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# Evaluation of Marking Procedures to Estimate Natural Reproduction of Chinook Salmon in Lake Michigan

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# EVALUATION OF MARKING PROCEDURES TO ESTIMATE NATURAL REPRODUCTION OF CHINOOK SALMON IN LAKE MICHIGAN

## FINAL REPORT

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## INTRODUCTION

In Lake Michigan, as in Lakes Huron and Ontario, chinook salmon is the dominant open-water predator. For more than 20 years, managers have wrestled with a perceived need to balance the number of predators and the forage fish that support them (Stewart et al. 1981, Stewart and Ibarra 1991). Current assessments of consumption by chinook salmon depend critically upon assessment of the level of wild production and hatchery recruitment.

Previous attempts to quantify natural recruitment of chinook salmon in Lake Michigan through oxytetracycline (OTC) marking were compromised by variable mark quality and quality control, and uneven effort for mark recovery. Because of limited information on wild production of Chinook salmon, managers on Lake Michigan cooperated to mark or tag hatchery fish during 1990-1995. Results suggested from 20 to 40 % of the entire Chinook salmon recruitment was naturally produced (Hesse 1994). Unfortunately, during this earlier work, a consistent lake-wide approach to marking fish, quality control on the marking, and sampling fish in the lake was not in place. During 1990-1993 most hatchery fish were marked, with the primary method of marking being feeding with OTC, supplemented by CWT and fin-clips for various lots of planted fish. In some cases no attempt was made to mark a given lot of fish, in others evaluation of quality control samples indicated that no distinguishable mark was present, or was only present on a minority of fish, whereas in other lots nearly all fish had distinguishable marks. Further complicating the issue, quality control samples are not available from many lots of fish. The proportion of hatchery fish that were effectively marked appears to have declined substantially during 1994-1995, in comparison with the earlier years. Likewise, regular lake-wide sampling was not in place during the entire period. During some years substantial samples from the sport fishery, which allow calculation of the proportion of hatchery fish, were not collected or were only along the eastern shore. In other years, the only source of information on the proportion wild comes from limited sampling from a research survey. Problems with marking and the limited extent of the recovery effort make the calculation of wild recruitment difficult in some years, and increase the uncertainty associated with all the estimates. Many of these problems could have been alleviated with established and agreed-upon guidelines for mark application and quality control, and an extensive sample-recovery program.

There is clearly a need for more information on the magnitude of wild recruitment of Chinook salmon in Lake Michigan and throughout the Great Lakes. Previous marking studies of wild chinook salmon in Lake Michigan indicated recruitment varied greatly from year to year. These estimates were generated 10 years ago for only a few year classes, thus obscuring relationships between recruitment variability and potential environmental factors. There is virtually no quantitative information on the magnitude of recruitment of wild-born chinook salmon in Lake Huron. As a consequence, plans are underway for management agencies in both Lakes to begin mass-marking chinook salmon using OTC.

The objectives of this paper are to use literature review, data analysis and interviews to: 1) evaluate past marking procedures for chinook salmon in the Great Lakes, and report estimates of mark readability, precision and accuracy where possible; and 2) evaluate marking programs and procedures for salmon outside the Great Lakes, and suggest alternative or complementary methods for marking individuals. In a companion analysis, Dr. James Bence and Emily Smith of Michigan State University have produced an analysis and sampling program to estimate natural recruitment of chinook salmon from samples of marked and unmarked fish in the lake population.

## METHODS

Our study consisted of a thorough, but not exhaustive literature review and personal communications with various experts on different marking procedures. The literature review began with internet searches using the Web of Science, Cambridge Science Abstracts, Ovid, and other web search engines. Further articles were obtained by checking referenced articles from those found via search engines, as well as from various individuals and agencies involved in researching and practicing the different mass-marking techniques. Individuals from each federal or state agency responsible for Lake Michigan or Lake Huron salmonid fisheries were queried, and specific hatcheries also were contacted when possible. A few independent researchers that had studied OTC marking in the past, or were currently doing so, also were contacted. State resource agencies in Alaska and Pacific coast states were contacted about mass-marking programs for salmonids and the development of thermal marking protocol in Alaskan state fisheries.

We purposely restricted our analysis in several ways. We limited our focus to marking techniques that would be most practical in a mass-marking program whose primary goal is to quantify natural recruitment of chinook salmon. Thus, we ignored much of the recent literature on analysis of stable isotope or trace element composition of fish otoliths and scales which relates to environmental histories or natal origins of wild fish (eg. Thorrold et al. 2001). Analytical costs of these exciting new techniques are high, and must be ground-truthed each year because of annual environmental variability in isotopic or elemental signatures (Gillanders 2002). We also ignored the use of genetic techniques to separate hatchery vs wild fish, because genotypes are homogenized by Great Lakes hatchery practices which continually mix chinook salmon eggs and sperm from naturalized and hatchery adults. We paid more attention to techniques suitable for mass marking groups of fish, not individuals. Thus, we ignored literature on dart tags, transbody tags and internal anchor tags which are not practical for mass marking smaller chinook salmon because of time involved in implementing the mark, cost, size of fish, and effects on mortality, growth and behavior McFarlane et al. (1990). In contrast to previous literature reviews that reviewed marking techniques for one bony structure (e.g. otoliths – Brothers 1990), or one type of marking method (thermal marking - Volk and Hagen 2001), we considered all body structures and marking methods. We focused most attention on mass-marking techniques used for Pacific salmonids, but included information for other species groups where relevant. We included a cost analysis of various marking techniques based on recent published estimates and Michigan DNR reports on marking costs. Finally, we focused attention on characteristics relevant for any effective fish marking program: marks are easy to apply, easy to identify, inexpensive, cause minimal mortality or non-lethal impacts on tagged individuals, provide high tag retention rates, and minimize risk to humans due to consumption.

# RESULTS

## Internal Marking Methods

*Oxytetracycline hydrochloride (OTC) Marking:*

Different types of tetracycline (TC) antibiotics have been used to mark fish bony structures, most commonly TC hydrochloride and Oxytetracycline (OTC) hydrochloride. Weber and Ridgway (1967) found that all five TC antibiotics tested in Pacific salmon were equally well deposited in fish bones. Demthylchlortetracycline (DCTC) had better fluorescence overall, but is expensive and more difficult to obtain than other TC antibiotics. Currently, OTC is being used most often due to its availability.

Fish can be marked successfully with TC antibiotics by injection, feeding, and immersion techniques. All three of these methods can successfully mark various bones (generally otoliths or spinal vertebrae) with a fluorescent mark that can be detected with special equipment and training. The marks, when properly read, also can help determine the age of the fish at marking, growth rate, and relative age at capture. Best marking results are obtained if TC is applied when the fish are growing at a fast rate (Conover and Sheehan, 1998). This applies to times of the year (spring and summer versus winter months), as well as life stages (early in life versus adult stages). Younk and Cook (1991) found through literature review that marks were always more accurate and longer-lasting in juvenile walleyes over any other ages for all three marking methods. Most articles on marking walleye described techniques for immersion marking in solutions ranging from 200-500 mg/L for 6h to 24h, with a mean of 319 mg/L for 11h. Marking success rates ranged from 70-100%, mean=96%. Articles on OTC marking of salmonids through feed report a range of concentrations of 59 mg OTC/kg fish per day to 1000 mg OTC/kg fish per day, and durations from 2 to 54 days (mean 164 mg OTC/kg fish per day, 17 days). Marking success of salmonids through feed ranged from 68-100%, with a mean of 95% at concentrations ?250 mg/kg OTC. Immersion marking studies indicated 100% marking rates at concentrations of 55 mg OTC/L for 23 h, and 250 mg/L for 24h.

