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South Dakota Fisheries: An Evaluation of a Chemical Immersion Marking Technique for Juvenile Yellow Perch and Application to a Stocking Assessment of Marsh-Reared Yellow Perch into Eastern South Dakota Lakes

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An Evaluation of a Chemical Immersion Marking Technique
for Juvenile Yellow Perch and Application to a
Stocking Assessment of Marsh-Reared Yellow Perch into
Eastern South Dakota Lakes

AN EVALUATION OF A CHEMICAL IMMERSION MARKING TECHNIQUE FOR JUVENILE YELLOW PERCH AND APPLICATION TO A STOCKING ASSESSMENT OF MARSH-REARED YELLOW PERCH INTO EASTERN SOUTH DAKOTA LAKES

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Completion Report

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An Evaluation of a Chemically-induced Mark in Yellow Perch Fingerlings

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Abstract. – Currently, yellow perch *Perca flavescens* stocking needs in South Dakota are met by intensive trap and transfer of juvenile and adult perch. The success of these stocking efforts is largely undocumented, primarily due to problems in distinguishing yellow perch produced within the recipient water body and stocked perch. We first developed a transfer-tank marking protocol to determine immersion duration and optimal concentration of oxytetracycline (OTC) hydrochloride needed to produce an effective mark. Then we validated the protocol for adult yellow perch and determined the persistence of OTC in edible muscle tissue. Marking results indicated that satisfactory OTC marks may be obtained in juvenile yellow perch using 600- to 700-ppm OTC for an immersion period of 6 to 8 h. OTC marks were evident in juvenile yellow perch otoliths and dorsal spines checked at 3 months post-immersion. Mark quality was observed to be slightly better in juvenile dorsal spines than otoliths. OTC marks in adult yellow perch were detectable at otolith margins at 9 d post-immersion. Adult muscle tissues were analyzed with high pressure liquid chromatography to quantify OTC residues. A nonlinear model (ln epi-OTC [$\mu g\ g^{-1}$] = 0.960 - 0.389*ln time [h]; r^2 = 0.99) describing the combined OTC base/epi residue relation to time indicated that no more than 0.5 μg OTC g^{-1} should be present at 73 h following immersion.

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Introduction

Chemical batch marking appears to be the most viable approach for stocking evaluations. A variety of chemicals have been evaluated for the marking of embryo to adult life stages of various fish species (Behrens Yamada and Mulligan 1990). The use of fluorescent chemicals, such as tetracycline antibiotics (e.g., tetracycline [TC] and oxytetracycline [OTC]), appears to provide the best performance by providing convenient modes of induction, relatively long-term marks, and low-cost detection in comparison to other approaches (Younk and Cook 1991; Nielsen 1992; Guy et al. 1996). OTC is an antibiotic substance isolated from the elaboration products of the actinomycete *Streptomyces rimossus*. The hydrochloride form is highly soluble in water (1 g mL^{-1}). The TC family of antibiotics, which fluoresce yellow to green, may be detected in calcified structures under ultraviolet light.

Several studies have dealt with mass-marking of juvenile fishes using TC compounds (e.g., Younk and Cook 1991). Weber and Ridgeway (1962) were the first investigators to provide information on marking fish with TC. A review of the literature indicates that there have been over 40 studies published on marking with TC compounds since that study. Successful results have been achieved by administering tetracyclines by direct injection, feeding diets containing the chemical, or by immersing the fish in a solution. The most appropriate induction method is dictated by the size and life stage of the fish, quantity of fish to be marked, facilities and equipment required, time expense, and any other factors unique to a particular study.

Variables such as the age of the fish at the time of immersion, concentration, immersion duration and water chemistry (Hettler 1984; Beckman et al. 1990; Younk and Cook 1991; Brooks et al. 1994) can all play important roles in obtaining detectable marks. Several life stages of many fish species have been successfully immersion-marked with OTC concentrations ranging from 100 to 700 ppm over periods of 3 to 24 h. (Weber and Ridgeway 1962; Choate 1964; Scidmore and Olson 1969; Hettler 1984; Lorson and Mudrak 1987; Secor et al. 1991; Brooks 1994; Fielder 1994). For example, Brooks et al. (1994) determined that optimal results were observed by marking larval and juvenile walleyes *Stizostedion vitreum* with a sodium phosphate-buffered solution (0.297 g L^{-1} dibasic, 0.016 g L^{-1} monobasic) and of 500 mg OTC L^{-1} for a period of 6 h. That procedure resulted in 100% fluorescent marks on otoliths with minimal mortality. Fielder

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(1994) applied the same procedure to walleye in South Dakota, which resulted in marks of 85% and 65% for fry and fingerlings, respectively. However, Lucchesi (1996) observed 100% marks on walleye fry and fingerlings at concentrations of 492 mg L^{-1} to 597 mg L^{-1} for an 8-h period.

Unkenholz et al. (1997) were able to detect visible marks in 100% of juvenile yellow perch *Perca flavescens* fingerlings immersed for a minimum 6 h in 534- and 748-ppm OTC (total hardness, 380 ppm as $CaCO_3$). They were unable to consistently detect marks in fish immersed up to 12 h at 309-ppm OTC. Thus, they recommended an immersion treatment for a minimum of 6 h of at least 500 ppm buffered OTC to produce a detectable mark in yellow perch fingerlings. Optimal concentrations for specific time periods must be defined to provide reliable marks for stocking assessments of fish that are trapped and transferred. Because it is impractical to hold fingerlings up to 12 h during trap and transfer operations, concentrations and holding time periods require further investigation. Stress-related mortality associated with higher temperatures in late summer/early fall when transfer operations occur (along with other logistics of hauling operations) requires that fish be collected and transferred as quickly as possible. Therefore, our objectives were: 1) to validate the OTC marking protocol in transfer tanks; 2) to evaluate mark induction for adult yellow perch; 3) to visually compare marks produced in spines and otoliths; and 4) to conduct temporal assays of OTC residues in adult yellow perch muscle tissue.

Methods

Transfer-tank Marking Protocol

Age-0 yellow perch were collected with trap nets from a natural rearing pond (Little Brushy WPA-NRP) in Brookings County, and placed in one of two transfer tanks containing 757 L of lake water. The tanks contained a calculated concentration of either 500- or 700-ppm OTC hydrochloride ($C_{22}H_{24}N_2O_9 \cdot HCL$), buffered with sodium phosphate (dibasic, Na_2HPO_4). Chemicals were premixed with lake water in 19-L buckets, then added to the transfer tanks. Mixing was done with 12-VDC agitators and aeration was supplemented with pure oxygen. An anti-foaming agent (5% silicone base, food grade, Fritz Industries, Dallas, TX) was added (~60

mL) to each tank to minimize foam production. Fish were transported and held in the transport tanks at South Dakota State University (SDSU) for the duration of the marking period.

Temperature, dissolved oxygen (DO), and pH were monitored and recorded every 30 min. OTC crystals are fairly stable and show no loss in potency on heating for 4 d at 100 °C and less than 5% inactivation after 4 mos at 56 °C (Budavari et al. 1996). Aqueous solutions of the hydrochloride are less stable; at pH 1.0 to 2.5 the compound is stable for at least 30 d at 25 °C. Solutions at pH 3.0 to 9.0 have shown no detectable loss in potency during storage at 5 °C for at least 30 d. The half-life potency is 26 h for an aqueous OTC solution at 37 °C and a pH of 7.0. Our marking solution was at 18 °C and a pH of 7.3 and thus should have retained high potency for the 8-h marking period. Also, loss of potency in aqueous OTC solutions exposed to light indicates that photoreduction further facilitates breakdown of OTC. Therefore, tank hatch covers remained closed except when subsampling fishes.

At predefined time intervals (4, 6, and 8 h) 25 fish were randomly collected (N = 150) from each tank and given an identifying fin clip to denote the immersion time and marking concentration. After receiving the fin clip, fish were stocked into a 938-L circular tank and held for a three-month growout period. During the holding period, water quality was maintained with a flow rate of 3.75 L/min and supplemental aeration. The water source for fish holding was a municipal supply treated with sodium thiosulfate to remove chlorine. Water temperature was maintained near 24° C with thermostatically controlled heaters. Fish were fed to satiation once each day with a prepared salmonid grower diet (BioDiet, Warrenton, OR) for the first 30 d and then switched to a diet of fathead minnows *Pimephales promelas*. A 12-h light:12-h dark photoperiod was maintained throughout the holding period.

Otolith and Spine Processing

For mark evaluation, juvenile and adult fish were euthanized and sagittal otoliths were removed by dissection of the frontal bone. Otoliths were dried and mounted on glass slides with cyanoacrylic glue (liquid form) and allowed to dry in a dark environment for 24 h. Juvenile yellow perch dorsal spines were excised at the base of the articulating process at the inception of the pterygiophores. Spines were stored in scale envelopes until they were processed.

