Effects of simulated eutrophication on the growth of the invasive, biofouling hydrozoan *Cordylophora caspia*

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ABSTRACT

Agriculture-heavy areas such as the state of Illinois depend largely on fertilizer, a significant contributor of eutrophication, for mass production of crops. While a great deal of research has been done on the overall effects of eutrophication on entire ecosystems, little research has been done on the effects of eutrophication on the invasive, biofouling hydroid *Cordylophora caspia.* To examine the effects of Illinois-specific eutrophication on *C. caspia*, we simulated the eutrophic conditions of Illinois water systems in a laboratory setting to culture hydroid colonies and observed growth in terms of hydranth production. Our results showed that simulated eutrophic conditions significantly decreased the growth rates of *C. caspia*. Eutrophic conditions induced several incidences of morphological mutations in *C. caspia.* Based on our results, we suggest a variety of future studies, including what may need to be done for a potential field experiment in order to more closely examine the effects of eutrophication in Illinois on *C. caspia*.

INTRODUCTION

Eutrophication, or the introduction of excess nutrients into bodies of water, is a worldwide phenomenon (Carpenter et al., 1998, Hodgkin & Hamilton, 1993, Huang et al., 2017) that can occur as a side effect of nutrient runoff from fertilized agricultural areas. Eutrophication typically leads to an excessive growth of algae and phytoplankton in bodies of water (Turner & Rabalais, 1994, Burkholder et al., 2007, Gilbert et al., 2005, Heisler et al., 2008) and a decrease

in most animal growth (Pieczyńska et al, 1998, Carpenter, 2008, Seehausen et al., 1997) due to oxygen deficiency (Rosenberg & Loo, 2012). However, a small subset of animals are able to thrive in eutrophic conditions, including some macrozoobenthos such as the clam *Macoma balthica* (Beukema & Cadée, 1986) and other filter-feeding animals (Soto & Mena, 1999, Rice, 2001, Officer et al., 1982), some of which are known biofoulers (Nakano & Strayer, 2014), such as *Cordylophora caspia*, an invasive colonial hydroid found worldwide in a variety of water systems.

Cordylophora caspia, a member of the phylum Cnidaria and class Hydrozoa, is native to the Ponto-Caspian region but has been introduced to various freshwater and brackish ecosystems worldwide (Obolewski et al., 2015, Arndt, 1984). Its definitive morphological characteristic is the formation of feeding polyps, or hydranths, with tentacles to capture prey flowing in the water nearby (Fulton, 1962); these hydranths can vary in size and tentacle length (Kinne, 1958), and the number of hydranths on a colony have been used as a measure of growth in various experiments (Fulton, 1962, Folino-Rorem & Renken, 2018). While *C. caspia* is an unfamiliar organism to many, it poses a problem to many humans in the form of biofouling, or growing on man-made structures and obstructing water flow (Bixler & Bhushan, 2012, Melo & Bott, 1997). *C. caspia* has been observed to be a biofouling organism within water intake pipes and other man-made structures (Folino-Rorem & Indelicato, 2005), which contributes to a global cost of potentially more than $277.1 million USD annually (Nakano & Strayer, 2014). While it is likely that eutrophication leads to the expansion of biofoulers as a whole, little research has been done specifically on eutrophication affecting *C. caspia* specifically. Considering the massive impact this organism could have on a wide variety of human industries, this lack of research is especially concerning.

One of the reported incidences of *C. caspia* biofouling occured in Illinois (Folino-Rorem & Indelicato, 2005), a US state with heavy dependence on agriculture. According to the Illinois Department of Agriculture, Illinois is one of the top producers of agricultural products among all states, generating billions of dollars to the Illinois economy. The two major crops in Illinois are corn and soybeans as per the US Department of Agriculture (USDA, 2018). Among the total of 27,000,000 acres of operating farmlands in Illinois, corn farming occupies 10,500,000 acres while soybean farming occupies 9,950,000 acres. Corn and soybean farming accounts for approximately 75.5% of the total farmland in Illinois and value over $12 billion USD. In fact, as data from USDA National Agricultural Statistics Service (USDA NASS, 2018) indicate, Illinois ranks first in soybean production and second in corn production among all US states.

