

CLASSIFYING HATCHERY STEELHEAD TROUT STOCKS USING OTOLITH  
CHEMISTRY: SPATIAL AND TEMPORAL DISTRIBUTION OF ADULT STEELHEAD  
TROUT

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## ABSTRACT

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The salmonid fishery in Lake Erie is sustained by the stocking of ~2 million steelhead trout smolts (*Oncorhynchus mykiss*; MI 60,000; NY 250,000; OH 450,000; & PA 1,100,000) annually into tributaries of the four adjoining states. To better understand the mixed stock distribution in the lake and the dynamics of returning adult steelhead to their release tributaries, large scale marking of hatchery smolts is needed. Microchemical signatures of smolt otoliths measured by laser-ablation-inductively-coupled-plasma-mass-spectrometry (LA-ICPMS) were used to identify each hatchery stock. The state-specific hatchery stocks were identified with high confidence using discriminant analysis (included Sr and Ba concentrations in three otolith regions) and jackknifed validation (OH 100.0%, MI 93.1%, NY 96.3%, & PA 93.9% correct assignment). One Lake Erie tributary, Conneaut Creek, provides a unique opportunity to determine the extent of site fidelity of adult steelhead trout because it is equally stocked by both OH and PA each spring. Returning steelhead trout (N = 174) were collected from two different Conneaut Creek sites, Conneaut OH (2 km from Lake Erie) and Albion PA (40 km from Lake Erie), in the spring (April) and fall (November) of 2009. The hatchery stocks from different states were identified using the microchemistry of their otoliths. Classification results of a discriminant analysis (DA) based on elemental concentrations (ppm) of both Sr and Ba from returning adult steelhead are as follows: 32.8% OH, 60.9% PA, 1.7% NY, 0.6% MI, and 4.0% unknown (Total N = 174). Ohio stocked steelhead were collected in both the spring and fall at the Conneaut OH site, but no OH stocked steelhead were collected at the PA site in either the

spring or fall. This demonstrates strong within stream stock partitioning between the OH and PA stocked steelhead. Percentages of vagrancy of the total classified adult steelheads collected from hatchery sources not stocked in Conneaut Creek were 2.3% (1.7% NY and 0.6% MI). Because the state-specific hatchery stocks could be accurately distinguished an opportunity is provided for fisheries biologists to gain knowledge of the mixed stock distribution and site fidelity of steelhead trout in Lake Erie.

Dedicated to my family, especially my inspiring and loving wife.

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## CHAPTER I

### **Classifying hatchery stocks of steelhead trout (*Oncorhynchus mykiss*) in Lake Erie using otolith chemistry**

#### **ABSTRACT**

The salmonid fishery in Lake Erie is sustained by the stocking of ~2 million steelhead trout smolts (*Oncorhynchus mykiss*; MI 60,000; NY 250,000; OH 450,000; & PA 1,100,000) annually into tributaries of the four adjoining states. To better understand the mixed stock distribution in the lake and the dynamics of returning adult steelhead to their release tributaries, large scale marking of hatchery smolts is needed. Microchemical signatures of smolt otoliths measured by laser-ablation-inductively-coupled-plasma-mass-spectrometry (LA-ICPMS) were used to identify each hatchery stock. The state-specific hatchery stocks were identified with high confidence using discriminant analysis (included Sr and Ba average concentrations in three otolith regions) and jackknifed validation (OH 100.0%, MI 93.1%, NY 96.3%, & PA 93.9% correct assignment). Because the state-specific hatchery stocks could be accurately distinguished, along with the benefit of being naturally mass marked, an opportunity is provided for fisheries biologists to gain knowledge of both the mixed stock distribution and release site fidelity of steelhead trout in Lake Erie. Additionally, fishery managers may use this identification technique to modify stocking strategies that will optimize hatchery operation and return rates.

## INTRODUCTION

Steelhead trout (*Oncorhynchus mykiss*) are the potamodromous (migratory) life-history variant of the rainbow trout (Daugherty et al., 2003), that were introduced into the Great Lakes during the late 1800's for sport fishing (MacCrimmon & Gots, 1972). Subsequently, naturally reproducing populations in the Great Lakes have been established in tributaries with consistent cold water conditions, such as in northern Michigan, Ontario, and some New York tributaries. Adult steelhead in the Great Lakes return to spawn after 1-4 years in the lake, and many survive to spawn again in subsequent years (Seelbach, 1993). Successful steelhead trout reproduction and recruitment is dependent on several environmental conditions, such as water velocity, minimum water depth, and temperature (Nielsen et al., 1994; McEwan, 2001). Of these requirements, temperature is arguably the most critical factor limiting successful natural reproduction in Lake Erie tributaries. Many of Lake Erie's tributaries are designated "warmwater habitat" (WWH) or "seasonal salmonid habitat" (SSH), meaning these streams are capable of supporting the passage of salmonids from October to May, but not year-round (Ohio Environmental Protection Agency, 2008). Therefore, the steelhead fishery in Lake Erie relies heavily on hatchery-stocked fish.

Annually, there are ~2 million age-1 steelhead trout smolts, 15-22 cm total length (Kelch et al., 2006; Ohio Division of Wildlife, 2008), stocked in Lake Erie tributaries, with all adjoining states contributing annually: Michigan (5%), New York (15%), Ohio (25%), and Pennsylvania (55%). Smolts are released into tributaries as much as 40 km from the river mouth to as little as several hundred meters depending on tributary size, dam location, and stocking access points. Because little natural reproduction occurs in most Lake Erie tributaries, with exceptions being

Cattaraugus Creek and Chautauqua Creek in New York and the Grand River in Ontario, this study focused on the identification of hatchery-stocked fish.

The process of raising steelhead trout smolts is well understood and mechanized to maximize growth and survival. However, the fidelity of spawners to their release tributaries and hatchery-specific distribution once released are not as well known in the Great Lakes, especially Lake Erie, as it is in the western United States. There are several ways to approach the issue of stock discrimination. Identification of fish stocks (i.e. the hatchery-specific cohorts in this study) may require extensive laboratory techniques (genetics) and complex statistical analyses (Begg & Waldman, 1999). More traditional marking techniques, such as tagging and fin clipping, can be costly and time consuming because each fish must be handled, although mechanized approaches are being tested and implemented. For example, wire-coded tags have been used to study movements of Chinook salmon in Lake Huron (Adlerstein et al., 2007).

