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# Dissolved Copper Distributions in the Western Mississippi Sound: Characterizing an Unrecognized Endmember

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# Dissolved Copper Distributions in the Western Mississippi Sound: Characterizing an Unrecognized Endmember

by

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# ABSTRACT

This study examined the distribution of dissolved copper (Cu) in the western Mississippi Sound, as the element can be beneficial or harmful to organisms depending on its concentration and its chemical form. A mixing experiment eliminated flocculation as a control on Cu distributions during estuarine mixing in both dissolved and colloidal fractions. Field sampling of the western Mississippi Sound occurred during a low river discharge period, and distributions of Cu indicated conservative mixing between fresh ($[\mathrm{Cu}] \approx 17\ \mathrm{nM}$) and saline ($[\mathrm{Cu}] \approx 2\ \mathrm{nM}$) sources in mid-to-high salinities. Storm outfalls were sampled after rain events to evaluate the impact of road runoff, with inconclusive results. However, stable isotope ratios of water ($^{18}O/^{16}O$ for oxygen and $^{2}H/^{1}H$ for hydrogen) in field samples showed a fresh source with an isotopic signature ($\delta^{18}O$ = -2.2‰) unique from prior analyses of both the Mississippi River ($\delta^{18}O$ = -6.6‰) and local rivers ($\delta^{18}O$ = -3.9‰). This isotopic anomaly was not found to be significantly influenced by evaporation, indicating a freshwater source other than local rivers. Prior western Mississippi Sound work has shown groundwater with high Cu concentrations and a similar isotopic signature ([Cu] ≈ 20 nM; $\delta^{18}O$ ≈ -2.6‰), and other work has found that submarine groundwater discharge (SGD) is a seasonally important source of water to the region. Using the observations collected here, this study proposes SGD as an unrecognized source to the western Mississippi Sound's Cu mass balance.

***Keywords***: dissolved copper, Mississippi Sound, flocculation, estuary, trace elements

# ACKNOWLEDGMENTS

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF ILLUSTRATIONS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| BCS | Bonnet Carré Spillway |
| Cu | Copper |
| [Cu] | Copper concentration |
| $^{63}$Cu | Copper-63 |
| $^{65}$Cu | Copper-65 |
| dCu | Dissolved copper |
| [dCu] | Dissolved copper concentration |
| DOC | Dissolved organic carbon |
| EPA | Environmental Protection Agency |
| ESI | Electro Scientific Industries |
| HCl | Hydrochloric acid |
| HDPE | High-density polyethylene |
| HR-ICP-MS | High resolution inductively coupled plasma mass spectrometry |
| MS Sound | Mississippi Sound |
| NCEI | National Centers for Environmental Information |
| PVC | Polyvinyl Chloride |
| ROS | Reactive oxidative species |
| SGD | Submarine groundwater discharge |
| USGS | United States Geological Survey |
| wMS Sound | Western Mississippi Sound |
| $\delta^{18}$O | Oxygen isotope ratio |
| $\delta$D | Hydrogen isotope ratio |

# CHAPTER I: INTRODUCTION

Copper (Cu) is an essential micronutrient to most organisms, including both prokaryotes and eukaryotes (Burkhead et al., 2009; Peña et al., 1999). Copper's micronutrient status comes from its use as a cofactor for several enzymes essential to homeostasis, specifically for enzymes involved in respiration and cellular development (Balamurugan and Schaffner, 2006; Burkhead et al., 2009). However, Cu is not exclusively beneficial, as Cu becomes toxic at high concentrations (Balamurugan and Schaffner, 2006; Wang and Wang, 2021). This balancing act is particularly evident in aqueous environments, since Cu exposure is controlled by the ambient concentration for many organisms (Arnold et al., 2010). For economically and ecologically valuable estuaries like the Mississippi Sound (MS Sound), a thorough understanding of parameters like Cu can help in evaluating the estuary's overall health.

Estuaries are biodiverse communities containing strong chemical and physical gradients that occur when river and sea water mix (Gaston et al., 1998). As with any ecosystem, the presence of heterogeneity – or structure – supports biodiversity, and the mixing gradients found in estuaries are a form of structure (Gaston et al., 1998). Beyond gradient structure, foundational organisms can alter the nearby habitat physically or chemically to create additional structure (Grabowski et al., 2012). Oysters, as foundational organisms, create physical structure in their habitats through reef building, which provides shelter for many species of fish and invertebrates (Grabowski et al., 2012). In addition to creating physical structure, oysters create chemical structure by improving the overall water quality via filtration, denitrification, and nutrient recycling

(Grabowski et al., 2012). These structure-generating processes allow a healthy oyster population to promote biodiversity in estuaries (Grabowski et al., 2012).

In addition to ecosystem services, oysters are an economically valuable species to fisheries, including the fishery associated with the MS Sound (National Marine Fisheries Service, 2023). The MS Sound fishery is an important component of the local economy, bringing in nearly $400 million in total sales for the 2020 season (National Marine Fisheries Service, 2023). *Crassostrea virginica* (*C. virginica*), the local oyster species, is the third highest valued species in MS Sound fisheries production, following shrimp and menhaden (National Marine Fisheries Service, 2023). Though oyster reefs are found across the MS Sound, the largest reported oyster reefs are found in the western MS Sound (wMS Sound), just outside of St. Louis Bay (Mississippi Department of Environmental Quality, 2016). Because oysters are sedentary, their populations are often more greatly affected by events like flooding than mobile species (Pruett et al., 2021). The recovery of oyster populations is slow, with the high mortality of a 2019 flooding event (Gledhill et al., 2020) still felt in the population three years later (National Marine Fisheries Service, 2023). Consequently, oysters have not been landed at all in the 2019 and 2020 seasons, bringing a significant loss to the MS Sound fishery (National Marine Fisheries Service, 2023).

With the ecosystem and economic benefits of a healthy oyster population as described above, understanding the behavior of water parameters important to oysters is clearly invaluable to fisheries management (Walton and Swann, 2021). One parameter to consider is the dissolved Cu (dCu) concentration. Estuaries have several sources of Cu, both natural and anthropogenic. Natural sources include rivers (Gaillardet et al., 2014), seawater (Bruland and Franks, 1983), and groundwater (Kalnejais et al., 2015), while anthropogenic sources include industrial wastewater and road runoff (Crabtree et al., 2006). Anthropogenic sources are not limited to direct contamination, as rivers, sediments, and groundwater can also become enriched in dCu by human contamination (Casper et al., 2004). Each of these sources can vary spatially and temporally in dCu concentration and water volume flux, resulting in a dynamic picture of estuarine dCu distributions. Since Cu can be either beneficial or toxic to organisms depending on ambient concentrations, defining this picture in the wMS Sound can help predict how Cu will impact oyster and, subsequently, estuary health. As such, this work seeks to constrain dCu in the wMS Sound and the influence of mixing on Cu in the estuary, guided by the context provided in the following chapter.

# CHAPTER II: LITERATURE REVIEW

## 2.1 Impact of Copper on Oysters

Oysters are significant to estuarine health, as outlined in the previous chapter (Grabowski et al., 2012). Therefore, understanding the effects of ambient water conditions on oysters can yield more effective fisheries management practices (Walton and Swann, 2021). Copper (Cu) is an essential micronutrient for both prokaryotes and eukaryotes (Burkhead et al., 2009; Peña et al., 1999) and can be found in rivers (Gaillardet et al., 2014), seawater (Bruland and Franks, 1983), and groundwater (Kalnejais et al., 2015). Oysters have also been known to improve the surrounding water quality during filtration by removing metals and contaminants, including Cu (Engel, 1999). Copper can be hyper-accumulated in oyster tissues, with blue-green specimens observed in extreme cases of Cu accumulation (Wang et al., 2011). Though this accumulation poses a higher risk for toxicity, *Crassostrea virginica* (*C. virginica)* employs metallothionein proteins to sequester and detoxify Cu, decreasing the threat significantly (Engel, 1999). Figure 2.1 demonstrates the general preference metallothionein proteins have for copper ions over zinc ions, though specific metallothioneins vary in metal preferences (Calvo et al., 2022; Irving and Williams, 1953; Krezel and Maret, 2021).

