# The use of oxytetracycline marking to monitor stocking success of walleye fry in eastern Manitoba

by

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# ABSTRACT

Walleye fry stocking is common practice but success is rarely monitored. Use of oxytetracycline (OTC) can be used as a marking tool to identify stocked fish from naturally produced fish and determine stocking success. Through a series of experiments, parameters in the marking methodology were assessed for their effect on mark quality and retention of the mark over time. Mark quality was improved by marking fry at three days post hatch. Water source also significantly affected OTC mark quality. The use of powdered OTC produced higher quality marks than the use of liquid OTC. Retention of OTC marks was related to the quality of the OTC mark. Electrofishing surveys conducted on five lakes found high recruitment (>80%) among stocked walleye on two lakes, with more 40% recruitment on a third lake. This study found that stocked walleye fry were successfully recruited into the age 0+ year class.

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# 1.0 Introduction

## *1.1 Walleye in Manitoba*

The walleye (*Sander vitreus*) is Manitoba's most sought after fish species, intensively fished by commercial, recreational and subsistence fishermen (Stewart and Watkinson 2004). The commercial walleye fishery on lakes Winnipeg and Manitoba is the second largest and most profitable inland fishery in Canada, next to the Great Lakes commercial fishery (Lemm 2002, Stewart and Watkinson 2004). The fishery employs over 3,500 licensed fishers and hired help, with an annual quota of 13 million kg for commercially viable species and an average landed value of 27.9 million dollars (Manitoba Water Stewardship 2012, Manitoba Water Stewardship 2013). Walleye contribute the highest proportion, approximately 44%, to the annual commercial fishing quota by weight and more than 70% to the average annual landed value of the industry in Manitoba (Manitoba Water Stewardship 2013). Recreational walleye fishing is popular with both resident and non-resident anglers and contributes more than 100 million dollars annually to the Manitoba economy (Manitoba Water Stewardship 2005). Walleye also play a critical role in the culture of aboriginal peoples in Manitoba and are a significant component of First Nations subsistence harvest (Green and Derksen 1984, Berkes 1990, Warkinton 1995).

## *1.2 Brief History of walleye stocking in Manitoba*

The popularity of walleye in Manitoba and throughout North America has created consistently high fishing pressure and the need for careful management of both the recreational and commercial fisheries. Consequently, localized stocking practices are utilized as a management tool. Walleye stocking in Manitoba dates back to 1913, when this species was first stocked into lakes in the southwest region of the Province with the goal of improving catches for both recreational and subsistence fisheries. When this study commenced in 2011, Manitoba stocked between 30 and 70 million fry annually, originating

from three hatcheries; the Swan Creek Walleye Hatchery, the Whiteshell Fish Hatchery, and the Grand Rapids Fish Hatchery (J. Long, Manager of Fisheries Science and Fish Culture, Manitoba Fisheries Branch, pers. comm., 2011). In October 2012, the Grand Rapids Hatchery moved under the direction of Manitoba Hydro, however this shift did not affect any of the numbers quoted in this study. Currently, more than 950 million walleye are stocked into water bodies across Canada (Kerr 2008).

The first dedicated walleye hatchery, the Swan Creek Hatchery, was built in 1929 on the southeast shore of Lake Manitoba (Figure 1), with the goal of providing additional fish for the growing commercial fishery (Smith 1990). The hatchery is operated on a seasonal basis, from early April to early June and largely produces walleye destined for Lake Manitoba (J. Long, pers. comm., 2011). Annually between 2010 and 2012, the hatchery stocked between 12 and17 million walleye fry into Lake Manitoba (Manitoba Water Stewardship 2010 - 2012). The hatchery also produces walleye for recreationally fished lakes in Duck Mountain Provincial Park, as well as other lakes in southwestern Manitoba (K. Dyck, Acting Hatchery Manager, Manitoba Fisheries Branch, pers. comm., 2011). In the decades following the construction of the Swan Creek Hatchery, the increasing popularity of sport fishing among growing rural communities encouraged fisheries managers to develop new angling opportunities (Smith 1990). With this in mind, the Whiteshell Fish Hatchery was built in 1942, and began producing a variety of trout species to be stocked into streams and lakes in western and southwestern Manitoba (Figure 1) (Smith 1990). In 1982, the hatchery began producing walleye for recreationally fished lakes, largely in southeastern Manitoba. The hatchery continues to rear walleye, and currently stocks fish in the Whiteshell Provincial Park, Nopiming Provincial Park, and Duck Mountain Provincial Park (K. Dyck, pers. comm., 2011).

Most recently, the Grand Rapids Fish Hatchery was built in 1967, in conjunction with the construction of the Grand Rapids Generating Station (Figure 1). The original goal of the hatchery was to stock both walleye and lake whitefish (*Coregonus clupeaformis*) into Cedar Lake in the effort to mitigate

negative effects of the generating station on the commercial fishery. The Grand Rapids Hatchery has also produced trout for stocking recreationally fished lakes in the northeast and northwest regions of the Province. Currently, the hatchery focuses on producing lake sturgeon (*Acipenser fulvescens*), which are stocked largely into the Nelson River.

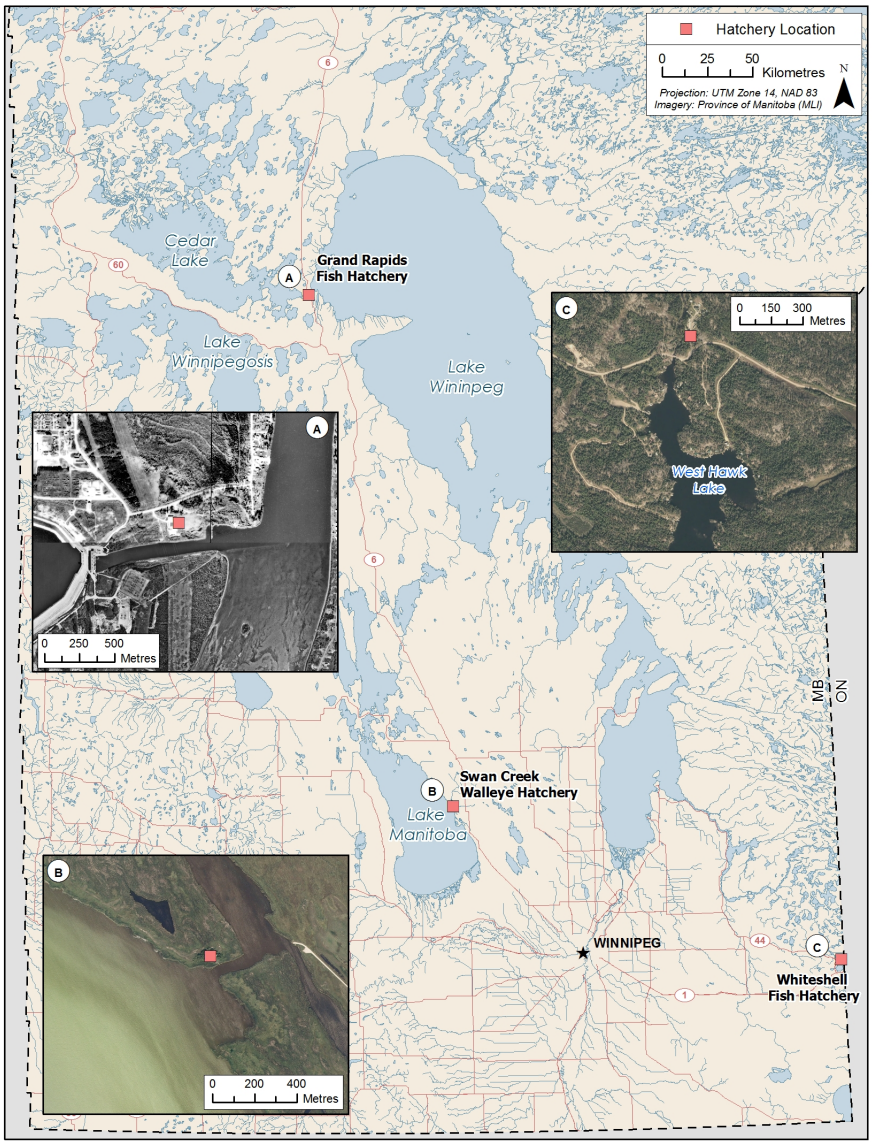


FIGURE 1. Location of operational fish hatcheries (Grand Rapids Fish Hatchery, Swan Creek Walleye Hatchery and the Whiteshell Fish Hatchery) in Manitoba as of May, 2014.

*1.3 The role of stocking in fisheries management*

Hatchery stocking is an important tool in fisheries management, alongside quota management and habitat rehabilitation (Molony et al. 2003). Each of these tools focuses on a different factor in fisheries management: hatchery stocking works to supplement fish stocks - either through increased abundance of existing stocks or the introduction of species, quota management works to maintain current stocks, and habitat management works to maintain/rebuild/create habitat for current stocks. Of these methods, hatchery stocking remains popular with both extractive and non-extractive stakeholders, due to its active approach to fisheries management, rather than a passive measure such as habitat conservation or a restrictive approach such as quota management. Hatchery stocking is also more visible and intuitive to the public imagination (put fish in, take fish out) than other management techniques, further fueling the popularity of stocking with the general public. The resulting popularity of hatchery stocking can lead to a lenient approach to monitoring the success of stocked fish, as it is a "win/win proposition" in which stocked fish either contribute to fishing opportunities for stakeholders, or do not, but still represent an active management style that is popular with stakeholders. However, for a stocking program to be truly successful, it has to meet the goals set out for the program, and it must do so without impeding the goals of other management practices (Waples 1999).

Stocking programs in Manitoba are designed to meet four goals, as stated by the Manitoba Fisheries Branch : the establishment of desirable species where there were none before, maintain fish numbers where natural reproduction cannot, supplement declining or over-harvested populations so that they can recover, and diversify fishing opportunities in areas where demand exceeds supply (Biggin 2009). The majority of walleye stocking into recreationally fished lakes in Manitoba are stocked in order to supplement existing populations, and would be defined as enhancement stocking. These stocked lakes either rely on stocking to maintain walleye populations throughout the year in lakes which experience winterkill, or use stocking as a tool to improve recreational fishing experiences. Walleye stocking into the

larger lakes in Manitoba, such as Lake Manitoba and Lake Winnipegosis would be a way to supplement harvest pressure on those fish populations, as well as tempering the impact of habitat degradation.

### *1.4 Stocking walleye for commercial fishing*

The supplementation of declining or over-harvested populations specifically targets those populations that are fished commercially. The majority of walleye reared in Manitoba are stocked into large commercially fished Manitoba lakes, such as Lake Manitoba and Lake Winnipegosis. For example, between 2010 and 2012, an average of 700,000 walleye fry were stocked into Lake Winnipeg at the Saskatchewan River annually, as well as 15 million into Lake Manitoba, 1.4 million into Lake Winnipegosis, 500,000 in Dauphin Lake and 1 million into Chitek Lake (Manitoba Water Stewardship 2010-2012). A number of small hatcheries have been operated by Fishing Co-operatives and First Nations communities over the past fifty years to supplement the stocking done by provincially-run hatcheries.

All of the stocked commercially fished lakes in Manitoba have some level of natural reproduction, with the exception of Chitek Lake (B. Galbraith, Manager of Commercial Fishing, Manitoba Fisheries Branch, pers. comm., 2011). Therefore, stocking is generally not done with the purpose of creating a population to be exploited by commercial fishing, but rather to supplement the population and increase recruitment. The spawn used to rear stocked fish are often are taken from lakes with large spawn runs, such as Falcon Lake and Lake Manitoba. Therefore, some of the stocking is seen as a way to recompense for spawn collection. For example, the Provincial Fisheries Branch has an informal policy that 10% of the number of fry produced from the spawn camp on the southeastern shore of the lake are stocked back into Lake Manitoba (J. Long, pers. comm., 2011). There is a strong feeling among commercial fishermen in Manitoba that stocking is effective, and therefore there is pressure to continue stocking commercially fished lakes, despite evidence that the practice may not be effective (Mathias et al. 1992).

*1.5 Stocking walleye for recreational fishing*

Walleye are Manitoba's most popular recreational fish (Department of Fisheries and Oceans 2010, Manitoba Fisheries Branch 2005). In 2005, walleye made up 51% of the twelve million fish caught in Manitoba and were the species most often retained by anglers (Manitoba Water Stewardship 2005). In order to meet this high demand, walleye are stocked into more than 50 recreationally fished lakes in Manitoba, most of which are located in the southeastern, western and southwestern parts of the province.

Over ten million walleye fry are stocked on an annual basis in Manitoba into more than fifty recreationally fished lakes (Manitoba Water Stewardship 2010-2012). Annually, angling contributes approximately $106.3 million to Manitoba's economy through spending on material and activities directly related to angling, such as boat rentals, fishing supplies and licenses. Anglers spent another $256 million on major purchases and investments related to recreational fishing, such as boating equipment, camping equipment and fishing equipment (Manitoba Water Stewardship 2005). The direct benefits of stocking are currently being studied in the RM of Rossburn, in western Manitoba (B. Bruederlin, Biologist, Manitoba Fisheries Branch, pers. comm., 2013) The stocking and aeration of four lakes (Rossman, Arrow, Tokaryk and Patterson), the first two of which are stocked with walleye, have considerably increased economic development in the area and led to benefits for the surrounding communities. The appeal of these stocked lakes extends not only to Manitobans, but draws anglers from throughout Canada and the United States (B. Bruederlin, pers. comm., 2011).

Anglers in Manitoba approve of stocking as a method of improving fishing activities. The Manitoba Angling Report (Manitoba Water Stewardship 2005) conducted a survey of residents who had been fishing in Manitoba for more than five years and of those surveyed, 12% felt angling had improved, 41% felt angling had stayed the same, and 39% felt angling had declined over the past five years. Those surveyed who felt angling had declined suggested that more stocking programs, closely followed by more

habitat improvement were the most important methods through which angling could be improved (Manitoba Water Stewardship 2005). This support for stocking amongst the angling public provides impetus for stocking to be relied on as a way of improving recreational angling in Manitoba.

### *1.6 The importance of monitoring hatchery programs*

Stocking programs are expensive, and unsuccessful stocking programs can be a drain on already limited resources in government departments. By monitoring the success of stocking, cost-benefit calculations can be made that can help determine the true contribution of stocking to commercial, recreational and domestic fishing opportunities. It is also important to follow the effect of walleye stocking on naturally reproducing populations, as stocking can depress surrounding year-classes and result in the decreased growth of stocked year classes (Li et al. 1996a, 1996b ). As well, highly publicized stocking programs have been shown to increase significantly recreational fishing pressure and could result in the reduction of total population abundance (Johnson and Carpenter 1994). Therefore, responsible stocking practices must include monitoring, in order to determine the relative success of the program and the effect on the population as a whole.

Of the methods used to monitor stocking success, the most common is using a marking technique to identify which fish have been raised in a hatchery environment and which were produced naturally. A number of marking techniques have been used over the years to measure stocking success, including fin clips, coded-wire tags, genetic marking, the chemicals calcein and alizarin red, and strontium chloride (Fielder 2002, Brooks et. al. 1994, Shroder et.al. 1994). Of these, marking with oxytetracycline has become the most common, likely due to its relative ease of use and low cost (Brown et al. 2002).

Oxytetracycline (OTC) is an antibiotic with fluorescent properties which can be absorbed by fish aging structures, such as otoliths. Otoliths, also known as earstones, are calcified structures located in the

inner ear and are used by the fish for both hearing and balance (Campana 1999). Otolith growth occurs through accumulation of calcium carbonate from the blood stream, as well as accumulation of other environmental elements (Campana and Nielson 1985). Oxytetracycline is applied to otoliths by immersing fish in a bath of OTC for four to six hours, during which time the OTC becomes permanently incorporated into the calcium carbonate matrix of the otolith and bone matrixes. In Manitoba, all stocked walleye have been marked with OTC since 2006. There has been some monitoring of stocked walleye since that time; however, mark recovery has been sporadic, even on those fish known to be marked with OTC. Variation in mark quality is fairly common among OTC marking studies, but the analysis of preliminary data found such low mark recovery rates from the Whiteshell Hatchery that the majority of the samples had no *functional* mark. Personal accounts from Fisheries Branch employees suggest that high mark rates have been achieved at the Swan Creek Hatchery in recent years, but these results have not been well documented. The low rate of mark identification among known marked walleye (i.e. false negative identification of marks) from the Whiteshell Hatchery, combined with the lack of data regarding the marking success at other hatcheries in Manitoba, indicate that the marking program in Manitoba needed to be reassessed in order to confirm the ability to consistently produce viable marks that correctly identify hatchery reared fish.

### *1.7 Objectives*

The objective of this thesis is to develop an oxytetracycline marking protocol for Manitoba that meets the five criteria originally set out in Everhart and Youngs (1981, cited by Younk and Cook 1991), apply this protocol effectively and use the subsequent identification of stocked fish to measure the success of Manitoba's stocking program in selected stocked water bodies in the eastern region of the province. The criteria for a successful marking protocol are: 1) relative ease of mark detection and identification; 2) high mark retention over time; 3) rapid application of mark; 4) ability to accurately identify unique groups of fish; and 5) minimal effect on fish behavior and wellbeing (Everhart and Youngs 1981, cited by Younk

and Cook 1991). The following chapter will provide a review of literature regarding walleye stocking, marking and investigations into stocking success. Chapter three describes an experimental investigation of factors in the OTC marking process to determine the influence that these factors have on OTC mark quality. This chapter also includes an examination of OTC treatment on walleye fry mortality. Chapter four investigates the retention of OTC marks over a ten week period, as applied through a series of experimental treatments. Chapter five tests the ability of OTC marking to differentiate stocked fish from naturally reproduced fish, and provides an estimate of the contribution of hatchery-reared fish to stocked water bodies. The goal of this thesis is to develop the means with which to measure stocking success, through oxytetracycline marking, as well as conduct an analysis of the current level of stocking success. Hopefully, this research will provide both the data and structure needed to develop an effective oxytetracycline marking program for Manitoba hatcheries and provide verification of the marking and stocking programs' effectiveness.

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# 2.0 Literature Review

## *2.1 Walleye stocking in North America*

Current reports suggest that walleye stocking continues to be a popular management tool (Fenton et al. 1996, Kerr 2008). In 2006, more than 850 million walleye were stocked throughout five Canadian provinces and 36 states (Kerr 2008). In Minnesota alone, the Department of Natural Resources (DNR) stocked more than 900 lakes with walleye fry and fingerlings (Jacobson and Anderson 2007). The Wisconsin DNR stocks more than 30 million walleye annually into over 170 lakes and 11 rivers (WDNR unpublished *in* Fayram et al. 2006, Kerr 2008). The majority of the walleye stocked in North America are released as fry (Kerr 2008); almost 98% of walleye stocked in Minnesota since the 1950's were stocked as fry (Li et al. 1996). However, despite the prevalence of walleye stocking throughout the North America, there are few documented successes. In the case of stocking walleye fry, as opposed to fingerlings, the evidence for success is particularly lacking. In addition, reviews of walleye stocking suggest although stocking can be used to rehabilitate struggling stocks or to introduce walleye into new areas, stocking to bolster a population with existing natural reproduction is generally unsuccessful (Laarman 1978, Kampa and Jennings 1998). Yet, the low number of success stories has not deterred the majority of fisheries departments from continuing not only to stock walleye, but to stock largely with the goal of creating and supplementing recreational fisheries (Fenton et al. 1996, Kerr 2008). The reason for this incongruence appears to be that individual fisheries management agencies remain under political pressure to stock walleye due to belief among anglers that stocking is an effective tool to bolster walleye stocks (Quinn 1992, Welcomme and Bartley 1998, Parsons et al. 2001). In light of this contradiction in theory and practice, it is valuable to evaluate past success and failures with stocking walleye, and determine whether Manitoba waterbodies can benefit from this practice, and how to tailor this practice to ensure success.

*2.2 Defining stocking success*

Stocking success is and yet, is not, a goal in and of itself. Ultimately, success must be measured as the ability to meet a goal, therefore, stocking success must be measured as the ability to meet a stocking goal. Therefore, in order to measure the success of a stocking program, there must be a goal which the program is striving to meet, and only once that has been defined, can one measure the relative success of the program as a function of the ability to meet that goal (Waples 1999). However, stocking success has not always been measured as such. Li et al. (1996a) summarized the ways in which a number of fisheries agencies measured the success of their stocking programs. Koppleman et al. (1992), Mathis et al. (1992) and Mitzner et al. (1992) measured success as the rate of survival of stocked fish, with high rates of survival suggesting a successful stocking program. Brooks et al. (2002) measured stocking success as the survival of one size of stocked walleye relative to another. Many researchers have considered stocking programs which contributed to walleye year-class abundance to represent a successful stocking program (Johnson 1971, McWilliams and Larsceid 1992, Parsons 1994, and Kampa and Jennings 1998). When the target population has low reproductive capacity, Bennet and McArthur (1990) considered the ability to establish a naturally reproductive and hence self-sustaining population to indicate the success of a stocked program. A number of studies have looked at quantifying the contribution of stocked fish, and ultimately the success of stocking these fish, by investigating survival and growth during the first year after stocking (Fielder 1992a, Fielder 1992b, Kayle 1992 and LaJeone 1992). The benefit of studying the contribution at this early stage is two-fold: first, survival from egg to fall age 0 is believed to be a good indicator of survival in future years (Quist et al. 2004, Lorenzen 2005), and second, it provides an estimate of success that can be quantified within the same year as stocking. The immediacy of this estimate is beneficial when the results could affect the goals of the stocking program for the following year. Ultimately, the majority of stocking programs, including those in place in Manitoba, do not have clearly outlined goals and therefore seem to work towards the goal of providing walleye for future harvest (Brooks et al. 2002).

*2.3 Creating meaningful goals for stocking programs*

Ultimately, the success of any stocking program depends on the program's ability to meet clearly defined goals while not impeding the progress of other management practices and public interests (Waples 1999). In order to develop goals, cost-benefit analysis can be conducted to assess the benefits and risks of any policy decisions within a structured framework. Waples (1999) suggests three main areas that could be assessed for cost-benefit analysis: 1) fiscal expenditure (measured in currency, such as dollars); 2) society at large (only some of which is valued in currency); and 3) natural populations and ecosystems (which cannot be measured effectively by dollars). The net benefit of any particular program can be determined by the general equation:

Net benefit = (benefit to society + benefits to natural population + benefit to ecosystems) – (costs to society + costs to natural populations + costs to ecosystems)

Common benefits of hatchery programs include reducing the threat of extinction or fishery collapse (conservation), the creation of more fish for both commercial and recreational harvest (enhancement), jobs for fishers/fish culturists/fish biologists, the ability to meet legal and treaty obligations, as well mitigating the negative effects of habitat degradation and loss (Waples 1999). Cost may include financial expenditures, loss of genetic diversity/fitness to native populations, possible transfer of disease and parasites, as well as the costs of not stocking, such as the potential of fishery collapse or extinction where stocks are not supplemented by hatchery production if stocking programs are not present or discontinued (Waples 1999).

For many stocking programs, this type of cost / benefit analysis has not been used to define goals, but rather the focus has been on producing large quantities of fish (Molony et al. 2003). This focus may be largely due to the fact that many stocking programs have been in place for over 50 years, or over 100 years, as is the case in Manitoba, and that the momentum from years of experience is driving the program

rather than a detailed cost benefit analysis and careful examination of the goals set out by the program (Waples 1999). Thus, the focus on hatchery production numbers is in most cases a relic of an older style of hatchery system, in which stocking was used rather indiscriminately to deal with issues of overfishing, habitat degradation and other causes of population decline. The focus on fish production numbers in many hatchery systems has led to a focus in hatchery research and budgeting on husbandry techniques and equipment, however there has been little effort in many jurisdictions to monitor the success of stocked fish (Waples 1999). Thus, most hatchery programs in North America do not have long term monitoring programs, and are largely running on the assumption that increasing production numbers equals increasing success of stocked fish (Welcomme and Bartley 1998).

### *2.4 Unsuccessful walleye stocking*

It may not be a surprise, in light of the focus on producing large numbers of fish and with limited monitoring of the success of stocking programs, that a large majority of stocking programs assessed for relative success were ultimately deemed unsuccessful. A review of stocking success was done by Laarman (1978), who classified stocking into three categories: introductory, maintenance and supplemental. He found that introductory stocking, defined as the introduction of walleye to new waterbodies, was relatively successful, with 48% of those studies reviewed reporting a favorable outcome. Maintenance stocking, defined as stocking walleye into water bodies without existing reproductive populations, but which have been previously stocked, was successful on average 32% of the time (Laarman 1978). However, supplemental stocking of walleye into waterbodies with naturally reproductive populations was deemed successful only five percent of the time (Laarman 1978). In their review of walleye stocking, Ellison and Franzin (1992) similarly found that 32 % (11 of 34 studies in question) reported success in stocking walleye fry, the majority of which would be classified as maintenance stockings. This is valuable to note as the majority of walleye stocking in Manitoba for recreational purposes would be defined by Laarman (1978) as supplemental stocking.

In their review of over 1700 walleye stocking records from Minnesota, Li et al. (1996a) found that stocking into lakes with existing natural reproduction had no effect on population abundance. Worse yet, stocking fry into lakes with natural reproduction decreased the mean weight of fish in that year class (Li et al. 1996a). In a similar study, Li et al. (1996b) found that although stocking walleye fry did increase the strength of the stocked year-class in lakes with existing natural reproduction, the surrounding year classes were significantly decreased. They suggested that the higher densities of walleye in the stocked year class could increase density dependent effects, thus negatively impacting the strength of surrounding year classes (Li et al. 1996b). Density dependent effects have also been shown to negatively impact growth in wild trout, when population density was increased by stocking hatchery trout (Bohlin et. al. 2002). It is well understood that populations cannot grow without limit, as growth at some point will be met with density dependent effects, such as decreased survival, growth, and fecundity, or increased migration (Bohlin et. al. 2002). However, stocking often occurs with the assumption that the population can simply be increased by adding more fish, despite the understanding of density dependent effects, which ultimately maintain a population within the carrying capacity of the current environment.

### *2.5 Relative stocking success*

Despite the suggestion that supplemental stocking in particular is less than successful, there have been individual cases in which supplemental stocking proved successful, according to the criteria set out by the researchers. One important example of stocking success, which later became the basis for the evaluation of fry stocking in Manitoba, is a study conducted by Lucchesi (2002) (W. Biggin, Information Technologist, Manitoba Fisheries, pers. comm., 2011). Lucchesi (2002) reported high contribution (93 %) of stocked fry to the fall age-0 year class, resulting in moderately to strong year classes for 11 out of 12 lakes stocked, all of which contained naturally reproducing walleye populations and had previously been stocked with either fry or fingerlings. Lucchesi (2002) also reported stronger year classes in lakes

which were stocked with fry relative to those stocked with fingerlings, although he suggests that differences in stocking densities between fry and fingerlings may explain this observation. Despite the relative success of this stocking program, Lucchesi (2002) admits that large year classes of fall age-0 walleye do not necessarily translate into large adult walleye populations. After further analysis of gill-net CPUE, Lucchesi (2002) found that contribution of stocked walleye fry to the adult year class would be more in line with previous literature, such as that of Laarman (1978) and Ellison and Franzin (1992).

Logsdon (2006) documented the effective use of stocking fry in Red Lake, Minnesota. Stocked fry contributed more than 80% to the stocked year class in two years of the study. The first year in which walleye fry were stocked was 1999 during which the study documented the largest CPUE in the study lake in fifteen years (Logsdon 2006). Of this year class, 86% had observable oxytetracycline marks, thus identifying them as stocked walleye (Logsdon 2006). After five years of stocking walleye fry, hatchery-reared walleye had been recruited into the naturally reproductive population, the study was concluded and considered highly successful (Logsdon 2006). However, despite stating a successful conclusion to the Red Lake stocking program, he did acknowledge a few weaknesses observed in stocked walleye, including significantly different relative growth rates and slightly lower maturity rates when compared with naturally produced fish (Logsdon 2006). Overall, the Logsdon study (2006) supports the hypothesis that stocked walleye can contribute to the total walleye population and aid in the recovery of walleye populations in lakes with depleted populations, as well as illustrates some of the potential negative impacts of stocking.

