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Contribution of Fry Stocking to the Recovery of the Walleye Population in the Red Lakes[1]

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Abstract.— Newly hatched walleyes were successfully marked by immersion in a solution of 700 mg oxytetracycline hydrochloride (OTC)/L for 6 h without adverse effects on growth or mortality, and these marks were still visible on over 70% of the walleyes sampled up to 4 years after treatment. The 1999 and 2001 year classes in the Red Lakes were the strongest year classes in over 20 years. Stocked fry contributed over 80% to the 1999 year class, over 50% to the 2001 year class, over 80% to the 2003 year class, and less than 10% to the 2004 year class. By 2004, stocked walleyes contributed over 68% of the total walleye biomass in the Red Lakes and, due to recruitment of stocked walleyes, stocking was no longer necessary to supplement natural reproduction to rehabilitate the walleye population.

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Introduction

Decades of overexploitation caused a severe decline in the walleye *Sander vitreus* population of the Red Lakes, Minnesota. Gill net catches during the late 1990s reached historic lows, and estimates of mature female biomass were below levels believed necessary to produce a moderate year class (Ekstrom 1997). In response to low walleye population levels, the Red Lake Fisheries Technical Committee was formed by State, Tribal, and Federal officials to establish a recovery plan for the walleye population in the Red Lakes. The plan included a closure of all walleye harvest, increased enforcement of fishing regulations, increased information exchange, and an aggressive, short-term rehabilitation stocking effort. This study evaluated the contribution of stocked fry to the walleye population of the Red Lakes so that stocking levels could be adjusted to changing conditions in the lakes, and discontinued when natural reproduction became sufficient for establishment of strong year classes.

Because natural reproduction was expected to recover in the Red Lakes, a method to discern between stocked and natural fry was necessary to evaluate the contribution of stocking. Conventional fish marking methods such as fin clips or tags; however, were not practical due to the small size of walleye fry at the time of stocking.

Production of a chemical mark by immersion in oxytetracycline hydrochloride (OTC) showed promise as a practical alternative for marking larval walleyes (Younk and Cook 1991; Brooks et al.1994; Peterson and Carline 1996; Fielder 2002; Lucchesi 2002). Younk and Cook (1991) first described a method of marking larval walleyes by immersion in a 350 mg OTC/L solution for a period of 8 h. Although species other than walleye have been marked as newly hatched fry or even as eggs (Dabrowski and Tsukamota 1986; Muth and Bestgen 1991), Younk and Cook (1991) reported complete marking success only with walleye fry that were at least 5 d post hatch at the time of treatment. Brooks et al. (1994) similarly reported a minimum age limitation of 4 d post hatch during immersion trials at 500 mg OTC/L. Restricting treatment to walleyes that were at least 4 d old would not integrate well with the existing configuration and operations of the Bemidji State Fish Hatchery. A single day's hatch at the Bemidji hatchery often approaches the carrying capacity of the fry tanks. Consequently, the hatchery generally needs to stock the walleye fry within 24 h of hatching. Insuring that all walleyes were at least 4 d old prior to treatment would have required additional fry holding facilities, and possible modifications in the timing of the egg take (Fielder 2002) or incubation temperatures to insure that the fry hatched daily did not exceed the carrying capacity of the fry tanks. Consequently, the first objective of this study was to develop and evaluate an OTC immersion procedure that would allow marking large numbers of newly hatched fry so that the hatchery's entire annual production of fry could be marked with little interruption in normal operations. I hypothesized that a higher concentration of OTC would be effective at producing marks on younger fish so that the holding period for fry prior to treatment could be reduced or eliminated. Fielder (2002) recommended routine use of 700 mg OTC/L for marking 3 to 5 day old walleye fry, while Lucchesi (2002) recently reported success in marking walleye fry as young as 2 d post hatch in raceways by increasing the treatment concentration to 700 mg OTC/L. In this study, I investigated the efficacy of marking newly hatched walleye fry (< 24 h post hatch) by immersion in 700 mg OTC/L during transport in fry bags, the effects of this treatment on survival and growth of the walleyes, and the retention of the mark for up to 5 years post immersion.

Methods

OTC treatment and mark detection

Newly hatched walleye fry (<24 h post hatch) were treated by immersion in an oxytetracycline solution following a modification of the procedure described by Brooks et al. (1994). Terramycin-343® was used as the source of OTC during 1999, 2001 and 2003, while Oxymarine® was used during 2004. A bulk solution of 700 mg OTC/L was mixed (Fielder 2002; Lucchesi 2002), then buffered to pH 6.8 with sodium phosphate dibasic. A silicon-based surfactant was also added to the solution at a rate of 0.04 mL surfactant/L to reduce foaming.

To reduce handling stress, the fry were treated directly in the transport containers en route to the waters being stocked. The containers, which are commonly used by the Minnesota Department of Natural Resources (MNDNR) to transport walleye fry, consisted of collapsible 19 L clear plastic water jugs with the caps modified by the addition of valve stems to facilitate inflation with oxygen. The fry were enumerated by weight and added to the OTC solution in each container at a density of approximately 4,400 fry/L. To allow room for oxygen inflation, a maximum of 50,000 fry and 11.4 L of OTC solution were placed in each container. The fry remained immersed in the OTC solution during and after transport to their destination for a total of at least 6 h. Care was taken to reduce fry exposure to sunlight and temperature changes during the process. After 6 h of immersion, the fry were immediately stocked into receiving waters in accordance with U.S. Food and Drug Administration guidelines under Investigational New Animal Drug (INAD) exemption No. 9033.

Inspection of both walleye fry and fingerlings for the presence of a mark was conducted following the methods of Secor et al. (1991) and Brooks et al. (1994). The otoliths were first removed from the specimens and wiped dry. They were secured to a microscope slide with cyanoacrylate cement and polished with either 600 or 1,200 grit sandpaper until the inner growth rings became visible with transmitted light under 100 X magnification. Inspection for a mark was conducted under an epi-fluorescent microscope with fluorescent lighting and filter blocks designed to optimize tetracycline fluorescence (Bumguardner 1991; Brooks et al. 1994). The specific system employed was a Nikon Eclipse E-400 microscope with B-3A filter cube (505 nm dichroic mirror, 420-490 nm exciter filter, and 520 nm barrier filter), 10 X and 20 X objectives, and a 100 W mercury UV light source. The intensity of the mark observed under 200 X was categorized using a rating system similar to that described by Weber and Ridgeway (1967) where: absent = no mark evident; faint = the mark is present but not clearly visible; clear = the mark is readily visible but not vivid; and intense = the mark is both readily visible and vivid.

Oxytetracycline Treatment Validation

OTC immersion treatment of newly hatched walleye fry was evaluated with an integrated sampling design that utilized samples from six separate treatment episodes, and untreated control walleyes in a variety of laboratory and pond experiments (Table 1). Supplemental information on mark efficacy and retention was also obtained through genetic analysis and inspection of the recaptured walleyes from the Red Lakes.

The first treatment episode was 2 May 1999 with 800,000 walleye fry at the MNDNR hatchery in Waterville, Minnesota to test the marking procedure under large-scale hatchery production conditions. Four subsequent treatment episodes (numbers 2, 3, 5, and 6) were conducted at the MNDNR hatchery in Bemidji, Minnesota to mark fish for stocking into the Red Lakes. Because all five of these treatments were conducted under production-level conditions, the exact age of the walleye fry at the time of treatment is unknown. Behavioral characteristics of larval walleyes and the characteristics of the hatching procedure, made it likely that most treated walleyes were < 24 h post hatch. The walleye fry at the two hatcheries were hatched in 6.6 L incubation jars. Since walleye fry are positively phototactic (McElman and Balon 1979), they swim towards the water surface of the incubation jars when hatched in a lighted room. This swim-up phase begins immediately after hatching (McElman and Balon 1979), and once the fry reach the water's surface, often with the anterior portion of their body still enveloped in the egg membrane, they become entrained in the out-flowing water and are passed out of the jars into a fry tank. The fry tanks were seined daily and the collected fry were treated with OTC.

Table 1. Abundance, source, and disposition of stocked walleye fry.

Year	Hatchery	Egg source	Treatment episode	Stocking dates	Number stocked	Waters stocked	Use
1999	Waterville	Pine River	1	2 May	800,000	Rice Lake	Blind efficacy
			control	3 May	2,500	Pond 8	Blind efficacy
			1	3 May	1,937	Aquaria	Acute mortality Trial 1
			control	3 May	1,770	Aquaria	Acute mortality Trial 1
	Bemidji	Pike River	2	10-18 May	41,127,201	Red Lake	Supplement population and Peterson abundance estimates
			2	14 May	2,500	Pond 3	Blind efficacy
			control	14 May	2,500	Pond 1	Blind efficacy
			2	14 May	25,000	Clearwater Pond	Efficacy
			2	14 May	12,500	Pond 12	Growth and mortality
			control	14 May	12,500	Pond 12	Growth and mortality
			2	14 May	12,500	Pond 13	Growth and mortality
			control	14 May	12,500	Pond 13	Growth and mortality
			2	14 May	554	Aquaria	Acute mortality Trial 2
			control	14 May	611	Aquaria	Acute mortality Trial 2
			2	17 May	1,415	Aquaria	Acute mortality Trial 3
			control	17 May	1,611	Aquaria	Acute mortality Trial 3
			2	6 Oct	903	Pond 3*	Retention
			control	22 Oct	1,150	Pond 1**	Retention
2001	Bemidji	Pike River	3	16-21 May	31,536,972	Red Lake	Supplement population and Peterson abundance estimates
			3	19 May	25,000	Pond 12	Blind efficacy
			control	19 May	25,000	Pond 13	Blind efficacy
2002	Waterville	Pine River	4	14 May	10,000	Raceway	Efficacy of treatment on walleye <3hr old
2003	Bemidji	Pike River	5	9-16 May	32,641,725	Red Lake	Supplement population and Peterson abundance estimates
			5	14 May	12,500	Pond 14	Efficacy
2004	Bemidji	Red Lake	6	14-20 May	10,000,844	Red Lake	Peterson abundance estimates
			6	18 May	25,000	Pond 12	Efficacy
			6	18 May	25,000	Pond 13***	Efficacy

* Walleyes in Pond 3 died after sampling for efficacy testing in 1999 and were replaced with OTC treated walleyes originally stocked into Clearwater Pond.
** Many of the walleyes in Pond 1 died after sampling for efficacy testing in 1999 and the number of surviving walleyes was supplemented with walleyes originally stocked into Frank Pond.
*** No walleyes were recovered from Pond 13 during 2004.

An additional treatment (Treatment episode 4) was conducted under more controlled conditions at the Waterville Hatchery during May 2002 to assess whether walleye fry known to be less than 3 h post hatch could be treated with OTC to produce a discernible fluorescent mark on their otoliths. During a period of active hatching on 14 May 2002, all visible fry were removed from 3 cannon-type 50 L hatching vessels (May 2002) by siphoning. After 3 h of continuous hatching, the jars were again emptied of newly hatched fry (< 3 h old). Approximately 10,000 fry were treated with OTC while another 10,000 fry were retained as untreated controls.

Treatment efficacy.— Samples of walleye fry from each treatment episode were used to evaluate the effectiveness of the OTC treatment to produce fluorescent marks in the otoliths of newly hatched fry. In the first efficacy experiment conducted in 1999, approximately 800,000 of the walleye fry from Treatment Episode 1 (Table 1) were stocked into a chemically reclaimed pond (Rice Lake) immediately after treatment, and approximately 2,500 untreated fry were stocked into Pond 8 at the Waterville Hatchery to serve as controls. After 5 months, 100 fingerlings were removed from each pond and inspected for marks in blind trials, where prepared otoliths from treated and untreated fish were randomly chosen and their identities concealed. The mark assignments were compared to the known treatment status of the fish, and percent marking success calculated. Additional tests of mark efficacy on walleye fry inferred to be < 24 h post hatch at the time of treatment were conducted with samples of the walleye fry hatched at the Bemidji hatchery for stocking into the Red Lakes. During 1999, Pond 3 at the Waterville Hatchery was stocked with approximately 2,500 treated walleye fry from Treatment Episode 2 (Table 1), while Pond 1 was stocked with untreated controls. Clearwater Pond, in the Bemidji Area, was also stocked in 1999 with 25,000 OTC treated fry from Episode 2 (Table 1). During 2001, approximately 12,500 treated walleye fry from Treatment Episode 3 (Table 1) were stocked into Pond 12, while Pond 13 was stocked with untreated controls. During 2003, approximately 12,500 treated fry from Treatment Episode 5 (Table 1) were stocked into Pond 14 and in 2004, Ponds 12 and 13 were each stocked with approximately 25,000 treated walleye fry from Treatment Episode 6 (Table 1). Evaluation of the treatment efficacy on walleye fry treated at < 3 h post hatch was conducted with walleye fry hatched at the Waterville Hatchery. Approximately 10,000 walleye fry from the Treatment Episode 4 (Table 1), and an equal number of untreated controls were held in separate fry tanks at the Waterville Hatchery for 9 days. After 1, 3, and 6 days, samples of treated walleye fry were collected and the otoliths examined for the presence of a mark. After 9 days, otoliths from both treated and control groups were examined for marks in blind trials.

Treatment effects on mortality and growth.— Samples of walleyes from Treatment Episodes 1 and 2 were held in aquaria to evaluate the level of acute mortality induced by OTC treatment. Samples of OTC-treated walleyes were separated from the OTC solution after the 6 h immersion period by using a larval concentrator similar to that described by Secor et al. (1991). The fry remained suspended in liquid while the OTC solution was replaced with untreated hatchery water by first pouring the solution with fry into the concentrator, then sequentially submersing the concentrator in buckets of untreated water until the yellow color of the OTC solution was no longer visible. The treated fry were distributed among three randomly assigned aquaria. Untreated fry from the same source as the treated fry were distributed among three aquaria to serve as controls. The aquaria were placed in a raceway and a constant water temperature was maintained by bathing the aquaria in flowing water. Continuous aeration was also provided by releasing compressed air through stone diffusers in each of the aquaria. Visual estimates of the number of dead fry were conducted daily, and total mortality rates were calculated from counts of dead and live fry at the end of the 3 d test period. Differences in mortality between treated and untreated walleyes were tested by chi-square analysis. Three replicates of this experiment were conducted. The first trial was conducted with walleye fry from Treatment Episode 1 (Table 1) and untreated controls from the Waterville Hatchery, whereas the second and third trials were conducted with walleyes from the Treatment Episode 2 (Table 1) and untreated controls from the Bemidji Hatchery.

An additional mortality and growth experiment was conducted by stocking Ponds 12 and 13 at the Waterville Hatchery with equal amounts of both OTC-treated fry from Treatment Episode 2 (Table 1), and untreated fry from the Bemidji Hatchery. To maximize parity in numbers between the treated and control fry at stocking, the fry were measured volumetrically. Each of the two 4 ha drainable ponds received 57 mL (approximately 12,500) treated walleye fry and the same volume of control walleye fry. After 5 months, the ponds were harvested and 100 fingerlings from each pond were collected. Total length of each walleye was measured to the nearest mm, and the otoliths were inspected for the presence of a mark. Differences in mortality between the treated and untreated walleyes were determined through chi-square comparison of the ratios of marked fingerlings in the samples to the ratio of treated fry at stocking. Growth was analyzed by t-test comparison of the mean total lengths of treated and untreated groups within each pond.

Mark retention and recognition.— Samples of the walleyes from Treatment Episode 2 (Table 1) were held in rearing ponds at the Waterville hatchery for evaluation of long-term retention and recognition of the OTC mark. The 0.4 ha ponds were initially stocked on 14 May 1999 with approximately 2,500 fry each. Pond 3 received fry treated with OTC, whereas Pond 1 received untreated fry and served as a control. Enough walleyes were present in both ponds on 30 June 1999 to provide samples for initial mark efficacy testing. A lack of visible feeding activity later in the summer; however, raised suspicions of poor survival. Consequently, each pond was drained during the week of 19 September 1999, and the water filtered through screen retention boxes to capture the remaining walleyes. Only 21 walleyes were recovered from Pond 3. These fish were disposed of and replaced on 6 October 1999 with 903 OTC-treated fingerlings that had been reared by Bemidji Area fisheries staff in Clearwater Pond. The replacement walleyes also originated from the walleye fry that were hatched at the Bemidji hatchery for stocking into the Red Lakes, and had been treated with the same batch of OTC solution as the fry originally stocked into Pond 3 (Treatment Episode 2, Table 1). Draining of Pond 1 resulted in the recovery of 336 walleye fingerlings. These fish were held in a raceway until the pond was refilled, then restocked into Pond 1. To supplement the number of unmarked controls, an additional 1,150 similar-sized walleye fingerlings were stocked into Pond 1 on 22 October 1999. These fish were also from the Pike River egg source, and were reared in Frank Pond by Spicer Area fisheries staff. After these changes, Pond 3 continued to hold OTC-treated walleyes from the 1999 year class of Pike River eggs while Pond 1 held untreated control walleyes of the same age and spawning stock.