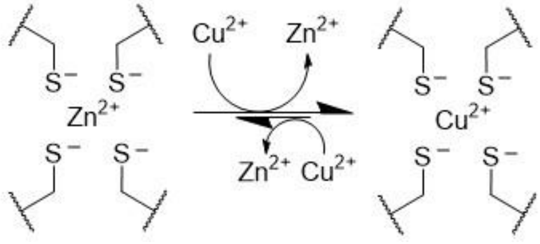

*Figure* ***Error! No text of specified style in document.****.1 A generic metallothionein-metal complexation. Sulfur atoms on the cysteine groups of metallothioneins coordinate with metal ions (Calvo et al., 2022).*

*The Irving-Williams series shows that divalent copper forms more stable complexes than divalent zinc due to crystal field stabilization energies as well as the Jahn-Teller effect (Irving and Williams, 1953). Copper (II) can also be oxidized to Cu (I) by the cysteine ligands, creating a softer metal to coordinate more strongly with the soft sulfur ligands (Calvo et al., 2022; Krezel and Maret, 2021).*

After being accumulated, Cu is used in oysters as a cofactor for several enzymes, including superoxide dismutase and cytochrome *c* oxidase (Balamurugan and Schaffner, 2006; Burkhead et al., 2009). Superoxide dismutase converts superoxide into oxygen and hydrogen peroxide, while cytochrome *c* oxidase is a mitochondrial enzyme involved in ATP production (Balamurugan and Schaffner, 2006; Bernal et al., 2004; Burkhead et al., 2009). Copper demand varies throughout *C. virginica*'s lifespan, with some early developmental stages requiring more Cu than others. Oyster larvae are pelagic for a short window before metamorphosizing into a sedentary, benthic adult form (Li et al., 2021; Prytherch, 1934).

In larval stages, experimentally controlled Cu concentrations of over 150 nanomolar (nM) have been shown to promote growth rates and settlement of *Crassostrea angulata (C. angulata)*, and Cu has a higher demand immediately prior to settlement (Li et al., 2021). Copper was shown to promote the production of chitin and chitin binding proteins, which are secreted to attach the individual to substrate and begin shell development (Li et al., 2021). In adult stages, Cu is utilized in oysters' immune response through the generation of reactive oxidative species (ROS) (Shi et al., 2019). Reactive oxidative species include superoxide and hydroxyl radicals that indiscriminately react with cellular receptors and damage tissues, leading to biocidal effects (Babior et al., 1973; Cao and Wang, 2017; Kaushik et al., 2015). By briefly concentrating Cu into hemolymph—the blood equivalent of invertebrates—near infection sites, ROS's biocidal behavior is leveraged against pathogens (Shi et al., 2019). Copper oxidation is paired with the generation of ROS as depicted in Figure 2.2 (Kaushik et al., 2015), but Cu also regulates ROS toxicity, as superoxide dismutase uses Cu as a cofactor to reduce ROS (Balamurugan and Schaffner, 2006).

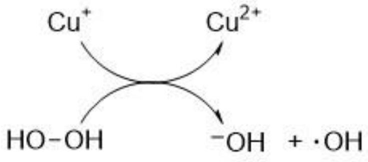

*Figure **Error! No text of specified style in document.**.2 Formation of ROS from hydrogen peroxide via copper oxidation (Kaushik et al., 2015). The hydroxyl radical is responsible for biocidal effects and is countered by superoxide dismutase (Balamurugan and Schaffner, 2006; Shi et al., 2019).*

Copper becomes toxic when metallothionein proteins are overwhelmed and ROS formation goes unchecked (Balamurugan and Schaffner, 2006; Wang and Wang, 2021). Experimental studies have found that Cu concentrations of 15,000 nM cause near-immediate mortality in *C. angulata* larvae, while concentrations of 300 nM cause larval mortality during chronic exposures (Li et al., 2021). Because Cu toxicity is dependent on the exposure concentration, other parameters can influence the proportion of ambient Cu that contributes to organism exposure (Arnold et al., 2010). For example, dissolved organic carbon (DOC) concentrations affect toxicity thresholds by reducing the bioavailability of Cu, thus increasing Cu tolerance (Arnold et al., 2010). In low DOC conditions, Cu toxicity for *C. virginica* occurred at 176 nM Cu, but this threshold increased significantly as DOC concentrations increased (Arnold et al., 2010). The difference in toxicity thresholds between the *C. angulata* and *C. virginica* studies are likely due to both differences in target species' tolerances of Cu and differences in DOC concentrations between studies (Arnold et al., 2010; Li et al., 2021). Because Cu falls on a balance between beneficial and toxic effects on oysters, monitoring Cu concentrations in estuaries can be useful to fisheries management (Walton and Swann, 2021).

## 2.2 Dissolved Copper Behavior

### *2.2.1 Sources of Copper to the Mississippi Sound*

To illustrate the expected behavior of dissolved Cu (dCu) in an estuarine environment, the various endmembers of the estuary should be first defined. An estuary primarily has riverine and oceanic endmembers, but many estuaries are also influenced by submarine groundwater discharge (SGD) (Burnett et al., 2003). Rivers can vary greatly in dCu concentrations, even seasonally within the same river (Shiller, 1997), but the global average of river dCu is around 23 nM (Gaillardet et al., 2014). This is significantly higher than the average surface sweater concentration, 0.54 nM (Bruland and Franks, 1983), creating a dCu gradient when rivers and seawater mix. Groundwater dCu is much less predictable, and interactions between porewater and sediment during discharge and recharge must be constrained. Groundwater dCu can be comparable to riverine concentrations, or it can be lowered in dCu due to mixing with low dCu seawater and due to removal processes like sedimentary adsorption (Beck et al., 2010; Kalnejais et al., 2015). Additionally, groundwater dCu is influenced by redox potential, with seasonal variations in porewater dissolved oxygen altering Cu's dominant phase (Kalnejais et al., 2015). Kalnejais et al. (2015) found that porewater was more deeply oxygenated in winter for Massachusetts Bay sediments, correlating with a higher flux of dCu into the overlying water column. Thus, seasonal redox conditions should be also considered when evaluating the dynamics dCu in groundwater and porewater.

These general descriptions of estuarine endmembers apply to the western Mississippi Sound (wMS Sound), but dCu concentrations have been more specifically constrained for the wMS Sound's endmembers. The Mississippi Sound (MS Sound) is bounded by the shoreline to the north and by a series of barrier islands to the south, as shown on Figure 2.3 below. Stable isotope ratios of water ($\delta^{18}O$ and $\delta D$) have been used to determine that the dominant sources of freshwater to the wMS Sound are generally local rivers rather than the Mississippi River (Sanial et al., 2019). An exception to local river dominance occurs when the Bonnet Carré Spillway (BCS) is open, diverting Mississippi River water through Lake Pontchartrain and into the MS Sound (Ho et al., 2019; Sanial et al., 2019).

Stable isotope ratios of an element are the ratios of two target isotopes, typically an uncommon isotope and an abundant isotope, and these ratios can be influenced by mass fractionation during a variety of kinetic processes (Craig, 1961). For water, oxygen and hydrogen isotopes can be fractionated by evaporation and precipitation, and because rates of evaporation and precipitation vary spatially and temporally, the stable isotope ratios of water sources can be spatially and temporally unique (Dansgaard, 1964; Craig, 1961; Kendall and Coplen, 2001). This isotopic signature can be measured and used to calculate relative contributions of endmembers to an estuary, as done in Sanial et al. (2019) to determine local river dominance over the wMS Sound.

The rate of fractionation can also vary between hydrogen and oxygen isotopes during evaporation and precipitation, allowing for better identification of these processes using stable isotope ratios (Dansgaard, 1964). For example, Sanial et al. (2019) used *d-excess*, a parameter giving the deviation of the hydrogen isotope ratio from the global meteoritic $\delta^{18}O$ and $\delta D$ relationship (Equation 3.4 below), to evaluate the influence of evaporation in the wMS Sound, where low *d-excess* indicates evaporative influence on a sample set (Dansgaard, 1964). Low *d-excess* is determined by a significant negative deviation from a mixing line between fresh and saline endmembers, which sets a baseline *d-excess* across a range of salinities for comparing samples to. (Sanial et al., 2019).

The major local rivers influencing the wMS Sound are the Pearl River, the Jourdan River, and the Wolf River (Figure 2.3). The Pearl River has been measured at 23 nM dCu (Shiller, 2003), though this value could vary seasonally as discharge rates change (Shiller, 1997; Shim et al., 2017). The Jourdan River and the Wolf River are expected to be lower in dCu than the Pearl River because their watersheds are composed of primarily residential areas, which typically contribute less Cu than agricultural and industrial areas (Genova et al., 2022). This expectation has been confirmed in preliminary work, where the Jourdan River has been measured to peak at ~16 nM dCu and the Wolf River at ~10 nM dCu (Shiller, unpublished).

The significance of SGD's contribution to both the MS Sound and the nearby Mobile Bay has been confirmed through the employment of several tracers, including methane, radium, and radon (Montiel et al., 2019a, 2019b; Moody, 2022). Submarine groundwater discharge in the wMS Sound has been found to have a seasonal component, with a higher fresh component of the overall volumetric flux in the dry season, when river discharge is low (Moody, 2022). Though water fluxes from SGD are an order of magnitude lower than river fluxes, SGD should be taken into consideration for mass balances of many parameters, including trace elements like Cu. The concentrations of parameters like Cu could be enriched enough to rival or outcompete river parameter fluxes, like seen in the nutrient SGD fluxes determined by Moody (2022). Alternatively, low SGD concentrations could dilute parameter concentrations by roughly 10% with a single order of magnitude difference, which is still a significant control on the parameter's distribution.

