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Thesis of
Ashley K. Le

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science
Marine Science

Nova Southeastern University
Halmos College of Arts and Sciences

January 2021

Approved:
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Committee Member: George Duncan, Ph.D.

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NOVA SOUTHEATERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

Assessment of Monochloramine Toxicity on Three Small Coastal Organisms of South Florida

By

Ashley K Le

Submitted to the Faculty of
Halmos College of Arts and Sciences
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

01/08/21

Abstract:

Monochloramine (MCA) is a secondary disinfectant used by water treatment facilities to eliminate lingering bacteria in basins, filters, and pipelines. While an effective disinfectant, monochloramine can have negative effects on aquatic organisms. Organisms affected by the chemical can include species whose environment is near to effluent sites and aquaculture facilities that use tap water lines or has water intake pipes near to effluent sites. Three species commonly found in south Florida that are likely exposed to MCA by effluent sites or aquaculture facilities are mosquitofish (*Gambusia affinis*), pink shrimp (*Farfantepenaeus duorarum*), and the hard clam (*Mercenaria mercenaria*). These species were acutely exposed to MCA over a 48-hour trial at concentrations below the maximum legal residual disinfectant level of 4.0 mg/l (ppm). The probability of mortality for each species was determined using standard toxicology protocols. All species were found to have a greater probability of death at higher MCA concentrations. The probability of death drastically decreased over time at moderate to low MCA concentrations. *Gambusia affinis* and *Farfantepenaeus duorarum* exhibited extreme signs of stress when exposed to MCA in the form of erratic swimming and loss of buoyancy. *Mercenaria mercenaria* was the only species to survive the 48-hour trials and had the greatest probability of survival. Based on these results, marine and freshwater species are sensitive to monochloramine and the chemical should be removed from the water prior to aquaculture or aquarium use. Although monochloramine is a threat to individuals kept in tanks, it may not pose a threat to wild individuals due to MCA's instability and decay over time.

Keywords: Monochloramine, Chlorine, Ammonia, Toxicity, Acute Toxicity, Water Quality, Mosquitofish, Pink Shrimp, Hard Clam,

Acknowledgments:

I would like to thank my committee members: Dr. David Kerstetter, Dr. Nicole Kirchhoff, Dr. Donald McCorquodale, and Dr. George Duncan for their support and guidance throughout this project. I have gained great experience and have developed a passion for aquaculture because of this project. I could not thank each of you enough for helping me. I would also like to thank my lab mates and everyone who helped collect samples: Ashley Vettese, Abby Ehlers, Ryan McGonagle, Emma Brennan, Sydney Panzarino, Matthew Rojano, and Thomas Ingles. A special thanks to my husband and family who provided me boundless support.

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Introduction

Chloramines have been used as a water disinfectant since the 1930's. The chemical has been cleared by the Environmental Protection Agency's (EPA) regulatory standards and is safe for drinking, bathing, and cooking uses. Although chlorine is primarily used as the main disinfectant for drinking water, many water treatment facilities have switched to monochloramine as their secondary water disinfectant (Environmental Protection Agency 2020).

Monochloramine is a species of organic chloramine that forms when free chlorine and ammonias combine, as well as smaller amounts of dichloramine and trichloramine depending on factors such as temperature, pH, and the amount of free chlorine and ammonia present (Pasternak et al. 2003). In an aqueous solution, chlorine reacts with ammonia to form chloramines (LeChevallier and Au 2004).

$$HOCl + NH_3 \rightarrow NH_2Cl \text{ (monochloramine)} + H_2O$$

The primary anthropogenic use for monochloramine is to disinfect drinking and wastewater. Monochloramine is favored over chlorine because of lower volatility in water relative to chlorine (Farrell et al 2001). Lower volatility allows monochloramine to eliminate lingering bacteria in basins, filters, and pipelines (Shull 1981). While chlorine has been a choice disinfectant previously, it has several drawbacks from a municipal water treatment perspective, including odor and difficulty in maintaining effective concentrations. In contrast, monochloramine's advantages stem from its greater effect on bacterial cell penetration (Coventry et al. 1935). Turetgen (2004) demonstrated that monochloramine could penetrate biofilms in cooling tower water systems more effectively than free chlorine. Monochloramine is often used when disinfection by-products (DBPs) are in excess (Vikesland et al 2001). DBPs can include trihalomethanes (THMs), halocetic acids, bromate, and chlorite. THMs include chemicals such as chloroform, bromodichloromethane, dibromochloromethane, and bromoform, and these chemicals have been previously linked to cancer (Shafiee and Lobat 2012). DBPs form when chlorine reacts with organic compounds and has also been linked to cancer and reproductive defects (Hua and Yeats 2010). Used as a primary or secondary disinfectant, monochloramines produce less DBPs, helping water treatment facilities stay under the regulatory limits of DBPs (Hua and Reckhow 2008). Monochloramines also help prevent the formation of trihalomethanes (Brodtmann & Russo 1979). Although monochloramine is more stable than chlorine, it is still a relatively unstable chemical, with varying half-lives depending on temperature and pH.

Monochloramine can auto-decompose, meaning that it will begin to decay without the presence of any other sources (Sacher et al. 2019). In an aqueous solution, when monochloramine decays, it decomposes into dinitrogen and ammonium chloride:

$$3\ NH_2Cl \rightarrow N_2 + NH_4Cl + 2\ HCl$$

The residual ammonia produced by monochloramine decay can cause nitrification, a process where ammonia is oxidized to nitrite and then to nitrate by bacteria (Kulkarni et al. 2018)

Many counties within the United States use monochloramine as their secondary wastewater disinfectant. A survey taken in 2004 saw that 29% of community water systems used monochloramines as its secondary disinfectant, 3% were in the process of changing to monochloramines, and an additional 12% were considering monochloramine as a secondary disinfectant (Seidel et al. 2005). Seidel et al. (2005) also surveyed water treatment facilities around the United States, asking why the facilities used chlorine or monochloramine. The survey was advertised nationwide, using a variety of forums such as emailing utility managers and workers, the American Water Works Association e-journal subscribers, and subscribers to a safe drinking water alert service. The results of the survey are shown in Table 1.

Table 1: Results from Seidel et al. (2005) survey of water treatment facilities across the United States showing explanations by the facility managers or other supervisory bodies for current disinfection practices.

Practice	**Chloramine**		**Chlorine**		**None**	
	Responses	%	Responses	%	Responses	%
Distribution System residual maintenance	96	90.6	201	81.7	0	0.0
Additional secondary contact time is necessary to achieve primary disinfection contact time	9	8.5	31	12.6	0	0.0
Disinfection by-product minimization	81	76.4	47	19.1	1	9.1
Taste and odor control	31	29.2	41	16.7	0	0.0
State requirement	22	20.8	112	45.5	1	9.1
Other	13	12.3	11	4.5	1	9.1

The results from the surveys in Seidel et al. (2005) included here as Table 1 shows that water treatment facilities primarily use monochloramine as disinfectant either when doing distribution system residual maintenance, to minimize DBPs, control taste and odors, or because it was a state requirement. It should be noted that drinking water suppliers are required by the U.S. Environmental Protection Agency (EPA) to maintain a residual disinfectant throughout the drinking water distribution system to rid the water of bacterial growth (Virginia Department of Health, 2020). The survey also asked water treatment facilities who used chloramines if they had experienced any problems. Some facilities reported chloramine-related problems such as positive coliform bacterial samples, corrosion control problems, taste and odor complaints, gasket material failure, and difficulty meeting disinfectant concentration times contact time (C x T) requirements (Seidel et al. 2005).

In the United States, the federal Safe Drinking Water Act (SDWA) of 1974 had the intention of creating national drinking water standards. Creating national primary drinking water regulations established maximum contaminant levels for various disinfectants. The maximum residual disinfectant level (MRDL) of monochloramine is 4.0 mg/ L or 4 ppm, a concentration with no known negative effects on humans (City of Fort Lauderdale 2019). A review of the annual water quality reports in 2019 from several different cities in southern Florida (Monroe, Miami-Dade, Broward, Collier, and Palm Beach Counties) found that chloramine concentrations were usually between 2.50-2.90 ppm (mg/L). In addition, these reported concentrations were not consistent throughout the year, with high and low ranges provided for each location. The highest concentration was 4.30 ppm (mg/L) in Stuart, Florida, which is 0.30 ppm (mg/L) higher than what is accepted by the EPA (City of Naples Utilities Department 2019).

Other than being redistributed into cities and used for drinking water, treated wastewater is also distributed back into the environment by way of effluent sites. After wastewater completes primary and secondary disinfection, it is often released into nearby rivers, streams, estuaries, and bays (EPA 2004). Being that monochloramine is used as a secondary disinfectant and has proven beneficial in preventing bacteria and pathogens from growing in water, it also has negative effects on drinking water and the environment. Monochloramine has been linked to corrosion of pipes, leading to an increased copper concentration in drinking water (Switzer et al. 2006). When released into freshwater environments by way of effluent or leakage, it can cause physiological damage to aquatic life. A study by Travis and Heath (1981) determined that rainbow trout exposed to small amounts of monochloramine (average concentration of 0.16-0.23 mg/l) exhibited an increase in methemoglobin, a form of hemoglobin unable to carry oxygen to tissues (Travis and Heath 1981).