Ponds 1 and 3 were drained into screen retention boxes again during 2000, 2001, 2002, and 2003 to provide samples of the 1999 year class for evaluation of mark retention and recognition. The pond sampling was conducted during the fall each year so that the results of mark validation could be applied to walleyes of a similar life stage as those collected from the Red Lakes during fall gill netting. Each year, 120 walleyes were retained from each pond for analysis, and during 2000, 2001 and 2002, the remaining fish were returned to the ponds after refilling. Fall 2003 was the last scheduled sample period, so the ponds were not restocked after sampling. The otoliths from 100 of the retained walleyes from each pond were inspected for marks in blind trials. The mark assignments were then compared to the known treatment status of the fish, and marking success was calculated.

The marks observed on walleyes sampled from the Red Lakes also served to help validate both efficacy and retention of fluorescent marks in OTC treated fish. The initial return rate of marked fish from the Red Lakes indicated the minimum possible efficacy levels for the OTC marking. Subsequent return rates, though sensitive to differences in mortality between stocked and wild walleyes, provided tacit evidence of mark retention.

Additional evidence of mark retention was provided by monitoring the brightness of the marks across time so that the fading of marks prior to mark loss could be identified.

Further evaluation of OTC mark efficacy and retention was provided through genetic analysis of the walleye samples from the Red Lakes. Walleyes from the 1999 and 2001 stockings were suitable for genetic assignment because they originated from the Pike River egg source, and were stocked prior to maturation and subsequent genetic contribution of other stocked walleyes. Walleyes from the 1999 and 2001 year classes were inspected for the presence of fluorescent marks, and a sample of their tissues provided to Dr. Loren Miller, University of Minnesota (U of M), for genetic analysis. Dr. Miller conducted maximum-likelihood analysis to discriminate between the Pike River strain and the native Red Lake walleyes by comparing the allele frequency of microsatellite DNA markers (Banks and Eichert 2000). Genetic stock assignments by Dr. Miller were compared to the occurrence of fluorescent marks to determine mark efficacy rates. To increase the confidence in the genetic assignments, only individuals that were 50 times more likely to be assigned to one stock over the other (LR>50) were used in the analysis. Genetic analysis was also conducted on walleyes from Ponds 3 and 12 in an attempt to confirm suspected contamination of these ponds with unmarked walleyes.

Contribution of Fry Stocking in the Red Lakes

Stocking.— The Red Lakes were stocked with walleye fry four times during this study, and all fry stocked were treated by immersion in OTC. The rehabilitation stockings were conducted with fry hatched from eggs obtained from the Pike River. Approximately 41 million Pike River strain fry were stocked in 1999, 31 million in 2001, and 32 million in 2003. The success of these three stockings met the criteria for discontinuing rehabilitative fry stocking in the Red Lakes. The Red Lake Fisheries Technical Committee, however, recognized the importance of the fry abundance estimates for evaluating natural reproduction, and agreed to conduct another stocking of OTC treated walleye fry to provide marked fish for Peterson population estimates (Gary Barnard, MNDNR, personal communication 2006). The additional stocking was conducted in 2004 with 10 million fry hatched from eggs collected from the Black Duck River in Lower Red Lake.

Collection and identification of stocked walleyes.— Young-of-the-year (YOY) walleyes were sampled annually by seining during a 6-week period between early July and late August each year. Red Lake Department of Natural Resources (RL DNR) fisheries staff seined four sites each in Lower Red Lake and West Upper Red Lake, and MN DNR fisheries staff seined five sites in East Upper Red Lake. Weekly sampling at each site resulted in a lake-wide annual sampling effort of 78 seine hauls. The fingerlings collected during seining were forwarded to the MN DNR fisheries research station at Waterville for OTC mark inspection. Upon completion of the OTC mark inspection, tissue samples from the inspected walleyes from the 1999 and 2001 year classes were forwarded to Dr. Loren Miller for genetic analysis. Tissue samples from the 2003 year class were not suitable for genetic analysis because many of Pike River strain walleyes that were stocked into the Red Lakes in 1999 had recruited to the spawning stock, and the subsequent likelihood of mixed stock parentage (Pike River/Red Lake) precluded differentiation of stocked fish through genetic analysis.

Walleyes ≥ age 1 were sampled each September by experimental gill netting. RL DNR fisheries staff set 32 nets annually in Lower Red Lake and 16 sets annually in West Upper Red Lake. MN DNR fisheries staff set 20 nets annually in East Upper Red Lake. Gill nets used by the two jurisdictions were identical, and consisted of 15.3 m panels of 19 mm, 25 mm, 32 mm, 38 mm, and 51 mm bar measure mesh. Nets were each set for approximately 24 hours before being fished. MN DNR fisheries staff collected data from the walleyes according to standard large lake monitoring guidelines (Wingate and Schupp 1985). This included measuring total length (TL) in millimeters and weight in grams, determining the gender and maturity, and collecting scales, otoliths and spines for age analysis. RL DNR fisheries staff collected the same data from their catch except the lengths of the walleyes were measured as fork length (FL) in millimeters and later converted to total length by multiplying fork length by 1.05 (Pat Brown, Red Lake DNR, personal communication 2006).

Walleye ages were determined by inspection of dorsal spine or otolith cross-sections. The scale samples and otoliths from the stocked year classes were forwarded to the Waterville Fisheries Research Station for OTC mark inspection. Upon completion of the mark inspection, the scale samples were then transferred to Dr. Loren Miller for genetic analysis.

Data analysis.— The abundance of fry in the Red Lakes immediately following stocking (N) was estimated using the Chapman modification of the Peterson single-census method (Ricker 1975):

$$N=(M+1)(C+1)/(R+1)$$

where M is the number of marked walleye fry stocked into the Red Lakes, C is the number of YOY walleyes from the seine catches that were inspected for the presence of an OTC mark, and R is the number of inspected walleyes with a visible mark. Confidence limits were then calculated for R and substituted in the formula to estimate the confidence limits of the abundance estimate (Ricker 1975). Only whole lake estimates of fry abundance were conducted due to the likelihood of movement of the stocked walleyes between basins during the period between stocking and seining. Estimates of the abundance of wild fry at the time of stocking were calculated by subtracting the number of stocked fry from the total fry abundance estimate.

The abundance of age 1 and older walleyes was estimated from the length frequency distribution of the net catches using Anderson's (1998) gill net catchability model (q_{abg} model). Catchability $q(l)$ was first calculated for each 10 mm length group using the formula:

$$q(l)=\alpha(l)\sum_m\beta_m\gamma(x)$$

where l is the midpoint of the length group, $\alpha(l)$ is the encounter probability per length group for the entire gill net, β_m is the contact coefficient per mesh size, and $\gamma(x)$ is the retention function for each mesh size where x is the fish/mesh perimeter ratio. The values used for α, β and γ were those updated by Anderson in 1999 (Charles Anderson, Minnesota DNR, personal communication 2006). The abundance estimate (N) was then calculated for each basin with the formula:

$$N=\sum[(CPE/q(l)(acres/132,516)(10^5)]$$

where CPE is the catch per effort of walleyes per 10 mm length interval, $q(l)$ is the catchability coefficient, the values *132,516* and 10^5 are constants, and *acres* is the area in acres of the basin where the netting took place. Biomass estimates (B) were also calculated from the q_{abg} model for each basin using the formula:

$$B=\sum_l N_l a l^{b0}$$

where N_l is the population estimate of walleyes at each length group, a is the antilog of the y intercept from the regression of log(weight) on log(total length), and b is the regression coefficient from the regression of log(weight) on log(total length). Total lake estimates of both abundance and biomass were calculated as the sum of the individual basin estimates, and confidence intervals of the estimates are calculated using bootstrap methods described by Hadden (2001) where population and biomass estimates calculated from individual net catches served as the pool for resampling. This pool of values was resampled with replacement to produce bootstrap samples of size n, where n = the number of nets fished during the survey. One thousand bootstrap estimates were generated, and the confidence intervals were calculated as the 5th and 95th percentiles of the distribution of bootstrap estimates.

Survival rates (S) from fry (at the time of stocking) to each age sampled were calculated by dividing the annual q_{abg} abundance estimate of the stocked year class of interest by its Peterson abundance estimate as fry. Instantaneous mortality estimates (Z) over the time periods between stocking and later sampling were calculated using:

$$Z=(\log_e N_t - \log_e N_{t+n})/n$$

where N_t is the abundance of fry immediately after stocking, N_{t+n} is the abundance of walleyes from the year class of interest at some time ($t+n$) later, and n is the time elapsed between sample periods. The variable n is included in the formula because the time between sample periods differs from one year (Ricker 1975).

To obtain annual abundance and biomass estimates of stocked walleyes age 1 and older in the Red Lakes, the q_{abg} model was first applied to the gill net catch of walleyes from the stocked year class in question, then multiplied by the proportion of marked walleyes in that year's sample. Annual abundance and biomass estimates of stocked walleyes from each of the stocked year classes were then summed to determine the total contribution of the stocked walleyes to the population during each year of the study. Abundance and biomass estimates of mature female walleyes in the Red Lakes were similarly obtained by applying the q_{abg} model to the gill net catches of mature females.

The Peterson abundance estimates of wild fry present at the time of stocking served as the measure of natural reproduction in the Red Lakes. Total egg production was estimated by multiplying total mature female biomass by a fecundity rate of 5,510 eggs/kg mature females (Bushong 1990). Hatch rate was then estimated by dividing the abundance of wild fry by the egg production estimate. An equation describing the relation between egg production and hatch rate was defined by fitting the hatch rate data with a least squares regression line forced through the origin. This equation was then used with fall gill net data to predict the level of natural reproduction the following spring.

The fitness of Pike River strain walleyes was evaluated by comparing maturity rates, growth, and survival of stocked walleyes to those of the native Red Lake walleyes. Differences in maturity rates between stocked and wild walleyes were identified by plotting the proportion of mature marked fish with the proportion of mature unmarked fish, by sex and year class, at each age. Maturity at length ogives were also fitted through logistic regression, by sex and year class, to identify differences in maturity rates between stocked and wild walleyes by total length. Differences in growth between stocked and wild walleyes were tested by defining a linear model incorporating age, lake basin, sex, mark status, and age*sex interactions terms to explain differences in total length between marked and unmarked walleyes by age group at ages 1 and above. Plotting the residuals identified heteroscedasticity among total lengths with age, consequently a second model using log transformed total length data was defined. Relative survival (RS, Heidinger et al. 1987) between stocked and wild walleyes was calculated annually using the formula:

$$RS = (p_{t+n}/p_t)/\left((1- p_{t+n})/(1- p_t) \right)$$

where p represents the proportion of marked fish in the sample, t is the year of the first sample, and n is the number of years between the first and second samples. Confidence intervals were calculated using bootstrap methods (Haddon 2001) where the mark status of inspected walleyes served as the pool for resampling. This pool of values was resampled with replacement to produce bootstrap samples of size n, where n is the number of walleyes inspected for a mark. One thousand bootstrap estimates were generated and the confidence intervals were calculated as the 5th and 95th percentiles of the distribution of bootstrap estimates.

The relative year-class strengths of walleyes in the Red Lakes were calculated with a model that has been used on other Minnesota lakes (Parsons and Pereira 2001). This model uses the equation:

$$log_e(CPE_{ij})=\mu+(\alpha_i)[age(i)]+(\beta_i)[yearclass(j)] + \varepsilon_{ij}$$

where CPE_{ij} is the number of fish age i from year class j caught divided by the number of nets, plus 1 to allow log of zero values, μ is the mean CPE across all ages for each year class, a_i and b_j are parameter estimates corresponding to each value of age and year class, *age(i)* and *yearclass(j)* are main effects of the model, and ε_{ij} is the residual sum of squares. The least square means for the year-class main effects represent age-adjusted gill net CPE for each year class, and were used as the indices of year-class strengths. Only ages 1 - 5 walleyes were used to remove potential ageing errors in older walleyes.

Results and Discussion

Oxytetracycline Treatment Validation

Treatment efficacy.— The results of the marking trials conducted in 1999, 2002, and 2003 demonstrated that walleye fry can be successfully marked with OTC immediately after hatching. Marking trails conducted in 2001 and 2004 failed to corroborate this success due to likely contamination of the rearing ponds.

The first otoliths inspected during this study were from a sample of known-treated fish from Clearwater Pond in 1999. The OTC-induced mark appeared as a golden yellow circular band against a green background when viewed under epifluorescent lighting (Figure 1). Even though some of the marks from Clearwater Pond were rather faint, all specimens exhibited a discernable mark (Table 2). Additional efficacy testing through blind trial inspection of the otoliths collected from walleyes reared in Ponds 1 and 3 during 1999 identified fluorescent marks on all treated walleyes after 3 or 5 months, and no false marks were observed on controls (Table 3).

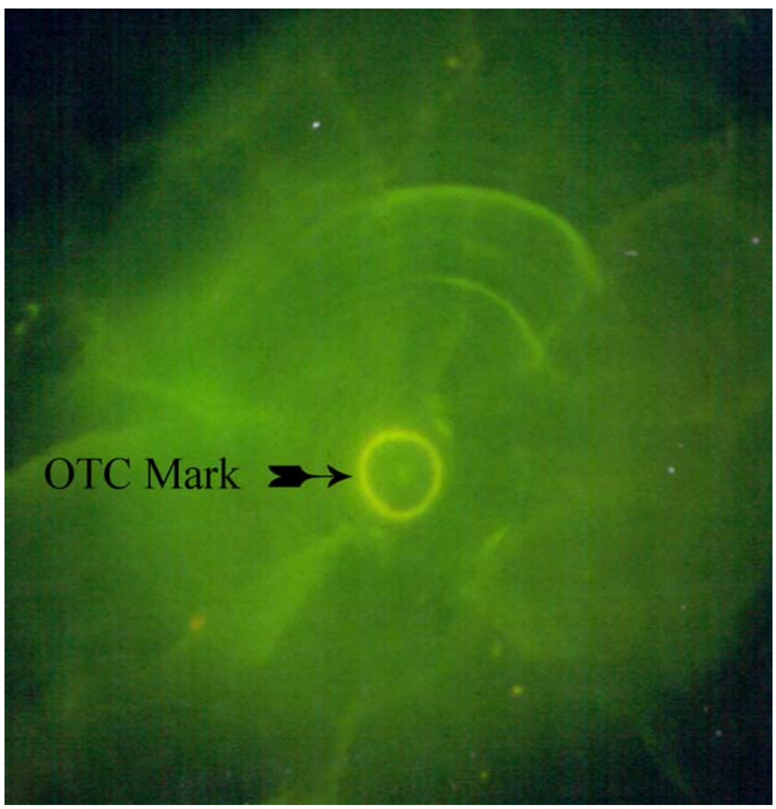

Figure 1. Oxytetracycline (OTC)-marked otolith of a walleye 3 months after treatment as a newly hatched (<24-h post hatch) fry. The otolith was polished along the sagittal plane and viewed at 200X under epifluorescent lighting.

Table 2. Frequency of fluorescent marks observed during examinations of otoliths from oxytetracycline (OTC)-treated walleyes that were stocked into Clearwater Pond near Bemidji, Minnesota or 2 rearing ponds at the state fish hatchery in Waterville, Minnesota.

Year	Treatment episode	Sample site	Age (d)	*n*	Percent marked
1999	2	Clearwater	38	35	100
2001	3	Pond 12*	47	57	9
		Pond 12*	86	98	3
2003	5	Pond 14	148	98	100
2004	6	Pond 12*	59	92	0

* This pond was likely contaminated in 2001 and 2004 with untreated fish from Lake Tetonka.

Table 3. Frequency of fluorescent marks observed during blind trial examinations of otoliths from oxytetracycline (OTC)-treated and untreated walleyes (controls) that were stocked into five rearing ponds and one chemically reclaimed lake near Waterville, Minnesota.

Year	Treatment episode	Sample site	Age (d)	*n*	Percent marked
1999	1	Rice Lake	152	100	100
		Pond 8 (control)	160	100	0
	2	Pond 3	53	100	100
		Pond 1 (control)	53	100	0
2001	3	Pond 12*	47	26	12
		Pond 13 (control)**	47	27	4

* This pond was likely contaminated with untreated fish from Lake Tetonka.
** This pond was likely contaminated with treated fish from Pond 12.

Inspection of the otoliths from YOY walleyes stocked for efficacy testing in 2001 showed results that were quite different than those observed during 1999. Fluorescent marks were observed on only 11 of the 181 walleyes inspected from Pond 12, which had been stocked with treated walleye fry 3 or 5 months earlier (Tables 2 and 3). Additionally, fluorescent marks were present on 2 of the 27 walleyes inspected from Pond 13, which was stocked with untreated controls (Table 3).