CONANP, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS | CONANP, Esri, HERE, Garmin, FAO, NOAA, USGS, EPA, NPS

*Figure* ***Error! No text of specified style in document.****.3: A map of the Northern Gulf of Mexico with key landmarks labeled (ESRI, 2023). The Mississippi Sound falls north of barrier islands and is marked by the blue shading.*

The saline endmember for the MS Sound is the northern Gulf of Mexico, which has been shown to have surface dCu concentrations of 1.4 nM in open waters (Wen et al., 2011). Several studies have been conducted to constrain trace element behavior off the Louisiana Shelf, and dCu was found to mix conservatively in the Mississippi River plume, though bottom inputs on the Louisiana Shelf may act as an additional dCu source (Joung and Shiller, 2016; Shiller, 1997). Conservative mixing occurs when a parameter's distribution is controlled only by the proportion of fresh and saline endmember contributions, without the influence of any other processes. However, as mentioned before, the wMS Sound is generally not dominated by the Mississippi River (Sanial et al., 2019), so it is important to study trace element behavior specifically in the MS Sound. Fewer studies have been done to constrain trace elements in the MS Sound itself, and those that have been done lack a thorough exploration of dCu behavior (Ho et al., 2019; Moody, 2022). For example, Moody (2022) explores the influence of SGD on trace metal distributions in the MS Sound and presents a Cu versus salinity plot of surface samples containing a low-salinity nonlinearity (Figure 2.4). This nonlinearity presents as a sharp initial decrease in Cu, which then levels off to linearly mix with a seawater endmember (Moody, 2022). The change in slope was not easily explained by spatial or temporal trends and drives this study to explore whether unexplored sources are controlling dCu distributions or if there are other processes at play (Moody, 2022).

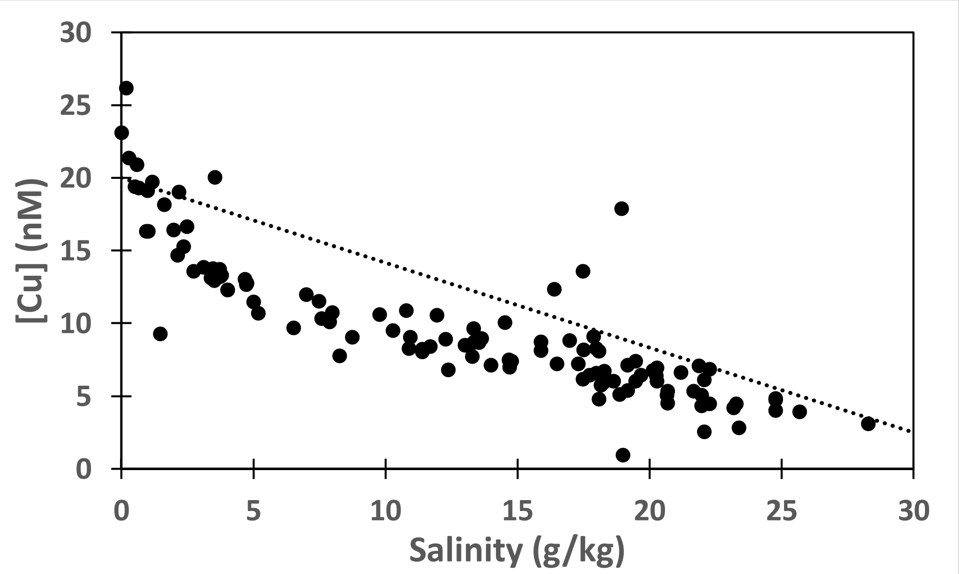

*Figure 2.4: Dissolved copper versus salinity for the MS Sound from Moody (2022). The dashed line represents mixing between the copper concentrations at the fresh and saline extremes to highlight the deviation observed.*

In addition to natural sources and controls of dCu concentrations, anthropogenic sources must be considered. Copper is a common pollutant (Crabtree et al., 2006), but there are no known direct anthropogenic contaminants of dCu to the wMS Sound. However, it is possible that road runoff contributes a significant amount of dCu to the MS Sound, as a major highway (US Highway 90) runs parallel to the beach for much of Mississippi Gulf Coast. This is a heavily trafficked roadway and is drained by numerous storm outfalls that directly empty into the MS Sound. Road runoff has been shown to pollute adjacent bodies of water, with a United Kingdom study observing over 300 nM dCu in road runoff (Crabtree et al., 2006). The total impact of runoff depends on many factors, including the volume and concentration of runoff, the frequency and intensity of rain events, and the connectivity of the road to a body of water, so the effect of road runoff on the wMS Sound cannot be easily predicted using current data.

### *2.2.2 Processes Affecting Copper Behavior*

Though conservative behavior of dCu has been shown in the Mississippi River plume (Joung and Shiller, 2016; Shiller, 1997), this is surprising for a few reasons, specifically biological effects, chemical speciation, and redox control. First, biological activity could reduce dCu concentrations because dCu is accumulated by most organisms during growth (Cabrita et al., 2020). Thus, areas of high productivity may experience lowered dCu (Cabrita et al., 2020). In an extreme example, the Sado estuary in Portugal was found to undergo near complete (98%) removal of bioavailable dCu during a phytoplankton bloom (Cabrita et al., 2020). However, the overall influence of biological activity on dCu concentrations is dependent on flux of dCu relative to biological demand, and dCu bioavailability is not universal among the chemical species present in water (Arnold, 2005).

This brings up the second factor influencing dCu behavior, chemical speciation. As with any element, Cu can be found as a variety of chemical species in the water column, including free ions, organically complexed dCu, and Cu adsorbed to particles. Free dCu is readily available for biological uptake, meaning the chemical species can behave non-conservatively due to biological activity, provided demand is comparable to dCu fluxes (Kogut and Voelker, 2001). Organically complexed Cu tends to be biologically unavailable, as the complexation can be strong, thus resisting dissociation of the Cu ion for biological use (Christl et al., 2001; Kogut and Voelker, 2001). Humic acids are a class of DOC that is particularly efficient at complexation, due to their large size and numerous carboxylic and phenolic groups that, when deprotonated, will chelate with metal ions as shown in Figure 2.5 (de Melo et al., 2016; Kogut and Voelker, 2001). Complexation with DOC is a pH dependent process, since protons will outcompete Cu ions for binding when pH is low (Mosley and Liss, 2020). Additionally, the composition of DOC will impact the magnitude of complexation, as binding constants and chelating abilities vary between organic chemical species (Mosley and Liss, 2020).

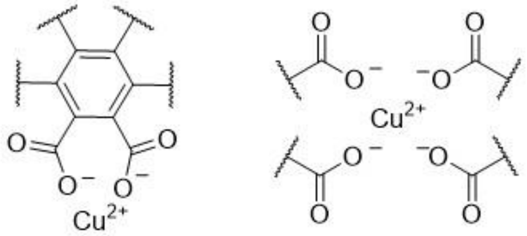

*Figure* ***Error! No text of specified style in document.****.5 Possible complexation schemes between carboxylic groups in humic acids and copper (II) (Xu et al., 2018). Phenolic groups also dominate copper coordination but are not pictured here. Additional monodentate (single attachment) and bidentate (double attachment) complexes are possible, but specific configurations depend largely on the structure of the humic acid, which varies greatly from compound to compound (Xu et al., 2018).*

While complexation affects bioavailability of dCu, adsorption onto large particles can cause Cu to settle out of the dissolved phase and into sediments. Additionally, the colloidal phase is an intermediate size fraction of suspended material that does not settle, and adsorption onto colloids would remove Cu from the dissolved phase but prevent settling to the sediments (Lu et al., 2021). Iron and manganese oxyhydroxides are particularly efficient at absorbing Cu and typically form large particles that settle, so systems rich in these chemical species are expected to exhibit non-conservative dCu behavior (Dang et al., 2015). Flocculation is a process that can produce particles from DOC, inducing non-conservative behavior in organically-complexed Cu (Waeles et al., 2005). When organically-rich freshwater mixes with seawater, it is met with a steep change in ionic strength due to the salinity increase and doubly charged ions found in seawater (e.g., $Ca^{2+}$, $Mg^{2+}$, and $Ba^{2+}$) (Mosley and Liss, 2020). This ionic strength increase compresses the negatively-charged outer layer of DOC as the doubly charged counter ions reduce the double-layer's width, and DOC molecules then aggregate into flocs (Mosley and Liss, 2020). As mentioned previously, Cu readily complexes with DOC, and the ions can remain attached to the flocs as they settle out of the water column, reducing dCu concentrations (Mosley and Liss, 2020). Because of the alkalinity and DOC composition differences between the Pearl River and Mississippi River (Savoie et al., 2022; Zhou et al., 2016), it is possible that dCu removal from flocculation occurs in the Pearl River plume despite not occurring in the Mississippi River plume (Joung and Shiller, 2016; Shiller, 1997).

