

ABSTRACT

Title of Dissertation: ANALYSIS OF MACROINVERTEBRATE
COMMUNITIES IN SEASONAL
WETLANDS THROUGH TIME, ACROSS
SPACE, AND USING SPECIES TRAITS

Elanor Dale Stevens Spadafora
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Dr. William O. Lamp
Entomology

Restoration of natural wetlands may be informed by macroinvertebrate community composition. Macroinvertebrate communities of wetlands are influenced by environmental characteristics such as vegetation, soil, hydrology, land use, and isolation. This dissertation explores multiple approaches to the assessment of wetland macroinvertebrate community composition, and demonstrates how these approaches can provide complementary insights into the community ecology of aquatic macroinvertebrates. Specifically, this work focuses on macroinvertebrates of Delmarva Bays, isolated seasonal wetlands found on Maryland's eastern shore. A comparison of macroinvertebrate community change over a nine years in a restored wetland complex indicated that the macroinvertebrate community of a rehabilitated wetlands more rapidly approximated the community of a reference site than did a

newly created wetland. The recovery of a natural macroinvertebrate community in the rehabilitated wetland indicated that wetland rehabilitation should be prioritized over wetland creation and long-term monitoring may be needed to evaluate restoration success. This study also indicated that characteristics of wetland vegetation reflected community composition. The connection between wetland vegetation and macroinvertebrate community composition led to a regional assessment of predaceous diving beetle (Coleoptera: Dytiscidae) community composition in 20 seasonal wetlands, half with and half without sphagnum moss (*Sphagnum* spp.). Species-level identifications indicated that wetlands with sphagnum support unique and diverse assemblages of beetles. These patterns suggest that sphagnum wetlands provide habitat that supports biodiversity on the Delmarva Peninsula. To compare traits of co-occurring beetles, mandible morphology and temporal and spatial variation were measured between three species of predaceous diving beetles. Based on mandible architecture, all species may consume similarly sized prey, but prey characteristics likely differ in terms of piercing force required for successful capture and consumption. Therefore, different assemblages of aquatic beetles may have different effects on macroinvertebrate community structure. Integrating community-level and species-level data strengthens the association between individual organisms and their ecological role. Effective restoration of imperiled wetlands benefits from this integration, as it informs the management practices that both preserve biodiversity and promote ecosystem services.

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TRAITS

by

Elanor Dale Stevens Spadafora

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Advisory Committee:
Dr. William O. Lamp, Chair
Dr. Daniel Gruner
Dr. Maile Neel
Dr. Paul Leisnham
Dr. Andrew Baldwin

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Dedication

I dedicate this work to my parents, who always encouraged my exploration of the natural world. I dedicate this work to my brother, who spent countless hours getting muddy with me in the backyard and at Irby Creek. I dedicate this work to my son, who I hope will spend as much time as I did pulling apart flowers, making potions out of berries and stream water, and watching spiders building their webs. And I dedicate this work to my husband, who went bug hunting with me on one of our first dates and has never looked back. I owe success in my graduate endeavors to their loving support.

Foreword

With the recommendation of the dissertation director (William O. Lamp), and with the endorsement of the Director of the BISI-BEES Program (Chuck Dewhiche), Chapter 2 of this dissertation is a previously published work. The citation for this publication is as follows:

Spadafora, E., Leslie, A. W., Culler, L. E., Smith, R. F., Staver, K. W., & Lamp, W. O. (2016). Macroinvertebrate community convergence between natural, rehabilitated, and created wetlands. *Restoration Ecology*.

As directed in the graduate catalog for chapters previously published as coauthored works, I state that I was responsible for the inception of the manuscript and the majority of the manuscript preparation. This work was reformatted to match the rest of the dissertation, but all other aspects of the published manuscript, including the use of “we” when referring to the authors, are used in this document. A letter will be sent to the Dean of the Graduate School certifying the Dissertation Committee’s determination that I made a significant contribution to this work, and that the inclusion of this previously published work in this dissertation has the approval of the dissertation committee, the dissertation advisor, and the Graduate Director.

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Chapter 1: Considerations for community ecology research of wetland macroinvertebrates: an introduction to the conceptual frame work and study system

Abstract

Wetlands provide important ecosystem services that land managers actively work to preserve and promote, especially in restored wetlands. These services are supported by the plants and animals found in wetland habitats, including aquatic macroinvertebrates. As a result, effective wetland restoration incorporates knowledge of macroinvertebrate community response to changes in environmental characteristics. Wetland management decisions, therefore, must also reflect the spatial scale, temporal scale, and measures of community composition through which restoration success is measured. The following chapters measure changes in macroinvertebrate communities of seasonal wetlands over varying temporal and spatial scales, and using different metrics to evaluate community composition. All research was conducted in Delmarva Bays, isolated, seasonal wetlands on the eastern shore of Maryland. Vegetation characteristics vary between wetlands, with the presence or absence of sphagnum moss (Sphagnales: Sphagnaceae: *Sphagnum*) causing important changes to habitat characteristics. Changes in habitat characteristics are likely to influence wetland macroinvertebrates, such as predaceous diving beetles (Coleoptera: Dytiscidae). These diverse and abundant beetles show changes in behavior, dispersal rates, and species richness in response to changes in wetland vegetation density and composition. In order to explore the relationship between wetland restoration and macroinvertebrate community composition, the following chapters evaluate changes in macroinvertebrate community composition both seasonally and using long-term data, examine changes in macroinvertebrate community composition relative to wetland vegetation at a regional scale, and consider changes in macroinvertebrate community composition using high-

resolution data and species traits. Together, these chapters address some of the complexity involved in using assessment of macroinvertebrate community composition to inform wetland restoration practices and suggest considerations that may improve wetland management.

Introduction

Worldwide, wetlands provide economically valuable ecosystem services, such as nutrient retention and flood mitigation (Ghermandi *et al.* 2008). As imperiled habitats, wetlands have been increasingly studied over the past 30 to 40 years (Zhang *et al.* 2010), often with an emphasis on understanding how to successfully create or restore them. Ensuring the establishment of natural plant and animal communities is integral to management that protects and promotes wetland services (Hansson *et al.* 2005), especially in restored wetlands (Mitsch & Wilson 1996; Whigham 1999; Erwin 2009). Aquatic macroinvertebrates are abundant inhabitants of wetlands, and their patterns of community composition can reflect characteristics of the soil (Armitage & Fong 2004), vegetation (Verberk *et al.* 2010), and hydrology (Culler, Smith & Lamp 2014b), which in turn are influenced by wetland restoration status. However, the relationship of macroinvertebrate community composition to these environmental factors is not always well understood (Batzer 2013). Ambiguous connections between macroinvertebrate communities and their habitats can be the result of the spatial scale, temporal scale, and community metrics under which these connections are studied. As a foundation for the objectives that follow, I will first examine how space, time, and species traits affect assessment of wetland macroinvertebrate communities and the restoration practices that influence community composition.

Conceptual Framework

Temporal Scale

Wetland macroinvertebrate communities change seasonally. Seasonal changes can be the result of differing life history strategies, which cause high turnover in community composition (Greig & Wissinger 2010), such that community composition may change dramatically even over short periods of time. Even macroinvertebrate species that occur in high abundances within

a community may only be detectable for short periods throughout the year (see Chapter 3). Seasonal changes in macroinvertebrate community composition can produce a strong enough signal that they obscure the effects of environmental characteristics (Tangen, Butler & Ell 2003). Similarly, the effects of environmental factors, such as hydrology, may be amplified during some parts of the year more than others as different macroinvertebrates become active (Molnár, Csabai & Tóthmérész 2009). Studies that include multiple time points can help to resolve the ambiguity created by seasonal change.

Factors that affect community composition change over varying temporal scales (Levin 1992). Some occur rapidly, such as the ecological disturbance caused by fluctuations in the cycles of wetting and drying that are common in wetlands. Multi-year studies can avoid potentially anomalous observations caused by short-term disturbance (Meyer & Whiles 2008). Regular disturbance, such as seasonal flooding, can drive macroinvertebrate community patterns both within years and across years (Golladay, Taylor & Palik 1997). Seasonal changes in macroinvertebrate community composition are characteristic in seasonal wetlands, which are dry for part of the year (Lundkvist, Landin & Karlsson 2002; Fontanarrosa, Collantes & Bachmann 2009). Species interactions such as competition and predation can be strongly mediated by these changes (Schneider & Frost 1996). The influence of these temporal factors can sometimes provide better explanations for patterns of macroinvertebrate community composition than intrinsic environmental characteristics, such as vegetation (Culler *et al.* 2014b).

Short-term changes in community composition can represent responses to more gradual changes of environmental characteristics. For example, macroinvertebrate community composition may reflect how long a habitat has existed, as has been observed in constructed wetlands (Stewart & Downing 2008). Studies of wetland macroinvertebrates that span multiple

decades require substantial resources, but they also provide important insights into the interaction between short-term community change and gradual environmental change. Short-term trends, such as increasing or decreasing species richness, may be misleading, which can be revealed by multiyear studies (Silvertown *et al.* 2006). Long-term studies can be particularly important when evaluating restoration success, as recovery or creation of desired ecological processes is not always immediate (Mitsch & Wilson 1996; Whigham 1999).

Temporal context will always affect assessment of macroinvertebrate community composition. Some research objectives, such as evaluating the success of conservation efforts, cannot be addressed at short time scales (Ruhí *et al.* 2013). Whether or not measuring temporal change is an explicit objective, research must be designed to assess wetland communities within a temporal context that accounts for short-term seasonal variation and long-term changes of habitat characteristics.

Spatial Scale

The space occupied by an organism during its lifetime is largely a product of dispersal ability, a characteristic that varies among wetland macroinvertebrate (Bie *et al.* 2012). This variation is one of the key factors that mediates the influence of local factors, like vegetation characteristics, relative to regional factors, like land use (Meutter, Meester & Stoks 2007). Some species can actively move between habitats, such as dragonflies or beetles. Others, such as isopods or gastropods, disperse passively, relying on transport from wind, water, or other organisms. Dispersal mode can act as a biotic filter that may explain landscape scale patterns of species occurrence among wetlands (Meyer & Whiles 2008). Clustering of macroinvertebrate species among wetlands, or spatial aggregation, can often be explained by dispersal ability (Briers & Biggs 2005; Meutter *et al.* 2007). The effects of dispersal ability can be even more

important in habitats isolated wetlands that are not readily accessible to passive dispersers (Tiner 2003). Wetland isolation can produce patterns of macroinvertebrate community composition that are evident at broad spatial scales (Briers & Biggs 2005). Inoculation of restored or created wetlands can help to establish a more natural macroinvertebrate community (Brady *et al.* 2002).

Processes that influence local patterns of macroinvertebrate community composition often occur at regional or even global scales (Turner 1989; Suren *et al.* 2008). The local community may not be responding to local environmental factors, but rather to large scale environmental gradients. Soil characteristics (Armitage & Fong 2004), vegetation (Batzer & Resh 1992; De Szalay & Resh 2000), land use (Foltz & Dodson 2009), and hydrology (Tarr, Baber & Babbitt 2005; Molnár *et al.* 2009) can all vary across the landscape, thereby altering wetland characteristics and influencing local macroinvertebrate community composition. Wetland macroinvertebrates may also respond to the effects of and proximity to anthropogenic activity (Gustafson & Wang 2002). Furthermore, different factors may explain macroinvertebrate community composition at different spatial scales. For example, wetland hydrology may best explain local patterns while landscape vegetation and geology are more important regionally (Schäfer *et al.* 2006). Thus, proximity of restored wetlands to different habitats may affect successful colonization of these wetlands by macroinvertebrates (Alsfeld, Bowman & Deller-Jacobs 2010).

Determining the spatial scale at which to measure wetland macroinvertebrates requires integrating factors acting at local and regional scales. Wetlands are found in heterogeneous landscapes. The spatial scale at which wetland macroinvertebrate communities are measured will directly influence the connections made between environmental characteristics and community

composition, and this in turn will affect management decisions regarding locations of restored wetlands.

Community Metrics and Species Traits

Obtaining measures of wetland macroinvertebrate diversity is challenging, as it requires identification and enumeration of many organisms. The difficulty of identifying macroinvertebrates is an inherent barrier to accurately describing wetland diversity. For this reason, substantial thought has been dedicated to the merits of low resolution taxonomic data compared to high resolution taxonomic data. There are two main opposing arguments. The first is that data are lost by grouping together taxa, therefore high resolution data are the most informative for measuring wetland macroinvertebrates (King & Richardson 2002). The second is that low resolution data group together similar organisms, therefore patterns observed with low resolution data are the same as those indicated by high resolution data (Mueller, Pander & Geist 2013; Martin, Adamowicz & Cottenie 2016). If different measures of diversity are correlated across taxonomic levels, fine scale identification may not add enough unique information to warrant the time investment needed for identifying to lower taxonomic levels (Bowman & Bailey 1997). However, the taxa that make up a community should affect choices regarding taxonomic resolution. For example, community measures may be underestimated in communities with highly abundant and diverse taxa that are treated as single group, as is often true for aquatic fly larvae (King & Richardson 2002). Choice of taxonomic resolution can also bias conclusions, as higher taxonomic resolution will typically increase the differences in composition observed between communities (Bailey, Norris & Reynoldson 2001).

When high resolution data are needed, collection and evaluation of these data can be made more efficient. Systematically subsampling the organisms collected can be effective (King & Richardson 2002). Likewise, choosing a single order or family to identify to species level can

also simplify data collection when the taxon chosen is representative of the community as a whole. In wetlands, aquatic beetles are typically good representatives of overall community composition (Bilton *et al.* 2006). When wetland bioassessment of restored sites is the goal, a combined taxonomic approach may be the best technique, using family level (or higher) identifications for most taxa, and species level identifications for select indicator taxa (Bailey *et al.* 2001). Ultimately, management objectives coupled with knowledge of the macroinvertebrates within the community should drive the choice of taxonomic resolution.

Increasingly, measurements of species traits to evaluate functional diversity are used in concert with taxonomic diversity. Functional diversity refers to variation in species traits that relate how species function within ecosystems, and can affect stability, productivity, nutrient balance, and ecosystems dynamics (Tilman 2001; Petchey & Gaston 2006). Taxonomic diversity has been used a surrogate for functional diversity (Tilman 2001), but the former usually overestimates the latter in macroinvertebrate communities (Díaz & Cabido 2001). Patterns of functional diversity may correspond to patterns of taxonomic diversity (Mueller *et al.* 2013), but relatedness alone may not be adequate to infer trait similarity (Moravcová *et al.* 2015). For the purposes of wetland restoration, measuring species traits to assess functional diversity may be more informative than measuring taxonomic diversity (Richards *et al.* 1997; Petchey & Gaston 2006; Estrada *et al.* 2016). In some cases, functional traits of macroinvertebrate community members are more clearly linked to wetland characteristics than are patterns of species occurrence (Townsend, Doledec & Scarsbrook 1997a) and conflicting explanations of species occurrence among wetlands can be resolved by adding trait information (Thompson & Townsend 2006). Measures of functional diversity can also improve our ability to predict how macroinvertebrate communities will respond to environmental change, as functional diversity

can suggest more about the ecology of a wetland community and therefore, wetland processes that may help to inform management decisions (Richards *et al.* 1997; Townsend, Scarsbrook & Dolédec 1997b; Verberk, Van Noordwijk & Hildrew 2013).

Study System

In the following chapters, I consider the ecological factors influencing the macroinvertebrate community composition of actively managed seasonal wetlands within different spatial and temporal contexts and using different measures of community composition. Here, I introduce the study system to provide context for this work. I include a discussion of the wetlands I surveyed, the wetland vegetation expected to alter habitat characteristics, and the macroinvertebrates used to evaluate community composition.

Delmarva Bays

The Delmarva Peninsula comprises a 170 mile stretch along the mid-Atlantic seaboard that includes Maryland's eastern shore, part of Virginia, and the state of Delaware. Delmarva Bays are seasonal wetlands found on the peninsula, and they are analogous to Carolina Bays, their better-known southern counterparts (Sharitz 2003). Aerial photography indicates that, historically, there may have been 1500 to 2500 of these Bays on the peninsula (Fenstermacher *et al.* 2014). The bays formed approximately 20,000 years ago as the result of wind blowing across the peninsula (Stolt & Rabenhorst 1987b).

Delmarva Bays are characterized by a sandy rim and elliptical shape and can be distinguished as either flat-bottom ponds or deep-basin ponds depending on amount of "basin fill" (silty sediments) they contain (Stolt & Rabenhorst 1987a). These seasonal wetlands dry annually, usually between July and September (Pickens & Jagoe 1996; Sharitz 2003). and Although a few bays connect to adjacent water ways when they are flooded, most are isolated (Tiner 2003). Delmarva Bays are typically acidic and oligotrophic (Pickens & Jagoe 1996), which is attributed

to the input of surficial groundwater and accumulated vegetative material (Newman & Schalles 1990).