Pond 13 was most likely contaminated with treated walleyes from Pond 12 during 2001. These ponds are adjacent, and it was observed after draining of the ponds that a corrugated steel culvert connecting the ponds was inadequately sealed. Treated walleyes from Pond 12 could have swum into Pond 13. It is unlikely, however, that movement of walleyes through the pipe could account for the large proportion of unmarked fish in Pond 12. Equal numbers of walleye fry were stocked into each of the ponds. So even if walleyes swam freely between the ponds, it would be unlikely for the ratio of control to treated walleyes to exceed 50:50. It is possible that the OTC treatment did not produce a mark on all the walleyes that were stocked into Pond 12 during 2001.

There were, however, observations that were inconsistent with what would be expected of an inadequate OTC treatment. The walleyes stocked into Pond 12 during 2001 were a sub-sample of the walleyes treated in Bemidji for stocking into Upper Red Lake, and 70% of the subsequent seine catches of YOY walleyes from the Red Lakes during 2001 were composed of marked walleyes. The 70% mark rate from the Red Lakes includes the return of stocked walleyes diluted by untreated walleyes that were produced naturally in the lake. This high return rate of marked walleyes would be unlikely if there was indeed an incomplete marking of walleye fry. In addition, the 70% mark rate exceeded the expected proportion of stocked fish in the sample, as determined by maximum-likelihood method of genetic stock discrimination. Additionally, differences in assignment between walleyes that were both inspected for an OTC mark and genetically analyzed fell within the 10% error rate associated with this type of genetic analysis (Loren Miller, U of M, personal communication 2006). The quality of the fluorescent marks that were observed also did not conform to what would be expected if the treatment was insufficient in producing OTC marks. The marks that were observed on the walleyes from Pond 12 were either clear or intense. Oxytetracycline produces a mark because it is absorbed by the fish and then deposited in calcified structures during their formation. All of the fry stocked into Pond 12 were treated together in the same fry jug. Therefore, if the treatment produced a mark on the otoliths of some of the walleyes but not others, the sample of marked otoliths would be expected to have more of a gradient of mark intensities. There should have been more faint marks in the sample if the walleyes did not absorb enough OTC to produce consistent marks.

A more likely explanation for the low proportion of marked walleyes in Pond 12 during 2001 is that it was contaminated with untreated walleye fry during the filling of the pond with water pumped from adjacent Lake Tetonka. There were several fish species besides walleye observed in Pond 12 during sampling, and fall electrofishing by the Waterville Area Fisheries staff revealed an unusually high abundance of naturally produced YOY walleyes in Lake Tetonka. To maximize zooplankton production, the ponds are generally filled after the lake water warms but before the walleyes hatch. Extended winter conditions during early 2001 prevented filling the ponds until a few days before they were stocked with walleyes for efficacy testing. Given the difference in latitude between Waterville (southern Minnesota) and Bemidji (northern Minnesota), it is certain that the Lake Tetonka walleyes had already hatched and begun exogenous feeding before the ponds were filled and subsequently stocked with newly hatched fry. Thus the stocked walleyes may have suffered high mortality due to cannibalism or competition for forage by walleyes hatched in Lake Tetonka that had been pumped into the pond during filling. The idea that Pond 13 was contaminated by fry from Lake Tetonka was provided tacit support by genetic analysis of Pond 3 walleyes (Loren Miller, U of M, personal communication 2006). Dr. Miller genotyped a sample of unmarked walleyes from Pond 3. Though he lacked adequate baseline samples for assignment tests, he reported that several of the unmarked walleyes possessed an allele at one locus that has never been found in Pike River walleyes. This allele was common in a small sample of walleyes analyzed from Lake Tetonka.

Walleyes inspected during efficacy testing of the 2003 treatment expressed clear or intense fluorescent marks on all 98 of the otolith samples inspected from Pond 14. Pond results from 2004, however, provided results more similar to 2001. During 2004, no walleyes were recovered from Pond 13 and none of the 92 walleyes inspected from Pond 12 possessed a discernable mark (Table 2).

In 2004, the fish stocked into Pond 12 were treated with the Oxymarine® formulation of OTC instead of the Terramycin-343® formulation that had been used during all the previous treatments. I was initially concerned that the treatment did not produce a mark on the walleyes that were stocked into Pond 12. Ortonville Area Fisheries staff, however, treated walleye fry with the same concentration of Oxymarine® and also stocked a sample of the treated fry into rearing ponds for efficacy testing. Although they also observed fainter marks then in the past, all fish examined were marked (Chris Domeier, Minnesota Dept. of Natural Resources, personal communication 2006). In addition, the walleyes stocked into Ponds 12 and 13 were a subsample of the walleyes treated in Bemidji for stocking into the Red Lakes. The OTC marks from the Red Lakes, while fainter than that observed in past years, were observed on some YOY walleyes from both basins of the Lake.

If the OTC immersion failed to produce a mark on a substantial percentage of the treated fish, then the number of marked fish observed in the recapture sample would have led to an underestimation of the true fraction of stocked fish in the recapture sample. The resulting abundance estimate, if uncorrected, would have led to an overestimation of the true population size. The projected number of fry produced in Red Lakes, based on the mean hatch rate of walleyes from 1999, 2001, and 2003, was approximately 147 million fry. The Peterson population estimate, however, resulted in a natural fry abundance estimate of only 104 million fry.

It is more likely that Pond 12 was again contaminated with untreated walleye fry during the filling of the pond with water pumped from Lake Tetonka, and the stocked walleyes displaced by the walleyes pumped into the pond during filling. Genetic analysis provided evidence that at least some of the fish sampled from Pond 12 were contaminated. The possible Red Lake vs. Pike River vs. mixed Red/Pike parentage of the stocked walleyes, plus a lack of baseline data for the contaminant walleyes complicated the analysis, and Dr. Miller was unable to confidently exclude all of the fish from being stocked fish. He did again, however, identify specimens with southern Minnesota alleles that have never been found in Pike River walleyes.

The results of the experimental OTC treatment of walleyes from 0 to 3 h post hatch (Treatment 4) demonstrated that walleye fry could be successfully marked with OTC immediately after hatching. The first visible marks were detected on the walleye fry at 3 d after treatment. These marks were quite faint and only present on about one-third of the fry (Figure 2). At 6 d post treatment all inspected fry possessed discernable marks on their otoliths. By 9 d post treatment, all otoliths inspected during blind trials had either clear or intense marks, and no false marks were observed on untreated controls.

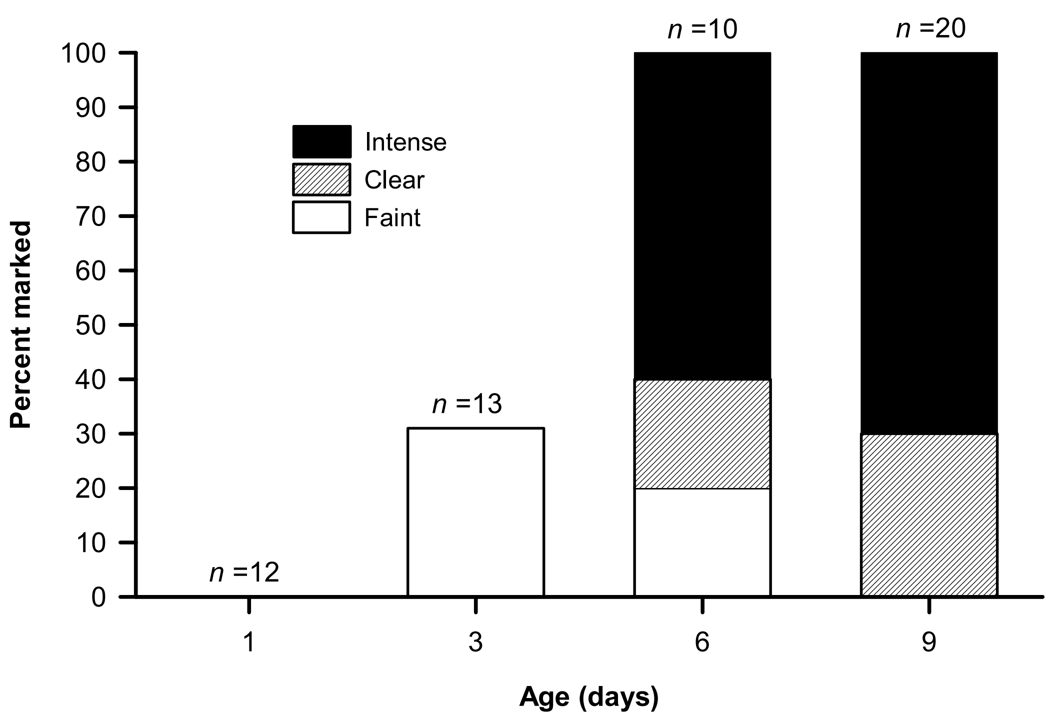

Figure 2. Quality of fluorescent marks observed on walleye otoliths sampled 1 to 9 d after treatment as 0 to 3 h post-hatch fry. Percent marked represents the percentages of walleyes that were assigned to each mark quality category based on the visibility of the mark under 200 X magnification.

Mark quality.― The quality of the marks observed during all marking trials varied among individuals. Most marks were readily visible under 100X magnification after polishing the otoliths for 1 to 3 min. The faintest marks required careful examination under 200X magnification, and the time expended polishing and examining these otoliths often exceeded the 5 min. reported by Brooks et al. (1994) as the maximum time required by a trained observer to prepare and detect a mark.

The quality of the marks did not appear to be a function of the age of the fry at marking. Walleyes that were known to be < 3 h post hatch at the time of treatment exhibited as high a quality of marks as those from groups that included fry up to 24 h post hatch at the time of treatment. Much of the variability in mark quality, instead, was likely due to variability in preparation technique. Fish marked by immersion incorporate OTC into a thin layer of calcium at the time of treatment (Secor et al. 1991; Brooks et al. 1994), and exposure of the mark requires removal of the overlying material. This material was removed by hand sanding with 600 or 1,200 grit sandpaper, periodically stopping throughout this process to look for a mark. This is not a very exacting method. Too little sanding left the mark unexposed or difficult to see, while too much sanding obliterated the mark.

The quality of marks seemed to improve with the experience of the observer, and whether or not a second otolith was available for inspection. Both sagittal otoliths per fish were mounted on the same microscope slide and prepared together for inspection unless an otolith was lost or broken upon removal from the walleye. Having both otoliths enabled mark confirmation in the event that the mark on the first otolith appeared faint or was removed by oversanding. Reinert et al. (1998) reported that the OTC detection rate for 1 to 3 year-old striped bass *Morone saxatilis* increased from 72.6% to 92.5% when both otoliths of a pair were examined. It is likely that some of the marks from walleyes in the current study would also have been overlooked or samples dismissed as unreadable if both sagittal otoliths were not routinely inspected during the periods that observers were gaining experience in processing the otoliths.

Treatment effects on mortality and growth.― The value of the OTC marking technique would have been negated if the marking process had adversely affected the survival or growth of the fish being marked. Researchers have generally reported little mortality associated with immersion marking of walleye fry in OTC solutions. Younk and Cook (1991) described mortality during the marking process as minimal, while Peterson and Carline (1996) reported mean mortality rates of 2.7% to 13% during 24 h simulated stocking bioassays. Brooks et al. (1994) reported that temperature had a significant effect on mortality during 3 d trials, but OTC concentration and immersion duration did not. Mean mortality at 10^{o} C was reported by Brooks et al. (1994) to be 9 %, while that at 15^{o} C increased to 43.7 %.

The fry treated during this study appeared healthy upon stocking. They were well pigmented, motile, and distributed uniformly within the transport containers. Surface water temperatures of the Red Lakes during stocking ranged from 10^{o} to 12^{o} C. Visual observations at the time of stocking into the Red Lakes indicate that less than 5% mortality had occurred during the immersion period, and dissolved oxygen concentrations in the containers that were monitored (n=4) exceeded 10 ppm. The walleye fry held for acute mortality testing in aquaria, however, suffered high mortality during the 72 h trial periods. Both the treated and control fry appeared healthy initially, but at least a few dead fry were observed in all the aquaria by day two. At the end of all the trial periods, both treated and untreated walleyes suffered 25% or more mortality, but the OTC-treated walleye fry held in aquaria suffered significantly higher mortality than the untreated fry (Table 4).

Higher mortality of treated walleyes was not observed during the pond experiments. The ratios of control fish to treated fish at harvest was 48:52 from Pond 12 and 49:51 from Pond 13 in 1999. The resulting P values from chi-square analysis do not indicate a deviation from the 50:50 ratio at stocking (Table 5). Equal survival rates were also reported by Lucchesi (2002) for OTC-treated

Table 4. Mortality of oxytetracycline (OTC)-treated walleye fry compared (Pearson chi-square analysis) to the mortality of untreated walleyes (controls) after being held for 72 h in aquaria at the state fish hatchery in Waterville, Minnesota during 1999.

	Control		OTC-treated			
Trial	*n*	Mortality (%)	*n*	Mortality (%)	χ^2	*P*
1	1,937	38	1,770	57	143.552	0.000
2	554	25	611	31	5.782	0.016
3	1,415	45	1,688	60	76.040	0.000

Table 5. Ratio of oxytetracycline (OTC)-marked walleye fingerlings to unmarked walleye fingerlings (controls) collected during harvest of two rearing ponds at the state fish hatchery in Waterville, Minnesota compared (Pearson chi-square analysis) to the ratio of OTC treated walleye fry to untreated walleye fry (controls) stocked into the ponds 5 months previous

	Ratio			
Pond	Stocked	Harvested	χ^2	*P*
12	50:50	52:48	0.160	0.689
13	50:50	51:49	0.040	0.841

and untreated walleyes reared in ponds at the Blue Dog State Fish Hatchery in South Dakota. If immersion in the OTC solution did cause higher acute mortality of treated walleyes; then, in order to produce mark ratios at harvest similar to those at stocking, it must have also caused a long-term survival advantage to those that survived the treatment.

It was more likely that the higher mortality of treated fish observed during the acute mortality trials was induced by handling stress during filtration with the larval concentrator and exasperated by elevated water temperatures (17^{o} to 20^{o} C). Handling was minimized as much as possible during this process, but it still required additional processing of treated fry that the untreated fry were not subjected to. Since both treated and untreated walleyes appeared healthy upon stocking and mortalities in the aquaria were somewhat delayed, it was assumed that mortality during Red Lake stocking was minimal. In addition, the pond studies provided evidence that the OTC treatment did not induce additional long-term mortality to stocked walleyes that would compromise the assumption of equal mortality between marked and unmarked walleyes in the Red Lakes for calculation of Peterson population estimates.

No apparent effect of the OTC treatment on the walleye growth was observed during the 1999 pond studies (Table 6). Mean total length of the treated walleyes harvested from Pond 12 during September was 127.4 mm while the controls from Pond 12 averaged 125.3 mm. Treated walleyes from Pond 13 (146.0 mm) were also similar in length to Pond 13 controls (148.2 mm).

Information on growth of walleye fry treated with OTC was unavailable, but evaluations of the growth of OTC treated walleye fingerlings (Scidmore and Olson 1969) and juvenile black crappie (Conover and Sheehan 1999) indicated no adverse effects of OTC on the growth of these fish. Their results agree with the findings of this study where OTC treated fish attained the same length as control fish by the end of the first summer. If the OTC treatment caused substantial stress, it would be expected to be expressed as reduced growth or higher mortality.

Table 6. Mean total length (TL) of oxytetracycline (OTC)-marked walleyes collected during harvest of two rearing ponds at the state fish hatchery in Waterville, Minnesota in 1999 compared (t-test) to the mean total length of unmarked walleyes (controls) reared in the same pond.

	Control			OTC-treated				
Pond	*n*	TL (mm)	SD	*n*	TL (mm)	SD	*t*	*P*
12	48	125.3	14.3	52	127.4	15.5	0.733	0.465
13	49	148.2	26.7	51	146.0	16.1	0.484	0.630

Table 7. Frequency of fluorescent marks observed during blind trial examinations of otoliths from oxytetracycline (OTC)-treated and untreated walleyes (controls) that were stocked into two rearing ponds at the state fish hatchery in Waterville, Minnesota. Pond 3 was possibly contaminated with untreated walleyes during re-stocking of the pond after the inspection of YOY walleyes in 1999.