The third factor influencing dCu behavior is redox control, particularly in porewater and sediments (Beck et al., 2010; Kalnejais et al., 2015). Both Cu and compounds that interact with Cu can be redox sensitive, so approximating the ambient redox conditions can help predict the behavior of dCu in an estuary (Baeyens et al., 1998). Compounds like sulfides, iron oxyhydroxides, and manganese oxyhydroxides can react with Cu and keep Cu in particulate or sedimentary phases (Baeyens et al., 1998). Copper is released from sulfides and DOC under oxidizing conditions and is released from oxyhydroxides under reducing conditions, creating a complex dynamic for Cu flux in porewater and sediments (Baeyens et al., 1998; Beck et al., 2010). Because oxygen, an oxidizer, has a limited depth of penetration into sediments, the dominance of sulfides and oxyhydroxides typically varies with depth (Beck et al., 2010; Kalnejais et al., 2015). Sulfides tend to dominate in deeper sediments, where persistent anoxic conditions preserve the compounds (Beck et al., 2010; Kalnejais et al., 2015). However, these reduced species do still form in shallower sediments and can be oxidized to release Cu and creating a positive correlation between dissolved oxygen and dissolved Cu concentrations (Baeyens et al., 1998; Kalnejais et al, 2015). In anoxic, reducing conditions, oxyhydroxides are dissolved, increasing porewater Cu (Beck et al., 2010).

Additionally, the redox potential of a particular site's sediments has a seasonal component, where the penetration of oxygen into the sediments can vary seasonally (Kalnejais et al., 2015). When oxygen penetrates further into the sediments, Cu flux from the sediments into the overlying water is increased, as diffusion within the sediments and porewater maintains the new equilibrium point (Kalnejais et al., 2015). Because SGD often involves transformations of groundwater as passes through sediments, it is possible that high dCu groundwater experiences removal of dCu before reaching the water column as it passes through anoxic, reducing coastal sediments (Beck et al., 2010). Dissolved Cu could also be enhanced if sediments are sufficiently oxic, depending on the dominance of sulfides or oxyhydroxides in the sediment (Beck et al., 2010; Kalnejais et al., 2015).

## 2.3 Expected Cu Behavior in the Western Mississippi Sound

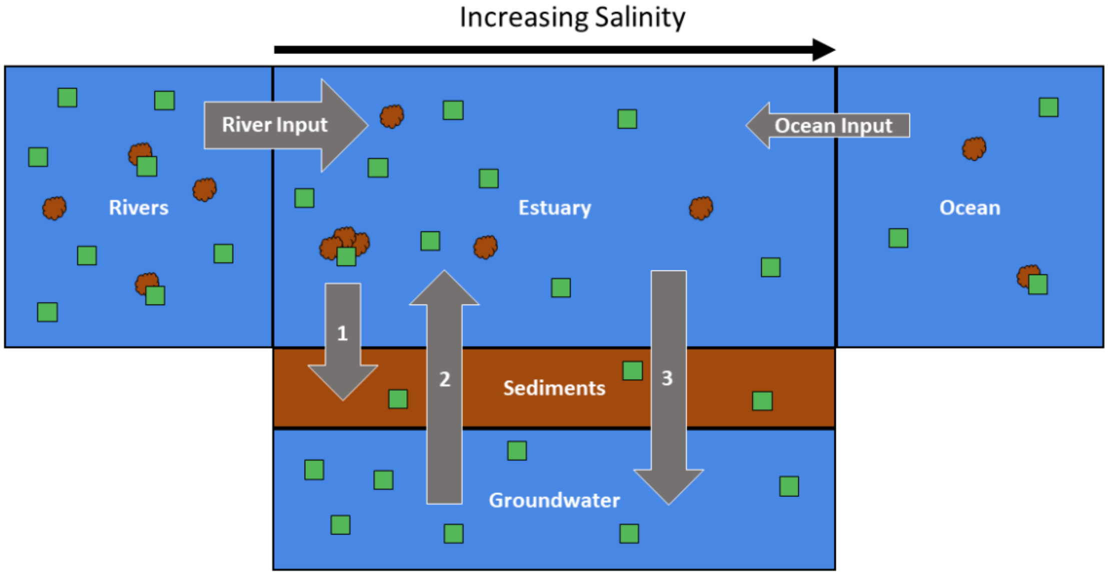

*Figure **Error! No text of specified style in document.**.6: A simplified representation of sources and sinks of copper in an estuary as discussed throughout this chapter. Green squares represent copper, and brown irregular circles represent DOC. This representation is not quantitative and simply demonstrates relative amounts of each at a given point in the estuary. Arrow 1 is representative of copper removal through flocculation. Arrow 2 is representative of SGD input. Arrow 3 is representative of removal through groundwater recirculation. Both estuary and groundwater salinity increase from left to right on the figure.*

Figure 2.6 summarizes the processes controlling dCu in an estuary influenced by groundwater as described throughout this chapter. As shown in the figure, river and groundwater are expected to contribute the most significant proportion of dCu, while seawater is expected to contribute little Cu. Flocculation can remove riverine dCu, provided the DOC composition prevents Cu displacement as ionic strength increases (Mosley and Liss, 2020). Additionally, dCu can be added or removed by sediment/redox interactions during SGD and groundwater recharge. This figure does not include anthropogenic sources like industrial wastewater and road runoff, which can be chronic sources of dCu or act as point contamination events.

This study investigates a few potential causes of the nonlinearity observed in Moody (2022) based on the above descriptions of Cu behavior in estuarine environments. The first is shifting source dominance, where various fresh endmembers with different [dCu] dominate the wMS Sound at different salinity ranges. These fresh endmembers to the wMS Sound are most likely local rivers and SGD, with Mississippi River influence occurring during BCS openings. The western MS Sound is known to have significant SGD rates (Moody, 2022), but only the fresh component has been shown to have a [dCu] on par with river concentrations, which could enrich dCu on the fresh extreme of Figure 2.4 above and cause the negative deviation. Conversely, low [dCu] SGD could dilute dCu across most of the salinity range, creating the observed negative deviation. Alternatively, road runoff could act as an additional fresh source of dCu, feeding into the MS Sound through storm outfalls. The dCu in storm outfalls should be comparable to or higher than river concentrations to cause a fresh-end increase, but runoff volumes are unlikely to be significant enough to dilute dCu throughout the salinity range. To investigate source dominance in the wMS Sound, stable isotope ratios of water can be employed. The second possible cause of the low-salinity negative dCu deviation is dCu removal due to flocculation, which can be tested by experimentally mixing fresh and saline endmembers to attempt to replicate the deviation. Other processes like biological removal are also possible controls but are not directly addressed here.

# CHAPTER III: METHODS

## 3.1 Study Site

The western Mississippi Sound (wMS Sound) (Figure 3.1) covers the region of the Mississippi Sound (MS Sound, Figure 2.3 above) west of Gulfport, MS, from the beachfront to the barrier islands (Eleuterius, 1978). The wMS Sound contains approximately 6.39 x $10^{12}$ liters of water across 2255 square kilometers of surface area when including Lake Borgne (Armandei et al., 2021). This portion of the MS Sound is fed by Lake Pontchartrain, the Pearl River, the Jourdan River, and the Wolf River. Lake Pontchartrain's freshwater sources vary depending on whether the Bonnet Carré Spillway (BCS) is open, as local rivers (Amite, Tangipahoa, Tchefuncte, and other rivers) dominate when BCS is closed, while the Mississippi River dominates during openings of BCS. When BCS is closed, the Pearl River is the dominant fresh endmember to the wMS Sound (Sanial et al., 2019).

In addition to river sources, submarine groundwater discharge (SGD) contributes freshwater to the wMS Sound, with a greater fresh output observed in the dry season (typically fall and winter) (Moody, 2022). These freshwater sources mix with northern Gulf of Mexico water entering through the passes between barrier islands, resulting in an estuarine salinity gradient. Wind conditions and tidal forcing can impact the residence time of water in the wMS Sound, but water in the MS Sound is typically expected to have a residence time of ~20 days in the summer, which encompasses the sampling period of this study (Bouchard, 2021; Moody, 2022).

