An Evaluation of Redd Counts as a Measure of Bull Trout Population Size and Trend

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ARTICLE

An Evaluation of Redd Counts as a Measure of Bull Trout Population Size and Trend

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Abstract
The use of redd counts to monitor abundance and trend of bull trout Salvelinus confluentus has been clouded by uncertainties concerning measurement error, life history variation, and correspondence of redd counts to adult population size. We compared census redd counts with population estimates of mature females for a migratory fluvial population of bull trout (primarily ≥ 300 mm fork length) and for a population of small (<200 mm), likely resident, bull trout. We also compared the measurement error of the experienced surveyors who conducted the redd counts to that of a group of inexperienced surveyors. Although the regression of redd counts on adult females for the migratory population was statistically significant, a large proportion of the variation in the relationship was unexplained ($r^2 = 0.47$). Despite that variation, redd counts accurately reflected a greater than 50% decline in the population over 10 years; however, 5-year trends in redd counts could be misleading. Power analysis parameterized by using the variation in the number of females per redd and measurement error of experienced surveyors indicated that minimum declines of 44–56% or increases of 78–118% over 10–15 years would be necessary for detection using traditional statistical criteria. Geometric mean abundance of migratory adults derived from redd counts and adults-per-redd values from the present study and published averages were similar to measured adult numbers in most cases. For both migratory and resident populations, redd counts by experienced surveyors were substantially more accurate and precise than those by inexperienced surveyors. Counts of migratory bull trout redds were more accurate and precise than counts of resident bull trout redds, which were significantly smaller and consistently underestimated. Thus, bull trout redd counts can be used to estimate abundance levels and to detect substantial longer-term changes in abundance, particularly for migratory populations. However, the reliability of the counts depends on the skill of the surveyors.

Estimates of abundance and trend (i.e., change in abundance over time) are critical in determining the status of species, particularly those that are suspected to be rare or declining. Bull trout Salvelinus confluentus are listed as threatened under the Endangered Species Act in the coterminous United States (USFWS 1999) and are classified as sensitive in Alberta, British Columbia, and the Yukon Territory, Canada (ASRD/ACA 2009). The draft bull trout recovery plan (USFWS 2002) contains numeric objectives for adult abundance in core areas or groups of populations (metapopulations) and objectives for maintaining stable or increasing trends in adult abundance. Consequently, monitoring abundance and trend is important for assessing recovery of the species.

Redd counts have been and continue to be the most widely used method for monitoring adult bull trout abundance (Rieman and Myers 1997; Dunham et al. 2001) and are used extensively for other salmonids, particularly anadromous forms (Gallagher et al. 2007). However, among salmonid species there are
considerable differences and similarities in the timing and
distribution of spawning, the size of adults and redds, and life
history forms, as well as in the physical characteristics and
conditions of the spawning areas (e.g., substrate and flow).
These factors can influence error in the redd counts and,
consequently, design of the surveys (Gallagher et al. 2007).

Redd counts offer several advantages for monitoring bull
tROUT abundance. Since redds are constructed by reproductive
fish homing to natal streams, they directly relate to the metric
of interest (adult bull trout) and generally reflect the spatial
distribution of breeding populations. Consequently, they do not
rely on additional assumptions or data regarding survival or
distribution, which are needed when using estimates of other
life stages. Bull trout spawn during early fall when flows and
turbidity are low, conditions that are conducive to detecting
redds (Rieman and Myers 1997). Redd surveys can also be
less expensive and more easily conducted than other inventory
methods, such as trapping or mark–recapture (Dunham et al.
2001). Redd counts can be used alone as an index of adult
abundance to determine population trends (Rieman and Myers
1997; Maxell 1999) or can be combined with information on
the ratio of redds to adults in order to estimate population size

Despite these advantages, the reliability of applying bull trout
redd counts to estimate adult abundance depends on several
factors influencing the precision and accuracy of these counts.
Such factors include measurement error in counting redds and
the relationship of redd counts to the number of adults. High
measurement error or a low correlation between redd counts
and the number of adults could, at best, obscure patterns in
abundance and reduce power to detect trends (Maxell 1999); at
worst, they could lead to erroneous conclusions.

In several previous analyses of bull trout redd counts, the
measurement error and adults-per-redd relationship were un-
known (Rieman and Myers 1997; Maxell 1999; Al-Chokhachy
et al. 2005). Dunham et al. (2001) found substantial measure-
ment error from high variation in counts among surveyors. Sur-
veyor counts were 28–254% of the best estimate of the "true"
number of redds. In a study limited to experienced surveyors,
Muhlfeld et al. (2006) found less measurement error (78–130% of
the best estimates) and applied detection efficiencies to cor-
rect redd counts for observer error and provide confidence
intervals (CIs).

Besides variability among surveyors, the timing, frequency,
and spatial coverage of redd surveys can profoundly influence
the ability to estimate total redd numbers (Dunham et al. 2001;
Courbois et al. 2008). For example, redd surveys are frequently
limited to "index" areas (e.g., Al-Chokhachy et al. 2005) rather
than randomly or probabilistically selected sites within the total
spawning distribution or complete surveys of the spawning dis-
tribution (Stevens et al. 2007; Jacobs et al. 2009). Variation in
the spatial distribution of spawning between index and unsurveyed,
nonindex areas may influence the detection of population trends
(Dunham et al. 2001; Isaak and Thurow 2006; Courbois et al.
2008). Different redd densities in nonindex areas could result
in inaccurate expansions of index counts for estimates of total
redd or population abundance (Jacobs and Nickelson 1998).

To generate estimates of population abundance, perhaps more
critical than the relationship of redd counts to true redd numbers
is the relationship of redd counts to numbers of adults. Com-
bined redd counts and corresponding independent abundance
estimates of spawning bull trout for the Wigwam River and
tributaries of Lake Pend Oreille and the Metolius River were
highly correlated but only on a log scale (Dunham et al. 2001).
The number of adults per redd varied substantially from 1.03
to 3.33, which Dunham et al. (2001) suggested could be due to
error in the estimates or to varying life history patterns. Ad-
ditional work has expanded the upper end of the range of adults
per redd to 4.3 (Taylor and Reasoner 2000). Thus, there is a need
to develop population-specific relationships, or at least regional
and life history-related relationships, to derive more accurate
abundance estimates.

Life history variation could affect both redd counts and es-
imates of adult abundance. Bull trout have been typified as
having two primary life history forms: migratory and resident
(Rieman and McIntyre 1993). Migratory juvenile bull trout rear
in tributaries, where spawning also occurs, for several years be-
fore moving downstream to larger rivers (fluvial forms) or lakes
(adfluvial) to mature. Later, as adults, they return to spawn in
natal streams, after which they move back down to the rivers or
lakes (Pratt 1992). Resident forms, on the other hand, remain
in upper reaches and smaller tributaries during all life stages.
Although there may be substantial size differences between resi-
dent and migratory adults (e.g., 150–300 and >300 mm, respec-
tively; Goetz 1989; Mullan et al. 1992; Pratt 1992), there is little
size differential between resident and migratory juveniles or be-
tween older migratory juveniles and resident adults (Rieman
and McIntyre 1993). Consequently, it is difficult to identify and
enumerate resident adults based on size or other external char-
acteristics. Although other authors have speculated about the
potential influence of differences in life history on redd counts
(Al-Chokhachy et al. 2005), factors related to classifying adults
and redds as resident or migratory (e.g., maturity, size at ma-
turity, and redd size) have not been evaluated and the influence
of life histories on redd counts has not been explicitly assessed.
Bull trout may also spawn either annually or in alternate years
(Pratt 1992). Failure to account for nonspawning adults could
result in underestimates of adult numbers based on redd counts.

Thus, while there is some evidence suggesting correlation
between redd counts and adults, there is also evidence of sub-
stantial variability in redd counts from measurement error and
differences in life history that could affect the utility of redd
counts for estimating abundance. Previous evaluations of bull
tROUT redd counts were lacking in (1) estimates of measurement
error in the redd counts, (2) estimates of the numbers of adults
for comparison, (3) estimates of the effect of life history dif-
fences on the redd counts, or (4) some combination of these.
The primary purpose of this study was to robustly evaluate the
relationship of redd counts to adult abundance for migratory and resident forms of bull trout by addressing possible sources of error in both estimates. Our objectives were to (1) compare estimates of adult population size generated from redd counts (redds per mature female or redds per adult) with those derived from direct estimates of adults, (2) compare trends in abundance based on adult estimates versus redd counts, (3) estimate the error in the redd counts due to variability among experienced versus inexperienced observers, and (4) determine the influence of the variability in redd counts and adult estimates on the detection of trends.

**STUDY AREA**

This study was conducted in the upper Mill Creek watershed (Walla Walla River subbasin) in northeastern Oregon and southeastern Washington (Figure 1). Previous redd surveys (Hemmingsen et al. 2001b, 2001c) indicated that 99% of the migratory bull trout population spawned upstream of a 2.5-m-high diversion dam, which was equipped with a fish ladder, at river kilometer 41. The Mill Creek watershed above the diversion dam is 8,798 ha (NPPC 2004). It was designated as the municipal water supply for Walla Walla, Washington, in 1918 and has been closed to public entry since 1974. The area is largely roadless and is closed to grazing and timber harvest. The tributaries to Mill Creek above the diversion dam are short (<6 km perennial flow) and contribute small amounts of flow (≤0.1982 m³/s [≤7 ft³/s] each) during summer base flow conditions (U.S. Forest Service, unpublished data). Besides bull trout, rainbow trout *Oncorhynchus mykiss*, steelhead (anadromous rainbow trout), sculpins *Cottus* spp., western brook lampreys *Lampetra richardsoni*, suckers *Catostomus* spp., and mountain whitefish *Prosopium williamsoni* also occur in upper Mill Creek. Spring Chinook salmon *O. tshawytscha* were historically present, and adults have been occasionally reintroduced to spawn.

**METHODS**

*Female abundance.*—Since redds are constructed by spawning females (Esteve 2005), we estimated numbers of mature bull trout females for the most direct comparison with the number of redds. To estimate the number of fluvial adult females, we used a combination of upstream trap counts and mark–recapture estimates. During 1998 through 2007, we operated a trap at the upstream end of the ladder at the diversion dam from mid-May through mid-October, which spanned the period when migratory fish moved above the dam (Hemmingsen et al. 2000). Bull trout trapped at the ladder were anesthetized with tricaine methanesulfonate (MS-222; 60 mg/L), measured for fork length (FL), weighed, and interrogated for a passive integrated transponder (PIT) tag; if no PIT tag was present, the fish was injected with one. In 2002–2007, we inspected each fish for maturity by using ultrasound to identify mature females. Ovaries and eggs can be readily identified with this technique (e.g., Evans et al. 2004). We used PIT tag recaptures to determine annual frequencies of upstream migration and gonadal maturity for individual fish. To estimate the number of females trapped during 1998–2001 prior to our use of ultrasound, we multiplied the total trap count of 300-mm or larger bull trout (see below) in those years by the mean fraction of females trapped in 2002–2007.

A telemetry study indicated that most fluvial adult bull trout in the Mill Creek drainage overwintered downstream from the dam; however, a few overwintered upstream of the dam (Hemmingsen et al. 2001b, 2001c). In 2003–2007, bull trout
captured in the trap were marked with a caudal punch to facilitate a mark–recapture (resight) estimate to account for any fluvial adults that had overwintered upstream from the dam and thus were not included in the counts at the ladder trap. The location of the caudal punch was changed each year so that marked fish from various release years could be distinguished.

To obtain mark–resight estimates, a single diver snorkeled all the pools and other habitats of sufficient depth to hold fluvial adult-sized fish on two to three consecutive days during mid- to late August prior to the onset of spawning. The diver recorded the number of marked and unmarked bull trout (300 mm or larger) that were observed. Trapping data for the previous 5 years indicated almost all of the migratory fish returning to spawn in the population were 300 mm or larger (Hemmingsen et al. 2001b, 2001c). We estimated the total number of unmarked, 300-mm or larger bull trout by incorporating the number of marked and unmarked bull trout observed while snorkeling and the number of marked bull trout released upstream of the trap at the time of snorkeling into Bailey’s modification of the Peterson mark–recapture formula (Ricker 1975), which accounts for replacement of marked fish into the population,

\[ N = \frac{M \times (C + 1)}{(R + 1)} \]

where \( N \) is the population size, \( M \) is the number of marked bull trout, \( C \) is the total number of 300-mm or larger bull trout that were observed (recaptured) by the snorkeler, and \( R \) is the number of marked bull trout observed by the snorkeler. We then multiplied the estimate of unmarked adults \( (N - M) \) by the female fraction of the bull trout trapped that year to determine the number of unmarked females above the trap. Since the percentage of marked fish in the snorkel count exceeded 10% of the total snorkel count, CIs were based on a binomial distribution (Seber 1982). To estimate the number of 300-mm or larger females that remained above the trap in 1998–2002 prior to our mark–recapture estimates, we multiplied the number of females trapped in each of those years by the mean proportion that the unmarked females from the mark–recapture estimates represented in the number of females trapped during 2003–2007.