These wetlands are typically set in forested landscapes and surrounded by *Acer rubrum* (red maple), *Liquidambar styraciflua* (sweetgum), and *Nyssa sylvatica* (black gum). Characteristic vegetation within the wetlands includes *Sphagnum* moss (Karlin, Andrus & Reed 1991; Halsey, Vitt & Gignac 2000) and a suite of shrubs such as *Cephalanthus occidentalis* (common buttonbush), *Clethra alnifolia* (sweet pepperbush), *Eubotrys racemosa* (fetterbush), sedges like *Carex striata* (Walter's sedge), and grasses such as *Scirpus cyperinus* (kunth woolgrass). Common aquatic macrophytes include *Utricularia* spp. (bladderwort) and *Proserpinaca pectinata* (mermaid weed). In addition to these common plants, Delmarva Bays provide habitat for many locally and globally rare plant species including seven rare species of *Carex* and six rare species of *Rhynchospora* (Tyndall 2000; McAvoy & Bowman 2002).

Long-term establishment of fish colonization in Delmarva Bays is inhibited by their hydrology and isolation (Sharitz 2003). As a result, macroinvertebrates are abundant predators and primary consumers (Culler *et al.* 2014b). Several rare insects species are known from these bays (Batzer & Wissinger 1996; Steiner *et al.* 2003), which they are generally considered to be insect diversity hotspots (Sharitz 2003). Many of the macroinvertebrates common in these wetlands aestivate in the soil and recolonize the Bays after they flood (Dietz-Brantley *et al.* 2002). Freshwater isopods (Isopoda: Asellidae) and non-biting midge larvae (Diptera: Chironomidae) and other dipterans are often the most abundant primary consumers and beetles (Coleoptera) and dragonflies and damselflies (Odonata) are the most abundant predators (Batzer & Wissinger 1996; Dietz-Brantley *et al.* 2002; Batzer *et al.* 2005).

Delmarva Bays are important habitats that support biodiversity in an otherwise human dominated landscape. Today, the livelihood of many of the residents of the Delmarva comes from chicken farming and a significant portion of the peninsula has been developed for this industry (Scott 1991). As a result, many Delmarva Bays have been impacted by anthropogenic development (Allen 2009). Agricultural activity on the Delmarva Peninsula has destroyed or degraded approximately 70% of Delmarva Bay habitat (Fenstermacher *et al.* 2014). Successful management and conservation of remaining Delmarva Bays will be improved by learning more about their ecology.



Figure 1.1 Delmarva Bay wetlands showing characteristic vegetation and illustrating forested surroundings. As pictured, these seasonal wetlands are commonly contain grasses, sedges, shrubs, and aquatic macrophytes. Pictures taken between 2012 and 2014.

Sphagnum Moss

Of the plants commonly associated with Delmarva Bays, sphagnum moss (Sphagnales: Sphagnaceae: *Sphagnum*) is likely one of the most ecologically significant in terms of changing habitat characteristics (Karlín *et al.* 1991; Halsey *et al.* 2000). The genus *Sphagnum* includes approximately 115 well described species, and up to several hundred more that are poorly known. Four species are common throughout the mid-Atlantic states: *S. cuspidatum*, *S. macrophyllum*, *S. recurvum* and *S. torreyanum* (Rydin & Jeglum 2013).

Sphagnum is widely recognized as an ecosystem engineer in that it alters wetland water chemistry, hydrology, and habitat structure (Tansley 1949; Moore 1995; Frankl & Schmeidl 2000). It is adapted to nutrient poor, low productivity habitats and will promote these conditions once established (Gunnarsson 2000; Granath, Strengbom & Rydin 2012). The living tissue of sphagnum contains cation exchange sites, which house uronic acids. These acids exchange hydrogen ions for cations, thereby lowering pH and conductivity of the water (Rydin & Jeglum 2013). By making the water acidic, sphagnum prevents microbial activity and slows decomposition. The result is the formation of peat, the layer of senescent plant tissue that is characteristic of sphagnum bogs. Both the living and dead tissue of sphagnum forms a thick mat that fills the water column, which substantially changes habitat structure (Andrus 1986). This increases habitat complexity and may provide important microhabitat, but the importance of sphagnum moss changing habitat structure is understudied.

The three main effects of sphagnum on habitat characteristics can then be summarized as acidification of the water, limiting microbial activity, and increasing habitat complexity. These are all likely to affect wetland communities, especially macroinvertebrates (Jackson & McLachlan 1991; Spitzer & Danks 2006; Suren *et al.* 2008) Changes to water chemistry caused by sphagnum have varying effects on macroinvertebrate taxa. Acidity is not likely to prevent

colonization of most macroinvertebrates (Batzer & Wissinger 1996). However, pH gradients do correspond to changes in community composition for some taxa (Nilsson, Elmberg & Sjöberg 1994) and acidic oligotrophic wetlands are usually associated with less diverse communities, both in wetlands and in general (Bedford, Walbridge & Aldous 1999; Worm & Duffy 2003). The habitat structure that sphagnum creates could also increase macroinvertebrate diversity (De Szalay & Resh 2000; Molnár *et al.* 2009) and provide refugia for prey and/or hunting substrate for predators (Henrikson 1993).

The role of sphagnum moss as ecosystem engineer merits further study, especially with reference to aquatic macroinvertebrates. Sphagnum moss is a common plant in isolated wetlands, such as Delmarva Bays. These, like all isolated wetlands, are high priority conservation sites, and their preservation and management can be improved by knowledge of the ecological factors that influence community structure.

Dytiscid Beetles

Predaceous diving beetles (Coleoptera: Dytiscidae) are ubiquitous macroinvertebrates in freshwater systems (Alarie & Leclair Jr 1988; Fairchild *et al.* 2003) and they are important predators (Yee 2014). They are a diverse group, with approximately 4,000 species found worldwide. Of these, roughly 500 species of the family Dytiscidae are found in North America (Larson *et al.* 2000).

Dytiscid beetle communities are influenced by a range of environmental characteristics. Species composition is affected by water chemistry, with pH and amount of dissolved oxygen in the water being particularly important (Eyre, Ball & Foster 1986; Foster *et al.* 1989). Species richness increases relative to abundance of potential prey items (Nilsson 1986). Community composition also changes in response to changes in vegetation (Gioria, Barcao & Schaffers

2010). Higher densities of aquatic macrophytes result in lower rates of dispersal of adult beetles (Yee, Taylor & Vamosi 2009) and lower rates of intraguild predation among larvae (Yee 2010)

As predators, dytiscid beetles are usually described as generalists. In laboratory settings, they will eat a wide range of prey items presented to them (Culler & Lamp 2009). They also have well documented consumptive and non-consumptive effects on wetland communities. Predation by dytiscid beetles can change density and demography of prey populations (Arts, Maly & Pasitschniak 1981). They also trigger trophic cascades that change community structure in terms of biomass and species composition (Cobbaert, Bayley & Greter 2010). As far as non-consumptive effects, they provide predator cues that deter oviposition by prey species (Ohba *et al.* 2012) and their presence also alters prey behavior and habitat use (Herwig & Schindler 1996). Additionally, intraguild predation is well documented in dytiscid beetles (Yee 2010; Culler, Ohba & Crumrine 2014a) and it is likely that this acts as a density dependent control on dytiscid population sizes (Juliano & Lawton 1990).

Although adults and larvae of dytiscid beetles are both aquatic, their ecology is quite different. Adult dytiscid beetles have primitive orthopteroid mandibles that are used to tear tissue from prey (Kehl 2014). They are opportunistic predators and they will often act as scavengers, with some species even preferring dead prey (Velasco & Millan 1998). Dytiscid larvae have three instars, all of which are obligate predators. Larvae have hollow, curved mandibles that are used to inject prey with hydrolytic enzymes, thereby utilizing extra-oral digestion to break down prey tissue (Holmen 1987). There are also some indications that the mandibles of dytiscid larvae are specialized both between species (Wall, Barman & Beals 2006; Barman *et al.* 2016) and between instars of the same species (Brannen, Barman & Wall 2005). Because of these

differences, adults and larvae are likely to have different prey species and different consumptive effects on ecological communities.

Despite their designation as generalists and high rates of intraguild predation, there are diverse communities of dytiscid beetles (e.g., Chapter 3). Diversity of dytiscid communities suggests that there may be factors that reduce competition and enable co-occurrence of different species of dytiscid beetles. One possibility is that prey regimes are more specialized than is currently thought (Nilsson 1986). Prey preference and foraging behavior are known to vary between genera (Formanowicz 1982; Lundkvist *et al.* 2003; Yee *et al.* 2009). This specialization is partly a function of body size, as both larval and adult dytiscid beetles range from 1 to 40 mm in total length. They also exploit different microhabitats. Some act as sit and wait predators (e.g., *Dytiscus*), others are pelagic (e.g., some *Agabus*), and others dig in the substrate for burrowing prey (e.g., Hydroporinae; Larson *et al.* 2000). Dytiscid beetles may be an important group for biocontrol of aquatic pests, as some species preferentially feed on mosquito larvae (Lundkvist *et al.* 2003; Culler & Lamp 2009). The mechanisms that facilitate diverse communities of dytiscid beetles and the characteristics that lead to specialized behavior and prey regimes merit further study.

Despite their ecological importance, their global distribution, and their abundance in freshwater systems, dytiscid beetles are a relatively understudied group. One substantial barrier to be overcome in studying their ecology is the difficulty of identification, especially for small species and early larval instars (Vamosi & Wohlfahrt 2014). The difficulty of identification can be resolved using techniques such as DNA barcoding, thereby gaining the taxonomic resolution needed to better understand an ecologically informative suite of species.

Objectives

Each of the following chapters provides insights into the ecological factors that structure macroinvertebrate communities of Delmarva Bays, thereby providing knowledge that can inform management decisions in these imperiled wetlands.

The second chapter seeks to address long term changes in macroinvertebrate community composition between Delmarva Bays with different management histories, including a natural wetland, a rehabilitated wetland, and a created wetlands. Given likely influences of management history, I hypothesized that the created and rehabilitated wetlands would develop different macroinvertebrate communities over the nine year period during which they were monitored. I also hypothesized that, after nine years, the macroinvertebrate community of the rehabilitated wetland would be more like that of the natural wetland than that of the created wetland. Conclusions from this work address the importance of providing adequate time to assess restoration success in wetlands.

The third chapter considers the influence of different habitat characteristics on predaceous diving beetle community composition by comparing 10 wetlands with sphagnum moss to 10 wetlands without sphagnum moss. Based on the changes to habitat characteristics caused by sphagnum, I hypothesized that beetle communities would be more diverse in wetlands with sphagnum compared to wetlands without sphagnum, that dytiscid community composition would be different between these two wetland types, and that some dytiscid beetle species would show high fidelity to sphagnum wetlands. As wetland vegetation is influenced by management history, this chapter examines an important link between wetland restoration, habitat characteristics, and beetle community composition.

The fourth chapter uses species traits to evaluate the potential ecological role of three different species of predaceous diving beetle that are abundant in Delmarva Bays, *Matus*

bicarinatus, *Matus ovatus* and *Agabus punctatus*. Cranial and mandibular morphology was characterized for all three species. As these beetles are important predators, knowledge of their feeding ecology helps to establish the necessity of encouraging colonization of restored wetlands by diverse predator communities. Based on phylogenetic relatedness of the species and similarity of body size, I hypothesized that mandible characteristics of *M. bicarinatus* and *M. ovatus* would be more similar to each other than either was to *A. punctatus*, that differences between the species would be due to differences in mandible shape rather than mandible size, and that differences between mandible morphology would be present in all instars. The resulting comparisons suggest that wetland restoration may need to be evaluated through species traits, which can provide specific insights into the community dynamics of wetland macroinvertebrates.

The following chapters evaluate changes in macroinvertebrate community composition both seasonally and using long-term data, in relation to varying habitat characteristics and at both local and regional scales, and using high-resolution data and species traits. In concert, these objectives connect several potential sources of environmental variation that may affect aquatic macroinvertebrates and provide insights into their ecology that may inform management of restored wetlands.

Chapter 2: Macroinvertebrate community convergence between natural, rehabilitated, and created wetlands

Abstract

Wetland restoration practices can include rehabilitating degraded wetlands or creating new wetlands. Empirical evidence is needed to determine if both rehabilitated and created wetlands can support the same macroinvertebrate communities as their natural counterparts. We measured long-term macroinvertebrate community change in seasonal wetlands known as Delmarva Bays in Maryland, U.S.A. We compared a rehabilitated, a created, and a natural Delmarva Bay. We hypothesized that the created and rehabilitated wetlands would develop different macroinvertebrate communities. We also hypothesized that the community composition of the rehabilitated wetland would become more similar to that of the natural wetland than to that of the created wetland over 9 years encompassed by this study. We monitored the macroinvertebrates, including both predators and primary consumers, and environmental conditions in the three wetlands from March to August in 2005, 2006, 2007, and 2012. Cluster analysis indicated that from 2005 to 2007, the macroinvertebrate community of the rehabilitated wetland and the created wetland were more similar to each other than to the natural wetland. In 2012, the rehabilitated wetland was more similar to the natural wetland than to the created wetland. This similarity was driven principally by changes in the composition of primary consumer taxa. Our results suggest that rehabilitated Delmarva Bays are more likely to support a natural macroinvertebrate community than are created wetlands. Restoration practices that rehabilitate existing wetlands may be preferred over practices that create new wetlands when restoration project goals include developing natural macroinvertebrate communities in a short period of time.

Note: This work is previously published as

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Introduction

Wetlands provide valuable ecosystem services such as flood regulation and nutrient retention/cycling, which are supported by a diverse community of wetland species (Hansson *et al.* 2005). Anthropogenic development threatens the services provided by wetlands and the ability of wetlands to maintain natural communities (Ghermandi *et al.* 2008). Thus, management of wetland ecosystems in human-dominated landscapes is necessary to protect wetland species and the ecosystem services they provide.

Wetland management approaches vary from protection and preservation to rehabilitation of existing wetlands or construction of new wetland habitat (Grenfell *et al.* 2007). These approaches have different results because they can yield different physical and biological conditions (Whigham 1999). This study focuses on the recovery of the macroinvertebrate community in response to wetland rehabilitation and creation, both of which we broadly refer to as restoration. Here, we refer to wetland rehabilitation as any process that reinstates specific soil, vegetation, and hydrology characteristics in existing but degraded wetland habitat. Wetland creation introduces these characteristics where a wetland does not currently exist.

Delmarva Bays are non-tidal depressional wetlands located on the peninsula of Delaware, Maryland, and Virginia, U.S.A. They typically dry during summer (Pickens & Jagoe 1996), which creates hydrologic conditions that support a suite of rare plant and animal species unique

to this specific type of wetland environment (McAvoy & Bowman 2002). Macroinvertebrates occupy all trophic levels (Culler et al. 2014), and fish are typically absent, as these are seasonal wetlands. Agricultural activity on the Delmarva Peninsula has destroyed or degraded approximately 70% of Delmarva Bay habitat (Fenstermacher et al. 2014), prompting wetland restoration to mitigate habitat loss. Restoration of Delmarva Bays has included both wetland creation and rehabilitation through measures such as plugging drainage ditches surrounding agricultural fields, girdling encroaching trees, and restoring natural hydrological fluxes from groundwater.

As part of a larger restoration effort at the Jackson Lane Preserve in Maryland, we compared the long-term aquatic macroinvertebrate community change of a created wetland and a rehabilitated Delmarva Bay to a natural Delmarva Bay (Figure 2.1). Hereafter, we refer to all the locations sampled as wetlands. The goal of our research was to determine if macroinvertebrate communities would respond differently to rehabilitation versus creation approaches. In addition, we wanted to determine if the rehabilitation of an existing wetland would result in a community more representative of a natural wetland than would occur through wetland creation. We hypothesized that the macroinvertebrate community developed during the 9-year period after restoration would differ between rehabilitated and created wetlands. We also hypothesized that, after 9 years, the macroinvertebrate community of the rehabilitated wetland would more closely mimic that of the natural wetland than that of the created wetland.

Methods

Site Description

The Jackson Lane Preserve is a 107 ha wetland complex in the Choptank River watershed in Caroline County, Maryland (39°03'11.9"N, 75°44'50.2"W). In the 1970s, several Delmarva

Bays were drained for use as cropland and cattle pasture. In 2003, The Nature Conservancy, in partnership with U.S. Fish & Wildlife Service, Maryland Department of the Environment, and the Natural Resource Conservation Service, restored the site.

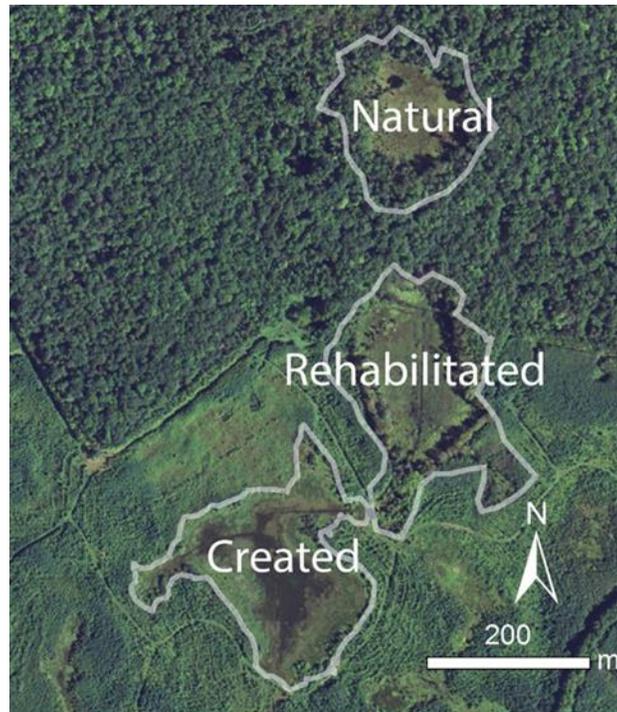


Figure 2.1 An aerial photograph of wetlands from the Jackson Lane Preserve sampled for this study.