Younk and Cook (1991) found that injection methods of TC marking produced a higher quality mark than did feeding and immersion techniques. Much lower concentrations of OTC (25-50mg/kg fish weight) also can also be used for direct injections as opposed to feeding or immersion (Babaluk and Craig 1990). However, TC injection involves directly handling of individual fish for marking, and can cause unnecessary stress, possibly affecting health. It also requires more time, equipment and facilities than other TC marking methods, thus is not a viable choice for mass-marking (Schmitt 1984).

Introduction of TC through feed has produced better long-term results than either injection or immersion. In general, mark quality improves with TC concentration and length of application. Weber and Ridgway (1967) found that the optimal concentration for Pacific salmon was 250 mg TC per kg of body weight per day for four consecutive days. Mark quality improved at a declining rate up to 500 mg/kg body weight, after which there was no improvement. Marks remained up to 3.5 years after feeding, after which the study was discontinued. Hendricks et al. (1991) found 88g TC or OTC per kg of feed per day for 3 days to be the optimal treatment. In both studies, fish were starved for two days prior to medicated feeding. Nordeide et al. (1991) used concentrations of 500mg OTC per kg body weight per day over the course of 18 days for marking cod. There was no decline in the mark after 35 months. Koenings et al. (1986) and Bilton (1986) also found that longer feeding periods coincide with better mark quality.

Potentiators have been added to feed to improve mark uptake and intensity, thus making it possible to use lower concentrations of TC antibiotic. Dry feed coated with various concentrations of TC antibiotics were most common, following the normal feeding procedures of the hatchery where the studies were conducted. Weber and Ridgway (1967) found that adding potentiators such as glucosamine or terepthalic acid to feed could increase mark intensity, making it possible to use lower concentrations of TC antibiotic. Hendricks et al. (1991) found concentrations of 1.65g glucosamine per kg of food had positive results.

Variable results with TC marks from feed are cause for concern. Not only do recommended dosages and length of treatment differ, but mark location has also been disputed. Brothers (1985) achieved poor mark quality on otoliths of lake trout fry marked by feeding techniques, while Hendricks et al. (1991) reported 100% success in marking

American shad otoliths through TC feed. Toften and Jobling (1996) found OTC feed to cause spinal deformities in Atlantic salmon, but not Arctic charr. In this study, 17% of treated salmon developed deformities, including spinal fracturing. They hypothesized that long-term ingestion of TC antibiotics may deplete ascorbic acid, which in turn impairs bone formation. Their study used doses of 150-200mg OTC per kg fish weight per day, but deformities were not detected until the 44th and 65th days. Both salmon and charr received doses several times higher than the normal recommended (250 mg OTC $kg^{-1}$) at which no adverse health effects have been reported.

Immersion marking is the most commonly-reported technique for TC application. It is the least time-consuming of the three techniques, which makes it the most popular for mass-marking procedures. Most studies deal with marking larval and juvenile fish (eg. Tsukamoto 1985, Secor et al. 1991), but there have been attempts to mark at fertilization, while in the "eyed" stage, and just prior to hatching (Beltran et al. 1995b). Reinert et al. (1998) found mark presence was high (92%) for striped bass larvae immersed in 350 mg OTC/L for 6-8 hrs, but mark retention was lower (80%). Brooks et al (1994) found that immersing larval and juvenile walleyes in 500mg/L OTC for 6 hours provided the most consistent marks. All fish older than 3 days retained marks, while marking success for younger fish was inconsistent. Post-stocking mortality was quite low at only 3%. Marking at 350 mg/L also was 100% successful, but the marks were less clear than at the higher concentration (500mg OTC/L). Colesante (1994) also found 500 mg/L OTC for 6 hours to be optimal for marking walleyes. Isermann et al. (1999), and Conover and Sheehan (1998) also found the 500mg/L concentration to be optimal for marking crappies. Immersion marking at lower concentrations generally requires longer immersion periods (Lorson and Mudrak 1987).

Opinions vary on the best structure to examine for marks. Swartz (1971) and Trojnar (1973) were able to produce long-lasting TC marks in salmon vertebrae through feeding. Peterson and Carline (1996) claimed lapillae require less preparation and care than do sagital otoliths, while Scidmore and Olson (1969) found that the pelvic bones had the most consistent marks. Otoliths tend to calcify before other bones (Lorson and

Mudrak 1987), thus age of the fish at treatment may affect the efficacy of examining different bones, as well as the treatment selected.

There is little variation in methods of sample preservation for OTC mark identification. Muth et al. (1988) found that preserving specimens in formalin buffered with phosphate degraded mark quality, while preservation in 95% ethanol kept the marks in relatively good condition. OTC mark quality will deteriorate in direct light (Brothers 1990). Freezing of specimens and storage away from direct light will not degrade OTC marks, and has become the most common preservation method by far.

Preparation of otoliths and other marked bones for examination also may affect mark detectability. Brooks et al. (1994) found that edges, cracks, stress marks, and connective tissue all can cause auto-fluorescence in unmarked fish, thus creating false positives. If heat is produced when mounting otoliths, poor marks may be further degraded. Sectioning and grinding can be slow and inconsistent, making it possible to grind through marks if not extremely careful (Weber and Ridgway 1967).

Fish hatchery conditions also may affect OTC mark quality. In weakfish, Paperno et al. (1997) found that varying feeding levels (from 100% to 17% of maximum rations) had a significant effect on increment widths (i.e. mark quality). Calcium and other divalent and trivalent metallic ions present in water or feed will chelate with tetracyclines and reduce absorption of the antibiotic (Weber and Ridgway 1967). Hesse (1994) reported that chinook salmon smolts fed at low raceway densities (<500 fish/kg body weight) had higher marking success (95%) than salmon fed at higher densities (85%). Radtke and Fey (1996) found similar results suggesting that higher water temperatures and better feeding increased marking success in Arctic charr. Stress from smolting may cause fish to decrease feeding, which directly affected OTC mark quality for salmonids. Monaghan (1993) also found reduced activity and a cessation of feeding in flounder fed only 25 mg/kg body weight OTC. On the other hand, Odense and Logan (1974) found that 250 mg OTC/kg feed for salmon was necessary for at least 5 days, with fish at least a year old for most efficient marks, which would suggest that the extended feeding techniques are necessary.

### *Calcein Marking*

Calcein is a chemical marker that has been used mainly in immersion studies as an alternative to OTC. Calcein application produces bright blue marks in fish otoliths, fins, and vertebrae in the same manner as OTC marks. Although calcein is more expensive by volume than OTC, lower concentrations are required for efficient marking, thus making total costs fairly equivalent. Concentrations used ranged from 1 to 900 mg/L for 15 min to 24h, with mean treatment application of 358 mg/L for 13h. Marking success was reported as 100% in most studies, but was variable for marking experiments with larval and juvenile sciaenids (Beckman et al. 1990). Mohler (1997) found Atlantic salmon fry had lower mortality when immersed in 125 mg/L calcein solution for 48 hours than when immersed in 250 mg/L OTC for the same duration, with similar marking success. Other immersion marking studies of non-salmonid species reported high marking efficiencies for fish immersed in 250 mg/L calcein for 24 h (Leips et al. 2001), or 125 mg/L calcein for 2h (Wilson et al. 1987).