Mathis et al. (1992) studied the effect of stocking walleye fry into Dauphin Lake in order to supplement the winter walleye fishery. This is the only example in the literature in which stocking was used to bolster a largely commercial fishery (Fenton et al. 1996). The results of this study estimated the survival of stocked fry to age 3 at less than 0.04% and suggested that over the ten year period during which stocked walleye would be susceptible to the commercial fishery, they would contribute only 80 kg

to the total annual harvest of approximately 13,000 kg (Mathias et al. 1992). The resulting cost benefit ratio of stocking walleye fry in Dauphin Lake was 2.6 : 1, for which every dollar of improvement in the commercial fishery would require an investment of $2.60 into stocking programs (Mathias et al. 1992, Lemm 2002). However, recalculating the cost benefit ratio of stocking walleye in Dauphin Lake for recreational fishing purposes yields a new cost benefit ratio of 1.2 : 1, based on an estimated value of $6.35/ kg walleye to the sport fishery in Canada (Mathias et al. 1992). The difference between these cost benefit ratios highlights that the value of stocking depends on the purpose of stocking, although in this case, stocking to contribute to both commercial and recreational fisheries had higher total financial costs than benefits.

### *2.6 Negative consequences of stocking*

In light of the relatively few examples of walleye fry stocking success, it must be acknowledged that there can be negative consequences to stocking beyond low success rates, and these consequences must be factored into the decision of whether to stock or not. Rearing walleye in hatcheries may reduce avoidance responses to predation (Wisendon et al. 2004). Juvenile walleye use chemical cues, such as the compounds given off by predators, as well as the "alarm" chemicals given off by conspecifics in the face of a threat, to format a response to predation (Wisenden et al. 2004). The ability to recognize these cues is a learned trait and requires exposure to the threat of predation (Wisenden et al. 2004). Therefore, walleye that are not exposed to predators during their developmental stage, such as those reared in a hatchery environment, may be more susceptible to predation than those raised in a natural setting.

Stocking also has the potential to affect the genetic fitness of the target population (Cena et al. 2006). In Ontario, hatchery operations often take walleye brood stock originating from a single lake and distribute the resulting fry to a series of lakes, sometimes on opposite ends of the province and in very different lake systems than the lake of origin (Cena et. al. 2006). Thus, there is the potential for hatchery

stocking to reduce the genetic diversity of walleye between lakes, as well as within lakes, however this has not been formally documented (Cena et. al. 2006).

### *2.7 Use of oxytetracycline as a marker*

The first recorded use of tetracycline drugs to mark fish was by Weber and Ridgeway (1962), who developed a marking technique that followed previous studies on the use of tetracyclines to mark bird and mammal bones. Weber and Ridgeway (1962) noted that the use of tetracyclines to mark fish circumvented two previous issues with fish marking. Firstly, the ability to mark fish by adding tetracycline to their diet or through an immersion process reduced the stress and mortality associated with handling. Secondly, creating an internal mark avoided the complications that arose with external marks, such as physical damage and potential for differential survival (Fry 1961 in Weber and Ridgeway 1962). In the years following the study by Weber and Ridgeway (1962), marking fish with tetracyclines, particularly oxytetracycline (OTC), has become a common and accepted technique for stock determination (Muncy et al 1990). OTC marking is now one of the most common ways of marking stocked walleye due to its relative ease of use, low associated mortality, and low cost (Fielder 2002).

Oxytetracycline can be applied to fish through a number of methods, such as injection, immersion in solution and incorporation in food, all which result in the uptake of OTC in proliferating bone tissue (Younk and Cook 1991). The most common technique for marking recently hatched fish is immersion in a solution of OTC, which allows hatcheries to mark large numbers of fish at a time, and is thus ideal for marking fish at early developmental stages (Guy et al. 1996). The parameters of this technique, such as the length of immersion time, age at which fish are marked and concentration of OTC are varied throughout the literature; however, in recent years, methods for marking walleye fry and juveniles have been largely based on early experiments by Brooks et al. (1994). In a series of experiments, Brooks et al. (1994) marked walleye fry and juveniles in various concentrations of OTC and varied the lengths of

immersion time. They reported the resulting mark quality, mark rate and survival post immersion, concluding from these experiments that immersion of walleye fry in 500 mg/L OTC for 6 hours at 4-5 days post hatch produced a high percentage of marked fry with low mortality and high quality marks on otoliths.

*2.7.1 Mark quality*

Brooks et al. (1994) reports that prior to the publication of their study, there had been limited use of oxytetracycline marking techniques in the literature. This absence was due to the high variability of OTC deposition and the resulting inconsistency of fluorescent intensity (Brooks et al. 1994). Variability in mark quality was also noted by Weber and Ridgeway (1962), Secor et al. (1991), Campana and Neilson (1982), Hettler (1984), Kayle (1992) and Dabrowski and Tsukamoto (1986), and by Conover and Sheehan (1996), Reinart (1998), Brown et al (2002), Denson and Smith (2008), D. Isermann (Professor of Fisheries, University of Wisconsin-Steven's Point, pers. comm., 2011), D. Logsdon (Fisheries Research Biologist, Minnesota Department of Natural Resources, pers. comm., 2011). Despite the conclusions of Brooks et al. (1994) and the subsequently recommended marking protocol, it is still unclear to what degree additional parameters in the marking methodology and mark observation process may result in mark variability. The parameters in question include the age of fish when marked, the density of fish in the oxytetracycline solution, the type of OTC chemical used, source of the water used in the marking solution, as well as the experience of the person analyzing structures, sensitivity of the florescence detection equipment and the length of time between marking and when the mark is observed.

*2.7.2 Age*

The age at which fish are marked can affect the mark rate, quality and retention over time. This has been noted by Dabrowski and Tsukamoto (1986), who observed increased mark retention when marking coregonid larvae compared with marked embryos. Brooks et al. (1994) attempted to mark

otoliths of walleye prior to hatch, but were unable to distinguish a mark. Brooks et al. (1994) also made a distinction between the success rate of fry marked at 1-3 days post-hatch, which was less than 100% (although exact numbers were not provided) and fry marked at 4-6 days post hatch, which had 100% mark rate. Fielder (2002) suggested marking fry at 5 days post-hatch for best results, but that younger fry (3-4 days) would still retain a mark. However, Fielder (2002) cautioned that 1-2 day old fry are "inadequate" for marking. Marking fry at 3-5 days requires that hatcheries have the holding capacity to retain fish post-hatch until they are old enough to mark; however, not all hatcheries have the space and time for the additional handling this process requires. In order to circumvent this problem, Logsdon et al (2004) experimentally marked fry at less than 24 hours post-hatch, in a concentration of 700 mg OTC/L and found that 100% of marked walleye fry otoliths had an observable mark, however these resulting marks were of variable quality.

*2.7.3 Density*

The density of fry in solution is an additional variable in the marking process that may account for the variation in mark rate and quality observed in the literature. Logically, high densities of fry in the marking solution compared with lower densities at a similar concentration of OTC would result in reduced availability of OTC, as it is taken up by fry. Recommendations have been made as to the density of walleye fry in solution that will produce a high rate and quality of mark. Lucchesi (2002) found a 100% of marked fry exhibited an identifiable mark when marked at a density of 2,000 fry/L at 700 mg/L OTC, but did not supply experimental results to support this as the upper density limit. A more conservative density was suggested by Fielder (2002) of 1,000 fry /L at 500 mg/L OTC, but similarly without experimental evidence. Logsdon (2006) marked fry at a comparatively higher density of approximately 4,400 fry/L, and a concentration of 700 mg/L OTC and reported high quality marks. OTC marking protocol in Manitoba hatcheries since 2006 has been to mark fry at a density of 6,600 fry/L at 1,400 mg/L, following the density recommended in Logsdon (2006). However, discussions with hatchery staff

suggest that the density in practice may be closer to 100,000 fry/L or more (D. Bilenduke, Retired Whiteshell Fish Hatchery Manager, Manitoba Fisheries Department, pers. comm., 2010).

### *2.7.4 Chemical Type*

The type and brand of oxytetracycline varies between studies, and although there has been little mention of the effect of the exact form of OTC used, communication with Isermann (pers. comm., 2011) and Logsdon (pers. comm., 2011) suggested that various brands and formulas of oxytetracycline influence the effectiveness of marking. For example, Logsdon (2006) observed a reduction in mark quality when fish were treated with Oxymarine (Alpharma, Bridgewater, NJ) formula OTC instead of the previously used Terramycin-343 (Pfizer Inc. New York, NY). The powdered form of OTC is the most common form used for marking (D. Isermann, pers. comm., 2011) but can cause issues in the marking procedure such as causing a lethal drop in pH and excess foaming of the solution. Both Fielder (2002) and Carty (2007) found that the drop in pH can be augmented by adding sodium dibasic in a 1:1 ratio with OTC. Fielder (2002) and Logsdon (pers. comm., 2011) recommend the use of No-Foam, a commercial anti-foaming agent, to prevent the solution from foaming. Manitoba hatcheries have been using a liquid pre-buffered form of OTC in order to avoid having to buffer the solution and use an anti-foaming agent. The use of pre-buffered liquid formula may influence the variability of mark quality and rate of marking.

### *2.7.5 DMSO*

Scidmore and Olsen (1969) reported that uptake of oxytetracycline was more consistent and less time was required to achieve a successful mark when dimethyl sulfoxide (DMSO) was added to the marking solution. They tested concentrations of 0, 2, 2.5 and 3% DMSO with 200 mg/L OTC and found that they could not produce good quality marks with concentrations of DMSO less than 2%, and that marks from solutions without DMSO produced less than 100% mark rate and that the quality of marks was highly variable. Since Scidmore and Olsen (1969) first reported the use of DMSO in OTC marking,

there has been no mention in the literature of its use until Kayle (1992), who had success in marking fish at 40 days post hatch with 0.81% DMSO and 200 mg/L OTC. The action of DMSO in the OTC marking solution is likely due to its ability to act as a solvent and to cross through cell membranes (Wood and Wood 1975).thus giving it the ability to potentiate and improve uptake of OTC from solution.

*2.7.6 Water source*

The water used at the Swan Creek and Whiteshell Hatcheries are significantly different from one another and may account for some of the variation in marking rate and intensity between hatcheries. The Swan Creek facility is located on Lake Manitoba (Figure 1), and uses a combination of lake and well water in its marking solution. The ratio of these water types varies from year to year, and throughout each spawning season in order to maintain temperatures ideal for fry and eggs. The Whiteshell Hatchery is located in the Whiteshell Provincial Park, between West Hawk and Caddy Lake, on the Canadian Shield (Figure 1). Water at the hatchery is taken from West Hawk Lake, and has relatively low alkalinity, metals and TDS compared with water from Swan Creek. The differences in water chemistry between these two hatcheries may account for the variation observed in preliminary data and reports.

The potential relationship between water source and the variability of OTC marks requires some understanding of how oxytetracycline is incorporated into otoliths. Otoliths are largely made up of calcium carbonate (96%), as well as 3% organic make up and less than 1% non-organic trace elements (Campana 1999). It has been suggested that tetracycline molecules bind to calcium on the collagen fibrils of the developing bone matrix via the naphthacene carboxamide nuclei of the tetracycline molecule (Milch et al 1957). The benefit of tetracycline molecules as antibiotics derives from their molecular structure and spatial arrangement which allows them to be "chemically promiscuous" and form complexes with macromolecules and cations such as $Ca^{2+}$ and $Mg^{2+}$ (Nelson 1998, Lunestad and Goksayr 1990, Arias et al. 2007). These complexes can act as ionphores and facilitate transport of $Ca^{2+}$ and $Mg^{2+}$

through lipid membranes, and can essentially act to deliver $Ca^{2+}$ to biological targets (Nelson 1998). Therefore it is likely that tetracycline is incorporated into newly developing bone as a complex with $Ca^{2+}$, rather than binding with $Ca^{2+}$ once incorporated into the otolith. This hypothesis is supported by the observation of tetracycline florescence not only in areas of bone mineralization, where calcium phosphate is added to the bone matrix, but also in diffuse regions of bone closely following the distribution of calcium from secondary mineralization and long term exchange (Harris et al. 1962).

However, despite the suggestion of Nelson (1998) that tetracycline $Ca^{2+}$ complexes may act to facilitate transport of cations such as $Ca^{2+}$ through biological membranes, Lambs et al. (1988) suggested that tetracycline bound in complexes with $Mg^{2+}$ and $Ca^{2+}$ have a reduced ability to diffuse through biological membranes, due to the resulting change in charge and decreased lipid solubility. The validity of this suggestion was confirmed by J. Betteridge (Phd Candidate, University of Manitoba, pers. comm., 2011), who asserted that a complex of oxytetracycline with some other cation would become polarized, such that diffusion through the non-polar cell membrane would be less likely. In addition, Lunestad and Goksayer (1990) found that the antibiotic activity of OTC was significantly reduced in sea water, likely due to complex formation of tetracycline with $Mg^{2+}$ and $Ca^{2+}$ (Lunestand and Goksayr 1990). The effect of salinity on OTC mark quality was investigated in the work of Thomas (1995) and Denson and Smith (2008), who both observed that high salinity OTC marking solution reduced the mark quality of larval red drum *Sciaenops ocellatus*. Similarly to Lunestad and Goksayr (1990), Denson and Smith (2008) felt the reduced activity of OTC was due to the formation of tetracycline $Mg^{2+}$ and $Na^{2+}$ complexes. This is in contradiction with views expressed by both Isermann (pers. comm., 2011) and Logsdon (pers. comm., 2011), who suggested that soft water, with low concentration of $Mg^{2+}$ and $Ca^{2+}$, resulted in poor mark quality. Isermann (pers. comm., 2011) also suggested that TDS seemed to play a role in mark efficacy, with increased levels of TDS generally resulting in higher mark efficacy rate and mark quality. A possible explanation for these seemingly contradictory accounts may be that the location in the pathway from solution to otolith in which the tetracycline molecule becomes bound with $Ca^{2+}$ may determine the

bioavailability of the tetracycline complex. If oxytetracycline becomes bound with $Ca^{2+}$ in the marking solution, it may be that the complex is rendered unable to cross biological membranes due to a change in charge in the complex. However, if this binding with $Ca^{2+}$ occurs further along the pathway, such as between the blood and the endolymph surrounding the otolith, the complex may pass cell membranes through some sort of active transport.

*2.7.7 Objectivity and the art of mark identification*

Despite the many possible parameters within the marking methodology, a large source of the variation in mark quality and rate may be the process of mark identification. It has been acknowledged that marks can be misidentified, both by observing a mark where there is none (false positive), or incorrectly judging an otolith as unmarked, when it was marked (false negative) (Brooks et al 1994, Reinert 1998, Fielder 2002). The ability to differentiate accurately between marked and unmarked fish is essential when using oxytetracycline marking to determine fish origin (Reinert 1998). As discussed previously, the marking methodology must be such that a fluorescent mark is produced and that the mark is sufficiently intense as to be identified. However, even if the mark is present, other factors in the observation process can affect the accuracy of mark identification. The presence of a stocking stress mark, a dark band with greenish fluorescence which appears to be created while fish are stocked, was noted by Fielder (2002) to have the potential to be misidentified as an OTC mark. Other studies also observed a darker ring near the center of the otolith that corresponded with an OTC mark, but did not display the distinct gold color of tetracycline fluorescence (Logsdon et al. 2009, Dabrowski and Tsukamoto 1986).

Misidentification of the stress mark for an OTC mark is not a significant error, as the stress mark also indicates the fish are of hatchery origin. However, wild fish can also display similar stress marks for unknown reasons, which could then represent false OTC marks (Fielder 2002). Therefore it is essential for the reader to be able to recognize the distinct golden color of an OTC mark as separate from the

greenish color of autofluorescence caused by checks or impurities in the otolith. The more experienced a reader is, the more likely they will be able to correctly identify marked otoliths (Hawkins 2002). This correlation between experience and correct identification is in part related to the knowledge and application of otolith preparation techniques. Denson and Smith (2008) demonstrated the connection between the level of otolith preparation and the resulting intensity of the OTC mark. Due to these various factors, Reinert (1998) stresses the importance of supporting the use of oxytetracycline as a marking technique with experimentally determined mark detection, such that the error rate when differentiating between wild fish and marked hatchery fish can be properly accounted.

### *2.7.8 Mark retention*

Another factor in the variability of OTC mark quality is the time between when the fish is marked and when that mark is observed. Denson and Smith (2008) evaluated mark retention on larval red drum otoliths over a twenty month period, and found that mark detection diminished over time, from 100% mark detection at 3 months post marking, to 20-30% mark detection at 20 months post marking. The authors state the difficulty of reaching the nucleus of the otolith, where the mark will be present, in older fish, and suggest this may have been why mark detection decreased with fish age. They note that other researchers, such as Jenkins et al. (2002), have had higher mark detection at older ages by marking fish as juveniles, such that the OTC mark is larger and more easily visible. However, mark retention does not always decrease over time, as Mauk (2008) found that mark rate increased from 74% to 95% respectively on fingerling palmetto bass at 14 days and 344 days post-mark.

## *2.8 Conclusion*

As long as fisheries agencies continue to use walleye stocking as a management tool, there will be value in measuring the success of stocking programs. Walleye stocking has not been successful in all cases and has even been associated with such negative impacts as decreasing the weight and strength of

surrounding year classes (Li et al. 1996 a, b), as well potentially impacting the genetic diversity of target populations (Cena et al. 2006). Even when stocking has been successful, there is often little thought as to what our definition of success is, and whether stocking has truly met the intended goals. Hilborn (1992) suggests that it can be difficult for fisheries management agencies to learn from experiences, due to an inability to retain lessons learnt in the institutional memory. What we are reiterating in this study is the necessity of measuring the contribution of stocked fish at a local level, in order to provide valuable data with which to inform management decisions.

Effective monitoring of walleye stocking requires a proven marking technique, in order to correctly identify stocked fish. In Manitoba, there have been inconsistencies in the marking technique, which uses OTC to produce a fluorescent mark on calcified structures, such as otoliths. Differing levels of mark quality may be caused by a number of factors, including the age at which fish are marked, the type of OTC used, and the water source of the marking solution. Objectivity of mark observation can also be an issue in correct identification of stocked fish, as is the ability for the mark to be retained throughout the length of the monitoring program.

The focus then for this study is to first ensure that the marking technique used to identify stocked fish is effective and that marks applied will be retained throughout the study period. This marking technique will then be used to determine the contribution rate of stocked walleye in Manitoba and will provide the first step for a continual monitoring program of stocked walleye in Manitoba.

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# 3.0 An evaluation of oxytetracycline marking techniques for larval walleye (*Sander vitreus*)

## *3.1 Abstract*

Marking larval walleye with oxytetracycline (OTC) is a common technique used to identify hatchery reared fish from naturally produced fish, in order to determine the success of stocking. Manitoba hatcheries have been marking walleye with oxytetracycline for a number of years, however, a preliminary study done in 2010 found that stocked walleye had either undetectable or poor quality marks, making it difficult to correctly identify stocked walleye in the wild. The focus of this study was to identify variables in the OTC marking methodology used in Manitoba that may affect mark quality, with the end goal of developing a marking methodology that produces high quality marks on larval walleye. A series of experiments was designed to study the effect of six parameters – age at mark, water source, type of OTC, concentration of OTC, fry density, and DMSO - on OTC mark quality on 14 day old larval walleye. Walleye marked at 4 d post hatch had higher quality marks than those marked at 1 d post hatch (Wilcoxon, $Z=6.6256$, $P<0.0001$). Water source significantly affected mark quality when using both liquid OTC (Kruskal-Wallis, $\chi^2=30.8264$, $P<0.0001$) and powder OTC (Kruskal-Wallis, $\chi^2=16.8819$, $P=0.0002$). The use of powdered OTC in combination with sodium dibasic as a buffer produced higher quality marks than use of pre-buffered liquid OTC (Wilcoxon, $Z=13.9093$, $P=<0.0001$). Addition of dimethylsulfoxide, concentration of OTC and fry density in solution did not significantly affect mark quality. The combination of factors which was most effective at producing high quality marks was the use of 700 mg OTC/L powder OTC on walleye fry 3-5 day post hatch with either Swan Creek or Whiteshell Fish Hatchery water.

## *3.2 Introduction*

Manitoba Fisheries Branch stocks approximately 30 million walleye fry each year into water bodies across the province (Manitoba Water Stewardship 2010-2013). The majority of these stockings are into lakes with existing walleye populations in which some amount of natural reproduction occurs.

Stocking success in lakes with natural reproduction is variable, and the ability to estimate this success is required to maximize use of hatchery reared fish. Manitoba has attempted to differentiate between hatchery reared and naturally reproduced walleye by marking hatchery fish with oxytetracycline (OTC), with the goal of evaluating stocking success.

OTC is an antibiotic in the tetracycline family, all of which are flourochromes (i.e. fluoresce yellow-green under ultraviolet light) (Mitscher 1978). OTC can be applied to fish in a number of ways, such as incorporation into feed or by injection, yet the most commonly used method for marking fish is by immersion in a solution of OTC. This method allows for large numbers of fish to be marked at a time. The action of OTC that allows for it to be used as a marker is its uptake into the bloodstream, from where it becomes incorporated into developing calcified structures, such as bones, and creates a golden mark which is visible under UV light.

Manitoba hatcheries began marking larval walleye with OTC in 1998 (Biggin 2008). These early trials were based on protocol described in Brooks et al. (1994), in which larval walleye, 4-5 days post hatch, were mass marked by immersion in 500-700 mg OTC/L for 6 hours. The technique has been documented to produce high percentages of marked fry, possessing high quality marks while experiencing low mortality rates (Brooks et al. 1994, Fielder 2002; Lucchesi 2002). Brooks et al. (1994) used a mixture of sodium dibasic and sodium monobasic to buffer the solution, as OTC is highly acidic and must be buffered to maintain a neutral pH to prevent fry mortality during the immersion process. In 1998, fisheries managers in Manitoba attempted to buffer the OTC solution, but experienced difficulty maintaining a neutral pH and subsequently experienced high fry mortality (Biggin 2008). Therefore, in 1999, Manitoba hatcheries switched to using a pre-buffered form of OTC called Oxyvet 100 Lp, a veterinary grade liquid OTC, rather than the previously used, non-buffered, powdered OTC Neoteramycin (Biggin 2008). Following these amendments to the marking protocol, there were no documented mark efficacy trials from 1998 through 2004 (Biggin 2008). However, mark efficacy trials in 2005 found low mark rates on

known marked fish. Although all fish were marked with Oxyvet 100 LP at 700 mg/L, only 7 - 29% of marked fish had an identifiable OTC mark. As a result, in a 2006 trial, OTC concentration was increased from 700 to 1,400 mg OTC/L, which increased the rate of identifiable marks on known marked fish to between 86 and 100 % (Biggin 2008).

From 2006 to 2009, all walleye reared in Manitoba were marked with 1,400 mg Oxyvet 100 LP/L (Biggin 2008). During this period, there was limited monitoring and quality control of OTC marking. In 2010, a preliminary study of known marked walleye from the Whiteshell Hatchery in southeastern Manitoba found OTC marks on 33% of fish (Groening, unpublished). This low mark recovery rate from known marked fish suggested that although all walleye were being marked, only a small percentage of those fish retained a functional mark. The preliminary study in 2010 was conducted at the Whiteshell Fish Hatchery, whereas the study in 2006, as documented in Biggin (2008), had been conducted at the Swan Creek Hatchery (Groening, unpublished). The marking protocol used at both hatcheries is the same; however water chemistry between these locations is quite different. The Swan Creek Hatchery is located on the eastern shore of Lake Manitoba, along the prehistoric lake bed of Lake Agassiz, whereas the Whiteshell Hatchery is in the far east of the province, and overlies the Canadian Shield. The unique geographies of these two hatchery locations result in differences in water chemistry which may affect the uptake and incorporation of OTC into calcified structures (Lunestad and Goksayr 1990). Therefore, it became critical to hatchery operations that sources of variation were in the marking protocol were identified and reduced in order to create an effective marking protocol.

A review of literature on oxytetracycline marking was conducted in order to identify potential sources of variation in the marking protocol used by the Province of Manitoba. This full review will not be repeated here, but can be found in chapter two of this thesis. The results of this review suggested five main source of potential variation, which were then identified as factors to be studied as to their ability to influence final OTC mark quality.

The first potential source of variation identified in this review was: 1) the age at which fry were marked. It was suggested in the literature that walleye fry should be marked at no earlier than 4-5 days post hatch (Brooks et al. 1994, Fielder 2002), however, operational demands at the three Manitoba hatcheries often resulted in marking walleye fry within 24 hours post-hatch. Therefore, the age at which fry were being marked was determined to be a potential source of variation that may influence the quality of the final OTC mark. Another potential source of variation identified in the review was: 2) differences in water chemistry between the two main walleye hatcheries in Manitoba. Both alkalinity and salinity can affect the effectiveness of oxytetracycline (Denson and Smith 2008, Lunestad and Goksayr 1990), and therefore have the potential to impact OTC mark quality. A third factor identified as having the potential to impact mark quality was: 3) the type of OTC used. The existing protocol in Manitoba calls for the use of liquid OTC, however powdered OTC is more commonly used for marking fish (Logsdon 2004, Lucchesi 2002). In addition, 4) The concentration of OTC in the marking solution was also identified as a potential source of variation. The Province of Manitoba previously used 1,400 mg/L OTC in the marking solution, rather than the 700 mg/L suggested in Brooks et al. (1994). Finally, 5) fry densities in the marking solution were estimated by hatchery managers to be much higher (~100,000 fry/L) than the density of 2000 fry/L suggested in Lucchesi (2002, D. Bilenduke, pers. comm., 2011). In addition to these five potential sources of variation, there were a few references to the use of dimethylsulfoxide (DMSO), which was stated as having the potential to increase mark quality (Scidmore and Olsen 1969, Kayle 1992).

The objectives of this study were to examine the effect of these five factors; age at which fish were marked, water source used in the marking solution, type of OTC used, concentration of OTC in solution and density of fry in solution, on OTC mark quality.

## *3.3 Methods*

### *3.3.1 Fry acquisition*

In the spring of 2011 and 2012, walleye eggs were collected during a spawn-taking operation on Falcon Creek, a tributary to Falcon Lake, Manitoba. The eggs were incubated at the Whiteshell Fish Hatchery (West Hawk Lake, Manitoba) for approximately two weeks. In 2011, fry used in experiments were collected as eggs over a series of 3-5 days. Hatchery infrastructure did not allow for fry to be isolated from each day of collection once hatched, therefore, we were only able to estimate the collection date within the 3 to 5 days. However, in 2012, changes were made to hatchery infrastructure that allowed us to isolate fish based on the day of collection and the day of hatch. Therefore, in 2012, all fish used in the study were taken from the single day of the spawn taking operation to reduce variability in survival between different days of the spawning run. It was assumed for this study that the variation in age between 3 and 5 days did not impact mark quality.

### *3.3.2 Experimental design*

In order to test the effect of the six potential sources of variation identified in chapter 2 (age at which fry were marked, source of water in which fry are marked, type of OTC, concentration of OTC in solution, density of fry in solution and the use of DMSO in the marking solution), fry were marked in a series of experiments in 2011 and 2012 at the Whiteshell Fish Hatchery. In 2011, a series of eight experimental treatments was developed to test the main effects of four factors; 1) density, at either 4,000 or 10,000 fry/L, 2) age at which fry were marked, at either one day or three to five days old; 3) water source, either from the Whiteshell Fish Hatchery or from Transcanada Pond; and 4) Type of OTC used, either liquid or powder (Table 1). Each of these treatments were applied to three replicate groups of fry (n=200). Each group of fry was hand counted and placed in 28 mL of water in a 188 mL whirlpak bag, so fry were at a density of approximately 3,571 fry/L, which was rounded up to 4,000 fry/L. Despite hand

counting fry, the number of fry in each bag still varied and therefore all densities should be taken as approximate values rather than set values. Oxytetracycline was then added to 28 mL of water in a separate container, which was then added to the fry bag.

