12-15-2023

DON'T PUT ALL YOUR EGGS IN ONE BASKET: INCREASING SURVIVORSHIP IN A HEADSTARTING PROGRAM FOR THE ENDANGERED HINE'S EMERALD DRAGONFLY (SOMATOCHLORA HINEANA)

Lauren Michelle Morris
University Of South Dakota

DON'T PUT ALL YOUR EGGS IN ONE BASKET: INCREASING SURVIVORSHIP IN A HEADSTARTING PROGRAM FOR THE ENDANGERED HINE'S EMERALD DRAGONFLY (*SOMATOCHLORA HINEANA*)

By

Lauren Michelle Morris

B.S., Western Kentucky University, 2019

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of Master of Science

Department of Biology

Conservation and Biodiversity Program
In the Graduate School
The University of South Dakota
December 2023

The members of the Committee appointed to examine
the Thesis of Lauren Michelle Morris
find it satisfactory and recommend that it be accepted.

DocuSigned by:
Daniel Soluk
63CC8A5C1AB94B1...
Chairperson

DocuSigned by:
DD3E178E79BE497...

DocuSigned by:
Mark Dixon
1C4CF6FFB73E4A8...

ABSTRACT

In the age of the Anthropocene, wildlife is impacted by the presence of humans in both positive and negative ways. Many humans desire to conserve wildlife and the associated resources that come with it, while simultaneously the ever-expanding human population demands the land, food, and water resources, as well as other materials sourced from wildlife. Insects, a diverse and necessary group of animals are no exception to the impacts currently being experienced by wildlife. Because of this, some species are threatened or endangered, and human intervention is necessary. The federally endangered Hine's Emerald dragonfly (*Somatochlora hineana*) is receiving intervention to augment wild populations via a captive rearing program based primarily at the University of South Dakota, using an approach known as headstarting. In the program, eggs are wild collected from adults in native habitat and brought to a laboratory setting until they are teneral adults, at which time they are released back into the area from which they were sourced. By diminishing potential sources of mortality that newly hatched larvae experience (e.g., competition, predation, and access to adequate prey resources), we increase survivorship, and the number of Hine's emerald dragonflies introduced back into their native habitat. Laboratory experiments in microcosms evaluated the role of density and detrital community in growth and survivorship of hatchlings. Microbenthic community samples were collected in productive *S. hineana* habitat and contrasted with samples collected from microcosms. Results indicate that microbenthic communities from natural habitat were more abundant than laboratory microcosms. Density and components of the microbenthic community, such as Amphipoda and edible prey types, negatively and positively impact survivorship and growth of larvae in microcosms. These findings demonstrate the importance of evaluation of captive rearing protocol in efforts to conserve a species.

Thesis Advisor DocuSigned by: Daniel Soluk 63CC8A5C1AB94B1...

Dr. Daniel A. Soluk

Acknowledgements

Funding for this study was provided by grants to Daniel A. Soluk from the U.S. Fish and Wildlife Service, the Illinois Department of Natural Resources, and the Forest Preserve District of DuPage County Illinois. This project would not have been possible without the housing provided by The Nature Conservancy of Northeast Wisconsin and Michael Grimm. I acknowledge all of those involved through the Hine's emerald dragonfly captive rearing program, including Kristopher Lah and Joe Kath. I'd like to also acknowledge those who made lab and field work possible: Rodrigo Meza, Alyssa Zirpel, Naomi Bingham, Jacob Woelber, and Nikki Kelly. Thank you to my committee members for their assistance through the project development and writing process. A special thank you to Tristan and Laureen Soluk for your extra help and support, especially during breaks and the long field seasons during the summer. Finally, a huge thank you to my parents, my brothers, my cats, my fiancé, and my dear friends for all the love and support through this entire process; I could not have done it without you all.

Dedication

This thesis is dedicated to my parents. My parents have always emphasized to me the importance of higher education and wanted nothing more than for me to pursue my academic and career goals at the University of South Dakota, even if it was a long 900 miles away from home. They have always encouraged me to be independent, follow my dreams, work hard, and to never give up. I love you both more than words can describe, and I cannot thank you enough for your support.

Table of Contents

List of Tables

List of Figures

Thesis Introduction

Conservation of biodiversity is important for maintaining the health of most ecosystems and can benefit humans (Cox and Underwood 2011, Guo, Zhang, Li, 2020). However, the world is facing an unprecedented rate of extinction driven by human activity (Winfree *et al.* 2009, Hallmann *et al*. 2017, Forrister *et al*. 2019). A loss of just a single species can drastically impact an entire ecosystem, creating an extinction vortex, where many other species may also go extinct (Gilpin and Soulé 1986). This is because a loss of a species may result in a loss of its ecosystem role as a potential food source or biotic control. Loss of biodiversity can also impact humans, as there is often a loss of ecosystem services provided by that organism, causing an economic cost to society. Natural extinctions are common and expected, though the rate of anthropogenic-influenced extinction is significantly higher than normal (Turvey and Crees 2019). In the current era, sometimes referred to as the "Anthropocene", extinction rates are as high as 1,000 times the estimated rates prior to the presence and domination of humans (Pimm *et al.* 1995). Observed causes for current declines in biodiversity include global climate change, habitat fragmentation and loss, extirpation through overexploitation and others (Brook, *et al*. 2008, Butchart *et al.* 2010).

Preservation and conservation of insects is becoming increasingly recognized as critical since insect biodiversity losses impact ecosystems of all kinds. In recent years, there have been drastic declines in overall insect biomass and diversity in many parts of the world (Potts, *et al.* 2010) that will undoubtedly be detrimental for many ecosystems. These losses are likely driven by anthropogenic factors such as climate change, habitat destruction, pollution, and widespread use of insecticides (Nicholls *et al.* 2018, Goulson 2019, Hallmann *et al*. 2020). Loss of insect

biomass is less pronounced in areas where land is protected, compared to those where the land is unprotected (van Klink *et al.* 2020), suggesting that there is an association between insect declines and land use intensification. Conversely, other studies suggest a positive trend in freshwater insect abundance coinciding with the protection of waterways (van Klink, *et al*. 2020).

Scientists are beginning to understand how the presence of human-built structures, habitat degradation, and the harvesting of organisms is inducing selection pressures on dispersal and genetic diversity in organisms (Otto 2018). Human intervention can help and through the habitat protection and other conservation efforts a threatened species can return to health once more (Frampton and Dorne 2007, Tonietto and Larkin 2017). Given the environmental shifts, gathering additional data and observations are increasingly necessary to determine species sensitivity and resilience in the Anthropocene.

Under the provisions of the Endangered Species Act of 1973, an "endangered species" is a species that is likely to go extinct in the near future without intervention to stop or slow its rate of decline. A "threatened species" is a species that is of particular concern, as a lack of intervention will likely lead it to become endangered. A species is listed when it experiences: 1) "Present or threatened destruction, modification of curtailment of its habitat", 2) "Overutilization for commercial, recreational, scientific, or educational purposes", 3) "Disease or predation", 4) Impacts because of the inadequacy of existing regulatory mechanisms", and 5) "Other natural or manmade factors affecting its survival" (United States 1983). Because of a combination of issues an endangered species may be facing, human intervention is necessary to preserve many

wild populations. Such intervention could include treatment for disease, or protection of natural habitat or the augmentation of natural populations. Individuals may be protected as well, with some being individually tracked, and with laws and fines being put in place for actions causing injury or death of an organism or damage to its habitat. Ultimately, the goal of human intervention is to grow the wild population to a self-sustaining size that is unlikely to go extinct in the foreseeable future. In the United States, relatively few aquatic and terrestrial insects are currently listed as threatened or endangered species, and far more need to be listed as populations dwindle year by year (Hallmann, *et al.* 2020).

One of the most effective ways to grow a wild population or recover one previously extirpated is through the addition of individuals that have either been extracted from other healthy populations or generated through *ex-situ* conservation measures such as "captive breeding", or "captive rearing" (also known as headstarting). Captive breeding is the process of collecting adults to be bred in captivity; however, this approach is limited in its success as it requires the cooperation of these organisms in an unnatural setting. This method may be utilized to generate individuals for release or to preserve genetic diversity within a vulnerable population (Utter and Epifanio 2002). In contrast, "headstarting" is a method that removes eggs or immature individuals temporarily into captivity allowing them to reach maturity in a controlled, protected, and quicker way (Wijewardena *et al.* 2023). By decreasing their vulnerability, typically to predation, this can significantly decrease the mortality of young individuals (Heppell *et al*. 1996). Headstarting has been successful with animals, such as turtles and birds, resulting in quicker increases in size, reduction in mortality, and shorter time to sexual maturation (Heppell *et al*. 1996, Elliot *et al*. 2001, Collins *et al*. 2016). Minimal research has been conducted on the effectiveness and

impacts of headstarting on insects (USFWS 2003) and other arthropods for conservation purposes.

Hine's emerald dragonfly (*Somatochlora hineana*) is a federally-listed endangered species, found in isolated populations in wetland habitats in Illinois, Wisconsin, Michigan, Missouri, and the province of Ontario in Canada. Much of the cause of its decline is due to a variety of anthropogenic impacts, including habitat fragmentation, loss of wetland habitat, global climatic change, groundwater extraction and contamination, and both the direct (vehicle collisions) and indirect (habitat modification) effects of roadway development (Soluk *et al.* 2011, Furness and Soluk 2015). *Somatochlora hineana* has a complicated life history in which it can take up to five years to reach adulthood after 11-12 larval instars (Soluk and DeMots unpublished data). Adult *S. hineana* are active in the summer months from June to August and eggs will go into dormancy for winter months, hatching in early to mid-Spring (Foster and Soluk 2004, Pintor and Soluk 2006). This species is unusual in that it depends on a predator, the plains devil crayfish (*Lacunicambarus nebraskensis*), and the burrows it creates for habitat during winter and when its fen habitat dries during the summer (Pintor and Soluk 2006).

Although *S. hineana* is endangered range wide, there are some populations that are especially threatened. One of these is the Illinois population centered in the Des Plaines River Valley in the greater Chicago metropolitan area; the population has been estimated to produce only between 100-300 adults per year (Soluk and Mierzwa 2012). These numbers are excessively low and threaten the ability of this population to persist at a self-sustaining rate, especially given the rapid development in this urbanized area. In addition, the probability of loss of genetic diversity is

significantly enhanced in small populations that can experience inbreeding depression, decreased fitness, and increased susceptibility to disease (Booy *et al.* 2000). It is clear that intervention and protection are necessary for this population and possibly for other populations of *S. hineana*.

The goal of recovery for *S. hineana*, as with any endangered species, is to generate sustainable populations through augmentation and whatever other ways that are necessary, including identifying consequences of human activities on the stability of populations (U.S. Fish and Wildlife Service 2001). Unlike many other species, there are only limited opportunities for *ex-situ* conservation of *S. hineana*. Captive breeding has proved to be unsuccessful at a practical scale since adult *S. hineana* will not forage or mate properly even in relatively large enclosures (Soluk unpublished data). Thus, *ex-situ* augmentation of populations is only possible through "headstarting" wherein eggs are collected in the wild, allowed to hatch, and then the larvae are reared in captivity until they can be released either as mature pre-emergent larvae or as adults.

To achieve the goal of augmentation with the minimal amount of disturbance to natural populations, it is critical to minimize the number of eggs removed from the wild by maximizing the survivorship of larval *S. hineana* in the laboratory. Survivorship of *S. hineana* reaching adulthood in the wild is not directly known, however, studies of other dragonflies, even species in the same family (Corduliidae) suggest that survivorship could be as low as 0.2% (Corbet 1999). While these numbers may appear low, this is a common demographic pattern in many insects and other "r-selected" organisms which produce a high number of offspring with few reaching adulthood (Pianka 1970).

Survivorship of larvae in the current captive rearing program at the University of South Dakota averages under 10% from hatching to the time of release (Soluk 2020). Although this demonstrates that survivorship can be much higher in the lab than in the wild, demonstrating the value of the current program, there is much room for improvement and the generation of higher survivorship. Based on laboratory observations at the University of South Dakota, the greatest mortality of *S. hineana* occurs in the first 4-6 weeks immediately after hatching. Larvae at this stage are very small (<1 mm) and this makes them vulnerable to predators and competition, as well as being limited in their prey resources. In addition, higher growth rates during captive rearing following hatching in *S. hineana* larvae can also significantly decrease the time spent to reach adulthood, to two to three years, instead of five.

Augmenting existing populations and generating enough captively-reared *S. hineana* individuals for possible reintroduction into previously extirpated sites may prove necessary for the long-term conservation of this species. In this thesis, I contribute to this effort: in Chapter 1, I examine the efficacy of rearing *S. hineana* individuals in groups and the impacts this can have on survivorship and growth; in Chapter 2, I examine the importance of detritus and the associated microbenthic community, and the impacts this can have on survivorship and growth. I introduce newly discovered techniques in captive rearing and with the intention of expanding the current understanding of early instar larval Odonata for future conservation efforts.

References

Booy, G., Hendriks, R.J.J., Smulders, M.J.M., Van Groenendael, J.M., and Vosman, B. (2000). Genetic diversity and the survival of populations. *Plant Biology:* 2(4): 379-395.

Brook, B.W., Sodhi, N.S., and Bradshaw, C.J.A. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution:* 23(8): 453-460.

Butchart, S.H., Walpole, M., Collen, B., Van Strien, A., Scharlemann, J.P., Almond, R.E., Baillie, Bomhard, J.E., Brown, C., Bruno, J. and Carpenter, K.E. (2010). Global biodiversity: indicators of recent declines. *Science:* 328(5982): 1164-1168.

Collins, S.A., Sanders, F.J., and Jodice, P.J.R. (2016). Assessing conservation tools for an at-risk shorebird: Feasibility of headstarting for American Oystercatchers Haematopus palliatus. *Bird Conservation International:* 26(4): 451-465.

Corbet, P.S. (1999). Dragonflies: Behavior and Ecology of Odonata. Cornell University Press: 829.

Cox, R.L., Underwood, E.C. (2011). The Importance of Conserving Biodiversity Outside of Protected Areas in Mediterranean Ecosystems. *PLoS ONE*. 6(1): e14508. doi:10.1371/journal.pone.0014508

Dodds, W.K., Whiles, M.R. (2020). Freshwater Ecology: Concepts and Environmental Applications of Limnology. Third Edition. *Academic Press*: 981.

Elliott, G.P., Merton, D.V., and Jansen, P.W. (2001). Intensive management of a critically endangered species: the kakapo. *Biological Conservation:* 99(1): 121-133.

Forister, M.L., Pelton, E.M., Black, S.H. (2019). Declines in insect abundance and diversity: We know enough to act now. *Conservation Science and Practice.* https://doi.org/10.1111/csp2.80

Foster, S. E., and Soluk, D.A. (2006). Protecting more than the wetland: the importance of biased sex ratios and habitat segregation for conservation of the Hine's emerald dragonfly, Somatochlora hineana Williamson. *Biological Conservation:* 127(2): 158-166.

Frampton, G.K., Dorne, J.L.C.M. (2007). The effects on terrestrial invertebrates of reducing pesticide inputs in arable crop edges: a meta-analysis. *Journal of Applied Ecology:* 44, 362-373.

Furness, A.N., and Soluk, D.A. (2015). The potential of diversion structures to reduce roadway mortality of the endangered Hine's emerald dragonfly (Somatochlora hineana). *Journal of Insect Conservation:* 19: 449-455.

Gilpin M.E., and Soulé M.E. (1986). Minimum Viable Populations: Processes of Species Extinction.

Goulson, D. (2019). The insect apocalypse, and why it matters. *Current Biology:* 29(19): R967-R971.

Guo Z., Zhang L., Li Y. (2010). Increased Dependence of Humans on Ecosystem Services and Biodiversity. *PLoS ONE*: 5(10): e13113. doi:10.1371/ journal.pone.0013113

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Muller, A., Sumser, H., Horren, T., Goulson, D., de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE*: 12(10): e0185809. https://doi.org/10.1371/journal. Pone.0185809

Heppell, S.S., Crowder, L.B., and Crouse, D.T. (1996). Models to evaluate headstarting as a management tool for long-lived turtles. *Ecological Applications:* 6(2): 556-565.

van Klink, R., Bowler, D.E., Gongalsky, K.B., Swengel, A.B., Gentile, A., Chase, Jonathan M. (2020). Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science:* 368, 417-420.

Nicholls, E., Botias, C., Rotheray, E.L., Whitehorn, P., David, A., Fowler, R., David, T., Feltham, H., Swain, J.L., Wells, P., and Hill, E.M. (2018). Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the UK during the transition from pre- to post-moratorium. *Environmental Science & Technology*: 52(16): 9391-9402.

Otto, S.P. (2018). Adaptation, speciation and extinction in the Anthropocene. *Proceedings of the Royal Society: B* 285(1891): 2018-2047.

Pianka, E.R. (1970). On r-and K-selection. *The American Naturalist*: 104(940): 592-597.

Pimm, S.L., Russell, G.J., Gittleman, J.L., and Brooks, T.M. (1995). The future of biodiversity. *Science:* 269(5222): 347-350.

Pintor, L.M., Soluk, D.A. (2006). Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. *Journal of Biological Conservation:* 130:584-591.

Potts, S.G., Biesmiejer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. (2010). Global pollinator declines: trends, impacts, and drivers. *Trends in Ecology and Evolution*: 25(6) 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Soluk, D.A., Mierzwa, K.S. (2012). An assessment of Hine's Emerald Dragonfly (Somatochlora hineana) Population Size in the Lower Des Plaines River Valley, Illinois. Submitted to the Habitat Planning Conservation Partners and the United States Fish and Wildlife Service: 26.