Calcein marks also have been produced through introduction in feed. Monaghan (1993) found 25 mg calcein/kg food to have high marking success in summer flounder. Unfortunately, not enough work has been done with salmonid species to find an acceptable standard for calcein marking. Leips et al. (2001) developed a portable new method of detection for calcein marks that may make calcein more economically viable in the future, as compared to OTC. Bart et al. (2001) have demonstrated that cavitation level ultrasound enhances permeation of calcein into rainbow trout, reducing absorption time to only 5-15 minutes. This new method may be able to reduce the negative effects caused by long exposure times (and could possibly be used with other chemical marking methods as well).

### *Alizarin Compound Marking*

Alizarin chemicals (most commonly alizarin complexone (ALC) and alizarin-red S (ARS)) produce well-defined scarlet bands in fish otoliths. ALC has been successfully used to mark larvae and juveniles of numerous species, including flatfishes (Takahashi

1994, Reichert et al. 2000, Lagardere et al. 2000), moronids (Secor et al. 1995), cyprinids (Beckman and Schulz 1996), osmerids (Tsukamoto 1988), and salmonids (Nagata and Irvine 1997, Kawamura et al. 2001). Eight articles described fish immersion in ALC at concentrations ranging from 25-200mg/L for 6-24h, mean=86 mg/L for 16h. All articles reported 100% marking success. The only ALC feed experiment used 100 mg/kg fish ALC for 10 days, with 100% success. In two studies, fish were immersed in ARS concentrations of 200 mg/L for 12h and 400 mg/L for 24h, both with 100% reported marking success. Lagardere et al. (2000) reported 100% marking success of juvenile turbot marked by immersion in an ALC bath of 30 to 120 mg/L for 18 to 24 hours, with no significant effects on growth or mortality. Fewer studies have been conducted with alizarin-red S, but similar results have been found (with concentrations of 200-400 mg/L ARS for less than 24 hours; Lagardere et al. 2000).

Japanese and American scientists have successfully used ALC to mass-mark salmonids and other species in the embryonic stage (Tsukamoto 1988, Kawamura et al. 2001, Secor et al. 1995). In one study, 11.5 million chum salmon eggs were marked at the embryo stage by immersion for 24 h in 200 mg/l solution of ALC, and released into the Sea of Japan (Kawamura et al. 2001). Recaptures of adults 5 years later confirmed individuals retained viable ALC marks. In one recent study of ALC diet in juveniles flounder, Takahashi (1994) found fish fed 100 mg ALC/kg for 15 days had 100% mark retention. Secor et al. (1995) used 25 mg/L ALC for 6 hrs to mass mark 6.5 million striped bass embryos and larvae for release into a Chesapeake Bay tributary.

### *Strontium Chloride or Isotope Marking*

Strontium is a naturally-occurring element that may be incorporated into fish bone, scale or other tissue to leave marks. Strontium chloride has been applied successfully to mass mark salmonid fry (Behrens Yamada and Mulligan 1982, 1987, Schroder et al. 1995). It can be applied at any life stage, even to gravid females who may pass the elevated strontium levels on to their eggs (Schroder et al. 2001). The appeal of strontium chloride is it can be used to mark wild fry in situations where thermal marking, OTC or some other technique is not practical. Strontium isotopes also have been used to mark and document habitat utilization by hatchery-released Atlantic salmon (Kennedy et al. 2000). Wave dispersive spectrometry is applied to marked areas of the otolith to produce electron backscatter and x-rays characteristic of elemental composition. Costs of application are slightly higher than for OTC, and analytical costs are significant (Table 1).

### *Thermal Marking*

Thermal marking of otoliths is now widely used to mass-mark millions of hatchery-released salmonids in the Pacific Ocean. Recent estimates indicate that nearly 1 billion salmonids, or 20% of total hatchery production in the North Pacific, are marked each year using thermal marking techniques (Urawa et al. 2001). The attractions of using this procedure to mass-mark salmonids are that it is chemical-free, in-expensive, non-destructive, and produces a permanent mark with success rates over 95%. Negative aspects of thermal marking are that initial costs of heating or cooling water may be high, relatively few marks can be used, the fish must be destroyed to obtain the mark, and time and expertise are required to process otoliths and read marks. Also, naturally-produced otolith marks resulting from environmental fluctuation can be mistaken for hatchery-reared marks in cases were the wild-stock was affected by varying water temperatures (Brothers 1990, Volk et al. 1999). This can lead to an overcomplicated marking system such as Morse code which may become expensive, because costs are associated with heating and cooling water and training and skill needed to identify marks in the otolith.

Thermal marks are created by varying short-term temperature fluctuations to induce distinctive structural marks onto the otoliths of incubating fish (Volk et al. 1999). Otolith growth patterns are characterized by two different zone types, the D zone (the dense, dark-rich zones of protein and calcium carbonate), and the L zone (light zone characterized by crystalline calcium carbonate (aragonite)) (Secor et al. 1995, Volk et al. 1999). The dense bands are produced from a rapid decline in temperature (Munk et al. 1993). Elevated temperatures shorter than 24 hours, as well as gradual increases or decreases are less effective at producing obvious marks. Depending on the variation in temperature and time exposure, bands can be produced in different patterns. Thermal marks are most distinctive when temperatures are reduced or elevated from ambient temperatures from as little as 2 hours (chilled water) to several days (heated water). Marks are distinctive at temperature changes from 2 to 5 ?C, with greater distinction at greater differences from ambient temperatures (eg. Figure 1) (Volk and Hagen 2001).

The process of thermal marking has been likened to maximizing a signal-to-noise ratio. The most recognizable marks are generated by maximizing differences between normal patterns generated by ambient events (noise), and patterns generated by specific temperature manipulation (signal). Volk et al. (1994) varied water temperature and duration to ascertain the most visually distinctive marks. The best marks occurred when fish were kept in higher temperatures or the fish was held in the marking temperature for a longer period of time. Elevated temperatures shorter than 24 hours are less effective at producing obvious marks, as well as gradual temperature increases and decreases. Letcher et al. (1998) found strong thermal marks could be produced on Atlantic salmon otoliths by holding fish at the pre-mark temperature of 5 ?C for 4 days and at the marking temperature of 1 ?C for at least 24 hours.

Error recognition and misclassification rates of otolith thermal marks have been reported for Pacific salmonids and lake trout. Investigators agree that the presence of a pattern is more easily recognized than its absence (Bergstedt et al. 1990, Volk et al. 1999). Bergstedt et al. (1990) reported classification rates of 85-98% in marked and unmarked fish. Hagen et al. (1995) accurately identified 64-100% of known marked and non-marked adult pink salmon otoliths. Volk et al. (1999) reported a mean error rate of 2% for known marked fish, with a higher error rate (6-11%) for classifying known unmarked control fish.

Although thermal marks are most useful for a relatively low number of marks are needed, more complex marks have been created by inducing patterns in Morse code (Brothers 1990), bar code (Volk et al. 1994) and the RBr system (Munk and Gieger, 1998). By applying a 9-13 day hold at the ambient water temperature, more then one group of otolith rings can be induced (Bergstedt et al. 1990).