Given this massive crop production, extensive fertilizer usage in Illinois is commonplace. According to USDA NASS data, the usage of every primary component of fertilizers (nitrogen, phosphate, potash/potassium) on corn and soybean in Illinois is above the national average, and in many cases, Illinois fertilizer usage is substantially above the national average (Tables 1A and 1B). This can be a potential indicator of more severe fertilizer runoff and thus, increased likelihood of eutrophication. Since agriculture is tightly connected to eutrophication, it is reasonable to deduce that in an agriculture-intensive state like Illinois, the problem of eutrophication is potentially even more pronounced. However, there has not been much research done on the effects of eutrophication in Illinois specifically. This lack of location-specific research is rather worrying and needs to be addressed.

Table 1A. 2018 Illinois Fertilizer Usage on Corn as Compared to National Average. Unit: Pounds/Acre

Corn	Illinois	National Average

Nitrogen	172	149
Phosphate	108	69
Potash	135	87

Table 1B. 2018 Illinois Fertilizer Usage on Soybean as Compared to National Average. Unit: Pounds/Acre

Soybean	Illinois	National Average
Nitrogen	22	17
Phosphate	75	55
Potash	111	87

Due to a lack of research regarding the impact of eutrophication on aquatic organisms and ecosystems on a local level in Illinois, we sought to address this issue using the model biofouling organism *C. caspia*. More specifically, we looked into how eutrophication affects the growth of *C. caspia* in terms of hydranth (feeding polyp) production; i.e, whether eutrophic conditions increase or decrease the number of hydranths on *C. caspia* colonies. Based on the current literature (Soto & Mena, 1999, Rice, 2001, Officer et al., 1982, Roos, 1979) and that *C. caspia* is considered a filter feeder, our hypothesis is that eutrophication significantly increases the growth rate of *C. caspia* in terms of hydranth production.

MATERIALS & METHODS

Establishing Experimental Colonies

For our experiment, *C. caspia* colonies from the Des Plaines River in Joliet, IL were divided into two groups: control and treatment. The control group colonies were cultured in

water without nitrogen, phosphorus, and potassium ions (NPK), while the treatment group colonies were cultured in water with NPK present to simulate eutrophication. For both groups, we prepared water at a salinity of 0.6ppt, the salinity of *C. caspia*'s freshwater habitat in the Des Plaines River. Two 8L tanks with appropriate filters were used for each group. Nitrogen was introduced into the treatment group through sodium nitrate ($NaNO_3$) (0.0723g/L), phosphorus was introduced through sodium phosphate dodecahydrate ($Na_3PO_4 \cdot 12\ H_2O$) (0.1472g/L), and potassium was introduced through potassium chloride (KCl) (0.0286g/L). Concentration values for each nutrient were obtained by using USDA NASS data (Table 1) converted into grams and using NOAA data on rainfall levels in Joliet, IL to estimate the amount of water that flowed through a single acre in a year. Our values do not account for additional sources of irrigation or other nutrient sources.

C. caspia colonies were prepared for the experiment in several steps: firstly, segments of a single parent colony, each with two hydranths (their characteristic feeding polyps with tentacles) were prepared. Secondly, these segments were attached to glass microscope slides by use of rubber bands. Thirdly, these slides were placed into a plastic slide box (4 slides per box) with a large viewing hole cut into the top and bottom, and the slides were evenly spaced within the box. The box itself was placed with the colonies facing the bottom of the tank so as to prevent buildup of debris (Figure 1). A total of 24 replicate colonies for each group (control and treatment) were used, so 6 slide boxes were used per treatment group, and three slide boxes were used per tank.

