	Temp(°C)	pH	Temp(°C)	pH	Temp(°C)	pH	Temp(°C)	pH
County Bridge	N41° 40’ 40.5” W76° 56’ 31.1”	4.1	6.08	14.7	6.19	0.5	5.78	8.0	5.82
Fall Brook	N41° 40’ 41.0” W76° 59’ 21.0”	5.0	4.36	15.4	3.84	1.3	4.24	7.9	4.26
DFB099	N41° 40’ 18.1” W76° 59’ 20.3”	8.2	3.32	9.6	3.08	8.0	3.22	9.8	3.20
Coal Creek	N41° 40’ 34.8” W77° 03’ 0.54”	N/A	N/A	N/A	N/A	10.1	2.53	10.2	2.45

PCR amplification of 16s rDNA and T-RFLP

Bacterial 16S rDNA from each sample was amplified using 63f and 1387r primers (Marchersi et al. 1998). All primers were purchased from Integrated DNA Technologies (Coralville, IA). PCR amplification was performed using Thermo-Start PCR Master Mix (ABgene, Epsom, UK) and 10 pmol of each primer. The conditions used for PCR amplification were as previously described (Hay et al. 2001) with an annealing temperature range of 60°C to 50°C. After amplification, the PCR end products were visualized using gel electrophoresis in a 1% agarose gel run at 10 V cm^{-1} for 15 minutes in 0.5X TBE buffer and stained with ethidium bromide.

For T-RFLP analysis, the 63f primer was 5'-tagged with FAM and the PCR was run in a 50 µL reaction volume. PCR conditions were as described above. The PCR products were then cleaned with a QuickStepTM2 PCR Purification Kit (Edge BioSystems, Gaithersburg, MD) and quantified using a QubitTM Fluorometer (InvitrogenTM, Carlsbad, CA). Using samples with sufficient DNA concentration (CB: both samples in April 2006 and 2007); FB and DFB 099: both samples in April 2006, January 2007, and April 2007; CC: both samples in January and April 2007), a digest of 200 ng of PCR product was then performed using the restriction enzyme CfoI (Fisher Scientific, Pittsburgh, PA). No template negative controls were also run and examined on a 1% agarose gel stained with ethidium bromide to ensure that no peaks were due to lab contamination. After the digest, the products were cleaned again using the same kit as mentioned above, eliminating the SOPE resin step.

The lengths of the fluorescently tagged digest products were then determined using a 3730xl DNA Analyzer (Applied BioSystems, Foster City, CA) at the Core Life Sciences Center at Cornell University. The traces were then analyzed using Genemapper Software v. 3.0 (Applied BioSystems, Foster City, CA).

Identification of 305 bp peak from DFB099

PCR amplification of the 16S rDNA and restriction digest was performed as for T-RFLP on DNA extracted from 099 sediment in April 2007. The sample was separated on a 1.0% agarose gel with no ethidium bromide and the fluorescent band at approximately 300 bp was excised and purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA). Ligation-mediated PCR was then performed as previously described (Junker et al. 2006) using the adaptamers CfoI-linker1 (5'-TCAGGACTCATCGAC-3') and CfoI-linker2 (5'-GATGAGTCCTGAGCG-3'). Following ligation of the adaptamer to the gel-purified 300 bp fragment, the fragment was PCR amplified using the 63f 16s rDNA primer and CfoI-linker2. This PCR product was ligated into pGEM®-T Easy (Promega, Madison, WI) and the construct was confirmed using one of the vector-specific primers T7f or M13r in combination with the insert-specific primer 63f. PCR products from these both these confirmatory reactions on the ligation reaction were submitted for sequencing at the Core Life Sciences Center at Cornell University. A nucleotide-nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed to determine the putative identity of the organism responsible for the peak at 305 bp from the 099 samples.

Enrichment and Isolation of Dominant Species at DFB099

Water and sediment samples from 099 were taken at various intervals and pH of the samples were measured as described previously. N-free medium (100 mL) was inoculated with 099 sediment (1 g) and incubated with shaking at 8°C for four weeks. The N-free media contained, per liter: 0.03 g ferrous sulfate, 0.05 g calcium chloride, 1 g potassium dihydrogen phosphate, 2.5 g potassium monohydrate, 1 ml 1 M magnesium sulfate (added after autoclaving), and 0.1% glucose/dextrose. The media was brought to a pH of 3 by the addition of hydrochloric acid.

Following four weeks of incubation, samples from the enrichments were streaked onto plates of the same N-free medium to isolate colonies. In order to prevent hydrolysis of the agar by the low pH of the medium during autoclaving, plates were made with the same N-free media as described above by separately autoclaving 2X N-free medium and 3.0% agar, then mixing equal volumes of the two when the temperature reached approximately 55°C. The plates were incubated for several weeks at 8°C and inspected regularly for growth of colonies. All bacterial colonies that grew were subjected to PCR with primers specific to the 305 bp fragment identified as dominating 099 sediment (Beij24F – CTGCCTCCCGTAAGAGTCTG, Beij70F – GGTCATCCTCTCAGACCAGC, Beij205R – CGGTACGGAATAACTCAGGG, Beij305R – CTCAGGCCTAACACATGCAA).

Enumeration of bacteria

Sediment for enumeration was collected in June 2008. In order to prepare the sediment for direct counts, 4.5 g of sediment was suspended in a total of 45 mL 0.85% NaCl in 50 mL centrifuge tubes. The tubes were shaken horizontally at 150 rpm for one hour and the sediment was allowed to settle out for 30 minutes. The sediment suspension (1 µL) was then filtered onto a 0.2 micron filter of known area and stained with 4',6-diamidino-2-phenylindole (DAPI). Counts were performed in triplicate and 10 fields of view at 1000X magnification were averaged for each filter.

Statistical analyses

For T-RFLP statistical analyses, only DNA fragments longer than 60 bp were considered. For both T-RFLP and Biolog data, UPGMA cluster analysis was performed (Viti & Giovannetti 2004) and dendrograms were constructed using SPSS v. 15.0 (SPSS Inc., Chicago, IL). Biolog UPGMA analysis was performed using the average absorbance at 590 nm for each supplied substrate. Means were compared with ANOVA and differences among means were identified with the Student-Newman-Keuls multiple-range test at the 0.05 significance level. Biodiversity statistics were based on the data obtained through T-RFLP and were calculated using EcoStat (Trinity Software Inc., Port St. Lucie, FL).

RESULTS

Metabolic Analysis

A comparison of the number of substrates metabolized at each site as measured by Biolog EcoPlates^{TM} (Fig. 1) indicated that CB consistently metabolized significantly more ($p < 0.05$) substrates than any other site within any time point (67-89% of the supplied substrates). In the 2006 samples, FB metabolized 32-53% of the supplied substrates, showing the second largest metabolic range. In 2007, however, the number of substrates metabolized by FB dropped off appreciably to 1-12% of the supplied substrates. With the exception of April 2007 (4.67 ± 1.5), the number of substrates metabolized by the 099 sample was not significantly higher than zero ($p > 0.05$). CC did not show any metabolic activity at any timepoint.

The similarities between the patterns of metabolic ability (extent of metabolism of each substrate) of the sediment bacterial communities from each site at each timepoint were analyzed using UPGMA analysis in SPSS v. 15.0 (Fig. 2). The metabolic patterns from each sample were most closely related to the other samples from the same site. The cluster analysis (Fig. 2) also suggested that samples from 099 are the most distantly related to the other samples. CB and FB exhibited more similarity in their metabolic patterns, indicated by a shared branch on the dendrogram (Fig. 2). All CC samples and the 099 samples from 2006, which had no detected metabolism, were omitted from the analysis.