To identify mature resident females (<300 mm), we used endoscopy to examine bull trout (130–299 mm FL) collected by electrofishing a systematic sample of habitat units (23% of the pools and 14% of the riffles) of Mill Creek and its tributaries above the diversion dam in 2002. Endoscopy has been shown to be a highly accurate technique for determining maturity and sex in small salmonids (Swenson et al. 2007). Similar work we previously conducted in Silver Creek (Powder River subbasin, Oregon) indicated that mature resident females were 140–200 mm (Hemmingsen et al. 2001b). Therefore, to be conservative, we inspected every captured bull trout that was 130 mm or larger. Since no mature, resident-sized females were found that year in Mill Creek and tributaries where fluvial-sized fish had previously been observed to spawn, subsequent sampling of resident-sized females smaller than 300 mm was limited to Low Creek (Figure 1), where only resident-sized fish smaller than 300 mm had been observed to spawn.

In early August 2003 and 2005, we blocknetted and electrofished every third pool and riffle to obtain depletion estimates of mature resident females in Low Creek. We completed at least two passes until we achieved a 67% reduction from the previous pass. Fish were examined endoscopically for maturity by following the methods of Swenson et al. (2007). Densities were determined by dividing removal estimates of female adults by the surface area of the units sampled. These densities were expanded across the available habitat area in the reach where the density samples were collected to obtain a total abundance estimate of mature resident females using methods described by Hankin and Reeves (1988). Prior to the depletion estimates, habitat units throughout Low Creek were classified as pools or riffles and the unit areas were measured. Because of possible bias in electrofishing depletion estimates, in 2005 we evaluated bias in our estimates by following the methods of Peterson et al. (2004). In a subsample of eight pools and eight riffles longitudinally spaced throughout the distribution of bull trout in Low Creek, we applied a caudal fin clip to 130-mm or larger bull trout that were collected during the removal passes and then returned the marked fish to the blocknetted units. The next day, we completed electrofishing removal passes in these blocknetted units, and the marked and unmarked bull trout in the catch were measured and counted.

**Redd counts.**—We conducted three to four redd counts at 2–3-week intervals during September and October in Mill Creek and its tributaries upstream of the diversion dam to the upstream limits of fish distribution. Data for Mill Creek indicated that the spawning duration was from the last week of August through October (Hemmingsen et al. 2001b) and that 94% of the redds remained visible during that survey interval (Hemmingsen et al. 2001a). Thus, the spatial extent, frequency, and timing of our redd counts should have provided essentially a complete count or “census” of the number of detectable redds (after Gallagher et al. 2007; Jacobs et al. 2009).

On each survey, each new redd observed was flagged and uniquely numbered, and the combined area of the pit and tailspill was calculated using measurements of the maximum length and width (Zimmerman and Reeves 2000). Flagged redds from previous surveys that year were remeasured. We used the largest redd area measured during all of the surveys to ensure that the area represented a fully completed redd. Lengths of bull trout observed on or within 1 m of the redds were visually estimated to the nearest 50-mm length-class. For comparison of redd sizes, we also measured redds using the same methods in the Little Minam River (adjacent Grande Ronde River subbasin), where, like Low Creek, all bull trout observed during previous spawning surveys and other sampling (e.g., electrofishing) were smaller than 300 mm.

Surveys were conducted by three to six individuals with prior experience in counting bull trout redds, and over the course of
the study most of the surveys were done by the same individuals. Each of the three of the surveyors who conducted redd counts throughout the study had about 14 years of experience in conducting bull trout redd surveys. A surveyor added later had 9 years of experience in counting bull trout redds. Most of the surveyors had additional experience surveying redds of Chinook salmon, coho salmon *O. kisutch*, and summer steelhead. A group of inexperienced surveyors, who were conducting bull trout redd counts in other adjacent portions of the Walla Walla and Umatilla River subbasins, were also evaluated for measurement error for purposes of comparison with the experienced surveyors counting redds in Mill Creek and its tributaries. At the time of evaluation, the inexperienced surveyors had 2 weeks of training that covered redd characteristics and observation of bull trout redds and 6 weeks of experience in counting bull trout redds during regularly scheduled surveys.

To estimate potential observer error in our redd surveys, in 2004 we established three 1-km test reaches in a section of main-stem Mill Creek that was used by larger, fluvial adults and three 1-km reaches in Low Creek, which was used by smaller, resident-sized adults. Redds were identified and flagged during the first survey on 21–22 September. To determine the best estimate of the “true” number of visible new redds in the test reaches, we followed methods similar to those used by Dunham et al. (2001) and Muhlfeld et al. (2006). During the 3-week period after the first survey (a typical interval between surveys), redds were identified and flagged at weekly intervals by an experienced surveyor that was not responsible for routine surveys of those reaches. At the end of the 3 weeks, flags were removed from all redds identified after the first survey. Each of the surveyors responsible for the routine surveys of fluvial redds (i.e., all survey sections except Low Creek) and the inexperienced surveyors used for comparison independently counted unflagged redds in each of the test reaches on main-stem Mill Creek. The experienced surveyor responsible for routine surveys of redds of suspected resident fish in Low Creek and the same crew of inexperienced observers independently counted redds in the three test reaches in Low Creek. On the day after the evaluation, four experienced surveyors together resurveyed the test reaches to verify the best estimate of the number of new redds since the first survey. Only the experienced surveyors evaluated for observer error conducted the redd surveys in 2004–2007 and most of the surveys in prior years.

**Analysis**.—Trends in redd counts and adult females were initially analyzed using linear regression. Counts were log<sub>e</sub>-transformed based on an assumption of exponential changes in abundance (Maxell 1999) and to normalize the distributions. Because of apparent nonlinear patterns and autocorrelation in the linear regressions, we also used polynomial regression. To evaluate effects of error in the redd counts in detecting trends, we used TRENDS software (Gerrodette 1993), similar to the work by Maxell (1999). This allowed us to determine the minimum detectable proportional increase and decrease in abundance using coefficients of variation (CV = SD/mean) from female : redd ratios for Mill Creek and from observer error in redd counts from the test reaches. We compared minimum detectable differences in trends in 5-year increments from 5 to 25 years. We also tested varying assumptions about significance level (α) and one- or two-tailed tests for significance. Following the methods of Maxell (1999), an α level of 0.05 and a two-tailed test were compared with an α level of 0.20 and a one-tailed test to detect declines. The latter permits increased detection of declines (i.e., lower minimum decreases or earlier detection) but does not distinguish increasing trends from stable trends. However, this would meet monitoring needs for trends in abundance under the draft recovery plan. Power was held constant at 0.80, and the CV was assumed to be constant with respect to abundance.

We also compared estimates of adult population size based on direct methods to estimates from expansions of redd counts. To directly determine the total number of fluvial adults (females and males), we combined the numbers of all 300-mm or larger bull trout in our upstream trap counts and all unmarked, 300-mm or larger bull trout upstream of the trap from our mark–recapture estimates (see section on female abundance in Methods). For estimates of population size based on redd counts, we compared estimates from the 10 years of Mill Creek redd counts by using the mean adults-per-redd for Mill Creek and mean adults-per-redd values from Dunham et al. (2001) and Al-Chokhachy et al. (2005) as examples where population-specific data might not be available. We used geometric mean abundance for 5- and 10-year periods during 1998–2007 and related these to six abundance classes (1–50; 50–250; 250–1,000; 1,000–2,500; 2,500–10,000; and 10,000–100,000 adults) used by the International Union for Conservation of Nature (IUCN 2001) for its Red List of Threatened Animals and by the U.S. Fish and Wildlife Service (USFWS 2008) to assess status of core areas during its 5-year status review of bull trout. Similar to the trend analysis, geometric means are used when changes tend to be exponential, as in population growth.

**RESULTS**

**Female Estimates and Redd Counts**

In our systematic sample of Mill Creek and tributaries above the trap, we collected 54 bull trout (113–255 mm FL) to identify potentially resident mature females smaller than 300 mm that would not be accounted for in our trap counts and mark–recapture estimates. Ninety-six percent of the sampled fish were 125–255 mm; 81% of the sampled fish were from Mill Creek, where most of the redds in previous spawning surveys were counted. We did not collect any bull trout from the 256–299-mm size-class. We found 13 mature males (159–255 mm) but no mature females, suggesting that smaller, potentially resident females did not occur or were present at low densities.

We also examined redd size for evidence of smaller, resident adults. Pairwise comparisons of redd area indicated that redds in main-stem Mill Creek and the North Fork Mill Creek were significantly larger than redds in Low Creek and other
of bull trout redd area at the sites listed in Table 1.

TABLE 2. Results of Dunn’s test (\(Q\) test statistic) for pairwise comparisons of bull trout redd area at the sites listed in Table 1.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Difference of ranks</th>
<th>(Q)</th>
<th>(P &lt; 0.01)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill Creek versus Low Creek</td>
<td>397.89</td>
<td>21.12</td>
<td>Yes</td>
</tr>
<tr>
<td>Mill Creek versus Bull Creek</td>
<td>267.42</td>
<td>3.92</td>
<td>Yes</td>
</tr>
<tr>
<td>Mill Creek versus Paradise Creek</td>
<td>246.02</td>
<td>3.85</td>
<td>Yes</td>
</tr>
<tr>
<td>Mill Creek versus North Fork Mill Creek</td>
<td>35.70</td>
<td>0.86</td>
<td>No</td>
</tr>
<tr>
<td>North Fork Mill Creek versus Low Creek</td>
<td>362.18</td>
<td>8.48</td>
<td>Yes</td>
</tr>
<tr>
<td>Paradise Creek versus Low Creek</td>
<td>151.86</td>
<td>2.34</td>
<td>No</td>
</tr>
<tr>
<td>Mill Creek versus Low Creek</td>
<td>503.41</td>
<td>20.78</td>
<td>Yes</td>
</tr>
<tr>
<td>Mill Creek versus Little Minam River</td>
<td>503.40</td>
<td>21.95</td>
<td>Yes</td>
</tr>
<tr>
<td>Little Minam River versus Low Creek</td>
<td>0.01</td>
<td>0.00</td>
<td>No</td>
</tr>
</tbody>
</table>

Low Creek and the Little Minam River had nearly identically sized smaller redds and smaller spawners (<300 mm; Table 1).

During 2002–2007, the number of mature migratory adult females trapped in Mill Creek ranged from 32 to 90, and the mean proportion of mature females was 0.51 (SD = 0.02). The FL of mature females was 430 mm (\(N = 408\), SD = 38). Only five mature females were smaller than 300 mm, and they were all 289 mm or larger. From our PIT tag capture histories of Mill Creek adults, only 8% of the recaptures at the upstream trap (\(N = 576\)) were not in successive years, and all mature females initially captured had mature gonads when recaptured in following years. Unmarked, 300-mm or larger females in the mark–recapture estimate above the trap accounted for an average of 21% (SD = 5) of the total number of females. The average lower CI for the mark–recapture estimate of unmarked females was 17% (SD = 5) of the estimate, and the upper CI was 29% (SD = 5) of the estimate. Redd counts in Mill Creek and North Fork Mill Creek during 1998–2007 ranged from 56 to 180 (Figure 2). The mean number of females per redd during that period was 0.9 (SD = 0.2).

In 2003 and 2005, we estimated that there were 48 (95% CI = 27–69) and 97 (95% CI = 69–125) mature females, respectively, in Low Creek. Mature females ranged from 142 to 198 mm, and mature males ranged from 135 to 193 mm. Sex ratios were slightly skewed toward males (44–47% of the fish were female). The numbers of redds counted in Low Creek in those same years were 28 and 43, respectively, yielding 1.7 and 2.3 females/redd—about twice the ratio for Mill Creek.