Monochloramine toxicity is relatively well studied within fresh-water aquatic communities, as there are many more studies similar to the Travis and Heath (1981) research. Laboratory bioassays of the freshwater hornsnail (*Pleurocera unciale unciale*) resulted in 96 hour LC_{50} values of 0.252 ppm (mg/L) monochloramine (Goudreau et al. 1993), the same study found glochidia of the freshwater rainbow mussel (*Villosa iris*) to have a lower tolerance to monochloramine, with 24 hour LC_{50} values of 0.084 ppm (-mg l^{-1}). Farrell et al. (2001) determined LC_{50}s for juvenile chinook salmon and the freshwater invertebrate water flea (*Ceriodaphnia dubia*). They found that chinook salmon and water flea had an LC_{50} of 0.144 mg/L (ppm) and 0.056 mg/L (ppm), respectively after 96 hours of exposure. Table 3 summarizes monochloramine LC_{50} values of freshwater invertebrate and vertebrate species.

In a similar acute toxicity study by Roseboom and Richey (1977), bluegill and channel catfish were exposed to residual chlorine and ammonia, a by-product of monochloramine, for 96 hours. Median tolerance limits (TL_{50}) for each species were determined. TL_{50}s are the chemical concentration at which 50% of the test organisms survive for a specific exposure time (Rand 1995). Results showed that residual chlorine TL_{50}s for bluegill ranged from 0.18-0.33 mg/L (ppm) and ammonia TL_{50}s ranged from 0.40-1.3 mg/L (ppm) depending on temperature and weight. Channel catfish were more sensitive to residual chlorine with a TL_{50} of 0.09 mg/L (ppm) and an ammonia TL_{50} range of 1.5-3.0 mg/L (ppm) (Roseboom and Richey 1977).

Table 2: Summary of the 2019 Annual Water Quality Report for chloramines in several cities in Southeast Florida (City of Fort Lauderdale 2019; [Hollywood] Department of Public Utilities 2019; [Stuart] Martin County Utilities and Solid Waste Department 2019; City of Naples Utilities Department 2019; [Boca Raton] Utilities Services 2019; [Miami] Water and Sewer 2019).

Location	**Level Detected (ppm)**	**Range of Results (ppm)**
Hollywood, FL	2.58	1.00-3.90
Boca Raton, FL	2.80	0.74-3.50
Miami, FL	2.90	(0.70 – 3.0)
Stuart, FL	2.80	0.60-4.30
Ft. Lauderdale, FL	2.70	2.30-3.10

Table 3. Monochloramine LC_{50} summary of findings from three different monochloramine toxicity studies. Monochloramine (MCA), residual chlorine (RC), and inorganic chloramine (ICA) were measured. Chinook salmon with an LC_{50} of 2.56 ppm (mg/L) died after 0.167 hours (10 minutes)

Species	Fish/ Invertebrate	Chemica l	LC50 (mg/L; ppm)	Exposure Time (hours)	Study
Chinook Salmon *Oncorhynchus tshawytscha*	Fish	MCA	2.56	0.167	Farrell et al. 2001
Chinook Salmon *Oncorhynchus tshawytscha*	Fish	MCA	0.197	48	Farrell et al. 2001
Chinook Salmon *Oncorhynchus tshawytscha*	Fish	MCA	0.144	96	Farrell et al. 2001
Water Flea *Ceriodaphnia dubia*	Invertebrate	MCA	0.118	48	Farrell et al. 2001
Water Flea *Ceriodaphnia dubia*	Invertebrate	MCA	0.056	96	Farrell et al. 2001
Liver Elimia *Goniobasis livscens*	Invertebrate	MCA	0.045	96	Goudreau et al. 1992
Hornsnail *Pleurocera unicale unicale*	Invertebrate	MCA	0.252	96	Goudreau et al. 1992
Bladder Snail *Physa integra*	Invertebrate	MCA	>0.810	96	Goudreau et al. 1992
Pointed Campelona *Campelona decisum*	Invertebrate	MCA	>0.810	96	Goudreau et al. 1992
Coho Salmon-alevin *Oncorhynchus kisutch*	Fish	RC	0.083	96	Larson 1978
Coho Salmon-fry *Oncorhynchus kisutch*	Fish	RC	0.079	96	Larson 1978
Coho Salmon-Juvenille *Oncorhynchus kisutch*	Fish	RC	0.082	96	Larson 1978
Brook trout-alevin *Salvelinus fontinalis*	Fish	ICA	0.1055	96	Larson 1978
Brook trout-fry *Salvelinus fontinalis*	Fish	ICA	0.0818	96	Larson 1978
Brook trout-juvenille *Salvelinus fontinalis*	Fish	ICA	0.0906	96	Larson 1978
Cutthroat trout-juvenille *Oncorhynchus clarkii*	Fish	ICA	0.0745	96	Larson 1978

Other than natural freshwater systems, the effects of monochloramine can be seen in breweries and dialysis patients. For example, chloramine can add a medicinal taste to beer if not properly removed (Palmer 2006). The Center for Disease Control and Prevention (CDC) states that all dialysis centers treat their water to remove all chemical disinfectants before treating patients (CDC 2020). Failure to remove chloramines from water used for dialysis treatment can cause the patient's red blood cells to become more susceptible to oxidant damage (Klein 1986).

Similar to dialysis patients, monochloramine can cause oxidative damage to aquatic life in aquaculture facilities if not properly removed from the water. A recent study by Bakhiet et al. (2020) determined that residual chlorine causes tissue damage in the Nile tilapia, *Oreochromis niloticus*, in the form of severe congestion of blood vessels, edema of epithelial cells at the lamellae, hyperplasia of mucous cells, and gill filament damage. In aquariums and aquaculture facilities, chloramines are known to be dangerous because they enter the bloodstream directly, decreasing the oxygen-carrying ability of blood and thereby suffocating the fish (Skipton and Dvorak 2007).

Monochloramine's effect on aquatic life can affect aquaculture production and the aquarium industry if not properly removed from municipal water. The reliance on farmed marine organisms to meet the demand for seafood is shown through increases in aquaculture's total production. In 2011, aquaculture provided 40.1% of total world fish production and produced 62.7 million tons of fin fish, mollusks, and crustaceans (FAO 2011). Since 2011, the global fish, crustacean, and mollusk production has been rising, with fish production increasing at an average annual rate of 5.7% per year, crustacean production at an average annual rate of 9.92% per year, and mollusk production at 3.46% per year (Tacon 2020). As for aquariums, the industry continues to grow, with the American Pet Products Association (2020) reporting that 13% of households in the United States have freshwater or saltwater fish. Home aquarists often use municipal tap water as their main source of water for the aquariums, thus exposing aquatic organisms to monochloramine if not properly removed.

Understanding the effects of monochloramine on different organisms can aid in the cooperation between counties, water treatment plants, and aquaculture facilities. For example, after water is treated with monochloramine, it could be filtered through an activated carbon filter, shown by Perez-Garcia and Rodriguez-Benitez (1999) to an effective method for the elimination of residual chloramines. Another method of eliminating chloramines is to add the alkali metal formaldehyde bisulfite ($CH_4NaO_4S^+$), which neutralizes chloramines and is often used in aquaculture (Kuhns 1987). More recent methods for chloramine removal include reverse osmosis (RO) and nanofiltration membranes (Al Habobi et al. 2012). Aquariums and aquaculture facilities that use municipal tap water lines or draw water from sources near effluent sites need to remove chloramines and chlorines from the water before used. Clear knowledge about the chemical can result in improved water quality management practices at municipal levels. In addition, aquaculture facilities rearing marine organisms can improve their understanding of how monochloramine effects their stocks as well as improve preventative measures against mortality rates.

Water treatment facilities will likely not stop using monochloramine to disinfect the waters, as it remains a convenient and effective disinfectant, as well as being cost-effective for local taxpayers. However, it would be environmentally responsible for the treatment facilities to understand how the chemical effects organisms within their respective ecosystems. Some organisms that are affected by monochloramine are those found in bait shops, grocery stores, aquaculture species, ornamental fish, or organisms with habitats close to freshwater input, such as estuaries, mangroves, salt marshes, or marinas where municipal water is used to rinse boats. Pink shrimp, hard clams, and mosquitofish are species commonly found in southern Florida, used as bait and food sources, and inhabit environments that could be near effluent sites, making monochloramine exposure likely.

Mosquitofish (*Gambusia affinis*)

Mosquitofish are native to the southeastern region of North America and are known as one of the most widely distributed freshwater fish in the world (Pyke 2005). Being opportunistic feeders and residing in habitats such as ponds and streams, mosquitofish have a wide diet consisting of algae, crustaceans, and insects. Mosquitofish can tolerate various physical properties (temperature, salinity, and dissolved oxygen) as well as exposure to numerous toxic chemicals found in pesticides (Pyke 2005). Since mosquitofish have been known to tolerate various harsh water quality conditions, they have been introduced to aquatic habitats across the globe to help decrease mosquito populations (Nordlie 2006). Although mosquitofish are tolerant of many different water qualities, they can exhibit effects from water contaminant exposure, and have been used as biomarkers of contamination from pesticides and effluent sites (Pyke 2005).

Pink Shrimp (*Farfantepenaeus duorarum*):

Regionally, pink shrimp is often used for human food and bait in southern Florida. Shrimp landings, the amount of shrimp harvested, are often transported to stores or bait shops where they are held until sold. Estuaries and bays, specifically in southern Florida, are considered nursery habitats for pink shrimp and are an important stage in their life history (Roessler and Rehrer 1971). In 2005, annual landings of bait pink shrimp in Biscayne Bay alone were valued at over $1 million (Johnson et al. 2012). Shrimp landing values have only increased since then and is currently estimated over $17 million in Florida in 2020 (Florida Fish and Wildlife Conservation Commission 2020). In addition to its commercial value, the species was selected as an ecological indicator by the Comprehensive Everglades Restoration Plan (CERP) because freshwater management and restoration actions effect its distribution, growth, abundance, survival, and productivity. The organisms that feed on pink shrimp are also of great ecological and economic importance, making the shrimp a critical trophodynamic link (Browder and Robblee 2009; Zink et al. 2017).