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To view the spines for presence of a mark, the second and third dorsal spines were cut away from the others and placed on an acetate slide. Transparent cellophane tape was placed across the two spines to allow slicing without displacing sections. A Dremel moto tool, mounted to an articulating base, was equipped with a cut-off wheel (no. 409) to section spines. Beginning just distal to the basal process, cross sections were made approximately 1-mm thick. Two sections were removed from the dorsal spine and placed on a drop of cyanoacrylic glue (gel form) on a glass microscope slide. Spine cross sections were viewed immediately after mounting. However, if the cross section was too thick or uneven, the mount was stored in the dark until the glue was sufficiently dry at which time the section was ground with the flat surface of the cut-off wheel.

The equipment configuration used for mark detection in juveniles was an Olympus BH2 RFCA compound microscope equipped with 100-W ultraviolet (Hg arc) light source and fluorescent detection assessories (i.e., DMIB filter cube, 505 dichroic mirror, 450 to 495-nm excitation filter, and 515 IF barrier filter). Two readers examined and scored structures; otoliths and spine sections were viewed independently for mark presence and quality. Otoliths from adult fish sampled for OTC residue analysis were viewed with a Nikon E400 compound microscope equipped with a 100-W ultraviolet (Hg arc) light source and fluorescent detection assessories (i.e., B3 filter cube, 505-nm dichroic mirror, 420 to 490-nm excitation filter and 520 barrier filter). Mark quality of otoliths and dorsal spines was defined on a rank scale of 0 to 3 (0 = no mark, 1 = barely detectable, 2 = easily detected, but partial mark or not brilliant; 3 = bright, well-defined continuous mark). Relative distance of the mark from the otolith margin was defined on a rank scale of 0 to 5 (e.g., 0 = mark on the margin, 5 = mark farthest from margin). During mark evaluation, otoliths were lightly sanded with wet 1,000 grit sandpaper.

OTC Residue Analysis

Yellow perch (164 mm mean TL) were held for 6 h in a 1,250-L fiberglass raceway and immersed in 600-ppm OTC hydrochloride buffered to a pH of 7.2 with sodium phosphate. Approximately 80 mL of an antifoam agent was added to reduce foaming of the OTC solution caused by aeration.

Following marking, the fish were maintained in the raceway and fed a prepared salmonid grower diet (BioDiet, Warrenton, OR) to satiation by delivering the ration three times daily with a 24-h belt feeder. During the holding period, water quality was maintained with a flow rate of 3.75 L/min and supplemental aeration. A 13-h light:11-h dark photoperiod was maintained throughout the study. The marking and holding temperature was 19 °C. After the immersion marking period, normal water flows were resumed and the OTC-treated waters were flushed through an active carbon filter to remove the organic contaminant.

Treated fish (N=153) were subsampled (n = 9) at preselected time intervals (1 to 1,080 h) and sacrificed for tissue analyses. The samples were stored in a dark environment and frozen at -20 °C, pending preparation and analysis. Individual fish from each treatment were prepared by removing the fillet (skin off, no bones). The muscle tissue was pooled (n = 3) and homogenized to form three separate composite samples for each time period. Composite samples and OTC-treated waters were analyzed with high pressure liquid chromatography (HPLC) (Houglum and Larson 1999).

Data Analysis

Standard parametric procedures were applied to rank data (Conover and Iman 1980). Paired t-tests were conducted to determine whether differences consistently occurred between readers; when no difference was detected mean ranks were used for subsequent analyses. For calcified structures, comparisons were made among treatments (OTC concentrations and immersion periods) with analysis of variance. Otolith and spine mark quality were compared with the paired t-test. Simple (i.e., t-tests) and multiple comparisons were done using Bonferroni's adjusted probabilities.

Nonlinear regression models were developed to describe the relationship between OTC tissue residue (μg OTC g^{-1} tissue) and depletion time. All statistical analyses were conducted with SYSTAT (1999); an alpha level of 0.10 was used for all inferential tests.

Results and Discussion

Transfer-tank Marking Protocol

On September 10, 1996 approximately 2,428 juvenile yellow perch (89.4 mm mean TL) collected from Little Brushy Lake(Brush Lake WPA, Brookings County) were evenly distributed between the two transfer tanks containing OTC-treated water. Management of water temperature and DO concentrations in transfer tank water is frequently a primary concern when transporting fish. As ambient and tank water temperature increased and saturability of oxygen began to decrease during the marking period, we added 23 kg of bagged ice to each tank at 3 h. By floating bagged ice we were able to maintain tank water temperature within ± 2 °C of the initial water temperature. Oxygen was maintained at > 90% saturability by controlling temperature, agitation and diffusing 0.5 L pure O_2/min. The tank water was monitored for pH fluctuations because the acidic nature of OTC hydrochloride would cause stress. We observed no deviation from the initial pH after introduction of the slurry to the tanks.

Fish (marked at 500- or 700-ppm OTC for 8 h) that remained in the transfer tanks after subsampling were stocked into Lake Goldsmith (Brookings County). Fish marked at 500-ppm OTC received a left pelvic fin clip and fish marked at 700-ppm OTC received a right pelvic fin clip. Unfortunately, Lake Goldsmith winterkilled and we were unable to retrieve fish for mark evaluation the following spring.

Acute mortality (8 h) was determined to be 0.49 and 0.74 % for the 500 and 700-ppm OTC treatments, respectively. Because of this low mortality we attributed minor losses to handling and weighing stress, not to any potential marking or holding stresses. Overall, the protocol used for OTC-marking yellow perch in transfer tanks proved to be relatively simple, requiring little additional effort beyond a normal trap and transfer episode. The primary concern would be the loss of work time while fish must be held in the marking solution. Specifically for trap and transfer operations, further research should be directed toward the use of potentiators that accentuate OTC uptake and thus reduce holding time.

Otolith and Dorsal Spine Evaluation

Assays of OTC-treated transfer tank waters sampled at the midpoint (4 h) revealed soluble concentrations of 300- (567 ppm total) and 279- (796 ppm total) ppm OTC for the 500 and 700 ppm treatments, respectively. A number of factors such as water hardness, level of mixing, OTC activity, etc., could have influenced the amount of OTC detected in the solutions. Additionally, the actual amount of OTC extracted from the solution by yellow perch would be difficult to accurately assess. We suspected that both moderately high water hardness (~440 ppm as $CaCO_3$) and reduced mixing action (cube-shaped tanks) reduced solubility.

No difference was detected between mark ranks assigned by readers (P = 0.89). Overall, immersion time (P = 0.06) proved to be a greater influence on mark detection and quality in juvenile otoliths than did concentration (P = 0.47). This is likely because the solute component of the two treatment concentrations were similar. Within the 500-ppm OTC treatment, immersion time did not significantly differ (P = 0.15); however, mark quality did increase with immersion time (Table 1.1). Within the 700-ppm OTC treatment, mark quality differed little after 6 h (P = 0.33).

Similar patterns in the quality of marks were observed for dorsal spines. Mark quality improved over time (P = 0.07), more so than with increased concentration (P = 0.82). Within the 500-ppm OTC treatment, mark quality increased with time, but not significantly (P = 0.33). Likewise, within the 700-ppm OTC treatment mark quality increased with time, but not significantly (P = 0.23).

HPLC analysis of OTC-treated water (600 ppm) from the adult marking experiment showed 197 ppm in solution and 587 ppm total. Again, previously mentioned factors may have influenced the amount of OTC in solution. Water hardness at the SDSU lab is ~380 ppm as $CaCO_3$. Otoliths were collected (n = 5 per period) beginning 9 d following the marking period (216-h post immersion) through day 63 (Table 1.2).

Marks were consistently detected on all adult otoliths, but there was no significant difference (P = 0.91) in mark quality found among the 11 samples. Although there was minimal change in mark brightness over time, greater distances between the mark and the margin facilitated quicker detection. We were unable to quantify body and otolith growth because of

advanced fish size and short duration of the experiment. Although subjective, we were able to assign a rank score for the relative distance of the mark from the otolith margin. Based on those scores, there was a significant (P < 0.001) increase in relative distance with time (Table 1.2). Thus, although marks were detectable in adult otoliths at 9 d, the likelihood of detection would be greater at ≥51 d, particularly for an inexperienced reader.

Several calcified structures (e.g., dentary and maxillary bones, spines and fin rays, vertebrae, and teeth) have been evaluated for OTC or TC marks. Retention of marks in external structures such as scales did not exceed 3 months in walleyes (Brooks et al. 1994), but were present in the scales of red drum *Sciaenops ocellatus* after 10 months (Bumgardner 1991). Because tetracycline antibiotics are sensitive to natural light, internal bony structures are not as subject to degradation (Muth and Bestjen 1991). As such, the most common calcified structures examined for marks are sagittal otoliths. Regardless of dosage applied, tetracycline drugs were deposited in the growing surfaces of all internal bones except the skull (Weber and Ridgeway 1962).