We recaptured 56% of the marked bull trout (61% in pools, 46% in riffles) during the second electrofishing depletion estimate from the subsampled units where we used mark–recapture to evaluate potential bias in the depletion estimates on which our population sizes for Low Creek were based. However, we captured only one unmarked bull trout, which was smaller than 130 mm. The absence of any unmarked fish in the size range of mature females during the recapture and the generally higher...
FIGURE 3. Mean redd counts, expressed as a percentage of the best estimate of the true number of redds, as determined by experienced and inexperienced surveyors in test reaches containing redds of large, fluvial adult bull trout (Mill Creek) and redds of small, resident bull trout (Low Creek).

electrofishing efficiencies for larger-sized fish (Peterson et al. 2004) suggest that our depletion estimates of mature females were not substantially biased.

**Redd Count Measurement Error**

Redd counts of individual experienced surveyors used to conduct spawning surveys in Mill Creek were 67–122% of the best estimate of the number of fluvial bull trout redds in the three test reaches (Figure 3). The grand mean proportion of the best estimate counted by all experienced surveyors, which represents how redds counts would be totaled in a typical survey, was 99%. In Low Creek, where only smaller, suspected-resident-sized fish spawned, redd counts of the experienced surveyor, who conducted all of the annual redd counts in that stream during the study, underestimated the total redds in the test reaches by an average of 45% (Figure 3). Adjusting the redd counts by that amount produced females-per-redd ratios (0.9, 1.2) similar to those in Mill Creek. Redd counts of inexperienced surveyors for
the test reaches in Mill Creek, which are used here only for comparison purposes, deviated substantially more from the best estimate, were consistently positively biased (mean = 161%, range = 117–211%), and were more variable (experienced: CV = 0.25; inexperienced: CV = 0.49; Figure 3). In Low Creek, inexperienced surveyors consistently underestimated the number of redds to a greater extent (68%) than the experienced surveyor, and counts were more variable (CV = 0.56) than those for the redds of fluvial bull trout.

**Population Size and Trend**

Linear regression indicated the relationship between redds and females in Mill Creek was significant but not highly correlated (Figure 4). However, separate linear regressions for redds and females over time revealed highly similar, significant declining trends in the population (Figure 5); Durbin–Watson statistics for females (1.32, P = 0.049) and redds (0.96, P = 0.008) suggested that the data were autocorrelated. Quadratic polynomial regressions reduced apparent autocorrelation (Durbin–Watson statistic; females: 2.11, P = 0.233; redds: 2.29, P = 0.344), significantly improved the fit of the data (F-statistic for the increase in \( r^2 \); females: P = 0.010; redds: P = 0.003), and likewise showed very similar trends (analysis of covariance: P = 0.623).

The variation in the annual number of females per redd in Mill Creek and among test counts of the surveyors was similar (CV in Table 3). The minimum detectable changes in abundance over 15 years of monitoring based on that variation would be a decrease of 44–47% or an increase of 78–87%, assuming typical statistical criteria (\( \alpha = 0.05 \), power = 0.8, and the two-tailed test) and no change in CV with abundance (Table 3). The trend in Mill Creek redd counts from the linear regression (Figure 5) was equivalent to a 55% decrease in abundance over 10 years, which is consistent with the minimum detectable decline of 52% over 10 years predicted in Table 3 and similar to the 52% decline from the linear regression for female counts. If the significance criteria are relaxed by using an \( \alpha \) of 0.20 with lower certainty and a one-tailed test to be able to distinguish between declines versus stable or increasing trends, detection of declines of 28% over 15 years are possible using the variation in females per redd observed in Mill Creek. Use of inexperienced surveyors would require a minimum decline of 70% or an increase of 224% over 15 years. Minimum detectable changes over 5 years are quite large, particularly using counts of novice surveyors, which require almost a total collapse of the population or an 11-fold increase.

Redd counts and abundance estimates for Mill Creek support the difficulty of accurately assessing trends over a period of 5 years. If we examine the data for 1998–2005, some of the potential short-term discrepancies between redd and female counts become apparent. Females estimates during 1998–2005 were stable based on log-linear regression (\( r^2 = 0.83, P = 0.59 \), coefficient = −0.01). However, the trend in the redd counts during the first 5 years of this period and the trend during the last 5 years suggest different conclusions. The trend in redd counts for 1998–2002 indicates a significant, steeply increasing trend (\( r^2 = 0.84, P = 0.03, \) coefficient = 0.14), whereas the trend in redd counts for 2001–2005 conversely indicates a significant, steeply declining trend with similar statistics (\( r^2 = 0.85, P = 0.03, \) coefficient = −0.19). Thus, neither of these 5-year trends in redd counts is consistent with the population trend.

In terms of abundance, geometric means of the Mill Creek population size predicted from redd counts based on mean adults per redd observed during 1998–2007 were similar to direct
### TABLE 3. Minimum detectable changes (%) in adult bull trout abundance for a given number of years of monitoring, based on fluvial redd counts in Mill Creek. Parameters in bold italics highlight the differences in the scenarios (CV = coefficient of variation).

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Parameter or condition</th>
<th>Type of change</th>
<th>Monitoring period (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Variation in females/redd</td>
<td>CV = 0.23</td>
<td>Increase</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>α = 0.05</td>
<td>Decrease</td>
<td>−68</td>
</tr>
<tr>
<td>Variation in redd counts by experienced observer in test reaches</td>
<td>CV = 0.25</td>
<td>Increase</td>
<td>276</td>
</tr>
<tr>
<td></td>
<td>α = 0.05</td>
<td>Decrease</td>
<td>−74</td>
</tr>
<tr>
<td>Variation in redd counts by novice observer in test reaches</td>
<td>CV = 0.49</td>
<td>Increase</td>
<td>1,115</td>
</tr>
<tr>
<td></td>
<td>α = 0.05</td>
<td>Decrease</td>
<td>−92</td>
</tr>
<tr>
<td>Variation in females/redd and a one-tailed test with reduced significance level</td>
<td>CV = 0.23</td>
<td>Increase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>α = 0.20</td>
<td>Decrease</td>
<td>−41</td>
</tr>
</tbody>
</table>

Mean adult estimates for Mill Creek would not be statistically different from the direct adult estimate, except for 1998–2002 when using the highest value of adults per redd (Holm–Sidak multiple-comparison test). Likewise, the population estimate relative to the abundance classes used to evaluate status would generally be similar except in some cases when using the highest adults-per-redd value from the literature (Figure 6).

**DISCUSSION**

There are a number of possible factors influencing variability in the relationship of redd counts to female or adult counts and the reliability of using redd counts to estimate adult abundance and trend. First, there is measurement error in both counts. We attempted to minimize error in the adult female count to the extent possible. There was likely little error in our trap counts of migratory adults moving upstream prior to spawning, which accounted for an average of 79% of the female adults, or in our identification of adult females by use of ultrasound (Evans et al. 2004). Our snorkel counts used to estimate the number of nonmigratory adult females (≥300 mm FL) in the spawning population were largely a complete survey of potential holding habitat above the dam, so there was no error associated with sample reach selection. There is error associated with estimating the size of fish while snorkeling (O’Neal 2007). However, we used only two size-classes (<300 and ≥300 mm FL). Data from trapping, electrofishing, and other snorkeling indicated

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**FIGURE 6.** Geometric mean (±95% confidence interval) adult bull trout abundance in Mill Creek during three periods (1998–2002, 2003–2007, and 1998–2007) from direct estimates and from predictions using redd counts for Mill Creek (1.76 adults/redd) and averages from Dunham et al. (2001; 2.16 adults/redd) and Al-Chokhachy et al. (2005; 2.68 adults/redd). Dashed lines delineate abundance classes (0–50, 50–250, and 250–1,000 adults; categories from IUCN 2001 and USFWS 2008).
that there were few 200–299-mm bull trout in the area sampled, so there should have been little uncertainty in classifying the fish observed into the two size-groups since most fish would have been at least 300 mm or would have been substantially smaller. Our maturity data also indicated that very few mature, nonmigratory females smaller than 300 mm were excluded from our mark–recapture estimates.

Measurement error in redd counts can be substantial (Dunham et al. 2001). As suggested by Muhlfeld et al. (2006), we attempted to minimize this source of error by primarily using a consistent crew of experienced surveyors. The range in average differences in individual surveyor counts from the best estimate of redds (67–122%) in test reaches in Mill Creek was similar to that of experienced surveyors in Montana (78–130%; Muhlfeld et al. 2006). The deviation of the combined counts of the surveyors from the best estimate (–1%) suggested little bias in the total redd counts, assuming the errors were similarly distributed among years. Differences among surveyors in errors from undercounting (missed redds) and overcounting (false identifications) tended to cancel each other out, as occurred for Dunham et al. (2001). In the Swan River, equal compensation in the two types of error occurred at densities of about 5 redds/km (Muhlfeld et al. 2006). Average redd density in the Mill Creek test reaches was 6 redds/km.

Our power analysis assumes lognormal measurement error in the redd counts. That may not be the case (Muhlfeld et al. 2006), which could lead to overestimating the power of linear regression to detect trends. Since we did not have separate estimates of missed redds versus falsely identified redds, we could not determine the error structure in a manner similar to that described by Muhlfeld et al. (2006).

One factor related to surveyor error that was not evaluated in this study or in previous studies is the year-to-year variation in surveyor error. For example, estimates of observation error in redd counts for this study and the study by Muhlfeld et al. (2006) are based on a single evaluation and assume that there were no differences in error among years. However, the variation in the females per redd over 10 years (CV = 0.23) in Mill Creek, which includes all sources of measurement error plus any true annual variation in the number of redds constructed by females, was similar to the variation among the experienced surveyors in the 1 year of test counts (CV = 0.25), suggesting little year-to-year variation in surveyor error.

There was substantially more measurement error in the counts by inexperienced surveyors of both fluvial and resident bull trout redds. These were not totally novice surveyors, however. In fact, the 8-week period of training and experience of this group at the time of evaluation was considerably greater than the training period provided to many surveyors that are hired to conduct redd surveys of other bull trout populations in the region (Moore et al. 2006). This suggests that the bias and imprecision of the inexperienced surveyors in our study were not higher, and could have even been lower, than would be expected for other inexperienced surveyors.

Other studies have suggested higher correlation between redds and spawning adults than we observed (Dunham et al. 2001; Johnston et al. 2007). This may be at least partially due to differences in the range of redd counts and spawner abundance used in the analyses. In Mill Creek, there was a threefold range in both redd and female counts, whereas there was a 10-fold range in the counts used by Dunham et al. (2001; <100 to >1,000), although the variation in spawners per redd in that analysis (CV = 0.46; J. Dunham, U.S. Geological Survey, unpublished data) was much greater than for Mill Creek (CV = 0.23). Spawners and redds in Smith-Dorrien Creek, Alberta, where there was a 1:1 relationship between spawning females and redd counts (Johnston et al. 2007), increased more than 20-fold from 1992 to 1998 (Johnston 2005). As indicated by the power analysis, the detectability of changes in abundance based on redd counts depends on the magnitude of change.

The size of the spawning fish and corresponding redd size and detectability may also account for some of the variability in redd : adult relationships among populations. For example, adfluvial bull trout spawners in Smith-Dorrien Creek (Johnston et al. 2007) average about 600 mm when they first spawn (Stelfox 1997) and create redds that are approximately 2 m² (Johnston 2005). Likewise, for the primarily adfluvial bull trout spawning in portions of the Metolius River and tributaries (included in the Dunham et al. 2001 analysis), redd area averaged 3–5 m² (Higgins et al. 2005) and female spawners averaged 578 mm (Riehle et al. 1997). For adfluvial bull trout in the Flathead River, Montana, redd area averaged 2.3–3.7 m² and spawners averaged 611 mm (Shepard et al. 1984). In Mill Creek, redds and spawners were smaller (mean redd area = 1.4 m²; mean FL of mature females = 430 mm). As was evident in Low Creek, where redds averaged 0.2 m² and where adults were smaller than 200 mm, smaller redds probably increase the error associated with redd counts. We previously examined the relationship between small redds and adults (132–214 mm) in another apparent resident population (Hemmingsen et al. 2001b). A population estimate and redd counts based on methods similar to those used for Low Creek resulted in 42 adults/redd, suggesting very high negative bias in the redd counts. This was attributed to a small redd size coupled with a fine, granitic spawning substrate that made the redds very difficult to detect.