TABLE 1. Experimental oxytetracycline marking treatments applied to larval walleye in the spring of 2011 at the Whiteshell Fish Hatchery.

| Density (fry/L) | Age at mark (days) | OTC concentration (mg/L) | Water source | OTC type | Replicate treatments | n (fry) |
|---|---|---|---|---|---|---|
| 4000 | 1 | 1400 | Whiteshell | Liquid | 3 | 200 |
| 4000 | 1 | 1400 | Transcanada | Liquid | 3 | 200 |
| 4000 | 3-5 | 1400 | Transcanada | Liquid | 3 | 200 |
| 4000 | 3-5 | 1400 | Whiteshell | Liquid | 3 | 200 |
| 4000 | 3-5 | 1400 | Whiteshell | Powder | 3 | 200 |
| 10000 | 3-5 | 1400 | Whiteshell | Liquid | 3 | 200 |
| 4000 | 3-5 | 0 | Whiteshell | None | 3 | 200 |
| 4000 | 3-5 | 1400 | Whiteshell | Liquid | 3 | 200 |

In 2012, each treatment was applied to groups of fry (n=300) in a factorial design approach (Table 2). A series of eleven treatments were developed to test the main and interaction effects of four factors; 1) water source, at three levels, either from the Whiteshell Fish Hatchery, the Swan Creek Hatchery or deionized water, 2) the type of OTC used, at two levels, either liquid or powder, 3) the concentration of OTC used, at three levels, 0 mg/L, 700 mg/L and 1,400 mg/L and 4) DMSO, at two levels, 0% and 2% of the marking solution (Table 2, Table 3). These factors were arranged into four factorial experiments, which are mapped out in Table 2. The first experiment (a) is a three by two factorial design, to test effect of water source at three levels, Whiteshell Fish Hatchery, Swan Creek Hatchery, and deionized water, and OTC type, at two levels, liquid and powder, on OTC mark quality, and was applied to nine replicate groups of n=300 fry. Experiment b) set out to test the effects of OTC concentration at three levels, 0 mg/L, 700 mg/L and 1,400 mg/L on OTC mark quality, on a single group of 300 fry for each treatment. Experiment c) was a two by two factorial design, which set out to test the

effect of DMSO at two levels, 0% and 2% of the marking solution, and OTC type at two levels, liquid and powder, on OTC mark quality, and was applied to three replicate groups of n=300 fry. Experiment d) was a two by two factorial design, which tested the effect of two levels of water source, Whiteshell Hatchery and Swan Creek Hatchery, on two levels of OTC concentration, 700 mg/L and 1,400 mg/L, on a four groups of n=300 fry.

TABLE 2. Number of replicate groups of n=300 larval walleye treated with the following experimental OTC treatments at the Whiteshell Fish Hatchery in 2012.

a)

| 3 X 2 | **OTC Type** | |
|---|---|---|
| **Water Source** | Liquid | Powder |
| Whiteshell | 9 | 9 |
| Swan Creek | 9 | 9 |
| Deionized | 9 | 9 |

b)

| 1 X 3 | **OTC concentration (mg/L)** | | |
|---|---|---|---|
| **Water Source** | 0 | 700 | 1,400 |
| Whiteshell | 1 | 1 | 1 |

c)

| 2 X 2 | **OTC Type** | |
|---|---|---|
| **DMSO (% in solution)** | Liquid | Powder |
| 2% DMSO | 3 | 3 |
| 0% DMSO | 3 | 3 |

d)

| 2 X 2 | **OTC concentration (mg/L)** | |
|---|---|---|
| **Water Source** | 700 | 1,400 |
| Whiteshell | 1 | 1 |
| Swan Creek | 1 | 1 |

TABLE 3. Experimental oxytetracycline marking treatments applied to larval walleye in the spring of 2012 at the Whiteshell Fish Hatchery.

| Density (fry/L) | Age at mark (days) | OTC concentration (mg/L) | Water source | OTC type | DMSO (%) | Replicate treatments | n (fry) |
|---|---|---|---|---|---|---|---|
| 4000 | 3 | 700 | Whiteshell | Liquid | 0 | 9 | 300 |
| 4000 | 3 | 700 | Whiteshell | Powder | 0 | 9 | 300 |
| 4000 | 3 | 700 | Swan Creek | Liquid | 0 | 9 | 300 |
| 4000 | 3 | 700 | Swan Creek | Powder | 0 | 9 | 300 |
| 4000 | 3 | 700 | Deionized | Liquid | 0 | 9 | 300 |
| 4000 | 3 | 700 | Deionized | Powder | 0 | 9 | 300 |
| 4000 | 3 | 700 | Whiteshell | Powder | 2 | 3 | 300 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 2 | 3 | 300 |
| 4000 | 3 | 1,400 | Whiteshell | Powder | 0 | 1 | 300 |
| 4000 | 3 | 1,400 | Swan Creek | Powder | 0 | 1 | 300 |
| 4000 | 3 | 0 | Whiteshell | None | 0 | 1 | 300 |

Each group of fry was counted volumetrically, and placed in 42 mL of water in a 188 mL whirlpack bag. Similarly to 2011, fry densities were to approximate 3, 571 fry/L, which was rounded up to 4,000 fry/L. OTC stock solutions were prepared in the laboratory at the Whiteshell Fish Hatchery within two hours prior to marking to facilitate speed the speed of mark application and reduce variation in OTC mark solutions. For each treatment, 42 mL of OTC stock solution was added to the fry bag. The concentration of each OTC solution prepared was not experimentally verified due to the prohibitive cost of lab analysis; rather, we relied on correct calculations and measurements made in the laboratory.

*3.3.3 Application of oxytetracycline treatments*

Fry were held in the marking solution for 6 hours in individual treatment bags in a series of coolers with the goal of maintaining consistent water temperature and minimal exposure to light. After 6 hours, fry were transferred into screened-in areas or rearing troughs equipped with flow through systems with a velocity of 0.01 m/s. The screened-in areas had volumes of 19.8 L and 14.9 L in 2011 and 2012, respectively. Each screened-in area within the rearing trough had an associated number and letter based

on its position in the rearing trough. These number-letter codes then became the way each experimental group of fry was identified, from the time each group was marked in the field until the otoliths were processed in the lab. The day on which fry were marked was the only point at which I, as the experimenter, was aware of which treatment was associated with each code. These were recorded in a master spreadsheet, which was then not consulted until after otoliths had been removed in the lab and analyzed for mark quality. Rearing troughs were located in a semi-outdoor laboratory setting such that fry experienced a temperature-photoperiod regime similar to natural conditions (see Appendix 3). Temperature of trough water was recorded daily, as was pH, using a Hanna pH meter (model H198128), total dissolved solids (TDS), using a Oakton TDS meter, and dissolved oxygen (DO), measured using a HACH DO meter (HQ 30d model), as well as the number of mortalities in each section, which were counted by hand. Each section of the rearing trough had an individual aquarium bubbler to maintain high levels of oxygen and prevent fry from gathering the screens which separated each section. Fry were fed 200 mL of concentrated zooplankton solution collected from West Hawk Lake using two 800 μm bongo plankton nets, graded to <600 μm, twice a day from day seven to day fourteen. After two weeks, fry were sacrificed and frozen until otoliths could be extracted and observed for marks in the lab.

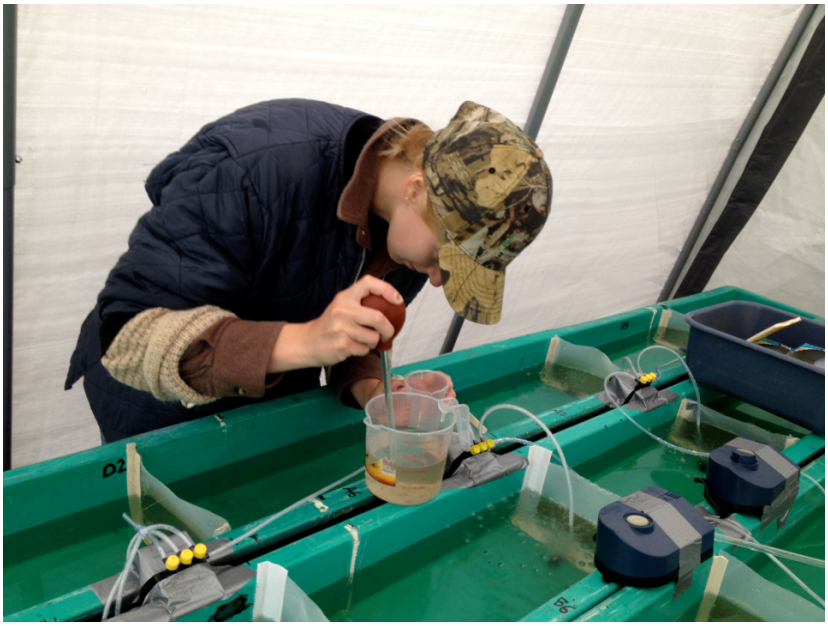


FIGURE 2. The author collecting walleye fry mortalities from the rearing troughs located at the Whiteshell Fish Hatchery in which oxytetracycline treated walleye were held for two weeks in early May of 2012.

There was some concern about the risk of transporting Koi herpesvirus, which has been found in the Swan Creek watershed, to the eastern part of the province where the Whiteshell Fish Hatchery is located, through the use of water from the Swan Creek for the experimental trials at the Whiteshell Fish Hatchery. Therefore, in order to prevent Koi herpesvirus transfer but still test the effect of relatively hard water, as is that used at the Swan Creek Hatchery, we tested a number of ponds within the Whiteshell Fish Hatchery watershed to find a water body of similar water hardness to that of Swan Creek Hatchery to use as a proxy (Table 4).In a preliminary study, water from the Transcanada Pond, which is located just outside the range of the Canadian Shield, but within the watershed of the Whiteshell Hatchery, was found to have similar water chemistry to water from the Swan Creek Hatchery, on Lake Manitoba (Table 4). Because of this similarity in water chemistry, water from Transcanada Pond was used as a proxy for water from Swan Creek, thereby eliminating the risk of transferring Koi herpesvirus from Swan Creek to eastern watersheds.

TABLE 4. Water chemistry of water sources used in 2011 and 2012 retention OTC marking treatments at the Whiteshell Fish Hatchery. Parameter measurements were determined by ALS Environmental Laboratories in Winnipeg, Manitoba.

| Water quality parameters | Deionized Water 2012 | Whiteshell Hatchery 2011 | Whiteshell Hatchery 2012 | Transcan pond 2011 | Grand Rapids Hatchery 2012 | Swan Creek Hatchery 2012 |
|---|---|---|---|---|---|---|
| Alkalinity (CaCO3) (mg/L) | 0 | 24.6 | 25 | 144 | 157 | 220 |
| Total calcium (mg/L) | 0 | 10.1 | 9.52 | 45 | 52.4 | 55.6 |
| Total magnesium (mg/L) | 0 | 1.48 | 1.42 | 13.4 | 19.4 | 57.7 |
| pH | 7 | 7.56 | 7.61 | 8.24 | 8.37 | 8.41 |
| TDS (mg/L) | 0 | 54 | 45 | 296 | 248 | 1130 |

*3.3.3.1 Age at time of mark*

*2011*

To test the effect of age at which fish were marked on mark quality, three replicate groups of fry (n=200) were marked less than 24 h post hatch. All other marking variables remained constant (Table 1).

*2012*

In 2012, all fry were held for 3 - 5 days prior to marking (Table 3).

*3.3.3.2 Oxytetracycline type*

*2011 and 2012*

To test the effect of OTC type on mark quality, two types of OTC were used; liquid OTC (Oxyvet 100 LP, 100 mg active OTC/ 1 mL) and powdered OTC (Onnacin 250, 100 g active OTC/ 1 mL). Powdered OTC was buffered by adding equal parts sodium dibasic to OTC to the marking solution. In 2012, sodium dibasic was first dissolved in 10 mL of hot water and added to the OTC marking solution (Table 1 and 2). This solution was then thoroughly mixed in a large container with a stirring tool attached to a drill for at least 10 minutes before using to mark fry.

*3.3.3.3 Concentration*

*2012*

In 2011, all treatment groups were marked at a concentration of 1,400 mg OTC/L (Table 1). In 2012, all treatments were marked at a concentration of 700 mg OTC/L, with the exception of two treatments, both marked with powdered OTC, at a concentration of 1,400 mg OTC/L, to test for the effect of concentration on mark quality, as well as one unmarked control treatment. (Table 2 and 3).

*3.3.3.4 Density*

To test the effect of relatively high density on mark quality, one of the treatment groups of walleye fry was marked at a density of 10,000 fry/L in 2011, which was the operational fry density in previous hatchery marking programs (Table 2). Following results from 2011, which can be reviewed in section 3.4.4 and found no difference between marking fry at 10,000 and 4,000 fry/L, all experiments in 2012 were conducted at an approximate density of 4,000 fry/L of OTC marking solution to maintain consistency.

*3.3.3.5 DMSO*

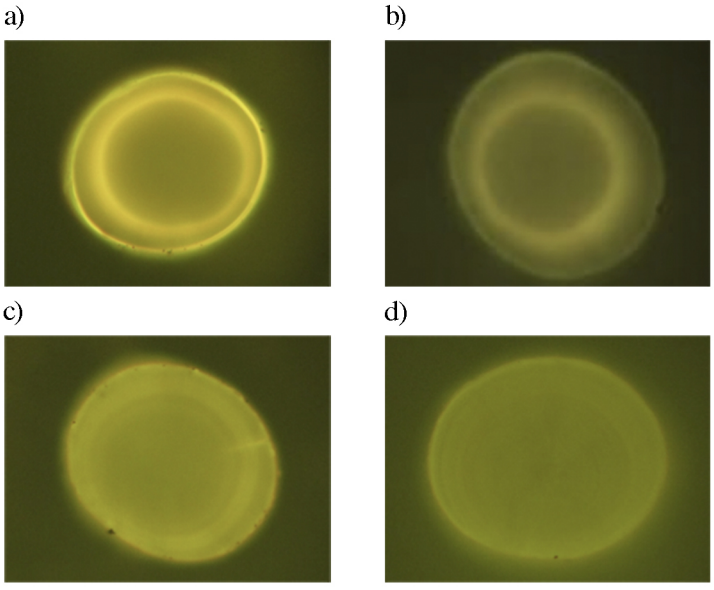
In 2012, the effect of DMSO was tested by marking two treatment groups with 2% DMSO in the marking solution and two treatment groups with 0% DMSO (Table 2 and 3). Three replications of each treatment were applied.

*3.3.4 Mark detection*

Otoliths from fry were removed under a dissecting microscope and placed on a glass slide for analysis. Otoliths from 3 month old fish were mounted on slides with super glue (Krazy Glue, Columbus, Ohio), concave side up, and polished with 600 and 1,200 wet grit sandpaper until the inner daily rings were observed under 100X magnification with transmitted light. The otoliths were analyzed for OTC marks using a Nikon Eclipse 50i Epifluorescent microscope with UV B-3A and 2B filter cubes, and C-

SHG1 super high pressure mercury lamp power supply. For each otolith, OTC mark intensity was assigned a grade using the following scale: 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark. This mark ranking system was based on that ranking system used by Isermann et al. in their work on OTC mark efficacy (1999, 2002). I was also trained by Dr. Dan Isermann (University of Wisconsin-Stevens Point) in OTC mark detection and analysis in November of 2011 and therefore have continued to use his ranking system to categorize the intensity of OTC marks. Examples of each mark intensity rank can be observed in Figure 3. Otoliths were deemed to have "no mark" only after several cycles of observation and sanding had produced no visible mark, and the otoliths were clearly sanded past the central annuli. If otoliths were over sanded without observation of the central annuli, the otoliths were scored as over sanded, and removed from analysis. A photograph was taken of each mark, viewed under the 50x oil immersion objective, with a small amount of immersion oil and the 2B filter cube. Camera settings were kept consistent at an exposure composition of -1 1/3 EV.

FIGURE 3. Examples of the four ranks (1-4) used to describe oxytetracycline mark intensity. Figure a) represents a rank of 1 = obvious mark with distinct gold color; figure b) represents a rank of 2 = clear mark, but lacking intensity; figure c) represents a rank of 3 = faint mark, yet appears present; and figure d) represents a otolith with no clear OTC mark, which would be ranked as 4 = no mark.



### *3.3.5 Mark quality control*

Marks were viewed and scored during the first observation blind, such that the reader did not know with which treatment the otoliths had been marked. Each mark was then scored a second time, also blind, based on the photograph alone. These scores were compared with the first, and if a discrepancy occurred between the first and second reading, the otolith was analyzed a third time, after which the modal age was used as the final reading.

### *3.3.6 Sample size*

In both 2011 and 2012, not all larval walleye subjected to experimental OTC treatments survived to the two week mark, at which point OTC mark quality was analyzed. In 2011, survival of treated larvae was quite low, and therefore the number of otoliths which could be analyzed for OTC mark quality was limited and varies for each treatment group based on the survivorship of the treatment group. The number of fry analyzed from each treatment is noted in Tables 5 and 6. In 2012, survival of treated larval walleye was higher than in 2011, and therefore, 5 larval walleye otoliths from each replicate group of treated walleye were analyzed for OTC mark quality (Table 6).

### *3.3.7 Statistical analysis*

As data (i.e. scores) were discrete and therefore not normally distributed, non-parametric statistics were applied. The Wilcoxon and Kruskal-Wallis rank sum tests were used to examine differences between mark quality ranks associated with each treatment variable. The proportional odds (PO) model, a generalized linear regression model, was used to test which experimental factors significantly predicted the quality of OTC marks (Agresti 2002). The level of significance was set at 0.05. All analyses were performed using SAS 9.3 statistical software (SAS Institute, Cary, NC).

## *3.4 Results*

### *3.4.1 Age at time of mark*

Two groups of fry were marked with liquid oxytetracycline at less than 1 d post hatch, one in water from the Whiteshell Hatchery and one in water from Transcanada pond. Both treatment groups had the lowest number of observable marks, defined as any mark above a rank of 3, (38% and 50%, respectively) and the lowest mark intensity (3.6 ± 0.6 and 3.4 ± 0.7) among all 2011 treated groups (Table 4). Walleye fry marked at less than 1 d post hatch had significantly lower OTC mark quality than those marked at 3-5 d post hatch (Wilcoxon, Z=6.6256, P<0.0001) (Figure 4).

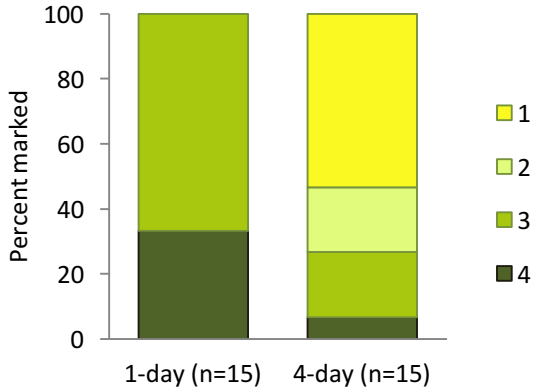



FIGURE 4. Percent frequency of oxytetracycline mark intensity in otoliths of two week old walleye fry marked in immersion in 700 mg liquid OTC/L at 1-day and 3-5-days old, administered at the Whiteshell Hatchery in 2011. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

*3.4.2 Water source*

*2011*

Water source was analyzed as a categorical variable, as it was affiliated with each hatchery from which the water was sourced. In this way, the interactions of the various water quality parameters were not investigated for their potential to effect OTC mark quality; rather, this study focused on studying the effects of parameters which reflected the variation experienced during the normal OTC marking process in previous years. Therefore, despite overlooking the potential impact of various water quality parameters, our use of Whiteshell, Transcanada and Swan Creek Hatchery water, compared with deionized water was decided to be representative of water sources that would be used in future years for marking larval walleye. Deionized water was used as a control variable as it contains no minerals or dissolved solids and

has a neutral pH. Individual water quality parameters such as pH, TDS, and alkalinity are listed in Table 4.

The intensity of OTC marks was significantly different between fry otoliths marked in Whiteshell, Transcanada and deionized water (Kruskal Wallis, $\chi^2$=15.8804, *P*=0.0004). Oxytetracycline marks were observable on 100 percent of fry otoliths marked in deionized water and Transcanada water, with observable marks on 89 percent of otoliths from fry marked in Whiteshell water (Figure 5). Median mark intensity was highest on fry otoliths marked in deionized water (1.1 ± 0.3) compared with mark intensity observed on otoliths marked in Whiteshell water (2.0 ± 1.0) and Transcanada water (2.0 ± 0.9) (Table 4).

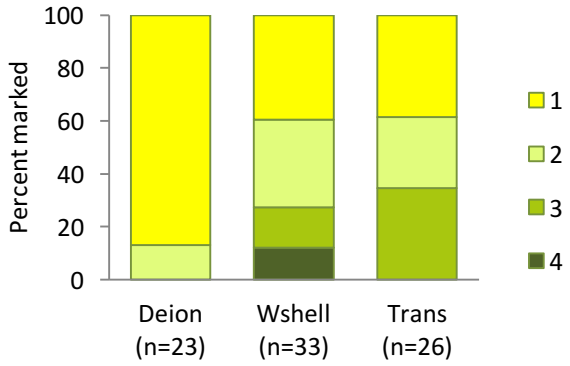



FIGURE 5. Percent frequency of oxytetracycline mark intensity in otoliths of two week old walleye fry marked when immersed in 700 mg liquid OTC/L of deionized, Whiteshell and Transcanada water administered at the Whiteshell Hatchery in 2011. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

*2012*

The effect of water source on OTC mark quality was not significant when those marked with powdered OTC were included in the analysis with fry marked with liquid OTC (Kruskal-Wallis, $\chi^2$=2.6430, P=0.2666). However, when the effect of water source was analyzed on OTC mark quality with those fry just marked with liquid OTC (Figure 6), and those fry just marked with powdered OTC (Figure 7), there was a significant effect on water source for each (Kruskal-Wallis, $\chi^2$=30.8264, P<0.0001, and $\chi^2$=16.8819, P=0.0002, respectively).

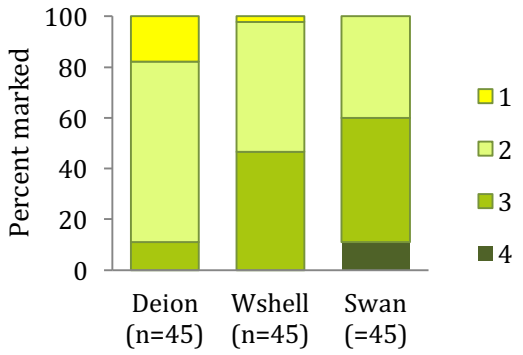



FIGURE 6. Percent frequency of oxytetracycline mark intensity in otoliths of two week old walleye fry marked when immersed in in 700 mg liquid OTC/L of deionized, Whiteshell and Swan Creek water administered at the Whiteshell Hatchery in 2012. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

### *3.4.3 Oxytetracycline type*

*2011*

Fry marked with powdered OTC had a higher percentage of observed marks than fry marked with liquid OTC, but the average intensity of marks was similar between the two groups (1.6 ± 0.7 and 2.0 ± 1.0, respectively) and the difference in mark quality was not significant (Wilcoxon, Z= -0.8216, P=0.4113).

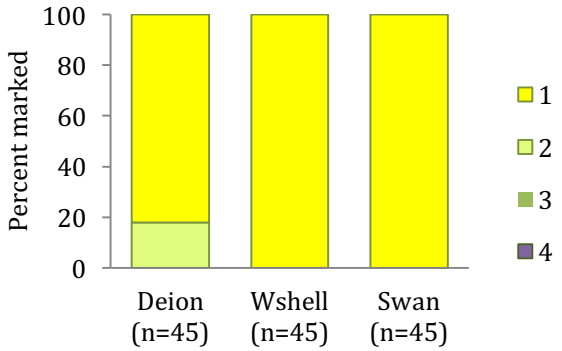



FIGURE 7. Percent frequency of oxytetracycline mark intensity in otoliths of two week old walleye fry marked when immersed in in 700 mg powdered OTC/L of deionized, Whiteshell and Swan Creek water administered at the Whiteshell Hatchery in 2012. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

*2012*

Otoliths marked with powdered OTC had significantly higher average intensity of marks than those marked with liquid, for treatments using Whiteshell, Swan Creek and deionized water in the marking solution (Wilcoxon, Z=13.9093, P=<0.0001) (Figure 8, Table 5).

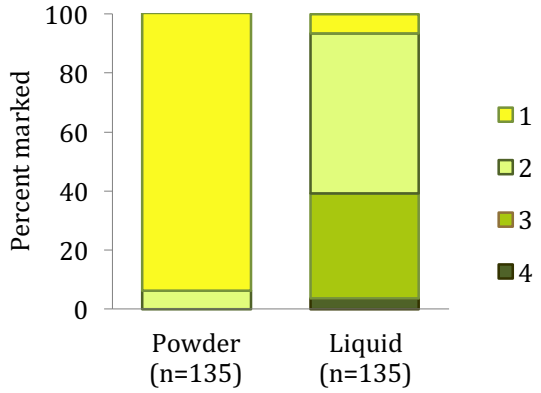



FIGURE 8. Percent frequency of oxytetracycline mark intensity in otoliths of two week old walleye fry marked in liquid and powdered oxytetracycline at a concentration of 700 mg/L in Whiteshell water, administered at the Whiteshell Hatchery in 2012. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

*3.4.4 Concentration*

There were no differences in mark intensity between those walleye marked at 700 mg/L and those marked at 1,400 mg/L, in either Whiteshell water or Swan Creek water. Both groups of walleye were marked with powdered OTC, which created such high intensity marks at both concentrations, that there was no variation between the mark intensity of the groups marked at different concentrations.

*3.4.5 Density*

Mark quality was not significantly different between treatment groups marked at a density of approximately 10,000 fry/L and 4,000 fry/L of marking solutions, which were both marked at a concentration of 1,400 mg/L(Wilcoxon, Z = 0.0862, P = 0.9313) and groups of fry marked at a density of

10,000 fry / L and 4,000 fry / L both had 100 percent observable OTC marks, with similar average mark intensity (1.9 ± 0.7 and 2.0 ± 1.0, respectively) (Table 4).

TABLE 5. The mean intensity of fluorescent marks on walleye fry otoliths two weeks post immersion in a series of oxytetracycline treatments administered at the Whiteshell Hatchery in 2011. All treatment groups were marked at a concentration of 1400 mg OTC/L. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

| Treatment | | | | *n of fish* | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|
| **Water source** | **OTC type** | **Age at mark (d)** | **Density (fry/L)** | | | |
| Whiteshell | Liquid | 1 | 4,000 | 24 | 38 | 3.6 ± 0.6 |
| TransCanada | Liquid | 1 | 4,000 | 16 | 50 | 3.4 ± 0.7 |
| Whiteshell | Liquid | 4 | 4,000 | 33 | 88 | 2.0 ± 1.0 |
| Deionized | Liquid | 4 | 4,000 | 23 | 100 | 1.1 ± 0.3 |
| Whiteshell | Liquid | 4 | 10,000 | 14 | 100 | 1.9 ± 0.7 |
| Whiteshell | Powder | 4 | 4,000 | 8 | 100 | 1.6 ± 0.7 |
| TransCanada | Liquid | 4 | 4,000 | 26 | 100 | 2.0 ± 0.9 |

TABLE 6. The mean intensity of fluorescent marks on walleye fry otoliths two weeks post immersion in a series of oxytetracycline treatments administered at the Whiteshell Hatchery in 2012. Intensity was measured on a scale of 1-4, with 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark.

| Treatment | | | | *n* | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|
| **Water source** | **OTC type** | **DMSO (%)** | **OTC (mg/L)** | | | |
| Whiteshell | Liquid | 0 | 700 | 45 | 100 | 2.4 ± 0.5 |
| Whiteshell | Powder | 0 | 700 | 45 | 100 | 1.0 ± 0.0 |
| Swan | Liquid | 0 | 700 | 45 | 89 | 2.6 ± 0.7 |
| Swan | Powder | 0 | 700 | 45 | 100 | 1.0 ± 0.0 |
| Deionized | Liquid | 0 | 700 | 45 | 100 | 1.9 ± 0.5 |
| Deionized | Powder | 0 | 700 | 45 | 100 | 1.2 ± 0.4 |
| Whiteshell | Liquid | 2 | 700 | 15 | 100 | 2.3 ± 0.5 |
| Whiteshell | Powder | 2 | 700 | 15 | 100 | 1.4 ± 0.6 |
| Whiteshell | Powder | 0 | 1,400 | 5 | 100 | 1.0 ± 0.0 |
| Swan | Powder | 0 | 1,400 | 5 | 100 | 1.0 ± 0.0 |

*3.4.6 DMSO*

Fry marked with liquid OTC with 2% DMSO in solution had no significant effect on mark quality (Wilcoxon, z = -1.2156, P = 0.2241). There was a significant effect of DMSO on mark quality in those fry marked with powdered OTC; however, rather than increasing the mark quality, those with 2% DMSO in solution had significantly reduced mark quality (Wilcoxon, Z = 3.9911, P < 0.0001).

*3.4.7 PO Models*

*2011*

The effect of OTC type, water source, age at which fry were marked with OTC and fry density were analyzed for their ability to predict mark intensity in a proportional odds (PO) model. Only the effects of water source and age when fry were marked were significant and included in the final effects model. The model converged and met the Proportional Odds Assumption (Proportional Odds Assumption, $\chi^2 = 10.9943$, P=0.0886). The final fitted model can be described as

Logit (Mark intensity) = 4.8523 + (-1.7791) * Water source + 0.7733 * Age when marked

The model indicates that the log of the odds of an increase in mark intensity is overall positively related to water source ($p<0.05$), and positively related to age when fry are marked ($p <0.05$). In other words, when fry are marked at older ages, it is correlated to an increased probability of higher mark quality. The shift from deionized water to Whiteshell sourced water is correlated with a decreased probability of higher mark quality, as is the shift from deionized water to Transcanada sourced water.