Thus, there are several benefits to using saggital otoliths for mark detection. The otolith is among the first calcified tissues formed in fish (McElman and Balon 1985), they do not appear to be re-absorbed during periods of stress, they are easily removed, and have the added benefit of daily growth rings for analysis of growth in young fishes. (Taubert and Coble 1977; Miller and Storck 1982; Schmidt 1984).

The detection of marks (presence or absence) was similar between otoliths and dorsal spines; thus, either structure may be adequate for stocking assessment of juveniles. In yellow perch the first dorsal fin is supported by 13 to 15 spines, while the anal fin has two spines (Craig 1987). The spines retain essentially the same form throughout life with cross sections resembling a heart with unequal lobes. Spine growth is accomplished by seasonal deposit of tissue on the margin that is proportional to otolith growth. In other spines, such as pectoral spines, deterioration around the lumen may obscure part of the first annual mark in older fish, which could present a problem in detecting marks in older fish. Therefore, dorsal or anal spines would likely be more appropriate for detection of marks for long-term assessments. Additionally, the dark tegument on the dorsal fin appears to limit photodegradation of the mark.

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Table 1.1. Mean ranks (0 to 3) for mark quality of otoliths and dorsal spines from juvenile yellow perch (N = 150) immersed in 500 (300 in solution, 567 total) or 700 (279 in solution, 796 total) ppm oxytetracycline hydrochloride for durations of 4, 6 or 8 h. Row P-values indicate the Bonferonni adjusted probability of a significant difference between calcified structures based on a paired t-test.

Treatment	Otolith	Spine	P- value
500/4 h	1.3	2.5	0.03
500/6 h	2.0	2.7	0.18
500/8 h	2.3	3.0	0.10
700/4 h	1.1	2.4	0.08
700/6 h	2.0	2.7	0.24
700/8 h	1.8	3.0	0.01

Table 1.2. Mean ranks for mark quality (0 to 3) of otoliths and relative distance (0 to 5) of the OTC mark from the otolith margin. Otoliths were aquired from adult yellow perch (5 per d, N = 55) immersed in 600 (197 in solution, 587 total) ppm oxytetracycline hydrochloride for a 6-h duration. Similar letters following mean distance ranks indicate no statistical difference (α = 0.10) based on a Bonferonni post hoc test.

Sample Day	Otolith	Distance
9	2.6	1.0a
12	2.9	1.0a
15	2.9	1.0a
21	2.8	1.2a
27	2.5	1.8b
33	2.7	2.0b
39	2.6	2.0b
45	2.7	2.0b
51	2.8	3.0c
57	2.9	3.0c
63	3.0	3.5c

OTC Residue Analysis

Before OTC marking and stocking evaluations in public waters using edible-sized fish can be safely done it is necessary to know the persistence of OTC in muscle tissues. HPLC analysis indicated that within hours the OTC base was rapidly depleted below 0.5 µg OTC g^{-1} (Table 1.3; Figure 1.1). The empirical data indicate that the 0.5 µg OTC g^{-1} level would be reached between 48 and 72-h post immersion (Table 1.3). A nonlinear model,

$$\ln \text{OTC base [µg g}^{-1}\text{]} = 0.932 - 0.499 * \ln \text{time [h]} \ (r^2 = 0.93),$$

describing the residue/time relation suggested that no more than 0.5 µg OTC g^{-1} should be present at 28 h following immersion. The OTC base and epi-OTC combined depleted below 0.5 µg OTC g^{-1} within 3 d (Table 1.3; Figure 1.2). (Epi-OTC [4-epioxytetracycline] is one of the most common breakdown products of OTC.) A similar nonlinear model,

$$\ln \text{total OTC [µg g}^{-1}\text{]} = 0.960 - 0.389 * \ln \text{time [h]} \ (r^2 = 0.99),$$

predicted no more than 0.5 µg OTC g^{-1} should be present at 73 h following immersion. Control fish samples were determined to contain 0.00 µg OTC g^{-1}. An untreated fish sample was spiked with 0.17 µg OTC g^{-1} and determined to contain that concentration.

Current Federal Drug Administration guidelines regarding OTC residues in food fish are specifically for feed use in salmonids and ictalurids. Mandatory withdrawal times are 7 d for Pacific salmon and 21 d for other salmonids and ictalurids (21 CFR 558.450). Tolerance in the flesh is 2.0 µg OTC g^{-1} (21 CFR 556.500). Based on tolerance guidelines, our results indicate that adult yellow perch immersed in OTC under a similar protocol (i.e., 600-ppm OTC, 6 h) could be safely consumed following a 14-h withdrawal period.

Table 1.3. Results of HPLC analysis of OTC residues in adult yellow perch muscle.

Week	Day	Hours	Time interval (h)	OTC base (mg L^{-1})	OTC base + Epi (mg L^{-1})
1	0	1	1	2.560	2.620
	0	14	14	0.217	0.943
	1	24	24	0.246	0.680
	2	48	24	0.321	0.604
	3	72	24	0.131	0.412
	6	144	72	0.320	0.355
2	9	216	72	0.171	0.356
	12	288	72	0.226	0.343
3	15	360	72	0.120	0.319
	21	504	144	0.186	0.231
4	33	792	144	0.048	0.174
6	45	1,080	144	0.017	0.059

Oxytetracycline (base)
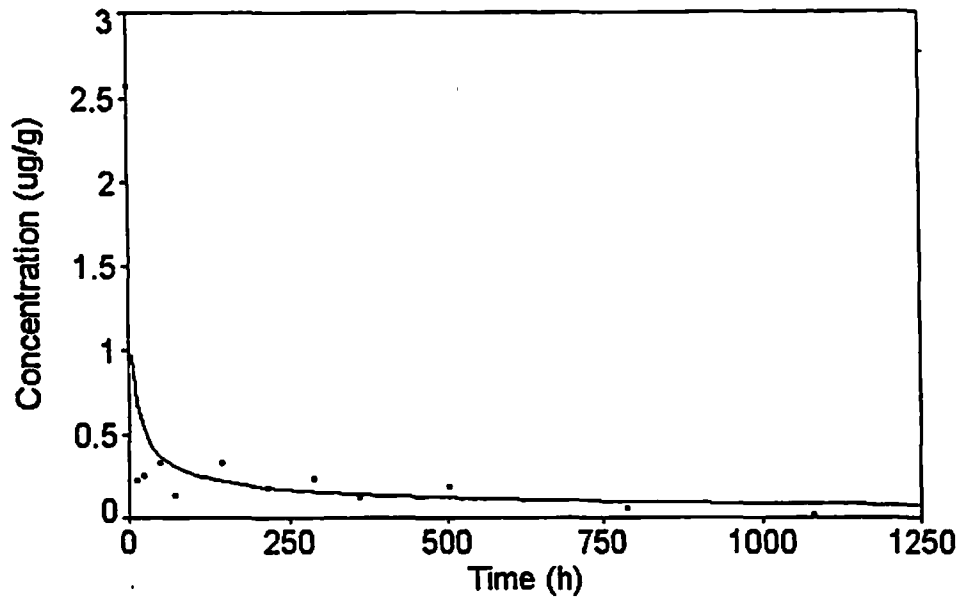
Figure 1.1. Temporal relationship of base OTC depletion in adult yellow perch.

Oxytetracycline (epi + base)
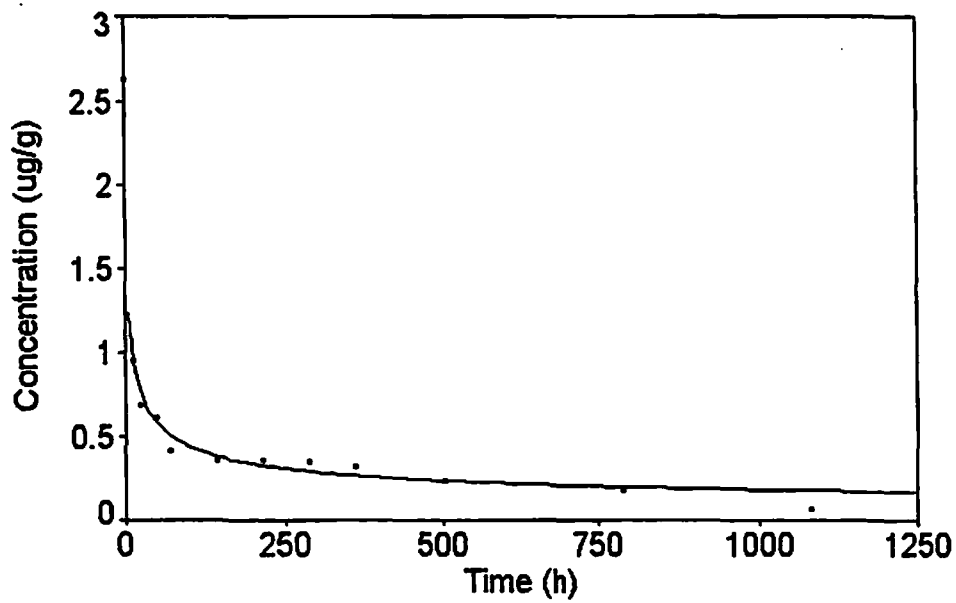
Figure 1.2. Temporal relationship of base and epi-OTC depletion in adult yellow perch.