Another way to determine the origin of stocked fish is by using otolith chemistry. Otolith chemistry is a well-established method for identifying stocks and life-histories of salmonids (Kalish, 1990; Volk et al., 2000; Barnett-Johnson et al., 2007; Donohoe et al., 2008). However, for otolith chemistry to be an effective tool in differentiating fish stocks, the otolith signatures must be unique. Otolith chemistry is primarily affected by water chemistry, although other environmental factors, like water temperature, can be influential (Elsdon et al., 2008). Steelhead trout hatcheries need water sources that remain at relatively constant and cool temperatures to raise steelhead trout optimally. Raising the trout at relatively constant temperatures reduces variation in the temperature effect (Negus, 1999), but requires that hatchery managers use various water sources during the year to maintain these thermal conditions. Most hatcheries rely

on combinations of wells and spring-fed streams whose contribution to the raceways are adjusted annually and consistently to optimize temperature for the fish. Within a hatchery, the different water sources can have varying concentrations of elements that get incorporated into otoliths, thus providing an ideal scenario for developing unique, discernable otolith microchemistry signatures for fish reared in hatcheries, especially if these hatcheries are also located in geologic regions with different water chemistry. Otolith chemistry has been proven to be a useful way to discriminate stocks originating in distinct geographical locations (Thorrold et al., 1997; Thorrold et al., 1998; Bacon et al., 2004; Barnett-Johnson et al., 2008). A major advantage of otolith chemistry is that it allows entire fish populations to be identified without artificially marking every individual, and it also allows the early life-history stages of fish to be tracked (Kennedy et al., 2002). Tracking millions of migratory fish seems impossible using conventional marking techniques (Kennedy et al., 1997), whereas with otolith chemistry every fish is naturally marked.

The reason that otoliths contain time-sensitive information about fish life history is because the material in the otolith is not resorbed as in other bone (Faure & Mensing, 2005). This is one reason why otolith biochronologies provide information with unparalleled precision and accuracy (Campana & Thorrold, 2001), especially when compared to techniques where the whole otolith is dissolved and the spatial resolution is lost. Techniques such as laser-ablation-inductively-coupled-plasma-mass-spectrometry (LA-ICPMS) used for analyzing sections of otolith allows the detection of small-scale differences in otoliths. This enables the ability to determine unique chemical tags for different life-history stages (Elsdon et al., 2008). This study utilizes differences in elemental concentrations of the otolith signatures in hatchery smolts to differentiate the steelhead trout stocks in Lake Erie.

## METHODS

### *Field sampling*

Smolts were collected by the hatchery managers/staff from every state hatchery in the United States that stocks steelhead trout into Lake Erie: Wolf Lake State Fish Hatchery (MI), Castalia State Fish Hatchery (OH), Salmon River Hatchery (NY), Linesville State Fish Hatchery (PA), Tionesta State Fish Hatchery (PA), Fairview State Fish Hatchery (PA), and from the private 3-C-U, Erie County Cooperative Trout Nursery (PA, private). Smolts from the 2008 and 2009 year classes (N = 12-19 per hatchery cohort) were obtained in spring (February-April) before stocking into Lake Erie tributaries (both years, except for NY and OH hatcheries where only one year class was collected). All fish were frozen and otoliths were removed upon return to Bowling Green State University. Water samples from each hatchery were also obtained to aid in interpretation of otolith signature profiles. Water (60 mL) was filtered through a 0.45  $\mu\text{m}$  syringe filter and transferred to an acid-washed polypropylene Nalgene bottle with 1.2 mL of trace metal-grade nitric acid. Water analyses were conducted at BGSU using a ThermoElectron iCAP 6500 ICP-OES following EPA protocol 3005A (Opfer, 2008).

### *Analytical methods*

Procedures for preparation of otoliths for chemical analysis were similar to those used by Hayden (Ph.D. dissertation, 2009). Sagitta otoliths were removed from the fish. The otoliths were placed into dilute hydrogen peroxide (3% V:V) to remove any organic tissue still attached to the otoliths, and were air dried to remove excess moisture. The otoliths were embedded in West System 105 epoxy resin and 206 slow hardener neither of which contain measurable

amounts of Ca, Sr, or Ba, elements analyzed in these otoliths. Embedded otoliths were sectioned in the transverse plane using a low-speed, diamond-tipped saw (South Bay Technology Inc., Model 650) while trying to retain the core in the section and limiting the amount of otolith that needed to be polished. Both sides of the otoliths were wet polished with 3M-brand silicon carbide sandpaper and 3M-brand lapping film (particle size: 20  $\mu\text{m}$ , 10  $\mu\text{m}$ , 6  $\mu\text{m}$ , 2  $\mu\text{m}$ ) to a thickness of  $\sim 200$   $\mu\text{m}$ . This allowed the core to be viewed using a light microscope. After polishing, the otoliths were mounted on standard petroscopic microscope slides ( $\sim 16$  otoliths per slide) using Krazy Glue (which is also free of Ca, Sr, and Ba). Mounted slides were triple-rinsed with Milli-Q (Millipore) ultrapure water and sonicated for five minutes in Milli-Q water. This rinsing and sonicating procedure was performed twice, and then the slides were covered and allowed to dry overnight. The slides were stored in clean Petri dishes until the analysis was performed.

Laser-ablation-inductively-coupled-plasma-mass-spectrometry (LA-ICPMS) was used for analyzing trace elemental concentrations in the otoliths. All of the analyses were performed at the Great Lakes Institute for Environmental Research (University of Windsor, ON). The laser source was a Quantronix Integra C femtosecond laser operating at a 100 Hz pulse rate producing 0.059 mJ/pulse at the sample, with a resulting laser crater size of 26-29  $\mu\text{m}$ . The laser was linked to a Thermo-Elemental X7 quadrupole ICPMS operating in low resolution peak-jumping mode (isotope dwell time: 10 ms, carrier gas: Ar). The laser ablation was performed in a sample chamber located on a computer controlled stage (X-Y-Z direction) of an Olympus BX-51 light microscope. The traverse rates of the laser were constant for any given traverse ( $\sim 5.0$   $\mu\text{m}/\text{sec}$ ). Observation during laser ablation was accomplished by using a Sony analog camera interfaced to a PC with a video capture card (Crowe et al., 2003).