Soluk, D.A., Tesar, D.E., Morris, L.M., Soluk, T.D. (2020). Captive Rearing and Augmentation of the Hine's Emerald Dragonfly: 2020 Performance Progress Report. Submitted to the United States Fish and Wildlife Service: 5.

Soluk, D.A., Zercher, D.S., and Worthington, A.M. (2011). Influence of roadways on patterns of mortality and flight behavior of adult dragonflies near wetland areas. *Biological Conservation:* 144(5): 1638-1643.

Tonietto, R.K., Larkin, D.J. (2017). Habitat restoration benefits wild bees: A meta-analysis. *Journal of Applied Ecology:* 55(2):582-590. https://doi.org/10.1111/1365-2664.13012

Turvey, S.T., and Crees, J.J. (2019). Extinction in the Anthropocene. *Current Biology:* 29(19): R982-R986.

United States. (1983). The Endangered Species Act as amended by Public Law 97-304 (the Endangered Species Act amendments of 1982).

U.S. Fish and Wildlife Service 2001. Hine's Emerald Dragonfly (Somatochlora hineana) Recovery Plan. Fort Snelling, MN. (120).

U.S. Fish and Wildlife Service. 2003. Final Recovery Plan for the Karner Blue Butterfly (*Lycaeides melissa samuelis*). U.S. Fish and Wildlife Service, Fort Snelling, Minnesota. 273

Utter, F., and Epifanio, J. (2002). Marine aquaculture: genetic potentialities and pitfalls. *Reviews in Fish Biology and Fisheries* 12: 59-77.

Winfree, R., Aguilar, R., Vazquez, D.P., LeBuhn, G., Aizen, M.A. (2009). A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology*: 90(8): 2068-2076.

Chapter 1: Factors determining initial post-hatching survivorship of captive-reared larvae of the endangered Hine's Emerald dragonfly (*Somatochlora hineana*): a model for the conservation of Odonata

Abstract

Conservation of endangered species frequently requires development of *ex-situ* approaches to augment or reintroduce populations. Dwindling populations of the endangered Hine's Emerald dragonfly *(Somatochlora hineana)* in parts of its range, had led to the development of an *ex-situ* captive rearing program to headstart individuals for later release. In the field, most mortality for odonates occurs within the first few instars following hatching. Reducing this mortality through headstarting in the lab generates individuals for reintroduction that would have died otherwise, however, even in the lab highest mortality occurs shortly after hatching. In this chapter, I evaluate the role of larval population density, size of rearing containers, and dietary components on early instar larval *S. hineana* survival and growth. These experiments indicated that group-rearing in larger containers generated higher survivorship than raising individuals separately in small containers, and that mortality rates did not seem to differ significantly between larger containers. Larval growth and development varied by many instars amongst treatments at various densities. This work helps to confirm that headstarting efforts for augmentation are a potentially important tool for the conservation of *S. hineana* and could be useful for the conservation of other species of Odonata.

Introduction

Ex-situ conservation, also known as off-site conservation, is an approach in which conservation practices are applied to organisms outside of their native habitats. Typically, "*ex-situ* conservation" is used to describe conservation research done in a zoological or laboratory setting (Saul-Gershenz 1995, Balmford 1996). This approach to conservation is especially valuable for tackling issues such as disease or habitat degradation faced by threatened and endangered species (Collins *et al*. 2016). *Ex-situ* conservation often includes efforts to "captive breed" or "headstart" a species whose numbers have drastically declined and require human intervention to maintain a healthy and self-sustaining population. In terms of conservation, "Captive breeding" is when organisms are bred in captivity and their young are subsequently reared in captivity, perhaps for reintroduction efforts, but can also include for genetic or research purposes. In contrast, "headstarting" is the process of collecting eggs or young juveniles from natural settings and rearing them in captivity for release at a later time. This approach (used predominantly with egg-bearing species) removes individuals from the wild when they are most vulnerable and releases them when they are at less risk thus increasing survivorship and generating a large number of individuals for population reintroduction or augmentation. This approach, with the goal of repatriation into the wild, is an effective way to strengthen populations and it has proven successful for many species including birds (Collins *et al*. 2016), mammals (Ross *et al*. 2021), and insects (U.S. Fish and Wildlife Service 2003, Crone *et al*. 2007).

Many insect groups are now experiencing an unprecedented rate of extinction, and extensive biomass loss (Goulson 2019). To address the decline of many insect populations will likely require the use of *ex-situ* conservation approaches. Already, captive breeding and rearing efforts

have been implemented for some insect groups to generate captive populations for conservation efforts (USFWS 2003), however, most captive reared insects are being farmed for the production of silk, carmine, protein, or other commercial products, including: Orthoptera (Pearce-Kelly 1998), Diptera (Kamden and Otomo 2023), and Lepidoptera (Lewis and Thomas 2001, Crone 2007).

One insect group that is showing declines is the Order Odonata, composed of the suborders Anisoptera (dragonflies) and Zygoptera (damselflies). Worldwide, many species of Odonata are already listed as threatened or endangered (IUCN 2022). Although there are relatively few odonates listed in the United States, there are many species that are threatened by habitat degradation and loss and threats to groundwater quality (Carle 1997, Watts 2007, Kiyoshi 2011, Dodds and Whiles 2020, USFWS 2023). As anthropogenic-influenced climate change continues to impact the extinction rate of species, it is likely that more Odonata are either already in need of intervention or will likely need intervention in the future. Overall, Odonata, because of their complicated life histories with both terrestrial and aquatic phases, will be sensitive to the challenges most insects will face during this extinction event, including habitat loss and degradation, threats to groundwater quality, pollution, and continued use of pesticides (Goulson 2019).

The Hine's emerald dragonfly (*Somatochlora hineana*) is the only federally listed endangered dragonfly (Order: Odonata, Suborder: Anisoptera) in the United States. It survives in isolated wetland areas in Illinois, Wisconsin, Michigan, Missouri, and the province of Ontario in Canada. The larval habitat of *S. hineana* is in flowing areas of calcareous fens, where they can live up to

five years as larvae, before emerging as adults (Soluk *et al*. 1998, Foster and Soluk 2006). From the time immediately following hatching through the first few months of life, these dragonflies are vulnerable, and most of the mortality occurs during this part of their life history. Additionally, these organisms take a long time to reach maturity, wherein Odonata larvae will reside in wetland habitat for only 1-2 years (Corbet 1999), with *S. hineana* taking up to five years to reach their adult stage. Overall, their complicated life history contributes to the species' endangered status and likely will contribute to future listing of additional Odonata.

To augment or reintroduce *S. hineana* populations, a headstarting program has been in development since 2010. The goal of this program is to effectively augment threatened populations in a manner that is minimally disturbing to wild populations. In this program, eggs are collected from wild-caught adults, held overwinter until they hatch in the spring and then the larvae are raised in captivity until they reach adulthood. As with other headstarting programs, the goal is to increase survivorship over what is seen in the wild and by protecting larvae when they are most susceptible to threats, such as starvation, disease, or predation. Such efforts are especially important for isolated populations of *S. hineana* in Illinois that have low numbers, likely due to habitat loss and degradation (Soluk and Mierzwa 2012).

The headstarting program has been able to generate survivorship of larval *S. hineana* of around 10% in the laboratory. Studies of insects in general suggest that most mortality occurs within the earliest larval instars, and this is generally assumed for most dragonflies, however, few studies have actually generated survivorship curves for dragonflies from hatching through emergence. For example, Ubukata, (1981) performed a study on survivorship from egg to adulthood on other

Corduliids (dragonflies in the same family as *S. hineana*) and found that survivorship in the wild for this family was low (~0.2%). If we assume that *S. hineana* larval survivorship follows a similar trajectory, then we can estimate that larval survivorship in the wild is likely around 0.2% from hatchling to adulthood, with the vast majority of the losses occurring within the first few larval instars. Thus, the headstarting program can be viewed as successful even if it generates improved larval survivorship of only 10%, however, it is also apparent that there is much room for improvement.

In the Captive Rearing Program at the University of South Dakota, survivorship following initial hatching under a variety of conditions has been quite variable ranging from 2% to 96% (Soluk unpublished data). This contrasts with wild averages as survivorship of larval *S. hineana* is estimated to be 0.2% in the wild (Corbet 1999). This strongly indicates that there is potential to improve the program and increase the number of individuals generated for augmentation by understanding the sources of this variability. For example, preliminary studies indicate that lab group-reared larvae in stock tanks containing ample prey sources generated survivorship of early instar larvae varying from 40% up to 96% (Soluk unpublished data). But why some tanks had high survivorship and some lower survivorship has remained unclear. Understanding the conditions that are the cause of mortality in larval individuals following hatching has great potential to increase survivorship and ultimately led to improvement of the captive rearing methods for this species. This includes identifying the causes of the high initial mortality of *S. hineana* larvae that occurs within the first 4-6 weeks of life.

In this chapter, I experimentally evaluate the role of density of larvae and size of container on early instar larval *S. hineana* survival and growth to improve headstarting efficacy and efficiency. The overall goal is to improve rearing techniques and approaches in the headstarting program to increase early hatchling survivorship and optimize their growth rates. Increasing our understanding should help reduce time and effort required in this program and minimize the number of eggs that need to be extracted from the wild population and the resulting disturbance required for the program by improving avoidance of potential competitors and other threats to larval survivorship. Successful culturing of *S. hineana* will contribute to insect conservation for other species as well, as the potential for more odonates to be listed is high and need for intervention and augmentation of populations may become more widespread.

Methods

Experiments were designed to evaluate optimal density and growth in laboratory microcosms by comparing rates of survivorship of larvae housed under varying conditions. Because we are working with an endangered species, experiments were intentionally designed to minimize overall mortality of *S. hineana* larvae. Collections and experiments were carried out under Native Endangered Species Recovery Permit (Permit Number: ES805269-16) issued by the United States Fish and Wildlife Service, as well as permits from the Illinois Department of Natural Resources and the Wisconsin Department of Natural Resources.

Experimentation took place at the University of South Dakota in “medium” microcosms (0.34 x 0.29 m black plastic dishwashing tubs) or “small” microcosms (50.8 mm diameter food storage tubs). These were used in comparison to previous lab rearing protocol and preliminary

experimentation, which took place in “large” microcosms (0.74 x 0.60 m oval black plastic stock tanks) and “tiny” microcosms (individual tissue culture wells, 20 mm diameter). Large microcosms provided plenty of space; however, it was difficult to monitor larvae in them following introduction. Medium microcosms function similarly to the large, although their smaller size allows for some monitoring. Both large and medium microcosms make monitoring of larvae challenging in that they are dark in color and provide many spaces for larvae to hide. Small microcosms are clear, and their small size makes monitoring of larvae during experimentation possible. Tiny microcosms are ideal for monitoring, though only a single individual can be housed in each culture well, making density studies impossible and making rearing of larvae much less efficient.

Egg collection occurs during the height of the breeding season for *S. hineana* adults at the end of July until the middle of August. Reproductively mature female adults were captured with aerial nets in natural habitat in Door County, Wisconsin and Will County, Illinois. To collect eggs for these adults, their abdomens were dipped into specimen cups with untreated well water. Specimen cups containing eggs are placed in coolers until they could be brought back to the Captive Rearing Program at the University of South Dakota. Once there, specimen cups containing eggs were placed into incubation systems where temperature were set to mimic seasonal temperatures in natural habitat in order to induce hatching at the appropriate time (approximately mid-February of the following year). Larvae were monitored and when hatching was observed, hatchlings were collected within approximately the first 48 hours.

Density Experiments in Medium Microcosms:

Medium microcosms were housed in a temperature-controlled room at 21.1°C. Microcosms were each provided with a LED lighting on a daily light regime of 12 hours of light and 12 hours of dark. Each microcosm contained similar amounts of detritus and had an air stone bubbler for increased water flow and oxygenation. Microcosms were stocked weekly with similar amounts of plankton (copepods and daphnids) and dead (cottonwood leaves) and live (elodea and duckweed) vegetation for up to a year prior to the initial experiment. To boost the community of microorganisms living within the detritus, prior to the start of the experiment, microcosms were stocked with approximately 237 mL of detritus about two months prior to the onset of the experiment and introduction of larvae; detritus was collected from a productive *S. hineana* habitat found in Door County, WI and cultured for many months in a lab setting prior to inoculation in medium microcosms. During experimentation, each microcosm was supplemented with 473 mL (the size of one small microcosm) of densely packed planktonic prey and approximately 0.08 grams of brewer's yeast and 0.08 grams of spirulina (blue-green algae) powder to supplement detrital communities and provide additional prey items present for *S. hineana*. An optimal density experiment was conducted using medium microcosms containing $50/m^2$, $100/m^2$, $200/m^2$, $400/m^2$ *S. hineana* larvae (densities of 5, 10, 20, and 40 per medium container). There were seven replicates for each treatment for 28 total microcosms. As dragonflies hatched out in the Spring (see above), broods were homogenized to eliminate potential genetic effects on microcosms, and larvae were randomly assigned to treatments.

The optimal density experiment was run once for ten weeks beginning in February 2022, following the beginning of the hatching period. The length of time that experiments were run

was set to surpass the initial mortality threshold the hatchlings face. Microcosms were broken down at the end of the experiment by decanting off excess water that was subsequently filtered through a fine mesh to collect any loose and floating larvae. The remaining detritus was separated into sorting trays and live-picked. Any *S. hineana* larvae found were collected and photographed under a dissecting photo microscope at 6.3X magnification. The distance between the eyes, measured using a ruler tool within photo analytic software, was used as an indicator of total larval head width for the small *S. hineana* larvae. These measurements were then used to determine S. *hineana* larval instar.

An additional optimal density experiment was run in medium mesocosms for ten weeks, beginning in February 2023. The purpose of this experiment was to determine a maximum threshold after which survivorship would begin to significantly decrease. This microcosm study was conducted using similar conditions and methodologies to the previous experiment; however, in this case larvae were experimentally introduced at density equivalents of 200, 400, 600, and 800 larvae/m^2 (20, 40, 60, and 80 larvae per medium microcosm). Overlap in densities from the 2022 and 2023 experiments was intentional to control for time effects.

Density Experiments in Small Microcosms:

Small microcosms were also used to evaluate the relationship between density and survivorship/growth of *S. hineana* larvae. These small microcosms were provided with equal amounts of detritus (~50 mL) and filled with approximately 237 mL of habitat water. Larvae were placed in densities of 5, 10, and 15/container (equivalent densities of 625, 1,250, and 1,875 *S. hineana* larvae/m^2). During experimentation, each microcosm was supplemented with one

pipette (1 mL) of densely packed planktonic prey (0.5g dry weight) and approximately 0.08 grams of brewer's yeast and 0.08 grams of spirulina (blue-green algae) powder to supplement detrital communities and provide additional prey items for *S. hineana*. This experiment ran for six weeks. At the end of the experiment, each microcosm was broken down, emptied into a sorting tray, and live picked for *S. hineana* larvae.

Statistical Analysis

All statistics were performed in Excel and RStudio version 2023.06.1using the following packages Tidyverse, brms, ggplot, and janitor (Wickham 2016, Wickham *et al*. 2019, Microsoft Corporation, 2018). Spreadsheets were used to organize data, as well as calculating basic statistics and preforming linear regression analysis.

Results

Density Experiments in Medium Microcosms

It was initially hypothesized that survivorship would decrease as the number of *S. hineana* larvae present in the microcosm increased. Although larval survivorship (Figure 1) initially appeared to decrease as larval density increased in the first experiment, this was not true for the highest density ($400/m^{2)}$ treatment, which had survivorship (61%) comparable to that in the lowest density treatment (74%). Single factor ANOVA found no significant difference ($p=0.19$) in survivorship amongst all treatments. Overall mean survivorship of this experiment across all densities was 57%.

Density Experiments in Medium Microcosms and Growth Rate

It was also hypothesized that size of *S. hineana* larvae would decrease as the number of larvae present in each microcosm increased. Median head width of surviving larvae in each microcosm (Figure 2) appeared to decrease only slightly across treatments. Single factor ANOVA comparing median head width amongst density treatments indicated no significant difference ($p=0.55$) in growth rate of larvae among initial density classes. However, regression analysis indicated a weak positive relationship between initial density and median head width with the correlation coefficient ($R^2=0.32$, $p<0.001$) explaining 32% of the variance. The average median head widths were 2.8, 2.8, 2.1, and 2.0 mm for the larval densities of 50, 100, 200, and 400 larvae/m^2, respectively. In terms of average instar values, these larvae ranged between f-3 and f-4 in these treatments. The majority of larvae in lower density treatments (50 and 100 larvae/m^2) were at least one instar further along in their development than those in higher density treatments.

Time of death of dragonfly larvae was not monitored in the treatments, so it is possible that the density experienced by larvae in the microcosms may be more reflective of density at the end of the experiment, rather than just initial density. This could happen if many of the larval deaths occurred shortly after the start of the experiment. Median head width of surviving larvae (Figure 3) decreased with final density ($R^2= 0.45$), suggesting that although the initial density of *S. hineana* in a treatment did not strongly influence growth, the actual number of larvae that were in the microcosms at the end of the experiment did. Median instar values in final density groupings ranged from f-3 to f-6, indicating a difference of as great as three instars in larval development of *S. hineana* across the entire experiment.

Unfortunately, no meaningful data could be collected from the high-density microcosm study conducted in 2023 since all but 2 of the 28 medium microcosms had 0% survivorship. Why this occurred was unclear, but was likely not a function of larval density.

Density Experiments in Small Microcosms:

Optimal density experiments that were run in the small microcosms yielded 0% survivorship at all densities. Thus, it provided no conclusive information on the effect of small microcosms and density on larval survivorship and growth rates.