TABLE 7. The effect of water source and age at which fry were marked in OTC treatment on mark quality observed on walleye fry marked at the Whiteshell Hatchery in 2011 (PO, P≥0.05).

| Variable | β | *SE* β | Wald $\chi^2$ | *df* | *P* | $e^{\beta}$ (odds ratio) |
|---|---|---|---|---|---|---|
| Water source (whiteshell, transcanada, deionized) | | | 19.9474 | 2 | <.0001 | |
| Water source (deionized to whiteshell) | -1.1606 | 0.2679 | 18.7691 | 1 | <.0001 | <.0001 |
| Water source (deionized to transcanada) | -0.6185 | 0.2979 | 4.3095 | 1 | <.0001 | 0.0379 |
| Age at mark (1, 4 d) | 0.7733 | 0.1344 | 33.1218 | 1 | <.0001 | <.0001 |

| Test | $\chi^2$ | *df* | *P* |
|---|---|---|---|
| Overall model evaluation | | | |
| Likelihood ratio test | 80.6677 | 3 | <.0001 |
| Score test | 65.7965 | 3 | <.0001 |
| Wald test | 55.3159 | 3 | <.0001 |
| Goodness-of-fit test | | | |
| Deviance | 2.4373 | 9 | 0.0091 |
| Pearson | 1.9117 | 9 | 0.0456 |

Based on the observations from experimental OTC treatments used in 2011, it was determined that all marking experiments in 2012 would be conducted on fry at least 4 days post hatch. The experiments in 2011 suggested that there was some influence of water source on the rate and intensity of OTC marks. The results also suggested that the type of OTC (liquid or powdered formula) may have some influence on mark rate; however the sample size in 2011 was likely too small to observe a significant effect on average mark intensity.

*2012*

The effect of OTC type and concentration, water source, and DMSO were analyzed for their ability to predict mark intensity in a proportional odds (PO) model. However, although the model converged, the data did not fit the Proportional Odds Assumption, and therefore could not be appropriately modelled by this regression (Proportional Odds Assumption, $\chi^2 = 16.7527$, P=0.0102).

*3.4.8 Mortality*

The current study was not designed to measure the effect of OTC treatment on mortality, and therefore although data on daily fry mortality was recorded, the limited observation of surrounding environmental factors would make it unreasonable to use this data to determine whether the range of treatments used was associated with the mortality. The average mortality rate over the two week experimental period in 2012 was 12.4 fry/day, with the lowest average mortality rate at 8.5 fry/day and the highest average mortality rate at 19.2 fry/day. The majority of mortality occurred within the first four days post treatment. Daily mortality and average mortality by treatment have been documented in Appendix 2.

## *3.5 Discussion*

The present study suggests that oxytetracycline marking can be affected by the age at which walleye fry are marked, the source of water used in the OTC solution, and the type of OTC used (liquid or powdered formula).

*3.5.1 Age at time of mark*

The Whiteshell Hatchery had in previous years marked walleye fry between the range of less than 24 hours to two days post hatch, largely due to space restrictions on-site. In order to hold fry for a number of days after they hatch from the incubation batteries, it requires a series of holding tanks in which fry could be kept prior to applying the OTC mark, and these logistical issues have kept holding fry for a numbers of days prior to marking from becoming part of the marking methodology. Our results suggest that holding fry for 3-5 days post-hatch prior to marking with liquid OTC can significantly improve the OTC mark quality. Therefore, despite logistical barriers, holding fry for at least 3 days post hatch should be incorporated into the practice of OTC marking in order to improve overall mark quality.

This finding is consistent with the recommendations of both Brooks (1994) and Fielder (2002), who suggested that walleye fry should be marked at no less than three days post hatch in order to ensure a

high quality, and therefore functional OTC mark. Neither of these studies, nor the study at hand, investigated why mark quality is improved by marking older fry, but there are a few logical explanations. The first is that the age at which fish are marked dictates where on the otolith the mark will be located. Fish marked as fry will have a mark close to the nucleus, and the observation of this mark may be confounded by the appearance of the nucleus itself, which has a slightly florescent property. In addition, observation of a mark close to the nucleus requires a large amount of sanding before it can be viewed, which increases the potential for missing the mark. These factors are present whenever an otolith is analyzed for mark presences, however, the older fish are when marked, the less impact they have on accurate mark detection, as the nucleus will be further from the mark, and less sanding will be be required.

It should be noted however, that these results were observed in 2011, when all fry were marked with liquid OTC, as opposed to powdered OTC. Marking with liquid OTC resulted in lower quality marks overall than marking with powdered OTC, and the compounded factors of marking with liquid OTC on walleye fry that were less than 24 hours post hatch may have resulted in the extremely low quality marks. Logsdon (2004) found that fry could be effectively marked with powdered OTC at less than 24 hours of age. It is possible that the use of powdered OTC may have been able to create identifiable marks on fry less than one day old, rather than the liquid OTC used in these trials.

*3.5.2 Water source*

The use of water from difference sources - deionized, Whiteshell Hatchery and Transcanada pond in 2011 and deionized, Whiteshell Hatchery and Swan Creek in 2012 – did have an effect on mark quality, although the degree to which it affected mark quality was dependent on the type of OTC used. Water source was included as a variable in the study due to the presumption that the differences observed between OTC marks from fry marked at the Whiteshell Hatchery and those marked at the Swan Creek Hatchery may be different due to the contrasting water quality parameters. As mentioned in the introduction, the Swan Creek Hatchery is located on the eastern shore of Lake Manitoba and has

relatively high values of alkalinity, total dissolved solids (TDS) and pH compared with water from the Whiteshell Fish Hatchery, which overlies the Canadian Shield and has relatively soft water. Within the literature review, there were a few studies (Lunestad and Goksayer 1990, Mauk 2008) which suggested that differences in water chemistry such as the amount of hardness, specifically the presence of $Ca^{2+}$ and $Mg^{2+}$ ions could possibly affect the uptake and incorporation of OTC into calcified structures (Lunestad and Goksayr 1990).

Otoliths are largely made of calcium carbonate (96%, $CaCO^2$), as well as 3% organic make up, largely consisting of proteins, and less than 1% non-organic trace elements, which can include up to 31 elements such as Na, Sr, K, S and Cl, depending on which elements the fish has been exposed (Campana 1999). Otoliths have long been used to determine the age of fish by counting the annuli, which represent layers of $CaCO^2$ accumulated during seasonal growth. In addition to this application, otoliths have the unique property of being metabolically inert, such that material incorporated into the otoliths cannot be reabsorbed, allowing the otolith to be used as a sort of "environmental recorder" of all the compounds to which the fish has been exposed (Campana and Neilson 1999, Campana 1999). It is this property of otoliths that makes them ideal structures for marking applications, as any chemical exposure will be permanently recorded in the otolith. Oxytetracycline is incorporated into the otolith as the tetracycline molecules bind to calcium on the collagen fibrils of the developing bone matrix via the naphthacene carboxamide nuclei of the tetracycline molecule (Milch et al 1957).

These findings suggest that the source of water used to mark otoliths can have a significant effect on mark quality. In 2011, mark quality was significantly different between treatment groups marked in deionized, Whiteshell and Transcanada water with liquid OTC, and a similar result was seen in 2012 when marking with liquid OTC, with the exception of replacing water from the Transcanada pond with water from the Swan Creek Hatchery, which has similar levels of hardness, alkalinity and pH (Table 4). However, the average mark quality was higher for all treatment groups in 2011 than in 2012, which may

reflect a bias in the determination of marks, as the increased use of powdered OTC in 2012 created much higher quality marks than seen with the use of liquid OTC. As mark determination is highly subjective, as discussed in Section 2.7.7, there is a possibility for this type of shift in mark determination to occur. Unfortunately, there does not currently exist a more objective method for ranking the quality of OTC marks. The assistance of a computer engineer was recruited to investigate the possibility of developing a computer program to quantify the brightness observed on an OTC mark under the microscope. However, due to the quality of the mark being both related to the brightness and color of the mark, with OTC having a distinct golden tone above the green autoflorescence of the background, it did not seem possible to develop a program that could account for the autofluorescence in a way that would make the rankings truly comparable.

In 2012, the effect of water source on mark quality between treatment groups which had been marked with liquid OTC and powder OTC, although significant when analyzed separately, was not significant when analyzed together, as the strong effect of powder OTC on mark quality overpowered the effect of water source.

The potential effect of water source on OTC mark quality is likely related to the propensity for tetracycline molecules to bind to cations in solution, such as $Ca^{2+}$ and $Mg^{2+}$ . The benefit of tetracycline molecules as antibiotics derives from their molecular structure and spatial arrangement which allows them to be "chemically promiscuous" and form complexes with macromolecules and cations such as $Ca^{2+}$ and $Mg^{2+}$ (Nelson 1998, Lunestad and Goksayr 1990). Lambs et al. (1988) suggested that tetracycline bound in complexes with $Mg^{2+}$ and $Ca^{2+}$ have a reduced ability to diffuse through biological membranes, due to the resulting change in charge and decreased lipid solubility. Lunestand and Goksayer (1990) found that the antibiotic activity of OTC was significantly reduced in sea water, likely due to complex formation of tetracycline with $Mg^{2+}$ and $Ca^{2+}$ (Lunestand and Goksayr 1990). The effect of salinity on OTC mark quality was investigated in the work of Thomas (1995) and Denson and Smith (2008), who

both observed that high salinity OTC marking solution reduced the mark quality of larval red drum *Sciaenops ocellatus*. Similarly to Lunestad and Goksayr (1990), the authors felt the reduced activity of OTC was due to the formation tetracycline $Mg^{2+}$ and $Na^{2+}$ complexes. If OTC becomes bound with $Ca^{2+}$ in the marking solution, it may be that the complex is rendered unable to cross biological membranes due to a change in charge in the complex.

For this study, I was unable to isolate the effect of any particular parameter with the range of water quality parameters and focused instead on the differences in a number of water quality parameters in the water from the different hatcheries where marking takes place. However, it does appear that water source had an effect on mark quality, in particular that those samples which were marked in deionized water, which contains no $Mg^{2+}$ and $Ca^{2+}$ cations, had some of the highest scores among all treatment groups in both 2011 and 2012 (see tables 4 and 5). Although marking in deionized water is not practical for large scale marking practices, it does suggest that there is some effect of water quality variables in the marking process, but that this effect can be largely suppressed by switching from liquid OTC to powder OTC.

*3.5.3 Oxytetracycline type*

We found a striking difference in the mark quality of those otoliths marked with powdered OTC and those marked with liquid OTC. Fry marked with powdered OTC had a higher percentage of observed marks than those marked with liquid OTC in both 2011 and 2012 (Figure 7 and 8), as well as significantly higher quality of marks in 2012. The type and brand of oxytetracycline used in other studies across North America has varied and yet there has been little mention of the effect of the exact form of OTC used. Despite a lack of this analysis in the formal literature, discussions with researchers suggests that the brand and formula of oxytetracycline used does seem to influence the effectiveness of marking (D. Isermann, pers. comm., 2011, D. Logsdon, pers. comm., 2011). The powdered form of OTC is the most common form used for marking in the United States (D. Isermann, pers. comm., 2011) but can cause problems in the marking operation, such as causing a lethal drop in pH and excess foaming of the solution.

Both Fielder (2002) and Carty (2007) found that the drop in pH could be augmented by adding sodium dibasic in a 1:1 ratio with OTC. Fielder (2002) and Logsdon (pers. comm., 2011) recommend the use of No-Foam, a commercial anti-foaming agent, to prevent the solution from foaming. Manitoba hatcheries have been using a liquid pre-buffered form of OTC in order to avoid having to buffer the solution and use an anti-foaming agent. It is possible that the pre-buffered liquid formula influences the variability of mark quality and rate of marking. During the process of marking in 2012, we developed a new technique for mixing the powdered OTC into the marking solution which helped to keep the OTC powder in solution and likely created a more effective marking solution as less of the OTC may have precipitated out of solution (Section 3.3.2.3).

*3.5.4 Density*

At the time that this study took place, there was no reproducible measure of the density of fry in a treatment - rather, experienced hatchery staff measured density of fry visually. Unfortunately, this is common practice amongst hatcheries. Among the literature reviewed in preparation to this study, only one paper explained their methodology for measuring density; Logsdon (2006) enumerated walleye fry by weight, but still admitted that the resulting measurement was approximate at best. Therefore, the results of mark quality as it is related to fry density in the marking solution should be taken in context, and seen as an approximate measure rather than a truly quantitative measurement. However, we did not find a significant effect of density on the quality of OTC marks, therefore despite the highly variably density likely used in marking solutions, it likely has little effect on the end mark quality.

*3.5.5 Concentration*

Previous studies have observed an increase in mark intensity with increasing concentration of OTC in the marking solution (Brooks 1994, Lucchesi 2002, Denson and Smith 2008). The highest dose of OTC used in those studies was 700 mg/L, which is also the maximum approved dosage under the US Food and Drug Administration (FDA) legislation for the chemical marking of fin fish (Carty et al. 2007).

In this study, there was no increase in mark intensity associated with an increase in dosage from 700 to 1,400 mg/L OTC, when using powdered OTC (Onnacin 250). Whether this concentration represents the upper limit of OTC that can permeate biological membranes (suggesting a progressive relationship between OTC amount and stain intensity) or whether a concentration of 700 mg/L is simply the approximate dosage required to be taken up and stain the otolith is not entirely clear from this work. However, it would appear suggestive, that a concentration of 700 mg/L is sufficient to stain the calcified structure. As a result, concentrations beyond what is required to mark the calcified structure simply represent increased risk associated with the use of OTC to fry being marked.

*3.5.6 DMSO*

Dimethyl sulfoxide (DMSO) has previously been used to improve the uptake of OTC (Scidmore and Olsen 1969, Kayle 1992). This potentiating action is due to DMSO's solvent properties, which can facilitate transport of drugs, such as OTC, across cell membranes (Wood and Wood 1975, J. Betteridge, pers. comm., 2011). The use of DMSO was designed to test the hypothesis that mark intensity was low due to the inability for OTC to pass from the marking solution to the otoliths. However, the use of DMSO was not able to improve significantly the mark intensity of samples treated with liquid OTC. In fact, the use of DMSO had the opposite effect on mark intensity of fry treated with powdered OTC and significantly reduced mark intensity. Inclusion of DMSO in the marking solution was not able to improve mark intensity, therefore suggesting that the increased transportation of OTC from the marking solution to the otolith did not improve mark intensity. This suggests that the reduced mark intensity observed with liquid OTC compared with powdered OTC is not due to reduced transport of OTC from the marking solution to the otolith. The reduced mark intensity observed when DMSO was used in conjunction with powdered OTC suggests that DMSO does have an effect on OTC, albeit not the expected effect in this situation.

*3.5.6 Mortality*

During this study, high levels of mortality were observed in walleye fry. However, the experimental design of this study was not developed to include analysis of mortality, and such, it is not possible to determine whether the mortality occurred was associated with oxytetracycline treatments, holding conditions, or environmental factors.

Walleye fry pass through a number of stages until they begin exogenous feeding, and each of these stages is associated with a certain amount of mortality. When fry are first hatched, they are referred to as pro-larva, and are fed solely from their yolk sac and oil globule, which provide nutrition until their gut and jaws develop further (Li and Mathias 1982). The pro-larvae stage lasts from hatch until approximately day 5, at which point the yolk sac has been used up and fry begin to feed exogenously while still relying on their remaining oil globule. The oil globule provides a nutritional buffer, while fry begin exogenous feeding, however by day 7 and 8, fry that have not begun exogenous feeding will starve. The second stage of post-larva begins when fry develop a more complex digestive system, and this transition lasts from approximately day 10 to day 18 post-hatch (Li and Mathias 1982). As mortality largely occurred within the first four days of treatment, which span the first four to seven days post-hatch for the larvae in this experience, it seems possible for the observed mortality to be largely explained as a result of this natural transition to exogenous feeding.

However, high fry mortality was observed during preliminary trials with powdered OTC. One potential cause for this mortality may be related to the tendency for powdered OTC and sodium dibasic buffer to flocculate out of solution, forming a precipitate on the bottom of the stocking bags. In one instance, so much flocculate developed in the solution that the majority of fry appeared to be caught in the loose powder at the bottom of the bag, and may have suffocated. However, this issue was solved by dissolving the buffer in hot water before creating the marking solution, eliminating the formation of precipitate as a potential cause of mortality in marked fry.

Other studies have seen high OTC treatment related mortality. Logsdon et al (2004) found OTC treated larval walleye analyzed for acute post-treatment mortality had significantly higher rates of mortality than untreated larval walleye. However, all larval walleye held in aquaria post hatch experienced high mortality; even those larval walleye which were not treated with OTC had mortality rates of up to 45%. Mortality rates between treated and untreated walleye were not significant when stocked directly in a pond environment post marking, highlighting the difficulty of determining whether mortality is associated with the holding environment, handling conditions, or the actual treatment experienced. Lucchesi (2002) similarly found that OTC treated walleye fry did not have a significantly different survival rate than untreated walleye when stocked directly into a pond environment post-marking.

Although this study was not able to investigate treatment related mortality, it would be valuable for future studies to examine the effects of oxytetracycline treatment on mortality.

### *3.5.7 Recommendations*

The results of this study suggest that water source, OTC type and the age at which fry are marked have an effect on the quality of the OTC mark applied to larval walleye. The combination of factors which was most effective at produced high quality marks was the use of 700 mg/L powder OTC on walleye fry 3-5 day post hatch with either Swan Creek or Whiteshell Fish Hatchery water.

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# 4.0 Evaluation of retention and detection of oxytetracycline marks applied to larval walleye otoliths


## *4.1 Abstract*

Hatchery reared walleye (*Sander vitreus)* were marked in 2011 and 2012 in Manitoba to test whether oxytetracycline (OTC) marks could be observed up to ten weeks after a mark was applied. A series of OTC treatments was applied to two groups of larval walleye. One group was sampled two weeks after marking, while the other group was stocked into retention ponds and sampled ten weeks after marking. The goal of this work was to determine whether OTC marks would be retained over the ten week duration. Mark retention was measured as the mark rate, as well as the quality of marks observed. OTC mark quality decreased significantly over the ten week period in both 2011 and 2012 (Wilcoxon, $Z = 4.2152$, $P < 0.0001$ and $Z = 4.0270$, $P < 0.0001$, respectively). However, in 2012, marks were identifiable on 100% of fry marked with powdered OTC after the ten week period. Mark rates at ten weeks for fry marked with liquid OTC (700 mg/L) ranged from 38-71%. Fry marked with 1,400 mg/L powdered OTC maintained high quality of marks from two weeks and ten weeks (Wilcoxon, $Z = -1.1375$, $P = 0.2553$), whereas decreased mark quality was observed for fry marked in 700 mg/L powdered over the same time period (Wilcoxon, $Z=5.4936$, $P<0.0001$). Subsequently, fry marked in 1,400 mg/L OTC had higher quality marks than those marked in 700 mg/L at end of the ten week period (Wilcoxon, $Z = -3.2790$, $P = 0.0010$). Fry marked with Grand Rapids water had significantly higher quality marks ten weeks post immersion than those marked in Whiteshell water, for both treatment groups marked in 700 mg/L powdered OTC and liquid OTC (Wilcoxon, $Z = -4.5368$, $P < 0.0001$ and $Z = -2.4438$, $P = 0.014$, respectively. Oxytetracycline is an effective tool for tracing hatchery progeny. In order to maintain mark quality during the first growing season in Manitoba, a higher concentration of powdered OTC (1,400 mg/L) may be required than previously thought.

*4.2 Introduction*

Oxytetracycline (OTC) in an antibiotic in the tetracycline family, all of which are flourochromes, and fluoresce yellow-gold in ultraviolet light (Mitscher 1978). When applied to fish, either through feed, injection, or immersion, oxytetracycline becomes incorporated into the calcium carbonate matrix of developing bone (Milch et al. 1961). The resulting mark is visible on fish bones as a yellow-gold ring and can be used as an internal marker to differentiate hatchery origin fish from naturally produced fish (Logsdon 2006, Lucchesi 2002, Scidmore and Olson 1969). The ability to correctly identify hatchery reared fish is essential to monitoring the contribution of stocked fish into native populations. Immersion marking is largely used for marking larval fish, as millions of fish can be easily marked, with minimum handling and cost.

In Manitoba, preliminary marking of larval walleye with OTC began in 1998 (Biggin 2008). However, the marks recovered from known marked fish were found to be of varying quality, depending on the year and the specifications of the treatment applied (Biggin 2008). The absence of consistently high quality OTC marks on marked larval walleye has made it difficult to distinguish between stocked and naturally reproduced fish. Due to the observed variability of OTC marks in previous years, it was clear that in order to determine stocking success of walleye, it would be necessary to retain fish for a number of months in order to determine the percentage of marked fish we could expect to attribute as hatchery progeny from post-stocking lake surveys. Therefore, the rate of OTC marking observed in walleye marked as fry and held for ten weeks could be used to determine the correction factor which would be applied to the percentage of marked fish observed in lake samples (Mauk 2008).

In 2011 and 2012, a series of experiments was done to test the effect of a number of variables within an OTC marking methodology on mark quality and are discussed in chapter 3 of this volume). The goal of this work was to inform and develop a marking protocol for Manitoba hatcheries that would produce consistent, high quality marks. The marks from these experiments were viewed two weeks

following the application of OTC. The goal of this current study is to follow the retention of the OTC marks applied to larval fish from two weeks to ten weeks post-immersion, to determine the potential level of mark quality, and therefore mark rate, that we could expect to observe in age 0 stocked walleye sampled in the fall of the year they were stocked.

Manitoba Fisheries Branch performs annual to bi-annual lake assessment surveys on stocked walleye lakes in the eastern region of the province. These surveys are done by shoreline electrofishing, and focus on collecting baseline data on species composition and shoreline habitat, as well as collecting ageing structures for population assessments. The Provincial Fisheries Branch is currently attempting to incorporate the assessment of walleye stocking success into these annual surveys. In order to have these surveys be successful, hatchery reared walleye need to retain the OTC marks applied in the spring.

A series of variables in the OTC marking process was identified as potentially influencing both mark quality at two weeks and ten weeks post-immersion, as well as the retention of mark quality over this time period. Walleye fry are marked in Manitoba at Swan Creek, Grand Rapids and Whiteshell Fish Hatcheries. The water used to mark fry at each of these facilities has different water chemistry, and it is possible that the differences in water alkalinity may influence the effectiveness of oxytetracycline (Lunestad and Goksayr 1990) and the resulting mark quality (Denson and Smith 2008, Mauk 2008). In addition, the marking protocol in Manitoba originally used liquid OTC for fry immersion marking, but recently switched to using powdered OTC in 2012, as mentioned earlier in Section 3.2. There may be some difference in the action of powdered and liquid OTC that influences mark quality, and subsequently, mark retention. Hatcheries in Manitoba have previously marked fry at a concentration of 1,400 mg OTC/L, however it would reduce hatchery costs as well as reducing potential harm to larval walleye to mark at a concentration of 700 mg OTC/L, as used in Brooks et al. (1994) and Luchesi (2002). Before reducing the OTC concentration in the methodology used in Manitoba, mark quality needed to be confirmed as consistent over the ten week time period, therefore differences in mark retention between

groups marked at 1,400 and 700 mg OTC/L were tested. The influence of dimethylsulfoxide (DMSO) on mark quality, which has been used by both Scidmore and Olsen (1969) and Kayle (1992) as a potentiator to improve OTC uptake into otoliths, was also tested for the ability to improve mark quality and retention.

Long-term persistence of OTC marks has been observed in injected whitefish for over 4.5 years post mark (Nagiec 1992) and over a period of 56 days and 8 months in immersion-marked fingerling yellow perch (Unkenholz et al 1997). Mauk (2008) found that palmetto bass, marked by immersion as fingerlings, had a higher mark rate at 344 days than at 14 days post-immersion. However, larval fish marked by immersion have not always had high retention rates such as those seen in fish marked as fingerlings. Larval striped bass had a retention rate of 80% after one year (Reinart 1998), whereas larval red drum may only have reliable OTC marks up until three months post-immersion (Denson and Smith 2008). However, Lucchesi (2002) observed 100% mark retention rate for walleye otoliths marked as fry after 85-93 days post immersion. There appears to be no trend in decreasing intensity over time among marked larval walleye between three months and five years, suggesting that marks were retained throughout this time period (Logsdon 2009). However, what is not clear is whether there is some loss of mark quality within the first three months that may affect the ability to identify this mark in later years.

The objective of this study is to test a variety of OTC marking methods for larval walleye and determine the level of mark retention from two weeks to ten weeks post-immersion. The marking rate observed on larval walleye otoliths after the ten week period will provide a baseline marking rate which can be expected when hatchery origin walleye are sampled in fall of the stocking year.

### *4.3 Methods*

The methods described in this paper are similar to those outlined in Section 3.3, but are repeated here in full to describe the context in which oxytetracycline mark retention was studied.

*4.3.1 Fry acquisition*

In the spring of 2011 and 2012, walleye eggs were collected during a spawn-taking operation on Falcon Creek, a tributary to Falcon Lake, Manitoba. The eggs were incubated at the Whiteshell Fish Hatchery (West Hawk Lake, Manitoba) for approximately two weeks. In 2011, fry used in experiments were collected as eggs over a series of 3-5 days. Hatchery infrastructure did not allow for fry to be isolated from each day of collection once hatched, therefore, we were only able to estimate the collection date within the 3 to 5 days. However, in 2012, changes were made to hatchery infrastructure that allowed us to isolate fish based on the day of collection and the day of hatch. Therefore, in 2012, all fish used in the study were taken from the single day of the spawn taking operation to reduce variability in survival between different days of the spawning run. It was assumed for this study that the variation in age between 3 and 5 days did not impact mark quality.

*4.3.2 Experimental design*

Fry were marked with OTC in a series of experiments in 2011 and 2012 at the Whiteshell Fish Hatchery (Table 8 and 9). One experimental group of fry was treated in small batches to be held for two weeks, and another group of fry was treated en mass, in stocking bags, to be held for three months. In 2011, all treatments were applied to three replicate groups of fry (n=200). Each group of fry was hand counted and placed in 28 mL of water in a 188 mL whirlpak bag. Oxytetracycline was then added to 28 mL of water in a separate container, which was then added to the fry bag. In 2012, nine experimental marking treatments were developed to study the influence of water source, OTC type, DMSO and OTC concentration on the quality of OTC marks (Table 9).

TABLE 8. Experimental oxytetracycline marking treatments applied to larval walleye in the spring of 2011 at the Whiteshell Fish Hatchery, and held for 10 weeks in retention ponds.

| | | | | | Age in weeks | |
|---|---|---|---|---|---|---|
| | | | | | 2 | 10 |
| **Density (fry/L)** | **Age at mark (days)** | **OTC concentration (mg/L)** | **Water source** | **OTC type** | **Otoliths analyzed (n)** | **Otoliths analyzed (n)** |
| 4000 | 3 | 1,400 | Whiteshell | Liquid | 37 | 19 |
| 4000 | 3 | 1,400 | Whiteshell | Powder | 19 | 20 |
| 4000 | 3 | 1,400 | Transcanada | Liquid | 36 | 25 |
| 4000 | 1 | 1,400 | Grand Rapids | Liquid | 0 | 20 |

TABLE 9. Experimental oxytetracycline marking treatments applied to larval walleye in the spring of 2012 at the Whiteshell Fish Hatchery, and held for 10 weeks in retention ponds.

| | | | | | | Age in weeks | |
|---|---|---|---|---|---|---|---|
| | | | | | | 2 | 10 |
| **Density (fry/L)** | **Age at mark (days)** | **OTC concentration (mg/L)** | **Water source** | **OTC type** | **DMSO (%)** | **Otoliths analyzed (n)** | **Otoliths analyzed (n)** |
| 4000 | 3 | 700 | Whiteshell | Liquid | 0 | 45 | 18 |
| 4000 | 3 | 700 | Whiteshell | Powder | 0 | 45 | 32 |
| 4000 | 3 | 700 | Swan Creek | Liquid | 0 | 45 | 25 |
| 4000 | 3 | 700 | Swan Creek | Powder | 0 | 45 | 0 |
| 4000 | 3 | 700 | Deionized | Liquid | 0 | 45 | 31 |
| 4000 | 3 | 700 | Deionized | Powder | 0 | 45 | 46 |
| 4000 | 3 | 700 | Whiteshell | Powder | 2 | 15 | 12 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 2 | 15 | 15 |
| 4000 | 3 | 1,400 | Whiteshell | Powder | 0 | 5 | 30 |
| 4000 | 3 | 700 | Grand Rapids | Liquid | 0 | 0 | 7 |
| 4000 | 3 | 700 | Grand Rapids | Powder | 0 | 0 | 33 |

Six treatments (Whiteshell liquid, Whiteshell powder, Swan Creek liquid, Swan Creek powder, deionized liquid, deionized powder) were applied to nine replicate groups of fry (n=300). Two treatments (Whiteshell liquid DMSO and Whiteshell powder DMSO) were applied to two replicate groups of fry (n=300). One treatment (Whiteshell powder 1,400) was applied to a single group of fry (n=300). Each group of fry was counted volumetrically and placed in 42 mL of water in a 188 mL whirlpack bag. OTC

stock solutions were prepared in advance for each experimental treatment. For each treatment, 42 mL of prepared OTC stock solution was added to the fry bag.