Duration and recognition of thermal marks lasts as long as other marks. Duration of thermal marks on lake trout sac fry lasted for at least seven years with 100% mark recognition (Negus 1999). Thermal marks made on salmonid embryos were recognized 5 years later on adults. Marking can be conducted according to the water temperatures sources available, but if water sources are not available then heating and cooling systems (including generators) must be purchased. In one study, a modular water chilling system was used consisting of three 10,000 BTU electronic water chillers mounted in an insulated box with an inlet at one end and a pump at the other to move chilled water out of the box (Volk et al. 1999). When marking millions of fish, non-mobile bigger units are more beneficial but also are costly.

### *Otolith Dry Marking*

Otolith marks also can be produced by exposing eggs or young embryos to periods of low or no water. The change in ambient conditions results in physiological reactions that alter the normal deposition of protein and calcium carbonate material in otoliths that generates dark and light bands. In Russia, the dry method has been used in conjunction with thermal marking to mass mark salmonids. Recently, 4.5 million salmon were marked using the dry method by alternating dry and wet conditions for eggs. Drying eggs for 12-24 hours results in no increase in mortality of eggs compared to controls (Rogatnykh et al. 2001). One advantage of dry marking over thermal marking is that it involves no special electrical equipment or cost. A disadvantage is that it can only be applied to salmon eggs, not to alevin or fry stages.

### *Coded Wire Tags*

The most common method for tagging individual fish has been the coded-wire micro tag (CWT) (Jeffries et al 1963; Volk 1999). Tags were introduced in the 1960s as an alternative to fin clipping methods, and by 1996 cwt releases totaled >96 million salmonids in the Pacific Northwest. Tags originally were 1.5mm long and provide five bars with a range of colors that allowed for five thousand codes to characterize fish individuals and populations. Later, binary codes were introduced measuring 1mm and presenting 250,047 different codes. With small changes in the wire, this number can be dramatically increased (PSMFC 1999). CWT's are made of a magnetized stainless steel wire that is injected into the cartilaginous wedge of chondro-cranium located in the fish's snout anterior to the eyes (Elliott et al. 2001). CWT marking of larger salmonid parr such as chinook salmon is more effective and retention time is longer than for smaller parr such as pink salmon (Kaill et al. 1990). In largemouth bass populations, retention of tags was 100 percent for individuals examined after 9 weeks (Fletcher et al. 1987). Sequentially-coded wire tags are now being used in conjunction with computer programs to cut tag-reading time, and eliminate mis-identified tagging and rejected tags (Unwin et al. 1997).

Studies of fish marked with CWT's appear to demonstrate more problems than have been found with other methods (thermal marking, fin clip, etc. Elliott and Pascho (2001) found that salmon injected with CWT had increased infection rates of Renibacterium salmoninarum, which causes Bacterial Kidney Disease (BKD) as well as lesions and the destruction of tissues of one or both olfactory organs. The infections likely occurred through contamination in the snout from infected fish, or by facilitating entry by pathogens through the injection wound. Because of these results, fish tagged by CWT's must also go through an extensive sanitary system that prevents these diseases from occurring, thereby increasing costs. The deleterious effects of CWT's on olfactory organs also has raised suspicion that fish injected with the tags have damaged homing abilities, which would bias population, growth, and mortality statistics. CWT-tagged pink salmon fry showed a significant increase in straying rates over fin-clipped fry in Prince William Sound, Alaska. The difference in straying rates was significant for fish stocked at the tributary mouth, but was negligible for fish planted upstream (Thedinga et al. 2000). Habitch et al. (1998) found that CWT-tagged fish that strayed from the stocking location were more likely to have tags near or in olfactory organs, their associated nerves, and the brain than tagged fish which homed. Factors such as untrained tagging personal, variable release techniques, and variable hatchery environments also may cause lower success with CWT marking. With smaller fish (0.85g), tag loss rates can approach 40% and losses continue to accrue some 98 days after tagging. In this case, half-length tags are used but reduce the ease of marking (Blankenship et al. 1990). Although short-term (40-day) retention rates of CWT-tagged pink salmon exceeded 93%, long-term (2-year) retention rates were lower, ranging from 49-84% (Kaill et al. 1990). In contrast to the smaller pink salmon, reported tag loss rates for coho and chinook salmon were relatively low (<5.3%) and occurred within the first 20 days (Blankenship 1990). CWT retention rates reported for other fish species are lower. Retention rates of tags implanted in the cheek or nape of striped bass juveniles were much higher (range 85-94%) than for nose implanted fish (65%). Bumguardner et al. (1990) found that only seven percent of CWT-tagged red drum juveniles survived and retained CWTs. CWT tagging and detection costs are relatively expensive compared to other methods (Table 1).

*Passive Integrated transponder (PIT) Tagging*

PIT tags were introduced during the 1980s as an alternative to CWT and freeze brand tags. The PIT tag consists of an integrated microchip bonded to an antenna coil which is inserted into the body cavity. Electronic components of the tag are encapsulated in a glass tube about 12 mm long x 2 mm diam. Each tag is programmed at the factory with one of 34 million unique code combinations. The tag is energized by a 400-khz external signal that enables the tag to transmit a unique 40-50 KHz signal to the interrogation equipment, where the code is immediately processed (decoded), displayed, and stored on a computer. The PIT tag allows for passive (in situ) collection of a tag code from an individual without handling the fish (Prentice et al. 1990a).

The PIT tag appears to be an attractive tagging method for relatively small releases of salmonids. It causes little to no mortality in tagged individuals, has no effects on fish growth, movements or respiration rate, and can be inserted into fish ? 55 mm, however Elliott and Pascho (2001) caution that insertion of PIT tags may increase likelihood of BKD infection. PIT-tag retention in salmonids ? 55 mm was near 100 %. The tag's longevity is estimated at 10 or more years (Prentice et al. 1990b). Tag detection efficiency measured at hydroelectric dam facilities was 93% at a passage rate of 20,000 fish/hr, and reading accuracy exceeded 99% (Prentice et al. 1990b). Tagging rates are relatively slow (Prentice et al. 1990c) at 150-300 fish/hr compared with CWT tagging rates that may exceed 600 fish/hr.

## External Tags

### Fin Clips

Mutilation of fins by clipping has been practiced for over 100 years, and is still considered a viable way to mass mark fish. Drawbacks are a limited number of fin clip combinations are possible, with unknown consequences to the marked individuals. All fins except the adipose fin will not regenerate if cut back to the bone. The adipose fin will occasionally grow back, or will sometimes be lost, creating a false mark (McFarlane et al. 1990). In the Great Lakes as elsewhere, fin clips have been used to designate CWT-marked fish released by different states or agencies, and the adipose fin has been used to designate presence of a CWT-marked fish. Clipping rates of approximately 800 fish/hr are reported for large marking operations on Pacific salmonids, making this a rapid marking approach. Costs of clipping are relatively low compared to some other marking approaches (Table 1).

### Photonic Paint Marks

Hayes et al. (2000) evaluated the use of photonic paint to mark salmonids. In this study, paint was injected into three areas of the fish to identify the best possible marking procedure. The three positions included the pectoral fin, pectoral girdle, and the dorsal fin. Fish marked were mostly juveniles averaging between 701-956 mm and the paint injections were polymethylmethacrylate fluorescent pigment encapsulated in latex microscopes (Hayes et al. 2000). The paint used was visible in regular light although marking techniques have been conducted with cryptic colors. The marking was conducted with a BMX1000 marking gun with a pressure of 42.2 kg/cm2 and consisted of .15mL (Hayes et al. 2000). Marking ratings were conducted using a good, fair, poor and unmarked system. Marks applied to the dorsal fin were mostly unmarked or poor, and those applied to the pectoral fin were mostly fair to poor. Marks applied to the pectoral girdle, however were good. The study found that pink coloring was the easiest to identify. Results indicated that average retention time of photonic paint marks was forty-five days.