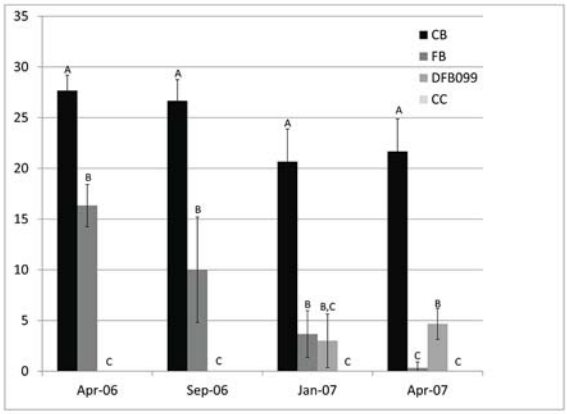

Fig. 1. Average Number of Biolog EcoPlate Substrates Metabolized by Microbial Populations. A substrate was considered to be metabolized if the average blanked absorbance at 590 nm was greater than 0.4. Error bars are standard deviation. Letters indicate significance groups at $p < 0.05$.

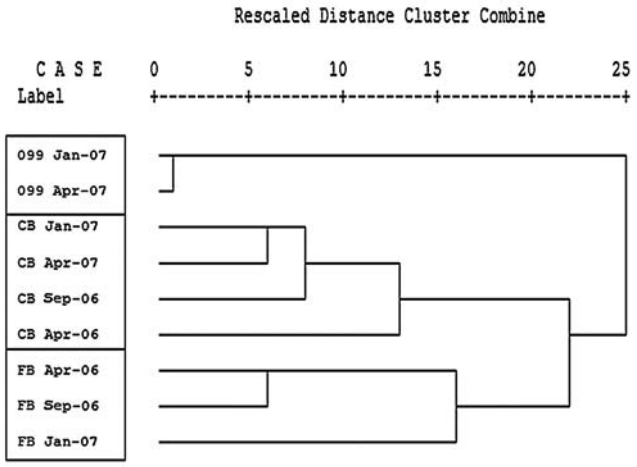

Fig. 2. Dendrogram of Biolog patterns. Constructed using UPGMA analysis in SPSS v. 15.0. Patterns of metabolic ability of the microbial communities at each site as measured by average blanked absorbance after three days of incubation at room temperature for each substrate in Biolog EcoPlates were compared. Samples for which no metabolic ability was detected (absorbance @ 590 nm < 0.4) were eliminated from the analysis. Boxes indicate Biolog patterns from the same sites clustering on the same dendrogram branch.

T-RFLP Analysis

The bacterial community structures at each site were examined and compared using T-RFLP performed on DNA extracted from the sediment at each site. The biodiversity and evenness of the bacterial communities were calculated from the T-RFLP electrophoretograms, utilizing each unique

Table 2. Microbial Diversity. Diversity and evenness were calculated from T-RFLPs of samples from April 2006 to April 2007. Beginning at 60 bp in length, peaks were defined as an operational taxonomic unit (OTU) and peak height as abundance. Standard deviation is in parentheses. Letters indicate significance groups at $p < 0.05$.

		Diversity		Evenness		
	OTUs	Simpson (D)	Shannon (H')	Simpson	Shannon (J%)	n
County Bridge	78 (13)[a]	0.96 (0.02) [a]	1.61 (0.12)[a]	0.97 (0.02)[a]	0.85 (0.03)[a]	4
Fall Brook	56 (10) [b]	0.93 (0.01) [b]	1.40 (0.06)[b]	0.95 (0.01)[b]	0.81 (0.03)[b]	6
DFB099	44(10)[c]	0.81 (0.13)[c]	1.09 (0.22)[c]	0.83 (0.14)[c]	0.67 (0.12)[c]	6
Coal Creek	24 (10) [d]	0.85(0.02)[c]	1.02 (0.06)[c]	0.89 (0.02)[c]	0.76 (0.07)[b, c]	4

fragment size as an operational taxonomic unit (OTU) and peak height (relative fluorescence) for each OTU as an estimate of abundance. The numbers of OTUs detected by T-RFLP from all sites were significantly different from each other, with the number of OTUs lower at sites with lower pH (Table 2). A mean of only 24 OTUs were detected at CC (mean pH 2.49), approximately one third the mean of 78 OTUs detected at the control site CB (mean pH 5.97). FB (mean pH 4.18) and 099 (mean pH 3.21) fell in between these values with mean OTUs of 56 and 44, respectively.

As indicated by the Simpson (D) and Shannon (H') indices, the biodiversity and evenness at all sites were relatively high (Table 2). For the Simpson metrics, no value was below 0.80. The lowest mean H' was 1.02 (CC) and the lowest mean J% was 0.67 (099). A trend similar to that of OTUs was seen in the diversity indices. The greatest bacterial biodiversity ($D = 0.96$, $H' = 1.61$) and the highest evenness (Simpson = 0.97, J% = 0.85) was found at the control site, CB ($p < 0.05$). The biodiversity ($D = 0.93$, $H' = 1.40$) and evenness (Simpson = 0.95, J% = 0.81) of the bacterial community at FB was also high, although significantly lower ($p < 0.05$) than at CB. CC and 099 were significantly less diverse than both CB and FB ($p < 0.05$) and exhibited the lowest evenness.

Although the values of the diversity metrics for CC and 099 were not significantly different ($p > 0.05$), visual inspection of the T-RFLP electrophoretograms revealed one striking difference: a dominant peak at 305 bp in the 099 samples which was absent or minor in all samples from the other sites. This peak accounted for up to 70% of the fragments detected by T-RFLP at the 099 site, with a mean representation of 38% (± 19%). No other single OTU in any site accounted for such a high fraction of the bacterial community.

Analysis of the T-RFLP data by UPGMA was performed, in this case to compare the taxonomic structures of the bacterial communities at the sites. It was noticed that a similar pattern is seen within the T-RFLP dendrogram (Fig. 3) as within the Biolog dendrogram (Fig. 2). In general, the T-RFLP patterns from each sample were most closely related to the other samples from the same site, with the exception of CB and two FB samples (one from January 2007 and one from April 2007). There is no striking seasonal pattern in the data, although in most cases the samples from the same date and site cluster most closely together.

As with the Biolog cluster analysis, the 099 samples are most distantly related to CB and FB. The T-RFLP cluster analysis, however, which included CC, revealed that the taxonomic bacterial community structures at 099 and CC are more similar to each other than either 099 or CC are to CB or FB. The CB and FB samples, on the other hand, frequently cluster on the same branches indicating a high similarity between the bacterial communities at the two sites. CB can be found on three distinct branches whereas FB is only on two, suggesting that there is more variation in the community structure at CB than at FB.

Identification of the dominant OTU at DFB099. Due to the dominance of the 305 bp OTU in the 099 samples, and therefore its potential ecological significance at the site, this OTU was selected for identification. The fluorescence from this fragment was easily visualized when the CfoI-digested FAM-labeled 16s rDNA PCR product was separated by agarose gel electrophoresis. The fragment was gel-extracted, amplified using ligation-mediated PCR, and ligated into pGEM-T® Easy as described above. Sequencing of four cloned fragments and subsequent BLAST analysis revealed that the fragment was from bacterial 16s rDNA. Although the closest matches for the sequence were from unidentified, uncultured clones of bacterial 16s rDNA, there were numerous sequences with similarity >90% from the family Beijerinickiaceae. The Beijerinickiaceae genera Beijerinckia and Methylocella were both represented in the matches returned by BLAST. No other distinct taxonomic classifications were reported. The narrowest taxonomic classification into which the sequenced fragment may be placed is therefore the Beijerinckiaceae. Attempts to enrich for and isolate this organism were unsuccessful.

