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Converting Chemical Signatures in Vaterite Otoliths to Aragonite Otoliths in Steelhead Trout:
Developing a Partition Coefficient

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Honors Thesis

Abstract

Otoliths are small bones near the brain of teleost fish that aid in hearing and balance. Otoliths are typically composed of a polymorph of calcium carbonate called aragonite; however, when stressed, fish may transition to producing a less dense polymorph called vaterite. Otoliths are typically used in studies of fish natal origin or migration patterns because 1) otoliths grow concentrically around an origin (birth point), 2) the material in the otolith is not replaced over time, and 3) microelements (Sr and Ba) can be incorporated into the otoliths in proportion to elemental ratios in the water (i.e., when fish move to a different water mass the new otolith material will change elemental ratios to match the water chemistry). Thus, fishery biologists have a powerful tool to track origins or movements of fish. However, these analyses only use aragonitic otoliths rather than vaterite because of the vast differences in elemental uptake rates between the two polymorphs. A large fraction of samples are thus wasted because many fish contain otoliths with vaterite. For steelhead trout (*Oncorhynchus mykiss*) it is important to track the migration patterns of vateritic individuals because individuals returning from Lake Erie to their natal stream to spawn tend to stray away from their natal stream to streams in different states. To be able to use vateritic individuals in microchemical analyses, a partition coefficient (K_d) must be developed to convert vaterite elemental concentrations to aragonite elemental concentrations. Developing a partition coefficient that can be used without knowledge of water chemistry elemental concentrations can allow migration patterns or origin of vaterite individuals to be determined. This would allow managers to better understand the contribution of their hatchery to the sport fish population in Lake Erie and identify if potential environmental stressors are present at their hatchery that need to be addressed to increase the survival of and return rate of individuals from their hatchery. My research compared elemental signatures in regions of aragonite and vaterite to develop this conversion factor (partition coefficient). My results demonstrated that a common partition coefficient could be developed for fish.

Introduction

Otoliths are small calcium carbonate structures found in the brains of teleost fish that aid in balance and hearing (Reimer et al 2016). There are three different types of otoliths: the sagittal, asteriscus, and lapillus. Sagittal otoliths are most commonly used for otolith microchemistry analyses, such as laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), because they are often the largest of the three and are the primary auditory receptor in many species (Oxman et al 2007). Sagittal otoliths are composed of two main calcium carbonate polymorphs, vaterite and aragonite. Otoliths are most commonly composed of aragonite, but vaterite replacement occurs in approximately one-third of individuals (Melancon et al 2005). To determine orientation, each otolith has a sensory epithelium with mechanoreceptive hair cells overlain by the otolith (Oxman et al, 2007), when the otolith moves between the sensory epithelium and the hair cells, information about body position and the surrounding environment is relayed to the brain. Typically the inertia of aragonite otoliths result in differential movement between the sensory epithelium, which shears sensory hair bundles and stimulates the auditory nerve. However, because vaterite otoliths are less dense, it takes a more intense stimulus to move the otolith across the epithelial membrane and stimulate the hair cells producing a neural response, which decreases the auditory acuity of the fish. This decrease in auditory acuity could affect a fish's ability to survive by decreasing its ability to locate food and avoid predators (Oxman et al 2007).

Otoliths grow continually and form distinctive yearly rings, or annuli, each year (Melancon et al 2005). Because otoliths grow throughout a fish's life, an otolith elemental fingerprint is formed, and can be used to distinguish between fish that have experienced different overall environmental exposures (Campana et al., 2000; Elsdon et al 2008). Thus, the generally concentric growth pattern of otoliths preserves a chronological sequence of changes in water chemistry or other environmental factors that influence trace element uptake (e.g., temperature or stress) during the fish's life (Elsdon and Gillanders, 2002). Otoliths may also provide a record of the duration of time fish spend in different environments (Whitledge et al. 2007), and a time-correlated record of changes in water chemistry (pollutants/nutrients) in a specific water body. These changes in water chemistry are determined through microchemical analyses such as LA-ICP-MS. Such analyses can also be used to detect the presence of vaterite as well as regions where a switch from aragonite to vaterite has occurred. Otolith chemistry is commonly used to track migration of individuals between different water masses. For instance, steelhead trout (*Oncorhynchus mykiss*) in Lake Erie are stocked from a hatchery into a local stream and migrate into the lake where they reside for a year to a few years. Steelhead trout then migrate back to streams to spawn, usually selecting the same stream in which they were stocked. However, individuals often stray to other streams in different states as opposed to their natal streams. Therefore, otolith chemistry can be used to track the hatchery an individual was stocked from, when it entered the lake, and which stream it returned to as each water mass will have a different chemical concentration of elements, particularly Strontium and Barium. Tracking migration patterns will allow managers to understand the contribution of their hatchery to the sport fishing population in Lake Erie by determining return and survival rates of individuals from the hatchery.