Year	Treatment episode	Sample site	Age (years)	Percent marked
1999	2	Pond 3	0	100
		Pond 1 (control)	0	0
2000	2	Pond 3	1	98
		Pond 1 (control)	1	0
2001	2	Pond 3	2	99
		Pond 1 (control)	2	0
2002	2	Pond 3	3	72
		Pond 1 (control)	3	0
2003	2	Pond 3	4	91
		Pond 1 (control)	4	9

Mark retention and recognition.— Most of the walleyes held in Pond 3 for testing mark retention exhibited fluorescent marks on their otoliths 1 to 4 years after treatment. Blind trial examinations of walleyes from Ponds 1 and 3 detected marks on 72% to 99% of the walleyes from Pond 3 and from 0% to 9% of the walleyes from Pond 1 (untreated controls, Table 7). The actual presence of the marks on control fish is suspect, as they were reported by a laboratory assistant with inadequate training on the use of the epifluorescent microscope. Further, interpretation of the Pond 3 data is complicated due to possible contamination of untreated walleyes during restocking of the pond in September, 1999. After inspection of the YOY walleyes from Pond 3 in 1999, the walleyes in the pond died due to oxygen depletion and had to be replaced with fingerlings that had also been treated with OTC as fry. The replacement fingerlings were initially reared in Clearwater Pond, and were fed minnows seined from Lake Bemidji. Several young-of-the-year (YOY) walleye were observed in the seine catch by Minnesota DNR Fisheries staff (Carl Moen, Minnesota DNR, personal communication 2006), so it is possible that some YOY walleyes from Lake Bemidji were stocked into Clearwater Pond along with the minnows, and subsequently transferred to Pond 3 at Waterville. Tissue samples were forwarded to Dr. Miller to differentiate between the treated Pike River strain and the potential contaminates from Lake Bemidji, but the results were inconclusive because the unmarked fish did not assign to either population with high probability. Since Pond 3 was likely contaminated with untreated fish but independent confirmation of the individual contaminates could not be confirmed with genetic analysis, the percent of marked walleyes observed from Pond 3 should be considered estimates of minimum mark retention/recognition rates rather than estimates of absolute retention rates.

The intensity of marks observed on walleyes sampled from both Pond 3 and the Red Lakes provides additional evidence of high retention of OTC marks. Since OTC is a chemical mark that is absorbed and deposited into the calcified structures of fish, it cannot be detached like a coded wire tag or anchor tag. Loss of an OTC mark would have to occur through re-absorption of the chemical into the surrounding tissue. One would expect this to be a gradual process and, consequently, that the quality of the marks observed would decrease slowly over time. The marks observed during this study lacked the presence of a trend to suggest that mark loss had occurred (Figures 3 and 4) and the variability in the percentage of marks observed at each mark intensity grade was likely nothing more that sampling variability.

The genetic analysis of OTC inspected walleyes also provided supporting evidence for the high rate of OTC mark retention of walleyes marked as newly hatched fry. There was disagreement in assignments between OTC mark observation and genetic analysis for all samples, but not the increasing disparity that would be indicative of OTC mark loss. The percent of inspected walleyes genetically assignable to Pike River strain at LR>50 that did not possess visible OTC marks (putative missed OTC marks) ranged from 0% to 20% for samples of walleyes from the 1999 year class, and from 3% to 24% for samples from the 2001 year class (Figure 5). The percent of walleyes genetically assignable to Red Lakes strain at LR >50 that possessed a visible OTC mark (putative false marks) ranged from 0% to 75% for samples from the 1999 year class and 0% to 11% for samples from the 2001 year class (Figure 5). Sample sizes were extremely low and disagreement across time was quite variable for samples from the 1999 year class that were genetically assigned to Red Lakes strain. The difference between the overall percent of the sample genetically assigned to Pike River strain and the overall percent of the sample with visible OTC marks was less than the sum of the individual error rates because walleyes with putative false marks had a canceling effect on the error rate of putative missed OTC marks. The difference between the overall percent of the sample identified as stocked fish (Pike River strain at LR>50), and the overall percent of the sample identified as stocked fish by identification of an OTC mark ranged from 1% to 12% for the sample from the 1999 year class and from 3% to 10% for the 2001 year class (Figure 6). In general, the estimate of stocked fish derived from OTC mark inspection was less than that of genetic analysis, but no trends in the magnitude of the differences over time were evident.

The current study indicates that newly hatched walleye fry can safely and successfully be marked by immersion in 700 mg/l OTC for 6 h. The ability to OTC-mark newly hatched fry with little disruption to routine hatchery operations allowed the entire annual production of walleye fry at the Bemidji hatchery to be marked during 1999, 2001, and 2003. Treating newly hatched walleye fry instead of 3 to 4 d old fry also lessened their time in the hatchery, and reduced the risk of catastrophic loss due to equipment failure, low dissolved oxygen levels, or impingement upon retaining screens.

This study also shows that OTC marks produced on newly hatched walleye fry can be retained up to 4 years, but that recognition may not be 100%. The mark retention rate is likely very high but, even with the multifaceted experimental design used during this study, identification of the exact mark retention rate of walleyes treated with OTC has remained elusive.

Contribution of the Stocked Year Classes

1999 year class.— Of the four fry stockings conducted during this study, the 1999 stocking provided the largest contribution to the walleye population in the Red Lakes. The first opportunity to sample stocked walleyes from the 1999 year class occurred during seining in July 1999. The seine CPE of 49.5 YOY walleyes per lift in 1999 was the largest catch rate in the Red Lakes during the past 15 years (Figure 7), and far exceeded the 10/haul target that was established by the Red Lakes Fisheries Technical Committee as an indicator of a strong year class (Gary Barnard, MN DNR, personal

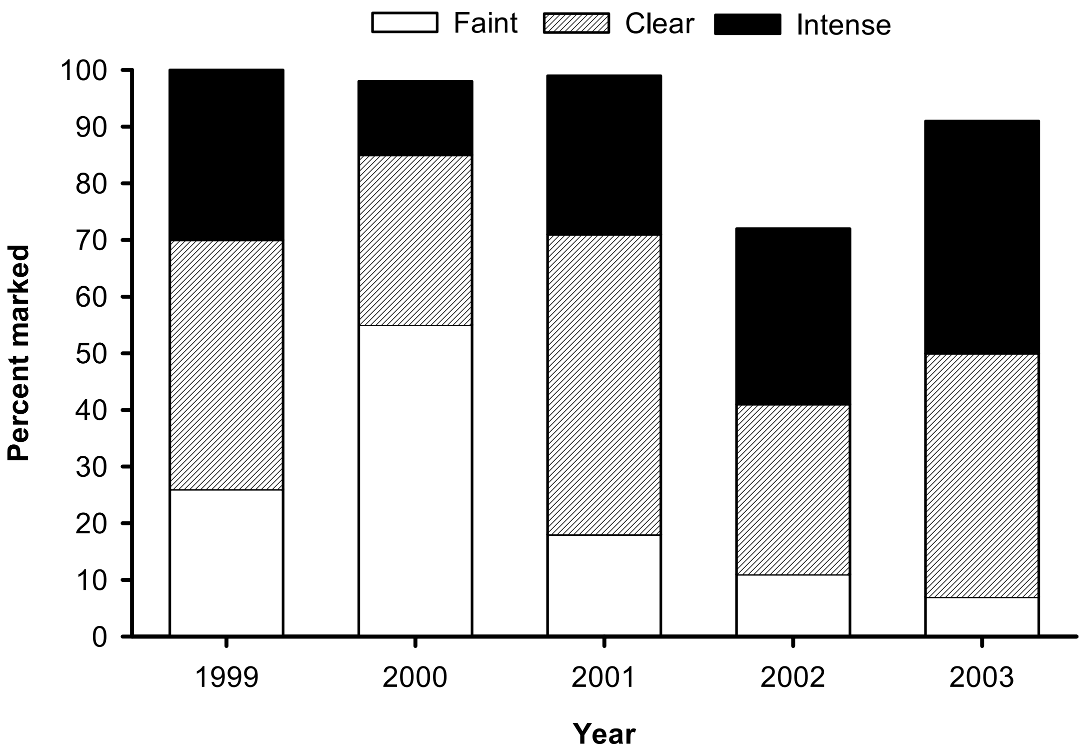

Figure 3. Quality of fluorescent marks observed on otoliths from the 1999 year class of walleyes sampled annually from Pond 3 during 1999 - 2003. Percent marked represents the percentages of walleyes that were assigned to each mark quality category based on the visibility of the mark under 200 X magnification.

communication 2006). Oxytetracycline marks were detected on 86% of the inspected walleyes, confirming that a strong majority of the YOY walleyes in the Red Lakes during 1999 had been stocked as fry.

The walleyes from the 1999 year class began recruiting to the gill nets at age 1, and were subsequently sampled through age 5 during annual gill net surveys. High gill net catch rates of walleyes from the 1999 year class confirmed that the 1999 year class was abundant and had survived well through their first winter (Table 8). The results of q_{abg} modeling of the gill net catch estimated the range of abundance of the 1999 walleye year class from a high of 6.5 million in 2001 to 1.2 million in 2004 (Table 9). The percentage of marked fish in the sample consistently remained over 80% (Figure 4, Table 8).

Survival rates calculated from the Peterson and q_{abg} abundance estimates indicate that approximately 12% of the total walleye fry present in the Red Lakes at the time of stocking in 1999 survived until September the following year, and 3% of the total fry survived until the end of the study at age 5 (Table 10). A higher estimated survival rate to age 2 (14%) than to age 1 suggests that the abundance estimates of age 1 walleyes may have been underestimated by the q_{abg} model.

2001 year class.— The 2001 fry stocking also contributed to the formation of a strong year class of walleyes in the Red Lakes. The 2001 seine CPE of 46.5 YOY walleyes per haul overwhelmingly surpassed the target catch rate of 10/haul and was exceeded during the last 15 years only by the catch rate in 1999 (Figure 7). The mark rate of 70% was also

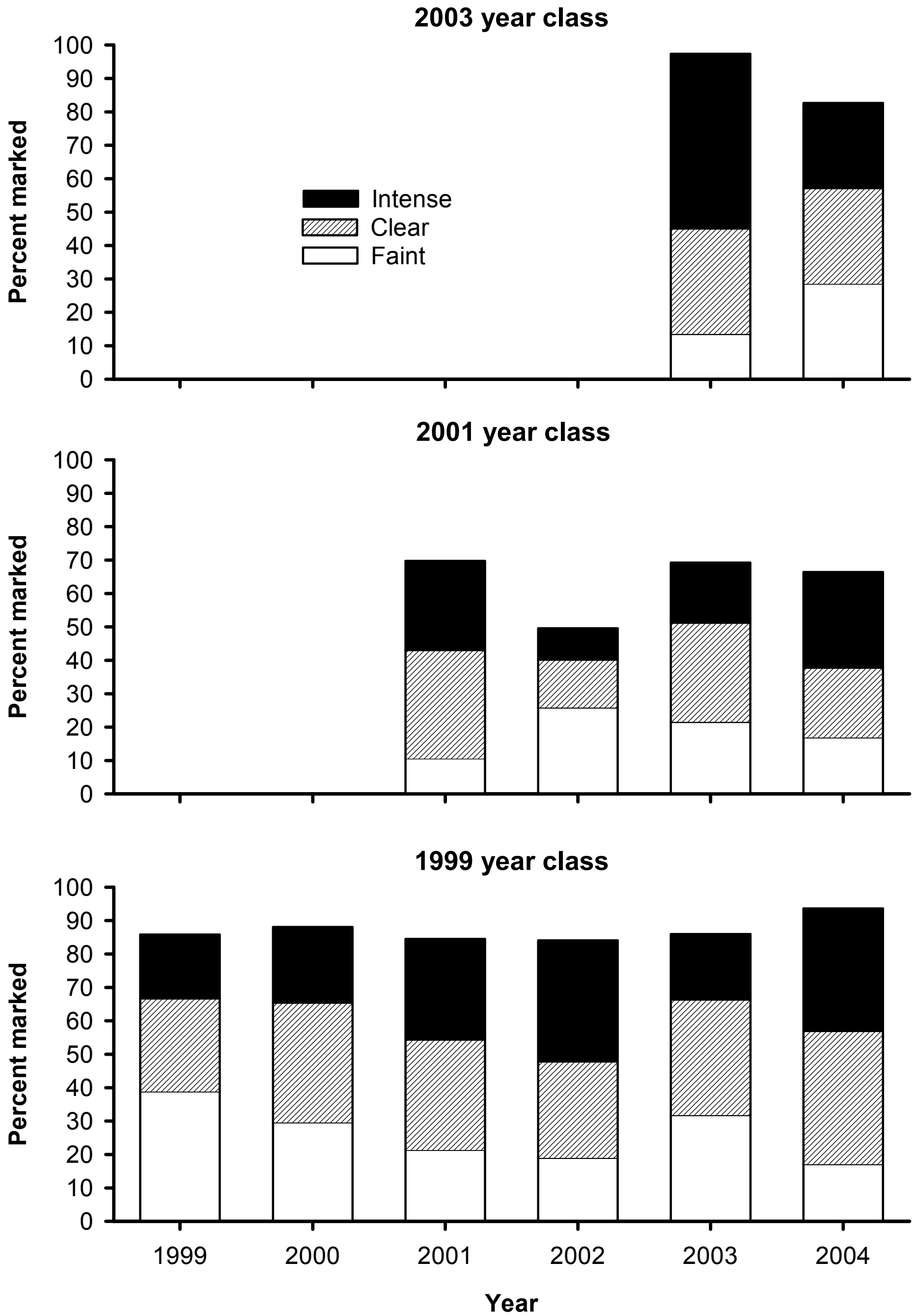

Figure 4. Quality of fluorescent marks observed on otoliths from the 1999, 2001, and 2003 year class of walleyes sampled annually from Red Lake during 1999 - 2004. Percent marked represents the percentages of walleyes that were assigned to each mark quality category based on the visibility of the mark under 200 X magnification.

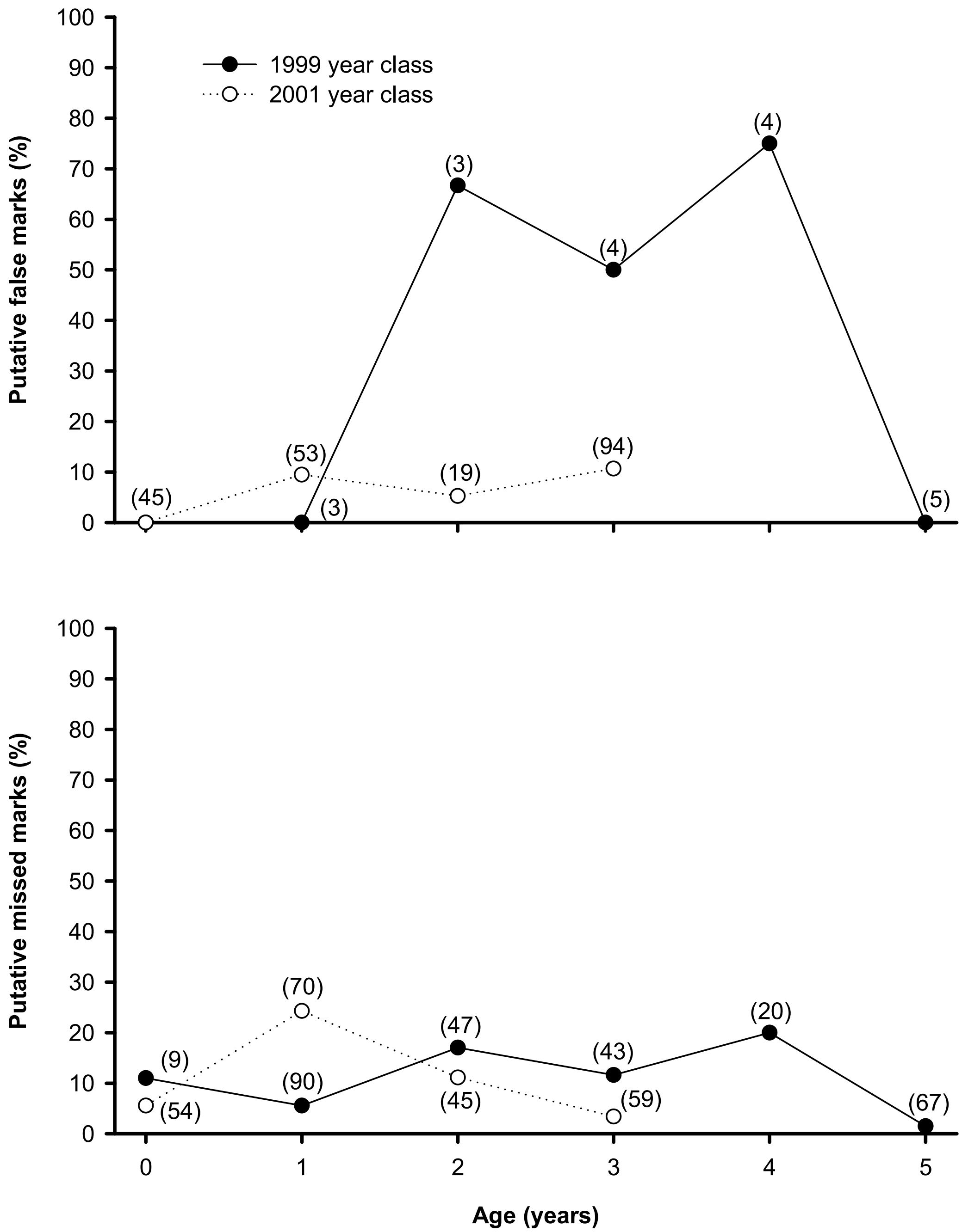

Figure 5. Percent of the 1999 and 2001 walleye year classes genetically assignable at LR>50 to Red Lake strain that possessed a visible OTC mark (putative false marks) and to Pike River strain that did not possess a visible OTC mark (putative missed marks). Sample sizes of walleyes genetically assigned to Red Lake strain (upper graph) and Pike River strain (lower graph) are presented in parentheses.