For this study, we were fortunate to have discrete resident versus migratory populations to evaluate separately. This would be more complicated for populations consisting of a mix of migratory and resident females. Small, presumably “sneaker” males (~150–250 mm) were observed near redds occupied by larger (>300 mm) females and males in Mill Creek. Similar breeding behavior involving small males has been observed in other migratory bull trout populations (McPhail and Baxter 1996) and is often observed in other large, migratory salmonoids (Esteve 2005). Small, mature males were also well distributed in our sample from Mill Creek and its tributaries. However, their presence would not in itself constitute a resident population, which would also have to include resident adult females.
Understanding not only the relationship between redds and spawners but also the relationship between redds and the total number of adults can be useful in estimating effective population size and determining abundance criteria for recovery plans (Rieman and Allendorf 2001). For example, bull trout may spawn in successive years or in alternate years (Pratt 1992). Consequently, any alternate-year spawners that did not migrate above our trap in years when they did not spawn would not be accounted for in our adult estimates. However, our data indicated that in Mill Creek, almost all of the adults that overwintered below the trap migrated upstream to the spawning area and were mature annually. In Flathead Lake, on the other hand, where alternate-year spawning is more prevalent, about 40% of the adult population remained in the lake and did not spawn in a given year (Fraley and Shepard 1989). Consequently, adult estimates based solely on spawner : redd relationships or trap counts might exclude the nonspawning and nonmigratory adults that are present below or above the trap, as occurred in Mill Creek.

Conclusions

Variation in the relationship between redd counts and adult abundance can limit the use of redd counts in detecting trends in abundance or estimating adult abundance. Detection of short-term trends based on redd counts (e.g., 5 years) can be misleading and likely requires extreme changes in abundance to be accurate. Guidelines for evaluating trends for status assessment (Morris et al. 1999; IUCN 2001; USFWS 2008) recommend a minimum of 10 years of data and preferably data for three generations, which for bull trout has been interpreted as a 15-year period based on 5 years/generation (Maxell 1999). From our results, detection of trends over 10–15 years by using traditional statistical criteria would be possible for only the higher-risk status categories defined by the IUCN (2001) and the USFWS (2008). However, our study also suggests greater potential power of redd counts to detect population trends than previously reported. For example, the CVs in the redd counts for the Flathead and Swan rivers were considerably higher (0.42–0.67; Maxell 1999) than we observed and would require correspondingly larger changes or a longer time to detect. However, this difference was not because redds counts for Mill Creek were “better” but because the CVs used for Mill Creek were directly determined from variation in the female: redd ratios and measurement error in the redd counts, whereas the CVs for the Flathead and Swan rivers were based on annual changes in redd counts, which included large declines.

Abundance estimates derived from redd counts and the number of adults per redd can provide a confidence band that includes the actual size of the population. Like trend analysis, longer-term data sets were more informative. Ten-year geometric means have been similarly used to assess abundance of salmon and steelhead populations listed under the Endangered Species Act in the interior Columbia River basin (ICTRT 2007). Where direct estimates of adults per redd are not available for the population of interest, values from other populations that have similar life history characteristics (e.g., migratory versus resident, alternate-year spawning) should be used.

Error in redd counts can come from a variety of sources, including aspects of survey design (e.g., spatial and temporal distribution) and observer variability. Consequently, estimates of that uncertainty should be included with redds counts similar to other methods for estimating abundance, such as depletion and mark–recapture, to help with their interpretation.

Given the limitations of redd counts, a precautionary approach in using them to assess status, particularly for an imperiled species like the bull trout, appears warranted. For example, using lower levels of statistical significance and a one-tailed test for trend analysis, as suggested by Maxell (1999), would permit detecting lower rates of decline or reduce the time needed to detect those declines, while still being consistent with the recovery objective of distinguishing between declines and stable-to-increasing populations. Likewise, use of conservative adult : redd conversion factors for estimating abundance where population-specific data are lacking will reduce the likelihood of overestimating the abundance of the population. This would be particularly important in the case of small, depressed populations since they are more vulnerable to extirpation and since redds counts at low redd densities tend to be inflated (Muhlfeld et al. 2006).

Two important factors influencing the variability of redd counts are redd size, which is related to adult size and life history form, and measurement error in redd surveys. Counts of redds produced by small, presumably resident adults greatly underestimated the abundance of redds and adults and can be more variable. Consequently, redd counts are likely to be a more reliable and sensitive indicator of abundance for larger, migratory forms than for small, resident forms. Redd surveys that include estimates of redd area and spawner lengths could be used to sort migratory versus resident redds and to describe the size and distribution of life history forms, which are also useful attributes in assessing the status of populations.

Previous studies (Dunham et al. 2001; Muhlfeld et al. 2006) and the data from this study point out the importance of using skilled redd surveyors to reduce measurement error. Redd surveys are not a purely mechanical, objective method that is immune to operator error; and skill is not solely determined by experience. Although experienced surveyors generally produced redd counts that were more accurate and precise in the test reaches in this study, a mix of experienced and inexperienced surveyors had the fewest counting errors in the study by Dunham et al. (2001). The use of skilled surveyors across the range of the species may be very difficult. Hiring, training, and retaining skilled surveyors will clearly be a challenge.

Regardless of the skill of the surveyors or the approach that is used to analyze redd counts, the reliability of redd counts in assessing status is dependent on a well-designed and executed sampling plan. Bias in selecting areas to survey (e.g., index reaches) or in the timing and frequency of surveys (e.g., peak counts) may increase the error in the counts and the variability
in their relationship to abundance. Other abundance monitoring techniques may be helpful in evaluating assumptions made using redd counts (e.g., adults per redd) and may be more suitable for some bull trout populations (e.g., those occurring sympatrically with brook trout *Salvelinus fontinalis* and for certain spawning conditions (e.g., substrate type). Likewise, monitoring approaches that assess the risk of decline rather than measure abundance or trend can be useful in situations where the error in abundance estimates is high and the power to detect trends is low (Staples et al. 2005).

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Dam Removal and Implications for Fish Health: *Ceratomyxa shasta* in the Williamson River, Oregon, USA

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**Abstract**

The removal of dams on a river is one potential tool for the ecological restoration of native salmonid fishes. However, the removal of barriers also introduces risks, such as the introduction of fish pathogens into previously isolated populations. The proposed removal of four dams on the Klamath River, Oregon–California, provides an opportunity for examining the disease risks associated with dam removal. A salmonid pathogen endemic to the region, *Ceratomyxa shasta*, is responsible for high mortality in juvenile Chinook salmon *Oncorhynchus tshawytscha* and coho salmon *O. kisutch* below the dams. Above the dams, parasite densities are lower and not implicated in salmonid mortality, except in the Williamson River tributary, where high parasite densities raise concerns over the restoration of anadromous fish that are likely to take advantage of spawning habitat in that river. In the current study, baseline information on parasite density, distribution, and genotype composition in the Williamson River was gathered to determine how salmonid reintroduction might be affected by parasite dynamics. Assay of water samples highlighted two areas of high parasite density: between the mouth of the Williamson River and the confluence of the Sprague River tributary, and above the Spring Creek confluence. Despite these high parasite densities, mortality did not occur in sentinel coho or Chinook salmon. Genetic analyses of parasites from water samples and infected fish demonstrated that *C. shasta* genotype II was dominant and was associated with stocked nonnative rainbow trout *O. mykiss*. The absence of pathogenicity of this parasite genotype for Chinook and coho salmon suggests that reintroduction plans will not initially be adversely affected by the high parasite densities in the Williamson River. However, following dam removal, returning adult salmon will transport parasite genotypes present below the dams upstream. These genotypes are likely to become established and may reach densities that could affect juvenile Chinook and coho salmon.

Anadromous salmonids require both freshwater and marine environments for the completion of their life history with freshwater environments providing critical spawning and early life stage rearing habitats. Over the last century, a variety of factors have led to the decline of anadromous salmonids in the Pacific Northwest, including habitat degradation brought about by agriculture, the raising of livestock, forest usage, urbanization, and dams. These effects have been further compounded by overharvest, disease, and ocean conditions (Fresh 1997; Mundy 1997; Pearcy 1997). One solution proposed for mitigation of these effects is dam removal, which would return the river to a more natural hydrograph and provide access to historically utilized habitats (Bednarek 2001).

Positive effects of dam removal on fish populations have been demonstrated in several rivers. On the Milwaukee River, Wisconsin, removal of the Woolen Mills Dam resulted in improved habitat and increased numbers of the native smallmouth bass while nonnative common carp *Cyprinus carpio* declined (Kanehl et al. 1997). Similarly, colonization of habitat post dam removal by anadromous salmonids occurred in the Mad River, California; Rogue River, Oregon; and Clearwater River, Idaho (Winter 1990). However, there are also risks associated with dam removal. The release of large amounts of sediment from reservoirs could negatively affect fish populations, especially if toxins have accumulated in the sediment (Stanley and Doyle 2003), and introduction or amplification of pathogens may...
occur as fish populations separated by the dams come into contact (Brenkman et al. 2008; Zielinski et al. 2010).

Few case studies have compared pathogen dynamics and associated disease risks prior to and following dam removal or fish passage. One study on the Deschutes River, Oregon, examined the risk of introducing Myxobolus cerebralis as a result of fish passage above the dam (Zielinski et al. 2010, 2011). Prepassage monitoring showed that M. cerebralis was established in at least one tributary below the dams, thus increasing the risk of the parasite being introduced above the dams. To improve the long-term success of fish passage, the authors recommended transfer of eggs or fry raised in pathogen-free water rather than passing naturally reared juveniles (Zielinski et al. 2010) and continued monitoring for M. cerebralis in areas where the pathogen was detected (Zielinski et al. 2011). On the Cowlitz River, Washington, passage of adult spring Chinook salmon Oncorhynchus tshawytscha above the Mayfield Dam began in 1996. Although no studies were conducted to assess changes in pathogen effects before and after fish passage, observational data suggests that prevalence of Ceratomyxa shasta has increased. Sentinel studies conducted in the late 1990s (shortly after reintroduction began) demonstrated a less than 10% prevalence of C. shasta in juvenile spring Chinook salmon upstream of the dam, while 70–84% infection prevalence occurred in juvenile spring Chinook collected from traps in 2010. In addition, mortality of spring Chinook salmon in Cowlitz Hatchery, located below the dam, has increased since fish passage (E. Ray, Washington Department of Fish and Wildlife, personal communication). A third study in the Elwha River, Washington, collected baseline information on multiple fish pathogens prior to dam removal and will also assess changes in pathogen dynamics post dam removal (Brenkman et al. 2008). The researchers hypothesize that the prevalence of endemic pathogens (such as Renibacterium salmoninarum) may increase above the dams and that nonendemic pathogens (such as infectious hematopoietic necrosis virus) could be introduced. In addition, pathogens that co-evolved with resident fish above the dams may be more virulent in naïve, reintroduced stocks (Brenkman et al. 2008). Thus, pathogens may be influenced by interactions between resident and reintroduced populations and could hinder salmonid recovery in spite of dam removal.

In the Klamath River, Oregon–California, dam removal and fish passage have been proposed for increasing salmonid habitat in the basin. However, the success of salmonid reintroduction in the Klamath River basin is contingent, in part, on their ability to cope with the endemic parasite C. shasta. This parasite causes ceratomyxosis, a disease of salmon and trout characterized by severe inflammation, hemorrhage, and necrosis of the intestine (Bartholomew et al. 1989). Infection often results in death, although fish native to rivers where the parasite is endemic are less likely to develop the disease (Zinn et al. 1977; Bartholomew 1998). Ceratomyxa shasta has a complex life cycle that requires two hosts, the salmonid host releasing the myxospore stage of the parasite and the polychaete Manayunkia speciosa releasing the actinospore stage (Bartholomew et al. 1997). Parasite distribution is seasonal, with infection occurring in sentinel fish exposed between April and mid-December (Ratliff 1981; Ching and Munday 1984; Hendrickson et al. 1989), coinciding with the presence of adult spring Chinook salmon, the fish most likely to utilize upper basin habitat (Hamilton et al. 2005).

In the upper Klamath basin, high densities of both C. shasta and the polychaete host occur in the Williamson River, an area of critical spawning habitat. From the mouth of the Williamson River to the Sprague River confluence (river kilometer [km] 19), 90% parasite-induced mortality has been observed in nonnative (naïve) rainbow trout O. mykiss (RBT; freshwater form) exposed in cages in the river (Hemmingsen et al. 1988; Buchanan et al. 1989; Hallett and Bartholomew 2006; Stocking et al. 2006). Assay of water samples collected during sentinel studies in 2004 demonstrated that parasite densities in the Williamson River were similar to those in the lower Klamath River where severe disease occurs (Hallett and Bartholomew 2006). In addition, a survey for the polychaete host identified a large population at the Williamson River confluence with Klamath Lake (Stocking and Bartholomew 2007). These studies demonstrated that appropriate conditions exist for C. shasta to persist in the Williamson River.