Discussion

Density Experiments in Medium Microcosms and Survivorship

Results from the medium microcosms did not support the prediction that survivorship would negatively correlate with the increasing density of *S. hineana* hatchlings. In fact, survivorship in the highest density treatment was comparable with those in the lowest density treatment. This contradicts evidence reported by others evaluating the effects of density stress or overcrowding on the survivorship of larval aquatic organisms (Semlitsch and Caldwell 1982, Petranka 1989, McIntosh 2022). Compared to other, more voracious dragonfly species, *S. hineana* larvae are relatively docile and passive predators. Given their foraging behavior it is even possible that the presence of additional larvae may contribute to higher rates of prey capture, as more larval movement could stir up prey, leading to more encounters and resulting in higher success); this is known as "predator facilitation" (Soluk and Collins 1988, Kotler *et al.* 1992).

Density Experiments in Medium Microcosms and Growth Rates

It is not known whether microcosms experienced the effects of initial density or final density,

since mortality was not recorded during experimentation to minimize disturbance. Although survivorship did not change consistently as an effect of initial density, growth rates did appear to change as the initial density of *S. hineana* larvae was increased. However, the effect of density was much clearer when growth was plotted against the final density of larvae and was consistent with other studies of aquatic larval organisms in density-controlled settings (Semlitsch and Caldwell 1982, Petranka 1989, Warner *et al*. 1991). The slight decline in median head width in the highest density treatment could be explained by intraspecific interference competition. Not only are hatchling *S. hineana* competing with other microbenthic predators, but they are also competing with one another for the same prey sources, especially as the number of larvae in the microcosm increase. A preliminary study using tiny microcosms found some evidence of interference in the form of intraspecific aggression resulting in the maiming or death of some individuals when there was a difference in the size of individuals of at least one instar (Meza and Soluk unpublished data).

Insects follow a stepwise pattern of development with discrete instars, as opposed to a continuous growth. Final density indicated a negative relationship between the final density and growth rate of larvae, with differences in median size as great as three larval instars. For captive rearing efforts, this indicates a considerable difference in development rate and suggests a clear trade-off between the number of larvae reared past the initial mortality threshold and their size. Implications of this include the need for additional efforts immediately following hatching, since more microcosms, and human resources are required to maintain larvae at lower densities. These contrast with the benefits of having significantly more developed larvae which require less time before being released into the wild. In contrast, rearing at higher densities requires less time and

effort to potentially yield a higher number of larvae.

As previously dealt with in the small microcosms, the same detrital content and associated microbenthic communities were used in other experiments and were unknowingly overrun with the presence of planaria, and colonization of prey communities was weak and unsuccessful. As is the case with culturing wild-collected microbenthic organisms, it is difficult to predict what will establish itself and flourish and what organisms may be hiding within the initial collected samples. This makes culturing of naturally collected detrital communities challenging as it is difficult to prevent unwanted organisms from establishing themselves, and there is some level of randomness in composition of communities (Farnon-Ellwood *et al*. 2009). It is hypothesized that the study in 2023 failed for similar reasons, as it appeared that microbenthic communities were overrun with potential competitors and predators, such as planaria and Amphipoda. This variability demonstrates the importance of spreading the risk by culturing larvae in many different microcosms. Though hatchlings are small, and it is feasible to place them in microcosms at high densities, this variability and unpredictability of cultures demonstrates that you should not "put all of your eggs in one basket".

Microcosm Size Differences

Survivorship data in the various container sizes was collected from a series of studies. Initial survivorship observed in tiny microcosms (individual tissue culture wells) was based on data collected over the course of multiple years in which dragonflies were reared on an individual basis in the laboratory. Average survivorship for these tiny containers was approximately 4% for the laboratory average prior to the implementation and experimentation of group-rearing efforts

(Soluk *et al*. 2020). For small microcosms, the average density experiment performed in the current study (see above) yielded abnormally high mortality (100%), and so was not included in survivorship comparisons; however, a series of similar small container experiments performed at similar densities demonstrated that small microcosms were capable of yielding high survivorship. It is worth nothing that there was high variation in the quality of detritus and the associated microbenthic community provided in each small microcosm, and much of this detritus was sourced from lab-cultures and not natural habitat to be cultured in the lab. The importance of the microbenthic community to survivorship and development of *S. hineana* larvae is explained further in Chapter 2. Rearing attempts in the large containers (stock tanks) at densities ranging from 91 larvae/m^2 (40 per stock tank) to 182 larvae/ m^2 (80 per stock tank) yielded survivorship as high as 96% and over 60% respectively. Measures were taken to evaluate the effectiveness of headstarting larvae in microcosms at different and larger sizes, while also in group settings. Though the data did not yield promising results for *S. hineana* larvae reared in small microcosms, it is worth acknowledging that high degrees of survivorship are still capable of being achieved in densities ranging from 250/m^2 to 3,750/m^2 (Soluk unpublished data).

Small microcosms have the benefit of easy storage and transportation, are relatively inexpensive, and clear, providing ease of monitoring larvae throughout an experiment (once they are of an appropriate size). Medium microcosms tend to yield the highest survivorship and growth rates of *S. hineana* larvae, though monitoring of larvae is much more challenging. Large microcosms (stock tanks) utilized in preliminary experiments yielded variable survivorship and were often negatively impacted by the presence of planaria (Soluk unpublished data). Further experimentation of the utility of large microcosms should be addressed, as these stock tanks can

hold a much greater magnitude of larvae at one time, though they have the disadvantage of taking up significant space and are difficult to evaluate for the presence of larvae throughout the duration of the experiments. Overall, medium microcosms tend to be the most beneficial; in that they are affordable, easy to break down, and can consistently yield higher rates of survivorship than other rearing containers.

Overall, little information is known about early instar larval odonates, and this is especially true for *Somatochlora hineana*. Various rearing methods and informal experiments conducted in the lab have yielded survivorships ranging from the average observed in the lab (4%) to significantly higher levels (96%). Further experimentation is necessary to determine optimal density for survivorship, as low survivorship in a previous experiment was likely due to external factors (colonization of predator and prey types). Further evaluation and consideration are necessary, as it is unknown how these densities compare to larval density in natural habitat of *S. hineana*. Though it is possible to yield high survivorship in small microcosms (see Chapter 2), when calculating cost considering the number of people and hours required to care for these microcosms, medium microcosms greatly increase efficiency for captive rearing, as well as surpass survivorship and growth observed in the lab previously. This provides useful insight for future captive rearing efforts that prioritize the maximization of survivorship of larval odonates. The challenges of headstarting endangered species like *S. hineana* include moral and ethical concerns about the various treatments they could be subjected to in studies. The ultimate priority should be the protection of *S. hineana* and the minimization of their death of any kind. In Chapter 2, I evaluate the role of the detritus and associated microbenthic community provided in these microcosms to determine if they are a critical component for understanding the variability

observed in these experiments.

Acknowledgments

Funding for this study was provided by grants to Daniel A. Soluk from the U.S. Fish and Wildlife Service, the Illinois Department of Natural Resources, and the Forest Preserve District of DuPage County Illinois. This project would not have been possible without the housing provided by The Nature Conservancy of Northeast Wisconsin and Michael Grimm. I acknowledge all of those involved through the Hine's emerald dragonfly captive rearing program, including Kristopher Lah and Joe Kath. I'd like to also acknowledge those who made lab and field work possible: Rodrigo Meza, Alyssa Zirpel, Naomi Bingham, Jacob Woelber, and Nikki Kelly. Thank you to my committee members for their assistance through the project development and writing process.

References

Balmford, A., Mace, G.M. and Leader-Williams, N. (1996) Designing the ark: setting priorities for captive breeding. *Conservation Biology:* 10, 719–27.

Carle, F. L. (1979). Environmental monitoring potential of the Odonata, with a list of rare and endangered Anisoptera of Virginia, United States. *Odonatologica*: 8(4): 319-323.

Collins, S., Sanders, F., & Jodice, P. (2016). Assessing conservation tools for an at-risk shorebird: Feasibility of headstarting for American Oystercatchers *Haematopus palliates*. *Bird Conservation International:* 26(4): 451-465.

Corbet, P.S. (1999). Dragonflies: Behavior and Ecology of Odonata. Cornell University Press: 829.

Crone, E. E., Pickering, D., Schultz, C. B. (2007). Can captive rearing promote recovery of endangered butterflies? An assessment in the face of uncertainty, *Biological Conservation*: 139(1–2): 103-112, ISSN 0006-3207.

Dodds, W. K., Whiles, M. R. (2020). Freshwater Ecology: Concepts and Environmental Applications of Limnology. Third Edition. Academic Press.

Farnon-Ellwood, M. D., Manica, A., and Foster, W.A. (2009). Stochastic and deterministic processes jointly structure tropical arthropod communities. *Ecology Letters:* 12(4): 277-284.

Foster, S. E., and D. A. Soluk. (2006). Protecting more than the wetland: the importance of biased sex ratios and habitat segregation for conservation of the Hine's emerald dragonfly, Somatochlora hineana Williamson. *Biological Conservation:* 127(2): 158-166.

Goulson, D. (2019). The insect apocalypse, and why it matters. *Current Biology:* 29(19): R967-R971.

IUCN. (2022). The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org.

Kamdem, M.M., and Otomo, P.V. (2023). Developmental performance of Eristalis tenax larvae (Diptera: Syrphidae): Influence of growth media and yeast addition during captive rearing. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology:* 339(5): 503-513.

Kiyoshi, T., Takahashi, J.I., Yamanaka, T., Tanaka, K., Hamasaki, K., Tsuchida, K. and Tsubaki, Y. (1948). Taxonomic uncertainty of a highly endangered brook damselfly, *Copera tokyoensis* Asahina, (Odonata: Platycnemididae), revealed by the mitochondrial gene genealogy. *Conservation Genetics:* 12: 845–849.

Kotler, B.P., Blaustein, L. and Brown, J.S. (1992). Predator Facilitation: The Combined Effect of Snakes and Owls on the Foraging Behavior of Gerbils. *Annales Zoologici Fennici* (29:4): 199–206. *JSTOR*, http://www.jstor.org/stable/23735621.

Lewis, O.T., and Thomas, C.D. (2001). Adaptations to captivity in the butterfly Pieris brassicae (L.) and the implications for ex situ conservation. *Journal of Insect Conservation* 5: 55-63.

McIntosh, A.R., Greig, H.S., and Howard, S. (2022). Regulation of open populations of a stream insect through larval density dependence. *Journal of Animal Ecology*: 91: 1582–1595.

Microsoft Corporation. (2018). *Microsoft Excel*. Retrieved from https://office.microsoft.com/excel.

Pearce-Kelly, P., Jones, R., Clarke, D., Walker, C., Atkin, P., and Cunningham, A.A. (1998). The captive rearing of threatened Orthoptera: a comparison of the conservation potential and practical considerations of two species' breeding programmes at the Zoological

Society of London. *Journal of Insect Conservation:* 2: 201-210.

Petranka, J.W. (1989). Density-Dependent Growth and Survival of Larval Ambystoma: Evidence from Whole-Pond Manipulations. *Ecology*: 70(6): 1752–67.

Ross, A. K., Lawes, J. C., Elphinstone, A., Stutsel, S., Letnic, M. (2021). Headstarting as a cost-effective conservation strategy for an endangered mammal, *Current Biology*: 31(10): R465-R466, ISSN 0960-9822.

Saul-Gershenz, L.S., Arnold, R.A., Scriber, J.M. (1995). Design of Captive Environments for Endangered Invertebrates. Conservation of Endangered Species: An Interdisciplinary Approach. 483.

Semlitsch, R. D., and Caldwell, J.P. (1982). Effects of Density of Growth, Metamorphosis, and Survivorship in Tadpoles of Scaphiopus Holbrooki. *Ecology*: 63(4): 905–11.

Soluk, D.A., and Collins, N.C. (1988). Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. *Oikos*: 94-100.

Soluk, D. S., and Mierzwa, K.S. (2012). An assessment of Hine's emerald dragonfly (Somatochlora hineana) population size in the lower Des Plaines River Valley, Illinois. Report submitted to the Habitat Conservation Plan Partners and US Fish and Wildlife Service, Barrington, Illinois.

Soluk, D.A., Swisher, B.J., Zercher, D.S., Miller, J.D., and Hults, A.B. (1998). The ecology of Hine's emerald dragonfly (Somatochlora hineana): Monitoring populations and determining patterns of habitat use. Activity summary and report of findings (September 1996-August 1997). IL Nat. History Survey, Champaign, IL: 111.

Soluk, D.A., Tesar, D.E., Morris, L.M., Soluk, T.D. (2020). Captive Rearing and Augmentation of the Hine's Emerald Dragonfly: 2020 Performance Progress Report. Submitted to the United States Fish and Wildlife Service: 5.

Ubukata, H. (1981). Survivorship curve and annual fluctuation in the size of emerging population of Cordulia aenea amurensis Selys (Odonata: Corduliidae). Jap. J. *Ecology:* 31: 335-346.

U.S. Fish and Wildlife Service. (2003). Final Recovery Plan for the Karner Blue Butterfly (*Lycaeides melissa samuelis*). U.S. Fish and Wildlife Service, Fort Snelling, Minnesota. 273 pp.

Warner, S.C., Dunson, W.A. and Travis, J. (1991). Interaction of pH, Density, and Priority Effects on the Survivorship and Growth of Two Species of Hylid Tadpoles. *Oecologia*: 88(3): 331–39.

Watts, P.C., Saccheri, I.J., Kemp, S.J. and Thompson, D.J. (2007). Effective population sizes and migration rates in fragmented populations of an endangered insect (Coenagrion mercuriale: Odonata). *Journal of Animal Ecology*: 790-800.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Wickham H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open-Source Software*: **4**(43): 1686.

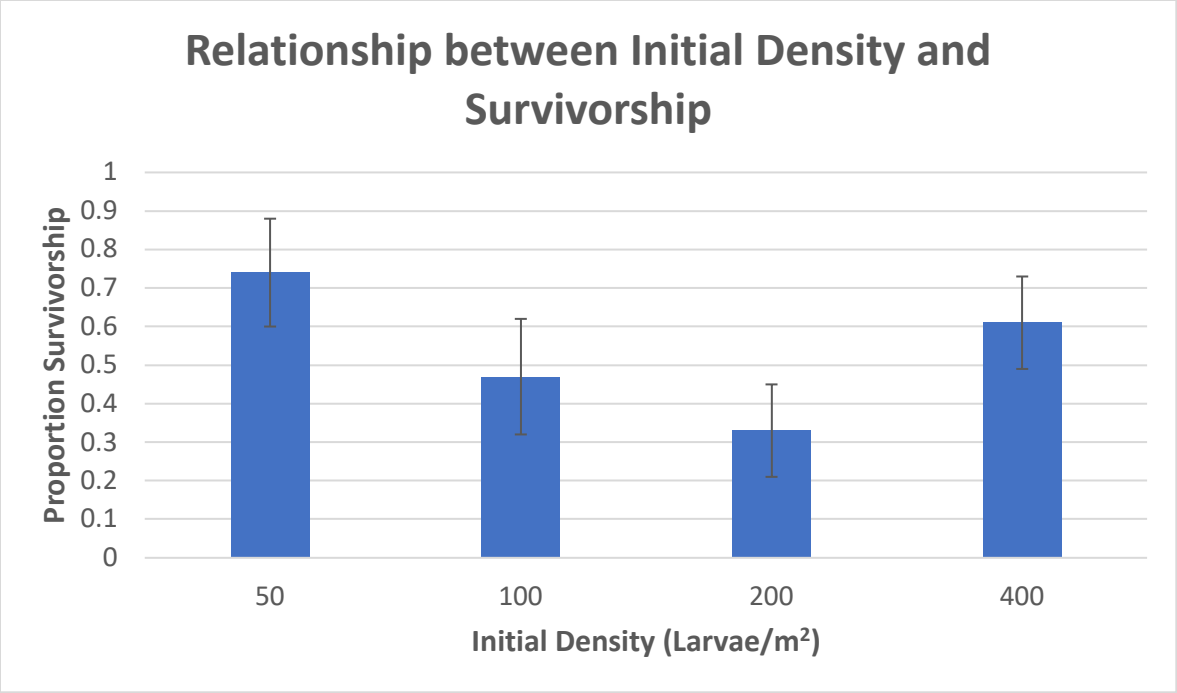

Figure 1. Mean survivorship of *S. hineana* larvae after 10 weeks as a function of initial larval density in the medium-sized ($0.1m^2$) microcosms. Error bars indicate standard error of the averages for each treatment. Analysis of variance indicated no significant differences in proportional survival of larvae in microcosms with different initial densities.