Restoration included two components. The first involved rehabilitating a degraded Delmarva Bay, formerly used as a cattle pasture (Figure 2.2). This pond was ditched and partially drained in the 1970s, which reduced its overall size, altered its hydrology, and facilitated encroachment by surrounding trees. Rehabilitation included plugging drainage ditches and girdling or removing encroaching trees and vegetation. Size of this wetland after rehabilitation was 3.3 ha. The dominant species at this wetland before rehabilitation included *Carex striata* (walter's sedge), *Bidens frondosa* (devil's beggartick), *Chasmanthium laxum* (slender woodoats), and *Rubus hispidus* (bristly dewberry). These species were replaced by *Ludwigia sphaerocarpa* (globe-

fruited false-loosestrife), *Proserpinaca pectinata* (mermaid weed), and *Polygonum hydropiperoides* (swamp smartweed) after rehabilitation (Samson, Wilson & Zimmerman 2011). *Sphagnum cuspidatum* (toothed sphagnum) is commonly associated with Delmarva Bays and was first recorded in 2009 (6 years after the project began).

The second component of the Jackson Lane restoration project involved creating 30 new wetlands. Locations for created wetlands were selected by using topographic maps to identify natural depressions. The single created wetland included in our study (Figure 2.3) was constructed using an earthen berm to block the drainage ditch adjacent to the wetland. Microtopography was created within the wetland using a backhoe. Seedling trees were planted along edge habitat and straw was added to prevent cattail (*Typha* sp.) colonization. Coarse woody debris was added to increase habitat heterogeneity. The total size of this wetland after restoration was 3.7 ha. Vegetation transects indicate that wetland plants did become established. Dominant plant species included *Scirpus cyperinus* (kuntz woolgrass), *Ludwigia palustris* (marsh seedbox), *Eleocharis obtusa* (blunt spikerush), and *Lemna minor* (common duckweed; Samson et al. 2011).

We also sampled an existing natural wetland in the Jackson Lane Preserve (Figure 2.4). Its vegetation, soil, and hydrology were characteristic of a Delmarva Bay prior to the project, and aerial photography indicated that it was not altered by human activity. The soil, classified as Corsica mucky loam (fine-loamy, mixed, active, mesic Typic Umbraquults), is saturated most of the year, but drying often occurs in summer with refilling in autumn. The size of this wetland is 1.3 ha. The dominant plant species at this wetland between 2005 and 2012 were *C. striata*, *Cephalanthus occidentalis* (common buttonbush), *Acer rubrum* (red maple), *Triadenum*

virginicum (virginia marsh St. Johnswort), and *Liquidambar styraciflua* (sweetgum; Samson et al. 2011). The bryophyte *S. cuspidatum* was present at the natural wetland throughout the study.



Figure 2.4. Pictures of the natural wetland from June 2003 (left) to August 2012 (right).



Figure 2.2. Pictures of the rehabilitated wetland from May 2003 (left) to June 2012 (right).



Figure 2.3. Pictures of the created wetland from August 2003 (left) to 2012 (right).

Sampling

Following restoration, we took monthly samples at the natural, rehabilitated, and created wetlands from March through August, as long as they retained water, during 2005, 2006, 2007, and 2012 (Figure 2.1). In total, we collected 22 samples at the created wetland, 20 samples at the restored wetland, and 18 samples at the natural wetland. We designed this research as a case study, similar to a before-after control-impacted (BACI) experiment, focused on examining temporal changes in these three wetlands. However, the characteristics of the natural wetland are consistent with those of a Delmarva Bay, including water chemistry (Pickens & Jagoe 1996) and macroinvertebrate community composition (Batzer et al. 2005). Within this context, the changes that took place at both the rehabilitated and created wetlands are likely to be informative beyond this study system.

Our sampling procedures follow the protocol described by Culler et al. (2014). Summarized briefly, we measured pH and conductivity with a YSI 63 Model Probe (YSI Inc., Yellow Springs, OH, U.S.A.) and analyzed water samples for total nitrogen (TN), total phosphorus (TP), and chloride (Cl). TN and TP were measured as the total amounts of either nitrogen or phosphorus present in the sample on a mass basis, including inorganic forms as well as in dissolved and particulate matter. Concurrently, we sampled macroinvertebrates by conducting 20 sweeps at each wetland with a 500 μm D-net with a cross-sectional area of 622 cm^2 . One sweep constituted disturbing a 1m long section of sediment and vegetation and passing the net through the water to capture macroinvertebrates. Samples were washed to remove debris and then preserved in 80% ethyl alcohol. Macroinvertebrates were removed from sub-samples until we reached ≥ 200 individuals (King & Richardson 2002). We identified macroinvertebrates to the lowest practical taxonomic level (typically genus).

Data Analysis

Values for pH, conductivity, Cl, TN, and TP were averaged over each year to compare changes within and among wetlands among years. We also used the function *prcomp* (R Core Team 2014) to perform a principal components analysis (PCA) on centered and standardized monthly water chemistry samples to evaluate how pH, conductivity, Cl, TN, and TP contributed to differences between wetlands.

All analyses of macroinvertebrate community composition were performed on data summed across monthly samples for each year for each wetland. We calculated relative abundances for each taxon and used these abundances to compare the five most abundant taxa for each year from the natural wetland to their abundances in the rehabilitated and created wetlands. We also calculated Shannon diversity, taxa richness, primary consumer relative abundance, and predator relative abundance (Merritt & Cummins 1996).

We used the *hclust* function from the stats package in R (R Core Team) to perform hierarchical cluster analysis, which grouped yearly samples based on Bray-Curtis dissimilarities to assess changes in the macroinvertebrate communities through time. Samples were designed to be representative of each wetland, and we had a systematic sampling design. Thus, groups were formed using the unweighted pair group method with arithmetic mean (Legendre & Legendre 2012).

Similarity percentage was calculated between the main groups identified by cluster analysis using the *simper* function from the vegan package in R (Oksanen et al. 2015), which provides the average contribution of each individual taxon to Bray-Curtis dissimilarities between assigned groups (Clarke 1993). Based on the cluster analysis, we calculated similarity percentage based on the groups identified by the first split in the dendrogram (Figure 2.6).

Results

Water Chemistry

In general, conductivity, pH, Cl, TN, and TP were lowest in the natural wetland, highest in the created wetland, and intermediate in the rehabilitated wetland (Table 2.1). Seasonal variation appeared consistent within each wetland across years. The PCA biplot has two axes that account for 87% of the variance between samples (PC1 = 65%, PC2 = 22%, Figure 2.5). All water chemistry measures are negatively correlated with PC1, whereas pH, conductivity, and Cl are negatively correlated with PC2, and TN and TP are positively correlated with PC2.

Table 2.1. Yearly means and standard deviations of water chemistry characteristics for the three wetlands.

	<i>Year</i>	<i>Conductivity ($\mu\text{S}/\text{cm}$)</i>	<i>pH</i>	<i>Chloride (ppm)</i>	<i>Total Nitrogen (ppm)</i>	<i>Total Phosphorus (ppm)</i>
Natural	2005	40.2 \pm 6.9	4.6 \pm 0.2	1.6 \pm 0.8	1.9 \pm 0.5	0.07 \pm 0.05
	2006	36.2 \pm 8.5	4.6 \pm 0.1	1.6 \pm 1.1	1.8 \pm 0.3	0.06 \pm 0.02
	2007	27.6 \pm 16.5	4.9 \pm 0.1	2.7 \pm 1.7	1.3 \pm 0.7	0.06 \pm 0.06
	2012	24.4 \pm 2.0	4.8 \pm 0.0	1.8 \pm 0.3	2.6 \pm 2.3	0.15 \pm 0.21
Rehabilitated	2005	37.1 \pm 10.4	5.4 \pm 0.3	2.3 \pm 1.5	2.1 \pm 0.8	0.07 \pm 0.04
	2006	52.8 \pm 11.2	5.5 \pm 0.1	4.3 \pm 2.3	2.5 \pm 0.5	0.10 \pm 0.03
	2007	28.8 \pm 3.6	5.2 \pm 0.3	1.7 \pm 1.1	4.4 \pm 6.4	0.24 \pm 0.42
	2012	25.0 \pm 4.6	5.1 \pm 0.1	2.0 \pm 0.9	2.4 \pm 1.8	0.07 \pm 0.08
Created	2005	65.5 \pm 11.4	7.0 \pm 0.3	3.1 \pm 1.8	2.1 \pm 0.7	0.12 \pm 0.06
	2006	80.6 \pm 23.0	7.1 \pm 0.2	5.6 \pm 2.9	2.6 \pm 0.8	0.17 \pm 0.06
	2007	68.9 \pm 46.5	6.9 \pm 0.2	6.9 \pm 7.5	9.3 \pm 11.3	0.70 \pm 0.84
	2012	60.7 \pm 37.1	6.8 \pm 0.2	5.6 \pm 6.0	4.0 \pm 3.3	0.26 \pm 0.25

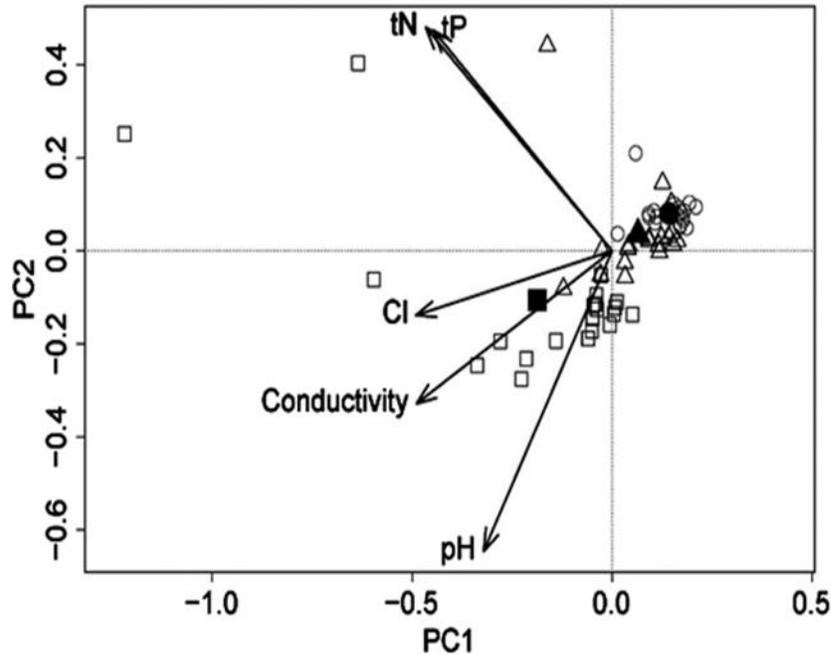


Figure 2.5. Principle components analysis showing water chemistry data from created, rehabilitated, and natural wetlands. Triangles represent the rehabilitated wetland, squares represent the created wetland, and circles represent the natural wetland. Open shapes represent individual monthly samples, and solid shapes represent the centroid for the monthly samples for each wetland.

Macroinvertebrate Community

A total of 13,801 individuals, representing 12 macroinvertebrate orders and 30 insect families were processed during the study. Shannon diversity was lowest at the natural wetland in each year, highest at the rehabilitated wetland in 2005 and 2006, and highest at the created wetland in 2007 and 2012 (Table 2.2). The lowest proportion of predators (4%) was observed at the natural wetland in 2012, whereas the highest proportion (46%) was observed at both the natural and rehabilitated wetland in 2007 (Table 2.2).

Caecidotea (freshwater isopod) was the most abundant taxon at the natural wetland in all years and was absent from the rehabilitated and created wetlands until 2012. In 2012, *Caecidotea*

represented 75% of the community of the natural wetland, 59% of the rehabilitated wetland, and 9% of the created wetland (Table 2.3). In 2005, the five most abundant taxa at the natural wetland comprised 94% of that community. These same five taxa represented 32 and 40% of the communities of the rehabilitated wetland and created wetland, respectively. In 2012, the five most abundant taxa at the natural wetland comprised 96% of that community. These taxa represented 77% of the rehabilitated wetland community, but only 42% of the created wetland community (Table 2.3).

Cluster analysis separated the yearly community samples into two main groups (Figure 2.6). One group included all years of the natural wetland as well as 2012 data from the rehabilitated wetland. The other group included all years from the created wetland as well as 2005 – 2007 from the rehabilitated wetland. *Caecidotea* contributed 37% to the Bray – Curtis dissimilarities between the two main groups. The next most important taxa were Chironomini (9%), Tanytarsini (6%), Orthoclaadiinae (5%), and Tanypodinae (5%). All other taxa contributed less than 5% to the taxonomic differences among sites.

Table 2.2. Diversity metrics for each wetland based on the sum of each taxon across monthly samples for each year. Relative abundance for predators and primary consumers is included.

	<i>Year</i>	<i>Diversity</i>	<i>Taxa Richness</i>	<i>Predator (%)</i>	<i>Primary Consumer (%)</i>
Natural	2005	1.42	27	17	83
	2006	1.68	36	25	75
	2007	1.50	19	46	54
	2012	1.00	20	4	96
Rehabilitated	2005	2.66	40	38	62
	2006	2.22	35	39	61
	2007	1.63	29	46	54
	2012	1.67	31	21	79
Created	2005	2.48	40	23	77
	2006	1.89	33	22	78
	2007	1.96	28	39	61
	2012	1.79	25	36	64

Table 2.3. The five taxa with the highest relative abundance from the natural wetland compared to their relative abundances for the rehabilitated and created wetlands. Abundances are presented as percentages.

	<i>Order: Taxon</i>	<i>Natural</i>	<i>Rehabilitated</i>	<i>Created</i>
2005	Isopoda: <i>Caecidotea</i>	59	0	0
	Diptera: Chironomini	16	18	27
	Diptera: <i>Bezzia</i>	10	8	8
	Diptera: Sciaridae	6	1	0
	Diptera: Tanypodinae	3	5	5
	Other	6	68	60
2006	Isopoda: <i>Caecidotea</i>	58	0	0
	Diptera: <i>Bezzia</i>	11	10	4
	Diptera: Tanypodinae	9	14	10
	Diptera: Orthoclaadiinae	6	37	5
	Diptera: Chironomini	4	8	3
	Other	12	31	77
2007	Isopoda: <i>Caecidotea</i>	44	0	0
	Diptera: <i>Bezzia</i>	35	29	5
	Diptera: Tanypodinae	7	7	29
	Diptera: Chironomini	3	47	28
	Amphipoda: <i>Gammarus</i>	3	0	0
	Other	8	16	38
2012	Isopoda: <i>Caecidotea</i>	75	59	9
	Gastropoda: Ancyliidae	12	11	0
	Diptera: Chironomini	5	3	32
	Amphipoda: <i>Gammarus</i>	4	0	0
	Odonata: Coenagrionidae	2	4	1
	Other	4	23	58

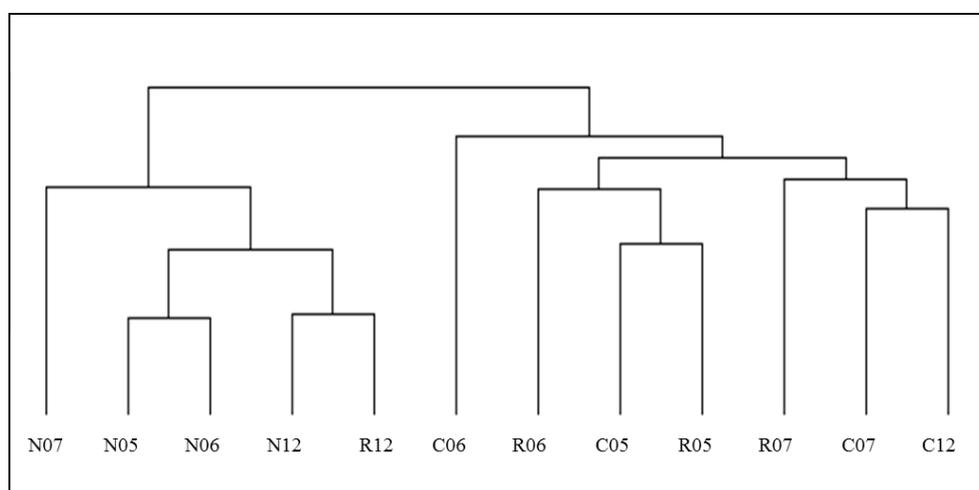


Figure 2.6. A dendrogram displaying the results of cluster analysis performed on Bray – Curtis dissimilarities calculated between macroinvertebrate community samples summed for each wetland for each year. Branches are designated N (natural), R (rehabilitated), or C (created) and labeled with the year (2005, 2006, 2007, or 2012).

Discussion

We found that (1) the macroinvertebrate communities differed between the rehabilitated and created wetlands and (2) the 2012 community of the rehabilitated wetland was more similar to the natural than created wetland community. This result suggests that different restoration processes produce different abiotic habitat conditions (Steven & Lowrance 2011) and rehabilitated wetlands likely support macroinvertebrate communities more typical of natural than created wetlands within a short-time period similar to the 9 years of this study (Whigham 1999).