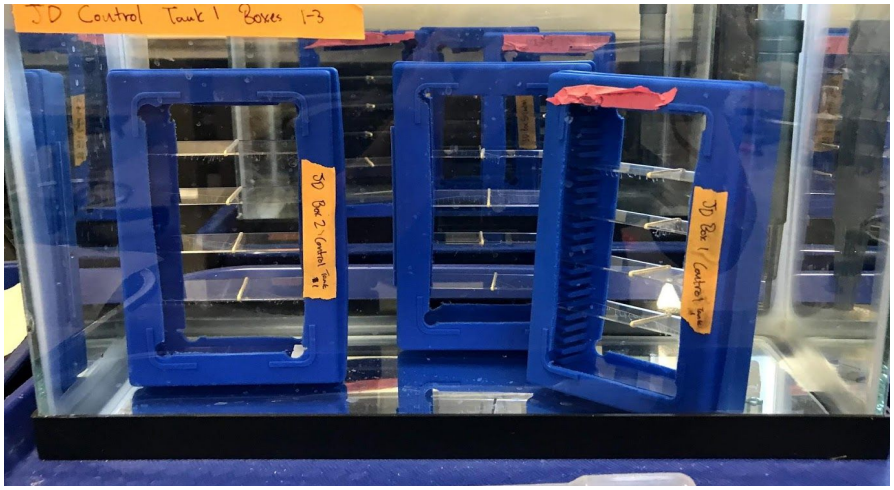

Figure 1: The experimental set up of one of our two control tanks. While the *Cordylophora caspia* colonies are not visible in the photo, it should be noted that they are attached to the slides via the rubber bands and are facing downwards so as to prevent buildup of debris among the colonies. Four similar tanks like this were used, each with three slide boxes, and two of these tanks were treated with nutrients and served as treatment tanks.

Culturing & Data Collection

After setting up the two groups, the number of hydranths on each colony was recorded over 26 days on days 2, 5, 7, 9, 12, 14, 16, 19, 21, 23, and 26. On each of these days, each colony was fed to saturation (until the hydranth tentacles could not catch any more food) with brine shrimp (genus *Artemia)*. In order to keep the concentration of nutrients in the experimental group constant, we changed the water out in every tank on days 9, 16, and 23.

To ensure that no anomalous conditions took place in the tanks, the pH values of the water in the control and experimental tanks were measured on day 12. Salinity and temperature of the tanks was also measured, but greater flexibility was taken with these values as *C. caspia* is known to tolerate a wide range of salinities (Kinne, 1958) and temperatures (Fulton, 1962). Fungal growth was also removed from the colonies on each day when hydranth counts were measured. Regardless, some anomalous conditions occurred, such as unexpected menont

formation and mutations within hydranths, and these were recorded in the form of qualitative observations.

Data Analysis

The rate of *C. caspia* growth, in terms of hydranth production over time, was determined by plotting a scatter plot of the day (x-axis) against the number of hydranths on each replicate colony (y-axis) and then fitting a best-fit line for each colony. The slope of this best-fit line was used as the growth rate, and the unit for our growth rate was new hydranths produced per day. A two-sample t-test was then used to assess whether there was a significant difference between the growth rates (mean slopes) of the different groups.

RESULTS

After the data were analyzed, the average growth rate for treatment was determined (Table 2, Figure 2). For the control group, the average growth rate was 0.844 hydranth/day (SE=0.063). For the treatment group, the average growth rate was 0.577 hydranth/day (SE= 0.083). This difference in growth rates can be visualized in Figure 2; throughout the experiment, the mean number of hydranths on the control colonies was consistently higher than the mean number of hydranths on the treatment colonies.

The 2-sample t-test suggested that there was a significant difference in hydranth growth rate between the control group and the treatment group (t= -2.550, df=23, p=0.0144) (Table 3). The treatment group cultured in eutrophication settings had significantly lower growth rate as compared to the control group which was cultured in 0.6ppt water without nutrients added .