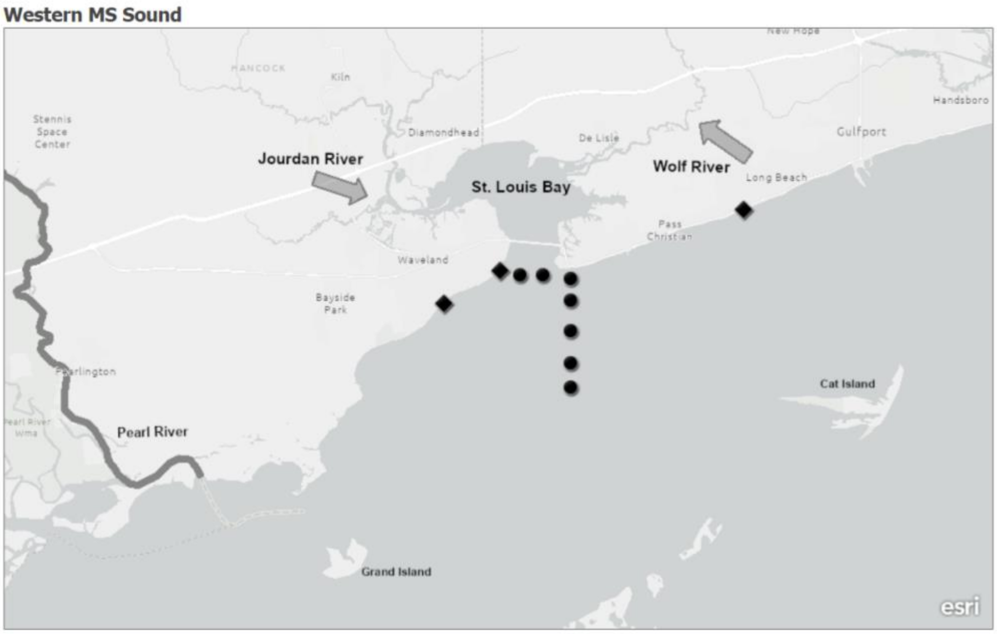

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*Figure* ***Error! No text of specified style in document.****.4: Map of the wMS Sound with relevant rivers labelled (ESRI, 2022). Offshore sampling sites are marked with circles, and storm outfall sampling sites are marked with diamonds. Samples were taken at a depth of ~0.3 meters to avoid surface slicks. Sampling occurred in late July (07/12/2022, 07/23/2022, and 07/29/2022), during a period of frequent afternoon storms. Maximum bottom depth of the offshore samples was ~6 m (NCEI, 2018).*

## 3.2 Field Sampling

Materials for all trace metal samples were prepared in advance by washing with hydrochloric acid (HCl). High density polyethylene (HDPE) bottles (Nalgene$^{TM}$, various volumes) and syringes (polyethylene, 50 mL, ThermoFisher) were acid washed by soaking in hot HCl (10% v/v, ~1.2 M, reagent grade, ThermoFisher) overnight, then rinsing thoroughly with distilled, deionized water (18.2 MΩ·cm, Millipore) (Shiller, 2003). Filters (25 mm diameter, polypropylene, Whatman Puradisc) were also acid washed by passing 20 mL of 10% v/v HCl through the filter, then rinsing with 40 mL of distilled, deionized water (Shiller, 2003).

A set of Cu field samples were collected from wMS Sound surface waters just outside of St. Louis Bay on 07/25/2022 (Figure 3.1, circles) during a low river discharge period. This period also corresponded with frequent afternoon thunderstorms, which likely mixed the shallow water column. Samples were collected from just below the surface by attaching a 250 mL high density polyethylene (HDPE) Nalgene™ bottle to a PVC pole, which was then extended over the water surface and submerged to collect the sample (Shiller, 2003). Samples were then filtered in the field through a pore size of 0.45 µm shortly after collection in a plastic "bubble" to prevent dust contamination (Shiller, 2003). A polyethylene, rubber-free syringe, a 0.45 µm filter, and a sample bottle were pre-rinsed with sample water, then 60 mL of filtered sample was collected using these materials (Shiller, 2003). Samples from the ends of storm outfalls along the western Mississippi Gulf Coast were collected to explore the concentrations of copper (Cu) in runoff from adjacent roadways. These samples were collected and filtered following the procedure described above (Shiller, 2003). Samples were collected from storm outfalls on both sides of St. Louis Bay on 07/13/2022 and 07/29/2022 (Figure 3.1, diamonds). The first sampling occurred when the tide was rising, resulting in saline samples. The second sampling occurred when the tide was ebbing, but samples were still saline.

Water stable isotope ratio ($\delta^{18}O$ and $\delta D$) samples were collected as part of other work performed alongside to this project from all locations (Magbitang, unpublished). These samples were collected into clean, airtight, 30 mL amber glass vials, then filtered to 0.45 µm and sealed with Parafilm® M (Bemis Company) to prevent evaporation before analysis (Magbitang, unpublished; Sanial et al., 2019).

### 3.3 Mixing Experiment

In addition to field samples, a mixing experiment was performed with Pearl River water and seawater (unfiltered, salinity 31.2 g/kg, pH 7.7) to determine if flocculation was a control on Cu. The in-house seawater was collected on a previous cruise independent of this study, but the exact conditions of collection are of low importance. The water was sufficiently saline, uncontaminated by trace metals, and of a natural pH, which are the key parameters to studying flocculation. Pearl River water was collected from just below the surface with the previously mentioned PVC pole and returned to the lab for mixing. Mixing was done into acid-washed 125 mL HDPE Nalgene™ bottles with varying proportions of Pearl River water and seawater to generate 14 samples covering a range of salinities, shown in Table 3.1. These mixtures were incubated overnight in the dark with gentle shaking at room temperature before filtration. Two size fractions were collected, a < 0.45 μm fraction and a < 0.02 μm fraction. The 0.02 μm fraction was first filtered through the 0.45 μm filter then again through the 0.02 μm filter (25 mm diameter, polypropylene, Whatman Anotop) to prevent clogging of the smaller pore size. These size fractions are the operationally defined colloidal and dissolved size boundaries, as done in previous work in the area (Joung and Shiller, 2016).

***Table** Error! No text of specified style in document.**.1:** Mixing ratios for samples with resulting salinities. A larger proportion of samples were generated in low salinities because the greatest influence of flocculation is observed in low salinities (Mosley and Liss, 2020). Final volumes were approximately 125 mL.*

| *Sample* | Freshwater (g) | Saltwater (g) | Salinity (g/kg) |
|---|---|---|---|
| *1* | 125.96 | 0.00 | 0.0 |
| *2* | 122.99 | 2.05 | 0.6 |
| *3* | 120.93 | 4.09 | 1.1 |
| *4* | 118.99 | 6.09 | 1.6 |
| *5* | 116.97 | 8.13 | 2.1 |
| *6* | 112.98 | 12.25 | 3.1 |
| *7* | 109.16 | 16.24 | 4.1 |
| *8* | 104.82 | 20.39 | 5.1 |
| *9* | 84.96 | 40.67 | 10.1 |
| *10* | 64.83 | 60.86 | 15.1 |
| *11* | 45.00 | 80.26 | 20.0 |
| *12* | 24.89 | 100.50 | 25.0 |
| *13* | 4.96 | 120.37 | 30.0 |
| *14* | 0.00 | 125.16 | 31.2 |

## 3.4 Sample Analysis

### *3.4.1 Copper Concentrations*

Upon arrival at the laboratory, filtered copper samples were acidified to a pH of 2 using 6 M ultra-pure HCl (Optima grade, ThermoFisher) and stored until analysis (Shiller, 2003). All preparations were performed under a laminar flow bench in a workspace free of metals to prevent contamination. A seaFAST SC-4DXS pico (ESI) was used to concentrate samples (4-fold concentration) and remove the seawater matrix. The dCu concentration was then determined via high resolution-inductively coupled plasma-mass spectrometry (HR-ICP-MS, Element XR, ThermoFisher), using isotope dilution to calculate element concentrations from isotope ratios (Shim et al., 2012). The limit of quantification for $^{63}$Cu and $^{65}$Cu in medium resolution using this method were 0.09 nM and 0.17 nM, respectively. To perform isotope dilution, an uncommon isotope ($^{65}$Cu) is added to a sample as an enriched spike, and the ratio of this isotope to the most common isotope ($^{63}$Cu) is measured (Shim et al., 2012). This new isotope ratio is compared to the natural isotope ratio to calculate the total concentration of the element in the sample through Equation 3.1, where V is the volume of each solution added, X is the mole fraction of each isotope in the enriched spike and the natural sample, and R is the new ratio of copper isotopes in the resulting sample (Shim et al., 2012).

$$\text{Equation 3.1: } [\mathrm{Cu}]_{\mathrm{sample}} = [\mathrm{Cu}]_{\mathrm{spike}}\left(\frac{V_{sample}}{V_{spike}}\right)\left(\frac{\mathrm{X}_{spike}^{63Cu} - R\mathrm{X}_{spike}^{65Cu}}{R\mathrm{X}_{sample}^{65Cu} - \mathrm{X}_{sample}^{63Cu}}\right)$$

### *3.4.2 Stable Isotope Ratios*

Water samples were analyzed via stable isotope laser spectroscopy (L2120-i cavity ring-down spectrometer, Picarro) as a part of ongoing work (Magbitang, unpublished). The data generated from this analysis was calibrated to VSMOW using laboratory reference water and corrected for both memory and drift effects (Magbitang, unpublished; Sanial et al., 2019; van Geldern and Barth, 2012). Equations 3.2 and 3.3 show how both ratios are calculated, where the final value is the per mille deviation of the sample ratio from the reference ratio. Then *d-excess* was calculated from the $\delta^{18}$O and $\delta$D values (Equation 3.4) to explore evaporative effects (Magbitang, unpublished; Sanial et al., 2019; van Geldern and Barth, 2012).