Hard Clam (*Mercenaria mercenaria*)

Clam aquaculture has been a successful industry in the eastern United States. In 2016, the United States produced 9.7 million pounds of clams, estimated at a value of $138 million (NOAA 2019). In 2018, bivalve sales reached over $15.5 million in Florida, ranking Florida third in the nation for total clam sales (United States Department of Agriculture 2019). Hard clams are found in environments such as estuaries and lagoons with various temperatures and sediment types and have been known to live for decades. Due to their wide distribution of environments and long lifespan, clams can provide insight on the overall health of its habitat (Bricelj et al. 2017). Generally, bivalves have been useful in marine pollution monitoring and water quality due to their ability to accumulate various kinds of contaminants. Clams play an important role in their ecosystem by filtering excess nutrients such as nitrogen and phosphorus from the water (Reyna et al. 2019).

Objectives:

The purpose of this study was to determine the toxicity of monochloramine on species that are likely to be exposed to the chemical. Exposure can come from environments near to effluent sites and aquaculture facilities that use chloraminated tap water lines or have intake pipes near to effluent sites. Based on prior research listed in Table 3, pink shrimp, hard clam, and mosquitofish are expected to be less tolerant to acute monochloramine exposure. Along with determining monochloramine toxicity, behavior of the species during exposure was observed. This study hopes to provide insight on the effects of monochloramine on inshore environments and aquaculture facilities.

Materials and Methods

Specimen Acquisition and Husbandry

Live hard clams were purchased from the local Publix grocery store. Clams were housed in fifteen-gallon tanks filled with artificial seawater from the brand Instant Ocean (Instant Ocean; Spectrum Brands; Blacksburg, VA). Water temperature was maintained between 20-23^0 C and salinity between 30-35 practical salinity unit (PSU). Water changes were conducted every other day to keep water quality at optimal conditions. The tanks did not contain substrate or vegetation and the clams were fed Instant Algae (Reed Mariculture; CA) containing six types of microalgae at least three times before trials begin, but not proceeding 24 hours before they were exposed. Clams that were not actively feeding prior to trials were not used in the experiments and individuals chosen were not fed during a trial. To avoid acute health problems during trials, individuals were given a minimum of two days to acclimate to the laboratory environment (Rice et al 2012).

Pink shrimp were purchased from a Dania Beach bait shop (Angler’s Bait and Tackle) and kept in a five-gallon bucket with a portable aerator until arrival at the lab. Angler’s Bait and Tackle collects pink shrimp primarily from Biscayne Bay. Like the clams, shrimp were kept in a fifteen-gallon tank filled with artificial seawater. Water temperature was maintained between 20-23^0 C and salinity between 30-35 PSU. Water changes were conducted every other day to ensure optimal water quality. Hikari Crab Cuisine (Kyorin Food Industries; Japan) was purchased from Pet Supermarket or PetSmart and fed to the shrimp sparingly. The shrimp were acclimated for at least two days and fed once before trials began. Shrimp that were not actively feeding or responding to stimuli were not chosen for trials.

The mosquitofish were sampled from freshwater sources in Weston, Plantation, and Dania Beach, Florida. Fish were sampled from freshwater areas with high vegetation using a five-and-a-half-foot dip net and cast net. Fish were stored in a five-gallon bucket with aeration until transported back to the laboratory. All fish were sampled under Florida Fish and Wildlife Conservation Commission (FWC) freshwater fishing license number 1004037846. Once fish were brought to the laboratory, they were kept in a fifteen-gallon tank filled with reverse osmosis water. Water temperature was maintained between 20-23^0 C. Mosquitofish were slowly acclimated to artificial seawater with a low salinity of 20 PSU. They were given at least seven days to acclimate to the artificial seawater (Rice et al. 2012). Mosquitofish were fed Tetra Goldfish flakes purchased from Pet Supermarket or PetSmart at least four times before trials began. Similar to the invertebrates, mosquitofish were not fed during the trials.

Experimental Design

All experimental procedures were conducted in the Guy Harvey Oceanographic Center (GHOC) room 238, a pass-card protected wet lab at the NSU Oceanographic Center campus in Dania Beach, FL. Experiments took place in 1,000 mL beakers, filled with artificial seawater. Trials were in the form of an acute toxicity test, the relative toxicity of a chemical to aquatic organisms under short-term exposure at different concentrations. Due to the volatility of monochloramine, this project used a predetermined length of time (thus, a time-dependent acute toxicity test) of 48-hour trials. During trials, the temperature, salinity, pH, dissolved oxygen, monochloramine concentration, and mortality were recorded. To test the toxicity of monochloramine hard clams, pink shrimp, and mosquitofish, a static acute test was performed. In a static acute test, the organisms and the test solutions are kept in the same chamber for the duration of the trial. Acute toxicity tests do not usually exceed 96 hours because of issues that occur with longer duration tests, such as metabolic products increasing above desired concentrations or a significant decrease in test material concentrations due to the uptake by the test organisms (Ward and Parrish 1982). Although less volatile than chlorine, Figure 1 shows the volatility of open and closed containers of monochloramine. Silva et al. (2005) study aimed to determine the stability of monochloramine and found that in a closed container, monochloramine concentrations were almost constant. The same study also determined that monochloramine concentrations can decrease from evaporation. Beakers were covered to decrease the rate of evaporation.

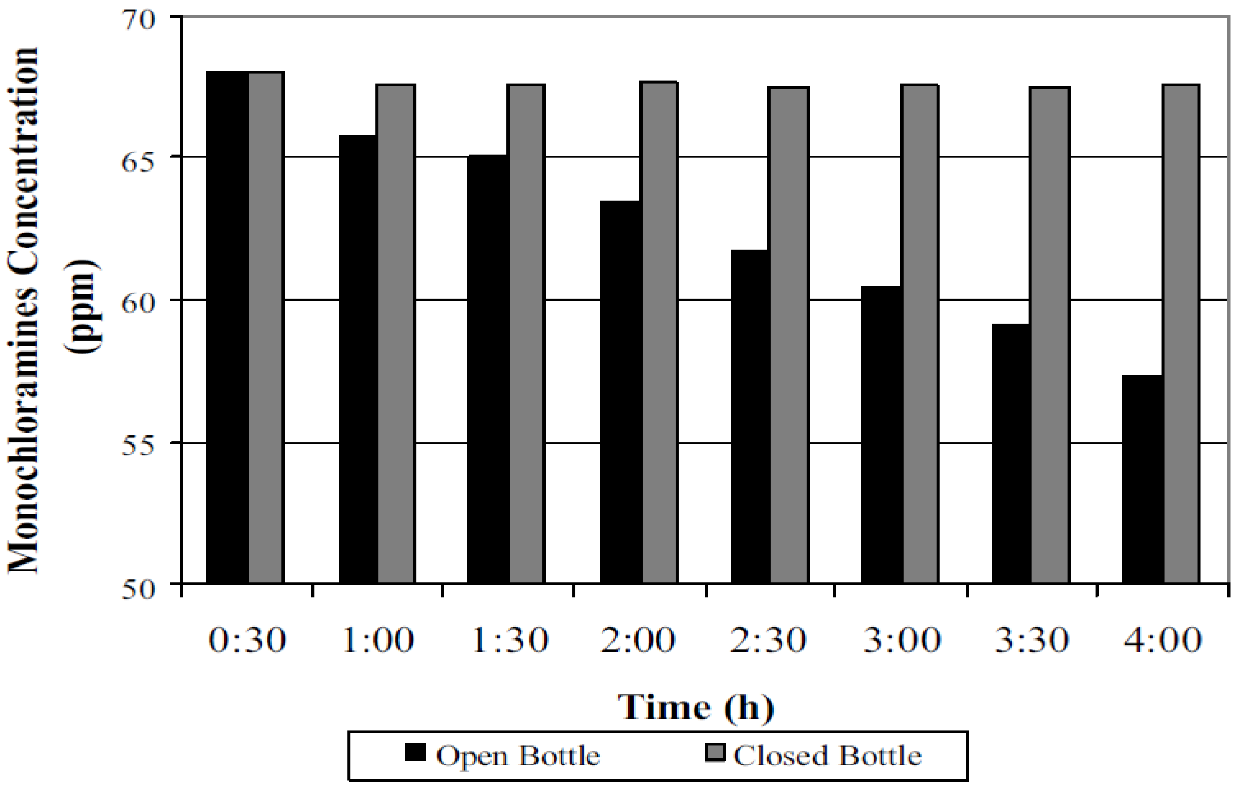


Figure 1: Silva et al. (2005) experiment comparing the volatility of monochloramine in an open versus closed container.

For each species, one individual was placed in a beaker, with a total of five beakers per trial. One beaker would act as the control, meaning that no monochloramine dosage was added during the experiment. Each beaker was gently aerated and maintained at the same temperatures and salinities as the species' holding tanks. During the 48-hour experiments, individuals were observed at set logarithmic checkpoints. After the initial measurement, the following measurements took place at 1.5, 3, 6, 12, and 24 hours. If the trial exceeded twenty-four hours, the second day consisted of the same measurement checkpoint times (Rice et al. 2012). The time of mortality and concentration of monochloramine within the 48-hour trial was recorded. Mortality of the hard clam was determined if the clam did not respond to needle stimuli or if the clam was unable to close. Pink shrimp and mosquitofish were considered dead if unresponsive to needle stimuli. Lack of operculum movement also determined death for mosquitofish. At the set times of measurement, the following parameters were assessed: temperature, salinity, dissolved oxygen, free and total chlorine (chloramine concentration), and mortality. Monochloramine levels were measured using the portable colorimeter, a model WL0020-ATC refractometer (Agriculture Solutions; Kingfield, ME) was used to measure salinity, a pH meter (7Pros) measured pH, and dissolved oxygen pen (Fisher Scientific; Waltham, MA) measured dissolved oxygen.

