Ecotoxicological Effects of Roundup© Herbicide on the Hatching Success and Naupliar Survival of *Acartia* spp.

Sydney Ulland[ac, 1], Alex Ascher[ad, 2], Katie Lankowicz[b, 2], Isabelle See[ac, 2]

[a] Quahog Bay Conservancy, Harpswell, ME

[b] Gulf of Maine Research Institute, Portland, ME

[c] University of Maine, Orono, ME

[d] Woods Hole Oceanographic Institute, Falmouth, MA

[1] Author

[2] Project Advisor

Abstract

Zooplankton are lower trophic level animals that are particularly vulnerable to anthropogenic effects. They are often found in surface waters feeding on phytoplankton, which is also where contamination from runoff begins in aquatic environments. This study aimed to test how an herbicide, a type of pesticide, affects the hatching success and naupliar survival of copepods, one of the most abundant zooplankton groups. This was done by observing the hatching success and naupliar survival of *Acartia* spp., extracted from Casco Bay, ME, across four increasing concentrations of dicamba, a common herbicide chemical. Both hatching success and naupliar survival of *Acartia* spp. were reduced when treated with dicamba. This indicates that this foundational species of the food web is susceptible to herbicide toxicity at multiple life stages. Contamination from runoff could therefore potentially impact the ecosystem as a whole through a bottom-up trophic cascade, regardless of how other trophic levels respond to contaminants.

Introduction

Chemical contamination in environmental systems is a growing issue in coastal marine and freshwater areas. Its effects on ecosystems can be immediate and obvious, or more discrete over time. Contamination can occur from both natural and anthropogenic sources. Anthropogenic sources come in the form of air, noise, or chemical pollution. When this pollution comes in the form of chemical contamination, or runoff, it can create “hotspots” of nutrient output into aquatic ecosystems. These contamination zones often involve runoff of pesticides or fertilizers from grassy areas like lawns. Grassy areas contribute to chemical contamination concentrations four times higher than those of other urban runoff areas (Müller et al., 2019).

Pesticides are common contributors to high pollutant concentrations. Pesticides are chemical substances that are used to prevent or destroy a variety of pests including insects, rodents, and weeds. Due to their wide range of applications, pesticide use has increased globally in recent decades with over 1 billion pounds annually used in the United States and 5.6 billion pounds worldwide (Alavanja, 2009). Pesticides have the potential to be the most serious of contaminants because of their intended effect of killing living organisms, whether that be the target organisms or unintended organisms (Hanazato 2001). Among pesticides, herbicides target weeds and plants in residential or commercial lawns and grassy areas. A common active ingredient in herbicides is dicamba, which this study focuses on. Herbicides with dicamba have become more widely used due to the emergence of weeds that have become glyphosate-resistant (another common active ingredient in herbicides). However, the issue with the increased use of dicamba-based herbicides is that they have been found to contaminate ground and surface waters due to their high mobility in soil and slow biodegradability (Homa et al., 2024).

Given the potential ecotoxicological impacts of nutrient runoff on aquatic ecosystems, studying lower trophic level animals is crucial for understanding possible cascading effects on the ecosystem as a whole (Hanazato, 2001). Zooplankton link the energy produced by primary producers (phytoplankton) to the organisms in higher trophic levels such as fish, whales, crabs, and seabirds. Common zooplankton include copepods, 1-2 mm long crustaceans, cnidarians, ctenophores, certain worms, and others (NOAA, n.d.). Studying their important role in the pelagic food web as prey to larger organisms and ichthyoplankton is key to understanding the processes and interactions of the marine ecosystem (Turner, 2004; Deschamps et al., 2024). In this study, *Acartia* spp. is the target study genus including primarily *A. clausi* and *A. tonsa*. Both species are prevalent in Casco Bay, where the study organisms were collected. Casco Bay, located in the mid-coast region of Maine, has detectable levels of dicamba from stormwater runoff (Friends of Casco Bay, 2024). Although herbicides with dicamba are not the only herbicides being used in the area, the biochemical and biophysical processes by which dicamba affects plants are very similar to other herbicides being introduced into coastal ecosystems.