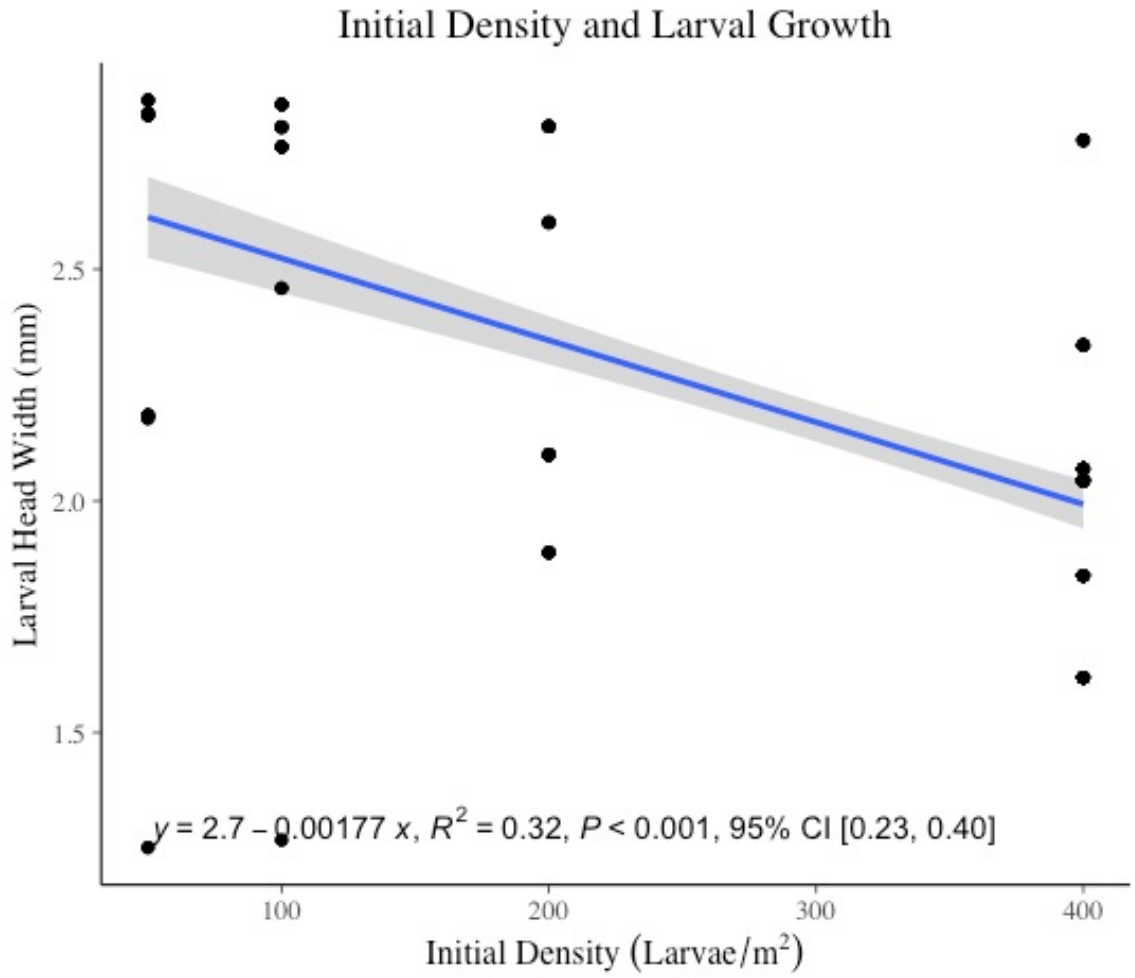

Figure 2. Distribution of median head widths of *S. hineana* larvae recovered at the end of the experiment as a function of initial density of larvae in the experimental mesocosm. Whiskers on the boxplots indicate the lower and upper quartiles. Points indicate raw data outliers. Line equation is y=2.7 – 0.00177x. R^2=0.32, p<0.001, 95% confidence interval (0.23, 0.40), where the correlation coefficient explains 32% of the variance.

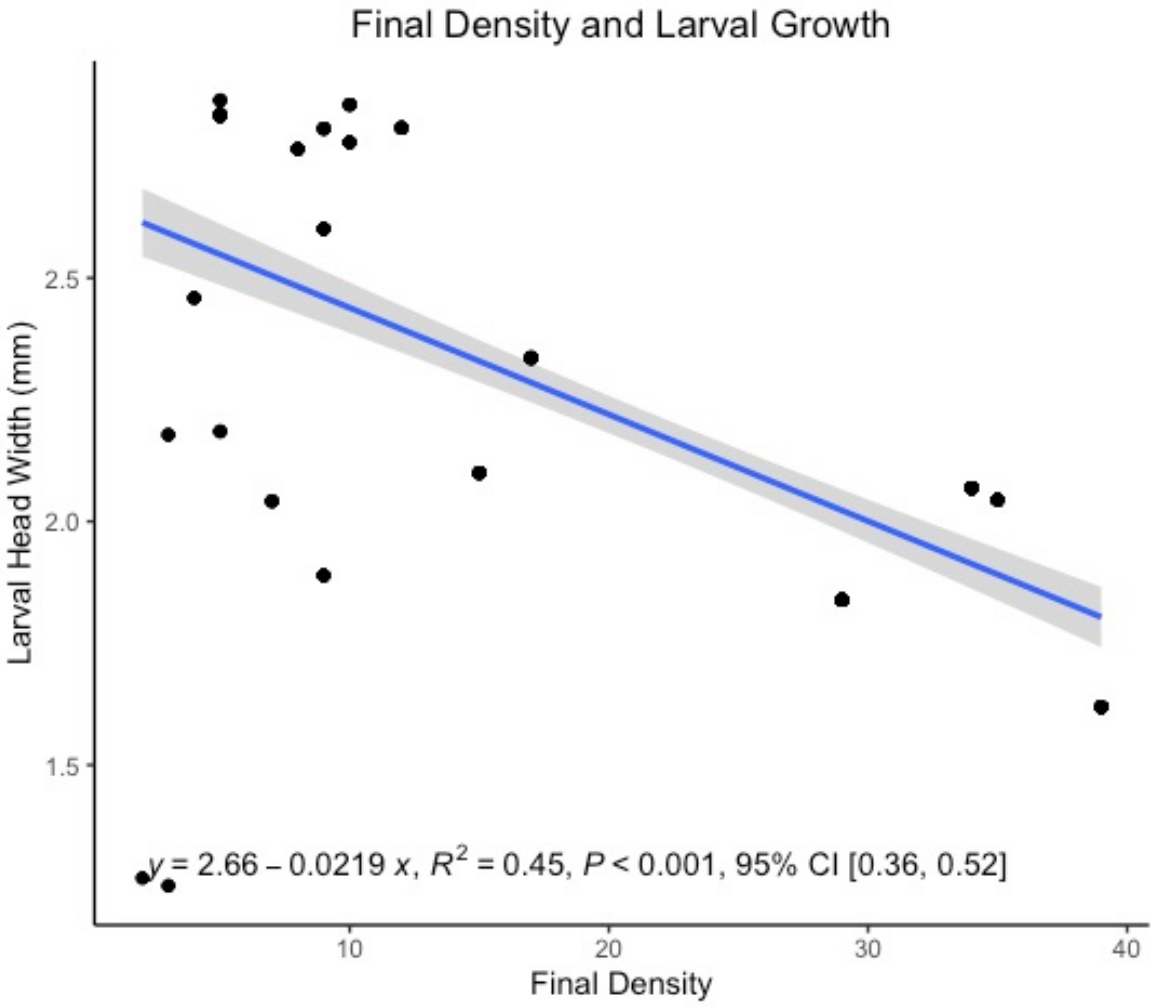

Figure 3. Median head width of *S. hineana* larvae recovered at the end of the experiment as a function of final density of larvae in the experimental mesocosm. Points indicate median head width for each microcosm in each treatment. R^2 value suggests a relationship between larval growth and larval density in microcosms. Line equation is y=2.66 – 0.0219x. R^2=0.45, p<0.001, 95% confidence interval (0.36, 0.52).

Chapter 2: Headstarting the endangered Hine's emerald dragonfly (*Somatochlora hineana*): the influence of detritus and other factors on post-hatching survivorship.

Abstract

Hine's emerald dragonfly (*Somatochlora hineana*) is a federally endangered species that occurs in wetland fens and ephemeral streamlets where a rich supply of detritus supports a diverse assemblage of microbenthic organisms. A headstarting program has been in development to create the ability to augment struggling populations of this species. There is need in this program to improve early instar survivorship and this can be done by improving dietary components and housing conditions. Detritus is an important component of the natural environment of hatchling *S. hineana* larvae, providing habitat structure and supporting a microbenthic community composed of small-sized prey. In this chapter, I describe this microbenthic community from samples collected in productive *S. hineana* habitat and contrast it with samples collected from microcosms used to house early instar larvae in the headstarting program. Initial sampling efforts indicated a much wider diversity and density of organisms is found in natural habitats when compared to those in the microcosms. Laboratory experiments in microcosms evaluated the role of detrital community in growth and survivorship of hatchlings under a range of densities. Early instar larval survivorship increased both when detritus was present and when detrital communities were supplemented. The density of amphipods present in the lab microcosms was the strongest negative indicator for larval survivorship and growth. This work provides a useful framework for the captive rearing of early instar larval Odonata from many species, which will become increasingly necessary as more species become vulnerable to extinction.

Introduction

Ex-situ conservation refers to efforts made to conserve species outside of their native habitat. Headstarting, a form of captive rearing, is one valuable method of intervention that augments wild populations of threatened and endangered species by collecting eggs or newly hatched individuals and holding them in captivity during their most vulnerable, early life stages. This method of conservation is growing in popularity and has had a high success rate in a wide variety of organisms, including insects (U.S. Fish and Wildlife Service 2003, Crone *et al*. 2007). Though headstarting can be successful for the augmentation of threatened populations, there are many challenges to developing such programs. For example, success of headstarting requires an adequate knowledge of nutritional needs of the species being raised. For many species in need of intervention, little is known about their food requirements and feeding habits that ultimately affect the survivorship in the headstarting programs (Pryor 2014, Francis *et al*. 2014). In these cases, further investigation is required to achieve the goal of returning healthy and hardy organisms back to the wild.

There have been relatively few studies specifically focusing on the captive rearing of insects for the purposes of returning them to their wild habitat. The majority of captive rearing efforts for insects are for commercial purposes, such as orthopterans (Baiano 2020, Christman *et al*. 2022), There have been some efforts to conserve threatened and endangered species, for example, the federally-listed endangered Karner Blue Butterfly was one of the first endangered insect species that was captively reared in order to augment wild populations (USFWS 2003).

Although dragonflies (Odonata, Anisoptera) are terrestrial in their adult life stage, their larval stages can be found in a large variety of aquatic ecosystems, including rivers, lakes, streams, and most types of wetlands. Dragonflies can persist in their larval forms for 1 to 5 years and have 9 to 17 instars (median of 12 instars, Corbet 1999) before going through incomplete metamorphosis to reach adulthood. The time it will take for dragonflies to reach their adult stage is dependent on the species, as well as the resources available and the stress they experience. This has important implications for their conservation, since larval Odonata can take many years to reach maturity. Such slow growth can cause populations to be vulnerable to extinction since they will take a long time to recover from frequent environmental perturbations.

All species of Anisoptera are carnivorous and often are referred to as apex predators in their systems. Larval odonates are typically generalist predators and their diet can vary significantly as they develop (Corbet 1999). Early instar larval odonates are thought to consume organisms such as large protozoans and Rotifera, though further research is necessary to fully understand consumption habits of these exceptionally tiny predators. Such research is especially difficult because the small size of the early instars make it difficult to obtain fecal pellets, and even if these are obtained, the body parts of many soft-bodied prey organisms do not survive digestion (Lawton 1970). Later instar larval odonates frequently feed on other macroinvertebrates, such as Amphipoda, Copepoda, and various insects, but it is also not uncommon for them to feed on vertebrates like small fishes and tadpoles (Dodds and Whiles 2020).

Detrital material is an important component in all aquatic food webs (Minshall 1967). Detritus is defined here as finely broken-down particles of various types of organic matter often found on

the bottom of aquatic systems; it is composed of decaying plant material, dead aquatic and terrestrial organisms, and fecal material (Tenore *et al.* 1982, Dodds and Whiles 2020). Detrital material is often dependent on the presence and abundance of shredders in a system (Tenore and Hanson 1980, Obernborfer 1984), and it accumulates at the bottom of aquatic systems over time, sometimes taking many months to artificially culture in a laboratory setting. Detritus, as it is broken down, provides nutrients that support a diverse assemblage of microbenthic organisms, macroinvertebrates, and even some vertebrates. Typical benthic detritus feeders include: various insects, Amphipoda, Ostracoda, some copepods, bacteria, protozoa, fungi, various fishes, and larval amphibians (Minshall 1967, Rich and Wetzel 1978, Anderson and Sedell 1979, Dodds and Whiles 2020). In fact, many of the organisms that reside in the benthos that are not predators or do not specialize in the consumption of algae or aquatic plants are dependent on detritus for nutrients (Dodds and Whiles 2020). There are even some keystone species in aquatic systems that are almost entirely dependent on the consumption of detrital material and these, as well as many other organisms contribute to the flow of nutrients in these ecosystems (Rich and Wetzel 1978, Wallace 1982, Dodds and Whiles 2020).

In an aquatic system, detritus may have multiple functional roles, including acting as a 3-dimensional structure that floats above settled sediments at the bottom of ecosystems and provides habitat and cover for various organisms. Detrital material can also play an important role in creating structured environment for benthic species. Many Odonata use “hide and wait” or “sit and wait” strategies where they stalk and ambush their prey, detritus may act as a perfect environment for concealment during these times. Additionally, larval odonates are often preyed upon by other organisms like fish, birds, and even older, larger larval odonates. Detritus likely

acts as cover to protect larvae from such predators and some larvae are even covered with fine hairs that allow detritus and silt to stick to and cover their bodies. Detritus also may provide structural elements that allow larval odonates to avoid aggressive intraspecific interactions, reducing the chance of injury that may ultimately lead to death of the individual (Johnson *et al*. 1985).

There is little information regarding the effective headstarting or captive rearing of Anisoptera (dragonflies) in early larval stages, with much of the available information being published only in recent years (Rice 2008, Palacino-Rodríguez *et al*. 2022). In this chapter, I evaluate the role of detritus and the microbenthic community it supports in determining survivorship and growth of early instar larval *S. hineana* in experimental microcosms. Determining the role of detritus and the microbenthic prey assemblage for successful captive rearing of this species may prove useful as the potential for more odonates to be listed is high, and further intervention and augmentation of populations may be necessary.

Methods

Assessing the Role of Detritus:

Experiments were designed to evaluate the role of detritus and its associated microbenthic communities for the headstarting of hatchling *S. hineana* using a series of laboratory microcosm experiments that manipulated various aspects of the captive rearing protocol. All activities for *S. hineana* are under United States Fish and Wildlife Service Native Endangered Species Recovery Permit (Permit Number**:** ES805269-16). Larvae were sourced from eggs collected from adults from the Mud Lake North Natural Area of Door County, Wisconsin. Eggs were stored in

temperature-monitored and controlled refrigeration units. Temperature was adjusted on a schedule that mimicked water temperature condition throughout the Fall, Winter, and Spring in Wisconsin.

The first experiment evaluated the role of detritus and its contribution to survivorship of *S. hineana* larvae. Detritus had previously been provided to larvae as a part of headstarting protocol, but the necessity of it was unknown. The experiment was conducted in 11 small (50.8mm diameter food storage tubs) microcosms (Figure 1A) each provided with 3 full 10 mL pipettes of suspended sediments, to create a thick enough layer to cover the bottom of the microcosm. Approximately 120 mL of water was then added before 10 hatchlings (equivalent to 1,250 dragonflies/m^2) were added to each microcosm. Of the 11 microcosms, there were 6 replicates with detritus and 5 containing no detritus. Microcosms were also given ample air space to allow for oxygenation. All microcosms were supplemented with 0.5 g dry weight of planktonic prey (equivalent to 10 mL densely packed live prey) to supplement dragonfly feeding once a week. Roughly 50 mL of detritus that was wild-collected and cultured in the laboratory for many months was provided to detritus treatments. This experiment ran for approximately 6 weeks before microcosm contents were live picked to collect surviving *S. hineana* larvae. Qualitative observations of prey assemblages and water quality were also made.

Detritus Microbenthic Community vs. Structure:

The second experiment evaluated whether the role of detritus was primarily structural for *S. hineana* larvae, by using an artificial 3-dimensional structure as a substitute for the structural component of detritus. Each microcosm was supplemented weekly with approximately 0.5 g dry

weight of planktonic prey as a food source for juvenile *S. hineana* larvae. Traditionally in the Captive Rearing Program at the University of South Dakota, feeding of *S. hineana* larvae has included the provision of wild-collected daphnids and copepods, though fish foods (composed of densely packed and nutritious dried, planktivorous organisms) were also used in later experiments. Treatments for this small microcosm experiment included: 1) a control treatment containing approximately 120 mL of "habitat water" (groundwater water collected near high quality larval habitat) without detritus or pond mesh; 2) a detritus treatment containing approximately 50 mL of wild-collected and lab-cultured detritus and habitat water; and 3) a structure-only treatment containing pond filter media (pond mesh) from The Pond Guy® (6.5 cm x 6.5 cm x 3.5 cm) and habitat water. These experiments were carried out in small microcosms (Figure 1) that each contained two dragonfly larvae (250 dragonflies/m^2). There were 9 controls, 10 detrital microcosms, and 14 structure only replicates. Because microcosms were started based on the availability of hatchlings during the Spring hatch, not every treatment had an equal number of replicates as some replicates were started at later times. This experiment ran for approximately six weeks before microcosms were live-picked, and larvae collected and counted.

Supplementation of the Microbenthic Community:

Based on information gathered from previous experiments in small microcosms, it was hypothesized that providing copepods, daphnids, and other planktonic prey was feeding and directly benefitting the microbenthic community, rather than directly providing significant food resources for the dragonfly larvae. To test this hypothesis, another experiment was set up using the small microcosms with detritus. There were three separate treatments: 1) Control microcosms with detritus and benthic microfauna, 2) Microcosms with detritus, benthic

microfauna, and weekly supplementation of live planktonic prey (0.5 g dry weight), and 3) Microcosms with detritus, benthic microfauna, and weekly supplementation of fish food flakes (0.5 g dry weight). Live plankton was composed of various daphnids, copepods, and rotifers. Fish food provided was from the brand Omega One™. There were 16 replicates for each of the plankton and fish food treatments; however, because the control was likely to exhibit high mortality only 5 replicates were run of this treatment. Five larvae were added to each small microcosm. This experiment ran for approximately six weeks before microcosms were emptied into sorting trays and larvae were collected and counted.