Enumerations

The sizes of the microbial populations at all four sites were on the order of 10^8 cells per g sediment as determined by direct counts of DAPI-stained cells (Table 3). In contrast to the results of most of the other analyses performed, the smallest microbial population ($7.84 \pm 2.50 \times 10^7$ cells per g sediment) was detected at the control site CB and the largest ($2.31 \pm 0.50 \times 10^8$ cells per g sediment) was detected at 099. These values were significantly different ($p < 0.05$), with the mean cells per g sediment at CB approximately one-third that at 099. The population counts for FB and CC were intermediate between the two extremes but were not significantly different from each other ($p > 0.05$). As in other analyses, however, the values at CC were not significantly different from those at 099 ($p > 0.05$) and FB and CB were more similar in value.

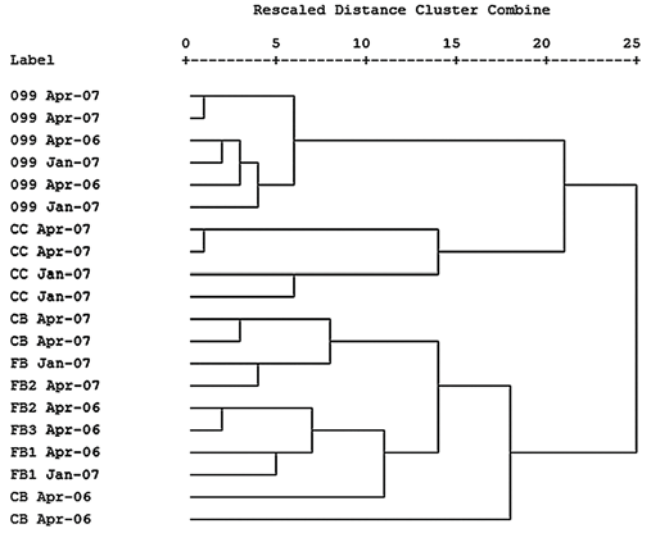

Fig. 3. Dendrogram of TRFLP patterns. Constructed using UPGMA analysis in SPSS v. 15.0. Each sample represents a unique DNA extraction from sediment from sites.

DISCUSSION

The results of this study indicate that the bacterial communities in the sediments of streams receiving AMD differ from those at a non-impacted site. Most of the analyses of the bacterial communities reveal a similar trend, with FB, which is farther downstream of mine outflows and has a higher pH than the other impacted sites, most resembling (but significantly different from) the control site CB. The sites nearest mine outflows, 099 and CC, on the other hand, are more similar to each other than to either FB or CB. This pattern holds for the Biolog samples (numbers of substrates metabolized in 2006 and cluster analysis), biodiversity indices, T-RFLP cluster analysis, and enumerations. These results are only in partial agreement with the expected outcome based on our hypothesis. We have seen from the impact of AMD on other organisms such as the benthic macroinvertebrates and meiobenthic fauna that species have either been eliminated in streams receiving AMD or the biodiversity in such streams has been greatly impacted with only a few dominant species (Moase et al. 1999; Nicomrat et al. 2006). Within the prokaryotic communities however, we noticed from the cluster analysis performed on the Biolog and T-RFLP data that both FB and CB were indicated as having similar community structure by the two sites clustering together and sharing a dendrogram branch (Figs. 2 and 3). These results are particularly surprising because we would have expected FB to cluster more closely with other impacted sites such as 099 or CC rather than the control site. This may indicate that microbes are more tolerant to AMD than we had first speculated based on the impact AMD had on macroscopic organisms.

Similar to what was observed in other studies (Lear et al. 2009; Nicomrat et al. 2006), one of the sites closer to the mine outflow, 099, was apparently dominated by one taxonomic group, as indicated by the peak at 305 bp in the T-RFLP analysis. Whereas Nicomrat et al. (2006) found *Acidithiobacillus species* and Lear et al. (2009) found *Gallionella* dominating, the dominant organism in the sediment at 099 has been tentatively identified as a member of the Beijerinckaceae family. Unlike *Acidithiobacillus* and *Gallionella*, Beijerinckaceae are not iron oxidizers. Although the identification of the dominant bacteria as Beijerinckaceae needs to be confirmed in situ, characteristics of the Beijerinckaceae make its dominance at 099 ecologically plausible. In the oligotrophic environment of the AMD-impacted stream, it may be an advantage that Beijerinckaceae are known to be capable of nitrogen fixation (Becking 2006). In addition, Beijerinckaceae are acid-tolerant (Becking 2006) and can use a wide range of organic compounds as carbon sources (Dedysh et al. 2005). Beijerinckaceae have been detected in acidic, hydrocarbon-rich environments (Hamamura et al. 2005). In the future, the isolation of this organism will allow us to better ascertain its ecological role at DFB099.

Table 3. Bacterial Enumerations. Direct counts were performed on DAPI-stained cells from sediment from each site. Standard deviation is in parentheses. Letters indicate significance groups at $p < 0.05$.

	10^7 Cells per g sediment
County Bridge	7.84 (2.50)c
Fall Brook	11.7 (4.29)b,c
DFB099	23.1 (5.01)a
Coal Creek	18.6 (3.65)a,b

In contrast to macroscopic populations, the results from the bacterial enumerations showed no decrease in population size in the AMD-impacted streams. In fact, the bacterial population as measured by direct DAPI counts was significantly higher in 099 compared to the control site CB. This result could be an artifact, due to confounding fluorescent debris present in the sediment samples. Many bacteria, however, are well suited to living at low pH and can proliferate under such conditions, reaching populations comparable to (or exceeding) the populations at neutral pH. Therefore, even though the population size at lower pH can be comparable to the control site, there are fewer OTUs and lower diversity because only select organisms (such as the *Beijerinckia* at 099 or *Acidithiobacillus* as found by Nicomrat et al. (2006)) can thrive under these conditions.

It is surprising, considering the population enumerations, that little or no metabolic activity was detected with the Biolog plates in the lowest pH sites (099 and CC). This may be due to the fact that the assay is performed at neutral pH. The organisms found in the most acidic streams may not have detectable metabolic activity at the pH at the assay. In addition, the Biolog Ecoplates only detect heterotrophy of 31 common substrates. The organisms in the most impacted streams could be either growing autotophically or utilizing substrates which are not provided in the Ecoplates. For example, members of the Beijerinckaceae have been shown to grow methylotrophically (Dedysh et al. 2005), which would not be detected by the Ecoplates. Still, the Biolog Ecoplates were valid for the purposes of this study: to measure relative metabolic abilities at the sites by comparing the metabolic profiles to each other and the control site. Determining the absolute metabolic profile at each site was not the goal of this study, although it would be desirable to pursue this line of investigation.

This study focused mainly on pH, which was identified by Lear et al. (2009) as the primary variable determining bacterial communities in AMD. AMD, however, alters the environment in many other ways. In the future, other environmental factors, such as temperature, metal concentrations, sulfate levels, and suspended solids, should be investigated to determine their influence on the bacterial community structure at AMD-impacted sites. For instance, studies by Janzen et al. (2008) in the Shamokin Creek Watershed suggested that AMD sites with high levels of iron are dominated by members of the phylum Bacteroidetes. Also, the T-RFLP technique relies on pooling all amplified 16S fragments of the same size into a single OTU, which may underestimate the true biodiversity of a site. Advances in high-throughput sequencing may make future investigations using metagenomic approaches possible.