In other studies, copepods in the *Acartia* genus have been exposed to fluctuations in environmental variables beyond their optimal conditions. These environmental variables include salinity and temperature, which have been shown to affect fecundity and hatching success. Significant changes from optimal conditions altered the population's lower egg production rates at low temperatures and salinity (Castro-Longoria, 2003). Another study that looked at zooplankton egg banks and the effect of glyphosate introduction found that dormant communities, such as those in egg banks, were susceptible to toxicity effects from contamination, ultimately reducing the abundance and diversity of hatchlings (Gutierrez et al., 2017). Early life stages of zooplankton are often considered the most sensitive to pesticides, making populations composed of those early post-hatch organisms inherently more vulnerable to decline (Hanazato, 2001).

With the assumption that *Acartia* spp. populations are affected by alterations in their optimal conditions caused by natural or anthropogenic forces, this study investigates the effect of anthropogenic chemical contamination in the form of dicamba on hatching success and naupliar survival. The herbicide used in this study was Roundup for Lawns, which has active ingredients including Dicamba, MCPA, Quinclorac, and Sulfentrazone. This test principle was chosen because Dicamba is found to be harmful to aquatic life with lasting impacts (Albaugh, 2021). Within this study, it is hypothesized that egg hatching rates and naupliar survival will decrease with more concentrated levels of dicamba contamination. Measuring the effects of dicamba on *Acartia* spp. hatching success and naupliar survival could aid in modeling population dynamics in areas with high anthropogenic activity.

Methods

Copepod Sampling

Eggs, nauplii, and copepods were collected during the day from Casco Bay, ME in July 2024 by plankton net. Boat tows were the primary method of extraction, although dock tows were occasionally utilized for early parts of testing. The mesh size was 100 microns and after pulling horizontally for 10 to 15 minutes, water was filtered out of the net and transferred into capped bottles that remained in a cooler with ice packs to maintain cool, tolerable temperatures required for survival until transferred to the lab. Once in the lab, the samples were filtered through a 180-micron sieve to catch copepods and larger organic material, and then a 53-micron sieve to catch eggs and nauplii. *Acartia* spp. eggs were identified by size (~80 µm) and separated from the filtrate using a 100µL pipette.

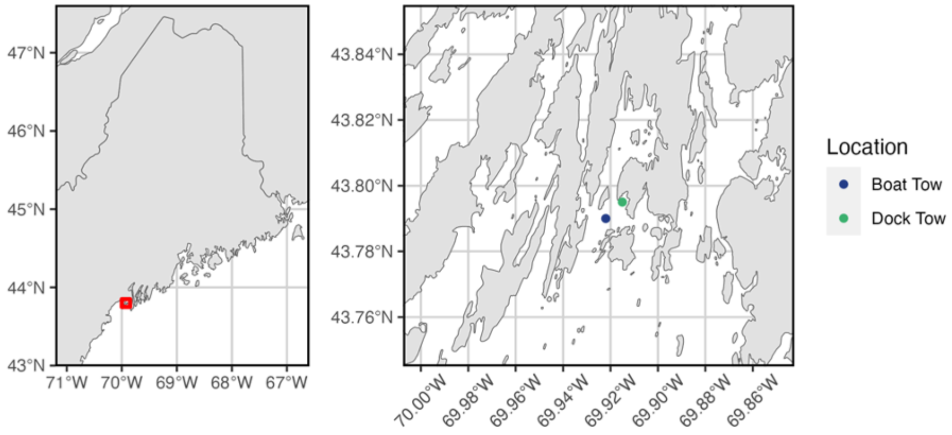

Figure 1. Location of boat and dock tow in Casco Bay

Concentrations

Four concentrations of dicamba were tested: 0, 1, 10, and 100 ppb. These concentrations were decided based on values found in a Friends of Casco Bay Residential Pesticide Study (2003). That study found concentrations of dicamba from 2 to 4 ppb in the test sites, so to understand potential differences in concentrations from the past 20 years we chose concentrations below and higher than the study's published values. Desired concentrations were obtained through serial dilution of a stock solution of Roundup for Lawns© which contains dicamba at a concentration of 0.029%.