While the causes of vaterite replacement are unknown, environmental stressors may play a role. Changes in temperature, density effects, and starvation from decreased resources are known to increase stress and may lead to the formation of vaterite (Oxman et al 2007). Because these stressors are more prevalent in hatcheries, with fluctuations in temperature, pressure from crowding, and increased competition for resources, hatchery raised fish are more likely to contain vaterite than naturally reproduced individuals (Melancon et al 2005).

The presence of vaterite also has an economic impact on sampling, as otoliths that contain vaterite are not used in microchemistry analyses. This is very wasteful, especially when sampling farm raised fish, as hatchery fish are 10.4 times more likely to contain vaterite than naturally produced fish (Reimer et al. 2015). Specifically, Brown et al. (2013) found that for, steelhead trout, 50% of the otoliths collected from hatcheries contained vaterite, while only 5% of wild fish contained vaterite. This means that 50% of samples from hatcheries were not able to be used in microchemistry analyses. To be able to utilize as many samples as possible, a partition coefficient could be used to convert elemental concentrations in vaterite otolith sections to an aragonite equivalent. A partition coefficient, as defined by Melancon et al (2005), is a ratio of the concentration of elements in one phase to the concentration of elements in a coexisting phase. Typically, a ratio of elemental concentrations in water relative to those in aragonitic otoliths is used to determine the origin of an individual or population of fish, however, because of the differences in elemental uptake rates in vaterite and aragonite, vateritic otoliths cannot be used in the same ratio. Thus, it would be beneficial to develop a partition coefficient, which would allow the origin of fish with vaterite otoliths to be determined not dependent upon the origin of the individual. This study focuses on the development of a partition coefficient to convert vaterite values to aragonite values and to determine whether a universal partition coefficient for steelhead trout can be developed, which would allow vaterite otoliths to be used in microchemistry analyses.

Methods

Sample Collection

Juvenile steelhead trout were collected from 4 state hatcheries around the Lake Erie region (Salmon River, NY, Castalia, OH, Fairview, PA, and Wolf Lake, MI).

Laser Ablation Inductively Coupled Plasma Mass Spectrometry

After collection of steelhead was completed, sagittal otoliths were removed and prepared for microchemical analysis. Organic material was removed from the otoliths by sonication in hydrogen peroxide (3% V:V), and were then air dried. Otoliths were embedded in a two-part epoxy (West System 105 Epoxy Resin® and 206 Slow Hardener®) and sectioned in the transverse plane using a low-speed wafer saw with a diamond-tipped blade. Using ultrapure (Milli-Q™) water with 3M™-brand silicon carbide sandpaper and lapping film (particle size: 20 µm, 10 µm, 6 µm, 2 µm) the cut otoliths were wet polished on both sides to a thickness of approximately 200 µm. Using epoxy, the polished otoliths were mounted on standard petroscopic microscope slides. Mounted slides were triple-rinsed and sonicated for five minutes with

ultrapure water, then covered, allowed to dry overnight, and stored in clean Petri dishes until analyses were performed.