Table 2. Summary of the Experimental Data

	Replicate Number	Mean Rate (hydranths/day)	Standard Error
Control	24	0.844	0.0630
Treatment	24	0.577	0.0835

Table 3. Summary of the t-Test Statistics

Difference	-0.26693
t Ratio	-2.55028
p-value	0.0144

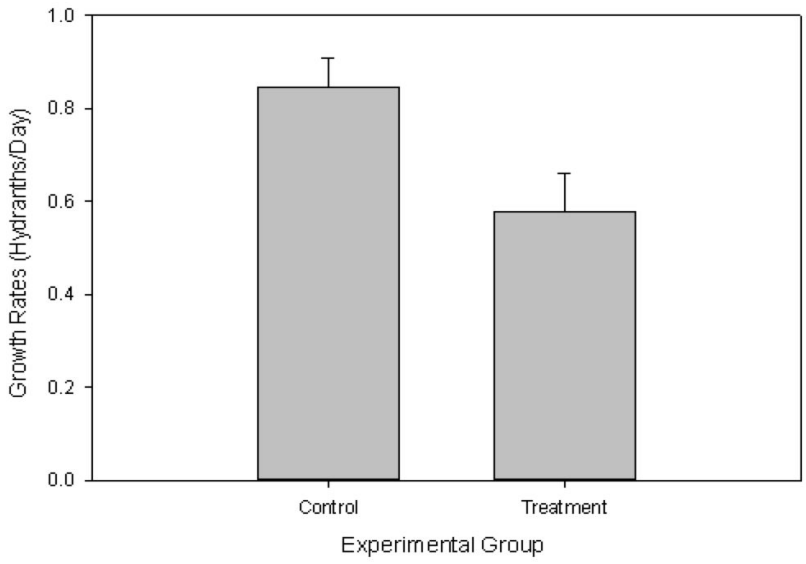

Figure 2: Mean growth rates of *Cordylophora caspia* colonies in terms of hydranths/day in the control group and the treatment group. Both groups contain the same number of replicates (n=24). The error bars represent one standard error.

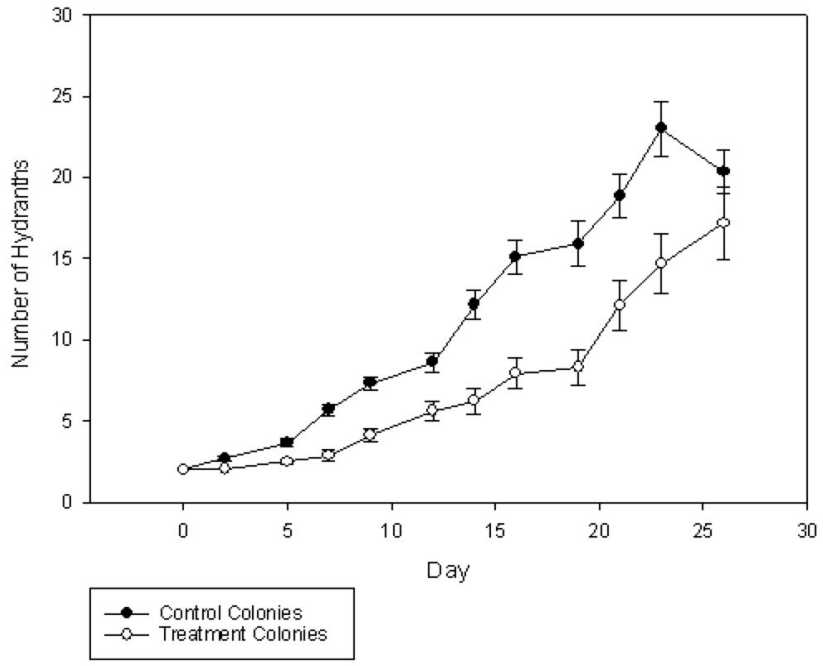

Figure 3: Mean number of *Cordylophora caspia* hydranths in the control and treatment group over the duration (26 days) of the experiment. The error bars represent one standard error.