Natural prey assemblages in S. hineana larval habitat:

Somatochlora hineana can be found in high densities in Door County, Wisconsin, and in lower densities in the greater Chicago area in Illinois at sites where adults are caught for egg collection for the headstarting program. To characterize microbenthic communities and abundance in natural habitats, sampling occurred in natural habitat where *S. hineana* larvae and adults can be found in both Mud Lake North Natural area in Door County, Wisconsin, and the Lockport Prairie in Lemont, Illinois. Detritus samples were also taken in the laboratory to describe prey composition and density in the lab and make comparisons with the field to inform changes in lab culturing when necessary. Microbenthic detrital samples were also collected to be cultured in the lab from locations in Wisconsin and Illinois where *S. hineana* are found to provide prey most similar to those found in wild habitat.

All samples were collected using a coring method and a syringe to collect cores of detritus in wetland environments to approximately 20 mm in depth. A PVC square (approximately 0.09

m^2) acted as a boundary in which 7 cores were taken randomly. Samples were collected from streamlets along Lime Kiln Road in the Mud Lake North Natural Area in Door County, WI in the summer of 2021 and 2022, and from streamlets in the Lockport Prairie Nature Preserve (LP) in Will County, IL in the summer of 2022. Samples collected in 2021 (Table 1) were collected over the course of a week within two streamlets in the Mud Lake North (MLN) area of Wisconsin. The Mud Lake North area in Door County, Wisconsin was chosen as the primary location for sampling efforts because the area hosts habitats with some of the highest densities known for *S. hineana.* Samples were conducted in the same location on two individual streamlets, referred to as Streamlet 1 and Streamlet 2, approximately 15 m upstream of the confluence with a larger stream that both streamlets feed into. Living samples were evaluated within a 24-hour period following collection. Taxa identification to order or family, measurement, and counts were made using a plankton counter petri dish under a digital dissecting microscope (Leica DMS 1000) displaying on 109 cm widescreen monitor at an overall magnification of 39X. Organisms observed were classified into two size groupings, "big" and "small", with "big" organisms being too large (>0.5 mm) to be consumed by early instar *S. hineana*, while "small" organisms (<0.5 mm) were considered to be possible prey.

Lab Samples and Successful Microcosms:

As later instar *S. hineana* highly favor larval chironomids as a prey source when they are large enough to consume them, it was worth considering that soft-bodied worm-like organisms may be an optimal prey type. Because of this, it was hypothesized that a presence of size-appropriate soft-bodied, worm-like organisms would result in higher survivorship of larval *S. hineana.* Data collected during the experiments evaluating survivorship of larvae from medium microcosms at

varying densities (see Chapter 1). During that experiment microbenthic productivity and colonization changes over the ten-week study was tracked. To do this, one sample was taken from each medium microcosm (28 in total) before the dragonflies were introduced, during their presence (at approximately 5 weeks of runtime) and following their removal to characterize the change of microbenthic fauna over time. These 3 microbenthic organism samples across time were averaged to create one representative sample for each taxonomic group in each medium microcosm. Individual larvae that were recovered from treatments were placed in a clear tissue culture well and photographed using a Leica microscope. Using the Leica program to analyze photos, head width (distance measured between the eyes indicative of instar) of each *S. hineana* individual was measured and recorded in a spreadsheet. Because of noticeable size differences in the larvae upon recovery, I wanted to see if there was a relationship between head width differences, the prey distributions in the individual's associated tub, and the initial density treatments (5, 10, 20, 40).

Statistical Analysis:

All statistics were performed on Excel (Microsoft Corporations 2018) and RStudio version 2023.06.1 using the following packages to build models for Bayesian statistics, *Tidyverse* (Wickham *et al*. 2019), *brms* (Bürkner 2021), *ggplot* (Wickham 2016), *tidybayes* (Kay 2023), *ggpmisc* (Aphalo 2016), and *janitor* (Firke 2021). Analyses of variance (ANOVA) were run to determine if there were any significant differences in survivorship or median growth (assessed as head width) amongst treatments. To evaluate prey availability and composition in laboratory settings, Bayesian models were built to evaluate co-variates and make predictions about certain competitors and prey types and their effect on survivorship and growth of *S. hineana* larvae.

Edible prey types, or microbenthic organisms likely consumed by larvae, were grouped together for some of these analyses and included amphipods, nematodes, oligochaetes, large protozoans, copepods, daphnids, and rotifers. To test the hypotheses that dragonfly survival varied in response to the abundance of oligochaetes, the density of edible prey types, and the density of amphipods, models evaluating the relationship between survivorship and the density of organisms were used to fit generalized linear mixed models. These models had the number of initial dragonflies surviving as the response variable (the probability of survival as a proportion), the mean number of organisms (i.e., oligochaetes, edible prey types, or amphipods, depending on the model) in a sample as the predictor variable, with their interactions with the number of dragonflies per treatment, and microcosm as a varying intercept. The likelihood was Binomial, a logit link function was used, and the mean number of organisms were standardized with a z-score. To test the hypothesis that dragonfly growth varied in response to the abundance of oligochaetes, the density of edible prey types, and the density of amphipods, models evaluating the relationship between growth and the density of organisms were used to fit a generalized linear mixed model with head width as the response variable with mean number of organisms in a sample, their interactions with the number of dragonflies per treatment, and microcosm identification as a varying intercept. Because the data were continuous and positive, we used a Gamma likelihood, and the mean numbers of organisms were standardized with a z-score. Along with the models mentioned above, we also fit other models with the number of larvae recovered interacting with the mean number of organisms, the number of larvae per treatment, and tub effects, while also considering different times in which samples were collected (before, during, after, and average) and compared the models for each analysis using WAIC (Watanabe-Akaike Information Criteria), an information criterion that estimates expectations by computing

the log pointwise posterior predictive density and then corrects to avoid overfitting (Gelman, Hwang, Vehtari 2014).

Results

Assessing the Role of Detritus:

The initial microcosm experiment clearly indicated that the presence of detritus had a highly beneficial impact on early instar *Somatochlora hineana* larvae (Figure 2). All of the controls (containing no detritus) had no larval survivorship, although prey for larvae was provided. Variation in survivorship was observed in the treatment with detritus, including one of the six small microcosms resulting in 0% survivorship. The rest of the small microcosms had survivorship values ranging from 40% up to 70%. This initial experiment informed subsequent experiments on the necessity of detritus in laboratory cultures, though the role of detritus at this point was still unclear.

Detritus Community vs. Structure:

This experiment evaluated the effect of detritus as a 3-dimensional structure that may be providing cover and habitat for juvenile *S. hineana* larvae and compared this to the effect of an artificial 3-dimensional structure (pond mesh), on the survivorship of recently hatched larvae. No survivorship was observed in the controls containing neither pond mesh nor detritus, highlighting the importance of sediment, or a sediment-like structure for the captive rearing of larval dragonflies. As indicated in Figure 3, survivorship was only observed in the treatment containing detritus, since no larvae survived across microcosms in the treatment containing pond mesh. Survivorship in small microcosms containing detritus ranged from 0% to 100%, with approximately half of the microcosms yielding no survivors, and the other half resulting in survivorship of either 50% or 100%. Interestingly, survivorship of larvae was far more

consistent and higher on average in treatments of the previous experiment at densities of 1,250 dragonfly larvae/m^2 (10 larvae per small microcosm) than those at densities of 250 larvae/m^2 (2 larvae per small microcosm) in this experiment, indicating the possible value of raising newly hatched larvae at higher densities.

Supplementation of the Detrital Community:

It was hypothesized that using fish food flakes would greatly improve captive rearing by reducing efforts and cost required to source and culture planktonic organisms as prey for captive-reared *S. hineana*. We tested whether the provision of planktonic prey, both live and dried, would result in higher larval survivorship than those treatments containing only detritus. Results yielded 0% survivorship observed in the small microcosm treatment containing detritus alone (Figure 4). Survivorship is small microcosms containing detritus and supplemented with live planktonic prey averaged 13% amongst containers that contained larvae at the end of the experiment. Most of the small microcosms in this treatment yielded 0%, bringing the mean across the entire treatment down to 3.8%, which is equivalent to the approximate lab average. Survivorship in small microcosms containing detritus and supplemented with fish food flakes was approximately 60% amongst containers that contained larvae by the end of the experiment. Overall survivorship within this treatment, including the many microcosms that had 0% survivorship, was approximately 19%. As indicated in Figure 4, the standard error bars for the fish food treatment were high, as survivorship in microcosms containing surviving larvae ranged from 40% up to 90%. An analysis of variance conducted between the two treatments resulting in survivorship showed that mean survivorship was significantly higher in the microcosms supplemented with fish food than in those supplemented with live planktonic prey ($df=2$, $F=5.99$, $p=0.0087$). A t-test of unequal variances was also conducted between the two treatments

resulting in survivorship, fish food and planktonic prey, and it indicated qualitatively similar results to the ANOVA.

Sample Comparisons of Microbenthic Communities in S. hineana Larval Habitat:

During initial sampling to characterize microbenthic communities in high quality prey habitat, we observed that prey assemblages in the field contained a wide variety of soft-bodied oligochaetes such as Tubificidae, as well as large protozoa, Nematoda, and other worm-like organisms. Many of the soft-bodied organisms fell into the "big" size class (>0.5 mm) indicating that they are generally too large for early instar larval consumption; however, many hundreds, or even thousands of small organisms were still present in a given square meter in stream systems containing *Somatochlora hineana* larvae. Additional microbenthic taxa observed included: Amphipoda, Copepoda, Anomopoda, Sphaeriidae, and various Hexapoda, such as Ephemeroptera and Collembola.

In the samples collected first, there was a high density of microbenthic organisms, approximately 4,500-6,000 microbenthic organisms/m^2 on average. As air and water temperatures continued to warm and the onset of summer approached, edible and non-edible microbenthic organism density (see Table 1) increased as great as 10 times. The highest density of microbenthic organisms was calculated at over 42,000 individuals/m^2, indicating that early instar larval *S. hineana* are likely frequently encountering prey organisms in this habitat.

In 2022, we decided to compare microbenthic organismal abundance in the high-quality natural habitat (Mud Lake North) sampled in 2021 with that in a more disturbed environment associated

with a smaller population of *S. hineana.* Samples collected in Mud Lake North (MLN) at the same locations as those in 2021. Samples collected at Lockport Prairie (LP), Illinois were collected from two separate sites in the same streamlet, one downstream from the other. Initial samples in 2022 in Wisconsin include a much higher abundance of microbenthic organisms earlier in the year, as compared to the samples collected in 2021 at the same sites. After just a few days, additional samples were collected that indicated an abundance of nearly 100,000 total microbenthic organisms occurring in just one square meter of a streamlet. In comparing MLN to LP, it is clear that the abundance of microbenthic organisms is markedly lower in the Illinois streamlets. Samples in Lockport Prairie were collected well into the summer (mid-July) and only approximately 5,000 microbenthic organisms/m^2 were observed. This is comparable to the means calculated in late Spring of 2021 in high quality Wisconsin habitat, where *S. hineana* larvae are abundant.

Lab Samples and Successful Microcosms:

When initially comparing abundance amongst natural setting samples and laboratory collected samples, it was clear that lab sample means in 2022 were comparable to the natural setting samples with the abundance of organisms present in samples (See Table 1).

Microbenthic Organism Abundance and Larval Survivorship:

Bayesian analysis of the relationship between oligochaete density and survivorship of *S. hineana* larvae (Figure 5), indicates the line has a slope of 0.05 with a 95% credible interval=(-0.23,0.23). This means that there is a 95% probability that the slope of the line associated with this model will fall within this range. The lack of slope indicates that there is no evidence of a relationship

between the density of oligochaetes and the survivorship of *S. hineana*. For the relationship between the density of all edible prey and survivorship of *S. hineana* larvae (Figure 6), the line has a slope of 0.08 with a 95% credible interval (CrI) =(-0.20,0.28). This slope and wide credible interval indicate that while there is evidence of a slightly positive relationship between edible prey density and larval survivorship, there is also the possibility that this relationship can vary substantially. The model evaluating the relationship between amphipod density and survivorship of *S. hineana* larvae (Figure 7) has a slope of -0.24 with a 95% CrI=(-0.37,-0.08). This negative slope and credible interval indicate a strongly negative relationship between the density of amphipods and the survivorship of *S. hineana*.

For the models evaluating the relationship between oligochaete density and head width of larvae, the WAIC (Watanabe-Akaike Information Criteria) score indicated that the first model where head width was the response variable, and treatment, mean number of oligochaetes and their interactions as the predictors, along with microcosm identification as a varying intercept best described the data. All models (Table 2) were selected based on their WAIC score, which indicates how well the model fits the data. The line appears to be relatively flat (Figure 8) with a slope of -0.05, and 95% CrI=(-0.29, 0.22). This means that there is a 95% probability that the slope of the line associated with this model will fall within this range. This indicates that there is likely no relationship between the density of oligochaetes and the head width (growth) of *S. hineana* larvae in this experiment.

For the model evaluating the relationship between edible prey density and head width of larvae, the WAIC score indicated that the first model generated considering head width as the response

variable, and the treatment, mean number of edible prey in a sample and their interactions as the predictors, along with microcosm identification as a varying intercept best described the data. The line generated (Figure 9) appears to be slightly positive in slope (0.08) and a 95% CrI=(-0.18, 0.38). This indicates that there is a 95% probability that the slope of the line associated with this model will fall within this range. This would indicate that there could be a weakly positive relationship occurring between the density of edible prey and the head width (growth) of *S. hineana* larvae.

For the model evaluating the relationship between large amphipod density and head width of larvae, the WAIC score indicated that the first model generated considering head width as the response variable, and the treatment, mean number of big amphipods in a sample and their interactions as the predictors, along with microcosm identification as a varying intercept best described the data. The line generated for this model has a more constrained posterior (Figure 10) and seems to be slightly negative in slope (-0.25) with a 95% CrI=(-0.43, -0.02). This means that there is a 95% probability that the slope of the line associated with this model will fall within this range. This would indicate that there is probably a weakly negative relationship occurring between the density of big amphipods (likely competitors or predators) and the head width (growth) of *S. hineana* larvae.

Discussion

Results of the experiment evaluating the role of detritus on survivorship in microcosms clearly indicated that the presence of detritus increased survivorship of early instar larval *S. hineana*. However, it is worth considering that although no survivorship occurred in the treatment that

contained no detritus in small microcosms, comparing previous lab protocols of rearing larvae in individual tissue culture wells in which very little detritus is present was able to consistently generate low survivorship (~4%). Impressive rates of hatchling survivorship could be found in microcosms containing detritus, which would be consistent with the understanding that detrital communities are an important part of aquatic food webs (Minshall 1967, Odum and Heald 1975). that ultimately dictate the predacious fauna that can ultimately colonize a stream system (Flecker 1984). However, there may also be a survivorship benefit from the presence of additional larvae in a microcosm. As mentioned previously, *S. hineana* larvae, unlike most other dragonfly larvae (Corbet 1999), utilize a "sit and wait" predation strategy, and it is possible that the presence of additional larvae may be causing additional movement within the system, stirring up prey, which in turn increases prey encounter rates, a theory known as predator facilitation (Soluk and Collins 1988, Kotler *et al.* 1992, Soluk 1993). Further experimentation would be necessary to determine if this is the case.

The second experiment indicated that detritus benefits larval survivorship primarily by providing prey resources rather than a direct structural benefit. When larvae were just provided structural components to hide from one another, there was no survivorship. It is worth considering that the exceptionally small size of early instar larvae likely meant that few encounters were occurring in larger microcosms. Additionally, all treatments were provided with planktonic prey, though these prey were either not abundant enough or were the wrong size for these early instar *S. hineana* larvae, which were significantly smaller than some of the copepods or daphnid provided for consumption. However, preliminary experiments of individually raised larvae did indicate that survivorship is still higher when planktivorous prey is provided in early life stages.

If most planktonic prey are too large to be a prey source for young hatchling *S. hineana* larvae, then why would the addition of plankton contribute to higher larval survivorship at these stages? We hypothesized that instead of providing nutritional benefits directly to early instar larvae, the plankton was likely contributing to the microbenthic community when they died. This could explain why improved survivorship was observed in microcosms where detritus is provided. Because of this, we questioned the efficiency of providing live planktonic prey, as it is very costly in time and manpower. With frequent wild collection necessary from freshwater lakes many miles away, and often unfavorable weather conditions at the locations of collection sites, including wind, cold or ice, wild collection of planktonic prey can be challenging. Alternatively, plankton cultures maintained in the lab frequently collapse and planktonic prey may not be able to breed fast enough to feed thousands of dragonfly larvae in a headstarting program. Most preliminary studies indicated that the provision of planktonic prey generally yielded high survivorship. The difference between preliminary experiments that provided planktonic prey and the experiments listed above was likely the presence of detritus. Based on these findings, we hypothesized that detritus likely contained microbenthic organisms that newly hatched *S. hineana* larvae could easily prey on, in contrast to the planktonic prey types typically provided many of which were much too big to be consumed by newly hatched and early instar *S. hineana* larvae. We hypothesized that planktonic prey, given their high death rate, might be providing nutrients to the microbenthic organisms present in the detritus, which in turn would provide more abundant prey for the juvenile *S. hineana*. Given this, we investigated the effectiveness of providing a different form of supplementation for the microbenthic community (i.e. fish food flakes), which are primarily composed of dried planktonic prey. Experimental results indicated that survivorship was higher on average in small microcosms provided with fish food than those

with live planktonic prey. This makes sense, as the fish food flakes we used were largely dried, concentrated plankton, providing the microbenthic community with the same type of nutrients they would get from live plankton, when they died. Detrital material, composed of dead organic matter, sustains an associated microbenthic community that processes and breaks down organic materials (Bowen 1987). Since these microcosms are functionally closed systems, they require a constant input of such materials to sustain the organisms living in the detritus that may then feed early instar larval odonates. We hypothesized that provided zooplankton and fish food flakes act as this artificial allochthonous input. However, fish food has its drawbacks in comparison to the use of live planktonic prey, as its high concentration of nutrients can rapidly lead to a highly eutrophic environment if too much is added. This may be why there were small microcosms in the fish food treatment that yielded low survivorship. Often, these microcosms had excessive bacteria growth and emitted a foul smell, indicating anoxic conditions. Because of this experiment, we can now infer that the provision of live and dead plankton benefits the microbenthic community, as survivorship is higher in fish food treatments, and early instar dragonfly larvae do not directly consume non-living prey.