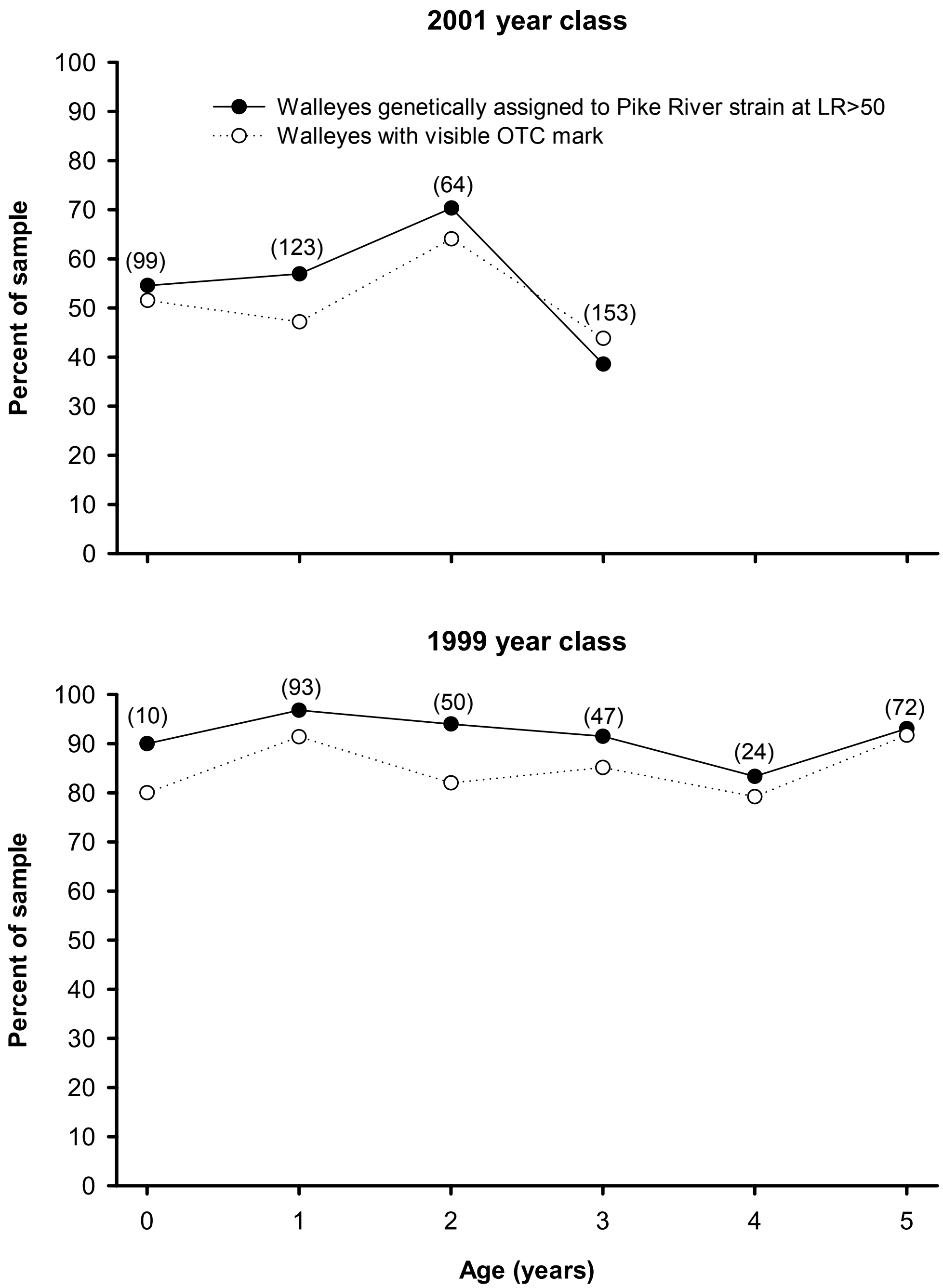

Figure 6. Overall percent of the 2001 and 1999 walleye year classes genetically assignable at LR>50 that were assigned to Pike River strain compared to the overall percent of genetically analyzed walleye that possessed a visible OTC mark. Sample sizes of the number of walleyes that were both inspected for a mark and assignable to a genetic strain are presented in parentheses.

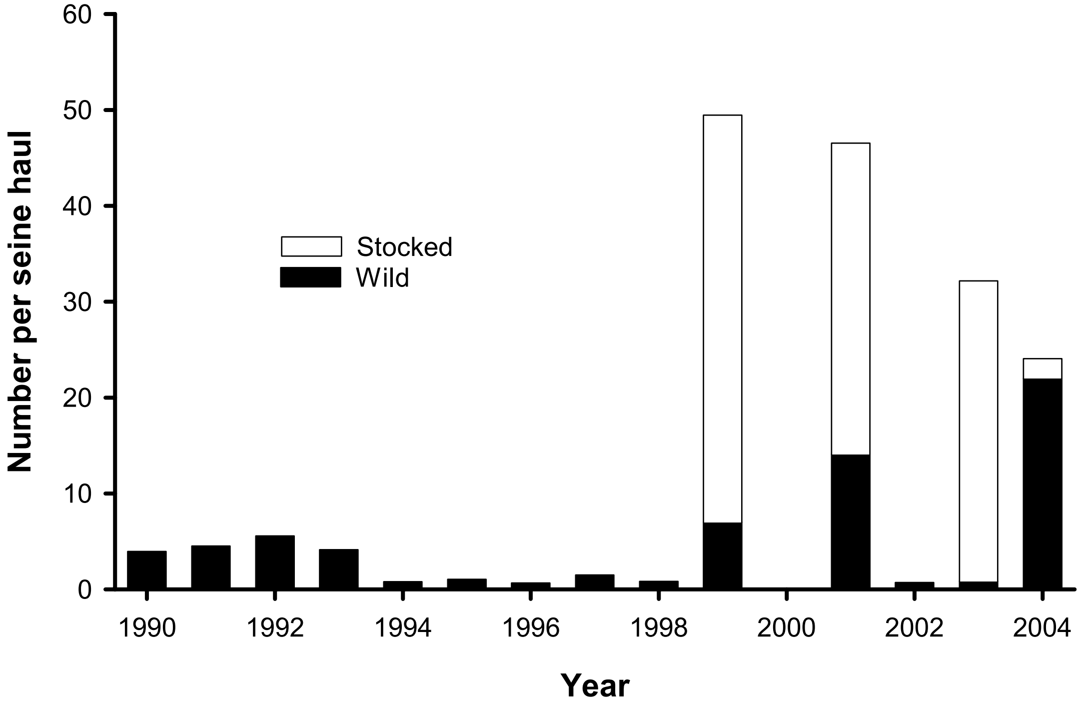

Figure 7. Seine catch-per-unit-effort of young-of-the-year walleyes in the Red Lakes, 1990 – 2004. Stocked walleyes in 1999, 2001, 2003, and 2004 were identified by the presence of a fluorescent OTC mark on their otoliths.

Table 8. Gill net catch per effort (CPE) and percent of marked walleyes collected from the Red Lakes during 2000 – 2003.

		Lower Red Lake			Upper Red Lake			Total lake		
Year class	Age	CPE	Number inspected	Percent marked	CPE	Number inspected	Percent marked	CPE	Number inspected	Percent marked
1999	1	6.6	120	88	5.0	140	88	5.7	260	86
	2	15.7	180	84	12.5	175	85	14.0	355	85
	3	7.7	118	78	23.8	114	90	16.2	232	84
	4	19.8	* 0		32.9	107	86	26.7	107	86
	5	6.1	182	95	14.5	180	92	10.6	362	94
2001	1	4.9	120	43	7.6	104	56	6.3	224	50
	2	16.7	181	71	10.6	186	68	13.5	367	69
	3	9.4	180	70	13.9	180	63	11.8	360	66
2003	1	1.4	42	86	1.8	56	80	1.6	98	83

*Data omitted because quality of data was suspect due to misalignment of microscope.

Table 9. Q_abg estimates for abundance of the 1999, 2001, and 2003 year classes of walleyes in Lower and Upper Red Lakes from 2000 through 2004.

Year		Lower Red Lake		Upper Red Lake		Total lake	
class	Age	N	95% Confidence limits	N	95% Confidence limits	N	95% Confidence limits
1999	1	3,555,785	1,990,528 – 6,054,040	2 296 182	1 813 904 – 2 807 023	5,851,967	4,124,789 – 8,465,142
	2	3,936,408	2,934,923 – 5,153,703	2,566,946	2,044,735 – 3,066,297	6,503,354	5,388,788 – 7,848,738
	3	863,416	633,517 – 1,916,878	2,632,883	2,028,086 – 3,252,498	3,496,299	2,830,614 – 4,144,157
	4	1,089,821	788,703 – 1,437,105	2,099,132	1,775,966 – 2,478,514	3,188,953	2,744,115 – 3,687,894
	5	324,726	237,515 – 418,604	876,463	707,616 – 1,047,577	1,201,189	1,004,619 – 1,394,846
2001	1	2,849,884	1,909,269 – 3,835,427	3,318,979	2,549,542 – 4,151,732	6,168,863	4,913,105 – 7,528,954
	2	3,308,464	2,642,686 – 4,068,783	2,307,466	1,958,268 – 2,704,292	5,615,930	4,920,724 – 6,460,865
	3	1,184,632	946,475 – 1,454,479	2,016,615	1,658,242 – 2,392,984	3,201,247	2,800,955 – 3,620,060
2003	1	1,226,963	836,196 –1,622,482	1,296,485	827,330 – 1,769,059	2,523,448	1,905,585 – 3,149,092

Table 10. Survival (S) and instantaneous mortality (Z) rates from fry at the time of stocking to each age sampled of the 1999, 2001, and 2003 year classes of the Red Lakes walleyes collected by gill nets during 1999 – 2004.

	1999 year class		2001 year class		2003 year class	
Age	S	Z	S	Z	S	Z
1	0.12	1.62	0.14	1.53	0.12	1.62
2	0.14	0.87	0.12	0.91		
3	0.07	0.79	0.07	0.80		
4	0.07	0.63				
5	0.03	0.70				

less that that of the 1999 year class, but still indicated that stocking contributed the majority of walleyes to the 2001 year class.

The high abundance of walleyes from the 2001 year class was also reflected in the gill net catches for ages 1 - 3. The overall CPE for the 2001 year class in the Red Lakes ranged from 6.3 to 13.5 walleyes per lift, with the highest catch occurring in 2003 (Table 8). The q_{abg} abundance estimates of the 2001 year class showed a steady decline from 6.2 million walleyes at age 1 to 3.2 million walleyes at age 3 (Table 9). The mark rate of walleyes captured in gill nets was more variable than that of the 1999 year class, and ranged from 50% to 69% (Figure 4, Table 8).

Survival of walleyes from the 2001 year class was similar to that of the 1999 year class. First year survival was estimated to be 14%, and survival to age 3 at the end of the study was 7% (Table 10). Even though the survival estimate to age 2 was lower than the estimate to age 1, it is similar enough to the age 1 survival rate to suggest an underestimation of the age 1 population.

2003 year class.— The 2003 stocking was also successful in the production of a potentially strong walleye year class in the Red Lakes. Even though the 2003 fry stocking was the final stocking intended as a rehabilitation stocking, the percentage of marked YOY walleyes in the 2003 seine catches (97%) exceeded that of either 1999 or 2001. The seine CPE of 32.2 YOY walleyes per haul, while 30% lower than the catch rates of either 1999 or 2001, was the third highest for the Red Lakes in the last 15 years (Figure 7).

The gill net CPE of age 1 walleyes from the 2003 year class was 1.6 per lift and the resulting q_{abg} modeling estimated the abundance of age 1 walleyes to be approximately 2.5 million. Survival of the 2003 year class was similar to that of the 1999 and 2001 year classes, with an estimated 12% of the walleye fry present at the time of stocking surviving to age 1.

The percentage of marked fish in the sample decreased from 97% at YOY to 83% at age 1. The reason for this decrease is unknown, but a similar decrease in the percentage of marked fish at age 1 was observed for the 2001 year class. Mark percentages for the 2001 year class at ages 2 and 3 did, however, return to a level similar to what was observed on YOY fish. Future sampling will be necessary to see if the 2003 year class follows the same trend in mark percentages as the 2001 year class.

2004 year class.— Because of the potential for substantial natural reproduction in 2004, the walleyes stocked in 2004 were not intended to supplement or rehabilitate the population, but rather provide marked fish for Peterson population estimates. Consequently, stocked walleyes made up a much lower percentage of the seine catch of YOY walleyes in 2004 than any of the other stocked years. Marks were identified on only 9% of the samples inspected from the Red Lakes. Even with the low percentage of stocked walleyes in the samples, the CPE of 24.5 per seine haul exceeded the catch rates during all the non-stocked years since 1990 (Figure 7).

Population abundance and biomass.— The walleye population in the Red Lakes is recovering. Gill net CPE has risen from a low of 0.6 fish per lift in 1996 to a high of 46.9 per lift in 2003. The gill net catch dropped to 26.3

per lift in 2004, but CPE in 2004 was still higher than the catch rates experienced during 1988 - 2002 (Figure 8). Much of the increase in gill net CPE was due to the strong year classes of 1999, 2001, and 2003. The 1999 and 2001 year classes were, by far, the strongest year classes in the past 26 years. The relative strength of the 2003 year class was less than one-half of the 1999 and 2001 year classes, but still exceeded the majority of the year classes since 1979 (Figure 9). Much of the reduction in gill net catch between 2003 and 2004 was due to the reduced abundance of the 450 to 650 mm walleyes from the 1997 year class. In addition to continued natural mortality of the 1997 year class, the decline was also reflective of natural mortality of the strong 1999 and 2001 year classes as well as a reduction in recruitment of new walleyes to the gill nets due to a somewhat weaker 2003 year class.

The q_{abg} estimates of standing stock biomass followed a similar trend to the gill net CPE. Biomass estimates of age 1 and older walleyes increased from a low of 36,000 kg in 1996 to 4.6 million kg in 2003 (Figure 10). Application of the mark recapture data to the biomass estimates suggests that, beginning in 2000, over one-half of the biomass of age 1 and older walleyes in the Red Lake consisted of stocked fish. The 1999 year class has contributed the most to the standing stock biomass of Red Lakes, followed by the 2001, and the 2003 year classes.

Maturation and contribution to the spawning stock.— Precocious males from the 1999 stocking began to appear in the gill nets at age 2. By age 3, over 90% of the males collected in the nets were mature. Precocious females from the 1999 stocking were first netted at age 3, and by age 4 over 60% of the females from the 1999 stocking were mature. Male walleyes from the 2001 year class matured at a slightly slower rate than that of the 1999 year class. Precocious males from the 2001 year class also appeared in the nets at age 2, but at age 3 only 53% of the males were mature. Two percent of the females from the 2001 stocking were mature at age 3 (Figure 11).

The total biomass estimate of mature females in the Red Lakes also followed a trend similar to fall gill net CPE. Estimates of mature female biomass in the Red Lakes rose from a low of 6,000 kg in the fall of 1997 to over 1 million kg in 2003, then dropped to 286,000 kg in 2004 (Figure 12). Much of the increase in mature female biomass in the fall of 2003 was due to maturation of walleyes from the 1999 stocking. Estimates from the previous fall's nettings indicated that stocked females contributed 7% of the available spawning biomass in 2003, 54% in 2004, and 83% in 2005 (Table 11). Male walleyes from the fry stockings had a more rapid affect on the spawning stock in the Red Lakes. Even though only 1% of the 1999 stocked males were mature during fall netting in 2001, they were so abundant that they composed 11% of the total mature male population available for spawning in the Red Lakes during spring of 2002. Contribution of stocked fish to the male spawning stock then increased to 84% in 2003, and decreased to 74% in 2004, and 75% in 2005.

Natural Reproduction

A substantial increase in natural reproduction followed the recruitment of the 1999 year class to the spawning stock. Peterson population estimates indicated that there were approximately 104 million naturally reproduced walleye fry in the Red Lakes in 2004. This is substantially higher that the estimates from 1999, 2001 and 2003 (Table 12), and suggests that the walleye population is recovering.

Estimated walleye egg hatch rate in 2004 was 0.18%. The 2004 hatch rate was higher than that of 2001 and 2003, but less than that of 1999 (Table 13). The 1999 hatch rate was substantially higher than the other 3 years that Peterson estimates were conducted, and had a strong influence on the overall mean hatch rate across years. A plot of the mean (0.24%) against the overall estimates shows that the mean likely over estimates the long-term relationship between egg production and fry abundance in the Red Lakes, while a least squares regression line forced through the origin provides a much better fit to the data (Figure 13).