DISCUSSION

Conclusions and Sources of Error

While we initially expected to see that *C. caspia* would be able to adapt to the eutrophic condition quickly as suggested in Roos (1979) and demonstrate greater hydranth production than control colonies, the exact opposite occurred. Our results showed that eutrophic conditions have long-lasting negative impacts on the growth of *C. caspia*, even after weeks of adaptation. This long-term effect has several possible explanations: firstly, the ions in eutrophic water could have imposed extra osmotic stress on the *C. caspia* colonies, thus requiring more energy to maintain homeostasis, and therefore leading to decreased growth.

Secondly, the phosphate ion (PO_4^{3-}) is a relatively strong conjugate base of phosphoric acid, which leads to the pH of the water increasing. This was observed in our pH measurements; the treatment tanks were slightly alkaline (7.78 compared to 7.06 for control tanks). While

current literature suggests that *C. caspia* can tolerate slight pH changes, *C. caspia* growth is inhibited in the basic conditions present in our treatment tanks (Fulton, 1962). Thus, the basic conditions of the treatment tanks could have reduced the growth rate of the treatment colonies.

Thirdly, a considerable amount of fungus was present on the colonies. While we did not quantitatively measure the fungal growth on the colonies, we generally observed more fungus on colonies in the treatment group. This fungal growth could also have been a source of error, and even though we tried to remove most of this growth, it was generally impossible to completely remove the fungus, which could have inhibited the growth of our treatment colonies.

Fourthly, in order to maintain nutrient concentration in our tanks at our prescribed level, we performed water changes every week. This could have added stress to the colonies and reduced their growth. However, both groups showed slower growth immediately after a water change (Figure 3), particularly the control group towards the latter end of the experiment. While we cannot completely eliminate the possibility of water changes being the main reason for our unexpected results, it is less likely to be the main reason than the previously mentioned sources of error, those being excess ion concentrations, higher pH, and fungal growth.

Based on our results, the main takeaway we can propose is that more research in the area of eutrophication on *C. caspia* is necessary to truly grasp the effects of eutrophication of the growth of *C. caspia.* In addition, greater research is necessary to limit the effects of Illinois fertilizer usage on Illinois water systems so that farmers and conservationists can agree on action taken to both maximise crop yield and protect local ecosystems. The research performed in this experiment is one small piece of the overall puzzle.

Suggestions for Future Studies

Our primary suggestions for future studies simply aim to circumvent the sources of error found in our experiment. Firstly, more time to culture the colonies might produce results more reflective of long-term effects of eutrophication on *C. caspia* colonies. Towards the latter end of the experiment, the control colonies seemed more variable in their hydranth counts and their growth seemed to slow (Figure 3). Given more time, the treatment (eutrophic) colonies could very well have adapted to the eutrophic conditions and grown to be even bigger than the control colonies. Hence, the first suggestion for a future study would be to use similar nutrient concentrations, but to culture the colonies for a longer time. This would determine if, in the long run, *C. caspia* thrives in eutrophic conditions.

A replication of our experiment, but over a longer timespan, would also have the added benefit of reducing the likelihood of fungal growth on the colonies, eliminating another confounding factor. However, if this experiment were to be performed again, one crucial step would be to add NaOH or some other non-NPK base to the control tanks until the pH of the control tanks is the same as the pH of the treatment tanks. This would eliminate the confounding factor of pH on colony growth, leading to results that are more reflective of the effects of eutrophication.

It is also worth noting that a few mutated hydranths were observed in the eutrophication treatment group while none was observed in the control group. One hydranth in the treatment group had two mouths/anuses on the same hydranth (Figure 4a). Another hydranth merged itself into a nearby hydranth (Figure 4b). This suggests that eutrophication might have the ability to induce mutations, potentially due to the physiological shock it exerts onto the organisms. An area of future research would be to focus on the connection between eutrophication and physiological features and mutations through DNA analysis.