A collection of background counts were taken for 60 seconds prior to analysis of the otolith or reference material. Analysis of a certified standard reference glass (National Institute of Standards and Technology 610) was conducted twice, both before and after each group of ~16 otoliths. The NIST 610 was used to calibrate concentrations and correct for instrument drift. The theoretical concentration of calcium in calcium carbonate ( $400,432 \mu\text{g Ca g}^{-1} \text{CaCO}_3$ ) was used as an internal standard. All data processing and calculations of detection limits were performed using a Microsoft Excel spreadsheet macro (Yang, 2003) based on algorithms developed by Longerich et al. (1996).  $^{86}\text{Sr}$  and  $^{138}\text{Ba}$  were used to discriminate fish stocks. Both  $^{86}\text{Sr}$  and  $^{138}\text{Ba}$  are robust, very abundant, and commonly replace Ca in the calcium carbonate ( $\text{CaCO}_3$ ) crystal matrix of otoliths.

### ***Data analysis***

Average concentration (ppm) values of the hatchery smolt otoliths for both Sr and Ba were calculated for three different regions within the otoliths. The three regions included in the analysis were the edge region of the otolith (outer 75-100% from the identified core or primordium), middle region of the otolith (40-60%), and inner region of the otolith (15-40%, Figure 1). These regions were selected by observing elemental patterns in the hatchery smolt otoliths and choosing regions that appeared to maximize differences. There was some uncertainty as to whether or not the core, primordium, of the otolith could be accurately analyzed in every otolith because of cutting and polishing effects; because of this, the 0-15% region of the otolith was avoided in the analysis. The other region not included in the analysis, 60-75%, was avoided because of considerable variability among fish at the Ohio fish hatchery in Castalia.

A multivariate analysis of variance (MANOVA) was used to determine if the state hatchery stocks had significantly different chemical signatures (Coghlan et al., 2007). Discriminant analysis was used to assign a likelihood probability that each fish belonged to its known state hatchery system (grouping the four hatcheries in PA, Table 1), and the classification accuracy was determined by performing a jackknife validation procedure on the state-specific smolt otoliths (Walther & Thorrold, 2008; Walther et al., 2008). All statistical analyses were conducted using JMP® 8.0.2, copyright © 2009 SAS Institute Inc.

## RESULTS

The discriminant analysis (Figure 2) included six variables in a model (overall MANOVA: Wilk's  $\lambda$   $F_{18,397} = 106.87$ ;  $P < .0001$ ). The first canonical axis explained 95.8% of the total variance, and this axis was composed primarily of the edge variables, especially Sr, which was the most important component for separating the OH smolts from fish at the other state hatcheries. Canonical axis 2 explained 4.1% of the total variance, and was primarily composed of the middle and inner variables for both Sr and Ba. Although the Ba-middle and Ba-inner regions were not large components in the overall model, they were necessary for maximizing the differences between and classification accuracy of the PA smolts and the NY hatchery smolts, with the PA fish having higher Ba concentrations. A classification accuracy assessment of the discriminant analysis was generated by jackknifing the hatchery steelhead smolt otolith data (Table 2). Jackknifing allowed each fish to be treated as if its origin is unknown, and this gives it an un-biased classification probability that was based on its distance from the state centroids. The average jackknifed classification accuracies ranged from 93.1-

100.0%, with the jackknifed-discriminant analysis misclassifying only 1.3% (2 of 149) hatchery smolts.

Sr concentrations in the edge region of the otolith for fish from Ohio (Castalia hatchery) were four times greater than in smolts from any other state (Figure 3A), which is consistent with the much higher Sr content in Castalia hatchery water (Table 1). Using Sr and Ba concentrations from multiple otolith regions allowed for changes in hatchery smolt otolith profiles to aid in stock discrimination. For example, the NY smolts had a spike in Sr concentrations in the middle region of the otolith signature (Figure 3B), which aided in distinguishing the NY hatchery fish from both the PA and MI hatchery fish. The PA hatchery smolts had consistently higher Ba than fish from all of the other states (Figure 4).

Determining the year-to-year consistency of hatchery signatures is important because it takes several years for smolts to become sexually mature and it would be useful if a signature from one cohort could be used to identify hatchery stocks from any year. To address this, I compared hatchery signatures of two cohorts (2008 & 2009) from four of the hatcheries (Wolf Lake MI, Fairview PA, Linesville PA, Tionesta PA, i.e., available data as I had only single cohorts from the other hatcheries). The otolith signatures were consistent enough between the cohorts not to cause any fish to be misclassified due to year-to-year differences in the signatures (misclassified fish were from the same cohort at the 3-C-U, Erie County Cooperative Trout Nursery).

## DISCUSSION

The discriminant analysis method employed for separating the stocked steelhead trout by state proved to be highly accurate, as it has for others (Coghlan et al., 2007; Walther et al., 2008; Walther & Thorrold, 2008). Initially, an attempt was made to classify fish by individual hatchery rather than state; however the smolts from the four PA hatcheries were so similar in otolith chemistry that our classification accuracy was poor for them (as low as 50%). One reason for the similarity in the otolith signatures from trout in PA hatcheries was that some of the PA fish spent time at more than one of the PA hatcheries, as the state optimizes use of their systems (Craig Vargason, PA Hatchery Manager for Tionesta and Fairview State fish hatcheries). In contrast, there was no overlap of data points (canonical scores) when fish were grouped by state, and 98.7% of steelhead trout smolts were correctly classified using the jackknifed approach. The model was sufficiently robust using only two elements, Sr and Ba, over three different regions of the otolith. Although we collected data for a suite of elements, including Mg and Mn, these elements were not included in the analysis. Additionally, it was not necessary to use other available statistical methods for distinguishing signatures, such as curve fitting and clustering (Wang et al., 2006; Shima & Swearer, 2009). The regions utilized in the analysis produced consistent results, meaning that using larger regions aids in reducing “noise” in the signatures caused by small changes in otolith chemistry, or analytical methods and processing effects.

The ability to accurately differentiate these state-stocked steelhead trout utilizing otolith chemistry can give fishery biologists and managers knowledge about stock distribution in Lake Erie. This could potentially provide information about adult steelhead trout when they return to the streams for spawning without taking the manpower and resources to artificially mark fish in

advance. The model generated with these hatchery fish also allows for the investigation of vagrancy, or the non-release site use by a spawning adult steelhead trout, in Lake Erie tributary steelhead trout streams. This is especially of interest for managers of stocked streams nearest to neighboring states that also stock steelhead trout because neighboring streams with different stocks may increase mixing potential. I took special interest in applying this model to Conneaut Creek, because it is stocked by both the Ohio Division of Natural Resources and the Pennsylvania Fish and Boat Commission (see companion chapter), but at different locations in this 80 km-long tributary. Studying Conneaut Creek allows me to ask important ecological questions pertaining to within-stream site fidelity and vagrancy, and also allows me to ask management questions that may have economic implications.