At the Great Lakes Institute for Environmental Research (GLIER; University of Windsor, ON), the otoliths were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). A description of and operating conditions for the LA-ICP-MS can be found in Boehler et al. (2012). The theoretical concentration of calcium in calcium carbonate ($400,432 \mu\text{g Ca g}^{-1} \text{CaCO}_3$) was used to correct for ablation yield differences and the occurrence of mass 120 (measured as ^{120}Sn , tin isotope), a contamination indicator, was also quantified (Ludsin et al. 2006; Reichert et al. 2010). A Microsoft Excel spreadsheet macro based on algorithms by Longerich et al (1996) was used to perform data processing and calculations of detection limits (Yang 2003). Although only ^{86}Sr and ^{138}Ba (reported as total Sr and Ba) were used in the discrimination analysis, a total of 20 isotopes of 15 elements (^7Li , ^{25}Mg , ^{33}S , ^{39}K , ^{43}Ca , ^{44}Ca , ^{55}Mn , ^{57}Fe , ^{63}Cu , ^{65}Cu , ^{66}Zn , ^{67}Zn , ^{86}Sr , ^{87}Rb , ^{88}Sr , ^{120}Sn , ^{133}Cs , ^{137}Ba , ^{138}Ba , ^{208}Pb) were collected. This approach was used because Sr and Ba have previously shown to be important discriminators of natal origins in the Great Lakes (Brazner et al. 2004; Ludsin et al. 2006; Hand et al. 2008; Pangle et al. 2010; Boehler et al. 2012), and because Sr and Ba had concentrations with lower variance within hatcheries compared to other elements analyzed. Sr and Ba concentrations were always greater than the limits of detection (LOD; mean LOD concentration in ppm and standard error: Sr 1.24 ± 0.03 and Ba 0.044 ± 0.002).

Elemental concentrations of otolith regions were standardized using procedures outlined by Boehler et al. (2012). Briefly, otoliths from steelhead hatchery yearlings were standardized from core (0%) to edge (100%), and otoliths from wild stocks (Grand River and Cattaraugus Creek) were standardized from core (0%) to first annulus (100%). Average concentrations for Sr and Ba were calculated for each 5% otolith increment, but the inner 10% (50-60 μm) of all otoliths were excluded from subsequent analyses to minimize potential maternal influences (Chittaro et al. 2006; Macdonald et al. 2008; Wolff et al. 2012). The remaining 18 Sr and 18 Ba variables were entered into a linear discriminant analysis (LDA) and systematically removed with backwards stepwise variable selection, until the combination of variables yielding the lowest number of misclassified fish was obtained. To remove impacts of multicollinearity, any otolith regions that were continuous and highly correlated (pairwise correlation >0.70) were pooled (McGarigal et al. 2000).

Identification of vaterite individuals

The chemical signatures developed by LA-ICP-MS for Sr, Mg, and Mn for each individual was used to identify individuals that transitioned to forming vaterite. For aragonite individuals it was expected that the Sr signature would remain at a fairly constant and elevated level and a sudden drop in Sr would indicate the formation of vaterite (Melancon et al 2005). Sr is taken up at a different rate in vaterite compared to aragonite and can be used to differentiate between the two polymorphs. The formation of vaterite was confirmed by looking at the Mg and Mn signatures, if there was a sudden increase in Mg and Mn along with the sudden decrease in Sr, then that individual was identified as a vateritic individual (Melancon et al 2005).

Calculation of Partition Coefficient

To develop a partition coefficient (K_d) between vaterite and aragonite, average values from the outer 10% of the otolith, a region known to contain vaterite in all vateritic individuals in the study, was used for both vateritic and aragonite individuals. 20 juvenile individuals with vateritic otoliths were identified (2 from Castalia, 6 from Fairview, 8 from Salmon River, and 4 from Wolf Lake) and 20 individuals with aragonite otoliths were randomly selected from each hatchery (corresponding to the number of vaterite individuals from each hatchery). The coefficient was calculated using the following equation:

$$K_d = [\text{metal/Ca}]_{\text{aragonite}} / [\text{metal/Ca}]_{\text{vaterite}}$$

The K_d was calculated for the 90-95% and 95-100% regions of the otolith for Sr/Ca as there is a large difference in uptake rates between vaterite and aragonite for Sr. This value was calculated for individuals from each hatchery independently and the average values for each hatchery were then run through an ANOVA and Tukey's all pairs test to determine if the values were significantly different between hatcheries.