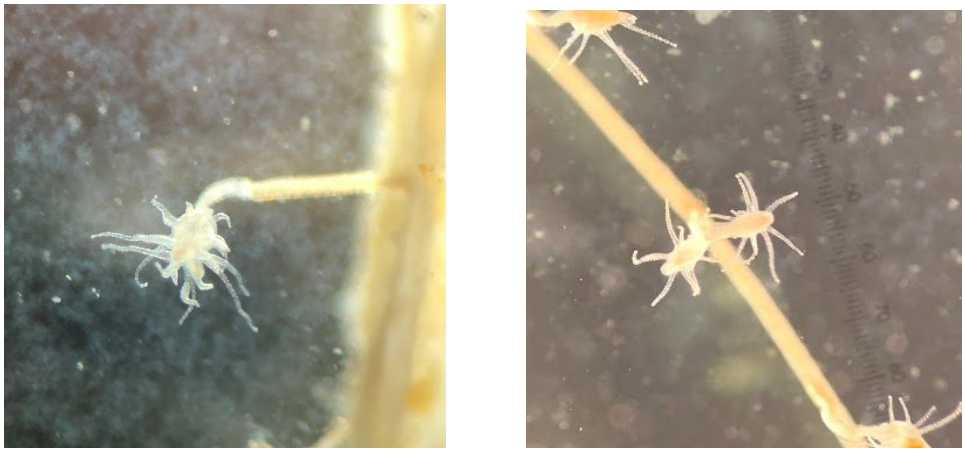

Figure 4: Examples of mutated hydranths found on *Cordylophora caspia* colonies cultured in eutrophic conditions. (left, Figure 4a): A mutated hydranth with two mouths/anuses found in the treatment group of our experiment. (right, Figure 4b): A mutated hydranth that merged into a nearby hydranth.

In order to collect data and derive results even more specific to Illinois, a field experiment might be an option as a field experiment can more accurately represent eutrophication levels in Illinois water systems. However, a field experiment could also introduce many more confounding variables, such as varying levels of algae, varying levels of oxygen, and a possible presence of predators of *C. caspia* (for example, experiments with *C. caspia* in the San Francisco Estuary found several species of fish that fed on hydroids, e.g. shimofuri and shokihaze gobies (Wintzer et al., 2011)). We opted to not perform a field experiment due to the potential abundance of confounding variables, but it is possible for a field experiment to be performed if a highly eutrophic and a non-eutrophic area were found in the same water system. If such areas were found, a field experiment would provide even more insight into how eutrophication affects *C. caspia* in Illinois, as well as how eutrophication affects water systems in Illinois as a whole.

REFERENCES

Arndt, E. A. (1984). The ecological niche of *Cordylophora caspia* (Pallas, 1771). *Limnologica, 15,* 469-477.

Beukema, J. J., & Cadée, G. C. (1986). Zoobenthos responses to eutrophication of the Dutch Wadden Sea. *Ophelia, 26*, 55-64. doi:10.1080/00785326.1986.10421978

Bixler, G. D., & Bhushan, B. (2012). Biofouling: lessons from nature. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 370*, 2381-2417. doi:10.1098/rsta.2011.0502

Burkholder, J. M., Tomasko, D. M., & Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology, 350*, 46-72. doi:10.1016/j.jembe.2007.06.024

Carpenter S. R., Caraco N.F., Correll D. L., Howarth R. W., Sharpley A. N., & Smith V. H (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*. 8, 559–568.

Carpenter, S. R. (2008). Phosphorus control is critical to mitigating eutrophication. *Proceedings of the National Academy of Sciences, 105*, 11039-11040. doi:10.1073/pnas.0806112105

Fulton, C. (1962). Environmental factors influencing the growth of *Cordylophora*. *Journal of Experimental Zoology, 151,* 61-78.

Folino-Rorem, N., & Indelicato, J. Controlling biofouling caused by the colonial hydroid *Cordylophora caspia. Water Research, 39,* 2731-2737. doi:10.1016/j.watres.2005.04.053

Folino-Rorem, N., & Renken, C. J. (2018). Effects of salinity on the growth and morphology of the invasive, euryhaline hydroid *Cordylophora* (Phylum Cnidaria, Class Hydrozoa). *Invertebrate Zoology, 137.* 78-90. doi:10.1111/ivb.12207