At the end of the 48-hour trial, all individuals were humanely euthanized. All methodology met the guidelines and regulations of NSU's Institutional Animal Care and Use Committee (IACUC), including protocol approval prior to experimentation with vertebrate fishes (NSU protocols 2019.03.-DK11 and 2020.03.-DK11). Mosquitofish were immersed in an ice-slurry to achieve death by hypothermia (Blessing and Balcombe 2010), while invertebrates underwent a two-step euthanasia procedure to confirm death, the first step being a chemical introduction (1-5% concentration of ethanol) and the second step an ice-slurry (Underwood et al. 2013).

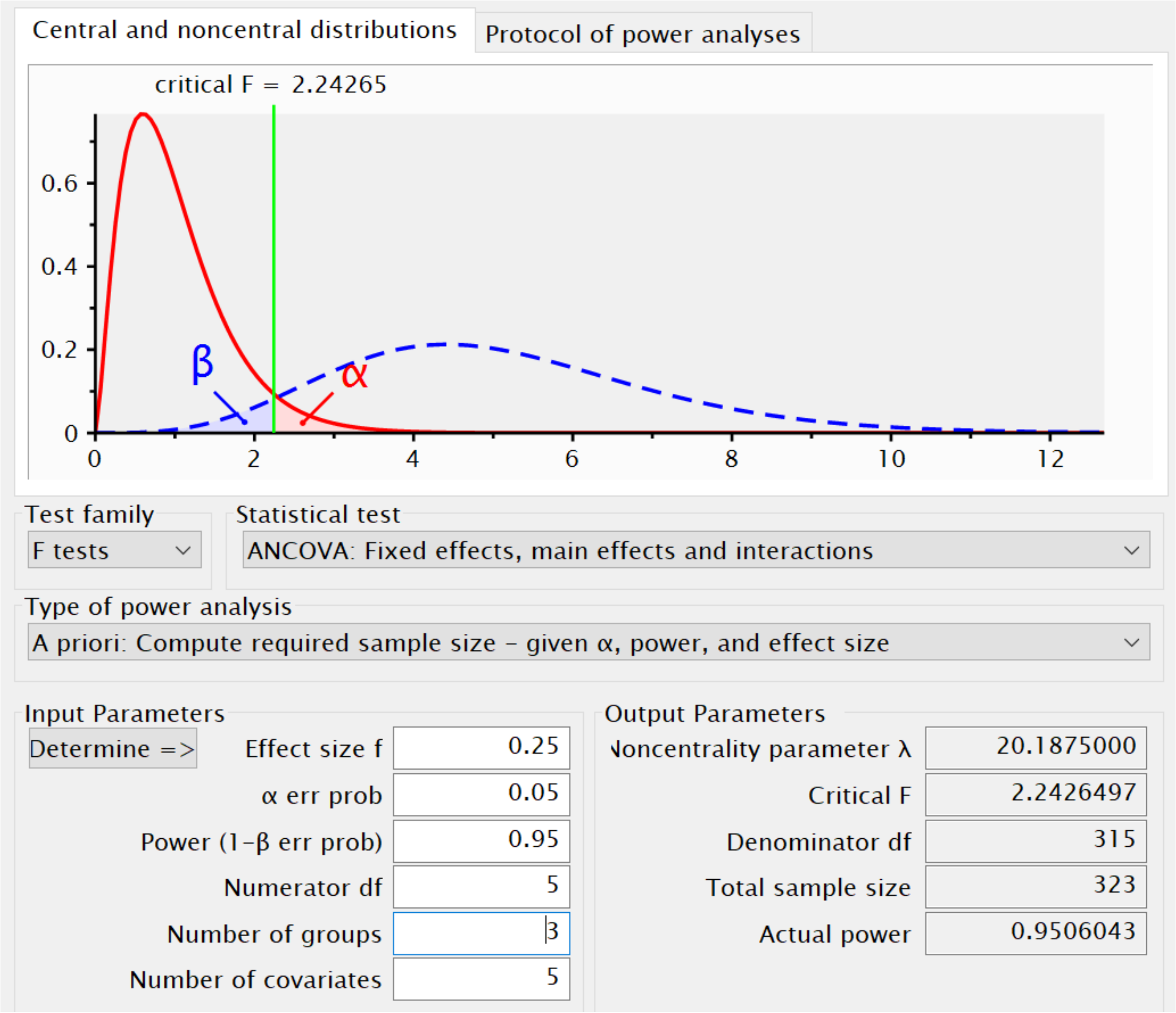
Monochloramine Synthesis

Monochloramine stock solution was prepared by the reaction of ammonium chloride solution with sodium hypochlorite. Three moles of ammonium chloride was mixed with one mole of sodium hypochlorite and sodium hydroxide used to buffer pH (Farrell et al. 2001). Concentrations of monochloramine in the stock solution were measured using a multi-parameter portable DR 900 colorimeter (Hach Inc.; Loveland, CO), that can detect levels of monochloramine as low as 0.02 ppm (mg/L). The colorimeter was calibrated by the manufacturer prior to use. Calibration was confirmed once the colorimeter was received using a secondary gel standards for monochloramine from Hach.

Statistical Analyses

A power analysis was accomplished using the software G*Power (v. 3.1; G*Power), with the results below in Figure 2. Power analyses calculate the minimum sample size needed to obtain a statistically significant result. The power of a statistical test is also the probability that the null hypothesis will be rejected (Cohen 1992). To calculate a statistical test's power, an effect size is need. An effect size is the magnitude of the effect of interest in the population (Salkind 2010), and based on previous monochloramine toxicity studies and their results, a medium effect size (0.25) was used for the power analysis calculations. Calculations also assumed three trial groups. Based on these parameters, minimal sample size of 323 was determined by the G*Power software. For each species, trials containing five organisms were repeated (22 trials per species until 110 individuals were tested, surpassing the minimum estimated for statistical significance.

A log-rank (Mantel-Cox test) was used to compare survival distributions between salinity, pH, temperature, dissolved oxygen, and monochloramine. Only the monochloramine provided a significant p-value, meaning that monochloramine was the only variable attributable to species' deaths. Since monochloramine was the only variable related to species' mortality, a Mantel Haenszel test was then used as it estimates the relation between monochloramine exposure and death. A Kaplan-Meier estimator was then used to show the survival function of monochloramine for each species. The monochloramine concentrations were divided into three groups (low, mid, and high; see Figure 4) to display a range of mortality probabilities. The plot of the Kaplan-Meier estimator shows the probability of mortality of a species over time at the three ranges of monochloramine concentrations.



Test family: F tests

Statistical test: ANCOVA: Fixed effects, main effects and interactions

Type of power analysis: A priori: Compute required sample size – given α, power, and effect size

Input Parameters

Effect size f	0.25
α err prob	0.05
Power (1–β err prob)	0.95
Numerator df	5
Number of groups	3
Number of covariates	5

Output Parameters

Noncentrality parameter λ	20.1875000
Critical F	2.2426497
Denominator df	315
Total sample size	323
Actual power	0.9506043

Figure 2: The results of a power analysis to determine sample size using G*Power 3.1 software.

Results

While most of the pink shrimp and mosquitofish died, most clams survived (Table 4). Monochloramine concentrations were attributable to the death of pink shrimp (chisq = 112, df = 4, p = <2e-16), hard clams (chisq = 61.7, df = 2, p = 4e-14) and mosquitofish (chiq =46.4, df=2, p=9e-11). All p-values were below 0.05, indicating that MCA concentrations were significant to the death of individuals. Mosquitofish showed a strong response to the addition of monochloramine with many of the deaths occurring within the first thirty minutes of the trial (Figure 3 and Table 4). Figures 3, 5, and 7 plot the monochloramine concentrations against the time of death for each species. In addition to the log-rank test, a Kaplan-Meier mortality curve showing the probability of mortality of different levels of monochloramine concentrations was used. Figures 4, 6, and 8 show that the probability of death for each species decreased as time elapsed. Higher concentrations of monochloramine were more likely to cause death during the trial. Mid to low monochloramine concentrations showed a drastic decrease in the probability of death over time.

Behavioral Observations

When monochloramine was added to the beakers, pink shrimp and mosquitofish reacted immediately to the chemical showing high signs of stress in the form of erratic swimming. Most individuals began flailing, hitting the plastic covering the beakers. After the erratic swimming and flailing ceased, individuals became lethargic, either sinking to the bottom or floating to the top of the beaker. In mosquitofish, operculum movement was rapid at first but slowed once the fish became lethargic. The clams also reacted immediately to the addition of monochloramine during the trials. Once the monochloramine was added, most clams closed, and remained so for the duration of the trial. Clams exposed to a low level of monochloramine opened occasionally but remained closed during most of the trial.

Table 4: Descriptive statistics showing the average weight, observed mortality, and average time of death for each species.