Just as there were issues with the plankton experiment yielding low to no survivorship in some of the small microcosms due to anoxic conditions, other experiments had similar outcomes. Ultimately, the source of detrital material and the associated microbenthic community varied, as communities can often develop in unpredictable and random ways (Bellisario *et al*. 2011). Though special care was taken to sample and observe microbenthic communities to ensure similarities in colonization, Tricladida (planaria) flourished in some microcosms, and it is

hypothesized that Tricladida are dangerous competitors, especially to fragile early instar *S. hineana*, and that they may even prey upon young larvae.

Microbenthic communities sampled in habitat where *Somatochlora hineana* adults and larvae are found had lesser abundance than those observed in laboratory settings. Microbenthic communities in high quality larval *S. hineana* habitat within streamlets in Door County, Wisconsin supported abundant prey, though there was some variation between years and streamlets. Further evaluation of microbenthic organisms in high quality habitat is encouraged to better understand how colonization rates differ within and amongst streamlets utilized by *Somatochlora hineana,* especially in varying conditions.

Along with sampling conducted in high quality prey habitat, benthic sampling was also conducted in habitat where populations are much lower in the Lockport Prairie Nature Preserve in Will County, Illinois. Though the streamlet sampled in 2022 in Illinois had a greater depth and better flow than the streamlets in Wisconsin, and the samples were collected well into the summer; microbenthic community abundance was a magnitude lower than in the Wisconsin sites. It is possible that microbenthic organismal abundance is lower in Illinois because this streamlet is in a highly developed area and faces threats from pollution and widely fluctuating water levels. Further experimentation is necessary to test these hypotheses to better understand aquatic microbenthic community assemblages and the impact they may have on endangered species like *S. hineana*.

In laboratory microcosms, it was hypothesized that the presence of oligochaetes and other edible prey types, would have a strongly positive relationship on the survivorship of larvae. I did not find evidence indicating that oligochaete abundance had any significant impact on survivorship, although they were far more abundant than all other prey types, which is typical of many stream communities (Odum and Heald 1975, Flecker 1984). There was a weak positive relationship between larval survivorship and the abundance of all edible prey types combined, perhaps indicating that the microbenthic community interactions are more important to ultimate larval success. This could be due to the interactions between organisms on one another, which in turn can influence the dragonfly larvae. It was Amphipoda that had the greatest apparent effect on *S. hineana* hatchling survivorship, and this was in a negative direction. Most freshwater amphipods are omnivorous, so their influence on *S. hineana* hatchlings may be because they compete with them or possibly because they can consume them, especially when the larvae are vulnerable after molting. The relationship between amphipods and *S. hineana* larvae is given further complexity by the observation that they are a commonly consumed prey used to feed larger *S. hineana* larvae in the Captive Rearing Program. Further studies are recommended to better understand the complexity of how some of these key benthic taxa impact early instar juvenile Odonata survival.

Bayesian models proved useful in the determination of impacts of densities of varying microbenthic organisms on the growth and development of *S. hineana* larvae. As indicated in the Chapter 1, we know that the density at which larvae are reared substantially impacts the development rate and their time in the headstarting program. Additionally, it is likely that the structure of the microbenthic community assemblage that these larvae are living with is also having a direct impact on their overall development. It was hypothesized that the presence of

soft-bodied organisms, like oligochaetes, would increase growth rates of dragonfly larvae; however, even the model that best described the data does not seem to indicate that there was a clear relationship between oligochaete abundance and *S. hineana* growth rate. It is worth considering that the entire community of organisms available for consumption, as demonstrated in the second model, is having more of an impact than simply just the predominant, soft-bodied organisms. One reason for this outcome may be that oligochaetes were often much larger than the hatchlings and they could not subdue them, even though these soft-bodied prey are readily consumed by larger larvae. There was a slightly positive relationship between the entire edible prey community and larvae, indicating that perhaps a wider diversity of prey or sizes of prey may prove more beneficial, or that perhaps nutritional diversity for larvae may play an important role in maintaining growth and development. Studies and models suggest that biodiversity inevitably plays an important role on the functioning of food webs (Thebault and Loreau 2006). Further investigation of these hypotheses should be considered and to allow for continued improvement of the headstarting program. The strongest relationship observed between larval growth and the microbenthic assemblage only considers Amphipoda that are too large to be consumed by early instar *S. hineana*. These relatively large invertebrates likely act as competitors or predators negatively impacting development of young and vulnerable *S. hineana*. Whether these invertebrates as acting predominantly as predators or competitors, or if it is some combination of the two is unknown. Amphipods are commonly referred to as opportunistic generalists (MacNeil, Dick, and Elwood 1997) and when contained within microcosms, are likely resource limited and will prey upon organisms similar to larval *S. hineana*.

Microbenthic organismal abundance in laboratory cultures was generally similar in abundance to natural microbenthic communities, and since cultures were recently sourced from natural habitat it resembled natural community assemblages. However, lab-cultured detrital communities can often turn into monocultures composed of only Nematoda, Amphipoda, or Ostracoda, or perhaps a combination of these three. This is of particular concern because Ostracoda are typically too large and even small ones are too armored to be consumed easily by early instar *S. hineana* larvae. Additionally, Tricladida and Amphipoda, which are likely competitors and/or potential predators on *S. hineana* hatchlings, frequently flourished in lab cultured detritus. It should also be recognized that because of modified sampling protocol used for the lab samples (fewer replicates due to time constraints and reluctance to disturb the experimental microcosms), there is a possibility that the high variability of lab samples masked important differences. Following initial natural sampling efforts in the summer of 2021, additional efforts were taken to collect and culture detritus and the associated microbenthic organisms to use for inoculation in microcosms for future experimentation. Following these efforts, the number of unsuccessful microcosms drastically decreased, and survivorship increased on average, resulting in successful microcosm experiments, such as the medium microcosm experiment performed in Spring of 2022. The presence was Tricladida was common in failed microcosms that resulted in no survivorship. It is recommended that additional efforts be made to better understand how to properly culture detritus and the associated communities in a laboratory setting, while avoiding the creation of monocultures or culturing and flourishing of potential competitors and predators.

Describing microbenthic communities in the wild and understanding the impacts of lab-cultured communities that resemble these will enhance the headstarting program. As mentioned

previously, special consideration was taken in experiments to reduce the number of replicates in the treatments resulting in high mortality, as we would expect low survivorship. When working with an endangered species, there are ethical concerns that limit use of replicates that would potential have high mortality. With the goal of increasing survivorship, microcosm studies can effectively improve protocols for rearing early instar larval *S. hineana* by manipulating the structure of microbenthic communities. It is important to consider the trade-offs of group-rearing under particular conditions that reduce mortality, while also impacting larval development and the time larvae take to reach maturity and can be returned to the wild. These methods can effectively improve the success of the program, while lowering the overall time, money, and manpower necessary. Through an increased understanding of the role of detritus and its associated microbenthic communities, we can advance our understanding of how to captively rear aquatic insects, especially dragonflies. This is an important effort to help mitigate the impacts of the insect decline and aid in the conservation and protection of many species, and in turn preserving the biodiversity and subsequent health of ecosystems.

Acknowledgments

Funding for this study was provided by grants to Daniel A. Soluk from the U.S. Fish and Wildlife Service, the Illinois Department of Natural Resources, and the Forest Preserve District of DuPage County Illinois. This project would not have been possible without the housing provided by The Nature Conservancy of Northeast Wisconsin and Michael Grimm. I acknowledge all of those involved through the Hine's emerald dragonfly captive rearing program, including Kristopher Lah and Joe Kath. I'd like to also acknowledge those who made lab and field work possible: Rodrigo Meza, Alyssa Zirpel, Naomi Bingham, Jacob Woelber, and Nikki Kelly. Thank you to my committee members for their assistance through the project development and writing process.

References

Anderson, N.H. Sedell, J.R. (1979). Detritus Processing by Macroinvertebrates in Stream Ecosystems. *Annual Review of Entomology*: 24(1): 351-377. Annual Reviews 0066-4170. doi: 10.1146/annurev.en.24.010179.002031 https://doi.org/10.1146/annurev.en.24.010179.002031

Aphalo, P.J. (2016). Learn R ...as you learnt your mother tongue. Leanpub, Helsinki.

Baiano, A. (2020). Edible insects: An overview on nutritional characteristics, safety, farming, production technologies, regulatory framework, and socio-economic and ethical implications, *Trends in Food Science & Technology*: 100: 35-50. ISSN 0924-2244, https://doi.org/10.1016/j.tifs.2020.03.040.

Bellisario, B., Cerfolli, F., and Nascetti, G. (2011). Pattern of species occurrence in detritus-based communities with variable connectivity. *Web Ecology:* 11(1): 1-9.

Bowen, S.H. (1987). Composition and nutritional value of detritus. *Detritus and Microbial Ecology in Aquaculture:* 14: 192-216.

Bürkner, P.C. (2021). Bayesian Item Response Modeling in R with brms and Stan. *Journal of Statistical Software*: 100(5): 1–54. doi:10.18637/jss.v100.i05.

Christman, M.E., Spears, L.R., Koch, J.B.U., Lindsay, T.T., Strange, J.P., Barnes, C.L., Ramirez, R.A. (2022). Captive Rearing Success and Critical Thermal Maxima of *Bombus griseocollis* (Hymenoptera: Apidae): A Candidate for Commercialization? *Journal of Insect Science*: 22(6) https://doi.org/10.1093/jisesa/ieac064

Corbet, P.S. (1999). Dragonflies: Behavior and Ecology of Odonata. Cornell University Press: 829.

Crone, E.E., Pickering, D., Schultz, C.B. (2007). Can captive rearing promote recovery of

endangered butterflies? An assessment in the face of uncertainty, *Biological Conservation*: 139(1–2): 103-112. ISSN 0006-3207, https://doi.org/10.1016/j.biocon.2007.06.007.

Dodds, W.K., Whiles, M.R. (2020) Freshwater Ecology: Concepts and Environmental Applications of Limnology. Third Edition. Academic Press. 981

Firke, S. (2021). janitor: Simple Tools for Examining and Cleaning Dirty Data. R package version 2.1.0. https://CRAN.R-project.org/package=janitor

Flecker, A.S. (1984). The effects of predation and detritus on the structure of a stream insect community: a field test. *Oecologia:* 64: 300-305.

Francis, D.S., Salmon, M.L., Kenway, M.J. and Hall, M.R. (2014). Palinurid lobster aquaculture: nutritional progress and considerations for successful larval rearing. *Reviews in Aquaculture*: 6: 180-203. https://doi.org/10.1111/raq.12040

Gelman, A., Hwang, J., Vehtari, A. (2014). Understanding Predictive Information Criteria for Bayesian Modeling. *Statistical Computation:* 24: 997–1016. https://doi.org/10.1007/s11222-013-9416-2

Johnson, D.M., Crowley, P.H., Bohanan, R.E., Watson, C.N. and Martin, T.H. (1985). Competition among Larval Dragonflies: A Field Enclosure Experiment. *Ecology*: 66(1): 119–28.

Kay, M. (2023). *tidybayes: Tidy Data and Geoms for Bayesian Models*. doi:10.5281/zenodo.13081513, R package version.0.6, http://mjskay.github.io/tidybayes/.

Lawton, J.H. (1970). Feeding and Food Energy Assimilation in Larvae of the Damselfly Pyrrhosoma Nymphula (Sulz.) (Odonata: Zygoptera). *Journal of Animal Ecology*: 39(3): 669–89. *JSTOR*, https://doi.org/10.2307/2859.

Microsoft Corporation. (2018). *Microsoft Excel*. Retrieved from https://office.microsoft.com/excel

MacNeil, C. Dick, J.T.A., and Elwood, R.W. (1997). The trophic ecology of freshwater Gammarus spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews:* 72(3): 349-364.

Minshall, G.W. (1967). Role of Allochthonous Detritus in the Trophic Structure of a Woodland Springbrook Community. *Ecology*: 48(1): 139–49. *JSTOR*, https://doi.org/10.2307/1933425.

Odum, W.E., and Heald, E.J. (1975). The detritus-based food web of an estuarine mangrove community. *Estuarine Research: Chemistry Biology, and the Estuarine System*: 265.

Obernborfer, R.Y., McArthur, J.V., Barnes, J.R. and Dixon, J. (1984). The Effect of Invertebrate Predators on Leaf Litter Processing in an Alpine Stream. *Ecology*: 65(4): 1325–31. *JSTOR*, https://doi.org/10.2307/1938337.

Palacino-Rodríguez, F., Cordero-Rivera, A., Palacino, D.A. & Penagos, A.C. (2022). Captive breeding of odonate larvae: effects of type of water, handling, and sex. *Entomologia Experimentalis et Applicata:* 170: 505-512. https://doi.org/10.1111/eea.13169

Pryor, G.S. (2014), Tadpole nutritional ecology and digestive physiology: Implications for captive rearing of larval anurans. *Zoo Biology*: 33: 502-507. https://doi.org/10.1002/zoo.21152

Rice, T.M. (2008). A review of methods for maintaining odonate larvae in the laboratory, with a description of a new technique. *Odonatologica*: *37*(1): 41–54.

Rich, P.H., and Wetzel, R.G. (1978). Detritus in the Lake Ecosystem. *The American Naturalist*: 112(983): 57–71.

Soluk, D.A. (1993). Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. *Ecology:* 74(1): 219-225.

Soluk, D.A., and Collins, N.C. (1988). Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. *Oikos*: 94-100.

Tenore, K R., Cammen, L., Findlay, S.E., and Phillips, N. (1982). Perspectives of research on detritus: Do factors controlling the availability of detritus to macroconsumers depend on its source? *Journal of Marine Research:* 40(2).

Tenore, K.R., and Hanson, R.B. (1980). Availability of detritus of different types and ages to a polychaete macroconsumer, *Capitella capitata*. *Limnology and Oceanography:* 25: 553–558.

Thebault, E., and Loreau, M. (2006). The relationship between biodiversity and ecosystem functioning in food webs. *Ecological Research:* 21(1): 17-25.

U.S. Fish and Wildlife Service. 2003. Final Recovery Plan for the Karner Blue Butterfly (*Lycaeides melissa samuelis*). U.S. Fish and Wildlife Service, Fort Snelling, Minnesota. 273.

Wallace, J.B., Webster, J.R. and Cuffney, T.F. (1982). Stream Detritus Dynamics: Regulation by Invertebrate Consumers. *Oecologia*: 53(2): 197–200.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org

Wickham, H., Averick M., Bryan J., Chang W, McGowan L.D., François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). Welcome to the tidyverse. *Journal of Open-Source Software*: 4(43): 1686. doi:10.21105/joss.01686

Date Collected	Location	Mean Microbenthic Organisms/m^2	SE
Summer 2021	MLN-Steamlet 1	1,093,000	684,000
Summer 2021	MLN-Steamlet 2	991,000	375,000
Spring 2022	Lab-SD	1,078,000	204,000
Summer 2022	MLN-Steamlet 1	490,883	195,000
Summer 2022	MLN-Steamlet 2	5,340,000	1,720,000
Summer 2022	LP-IL	266,000	68,900

Table 1. Mean abundance of microbenthic organisms in natural *Somatochlora hineana* habitats in the Mud Lake Natural area along Lime Kiln Road in Door County, Wisconsin and in the Lockport Prairie Nature Preserve in Will County, Illinois, as well as in a laboratory setting in medium microcosms cultured in South Dakota. This indicates that the overall abundances in the field and laboratory were generally similar, except for the Lockport Prairie streamlet (see text).

Model Name	Model	Mean WAIC Score	SE WAIC Score
SurvivorshipOligochaete1	final density|trials(initial density) ~ average oligochaete+ (1|tub_int)	Only 1 model	Only 1 model
SurvivorshipEdiblePrey1	final density|trials(initial density) ~ average edible prey+ (1|tub_int)	Only 1 model	Only 1 model
SurvivorshipAmphipod1	final density|trials(initial density) ~ average amphipod+ (1|tub_int)	Only 1 model	Only 1 model
HeadWidthOligochaete1	head width ~ initial density*average oligochaete + (1|tub_int)	0	0
HeadWidthOligochaete2	head width ~ final density+initial density+average oligochaete+(1|tub_int)	-0.6	0.7
HeadWidthOligochaete3	head width ~ final density+average oligochaete+(1|tub_int)	-0.6	0.6
HeadWidthEdiblePrey1	head width ~ initial density*average edible prey + (1|tub_int)	0	0
HeadWidthEdiblePrey2	head width ~ final density+initial density+average edible prey+(1|tub_int)	-0.8	0.4
HeadWidthEdiblePrey3	head width ~ final density+average edible prey+(1|tub_int)	-0.4	0.5
HeadWidthAmphipod1	head width ~ initial density*average amphipod + (1|tub_int)	-0.3	0.5
HeadWidthAmphipod2	head width ~ final density+initial density+average amphipod+(1|tub_int)	-0.6	0.2
HeadWidthAmphipod3	head width ~ final density+average amphipod+(1|tub_int)	0	0

Table 2. Bayesian models built and compared using WAIC. Survivorship models had one version, while growth models evaluating head width had three models. The lower the WAIC score, the better the model describes the data.