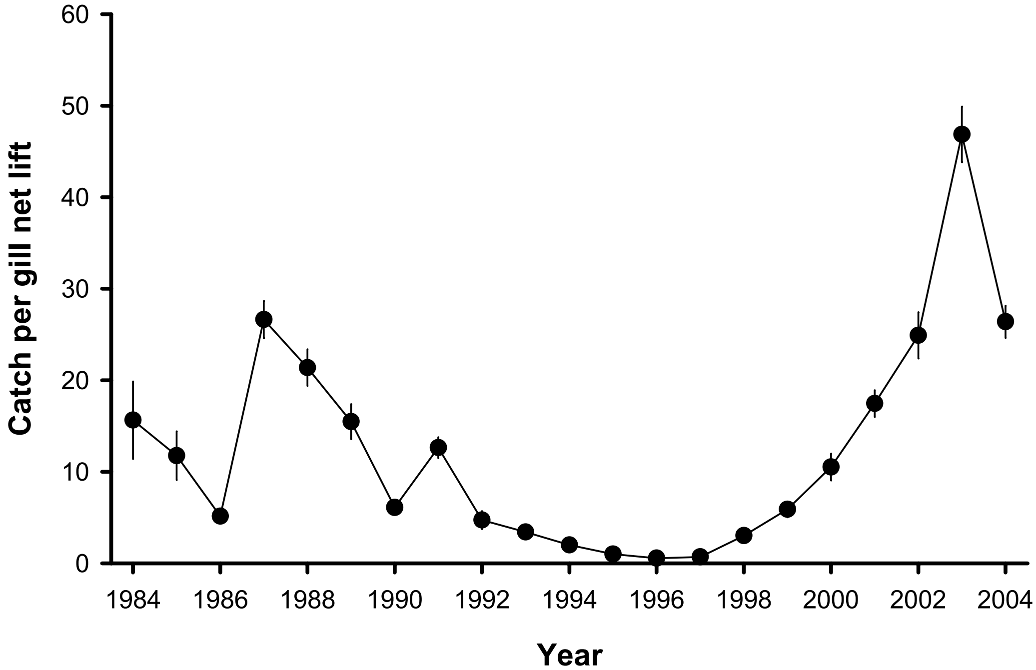

Figure 8. Total catch per effort of walleyes collected by gill nets from the Red Lakes during 1984 - 2004. Error bars represent standard errors.

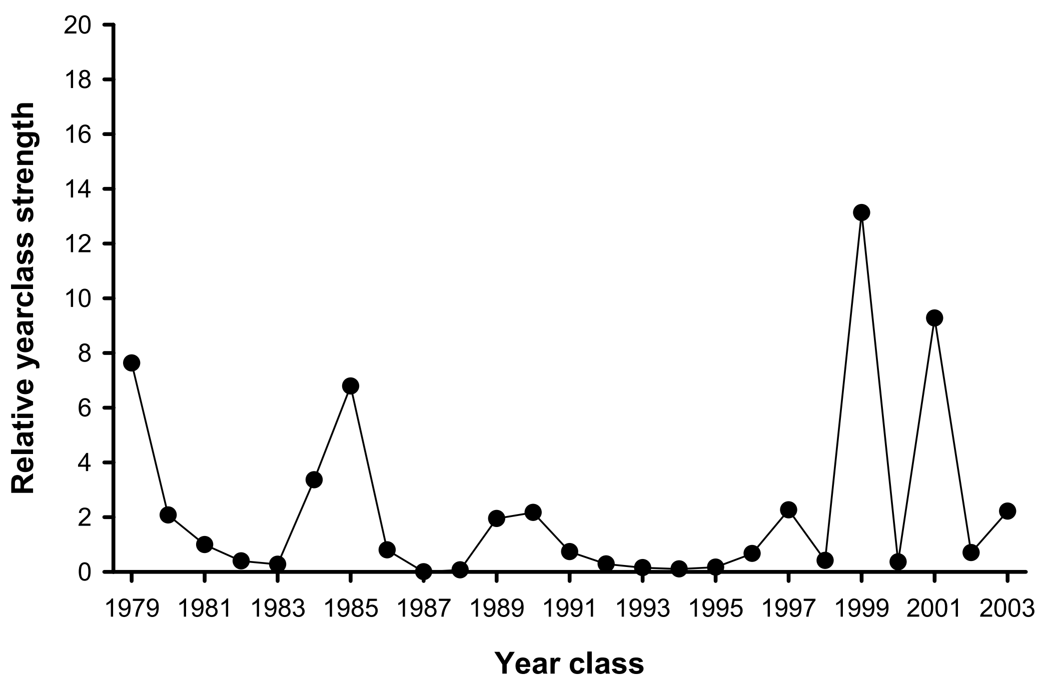

Figure 9. Pereira's relative strength index of the 1979 - 2003 year classes of walleyes in the Red Lakes. Data prior to 1994 are from East Upper Red only.

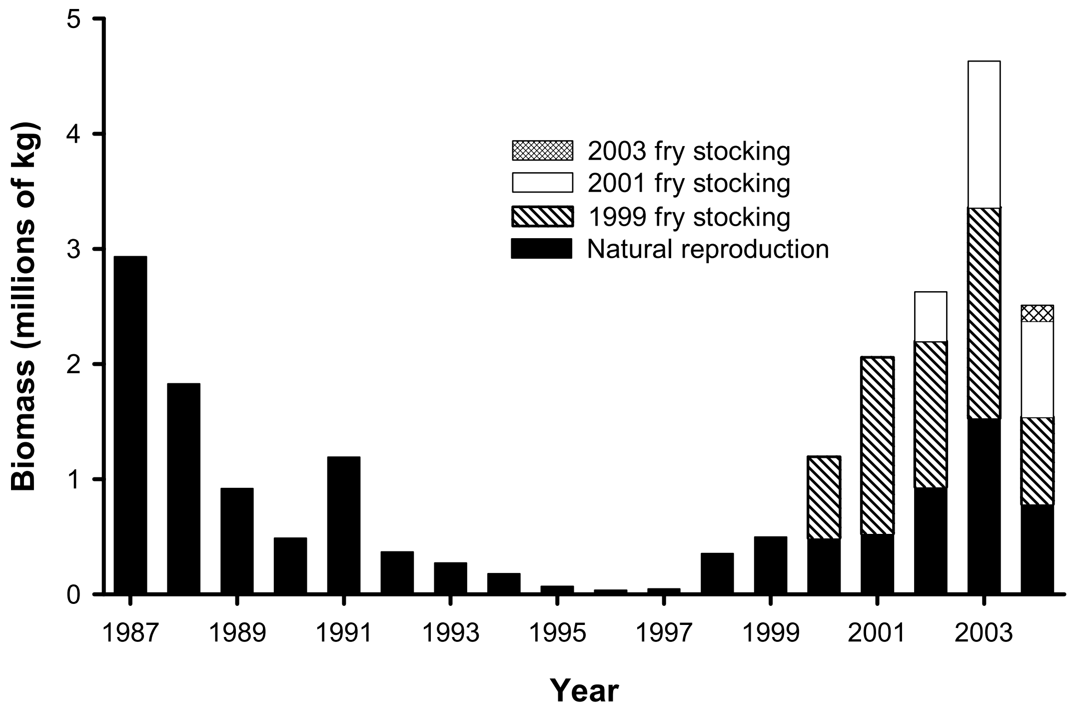

Figure 10. Q_{abg} estimates of the biomass of age 1 and older walleyes in the Red Lakes from 1987 - 2004.

Table 11. Estimates of the potential contribution of Pike River strain walleyes to natural reproduction in the Red Lakes from 2002 - 2005. These estimates were calculated from the number of mature walleyes that were collected by gill netting during the preceding fall sample periods. Male contribution was estimated as the percentage of mature males in the population that were of Pike River origin, whereas female contribution was estimated as the percentage of egg production by females that were of Pike River origin.

	Contribution of Pike River gametes (percent)	
Spawning year	Males	Females
2002	11	0
2003	84	7
2004	74	54
2005	75	83

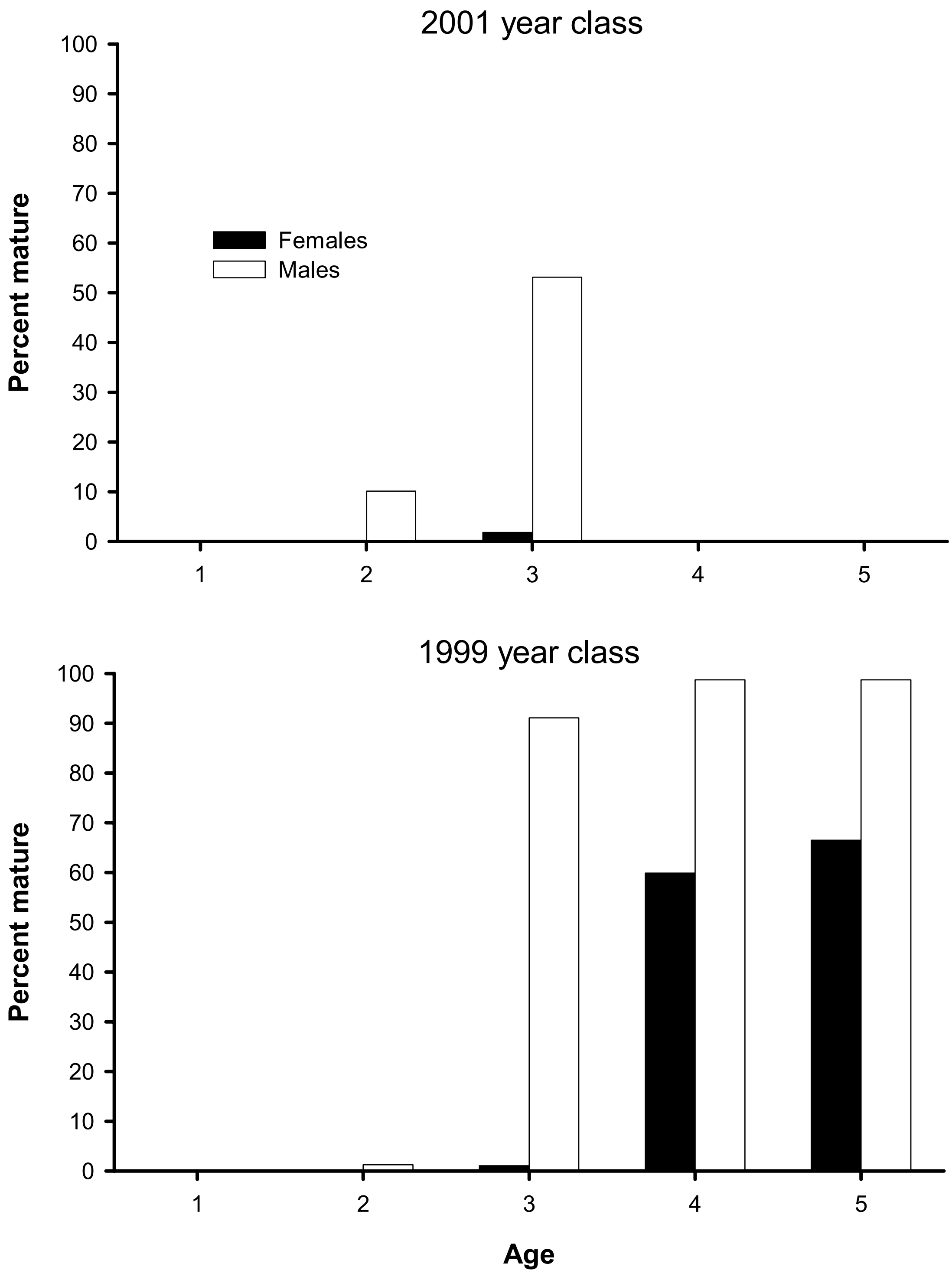

Figure 11. Percent mature, by age, of the samples of OTC marked walleyes collected by gill netting in the Red Lakes from 2000 - 2004.

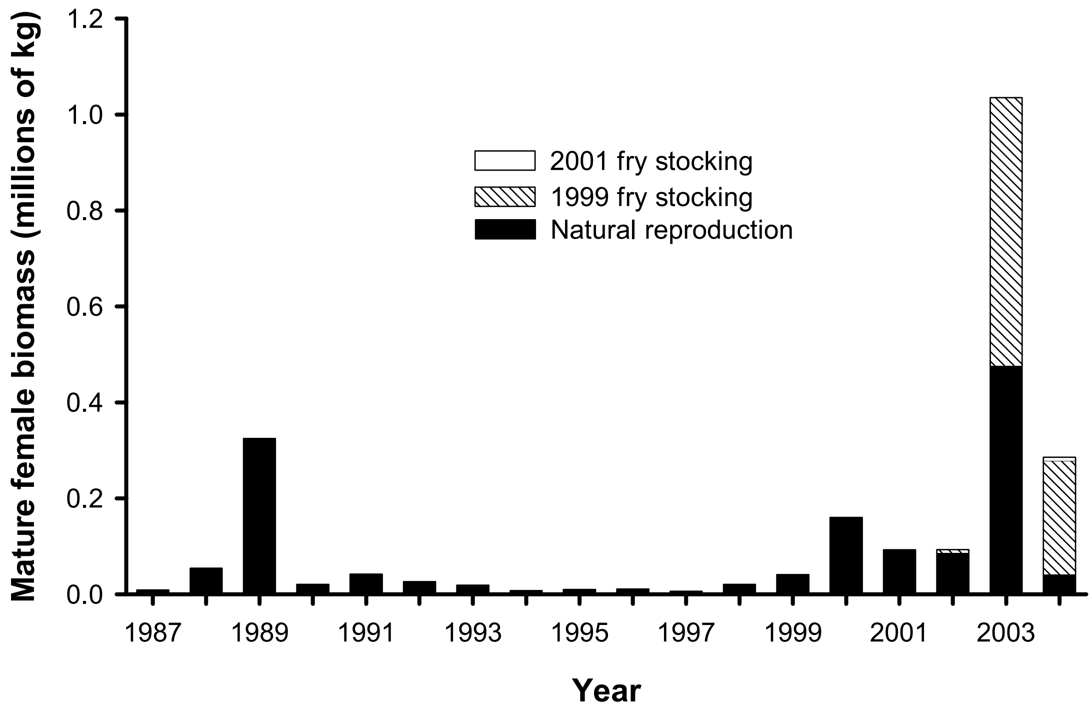

Figure 12. Q_{abg} estimates of the biomass of mature females in the Red Lakes from 1987 - 2004.

Table 12. Peterson population estimates of wild fry abundance in the Red Lakes at the time of stocking in 1999 – 2004.

	Wild fry abundance	
Year	*N*	95% confidence limits
1999	6,766,089	1,729,790 - 12,392,447
2001	13,754,830	9,990,468 - 17,859,875
2003	879,239	8 - 4,906,938
2004	104,486,441	80,281,065 - 135,065,828

Table 13. Hatch rate estimates of natural walleye reproduction in the Red Lakes during 1999 - 2004.

Year	Hatch Rate (%)
1999	0.60
2001	0.16
2003	0.02
2004	0.18
Mean	0.239

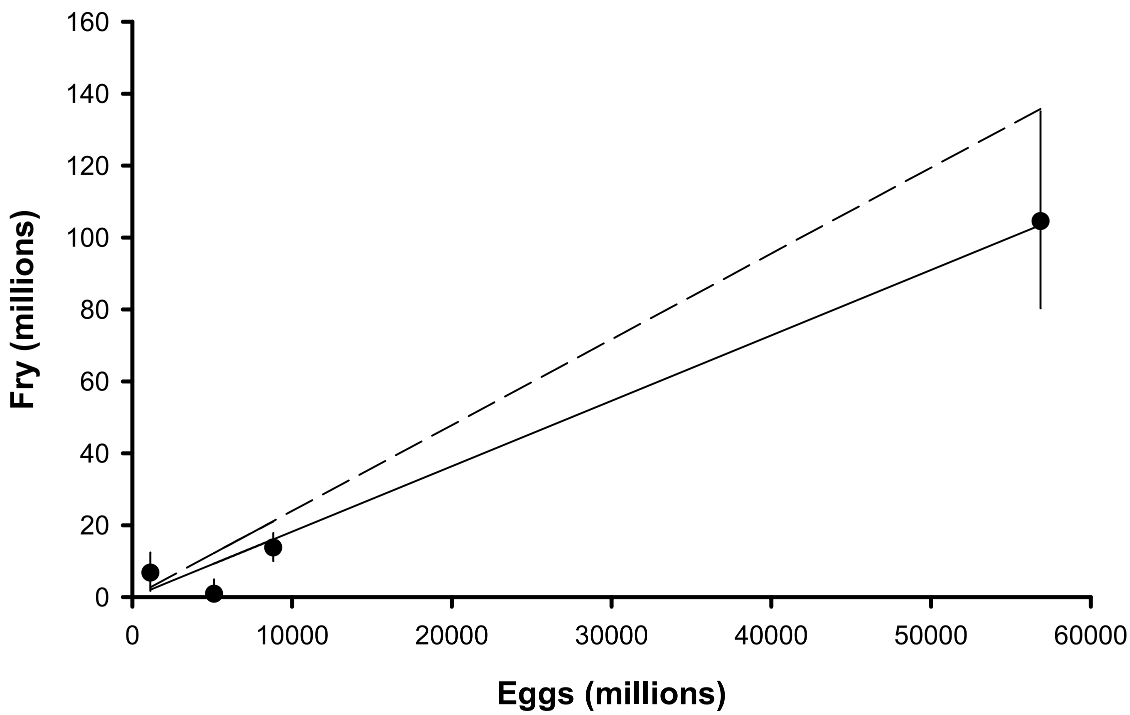

Figure 13. Hatch rate estimates from 1999, 2001, 2003, and 2004. The dashed line represents the mean hatch rate across all years (0.239%), the solid line represents the least squares regression ($y=0.0018x$, $r^2=0.9861$), and the error bars represent 95% confidence limits of the fry estimates.