Egg Hatching Experiment

Acartia spp. eggs caught during plankton net tows were placed into petri dishes with 10mL of seawater at a salinity of 25 ppt. Each dish contained 10 eggs, and a concentration of Dicamba as outlined above. Eggs were monitored in their treatment concentrations for 72 hours. When checked, it was noted if they had hatched.

Naupliar Survival Experiment

Nauplii were obtained from a culture of copepods in the lab which were initially collected from Casco Bay as described above. We divided 10 nauplii into each treatment (0, 1, 10, and 100 ppb dicamba) for 72 hours. Nauplii were monitored *ad libitum* under a stereomicroscope for the duration of the experiment with a total of 8 monitoring times. They were monitored more frequently earlier in the experiment to capture early mortalities. Nauplii which lacked obvious signs of life, such as motor function, were recorded as mortalities.

Statistical Methods

All statistical calculation and graphing was done with R software (R Core Team, 2024). To compare hatching success at the different concentration levels, percent hatching success at each observation was calculated by total hatched eggs divided by total eggs in the treatment. The calculated hatch rate over time was then visualized as a stepwise function. To determine if there was a statistically significant difference between the treatments, a generalized linear model with binomial distribution was run. A two-sided power analysis was conducted to quantify the number of samples theoretically needed to identify significant differences between treatment levels paired with the control at a 95% confidence level given the observed differences between means.

For the naupliar survival experiments, the data were modeled as Kaplan-Meier survivorship curves. A Cox Proportional Hazards model was used to test for significant differences in survivorship between the treatment levels and the control. The assumptions for a Cox proportional hazards model were tested and validated prior to modeling.

Results

Hatching Success

After calculating the percent hatching rate at each time the eggs were counted, a stepwise function of hatching success (Figure 2) showed the Dicamba concentration of 100 ppb had the lowest hatching rate and the control (0 ppb) had the highest hatching rate at the end of the experiment. 1 ppb had a lower hatching rate than 10 ppb throughout the duration of the experiment.