The results of this study indicate that exposure to AMD does effect the structure of bacterial communities in receiving streams, although the effect is less extreme than for the macrobiota at these sites. As would be expected, the bacterial communities within 100 m of mine outflows show the greatest deviation from the community structure at the non-impacted site. The similarity in community structure (as determined via T-RFLP analysis) between the two sites closest to mine outflows, however, was less than between FB (farther downstream of a mine outflow) and CB (the non-impacted site). This suggests that the shifts in community structure at different sites due to high levels of AMD exposure can vary greatly, however at lower AMD exposures the bacterial communities retain many of the features of the original bacterial population. These results inform the potential design of studies to monitor AMD impact as well as the improvement of water quality as a result of remediation. Whereas no characteristic response of the bacterial community to high levels of AMD exposure was detected, improved water quality would be indicated by a bacterial community structure more similar to non-impacted sites within the same watershed.

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IMPACT OF CHEMICAL FERTILIZER AND PESTICIDES ON AQUATIC MICROCOSMS[1]

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ABSTRACT

Four commonly used greenhouse chemicals: 10:10:10 Peters fertilizer, two fungicides and an insecticide/ nematicide, were analyzed to determine their effects on water quality and productivity. Each of these chemicals in varying concentrations were added to three microcosms and assessed for their impact on pH, total dissolved solids (TDS), specific conductance, and dissolved oxygen, nitrate, ammonia, phosphorous, algae and chlorophyll a. In general, the addition these of chemicals did not affect pH, TDS, specific conductance or the dissolved oxygen. In all microcosms, the addition of fertilizer increased phosphorus, nitrate-N, and ammonia-N concentrations which in turn increased the size of algae communities and chlorophyll-a concentrations. There was a significant correlation between the size of the algae communities and chlorophyll a concentrations in all microcosms. The effect of fungicides and insecticides/nematicides varied among the different chemical concentrations, but in general, there was a reduction in nutrient concentrations with increasing concentrations of the chemicals. But when these chemicals were combined with fertilizer, nutrient concentrations, algae communities and chlorophyll-a exhibited similar increases as the microcosms receiving fertilizer alone. Based on the results of this study,a hydroponic system was designed to reduce the influx of nutrients into receiving fresh water systems. [J PA Acad Sci 87(1): 42-49, 2013]

INTRODUCTION

Since laboratory microcosms were first introduced by Odum and Hoskins (1957), they have been used to assess a variety of ecological processes including diurnal metabolism (Beyers 1962, 1963 a,b, 1965), plankton dynamics (Odum et al. 1963), nutrient cycling (Beyers and Odum 1963), as well as the impact of chemical contaminates on ecological systems (Williams and Mount 1965, Rose and McIntire 1970, Mitchell 1971, Ausmus et al. 1980, Gidding and Eddlleman 1987). Although it is difficult to define microcosms since they have a variety of shapes, sizes and compositions, Wimpenny (1988), defined microcosms as isolated systems of varying sizes, derived from natural ecosystems that possess genotypic, spatial and temporal heterogeneity.

Since laboratory microcosms are designed to simulate natural systems, they are used to provide rapid assays on the impact of potential pollutants on natural ecosystems. For example, Chen, et al. (2009) used laboratory microcosms to evaluate trimethylbenzene (TMB) as a tracer to determine the biodegradability of TMB in groundwater contaminated by gasoline under anaerobic conditions and the bioremediation of uranium in contaminated sediments (Madden et al. 2009). Both of these studies provide further insight into the use of microcosms to evaluate the possible impacts of potential pollutants on natural ecosystems.

Microcosms have also been used to evaluate the potential impacts of chemical fertilizers on aquatic systems. Fertilizers containing ammonia generally reduce soil pH while preventing severe micronutrient deficiencies and poor growth. Excess water containing fertilizers, insecticides and fungicides are often discharged into aquatic habitats, thereby adversely impacting natural systems. Although these chemicals are widely used by the greenhouse industry, there is little research on their impact on receiving aquatic systems. Van den Brink et al. (2009) concluded that the application of a mixture of the herbicide atrazine and the insecticide lindane to freshwater plankton-dominated microcosms resulted in a shift in the functional parameters including dissolved oxygen, pH, alkalinity, and specific conductance.

The current study investigated the potential impact of two commonly used fungicides Clearys (W.A. Cleary Chemical Corporation) and Subdue(CIBA-GEIGY), and an insecticide/ nematicide Vydate (Valent Corporation) and a 10 Nitrogen (N): 10 Phosphorus (P): 10 Potassium (K) Peters fertilizer on aquatic ecosystems using laboratory microcosms.

[1]Accepted for publication November 2012.

METHODS

Several 44 liter microcosms were created using water obtained from a local pond three weeks prior to the study and maintained at 16:hr L:8 hr D cycle throughout the study. Distilled water was added to each microcosm to maintain a constant water level. A Subdue (Methoxyacetyl Alanine Methyl Ester 25%), Clearys (W.A.Clearys Chemical Corporation: 4,4'-0-phenylenebis – 3 thioallophenate) and an insecticide/nematicide Vydate (Valent Corporation: active ingredient Acephate [O,S- Dimethyl acetylphos-phosphoramidothioate] 75%, inert ingredients 25%) were tested individually for their impact on water quality. Triple microcosms (12 experimental and 12 controls) were established and each set of three microcosms received either a 0.4, 2.0 and 4.0 percent concentration of each pesticide and Peters fertilizer. The percent concentration in the 44 liter microcosms receiving 100 ml of 0.4 percent solutions was 0.4 x 10^{-5} and 4.54 x 10^{-4} and 9.05 x 10^{-4}, respectively. After 24 hours, 7 and 14 days, triplicate samples of each microcosm were analyzed for pH, total dissolved solids (TDS), specific conductance, dissolved oxygen (DO), phosphate (PO_4), nitrate (NO_3-N), and ammonia (NH_3-N), along with the number of phytoplankton/ml and chlorophyll a/l (chlor.a/l). All samples were collected and analyzed between 1200 and 1400 hr. After the completion of this phase of the study, triplicate microcosms were established and 0.4, 2.0, and 4.0 percent concentrations of each pesticide were combined with the same concentrations of Peters Fertilizer and triplicate samples from each microcosms were analyzed for the parameters defined previously after 24 hours, 7 and 14 days.

The pH of all samples was determined using a model 290A Orion pH meter, conductivity and TDS was determined by a Fisher Scientific model 09-328-2 conductivity and TDS meter and dissolved oxygen (DO) concentrations were determined using a Yellow Springs Instrument Model 57 Dissolved Oxygen Meter. All meters were standardized after each series of analyses. Ammonia concentrations were determined using the Nesslerization methodology as defined in Standard Methods Procedure 4500-NH_3 C and nitrate concentrations were determined using the Ultraviolet Spectrophoto- metric Screening Procedure (Standard Methods procedure 4500-NO_3 –B) Phosphate concentrations were determined by the Ascorbic Acid methodology according to the procedure set forth in Standard Methods Procedure P E. The number of phytoplankton was determined according to the procedures described by Edmondson (1960), Vollenweider (1969) and Brenner et al. (1989). Triplicate subsamples (0.1 ml) were placed on a microscopic slide under a 22 mm x 22 mm cover glass. The number of algae was calculated as the area (484 mm^2) of the coverslip X counts/ transect X the area of the transect (22 mm). Chlorophyll a (chlor. a) was determined according to the procedures described by Richards and Thompson (1952), Parsons and Strickland (1968), Strickland and Parsons (1960), Vollenweider (1968) and Brenner et al. (1989). A one liter sample was filtered through a 0.8 µm membrane filter and re-filtered through a 0.45 µm membrane filter. Chlorophyll a was extracted with 90 percent acetone at 5°C for 24 hours. A 10 ml sample was then removed and the optical density determined using a spectrophotometer at wavelengths of 630 OD 645 OD and 665 OD. Chlorophyll a concentrations were determined according to the equation: Chlor. a = OD645 (15.6) – 0.14 x OD665 (0.8)-1.31 x OD665 (2.0) (Parsons and Strickland 1963). Data were analyzed using Analysis of Variance, a t-distribution, and Pearson Correlation analysis using Sigma Plot 12 Statistical Program. All data sets were normalized prior to the application of all statistical procedures and an alpha level of <0.05 or less was used as significant.