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Future Needs

Potentiators can be used to accentuate marks, but investigators have reported variable degrees of success (e.g., Weber and Ridgeway 1967; Scidmore and Olson 1969; Odense and Logan 1974; Hettler 1984; Wahl and Stein 1987). Determination of an effective, nonstressful potentiator that provides consistent results would greatly benefit trap and transfer operations by reducing holding periods.

OTC uptake is apparently variable depending upon water quality parameters because considerable differences in mark quality have been observed among studies that have marked similar life stages of the same species for similar durations and concentrations. Water hardness has been suggested as a primary factor influencing OTC uptake. Thus, correction factors could likely be developed that would estimate the increase in the OTC concentration required to offset elevated calcium concentrations and provide a quality mark.

Most protocols for OTC detection in fishes require sacrifice and removal of saggital otoliths. This quickly becomes a concern when sampling populations of small size. Additionally, this approach limits managers to a one-time utilization for stock contribution assessment. Thus, use of spines for mark detection should be explored further. This approach would allow managers to monitor populations over a period of years providing the opportunity to conduct population trend analyses. Futhermore, an evaluation of mark longevity in otoliths and spines needs to be done.

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Stocking Assessment of Marsh-reared Yellow Perch in Eastern South Dakota Lakes

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Abstract.— The success and value of yellow perch *Perca flavescens* stocking programs are largely unknown due to the difficulties in differentiating between naturally produced and translocated stocks. To determine stock contributions of yellow perch, fingerlings and adults were collected (primarily during fall) from natural rearing ponds in southeastern South Dakota. Prior to stocking, all fish were marked for 6 h in transfer tanks containing 700-ppm OTC. Seven lakes with existing yellow perch populations were supplemented at densities of about 25, 135 or 200 fish/ha. Yellow perch populations were subsequently sampled with experimental gill nets during late summer; two lakes were resampled with additional gears (i.e., electrofishing, trap nets, and cloverleaf traps). Yellow perch were processed for routine measurements and saggital otoliths were removed to determine the origin of the fish. Stock contribution analysis was done to determine if stocked (marked) yellow perch had a measurable impact on increasing densities in existing perch populations. Stock contribution estimates for cohorts determined from gill-net samples for Island, Oak, Twin, and Wall lakes were approximately 18, 5, 41, and 38 %, respectively. Samples from other gears provided stock contributions of 15 and 10 % for Cavour Lake and 41 % for Diamond Lake. Although these results showed that supplemental stockings were successful, variability in stock contributions among populations indicates a need for further assessment of variables that may influence the stocking success of yellow perch.

Introduction

Panfish compose an important component of South Dakota's sport fisheries, ranking second only to walleye *Stizostedion vitreum* and sauger *S. canadense* for resident and nonresident angler days (USDI 1991). Statewide surveys (McPhillips 1988; Mendolsohn 1994) have indicated that, of the panfish group, yellow perch *Perca flavescens* are a highly preferred sportfish. Although this species occurs in over 80% of eastern South Dakota lakes and several other water types, few lakes consistently support quality yellow perch fisheries.

Many eastern South Dakota glacial lakes are shallow, windswept and eutrophic. As a result, these lakes do not thermally stratify and are prone to both dense summer algal blooms and winterkill (Unkenholz 1976). Further, many populations exhibit low or inconsistent recruitment (Lott 1991; Fisher 1996). As a consequence, some glacial lake yellow perch populations require stockings of juveniles or adults from rearing ponds or populations having consistent recruitment (Unkenholz 1976). Currently, most stocking requests are fulfilled by trap and transfer operations, and to a lesser extent, through occasional extensive culture at the Blue Dog State Fish Hatchery.

The success and value of these stocking efforts is largely undocumented, primarily due to problems in distinguishing between yellow perch produced within the recipient water body and those that are stocked. Accordingly, South Dakota Department of Game, Fish and Parks (SDGFP, Large Lakes and Reservoirs Planning Team, 1994) identified effectiveness of panfish stockings to be an important research need. Thus, the objective of this study was to apply a mass-marking field technique to yellow perch trapped from natural rearing ponds (NRP) and then provide a stocking assessment following introduction into natural populations.

Methods and Materials

Study Sites

The six study lakes were located in eastern South Dakota (Figure 2.1). These lakes were characterized as shallow, windswept glacial lakes prone to winterkill. All lakes periodically receive stockings of yellow perch to supplement populations that are subject to recruitment problems.

Cavour Lake (Beadle County; T. 111, R. 60, Sec. 20-22) was classified by SDGFP as a warmwater marginal, meandered public water. The lake has a surface area of 93 ha with mean and maximum depths of 1.2 and 2.4 m, respectively. Primary fish species present were northern pike *Esox lucius*, black crappies *Pomoxis nigromaculatus* and black bullhead *Ameiurus melas*. Secondary species were yellow perch, common carp *Cyprinus carpio* and saugeye *S. vitreum x S. canadense*. Coontail *Ceratophyllum* sp. was the primary submergent vegetation present.

Diamond Lake (Minnehaha County; T.104, R 52 , Sec. 5) was classified by SDGFP as a warmwater, marginal, meandered public water. The lake has a surface area of 104 ha, with mean and maximum depths of 1.8 and 3.5m, respectively. Primary fish species present included northern pike, yellow perch and bluegill *Lepomis macrochirus*. Secondary species were black bullhead and green sunfish *L. cyanellus*. Coontail and cattail *Typha* sp. were the only vegetation present.

Oak Lake (Brookings County; T. 112N, R. 47-48, Sec. 1,7,12-13,18) was classified by SDGFP as warmwater marginal, meandered public water. The lake has a surface area of 160 ha with mean and maximum depths of 0.9 and 1.8 m, respectively. Primary fish species present were northern pike, yellow perch and black bullhead. Secondary species were white sucker *Catostomus commersoni*, orange-spotted sunfish *L. humilis* and common carp. Aquatic vegetation was primarily coontail and cattail.

Island Lake (Minnehaha County; T. 104N, R. 52W, Sec. 19: McCook County; T. 104N, R. 53W, Sec. 24) was classified by SDGFP as warmwater marginal lake. North Island is not a meandered water and South Island was a meandered water that has been relicted. The lake has a surface area of 101 ha with mean and maximum depths of 2.4 and 4.5 m, respectively. Primary fish species present were walleye, yellow perch and black bullhead. Secondary species included northern pike and black crappie. Aquatic vegetation consisted of coontail and pondweed *Potamogeton* sp..

Twin Lake (Sanborn County; T. 106N, R. 62W, Sec. 30, 31) was classified by SDGFP as a warmwater semi-permanent, but not meandered. The lake has a surface area of 102 ha with mean and maximum depths of 1.8 and 3.8 m, respectively. Primary fish species present were northern pike, black crappie, white crappie and walleye. Secondary fish species included

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largemouth bass *Micropterus salmoides*, yellow perch, white sucker, black bullhead, and common carp. Aquatic vegetation consisted of coontail and cattail.