Equation 3.2: $\delta^{18}O = 1000\left(\frac{({}^{18}O/{}^{16}O)_{sample} - ({}^{18}O/{}^{16}O)_{reference}}{({}^{18}O/{}^{16}O)_{reference}}\right)$

Equation 3.3: $\delta D = 1000\left(\frac{({}^{2}H/{}^{1}H)_{sample} - ({}^{2}H/{}^{1}H)_{reference}}{({}^{2}H/{}^{1}H)_{reference}}\right)$

Equation 3.4: d-excess = $\delta D - 8(\delta^{18}O)$

# CHAPTER IV: RESULTS

## 4.1 Mixing Experiment

The mixing experiment emulated the behavior of dissolved and colloidal copper (Cu) as the Pearl River mixes with seawater in the western Mississippi Sound (wMS Sound). The colloidal phase is defined as between 0.45 µm and 0.02 µm, while the dissolved phase is smaller than 0.02 µm. The Cu concentrations ([Cu], indicating a measured value for Cu) were measured in each fraction as described in Chapter 3 via HR-ICP-MS and are shown in Figure 4.1. Conservative mixing for an element is shown when its concentration is linearly proportional to salinity, indicating that the primary control of that element is the amount of river and seawater in the sample. This parameter-versus-salinity relationship does require an assumption that processes like upwelling do not contribute to the parameter, which is true of the shallow MS Sound. Since this experiment was designed to simulate estuarine mixing without the influence of additional processes, a linear correlation can be interpreted as conservative behavior.

The dissolved [Cu] ([dCu]) obtained from the mixing experiment has a linear correlation ($R^2 = 0.970$) and indicates that Pearl River dCu should mix conservatively with seawater in the wMS Sound. The colloidal fraction was minimal (< 20%) for all samples, and while colloidal Cu had a lower linear correlation ($R^2 = 0.614$), there is no obvious trend in either direction, with eight positively deviant samples and six negatively deviant samples. The colloidal [Cu] is the result of the difference between two separate measurements, increasing error from ±0.05 nM to ±0.07 nM, which could explain part of the scatter in these samples. Deviations alternated between positive and negative at irregular salinity intervals but were often greater than the propagated error. This scatter is likely further increased by the low colloidal [Cu], as the concentrations were closer to the quantification limit of 0.4 nM. Nearly all the samples fall within ±0.4 nM of the linear regression, barring the sample at a salinity of 10.13 g/kg.

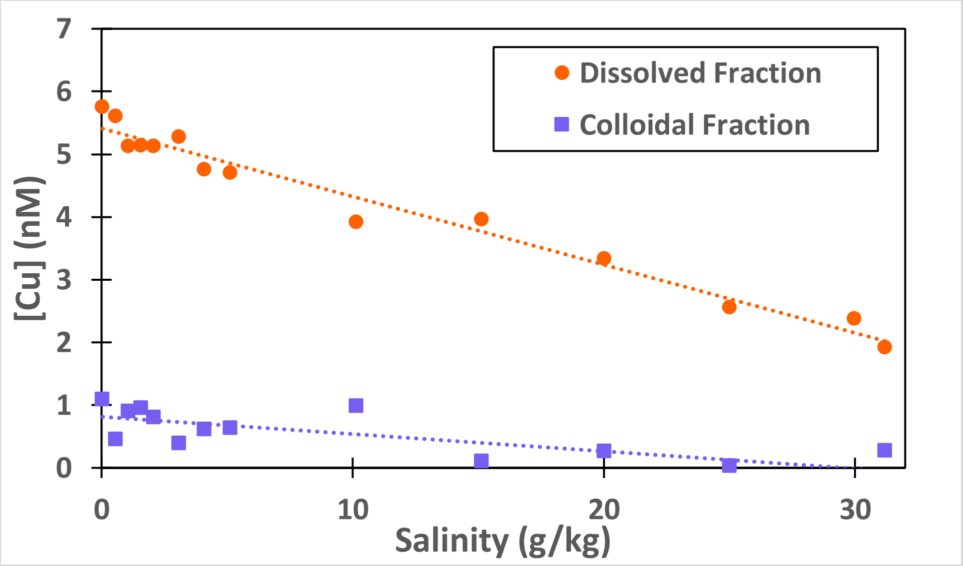

*Figure 4.1: Mixing experiment [Cu] for both the dissolved and colloidal size. The dissolved fraction consists of the < 0.02 µm size class, and the colloidal fraction consists of the 0.02 µm - 0.45 µm size class. Standard deviation on dissolved [Cu] was ± 0.05 nM, while standard deviation on colloidal [Cu] was ± 0.07 nM.*

## 4.2 Field Samples

Measured [dCu] was plotted versus salinity in Figure 4.2. Offshore [dCu] in the wMS Sound showed a linear correlation ($R^2 = 0.815$) across the limited salinity range collected. More scatter was seen in the outfall samples, and an overall trend was not evident. A linear regression was generated from the offshore samples (solid line) and projected to the fresh and saline endmembers (dashed line) to estimate endmember [dCu], assuming conservative mixing. This regression indicates that the average fresh endmember to the wMS Sound had a [dCu] of about 18 nM, while the saline endmember had a [dCu] of about 3 nM at a salinity of 32 g/kg. Some of the outfall samples followed the overall trend presented by the offshore samples, though there was less agreement in these samples.

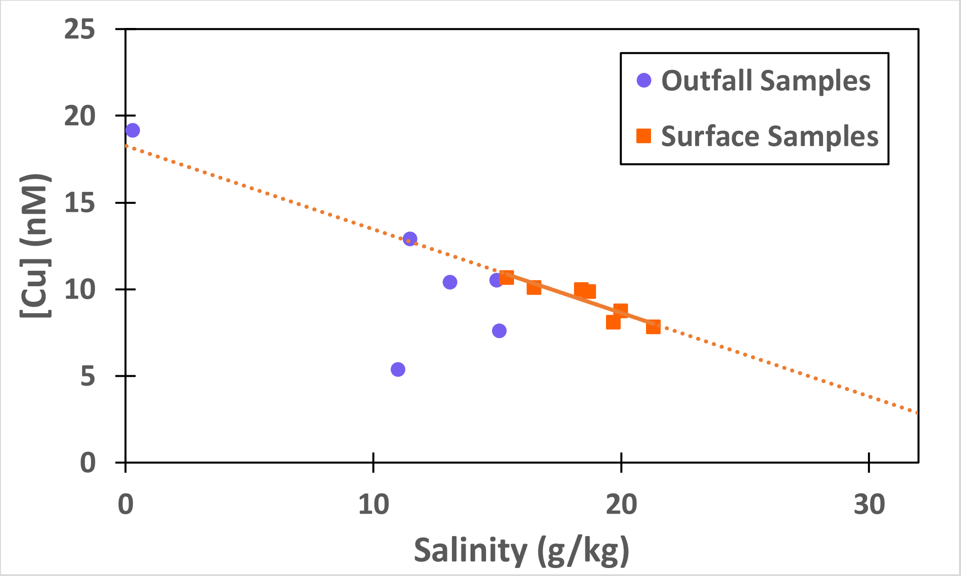

*Figure 4.2: Salinity versus [dCu] for all field samples. Samples were collected during a low river discharge period and from a presumably well mixed water column, as described in Chapter 3. All samples were filtered to 0.45 µm, and error on these measurements is ± 0.05 nM.*

Comparing the freshwater endmembers projected by both the field samples and the mixing experiment reveals a difference in [dCu] for the systems. The [dCu] of the field samples indicated a freshwater endmember of about 18 nM, while the mixing experiment showed that the Pearl River was roughly 6 nM during a low discharge period. Prior work has shown that the Pearl River does have seasonal [dCu] variations (Shiller, unpublished; Shim et al., 2017), but the field samples and Pearl River water used in the mixing experiment were both collected in the summer, during low discharge periods. The discrepancy between [dCu] for these sample sets is more likely to be explained by the collection location for the mixing experiment. This will be discussed in the next chapter, but, in short, the Pearl River water used for the mixing experiment was collected north (approx. 70 kilometers) from the river's mouth, missing a significant section of the floodplain that could increase [dCu] (Shim et al., 2017).