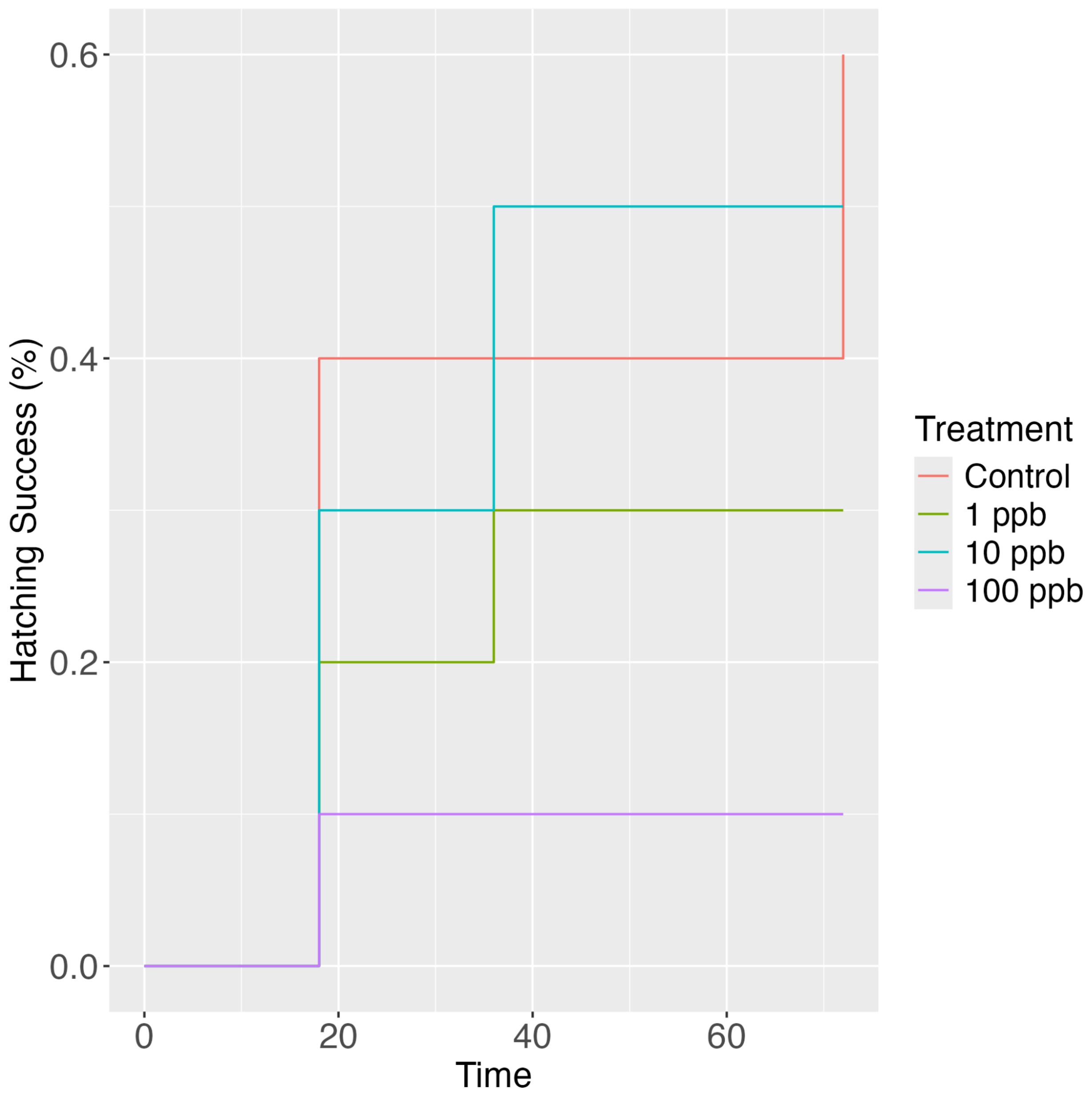

Figure 2. Stepwise function of hatching success over 72 hours

After running a binomial-distribution generalized linear model, the data showed that only the treatment with 100 ppb was significantly different from the control treatment with a p-value of 0.035. Both 1 (p=0.185) and 10 ppb (p=0.654) did not show significant differences from the control, with 10 ppb being the most similar to the control. Figure 3 shows the predicted probability of hatching success with 95% confidence intervals as indicated by red dashed lines. Confidence intervals overlap between all treatments, but there is less overlap between 100 ppb and control which was the only statistically significant difference.

Power analysis showed that egg counts would have to be larger in order to identify significant differences with 95% confidence between each treatment and the control. The two-sided comparison gave an n-value of 69 for comparison between 1 ppb and control, indicating that to see a distinct difference between treatments 69 eggs would have had to be the total egg count in each treatment. The other treatments similarly required larger egg counts to show a difference between the treatment and control groups: 640 eggs for the 10 ppb concentration and 21 eggs for the 100 ppb concentration.