Figure 1A Figure 1B

Figure 1. An example of a small “Ziploc” microcosm (A) and a medium “dishwashing tub” microcosm (B). A ruler is used to compare scale of small and medium microcosms.

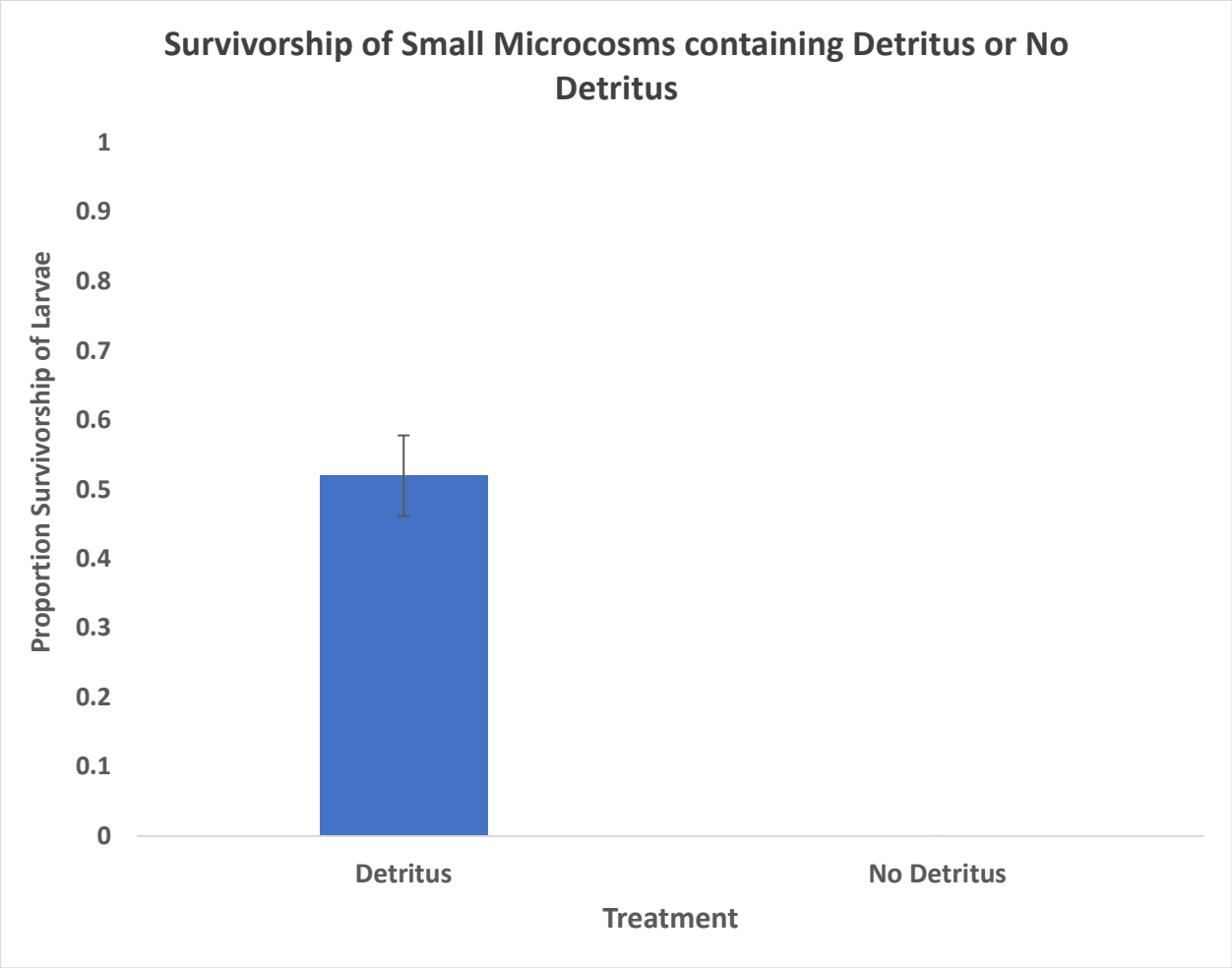

Figure 2. Mean survivorship (±standard error) of hatchling *S. hineana* larvae with or without detritus in small microcosms. This indicates that there is a relationship between *S. hineana* larval survivorship and the presence of detritus in microcosms.

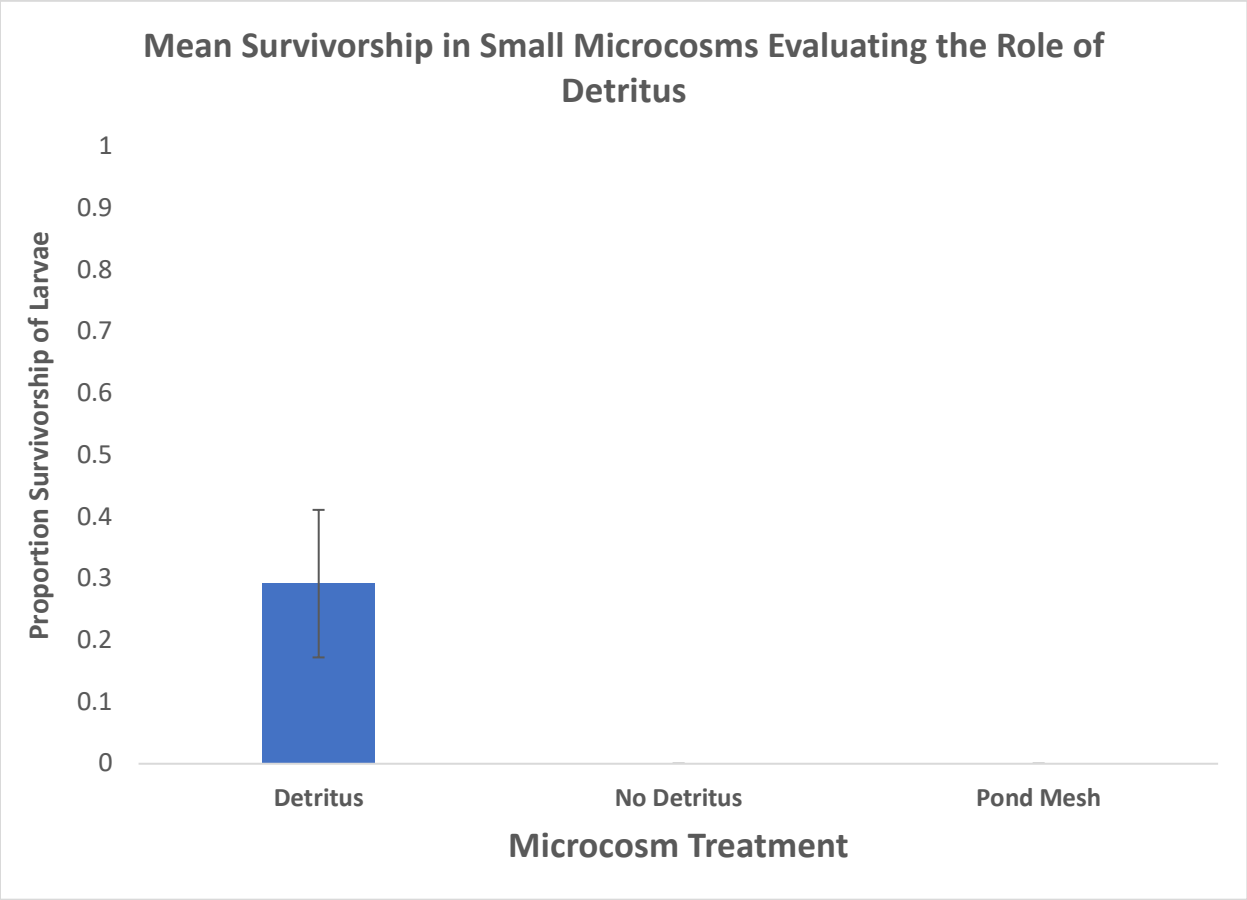

Figure 3. Mean survivorship (±standard error) of hatchling *S. hineana* larvae in microcosms with detritus, without detritus, or with pond mesh alone. Error bars indicate standard error for the calculated mean. This indicates that larval survivorship is higher in microcosms containing detritus, and that this is likely due to a nutritional component.

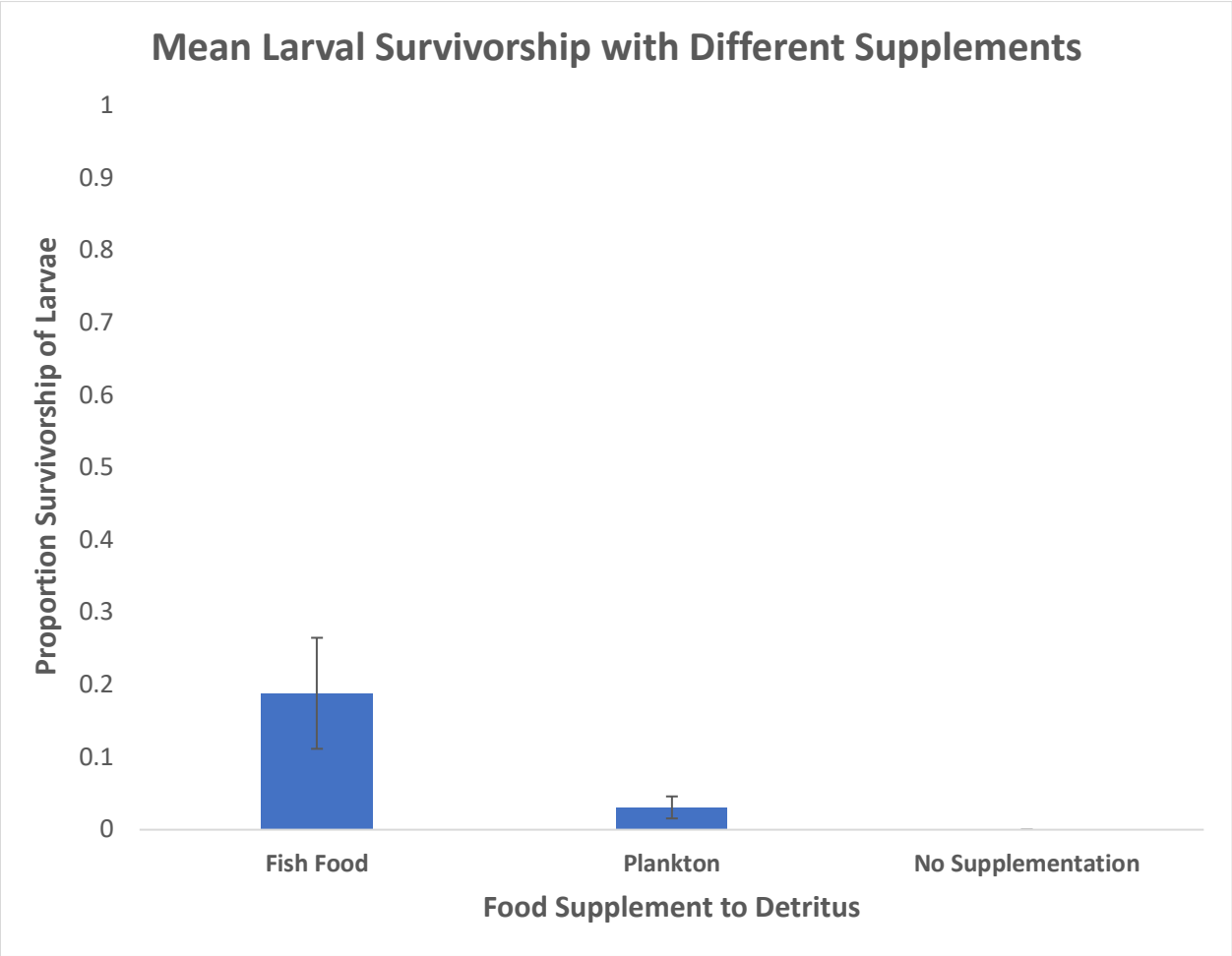

Figure 4. Mean survivorship (±standard error) of *S. hineana* larvae in small microcosms with fish food, plankton, or no supplements added. Error bars indicate standard error. This indicates that larval survivorship is higher in microcosms when the microbenthic community is provided additional supplementation.

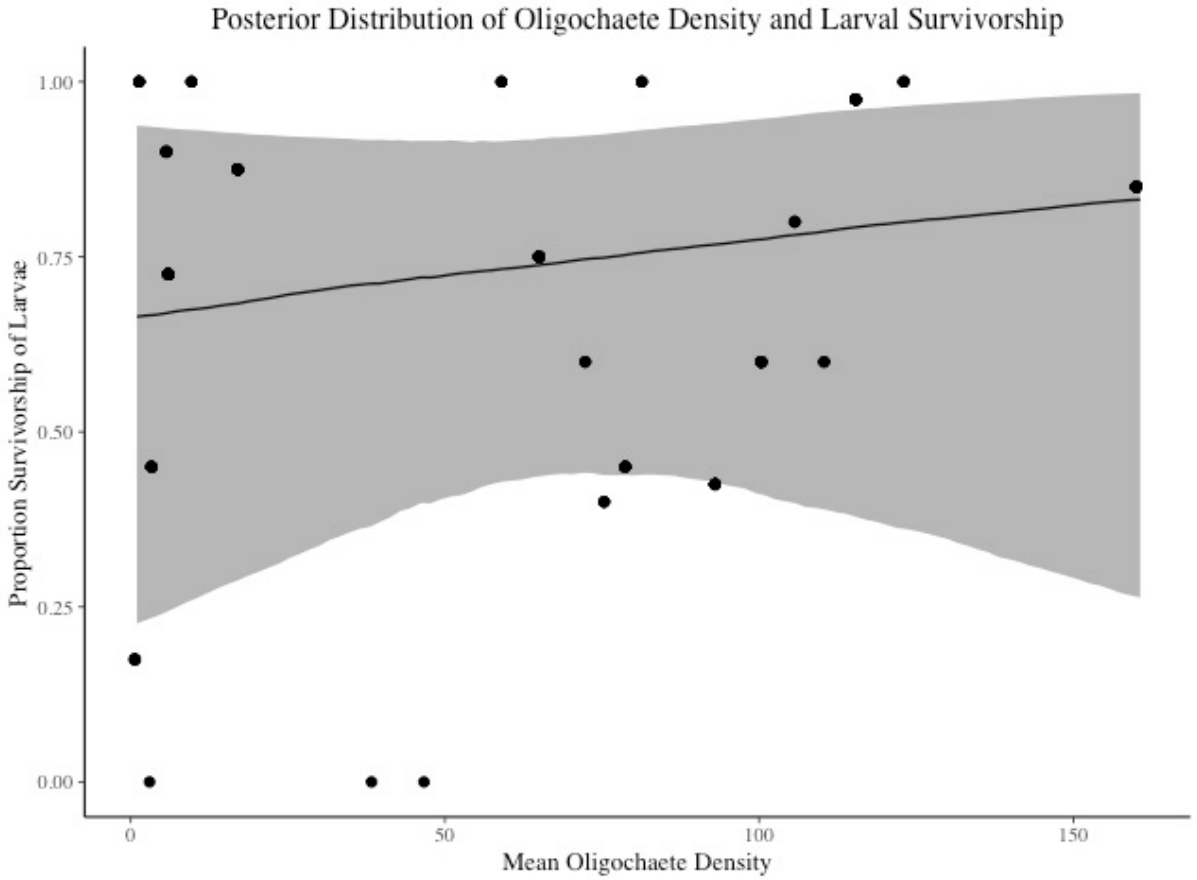

Figure 5. Relationship between mean survivorship of *S. hineana* larvae and mean oligochaete density in medium microcosms. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is 0.05, with a 95% CrI=(-0.23, 0.23). This suggests that there is likely not a relationship observed between *S. hineana* larval survivorship and oligochaete density.

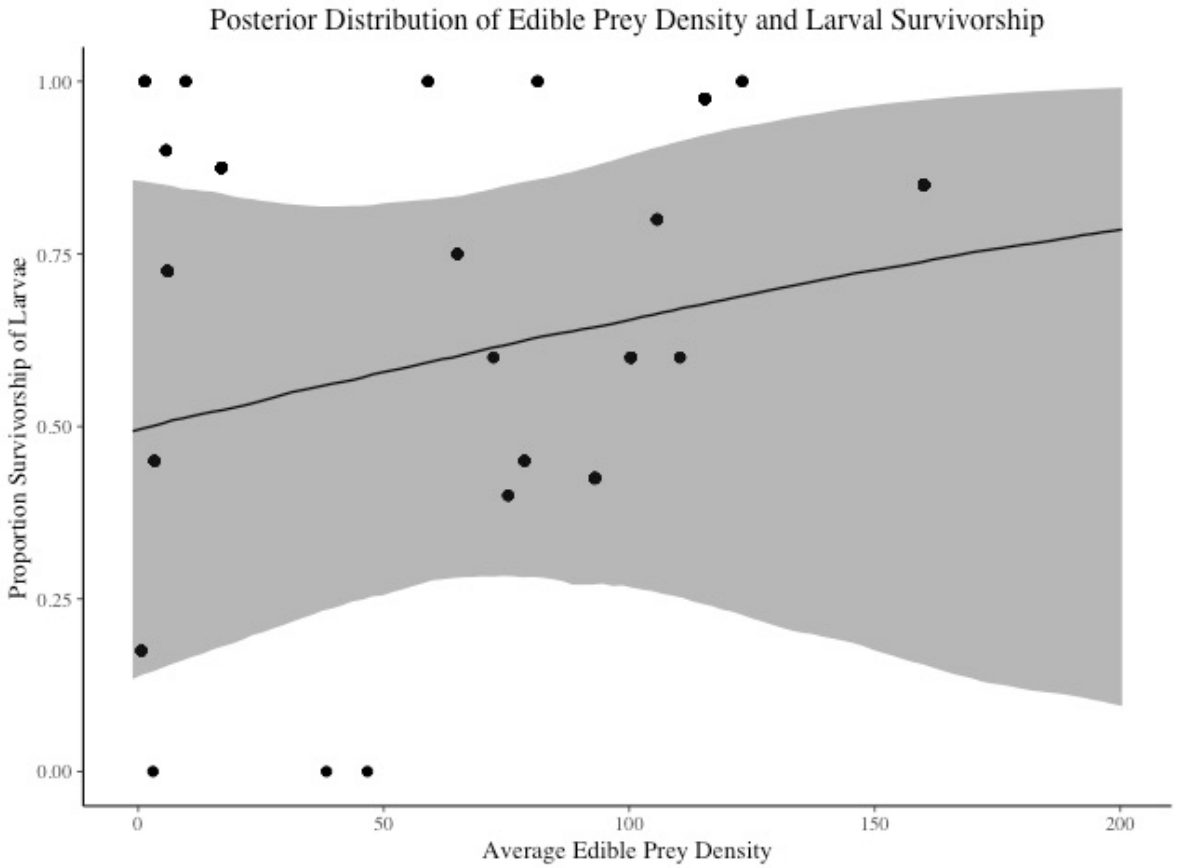

Figure 6. Comparing expected survivorship of *S. hineana* larvae in medium microcosms and mean edible prey density. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is 0.08, with a 95% CrI=(-0.20, 0.28). This indicates there is likely not a relationship associated between *S. hineana* larvae survivorship and edible prey density.