### 4.3 Stable Isotope Ratios

To further constrain [dCu] in the freshwater endmembers, stable isotope ratios of water ($\delta^{18}O$ and $\delta D$) were employed. As mentioned previously, water isotope samples were collected and analyzed as part of ongoing work in the group, and $\delta^{18}O$ is plotted against salinity in Figure 4.3 (Magbitang, unpublished). The fresh endmember of this sample set was characterized by projecting a linear regression from the existing sample set. The linear regression excluded outfall samples due to the uncertainty in sample composition. Because a linear regression assumes a single fresh endmember, and the wMS Sound is known to have multiple freshwater sources (Pearl River, Jourdan River, Wolf River, groundwater), exact definitions of the fresh endmembers cannot be calculated from this sample set alone. Instead, the endmember contributions can be estimated using previous $\delta^{18}O$ definitions.

In the offshore samples, the averaged fresh endmember had a $\delta^{18}O$ of about -2.2 ‰. This value is heavier (greater $\delta^{18}O$) than any of the river endmembers defined previously, indicating that none of these rivers are the sole source of water to the samples (Sanial et al., 2019). This projection does, however, agree better with the -2.7 ‰ groundwater nearshore endmember $\delta^{18}O$ definition (Moody, 2022; Gilbert, unpublished). Because all the samples fall above a mixing line from any of the currently defined endmembers to seawater, any contribution calculation would project 100% groundwater contribution, since this is the heaviest (greatest $\delta^{18}O$) endmember defined by previous work (Gilbert, unpublished; Sanial et al., 2019). This is highly unlikely to be accurate, as the rivers do still have some outflow during the sampling period and interpretations of this finding will be discussed in the next chapter.

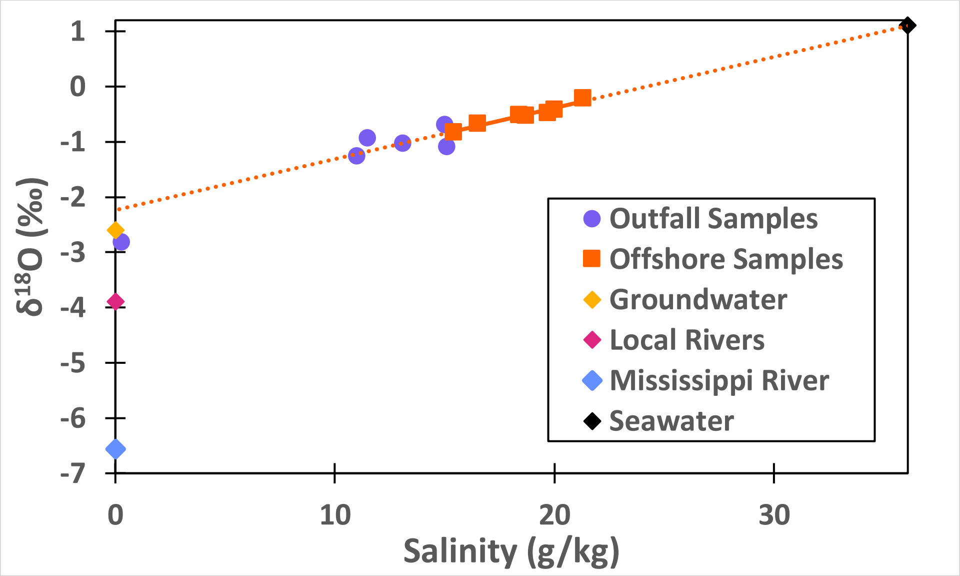

*Figure 4.3: $\delta^{18}O$ isotope ratios for field samples plotted alongside well-constrained local endmembers and Gulf of Mexico seawater (Magbitang, unpublished). A linear regression was generated from solely the offshore samples (solid line) and projected to the fresh and saline endmembers (dashed line). The linear regression of field samples projects to an endmember with an oxygen isotope ratio of -2.2 ‰, indicating groundwater as the primary freshwater contributor when using current endmember definitions (Sanial et al., 2019).*

A possible cause of heavy $\delta^{18}O$ is evaporation, as the lighter isotope ($^{16}O$) will evaporate at a higher rate than the heavier isotope ($^{18}O$), as described before. To evaluate the effects of evaporation, *d-excess* was calculated from $\delta^{18}O$ and $\delta D$ measurements. While this value is difficult to interpret independently, comparing it to a linear regression of *d*-excess versus salinity across a large, local sample set can reveal deviations from "normal" conditions that can be attributed to evaporative effects. The field sample set of this study was relatively small, so previous work was referenced for a linear regression that characterizes the MS Sound (Sanial et al., 2019). The linear regression of *d-excess* across the full salinity range is shown in Figure 4.4, as well as a range of 2σ on either side denoting a significance. Points that fall within the dashed lines were not significantly deviant from the linear regression of MS Sound samples. For evaporation to be significant, the sample's *d-excess* should fall below the range of significance, which does not occur in any case. In general, *d-excess* is slightly or significantly above the regression, which eliminates evaporation as a control over these samples. Precipitation is more difficult to discern, as the rivers of the area are largely fed by precipitation, making them isotopically similar to rainfall (Lambert and Aharon, 2010). Due to this similarity, the two sources cannot be easily distinguished using current methods and data sets, but a high *d-excess* generally indicates a precipitative contribution (Lambert and Aharon, 2010).

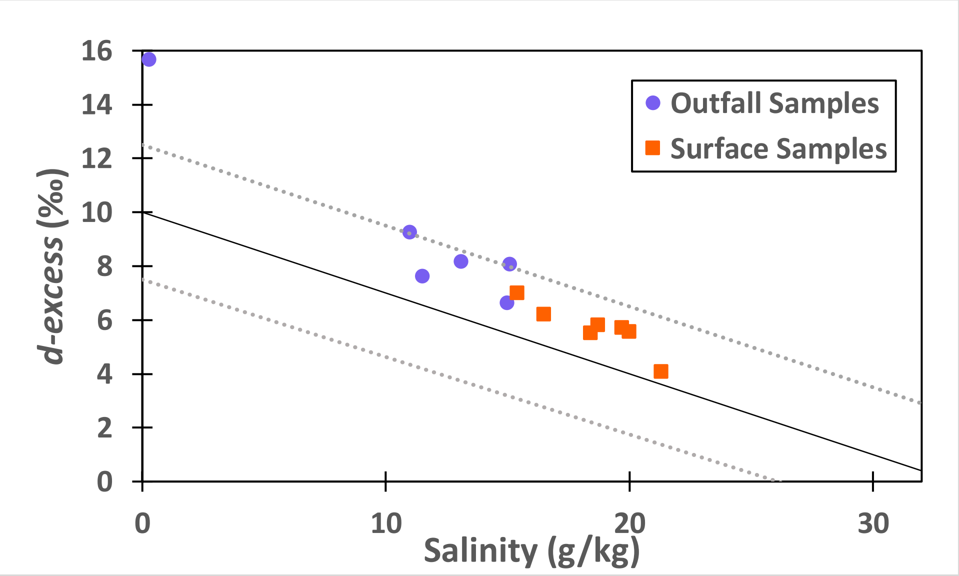

*Figure 4.3: The calculated d-excess of field samples plotted versus salinity to determine effects of evaporation. Equation 3.4 shows how d-excess was calculated, with a low d-excess indicating a high likelihood of evaporative effects. The solid trendline serves as an average of a large set of MS Sound samples, while the dashed trendlines show the range of significance (2σ) in either direction (Sanial et al., 2019).*

# CHAPTER V: DISCUSSION

Copper (Cu) distributions in estuaries can be controlled by a variety of endmembers and non-conservative processes. In the western Mississippi Sound (wMS Sound), sources of Cu can include rivers, groundwater, and contamination from road runoff or industrial wastewater (Beck et al., 2010; Crabtree et al., 2006; Gaillardet et al., 2014). The northern Gulf of Mexico is indeed an additional source of copper, but since surface Cu concentrations ([Cu]) are low and wind patterns tend to flush water from north to south in the MS Sound, the saline endmember is not a focus of this study (Bruland and Franks, 1983; Wen et al., 2011). Copper removal processes include biological uptake and flocculation, with the additional possibility of removal during groundwater recirculation (Beck et al., 2010; Cabrita et al., 2020; Kalnejais et al., 2015; Mosley and Liss, 2020). In this study, the sources and sinks of Cu to the wMS Sound are explored to investigate non-conservative behavior seen in dissolved copper concentrations ([dCu]) across low salinity ranges (Moody, 2022).