Wall Lake (Minnehaha County; T. 101N, R. 51W, Sec. 21, 28) was classified by SDGFP as a warmwater semi-permanent, meandered public water. The lake has a surface area of 84 ha with mean and maximum depths of 3 and 5.5 m, respectively. Primary fish species present were walleye, black crappie and yellow perch. Secondary species included northern pike, black bullhead, white sucker, common carp, channel catfish *Ictalurus punctatus*, and bigmouth buffalo *Ictiobus cyprinellus*.
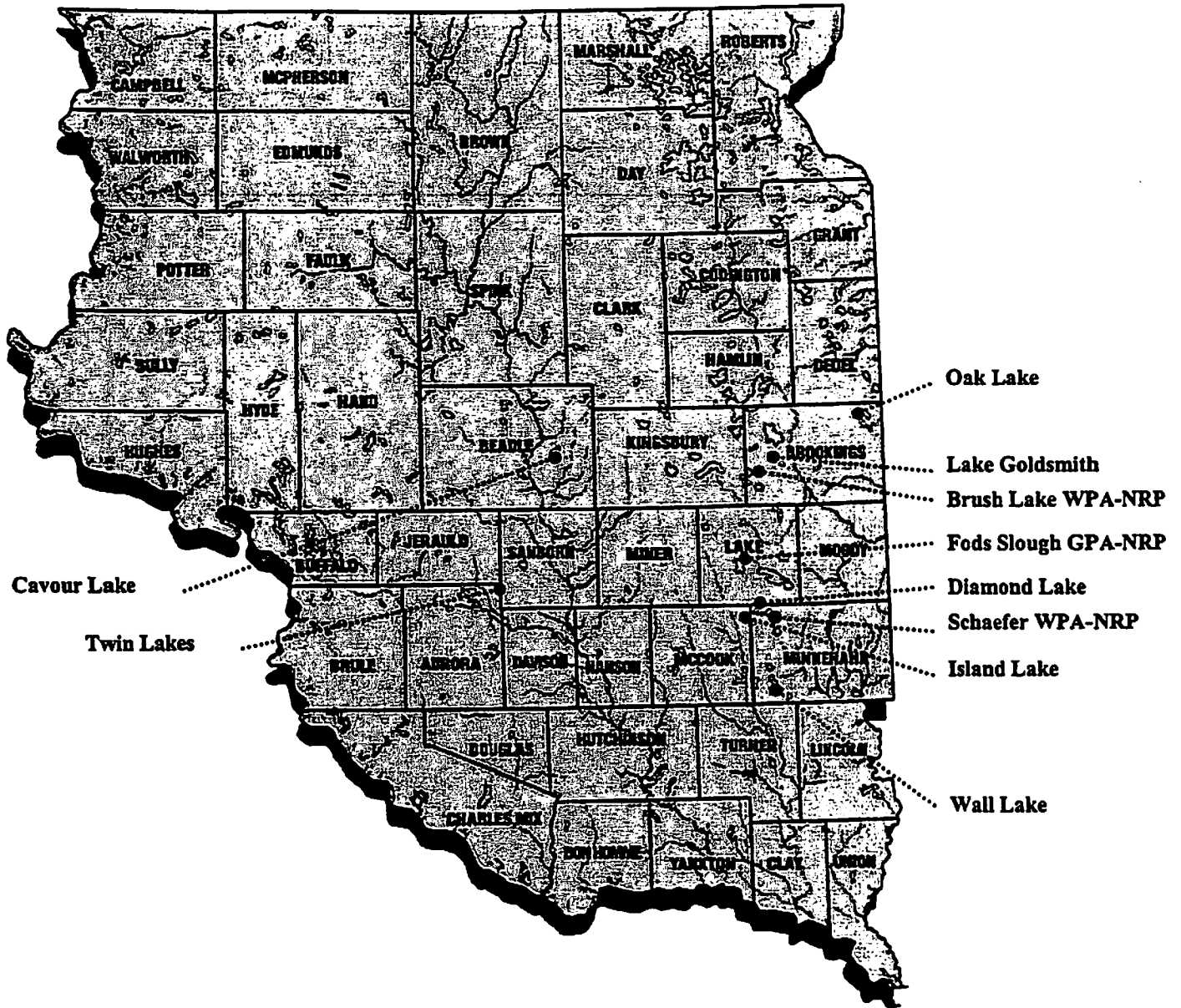
Figure 2.1. Geographic locations of natural rearing ponds (NRP) and lakes used in the current study.

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Harvest and Marking

Yellow perch fingerlings and adults were collected with trap nets from NRPs in Region 3 during fall 1996 and 1997 or spring 1997. NRPs were palustrine-emergent semi-permanent wetlands that were located within U.S. Fish and Wildlife Service (USFWS) Waterfowl Production Areas (WPA) and SDGFP Game Production Areas (GPA) closed to recreational fishing (Figure 2.1). These rearing ponds frequently winterkill allowing a monoculture to be established the following spring by stocking brood fish.

Trap-netted fish were subsampled to determine mean total length (TL, mm) and mean weight (g). All fish were weighed as they were transferred into a 757-L truck-mounted stocking tank to determine total number of kilograms (kg) marked and stocked.

The marking protocol followed that suggested in the previous study (Brown et al. 2000). The water source for marking and holding was the rearing ponds. Yellow perch were immersed for 6 h in a calculated 700-ppm solution of OTC hydrochloride ($C_{22}H_{24}N_2O_9$ •HCL), buffered to a pH of above 7.0 with sodium phosphate (dibasic, Na_2HPO_4). An anti-foam agent (5% silicone base, food grade) was added to the tank to reduce foaming of the OTC solution. The hauling tank was equipped with bottled oxygen and two 12-VDC agitators. Temperature and dissolved oxygen (DO) levels were monitored to ensure that water quality parameters were kept within a normal range for yellow perch. Bagged ice was periodically used on warm days to control water temperature.

Upon completion of the holding period, marked fish were netted out of the stocking tank and released into the designated study lake. Mortalities were removed and enumerated to adjust stocking records. A sample of the marking solution was taken from the tank for to assay the OTC concentration in solution. The remaining OTC solution was flushed out of the tank into a clay-lined pit and allowed to naturally photodegrade.

To determine a marking fraction, a control group containing age-0 and age-1 OTC-marked and unmarked fish was held in the fish holding laboratory at SDSU. Fish were fin clipped to later verify their immersion in OTC. Control fish were kept in a circular tank for a period of 4 months to achieve some growth so that the OTC mark on the otolith would be more identifiable. Thermostat-controlled heaters were used to maintain temperatures within 23 to 28

°C (Hokanson 1977). Fish were fed a prepared grower diet (BioDiet, Warrenton, OR) once each day to satiation for the first 30 d. For the remaining 3 months fish were fed fathead minnows to satiation. Throughout the growth period individuals were periodically euthanized and otoliths were extracted for analysis. Mark presence/absence was determined by blind trial.

Sampling and Processing

Yellow perch populations were sampled with monofilament experimental-mesh gill nets, which are commonly used as standard gear for sampling yellow perch (Lott and Willis 1991). For example, mean CPUE of yellow perch was greater for gill nets in five eastern South Dakota lakes sampled with both frame nets and gill nets (Lucchesi 1991). Lott (1991) found mean gill net CPUE highest in August in a survey of six eastern South Dakota lakes. Although most gill netting was done mid July to mid August, two lakes were sampled in late May.

Gill-net dimensions in the current study were 38 x 1.8 m and contained five panels with bar mesh sizes of 13, 19, 25, 38 and 51 mm. Four nets were set concurrently, approximately 1 h before sunrise and checked at approximately 2-h intervals. Netting continued until 32 h of total netting effort was expended. Relative abundance of yellow perch was indexed with catch per unit effort (CPUE), defined as the number of yellow perch captured per unit of sampling (number per net hour). Yellow perch were kept separate by net of capture and mesh size entangled, measured and weighed.

Scales were removed from the location below the lateral line and at the tip of the pectoral fin (Al-Absy and Carlander 1988). Scales were pressed onto acetate slides and aged using a microfiche projector. Scale focus, annuli, and edge locations were entered into the DISBCAL software program (Frie 1982) using a digitizing pad for back-calculations of length. A standard value of 30-mm was used for scale origination (Carlander 1982).

To determine the origin of the fish, saggital otoliths were extracted, cleaned and dried. The otolith was affixed concave side down on a glass slide with cyanoacrylic glue then allowed to dry in dark storage for 24 h. Otoliths were wet-sanded with 1,000 grit sandpaper and viewed periodically with an Olympus BH2 RFCA compound microscope equipped with 100-W ultraviolet (Hg arc) light source and fluorescent detection assessories (i.e., DMIB filter cube, 505

dichroic mirror, 450 to 495-nm excitation filter, and 515 IF barrier filter). Two independent readers examined otoliths for mark presence.

To assess fish condition, relative weight (Wr; Wege and Anderson 1978) values were determined by length category (Gabelhouse 1984). Relative weight is defined as $Wr = (W/Ws)$ x 100, where W is an individual fish weight and Ws is the specific standard weight (Willis et al. 1991) for a yellow perch of that length. Length categories containing yellow perch sizes assessed in this study included substock (100 to 129 mm), stock to quality (130 to 199 mm), and quality to preferred (200 to 249 mm).

Abiotic and Biotic Parameters

Water quality parameters and primary productivity modify fish growth and densities in natural water bodies. Therefore, water temperature, conductivity, DO, pH, water transparency, and chlorophyll a were measured to characterize each lake in July 1997.

Water temperature and DO were measured at 0.5-m intervals (surface to bottom) with a YSI model 55 portable meter (Yellow Springs Instruments). Conductivity and pH were measured with handheld electronic meters (Oakton Testr series). Water transparency was measured with a Secchi disk.

Subsurface chlorophyll samples were collected from the middle of each lake. Water samples were premeasured and filtered through a 0.8-µm glass microfiber filter of 47-mm diameter. Filters were desiccated, enclosed in aluminum foil, and then frozen for up to 2 weeks, pending analysis. Chlorophyll extractions were done according to the method described by Lind (1985).

Triplicate zooplankton and benthos samples were collected at three separate stations on each lake. Zooplankton were sampled with vertical tows using a Wisconsin plankton net having a 153-µm mesh. The entire water column was sampled in each tow. Zooplankton was preserved in 5% buffered formalin solution. In the lab, zooplankton were concentrated to 40 mL in a graduated cylinder and three subsamples were drawn with a 1-mL Hensen-Stemple pipette. Each subsample was placed in a Segwick-Rafter cell and examined under a dissection microscope (20-

40x). Zooplankton were identified to the lowest taxon possible (Pennak 1989) and expressed as number per m^3 by taxon.