Conversion

As a check for the K_d value, the elemental concentrations of Sr/Ca from the otolith of each vaterite and aragonite individual was plotted against the water chemistry concentrations for Sr/Ca for each hatchery.

Results

| Hatchery | Hatchery Sr95-100 K_d | Hatchery Sr90-95 K_d |
|--------------|-------------------------|------------------------|
| Castalia | 6.368±0.017 | 6.658±0.0218 |
| Fairview | 9.681±0.888 | 9.989±0.546 |
| Salmon River | 7.470±0.684 | 7.845±0.454 |
| Wolf Lake | 6.864±0.893 | 6.475±0.523 |

Table 1. Average K_d values for each hatchery ± the standard error for the two outer 5% regions for Sr.

Average K_d values for Sr varied from 6.4-9.6 which is a fairly narrow range, suggesting that these values may not differ significantly. Fairview had the highest value of 9.68 and 9.98 for the 95-100 and 90-95% regions respectively, which is the highest value of all of the hatcheries. The other 3 hatcheries (Castalia, Salmon River, and Wolf Lake) only vary from each other by approximately 0.5-1.1 as opposed to Fairview, which differs from the other hatcheries by 2.2-3.3, twice the difference between the other 3 hatcheries. The larger difference between Fairview and the other hatcheries may result in it being significantly different from the other hatcheries, which is the case for the 90-95% region as shown in Figure 1, but not the 95-100% region (Figure 2).



Figure 1. Average K_d value for each hatchery of the 90-95% region for Sr \pm the standard error. Letters indicate results from the means ANOVA and Tukey's all pairs tests. Different letters indicate significant differences among hatcheries, $p=0.0015$

The ANOVA and Tukey's all pairs tests for the 90-95% region in Figure 1 show that the K_d value for Fairview is significantly different from the other hatcheries as the p value is less than 0.05. It also shows that aside from Fairview, there is not much variation among the K_d values for the 90-95% region with values ranging from 6.6 to 7.8. Fairview had the largest value and the difference between Fairview and the other hatcheries was the greatest at 2.1-3.5, while the other hatcheries differed from each other by about 0.4-1.4.



Figure 2. Average K_d value of the 95-100% region for Sr for each hatchery \pm the standard error. Letters indicate the results of means ANOVA and Tukey's all pairs tests. Different letters would indicate significant differences between the hatcheries, which is not the case here, $p=0.0854$.

The results from the ANOVA and Tukey's all pairs tests shown in Figure 2 show that the K_d values for each hatchery for the 95-100% region are not significantly different. This also shows that there is little variability among the K_d values for this region with values ranging from 6.3 to 9.6 when including Fairview. These values are similar to the values from the 90-95% region shown in Figure 1, with the K_d for Fairview 0.3 lower in the 95-100% region. This lowered value may be why the K_d for Fairview is not significantly different from the other hatcheries in the 95-100% region compared to the 90-95% region.