Application of the long term hatch rate from the least squares regression (0.18%) to the estimates of egg production from the 2004 netting predicted approximately 29 million wild fry would be produced in 2005. While the predicted fry production for 2005 is less than one-half of that observed in 2004, it is over twice as much as the other three years that Peterson estimates were conducted. Application of the 0.18% hatch rate to mature female egg production estimates for previous years suggests that the level of natural reproduction predicted for 2005 was the third highest in the past 18 years (Figure 14).

Fitness of Stocked Walleyes

Relative survival of stocked walleyes.— The proportion of marked walleyes from the 1999 year class has shown little variability among sample periods (Figure 4, Table 8), and estimates of the relative annual survival rates calculated from these data indicate that stocked walleyes from the 1999 year class survived at rates similar to that of the native Red Lake walleyes. Estimates of relative annual survival of the 1999 year class of stocked walleyes exceeded 1 (equal survival of stocked and wild walleyes) in 3 of the 5 years of life examined during this study, and fell slightly below 1 during the remaining 2 years (5). No strong trends in relative annual survival were evident, and the equal survival value of 1 fell within the 95% confidence limits for all estimates. The relative annual survival estimates for the 2001 and 2003 year classes, however, showed more variability than that of the 1999 year class. The 95% confidence intervals for first year relative survival fell below 1 for both the 2001 and 2003 year classes, and 95% confidence intervals for second year relative survival exceeded 1 for the 2001 year class (Figure 15). These values are reflective of the decrease in the proportion of marked walleyes from YOY to age 1 in the samples of the 2001 and 2003 year classes of walleyes (Figure 4). The proportion of walleyes in the 2001 year class dropped from 70% to 50% during the first year, then increased to 69% by age 2. The 2001 year class dropped from 97% marked fish to 83% marked fish during the first year. The dramatic drop in the proportion of marked fish between the first and second years, and then returning to similar proportion during the third year suggests either a survival disadvantage of stocked walleyes at age 1 and a survival advantage at age 2, or some sort of sampling error associated with incomplete mixing or unequal catchability between stocked and wild walleyes at age 1. Sampling error seems more likely, but I was unable to determine its cause.

Relative growth of stocked walleyes.— The stocked Pike River strain walleyes grew nearly as well as the native Red Lake walleyes. Linear modeling of length at capture data from age 1 and older walleyes from the 1999, 2001, and 2003 year classes indicates significant effects of basin (P<0.001), age (P<0.001), year class (P<0.001), sex*age at ages 2 (P=0.026), 4 (P<0.001), and 5 (P<0.001), as well as strain (P=0.026) on log transformed total length at capture. The difference in growth between strains, while statistically significant at α=0.05, was very small and inconsistent among samples (Figures 16, 17, and 18).

Relative maturity of stocked walleyes.— The stocked Pike River strain walleyes matured at a slightly slower rate than the native walleyes in the Red Lakes. The percentage of mature walleyes in the samples was consistently lower for stocked walleyes than wild walleyes for both males and females at each age group sampled (Figure 19). Differences in the percentage mature walleye between stocked and wild ranged from 1% to 17% for females, and 1% to 26% for males. The greatest difference was between age 3 males from the 2001 year class.

Many of the stocked Pike River strain walleyes matured at a larger size than the native Red Lake walleyes (Figure 20). The males from the 1999 year class generally matured at the same rate, stocked females from the 1999 year class and stocked males from the 2001 year class matured at a larger size than the native fish.

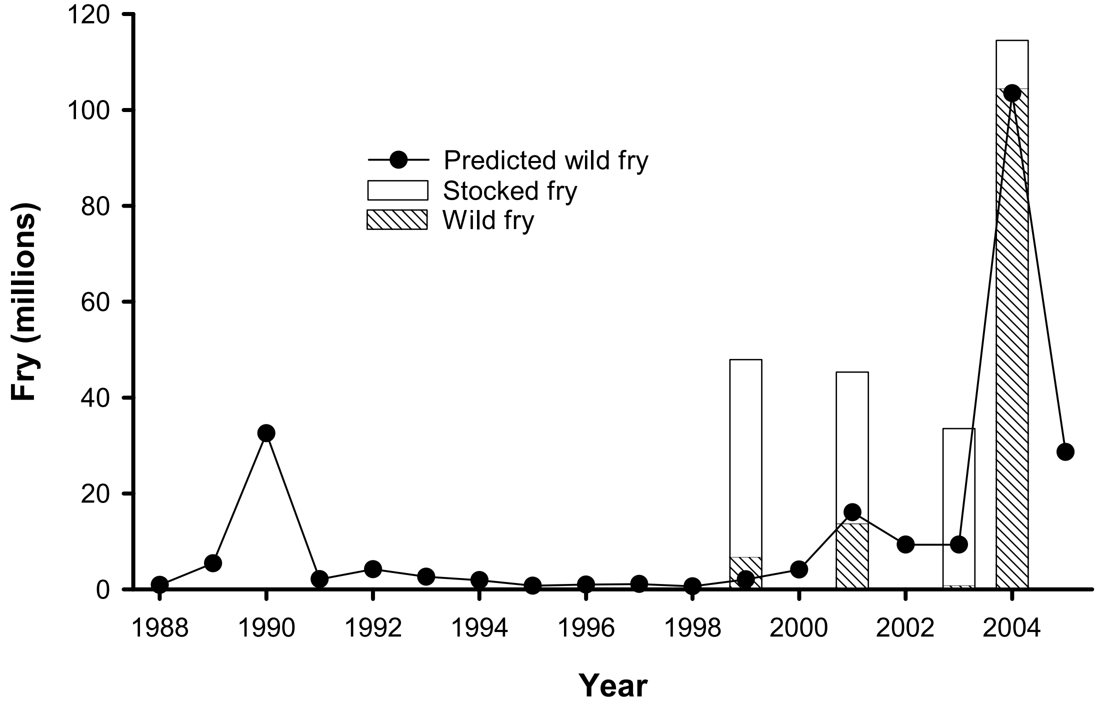

Figure 14. Fry abundance predicted by applying a 0.18% hatch rate to estimates of egg production from q_{abg} estimates of mature female biomass compared to abundance of stocked and wild fry estimated from Peterson population estimates during years that were stocked with marked fry.

Management Implications

Fry stocking proved to be a successful tool for accelerating the recovery of the walleye population in the Red Lakes. The stocked fry helped to produce three strong year classes, and returned the spawning stock to a level adequate to produce enough fry for strong natural year classes. It is unlikely that stocking alone would have produced the increase in spawning stock abundance observed during this study. If harvest had been allowed to continue, it is likely that many of the walleyes would have been removed from the system by either the sport or commercial harvest prior to reaching maturity.

The success of fry stocking in the Red Lakes may not be attainable on other lakes. The Red Lakes are large shallow wind-swept lakes that provide excellent conditions for walleye reproduction and survival. The overexploitation of the walleye stock in the Red Lakes simply left a reproductive void that was easily filled by stocked fry. Lakes with population declines due to environmental conditions such as inadequate forage, heavy predation, or habitat degradation would not be expected to respond as favorably to fry stocking without first addressing those conditions responsible for the decline.

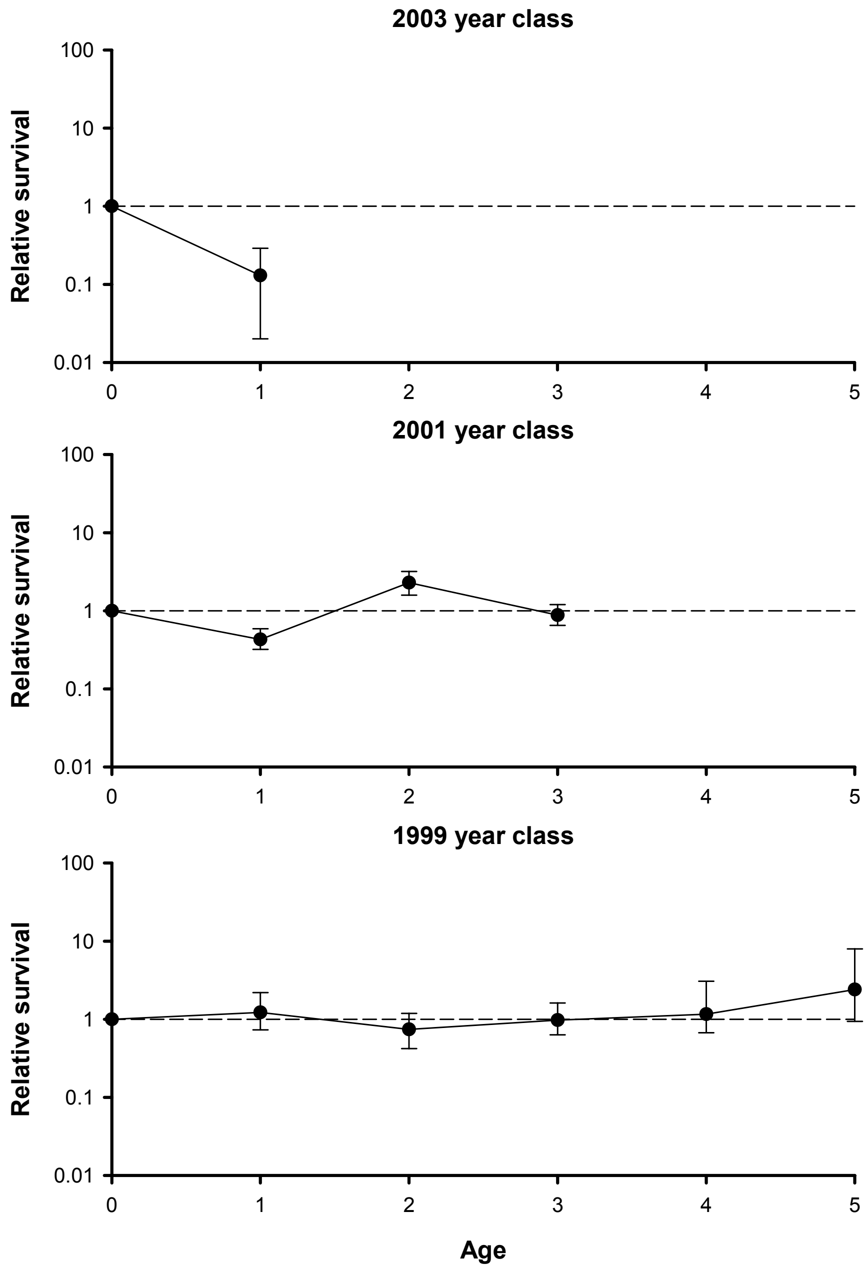

Figure 15. Relative annual survival from 1999 - 2004 of the 1999, 2001, and 2003 year classes of stocked walleyes in the Red Lakes. Relative survival is expressed as the annual survival rate of stocked (marked) walleyes divided by the annual survival rate of wild (unmarked) walleyes. Error bars represent 95% confidence intervals and the dashed line represents equal survival between stocked and wild fish.

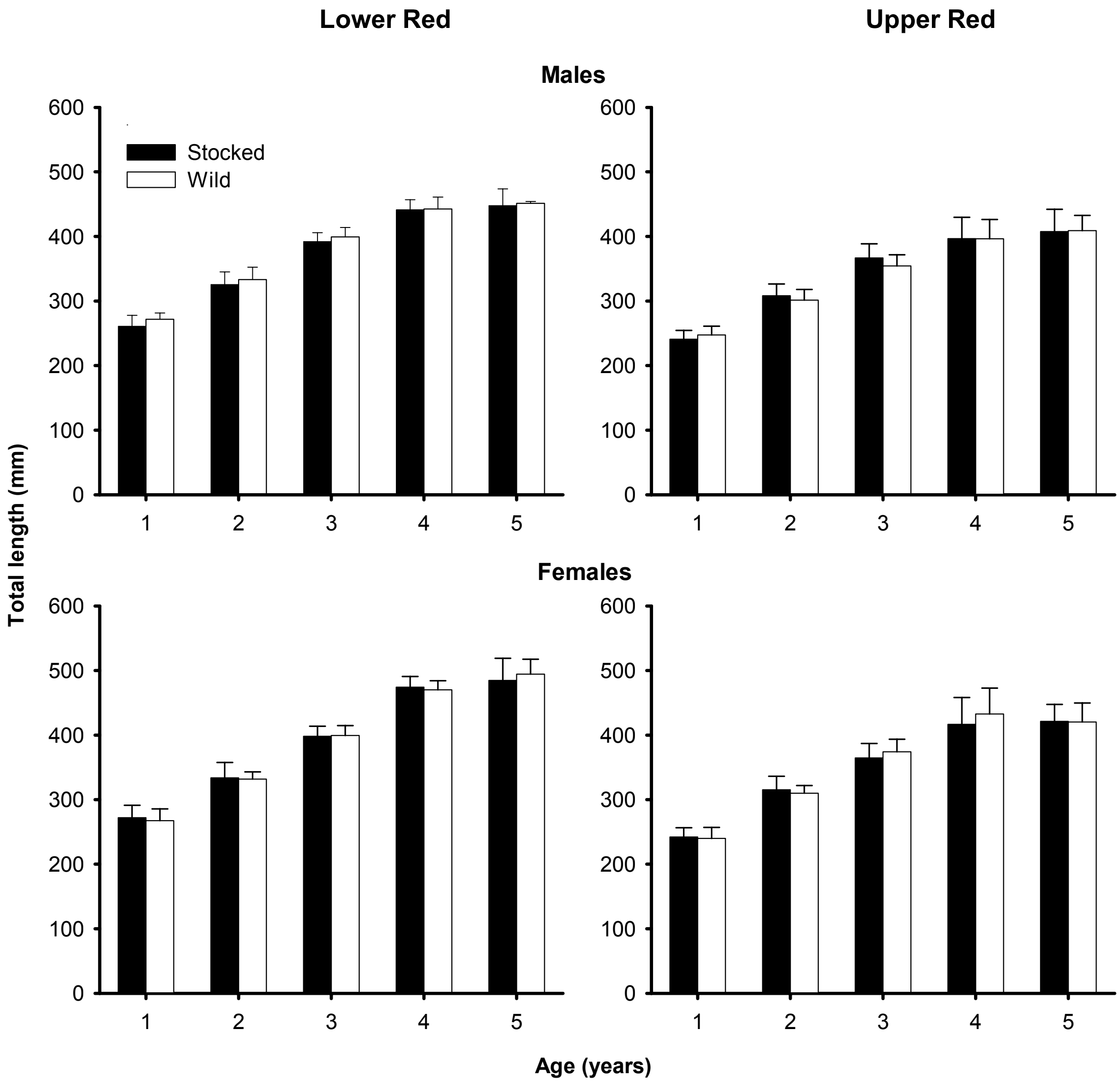

Figure 16. Total length, by sex, and basin of age 1 and older stocked and wild walleyes from the 1999 year class in the Red Lakes. Error bars represent standard deviations.