Flocculation was addressed in this study through a mixing experiment. Because the dissolved concentrations maintained a strong linear correlation after mixing (Figure 4.1, $R^2 = 0.970$), flocculation is unlikely to alter dissolved Cu (dCu) distributions during estuarine mixing. However, as mentioned previously, the Pearl River water used in the mixing experiment was collected near Bogalusa, LA, more than 70 kilometers upstream of the river’s mouth. This was done to avoid saltwater intrusion during collection, as river discharge was very low on the date of sampling (2530 $ft^3s^{-1}$, 6/27/2022, USGS). Because of the collection site used, a significant portion of the Pearl River floodplain was bypassed. Previous work has shown that interactions with the floodplain can alter trace metal concentrations, but the connectivity of the floodplain to the river varies seasonally and with discharge rates (Shim et al., 2017). In periods of low discharge, connectivity appears to be low, preventing floodplain interactions with both trace elements and organic material that would otherwise occur in higher discharge periods (Shim et al., 2017).

For Cu specifically, floodplain inputs of complexing dissolved organic carbon (DOC) are likely reduced by a low discharge rate. Consequently, the mixing experiment DOC composition is likely different from the DOC composition observed at the mouth of the river when discharge is sufficiently high. However, Pearl River discharge was low during both collection for the mixing experiment and offshore sampling, so it is possible that DOC in the wMS Sound sourced from the Pearl River is similar across both sample sets. Conservative mixing of dCu between rivers and the northern Gulf of Mexico has been observed in other systems as well (Joung and Shiller, 2016; Shiller and Boyle, 1991), so it is likely that reduced floodplain contact is not altering the observed conservative behavior. However, floodplain inputs of Cu directly are certainly limited by the low discharge rates, increasing temporal variation of dCu distributions.

Other dCu removal processes include biological uptake and groundwater recirculation. Biological uptake certainly does take place in the wMS Sound, but it is unlikely that the rate of uptake is high enough to observe a significant decrease of dCu in the estuary. Despite its role as a micronutrient, [dCu] does not often have a relationship with nutrients like phosphates and nitrates, indicating that biological activity does not significantly alter distributions (Windom et al., 1991). A study of DOC composition, as well as dCu speciation, can be done to clarify the bioavailability of dCu and can further support the assumption that dCu experiences minimal biological interference. Groundwater recirculation can change [dCu] when sediments and dCu interact, such as when sedimentary DOC complexes with Cu or when Cu-bound sediments are oxidized. Ultimately, this study does not elucidate the behavior of dCu during recirculation, but recirculated groundwater is salty and has a unique water stable isotope ratio signature, allowing for its identification if studied (Moody, 2022). Collecting bottom water would be ideal for investigating the influence of recirculation, as dCu would be lowest at the bottom if recirculating groundwater acts as a removal mechanism. Additional study of the redox potential of sediments can also help elucidate whether recirculation alters dCu as porewater interacts with the sediments (Beck et al., 2010; Kalnejais et al., 2015).

Without evidence of removal processes to alter surface [dCu], varying endmember dominance over the estuary may be a culprit of the nonlinearity observed in previous work (Moody, 2022, Figure 2.4 above). Since the deviation occurs in low salinity ranges, a fresh endmember dominance shift is most likely. Fresh or low salinity sources of dCu include rivers, groundwater, and road runoff/industrial wastewater. An industrial wastewater source of dCu is unlikely as the observed [dCu] values agreed with natural inputs, and spatial ‘hotspots’ of dCu did not occur. Without these indicators of industrial contamination, the possibility of wastewater inputs of dCu is not explored further here. Introduction of dCu from road runoff is less constrained, as this study was unable to collect adequate runoff samples. One fresh (0.3 g/kg) sample was obtained from the outfall sampling, which had a [dCu] of 19.2 nM. This [dCu] follows values seen in freshwater endmembers, so it is unclear whether runoff has been collected. Though the sampling areas and adjacent roadways were visually wet at the time of sampling, intrusion of saltwater into the outfalls likely reduced or muddled any runoff signal in the collected samples. To better characterize the influence of road runoff through storm outfalls, a time series sampling regime should be done to determine if [dCu] is increased following a rain event, particularly after a rain event preceded by a dry period. Ideally, runoff could be captured directly at the drain to prevent dilution within the outfall, or a monitoring station could be established at an outfall to watch for [dCu] spikes. Currently, the effect of road runoff on [dCu] in the wMS Sound is uncertain.

The remaining, and primary, fresh sources of [dCu] to the wMS Sound are rivers and groundwater. As established previously, the rivers that can potentially influence the wMS Sound are the Mississippi, Pearl, Jourdan, and Wolf Rivers. Previous work has shown that the Mississippi River only influences the wMS Sound during Bonnet Carré Spillway (BCS) openings (Sanial et al., 2019). Since BCS was closed during this study's sampling period, the Mississippi River is not a heavy contributor of water to the wMS Sound for this sample set. This is supported by the $\delta^{18}O$ measurements, as the Mississippi River trends very light (-6.64 ‰ $\delta^{18}O$, (Sanial et al., 2019)) compared to the range of samples collected (-0.22 ‰ to -2.82 ‰ $\delta^{18}O$). The offshore samples' $\delta^{18}O$ could be made heavier by evaporation, thus falsely indicating a heavy endmember, but calculation of *d-excess* indicates that evaporation was not a significant factor here. In evaporatively influenced samples, *d-excess* is low, since evaporation favors the lighter isotopes, reducing the $\delta D$ and $\delta^{18}O$ values (Dansgaard, 1964). The effect of precipitation on the wMS Sound is expected to be minimal (Dinnel and Wiseman, 1986), but no estimation of precipitation's influence is calculated here.

Because there was a difference between river and sample $\delta^{18}O$, groundwater must be explored as a significant endmember to these samples. The reported average $\delta^{18}O$ of fresh groundwater across the MS Sound is -2.66 ‰ (Moody, 2022). This value is still lighter than the fresh endmember projected by the linear regression of the offshore samples (-2.24 ‰ $\delta^{18}O$) but falls within the ±0.53 standard deviation of the endmember sample set (Moody, 2022). While this agreement in $\delta^{18}O$ could lead to the assumption that the mid-salinity surface samples are fully dominated by groundwater during low discharge periods, this is highly unlikely. The Pearl River, Jourdan River, and Wolf River still provide water volume flux to the wMS Sound even when discharge is low, contributing lighter isotope ratios (-3.95, -3.21, and -3.70 ‰ $\delta^{18}O$, respectively (Sanial et al., 2019)). As such, it is more likely that a fresh endmember heavier than -2.24 ‰ is contributing to the wMS Sound in addition to these rivers, with the relative contributions of each endmember depending on the exact $\delta^{18}O$ of the heavy source.

With the assumption that the surface samples collected in this study have the fresh endmembers of local rivers and groundwater, the nonlinear behavior of dCu should be explained by their relative contributions. River [dCu] varies seasonally with discharge rates (Shiller, unpublished; Shiller and Boyle, 1991; Shim et al., 2017), and SGD rates in the wMS Sound vary seasonally as well (Moody, 2022). Considering that the Pearl River trends to lower [dCu] during low discharge ([dCu] = 4.5 nM, discharge = 1520 $ft^3s^{-1}$, 10/02/2015 (Shiller, unpublished)) and that SGD flux is higher in the dry season (Moody, 2022), it is likely that [dCu] is more heavily influenced by groundwater when river discharge is low. This groundwater source should be isotopically heavy ($\delta^{18}O > 2.24$ ‰) and rich in dCu ([dCu] > 20 nM) to cause the behavior shown in Moody (2022).

However, high dCu groundwater has only been observed in the fresh component in SGD, which does not dominate SGD in the MS Sound, meaning that an SGD source of dCu is unlikely to be the sole explanation for this behavior. Alternatively, low [dCu] SGD in high volumes could dilute river [dCu] and cause the negative deviation seen in Moody (2022). Because SGD fluxes are more significant in the dry season, when these samples were collected, it is possible that SGD is contributing enough water volume to dilute [dCu], despite still being an order of magnitude below river fluxes (Moody, 2022). However, this does not explain the persistence of the nonlinearity into the wet season, when river dCu fluxes should compensate for SGD dilution.

Overall, dCu in the wMS Sound shows generally conservative behavior, with the nonlinearity at low salinities seen in Moody (2022) best explained by a shifting source argument, though the exact dynamics are still uncertain. As such, this study suggests SGD as a significant contributor to the dCu mass balance. The mixing experiment showed that flocculation is not a control on [dCu], which agrees with other work done in the area (Joung and Shiller, 2016; Shiller and Boyle, 1991). The [dCu] measured in this study agrees with Moody (2022), showing predictability in dCu behavior despite seasonal variations in endmember contribution. Copper concentrations fall well below EPA standards (>20,000 nM, EPA) but are high enough to support biological activity, raising no concern for the estuary’s health. Further work should seek to constrain and quantify the Cu mass balance in the wMS Sound to best inform fisheries management and better understand the intricacies of trace element behavior in the estuary.

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