RESULTS AND DISCUSSION

The first phase of the study involved the impact of the addition of 100 ml of 0.4 %, 2.0% and 4.0% concentrations of Peters fertilizer and the insecticide/nematicide Vydate, and fungicides Clearys and Subdue on pH, specific conductance, TDS, and nutrient concentrations. The pH ($F = 0.19$, $P > 0.1$) (TDS ($F = 1.70$, $P > 0.1$), specific conductance ($F = 0.78$, $P > 0.10$) (Table 1) or DO ($F = 1.75$, $P > 0.10$) (Table 2) did not vary significantly among the microcosms after the addition of increasing fertilizer and pesticide (Vydate, Subdue and

Table 1. Changes in water chemical parameters in microcosms following the addition of varying concentrations of fertilizer and pesticide solutions.

Parameter	Fertilizer N 27		Vydate N 27		Clearys N 27		Subdue N 27		Control N 27	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
pH	8.1	0.37	8.2	0.10	8.41	0.29	8.2	0.16	8.5	0.2
Conductance Micro-ohms/cm	270	8.66	279	4.10	253	4.73	253	4.73	260	2.5
TDS mg/l	133	3.79	125	4.08	123	4.73	127	2.48	130	1.3
Dissolve O2 Mg/l	8.9	0.49	7.9	0.17	8.1	0.32	7.4	0.16	7.9	0.37

Table 2. Changes in dissolved oxygen concentrations (mg/l) following the addition of varying concentrations of fertilizer and pesticide solutions.

	Fertilizer and Pesticide Concentration							
	N 9		N 9		N 9		N 9	
Fertilizer	0.4 Percent		2.0 Percent		4.0 Percent		Control	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Vydate								
24 hours	7.0	0.11	6.8	0.23	7.5	0.13	8.0	0.10
7 days	7.0	0.12	7.2	0.14	7.0	0.12	7.5	0.08
14 days	6.3	0.07	6.0	0.10	6.8	0.09	7.7	0.07
Clearys								
24 hours	7.2	0.06	7.4	0.14	7.4	0.09	7.4	0.08
7 days	6.6	0.15	6.4	0.10	6.5	0.11	7.2	0.09
14 days	6.5	0.07	6.3	0.08	6.3	0.05	7.2	0 .12
Subdue								
24 hours	7.2	0.13	7.1	0.07	7.0	0.08	7.2	0.11
7 days	7.4	0.09	7.0	0.14	7.2	0.12	7.2	0.07
14 days	7.2	0.13	7.2	0.08	6.7	0.10	7.1	0.09

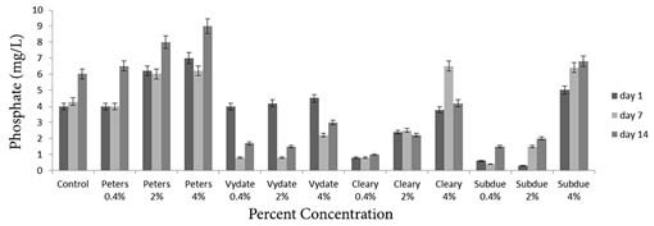

Figure 1. Changes in phosphate concentrations in microcosms following the addition of different concentrations of 10:10:10 Peters fertilizer, two fungicides (Subdue and Clearys) and an insecticide/ nematicide (Vydate).

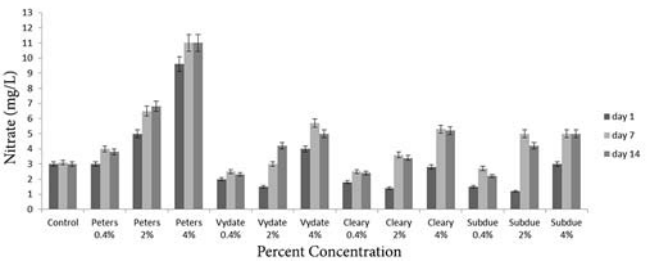

Figure 2. Changes in nitrate-N concentrations in microcosms following the addition of different concentrations of 10:10:10 Peters fertilizer, two fungicides (Subdue and Clearys) and an insecticide/ nematicide (Vydate).

Clearys) concentrations (Table 1) and as with natural aquatic systems, there was a significant correlation between TDS and specific conductance (r^2 = 91.3, P < 0.001).

However, nutrient concentrations varied significantly over the 14 days following the addition of these chemicals (F = 5.85, P <0.05). Within 24 hours following the addition of 0.4% fertilizer solution, nutrient concentrations did not differ significantly from the control systems (t= 1.10, P > 0.1) but there was a significant increase in all three nutrients in microcosms receiving 2.0% (PO_4 t = 3.12, P <0.05; NO_3-N t = 5.45, P < 0.01; NH_3-N t= 5.33, P< 0.01) and 4.0% (PO_4 t= 3.61, P <0.05; NO_3-N t = 5.45, P< 0.001; NH_3-N t= 8.21, P< 0.001) fertilizer solutions. Phosphate (PO_4) concentrations in all microcosms, including the controls, increased during the 14 day experimental period but nitrates (PO_4) increased for the first 7 days and remained relatively stable for the next 7 days (Fig. 1). But both phosphate and nitrate-N concentrations were significantly higher in microcosms receiving 2.0% (PO_4 t = 4.20, P < 0.01; NO_3-N) and 4.0% (PO_4 t = 4.85, P = < 0.01; NO_3-N t= 7.21, P < 0.001) fertilizer solutions after 7 and 14 days compared to the control samples (Fig. 2). Likewise, ammonia-N (NH_3) inceased significantly following the addition of a 2% (t=5.33, P<0.001) and 4% (t=8.21, P<0.001) (Fig. 3).

The response of microcosms to the addition of pesticides varied among the different treatments. Twenty four hours after treatment there were not significant differences in phosphate concentrations between microcosms receiving 0.4% and 2.0% Vydate solutions and the controls (t = 1.22, P<0.10), but after 7 and 14 days phosphate concentrations

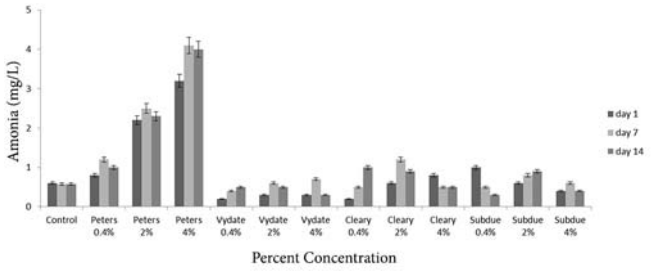

Figure 3. Changes in ammonia-N concentrations in microcosms following the addition of different concentrations of 10:10:10 Peters Fertilizer, two fungicides (Subdue and Clearys) and an insecticide/ nematicide (Vydate).