Chinook salmon and steelhead trout are probably the two most important non-native sport fish in the Great Lakes (Johnson, 2007), and angling for salmonids can bring great economic assets to many communities (Hubbs & Lagler, 2004). In Lake Erie for example, anglers take an average of 44 trips per year, and the annual value of steelhead trout fishing is \$12-\$15 million per year (Kelch et al., 2006). It is clear that steelhead trout fishing is becoming increasingly popular, as angler efforts tripled from 1993 to 2003 (72,413 to 200,816 trips). Locally, steelhead trout fishing activity generates \$5.7 million in new value-added economic activity in Erie County (PA), which supports 219 jobs (Murray & Shields, 2004). Since the steelhead trout fishery in Lake Erie has such an important economic impact on the local economy, it is important for steelhead trout managers to know whether or not they are seeing optimal returns from their stocking efforts.

The model employed in this study greatly increases the opportunity to understand stock distribution of Lake Erie steelhead trout and may provide managers with a tool to address

stocking practices that could lead to more efficient hatchery production and release strategies.

Given the costs and benefits associated with this fishery in Lake Erie (and elsewhere), using stock discriminating tools like otolith chemistry signatures may lead to optimizing fish production/release and understanding return dynamics of adults to the fishery.

## CHAPTER II

### **Spatial and temporal distribution of returning adult steelhead trout in Conneaut Creek, a Lake Erie tributary**

#### **ABSTRACT**

The Lake Erie steelhead fishery is maintained by hatchery stocking by four states (MI, NY, OH, & PA). Conneaut Creek is equally stocked by both OH and PA each spring and provides a unique opportunity to determine the extent of site fidelity of adult steelhead trout in Lake Erie. Returning steelhead trout (N = 174) were collected from two different Conneaut Creek sites, Conneaut OH (2 km from Lake Erie) and Albion PA (40 km from Lake Erie), in the spring (April) and fall (November) of 2009. The hatchery stocks from different states were identified using the microchemistry of their otoliths. Classification results of a discriminant analysis (DA) based on elemental concentrations (ppm) of both Sr and Ba for multiple regions of the otoliths from returning adult steelhead are as follows: 32.8% OH, 60.9% PA, 1.7% NY, 0.6% MI, and 4.0% unknown (Total N = 174). Ohio stocked steelhead were collected in both the spring and fall at the Conneaut OH site, but no OH stocked steelhead were collected at the PA site in either the spring or fall. This demonstrates strong within stream stock partitioning between the OH and PA stocked steelhead. Percentages of vagrancy of the total classified adult steelheads collected from hatchery sources not stocked in Conneaut Creek were 2.3% (1.7% NY and 0.6% MI).

## INTRODUCTION

Steelhead trout fisheries can play an important role in a region's economy due primarily to sports fishing (Hubbs & Lagler, 2004). For example, in Lake Erie, the annual value of steelhead fishing is \$12-\$15 million per year (Kelch et al., 2006). For this reason, it is important for the steelhead fishery biologists and managers in Lake Erie to better understand the site fidelity of returning adult steelhead.

Salmonids have been extensively studied in the Pacific Northwest (Welch et al., 2000; McEwan, 2001; Stewart et al., 2003; Keefer et al., 2006), and to some extent in the Great Lakes (Seelbach, 1993; Workman et al., 2002; Daugherty et al., 2003), but much less is known about their behavior in Lake Erie. There are many different stocking patterns in Lake Erie tributaries in terms of both tributary size and stocking location within the stream relative to distance from the lake. Stocking location can depend on many factors, such as dam location and stocking access points. It is not well understood in this system whether Lake Erie steelhead tributaries, especially smaller ones stocked near to the lake, have high vagrancy rates of the returning adults, thus reducing the economic return for the region.

The Lake Erie steelhead trout fishery offers an ideal situation to investigate stock discrimination. Stock discrimination is the classifying of fish specimens (individually or as groups) to identify stocks, and stock composition analysis is the estimation of the relative contributions of individual stocks to a mixed stock (Waldman, 1999). There are four states (MI 60,000; NY 250,000; OH 450,000; & PA 1,100,000) that combined stock ~2 million steelhead trout annually. The stocked steelhead trout in Lake Erie have ample opportunity to mix, because steelhead are known to migrate an average of 12 km/d (Haynes et al., 1986). It has been

demonstrated that the stocked steelhead trout from MI, NY, OH, and PA can be accurately differentiated based on the unique elemental signatures in the otoliths of the steelhead trout smolts at these hatcheries (see companion chapter). Because of its reliability, otolith chemistry has become a well-established method for identifying stocks and life-histories of salmonids (Kalish, 1990; Volk et al., 2000; Barnett-Johnson et al., 2007; Donohoe et al., 2008).

There is one Lake Erie tributary, Conneaut Creek, that is of particular interest economically because it is the only stream in Lake Erie that gets stocked by and flows through multiple states (OH & PA). It is also of interest ecologically because it is stocked at significantly different distances from the lake (2 km from the lake in OH and 40 km from the lake in PA). Conneaut Creek flows from Pennsylvania into Ohio, where it empties into Lake Erie (Figure 5). Both Ohio and Pennsylvania stock steelhead into Conneaut Creek. The steelhead strain stocked by Pennsylvania spawn in both the fall and spring. This is because Pennsylvania selects mature adults for brood stock in both early fall and late spring in an effort to extend the fishing season. Ohio stocks the Little Manistee strain of steelhead into its Lake Erie tributaries. The period of peak abundance for the Little Manistee strain of steelhead is in the late spring, but some individuals of this strain spawn in the fall. The annual Ohio-to-Pennsylvania stocking ratio for Conneaut Creek is approximately 1:1 (~75,000 by each state every spring). Investigating the numbers of returning adults from both the Ohio and Pennsylvania stocked steelheads in Conneaut Creek allows for a unique opportunity to study the stream partitioning/site fidelity and the vagrancy of returning adult steelhead. It is not known whether Ohio stocked steelhead travel upstream to utilize spawning habitats in Pennsylvania waters. The Ohio stocked smolts are released near the stream mouth (~2 km), and likely spend less time in the river system before entering Lake Erie than the Pennsylvania stocked smolts. The Pennsylvania stock must travel

greater distances from where they are released (~40 km) to reach Lake Erie, which likely increases the time they spend in the river system, although it may also increase mortality. This study employs the use of a discriminant analysis, based on highly distinguishable elemental signatures of hatchery steelhead smolts, to investigate the spatial and temporal distribution of returning adult steelhead from Conneaut Creek.