Species	Avg. Weight (g)	Total Number	Observed Mortality	Avg. Time of Death
Pink Shrimp	12.72	110	88	1.00 h
Hard Clam	23.30	110	10	11.70 h
Mosquitofish	< 1.00	110	87	30.18 min

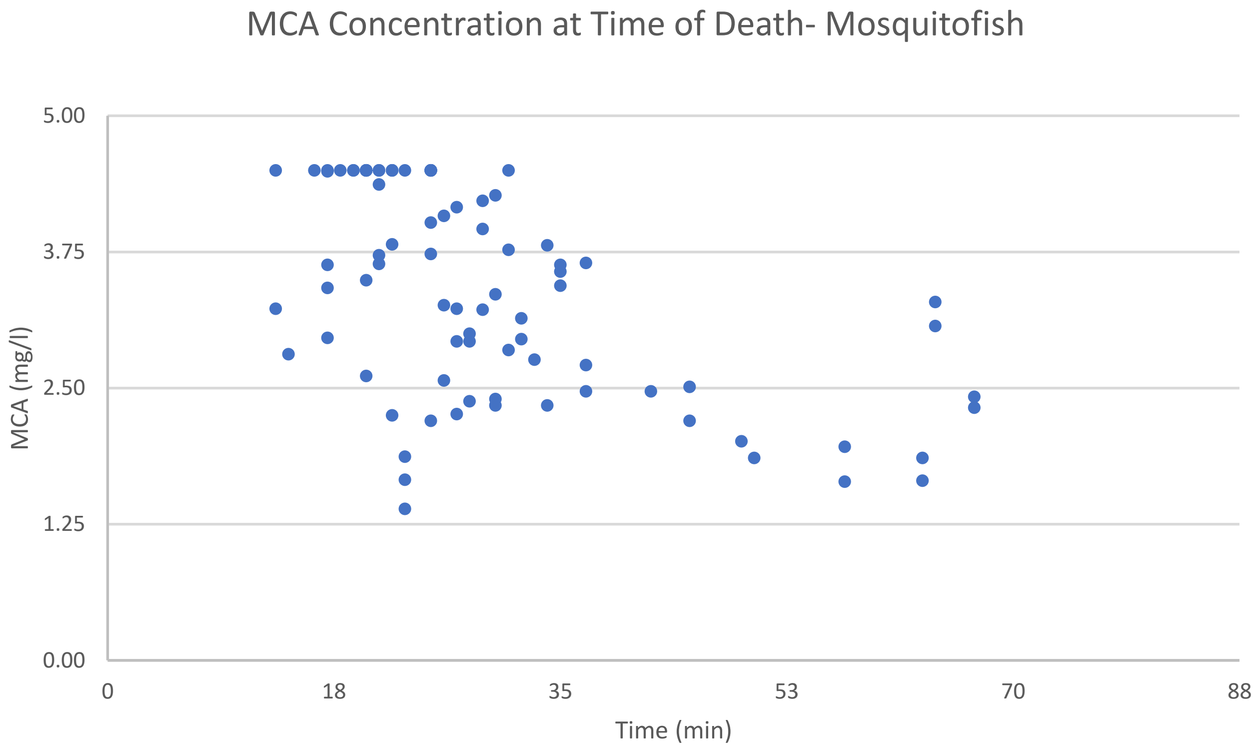


Figure 3: Monochloramine concentration at time of death during mosquitofish trials. Due to the instability of monochloramine, some concentrations exceeded the colorimeter's maximum reading, thus these readings are indicated at 4.5 mg/l.

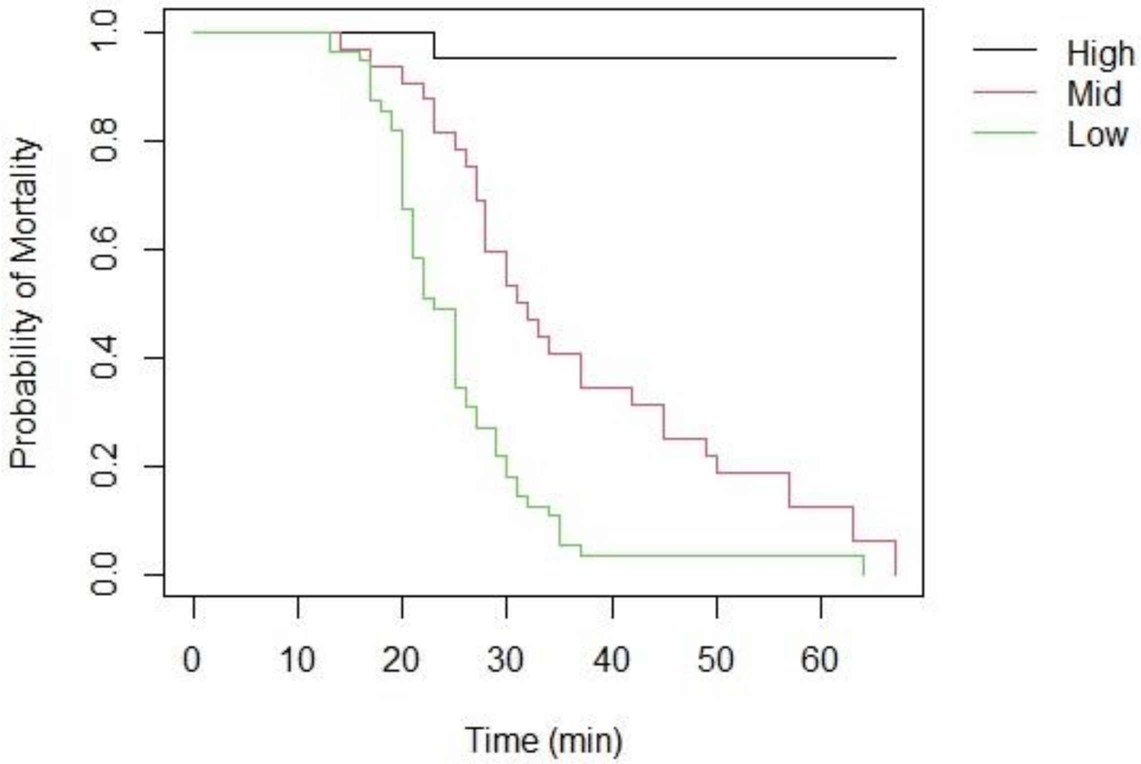


Figure 4: Mortality curve depicting the probability of death of *G. affinis* at different monochloramine concentrations indicated by high (3.1-4.5 mg/l), mid (1.6-3.0), and low (0.0-1.5 mg/l) levels.

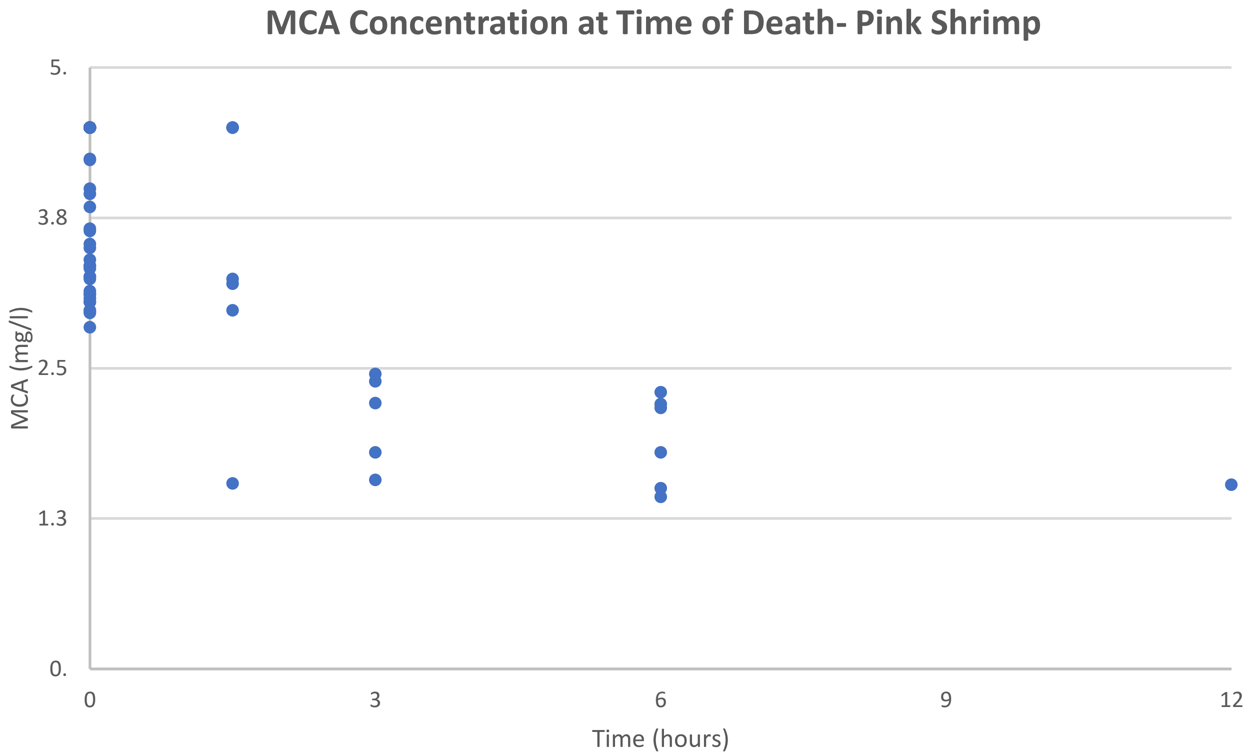


Figure 5: Monochloramine concentration at time of death during pink shrimp trials. Most individuals died within the first hour, shown by points on the 0 marker. Due to the instability of monochloramine, some concentrations exceeded the colorimeter's maximum reading, thus these readings are indicated at 4.5 mg/l.