Water chemistry varied seasonally at all three wetlands. Water chemistry characteristics of the rehabilitated wetland appear to be more similar to those of the natural wetland than to the created wetland. Both the natural and rehabilitated wetlands had the high acidity characteristic of Delmarva Bays (Pickens & Jagoe 1996), which is attributed to surficial groundwater and accumulated vegetative material (Newman & Schalles 1990). This may indicate that the habitat characteristics of the rehabilitated wetland were more similar to those of the natural wetland. However, the water chemistry conditions of the three wetlands were unlikely to prevent colonization or establishment of most wetland macroinvertebrates (Gorham & Vodopich 1992). Thus, we believe that differences in water chemistry among wetlands likely did not control composition of macroinvertebrate communities once established.

The macroinvertebrate community of the rehabilitated wetland was more similar to that of the created than the natural wetland from 2005 to 2007, but was more similar to that of the natural than created wetland in 2012. This change in community similarity was attributed primarily to a change in primary consumer taxa in the rehabilitated wetland between 2007 and 2012. In 2012, the rehabilitated wetland shifted from numerical dominance by chironomids to *Caecidotea*, an organism that may be an indicator of restoration success. The ecology of *Caecidotea* is not well understood, though they are typically abundant in southern forested

depressional wetlands such as Delmarva Bays (Batzer et al. 2005). We have observed them clustered on wetland grasses and believe that they were likely feeding on periphyton, which suggests they are primary consumers (E. Spadafora 2012, University of Maryland).

We found other community differences among the three wetlands, most notably that diversity was consistently lowest at the natural wetland. Although low diversity at the natural site may seem counterintuitive, it is not unexpected given that the natural wetland is acidic and oligotrophic. Although the created wetland consistently had the most diverse community, we do not consider this to be an indication of restoration success for our system as community composition differed substantially from the natural wetland. However, this result suggests that wetland creation may still result in a macroinvertebrate community that supports certain ecosystem services. Thus, the decision to utilize rehabilitation or creation approaches should consider the need for restored sites to match the conditions of the types of wetland being restored. In our case, replicating the conditions of natural Delmarva Bays was essential given their unique nature and widespread impacts from land development in the region.

At all wetlands, taxa richness and percent predators versus primary consumers varied considerably year-to-year. These differences could be explained by wetland vegetation composition (De Szalay & Resh 2000), hydrology (Dietz-Brantley et al. 2002), and disturbance (Tangen et al. 2003). For example, a drought in 2007 caused several nearby wetlands to dry earlier in the year (Culler et al. 2014). Continued monitoring of abiotic and biotic changes in rehabilitated and created wetlands is needed to determine how these factors influence macroinvertebrate diversity and succession as they relate to natural, unimpacted wetlands.

Our study was designed to focus on the long-term monthly and yearly changes that occurred in restored wetlands. This approach resulted in temporal but not spatial replication of wetland

restoration types. Thus, the inferences we make about different restoration approaches are limited. However, our overall conclusions about the effectiveness of rehabilitation versus creation are based on a change in community composition (i.e. the dominance of isopods) that was clearly documented and represented a long-term stable condition in the natural wetland. Although our study represented a substantial sampling effort, the conditions in the study wetlands will continue to change. The conditions in the created wetland may approach what is found in the natural and rehabilitated wetlands if given sufficient time to develop. Thus, the advantages of rehabilitation over creation may only apply to projects that seek to improve macroinvertebrate communities over short periods of time (i.e. about 9 years).

Our results suggest that rehabilitated wetlands are more likely to recover macroinvertebrate communities of their natural counterparts than are created wetlands, though recovery may not be apparent within the first two to four years after restoration measures are taken. As the composition of macroinvertebrate communities are linked to ecosystems services the wetland will provide, wetland rehabilitation should be prioritized over wetland creation, and both should be coupled with long-term monitoring programs to assess success.

Chapter 3: Predaceous diving beetle communities (Coleoptera: Dytiscidae) in relation to the presence of sphagnum moss in seasonal wetlands

Abstract

Wetland macroinvertebrate community composition is influenced by changes in aquatic vegetation, with some plants having a greater effect than others. Sphagnum moss (Sphagnales: Sphagnaceae: *Sphagnum*), a widely acknowledged ecosystem engineer, is likely to influence macroinvertebrate community composition by changing habitat structure and water chemistry. The influence of sphagnum moss merits further study, especially as it affects macroinvertebrate communities in imperiled and isolated wetlands. Using dytiscid beetles (Coleoptera: Dytiscidae) as representatives of overall community structure, I hypothesized that a) dytiscid beetles would be more diverse in wetlands with sphagnum compared to wetlands without sphagnum, b) dytiscid community composition would differ between these two wetland types, and c) some dytiscid beetle species would show high fidelity to sphagnum wetlands. I collected dytiscid larvae and adults in 20 Delmarva Bay wetlands, 10 with sphagnum and 10 without, on the Delmarva Peninsula in Maryland and Delaware. DNA barcoding was used to identify specimens to species level. I collected a total of 27 species of dytiscid beetles, half of which were only found in wetlands with sphagnum moss. Composition of sphagnum communities was significantly different than composition of non-sphagnum communities. Dytiscid diversity was also higher in sphagnum wetlands and two species had high fidelity to sphagnum wetlands. Habitat structure created by the presence of sphagnum moss may contribute to the more diverse dytiscid assemblages found in sphagnum wetlands. In general, dytiscid community structure may change predictably between wetlands with and without sphagnum, and sphagnum wetlands support unique dytiscid communities. With regard to management and conservation of

wetland habitat, sphagnum wetlands should be considered high priority conservation sites that maintain local and regional diversity.

Introduction

The richness, diversity, and composition of aquatic macroinvertebrates in wetlands are influenced by wetland habitat characteristics such as quality of the soil (Armitage & Fong 2004), cycles of wetting and drying (Golladay *et al.* 1997), and structure and composition of vegetation (Gioria *et al.* 2010; Yee 2010). Although these relationships are often difficult to interpret (Batzer 2013), linking community attributes with habitat features is important for informing management and conservation strategies for wetland habitats. For example, characteristics of wetland vegetation may influence richness and abundance of macroinvertebrate taxa (Batzer & Wissinger 1996), suggesting that diverse wetland plant communities may help to protect diverse macroinvertebrate communities.

One species that may have a prominent role in shaping macroinvertebrate communities is sphagnum moss (Sphagnales: Sphagnaceae), a widely recognized wetland ecosystem engineer that affects water chemistry, hydrology, and habitat structure (Tansley 1949; Moore 1995; Frankl & Schmeidl 2000). Sphagnum is both adapted to and creates nutrient poor, low productivity habitats (Gunnarsson 2000; Granath *et al.* 2012). Where it grows, sphagnum can form a thick mat that fills the water column (Andrus 1986). In this way, sphagnum creates microhabitat for macroinvertebrates (Henrikson 1993). These effects make it likely that sphagnum would influence wetland communities. Despite well-documented changes to habitat characteristics, research studying the relationship of sphagnum to other wetland organisms is lacking.

Sphagnum moss is commonly found in Delmarva Bays (Karlin *et al.* 1991; Halsey *et al.* 2000), which are seasonal wetlands found on the mid-Atlantic coast on the Delmarva Peninsula. These wetlands typically dry down in summer and refill in fall (Phillips & Shedlock 1993), creating hydrologic conditions that support a suite of rare species (Tyndall 2000; McAvoy & Bowman 2002; Tiner 2003). Delmarva Bays are typically acidic and oligotrophic (Pickens &

Jago 1996), making them optimal habitat for sphagnum. However, many bays do not have sphagnum, a condition which may be linked to management history, with impacted bays losing sphagnum (Spadafora *et al.* 2016). The absence of sphagnum in many bays may be caused by agricultural activity on the Delmarva Peninsula, which has destroyed or degraded up to 70% of these imperiled wetlands (Allen 2009; Fenstermacher *et al.* 2014). If further losses occur, it will be important to understand if impacted bays can support the same communities as wetlands with natural vegetation, such as sphagnum moss.

Predaceous diving beetles (Coleoptera: Dytiscidae) are important predators (Alarie & Leclair Jr 1988; Batzer & Wissinger 1996; Fairchild *et al.* 2003; Culler *et al.* 2014a) that have well-documented consumptive (Cobbaert *et al.* 2010) and non-consumptive (Ohba *et al.* 2012) effects on freshwater communities. In fishless wetlands they may become top predators and have a substantial role in suppression of mosquito populations (Culler & Lamp 2009b). Despite their ecological importance, they are relatively understudied, likely owing to the difficulty of identification for small adults and larvae (Vamosi & Wohlfahrt 2014).

Diversity and abundance of these beetles is known to change in response to vegetation diversity and density (Larson *et al.* 2000; Gioria *et al.* 2010) as well as acidity (Eyre *et al.* 1986; Foster *et al.* 1989). Therefore, the structural and chemical changes caused by the presence of sphagnum moss in Delmarva Bays are likely to affect dytiscid community composition. I expected dytiscid communities to differ between sphagnum and non-sphagnum wetlands. I hypothesized that a) if habitat structure provided by sphagnum is important, diversity of dytiscid beetles of sphagnum wetlands would be higher than diversity of non-sphagnum wetlands, b) the dytiscid community composition of sphagnum wetlands would be different from composition of non-sphagnum wetlands and c) some species of dytiscid would show high fidelity to sphagnum

wetlands. To test these hypotheses, I sampled dytiscid communities in wetlands with and without sphagnum and used DNA barcoding to identify dytiscid species.

Methods

To evaluate the influence of sphagnum moss on the composition of dytiscid beetle communities, I collected dytiscid beetles in 20 Delmarva Bays, 10 with sphagnum and 10 without. Dytiscid sampling was conducted during April, May, and June of 2013. Environmental characteristics were measured at the same time.

Environmental Characteristics

The Delmarva Peninsula extends along the Atlantic Coast of the United States and includes Maryland's eastern shore, part of Virginia, and the state of Delaware. It is covered in seasonal, depressional wetlands known as Delmarva Bays. These bays were thought to have formed roughly 20,000 years ago as the result of wind blowing across the Peninsula (Stolt & Rabenhorst 1987b). I located and surveyed potential field sites across Queen Anne's, Kent, and Caroline Counties in Maryland, and New Castle County in Delaware.

From all wetlands surveyed, I chose 20 to sample (Table 3.1). Ten wetlands had sphagnum moss naturally occurring, while it was absent in the other ten. Wetlands were chosen so that, when possible, sites with and without sphagnum were distributed evenly across the entire sampling area. As it was not possible to choose wetlands of uniform size, I included a range of wetlands sizes in each category. Within the wetlands chosen, I conducted vegetation surveys during June of 2013. This included walking a transect line taken at the widest point of the wetland and identifying all species found along this line.

I sampled each wetland once a month during April, May, and June of 2013 at a set of ten randomly chosen sampling points. Water chemistry measures were taken at the first point at each

wetland. Sampling included measuring pH and specific conductivity with a YSI 63 Model Probe (YSI Inc., Yellow Springs, Ohio). At all points I recorded depth and substrate type (e.g. sedges, leaf litter, or sphagnum), and collected dytiscid beetles. Previous work indicates that communities of natural Delmarva Bays are numerically dominated by of freshwater isopods (Isopoda: Asellidae ; see Chapter 2). For this reason, I also recorded presence/absence of isopods in sweep net samples.

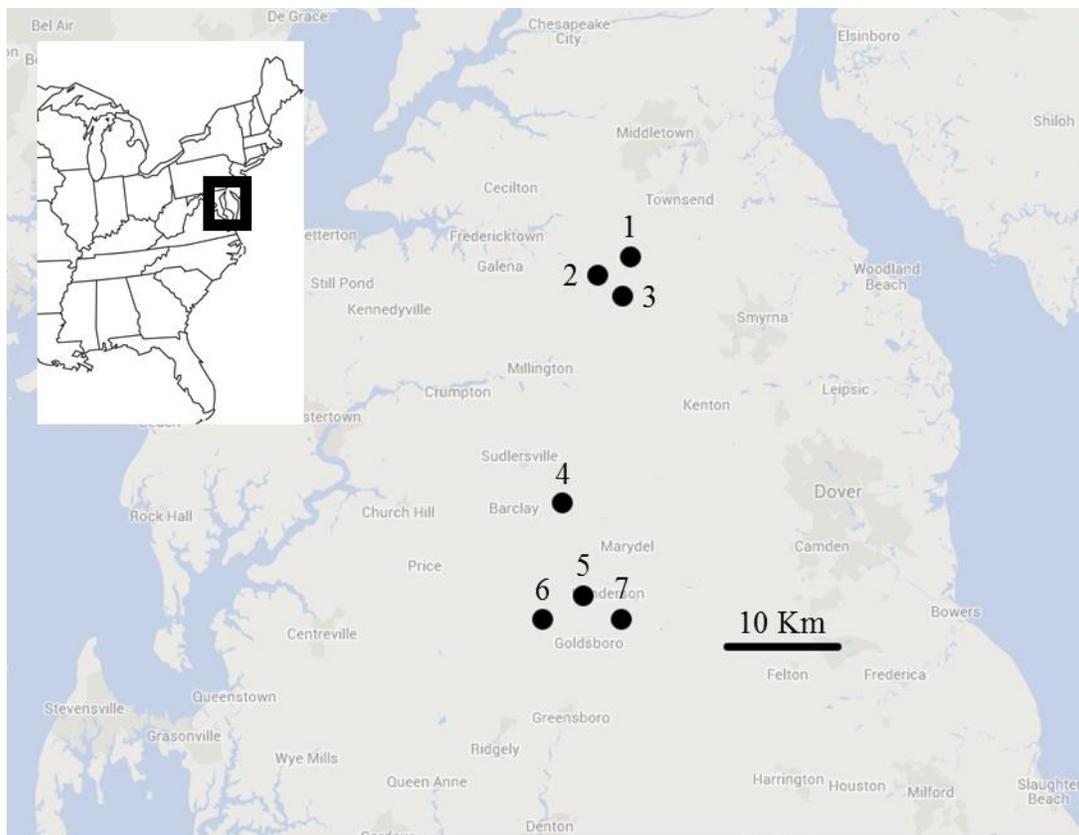


Figure 3.1. Location of wetland field sites on the Delmarva Peninsula. Each black dot represents one or more wetlands that were included in this study. Inset map shows location of the Delmarva Peninsula within the continental United States of America. Numbers correspond to locations listed in Table 3.1.

Macroinvertebrates

I collected dytiscid larvae and adults using a 500µm sweep net to disturb a one-meter-long section of sediment and vegetation. I then passed the net back and forth to collect material suspended in the water column. Contents of the sweep net were then transferred to a 500µm sieve inside of a plastic tub where they were rinsed to remove large piece of vegetation and debris. The rinsed sample from each individual point was then transferred to its own jar of 80% ethanol and transported to the lab. In the lab, samples were sorted under magnification. Dytiscid beetles were removed for identification and all other contents of the sample were discarded. Dytiscid beetles were then identified to lowest practical taxonomic level using local and regional keys. Each specimen was then transferred to an individual vial filled with 100% ethanol and stored in a -20°C freezer in preparation for DNA barcoding.

DNA Barcoding

I performed all DNA barcoding work at the Smithsonian National Museum of Natural History (NMNH) at the Laboratories of Analytical Biology (LAB). I used standard LAB high-throughput protocols for extraction, amplification, and sequencing of the 648 bp COI barcoding region. A total of 529 samples were prepared. I first placed tissue samples in 96-well plates for extraction. Depending on the size and quality of the specimens, I used one to three legs or a piece of the abdomen as the tissue sample. Tissue samples were then digested for at least eight hours in a lysing buffer. Following digestion, tissue samples were processed to remove cell material and retain DNA precipitate. Polymerase chain reaction (PCR) was then used to amplify the COI barcoding region of the DNA. I used Folmer primers (Folmer *et al.* 1994) during PCR as they have been shown to be effective in amplifying the COI barcoding region in dytiscid beetles (Pentinsaari, Hebert & Mutanen 2014). These primers include LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198

(TAAACTTCAGGGTGACCAAAAAATCA). Following PCR, amplified DNA strands were then stained with fluorescent dye using cycle sequencing. The product of cycle sequencing was then filtered through Sephadex plates to improve the product before sequencing.

Chromatograms produced by sequencing were analyzed using Geneious software (Kearse *et al.* 2012). Geneious was then used to assemble forward and reverse sequences to create a contig that was then edited to resolve ambiguities between sequences. Assembly included aligning sequences generated using the both the forward and reverse primers and then manually examining disparities between the sequences to create a single sequence that could be used in subsequent analyses. The contigs were then submitted to the BOLD system database, and they were checked against published DNA barcodes. I considered a 99% match conclusive for identifying a specimen. In instances where a match was not returned, I used published species descriptions or taxonomic keys for identification. Specimens that could not be identified through barcodes or taxonomy were given morphospecies designations.

Data Analysis

All analyses were conducted using R statistical software (R Core Team 2014). A one way analysis of variance (ANOVA) that included sphagnum presence or absence as the independent variable was performed first on measures of pH and then on measures of conductivity summed across each wetland for all months.