*4.3.3 Application of oxytetracycline treatments*

*4.3.3.1 Two weeks*

Fry were held in the marking solution for 6 hours in individual treatment bags in a series of coolers with the goal of maintaining consistent water temperature and minimal exposure to light. After 6 hours, fry were temperature acclimated and were transferred into screened-in areas or rearing troughs equipped with flow through systems with a velocity of 0.01 m/s. The screened-in areas had volumes of 19.8 L and 14.9 L in 2011 and 2012, respectively. Each screened-in area within the rearing trough had an associated number and letter based on its position in the rearing trough. These number-letter codes then became the way each experimental group of fry was identified, from the time each group was marked in the field until the otoliths were processed in the lab. The day on which fry were marked was the only point at which I, as the experimenter, was aware of which treatment was associated with each code. These were recorded in a master spreadsheet, which was then not consulted until time of data analysis after otoliths had been removed an analyses in the lab for mark quality. Rearing troughs were located in a semi-outdoor laboratory setting such that fry experienced a temperature-photoperiod regime similar to natural conditions. Temperature of trough water was recorded daily, as was pH, using a Hanna pH meter (model H198128), total dissolved solids (TDS), using a Oakton TDS meter, and dissolved oxygen (DO), measured using a HACH DO meter (HQ 30d model), as well as the number of mortalities in each section, which were counted by hand. Fry were fed 200 mL of concentrated zooplankton solution collected from West Hawk Lake using two 800 μm bongo plankton nets, graded to <600 μm, twice a day from day seven to day fourteen. After two weeks, fry were sacrificed and frozen until otoliths could be extracted and observed for marks in the lab.

*4.3.3.2 Three months*

*4.3.3.2.1 Oxytetracycline type*

*2011*

Fry (n=50,000) were placed in a large plastic tub filled with 7 L of water, and then added to a double bagged plastic fry bag. 200 mL of liquid OTC (Oxyvet 100 LP, 100 mg active oxytetracycline/ 1 mL) was then added to the fry bag with an additional 5 L of water, to make a total volume of 14 L marking solution at 1,400 mg/L OTC concentration. Bags were inflated with compressed air to provide adequate oxygen and held for 6 hours out of direct sunlight and heat and transported to stocking sites. Fry bags were floated in each pond to equalize the water temperature of bags to the temperature of the pond, and then released into a retention pond in South Eastern Manitoba. Age 0 fish were recovered from ponds in late August with 2, 1, and ¾" gill nets. Fish were sacrificed and frozen until otoliths could be removed and analyzed.

*2012*

*Powdered OTC*

A stock solution was mixed to create a twice concentrated solution at 1,400 mg/L, which was diluted in each fry bag, to make a total concentration in each bag of 700 mg/L OTC. 98 g of powdered OTC (Onnacin 250, 100 g active oxytetracycline/ 1 mL) was added to the stock solution, with 98 g of sodium dibasic dissolved in a small amount (10 mL) of hot water, in order to dissolve the buffer. A small amount of No Foam (Creative Marketing and Research, Fresno, California) was added to the stock solution as well to prevent foaming of the solution when mixed. The stock solution was mixed well. A mix of 70 L of concentrated fry solution is enough to mark 10 bags of fry, with 14 L of in each bag. Fry (n=50,000) were placed in a large plastic tub filled with 7 L of water, and then added to a double bagged plastic fry bag with 7 L of the stock solution, to make a total volume of 14 L marking solution.

*Liquid*

Fry (n=50,000) were placed in a large plastic tub filled with 7 L of water, and then added to a double bagged plastic fry bag. 200 mL of liquid OTC (Oxyvet 100 LP, 100 mg active oxytetracycline/ 1 mL) was then added to the fry bag with an additional 5 L of water, to make a total volume of 14 L marking solution at 700 mg/L OTC concentration.

Bags were inflated with compressed air to provide adequate oxygen, held for 6 hours, out of direct sunlight and heat, and transported to stocking sites. Fry bags were then floated in each pond to equalize the water temperature of bags to the temperature of the pond, and then released into a retention pond in South Eastern Manitoba. Age 0 fish were recovered from ponds in late August with 2, 1 and ¾" gill nets. Fish were frozen until otoliths could be removed and analyzed.

*4.3.3.2.2 Water quality*

*2011*

To test the effect that the water source of the OTC marking solution may have on mark quality, three water sources investigated; Whiteshell Fish Hatchery, Transcanada Pond, and laboratory grade deionized water. Each water source was used to mark three replicate groups of fry (n=200). Water from the Transcanada Pond, located just outside the range of the Canadian Shield, has similar water chemistry to water used at the Swan Creek Hatchery on Lake Manitoba (Table 4). Because of this similarity in water chemistry, water from Transcanada Pond was used as a proxy for water from Swan Creek, thereby eliminating the risk of transferring Koi herpesvirus from Swan Creek to eastern watersheds.

*2012*

To test the effect that the water source of the OTC marking solution may have on mark quality, four water sources investigated; Whiteshell Fish Hatchery, Swan Creek Hatchery, laboratory grade deionized water and Grand Rapids Hatchery. Each of these water sources were used to treat fry in conjunction with both powdered OTC (Onnacin 250) and liquid OTC (Oxyvet 100 LP). The water used in

each sample was that which used in operational marking protocol at the hatcheries, and from Swan Creek and Grand Rapids, this represents a mixture of well and lake water, which is adjusted daily to maintain optimum temperatures for walleye hatching. The water used from the Whiteshell Hatchery comes directly from the lake intake line, and fluctuates in water quality depending on lake conditions. Swan Creek Water was sterilized with UV light before using in marking to eliminate the potential for koi herpes virus transfer.

*4.3.3.2.3 Concentration*

*2011*

All treatment groups were marked at a concentration of 1,400 mg/L.

*2012*

All treatments were marked at a concentration of 700 mg OTC/L, except for two treatments, both marked with powdered OTC, which were marked at 1,400 mg OTC/L. One treatment used water sourced from the Swan Creek Hatchery and the other from the Whiteshell Fish Hatchery.

TABLE 10. Water chemistry of water sources used in 2011 and 2012 retention OTC marking treatments at the Whiteshell Fish Hatchery. Parameter measurements were determined by ALS Environmental Laboratories in Winnipeg, Manitoba.

| **Water quality parameters** | **Deionized Water 2012** | **Whiteshell Hatchery 2011** | **Whiteshell Hatchery 2012** | **Trans can pond 2011** | **Grand Rapids Hatchery 2012** | **Swan Creek Hatchery 2012** |
|---|---|---|---|---|---|---|
| Alkalinity (CaCO3) (mg/L) | 0 | 24.6 | 25 | 144 | 157 | 220 |
| Total calcium (mg/L) | 0 | 10.1 | 9.52 | 45 | 52.4 | 55.6 |
| Total magnesium (mg/L) | 0 | 1.48 | 1.42 | 13.4 | 19.4 | 57.7 |
| pH | 7 | 7.56 | 7.61 | 8.24 | 8.37 | 8.41 |
| TDS (mg/L) | 0 | 54 | 45 | 296 | 248 | 1130 |

*4.3.3.2.4 DMSO*

In 2012, the effect of DMSO was tested by marking two treatment groups with 2% DMSO in the marking solution. Three replications of each treatment were applied.

*4.3.4 Mark detection*

In the laboratory, walleye were measured for length and wet weight and otoliths were extracted. Otoliths were mounted on microscope slides with super glue (Krazy Glue, Columbus, Ohio), concave side up, and polished with 600 and 1,200 wet grit sandpaper until the inner daily rings were observed under 50X magnification with transmitted light. The otoliths were analyzed for OTC marks using a Nikon Eclipse 50i Epifluorescent microscope with UV B-3A and 2B filter cubes, and C-SHG1 super high pressure mercury lamp power supply. For each otolith, OTC mark intensity was assigned a grade using the following scale: 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark. This mark ranking system was based on that ranking system used by Isermann et al. in their work on OTC mark efficacy (1999, 2002). I was also trained by Dr. Dan Isermann in OTC mark detection and analysis in November of 2011 and therefore have continued to use his ranking system to categorize the intensity of OTC marks. Examples of each mark intensity rank can be observed in Figure 3. Otoliths were deemed to have "no mark" only after several cycles of observation and sanding had produced no visible mark, and the otoliths were clearly sanded past the central annuli. If otoliths were over sanded without observation of the central annuli, the otoliths were scored as over sanded, and removed from analysis. A photograph was taken of each mark, viewed under the 50x oil immersion objective, with a small amount of immersion oil and the 2B filter cube. Camera settings were kept consistent at an exposure composition of -1 1/3 EV.

*4.3.5 Mark quality control*

Marks were viewed and scored during the first observation by a single reader. These scores were done blind, such that the reader did not know with which treatment the otoliths had been marked. The same reader then scored each mark a second time, blind, based on the photograph alone. These scores were compared with the first, and if a discrepancy occurred between the first and second reading, the otolith was analyzed a third time, after which time, the modal age was used as the final reading.

*4.3.6 Statistical Analysis*

As data were discrete, and therefore not normally distributed, non-parametric statistics were applied. The Wilcoxon-Mann-Whitney test was used to examine differences between mark quality ranks associated with each treatment variable. The level of significance was set at 0.05. The analyses were performed using SAS 9.3 statistical software (SAS Institute, Cary, NC).

*4.4 Results*

Mark quality summarized across all treatments decreased significantly over the eight week period in both 2011 and 2012 (Wilcoxon, $Z = 4.2152$, $P < 0.0001$ and $Z = 4.0270$, $P < 0.0001$, respectively). However, despite an overall trend in decreased mark quality, there were a number of treatment groups which did maintain mark quality over the ten week period.

In 2011, the highest rate of mark identification was observed on fry otoliths which were treated with 1,400 mg powdered OTC/L in Whiteshell Hatchery water (88%, n=19) (Table 12). The second highest rate of mark identification was in fry treated with 1,400 liquid OTC/L in Grand Rapids Hatchery water (75%, n=20). The rate of mark identification was lower in those groups treated with 1,400 liquid OTC/L in both Transcanada water and Whiteshell water (58%, n=19 and 52%, n=25, respectively) (Table 12).

TABLE 11. Percent of oxytetracycline marks observed on otoliths from walleye immersed in experimental marking treatments at the Whiteshell Fish Hatchery in 2011 after fish were sampled two weeks post immersion.

| Density (fry/L) | Age at mark (days) | OTC conc. (mg/L) | Water source | OTC type | DMSO (%) | n | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|---|---|
| 4000 | 3 | 1,400 | Whiteshell | powder | 0 | 8 | 100 | 1.6 ± 0.7 |
| 4000 | 3 | 1,400 | Transcanada | liquid | 0 | 23 | 100 | 2.0 ± 0.9 |
| 4000 | 3 | 1,400 | Whiteshell | liquid | 0 | 33 | 88 | 1.6 ± 0.8 |

TABLE 12. Percent of oxytetracycline marks observed on otoliths from walleye immersed in experimental marking treatments at the Whiteshell Fish Hatchery in 2011 after fish were sampled three months post immersion.

| Density (fry/L) | Age at mark (days) | OTC conc. (mg/L) | Water source | OTC type | DMSO (%) | n | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|---|---|
| 4000 | 3 | 1,400 | Whiteshell | powder | 0 | 19 | 88 | 2.2 ± 1.0 |
| 4000 | 3 | 1,400 | Grand Rapids | liquid | 0 | 20 | 75 | 2.6 ± 1.1 |
| 4000 | 3 | 1,400 | Transcanada | liquid | 0 | 19 | 58 | 3.0 ± 1.1 |
| 4000 | 3 | 1,400 | Whiteshell | liquid | 0 | 25 | 52 | 3.2 ± 1.1 |

In 2012, all treatment groups of fry marked with powdered OTC had a 100 % mark rate and an average intensity between 1.8 and 1.1, while all treatment groups marked with liquid OTC had a median intensity of 2.3 and higher after three months (Table 14). Mark rates of fry marked with liquid OTC ranged from 38-71%, with the lowest mark rate observed in those fry marked with liquid OTC and Whiteshell water (Table 14). The fry which were marked with Swan Creek water and powdered OTC did not survive the ten week period, therefore there are no mark retention results from this treatment group.

TABLE 13. Percent of oxytetracycline marks observed on otoliths from walleye immersed in experimental marking treatments at the Whiteshell Fish Hatchery in 2012 after fish were sampled two weeks post immersion.

| Treatment | | | | | | *n* | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|---|---|
| Density (fry/L) | Age at mark (days) | OTC (mg/L) | Water source | OTC type | DMSO (%) | | | |
| 4000 | 3 | 1,400 | Whiteshell | Powder | 0 | 5 | 100 | 1.0 ± 0.0 |
| 4000 | 3 | 1,400 | Swan | Powder | 0 | 5 | 100 | 1.0 ± 0.0 |
| 4000 | 3 | 700 | Whiteshell | Powder | 0 | 45 | 100 | 1.0 ± 0.0 |
| 4000 | 3 | 700 | Deionized | Powder | 0 | 45 | 100 | 1.2 ± 0.4 |
| 4000 | 3 | 700 | Whiteshell | Powder | 2 | 15 | 100 | 1.4 ± 0.6 |
| 4000 | 3 | 700 | Deionized | Liquid | 0 | 45 | 100 | 1.9 ± 0.5 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 2 | 15 | 100 | 2.3 ± 0.5 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 0 | 45 | 100 | 2.4 ± 0.5 |
| 4000 | 3 | 700 | Swan | Liquid | 0 | 45 | 89 | 2.6 ± 0.7 |

TABLE 14. Percent of oxytetracycline marks observed on otoliths from walleye immersed in experimental marking treatments at the Whiteshell Fish Hatchery in 2012 after fish were sampled three months post immersion.

| Treatment | | | | | | n | % marked | Average intensity ± SD |
|---|---|---|---|---|---|---|---|---|
| Density (fry/L) | Age at mark (days) | OTC (mg/L) | Water source | OTC type | DMSO (%) | | | |
| 4000 | 3 | 700 | Grand Rapids | Powder | 0 | 30 | 100 | 1.1 ± 0.3 |
| 4000 | 3 | 1,400 | Whiteshell | Powder | 0 | 16 | 100 | 1.3 ± 0.4 |
| 4000 | 3 | 700 | Deionized | Powder | 0 | 45 | 100 | 1.4 ± 0.5 |
| 4000 | 3 | 700 | Whiteshell | Powder | 2 | 12 | 100 | 1.7 ± 0.5 |
| 4000 | 3 | 700 | Whiteshell | Powder | 0 | 44 | 100 | 1.8 ± 0.8 |
| 4000 | 3 | 700 | Grand Rapids | Liquid | 0 | 7 | 71 | 2.3 ± 0.5 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 2 | 13 | 46 | 3.3 ± 0.5 |
| 4000 | 3 | 700 | Deionized | Liquid | 0 | 30 | 57 | 3.4 ± 0.2 |
| 4000 | 3 | 700 | Swan Creek | Liquid | 0 | 16 | 44 | 3.4 ± 0.8 |
| 4000 | 3 | 700 | Whiteshell | Liquid | 0 | 16 | 38 | 3.6 ± 0.0 |

*4.4.1 Water source*

There was a significant decrease in mark quality over the two to ten week period post immersion for fry marked with in both Whiteshell and deionized water, in liquid and powdered OTC in 2012

(Wilcoxon, Z = 4.0270, P < 0.0001). There was no sample of fry marked with Grand Rapids water from two weeks post-immersion in either liquid or powdered OTC, therefore there is no comparison of mark quality over time. However, fry marked with Grand Rapids water in 2012 had significantly higher mark quality at ten weeks post immersion than those marked in Whiteshell water, for both treatment groups marked in 700 mg/L powdered OTC and liquid OTC (Wilcoxon, Z = -4.5368, P < 0.0001 and Z = -2.4438, P = 0.014, respectively). However, in 2011, there was no significant difference in mark quality between those marked in Grand Rapids water and those marked in either Whiteshell water (Wilcoxon, Z=-1.7642, P = 0.0777) or Transcanada water (Wilcoxon, Z = 0.9948, P = 0.3198). Yet, fry were marked in liquid OTC at a concentration of 1,400 mg/L in 2011 rather than the lower concentration of 700 mg/L in 2012.

Fry marked in deionized water in 2012 had significantly higher mark quality after ten weeks than fry marked in Whiteshell water, when both were marked with powdered OTC (Wilcoxon, Z = -2.5233, P = 0.0116). However, there was no significant difference in mark quality between fry marked in deionized water and those marked in Whiteshell water when marked in liquid OTC (Wilcoxon, Z = 1.2781, P = 0.2012).

*4.4.2 Oxytetracycline type*

Fry marked with 1,400 mg/L powdered OTC maintained high quality marks between two weeks and ten weeks in 2011 with no significant difference in mark quality over time (Wilcoxon, Z = -1.4257, P = 0.1540). However, fry marked in 1,400 mg/L liquid OTC had significantly reduced quality of mark after ten weeks post immersion (Wilcoxon, Z = 4.5940, P < 0.0001). In 2012, fry marked with both 700 mg/L liquid and powdered OTC had reduced quality of marks after the ten week period (Wilcoxon Z = 8.4772, P < 0.0001, and Wilcoxon, Z = -6.0929, P < 0.0001, respectively) however, fry marked in powdered had significantly higher marks than those marked in liquid OTC at ten weeks post immersion (Wilcoxon, Z = 9.2515, P < 0.0001).

*4.4.3 Concentration*

Fry marked with 1,400 mg/L powdered OTC maintained high quality marks between 2 weeks and 10 weeks (Wilcoxon, $Z = -1.1375$, $P = 0.2553$), whereas fry marked in 700 mg/L powdered OTC did not maintain mark quality over the same period, with marks decreasing in quality over time (Wilcoxon, $Z = 5.4936$, $P < 0.0001$). In addition, fry marked in 1,400 mg/L OTC had higher quality marks than those marked in 700 mg/L at ten week period (Wilcoxon, $Z = -3.2790$, $P = 0.0010$).

*4.4.4 DMSO*

There was no effect of DMSO on the quality of marks after ten weeks when compared to treatments without DMSO for both with powdered and liquid OTC treatments (Wilcoxon, $Z = -0.6559$, $P = 0.5119$, and $Z = -0.8754$, $P = 0.3813$, respectively). However, those marked with 2% DMSO and powdered OTC did not display a decrease in quality from two weeks to three months (Wilcoxon, $Z = 0.03085$, $P = 0.3789$), unlike those marked with 2% DMSO and liquid OTC and 0% DMSO and powdered OTC (Wilcoxon, $Z = 2.9399$, $P = 0.0033$, and $Z = 5.2521$, $P < 0.0001$, respectively)

## *4.5 Discussion*

The goal of this study was to observe the rate of mark retention, defined as the change in mark rate and quality, over an eight week time period. There are a number of ways to explain the loss of both mark quality and of the visibility of the mark as a whole. Logsdon et al. (2005) suggested there are three potential explanations for the loss of an oxytetracycline mark over time: poor efficacy of the OTC mark treatment, errors in mark identification, and true mark loss or dissipation. In the case of poor efficacy of the initial marking process, the source of the lost mark is the inadequate marking of the otolith at the time of marking, rather than the true loss of the mark over time. This study attempted to test for this type of mark loss by observing otolith marks two weeks after marking as well as at three months old. Without the inclusion of recently marked fry, it would be difficult to tell when observing marks after three months

post-marking, whether the mark was lost over time, incorrectly applied, or not fully incorporated during the marking procedure, and therefore never truly present. However, it is also possible that the oxytetracycline mark takes a number of days to become incorporated into the otolith after treatment. This has been confirmed by Logsdon et al. (2004), who observed walleye marked at less than 24 hours post hatch on the same day they were marked, as well as three six and nine days post-immersion. He found that high quality marks were not observable on otoliths until nine days post hatch (Logsdon et al. 2004). For this reason, it was decided that the two week time period at which fish were first observed for marks in this study should be able to serve as a good indication of the original applied mark.

In this study, the majority of fish from all treatment groups had appear to have "accepted" the initially applied OTC mark and these marks largely remained present and were thus correctly identified after ten weeks. However, mark quality was lower at ten weeks than at two weeks for every treatment studied in 2011 and 2012. This suggests that although OTC marks were initially incorporated into the otolith, as measured by the presence of a mark at two weeks post-immersion, there was a loss in mark quality between the initial marking procedure and ten weeks post-immersion. What is not clear is whether this loss of quality is due to dissipation of oxytetracycline within the otolith over time or whether there is some factor in the processing and observation of older otoliths that make an existing mark more difficult to identify .

There are a number of factors regarding otolith composition that suggest it is unlikely for oxytetracycline to dissipates over time after becoming incorporated into the otolith. Otoliths are acellular, and therefore once a compounded has been incorporated into the developing structure of the bone, it will be permanently retained (Campana and Neilson 1995), unless under anaerobic stress (Mugiya and Uchimura 1989). Tetracycline molecules bind to calcium on the collagen fibrils of the developing bone matrix via the naphthacene carboxamide nuclei of the tetracycline molecule (Milch et al 1957). Therefore, due to the largely metabolically inert structure of otoliths, oxytetracycline that had been once incorporated

in the otolith could be assumed to be continuously present, despite the passage of time. During the holding time in which fry were placed in troughs for two weeks, there was the potential for anaerobic distress, however, dissolved oxygen was continuously monitored and found to be at acceptable levels. As well, if the loss of mark quality observed in this experiment was induced by anaerobic stress, we would expect to see mark quality deteriorate over the two week period at the hatchery, rather than in those fish sampled after ten months. As it were, marks from fry held for two weeks within the hatchery were of significantly better quality than those viewed at ten weeks, which would have experienced less potential anaerobic stress. Therefore, it is likely that loss in mark quality may be better explained by the overall growth of the otoliths over time, rather than a loss or dissipation of oxytetracycline.

It has been suggested that mark retention may decrease over time due to additional growth of the otoliths making the oxytetracycline mark more difficult to observe. Ruhle and Grieder (1989) observed that the thin layer of OTC deposition in the otoliths can easily be ground off during processing, and result in a false-negative. It became clear over the course of the study how difficult it was observe nuclei on otoliths from larval fish, which require relatively little processing and have experienced only a small amount of growth covering the location where the OTC mark would be located. To locate the nucleus, and thus, the presence or absence of an OTC mark on the otolith of an older fish can require extremely careful analysis and processing to identify (Campana and Neilson 1985). Therefore, the potential cause of the observed decrease in mark quality over time may be simply the increased difficulty in detecting oxytetracycline marks, rather than the dissipation of the mark over time. However, this study is limited by having only two sampling periods (at two and ten weeks), which make it impossible to trace the trend of this relationship between time and mark quality. Notably, a study by Logsdon et al. (1999) was able to better follow this relationship by analyzing otoliths for OTC marks along a series of time periods, and observed a trend of decreasing mark quality over time. This may suggest that there is some truth to the dissipation of OTC over time, however, the trend observed in Logsdon et al. (1999) may also be due to

the increasing difficulty in observing an OTC mark as the otoliths continues to develop more layers of calcium carbonate over the original OTC mark.

Despite the observation that mark quality decreased when all treatments were pooled over the ten week period, treatments which used powdered OTC in 2012 maintained a mark rate of 100% over the ten week period. In comparison, treatment groups which were marked with liquid had a maximum mark rate of 88%, and lower, as well as having significantly lower mark quality than treatment groups marked with powdered OTC. These observations suggest that marking fry with powdered OTC produces higher quality marks both at two weeks and three months.

The observation of higher quality marks for those fry marked with powdered OTC at two weeks and ten weeks suggest that the determining factor of mark retention is mark quality at two weeks. It is reasonable to expect that any loss in mark quality can be attributed to the inability to observe a mark due to observation or processing error, rather than mark dissipation. If this is true, it would be expected that otoliths with higher quality marks at two weeks would have higher mark retention at ten weeks. This was the case for the majority of treatments which had the highest mark quality at two weeks, such as those marked in Whiteshell water at 1,400 mg powdered OTC/L and those marked in deionized water at 700 mg powdered OTC/L. The relationship between mark quality at two weeks and mark retention over time reinforces the interpretation that loss of mark quality is not due to dissipation, but rather, that observation of the mark gets more difficult over time due to otolith growth. The higher mark quality is at two weeks, the more likely the mark will observable at ten weeks, and will be correctly identified as hatchery origin.

In 2011, fry which were marked with 1,400 mg/L powdered oxytetracycline had no loss of quality over the ten week time period. This treatment group had the highest quality of marks of those marked in 2011. In 2012, fry marked with 700 mg/L powdered OTC in solution with 2% DMSO also showed no decrease in mark quality over time, as did the treatment group marked with 1,400 mg/L

powdered OTC. Those fry marked with DMSO showed no improvement in mark quality over those which were marked without DMSO, however those that were marked with powdered OTC and 2% DMSO did not show a decrease in mark quality over the period between two weeks and ten weeks as opposed to the decrease in mark quality observed in treatments without DMSO. This suggests that there may have been some action of DMSO that acted to "potentiate" the incorporation of oxytetracycline into the matrix of the otolith, such that the OTC remained visible after ten weeks.

Grand Rapids water has an alkalinity of 157 (mg/L) $CaCO_3$ and correspondingly high levels of calcium and magnesium (Table 4). The results discussed in section 3.4.2 of this document stated that walleye fry marked in deionized water had significantly higher mark quality than fry marked in Swan Creek and Whiteshell Hatchery water. Grand Rapids water is similar to Swan Creek water, in having high levels of alkalinity, as well as $Ca^{2+}$ and $Mg^{2+}$ The antibiotic properties of oxytetracycline are due to its ability to be "chemically promiscuous" and form complexes with macromolecules and cations such as $Ca^{2+}$ and $Mg^{2+}$ (Nelson 1998, Lunestad and Goksayr 1990). Lambs et al. (1988) suggested that tetracycline bound in complexes with $Mg^{2+}$ and $Ca^{2+}$ have a reduced ability to diffuse through biological membranes, due to the resulting change in charge and decreased lipid solubility.

Based on this hypothesis, it would be expected for mark quality to be lower when marked in Grand Rapids water than in both deionized and Whiteshell water. However, except for higher concentrations of OTC at 1,400 mg/L, Grand Rapids samples had higher quality marks than deionized water when marked with both liquid and powdered in 2012. In addition to this, Grand Rapids fry were sampled at a later date, such that they were sacrificed at 109 days, while fry from the Whiteshell were sampled for marks at 73 days old. The older age of Grand Rapids fish should have made the marks more difficult to observe, if the hypothesis was true regarding the effect of additional otolith growth on mark detection. It is unclear why higher quality marks were observed among those fry marked at the Grand

Rapids Hatchery but would be worthwhile pursuing in order to better understand the relationship of water source to OTC mark quality.

These results suggest marking larval walleye at a concentration of 1,400 mg powdered OTC/L creates high quality OTC marks that will be retained over the ten week summer period from stocking to collection in the fall. Water quality parameters, as designated by water source, had an effect of mark quality, such that the highest quality marks were observed when fry were marked in deionized water. However, without a cost-benefit analysis, it is not clear whether the improvement in mark quality is not great enough to justify the infrastructure costs required to mark mass batches of walleye fry with deionized water. In addition, although the use of deionized water improved mark quality, the use of powdered OTC created such high quality marks, that the differences in mark quality related to water source are negligible. Further work will be done to investigate the high quality of Grand Rapids fry marks and take suggestions from this work as to how to progress with OTC marking in Manitoba hatcheries in the future.