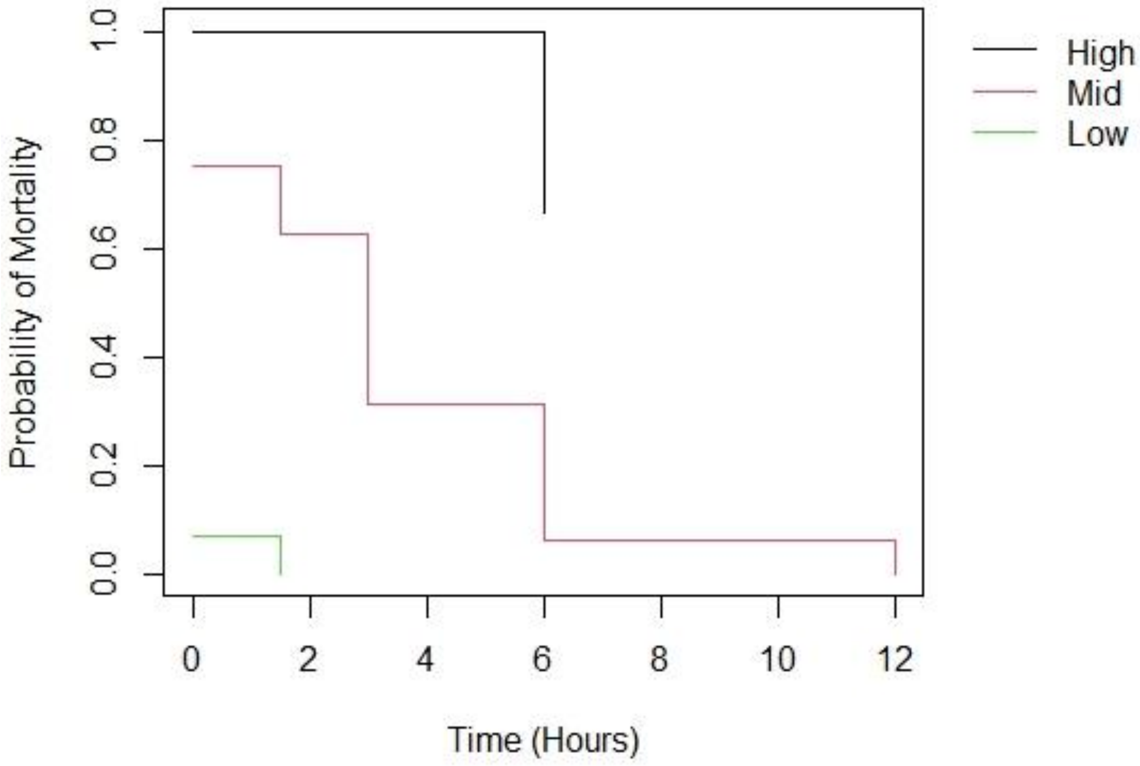


Figure 6: Mortality curve depicting the probability of death of pink shrimp at different monochloramine concentrations indicated by high (3.1-4.5 mg/l), mid (1.6-3.0), and low (0.0-1.5 mg/l) levels. The vertical drop at the end of each line indicates that there were no other individuals tested at the respective monochloramine concentrations (low, mid, and high), resulting in the end of the curve.

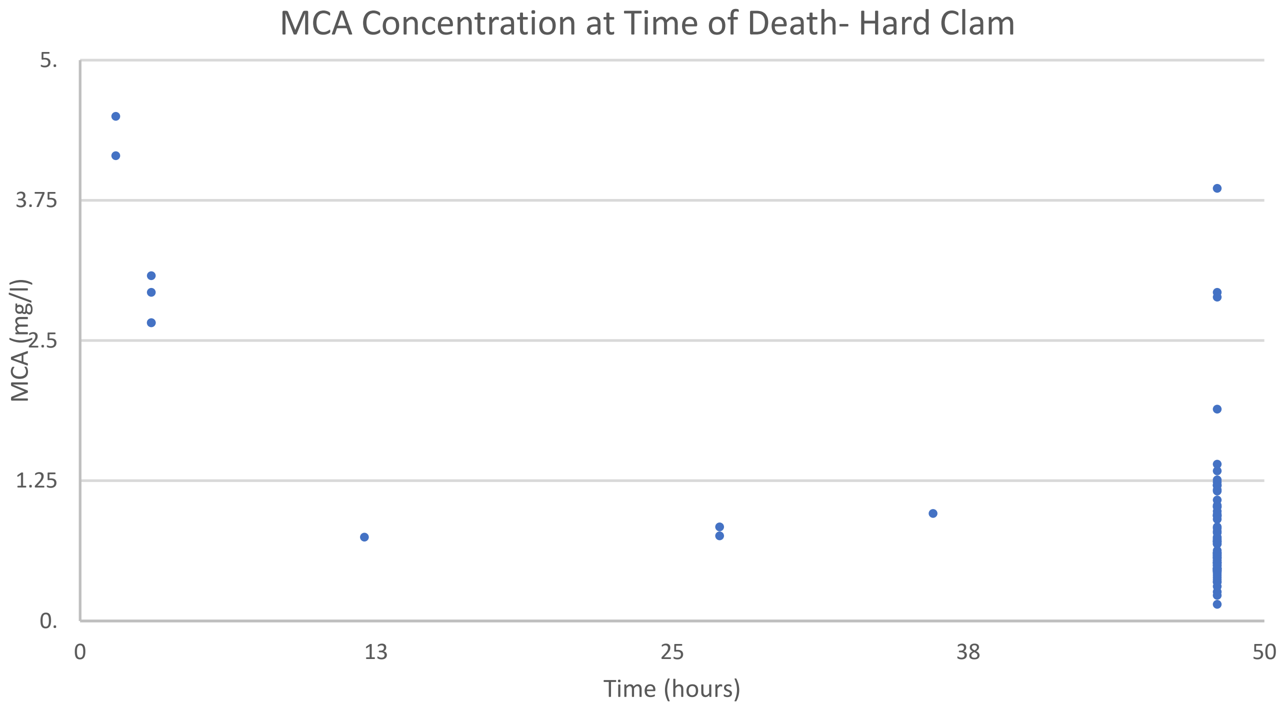


Figure 7: Monochloramine concentration at time of death during hard clams trials. Most individuals survived the 48-hour trial. Due to the instability of monochloramine, some concentrations exceeded the colorimeter's maximum reading, thus these readings are indicated at 4.5 mg/l

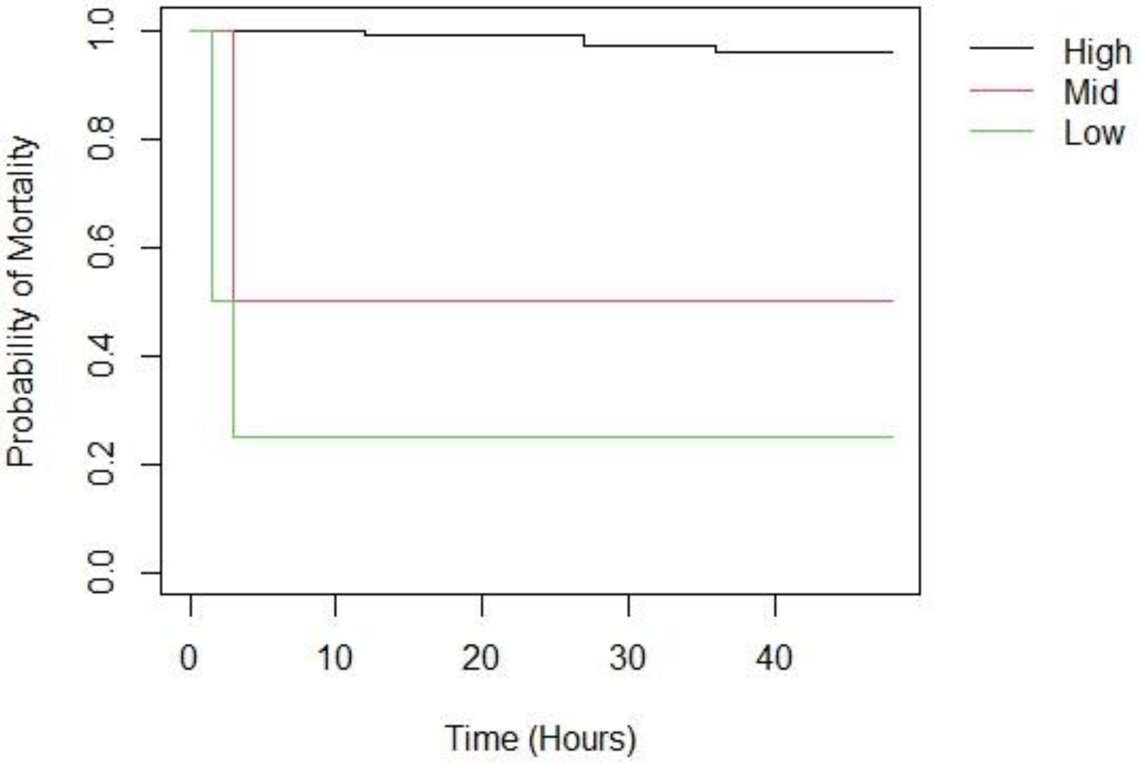


Figure 8: Mortality curve depicting the probability of death of the hard clam at different MCA concentrations indicated by high (2.0-3.0 mg/l), mid (1.1-2.0 mg/l) and low (0.0-1.0 mg/l) level

Discussion

Monochloramine was shown to be heavily toxic to mosquitofish and pink shrimp when tested at different concentrations under the MRDL of 4.0 ppm or mg/l. Low concentrations of monochloramine proved to be toxic to mosquitofish and pink shrimp, with most individuals dying within the first hour of the trial (see Table 4). It was observed that the addition of monochloramine caused great stress on mosquitofish and pink shrimp. Immediately after the dosage was added, both species began swimming erratically and suffered loss of buoyancy until death. Individuals exposed to lower monochloramine concentrations and thus survived longer than one hour experienced the same erratic swimming response when the chemical was first introduced but became more lethargic as time elapsed. Mosquitofish operculum movement was rapid at first but following the first hour of exposure, movement became slow until no movement was detected, and death was recorded. The erratic behavior of the mosquitofish and pink shrimp was most likely caused by monochloramine, as it decreased the blood's oxygen-carrying capability, resulting in suffocation and gill damage (Bakhiet et al. 2020; Skipton and Dvorak 2007). The hard clam showed to be more resilient to the chemical with most individuals alive by the end of the 48-hour trial. During trials, most clams stayed closed, rarely opening. The ability to remain closed during trials may have aided the clam in its survival. Bivalves' filter-feeding rate (clearance rates) is directly related to water quality. A study conducted by Galimany et al. (2017) determining the relationship between bivalve physiology and environmental variables found that hard clams in the Indian River Lagoon (IRL; Florida, USA) had decreased clearance rates in environments with poorer water quality, like environments near to freshwater releases (effluent).