Marking time averaged 30 seconds, and average cost was $0.24 per fish (Hayes et al. 2000).

### *Fluorescent Tags*

Fluorescent external marks also have been used to mark salmon populations alone or together with CWT's. The fluorescent tag is used specifically as an external marker indicating the wire tag or (CWT). The fluorescent tag can be implemented by using two separate methods, using visible implant fluorescent filament (VIF) or visible implant fluorescent elastomer (VIE). VIF is a monofilament polymer, while VIE consists of a two-component, biologically-inert silicone polymer that fluoresces under UV light. After the elastomer is mixed with a hardener, it is injected as a viscous liquid, filling the cavity creased by a hypodermic needle. Within hours, the material hardens into a pliable, rubber-like mass (Close 2000). Both tags are injected into the transparent adipose eyleid tissue of salmonids in the same manner as alphanumerically-coded visible implant (VI alpha) tags (Bailey et al. 1998).

Applying either of these tags involves anesthetization of individuals using tricaine methanesulfate (MS222), and tags were implanted with wire tags, using an NMT Mk IV injector with a fixed needle (Bailey et al. 1998). The average VIF tagger rate approximates 250 fish/h, which may not be conducive to large-scale studies. The filament material found in the fluorescent tags is not as stiff as CWT and often becomes jammed. In a marking study of coho salmon, VIF tag losses were around 5 %, while those from CWT-marked fish averaged 0.44% (Nelson et al. 1994, 1995). Bailey et al. (1998) evaluated retention and detection of VIE and VIF marks on coho salmon smolts. VIF tags were visible during the day, but require UV lighting to be seen at night (Bailey et al. 1998). VIE tagging rates averaged around 300 fish/hr. Average mortality averaged nearly 5%. VIE tag visibility was high, and no UV light was necessary. Although long-term tag loss of VIF tags was insignificant, VIE tag loss was high at 27% (Bailey et al. 1998). Close (2000) found that detectability and retention of VIE marks in rainbow trout fingerlings under UV light also were low, ranging from 57 to 87% of all marked fish after 195 days. Similar to most tags, the detectability of both VIE and VIF increases with size of fish marked (Close 2000).

Observations suggest this method could potentially increase predator detection of marked fish, thereby increasing their mortality (Bailey et al. 1998).

Advantages of using fluorescent implant tags are that fish do not need to be sacrificed as with CWT's or thermal marking, fish are also not harmed with open wounds from external tags or possibly chemical tagging. Compared with CWT or fin clipping, fluorescent implanting is more efficient and requires fewer people to conduct the procedure. Disadvantages include limitations to only batch-marking. Anesthetic use and fluorescent implanting also are susceptible to problems of mis-marking, jamming, high mortality or stress due to over-handling.

## Evaluation of Past Marking Procedures for Chinook Salmon in Lake Michigan and Lake Huron

In response to decreased survival of chinook salmon in Lake Michigan during the 1980s, Lake Michigan resource agencies initiated a mass-marking program to estimate natural reproduction and track movements of chinook salmon in lakes Michigan and Huron. Nearly all hatchery chinook salmon released into Lake Michigan were marked with OTC or were fin-clipped from 1990 to 1993, but marking rates declined during 1994 and 1995 (Table 2). (Approximately 5 million chinook salmon were implanted with CWT's and released in Lake Michigan and Huron during 1990-1995, but evaluation of this program is ongoing). In Lake Huron, nearly 80% of all hatchery chinook salmon were marked with OTC. Chinook salmon were marked with OTC through introduction in feed at doses of 350 mg OTC/kg body weight for 5 days. Fish were fed at 2% body weight daily rations approximately 4-5 weeks before release. Mark quality and control data were collected for Michigan hatcheries during all five years, and sporadically for other states' hatcheries during 1990-1992.

Hesse (1994) and Elliott (1994) estimated the percentage of wild fish in Lake Michigan based upon proportions of marked and unmarked fish. Their estimates of unmarked salmon ranged from 29-35% of all fish examined, producing an estimate of 2.5

to 3.8 million naturally produced chinook salmon from 1990-1991. Subsequent analysis of OTC marked chinook salmon by MDNR suggests the percentage of marked fish remained constant in 1992 and 1993, then declined probably due to the increase of unmarked fish in the population (David Clapp, MDNR, personnel communication).

Hesse (1994) and Elliott (1994) provided estimates of mark readability, precision and retention for cohorts of hatchery reared chinook salmon released in Lake Michigan from 1990-1992. OTC marking was evaluated using over 1,579 known hatchery chinook salmon of all ages from the 1990-1993 cohorts, including 269 hatchery smolts prior to stocking, and 179 young-of-year (YOY), 555 age-1, 451 age-2, and 125 age-3 hatchery chinook from the lake. Before stocking, samples of smolts were removed from raceways in each state, and frozen for later evaluation. Methods for detection of marks present on chinook salmon vertebrae are described by Hesse (1994). Smolts removed from hatchery raceways, and adults double-marked using OTC and fin clips were examined for marks to estimate marking success.

Results of the mark validation studies indicated that mark quality was highest for rapidly growing individuals at intermediate densities in raceways. Variation in brightness and quality of OTC marks correlated positively with the size of hatchery chinook at the time of marking. Easily recognizable marks were lacking in 8.9% of fish fed at a size of 500 fish/kg body weight, 5.2% of those fed at 350/kg, and <1% of those fed at 300/kg. Mark success averaged roughly 95% for these individuals, but was lower (85%) for chinook smolts fed in higher density raceways. If marked well initially, mark retention in chinook was good through age 3 provided certain methods for reading marks were used. Variation in sample collection, preparation, and reading methods used by different people was an important consideration in producing consistent marks (Hesse 1994, Elliott 1994).

Marking success reported for other states was variable. Although 100% of smolts from Wisconsin hatcheries were believed marked from 1990-1993 (Table 2), subsequent evaluations of double-marked individuals returning to spawn in Wisconsin rivers indicated that OTC marking success varied greatly among raceways. Mark success varied from 6 to 93 percent (average = 60%) for known-aged and double-marked (CWT, OTC) chinook salmon stocked at Strawberry Creek or in the Kewaunee River, and collected in spawning runs during fall 1992 at age 1+ and 2+. Quality control data were not available for Indiana or Illinois hatcheries after 1992.

Mark quality varied among hatcheries within states, and among states. In 1994 and 1995, for example, although 98 and 90% of all fish were marked respectively, respectively, the percentage of marks ranked as good or excellent from each hatchery ranged from 64-79% (average = 70%) in 1994, and from 32-77% average = 60%) in 1995 (Figure 2). No data on mark quality or success were available from other states during these years, but data available from a new mass-marking program initiated in 2000 suggested mark quality varied among states. As in 1990-1995, all hatchery chinook salmon released by Wisconsin and Indiana into Lake Michigan in 2000 and 2001 were marked, but at a lower concentration of 250 mg OTC/kg body weight for 4 days. (Illinois fin-clipped 33% of their hatchery fish, and have stopped using OTC to mark fish), Chinook salmon released by Michigan hatcheries were marked in 2000 at 350 mg OTC/kg for 4 days in 2000, then in 2001 at 250 mg OTC/kg for two periods of 4 days, separated by 1 day. Not surprisingly, OTC mark quality varied greatly among states. Average mark quality on a scale from 0 (no mark) to 4 (excellent mark) was high for Michigan (3.7), average for Indiana (2.4) and poor for Wisconsin (1.6). Variation in mark quality among hatcheries may be attributed to presence of calcium in feed which inhibits OTC uptake, and variable densities and sizes of fish at stocking which affects feeding and growth rates (Wisconsin DNR hatchery personnel, personnel communication).