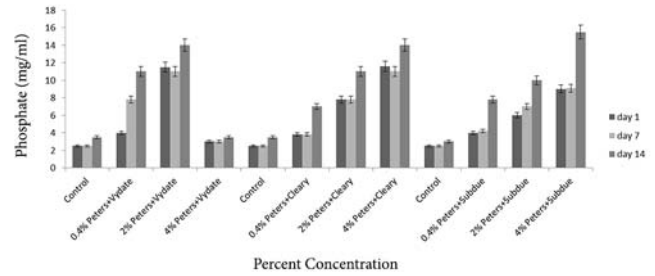

Figure 4. Changes in phosphate concentrations in microcosms following the addition of different concentrations of combinations of 10:10:10 Peters fertilizer, two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

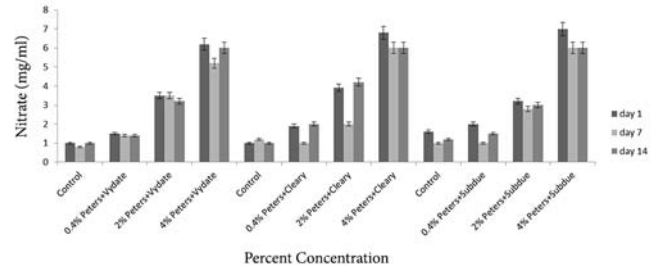

Figure 5. Changes in nitrate-N concentrations in microcosms following the addition of different concentrations of combinations of 10:10:10 Peters Fertilizer, two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

were significantly lower than the controls (t= 3.35, P < 0.05). Whereas within 24 hours, phosphates were elevated in microcosms receiving a 4.0% Vydate solution, but after 7 and 14 days, there was not a significant difference in phosphate concentratiosns between the experimental and control microcosms (t= 1.22, P > 0.10) (Fig. 1).

The response of microcosms to the additional of Clearys and Subdue varied among the different microcosms. In both systems receiving 0.4% and 2.0% Clearys and Subdue solutions, phosphate concentrations were significant lower than the controls after 24 hours (t =3.52, P <0.01), as well as 7 (t = 3.37, P <0.01) and 14 days (t= 6.21, P < 0.001) following treatment (Fig. 1). Whereas after 24 hours, phosphate concentrations in microcosms receiving a 4.0% Clearys were significantly lower than the controls (t = 2.56, P <0.05) but increased significantly after 7 days (t = 6.21, P < 0.001) and then decreased significantly during the next 7 days (t = 3.13, P < 0.01). Whereas in microcosms receiving a 4.0% Subdue solution, phosphate concentrations were significantly higher than the controls after 24 hours (t = 4.21, P < 0.01) and they remained evelated throughout the 14 day experimental period (7 days, t = 3.71, P < 0.01; 14 days, t= 2.64, P < 0.05) (Fig. 1). Although microcosms receiving a 4.0% Subdue solution, phosphate concentratons were significantly higher than both the controls (t = 5.34, P < 0.001 and Clearys microcosms (t = 4.86, P <0.001) after 24 hours and 14 days but were significantly lower than Clearys microcosms after 7 days (t= 3. 91, P< 0.01) (Fig 1).

Within 24 hours following the addition of a 0.4% Vydate solution, nitrate-N was significantly reduced (t = 3.86, P < 0.01 and it remained lower than the controls after 7 (t = 3.89, P < 0.0.01) and 14 days (t = 3.66, P <0.001). Whereas in microcosms receiving a 2.0% and 4.0% Vydate solutions, Nitrate-N intitially decreased (t = 4.11, P < 0.01), then increased (t = 4.11, P< 0.01) during the next 7 days but after 14 days there was not a significant difference in nitrate-N between the treatment and control microcosms (t = 1.83, P > 0.10) (Fig 2). Twenty four hours following the addition of all three concentrations of Clearys (t = 5.81, P < 0.001) and Subdue (t = 6.74, P < 0.001) solutions, nitrate-N was significantly lower than the controls. But in microcosms receiving 2% and 4%, Cleary (t =5.81, P < 0.001) and Subdue (t = 6.41, P < 0.001) solutions, nitrate-N was significantly higher than the controls after 7 and 14 days. Ammonia-N did not vary significantly in any of microcosms following the addition of varing pesticide concentrations (Fig. 3).

The increase in nutrient concentrations in response to the addition of combinations of Peters fertilizer and each of the three pesticides was similar to that when fertilizer was added alone. When 0.4% and 2.0% fertilizer-Vydate combinations was added to the microcosms, phosphate exceeded the controls with the highest concentrations occurring after 14 days but following the addition of a 4.0% solution, phosphate concentrations were similar controls throughout the 14 day treatment period. Whereas, except for the decline in phosphates after 7 days following the addition of a 0.4% solution, phosphates were significantly higher that the controls after 24 hours and continued to increase for 14 days following the addition of all three combinations of fertilizer-Clearys and Fertilizer-Subdue solutions (Fig. 4).

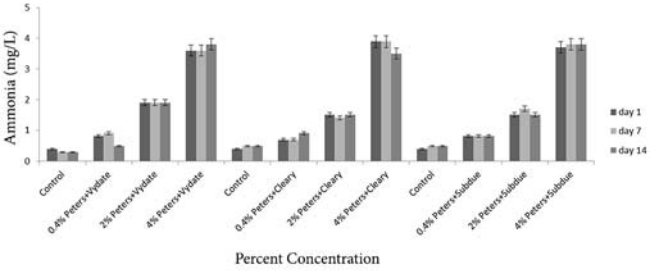

Figure 6. Changes in ammonia-N concentrations in microcosms following the addition of different concentrations of combined of 10:10:10 Peters fertilizer and two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

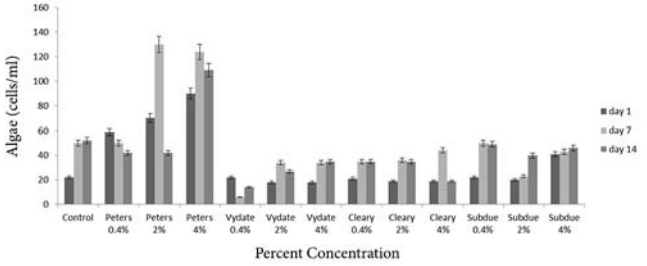

Figure 7. Changes in the size of the algae communities in microcosms following the addition of different concentrations of 10:10:10 Peters fertilizer, two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

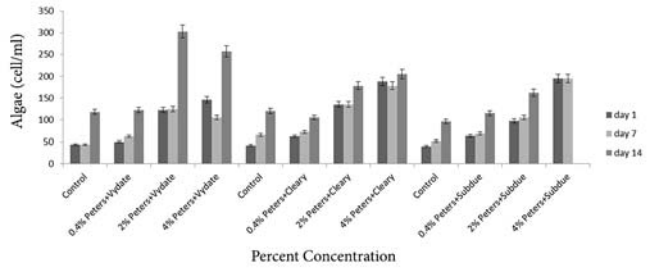

Figure 8. Changes in the size of the algae communities in microcosms following the addition of different concentrations of combinations of 10:10:10 Peters fertilizer, two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

Likewise, nitrate-N concentrations increased significantly in response to increasing concentrations of fertilizer and pesticide solutions with the highest nitrate-N concentrations occurring 14 days following treatment ($t = 5.98$, $P < 0.001$) (Fig. 5). With the exception of microcosms receiving 0.4% fertilizer-clearys and fertilizer-subdue solutions, after 24

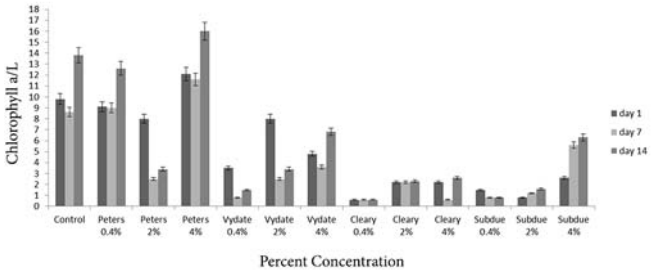

Figure 9. Changes in Chlorophyll a concentrations in microcosms following the addition of different concentrations of 10:10:10 Peters Fertilizer, two fungicides (Subdue and Clearys and insecticide/ nematicide (Vydate).