Benthic samples were collected with an Ekman dredge (232 cm^2). Samples were rinsed in a sieve bucket with a No. 30 mesh screen and preserved in a solution of 5% buffered formalin. In the lab, benthic samples were placed in an enamel pan and sorted using the flotation method (Lind 1985). Benthic invertebrates were identified (Merritt and Cummins 1984; Pennak 1989) and expressed as number per m^2 by taxon.

Data Analysis

Stock contribution analysis was done to determine the proportional contribution of stocked fish. The basic formula for each stocking was

$$C = \frac{X}{(S \times P)} ;$$

where C = stock contribution to year class (number of fish),

X = number of marked fish recovered,

S = sampling fraction, and

P = marking fraction (proportion of original stock marked).

Error corrections were done by applying an matrix correction procedure to provide nearly unbiased percent contribution estimates (Cook and Lord 1978). Confidence intervals (95%) for the estimates were derived using the method of Pella and Robertson (1978). Contribution estimates and confidence intervals were calculated using an Excel spreadsheet containing a series of macros developed by Joe Larscheid (Iowa Department of Natural Resources).

All data were tested for normality (Komolgorov-Smirnov test). If the data were normally distributed, a t-test or analysis of variance was used for simple or multiple comparisons, respectively. If the data were not normally distributed, the data were transformed to the ranks and parametric procedures were applied (Conover and Iman 1981). Scatter plots and correlation

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analysis was used to investigate relationships between yellow perch stock contribution and abiotic or biotic parameters. All data were analyzed using SYSTAT (1999). An alpha level of 0.05 was used as a reference level for statistical significance.

Results and Discussion

Yellow Perch Marking and Stocking

A total of 21 stockings (N ≅ 77,000) of juvenile and adult yellow perch occurred on seven lakes from 1996 to 1998 (Table 2.1). All fish were trap-netted from rearing ponds located in Brookings County (Little Brushy WPA-NRP, Knapper WPA-NRP), Minnehaha County (Schaefer WPA-NRP), and Lake County (Fodds Slough GPA-NRP) (Figure 2.1). Stocking rates were approximately 25/ha (low density), 135/ha (medium density), and 200/ha (high density). The medium and high density stockings primarily consisted of juveniles; low density stockings consisted of adult (age 1) fish. Lake Goldsmith was not included in any post-stocking evaluations because the fish community suffered a winterkill during the 1996-1997 winter. Observations from a shoreline survey of the lake immediately following ice-out indicated the fish community was likely eradicated. Oak Lake also experienced a partial winterkill during the same winter. Winterkill losses are primarily attributed to depleted DO concentrations. Yellow perch DO requirements vary with temperature. Yellow perch are somewhat tolerant of low DO levels under the ice by altering their behavior (Petrosky and Magnuson 1973). However, Kreiger et al. (1983) noted several studies of wintertime yellow perch survival that indicated DO levels from 0.2 to 1.5 mg L^{-1} were lethal.

Survival determined immediately following the 6-h marking period ranged from 81 to 100%. There was no apparent relationship between fish densities in transfer tanks and level of mortality, as four of five tank densities that exceeded 100 kg/757 L had 100% survival.

Tank water temperature consistently deviated from rearing pond water temperture by no more than 1.5 °C. Rearing pond and tank water pH ranged from 8.3 to 8.9 and 7.1 to 7.5, respectively. pH of the OTC-treated water did not deviate more than 1.4 units from ambient pH. Tank water DO content ranged from 6.7 to 8.6 ppm, with one exception. A faulty regulator (September 17, 1996) allowed DO levels to decrease to 3.5 ppm. Consequently, fish were

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stocked at 4 h to prevent further stress and potential loss. Otherwise, hourly temperature, pH, and DO readings for the 21 holding periods also did not indicate any apparent events that would contribute to mortality. A random subsample (n = 25) was taken from the group marked for 4 h and held overnight in a net pen at Lake Goldsmith to assess mortality. All fish appeared in good condition upon release.

One factor that could have influenced survival was holding fish in net pens for a period up to 3 d before marking and stocking. Yellow perch catch in the rearing ponds periodically declined; therefore, it was necessary to accrue fish over several days. Although these were atypical holding periods, some mortality was attributed to those stocking groups containing fish held over from previous trap days. We were unable to quantify this effect because net pens contained fish held for various times.

All fish were marked in precalculated concentrations of 700-ppm OTC. Subsequent HPLC analyses (Houglum and Larson 1999) of transport tank waters indicated a range of 422- to 671-ppm OTC in solution. Based on previous immersion marking results (Brown et al. 2000) considerably lower solute concentrations of OTC have produced easily detected marks in yellow perch.

Blind trials were conducted to determine mark presence/absence on yellow perch held at SDSU. Marked fish in the control group were subsampled from three separate stocking loads. Examination of otoliths from 178 (79 OTC marked, 99 unmarked) yellow perch provided a detection rate of 97%. There were no apparent mark detection differences among otoliths from the two age groups; therefore, the marking fraction determined in the control group was applied to all stock contribution estimates.

The quality of OTC marks on otoliths was consistent with those observed in the previous study (Brown et al. 2000). Except for two control fish (marked, but scored as unmarked), marks were readily visible in the remainder of the group. We believe that because of the poor condition of the two control fish, growth was negligible. Agreement between two independent readers was 100%, further indicating that marks were readily detected.

Table 2.1. Stocking summary for naturally reared yellow perch marked with OTC and stocked into eastern South Dakota glacial lakes from 1996 to 1998.

Lake	Stocking Date	Age	Mean TL	Mortality (%)	# Stocked	kg	#/ha
Cavour	September 1996	1	160	0	2,213	102.7	24
	September 1997	0	90	<1	7,271	65.9	
		0	90	<1	10,285	93.3	188
total					19,769	262	212
Diamond	September 1997	0	90	9	12,518	113.5	
		0	90	<1	8,217	74.5	
total					20,735	188	200
Goldsmith[a]	September 1996	0	89	0	2,428	19.9	
		0	89	0	3,624	29.6	
		0	92	0	3,302	26.4	
		0	92	0	1,067	7.7	
		0	110	0	4,250	59.0	
	October 1996	0	110	0	1,400	19.4	
total					16,071	162	138
Island	May 1997	1	169	<1	1,844	100.2	
		1	177	19	616	34.9	
total					2,460	135	24
Oak[b]	September 1996	1	149	0	2,819	104.4	
	October 1996	1	178	0	533	35.5	
		1	172	0	471	31.4	
total					3,823	171	24
Twin	May 1997	1	163	<1	2,150	95.6	
		1	177	<1	565	31.7	
total					2,715	127	27
Wall	October 1996	0	110	0	1,093	59.2	
		0	134	0	4,134	108.8	
		0	110	0	6,188	85.9	
total					11,415	254	136

[a] winterkilled during winter 1996-1997
[b] partial winterkill during winter 1996-1997

CPUE and Stock Contribution

Yellow perch population relative abundance was indexed with gill-net CPUE in four lakes and ranged from 5.4 to 29.6/net-h (Table 2.2). A partial winterkill in Oak Lake may have contributed to the low gill-net CPUE in that lake. A previous study of yellow perch in six eastern South Dakota lakes showed August gill-net catches of stock-length ($\geq$ 130 mm) yellow perch ranged from 1.3 to 37.3/h (Lott 1991); overall August CPUE ranged from 3.4 to 93.4/h in that study. In comparison to that study, we characterized our study populations as low to moderate density.

Gill-netted yellow perch ranged from a minimum of 107 mm to a maximum of 274 mm TL in the current study. Therefore, we assumed there was minimal size-selective bias associated with recapture of stocked fish because recaptured fish ranged from 137 to 257 mm TL. Peak capture efficiencies reported for 100, 140, 200, and 260-mm TL yellow perch were correlated with 13, 19, 25, and 38-mm bar meshes (Lott and Willis 1991).

Gill netting was ineffective in Cavour and Diamond lakes. Additionally, no fish from the 1996 stocking were recovered. Therefore, additional effort was expended to collect fish from the 1997 fingerling stocking using daytime boat electroshocking (Cavour Lake), trap nets (Cavour Lake), and cloverleaf minnow traps (Diamond Lake) to estimate stock contribution (Table 2.2). These gears were more successful, likely because yellow perch were using the littoral zone in these lakes more extensively than offshore areas during late May. Fisher et al. (1999) reported that daytime abundance of juvenile yellow perch was inversely correlated with depth in Pickerel Lake. Also, relative abundance was inversely related to substrate coarseness and positively related to chironomid density in that study. The nearshore distribution of yellow perch in Cavour and Diamond lakes could also have been influenced by several environmental factors.