Ability to detect OTC marks also may vary greatly among readers and have serious consequences for estimation of natural reproduction. I provided samples of adult chinook salmon collected in 1997 from spawning runs in the Muskegon and Manistee River tributaries to Lake Michigan to 3 different readers to estimate mark presence/absence. Two of three readers (Readers B, C) were experienced, and regularly examined chinook salmon vertebrae as part of MDNR’s program to evaluate natural reproduction of chinook in Lake Michigan. Reader A was relatively inexperienced, having been trained by myself and another biologist in MDNR who were experienced in mark detection and ageing. All readers had been trained to read marks using known samples of marked fish, and used essentially the same methods and equipment as in earlier studies (Hesse 1994) to process and read vertebrae for OTC marks. The sizes, ages, collection dates and locations of fish sampled were consistent among all 3 groups. Results indicated wide variation among readers in estimates of percentage of fish marked for a given year class. Reader A consistently found more marked individuals than did reader B, who found more marks than Reader C (Figure 3). The discrepancy in results may have serious implications for estimates of natural reproduction. For example, estimated abundance of wild smolts emigrating from the Muskegon River would vary from 400,000 to 7 million, depending upon the estimate of percent marked for a year class. As an aside, we subsequently estimated smolt abundance in the Muskegon River through trapping and electrofishing in earlier (1979, 1988) and later years (2000-2002), and found annual smolt abundances estimated from trapping or shocking agreed more with Reader C's estimates than the other readers.

Migration of unmarked chinook salmon from Lake Huron to Lake Michigan also may have complicated estimates of natural reproduction during these years. Unmarked hatchery chinook salmon stocked in Lake Huron ranged from 13 to 22 % of total hatchery fish released in 1990-1995 (Table 2), and some of these individuals, along with wild fish may have migrated to Lake Michigan. Of the 8,049 CWT-tagged individuals recovered that were planted in western Lake Huron during 1990-2000, 5 % were recaptured in Lake Michigan, although the true extent of migration is currently unknown due to biases in tag recapture and fishing effort (David Clapp, MDNR Charlevoix Lake Michigan Station, unpublished data).

## DISCUSSION

### What mark to use?

#### *The Losers*

Although it is clear that a number of methods are available for mass-marking salmonids in the Great Lakes, some ranked higher than others due to public or government acceptance, low cost, high mark quality or long duration. In contrast to CWT tags, fin clips or thermal marks, chemically-induced marks (OTC, alizarin, and calcein) all have been shown to fade with time. Both calcein and alizarin chemicals produce brighter, clearer marks in fish (bright blue and bright red, respectively) than the fainter yellow marks of OTC. These marks are less likely to be confused with natural fluorescence of scratches and other flaws under UV light, as can happen with OTC. Calcein marks can be detected using visible blue light as opposed to UV light necessary in detecting OTC marks, which can be harmful to the users (Monaghan 1993). Mohler (1997) also was able to detect calcein marks in caudal fin tissue of Atlantic salmon, meaning calcein could be used as a non-lethal sampling technique. However, calcein is much more expensive than OTC, and is yet to be approved by the Food and Drug Administration (FDA).

Immersion marking using alizarin compounds does not require the pH adjustments that OTC requires, and alizarin solutions do not foam as OTC solutions will (Secor et al. 1995). Both foaming and pH adjustments may induce stress in marked. Alizarin marks also have no effect on fish growth patterns, unlike OTC, which is also used as an antibiotic and can therefore bias estimates post-capture (Leips et al. 2001; Reichert et al. 2000; Lagardere et al. 2000). Alizarin-red S is similar in price to OTC, while requiring less handling and preparation than OTC (Secor and Houde 1992). But, as with calcein, alizarin has not yet been approved for use by the FDA. Although alizarin would appear to be a better method of marking for the future than OTC, work must be done to determine its non-toxicity in order to make it a viable alternative.

Some other techniques evaluated can be eliminated on the basis of high cost (strontium, PIT-tag), slow marking rate (VIE, VIF), relatively high mortality rate (calcein), or unacceptable tag detection loss rates (VIE). By process of elimination, we are fortunately left with just a few methods from which to choose, for which mark success, mark retention, mark readability were high and cost was relatively low. These methods are OTC marking, thermal marking, fin clipping and CWT marking.

### *The Contenders*

Although clearly superior to most other marking techniques, literature reviews of OTC-marking were not consistently favorable. Brothers (1990), Secor (personal communication) and others have found OTC marks on fish otoliths to be inferior to other marks (Alizarin, thermal marks). OTC marks on fish vertebrae appeared to be more consistent, but still varied greatly in quality and degrade with exposure to light. OTC mark success on chinook salmon vertebrae varied among the Great Lakes, being high in Michigan hatcheries and lower in other states. Under current FDA guidelines, mark retention is high and quality is adequate. Cost of OTC marking is relatively inexpensive.

Acceptance of OTC marks on salmon vertebrae is not uniform among the Great Lakes resource agencies. OMNR is forbidden by the Canadian government to use OTC to mark salmonids, and Illinois DNR has abandoned OTC as a marking tool. Wisconsin DNR has stated that they will abide by current FDA guidelines of 250 mg OTC/kg fish for 4 days. At present, Michigan DNR unilaterally has chosen to bend these guidelines by extending the marking period for 8 days: salmon are fed 250 mg OTC/kg fish for 4 days, followed by a 1 day break, then fed another 4 days at the same concentration. Given the inherent variability in mark quality and reader agreement mentioned above, it is urgent that agencies concerned choose a common protocal.

Marking large numbers of chinook salmon with CWT's can be practically and efficiently done. CWT's provide the additional advantage of being easily detected by low-cost metal detectors, resulting in negligible detection costs. Costs increase to remove and read tags. Mark success and retention rates are slightly lower than for thermal marks, but still are quite high, exceeding 90%. CWT's allow marking multiple groups, thereby providing additional knowledge of movement patterns. CWT's may be combined with other marking techniques or conducted alone. Resource agencies around the Great Lakes are experienced with CWT's. This method may not be the most efficient or cost effective to determine presence or absence of hatchery fish. Recent evidence suggests that it may facilitate the transfer of BKD disease (Elliott and Sascho 2001).

Fin clips are routinely used as a marking technique by resource agencies to mass-mark chinook salmon. Mark retention and detection rates are relatively high, despite regneration of clipped adipose fins or losses through natural processes. Marking costs are reasonable and detection costs are neglible.

### *The Winner*

Thermal marking appears to have high marking success and detection rates that exceed 90 %, with a reasonable low probability (10%) of false positive marks. The method is very inexpensive and easy to apply, but is expensive to decode. The mark should be readily acceptable by resource agencies and the public as it is chemical free and doesn’t deform the fish. The mark retention is 100%, and can be digitized and made available to multiple readers. Thermal marking is now the most common approach used by resource agencies around the North Pacific ocean for mass-marking salmonids. Given the ease of mark application, it is reasonable to assume resource agencies in the Great Lakes can mass-mark all chinook salmon released in Lake Huron and Lake Michigan at low costs. Thermal marking may be combined with other tagging methods (CWT) to maximize information about survival, reproduction and movement salmon stocks (Courtney et al. 2000). Dry marking techniques also show great promise and may be less expensive than thermal marks as they do not require manipulation of rearing temperatures.