This study showed that monochloramine is toxic to both mosquitofish and pink shrimp, but not very toxic to the hard clam. Both the mosquitofish and pink shrimp have a high probability of death when exposed to both low and high monochloramine concentrations. While the hard clam has a high probability of death at higher monochloramine concentrations, they are more likely to survive at lower concentrations. However, because many clams remained closed during the trials, it is likely that they were not exposed to monochloramine or had a lower exposure time, resulting in a need for further research.

The probability of mortality decreases over time most likely because of monochloramine decay. Species reared and raised in aquaculture facilities or home aquariums are sensitive and monochloramine should be removed from the water. Although monochloramine is a threat to individuals kept in tanks, it may not pose a threat to wild individuals because of monochloramine's instability. Monochloramine's volatility results in unstable and decreasing concentrations over time (Silva et al. 2005). Monochloramine may deteriorate at faster rates in large bodies of water such as estuaries, resulting in no effect on the wildlife. A study researching the dissipation of monochloramine in stormwater sewer systems found that natural organic matter (NOM) was the dominant factor controlling monochloramine dissipation, followed by ammonia (Zhang et al. 2016). NOM levels in estuaries or other bodies of water where monochloramine might enter (boat basins or stormwater drainage areas) could be high enough to cause monochloramine to decay quickly, therefore showing no effect on wildlife. A study by Sacher et al. (2019) found that monochloramine's half-life in river surface waters is short (0.06 to 1.50 hours) and mainly affected by presence of sediment and temperature.

Although the results of this study are important to understand how marine and freshwater organisms react when exposed to acute doses of monochloramine, the original goal of this study was to determine the LC_{50} of monochloramine for each species. Species would be acutely exposed to five different concentrations of monochloramine over 96-hours. The concentration and time of death would be recorded. The data collected from the trials would be analyzed using a probit model, a regression showing the relationship between a dose and a response. However, monochloramine concentrations were unable to be controlled, causing monochloramine exposure over various concentrations and failure to expose species for 96-hours due to rapid monochloramine decay, ultimately resulting in the inability to calculate LC_{50}s.

In March 2020, Nova Southeastern University mandated a COVID-19 campus-wide shut down. For several weeks, the university was closed without any access to the laboratory. Trials were delayed until students could get special permission from the department's Dean to come to campus and conduct research. Access was granted only if the student and their principal investigator followed a strict visitation schedule that provided proper supervision and social distancing. Once granted access, research was able to continue, and the first trials of this study were conducted in late May 2020.

As mentioned previously, monochloramine is a very unstable chemical and it was difficult to control. There are several parameters that are important to monitor when chloramine is added to water, including the chlorine to ammonia ratio, pH, chloramine species, and temperature. Residual loss of monochloramine can be caused by warmer temperatures, neutral and acidic pH, sunlight and wind exposure, and free ammonia (Kirmeyer 2004). During the first attempt at monochloramine synthesis, ammonium chloride and sodium hypochlorite solutions were kept at 1-4^0 C. Both solutions were mixed in a beaker and kept chilled until use. The solution formed monochloramine, but it was highly unstable and deteriorated quickly when put in artificial salt water. After further research, it was found that adding the ammonium chloride and sodium hypochlorite using a slow drip method allowed for monochloramine to form more successfully (Delalu et al. 2006). However, the same issue occurred when mixed and added to artificial sea water. It was first believed that the artificial salt, Instant Ocean, might be causing a reaction that stopped the formation of monochloramine. After checking the ingredients with an Instant Ocean customer service representative, it was confirmed that Instant Ocean did not have any chemical properties that were prohibiting monochloramine to form. Due to monochloramine's extreme sensitivity to temperature changes, the ammonium chloride and sodium hypochlorite solutions were kept at the same temperature as the artificial salt water (20-25^0 C). When the monochloramine solution was added to the sea water, there was no drastic change in temperature. This was successful and resulted in a more stable monochloramine solution.

Although the chemical was more stable than before, it was not stable enough to maintain specific concentrations throughout a trial. There was still a slow decaying of monochloramine during the 48-hour trials. Because of this, an LC_{50} value could not be obtained. Instead, this study's main objective was modified to determine the probability of mortality of the three species when introduced to monochloramine. The probability of mortality for each species was determined using the Kaplan-Meier estimate. The Kaplan-Meier estimate is used to analyze non-parametric data to determine survival functions. Once survival functions were determined, a Kaplan-Meier curve was created. The curve shows the probability of mortality of all three species used in this study over time.

In addition to the modification of this study's objective, it should also be noted that the mummichog (*Fundulus heteroclitus)* was the original species chosen for this study. Mummichog have been used in previous studies to determine water quality because it is known to tolerate various environmental conditions including temperature fluctuations and wide ranges of pH and salinity (Eisler 1986), as well as a wide geographic distribution in Atlantic coast regions. Its high tolerance of environmental conditions and previous use in water quality studies also made it a preferred choice for this study. However, no fish were caught despite numerous attempts in various locations using traps, seine nets, dip nets, and cast nets. Additional efforts to procure mummichogs from commercial bait suppliers were also unsuccessful. Mosquitofish were chosen as a replacement fish species because of both similar wide water quality tolerances and local abundance in southern Florida freshwaters.

Further studies would need to be conducted to determine how quickly monochloramine can deteriorate in salt water. Additional studies should include acute and chronic LC_{50} toxicity tests at different ages, sizes, and sex, gill analysis to determine if monochloramine causes internal damage, and species' ability to detect monochloramine.

References

Able, K. W., and Felley, J. D. (1986). "Geographical variation in Fundulus heteroclitus: tests for concordance between egg and adult morphologies."*American Zoologist* 26, no. 1: 145-157

Adams, C. M., Lazur, A. M., Zajicek, P., and Zimet, D. (1998). "An assessment of the market for livemarine baitfish in Florida." *Florida Department of Agriculture and Consumer Services, Bureau of Seafood and Aquaculture, Tallahassee*.

Ajana, A. M. (1980). "Fishery of the mangrove oyster, Crassostrea gasar, Adanson (1757), in the Lagos area, Nigeria."*Aquaculture* 21, no. 2: 129-137.

Al Habobi, N. A., Ismail, N. N., & Hamad, M. F. (2012). Chlorine removal with activated carbon using bubble column. *Engineering and Technology Journal*, 30(9), 1528-1537.

American Pet Product Association. (2020). "Pet Industry Market Size: Ownership Statistics." https://www.americanpetproducts.org/press_industrytrends.asp

Bakhiet, H. H. A., Omer, R., & Hashim, A. (2020) "Abnormal Tissues Changes Made by Chlorine in the Gills of Oreochromis niloticus Fish". *International Journal of Animal Biology*.

Blessing, J. J., Marshall, J.C., and Balcombe, S.R. (2010). "Humane killing of fishes for scientific research: a comparison of two methods." *Journal of Fish Biology* 76, no. 10: 2571-2577.

Bricelj, V. Monica, J. N. Kraeuter, and G. Flimlin. (2017). "Status and trends of hard clam, Mercenaria mercenaria, populations in a coastal lagoon ecosystem, Barnegat Bay–Little Egg Harbor, New Jersey." *Journal of Coastal Research* 78: 205-253.

Brodtmann Jr, N.V., and Russo, P.J. (1979) "The use of chloramine for reduction of trihalomethanes and disinfection of drinking water." *Journal (American Water Works Association)* 40-42.

Browder, J. A., and Robblee, M. B. (2009). Pink shrimp as an indicator for restoration of everglades ecosystems. Ecological Indicators, *Elsevier* 9(6), S17-S28.

Center of Disease Control and Prevention. (2020) "Water Disinfection with Chlorine and Chloramine". https://www.cdc.gov/healthywater/drinking/public/water_disinfection.html

Charles A., Sturmer, L., and Hodges, A. (2014). "Tracking the Economic Benefits Generated by the Hard Clam Aquaculture Industry in Florida." *EDIS New Publications RSS*. Accessed December 16, 2017. http://edis.ifas.ufl.edu/fe961.

City of Fort Lauderdale. (2019). "City of Fort Lauderdale 2019 Water Quality Report." https://www.fortlauderdale.gov/departments/public-works/water-quality-report-online-registration-form. 1-6.

Cohen, J. (1992). “Statistical power analysis”. *Current directions in psychological science,* 1(3), 98-101.

Coventry, F. L., Shelford, V. E., and Miller, L. F. (1935). "The conditioning of a chloramine treated water supply for biological purposes." *Ecology* 16, no. 1: 60-66.

Delalu, H., Duriche, C., Berthet, J., and Le Gars, P. (2006). *U.S. Patent No. 7,045,659*. Washington, DC: U.S. Patent and Trademark Office.

Eisler, R. (1986). "Use of Fundulus heteroclitus in pollution studies." *American Zoologist* 26, no. 1: 283-288.

Environmental Protection Agency. (2020). “Chloramines in Drinking Water” https://www.epa.gov/dwreginfo/chloramines-drinking-water

Environmental Protection Agency, Office of Wastewater Management. (2004). Primer for Municipal Wastewater Treatment Systems (pp. 5-7). Washington, DC: *United States Environmental Protection Agency*.