## METHODS

### *Field sampling*

Adult steelheads were collected using electro-shocking (Haynes et al., 1986; Thompson & Ferreri, 2002) in conjunction with personnel from the Ohio Department of Natural Resources and the Pennsylvania Fish and Boat Commission. Two locations (Figure 5), Albion PA (41° 53' 41.028" N, 80° 22' 13.314" W) and Conneaut OH (41° 57' 11.1414" N, 80° 32' 33.5538" W), were sampled during both the spring (March) and fall (November) runs of 2009. The length (Table 3) and sex (Table 4) of the adult steelhead samples were determined and recorded. All fish were frozen and otoliths were removed upon return to Bowling Green State University.

### *Analytical methods*

Procedures for preparation of otoliths for microchemical analysis are similar to those employed by Hayden (2009) as outlined above in chapter 1. Briefly, the sagitta otoliths were removed from the fish and placed into dilute hydrogen peroxide (3% V:V) to remove any organic tissue still attached to the otoliths. The otoliths were dried and embedded with epoxy before sectioning in the transverse plane. Both sides of the otoliths were wet polished with 3M

silicon carbide sandpaper and 3M lapping film (particle size: 20 $\mu$ m, 10 $\mu$ m, 6 $\mu$ m, 2 $\mu$ m) to a thickness of ~200 $\mu$ m, and then were mounted on standard petroscopic microscope slides. The otolith slides were triple-rinsed and then sonicated for five minutes in ultra-pure water.

Laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) was used for measuring the trace elemental concentrations in the otoliths. All of the analyses were performed at the Great Lakes Institute for Environmental Research (University of Windsor, ON). Laser traverses were run across the otoliths, normally from the rim through the core region. The data processing and calculations of detection limits were performed using a Microsoft Excel spreadsheet macro (Yang, 2003) based on algorithms developed by Longerich et al. (1996).

### *Data analysis*

After the otoliths were analyzed for elemental signatures using LA-ICPMS, the average concentrations (ppm) of Sr and Ba were calculated for three different regions within the hatchery portion (i.e. the region of the otolith signature representing the time period in the life-history of the fish where they were in the hatcheries) of the adult steelhead trout otoliths. Within the hatchery portion of the adult otoliths, three regions were included in the analysis: the edge region (outer 75-100%), middle region (40-60%), and inner region (15-40%) of the hatchery portion of the otolith. These regions were selected based on elemental patterns observed in the hatchery smolt otoliths. There is some uncertainty in whether or not the very core, or primordium, of the otolith can be accurately analyzed in every otolith because of cutting and polishing effects; thus the 0-15% region of the otolith was avoided in the analysis due to higher variance. The other region avoided in the analysis, 60-75%, also had high variance in some of the Castalia OH hatchery stock. This region on the otolith represents a time period when the fish arrived at the

Castalia hatchery from a different location, Wolf Lake MI, thus creating a large shift in the otolith chemical signature due to significant differences in water chemistries between these two hatcheries. Only the hatchery-specific otolith regions were analyzed on the adult steelhead otoliths, meaning any otolith material that grew after the steelhead left the hatchery was not used in the analysis.

A discriminant analysis based on otolith chemistry was used to classify the MI, NY, OH, and PA hatchery steelhead smolts (see companion chapter). A multivariate analysis of variance (MANOVA) was used to determine if the different hatchery stocks were significantly different (Coghlan et al., 2007). The classification accuracy was determined by performing a jackknife validation procedure (see companion chapter) on the hatchery smolt otoliths (Walther & Thorrold, 2008; Walther et al., 2008). A Tukey-Kramer HSD test was used to compare the means and test for significance of both the sex and length data among the collection sites. All statistical analyses were conducted using JMP® 8.0.2, copyright © 2009 SAS Institute Inc.

## **RESULTS**

Results of the classification of the returning adults (Figure 6) were generated using the discriminant analysis (overall MANOVA: Wilk's  $\lambda$   $F_{18,397} = 106.87$ ;  $P < .0001$ ). Overall, classification percentages of the returning adult steelhead from the discriminant model are as follows: 32.8% OH, 60.9% PA, 1.7% NY, 0.6% MI, and 4.0% unknown. Zero Ohio stocked steelhead trout were collected in either spring or fall at the Albion, PA site, but PA stocked steelhead were collected in the Conneaut, OH site. The spring Conneaut, OH adult steelhead collection had a similar ratio of OH to PA stocked adults as the original stocking ratio of

1OH:1PA. The fall Conneaut, OH sample collection was composed of a significantly higher (4:1) proportion of OH stocked steelhead than the original 1:1 OH:PA stocking ratio.

The fish collected in the spring Conneaut, OH sampling were significantly smaller on average (Tukey-Kramer HSD,  $P < .0001$ ) than both of the spring and fall collections at the Albion, PA site (Table 3). There were no significant differences among the sex ratios of the sexually mature adult fish from each of the sampled steelhead populations (Table 4). In the spring Conneaut, OH collection 25 smaller, sexually immature steelhead trout were collected, and this is the only collection where immature steelhead were collected.

## DISCUSSION

The most important factor leading to the evolution of homing is selection for individuals returning to appropriate breeding habitats (Neville et al., 2006). The results of this study demonstrate that the steelhead stocked in Conneaut Creek have strong within-stream site fidelity. The OH stocked fish are utilizing the lower portion of the watershed in both the spring and fall, and the PA stocked fish are in both the lower part of the watershed and in the upstream section. While no OH stocked fish were collected at the Albion, PA site (40 km upstream), it cannot be determined if and how far the OH stocked fish travel upstream of the Conneaut, OH site (i.e., do they utilize only the lower 5 km or 25 km of the stream).