## Other Considerations For a Mass Marking Program

Review of the Great Lakes experience in mass-marking chinook salmon argues for consistency of methods among readers, and for measurement of error in making and interpreting marks. Most of the better marking techniques can be implemented provided adequate resources are dedicated to obtaining sufficient samples of the lake population, to estimating marking success rates through quality control and multiple marks, and to quantifying reader error. If thermal marks are used, digital technology can record and archive marks for multiple readers.

Blick and Hagen (2002) discussed the use of agreement measures for evaluating precision of otolith mark determinations, but their considerations are relevant for any marking technique. For thermal patterns, accuracy of the mark pattern classification depends on the prominence of the pattern, the methods used to prepare and view the patterns, and the training and experience of the personnel who determine the presence or absence of a particular pattern. Estimating accuracy rates is problematic when no secondary marking is available and no error-free standards exist. Agreement measures, such as kappa (K), provide a relative measure of the reliability of the determinations when independent readings by two readers are available, but the magnitude of K can be influenced by the proportion of marked fish. If a third reader is used or if two or more groups of paired readings are examined, latent class models can provide estimates of the error rates of each reader. Blick and Hagen (2002) illustrate the applications of K and latent class models for estimating contributions of hatchery-reared chum and sockeye salmon in Southeast Alaska.

Based upon our experiences working with CWT and OTC mark data from Lake Michigan and Lake Huron, we recommend effort be dedicated to providing annual estimates of mark success, mark recapture for each lake. The marking database should be centralized, well documented and easily available and interpretable by others. We close by offering several recommendations applicable for any marking technique chosen.

1. Standardize mark application procedures for both lakes Michigan and Huron.
2. Implement Quality Assurance – Quality Control at all hatcheries, and sample all lots.
3. Obtain Measures of between-reader error. Have more than one reader read all marks.
4. Standardize equipment and evaluation procedures among readers.
5. Follow recommended sampling protocal for adults suggested by Bence.
6. Standardize marking data, and maintain them in a central database that is documented and easily available.

# CONCLUSION

This study was conducted to evaluate potential methods for mass marking chinook salmon to estimate their natural reproduction in the Great Lakes Region. After extensive review, we conclude that thermal marking provides the most promising and effective tool for mass marking chinook salmon populations. Thermal marking provides a safe, long lasting mark. Application of thermal marks is relatively easy and inexpensive. Application of this method will require new expenditures at some hatchery facilities for heating or cooling, and will require investments in research and training. Results should prove beneficial when weighed against the costly process of continued failure to agree on, and implement common marking protocols for other methods. Regardless of the method chosen, it is essential for the success of the marking program to standardize marking techniques, ensure quality control within and among state and federal agencies, conduct frequent, timely checking and estimation of error rates, and have adequate spatial and temporal coverage of sample areas in lake and tributary habitats.

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Table 1. Estimated costs of marking chinook salmon in Lake Michigan and Lake Huron. Costs were calculated for marking and for sampling and detecting marks. Data on marking costs for OTC provided by James Johnson and Jory Jonas, Michigan Department of Natural Resources, and Michigan DNR Fish Marking Committee. Costs all other tags estimates reported by Hammer and Blankenship (2001). Detection costs of PIT tags are unknown.

| Method | Marking Cost/fish | Detection Cost per fish sampled[1] | Cost per 1,000 sampled per million marked[2] |
|---|---|---|---|
| Fin Clip | 0.025 | 0.00 | 25,000 |
| OTC | 0.069 | 3.00 | 72,000 |
| CWT-AD clip | 0.136 | 3.00 | 125,000 |
| Elastomer | 0.060 | 0.00 | 60,000 |
| Thermal | 0.001 | 10.50 | 11,500 |
| Strontium/ALC | 0.003 | 100.00 | 103,000 |
| PIT | 5.50 | ??? | 5,500,000 |

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[1] Include collection by headhunters and laboratory costs.

[2] Assumes 0.01% recovery rates for all methods to allow comparisons of costs.

Table 2. Percentage of hatchery chinook salmon marked by fin clip or oxytetracycline (OTC) in Lake Michigan (MI) and Lake Huron (HU) from 1990 to 1995. Total percentage of chinook salmon marked (total), total number stocked (N, millions) in each lake is presented at bottom. Agencies include Michigan DNR (MI), Indiana DNR (IN), Illinois DNR (IL), Wisconsin DNR (WI), Ontario Ministry of Natural Resources (OM).

| Lake | Agency | 90 | 91 | 92 | 93 | 94 | 95 |
|---|---|---|---|---|---|---|---|
| MI | MI | 97 | 100 | 100 | 100 | 100 | 100 |
| | IN | 100 | 100 | 100 | 100 | 0 | 0 |
| | IL | 100 | 100 | 100 | 100 | 100 | 100 |
| | WI | 100 | 100 | 100 | 100 | 13 | 12 |
| | Total | 99 | 100 | 100 | 100 | 68 | 61 |
| | N | 7.1 | 6.2 | 5.8 | 5.5 | 5.8 | 6.1 |
| HU | MI | 100 | 100 | 100 | 97 | 100 | 100 |
| | OM | 15 | 27 | 34 | 16 | 12 | 12 |
| | Total | 83 | 87 | 85 | 78 | 83 | 84 |
| | N | 4.8 | 3.9 | 4.0 | 4.3 | 4.4 | 4.7 |