In order to measure dytiscid beetle diversity of individual sphagnum wetlands and individual non-sphagnum wetlands, I calculated effective species number for each wetland for all months. Effective species number was calculated as e raised to the power of the Shannon entropy index. Effective species number provides a meaningful measure of diversity that converts a diversity

index (e.g. Shannon diversity, Simpson diversity) into a number that can be more intuitively compared between communities (Jost 2006).

I calculated a Bray-Curtis dissimilarity matrix to evaluate beta-diversity of sphagnum wetlands compared to non-sphagnum wetlands. Dissimilarities were calculated based on the data summed across all sampling dates for each wetland. When calculating the dissimilarity matrix, I included a dummy species in the community data that occurred at a fixed abundance at all sites for all samples to correct for both low abundances of dytiscid beetles at some wetlands and rare and common species (Clarke, Somerfield & Chapman 2006). I used then used the *adonis* function from the *vegan* package to perform a permutational multivariate analysis of variance (PERMANOVA) to test for significant differences in community composition between the two wetland types. PERMANOVA is used to partition distance matrices and in this way can attribute variation within a distance matrix to experimental categories.

To capture seasonal variation among sites, I calculated a Bray-Curtis dissimilarity matrix that included all 60 samples collected as separate data points. To visualize this matrix, I used the *metaMDS* function in the *vegan* package in R (Oksanen *et al.* 2015) to perform an NMDS analysis on the Bray-Curtis distance matrix of individual sample points and then created a biplot of the data. NMDS is an appropriate ordination method to use in this case as it represents ranked distances and is robust when analyzing non-normal data. One goal of NMDS is to minimize stress, or lack of fit between matrices. Ordinations with stress levels less than 0.1 provide the best representation of the data, but they can be interpreted for stress levels up to 0.2 (Clarke 1993). An ordination based on three axes was adequate to achieve low stress (0.13). Having chosen an axis number, the NMDS analysis was run ten times using 100 iterations each, and the run with the lowest stress is presented in the results.

Finally, I used the *multipatt* function from the vegan package to perform indicator species analysis and determine the fidelity and exclusivity of each species to either wetland type. Indicator species analysis evaluates the relationship between species occurrence/abundance and grouped sites. This analysis can also be used to test the significance of patterns of species occurrence/abundance.

Table 3.1. Details of Delmarva Bay wetlands sampled and their associated environmental characteristics. County and geographic location of each wetland are included. Map area number corresponds to location of each wetland on the Delmarva Peninsula as provided in Figure 3.1. Mean and standard deviation for depth, pH, and conductivity for each wetland is provided. Diversity refers to effective number of dytiscid species for each wetland. The isopods column represents the mean number of times isopods were present out of 10 samples collected in each wetland. Depth, pH, conductivity, diversity, and isopod presence all represent mean values averaged across April, May, and June sampling dates.

Site	Location	Lat, Long	Map Area	Area (Ha)	Sphagnum	Depth (cm)	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Diversity	Isopods
S1	New Castle County, DE	39.31449, -75.75139	1	0.46	<i>S. palustre</i>	55 ± 5.4	4.4 ± 0.30	33.1 ± 4.8	7	9
S2	New Castle County, DE	39.31798, -75.75051	1	0.3	<i>S. palustre</i>	41 ± 14.1	4.2 ± 0.14	37.3 ± 6.1	4	3
S3	New Castle County, DE	39.31887, -75.7497	1	0.06	<i>S. palustre</i>	44 ± 4.6	4.8 ± 0.29	31.2 ± 3.5	5	5
S4	Caroline County, MD	39.05393, -75.83503	6	0.55	<i>S. cuspidatum</i>	53 ± 4.8	3.8 ± 0.72	38 ± 3.1	10	8
S5	Caroline County, MD	39.05533, -75.8333	6	0.18	<i>S. cuspidatum</i>	48 ± 4.3	4.1 ± 0.29	41.5 ± 2.7	2	6
S6	Caroline County, MD	39.05743, -75.75322	7	1.84	<i>S. cuspidatum</i>	44 ± 5.4	4.8 ± 0.36	26.1 ± 6.8	6	10
S7	Caroline County, MD	39.05465, -75.7533	7	2.03	<i>S. cuspidatum</i>	49 ± 6.1	4.6 ± 0.41	33 ± 9.0	6	10
S8	Queen Anne's County, MD	39.15003, -75.81287	4	1.53	<i>S. macrophyllum</i>	36 ± 3.9	4.3 ± 0.60	30.8 ± 5.2	4	9
S9	Caroline County, MD	39.07213, -75.79312	5	0.08	<i>S. palustre</i>	22 ± 13.2	3.9 ± 0.36	40.6 ± 10.9	6	6
S10	Caroline County, MD	39.07358, -75.79138	5	0.12	<i>S. palustre</i>	58 ± 5.2	3.6 ± 0.68	41 ± 7.0	2	0
			Mean			45 ± 6.7	4.25 ± 0.42	35.3 ± 5.9	5	7
N1	New Castle County, DE	39.34698, -75.74212	3	0.72	-	65 ± 5.5	4.7 ± 0.41	28.2 ± 2.7	5	4
N2	New Castle County, DE	39.34819, -75.74001	3	0.08	-	53 ± 3.3	4.4 ± 0.31	40.5 ± 9.6	4	3
N3	New Castle County, DE	39.34886, -75.74145	3	0.14	-	61 ± 5.7	4.1 ± 0.41	27.7 ± 2.4	2	2
N4	New Castle County, DE	39.35027, -75.74389	3	0.48	-	67 ± 5.4	4.5 ± 0.84	36.2 ± 2.3	5	4
N5	Kent County, MD	39.33367, -75.77654	2	0.43	-	57 ± 8.9	4.9 ± 0.29	41.4 ± 9.3	2	8
N6	Caroline County, MD	39.05223, -75.75521	7	2.9	-	47 ± 3.2	6 ± 0.48	47.6 ± 3.0	4	9
N7	Caroline County, MD	39.05134, -75.75837	7	0.42	-	51 ± 6.2	5.8 ± 0.40	40.8 ± 4.0	3	0
N8	Caroline County, MD	39.05378, -75.74861	7	0.4	-	34 ± 4.4	5.6 ± 0.12	38.9 ± 5.7	4	7
N9	Caroline County, MD	39.05427, -75.74661	7	0.07	-	83 ± 4.6	4.2 ± 0.70	33.3 ± 0.2	2	10
N10	Queen Anne's County, MD	39.14891, -75.81503	4	0.16	-	42 ± 5.3	4.3 ± 0.38	39.2 ± 8.5	3	3
			Mean			56 ± 5.3	4.85 ± 0.43	37.4 ± 4.8	3	5

Results

Wetland characteristics

pH and conductivity had a wider range across non-sphagnum wetlands than sphagnum wetlands. ANOVA indicated that the pH of sphagnum wetlands, which ranged from 3.6 to 4.8, was significantly different from the pH of non-sphagnum wetlands, which ranged from 4.1 to 6.0 ($p < 0.01$). Conductivity, which ranged from 26.1 to 41.5 $\mu\text{S}/\text{cm}$ in the sphagnum wetlands and 27.7 to 47.6 $\mu\text{S}/\text{cm}$ in the non-sphagnum wetlands, was not significantly different. The mean depth of each pond, averaged across all samples for all months, varied among wetlands, ranging from 22 to 83 cm (Table 3.1).

Species identified during vegetation transects were characteristic of Delmarva Bays, including shrubs such as *Cephalanthus occidentalis* (common buttonbush), *Clethra alnifolia* (sweet pepperbush), and *Eubotrys racemose* (fetterbush), and sedges such as *Carex striata* (walter's sedge), and *Scirpus cyperinus* (kunth woolgrass). The surrounding forested areas were predominantly *Acer rubrum* (red maple), *Liquidambar styraciflua* (sweetgum), and *Nyssa sylvatica* (black gum). Also common in many wetlands were *Utricularia* spp. (bladderwort) and *Proserpinaca pectinate* (Mermaid weed). Species of sphagnum collected included *S. cuspidatum* (toothed sphagnum), *S. macrophyllum* (largeleaf sphagnum) and *S. palustre* (prairie sphagnum; Table 3.1). In the wetlands I sampled, sphagnum was either absent or formed a dense ring around the periphery of each wetland.

Presence/absence of isopods in d-net samples was recorded at each of the 10 points sampled in each wetland. On average, in sphagnum wetlands, isopods were present in seven out of ten d-net samples. In non-sphagnum wetlands, isopods were present in five out of ten samples. Both groups of sites included at least one wetland where isopods were not detected in any sample during any month and at least one wetland where isopods were detected in all samples during all

months. Abundance of isopods did not show a clear correlation to presence/absence of sphagnum (Table 3.1).

Dytiscid beetles

I collected a total of 529 dytiscid beetles, including 496 larvae and 33 adults, representing 14 genera and 27 species. Through DNA barcoding, I was able to produce sequences for 19 of the dytiscid species that we collected, 12 of which had a match in the BOLD database. Species for which there were sequences but which did not have a match in the BOLD database could typically be identified using taxonomic keys and species descriptions. Those that could not be identified are designated by genus name and assigned a letter (Table 3.2).

Three of the dytiscid beetle species were found exclusively in wetlands without sphagnum, 14 were found exclusively in wetlands with sphagnum, and nine were found both at wetlands with and without sphagnum. Larvae and adults were collected for three species: *Matus bicarinatus*, *Desmopachria convexa*, and *Liodessus crotchi*. Adults and larvae of *M. bicarinatus* and *D. convexa* were found in the same wetlands. Adults and larvae of *L. crotchi* were always found in different wetlands. All other species were represented either by larvae or by adults, but not both.

The most abundant species across all months, *Agabus punctatus*, represented 28.0% of all dytiscid beetles collected and was found at both wetland types in a total of 13 wetlands. The next most abundant species were *Neoporus undulatus* (15.1%), *Matus bicarinatus* (11.7%), *Matus ovatus* (11.5%), and *Liodessus crotchi* (5.1%). All other species constituted less than 5% of the dytiscid beetles collected (Table 3.2).

Table 3.2. Dytiscid beetle species collected in Delmarva Bay wetlands. Data provided for each species includes abundance (%), life stages collected (L for larva, A for adult), and the wetland types at which each species was found (Non for wetlands without sphagnum, Sphagnum for with sphagnum, and Both for species found at both wetland types). All species were identified using DNA barcoding followed by taxonomic verification. Species listed with genus and a letter could not be identified further using DNA barcodes or available keys. DNA barcodes were not obtainable for species marked with a “*”, typically because there was not an adequate number of specimens collected to obtain a good tissue sample for barcoding. Therefore, the name given represents a morphospecies designation.

<i>Subfamily</i>	<i>Species</i>	<i>Number of Beetles</i>	<i>% of Community</i>	<i>Life Stages</i>	<i>Wetland Types</i>
Colymbetinae	<i>Agabus a</i>	4	0.8	L	Non
	<i>Agabus b</i>	3	0.6	L	Sphagnum
	<i>Agabus c*</i>	1	0.2	A	Sphagnum
	<i>Agabus d*</i>	1	0.2	A	Sphagnum
	<i>Agabus disintegratus</i>	20	3.8	L	Both
	<i>Agabus punctatus</i>	148	28.0	L	Both
	<i>Coptotomus longulus</i>	11	2.1	L	Both
	<i>Hoperius planatus</i>	4	0.8	A	Sphagnum
	<i>Matus bicarinatus</i>	62	11.7	L, A	Both
	<i>Matus ovatus</i>	61	11.5	L	Sphagnum
Copelatinae	<i>Copelatus a*</i>	1	0.2	A	Sphagnum
Dytiscinae	<i>Acilius sylvanus</i>	6	1.1	L	Both
	<i>Acilius mediatius</i>	13	2.5	L	Sphagnum
	<i>Dytiscus a</i>	15	2.8	L	Both
	<i>Dytiscus verticalis</i>	4	0.8	L	Both
	<i>Dytiscus faciventrus</i>	21	4.0	L	Sphagnum
	<i>Graphoderus liberus</i>	8	1.5	L	Both
	<i>Thermonectus a</i>	7	1.3	L	Sphagnum
	Hydroporinae	<i>Desmopachria convexa</i>	15	2.8	L, A
<i>Laccornis a*</i>		1	0.2	A	Non
<i>Laccornis b*</i>		1	0.2	A	Non
<i>Laccornis c*</i>		1	0.2	A	Sphagnum
<i>Laccornis d*</i>		1	0.2	A	Sphagnum
<i>Liodessus a*</i>		1	0.2	A	Sphagnum
<i>Liodessus crotchii</i>		27	5.1	L, A	Sphagnum
<i>Neoporus undulatus</i>		80	15.1	L	Both
	<i>Sanfilipodytes a</i>	12	2.3	L	Both

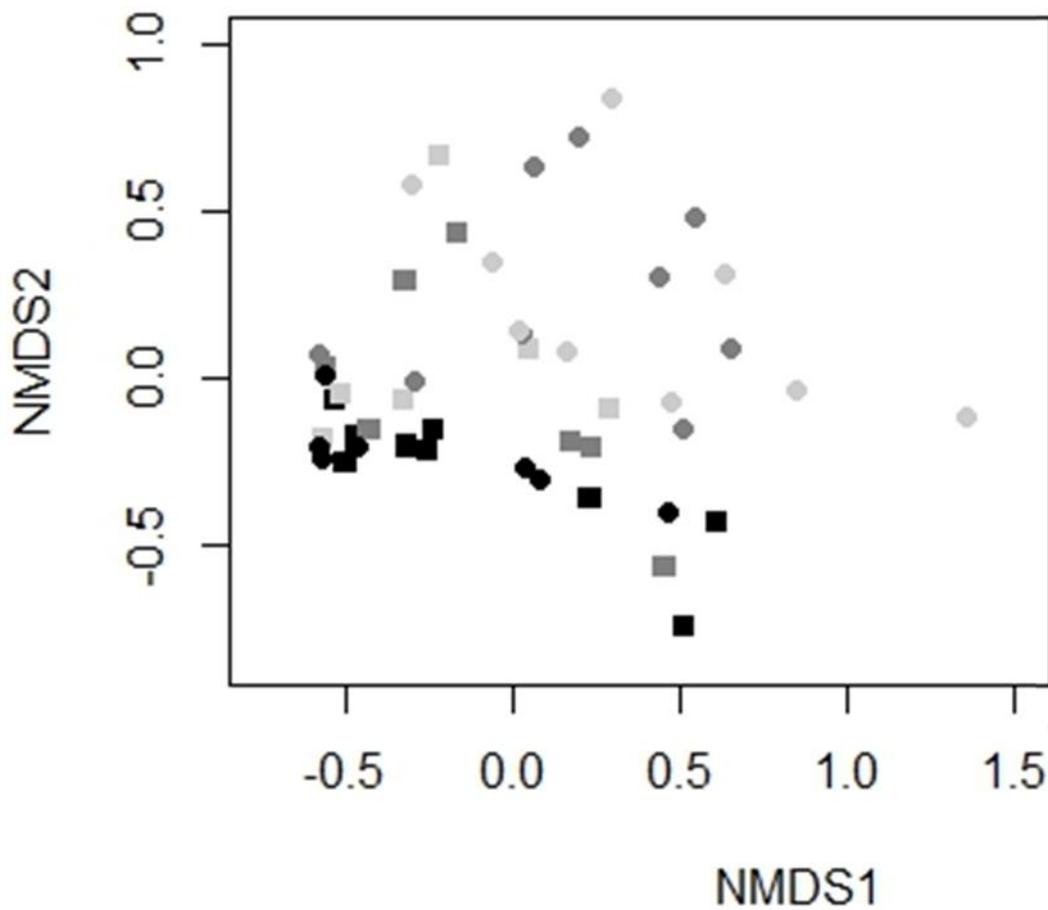


Figure 3.3. NMDS ordination (stress = 0.12, k = 3) showing pairwise distances between dytiscid beetle communities from each Delmarva Bay wetland during April, May, and June based on a Bray-Curtis dissimilarity matrix. Two wetland types- sphagnum and non-sphagnum wetlands are included. Sphagnum wetlands are represented by circles. Non-sphagnum wetlands are represented by squares. Black symbols represent dytiscid communities measured in April. Medium gray and light gray symbols represent communities from May and June respectively.

There was a temporal shift in species richness and composition from April through June (Figure 3.2). 12 species were collected during April, and 18 species were collected both during May and June (Figure 3.2). Of the 12 species collected in April, only four were represented by five or more individuals across all wetlands. In May, 10 of the 18 species collected were

represented by five or more individuals, and in June, seven of the 18 species collected were represented by five or more individuals. Of the most abundant species, *A. punctatus* occurred only during April and May, whereas *M. ovatus* and *M. bicarinatus* occurred only during May and June. *N. undulatus* and *L. crotchi* were collected on all three sampling dates, with peak abundances occurring during May and June, respectively. Seasonal changes across wetlands are further illustrated by the NMDS ordination (stress=0.12, k=3) that represented pairwise distances between communities of sphagnum non-sphagnum wetlands (Figure 3.3). In general, this ordination shows greater similarity of community composition across all sites in April, and greater distances between points in May and June.

The mean number of effective dytiscid species in the sphagnum wetlands was five, and these sites had a range in number of effective species from two to ten (Table 3.2). The mean number of effective species of the non-sphagnum wetlands was three, with a range from two to five. PERMANOVA analysis indicated wetland type (sphagnum vs. non-sphagnum) contributed significantly to the variation in overall community composition ($p < 0.05$). Indicator species analysis indicated that *Acilius mediatius* ($p = 0.032$) and *Dytiscus faciventrus* ($p = 0.003$) were both significantly associated with sphagnum wetlands. No indicator species were identified for non-sphagnum wetlands.