Complicating predictions of how C. shasta will affect reintroduction plans is our changing understanding of the parasite. Four distinct genotypes of C. shasta (0, I, II, and III) occur in sympathy with their salmonid hosts (Atkinson and Bartholomew 2010a, 2010b) and are host-specific (Hurst 2010). Thus, the ability of the parasite to establish and reproduce is influenced by the species and strain of salmonid hosts available. In the Williamson River, anadromous salmon have been extirpated since 1917 (Hamilton et al. 2005), leaving redband trout O. mykiss newberrii as the only native host for C. shasta. However, since 1925, nonnative RBT have been stocked into the Spring Creek tributary of the Williamson River to supplement recreational fishing. Fish stocking occurs during the peak months of parasite production—from May through August—and because of their high susceptibility to C. shasta, these fish are assumed to die if not caught by anglers (W. Tinniswood, Oregon Department of Fish and Wildlife [ODFW], personal communication). This strategy ensures that these fish will succumb to ceratomyxosis before interbreeding with native redband trout but may also amplify certain parasite genotypes in the Williamson River.

Understanding how biotic factors such as fish host species and polychaete host densities, and abiotic factors such as temperature and management practices influence parasite dynamics is important for predicting the success of reintroduced anadromous salmonids in the upper Klamath basin. The first objective of this study evaluates the risk of disease resulting from infection with C. shasta in the Williamson River. The approach was to determine distribution, density, and genotype composition of C. shasta in fish and polychaete hosts and water samples collected throughout the Williamson River. The second objective examines how stocking practices may alter parasite dynamics. Here
the approach was to compare parasite genotype composition, parasite density, and mortality of native and nonnative sentinel fish in the Williamson River and at a site downstream in the main-stem Klamath River where stocking does not occur.

**METHODS**

**Density, Distribution, and Genotype Composition of Ceratomyxa shasta in the Williamson River**

*Study location.*—The Klamath River is 480 rkm and extends from the Williamson River in Oregon to the Pacific Ocean in California. The upper and lower portions of the river are divided by a series of five dams. Copco I was completed in 1917, blocking fish passage above rkm 314. With installation of Iron Gate dam in 1962, upstream fish passage was further restricted to the lower 307 rkm (Hamilton et al. 2005). The Klamath Basin Restoration Agreement (Klamath Restoration 2010a) and Klamath Hydrologic Settlement Agreement (Klamath Restoration 2010b) propose increasing fish habitat in the upper basin by the removal of the four lower dams (Iron Gate, Copco I and II, and J. C. Boyle) and providing fish passage facilities at the fifth dam (Keno) beginning in 2020. Historically, the ranges of spring Chinook salmon and coho salmon *O. kisutch* and steelhead (anadromous rainbow trout) extended into the upper basin (Hamilton et al. 2005), and after conditions in the above agreements are met these fish are expected to repopulate their historic ranges, the Williamson River providing critical habitat.

The Williamson River is a headwater of the Klamath River basin and flows into upper Klamath Lake. This river is approximately 120 rkm and is predominately a spring-fed system with inputs from two major tributaries, Sprague River and Spring Creek, with confluences at rkm 19 and 27.5, respectively. Water sources for the Sprague River include a combination of snowmelt and cool springs, while Spring Creek is largely influenced by cool springs (Gannett et al. 2007). For this study, the main-stem Williamson River was divided into three reaches based on geomorphology. Reach 1 comprised the area from the mouth of the Williamson River (rkm 0) to the Sprague River confluence (rkm 19), reach 2 included the area between the Sprague River and Spring Creek (rkm 27.5), and reach 3 consisted of the area above Spring Creek to rkm 42. Twenty sites were selected within the three reaches: six sites in reach 1, three sites in reach 2, and seven sites in reach 3. In addition, two sites were sampled in each tributary. Sites were selected based on accessibility and spatial representation of the reaches.

*Water sampling.*—Four 1-L samples of water were collected from each site (Figure 1) in September 2008, June 2009, and May and June 2010. These months were selected because parasite density is highest from late spring to late summer (Hendrickson et al. 1989). Thermometers were placed about 6 in below the surface, and Global Positioning System latitudes

![FIGURE 1. Klamath basin in relation to the United States on the left; Williamson River and tributaries on the right, where oval boundaries indicate river reaches, filled circles depict water sample sites, triangles represent sentinel fish exposure sites, and hollow boxes indicate collection sites for polychaete (*Manayunkia speciosa*). [Figure available in color online.]]
and longitudes were recorded for each site. Sites were the same at all time points with the exception of rkm 36 in June 2009 to replace sites at rkm 36.3 and 36.6 as high water levels prevented access. All samples were kept on ice during transport to the John L. Fryer Salmon Disease Laboratory, Oregon State University, Corvallis, Oregon (SDL).

Water was filtered 24–48 h after collection, and the filter and its retentate were frozen at −20°C until processed (Hallett and Bartholomew 2006). The processing procedure was modified in 2010 to use acetone to dissolve the filter membrane instead of cutting the filters, leading to an increase in the sensitivity of the molecular assay described below. We extracted DNA from three of the four replicate samples from each site during each collection time. Each of the three samples was assayed in duplicate by quantitative polymerase chain reaction (qPCR; Hallett and Bartholomew 2006), along with negative (molecular grade water) and positive controls (C. shasta-infected tissue), using an ABI7300 qPCR sequence detection system. Interassay variability was assessed from the standard deviations of the positive control samples. Quantitative cycle threshold (Cq) values (a measurement of parasite DNA) for the three site replicates were averaged, and tests for inhibition were conducted on one sample from each site during each collection time (Hallett and Bartholomew 2009). Samples were considered inhibited if the sample fluoresced later than the negative control in the presence of a known amount of synthetic DNA. Inhibited samples were reanalyzed with a 1:10 dilution of parasite DNA to molecular grade water if a difference of greater than one cycle occurred between the sample and the negative control. We determined parasites per liter based on a one-spore standard reference sample with a Cq value of 32.5. Sites with less than 1 parasite/L were considered negative. Parasite density threshold values of 10 parasites/L and 100 parasites/L were extrapolated from a one-spore standard and on previous correlations (Hallett and Bartholomew 2006).

**Spore stage identification.**—To determine the parasite stage (myxospore or actinospore) at locations where water samples tested positive (the qPCR assay does not differentiate between spore stages), 5 L of water were collected at rkm 18.2 and 33 in June 2009, in conjunction with the water sampling. Sites were selected based on results from water samples collected in September 2008. Samples were transported on ice to the SDL, and water was poured into separate 25-L fiberglass tanks with air scrub pads used to dislodge benthic invertebrates and associated substrate contained within a Hess Sampler (256 cm²). Material was then collected in the sampler’s 80-µm aquatic mesh. Samples were transferred to Whirl-Pak plastic bags, immediately preserved with a 95% solution of ethanol, and placed on ice for transport to the SDL.

Preserved samples were emptied into a 30 × 20-cm² 180-D10 tray (Wildco, Yulee, Florida) subdivided into 15 sections. Three 5-cm² subsamples were randomly selected, and material within each section was collected using a disposable pipette and placed in 5-mL glass vials with a 1:3 ratio of Rose Bengal dye (20 mg/L 95% solution of ethanol) to a 95% solution of ethanol. After 24 h, the sample was filtered through an 80-µm sieve to remove dye and then stored in a 95% solution of ethanol. Polychaetes were sorted using a dissecting microscope at 100× magnification. This procedure was repeated for all three subsamples. Individual polychaetes were placed into 1.5-mL microcentrifuge tubes with ~100 µL of a 95% solution of ethanol and refrigerated until processing. Polychaete densities were calculated by averaging the three subsamples and extrapolating to polychaetes per square meter.

We extracted DNA from individual polychaetes according to Stocking and Bartholomew (2007) with the following modifications: DNA extraction buffer was reduced to 100 µL, proteinase K was reduced to 5 µL, and following the addition of RNase, sample incubation at 37°C was extended to 1 h. After boiling, pools of five polychaetes were created by adding 2 µL of each sample into a tube (10 µL total), then adding 2 µL of the pooled DNA into 198 µL of distilled water. Pooled DNA was assayed for C. shasta by qPCR (Hallett and Bartholomew 2006). Polychaetes from positive pools were then assayed individually by qPCR using a 1:100 dilution of the original sample to determine infection prevalence.

**Sequencing.**—We extracted C. shasta DNA from water samples, polychaetes, and fish intestinal tissues as above. Aliquots of 1 µL of the final eluate for water samples, 1 µL of a 1:100
dilution for fish samples, and 2 µL of a 1:100 dilution for polychaete samples was used in the genotyping polymerase chain reaction (PCR) assay (Atkinson and Bartholomew 2010a). Samples were purified using ExoSAP-IT (USB, Cleveland, Ohio) and submitted to the Center for Genomics and Bioinformatics at Oregon State University for sequencing using an ABI Prism 3100 genetic analyzer. Parasite genotype was determined according to the number of trinucleotide repeats in the internal transcribed spacer region I, and genotype proportions in mixed samples were identified by comparing chromatogram peak heights (Atkinson and Bartholomew 2010a).

Statistical analyses.—Statistical significance was determined with an α = 0.05 for all tests. Temperature differences among river reaches for June 2009 and 2010 were assessed using a one-way analysis of variance (ANOVA). Significant effects were examined using Tukey’s tests. The relationship between temperature and mean parasite density was examined using a polynomial regression. Both the one-way ANOVA and polynomial regression were performed using S-plus version 8.1 statistical software (Tibco, Palo Alto, California). Differences in parasite density (Cq values) among river reaches (1, 2, and 3) and sampling times (month–year combination) were examined using a nested ANOVA (PROC GLM, SAS version 9.2, Cary, North Carolina), where site was nested within reach, and genotype proportions in mixed samples were identified by comparing chromatogram peak heights (Atkinson and Bartholomew 2010a).

RESULTS

Density, Distribution, and Genotype Composition of Ceratomyxa shasta in the Williamson River

Parasite density differed between reaches and sites, and also by month–year combination (Table 1). Parasite density was highest in reach 1, there being greater than 10 parasites/L at all sites. Densities in reach 2 were less than 1 parasite/L, except at rkm 19.2 in June 2009 where parasite densities exceeded 1 parasite/L. In reach 3, parasite densities at two sites were 1–10 parasites/L; just above Spring Creek at rkm 28 and 33 (Tukey’s HSD: P < 0.05). At the remaining five sites, parasite densities were less than 1 parasite/L (Figure 2). The month–year variable also affected parasite density, densities in May 2010 being lower than in September 2008, June 2009, and 2010 (Tukey’s HSD: P < 0.05). In both the Spring Creek and Sprague River tributaries, parasite densities were less than 1 parasite/L during all sampling times. Variability between qPCR assays was low, as standard deviations for both reference samples were 1.1 and 0.9 cycles.

Water temperatures were significantly different (Figure 2) between river reaches but did not differ between June 2009 and June 2010 (one-way ANOVA: F2, 24 = 21.0, P < 0.001; F1, 24 = 2.8, P = 0.11). June temperatures in reach 2 ranged from 5°C.

Sentinel Fish Studies in the Upper Basin

Fish exposures.—Sentinel fish exposed as part of a comprehensive parasite monitoring study from 2006 to 2010 were used to assess differences in RBT mortality at rkm 7.6 in the Williamson River (42°30.820’N, 121°55.021’W), a system in which stocking of RBT occurs and at Keno eddy (42°8.9835’N, 122°0.9332’W), a site 47 rkm downstream of the Williamson River where stocking does not occur (Figure 1). In 2009, the Williamson River site was moved 6 rkm downstream for accessibility reasons (42°29.661’N, 121°56.095’W). Nonnative RBT (Roaring River Hatchery) were held at both sites in May and June 2006–2010. Chinook and coho salmon (Iron Gate Hatchery strains, Hornbrook, California) were exposed to determine the effects of upper basin parasite dynamics on native anadromous fish strains. Fall Chinook salmon were exposed at both sites in May and June 2006–2010. Coho salmon exposures were only conducted in the Williamson River and took place in May and June 2009. Fish exposures and subsequent monitoring followed methods in Stocking et al. (2006).

Water sampling.—To determine parasite density during sentinel fish exposures, three 1-L samples of water were taken at the beginning and end of fish exposure at each site, except in June 2008 when water samples were only collected at the beginning of the exposures. Water samples were processed and assayed using qPCR, as above.