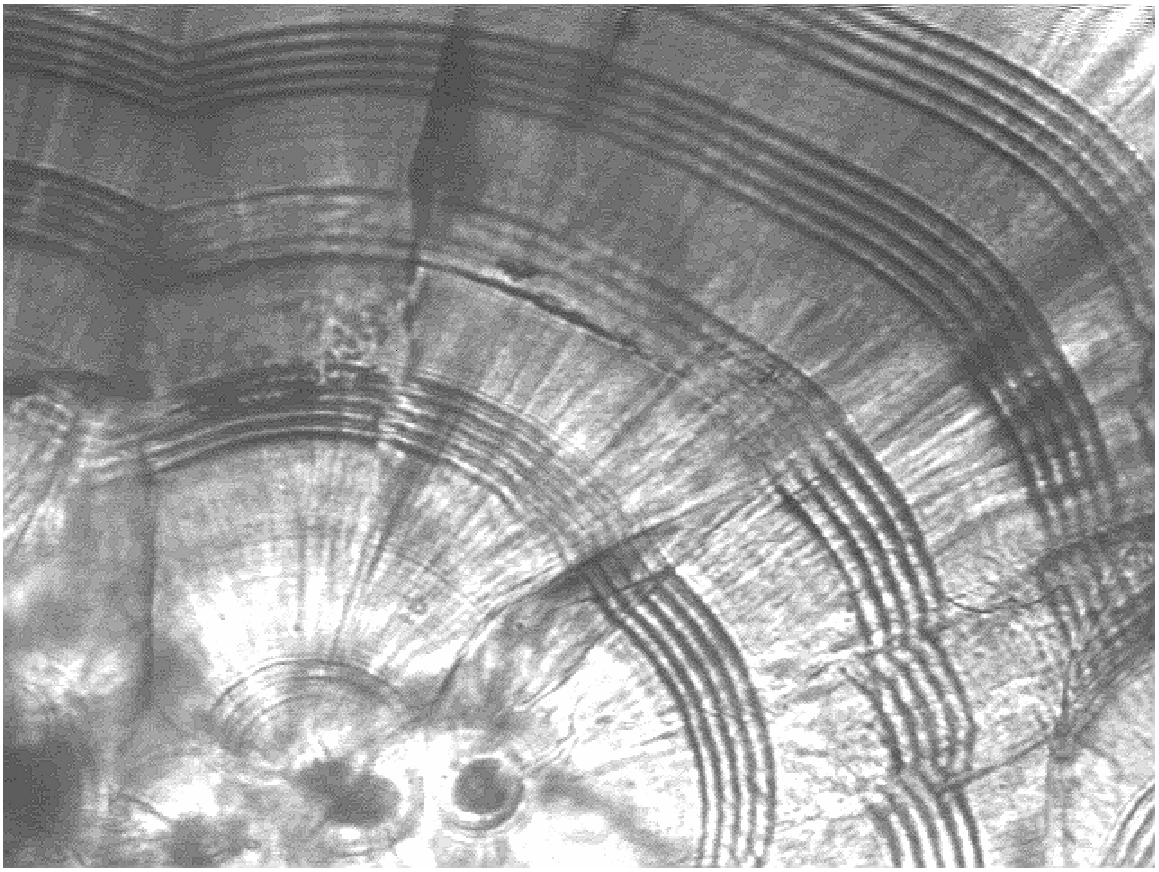

Figure 1. Thermal marks on otolith of chinook salmon. Banding patterns are five-day sequences of light otolith increments (generated by warm temperatures) followed by dark increments (generated by cold temperatures). The sequence of dark increments were produced by altering water temperatures from 16 hrs of ambient levels (53 ?F, or 11.7 ?C), to 8 hrs of colder temperatures (47 ?F, or 8.3 ?C). Light increments were produced under constant ambient conditions of 53 ?F.

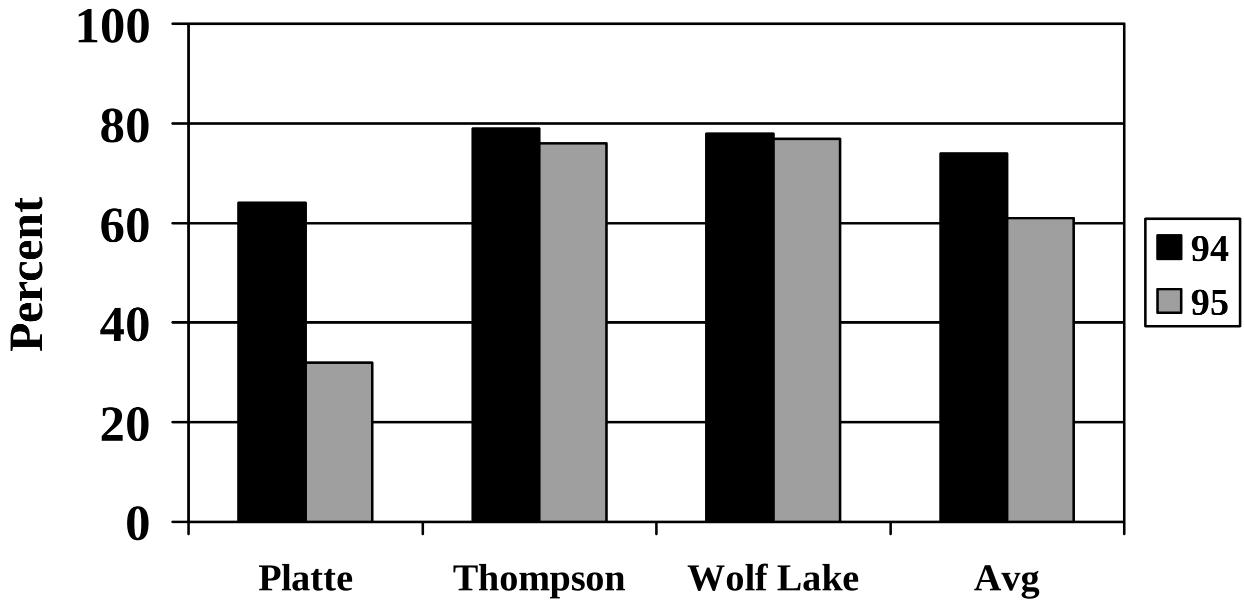

Figure 2. Variation in OTC marking success (% of total) of chinook salmon parr marked in Michigan hatcheries, 1994-1995. Avg = average percent marked.

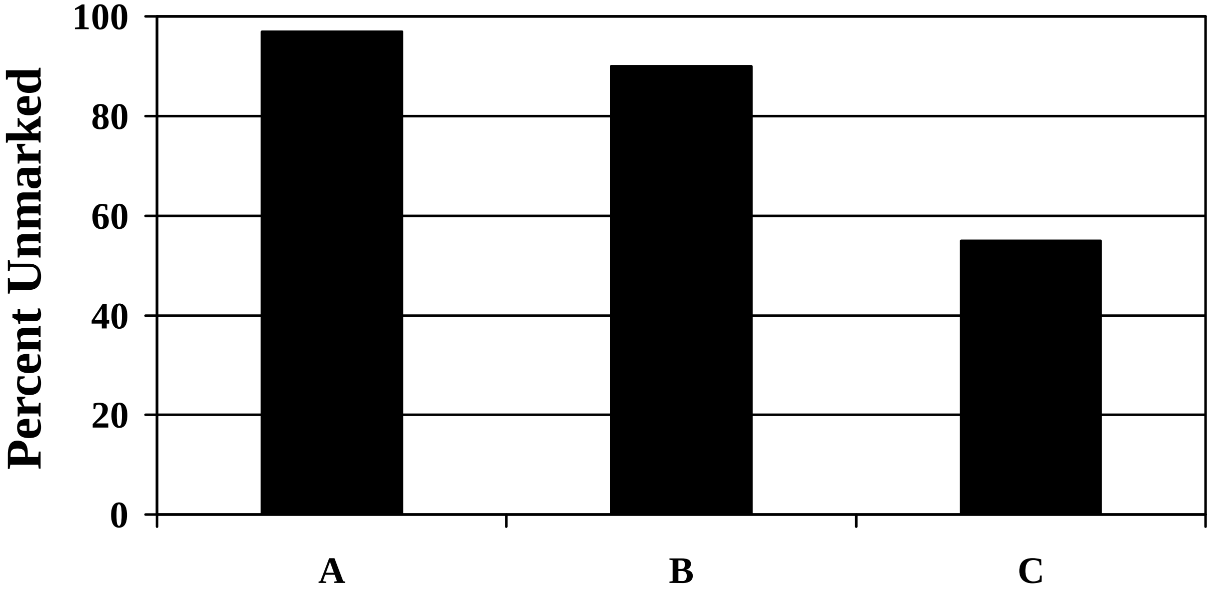

Figure 3. Variation among readers in percent of adult salmon captured in Muskegon River, 1997 that were unmarked by OTC. Reader A was a University of Michigan undergraduate student trained to detect OTC marked otoliths, readers B and C were Michigan DNR research technicians specializing in OTC mark detection.