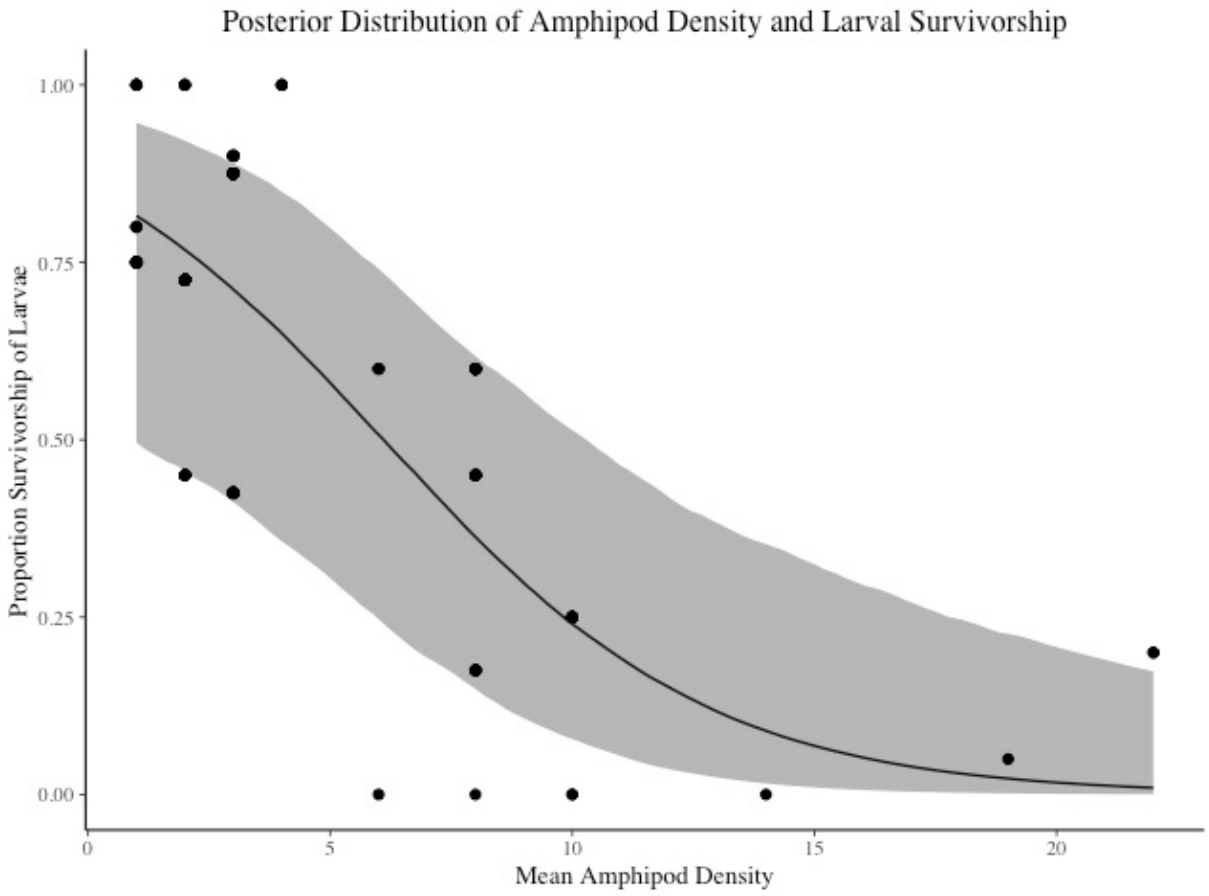

Figure 7. Comparing expected survivorship of *S. hineana* larvae and mean amphipod density in medium microcosms. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is -0.24, with a 95% CrI=(-0.37, -0.08). This suggests that there is a negative relationship between *S. hineana* larvae survivorship and amphipod density.

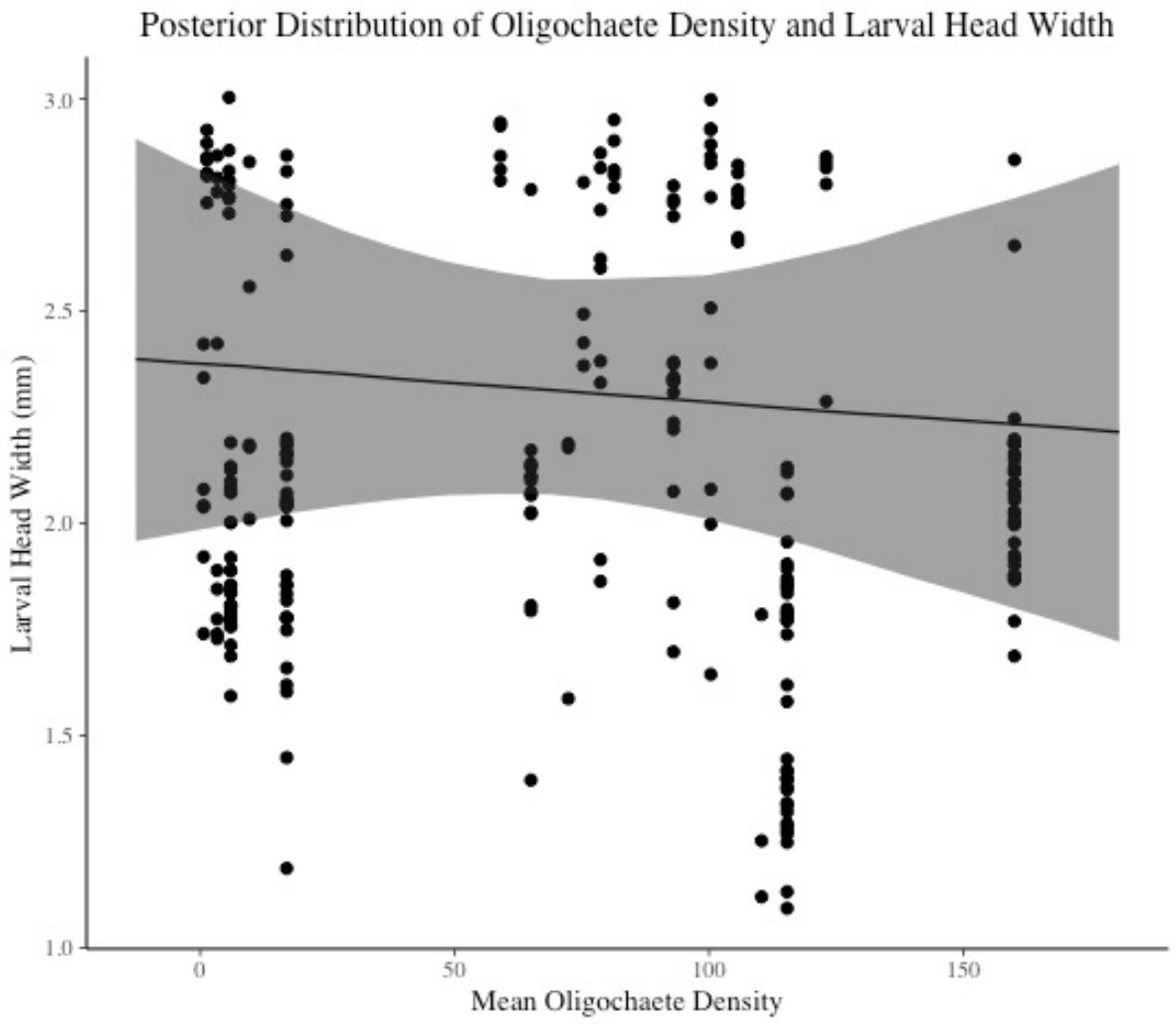

Figure 8. Relationship between individual larval head width and mean oligochaete density of *S. hineana* larvae in medium microcosms. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is -0.05 with a 95% CrI=(-0.29, 0.22). This suggests that there likely not a relationship observed between oligochaete density on the growth of *S. hineana* larvae.

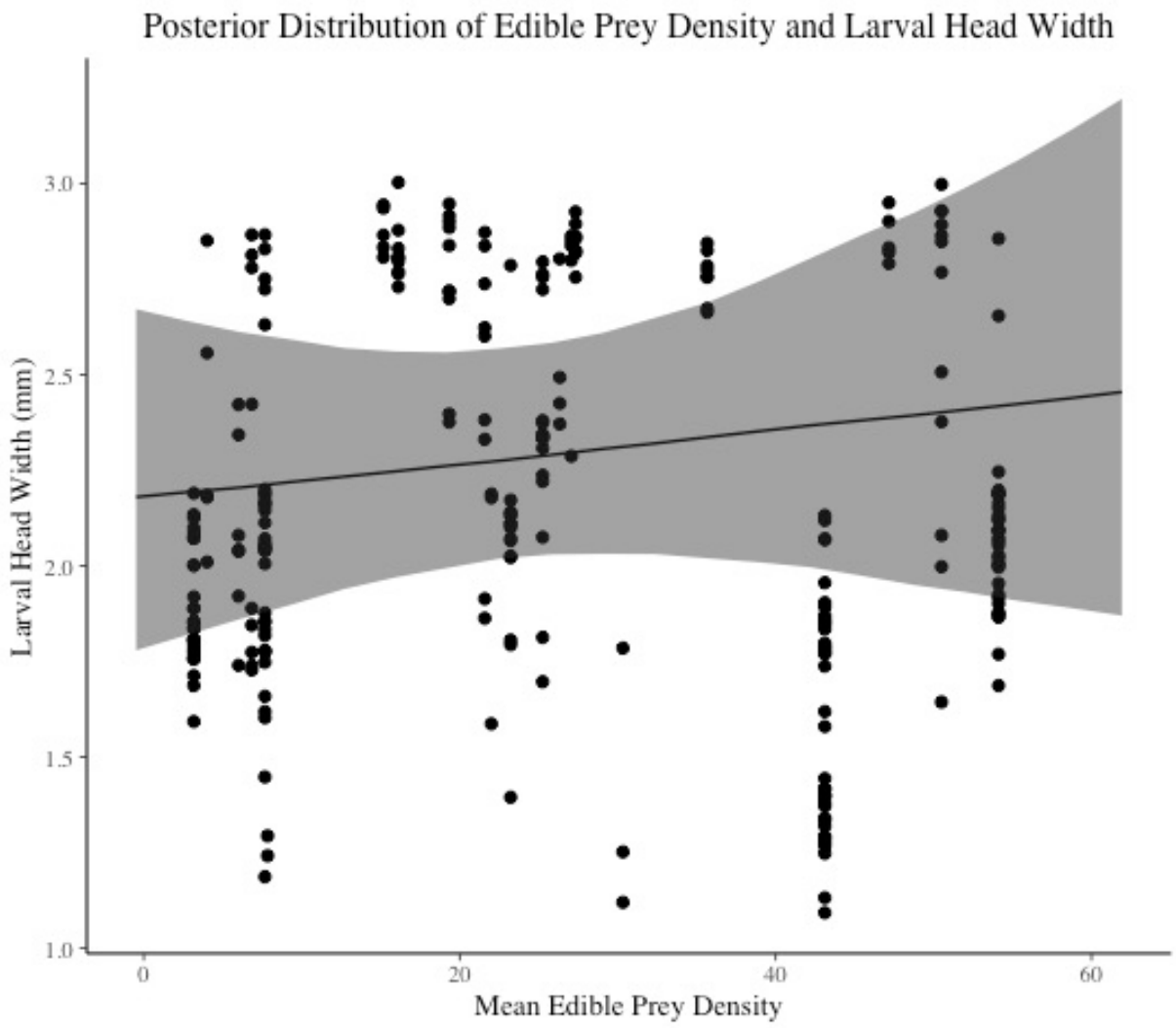

Figure 9. Comparing expected mean head width of *S. hineana* larvae in medium microcosms and mean edible prey density. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is 0.08, with a 95% CrI=(-0.18, 0.38). This indicates that there is a slightly positive relationship associated between *S. hineana* larvae and edible prey density.

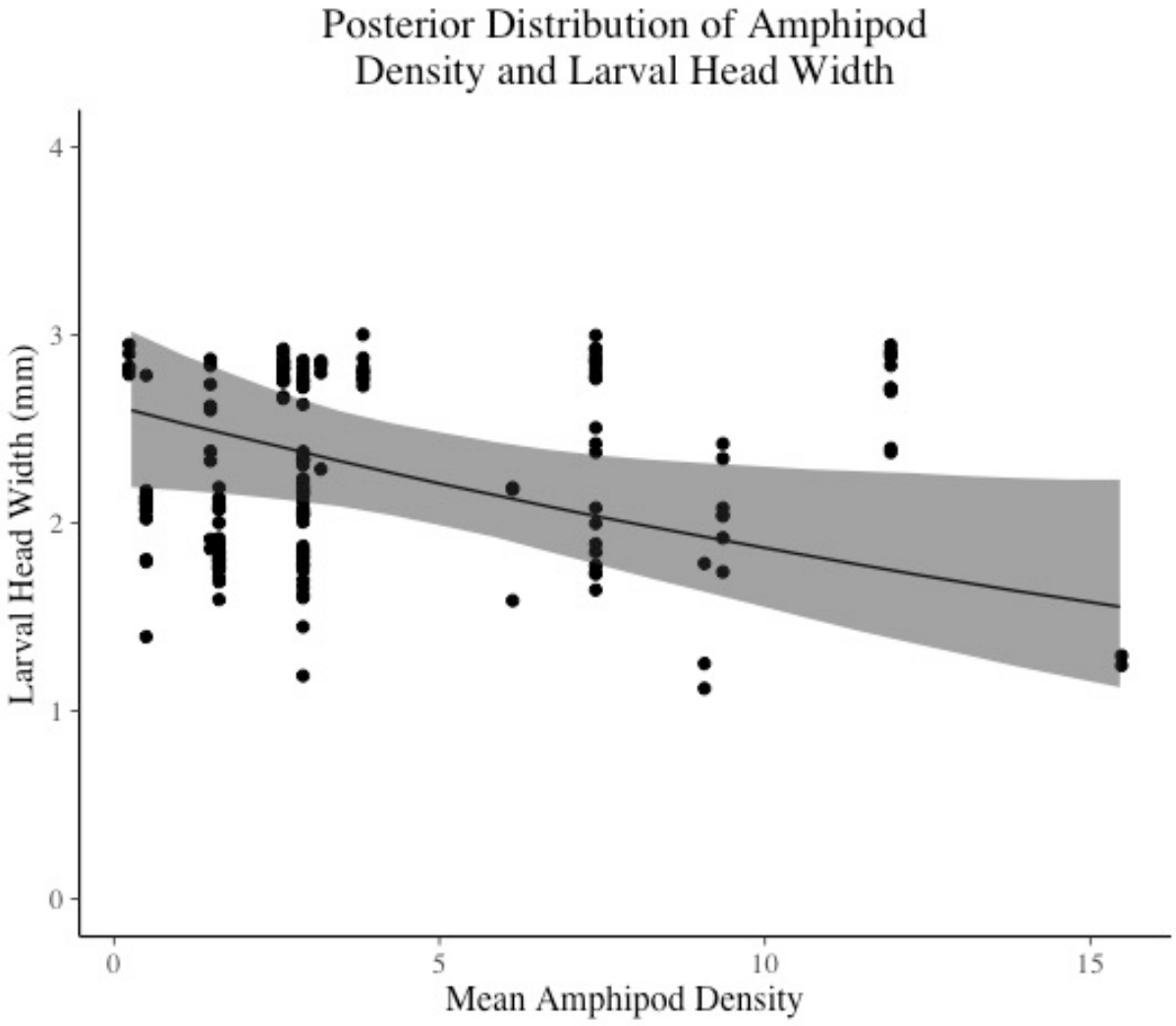

Figure 10. Comparing expected mean head width of *S. hineana* larvae in medium microcosms and mean Amphipoda density. Raw data is indicated with points. Shaded area is the credible interval. The slope of this line is -0.25, with a 95% CrI=(-0.43, -0.02). This indicates that there is a clear negative relationship between the presence of amphipods on the growth of *S. hineana* larvae.