There has been concern that Pennsylvania steelhead smolts have higher mortality due to the distance that they must travel to reach Lake Erie (~40 km). However, the results of this study suggest that if Pennsylvania stocked closer to the lake, such as where Ohio stocks their smolts (~2 km from Lake Erie), the Pennsylvania stocked steelhead may not travel into the

Pennsylvania portion of Conneaut Creek. It is not known whether the increased time that the PA smolts spend in the Conneaut Creek system due to the distance they must travel to reach Lake Erie reduces the likelihood that they will stray. When stocked greater distances from the lake, the smolts are likely to stay in the river system longer, rather than entering the lake soon after stocking, which may decrease the occurrence of straying.

This study provides the stocking source (by state) of the steelheads but not the specific tributary in which they were stocked. As such, this study can address the issue of vagrancy, but not philopatry, where vagrancy refers to individuals who appear to be outside of their normal range, specifically individuals who were found in Conneaut Creek but are not OH or PA stocked fish. Pacific salmon generally exhibit low levels of straying to non-natal spawning grounds (Bartron & Scribner, 2004). Of salmon with known origin, 2.5% were considered permanent strays (Keefer et al., 2008). The results of this study are consistent with the findings of Keefer et al. (2008) with overall percentage of vagrancy of 2.3% (1.7% NY and 0.6% MI) of the total classified adult steelhead identified as coming from hatchery sources that are not stocked in Conneaut Creek. However, the 2.3 % vagrancy value must be viewed as a minimum value as it does not include straying of fish identified as being from the OH or PA stocks that were stocked into tributaries other than Conneaut Creek.

Of the 4.0% of adult steelhead that could not be classified as belonging to one of the hatcheries, all of them have zero probability that they are Ohio stocked fish. The unknown origin steelhead showed equal probability of belonging to either the PA or NY stocks (combination of PA, NY, and MI in some cases). It is also possible the unclassified steelhead are not from any of the state hatchery stocks as some limited natural reproduction occurs in Lake

Erie tributaries (e.g., Cattaraugus Creek and Chautauqua Creek in New York). However, the main focus of this study was to identify hatchery-stocked fish, and the large numbers of stocked steelhead (> 2 million per year) likely masks any natural reproduction, especially in tributaries not in New York. Hence, only hatchery-specific otolith chemical signatures were used to discriminate adult steelhead in Conneaut Creek.

The size data of the steelhead collected from Conneaut Creek in spring 2009 do not fully agree with that of other steelhead studies in the same region of Lake Erie. Previous studies by Kayle (1996) and Thompson & Ferreri (2002) have reported steelhead runs in Conneaut Creek, Ohio were dominated by larger individuals (560-680mm) than in Trout Run and Godfrey Run, Pennsylvania (400-550mm). The fish collected for this study in spring 2009 at the Conneaut, Ohio site have a significantly smaller average length than fish sampled at the Albion, PA site in both spring and fall 2009. However, there were no significant differences in average length of the fish identified as OH stocked compared to PA stocked within the cohort of smaller individuals from the spring Conneaut, OH population. Hence, although many smaller, sexually immature fish were collected in the spring Conneaut, OH sampling, they were both OH (N = 11) and PA (N = 14) stocked.

In conclusion, the steelhead of Conneaut Creek exhibit vagrancy rates similar to other salmonid populations, and show strong within-stream site fidelity. This makes it important for the Pennsylvania steelhead managers to continue to stock the upper portion of the Conneaut Creek watershed to insure sufficient steelhead traveling into Pennsylvania waters. In addition, by employing discriminant analysis of the microchemistry of the hatchery portion of their otoliths, adult steelheads collected in Conneaut Creek were uniquely classified to their hatchery source.

The results show that a significant proportion (4:1) of the adult steelheads collected at Conneaut, OH in fall 2009 were OH stocked (Little Manistee strain) compared to PA stocked (PA strain) indicating there is a strong fall run for OH as well as PA stocked steelhead.

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**TABLES**

## Chapter I

Table 1. Water chemistry for state hatcheries that stock steelhead trout into Lake Erie.

Hatchery (Date)	Element	
	Sr (ppb)	Ba (ppb)
<b>Castalia, OH (6/7/07)</b>	7100	90
<b>Linesville, PA (4/30/07)</b>	140	405
<b>Fairview, PA (7/11/2010)</b>	130	115
<b>Tionesta, PA (6/18/07)</b>		
Tubbs Run	44	199
Raceway	69	266
Hatchery influent	68	209
Hatch house	75	282
<b>Salmon River, NY (6/12/07)</b>		
Well source	33	145
River source	31	149
<b>Wolf Lake, MI (6/26/07)</b>		
Well #4	155	172
Well #5	149	262
Well #6	116	257
Well #7	148	252
Spring source	44	470

Table 2. Jackknifed classification accuracy for the discriminant analysis of the steelhead trout hatchery smolt otoliths, N = 149.

DA(Jackknifed)	Hatchery Origin			
	OH	NY	PA	MI
<b>Classification Accuracy</b> (average p-actual)	100.0%	96.3%	93.9%	93.1%
<b>Number Misclassified</b>	0	0	2	0
<b>Total N</b>	16	16	93	24

**TABLES**

## Chapter II

Table 3. Results comparison of mean lengths with Tukey-Kramer HSD,  $P < .0001$ . Like letters (group) are not significantly different.

<b>Location</b>	<b>Season</b>	<b>Group</b>	<b>N</b>	<b>Mean Length(mm)</b>	<b>Std Error</b>
Albion, PA	Fall	A	42	640	11.1
Conneaut, OH	Fall	A	42	632	11.1
Albion, PA	Spring	A	41	610	11.2
Conneaut, OH	Spring	B	49	533	10.3

Table 4. Sex data for each sample location.

<b>Location</b>	<b>Season</b>	<b>Male</b>	<b>Female</b>	<b>N</b>	<b>p-value</b>
Albion, PA	Fall	22	20	42	0.758
Conneaut, OH	Fall	16	26	42	0.121
Albion, PA	Spring	26	15	41	0.084
Conneaut, OH	Spring	10	14	24	0.413

**FIGURES**

Chapter I

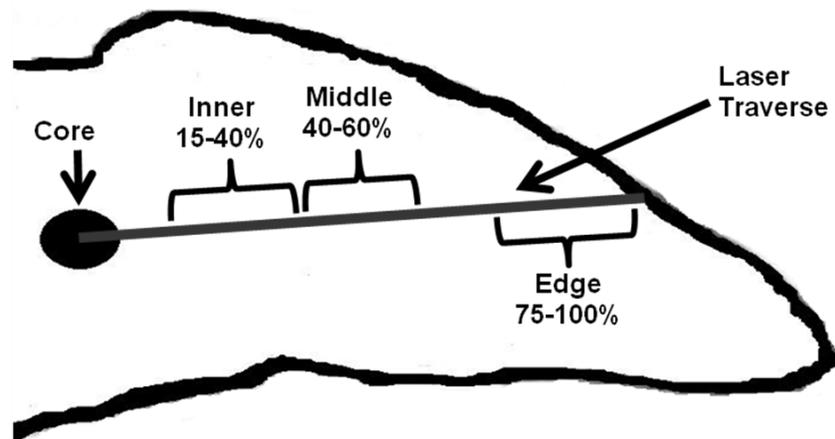


Figure 1. Diagram of otolith regions used for analysis. The three regions included in the analysis were the edge region of the otolith (outer 75-100%), middle region of the otolith (40-60%), and inner region of the otolith (15-40%). See methods sections for details concerning used/unused regions.