Although CPUE of the gears differed, stock contributions determined from electrofishing and trap net samples were similar. Stock contribution was positively related to CPUE of unmarked fish (r = 0.45) likely indicating that contributions were density independent across this group of study lakes. Overall, stock contributions to existing populations ranged from 5.1% to 41% (Table 2.2), indicating that transferred yellow perch successfully contributed to existing populations. Diamond and Wall lakes received high and medium density age-0 yellow perch

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Table 2.2. Age group stocked, recapture periods, catch per unit effort (CPUE), and year class stock contributions for marsh-reared yellow perch transferred into six eastern South Dakota lakes with existing yellow perch populations. Confidence intervals (95%) for the estimates were derived using the method of Pella and Robertson (1978).

Lake	Age group stocked	Recapture period	CPUE (#/h)	% contribution with 95% CI
Cavour[a]	age 0	May 1998	15.1	15.5 (7.2 to 23.8)
Cavour[b]	age 0	May 1998	3.3	10.4 (4.7 to 16.1)
Diamond[c]	age 0	May 1998	15.1	41.0 (25.9 to 56.2)
Island[d]	age 1	August 1997	7.2	17.7 (5.8 to 29.5)
Oak[d]	age 1	July 1997	5.4	5.1 (1.0 to 9.1)
Twin[d]	age 1	August 1997	10.2	41.0 (24.5 to 57.6)
Wall[d]	age 0	July 1997	29.6	37.9 (30.4 to 45.5)

[a] electrofishing
[b] trap net
[c] minnow trap
[d] gill net

stockings. Those stock contributions were about 40%; thus, stocking rates of at least 100/ha in low to moderate density receiving populations are likely to provide a significant increase to that age group. Low density yellow perch stockings also contributed to age-1 year classes in Island, Oak and Twin lakes. However, results were more variable (5 to 41%), likely due to natural variation in year-class strength among lakes and/or survival of stocked fishes.

Growth and Condition

Back-calculated lengths were used to determine length at age for stocked and naturally produced yellow perch for a growth comparison. Generally, growth of stocked yellow perch was relatively consistent with that of resident perch (Table 2.3). Slight differences in growth were observed; however, there were no consistent patterns of increased or decreased growth of stocked fish. For example, back-calculated length at age 1 for yellow perch stocked as fingerlings was slightly greater than that of resident fish in Cavour and Diamond lakes, but resident and stocked perch length at age 1 was identical in Wall Lake. Stocked yellow perch length at age 2 (age 1 at stocking) was slightly greater than resident perch in Twin Lake, but slightly less in Island and Oak lakes. Size differences observed in these older fish may have been conferred at an earlier age, prior to stocking. Additionally, females grow considerably faster and reach larger ultimate sizes than male yellow perch (Heidinger and Kayes 1986); secondary sex characteristics were not accounted for in our study.

Yellow perch growth is extremely variable, depending on a variety of factors but largely influenced by population density, habitat size and productivity (Scott and Crossman 1973). In eastern South Dakota lakes, yellow perch growth was strongly correlated with population size and density (Lott et al. 1996), mean lake depth, shoreline development, submerged aquatic macrophytes and availability of macroinvertebrates as a primary source of prey (Lott 1991). In a study of 20 South Dakota yellow perch populations, growth rates were determined to be highly variable, but with regional means similar to those cited by Carlander (1997) for ages 2, 3 and 4 (Willis et al. 1992). Across populations in the current study, length at age 1 was equal to or greater than the South Dakota mean. Mean length at age 2 ranged from 107 to 181 mm, encompassing the South Dakota mean.

Mean Wr of yellow perch was assessed by length category (Table 2.4). No significant differences were detected between marked and unmarked fish, and fish were therefore combined within length categories. In Cavour and Diamond lakes (May sample), stock to quality fish had the lowest condition. Low Wr values of reproductively mature fish is expected following the spawning period. However, quality to preferred fish in Cavour Lake had the highest mean Wr of all population segments. Alternatively, low Wr values are associated with scarce or inappropriately sized prey and high Wr values indicate an adequate abundance of appropriate-sized prey. Mean Wr values were lowest for the two length categories assessed for Island Lake sample during summer. For yellow perch captured during July and August, we assumed no effect due to reproductive status. The use of Wr as an indicator of prey availability has been demonstrated in other studies (Blackwell et al. 2000). Although prey utilization was not a component of the current study we assumed that differences in condition among length classes and lakes could be, in part, attributed to prey abundance.

Table 2.3. Sample size (N), mean back-calculated total length at age (mm) from scales, and standard error (±SE) for marked (X) and unmarked (UN) yellow perch in six eastern South Dakota lakes. South Dakota mean back-calculated lengths are the unweighted mean of means (Willis et al. 1992).

Lake	N	Mean back-calculated total length at age (mm)				
		1	2	3	4	5
Cavour (X)	14	89.7 (2.4)				
Cavour (UN)	58	79.3 (1.3)	121.3 (3.8)	168.6 (8.3)	215.9 (10.3)	242.1 (13.0)
Diamond (X)	11	95.9 (3.8)	107.2 (4.0)			
Diamond (UN)	15	76.0 (0.6)	108.0 (0.7)	131.2 (1.5)	149.3 (2.1)	
Island (X)	5	79.0 (2.1)	133.5 (8.6)	170.4		
Island (UN)	23	83.4 (2.2)	150.0 (2.4)	193.0 (0.2)		
Oak (X)	4	93.7 (7.3)	175.6 (14.5)			
Oak (UN)	84	110.5 (2.5)	178.4 (2.7)	211.4 (4.9)		
Twin (X)	10	87.3 (2.2)	159.0 (4.6)			
Twin (UN)	14	80.9 (3.7)	152.9 (3.6)	184.4		
Wall (X)	45	100.9 (1.8)	160.9 (7.0)			
Wall (UN)	76	100.1 (1.2)	181.6 (1.8)	216.2 (7.0)	232.9	
South Dakota mean		80	139	181	210	251

Table 2.4. Sample size (N), mean relative weight (Wr), and standard error ($\pm$SE) for yellow perch collected from six eastern South Dakota lakes. Length categories were sub-stock (SS) 100-129 mm, stock-quality (S-Q) 130-199 mm, and quality-preferred (Q-P) 200-249 mm. Dissimilar superscripts following mean Wr values indicate significant differences (P < 0.05) among length categories.

Lake	N	Mean Wr		
		SS	S-Q	Q-P
Cavour	28	101 (3.1)[a]	93 (1.6)[b]	115 (5.2)[c]
Diamond	87	106 (2.6)[a]	92 (2.3)[b]	
Island	28		80 (6.2)[a]	85 (5.2)[a]
Oak	82		103 (1.7)[a]	108 (1)[a]
Twin	25		103 (3.3)	
Wall	118		87 (1.7)[a]	92 (2.6)[a]

Environmental Factors affecting Stocking Success

Several abiotic and biotic factors were measured in an attempt to determine some of the limnological factors that may influence stocking success of yellow perch. Although physicochemical characteristics varied considerably among the study lakes (Table 2.5), these conditions were within acceptable ranges for yellow perch survival and growth (Heidinger and Kayes 1986). Conductivity was considerably lower in Oak Lake as compared to the other water bodies. Secchi disk transparency values in the study lakes ranged from 0.15 to 1.6 m. Oak and Cavour lakes had the lowest transparency and also contained a very limited amount of submerged aquatic vegetation. Conversely, Wall and Island lakes had the highest secchi transparency and contained the most aquatic vegetation of the study lakes. Chlorophyll a concentrations ranged from 8.7 mg L^{-1} to 130.5 mg L^{-1}, providing an indicator of primary productivity. Based solely on chlorophyll values, trophic states were mesotrophic (Cavour, Island, and Oak lakes), eutrophic (Diamond Lake), and hypereutrophic (Twin and Wall lakes).

August water temperature and DO profiles were similar among the study impoundments. Water temperatures in the six lakes ranged from 22.4 to 27.7 °C at the surface to 18.3 to 22.6 °C at the bottom. Although DO concentrations remained above 5 mg L^{-1} throughout most of the water column, low DO levels (i.e., <2 mg L^{-}) were detected in the bottom 0.5-m of water in each of the study lakes. Kreiger et al. (1983) indicated that DO concentrations <3.1 mg L^{-1} were lethal at 25 °C. The same authors concluded that the lower optimum DO limit for yellow perch would be 5 mg L^{-1} under conditions of long term exposure. However, no summerkills resulting from depleted oxygen levels were observed or reported.

Generally, yellow perch food habits in glacial lakes during the growing season reflect a diet of zooplankton, especially cladocerans, other aquatic invertebrates (primarily chironomids and corixids), and to a much lesser extent, prey fishes. Food habits also indicate that prey size, in addition to prey abundance, is also an important aspect of feeding ecology of yellow perch. Fishes are not often a primary summer diet component of yellow perch in glacial lakes, especially where invertebrate densities are characteristically high. The mean relative importance of fishes (e.g., fathead minnows *Pimephales promelas* in yellow perch diets sampled seasonally from six South Dakota populations did not exceed 3 (Lott et al. 1996).