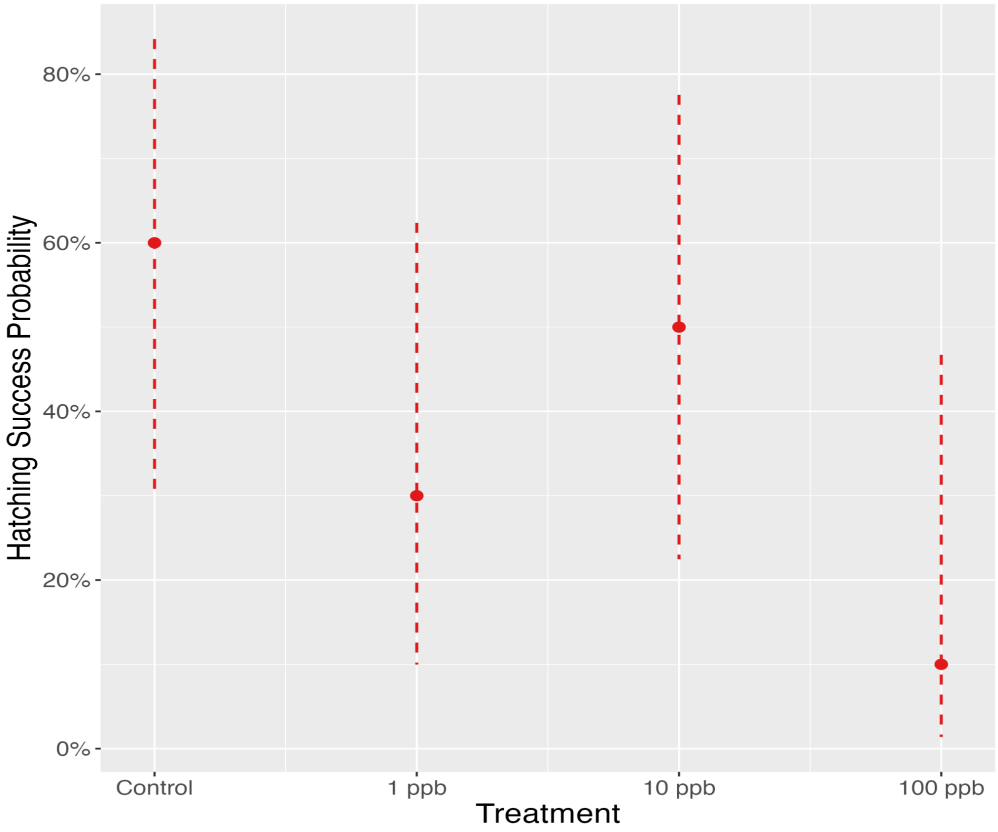

Figure 3. Hatching success probability at the four treatments

Naupliar Survival

Using the Kaplan-Meier survivorship curve (Figure 4), we saw that with higher dicamba concentration, survivorship probability decreased over time. There is a lot of overlap between the 95% confidence intervals (shown by the color-coded shading). The Cox model showed that there is no significant difference in naupliar survival between 1 ppb and control (p=0.697), a significant difference between 10 ppb and control (p=0.031), and a marginally significant difference between 100 ppb and control (p=0.105). Based on visual checks from the Kaplan-Meier curve, we can conclude that the control had the highest survival and 10 ppb had the lowest survival. Overall, the direction of these differences indicates that a higher concentration has a lower survival rate.