Discussion

Sphagnum wetlands supported different and more diverse dytiscid communities than non-sphagnum wetlands. In addition, two species (*A. mediatius* and *D. faciventrus*) exhibited high fidelity and exclusivity to sphagnum wetlands. Within these Delmarva Bays, species level identifications demonstrated that dytiscid community structure may change predictably between wetlands with and without sphagnum. Differences in diversity and the presence of certain

dytiscid species may be related to the effects of sphagnum moss as an ecosystem engineer, changing wetland water chemistry and habitat structure (Andrus 1986).

Sphagnum wetlands had lower pH, as was expected given the known effects of sphagnum on water chemistry (Gunnarsson & Rydin 2000). Sphagnum wetlands also exhibited pH and conductivity levels that are expected for acidic, oligotrophic Delmarva Bays (Newman & Schalles 1990). In some instances, the non-sphagnum wetlands had higher pH measures (>5) than is typical for a Delmarva Bay. High pH may indicate anthropogenic impacts (Gustafson & Wang 2002), potentially reflecting nutrient input and eutrophication of bays as a result of agricultural activity on the Delmarva Peninsula (Fenstermacher *et al.* 2014).

Sphagnum wetlands typically had higher dytiscid diversity than non-sphagnum wetlands. Higher diversity may be influenced by differences in pH between sphagnum and non-sphagnum wetlands (Eyre *et al.* 1986). Habitat structure created by sphagnum moss also likely influenced dytiscid diversity. The presence of aquatic vegetation reduces dytiscid dispersal (Yee *et al.* 2009) and intraguild predation (Yee 2010). *A. mediatum* and *D. faciventrus* were both identified by indicator species analysis as having a strong association to sphagnum wetlands. Similar species have been observed actively searching for prey when vegetation is absent, but switching to a sit-and-wait strategy and ambushing prey when vegetation is present (Henrikson 1993; Yee 2010). If dytiscid beetles are not actively searching for prey, they may be less likely to encounter one another and therefore less likely to consume other guild members. Thereby, habitat provided by sphagnum may mitigate density dependent effects of intraguild predation (Juliano & Lawton 1990). These more diverse assemblages may be important given that some species of dytiscid beetles are known to prey preferentially on pest species such as mosquitoes (Lundkvist *et al.* 2003).

Dytiscid diversity has also been shown to be positively correlated to abundance of freshwater isopods (Nilsson *et al.* 1994). Although not all dytiscid beetles are likely to encounter or exploit isopods as prey due to differences in foraging behavior (Yee 2010), high abundances of isopods may indicate overall changes of available prey species (See chapter 2) that may support diverse dytiscid assemblages. Across the wetlands sampled here, many with high dytiscid diversity also had abundant isopods.

Three of the most abundant species collected within both wetland types were *A. punctatus*, *M. bicarinatus* and *M. ovatus*. All are members of the subfamily Colymbetinae, and almost all individuals collected were larvae. The larvae of *A. punctatus* have swimming hairs on their legs, which suggests that they may be active hunters, moving through the water column to find prey (Barman 1996). The architecture of their mandibles suggests that they may be specialized for capturing prey that is relatively fragile and does not vigorously resist capture (Barman *et al.* 2016). *A. punctatus* may also be an important species for biocontrol of mosquitoes (Culler & Lamp 2009). By comparison, relatively little is known about the genus *Matus*. Larvae of some species have been described, but identification, especially of early instars, is difficult. One of the potentially significant morphological characteristics of both *M. bicarinatus* and *M. ovatus* is the presence of pseudocheilate tarsi on the pro- and mesothoracic legs (Alarie, Watts & Nilsson 2001). The function of these tarsi is unknown, but it suggests that *Matus* may utilize unique feeding strategies or exploit a different microhabitat than other dytiscids.

Patterns of seasonal abundance show that different species are present at different times, especially within subfamilies. For example, the highest abundance of the most abundant species, *A. punctatus*, occurred in April. By comparison, the highest abundances of *M. bicarinatus* and *M. ovatus*, the next most abundant species in this subfamily, occurred in June. Changes in

community composition over the season suggest temporal partitioning as another explanation for overall diversity of dytiscid beetles in both wetland types (Zimmerman 1960). Additionally, species richness increased from April to June. Increasing richness of dytiscid beetles may have been in response to availability of prey, which typically also increase in richness and abundance through late spring and early summer (Batzer & Wissinger 1996).

Of the dytiscid species collected, more than half were found only in sphagnum wetlands. Patterns of species occurrence may indicate an overall connection between changes in vegetation and changes in dytiscid community composition, which is suggested throughout the literature (Nilsson *et al.* 1994; Larson *et al.* 2000; Gioria *et al.* 2010; Yee 2010). Though this study focuses on dytiscid beetles, these patterns may be indicative of overall changes in macroinvertebrate community similarity, as beetle communities can be representative of community similarity between wetlands (Lancaster & Scudder 1987; Briers & Biggs 2005; Bilton *et al.* 2006).

My results suggest that Delmarva Bays with sphagnum support unique and diverse assemblages of dytiscid beetles. To the degree that these beetles are representative of the macroinvertebrate community as a whole, this may indicate that sphagnum wetlands support more diverse communities in general. In instances where macroinvertebrate diversity is a goal for land managers, sphagnum wetlands may have higher value as potential wetland restoration sites. Certain species of dytiscid beetles (e.g. *A. mediatum* and *D. faciventrus*) may also be useful indicators of wetland macroinvertebrate community composition characteristics. In cases where macroinvertebrate communities are not diverse, inoculation of degraded or created wetlands by sphagnum moss may help to restore habitat characteristics that promote diversity. For these reasons, sphagnum wetlands should be considered important aquatic habitats that support unique communities of wetland organisms and thereby maintain local and regional diversity.

Chapter 4: Differences in prey regimes of predaceous diving beetles (Coleoptera: Dytiscidae) indicated through mandible morphology, spatial patterns, and seasonal change

Abstract

Predaceous diving beetle larvae (Coleoptera: Dytiscidae) are important predators in freshwater systems. Because they are obligate predators, new insights into the feeding ecology of dytiscid larvae can be gained by studying the morphology of their mandibles. In this study, I compared first, second, and third instar larvae of *Matus bicarinatus*, *Matus ovatus*, and *Agabus punctatus*. I hypothesized that 1) mandible characteristics of *M. bicarinatus* and *M. ovatus* would be more similar to each other than either was to *A. punctatus*, 2) given similar body size, differences between the species would be due to differences in mandible shape rather than mandible size, and 3) differences between mandible morphology would be present in all instars. Sampling was conducted during April, May, and June of 2013 across 20 Delmarva Bay wetlands. Mandibles were dissected from each individual collected. Seven morphological characteristics were measured on each mandible and compared within and among species. *M. bicarinatus*, *M. ovatus*, and *A. punctatus* were found in 15 out of the 20 wetlands sampled. These three species co-occurred in 9 out of these 20 wetlands. *A. punctatus* was collected during April and May, and *M. bicarinatus* and *M. ovatus* were found during May and June. Morphometric analyses of the mandibles indicated that upper and lower limits of prey size for these three species are similar. However, mandible characteristics indicated differences in piercing force between genera, with the mandibles of *A. punctatus* likely better suited to fragile prey that is less resistant to capture. I also found that changes between instars of *Matus* are isometric, while changes in *Agabus* are allometric. These results indicate that different genera likely exploit different prey regimes. Because of these potential differences in prey between species, it is

possible that changes in dytiscid beetle community composition have corresponding changes on consumptive effects of prey species.

Introduction

Predaceous diving beetles (Coleoptera: Dytiscidae) are ubiquitous predators in lentic freshwater systems. They are the most diverse group of aquatic beetles with approximately 500 species occurring in North America (Larson *et al.* 2000). They also have well documented consumptive and non-consumptive effects on wetland communities. Predation by dytiscid beetles can change density and demography of prey populations (Arts, Maly & Pasitschniak 1981) and trigger trophic cascades (Cobbaert, Bayley & Greter 2010). Dytiscid beetles also provide predator cues that deter oviposition by prey species (Ohba *et al.* 2012) and can alter prey behavior (Herwig & Schindler 1996). Some species prey preferentially on aquatic pests such as mosquitoes, and dytiscids may be an important source of biocontrol (Lundkvist *et al.* 2003; Chandra *et al.* 2008; Culler & Lamp 2009)

Dytiscid beetles are generalist predators (Batzer & Wissinger 1996; Culler, Ohba & Crumrine 2014). In laboratory settings, they will eat a wide range of prey items presented to them (Culler & Lamp 2009). Additionally, intraguild predation is well documented in dytiscid beetles (Yee 2010), which may act as a density dependent control on dytiscid population sizes (Juliano & Lawton 1990). Despite potential for competition and intraguild predation, dytiscid beetles can co-occur in diverse communities (e.g. Chapter 3). Co-occurrence of dytiscid beetles is likely facilitated by factors that mitigate competition, such as the specialization of prey regimes (Nilsson 1986).

All dytiscid beetles have three larval instars, followed by pupation and emergence of adults. Adults have primitive chewing mandibles that are used to tear tissue from prey (Kehl & Dettner 2003). They are opportunistic predators, and some have been shown to prefer dead prey (Velasco & Millan 1998). By comparison, dytiscid larvae are obligate predators that have hollow, falcate

mandibles that inject prey with hydrolytic enzymes, thereby employing extra-oral digestion to break down prey tissue (Holmen 1987). Because of this feeding strategy, the architecture of larval mandibles may be closely related to the prey species that can be consumed (Wall, Barman & Beals 2006). Evidence suggests that mandibles of larvae may also be specialized between instars of the same species (Brannen, Barman & Wall 2005). Because of their obligate predatory behavior and high abundances in aquatic systems when compared to adults, this study focuses on larval dytiscid beetles.

The dytiscid genera *Agabus* and *Matus* commonly co-occur in wetlands of the eastern United States (see Chapter 3) and have similar body size (Barman 1996; Larson *et al.* 2000). *Agabus* includes a number of species that are known to be active predators that can move freely through the water column (Larson *et al.* 2000). They are often found in habitats with dense vegetation (Barman *et al.* 2016) and may prey preferentially on mosquitoes (Culler & Lamp 2009). Comparatively little is known about the ecology of *Matus*, though they are unique among the dytiscids in that they have pro- and mesothoracic tarsi modified into pseudo-chelae (Alarie, Watts & Nilsson 2001).

I compared morphological characteristics of mandibles as well as patterns of spatial and temporal abundance of *Agabus punctatus*, *Matus bicarinatus*, and *Matus ovatus*, three of the most commonly occurring dytiscid species in Delmarva Bay wetlands. Based on the phylogenetic relatedness of these species, I hypothesized that the two congeneric species of *Matus* would have more similar morphological characteristics and patterns of spatial and temporal distribution to each other than to *A. punctatus*. Because all three species have similar body size, I also hypothesized that differences in mandible morphology would be due to differences in mandible shape rather than mandible size. Finally, I hypothesized that these

differences in mandible morphology between genera would be present in first, second, and third instar larvae.

Methods

Sampling locations

The Delmarva Peninsula is a region of the mid-Atlantic Coast of the United States that includes Maryland's eastern shore, part of Virginia, and the state of Delaware. *A. punctatus*, *M. bicarinatus*, and *M. ovatus* were collected from Delmarva Bays, seasonal non-tidal wetlands located on the Delmarva Peninsula.

The Delmarva Bays included in this study were characterized by wetland plants common to this region, including shrubs such as *Cephalanthus occidentalis* (common buttonbush), *Clethra alnifolia* (sweet pepperbush), and *Eubotrys racemose* (fetterbush), sedges such as *Carex striata* (walter's sedge), and grasses such as *Scirpus cyperinus* (kunth woolgrass). Common aquatic macrophytes include *Utricularia* spp. (bladderwort), *Proserpinaca pectinata* (mermaid weed), and *Sphagnum* spp. (sphagnum moss). The hydrology of the sites included was driven principally by ground-water incursions, leading to drying in July or August and re-wetting through the fall.

I chose 20 sampling locations from wetlands across Queen Anne's County and Caroline County in Maryland, and Kent County and New Castle County in Delaware. Sites were chosen so that they would be accessible for regular sampling and to represent a range of different sizes and vegetative communities (see Chapter 3 for additional site details).

Dytiscid sampling

Sampling was conducted once a month during April, May, and June of 2013. A map of ten random points was generated for each wetland and sampling was conducted at these points each

month. I collected adult and larval dytiscid beetles using a 500 μ m sweep net. Field samples were stored and transported to the lab in jars of 80% ethanol. In the lab, samples were sorted under magnification and all dytiscid beetles were removed. As a preliminary step, dytiscid beetles were identified to genus, using local and regional keys. Each specimen was then transferred to an individual vial filled with ethanol and stored in a -20° C freezer. Dytiscid beetles were then identified using DNA barcoding at the Laboratories of Analytical Biology (LAB) at the Smithsonian National Museum of Natural History. Additional details on sampling and barcoding protocols are included in Chapter 3.

Mandible measurements

Heads of all specimens were removed and photographed under magnification. The right mandible was then dissected from each beetle and mounted in a plastic coin protector for preservation. In 16 instances, the right mandible was not usable and the left mandible, which is symmetrical to the right, was dissected. Photographs were taken using a Zeiss microscope and camera, and images were initially processed using Zeiss Zen software. Photos were then annotated in Adobe Photoshop. Annotations included adding guides such as the mid-point of the mandible and a line perpendicular to mandible apex. The ObjectJ plugin of ImageJ software (version 1.48) was then used to place nondestructive markers on each image. These markers were used to calculate chosen parameters for each image.

Based on the work of Wall et al. (2006) and Barman et al. (2014), I measured seven parameters on each specimen that were chosen because they have been shown to be biologically informative regarding feeding ecology. Two parameters were measured on the head, including head length, measured from the posterior edge of the coronal suture to the anterior edge of the frontoclypeus, and head width, which measured the widest part of the head capsule. On the

mandibles, I measured mandible length, medial arc, lateral arc, and angle of attack (Figure 4.1). Mandible length was defined as the distance from the mandible joint to mandible apex. Medial arc was defined as the angle formed by lines connecting the joint and apex of the mandible at the midpoint of the mandible on the medial edge. Similarly, the lateral arc represented the same measure at the midpoint of the lateral edge. Angle of attack was defined as the angle formed by a line perpendicular to the mandible apex and a line passing through the mandible apex and the lateral edge of the mandible at a distance of $1/10^{\text{th}}$ the total length of the mandible from the apex. Finally, I calculated proportional length of the mandible as mandible length divided by the length of the head. Pictures of representative mandibles of third instar larvae from each species are included in Figure 4.2.

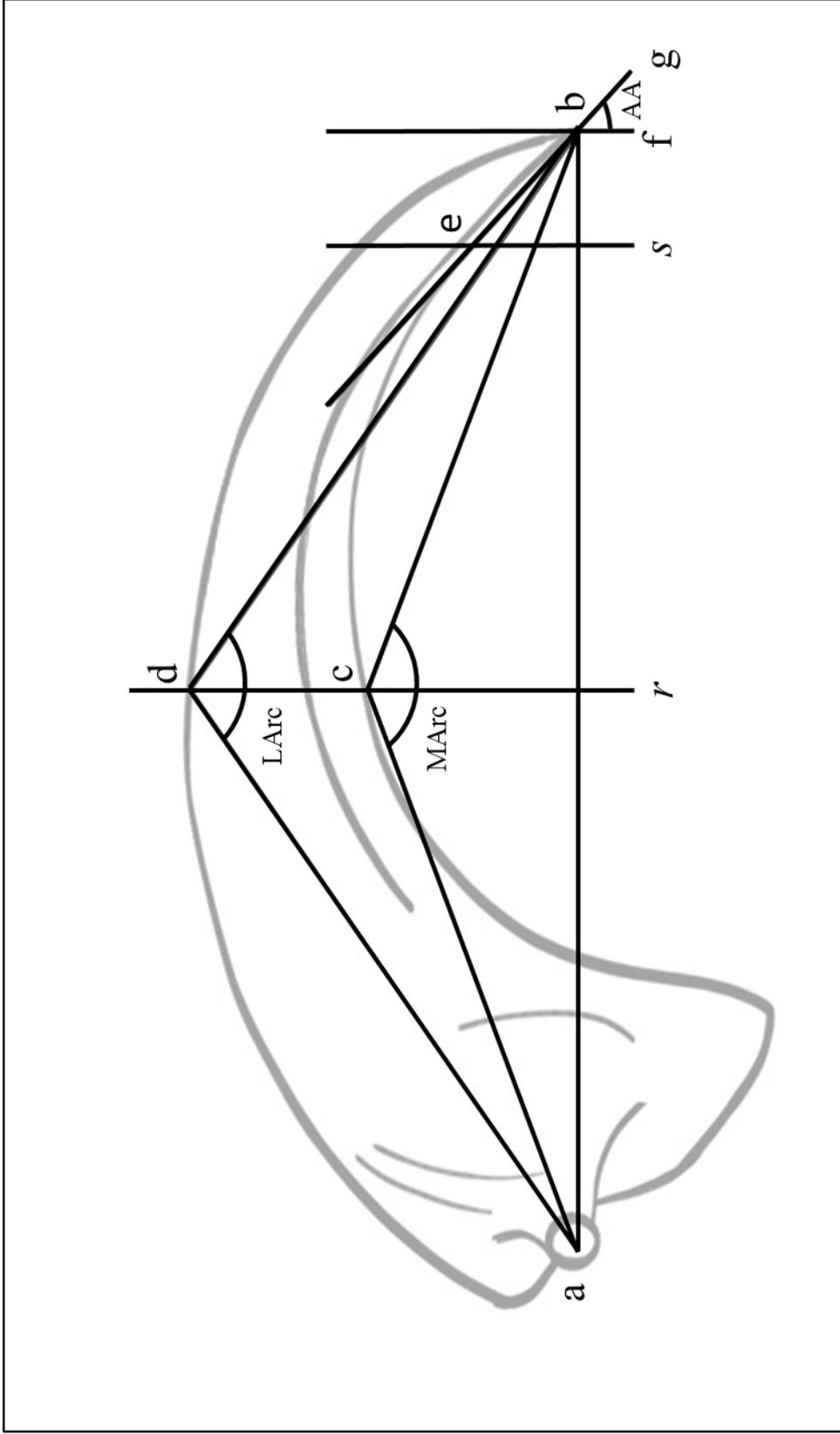


Figure 4.1. Diagram of measures calculated for each mandible from all dytiscid beetle larvae collected. The annotations depicted in the figure above were made to mandibles collected from each beetle in order to measure chosen morphological parameters. Point a represents the joint at which the mandible meets the head and point b represents the tip of the mandible. Points c and d represent the medial and lateral edges of the mandible at the midpoint of line ab. Line s is 1/10th the length of the mandible from the mandible tip, and e represents the point at which this line meets the medial edge of the mandible. The parameters measured included mandible length (length of line ab), medial arc (MArc = angle acb), lateral arc (LArc = angle adb), and angle of attack (AA = angle fbg).