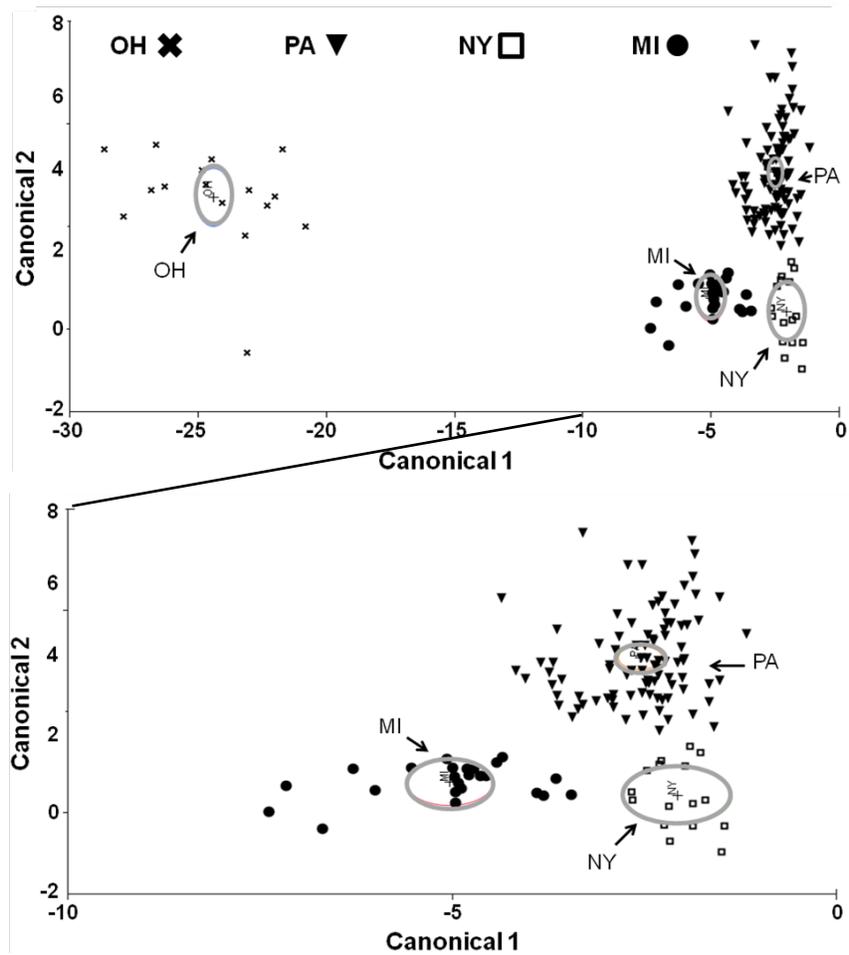


Figure 2. Results of the discriminant analysis for distinguishing hatchery steelhead smolts (overall MANOVA: Wilk's  $\lambda$   $F_{18,397} = 106.87$ ;  $P < .0001$ ). Top figure includes OH stock, and the bottom figure is expanded to better show the distribution of the other stocks. Centroids represent 95% confidence ellipses. See results section for description of Canonical Axes.

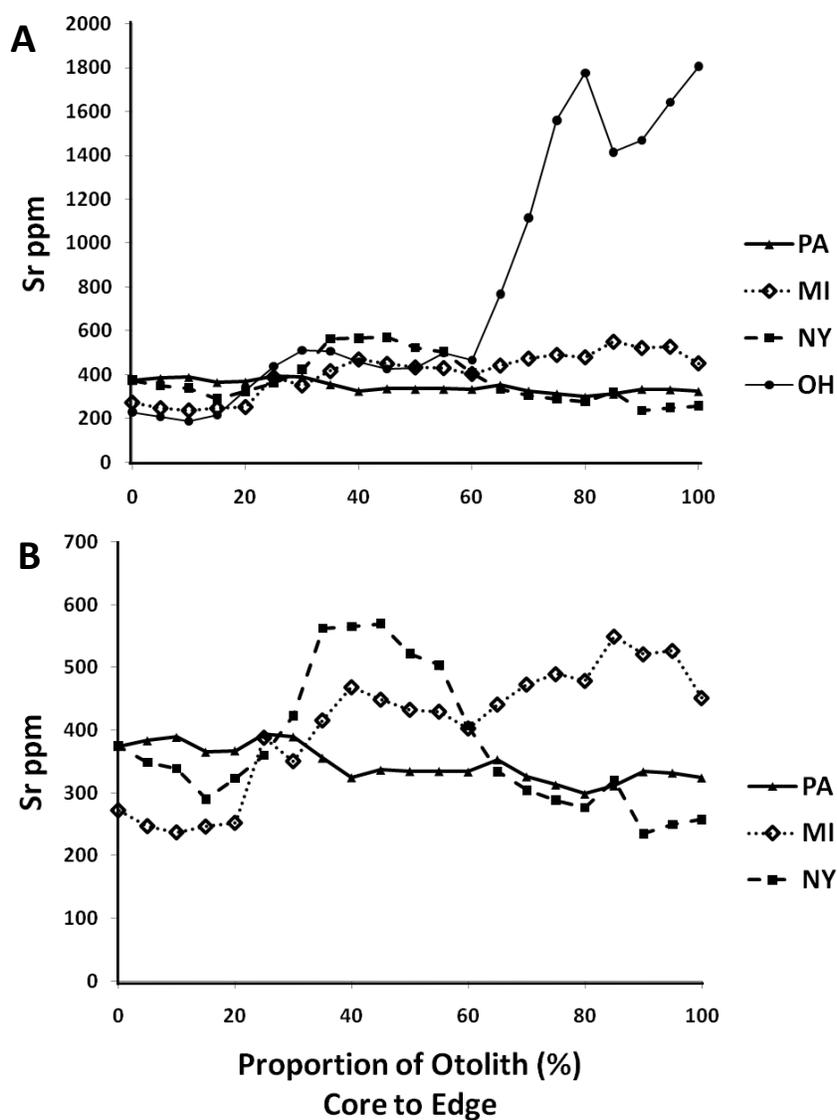


Figure 3. A) Sr otolith signatures of four hatchery stocks of steelhead smolts. Multiple otoliths ( $N = 5$ ) from each hatchery cohort were included in generating signature profiles. B) Sr otolith signatures of hatchery stocks excluding the signature of OH stock. Same data used as in part A.