Sequencing.—To determine if parasite genotype differed between the two upper basin sites, parasite DNA was sequenced from 10 C. shasta-positive RBT exposed at each location in June 2006–2010. When possible, fish were selected from both the mortality and survivor groups for parasite genotype comparison. Sequencing was performed as detailed above.

Statistical analyses.—Differences in Cq values between sentinel sites (Williamson River and Keno eddy), months, and years were analyzed using a one-way ANOVA with statistical significance determined with an α = 0.05. The data met the assumption of normality, and significant effects were tested using a Tukey’s honestly significant difference (HSD) test. Sentinel fish exposures were one component of a comprehensive monitoring study, and as a result we did not have an appropriate experimental design for statistical analysis of fish mortality between sentinel sites. We used S-plus version 8.1 (Tibco) to perform statistical analyses.

<table>
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<td>Error (whole plot)</td>
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Two of the four known genotypes of *C. shasta* were detected in the Williamson River. The proportions of parasite genotypes II (pathogenic for nonnative RBT) and 0 (associated with native redband trout) in water samples changed spatially and temporally, larger proportions of genotype 0 being detected in reach 1 than in reach 3. Genotype 0 generally accounted for 10–30% of the parasites present in water samples from all sampling times in reach 1, although in May 2010 water collected from rkm 18.2 contained 82% genotype 0. This was the only time when the proportion of genotype 0 was greater than genotype II. Genotype 0 was not detected at any sites in reach 3, except in June 2009 when it comprised approximately 10%. Parasite densities in reach 2 were below the sequencing threshold, except in June 2009 when genotype II accounted for 100% of the parasites detected.

Infection occurred in RBT held in water collected from either reach 1 (100%) or reach 3 (20%), demonstrating presence of the actinospore stage of the parasite. All fish were infected with genotype II; genotype 0 was not detected.

One polychaete assemblage was identified at rkm 18.3 in reach 1 and one assemblage at rkm 33.2 in reach 3. Polychaete density at the site below the Sprague River was calculated as 4.08 × 10^3 polychaetes/m², and infection prevalence was 1.1% (*n* = 185). Only genotype II was detected in the polychaetes. In the site within reach 3, polychaete density was estimated at 78 polychaetes/m² and infection prevalence could not be determined.

**Sentinel Fish Studies in the Upper Basin**

Mortality of sentinel nonnative RBT differed between the Williamson River and Keno eddy, while Chinook salmon mortality was consistently below 5% at both sites. No mortality occurred in coho salmon exposed in the Williamson River. From 2006 to 2010, mortality in RBT held at the Williamson River sentinel site during May and June ranged from 97% to 100%, and infection was from genotype II. Mortality in RBT exposed at Keno eddy ranged from 0% to 75% (Figure 4) and fish were infected with either genotype 0 (50%) or genotype II (50%), while exposure survivors were infected with genotype 0. Chinook salmon were infected with genotypes II and III (Atkinson and Bartholomew 2010a).
FIGURE 3. Polynomial regression plot of temperature versus *C. shasta* cycle threshold (Cq) values for all months and reaches. Main-stem Williamson River samples are indicated using filled squares, and tributary data are depicted with hollow squares. The dashed horizontal line indicates parasite production (1 parasite/L). [Figure available in color online.]

FIGURE 4. Mortality of nonnative RBT (bars) and average *C. shasta* cycle threshold (Cq) values (triangles) for the Williamson River and Keno eddy sentinel sites for years 2006–2010. The dashed line designates the Cq value that corresponds with 1 parasite/L. [Figure available in color online.]
Parasite density was affected by year and site, but we did not detect effects of month. Densities in 2006 and 2007 were higher than those in 2008–2010 (one-way ANOVA: $F_{4, 99} = 20.2$, $P < 0.0001$). Variation among reference samples on assay plates was up to three cycles or a 10-fold difference in parasite density and likely affected yearly comparisons in 2006–2007, whereas variation in 2008–2010 was generally less than 1 cycle and did not equate to a ten-fold change in density. Parasite density at Keno eddy was typically less than 1 parasite/L, which is 10–100-fold less than in the Williamson River (where density was usually > 10 parasites/L; one-way ANOVA: $F_{1, 99} = 232.3$, $P < 0.0001$).

**DISCUSSION**

The success of salmon restoration in the Klamath River is dependent on the availability of spawning and rearing habitat in the upper basin and on the level of exposure to pathogens that cause high mortality. In the Williamson River high densities of the myxozoan *C. shasta* reflect the influence of water temperature variability, location of polychaete populations, and movement of the fish hosts on parasite dynamics. Only two of the three parasite genotypes present in the upper basin were detected: genotype II (associated with nonnative RBT) was dominant in the Williamson River, and at Keno eddy (located 47 rkm downstream in the main-stem Klamath River) genotype 0, associated with native redband trout, was dominant. This genotype distribution likely reflects the presence of stocked nonnative RBT in the system, which has provided a new host for the parasite.

With dam removal, modification, or both, the migration of anadromous salmonids into the upper Klamath basin is likely to alter parasite dynamics by providing new hosts for existing parasite genotypes and introducing new genotypes. Chinook salmon that historically inhabited the Williamson and Sprague rivers (Hamilton et al. 2005) are the focus of reintroduction efforts (Hooten and Smith 2008). The short-term effects of the high parasite densities in the Williamson River are likely to be negligible for juvenile Chinook salmon as disease is not associated with exposure to the genotypes present (0 and II; Atkinson and Bartholomew 2010a; Hurst 2010). However, adult Chinook salmon will introduce genotype I as they migrate into new habitats (Atkinson and Bartholomew 2010a). As conditions in the Williamson River below the Sprague River confluence are already conducive to parasite propagation, this introduced parasite genotype is likely to become established. Thus in the longterm, we predict juvenile Chinook salmon that migrate through this area of the river after late May could encounter increased disease risk from *C. shasta*. Introduction of adult salmon infected with genotype I does not present a risk for the native redband trout as this species does not become diseased after exposure to this parasite genotype (Atkinson and Bartholomew 2010b).

In addition to risks from establishment of new parasite genotypes, reintroduction of anadromous fish into the upper Klamath basin potentially allows for an expansion of the distribution of *C. shasta*. Chinook salmon historically utilized spawning habitats in both the Williamson and Sprague rivers (Hamilton et al. 2005), and the recent removal of the Chiloquin Dam on the Sprague River in 2008 is expected to increase salmonid habitat. Although *C. shasta* was not detected in the Sprague River (this study), this may have been a result of warm temperatures that may limit both host utilization of the habitat (Cherry et al. 1977) and the parasite’s reproduction. However, Chinook salmon returning in the spring are likely to utilize the Sprague River when water temperatures are lower and are more favorable for both the parasite and the host.

The short-term effects of *C. shasta* on coho salmon reintroduction success are likely to be minimal. The historical distribution of coho salmon likely extended to Spencer Creek, located less than 1 rkm upstream of Keno eddy (Hamilton et al. 2005), suggesting it is unlikely these fish will migrate into the Williamson River. Even if migration into the Williamson River were to occur, the survival of coho salmon after exposure to high doses of genotype II in this study suggests that this genotype may be adapted to different host species in the upper and lower Klamath River basin. This differential pathogenicity has also been supported in laboratory challenges (Hurst 2010). The risk of disease from *C. shasta* for coho salmon migrating into the vicinity of Keno eddy is currently low based on low parasite densities and predominance of genotype 0 (Atkinson and Bartholomew 2010b). However, long-term effects of coho reintroduction would likely include the introduction and establishment of a lower-basin genotype II specific for coho salmon, potentially increasing disease risk over time.

Because of the specificity of *C. shasta* genotypes for their fish host species, the fish host(s) responsible for parasite amplification can be inferred from the genotype ratio. Native redband trout become infected with *C. shasta* genotype 0 but do not often develop ceratomyxosis (Buchanan et al. 1989; Atkinson and Bartholomew 2010b). In contrast, nonnative RBT are highly susceptible to infection and disease with genotype II (Atkinson and Bartholomew 2010a, 2010b). The low proportion of genotype 0 in the Williamson River suggests that native redband trout are not responsible for the high parasite densities. Thus, the stocking of nonnative RBT to supplement recreational fishing is the most likely explanation for the amplification of parasites in the Williamson River. This explanation is supported by higher mortality of RBT and higher density of genotype II in the Williamson River as compared with Keno eddy, where stocking does not occur. This result has prompted the ODFW to reevaluate the RBT stocking plan (Tinniswood, personal communication).

Patterns of fish movement can also be inferred from the genotype ratio. The presence of *C. shasta* genotypes 0 and II throughout the main-stem Williamson River suggests that both nonnative RBT and native redband trout are found throughout the main-stem river. Conversely, the inability to detect genotype II from water samples at Keno eddy indicates that the nonnative RBT are unlikely to migrate this distance downstream.
Following dam removal, lower basin genotypes I and II (associated with coho salmon) will likely be detected in the Williamson River and at Keno eddy as both Chinook and coho salmon migrate into this area.

Trends in parasite density and distribution are affected by temperature throughout the main-stem Williamson River and its tributaries. Water temperatures in the lower Williamson River (reach 1) are influenced by input from the Sprague River, where land use practices have decreased natural riparian shading, resulting in warmer temperatures (Boyd et al. 2002). Thus, in the Williamson River below the Sprague River confluence, temperatures rise above 10°C in April, peak in July, then continue to decline, falling below 10°C in October (USGS; http://waterdata.usgs.gov/or/nwis/rt; this study). Consequently, parasite densities below the Sprague River confluence were high during all sample times. Water temperatures above the Sprague River confluence were largely influenced by Spring Creek, a constant source of cold water (5–10°C) even during the summer months. Although both hosts may be present, temperatures are below the threshold of both actinospore production in the polychaete host (Bjork 2010) and parasite proliferation in the fish host (Schaefer 1968; Johnson 1975; Udey et al. 1975; Ratliff 1981; Hendrickson et al. 1989). Therefore, the cool-water input from Spring Creek likely limits parasite production in reach 2.

Parasite density above Spring Creek (reach 3) may be limited by water flow, as temperatures were similar to those recorded below the Sprague River confluence. The upper portion of reach 3, at rkm 42, is ephemeral, with no flow from late summer through winter (USGS; http://waterdata.usgs.gov/or/nwis/rt) and reduced flows occur further downstream where polychaetes were identified. This flow variability may naturally constrain polychaete populations, which require a certain amount of flow for filter feeding and to prevent desiccation (Stocking and Bartholomew 2007; Bjork and Bartholomew 2009). Thus, water flow may restrict the range of the parasite in the upper reaches of the Williamson River.

With dam removal proposed as a tool in many restoration efforts, it is important to consider how pathogen dynamics in a system may change once dams are removed (Brenkman et al. 2008). In cases where fish passage above the dams has never occurred, resident fish have been isolated from pathogens present below the projects and may have decreased resistance. Conversely, isolated resident fish could transmit novel pathogens to reintroduced fish stocks. Thus, when considering the disease risk from an endemic parasite such as Ceratomyxa shasta, some level of parasite-induced effects should be factored into reintroduction efforts. Dam removal may be expected to reduce some pathogen risks below dams as a result of improved flows and temperature as well as increased movement of fish. However, in areas upstream of the effects of dam removal, environmental conditions may not change significantly and reintroduction may have unintended consequences. Thus, disease risks should be evaluated by establishing baseline information on pathogens above and below the current barriers and by monitoring high-risk locations, such as the Williamson River, following reintroduction. Results of this study also suggest that current management practices, such as stocking nonnative fish, should be reevaluated as restoration plans progress.