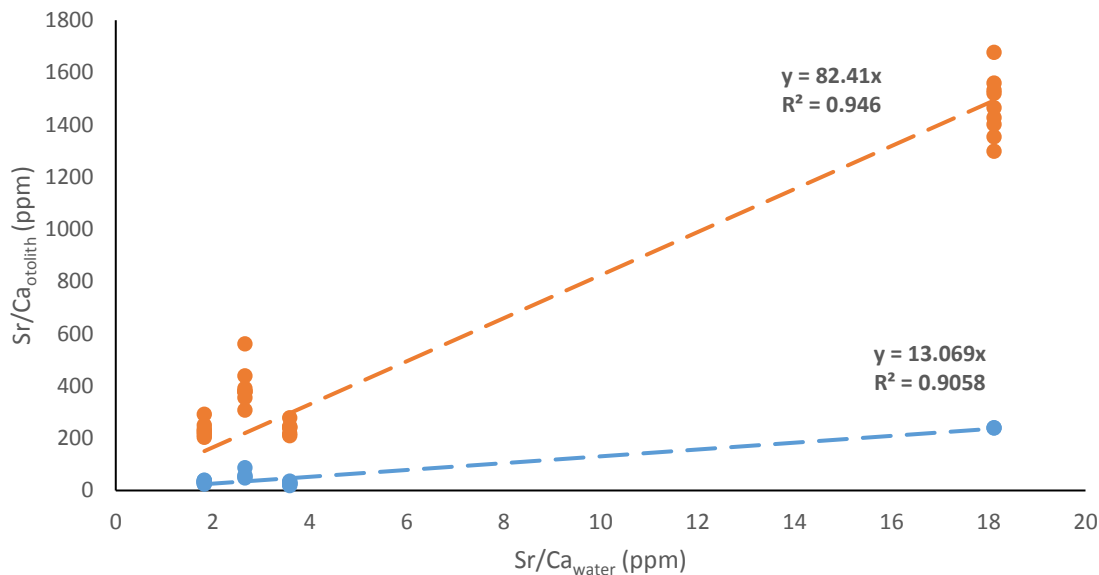


Figure 3. Comparison of water chemistry to otolith chemistry for both vaterite (blue) and aragonite (orange).

Figure 3 shows a linear relationship between otolith chemistry and water chemistry for both aragonite and vaterite. The r^2 value for aragonite was 0.946 and the r^2 for vaterite was 0.9058, which shows a tight linear relationship between water and otolith chemistry for both polymorphs. The equations for the lines are $y=82.41x$ and $y=13.069x$ for aragonite and vaterite respectively. This shows that aragonite has a steeper slope as would be expected because of the higher uptake rate of Sr in aragonite.

Discussion

A universal partition coefficient that could be used without knowledge of water chemistry values would be the most ideal situation as this coefficient would allow for the easiest conversion between vaterite and aragonite elemental concentrations. After calculating a partition coefficient for each of the four hatcheries, the coefficients were found not to be significantly different from each other. The K_d value for Fairview was significantly different from the other hatcheries for the 90-95% region as shown in Figure 2, which may be due to variation in the

water source used by the hatchery, leading to slight alterations of the water and otolith chemistry. Despite this difference, there was little variability among the K_d values for the outer two 5% regions and all of the K_d s for the 95-100% region were not significantly different. Therefore, a single partition coefficient could be used for any steelhead trout found in Lake Erie without knowledge of the water chemistry of the hatchery from which the individual originated.

The concentration of trace elements in the water an individual resides in is proportional to the concentration of trace elements in the otolith of that individual, whether that individual contains vaterite or aragonite (Bath et al 2000). This is because the higher the concentration of elements in the water, the more trace elements available for an individual to uptake into their otoliths. Although the concentrations of elements, such as Sr, are significantly lower in vaterite, vateritic individuals in water with a high Sr concentration will still have higher Sr concentrations than vateritic individuals in water with a low Sr concentration as shown in Figure 3. Because both aragonite and vaterite have a linear relationship with the water chemistry, the ratio between the two polymorphs should be constant. Thus a vaterite elemental concentration can be multiplied by this ratio to convert the vaterite value to an aragonite value. This is significant because vaterite individuals can be used in otolith microchemistry analyses, which typically only analyze aragonite due to the vast differences in elemental uptake rates of the two polymorphs. Therefore, individuals that contain vaterite do not have to be considered wasted or insignificant samples, as they were previously, adding more data points to a study. This allows for less samples to be collected as fewer samples would be wasted, for according to Brown et al (2013) about 50% of hatchery reared steelhead trout contain vaterite. Ultimately, being able to convert vaterite values to aragonite values allows for the determination of migration patterns of steelhead trout that have switched to producing vaterite, as typically migration patterns could not be determined because of the differences between vaterite and aragonite.