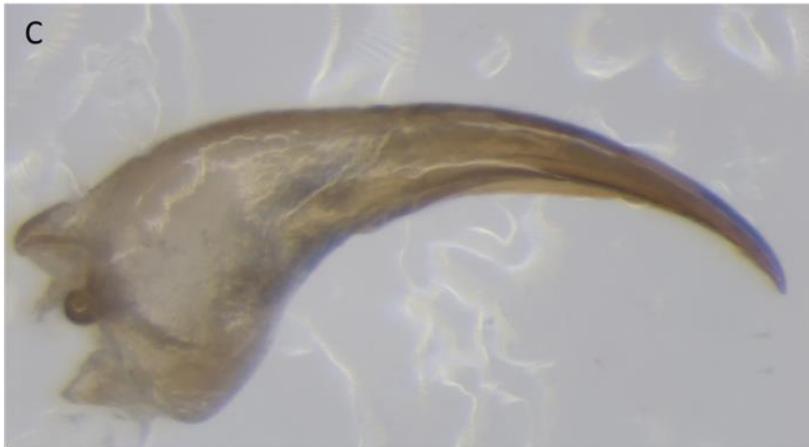
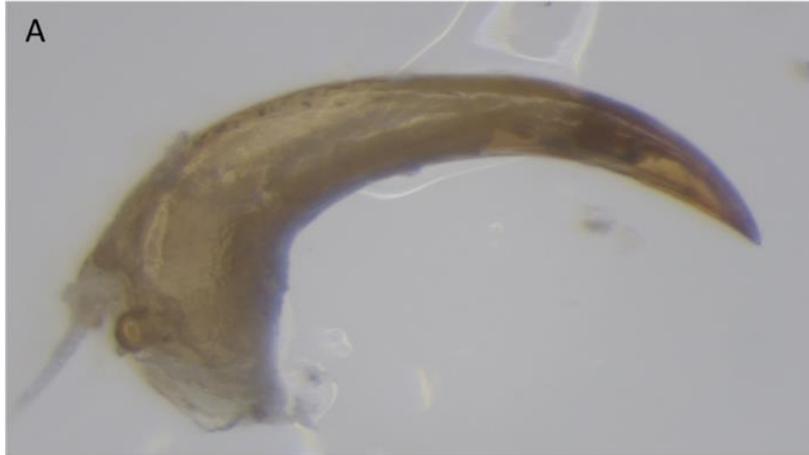


Figure 4.2. Mandibles dissected from dytiscid beetle larvae collected from Delmarva Bay wetlands in 2013. These mandibles represent third instar larvae from the three species collected, *Matus bicarinatus* (A), *Matus ovatus* (B), and *Agabus punctatus* (C).

Statistical Analyses

All analyses were performed using R statistical software (R Core Team 2014). Means and standard deviations for each parameter for each species for each instar were calculated. I used the *manova* function from the stats package (R Core team 2014) to perform a two-way multivariate analysis of variance (MANOVA) to compare morphological parameters both within and between species. Species and instar were included as independent variables and all morphological parameters measured were included as response variables. MANOVA was followed by a series of one-way analysis of variance (ANOVAs), performed using the *aov* function from the stats package (R Core team 2014) on each of the significantly different parameters. These ANOVAs were combined with Tukey-Kramer Multiple Comparisons Tests to examine individual mean difference for comparisons between instars of the same species and instars of different species. I also used the function *prcomp* (R Core Team 2014) to perform a principal components analysis (PCA) on centered and standardized data and graphed these results in a biplot. The PCA included all species and all instars.

Results

Spatial and Temporal Distribution

In the 15 out of 20 wetlands where at least one of these species was found, eight wetlands had more than one of the three target species. All three species co-occurred in seven wetlands. Six wetlands had *A. punctatus* only, one wetland had *M. ovatus* only, and one wetland had both species of *Matus* without *A. punctatus* (Table 4.1).

A total of 242 larvae were collected (132 *A. punctatus*, 56 *M. ovatus*, and 54 *M. bicarinatus*) and the abundance of these three species varied across sampling dates. *A. punctatus* was the only species collected during April and all three instars were collected. All three species were collected in May, and *A. punctatus* was the most abundant. All instars were collected for *A.*

punctatus and *M. ovatus* in May, and first and second instar larvae were collected for *M. bicarinatus*. In June, *M. ovatus* and *M. bicarinatus* were collected. All three instars were present for both species and *M. bicarinatus* was more abundant than *M. ovatus* (Figure 4.3).

Table 4.1. Sites where target species of dytiscid beetle larvae, *M. bicarinatus*, *M. ovatus*, and *A. punctatus*, were found. Wetland site names in the first column correspond to sites in Chapter 3. Additional details for each site can be found in Chapter 3. The county and geographical location for each site are provided, along with size and pH of the site, which represent overall site characteristics. Presence of these the three target species collected is indicated by an “x” in the corresponding column.

<i>Site</i>	<i>Location</i>	<i>Lat, Long</i>	<i>Area (Ha)</i>	<i>pH</i>	<i>M. bicarinatus</i>	<i>M. ovatus</i>	<i>A. punctatus</i>
S1	New Castle County, DE	39.31449, -75.75139	0.46	4.4	x	x	x
S7	Caroline County, MD	39.05465, -75.7533	2.03	4.6	x	x	x
S8	Queen Anne’s County, MD	39.15003, -75.81287	1.53	4.3	x	x	x
S9	Caroline County, MD	39.07213, -75.79312	0.08	3.9	x	x	x
N1	New Castle County, MD	39.34698, -75.74212	0.72	4.7	x	x	x
N4	New Castle County, MD	39.35027, -75.74389	0.48	4.5	x	x	x
N8	Caroline County, MD	39.05378, -75.74861	0.4	5.6	x	x	x
S6	Caroline County, MD	39.05743, -75.75322	1.84	4.8	x	x	
N5	Kent County, MD	39.33367, -75.77654	0.43	4.9		x	
S4	Caroline County, MD	39.05393, -75.83503	0.55	3.8			x
S5	Caroline County, MD	39.05533, -75.8333	0.18	4.1			x
N3	New Castle County, MD	39.34886, -75.74145	0.14	4.1			x
N6	Caroline County, MD	39.05223, -75.75521	2.9	6			x
N7	Caroline County, MD	39.05134, -75.75837	0.42	5.8			x
N10	Queen Anne’s County, MD	39.14891, -75.81503	0.16	4.3			x

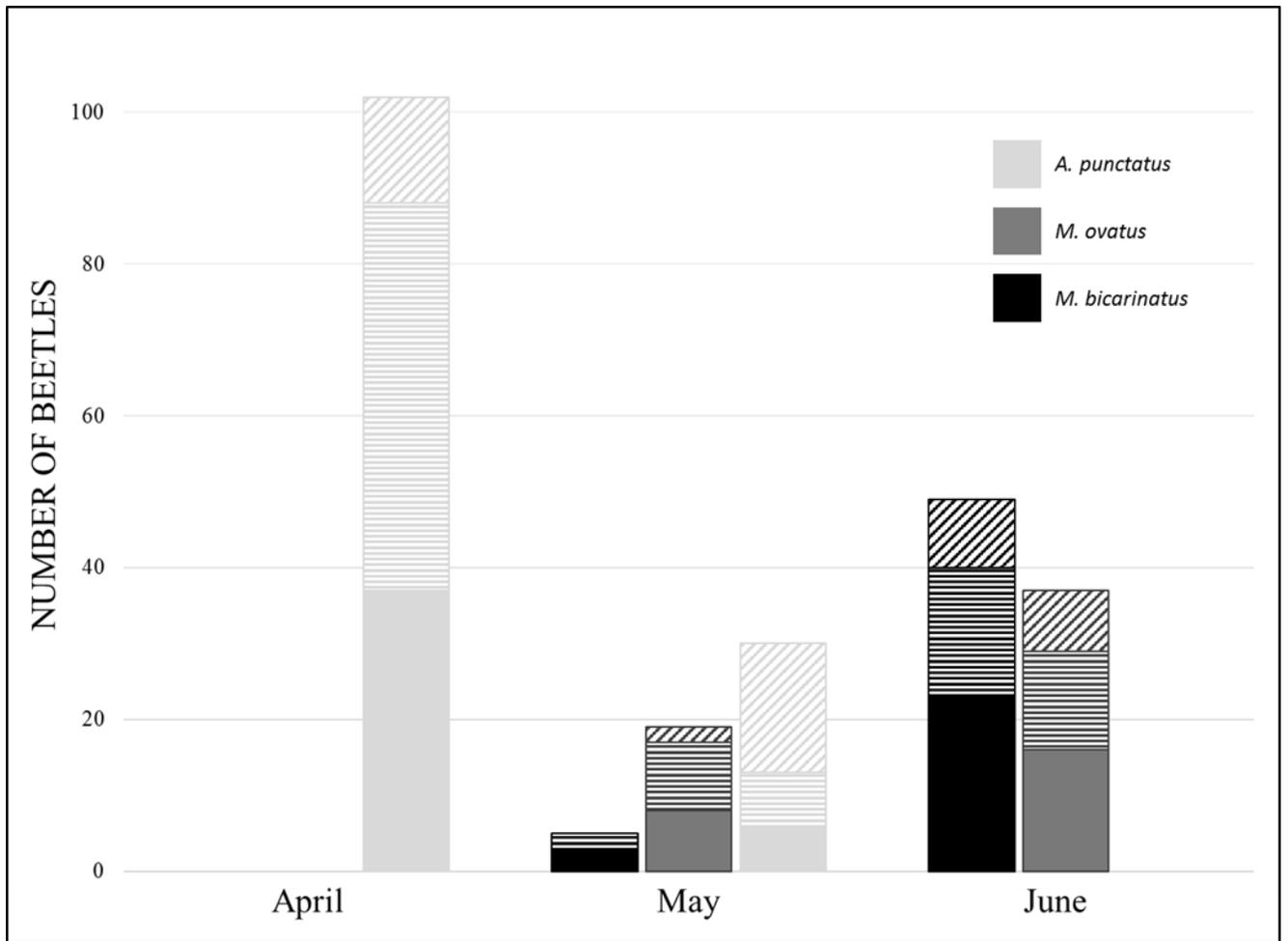


Figure 4.3. Abundance dytiscid beetle larvae of *Matus bicarinatus*, *Matus ovatus*, and *Agabus punctatus* collected from Delmarva Bay wetlands during April, May, and June of 2013. These abundances are presented as number of beetles found during each month. Each species is represented by a different color and each instar is represented by a different pattern. The solid section of each column represents first instar larvae. The horizontal lines and diagonal lines represent second and third instar larvae, respectively.

Morphological Characteristics

A summary of mean characteristics for each species for each instar can be found in Table 4.2.

A statistically significant MANOVA effect was obtained for the main effects of species (Pillais' trace=1.05, $p < 0.001$), and instar (Pillais' trace=1.31, $p < 0.001$). A significant effect was also found for the interaction between species and instar (Pillais' trace=1.03, $p < 0.001$). Because the interaction term was significant, the following analyses focus on the simple effects of species and instar.

Parameters that characterized shape (lateral arc, medial arc, and angle of attack) were not significantly different between instars of *A. punctatus*. An ANOVA indicated that angle of attack was significantly different between instars of *M. bicarinatus*, $F(2, 51) = 6.15$, $p < 0.01$, and the post hoc Tukey test indicated that first instar larvae had a significantly more obtuse angle of attack than second and third instar larvae ($p < 0.05$). ANOVA yielded significant differences in lateral arc between instars of *M. ovatus*, $F(2, 53) = 4.38$, $p < 0.05$, and the Tukey test indicated that second instar larvae had a more acute angle of attack than third instar larvae ($p < 0.05$) with first instar larvae intermediate between the two. Angle of attack was also significantly different between instars of *M. ovatus*, $F(2, 53) = 9.99$, $p < 0.001$, with third instar larvae having a more obtuse angle of attack than either second or first instar larvae ($p < 0.05$).

The following results are based on ANOVAs and post hoc Tukey tests. A full list of the test statistics can be found in Table 4.3. The mandibles of *A. punctatus* had a shorter proportional length in all instars relative to either *M. bicarinatus* or *M. punctatus*. The length of the mandibles of second and third instar larvae were not significantly different between *A. punctatus* compared to either species of *Matus*. However, the medial arc, lateral arc, and angle of attack were all significantly different between *A. punctatus* compared to either species of *Matus* for all instars. In all instars, the mandibles of *A. punctatus* had a more obtuse medial arc, lateral arc, and angle of attack. Cranial morphology of *Agabus* was also significantly different than that of *Matus* for all instars. In third instar larvae, *M. bicarinatus* had narrower heads and shorter mandibles than *M. ovatus*. There were no other significant differences between parameters measured on first and second instar larvae between species of *Matus*.

The first two axes of the PCA biplot for all first instar larvae accounted for 91% of the variance between parameters measured (PC1= 66%, PC2= 25%, Figure 4.4). Medial arc, lateral

arc, angle of attack, head length, head width and proportional length were all negatively correlated with PC1, while mandible length was positively correlated with PC1. Mandible length, head length, head width, and proportional length were negatively correlated with PC2; all other measures were positively correlated.

Table 4.2. Mean and standard deviation for each morphological characteristics for all dytiscid beetle larvae of *Matus bicarinatus*, *Matus ovatus*, and *Agabus punctatus*. These statistics are grouped by species and instar. All measures of length are given in mm and all angles are presented in degrees. Descriptions of all measures provided are included in

Species	Instar	Head Length (mm)	Head Width (mm)	Mandible Length (mm)	Proportional Length	Medial Arc	Lateral Arc	Angle of Attack
<i>M. bicarinatus</i>	I	0.56 ± 0.02	0.57 ± 0.02	0.46 ± 0.02	0.83 ± 0.04	136.23 ± 5.45	111.11 ± 4.13	81.21 ± 0.88
	II	0.85 ± 0.03	0.82 ± 0.03	0.61 ± 0.02	0.72 ± 0.03	137.07 ± 4.93	109.34 ± 2.96	81.31 ± 0.53
	III	1.18 ± 0.05	1.14 ± 0.06	0.74 ± 0.03	0.62 ± 0.03	138.33 ± 3.57	111.65 ± 1.77	82.18 ± 0.59
<i>M. ovatus</i>	I	0.56 ± 0.04	0.57 ± 0.03	0.46 ± 0.01	0.82 ± 0.07	136.65 ± 4.15	110.97 ± 2.86	81.18 ± 0.83
	II	0.86 ± 0.04	0.84 ± 0.05	0.62 ± 0.05	0.72 ± 0.06	137.96 ± 3.84	109.08 ± 2.54	81.44 ± 0.61
	III	1.26 ± 0.08	1.24 ± 0.08	0.80 ± 0.04	0.64 ± 0.04	139.79 ± 7.35	112.66 ± 5.37	82.55 ± 1.15
<i>A. punctatus</i>	I	0.89 ± 0.04	0.82 ± 0.04	0.42 ± 0.02	0.47 ± 0.02	151.29 ± 9.22	120.56 ± 5.85	83.73 ± 1.13
	II	1.32 ± 0.07	1.16 ± 0.06	0.60 ± 0.03	0.46 ± 0.02	150.65 ± 6.8	119.59 ± 4.64	83.81 ± 0.95
	III	1.69 ± 0.2	1.39 ± 0.17	0.76 ± 0.06	0.45 ± 0.04	149.57 ± 6.98	121.75 ± 3.86	83.77 ± 0.99

Table 4.3. The results of analysis of variance (ANOVA) and post hoc Tukey tests performed to compare seven different parameters characterizing mandible morphology of among three species of dytiscid beetle larvae. Tests were divided between instars. F values and degrees of freedom (df) for the ANOVA of each comparison are presented. One value is provided for degrees of freedom for each instar as the sample size for all ANOVAs within in each instar was the same. P-values from post hoc tests are also included to indicate significant differences between means of groups. The letters “ns” indicates that the comparison was not significantly different.