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Habitat for Age-0 Shovelnose Sturgeon and Pallid Sturgeon in a Large River: Interactions among Abiotic Factors, Food, and Energy Intake

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Habitat for Age-0 Shovelnose Sturgeon and Pallid Sturgeon in a Large River: Interactions among Abiotic Factors, Food, and Energy Intake

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Abstract
The main stems of large rivers throughout the world have been highly modified with little consideration for effects on fishes that rely on these areas to complete their life histories. Particularly important is the ability of riverine habitats to provide foraging opportunities for young fish. We explored how temperature, flow, and food availability influenced diet content, prey selection (Strauss’s linear selectivity index), and energy condition of age-0 shovelnose sturgeon Scaphirhynchus platyrhynchus and pallid sturgeon S. albus in major habitat areas (e.g., islands, channel borders, wing dikes, and side channels) of the middle Mississippi River during spring (March–May) and summer (June–August) 2008. Standardized diet mass (dry mass standardized for fish body mass) of the age-0 sturgeon peaked at about 19°C and at a flow velocity of 0.5 m/s. Although potential prey taxa were diverse, the diets for age-0 sturgeon of all sizes were dominated by mayflies (Ephemeroptera) and midge larvae (Chironomidae) across all habitats. As age-0 sturgeon grew, the relative energy return per habitat appeared to diverge; island tips upstream of the main channel and channel border areas behind wing dikes provided higher gains in standardized diet mass than other habitats. No differences in energy condition (kJ/g) occurred among habitats, although large (51–200 mm total length [TL]) age-0 sturgeon had higher energy densities than their small (≤50 mm TL) counterparts. Enhancement of areas with flow and substrates that facilitate the production and availability of midges and mayflies (e.g., instream island complexes) is critical for the recruitment of age-0 Scaphirhynchus sturgeon in large rivers.

The main channels of large rivers contain many species of fish (Dettmers et al. 2001) and may be important for allowing these organisms to complete key life history stages (Galat and Zweimuller 2001; Garvey et al. 2010). The habitats within the main channel and its adjacent areas are dynamic, creating heterogeneity that is probably critical for early growth and survival of fish, particularly for species that recruit in these areas, such as freshwater drum Aplodinotus grunniens, blue catfish Ictalurus furcatus, paddlefish Polyodon spathula, and North American sturgeons (Acipenseridae). Although extrachannel habitats (e.g., backwaters and floodplains) are often the focus of river conservation and restoration (Gutreuter 2004; Prato and Hey 2006), main-channel areas also have been altered in many ways for purposes such as flood control and navigation and require restoration attention (see Garvey et al. 2010). The impact of these changes on fishes that depend on these areas is not well understood (Dettmers et al. 2001). Identifying and quantifying key habitat types in these rivers are critical for understanding the effects of degradation and for determining restoration goals.

The middle Mississippi River (extending 320 river kilometers [rkm] between the confluences of the Ohio and Missouri rivers) has been highly channelized, leaving few remaining side channels, sandbars, and instream islands (Hurley et al. 2004; Koch et al. 2012). Loss of these habitat types may have negatively affected the resident sturgeons of the genus Scaphirhynchus. The more common species, the shovelnose sturgeon S. platyrhynchus, and its federally endangered congener, the pallid sturgeon S. albus, have high mortality rates and low recruitment due to a
multitude of anthropogenic impacts, such as overharvesting and habitat loss (Colombo et al. 2007; Tripp et al. 2009).

Recruitment in these sturgeon species is probably driven by the availability, quantity, and quality of habitat that is present in or lies adjacent to the main river channel after their larvae settle from the drift and become benthic-foraging juveniles (Galat and Zweimuller 2001; Braaten et al. 2008). The density of Scaphirhynchus larvae and juveniles differs among habitat types in the middle Mississippi River; small fish occupy areas of moderate flow that are sheltered from the main river channel, whereas larger, older individuals become more dispersed in the river (Phelps et al. 2010a). We evaluated the relative energetic value of major main-stem habitat types in the middle Mississippi River for small (≤50 mm total length [TL]) and large (51–200 mm TL) age-0 shovelnose sturgeon and pallid sturgeon. We hypothesized that habitats would differ in food availability and thus would differ in potential energy return, depending on flow and prey associated with each habitat area. We examined macrohabitats and other abiotic and biotic factors to evaluate whether they are more conducive for successful foraging and thus better growth and survival to advanced life stages.

METHODS

Once per week during May–September 2008, age-0 shovelnose sturgeon and pallid sturgeon were collected between 1000 and 1530 hours via 3-min tows with a mini-Missouri bottom trawl (see Herzog et al. 2005 for full gear description) at six island complexes in the middle Mississippi River: Mosenthein (rkm 298–303, where rkm is the distance upstream of the Ohio River confluence), Osborne Chute (rkm 233–236), Rockwood (rkm 161–166), Grand Tower–Cottonwood (rkm 126–129), Marquette (rkm 77–80), and Angelo (rkm 8). At each island complex, seven macrohabitats were sampled (Figure 1): channel border–dike (CBD), channel border–open (CBO), island–downstream tip (IDT), island–main channel (IMC), island–upstream tip (IUT), side channel (SC), and main channel. Sampling in each macrohabitat type included near-bank trawling (water depth <3 m) and off-bank trawling (water depth >3 m). Given that the highest densities and greater variability in lengths of young sturgeon occur near islands and SCs (Phelps et al. 2010a), about 70% of the sampling was concentrated in these areas. The remaining 30% of sampling effort was expended in the CBO and CBD strata. In addition, the three channel macrohabitats are homogeneous, whereas heterogeneity exists around island complexes; therefore, more sampling effort was needed around islands to capture the unique differences in abiotic and biotic factors. Trawl sites were excluded when river conditions (e.g., low water or the presence of obstructions) prevented sampling.

The TL (mm) and wet mass (g) of each age-0 sturgeon were recorded; each fish was placed into a vial that contained a 95% ethanol solution accompanied by specific trawl information (island, macrohabitat, date, water velocity, and water temperature).

At the sizes collected, it was impossible to morphologically distinguish shovelnose sturgeon from pallid sturgeon. Thus, this research focuses on patterns at the genus level, assuming that the shovelnose sturgeon predominated in the samples (Hrabik et al. 2007; Schrey et al. 2007). Each site, a Marsh–McBirney flowmeter was used to measure surface water velocity (m/s) and a Quanta or Hydrolab handheld meter or sonde was used to collect water temperature and dissolved oxygen data.

At each macrohabitat, 500 mL of trawl contents (consisting of a mixture of substrate, invertebrates, and organic matter) were randomly subsampled from a near-bank trawl tow and an off-bank trawl tow. The contents were then preserved in 95% ethanol, and macroinvertebrates were later extracted by hand for identification and quantification. The 500-mL subsample represented macroinvertebrate families in the entire trawl (D. Sechler, unpublished data). Prey densities were quantified as the number of individuals of each taxon per 3-min trawl tow.

The structure of Scaphirhynchus sturgeon stomachs may cause food to back up into the esophagus while other food is being processed (Held 1969). Thus, the esophagus was removed along with the stomach for studying the diet composition of each age-0 sturgeon. Stomach and esophagus contents were carefully removed and rinsed onto a gridded dish. Macroinvertebrates were enumerated and identified to the family level by using dichotomous keys (Merritt and Cummins 1996). Where head capsules were present, head capsule widths (mm) were measured for later calculation of biomass; such measurements were performed for Ephemeroptera (mayflies), Diptera (true flies) pupae, Chironomidae (midges; Diptera), Plecoptera (stoneflies), Trichoptera (caddisflies), and Odonata (damselflies and dragonflies). If other macroinvertebrates were encountered, they were measured according to the methods of Benke et al. (1999). In addition, if a macroinvertebrate was identified by a body part that was not used for measurement, it was counted to improve the accuracy of prey frequency of occurrence data (Chipps and Garvey 2007). Scion imaging software was used to measure all organisms at 500× magnification by using a digital camera (Cohu, Inc., San Diego, California) mounted on a Wild Model M5A dissecting scope.

Macroinvertebrate measurements were converted to dry mass (μg) values by using length–dry mass regressions (e.g., Benke et al. 1999; Chipps and Garvey 2007). For each age-0 sturgeon, diet mass was calculated in terms of micrograms of dry mass per gram of fish wet mass (i.e., μg/g) to standardize the amount across sizes of sturgeon sampled. After the diet analysis was completed (i.e., the esophagus and stomach were removed and the contents were identified and measured), the energy density (kJ/g) of age-0 sturgeon was determined to further investigate whether standardized diet mass was related to energy density. Each sturgeon was dried in an oven at 60°C to a constant mass and was then weighed. The dried sturgeon were pulverized, pressed into a pellet, and burned in a Parr 1425 semi-micro bomb calorimeter.
For each macroinvertebrate taxon, the frequency of occurrence and percent by number in diets and trawl samples were calculated. These data were used to calculate Strauss’s linear index of selectivity (Strauss’s $L$; Strauss 1979) for the four dominant prey categories: mayfly nymphs, chironomid larvae, dipteran pupae (including chironomid pupae), and other (i.e., all other prey consumed). Strauss’s $L$ was calculated as $L = r_i - p_i$, where $r_i$ is the relative abundance of prey item $i$ in the gut and $p_i$ is the relative abundance of prey item $i$ in the habitat. This index was used to determine prey size and macrohabitat diet selectivity by age-0 sturgeon. Strauss’s $L$ ranges from $-1$ to $1$; positive values indicate selection, negative values indicate avoidance or inaccessibility, and a value of 0 indicates that the prey type is consumed in proportion to its availability in the environment (Strauss 1979; Chipps and Garvey 2007).

Mean daily water temperatures ($^\circ$C) were compared among macrohabitat types by using a one-way analysis of variance (ANOVA) coupled with Tukey’s pairwise comparisons. Site temperature and surface water velocity (m/s) were also compared across macrohabitats with a one-way ANOVA combined with Tukey’s pairwise comparisons. Mean standardized diet mass ($\mu$g/g) was compared with water velocity and temperature by using nonlinear regression models; we assumed that a parabolic function (i.e., $Y = x + x^2$) would be appropriate for both comparisons. Previous research suggests that growth and diet intake should peak at some intermediate depth and temperature (Phelps et al. 2010b). Indices of macroinvertebrate abundance from trawls (mean number per 3-min trawl tow) also were compared across macrohabitats during each season via one-way ANOVA.

Mean standardized diet mass and age-0 sturgeon energy content across macrohabitats,
we used ANOVA with Tukey’s pairwise comparisons for both size-classes.

To further investigate whether size-related selectivity varied across macroinvertebrate taxa and macrohabitats, we conducted a multivariate ANOVA (MANOVA) on both sturgeon size-classes with each of the four prey groups as dependent variables and macrohabitat as the independent variable. Trends found with the MANOVAs were further evaluated by use of univariate ANOVAs. All analyses were conducted with the general linear models procedure in the Statistical Analysis System (α = 0.05).

RESULTS

In total, 404 age-0 *Scaphirhynchus* sturgeon were captured during 2008; 266 of these fish were categorized as small age-0 sturgeon (mean TL = 26.5 mm, SD = 0.03), and 138 were categorized as large age-0 sturgeon (mean TL = 103.1 mm, SD = 0.24). No age-0 sturgeon were caught in the main-channel macrohabitat; therefore, the main-channel habitat was excluded from the analyses. One-way ANOVA (F5,135 = 4.78, P = 0.0005) and Tukey’s pairwise comparisons indicated that water temperatures in the IMC and IUT macrohabitats were cooler than those in all other macrohabitats (Figure 2). Water velocity differed significantly across macrohabitats (F5,117 = 5.75, P < 0.0001; Figure 2). Tukey’s pairwise comparisons indicated differences in water velocity across macrohabitats; CBD and IUT macrohabitats had the lowest water velocities, and IDT habitat had the highest water velocities. The parabolic regression models of standardized diet mass in relation to water temperature (diet mass = [2.99 × temperature] − [0.078 × temperature2]; r² = 0.79) and velocity (diet mass = [83.2 × velocity] − [64 × velocity2]; r² = 0.73) were both significant (P < 0.0001), suggesting that an intermediate peak diet mass existed for both relationships. Macroinvertebrate abundance per trawl differed significantly among macrohabitats (MANOVA: Wilks’ lambda = 0.94, F20,1.705 = 1.61, P = 0.0400). However, the taxonomic categories did not significantly differ in abundance among habitat types (univariate ANOVAs: all P > 0.05; Figure 3); the exception was Ephemeroptera (univariate ANOVA: F5,517 = 3.41, P = 0.005). For three prey categories (dipteran pupae, chironomid larvae, and other prey), relative abundances were generally higher in areas associated with the channel border and IUTs.