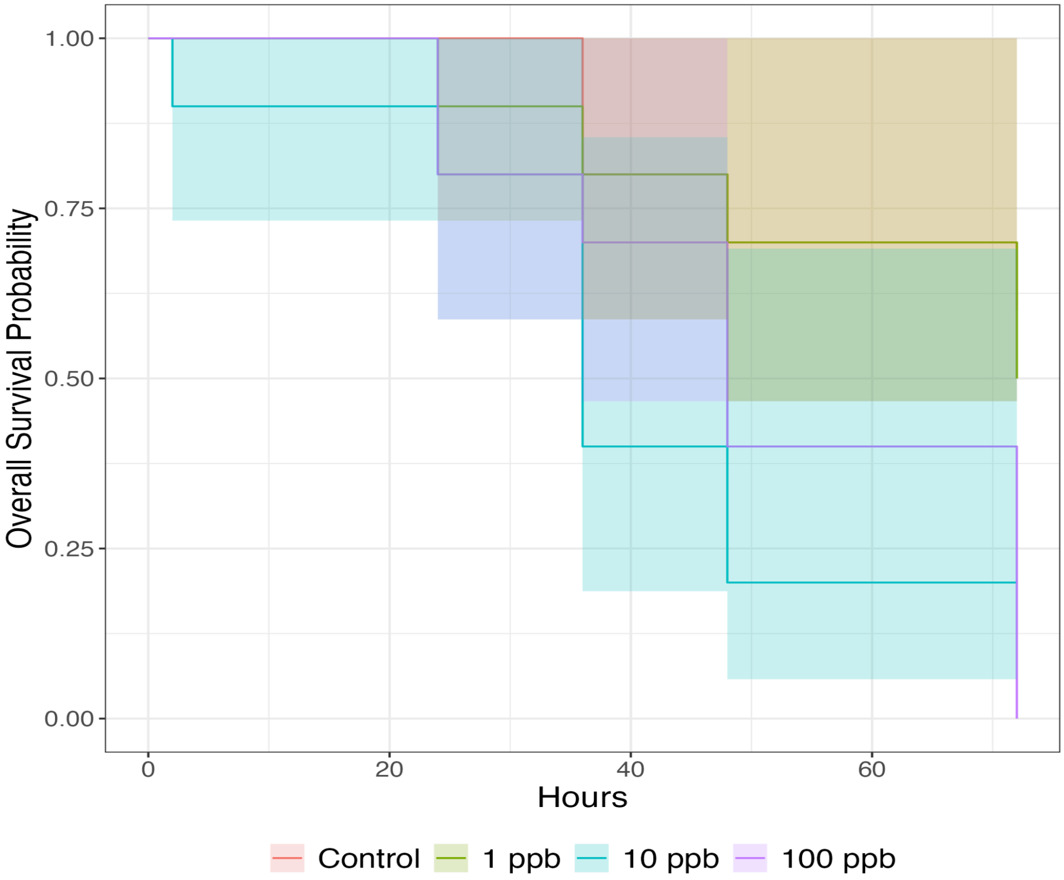

Figure 4. Kaplan-Meier survivorship curve of Acartia nauplii

Discussion

Previous work has suggested that copepods are highly sensitive to environmental changes and can alter metabolism and function such as hatching success and survival (Deschamps et al., 2024). As hypothesized, the 100-ppb treatment had the lowest hatching rate, and the control had the highest hatching rate. Hatching success was only significantly impacted by the highest concentration of dicamba (Figure 3). This indicates that hatching success is, in fact, reduced after higher exposure to contamination, which is consistent with patterns outlined in other papers (Gutierrez et al., 2017; Castro-Longoria, 2003; Hanazato, 2001).

The 1 ppb and 10 ppb treatments showed a similar pattern of higher egg mortality than the control. The 1 ppb treatment, however, experienced a lower hatching success than the 10 ppb treatment, which, following the line of reasoning outlined in the previous paragraph, is inconsistent with this study's expected results. Both treatments were not significantly different from the control. This could indicate that some other variable was hindering the egg's ability to hatch. Considering the eggs were all kept in the same area and with the same conditions during introduction to treatment and throughout monitoring, it is unlikely that temperature, salinity, or oxygen level were the variables that resulted in unexpected results. Extraction using the micropipette, however, could have resulted in injury or stress that may have resulted in the inability of certain eggs to hatch in these treatment levels. Another possibility is that the eggs were at different stages of development at the beginning of the experiment, which could have resulted in higher or lower susceptibility to chronic issues from contamination. For example, eggs at later stages of development may have a stronger tolerance against contamination whereas eggs in earlier stages may not yet have the basic protections against toxic stimuli affecting their developmental processes.

Naupliar survival followed the predicted pattern, where survivorship of nauplii decreased as dicamba concentration increased. Dicamba was hypothesized to affect the naupliar stage in a similar manner to egg hatching success where the toxicity would affect function and produce higher rates of mortality than would be observed in an uncontaminated treatment. Based on the data shown in Figure 4, the effect of increasing contamination is consistent with what was originally hypothesized. Naupliar survival rate was significantly lower in the 10 and 100 ppb treatments than the control.

This experiment had many variables that were not accounted for due to limits in time and space. If replicated or redone, a larger sample size, a longer monitoring period, and a greater number of replicates would be recommended to achieve more robust results. Despite these limitations, this study was still able to discern significant impacts of a common contaminant on biological factors which have important implications for zooplankton population health. It is therefore recommended that continued research be done to understand the long-term and broad-scale impacts of exposure to herbicides on zooplankton and other key organisms. Even if the contamination levels aren't lethal, sub-lethal effects of contamination could lead to slower growth and lower energetic content (Deschamps et al., 2024) This may reduce prey availability so fish and other organisms that eat zooplankton may be impacted by a lack of food availability as well as lower energetic content in contaminated zooplankton. These negative effects could cascade to impact human food production and economic activities within coastal communities.