	<i>M. bicarinatus</i> vs. <i>M. ovatus</i>		<i>M. bicarinatus</i> vs. <i>A. punctatus</i>		<i>M. ovatus</i> vs. <i>A. punctatus</i>	
	<i>F</i>	<i>df</i>				
Instar I						
Head Length	10.29	2, 90	ns	< 0.05	< 0.05	< 0.05
Max. Head Width	68.69		ns	< 0.05	< 0.05	< 0.05
Mandible Length	54.12		ns	< 0.05	< 0.05	< 0.05
Proportional Length	17.05		ns	< 0.05	< 0.05	< 0.05
Medial Arc	48.8		ns	< 0.05	< 0.05	< 0.05
Lateral Arc	45.96		ns	< 0.05	< 0.05	< 0.05
Angle of Attack	75.18		ns	< 0.05	< 0.05	< 0.05
Instar II						
	<i>F</i>	<i>df</i>				
Head Length	69.65	2, 96	ns	< 0.05	< 0.05	< 0.05
Max. Head Width	43.38		ns	< 0.05	< 0.05	< 0.05
Mandible Length	2.27		ns	ns	ns	ns
Proportional Length	67.92		ns	< 0.05	< 0.05	< 0.05
Medial Arc	58.53		ns	< 0.05	< 0.05	< 0.05
Lateral Arc	81.94		ns	< 0.05	< 0.05	< 0.05
Angle of Attack	10.54		ns	< 0.05	< 0.05	< 0.05
Instar III						
	<i>F</i>	<i>df</i>				
Head Length	48.88	2, 47	ns	< 0.05	< 0.05	< 0.05
Max. Head Width	12.21		< 0.05	< 0.05	< 0.05	< 0.05
Mandible Length	3.84		< 0.05	ns	ns	ns
Proportional Length	121.9		ns	< 0.05	< 0.05	< 0.05
Medial Arc	14.92		ns	< 0.05	< 0.05	< 0.05
Lateral Arc	34.74		ns	< 0.05	< 0.05	< 0.05
Angle of Attack	12.63		ns	< 0.05	< 0.05	< 0.05

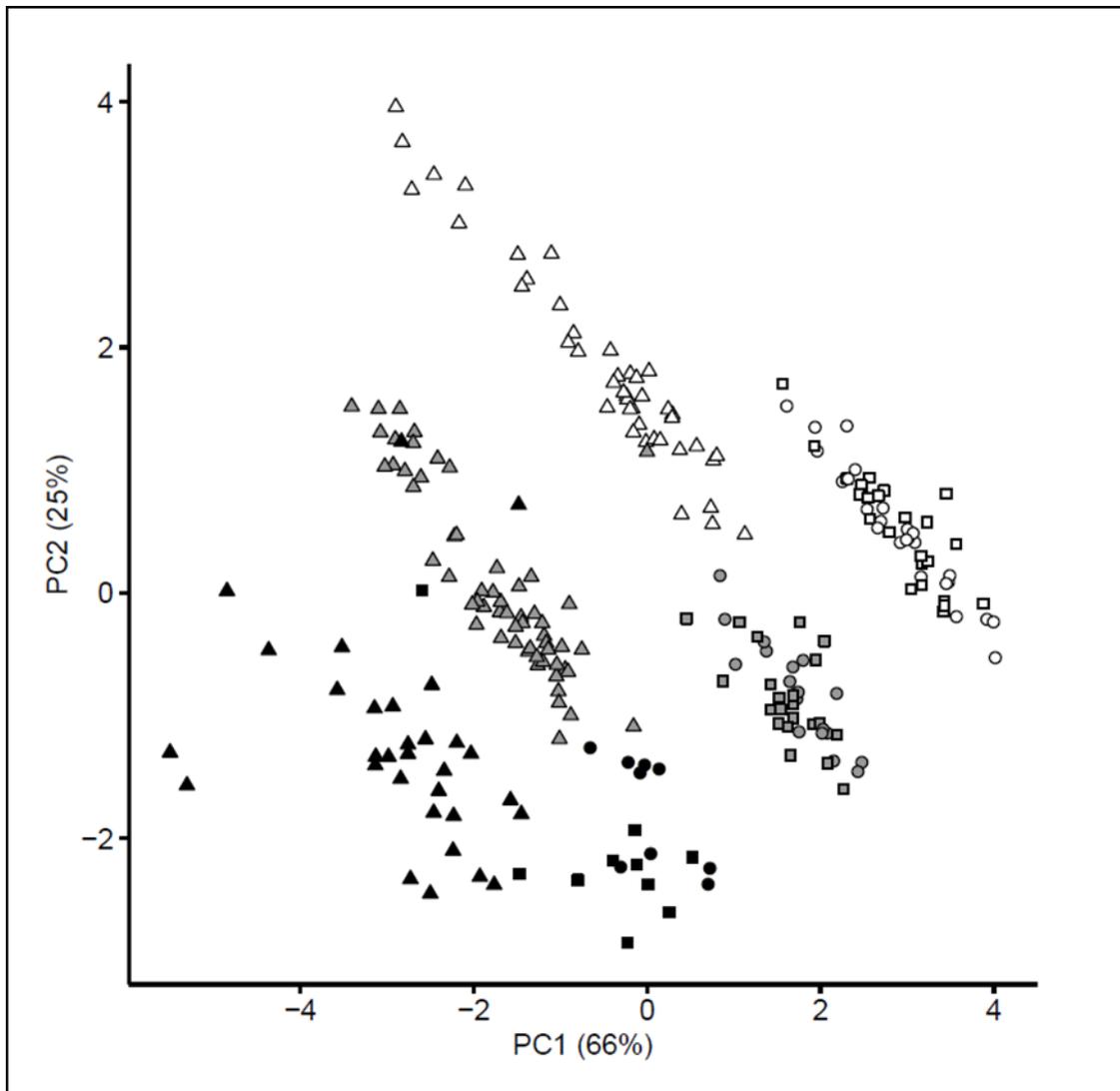


Figure 4.4. Principal components analysis (PCA) biplot representing variation between three species of dytiscid beetle larvae for seven morphological parameters measured. Triangles represent *Agabus punctatus*, circles represent *Matus bicarinatus*, and squares represent *Matus ovatus*. Points representing first instar larvae are black, second instar larvae are gray, and third instar larvae are white. The seven measures taken include medial arc, lateral arc, angle of attack, head length, head width, mandible length, and proportional length. Medial arc, lateral arc, angle of attack, head length, head width and proportional length were all negatively correlated with PC1, while mandible length was positively correlated with PC1. Mandible length, head length, head width, and proportional length were negatively correlated with PC2; all other measures were positively correlated.

Discussion

The aim of this study was to compare mandible morphology of three species of dytiscid beetle, *Agabus punctatus*, *Matus bicarinatus*, and *Matus ovatus*. As abundant wetland predators, dytiscid beetles alter prey community composition and are a potential source of biocontrol for

aquatic pests. Describing mandibular morphology of dytiscid beetles can aid in evaluating potential prey regimes and offer insights into their ecological role. As was hypothesized, a comparison between these species indicated more similar morphology within genera than between genera, though mandible size between these three species was similar for second and third instar larvae. Similarity in size may indicate similar upper and lower limits to prey size for all species at these life stages (Young 1967; Holmen 1987). The parameters that characterized mandible shape (i.e. medial angle, lateral angle, and angle of attack) were found to be different between *Agabus* and *Matus*, and these differences persisted across instars. Variation in morphological characteristics indicates that the mandibles of these two genera may be suited for handling different types of prey (Wall *et al.* 2006). As these two genera were also found to occur in peak abundances during different months, it is likely that they are exploiting different prey regimes.

Fifteen out of the 20 wetlands sampled contained one or more of these common predator species. Though *Agabus* was found at more wetlands than *Matus*, *Agabus* and *Matus* were found in seven of the same wetlands. Patterns of temporal partitioning were clear, with *Agabus* collected in highest abundances in April without *Matus*, and *Matus* being collected in highest abundances in June without *Agabus*. As the available community of available prey also changes seasonally (see Chapter 3) it is likely that these genera are encountering different macroinvertebrates in April compared to May and June.

Differences among instars of the same species likely reflected the general increase in size. The cranial shape of both species of *Matus* was approximately ovoid, with head length approximately equal to head width. An ovoid head shape was consistent across all instars, which suggests isometric growth between instars (Barman 1996). Isometric growth would suggest that

the larvae of *Matus* are consuming increasingly larger members of the same species across instars (Brannen *et al.* 2005). Possible changes in prey characteristics for different instars of *A. punctatus* are not as clear, as the head shape of *A. punctatus* varied from first to third instar. Similar measures of a closely related species, *A. disintegratus*, suggests that changes in cranial structure between instars are allometric (Brannen *et al.* 2005; Bridges, Wall & Barman 2009). Allometric changes would indicate that different instars of *Agabus* are consuming prey with different characteristics, and likely changing prey species (Bridges *et al.* 2009).

A comparison of mandibular morphology indicated clear differences between species, and these differences are likely to be linked to differences in feeding ecology (Sasakawa 2015). The mandibles of *A. punctatus*, have a larger medial arc, lateral arc, and angle of attack than either *Matus* species. As a result, the mandibles of *A. punctatus* have a straighter contour when compared to *Matus*. The more strongly curved mandibles of *Matus* are likely better suited to prey items with a relatively tougher integument that requires greater piercing force for consumption or that offer greater resistance to capture (Wheater & Evans 1989; Wall *et al.* 2006). Resistance of prey to capture is likely to be an important factor in prey selection as force at the mandible tip is required to hold onto prey after it has been captured (Gorb & Beutel 2000). Greater piercing force at the mandibular tip is represented by a smaller angle of attack (Wall *et al.* 2006), as seen in *M. bicarinatus* or *M. ovatus* compared to *A. punctatus*. However, there may also be trade-offs in these species between piercing force and piercing speed, such that *A. punctatus* may be able to capture prey that moves more quickly (Gronenberg *et al.* 1997; Paul & Gronenberg 1999; Levinton & Allen 2005).

The only significant difference in mandibular morphology between *M. ovatus* and *M. bicarinatus* based on characteristics that were measured in this study was a difference in

mandible length of third instar larvae, though this was small enough to call into question its biological importance. The similarity of these mandible characteristics is somewhat surprising, given that it suggests similarity of prey regimes (Sasakawa 2015), despite co-occurrence of both species in the same habitats and at the same time of year. The overall similarity of these species differs from research comparing congenics within *Agabus*, which indicated different mandibular morphology, and thereby different *in situ* prey regimes (Barman *et al.* 2016). Similarity between species of *Matus* may be explained in part by the fact that *Matus* is typically found in wetlands that have vegetation which increases habitat structural complexity (see Chapter 3). Such plants include sphagnum moss, (Sphagnales: Sphagnaceae) which are likely to provide important microhabitat for macroinvertebrates (Henrikson 1993). Additionally, wetland plant density is positively correlated with dytiscid abundance (Yee, Taylor & Vamosi 2009). For *Matus*, habitat characteristics may be more important for species co-occurrence than overlap in prey regime.

Other morphological features that differ between these species indicate that they are likely to encounter different prey. For example, the presence of robust natatory sensilla on *A. punctatus* (Barman 1996) suggests that these organisms may be active hunters that seek out prey in open areas of the water column. On *Matus*, the pro- and mesothoracic tarsi are modified into pseudo-chelae (Alarie *et al.* 2001). Though the function of the tarsi is not known, this unique characteristic is likely related to the ecology of *Matus*.

I found evidence that different dytiscid species exhibit distinguishable mandibular morphology that may allow them to make use of different resources than other similarly sized species. Though *M. bicarinatus*, *M. ovatus*, and *A. punctatus* may have similar upper and lower limits to prey size, they likely exploit different prey regimes, as indicated by the overall

difference in mandible architecture and differences in temporal abundance. Because dytiscid beetles are important wetland predators, this finding indicates that changes in the species composition of dytiscid communities may cause changes in consumptive effects on co-occurring prey species. These changes in consumptive effects may be especially important for biological control of aquatic pest species, as the ability of dytiscid beetles to suppress pests will change based on community composition. For dytiscid beetles, details of the feeding ecology of individual species may improve knowledge of their effect on aquatic macroinvertebrate communities.

Chapter 5: Synthesis, application, and future directions

Synthesis

Wetland macroinvertebrate communities change in response to environmental characteristics. As a result, monitoring macroinvertebrate communities can inform wetland restoration and management. Both holistic community monitoring and the use indicator species provide approaches to wetland bioassessment that can indicate establishment of desired ecological processes. Bioassessment using wetland macroinvertebrates enables land managers to evaluate restoration success for both long-term projects and at large spatial scales. The following summary of the previous chapters connects key insights from this work that can inform use of macroinvertebrate community monitoring in wetland restoration.

Chapter 2 examined long-term changes in macroinvertebrate community composition between a natural, a rehabilitated, and a created wetland. Results of this study indicated that a natural macroinvertebrate community may recover more quickly in an impaired wetland that has been rehabilitated than in a newly created wetland. In Delmarva Bays, recovery may be most evident in changes in primary consumer composition, with freshwater isopods being numerically dominant in natural wetlands and aquatic midge larvae being numerically dominant in impaired or created wetlands. Therefore, aquatic isopods may be appropriate indicator species for bioassessment in Delmarva Bay wetlands. Inoculation of restored wetlands by these organisms, as well as other passive dispersers, may also speed the establishment of a macroinvertebrate community that approximates that of a natural wetland. In the case of rehabilitated wetlands, establishment of a natural macroinvertebrate community may take up to 9 years, as was the case in this study. Nine years was not an adequate length of time for a natural macroinvertebrate to

establish in the created wetland. In similar created sites, restoration goals may not be met during the first decade following restoration.

Chapter 3 examines differences in macroinvertebrate community composition between wetlands with and without sphagnum moss. Sphagnum moss is an important ecosystem engineer that is commonly found in Delmarva Bays, though its absence can be an indication of habitat impairment. Wetlands containing sphagnum moss compared to wetlands without sphagnum moss had different and more diverse assemblages of dytiscid beetles. Sphagnum wetlands provided habitat for 14 dytiscid species that were exclusively found in sphagnum wetlands, as compared to the four species found exclusively in non-sphagnum wetlands. Higher diversity of dytiscids in non-sphagnum wetlands may be associated with changes in habitat characteristics caused by sphagnum, such as increased habitat complexity created by sphagnum growth. As a result, sphagnum wetlands may be high priority sites for protection and restoration. Similarly, inoculation of restored wetlands with sphagnum may promote macroinvertebrate diversity.

Chapter 4 uses species traits of closely related dytiscid beetles to evaluate potential differences in feeding ecology. Taxonomic diversity of macroinvertebrate communities does not always correspond to functional diversity, which is better addressed by comparing species traits. This chapter was based on a comparative analysis of mandible morphology between three species of dytiscid beetles. Results suggest that these species have significantly different mandibular architecture, which may lead to preference for and consumption of different prey items. Knowledge of species ecology for many macroinvertebrates is lacking, yet this knowledge is often indispensable for the purposes of successful wetland management. Studies like this one that measure traits related to the ecology of individual species can help land managers prioritize organisms and communities that will best support desired ecological processes.

Application

These findings regarding the importance of wetland history, wetland vegetation, and macroinvertebrate species traits have practical implications for wetland restoration and management. If the goal of wetland restoration is to promote the establishment of a macroinvertebrate community that approximates that of a natural wetland, rehabilitation of existing wetlands should be prioritized over wetland creation. In cases where rehabilitation is not possible, created wetlands may benefit from the introduction of plants species that create habitat characteristics of natural wetlands, such as sphagnum moss in Delmarva Bays. The presence of these plants may facilitate the establishment of diverse communities of macroinvertebrate predators, especially beetles. By promoting predator diversity, wetland managers may be able to enhance ecosystem services provided by wetlands, such as the suppression of pest species.

Future directions

The changes within and among macroinvertebrate communities of seasonal wetlands revealed by my research suggest compelling avenues for future study. In general, the predictive power and of my results regarding the relationship between changing wetland communities and changing environmental characteristics would be most advanced by a better understanding of the ecology of select taxa, such as the freshwater isopod *Asellus* and dytiscid beetles in the genus *Matus*. Knowledge of the ecology of these groups could be gained through experimental manipulations that assess changes in population structure, behavior, and feeding of these organisms under varying environmental conditions, and especially in response to the presence or absence of sphagnum moss.

Management goals for imperiled wetland habitats include preserving biodiversity and promoting ecosystem services. Integrating knowledge of community-level responses with species-level data can help to link biodiversity with function by connecting the traits of

individual species to their functional role. Successful wetland management will incorporate this integration, which can help to define specific management objectives and thereby protect the most important characteristics of wetland habitats.

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