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# 5.0 Contribution of supplemental fry stocking to walleye populations in Eastern Manitoba

## *5.1 Abstract*

Walleye are a valuable fish species in Manitoba, and the province has been augmenting their population through stocking for over 50 years. However, as in many other jurisdictions, monitoring of the success of these stocking programs has been limited. The objective of this study was to estimate the contribution of hatchery reared walleye to the age 0 year class of five Manitoba lakes. Hatchery reared walleye contributed to the age 0 year class in each of the five study lakes. Average contribution of hatchery reared walleye across study lakes was 48%, with a range of 11- 88%. Stocking effort, measured as the number of fry stocked per hectare lake area, was strongly related (OLS, $R^2$=0.627) to the catch per unit effort (CPUE) of marked walleye on each of the five study lakes. Stocked walleye fry contributed 82% to the age 0 year class of Barren Lake, 88% to Flanders Lake, more than 40% to Booster Lake, less than 15% to White Lake and less than 1% to Star Lake. Hatchery reared walleye were significantly smaller than naturally produced fish averaged across all study lakes (Length; One way ANOVA, F=73.78, P<0.0001; weight; One way ANOVA, F=47.31, P<0.0001). Considering the varied contribution rates observed in this study, continuing to monitor stocking success in Manitoba would enable fisheries managers to maximize stocking benefit and target appropriate water bodies for stocking.

## *5.2 Introduction*

Walleye (*Sander vitreus)* are one of Manitoba's most important fish species. With a natural distribution that covers all but the most northernmost parts of the province, walleye are fished by recreational, commercial, and domestic fishers (Stewart and Watkinson 2004). More walleye are retained by recreational anglers than any other species, making them the most popular food fish in Manitoba (Manitoba Water Stewardship 2005). Recreational fishing for walleye makes up a large part of the recreational fishing economy, creating more than 100 million dollars in revenue annually (Manitoba Water Stewardship 2005). Walleye also make up the majority of the commercial fishery in Manitoba and provide critical employment opportunities in rural and northern communities (Manitoba Water Stewardship 2013). In addition, walleye are critical to the domestic harvest of Manitoba's First Nations people (Green and Derkson 1984, Berkes 1990, Warkinton 1995).

Although walleye occur naturally in Manitoba, high fishing pressure, combined with high political pressure, has encouraged the Provincial Fisheries Branch to invest in walleye stocking programs. More than 30 million walleye are stocked each year into between 70 to 100 lakes in Manitoba from Provincially operated hatcheries. These stocking programs are largely designed to meet four goals: the establishment of desirable species where there were none before, maintain fish populations where natural reproduction cannot, supplement declining or over-harvested populations so that they can recover, and diversify fishing opportunities in areas where demand exceeds supply (Biggin 2009).

The presumed benefits of hatchery programs are clear: they can create more fish for commercial and recreational harvest (Seip 1995, Mathias et al. 1992, Bennet and McArthur 1990), create jobs for fishers, fish culturists and fisheries biologists (Waples 1999), allow governments to meet legal, treaty or other similar resource management obligations, as well as mitigate the effects of habitat degradation and loss (Waples 1999). However, the understandable benefits of stocking have led to a generalized

assumption that stocking always beneficial, rather than viewing stocking as a tool to be to be applied where and when it is needed. This assumption of inherent value in stocking programs has created a mindset in which many jurisdictions have neglected to study the effectiveness of stocking (Waples 1999, Welcomme and Bartley 1998). Manitoba has followed in this trend, and despite stocking large numbers of walleye each year, there has been relatively little monitoring of stocking success.

Monitoring of stocking success is valuable not only to better understand its effectiveness, but also to study any potential adverse effects. Although the effects of stocking fish are largely considered positive, there are can be negative consequences including the cost to fisheries departments for both hatchery infrastructure and transportation, as well as the potential for stocking to reduce the genetic diversity and fitness of native populations (Araki and Schmid 2010) and transfer diseases and parasites (Waples 1999, L. Janusz, Fisheries Biologist, Manitoba Fisheries Branch, pers. comm., 2012). Stocking has been shown to reduce the abundance of surrounding year classes, as well as result in the decreased growth of those year classes into which hatchery fish are stocked (Li et al 1996). As well, highly publicized stocking programs have been shown to increase recreational fishing pressure and could result in the reduction of total population abundance (Seip 1995, Johnson and Carpenter 1994). Therefore, it is valuable to monitor fish stocking programs not only to ensure the program is effective, but also to monitor the potential negative effects of stocking.

The goal of this project was to use the OTC marking methodology developed in Chapter 3 and tested for efficacy in Chapter 4 of this document and apply it to see if it could effectively be used to monitor stocking success of hatchery reared walleye. Five lakes in Eastern Manitoba were used as subjects and were stocked with walleye fry marked with oxytetracycline (OTC), in order to differentiate hatchery origin fish from those of natural origin. Although not an original goal of the study, we were able to make some inferences about factors which influenced walleye stocking success.

### *5.3 Methods*

The methods described in this paper are similar to those in section 3.2 of this document, and are re-stated here in detail to place the current study in an appropriate context.

#### *5.3.1 Fry acquisition*

In the spring of 2012, walleye eggs were collected during a spawn taking operation on Falcon Creek; a tributary to Falcon Lake, Manitoba. The eggs were incubated at the Whiteshell Fish Hatchery (West Hawk Lake, Manitoba) for approximately two weeks.

#### *5.3.2 Oxytetracycline marking*

Fry were held in a retention tank for three days after hatching, at which point, they were marked with OTC in stocking bags, just prior to release. Marking was done by mixing a 70 L batch of OTC solution at a concentration of 1,400 mg OTC/ L, which was then diluted to a final concentration of 700 mg OTC/L in each fry bag by adding 7 L of concentrated OTC solution to 7 L of water with approximately 50,000 fry in each stocking bag. The 1,400 mg OTC/L stock solution was mixed in a 80 L container, by adding 98 g of powdered OTC (Onnacin 250, 100 mg active oxytetracycline/ 1 mL) and 98 g of sodium dibasic dissolved in a small amount (10 mL) of hot water, in order to dissolve the buffer. A small amount of No Foam (Creative Marking and Research) was added to the stock solution to prevent foaming of the solution when mixed. The stock solution was mixed well. A mix of 70 L of concentrated fry solution is enough to mark 10 bags of fry, with 14 L of in each bag. Fry (n=50,000) were placed in a large plastic tub fry filled with 7 L of water, and then added to a double bagged plastic fry bag with 7 L of the stock solution, to make a total volume of 14 L marking solution. The above stock solution was used to mark fry stocked into White Lake, Flanders Lake and Booster Lake.

*5.3.3 Concentration of oxytetracycline*

Fry stocked into Barren Lake were marked in the technique described in above in Section 5.3.2, however a smaller batch of stock solution used at a concentration of 2,800 mg OTC/L. This stock solution was then diluted in stocking bags using the same method above, to create a final concentration of 1,400 mg OTC/L. This was done to test the difference in mark quality and retention between fry marked at 700 and 1,400 mg OTC/L.

*5.3.4 DMSO*

Fry stocked into Star Lake were marked in the technique described in above in Section 5.3.2, with a final concentration of 700 mg OTC/L, as well as adding 2% (280 mL) DMSO. The DMSO was added to test the influence of this potentiating chemical on OTC mark quality and retention. The different marking treatment applied to fry stocked into Barren Lake and Star Lake should have no effect of stocking success and for the purposes this study, no interaction between marking and stocking was assumed.

*5.3.5 Mark retention*

For each of the three OTC treatments used to mark fry stocked into study lakes (700 mg OTC/L, 1,400 mg OTC/L, and 700 mg OTC/L and 2% DMSO), a corresponding treatment group of 50,000 fry was marked in the identical protocol, and stocked into retention ponds in southeastern Manitoba. Age 0 fish were recovered from ponds in late August with 2, 1 and ¾" gill nets. Fish were frozen until otoliths could be removed and analyzed.

*5.3.6 Stocking*

Bags were inflated with compressed air to provide adequate oxygen, held for 6 hours out of direct sunlight and heat and transported to stocking sites. Before stocking, stocking bags containing fry were held in the lake until the temperature of the bag was equalized with the lake, to reduce temperature shock. Stocking locations were collected as GPS waypoints for Star and Barren Lake, and descriptively, as

relative to shore and boat launch locations, on White, Booster and Flanders Lake. Wind speed and direction, weather conditions, and lake temperature were recorded. Fry were stocked close to the windward shore to maximize the potential for the fry stocking location to overlap with the location of zooplankton.

*5.3.7 Contribution*

Nighttime boat electrofishing was used to sample the age 0+ walleye year-class from each of the five lakes. The electrofishing boat used was 20 ft. pontoon hull with a 200 horsepower jet motor, outfitted with a Smith-Root model SR-20EH electrofishing unit. Up to ten transects were surveyed on each lake, until a minimum sample size of 100 age 0 walleye was reached, or all ten transects had been surveyed (Appendix 2). Coordinates and start and end times were recorded for each transect, which followed the bi-annual transects conducted by Manitoba's Fisheries Branch non-lethal sampling protocol (K. Kansas, Manitoba Water Stewardship, unpublished). Electrofishing transects were conducted with two netters on the bow, and one netter on the shore side of the boat. All walleye observed during the surveys were netted and retained in the live well during the transect, however only walleye under 70 mm were kept for OTC analysis, as it was assumed all larger fish were from the previous year class, and therefore unmarked. Fish were frozen until otoliths could be extracted and observed for marks in the lab.

*5.3.8 Mark detection*

In the laboratory, walleye were measured for length and wet weight and otoliths were extracted. Otoliths were mounted on microscope slides with super glue (Krazy Glue, Columbus, Ohio), concave side up, and polished with 600 and 1,200 wet grit sandpaper until the inner daily rings were observed under 50X magnification with transmitted light. The otoliths were analyzed for OTC marks using a Nikon Eclipse 50i Epifluorescent microscope with UV B-3A and 2B filter cubes, and C-SHG1 super high pressure mercury lamp power supply. For each otolith, OTC mark intensity was assigned a grade using the following scale: 1 = obvious mark with distinct gold color; 2 = clear mark, but lacking intensity; 3 = faint mark, yet appears present; and 4 = no mark. This mark ranking system was based on that ranking

system used by Isermann et al. in their work on OTC mark efficacy (1999, 2002). I was also trained by Dr. Dan Isermann in OTC mark detection and analysis in November of 2011 and therefore have continued to use his ranking system to categorize the intensity of OTC marks. Examples of each mark intensity rank can be observed in Figure 3. Otoliths were deemed to have "no mark" only after several cycles of observation and sanding had produced no visible mark, and the otoliths were clearly sanded past the central annuli. If otoliths were over sanded without observation of the central annuli, the otoliths were scored as over sanded, and removed from analysis. A photograph was taken of each mark, viewed under the 50x oil immersion objective, with a small amount of immersion oil and the 2B filter cube. Camera settings were kept consistent at an exposure composition of -1 1/3 EV.

*5.3.9 Mark quality control*

Marks were viewed and scored during the first observation by a single reader. The first reading was completed blind, such that the reader had no knowledge of the treatment used. The same reader then scored each mark a second time, also blind, based on the photograph alone. The second scores were compared with the first, and if a discrepancy occurred between the first and second reading, the otolith was analyzed a third time, after which time, the modal age was used as the final reading. All marked young-of-year walleye were assumed to be stocked from the Whiteshell Hatchery, based on a marking success rate of 100% for all three oxytetracycline marking treatments applied during this study, as discussed in section 4.4 of this document (Table 11).

*5.3.10 Statistical analysis*

Fall electrofishing catches were used to determine abundance of hatchery and naturally produced fish. Catch per unit effort (CPUE) was calculated as the number of age 0+ walleye caught per hour of shoreline electrofishing. Length and weight values from all fish caught were transformed to a single value of condition, K; Fulton's condition factor (Froese 2006):

$$K = W*100{,}000 / (FL^3)$$

Where W = weight of the fish in grams, FL = fork length of the fish in millimeters.

Differences between age 0+ stocked and natural walleye condition factors were tested using one-way ANOVA. The quality of OTC marks was compared between treatment groups using a non-parametric one-way ANOVA by ranks (Kruskal-Wallis). The level of significance was set at 0.05. ANOVA and Kruskal-Wallis calculations were performed using SAS 9.3 statistical software (SAS Institute, Cary, NC).

## *5.4 Results*

### *5.4.1 Oxytetracycline mark efficacy*

Mark retention over ten weeks was studied by stocking walleye in retention ponds and is discussed in section 4.4 of this thesis. The goal of this study was to test OTC marking efficacy, so that we could appropriately determine whether fish stocked into lakes could be expected to be effectively marked. Mark efficacy was 100% for each of the three treatment groups; 700 mg OTC/L, 1,400 mg OTC/L and 700 mg OTC/L with 2% DMSO (see section 4.4, Table 12). There were significant differences in the quality of OTC marks observed between these three treatment groups (Kruskal-Wallis, $\chi^2 = 9.8627$, $P = 0.0072$), but despite this variation, the reader was correctly able to identify 100% of known marks from each of the treatments groups. Therefore, despite significant differences in mark quality between the groups, all fish which were marked had an identifiable mark, and therefore, all OTC marks observed on age 0+ fish from electrofishing samples were considered to correctly identify hatchery reared walleye.

### *5.4.2 Contribution*

Hatchery reared walleye contributed to the age 0 year class in each of the five study lakes. Average contribution across study lakes was 48%, ranging from only 11% contribution to White Lake to

88% contribution to Barren Lake (Figure 11, Table 15). Stocking effort, measured as the number of fry stocked per hectare lake area, appears to be related to the catch per unit effort (CPUE) of marked walleye (LR, $R^2$=0.62715) (Figure 9). Lake size, however, did not appear to be correlated with the catch per unit effort (CPUE) of marked walleye (LR, $R^2$=0.1502) (Figure 10). However, because we only have five study lakes, correlation coefficients were not significant for either correlation ($p>0.05$) (Figure 9 and 10).

TABLE 15. Contribution of oxytetracycline marked age 0+ walleye from night electrofishing samples collected in September 2012 from five lakes in Eastern Manitoba. CPUE calculated as the number of age-0 walleye caught per hour of fishing effort.

| Lake | Size (ha) | Stocking effort (fry/ha) | CPUE of age 0 walleye | Percent marked | CPUE of marked walleye |
|---|---|---|---|---|---|
| Barren | 27.9 | 7168 | 141 | 82 | 104 |
| Booster | 203.6 | 982 | 116 | 39 | 37 |
| Flanders | 73.7 | 2714 | 50 | 88 | 38 |
| Star | 63.1 | 3170 | 33 | 18 | 6 |
| White | 321.3 | 622 | 344 | 11 | 27 |

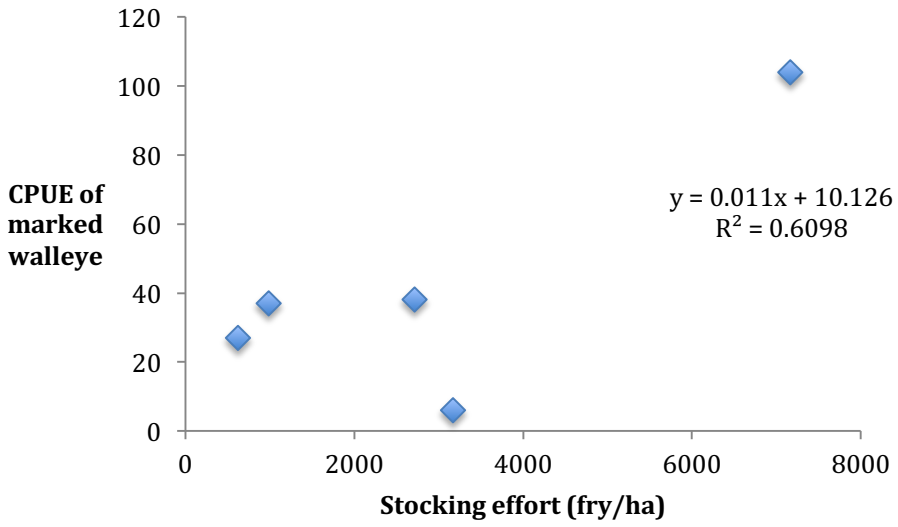


FIGURE 9. Catch per unit effort of marked walleye as a function of stocking effort. Catch per unit effort was measured as the number of age 0 fish identified as stocked due to the presence of an oxytetracycline mark, caught per hour shoreline electrofishing on five eastern Manitoba lakes. Lakes were surveyed from early to mid-September of 2012.

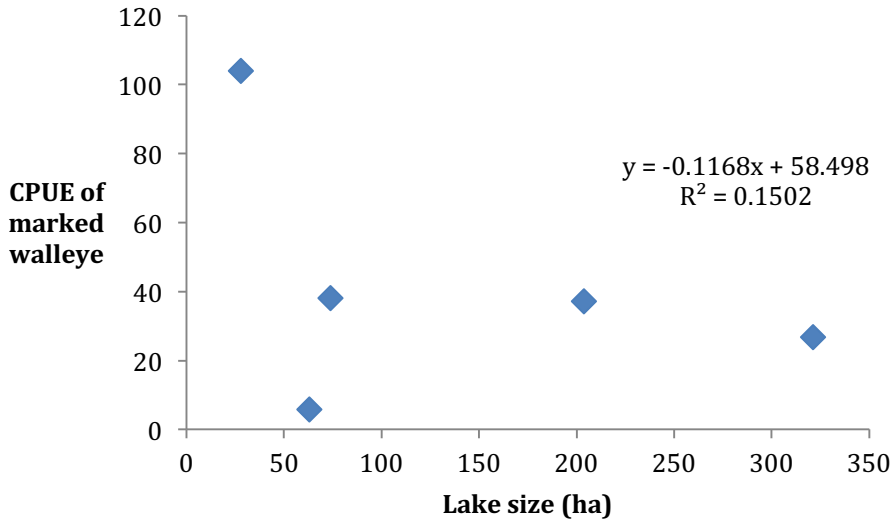


FIGURE 10. Catch per unit effort of marked walleye as a function of lake size. Catch per unit effort was measured as the number of age 0 fish identified as stocked due to the presence of an oxytetracycline mark, caught per hour shoreline electrofishing on five eastern Manitoba lakes. Lakes were surveyed from early to mid-September of 2012.

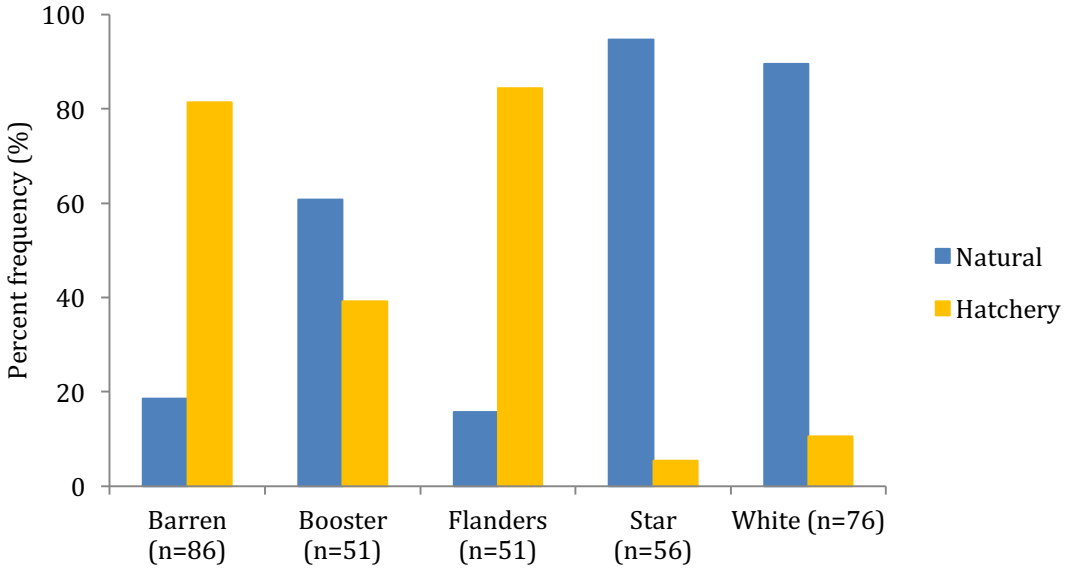


FIGURE 11. Percent frequency of natural fry vs. oxytetracycline-marked walleye, representing hatchery reared walleye, from electrofishing catch of age-0+ walleye in five Eastern Manitoba lakes in September, 2012.

*5.4.2 Catch per unit effort*

Total catch per unit effort (CPUE) was significantly higher on White Lake than all other study lakes at 344 fish per hour of shoreline electrofishing. Star Lake had a low total CPUE of only 33 fish per hour of shoreline electrofishing.

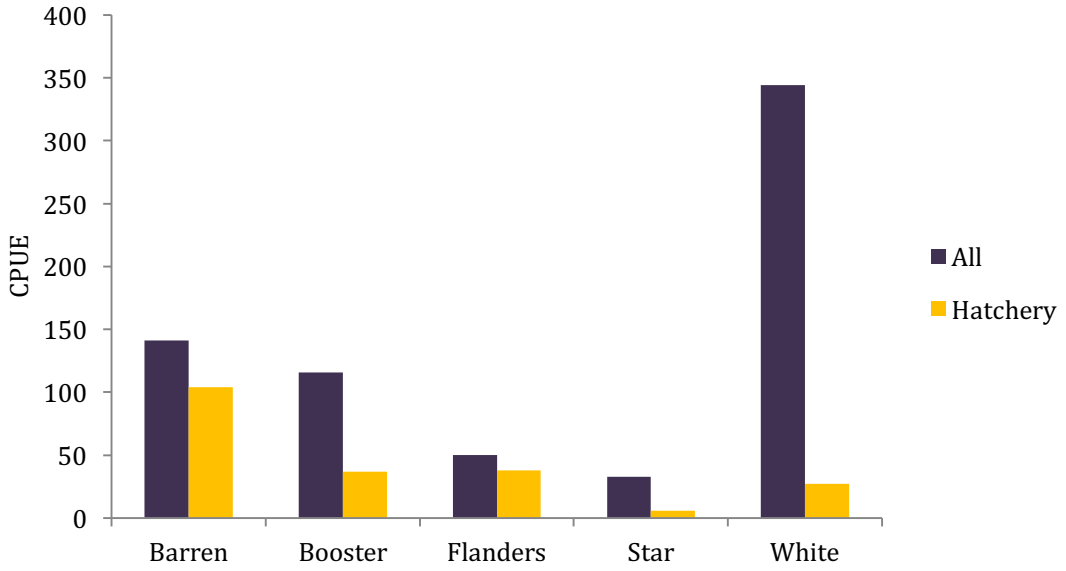


FIGURE 12. Catch per unit effort (CPUE), measured as the number of age 0+ walleye caught per hour of shoreline electrofishing, of all walleye vs. all hatchery origin walleye caught in five Eastern Manitoba lakes in September, 2012.

*5.4.3 Condition*

Naturally produced walleye were found to be significantly larger than hatchery origin walleye when compared across all lakes, as measured by their condition factor, K (K value; T test, $t = 8.16$, $P < 0.0001$) (Table 16). On a lake by lake basis, naturally produced walleye were significantly larger than hatchery origin fish in both Barren and Flanders Lake (K value; T test, $t=6.70$, $P<0.0001$, and K value; T test, $t=0.0003$, $P<0.0003$). (Table 16, Figure 13). Barren and Flanders Lakes also had the highest percentage of hatchery contribution (Figure 11).

TABLE 16. Results of a t test comparison of the mean condition factor (k) between hatchery reared and naturally produced age 0+ walleye sampled by fall shoreline electrofishing in five Eastern Manitoba lakes in 2012. The level of significance is considered $P \leq 0.05$.

| Lake | Hatchery or Natural Origin | n | Mean K | df | t value | P value |
|---|---|---|---|---|---|---|
| Barren | Natural | 15 | 1.0239 | 83 | 6.70 | <0.0001 |
| | Hatchery | 70 | 0.9168 | | | |
| Booster | Natural | 31 | 0.9543 | 49 | 1.21 | 0.2325 |
| | Hatchery | 20 | 0.9206 | | | |
| Flanders | Natural | 8 | 0.9553 | 49 | 3.86 | 0.0003 |
| | Hatchery | 43 | 0.889 | | | |
| Star | Natural | 49 | 0.9417 | 49 | 0.94 | 0.3519 |
| | Hatchery | 2 | 0.876 | | | |
| White | Natural | 68 | 1.0645 | 74 | -0.29 | 0.7691 |
| | Hatchery | 8 | 1.0735 | | | |
| All Lakes | Natural | 171 | 1.0007 | 312 | 8.16 | <0.0001 |
| | Hatchery | 143 | 0.9172 | | | |

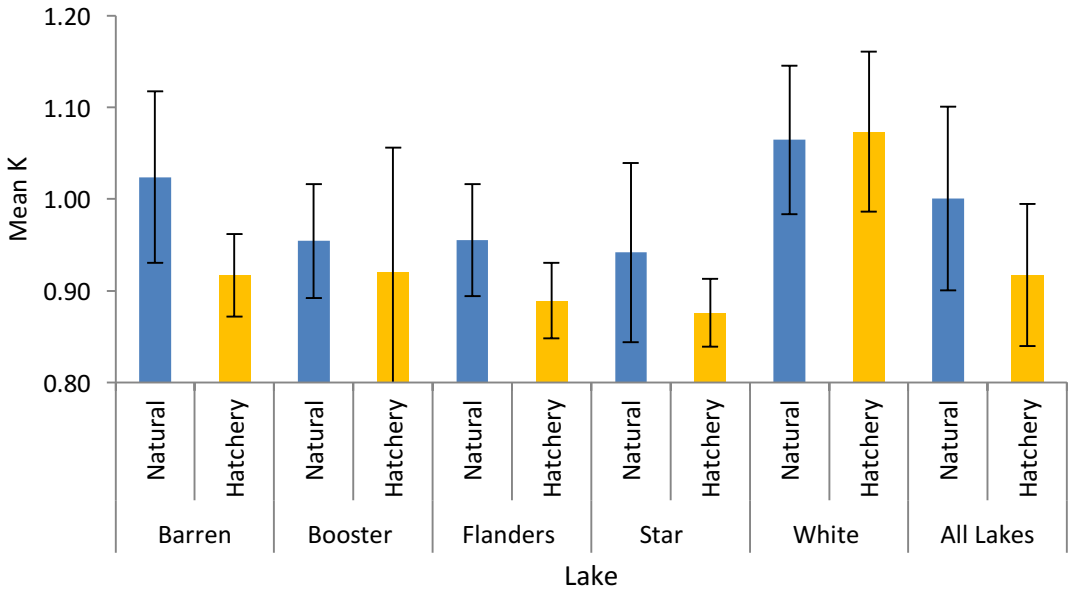


FIGURE 13. Mean value of Fulford's condition factor (k) for naturally produced and hatchery reared age 0+ walleye sampled by fall shoreline electrofishing in five Eastern Manitoba Lakes in September, 2012. Error bars display the standard deviation.

## *5.5 Discussion*

### *5.5.1 Study limitations*

The objective of this study was to estimate the contribution of stocked walleye fry to five study lakes through the use of OTC marking to determine hatchery origin. However, in order to correctly estimate contribution, the OTC marking protocol needed to be improved, which was discussed in chapter three of this volume, and it needed to be determined whether OTC marks could be retained over a three month period, as discussed in chapter 4 of this volume. Following these objectives, the third component of this study was to estimate contribution, however, the study period was reduced to a single field season, due to time and financial restrictions. Due to the limitations of a single year study, the results are thus subject to yearly variation of natural recruitment, environmental conditions, and food availability, for which this study was unable to account. Similarly, despite being aware of the potentially negative effects of stocking, such as the suppression of growth in surrounding year classes, this study was limited as to its scope, and as such, was not capable of studying whether these effects occurred. Rather, the focus was on providing baseline data as to the contribution of larval walleye in the five study lakes, and create momentum to encourage the future monitoring of walleye contribution.

### *5.5.2 Contribution*

Stocking larval walleye contributed to the fall age 0 populations in each of the five study lakes. Contribution rates ranged from 11% to 88%, with an average of 48%. Two of the study lakes had relatively high rates of contribution, with stocked walleye making up 82 and 88% of age 0+ year class sampled in Barren and Flanders Lake and in Booster Lake, stocked walleye contributed close to 40% of the age-0 year class. Similarly high stocking rates were observed by Lucchesi (2002), who reported an even higher contribution rate of stocked fry at 93% between eight study lakes. Logsdon (2006) reported detecting OTC marks on 86% of YOY walleye caught on Red Lake in 1999, and similarly high contribution of marked walleye in the following years, ranging from 43 – 94%. However, not all studies

have found walleye fry marking to be effective. Fielder (1992) determined that lakes which were stocked with walleye fry had no little to no increase in walleye population when compared with lakes which had not been stocked at all. Johnson et al (1996) also concluded that stocking success of walleye fry was negligible when compared with fingerling stocking success.