Zooplankton composition and densities varied among the lakes (Table 2.6). Copepod and cladoceran densities typically far exceeded rotifer density, except in Diamond Lake where a large number of *Keratella* were present. *Bosmina longirostris* were the dominant cladoceran in Diamond and Oak lakes; *Daphnia* spp. were the dominant cladoceran in samples collected from Cavour, Island, Twin and Wall lakes. Total abundance of zooplankton was most similar between Cavour and Twin, and Island and Oak. Copepod nauplii were most abundant in Diamond Lake. Lott et al. (1996) found that the relative importance of zooplankton (primarily *Daphnia* spp., *Bosmina* spp., and *Leptodora kindti*) ranged from 7 to 80% in six yellow perch glacial lake populations. Also that study reported that zooplankton were of higher relative importance in yellow perch populations having low size structures and slower growth. Similarly, Fisher et al. (1999) found that larval yellow perch abundance was correlated with zooplankton abundance (i.e., *Bosmina* and cladocerans), indicating the importance of zooplankton to early survival.

Estimates of total zoobenthos densities were highest in Cavour and Island lakes (Table 2.7). Benthic samples from all six lakes were dominated by chironomids. *Chaoborus* spp. were the only other taxa present in all lakes, however densities were consistently low. Chironomid densities were generally well under 1,000 organisms/m^2, with the exception of Island Lake (1,527 organisms/m^2). This taxon composed 39% (Twin Lake) to 87% (Island Lake) of total densities. These chironomid densities were similar to low to moderate July densities observed in a previous study on six glacial lakes (Lott et al. 1996), which ranged from 656 to 2,098 chironomids/m^2. In lakes having high chironomid densities, they were the major dietary component of yellow perch in those six lakes, with relative importance values ranging from 25 to 56. In another study, age-0 yellow perch distribution also was positively associated with chironomid densities in July (Fisher et al. 1999). Additionally, Lott et al. (1996) found that macroinvertebrate relative importance values ranged from 48 to 91, indicating the overall importance of benthic organisms to yellow perch populations. In comparison to the Lott et al. (1996) and Fisher et al. (1999) studies, it appears zooplankton were the more abundant prey item available to yellow perch in our study.

Contribution estimates were assessed in relation to measured variables that could conceivably affect stocking success. These factors included stocking variables (e.g., stocking

temperature, holding tank density, stocking density), abiotic variables (e.g., lake surface area, mean.depth, Secchi depth) and biotic variables (e.g., chlorophyll *a* concentrations, unmarked yellow perch CPUE, zooplankton density, benthic invertebrate density). Because of the different stocking rates, relative stock contribution estimates were calculated by correcting (weighting) for stocking rates. Adjusted, or relative, stock contribution estimates were 7.3, 20.5, 73.8, 21.3, 151.9, and 27.9 for Cavour, Diamond, Island, Oak, Twin, and Wall lakes, respectively. These relative contribution estimates indicate greater year class contributions in Island and Twin lakes, more so than in the other study lakes.

Scatterplots and correlation matrices were produced for each bivariate analysis to visualize linear or curvilinear factor relations to relative stock contributions. A total of 21 variables were evaluated for relationships. No stocking variable was apparently related to stock contributions.

Of the abiotic variables, only maximum depth ($r = 0.49$) and conductivity ($r = 0.79$) provided significant linear associations with relative stock contributions. Generally, yellow perch populations found in deeper lakes of eastern South Dakota are characterized as high density, slower growing, and having more constant recruitment. We anticipated a negative depth-contribution relationship; however, CPUE values were not greater in deeper lakes indicating that densities did not increase with maximum depth. Although the maximum depth range for study lakes was 0.9 to 3 m, it is possible that slightly deeper lakes provided more suitable environment for stocked yellow perch. Yellow perch are described as temperate mesothermal (Hokanson 1977) with seasonal vertical movements to suggest that they follow a 20 °C isotherm (Scott and Crossman 1973). Deeper study lakes were more likely to provide areas of thermal refugia during summer months. Similarly, the likelihood of winterkill diminishes in deeper water bodies.

Chlorophyll *a* concentrations ($r = 0.69$) and *Daphnia* spp. densities ($r = 0.71$) were two biotic variables significantly related to relative contribution estimates. From a energy transfer standpoint, it is reasonable to assume that these indicators of primary and secondary production would be linked to the effectiveness of stockings.

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Additional studies are required to determine the primary factors that influence stocking success in shallow glacial lakes. These studies should likely be done on a seasonal basis and focus heavily on abiotic characteristics (e.g., trophic state, oxygen and thermal constraints) across lake types and biotic characteristics (predator size structures and densities, prey availability, densities of conspecifics). Broader understanding of glacial lake characteristics and relationships to yellow perch ecology could lead to development of models that provide guidance for perch stockings.

Table 2.5. Morphometric and physiochemical characteristics of six eastern South Dakota lakes. Surface area and maximum depth were provided from SDGFP lake surveys; secchi disk transparency, chlorophyll a, pH, and conductivity were measured in July 1997.

Lake	Surface area (ha)	Maximum depth (m)	Secchi depth (cm)	Chlorophyll a (mg L^{-1})	pH	Conductivity (uS/cm)
Cavour	93	2.4	20	12.7	8.6	940
Diamond	104	3.5	35	48.2	8.8	1,100
Island	101	4.5	160	8.7	8.6	1,360
Oak	160	1.8	15	14.5	8.4	460
Twin	102	3.8	37	130.5	8.4	1,850
Wall	84	5.5	160	80.5	8.6	1,360

Table 2.6. Mean zooplankton densities expressed as (organisms/m^3) for the major zooplankton taxa present in six eastern South Dakota lakes sampled in July 1997. *Bosmina* species was *longirostris*, *Diaphanosoma* species was *birgei*, and *Leptodora* species was *kindti*. Standard errors are in parentheses (SE).

Taxa	Lake					
	Cavour	Diamond	Island	Oak	Twin	Wall
Copepoda						
Calanoid	21,337 (2,090)	50,442 (9,198)	200,889 (73,451)	17,182 (4,422)	6,790 (764)	10,634 (1,442)
Cyclopoid	13,153 (4,372)	23,623 (4,248)	11,321 (2,899)	27,336 (7,480)	4,554 (898)	63,893 (6,043)
Copepod nauplii	2,508 (540)	41,598 (6,949)	10,796 (3,547)	6,057 (2,165)	2,287 (466)	4,433 (797)
Total	36,998	115,663	223,006	50,575	13,631	78,960
Cladocera						
Bosmina	2,378 (3,275)	80,526 (14,798)	*	105,152 (35,028)	2,367 (582)	7,177 (3,493)
Daphnia spp.	33,270 (922)	32,423 (4,935)	33,332 (9,460)	50,342 (15,031)	70,784 (8,830)	14,115 (3,286)
Diaphanosoma	458 (176)	13,601 (1,337)	1,005 (565)	8,522 (2,496)	*	*
Leptodora	29 (29)	*	*	*	*	*
Total	36,135	126,550	34,337	164,016	73,151	21,292
Rotifera						
Keratella spp.	756 (225)	816,979 (203,461)	273 (180)	1,545 (542)	1,177 (464)	17 (17)
other rotifers	542 (279)	*	*	*	*	*
Total	1,298	816,979	273	1,545	1,177	17
Overall totals	74,431	1,059,201	257,616	216,136	87,959	100,269

* taxa not observed

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Table 2.7. Mean benthic macroinvertebrate densities (organisms/m^2) for major taxa sampled in six eastern South Dakota lakes sampled in July 1997. Asterisks indicate that taxa was not observed in the sample. Standard errors are in parentheses (SE).

Taxa	Lake					
	Cavour	Diamond	Island	Oak	Twin	Wall
Ephemeroptera	*	*	5	111 (89)	5	5
Diptera						
Chironomidae	932 (106)	237 (92)	1,527 (641)	693 (52)	386 (243)	420 (173)
Ceratopogonidae	10	*	*	*	*	*
Chaoboridae	5	24 (13)	5	5	5	5
Total	947	261	1,532	698	391	425
Pelecypoda						
Sphaeriidae	14	*	*	*	*	*
Gastropoda						
Physidae	92 (23)	*	96 (24)	*	275	*
Valvatidae	72 (65)	10	101 (59)	*	67	82 (62)
Lymnaeinae	*	14	*	5	164 (61)	*
Planorbidae	155 (79)	193 (172)	29	*	77 (39)	*
Total	319	217	226	5	583	82
Oligochaeta	39 (26)	5	*	*	5	38 (31)
Overall totals	1,319	483	1,763	814	984	550

Acknowledgments

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