Because of the linear relationship between Sr/Ca_{otolith} and $Sr/Ca_{\text{H}_2\text{O}}$, otolith elemental concentrations can be used to recreate to some extent the elemental concentrations of the ambient water an individual resided in at a given time. The trace element uptake of aragonite specifically, is very close to the trace element concentration in the water. Endolymphatic fluid in the inner ear of the fish, which contains bicarbonate, calcium, and trace metal ions, crystallizes to form aragonite (Farrell and Campana 1996). The bicarbonate, calcium, and trace metal ions in the endolymphatic fluid are derived from the ambient water the fish resides in. In particular, Strontium and Barium, among other divalent metals, are considered to replace Calcium ions in the orthorhombic crystal lattice because Sr and Ba have similar ionic radii to Ca (Bath et al 2000). According to Bath et al (2000), the relationship of trace metal concentration between ambient water ($[Me/Ca]_{\text{H}_2\text{O}}$) and aragonite ($[Me/Ca]_{\text{otolith}}$) can be described by a partition coefficient (D_{Me}) such that:

$$[Me/Ca]_{\text{Otolith}} = D_{\text{Me}} [Me/Ca]_{\text{H}_2\text{O}}$$

Because this relationship between aragonite and water trace metal concentrations can be developed, converting between vaterite and aragonite trace metal concentrations will allow for the conversion between vaterite and ambient water concentrations. Migration patterns across the otolith of an individual that forms only aragonite, only vaterite, or transitions from aragonite to

vaterite can then be tracked by converting otolith to water trace metal concentrations. This will allow for the recreation of water trace element concentration and will aid in the identification of the water body an individual resided in at a given time, including the point of vaterite formation on a transient individual. By identifying the water mass an individual resides in at the point of transition between aragonite and vaterite, the cause of vaterite formation can be narrowed, which if traced back to a particular water mass or hatchery can lead to the identification of environmental stressors. Upon further study, the cause of these stressors may be resolved to reduce the amount of individuals forming vaterite and increase survival rates.

Although transition from aragonite to vaterite can occur at any point in a fish's life, and thus in any region on an otolith, it was found that individuals consistently formed vaterite at the outer 10% (90-100% region) of the otolith. For juvenile individuals, this is the point at which they would be stocked, which suggests that as the fish grow larger, environmental stressors, such as overcrowding, are more likely to occur. For adults, this may suggest that they are experiencing some kind of stress during processes such as spawning or migration. Being able to convert vaterite elemental concentrations to aragonite and thus being able to track migrations can help determine at what point in a fish's life stress occurred and potentially locate where the vaterite formation of an individual began. From a management perspective, this is important because it can help managers understand the contribution of their hatchery to the sport fish population. If vaterite formation began when an individual was in a hatchery, then this indicates that there is some kind of stress present at the hatchery that should be investigated and corrected. Because the formation of vaterite reduces the hearing acuity in fish, their chance of survival, especially if formation begins in the juvenile stage, greatly decreases. Therefore, if the transition of aragonite to vaterite can be traced back to a hatchery, this stressor needs to be addressed to increase the rate of survival of individuals from that hatchery.

As well, because vaterite values can be converted to aragonite, specifically for steelhead trout, migration patterns can be further studied as steelhead trout tend to stray from their natal streams, returning to streams in a different state than they were stocked in to spawn. It has been suggested that because vaterite reduces hearing acuity it may also reduce the homing ability of individuals, which would prevent them from being able to return to their natal streams (Hasler and Scholz 1983). Thus, it would be economically beneficial to further study these migration patterns compared to the transition point from aragonite to vaterite to be able to determine what caused the formation of vaterite and where the formation began for states to be able to glean from the steelhead trout their money was used to stock for the sport fishing economy rather than losing individuals to a different state.

Conclusion

A single K_d value can be utilized to convert between vaterite and aragonite trace element concentrations and recreate the ambient water trace element concentrations a fish was in at a given time. Vaterite individuals do not have to be wasted samples, and can be utilized in otolith microchemistry analyses, cutting down on the amount of sampling that needs to be done and the amount of samples that are wasted. Identification of the location where vaterite formation began will enable managers to investigate and address potential environmental stressors and potentially

increase the survival rate and return rate of steelhead trout from their hatchery. The migration patterns of fish that form vaterite in their otoliths can be determined through these analyses, allowing managers to better understand the contribution of individuals from their hatchery to the sport fishing population in Lake Erie.

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