All lakes within this study have some level of natural walleye reproduction (K. Kansas, Fisheries Biologist, Manitoba Fisheries Branch, pers. comm., 2011) However, due to time and budget constraints, this study was not able to estimate values of natural reproduction. The existence of natural reproduction in the study lakes means that stocking in these lakes would be considered supplemental, for which the success rates have been predicted to be as low as 5%, according to Laarman (1979). Ellison and Franzin (1992) predicted a success rate of stocking walleye at 32%. Studies which boast comparatively high rates of contribution, such as Logsdon (2002), report stocking into lakes with almost no natural reproduction. Although these results are preliminary, they suggest that it is possible to have high return of stocked walleye fry in lakes where natural reproduction occurs.

One of the lakes in this study, Star Lake, was known to have a fairly low level of natural reproduction due to previous population estimates (Kansas, unpublished). Based on the results of Logsdon (2002) and Laarman (1979), it might be assumed that low levels of natural reproduction in a lake would result in higher contribution of stocked walleye. Instead, the second lowest level of stocked walleye contribution was observed on Star Lake at 18% and the lowest CPUE of age-0 walleye at 33 walleye per hour of electrofishing effort. Star Lake has a large population of centrachids, and this may make it difficult for larval walleye, stocked or natural, to survive to larger sizes (Kansas, unpublished). This is in contrast with Logsdon's (2002) results on Red Lake, who observed low numbers of age-0 walleye prior to stocking, and found that the population was significantly increased by walleye stocking. Logsdon (2002) suggested the low numbers of walleye observed in Red Lake in the initial study were a result of high fishing pressure, and that the effects of this harvest could be mitigated by stocking. The

differences between our results on Star Lake and the results Logsdon (2002) experienced on Red Lake are a good reminder that stocking has different effects depending on the characteristics of each lake, and that monitoring of stocked walleye can provide greater insight into the effectiveness of stocking on a particular water body.

Both Booster Lake and White Lake have healthy levels of natural spawning due to prolific amounts of natural spawning habitat (K. Kansas, pers. comm., 2011). The presence of existing populations of naturally reproducing walleye may explain the comparatively lower rates of contribution observed on these lakes. Booster and White Lake had the third and first highest catch of young of the year walleye, suggesting that both lakes have an existing healthy population of spawning walleye. Booster and White Lake are also the two largest lakes in the study and had the lowest levels of stocking effort (Table 12). Larger lakes tend to be stocked at lower densities than smaller water bodies (Welcomme and Bartley 1998). This may be due to the greater rate of control that can be managed on smaller lakes and higher rates of competition and predation that stocked fish inevitably face in larger lakes (Welcomme and Bartley 1998).

It is difficult to say why this study observed such high rates of contribution compared to the results of with Fielder (1992) and Johnson et al. (1996). One possibility is that both of these studies focused on comparing the effectiveness of fry versus fingerling stocking, and due to this focus, both studies primarily targeted walleye fingerlings in their collection methods and may not have had the resources needed to adequately observe age 0 fish in the fall. Johnson et al. (1996) used purse seine hauls to measure small pelagic fishes as well as small-mesh mini-fyke nets, and Fielder (1992) relied on small-mesh gill nets to sample age 0 walleye. Logsdon (2006) also used seine netting to measure age 0 walleye, whereas Lucchesi (2002) used night electrofishing, as did this study. Night electrofishing during the fall is a well-accepted method of monitoring age-0 walleye, as set out by Serns (1982) (in Borkholder and Parsons 2001).

In the absence of information about the level of natural production, we are unable to determine whether the stocked fry we observed in fall age-0 year class added to the total population, or whether they merely replaced naturally produced fish. Due to the high level of fishing pressure that occurs in all of our study lakes, it would be fair to make the assumption that these lakes are fished to a level below the carrying capacity, and therefore, stocking walleye fry is likely to contribute to the total walleye population, rather than simply replacing naturally produced walleye. We would advise continued marking of all stocked walleye and subsequent analysis of walleye otoliths, in order to see if stocked walleye survive to contribute to older year classes, and subsequently, to the recreational fishery. At this stage, we would then be able say if walleye stocking is truly meeting the goals set out for the stocking program. In addition, despite the suggestion of Quist et al. (2004) and Lorenzen (2005) that fall age 0 walleye populations can be a good indicator of survival at later stages of development, other studies, including Lucchesi (2002), who used fall age-0 walleye as an indication of stocking success, have admitted that the results of stocking were much lower when measured the summer after stocking. In this light, it would be extremely valuable to continue to monitor the contribution of stocked walleye in the following years to better understand how stocking contributes to the total population

*5.5.3 Condition*

Stocked walleye fry were smaller than naturally produced walleye fry on Barren and Flanders Lake. This same trend was also observed by Lucchesi et al. (2002), who noted significantly smaller lengths and weights of hatchery-reared fish. Considering the consistency of this trend, the difference in size between stocked and naturally produced walleye may be related to competition between these walleye of unique origins. It is also possible that there is some impact of the hatchery environment on the feeding ability or response in stocked walleye which may result in overall smaller size. There has been some suggestion that rearing fish in hatchery environments may reduce the ability for fish to detect and

capture food (Brown et al. 2003). However, this argument has largely been used when discussing survival and condition of fish stocked as juvenile or adults who have spent a significant portion of their developmental state in the hatchery environment. It seems unlikely that the four days in which larval walleye spend in the hatchery could impact the feeding ability, as they are essentially still feeding endogenously from the yolk sac and oil globule. (Li and Mathias 1982). However, it may still be that the initial shock of stocking creates a delayed feeding period and results in delayed growth.

Differences in size between hatchery and naturally reproduced walleye after the summer period may also be due to slow growth caused by cool water temperatures experienced at the hatchery during the first days post hatch. Due to current restricted hatchery operations, walleye batteries at the Whiteshell Fish Hatchery are treated/using water directly from the inflow line coming from West Hawk Lake. During previous years, the water used in the walleye batteries had been on re-circulation, such that the temperature can be controlled and maintained or increased to encourage fry growth and development. Water in West Hawk Lake remains cool long after spring temperatures begin to warm other lakes due to extreme depths of up to 115 m deep. The Whiteshell Hatchery, which was built in the immediate post-World War II era, is fed via gravity from two pipes transporting West Hawk Water, one mid-depth pipe and a deep water line. The water from the deep water line is used over the winter when the surface of the lake is frozen, and the deep water line was being used in the spring of 2012, while walleye fry were being raised in the hatching batteries. Construction is currently taking place at the hatchery and once completed, the hatchery will likely return to rearing walleye on re-circulated water.

Although walleye stocking can contribute populations in Manitoba lakes, it is not clear why stocking contributes to year classes in some water bodies, and not in others. The variable success observed in this study suggests that Manitoba Fisheries would benefit from continuing to monitor stocking success in order to: 1) maximize stocking benefit; and 2) target appropriate water bodies for

stocking. The provincial stocking program would also benefit from developing clear goals and targets for stocking programs.

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# 6.0 Summary, Conclusions and Implications

"Some folks have said that stocking fish is not biology, because it is done to satisfy public desires or "politics." By that logic, building bridges for public transportation would not be engineering, and running schools for public education would not be teaching. The truth is that fish stocking, like much of government work, is a blend of public service and science."

- Eric Palmer, Director of Fisheries, Vermont Fish & Wildlife Department

## *6.1 Introduction*

The goal of this thesis was to develop a marking technique using oxytetracycline (OTC) to identify hatchery-reared walleye and use this technique to estimate the contribution of hatchery-reared walleye to stocked water bodies in Manitoba. This goal was developed due to the discovery that the marking process previously used in Manitoba was not creating identifiable marks and therefore could not be used to differentiate hatchery reared walleye from fish which were naturally produced. The marking technique had a number of variables that may have resulted in the low mark rates observed in previously marked year classes. It was necessary to explore which variables in the existing OTC marking technique were affecting mark quality in order to use this information to refine the marking protocol. The ultimate goal then was to develop an OTC marking technique which produced consistent and high quality OTC marks on hatchery reared walleye. Following this, it needed to be determined whether the OTC marks produced by this technique could be retained over time and therefore provide consistent identification of hatchery reared walleye. Lastly, we used the ability to differentiate between hatchery and naturally produced walleye to determine the contribution of stocked walleye to five Manitoba water bodies.

## *6.2 Study Findings*

Investigation of the oxytetracycline marking process found three variables which did affect the resulting mark quality: age at which fry were marked, water source and type of oxytetracycline used.

Walleye fry which were marked at younger ages had significantly lower quality marks than those which were marked at older ages. It is possible that the lower quality marks on younger fish were due to the lack of otolith development at early stages of growth. Lower quality marks were also correlated with marking walleye in water which had higher levels of alkalinity, total calcium and total magnesium. This effect was noted on walleye marked in both powdered OTC and liquid OTC, and may be explained by the formation of oxytetracycline complexes with calcium and magnesium. Lastly, we noted that marking with powdered OTC resulted in much higher mark quality than marking with liquid OTC.

In all trials, OTC mark quality decreased over the three month study period. However, mark retention (marks were visible and correctly identified) was higher amongst those otoliths that were marked with higher levels of OTC (1,400 mg/L as opposed to 700 mg/L). Higher concentration of OTC in the marking solution was the most important variable leading to higher mark quality and retention. Marking with powdered OTC also influenced mark retention, and those otoliths that were marked with powdered OTC instead of liquid had a much higher mark quality and retention. Water source also affected mark retention, with those otoliths marked in lower alkalinities and pH levels having higher mark retention and quality after three months.

Stocked walleye contributed to the age 0 + year class in each of the five study lakes. Contribution was highly varied, ranging from 11 to 88% of the stocked year class. The two lakes, Barren and Flanders, in which the highest contribution of stocked fish was observed, we also noted significant size differences between those walleye identified as stocked by their oxytetracycline marks, and those that were naturally produced.

### *6.3 Research Implications*

The finding that the age at which fry are marked with OTC impacts the quality of the mark differs from that of Logsdon (2004), who had success in marking fry at less than 24 hours of age. It may be that

the differences observed are related to the type of OTC used, as Logsdon noted using a powdered OTC at a concentration of 700 mg/L whereas we used a concentration of 1,400 mg/L liquid OTC. The ability to mark fry at younger ages has a significant effect on the way marking takes place at the hatchery and will affect how this process occurs. This research suggests that fry must be held for up to four days prior to marking in order to apply an effective OTC mark. This means not only a longer time frame for the hatchery operation of walleye stocking and marking, but also the extended use of hatchery space, which can be extremely limited in the spring.

The impact of water source on OTC marking provides insight into the marking process and increases our understanding of how OTC moves from solution to become incorporated into otoliths. This work confirms observations made by Denson and Smith (2008) that OTC mark quality can be negatively impacted in the presence of magnesium, calcium, and higher levels of salinity. The effect of water source was noted largely at the Swan Creek Hatchery, where high levels of magnesium, calcium and total dissolved solids (TDS) may have impacted the ability for OTC marks to become incorporated into growing otoliths.

Mark quality was significantly affected by the type of OTC used, an effect which was previously undocumented in the literature. It remains unclear why marking with liquid OTC results in such lower quality marks than powdered OTC, however, it appears to be due to the buffering of liquid OTC, which perhaps has a similar action to that of increased alkalinity in water.

OTC marks can be retained for up to a three month period. Lucchesi (2002) noted that there was no significant decrease in mark rate in fish from age three months to five years. Therefore, it is reasonable to expect that these marks will continue to be visible indicators of hatchery origin for the next five years.

Stocking walleye fry does contribute to the fall age-0 walleye population in stocked lakes. The

degree to which stocked walleye contribute is highly variable and the factors that contribute to their survival are unclear. Despite the fact that stocked walleye contribute to the fall age-0 year class, they do not necessarily increase the total age-0 year class. Much work still needs to be done in this regard, in order to gain some understanding of natural reproduction in these lakes, as well as follow the survival of stocked walleye into the following years, in order to see if the provincial stocking program is truly meeting their goal of creating more fish for recreational fishing harvest. However, these results do suggest that monitoring can provide valuable information as to the level of success experienced on a local level and can inform future management decisions.

### *6.4 Recommendations*

The following management recommendations are based on the results of this study, and are put forward with the goal of increasing our understanding of how walleye stocking contributes to populations in Manitoba and how stocking can be best used to meet management goals.

1) Develop a monitoring program for stocked walleye. This would include continuing to mark all hatchery-reared walleye with the OTC methodology set out in this paper. Secondly, the monitoring program could be incorporated into the province's existing population assessment program, as well as the non-lethal sampling program in the Eastern Region. Finally, the results of the monitoring program could be used to adjust stocking priorities in order to maximize stocking effectiveness.

2) Determine the long-term retention of oxytetracycline marks by holding walleye in an experimental pond for up to five years. Although this study has provided proof that the OTC mark is retained over a three month period, it would be extremely valuable to have a sample of known marked fish that could be analyzed over a longer time period.

3) Gain some understanding of the natural reproduction rates in Manitoba lakes in order to better understand contribution rates. This would provide additional background information about the populations in stocked lakes and would help managers make informed stocking decisions.

4) Continue to support walleye stocking programs in Manitoba, conditional on the continuation of monitoring stocked walleye and increased focus on understanding natural reproduction in potential stocking lakes. Walleye stocking has the ability to contribute to stocked year classes but should be monitored in order to maximize benefit.

*6.5 References*

Denson, M. R. and T. I. J. Smith. 2008. Use of Oxytetracycline to Mark Larval Red Drum: Survival and Mark Persistence. North American Journal of Fisheries Management. 28: 1779–1789.

Logsdon, D. E., Pittman, B. J., and G. C. Barnard. 2004. Oxytetracycline marking of newly hatched walleye fry. North American Journal of Fisheries Management. 24:1071-1077.

Lucchesi, D. O. 2002. Evaluating the contribution of stocked walleye fry and fingerlings to South Dakota walleye populations through mass marking with oxytetracycline. North American Journal of Fisheries Management 22: 985-994.

Palmer, E. 2014. To stock or not to stock. Vermont Fish and Wildlife Department. http://www.vtfishandwildlife.com/fisheries_tostock.cfm. Accessed April 23, 2014.

# APPENDICES

Appendix 1.

Example of SAS 9.3 (SAS Institute, Cary, NC) program for Kruskal-Wallis rank-sum analysis of oxytetracycline mark quality data.

```
proc npar1way data = one wilcoxon;
  class wq;
  var qlt;
run;
```

Example of SAS 9.3 (SAS Institute, Cary, NC) program for Wilcoxon rank-sum analysis of oxytetracycline mark quality data.

```
proc npar1way data = one wilcoxon;
  class conc;
  var qlt;
run;
```

Example of SAS 9.3 (SAS Institute, Cary, NC) program for Proportional Odds analysis of oxytetracycline mark quality data.

```
proc logistic data = one;
  class wq /ref=first;
  model qlt (order=data descending)= wq  typeotcdv/ link=clogit scale=none
aggregate;
run;
```

Example of SAS 9.3 (SAS Institute, Cary, NC) program for one-way ANOVA analysis of fry mortality data.

```
proc glm data = one;
  class otctype;
  model permortd5 = otctype;
  means otctype;
run;
```

Appendix 2. Mortality of OTC treated larval walleye as measured daily during the two week holding period at the Whiteshell Hatchery, 2012.