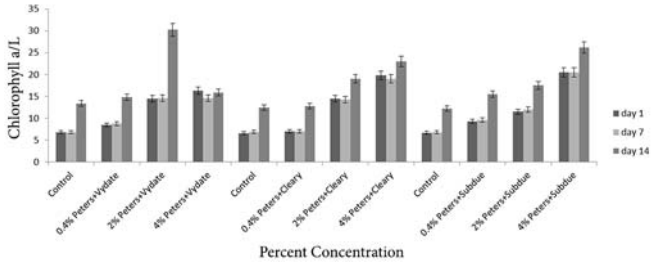

Figure 10. Changes in Chlorophyll a concentrations in microcosms following the addition of different concentrations of combinations of 10:10:10 Peters Fertilizer, two fungicides (Subdue and Clearys) and an insecticide/nematicide (Vydate).

hours, nitrate-N in all experimental microcosms exceeded the controls ($t = 6.58$, $P < 0.001$) (Fig. 5). As with the nitrate -N concentrations, ammonia-N also increased significantly in the microcosms receiving 2% ($t = 6.68$, $P < 0.001$) and 4% ($t = 7.59$, $P < 0.001$) solutions of fertilizer combined with the same concentrations of the other chemicals (Fig. 6).

The largest algae community (Fig. 7) and chlor. a concentrations (Fig. 9) occurred in microcosms receiving 2% fertilzer solutions. Whereas the smallest algae communities and chlor a concentrations occurred in microcosms receiving 4% fertilizer solutions (Fig. 7, Fig. 8). This decline in the size of the algae communities and the corresponding decline in chlor. a may be due to the possible toxicity of high nitrogen concentrations. In microcosms receiving a 0.4% fertilizer solution, there was an initial increase followed by a decline in both algae (Fig. 7) and chlor. a (Fig. 9) and after 14 days the the size of the algae communities and chlor a concentrations did not differ from the controls (Fig. 7, Fig. 9). In general microcosms receiving pesticide solutions, algae (Fig. 7) and chlor. a concentrations (Fig. 9) were either similar or reduced compared to control systems. This may be a result of a toxic effect of pesticide on algae/

ml as well as chlor. a concentrations compared to both the controls and microsoms receiving fertilizer solutions. But when pesticides were combined with Peters fertilizer, there were significant increases in both algae communities (Fig.8) and chlor a concentrations (Fig. 10) in response to increased nutrient concentrations. The increase in both the number of algae/ml (PO_4, $r^2 = 0.92$, $P < 0.001$; NO_3, $r^2 = 0.01$, NH_3, $r^2 = 0.82$, $P < 0.01$) and chlor a concentrations (PO_4 , $r^2 = 0.94$, $P < 0.001$; NO_3, $r^2 = 0.86$, $P < 0.01$,NH_3 $r^2 = 0.84$, $P < 0.01$) was positively correlated with increased nutrient concentrations and the size of the algae community was significantly correlated and chlor. a concentrations ($r^2 = 0.92$, $P < 0.001$).

Initially greenhouse waste water was going to be discharged into a farm pond that was experiencing algae blooms. But based on these microcosm studies, a hydroponic system was designed to recycle nutrient laden waste water thereby decreasing the frequency and magnitude of algae blooms in receiving natural systems. Likewise studies on natural systems also demonstrated the adverse impacts of fertilizers on freshwater ecosystems. According to Turner (1991), elevated nutrient concentrations in waste water discharges into aquatic systems results in the eutrophication that cannot be reduced without a reduction of fertilizer applications. The addition of a phosphorous-based fertilizers stimulates algae growth (Peterson et al. 1993), thereby adversely impacting aquatic systems by depleting dissolved oxygen, increasing water turbidity, and decreasing the diversity of endemic species. Furthermore, the addition of the fungicides and insecticides into an aquatic system may also result in the reduction aquatic life by impacting nutrients concentrations and depleting dissolved oxygen concentrations in the water column.

Although the use of microcosms was initially foccused on investigating the natural functions of aquatic systems (Beyers 1992, 1993 a,b, 1995, Beyers et al. 1993, Beyers and Odum 1993), they have also been employed to investigate the impact of a variety of agricultural and other chemical compounds on aquatic systems. For over 4 decades, investigaters have identified agriculture as the major contributor to the degradation of surface and ground waters in the Uniited States, as well as elewhere in the world (EPA 1983, 1987, Clark et al. 1985, Hill 1985, Macharis 1985 OECD 1985, Schaller and Bailey 1985, Raey et al. 1992, Tim et al. 1992, and Brenner and Mondok 1995). Brenner and Mondok (1995) indicated that 51 % of the farmers in the Shenango River Watershed did not take into account the nutrient availability of manure applications when applying commercial fertilizers to crop fields. Previous studies have used microcosms to address the source and presistence of fecal coliforms in freshwater streams (Brenner et al. 1999 a, b) as well the determination of the role of iron and managanese oxidizing bacterial in removal of metals from abandoned mine drainage (AMD) (Brenner et al. 1993, 1995, 2011). Laboratory microcosms provide a rapid and inexpensive method to study the potential impacts of a variety of agricultural and chemical compounds on freshwater ecosystems as wells as mechanisms operating within these systems.

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Wimpenny, J.T. 1988. Introduction. In: J.W. T. Wimpenny (ed). CRC Handbook or Laboratory model systems for microbial ecosystems. CRC Press, Boca Raton Florida. pp1-18Table 1. Changes in water chemical parameters in microcosms following the addition of varying concentrations of fertilizer and pesticide solutions.

Journal of the Pennsylvania Academy of Science 87(1): 50-52, 2013

CASE REPORT: OCCURRENCE OF THE GIANT KIDNEY WORM (*DIOCTOPHYMA RENALE*) IN LONG-TAILED WEASELS (*MUSTELA FRENATA*) FROM PENNSYLVANIA.[1]

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ABSTRACT

Three adult giant kidney worms (*Dioctophyma renale*) were found in the right kidneys of two male long-tailed weasels (*Mustela frenata*) from Pike County, Pennsylvania. At necropsy, both weasels showed no clinical signs of decreased fitness. The right kidneys of both animals were enlarged and contained nematodes. This is the first described infection of long-tailed weasels by giant kidney worms in Pennsylvania. A more thorough investigation of long-tailed weasels across their range is recommended to determine prevalence rates of this parasite and potential impacts on weasel populations. [J PA Acad Sci 87(1): 50-52, 2013]

INTRODUCTION

The long-tailed weasel (*Mustela frenata*) is the most widely distributed mustelid in the New World. Its range extends from southern Canada throughout the United States, south to Mexico, Central America, and northern South America (Sheffield and Thomas 1997). This species can be found throughout a diverse range of habitat types, including mature forests, woodlots, farmland, and wetlands (King and Powell 2007). Prey species include small mammals such as lagomorphs, rodents, and insectivores, as well as reptiles, amphibians, birds and fish (Merritt 1987; Sheffield and Thomas 1997; Fergus 2000; King and Powell 2007).