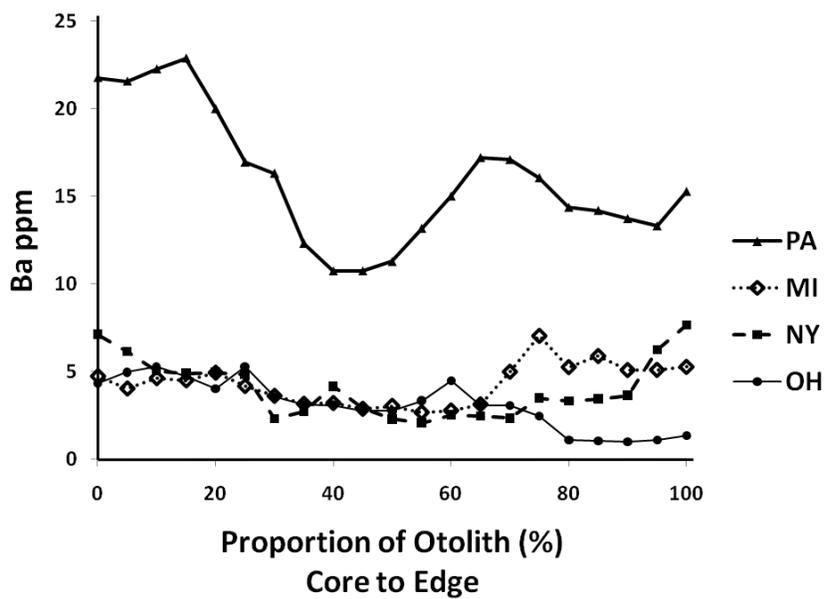


Figure 4. Generalized otolith signatures for Ba of four hatchery stocks of steelhead smolts. Multiple otoliths (N = 5) from each hatchery cohort were included in generating signature profiles.

**FIGURES**

## Chapter II

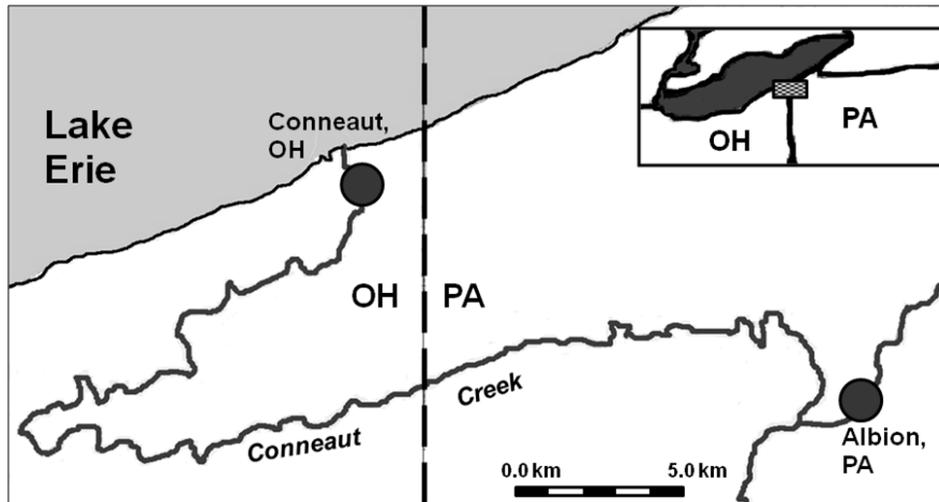


Figure 5. Map of adult steelhead trout sample collection locations, scale 1 : 110,000. Each location was sampled in both the spring and fall.

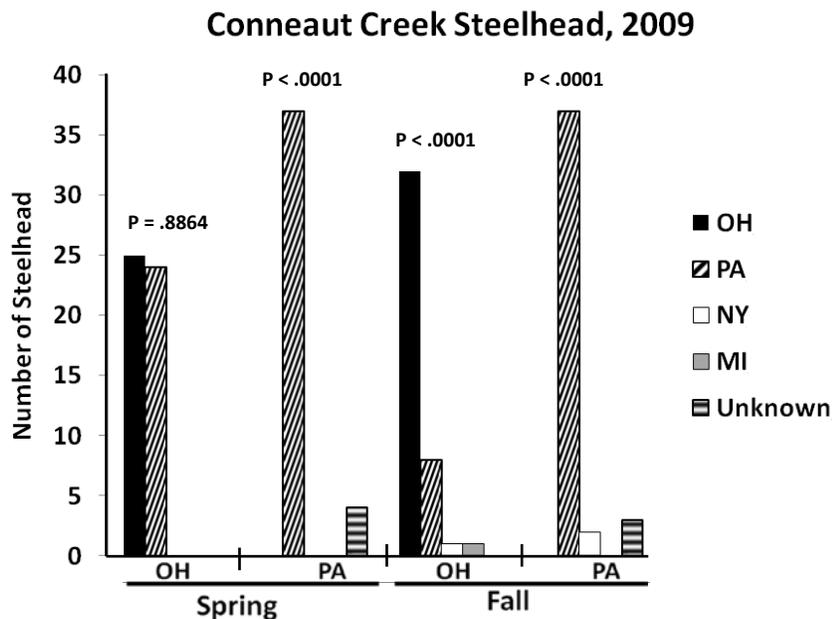


Figure 6. Discriminant analysis assigned hatcheries of the adult steelhead from Conneaut Creek, total N = 174 (total N by state: OH 57, PA 106, NY 3, MI 1, and Unknown 7). Figure shows number of steelhead classified to each stock by sampling date and location. Legend includes values of fish classified to each state. P-values based on 1 OH: 1 PA stocking.