To reduce the potentially negative impacts of chemical contamination, creating or enforcing herbicide application measures and being aware of potential impacts on fish stocks is recommended.

Acknowledgments

I would first like to thank Pat Scanlan and Quahog Bay Conservancy for giving me the opportunity to participate in this unique and enriching internship. I would then like to thank Isabelle See and Tait Nygaard for showing me the world of marine science applied in a way I hadn't seen before. I thank Katie Lankowicz and Alex Ascher for giving me the knowledge and confidence to feel like being a marine scientist isn't just a dream. I would also like to extend my gratitude to Dr. Walt Golet, the University of Maine School of Marine Science, and the entire Pelagic Fisheries Lab for making this a more vivid and hands-on learning experience. Finally, I would like to thank my fellow interns, Courtney Swenson, for making me laugh, Emma Archambault, for being a wonderful roommate and friend, and Reuven Frye, for putting up with all of it. All of you made this internship a once-in-a-lifetime experience, and I will value our memories and friendships forever.

Citations

Alavanja, M. C. R. (2009). Introduction: Pesticides Use and Exposure, Extensive Worldwide. *Reviews on Environmental Health*, *24*(4). https://doi.org/10.1515/REVEH.2009.24.4.303

BayScaping helps you grow green lawns that keep Casco Bay blue • Friends of Casco Bay. (n.d.). *Friends of Casco Bay*. Retrieved July 30, 2024, from https://www.cascobay.org/our-work/community-engagement/bayscaping/

Castro-Longoria, E. (2003). Egg Production and Hatching Success of Four Acartia Species under Different Temperature and Salinity Regimes. *Journal of Crustacean Biology*, *23*(2), 289–299. https://doi.org/10.1163/20021975-99990339

Deschamps, M. M., Boersma, M., Meunier, C. L., Kirstein, I. V., Wiltshire, K. H., & Di Pane, J. (2024). Major shift in the copepod functional community of the southern North Sea and potential environmental drivers. *ICES Journal of Marine Science*, *81*(3), 540–552. https://doi.org/10.1093/icesjms/fsad160

Ecology of the Northeast US Continental Shelf—Zooplankton: Ecosystem Considerations: Northeast Fisheries Science Center Ecosystem Assessment Program. (n.d.). Retrieved August 9, 2024, from https://apps-nefsc.fisheries.noaa.gov/nefsc/ecosystem-ecology/zooplankton.html

Gutierrez, M. F., Battauz, Y., & Caisso, B. (2017). Disruption of the hatching dynamics of zooplankton egg banks due to glyphosate application. *Chemosphere*, *171*, 644–653. https://doi.org/10.1016/j.chemosphere.2016.12.110

Hanazato, T. (2001). Pesticide effects on freshwater zooplankton: An ecological perspective. *Environmental Pollution*, *112*(1), 1–10. https://doi.org/10.1016/S0269-7491(00)00110-X

Homa, J., Stachowiak, W., Olejniczak, A., Chrzanowski, Ł., & Niemczak, M. (2024). Ecotoxicity studies reveal that organic cations in dicamba-derived ionic liquids can pose a greater environmental risk than the herbicide itself. *Science of The Total Environment*, *922*, 171062. https://doi.org/10.1016/j.scitotenv.2024.171062

Müller, A., Österlund, H., Marsalek, J., & Viklander, M. (2020). The pollution conveyed by urban runoff: A review of sources. *Science of The Total Environment*, *709*, 136125. https://doi.org/10.1016/j.scitotenv.2019.136125

R Core Team (2024). R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*. https://www.R-project.org

Turner, J. (2004). The importance of small planktonic copepods and their roles in pelagic marine food webs. In *Zoological Studies* (Vol. 43, p. 266).