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | B6 | Deionized | Liquid | 0 | 700 | 5 | |
| 23-May-12 | B6 | Deionized | Liquid | 0 | 700 | 39 | |
| 24-May-12 | B6 | Deionized | Liquid | 0 | 700 | 21 | |
| 25-May-12 | B6 | Deionized | Liquid | 0 | 700 | 18 | |
| 26-May-12 | B6 | Deionized | Liquid | 0 | 700 | 59 | |
| 27-May-12 | B6 | Deionized | Liquid | 0 | 700 | 18 | |
| 28-May-12 | B6 | Deionized | Liquid | 0 | 700 | 53 | |
| 29-May-12 | B6 | Deionized | Liquid | 0 | 700 | 2 | |
| 30-May-12 | B6 | Deionized | Liquid | 0 | 700 | 0 | |
| 31-May-12 | B6 | Deionized | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | B6 | Deionized | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 215 | 19.5 |
| | | | | | | | |
| 22-May-12 | C2 | Deionized | Liquid | 0 | 700 | 4 | |
| 23-May-12 | C2 | Deionized | Liquid | 0 | 700 | 65 | |
| 24-May-12 | C2 | Deionized | Liquid | 0 | 700 | 11 | |
| 25-May-12 | C2 | Deionized | Liquid | 0 | 700 | 21 | |
| 26-May-12 | C2 | Deionized | Liquid | 0 | 700 | 7 | |
| 27-May-12 | C2 | Deionized | Liquid | 0 | 700 | 12 | |
| 28-May-12 | C2 | Deionized | Liquid | 0 | 700 | 7 | |
| 29-May-12 | C2 | Deionized | Liquid | 0 | 700 | 13 | |
| 30-May-12 | C2 | Deionized | Liquid | 0 | 700 | 2 | |
| 31-May-12 | C2 | Deionized | Liquid | 0 | 700 | 4 | |
| 01-Jun-12 | C2 | Deionized | Liquid | 0 | 700 | 8 | |
| Total | | | | | | 154 | 14.0 |
| | | | | | | | |
| 22-May-12 | D4 | Deionized | Liquid | 0 | 700 | 1 | |
| 23-May-12 | D4 | Deionized | Liquid | 0 | 700 | 25 | ` |
| 24-May-12 | D4 | Deionized | Liquid | 0 | 700 | 1 | |
| 25-May-12 | D4 | Deionized | Liquid | 0 | 700 | 18 | |
| 26-May-12 | D4 | Deionized | Liquid | 0 | 700 | 7 | |
| 27-May-12 | D4 | Deionized | Liquid | 0 | 700 | 19 | |
| 28-May-12 | D4 | Deionized | Liquid | 0 | 700 | 8 | |
| 29-May-12 | D4 | Deionized | Liquid | 0 | 700 | 11 | |
| 30-May-12 | D4 | Deionized | Liquid | 0 | 700 | 4 | |
| 31-May-12 | D4 | Deionized | Liquid | 0 | 700 | 14 | |
| 01-Jun-12 | D4 | Deionized | Liquid | 0 | 700 | 3 | |
| Total | | | | | | 111 | 10.1 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | D7 | Deionized | Liquid | 0 | 700 | 4 | |
| 23-May-12 | D7 | Deionized | Liquid | 0 | 700 | 61 | |
| 24-May-12 | D7 | Deionized | Liquid | 0 | 700 | 13 | |
| 25-May-12 | D7 | Deionized | Liquid | 0 | 700 | 16 | |
| 26-May-12 | D7 | Deionized | Liquid | 0 | 700 | 5 | |
| 27-May-12 | D7 | Deionized | Liquid | 0 | 700 | 7 | |
| 28-May-12 | D7 | Deionized | Liquid | 0 | 700 | 17 | |
| 29-May-12 | D7 | Deionized | Liquid | 0 | 700 | 0 | |
| 30-May-12 | D7 | Deionized | Liquid | 0 | 700 | 0 | |
| 31-May-12 | D7 | Deionized | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | D7 | Deionized | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 123 | 11.2 |
| | | | | | | | |
| 22-May-12 | E1 | Deionized | Liquid | 0 | 700 | 0 | |
| 23-May-12 | E1 | Deionized | Liquid | 0 | 700 | 14 | |
| 24-May-12 | E1 | Deionized | Liquid | 0 | 700 | 8 | |
| 25-May-12 | E1 | Deionized | Liquid | 0 | 700 | 4 | |
| 26-May-12 | E1 | Deionized | Liquid | 0 | 700 | 1 | |
| 27-May-12 | E1 | Deionized | Liquid | 0 | 700 | 0 | |
| 28-May-12 | E1 | Deionized | Liquid | 0 | 700 | 1 | |
| 29-May-12 | E1 | Deionized | Liquid | 0 | 700 | 12 | |
| 30-May-12 | E1 | Deionized | Liquid | 0 | 700 | 1 | |
| 31-May-12 | E1 | Deionized | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E1 | Deionized | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 41 | 3.7 |
| | | | | | | | |
| 22-May-12 | E2 | Deionized | Liquid | 0 | 700 | 1 | |
| 23-May-12 | E2 | Deionized | Liquid | 0 | 700 | 35 | |
| 24-May-12 | E2 | Deionized | Liquid | 0 | 700 | 12 | |
| 25-May-12 | E2 | Deionized | Liquid | 0 | 700 | 22 | |
| 26-May-12 | E2 | Deionized | Liquid | 0 | 700 | 13 | |
| 27-May-12 | E2 | Deionized | Liquid | 0 | 700 | 6 | |
| 28-May-12 | E2 | Deionized | Liquid | 0 | 700 | 1 | |
| 29-May-12 | E2 | Deionized | Liquid | 0 | 700 | 3 | |
| 30-May-12 | E2 | Deionized | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E2 | Deionized | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E2 | Deionized | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 93 | 8.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | F6 | Deionized | Liquid | 0 | 700 | 0 | |
| 23-May-12 | F6 | Deionized | Liquid | 0 | 700 | 51 | |
| 24-May-12 | F6 | Deionized | Liquid | 0 | 700 | 10 | |
| 25-May-12 | F6 | Deionized | Liquid | 0 | 700 | 7 | |
| 26-May-12 | F6 | Deionized | Liquid | 0 | 700 | 9 | |
| 27-May-12 | F6 | Deionized | Liquid | 0 | 700 | 16 | |
| 28-May-12 | F6 | Deionized | Liquid | 0 | 700 | 5 | |
| 29-May-12 | F6 | Deionized | Liquid | 0 | 700 | 0 | |
| 30-May-12 | F6 | Deionized | Liquid | 0 | 700 | 0 | |
| 31-May-12 | F6 | Deionized | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | F6 | Deionized | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 98 | 8.9 |
| | | | | | | | |
| 22-May-12 | H2 | Deionized | Liquid | 0 | 700 | 2 | |
| 23-May-12 | H2 | Deionized | Liquid | 0 | 700 | 57 | |
| 24-May-12 | H2 | Deionized | Liquid | 0 | 700 | 7 | |
| 25-May-12 | H2 | Deionized | Liquid | 0 | 700 | 11 | |
| 26-May-12 | H2 | Deionized | Liquid | 0 | 700 | 25 | |
| 27-May-12 | H2 | Deionized | Liquid | 0 | 700 | 4 | |
| 28-May-12 | H2 | Deionized | Liquid | 0 | 700 | 7 | |
| 29-May-12 | H2 | Deionized | Liquid | 0 | 700 | 4 | |
| 30-May-12 | H2 | Deionized | Liquid | 0 | 700 | 2 | |
| 31-May-12 | H2 | Deionized | Liquid | 0 | 700 | 6 | |
| 01-Jun-12 | H2 | Deionized | Liquid | 0 | 700 | 3 | |
| Total | | | | | | 128 | 11.6 |
| | | | | | | | |
| 22-May-12 | C7 | Deionized | Liquid | 0 | 700 | 0 | |
| 23-May-12 | C7 | Deionized | Liquid | 0 | 700 | 29 | |
| 24-May-12 | C7 | Deionized | Liquid | 0 | 700 | 15 | |
| 25-May-12 | C7 | Deionized | Liquid | 0 | 700 | 31 | |
| 26-May-12 | C7 | Deionized | Liquid | 0 | 700 | 6 | |
| 27-May-12 | C7 | Deionized | Liquid | 0 | 700 | 26 | |
| 28-May-12 | C7 | Deionized | Liquid | 0 | 700 | 3 | |
| 29-May-12 | C7 | Deionized | Liquid | 0 | 700 | 8 | |
| 30-May-12 | C7 | Deionized | Liquid | 0 | 700 | 5 | |
| 31-May-12 | C7 | Deionized | Liquid | 0 | 700 | 14 | |
| 01-Jun-12 | C7 | Deionized | Liquid | 0 | 700 | 1 | |
| Total | | | | | | 138 | 12.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | A4 | Deionized | Powder | 0 | 700 | 31 | |
| 23-May-12 | A4 | Deionized | Powder | 0 | 700 | 33 | |
| 24-May-12 | A4 | Deionized | Powder | 0 | 700 | 12 | |
| 25-May-12 | A4 | Deionized | Powder | 0 | 700 | 7 | |
| 26-May-12 | A4 | Deionized | Powder | 0 | 700 | 16 | |
| 27-May-12 | A4 | Deionized | Powder | 0 | 700 | 13 | |
| 28-May-12 | A4 | Deionized | Powder | 0 | 700 | 24 | |
| 29-May-12 | A4 | Deionized | Powder | 0 | 700 | 5 | |
| 30-May-12 | A4 | Deionized | Powder | 0 | 700 | 15 | |
| 31-May-12 | A4 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | A4 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 156 | 14.2 |
| | | | | | | | |
| 22-May-12 | A8 | Deionized | Powder | 0 | 700 | 27 | |
| 23-May-12 | A8 | Deionized | Powder | 0 | 700 | 42 | |
| 24-May-12 | A8 | Deionized | Powder | 0 | 700 | 37 | |
| 25-May-12 | A8 | Deionized | Powder | 0 | 700 | 35 | |
| 26-May-12 | A8 | Deionized | Powder | 0 | 700 | 32 | |
| 27-May-12 | A8 | Deionized | Powder | 0 | 700 | 16 | |
| 28-May-12 | A8 | Deionized | Powder | 0 | 700 | 8 | |
| 29-May-12 | A8 | Deionized | Powder | 0 | 700 | 10 | |
| 30-May-12 | A8 | Deionized | Powder | 0 | 700 | 34 | |
| 31-May-12 | A8 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | A8 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 241 | 21.9 |
| | | | | | | | |
| 22-May-12 | B1 | Deionized | Powder | 0 | 700 | 7 | |
| 23-May-12 | B1 | Deionized | Powder | 0 | 700 | 15 | |
| 24-May-12 | B1 | Deionized | Powder | 0 | 700 | 4 | |
| 25-May-12 | B1 | Deionized | Powder | 0 | 700 | 1 | |
| 26-May-12 | B1 | Deionized | Powder | 0 | 700 | 0 | |
| 27-May-12 | B1 | Deionized | Powder | 0 | 700 | 7 | |
| 28-May-12 | B1 | Deionized | Powder | 0 | 700 | 19 | |
| 29-May-12 | B1 | Deionized | Powder | 0 | 700 | 9 | |
| 30-May-12 | B1 | Deionized | Powder | 0 | 700 | 6 | |
| 31-May-12 | B1 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | B1 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 68 | 6.2 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | C4 | Deionized | Powder | 0 | 700 | 46 | |
| 23-May-12 | C4 | Deionized | Powder | 0 | 700 | 48 | |
| 24-May-12 | C4 | Deionized | Powder | 0 | 700 | 27 | |
| 25-May-12 | C4 | Deionized | Powder | 0 | 700 | 38 | |
| 26-May-12 | C4 | Deionized | Powder | 0 | 700 | 19 | |
| 27-May-12 | C4 | Deionized | Powder | 0 | 700 | 15 | |
| 28-May-12 | C4 | Deionized | Powder | 0 | 700 | 5 | |
| 30-May-12 | C4 | Deionized | Powder | 0 | 700 | 1 | |
| 31-May-12 | C4 | Deionized | Powder | 0 | 700 | 8 | |
| 01-Jun-12 | C4 | Deionized | Powder | 0 | 700 | 0 | |
| 02-Jun-12 | C4 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 207 | 18.8 |
| | | | | | | | |
| 22-May-12 | C8 | Deionized | Powder | 0 | 700 | 5 | |
| 23-May-12 | C8 | Deionized | Powder | 0 | 700 | - | |
| 24-May-12 | C8 | Deionized | Powder | 0 | 700 | 3 | |
| 25-May-12 | C8 | Deionized | Powder | 0 | 700 | 2 | |
| 26-May-12 | C8 | Deionized | Powder | 0 | 700 | 0 | |
| 27-May-12 | C8 | Deionized | Powder | 0 | 700 | 3 | |
| 28-May-12 | C8 | Deionized | Powder | 0 | 700 | 3 | |
| 29-May-12 | C8 | Deionized | Powder | 0 | 700 | 0 | |
| 30-May-12 | C8 | Deionized | Powder | 0 | 700 | 0 | |
| 31-May-12 | C8 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | C8 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 16 | 1.5 |
| | | | | | | | |
| 22-May-12 | F4 | Deionized | Powder | 0 | 700 | 1 | |
| 23-May-12 | F4 | Deionized | Powder | 0 | 700 | 78 | |
| 24-May-12 | F4 | Deionized | Powder | 0 | 700 | 12 | |
| 25-May-12 | F4 | Deionized | Powder | 0 | 700 | 4 | |
| 26-May-12 | F4 | Deionized | Powder | 0 | 700 | 2 | |
| 27-May-12 | F4 | Deionized | Powder | 0 | 700 | 11 | |
| 28-May-12 | F4 | Deionized | Powder | 0 | 700 | 1 | |
| 29-May-12 | F4 | Deionized | Powder | 0 | 700 | 6 | |
| 30-May-12 | F4 | Deionized | Powder | 0 | 700 | 0 | |
| 31-May-12 | F4 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | F4 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 115 | 10.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | F8 | Deionized | Powder | 0 | 700 | 0 | |
| 23-May-12 | F8 | Deionized | Powder | 0 | 700 | 112 | |
| 24-May-12 | F8 | Deionized | Powder | 0 | 700 | 7 | |
| 25-May-12 | F8 | Deionized | Powder | 0 | 700 | 6 | |
| 26-May-12 | F8 | Deionized | Powder | 0 | 700 | 1 | |
| 27-May-12 | F8 | Deionized | Powder | 0 | 700 | 7 | |
| 28-May-12 | F8 | Deionized | Powder | 0 | 700 | 1 | |
| 29-May-12 | F8 | Deionized | Powder | 0 | 700 | 4 | |
| 30-May-12 | F8 | Deionized | Powder | 0 | 700 | 1 | |
| 31-May-12 | F8 | Deionized | Powder | 0 | 700 | 3 | |
| 01-Jun-12 | F8 | Deionized | Powder | 0 | 700 | 1 | |
| Total | | | | | | 143 | 13.0 |
| | | | | | | | |
| 22-May-12 | G6 | Deionized | Powder | 0 | 700 | 3 | |
| 23-May-12 | G6 | Deionized | Powder | 0 | 700 | 25 | |
| 24-May-12 | G6 | Deionized | Powder | 0 | 700 | 33 | |
| 25-May-12 | G6 | Deionized | Powder | 0 | 700 | 15 | |
| 26-May-12 | G6 | Deionized | Powder | 0 | 700 | 6 | |
| 27-May-12 | G6 | Deionized | Powder | 0 | 700 | 3 | |
| 28-May-12 | G6 | Deionized | Powder | 0 | 700 | 15 | |
| 29-May-12 | G6 | Deionized | Powder | 0 | 700 | 1 | |
| 30-May-12 | G6 | Deionized | Powder | 0 | 700 | 0 | |
| 31-May-12 | G6 | Deionized | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | G6 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 101 | 9.2 |
| | | | | | | | |
| 22-May-12 | H1 | Deionized | Powder | 0 | 700 | 4 | |
| 23-May-12 | H1 | Deionized | Powder | 0 | 700 | 75 | |
| 24-May-12 | H1 | Deionized | Powder | 0 | 700 | 13 | |
| 25-May-12 | H1 | Deionized | Powder | 0 | 700 | 3 | |
| 26-May-12 | H1 | Deionized | Powder | 0 | 700 | 4 | |
| 27-May-12 | H1 | Deionized | Powder | 0 | 700 | 3 | |
| 28-May-12 | H1 | Deionized | Powder | 0 | 700 | 5 | |
| 29-May-12 | H1 | Deionized | Powder | 0 | 700 | 3 | |
| 30-May-12 | H1 | Deionized | Powder | 0 | 700 | 0 | |
| 31-May-12 | H1 | Deionized | Powder | 0 | 700 | 3 | |
| 01-Jun-12 | H1 | Deionized | Powder | 0 | 700 | 0 | |
| Total | | | | | | 113 | 10.3 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | H6 | Deionized | None | 0 | 0 | 1 | |
| 23-May-12 | H6 | Deionized | None | 0 | 0 | 33 | |
| 24-May-12 | H6 | Deionized | None | 0 | 0 | 46 | |
| 25-May-12 | H6 | Deionized | None | 0 | 0 | 35 | |
| 26-May-12 | H6 | Deionized | None | 0 | 0 | 11 | |
| 27-May-12 | H6 | Deionized | None | 0 | 0 | 2 | |
| 28-May-12 | H6 | Deionized | None | 0 | 0 | 6 | |
| 29-May-12 | H6 | Deionized | None | 0 | 0 | 21 | |
| 30-May-12 | H6 | Deionized | None | 0 | 0 | 0 | |
| 31-May-12 | H6 | Deionized | None | 0 | 0 | 0 | |
| 01-Jun-12 | H6 | Deionized | None | 0 | 0 | 0 | |
| Total | | | | | | 155 | 14.1 |
| | | | | | | | |
| 22-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 5 | |
| 23-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 52 | |
| 24-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 35 | |
| 25-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 26-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 21 | |
| 27-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 28-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 15 | |
| 29-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 15 | |
| 30-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 30-May-12 | A3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 161 | 14.6 |
| | | | | | | | |
| 22-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 23-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 16 | |
| 24-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 5 | |
| 25-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 26-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 1 | |
| 27-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 28-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 29-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 30-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | B5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 22 | 2.0 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 1 | |
| 23-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 41 | |
| 24-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 14 | |
| 25-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 15 | |
| 26-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 22 | |
| 27-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 17 | |
| 28-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 10 | |
| 29-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 8 | |
| 30-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | D3 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 01-Jun-12 | D3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 137 | 12.5 |
| | | | | | | | |
| 22-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 23-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 75 | |
| 24-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 8 | |
| 25-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 42 | |
| 26-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 27-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 15 | |
| 28-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 29-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 30-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | D8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | D8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 158 | 14.4 |
| | | | | | | | |
| 22-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 1 | |
| 23-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 38 | |
| 24-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 15 | |
| 25-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 46 | |
| 26-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 14 | |
| 27-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 20 | |
| 28-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 29-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 3 | |
| 30-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 146 | 13.3 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 23-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 97 | |
| 24-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 7 | |
| 25-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 24 | |
| 26-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 12 | |
| 27-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 9 | |
| 28-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 7 | |
| 29-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 5 | |
| 30-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 4 | |
| 31-May-12 | G3 | Swan Creek | Liquid | 0 | 700 | 2 | |
| 01-Jun-12 | G3 | Swan Creek | Liquid | 0 | 700 | 2 | |
| Total | | | | | | 169 | 15.4 |
| | | | | | | | |
| 22-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 5 | |
| 23-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 35 | |
| 24-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 31 | |
| 25-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 21 | |
| 26-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 27 | |
| 27-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 4 | |
| 28-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 8 | |
| 29-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 4 | |
| 30-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | G4 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | G4 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 135 | 12.3 |
| | | | | | | | |
| 22-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 23-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 38 | |
| 24-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 60 | |
| 25-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 14 | |
| 26-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 17 | |
| 27-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 7 | |
| 28-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 10 | |
| 29-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 12 | |
| 30-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 3 | |
| 31-May-12 | G8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | G8 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 161 | 14.6 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 23-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 47 | |
| 24-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 60 | |
| 25-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 24 | |
| 26-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 8 | |
| 27-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 7 | |
| 28-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 6 | |
| 29-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 8 | |
| 30-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 31-May-12 | H5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | H5 | Swan Creek | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 160 | 14.5 |
| | | | | | | | |
| 22-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 55 | |
| 23-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 14 | |
| 24-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 12 | |
| 25-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 4 | |
| 26-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 20 | |
| 27-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 4 | |
| 28-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 38 | |
| 29-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 4 | |
| 30-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 0 | |
| 31-May-12 | A1 | Swan Creek | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | A1 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 151 | 13.7 |
| | | | | | | | |
| 22-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 1 | |
| 23-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 33 | |
| 24-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 0 | |
| 25-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 7 | |
| 26-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 10 | |
| 27-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 6 | |
| 28-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 9 | |
| 29-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 8 | |
| 30-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 2 | |
| 31-May-12 | B3 | Swan Creek | Powder | 0 | 700 | 3 | |
| 01-Jun-12 | B3 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 79 | 7.2 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 5 | |
| 23-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 49 | |
| 24-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 27 | |
| 25-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 8 | |
| 26-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 25 | |
| 27-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 12 | |
| 28-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 20 | |
| 29-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 7 | |
| 30-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 3 | |
| 31-May-12 | B4 | Swan Creek | Powder | 0 | 700 | 1 | |
| 01-Jun-12 | B4 | Swan Creek | Powder | 0 | 700 | 10 | |
| Total | | | | | | 167 | 15.2 |
| | | | | | | | |
| 22-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 13 | |
| 23-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 59 | |
| 24-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 5 | |
| 25-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 10 | |
| 26-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 3 | |
| 27-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 19 | |
| 28-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 4 | |
| 30-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 3 | |
| 31-May-12 | D5 | Swan Creek | Powder | 0 | 700 | 30 | |
| 01-Jun-12 | D5 | Swan Creek | Powder | 0 | 700 | 0 | |
| 02-Jun-12 | D5 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 146 | 13.3 |
| | | | | | | | |
| 22-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 3 | |
| 23-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 53 | |
| 24-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 0 | |
| 25-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 21 | |
| 26-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 1 | |
| 27-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 5 | |
| 28-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 1 | |
| 29-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 5 | |
| 30-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 0 | |
| 31-May-12 | D6 | Swan Creek | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | D6 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 89 | 8.1 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 0 | |
| 23-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 88 | |
| 24-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 17 | |
| 25-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 23 | |
| 26-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 7 | |
| 27-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 17 | |
| 28-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 6 | |
| 29-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 4 | |
| 30-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 4 | |
| 31-May-12 | F3 | Swan Creek | Powder | 0 | 700 | 3 | |
| 01-Jun-12 | F3 | Swan Creek | Powder | 0 | 700 | 1 | |
| Total | | | | | | 170 | 15.5 |
| | | | | | | | |
| 22-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 5 | |
| 23-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 109 | |
| 24-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 10 | |
| 25-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 22 | |
| 26-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 23 | |
| 27-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 6 | |
| 28-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 6 | |
| 29-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 3 | |
| 30-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 0 | |
| 31-May-12 | H3 | Swan Creek | Powder | 0 | 700 | 4 | |
| 01-Jun-12 | H3 | Swan Creek | Powder | 0 | 700 | 1 | |
| Total | | | | | | 189 | 17.2 |
| | | | | | | | |
| 22-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 3 | |
| 23-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 64 | |
| 24-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 31 | |
| 25-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 14 | |
| 26-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 9 | |
| 27-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 6 | |
| 28-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 19 | |
| 29-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 16 | |
| 30-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 0 | |
| 31-May-12 | H7 | Swan Creek | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | H7 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 162 | 14.7 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 5 | |
| 23-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 45 | |
| 24-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 23 | |
| 25-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 4 | |
| 26-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 8 | |
| 27-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 3 | |
| 28-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 6 | |
| 29-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 4 | |
| 30-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 0 | |
| 31-May-12 | H8 | Swan Creek | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | H8 | Swan Creek | Powder | 0 | 700 | 0 | |
| Total | | | | | | 98 | 8.9 |
| | | | | | | | |
| 22-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 1 | |
| 23-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 12 | |
| 24-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 8 | |
| 25-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 6 | |
| 26-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 22 | |
| 27-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 10 | |
| 28-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 14 | |
| 29-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 5 | |
| 30-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 4 | |
| 31-May-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 7 | |
| 01-Jun-12 | B2 | Swan Creek | Powder | 0 | 1,400 | 5 | |
| Total | | | | | | 94 | 8.5 |
| | | | | | | | |
| 22-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 5 | |
| 23-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 15 | |
| 24-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 12 | |
| 25-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 11 | |
| 26-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 14 | |
| 27-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 13 | |
| 28-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 39 | |
| 29-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 8 | |
| 30-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | A2 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | A2 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 117 | 10.6 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 7 | |
| 23-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 34 | |
| 24-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 15 | |
| 25-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 6 | |
| 26-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 20 | |
| 27-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 14 | |
| 28-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 26 | |
| 29-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 30-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | A5 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | A5 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 122 | 11.1 |
| | | | | | | | |
| 22-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 16 | |
| 23-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 56 | |
| 24-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 16 | |
| 25-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 12 | |
| 26-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 26 | |
| 27-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 7 | |
| 28-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 18 | |
| 29-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 3 | |
| 30-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 12 | |
| 31-May-12 | A6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | A6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 166 | 15.1 |
| | | | | | | | |
| 22-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 7 | |
| 23-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 33 | |
| 24-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 16 | |
| 25-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 30 | |
| 26-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 34 | |
| 27-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 19 | |
| 28-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 31 | |
| 29-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 9 | |
| 30-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 2 | |
| 31-May-12 | B8 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 01-Jun-12 | B8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 182 | 16.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 23-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 38 | |
| 24-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 26 | |
| 25-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 14 | |
| 26-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 18 | |
| 27-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 18 | |
| 28-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 9 | |
| 29-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 7 | |
| 30-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E3 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E3 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 131 | 11.9 |
| | | | | | | | |
| 22-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 23-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 42 | |
| 24-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 26 | |
| 25-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 26 | |
| 26-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 6 | |
| 27-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 10 | |
| 28-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 3 | |
| 29-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 10 | |
| 30-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E4 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E4 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 123 | 11.2 |
| | | | | | | | |
| 22-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 23-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 53 | |
| 24-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 17 | |
| 25-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 22 | |
| 26-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 8 | |
| 27-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 19 | |
| 28-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 29-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 30-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E6 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 120 | 10.9 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 6 | |
| 23-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 38 | |
| 24-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 7 | |
| 25-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 18 | |
| 26-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 9 | |
| 27-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 22 | |
| 28-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 18 | |
| 29-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 22 | |
| 30-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 31-May-12 | E7 | Whiteshell | Liquid | 0 | 700 | 19 | |
| 01-Jun-12 | E7 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 160 | 14.5 |
| | | | | | | | |
| 22-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 23-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 12 | |
| 24-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 25-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 2 | |
| 26-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 27-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 28-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 1 | |
| 29-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 30-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 31-May-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| 01-Jun-12 | E8 | Whiteshell | Liquid | 0 | 700 | 0 | |
| Total | | | | | | 17 | 1.5 |
| | | | | | | | |
| 22-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 0 | |
| 23-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 16 | |
| 24-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 11 | |
| 25-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 13 | |
| 26-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 10 | |
| 27-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 5 | |
| 29-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 4 | |
| 30-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 0 | |
| 31-May-12 | A7 | Whiteshell | Liquid | 2 | 700 | 0 | |
| 01-Jun-12 | A7 | Whiteshell | Liquid | 2 | 700 | 0 | |
| 02-Jun-12 | A7 | Whiteshell | Liquid | 2 | 700 | 0 | |
| Total | | | | | | 59 | 5.4 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 1 | |
| 23-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 44 | |
| 24-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 24 | |
| 25-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 21 | |
| 26-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 26 | |
| 27-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 28 | |
| 28-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 29 | |
| 29-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 13 | |
| 30-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 5 | |
| 31-May-12 | G1 | Whiteshell | Liquid | 2 | 700 | 8 | |
| 01-Jun-12 | G1 | Whiteshell | Liquid | 2 | 700 | 2 | |
| Total | | | | | | 201 | 18.3 |
| | | | | | | | |
| 22-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 4 | |
| 23-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 16 | |
| 24-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 2 | |
| 25-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 28 | |
| 26-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 19 | |
| 27-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 6 | |
| 28-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 16 | |
| 29-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 11 | |
| 30-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 1 | |
| 31-May-12 | G2 | Whiteshell | Liquid | 2 | 700 | 7 | |
| 01-Jun-12 | G2 | Whiteshell | Liquid | 2 | 700 | 2 | |
| Total | | | | | | 112 | 10.2 |
| | | | | | | | |
| 22-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 6 | |
| 23-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 44 | |
| 24-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 9 | |
| 25-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 26 | |
| 26-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 11 | |
| 27-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 12 | |
| 28-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 4 | |
| 29-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 9 | |
| 30-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 2 | |
| 31-May-12 | C3 | Whiteshell | Powder | 0 | 700 | 4 | |
| 01-Jun-12 | C3 | Whiteshell | Powder | 0 | 700 | 0 | |
| Total | | | | | | 127 | 11.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 1 | |
| 23-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 60 | |
| 24-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 4 | |
| 25-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 15 | |
| 26-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 10 | |
| 27-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 12 | |
| 28-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 5 | |
| 29-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 3 | |
| 30-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 0 | |
| 31-May-12 | C6 | Whiteshell | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | C6 | Whiteshell | Powder | 0 | 700 | 0 | |
| Total | | | | | | 110 | 10.0 |
| | | | | | | | |
| 22-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 2 | |
| 23-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 26 | |
| 24-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 5 | |
| 25-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 17 | |
| 26-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 4 | |
| 27-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 8 | |
| 28-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 6 | |
| 29-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 13 | |
| 30-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 2 | |
| 31-May-12 | F1 | Whiteshell | Powder | 0 | 700 | 24 | |
| 01-Jun-12 | F1 | Whiteshell | Powder | 0 | 700 | 1 | |
| Total | | | | | | 108 | 9.8 |
| | | | | | | | |
| 22-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 8 | |
| 23-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 30 | |
| 24-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 19 | |
| 25-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 3 | |
| 26-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 10 | |
| 27-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 14 | |
| 28-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 9 | |
| 29-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 2 | |
| 30-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 5 | |
| 31-May-12 | F2 | Whiteshell | Powder | 0 | 700 | 7 | |
| 01-Jun-12 | F2 | Whiteshell | Powder | 0 | 700 | 1 | |
| Total | | | | | | 108 | 9.8 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 0 | |
| 23-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 56 | |
| 24-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 17 | |
| 25-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 20 | |
| 26-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 14 | |
| 27-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 35 | |
| 28-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 19 | |
| 29-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 9 | |
| 30-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 2 | |
| 31-May-12 | F7 | Whiteshell | Powder | 0 | 700 | 18 | |
| 01-Jun-12 | F7 | Whiteshell | Powder | 0 | 700 | 0 | |
| Total | | | | | | 190 | 17.3 |
| | | | | | | | |
| 22-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 3 | |
| 23-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 28 | |
| 24-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 43 | |
| 25-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 13 | |
| 26-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 16 | |
| 27-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 9 | |
| 28-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 15 | |
| 29-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 12 | |
| 30-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 3 | |
| 31-May-12 | G5 | Whiteshell | Powder | 0 | 700 | 0 | |
| 01-Jun-12 | G5 | Whiteshell | Powder | 0 | 700 | 0 | |
| Total | | | | | | 142 | 12.9 |
| | | | | | | | |
| 22-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 2 | |
| 23-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 36 | |
| 24-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 24 | |
| 25-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 26 | |
| 26-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 10 | |
| 27-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 7 | |
| 28-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 6 | |
| 29-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 0 | |
| 30-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 1 | |
| 31-May-12 | G7 | Whiteshell | Powder | 0 | 700 | 2 | |
| 01-Jun-12 | G7 | Whiteshell | Powder | 0 | 700 | 1 | |
| Total | | | | | | 115 | 10.5 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 0 | |
| 23-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 38 | |
| 24-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 15 | |
| 25-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 14 | |
| 26-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 7 | |
| 27-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 4 | |
| 28-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 3 | |
| 29-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 5 | |
| 30-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 8 | |
| 31-May-12 | H4 | Whiteshell | Powder | 0 | 700 | 2 | |
| 01-Jun-12 | H4 | Whiteshell | Powder | 0 | 700 | 0 | |
| Total | | | | | | 96 | 8.7 |
| | | | | | | | |
| 22-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 10 | |
| 23-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 43 | |
| 24-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 2 | |
| 25-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 7 | |
| 26-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 5 | |
| 27-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 8 | |
| 28-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 5 | |
| 29-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 12 | |
| 30-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 10 | |
| 31-May-12 | C1 | Whiteshell | Powder | 0 | 700 | 9 | |
| 01-Jun-12 | C1 | Whiteshell | Powder | 0 | 700 | 2 | |
| Total | | | | | | 113 | 10.3 |
| | | | | | | | |
| 22-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 8 | |
| 23-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 48 | |
| 24-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 6 | |
| 25-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 18 | |
| 26-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 9 | |
| 27-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 8 | |
| 28-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 5 | |
| 29-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 3 | |
| 30-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 0 | |
| 31-May-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 3 | |
| 01-Jun-12 | D2 | Whiteshell | Powder | 0 | 1,400 | 4 | |
| Total | | | | | | 112 | 10.2 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 5 | |
| 23-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 39 | |
| 24-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 24 | |
| 25-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 32 | |
| 26-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 19 | |
| 27-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 11 | |
| 28-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 25 | |
| 29-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 11 | |
| 30-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 3 | |
| 31-May-12 | B7 | Whiteshell | Powder | 2 | 700 | 1 | |
| 01-Jun-12 | B7 | Whiteshell | Powder | 2 | 700 | 0 | |
| Total | | | | | | 170 | 15.5 |
| | | | | | | | |
| 22-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 2 | |
| 23-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 36 | |
| 24-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 20 | |
| 25-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 35 | |
| 26-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 10 | |
| 27-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 21 | |
| 28-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 15 | |
| 29-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 21 | |
| 30-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 0 | |
| 31-May-12 | C5 | Whiteshell | Powder | 2 | 700 | 0 | |
| 01-Jun-12 | C5 | Whiteshell | Powder | 2 | 700 | 0 | |
| Total | | | | | | 160 | 14.5 |
| | | | | | | | |
| 22-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 6 | |
| 23-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 43 | |
| 24-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 4 | |
| 25-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 17 | |
| 26-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 1 | |
| 27-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 7 | |
| 28-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 2 | |
| 29-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 14 | |
| 30-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 15 | |
| 31-May-12 | D1 | Whiteshell | Powder | 2 | 700 | 0 | |
| 01-Jun-12 | D1 | Whiteshell | Powder | 2 | 700 | 16 | |
| Total | | | | | | 125 | 11.4 |

| Date | Trough Location | Treatment | | | | Mortality | Average Daily Mortality |
|---|---|---|---|---|---|---|---|
| | | Water source | OTC type | DMSO (%) | OTC (mg/L) | | |
| 22-May-12 | F5 | Whiteshell | None | 0 | 0 | 0 | |
| 23-May-12 | F5 | Whiteshell | None | 0 | 0 | 28 | |
| 24-May-12 | F5 | Whiteshell | None | 0 | 0 | 27 | |
| 25-May-12 | F5 | Whiteshell | None | 0 | 0 | 42 | |
| 26-May-12 | F5 | Whiteshell | None | 0 | 0 | 8 | |
| 27-May-12 | F5 | Whiteshell | None | 0 | 0 | 48 | |
| 28-May-12 | F5 | Whiteshell | None | 0 | 0 | 22 | |
| 29-May-12 | F5 | Whiteshell | None | 0 | 0 | 16 | |
| 30-May-12 | F5 | Whiteshell | None | 0 | 0 | 11 | |
| 31-May-12 | F5 | Whiteshell | None | 0 | 0 | 6 | |
| 01-Jun-12 | F5 | Whiteshell | None | 0 | 0 | 3 | |
| Total | | | | | | 211 | 19.2 |

Appendix 3. Lake conditions and effort expended during shoreline electrofishing surveys for age 0+ walleye in fall, 2012.

| Lake | Lake temperature (C°) | Conductivity (μS) | Transect | Range of amperage (A) | | Pulse/sec | Voltage (V) | Percent voltage | Time fished/hour |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Low | High | | | | |
| Barren | 20.2 | 50 | BR-1 | 7 | 7.5 | 120 | 1000 | 80 | 0.00226 |
| | | | BR-2 | 8 | 8 | 120 | 1000 | 60 | 0.00377 |
| | | | BR-3 | 7.9 | 8.3 | 120 | 1000 | 65 | 0.0036 |
| | | | BR-4 | 7.9 | 8.5 | 120 | 1000 | 60 | 0.00363 |
| | | | BR-5 | 7.2 | 8.2 | 120 | 1000 | 70 | 0.00499 |
| | | | BR-6 | 7.2 | 8.2 | 120 | 1000 | 70 | 0.00375 |
| | | | BR-7 | 7.3 | 8.2 | 120 | 1000 | 70 | 0.0036 |
| Mean | | | | 7.5 | 8.1 | | | 68 | 0.00366 |
| Booster | 14.6 | 36 | BS-1 | 6 | 6.5 | 120 | 1000 | . | 0.00277 |
| | | | BS-2 | 7 | 7 | 120 | 1000 | . | 0.00314 |
| | | | BS-3 | 6 | 7 | 120 | 1000 | . | 0.00395 |
| | | | BS-4 | 6 | 7 | 120 | 1000 | . | 0.00247 |
| | | | BS-5 | 6 | 6.5 | 120 | 1000 | . | 0.00345 |
| | | | BS-6 | 6.5 | 7 | 120 | 1000 | . | 0.00307 |
| | | | BS-7 | 6 | 7 | 120 | 1000 | . | 0.00353 |
| Mean | | | | 6.2 | 6.9 | | | | 0.00319 |
| Flanders | 14.5 | 27 | FL-1 | 4.1 | 5.1 | 120 | 1000 | . | 0.00403 |
| | | | FL-2 | 6.5 | 7 | 120 | 1000 | . | 0.00444 |
| | | | FL-3 | . | . | 120 | 1000 | . | 0.00481 |
| | | | FL-4 | 4 | 5 | 120 | 1000 | . | 0.00436 |

Appendix 3. continued.

| Lake | Lake temperature (C°) | Conductivity (μS) | Transect | Range of amperage (A) | | Pulse/sec | Voltage (V) | Percent voltage | Time fished/hour |
|---|---|---|---|---|---|---|---|---|---|
| Flanders | 14.5 | 27 | FL-5 | 4 | 5 | 120 | 1000 | . | 0.0049 |
| | | | FL-6 | 5.5 | 6 | 120 | 1000 | . | 0.00451 |
| | | | FL-7 | 5.5 | 6 | 120 | 1000 | | 0.00434 |
| | | | FL-8 | . | . | 120 | 1000 | . | 0.0045 |
| | | | FL-9 | . | . | 120 | 1000 | . | 0.00454 |
| | | | FL-10 | 4.5 | 5 | 120 | 1000 | . | 0.0055 |
| Mean | | | | 4.7 | 5.4 | | | | 0.00459 |
| Star | 19 | 51 | ST-1 | 7.3 | 7.4 | 120 | 1000 | 70 | 0.0044 |
| | | | ST-2 | 7.2 | 7.9 | 120 | 1000 | 70 | 0.00983 |
| | | | ST-3 | . | . | 120 | 1000 | 70 | 0.0017 |
| | | | ST-4 | . | . | 120 | 1000 | 70 | 0.01544 |
| | | | ST-5 | 7.2 | 7.9 | 120 | 1000 | 70 | 0.00422 |
| | | | ST-6 | 7.9 | 8.3 | 120 | 1000 | 70 | 0.00459 |
| | | | ST-7 | 7.2 | 8 | 120 | 1000 | 70 | 0.00475 |
| | | | ST-8 | 6.8 | 7.5 | 120 | 1000 | 70 | 0.004 |
| | | | ST-9 | 7.8 | 7.9 | 120 | 1000 | 70 | 0.00983 |
| | | | ST-10 | 7.8 | 7.9 | 120 | 1000 | 70 | 0.00505 |
| Mean | | | | 7.5 | 8 | | | 70 | 0.00684 |
| White | 17 | 74 | W-1 | 6 | 7 | 120 | 1000 | 70 | 0.00372 |
| | | | W-2 | 8 | 8.2 | 120 | 1000 | 60 | 0.0035 |
| | | | W-3 | 8.1 | 8.2 | 120 | 1000 | 70 | 0.00525 |
| Mean | | | | 7.4 | 7.8 | | | 67 | 0.00416 |

Appendix 4. Experimental rearing trough set up at the Whiteshell Fish Hatchery in 2012.

Manitoba
Conservation and Water Stewardship

April 10, 2015

Director, Animal Care and Veterinary Services
University of Manitoba
208 - 194 Dafoe Road
Winnipeg, MB R3T 2N2

Re: Animal Care and Handling for Project: Use of oxytetracycline marking on larval Walleye (*Sander Vitreum*) in Manitoba

The project "Use of oxytetracycline marking on larval Walleye (*Sander Vitreum*) in Manitoba" was conducted while the student, Ms. Laura Groening, was also employed with Manitoba Conservation and Water Stewardship, Fisheries Branch. As an employee of the Department, Ms. Groening was obliged to comply with the requirements set out by the standard operating procedures at the Whiteshell, Swan Creek and Grand Rapids Hatcheries at the time of the study, that were consistent with North American practices for aquaculture and fisheries management. Her work did not undertake substantially new processes or treatments to live animals beyond the scope of work that is normally done at these facilities. Rather, her contributing work occurred from analysis done on dead specimens. For this reason, animal care approval was not sought from the University of Manitoba.

Please contact me with any questions or concerns.

Jeff Long, Ph.D. | Manager - Fisheries Science and Fish Culture | Fisheries Branch
MB Conservation and Water Stewardship | 200 Saulteaux Cres. | Winnipeg, Manitoba, |
R3J 3W3

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