In 1782, Goeze found worms in a canine kidney and described them as *Dioctophyma renale*, the giant kidney worm (Mace and Anderson, 1975). *Dioctophyma renale*, is most frequently observed in mink but also infects river otters, martens, short-tailed weasels, long-tailed weasels (*Mustela frenata*), wolverines (*Gulo gulo*), coyotes, gray wolves, red foxes, and raccoons (Fyvie 1971; Anderson 1992). Loukmas et al. (2010) reported on the prevalence, distribution, and health implications of the giant kidney worms in mink from New York. Gyoten and Nishida (1978) identified four kidney worms in three male Siberian weasels (*Mustela siberica*) from Japan. A male harbor seal (*Phoca vitulina*) was found moribund on the coast of New Jersey in 2003 and died a few hours later. Upon necropsy, a single female kidney worm was recovered from the peritoneal cavity, and a tissue mass was found adjacent to the pelvic urethra and urinary bladder. Within this tissue mass, two kidney worm ova were identified. This was the first reported case of a kidney worm infecting a harbor seal or any North American marine mammal (Hoffman et al. 2004). Humans are accidental definitive hosts (Acha and Szyfres, 1989). This nematode usually infects the right kidney of mammal host species, possibly due to its proximity to the duodenum (Woodhead 1950). Infection occasionally occurs in the left kidney (Woodhead 1950), both kidneys, or within the abdominal cavity (Davidson 2006).

Giant kidney worms have a complex life-cycle, in which adults develop and reside within the kidney of a mammalian host. Kidney worm eggs are deposited within the infected kidney and passed to the urinary bladder, later being expelled by the host during urination. Larvae begin development only after eggs are ingested by an intermediate annelid host. Infected annelids are consumed by paratenic fish, crayfish, or frog hosts, and larvae continue development until infected paratenic hosts are consumed by definitive mammalian hosts. Kidney worm larvae then move through the intestinal wall and occupy the kidney, where they develop into adults. Infective larvae may also be ingested by mammals via direct consumption of annelid hosts (Woodhead 1955). Potential mortality can be associated with kidney worm infection (Graves 1937; Meyer and Witter 1950; Mace and Anderson 1975), which may have implications on the population dynamics and ecology of long-tailed weasels.

MATERIALS AND METHODS

Two adult male long-tailed weasels were collected in Porter Township, Pike County, Pennsylvania in February 2010. The two adult weasels were identified as M1 and M2. The first infected male (M1) was trapped after being observed feeding

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on beaver pelts inside an outdoor storage facility. The second weasel (M2) was a vehicle strike. Weights and measurements were recorded for the nematodes and the weasel kidneys.

RESULTS

An external examination prior to pelt removal did not suggest decreased biological fitness in either individual. Weasel M1 weighed 202.8g; no weight was obtained for weasel M2. At necropsy, the right kidneys of both individuals were found to be abnormally enlarged (Figure 1) and weighed 7.0 g (M1) and 12.5 g (M2). One giant kidney worm was found in the kidney of M1 (Figure 2), and two individuals were found in M2. The left kidneys appeared normal and weighed 1.6 g (M1) and 1.3 g (M2). The right kidney from weasel M1 contained one female nematode which was 44.8 cm in length and weighed 3.8 g. The right kidney of weasel M2 contained both a male and female worm. The female nematode was 60 cm in length and weighed 5.5 g. The male nematode was 23.5 cm in length and weighed 0.6 g.

DISCUSSION

This is the first described occurrence of the giant kidney worm in long-tailed weasels in Pennsylvania. Reported weights of adult male long-tailed weasels range from 160 to 450 grams (Sheffield and Thomas 1997; King and Powell 2007), with weights up to 312 grams in Pennsylvania (Merritt 1987; Fergus 2000). The weight of weasel M1 fell within this range. In mink, female worms are usually 20–40 cm (8–16 inches) long (Fyvie 1971). One female nematode recovered from weasel M2 in this study exceeded this range with a length of 60 cm.

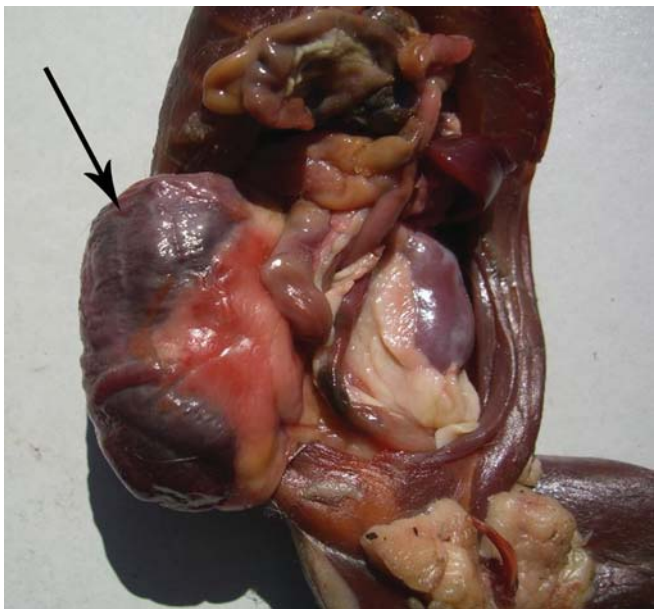

Figure 1. Infected (arrow) right kidney of weasel (M2). The kidney contained both a male and female kidney worm.

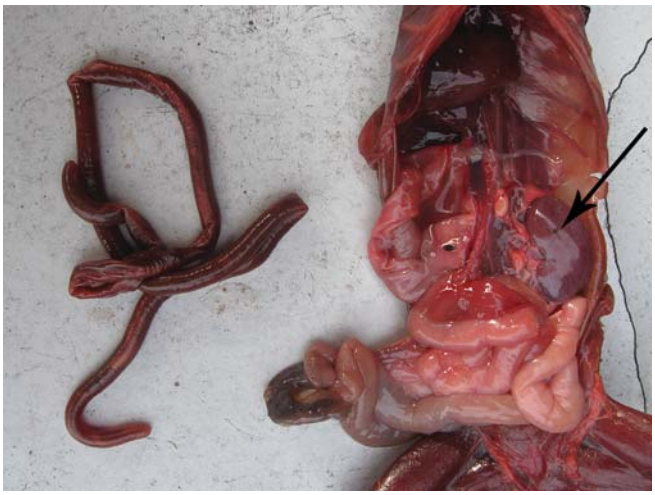

Figure 2. Giant kidney worm after removal from infected right kidney of M1 and normal left kidney (arrow).

Giant kidney worm infection of one kidney has little or no adverse effect on definitive mammalian hosts. If the left kidney remains intact, clinical signs of infection are often absent, however kidney function is limited. The infected kidney often remains only as a non-functional casing that houses the adult kidney worm. As a result, enlargement of the functional kidney often occurs to compensate for increased functional responsibility (Davidson 2006). If both kidneys contain kidney worms, infection could potentially be fatal due to renal failure.

The few cases in which humans have contracted this parasite likely result from the consumption of undercooked paratenic hosts (such as fish) containing kidney worm larvae (Davidson 2006). A fatal case of bilateral dioctophymatosis was reported in a 51-yr-old woman in China (Li et al. 2010).

Giant kidney worms are widely distributed throughout North America, but are abundant only in certain enzootic regions (Measures 2001). Although the sample size in this study was only two animals both were infected with the parasite. Localized clusters of infected mink were observed in the study by Loukmas et al. (2010) but the reasons for this was unknown. The elucidation of ecological factors that limit giant kidney worms and regulate parasite-host relationships might provide an explanation for the observed pattern of distribution in long tailed weasels. Further knowledge about the prevalence rates and potential impact of the parasite on long-tailed weasels would be beneficial for the management of this species.

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