Gilbert, P., Seitzinger, S., Heil, C., Burkholder, J., Parrow, M., Codispoti, L., & Kelly, V. (2005). The Role of Eutrophication in the Global Proliferation of Harmful Algal Blooms. *Oceanography, 18,* 198-209. doi:10.5670/oceanog.2005.54

Heisler, J., Gilber, P. M., Burkholder, J. M., Anderson, D. M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobbler, C. J., Heil, C. A., Humphries, E., Lewitus, A., Magnien, R., Marshall, H. G., Sellner, K., Stockwell, D. A., Stoecker, D. K., & Suddleson, M. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful Algae, 8*, 3-13. doi:10.1016/j.hal.2008.08.006

Hodgkin, E. P. & Hamilton, B. H. (1993). Fertilizers and eutrophication in southwestern Australia: Setting the scene. *Fertilizer Research,* 36, 95–103.

Huang, J., Xu, C., Ridoutt, B. G., Wang, X., & Ren, P. (2017). Nitrogen and phosphorus losses and eutrophication potential associated with fertilizer application to cropland in China. *Journal of Cleaner Production,* 159, 171–179.

Kinne, O. (1958). Adaptation to Salinity Variations - Some Facts and Problems. *Physiological adaptation*, 92-106.

Melo, L. F., & Bott, T. R. (1997). Biofouling in water systems. *Experimental Thermal and Fluid Science, 14*, 375-381. doi:10.1016/S0894-1777(96)00139-2

Nakano, D., & Strayer, D. L. (2014). Biofouling animals in fresh water: biology, impacts, and ecosystem engineering. *Frontiers in Ecology and the Environment, 12,* 167-175. doi:10.1890/130071

Obolewski, K., Jarosiewicz, A., & Małgorzata, O. (2015). Invasive Ponto-Caspian hydrozoan *Cordylophora caspia* (hydrozoa: Cnidaria) in southern Baltic coastal lakes. *Estuarine, Coastal, and Shelf Science, 167,* 147-153. doi:10.1016/j.ecss.2015.06.016

Officer, C. B., Smayda, T. J., & Mann, R. (1982). Benthic Filter Feeding: A Natural Eutrophication Control. *Marine Ecology Progress Series, 9*, 203-210. doi:10.3354/meps009203

Pieczyńska, E., Kołodziejczyk, A., & Rybak, J. I. (1998). The responses of littoral invertebrates to eutrophication-linked changes in plant communities. *Hydrobiologia, 391*, 9-21. doi:10.1023/A:1003503731720

Rice, M. (2001). Environmental Impacts of Shellfish Aquaculture: Filter Feeding to Control Eutrophication. Marine Aquaculture and the Marine Environment: A Meeting for the Stakeholders in the Northeast, University of Massachusetts, Boston, January 2001. Falmouth, MA: Cape Cod Press.

Roos, P. J. (1979). Two-State Life Cycle of a *Cordylophora* Population in the Netherlands. *Hydrobiologia, 62,* 231-239.

Rosenberg, R. & Loo, L.-O. (1988). Marine eutrophication induced oxygen deficiency: Effects on soft bottom Fauna, Western Sweden. *Ophelia, 29,* 213-225. doi:10.1080/00785326.1988.10430830

Seehausen, O., van Almphen, J. J. M., & Witte, F. (1997). Cichlid Fish Diversity Threatened by Eutrophication That Curbs Sexual Selection. *Science, 277,* 1808-1811. doi:10.1126/science.277.5333.1808

Soto, D., & Mena, G. (1999). Filter feeding by the freshwater mussel, Diplodon chilensis, as a biocontrol of salmon farming eutrophication. *Aquaculture, 171,* 65-81. doi:10.1016/S0044-8486(98)00420-7

Turner, R. E., & Rabalais, N. N. (1994). Coastal eutrophication near the Mississippi river delta. *Nature, 368,* 619-621. doi:10.1038/368619a0

Wintzer, A. P., Meek, M. H., Moyle, P. B., & May, B. (2011). Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary. Aquatic Ecology, 45, 151-161. doi:10.1007/s10452-010-9343-7