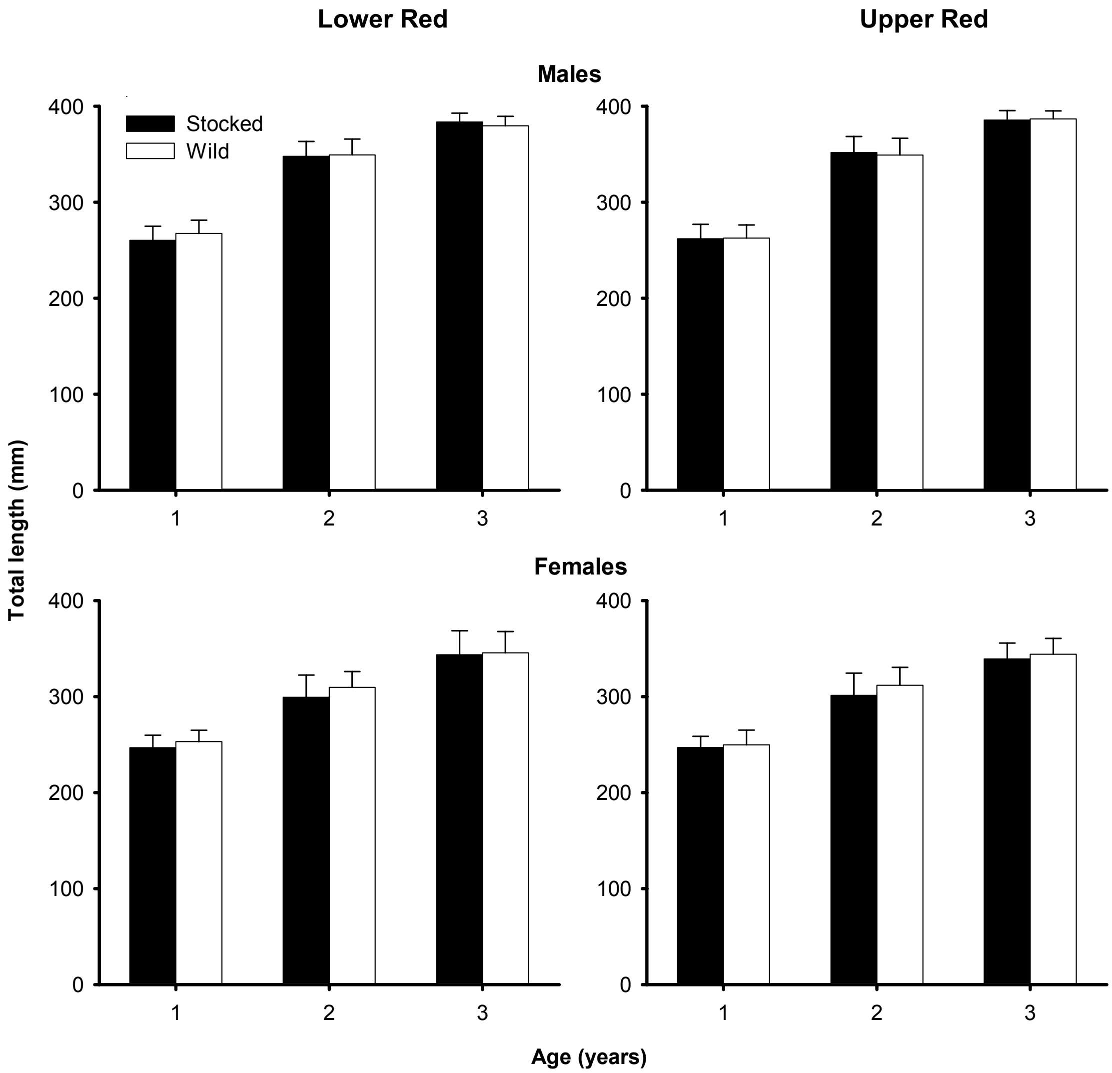

Figure 17. Total length, by sex, and basin of age 1 and older stocked and wild walleyes from the 2001 year class in the Red Lakes. Error bars represent standard deviations.

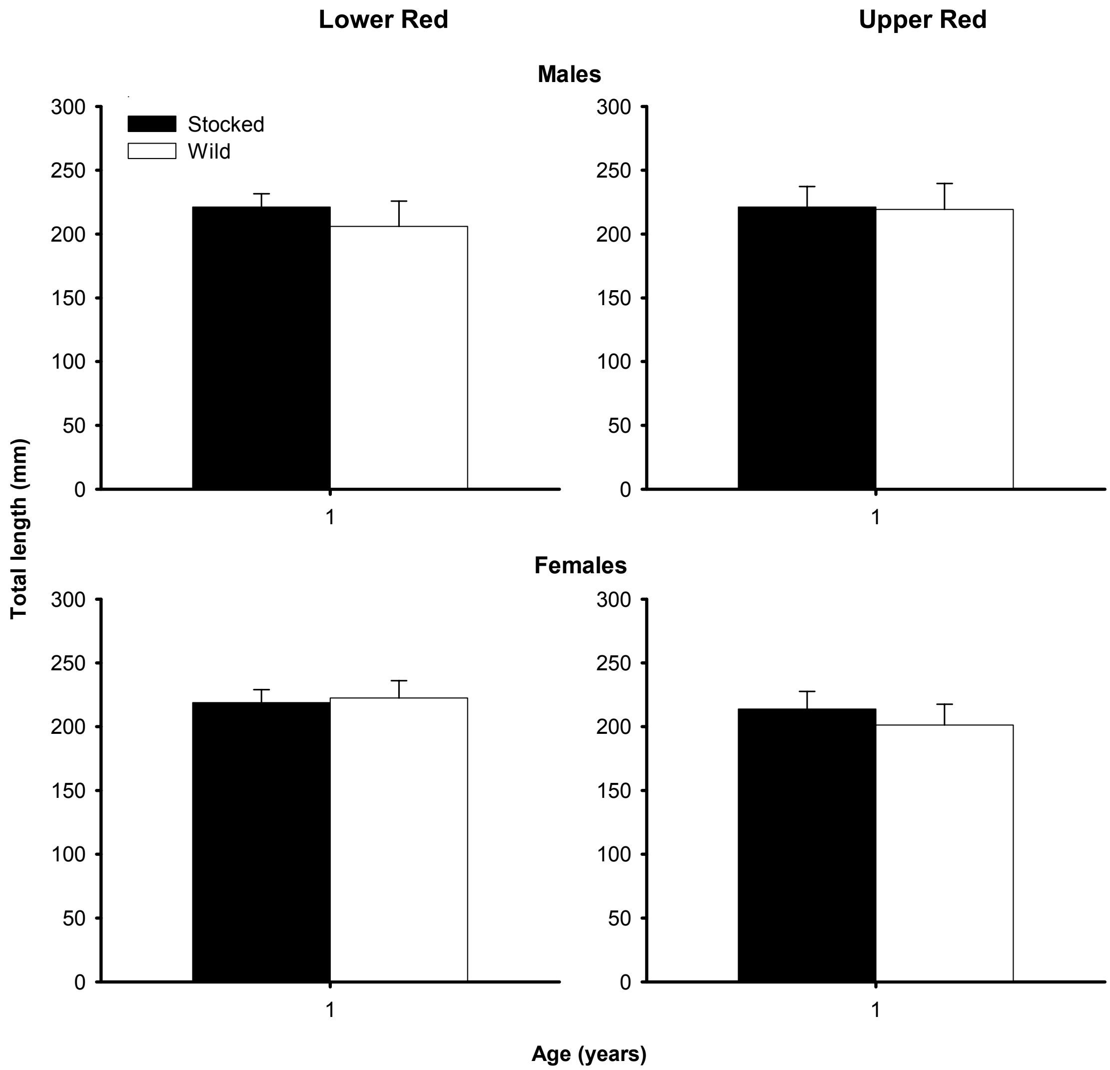

Figure 18. Total length, by sex, and basin of age 1 stocked and wild walleyes from the 2003 year class in the Red Lakes. Error bars represent standard deviations.

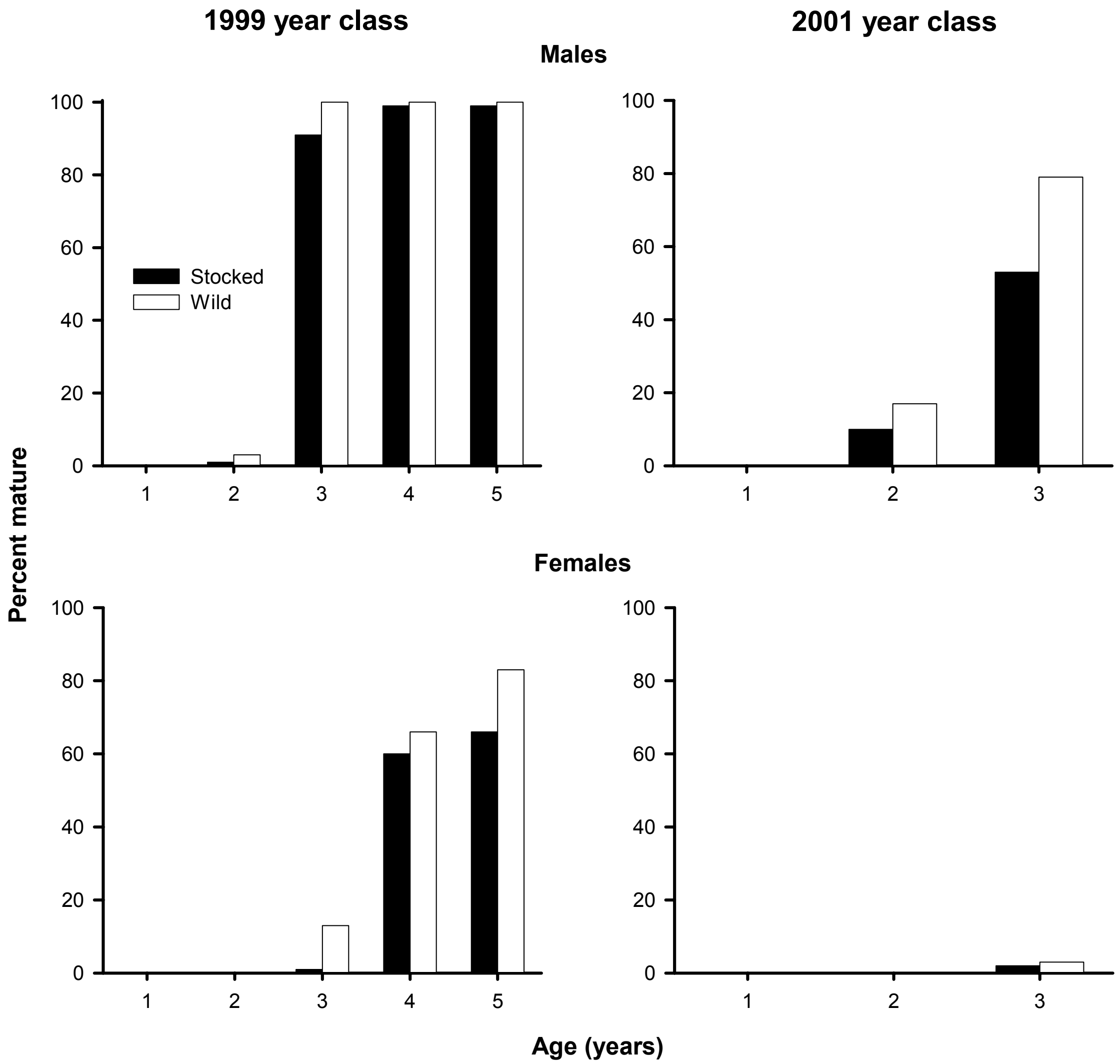

Figure 19. Maturity rates, by sex, of stocked and wild walleyes from the 1999 and 2001 year classes in the Red Lakes.

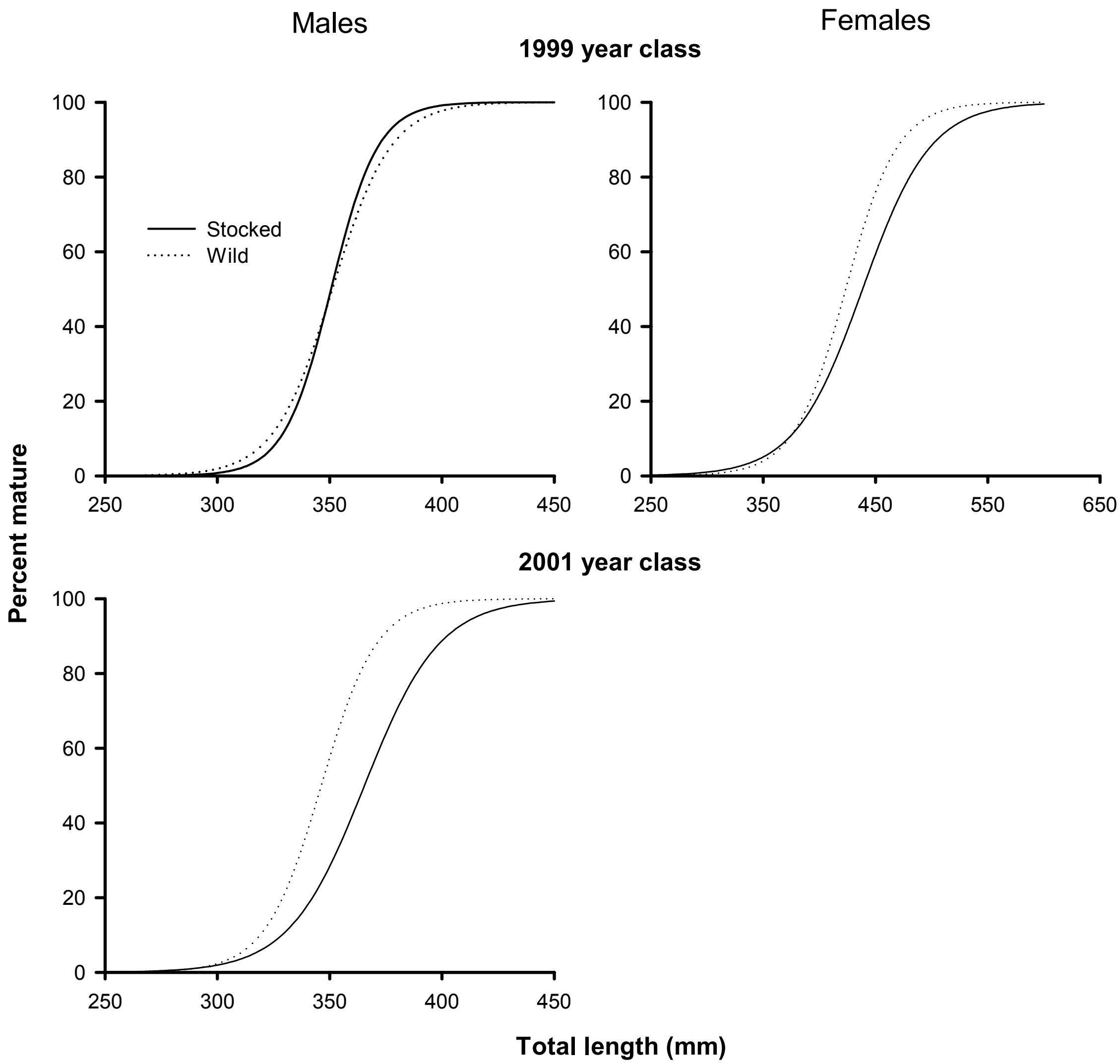

Figure 20. Logistic regression estimates of maturity rates, by total length, of stocked and wild walleyes from the 1999 and 2001 year classes in the Red Lakes. The low sample size of mature females from the 2001 year class prevented meaningful regression of their maturity rates.

Now that the spawning stock in the Red Lakes has recovered, further stocking is no longer necessary to maintain or enhance the resident walleye population. In fact, additional stocking could be detrimental to the population. Survival through the first winter is critical for the establishment of walleye year-class strength in northern latitudes, and those walleyes not reaching a suitable size by the end of the first growing season can suffer high winter mortality (Forney 1976, 1980). Since walleye growth can be density dependent (Carlander and Payne 1977; Fox and Flowers 1990), stocking walleyes into a lake with a high abundance of naturally reproduced walleyes could overwhelm the prey base and lead to poorer growth rates throughout the summer and fall. The net result could be fewer walleyes surviving through the first winter. It may also be possible that management for an overabundant brood stock would result in a high abundance of fry that experience slow growth and poor overwinter survival. Conversely, a return to previous harvest levels is unsustainable, and would lead to another crash of the population. Therefore it is important to maintain adequate brood stock levels through a well managed harvest. The fry projection model suggests that a spawning stock biomass of approximately 300,000 to 400,000 kg mature females would result in the level of fry densities that were produced through supplemental stocking during this study. The fry densities achieved through stocking appeared to produce a good compromise between abundance and growth that resulted in strong year classes; and, therefore, would seem to be an appropriate management goal.

Jurisdiction of the Red Lakes is divided between the State of Minnesota and the Red Lake Band of Chippewa, but the walleyes move throughout both basins. Marked walleye fry stocked into the upper basin during this study were later captured in the lower basin, and earlier studies by Smith et al. (1952) documented the widespread movement of adult walleyes throughout the lakes. The result of this movement is a rather homogeneous population (Smith et al. 1952) that requires the type of consistent management across basins that is achievable through continued cooperation under the auspices of the Red Lake Fisheries Technical Committee.

The long-term repercussions of the fry stocking on the overall fitness of the walleye population in the Red Lakes are unknown at this time. The Pike River strain of walleyes now compose 83% of the mature female biomass in the Red Lakes. Overall, the stocked Pike River strain walleyes have performed nearly as well as the native Red Lake walleyes in terms of growth and survival during this study. The lower maturity rates of the stocked walleyes, however, indicate that Red Lake fish do have at least a slight fitness advantage over the Pike River strain in the Red Lakes. If additional differences in fitness do occur between strains, they should be more apparent as time elapses, and differences that are not apparent at low densities may become evident when competition from higher densities forces natural selection.

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