Farrell, A. P., Kennedy, C., Cheng, W., and Lemke. A.M. (2001). "Acute toxicity of monochloramine to juvenile chinook salmon (Oncorhynchus tshawytscha Walbaum) and Ceriodaphnia dubia."*Water Quality Research Journal of Canada* 36, no. 1: 133-149.

Finney, D. J., and Tattersfield, F. (1952). “Probit analysis”. *Cambridge University Press*; Cambridge.

Fisheries, F.A.O. (2011). “Global Aquaculture Production Statistics for the year”. *Aquaculture Department*. http://www.fao.org/3/a-i4899e.pdf

Florida Fish and Wildlife Conservation Commission (2020). “Commercial Fisheries Landings Summaries”. Retrieved from https://public.myfwc.com/FWRI/PFDM/ReportCreator.aspx

Galimany, E., Lunt, J., Freeman, C. J., Reed, S., Segura-García, I., & Paul, V. J. (2017). “Feeding behavior of eastern oysters Crassostrea virginica and hard clams Mercenaria mercenaria in shallow estuaries”. *Marine Ecology Progress Series*, 567, 125-137.

Goudreau, S. E., Neves, R.J., and Sheehan, R.J. (1993). "Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA."*Hydrobiologia* 252, no. 3: 211-230.

Hansen, D. J. (1969). "Food, growth, migration, reproduction, and abundance of pinfish, Lagodon rhomboides, and Atlantic croaker, Micropogon undulatus, near Pensacola, Florida, 1963–65."*Fishery Bulletin* 68, no. 1: 135-146.

Hua, G., and Reckhow, D.A. (2008). "DBP formation during chlorination and chloramination: effect of reaction time, pH, dosage, and temperature."*Journal (American Water Works Association)* 100, no. 8: 82-95.

Hua, G., and Yeats, S. (2010). "Control of trihalomethanes in wastewater treatment."*Florida Water Resource Journal* 4. 6-12.

Jenkins, J.A., Bart Jr, H. L., Bowker, J.D., Bowser, P.R., MacMillan, J.R., Nickum, J.G., Rose, J.D. (2014). "Guidelines for the Use of Fishes in Research." *Bethesda, Maryland, USA: American Fisheries Society*.

Johnson, D.R., Browder, J.A., Brown-Eyo, P., and Robblee, M.B. (2012). "Biscayne Bay commercial pink shrimp, Farfantepenaeus duorarum, fisheries, 1986-2005. "*Marine Fisheries Review* 74, no. 4: 28-43.

Kirmeyer, G.J. (2004). "Optimizing chloramine treatment". *American Water Works Association.*
Klein, E. (1986). "Effects of disinfectants in renal dialysis patients." *Environmental health perspectives*. 69:45.

Kuhns, J.F. (1987). "Method and product for removal of chloramines, chlorine and ammonia from aquaculture water." *U.S. Patent 4,666,610*, issued May 19, 1987.

Kulkarni, V., Awad, J., van Leeuwen, J., Drikas, M., Chow, C., Cook, D., ... & Amal, R. (2018). "Impact of zinc on biologically mediated monochloramine decay in waters from a field based pilot scale drinking water distribution system." *Chemical Engineering Journal*, 339, 240-248.

Larson, G.L. (1978). "Toxicity of residual chlorine compounds to aquatic organisms." *Food and Agriculture Organization of the United Nations*.

LeChevallier, M.W., and Au, K. (2004). "Water treatment and pathogen control" *Iwa Publishing*.
Martin County Utilities and Solid Waste Department. (2019). "2019 Water Quality Report Martin County Utilities and Solid Waste." *Water Quality Test Results*. 1-10

NOAA-Hard Clam/Northern Quahog. (2019). Retrieved November 03, 2020, from https://www.fishwatch.gov/profiles/northern-quahog?_ga=2.115142272.604976477.1604419811-244553264.1604419811

Nordlie, F. G. (2006). "Physicochemical environments and tolerances of cyprinodontoid fishes found in estuaries and salt marshes of eastern North America". *Reviews in Fish Biology and Fisheries*, 16(1), 51-106.

Ohs, C., Lynn, R., Creswell, L., and DiMaggio. M.A. (2013). "Growing marine baitfish: a guide to Florida's common marine baitfish and their potential for aquaculture". Fort Pierce, FL: *University of Florida*/IFAS.

Palmer, J.J. (2006) “How to brew.” Mosher, R., and Labinsky, D., (3rd Edition)*EUA: Brewers Publications.*

Pasternak, J.P., Moore, D., and Teed, S.R. (2003). "An ecological risk assessment of inorganic chloramines in surface water." *Human and Ecological Risk Assessment* 9, no. 2: 453-482

Pérez-García, R., and Rodríguez-Benítez, P. (1999). "Chloramine, a sneaky contaminant of dialysate." *Nephrology Dialysis Transplantation* 14, no. 11: 2579-2582.

Pyke, G. H. (2005). “A review of the biology of Gambusia affinis and G. holbrooki” *Reviews in Fish Biology and Fisheries*, 15(4), 339-365.

Rand, G.M., (1995). “Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment.” *CRC Press*.

Reyna, P. B., Ballesteros, M. L., Albá, M. L., Bertrand, L., González, M., Miglioranza, K. S. B., and Hued, A. C. (2019). “A multilevel response approach reveals the Asian clam Corbicula largillierti as a mirror of aquatic pollution”. *Science of The Total Environment*, 692, 175-187.

Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). “Standard Methods for Examination of Water and Wastewater 2012”. Washington, DC: *American Public Health Association.*

Roseboom, D.P., and Richey, D. (1977). “Acute toxicity of residual chlorine and ammonia to some native Illinois fishes”. *Illinois State Water Survey*.

Sacher, F., Gerstner, P., Merklinger, M., Thoma, A., Kinani, A., Roumiguières, A., and Marolleau, F. (2019). “Determination of monochloramine dissipation kinetics in various surface water qualities under relevant environmental conditions-Consequences regarding environmental risk assessment.” *Science of The Total Environment*, 685, 542-554.

Salkind, N.J. (2010). “Encyclopedia of Research Design”. Thousand Oaks, Calif: *SAGE Publications*, *Inc*. eBook Collection (EBSCOhost), EBSCOhost (accessed May 3, 2018).

Scarpa, J., Sturmer, L., Adams, C.M., Creswell, R.L., and Nuñez, J. (2009). "Sunray Venus Clam: A New Species to Diversify the Florida Aquaculture Hard Clam Industry." *Florida Sea Grant Program.* 23.

Seidel, C.J., McGuire, M.J., Summers, R.S., and Via, S. (2005). "Have utilities switched to chloramines?." *Journal (American Water Works Association)* 97, no. 10: 87-97.

Shafiee, M.R., and Taghavi, L. (2012). "Health effects of Trihalomethanes as chlorinated disinfection by products: A review article." *World Academy of Science, Engineering and Technology.* 68: 2090-2096.

Shull, K. E. (1981). "Experience with chloramines as primary disinfectants." *Journal (American Water Works Association).* 101-104.

Silva, M., Tessaro, I.C., and Wada, K. (2005). "Study of the formation of stable high concentrated monochloramine solutions." *Mercosur Congress on Chemical Engineering (2.: 2005: Costa Verde, RJ).[Proceedings][recurso eletrônico]. Rio de Janeiro: 2005*.

Skipton, S., and Dvorak, B. (2007). "G07-1704 Chloramines Water Disinfection: Omaha Metropolitan Utilities District and Lincoln Water System." *University of Nebraska*.

Switzer, J. A., Rajasekharan, V. V., Boonsalee, S., Kulp, E. A., & Bohannan, E. W. (2006). Evidence that monochloramine disinfectant could lead to elevated Pb levels in drinking water. *Environmental science & technology*, 40(10), 3384-3387.

Tacon, A. G. (2020). “Trends in global aquaculture and aquafeed production: 2000–2017”. *Reviews in Fisheries Science & Aquaculture*, 28(1), 43-56.

Travis, T. W., & Heath, A. G. (1981). “Some physiological responses of rainbow trout (Salmo gairdneri) to intermittent monochloramine exposure”. *Water Research*, 15(8), 977-982.

Türetgen, I. (2004). "Comparison of the efficacy of free residual chlorine and monochloramine against biofilms in model and full scale cooling towers." *Biofouling* 20, no. 2: 81-85.

United States Department of Agriculture. (2019). “2018 Census of Aquaculture”. Retrieved from https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Census_of_Aquaculture/index.php

United States. (1992). “Organization for Economic Co-operation and Development”. *OECD Guideline for Testing of Chemicals*. 1-10.

Utility Services. (2019). "2019 Annual Water Quality Report." 2019 Annual Water Quality Report.

Vikesland, P.J., Ozekin, K., and Valentine, R.L. (2001). "Monochloramine decay in model and distribution system waters." *Water Research* 35, no. 7: 1766-1776.

Virginia Department of Health. (2020). “Chloramines”. Retrieved December 18, 2020, from https://www.vdh.virginia.gov/environmental-health/chloramines/

Ward, G. S., and Parrish, P. R. (1992). “Manual of methods in aquatic environment research-Part 6: Toxicity Tests (Vol. 185). *Food & Agriculture Org*.

Water and Sewer. (2019). "Miami-Dade County's 2019 Water Quality Report." *Water Quality Reports*.

Zhang, Q., Davies, E. G., Bolton, J., & Liu, Y. (2016). “A study on monochloramine dissipation in stormwater sewer systems.” *International Symposium on Outfall System*.

Zink, I. C., Browder, J. A., Lirman, D., & Serafy, J. E. (2017). Review of salinity effects on abundance, growth, and survival of nearshore life stages of pink shrimp (Farfantepenaeus duorarum). Ecological Indicators, 81, 1-17.