Empty stomachs were rare in all macrohabitats during 2008: the percentage of age-0 sturgeon with empty stomachs was 2% in IUT habitat (total n = 37 sturgeon); 1% in CBD habitat (n = 68); and 0% in the CBO (n = 33), IDT (n = 94), IMC (n = 129), and SC (n = 38) habitats. Roughly 2% of small age-0 sturgeon had empty stomachs, and approximately 1% of large age-0 sturgeon had empty stomachs. The standardized diet mass (µg/g) for small age-0 sturgeon did not vary across macrohabitats (F5,258 = 1.95, P = 0.0859; Figure 4). However, the standardized diet mass for large sturgeon was significantly different among macrohabitats (F5,129 = 47.13, P < 0.0001); large sturgeon sampled at CBD habitats had the highest mean diet mass, and those sampled at SC habitats had the lowest mean diet mass (Figure 4). Diet selectivity by both small age-0 sturgeon (Wilks’ lambda: F20,508 = 3.83, P < 0.0001) and large age-0 sturgeon (Wilks’ lambda: F20,231 = 2.78, P = 0.0001) differed significantly among macrohabitats (Figure 5). Selectivity by small sturgeon differed significantly among habitats for all four invertebrate prey categories (all P < 0.05): Strauss’s L varied from –1 to 1 for mayfly nymphs, from −0.02 to 1.00 for dipteran pupae, from −0.05 to 1.00 for chironomid larvae, and from −1.00 to 0.01 for the “other” category. The patterns were generally similar for the large age-0 sturgeon, although differences in mean Strauss’s L only occurred for mayflies and chironomid larvae. Energy density (kJ/g) of age-0 sturgeon did not significantly vary among macrohabitats for the small
FIGURE 3. Mean (±SE) number of macroinvertebrates in trawl samples from six macrohabitats (abbreviations defined in Figure 1) of the middle Mississippi River during May–September 2008. Invertebrates were aggregated into four primary prey groups: ephemeropterans (EPH; mayflies); dipteran pupae (DP; includes chironomid pupae); chironomid larvae (CH); and other (all other prey consumed). Sample sizes are the same as shown in Figure 2. Bars without a letter in common are significantly different (P < 0.05).

FIGURE 4. Mean (±SE) standardized diet mass (µg dry weight of stomach and esophagus contents per g of sturgeon wet mass) for small (≤50 mm total length) and large (51–200 mm) size-classes of age-0 Scaphirhynchus sturgeon across six macrohabitat types in the middle Mississippi River, May–September 2008. Sample sizes are given at the bottom of each bar. Bars without a letter in common are significantly different (P < 0.05).

DISCUSSION

We found that the main-stem areas of the middle Mississippi River varied in physical and biological characteristics during the 2008 growing season for age-0 shovelnose sturgeon and pallid sturgeon, and these differences may affect diet selection, energy density, and ultimately growth and survival during early life (J. E. Garvey and S. R. Chipps, in press). The effect of these habitats on age-0 sturgeon may depend on fish size. Although the small size-class of age-0 sturgeon appeared to forage in a consistent manner across macrohabitats, our results suggest that the larger age-0 sturgeon garnered different energy returns depending on the habitat they occupied. Our analysis indicated that habitats adjacent to the main stem near the channel border and behind wing dikes provided more food for large age-0 sturgeon. Standardized diet mass for large sturgeon in SC areas was considerably lower, suggesting that foraging opportunities are limited in these areas, particularly during low-flow periods late in the age-0 sturgeon growing season (e.g., fall), when these habitats...
FIGURE 5. Mean (±SE) values of Strauss’s linear selectivity index for small (≤50 mm total length) and large (51–200 mm) size-classes of age-0 *Scaphirhynchus* sturgeon across six macrohabitats (abbreviations defined in Figure 1) in the middle Mississippi River, May–September 2008. Selection is presented for four prey categories: ephemeropterans (EPH); dipteran pupae (DP; includes chironomid pupae); chironomid larvae (CH); and other (all other prey consumed). Sample sizes are given at the bottom of each bar. Bars without a letter in common are significantly different (P < 0.05).

can become disconnected from the main channel. Additionally, stage height (about 8 m) remained high throughout spring and summer during 2008, thereby potentially increasing available spawning habitats, nursery habitats, and catch rates of age-0 sturgeon (Phelps et al. 2010b) in comparison with the previous 4 years. A better understanding of the factors that made 2008 a more favorable year for growth, survival, and recruitment of age-0 *Scaphirhynchus* sturgeon will provide knowledge that is vital to enhancing the management of these declining populations.

Although we did find some habitat-specific differences in standardized diet mass, the energy density of the small and large size-classes of age-0 sturgeon did not depend on habitat. Small sturgeon had lower energy densities, probably because more body mass was allocated to energy-neutral materials, such as skeleton and bony scutes. In contrast, a greater proportion of energy was probably allocated to storage and energy-rich body structures in age-0 sturgeon of the larger size-class, leading to higher energy density. Energy density is often
FIGURE 6. Mean (±SE) energy density (kJ/g) of small (≤50 mm total length) and large (51–200 mm) size-classes of age-0 *Scaphirhynchus* sturgeon across six macrohabitats in the middle Mississippi River, May–September 2008. Sample sizes are given at the bottom of each bar. Bars without a letter in common are significantly different (*P* < 0.05).

An indicator of body condition and should be related to dietary intake.

Despite the wide variety and apparently high density of potential prey items available to age-0 sturgeon in the middle Mississippi River, only a few prey categories were consumed in different quantities than available in the habitats. These results are nearly identical to those found for age-0 shovelnose sturgeon in the upper Missouri River (Braaten et al. 2007). In particular, we found that both the small and large size-classes of age-0 sturgeon across habitats consistently consumed chironomids in proportions greater than their abundance in the trawls. Chironomids are often associated with fine substrates and may have been easily suspended in the river, thus entering the drift and becoming more accessible to foraging sturgeon. Dipteran pupae were fairly rare in trawl samples but still appeared in diets, again suggesting some preference for these benthic-oriented organisms. Mayfly nymphs, which probably embed in or cling to firm sand or gravel substrate, occurred similarly in all macrohabitats (i.e., based on trawl samples); this prey group often appeared in diets of both the small and large age-0 sturgeon, and selection often was positive. In summary, these results suggest that there is no apparent size-specific or age-specific dietary shift exhibited by age-0 *Scaphirhynchus* sturgeon among habitats. Although mayfly nymphs are larger and thus are the most energetically beneficial prey common in the diets, the clear preference of age-0 sturgeon for midge larvae suggests that they are an abundant and energetically efficient food source for young sturgeon in the middle Mississippi River. Our results are similar to those reported by Hoover et al. (2007) for older *Scaphirhynchus* sturgeon in the middle Mississippi River; in their study, mayflies and caddisflies were important diet components for adult *Scaphirhynchus* sturgeon. Pallid sturgeon have also been observed to consume fish as they age (Gerrity et al. 2006; Hoover et al. 2007). However, it is not known whether pallid sturgeon smaller than 200 mm TL will engage in piscivory; we did not document any fish in age-0 sturgeon diets during the present study.

The middle Mississippi River and other large rivers that harbor young *Scaphirhynchus* sturgeon and other river-obligate fish are continually modified for multiple uses; therefore, the quantity and quality of habitat must be defined accurately. Phelps et al. (2010a) found that the macrohabitat scheme used herein provided insight into the relative density of age-0 *Scaphirhynchus* sturgeon. Similarly, we found that food availability and energy density also varied across these habitat types in biologically meaningful ways. Areas of contrasting flow probably create substrate that is amenable to the production and perhaps suspension of midges and mayflies and appear to be important, particularly for older sturgeon life stages. Not surprisingly, the natural habitat types where these conditions occur include the IUT areas and channel borders. An artificial counterpart, the wing dike, creates alternating areas of scour and deposition that probably facilitate both invertebrate production and the foraging success of age-0 *Scaphirhynchus* sturgeon. At a finer scale, the parabolic functions we fit to our data suggest that standardized diet mass in age-0 sturgeon peaked in areas characterized by a temperature of about 19°C and a flow velocity of 0.5 m/s. These data indicate that management efforts to create heterogeneity in flow conditions will facilitate substrates favored by midges and mayflies, thereby improving *Scaphirhynchus* sturgeon foraging success, growth, and ultimately recruitment. Ideally, this could be accomplished by creating a series of island complexes in the main channel of the middle Mississippi River (Koch et al. 2012).

Future efforts should investigate shallow and deeper (>3 m) transects and the effects of depth, water temperature, water velocity, and sturgeon movements on diet composition and energy density of age-0 *Scaphirhynchus* sturgeon. Sturgeon movement is also of importance because large age-0 sturgeon are probably mobile in the river (Phelps et al. 2010a); therefore, diet contents
from a specific habitat sampled at one point in time may not reflect an individual’s actual foraging habitat because the sturgeon may have foraged elsewhere before moving into the habitat of capture. In addition, examination of both main-channel and SC habitats associated with island point-bar macrohabitats may provide further insight into how the aforementioned variables may differ in these additional macrohabitats. This resolution on a finer scale of macrohabitat sampling, when combined with trawling depth data, may provide more-specific information on which habitats are necessary for age-0 Scaphirhynchus sturgeon to successfully recruit to adult life stages. Lastly, this study provides baseline information that can be used to further explore crucial early life history stages of Scaphirhynchus spp. sturgeons.

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Assessing Red Drum Juvenile Stocking in a South Carolina Estuary Using Genetic Identification

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Abstract

The South Carolina Department of Natural Resources has been stocking red drum Sciaenops ocellatus since 1988 to evaluate parameters critical to their successful survival and recruitment in South Carolina estuaries. From 1999 to 2002, between 600,000 and 1,000,000 juvenile red drum were stocked each year in two tributaries of Charleston Harbor. The harbor and each tributary were partitioned into three independent strata and randomly sampled monthly for two decades, allowing population trends before, during, and after stocking to be evaluated. Using microsatellite-based parentage analysis, we examined the contribution of stocked age-0 juvenile red drum (15–60 mm total length) to the local population 1 year after release by using fishery-independent sampling. Analysis of these data showed that the highest contributions (88.9%) were close to the stocking site in years with low natural recruitment, whereas in years with high natural recruitment, contributions were lower and stocking was less effective in increasing catch per unit effort. The results of stocking 600,000 small juveniles/year from 1999 to 2001 in one of the study tributaries (Ashley River) indicated that stocked fish did not displace wild fish but had an additive effect on their abundance, supporting the hypothesis that trophic resources are not limiting for postlarval age-0 red drum within Charleston Harbor. The high observed variability in contribution among years of stocking similar-sized red drum suggests that (1) interpretation on a year-class-specific basis is necessary to fully understand the effects of stocking and (2) marine stock enhancement programs would benefit substantially from evaluation in the context of wild annual recruitment patterns.

The red drum Sciaenops ocellatus is an important recreational fish species that is distributed along the South Atlantic and Gulf coasts of the USA; populations of red drum have substantially declined, due in part to increased fishing pressure (e.g., Vaughan and Carmichael 2000). As a result, many states have enacted successively more-restrictive regulations on recreational anglers to allow a portion of the estuarine-dependent subadult population to reach sexual maturity and join the spatially separate offshore spawning aggregations (Peters and McMichael 1987; Ross et al. 1995; Rooker et al. 1998). In addition, Texas, Florida, and South Carolina have developed stock enhancement research programs to address specific questions about life history, stocking strategies, and tagging methods (McEachron et al. 1995; Arnold et al. 1996; Jenkins et al. 1997, 2004, 2005; Serafy et al. 1999). Historically, marine stock enhancement efforts have been perceived as unsuccessful due to their inability to demonstrate a quantitative contribution to the fisheries (Shelbourne 1964; Blaxter 2000; Chan et al. 2003). In many cases, however, these enhancement programs were targeted at commercially harvested species (Atlantic cod Gadus morhua and plaice Pleuronectes platessa), making it unlikely that small-scale stocking programs could impact catches with low numbers of stocked fish (Shelbourne 1975; Danielsen and Gjosaeter 1994). Typically, research-scale stocking programs release too few fish to be detected in open systems when evaluating changes in fisheries-dependent or fisheries-independent catch per unit effort (CPUE) as a measure of success (Tveite 1971; Blaxter 2000; Scharf 2000). In addition, historic egg, larvae, and small juvenile stocking programs implemented in both Europe and the
USA released fish that were not tagged or marked in a manner permitting the qualitative assessment of their impacts (Blaxter 1976; Solendal et al. 1984).

During the last 30 years, more sophisticated marking techniques (e.g., chemical or thermal marking of otoliths) have allowed researchers to mark larval fish prior to their release (Tsukamoto 1988; Volk et al. 1990; Brooks et al. 1994; Beckman and Schulz 1996). Since its inception in 1988, the red drum stocking program administered by the South Carolina Department of Natural Resources (SCDNR) has marked all fish prior to release to facilitate quantitative evaluation of the efficacy of stocking. During this time, SCDNR has used a variety of marking methods, including external anchor tags embedded in the dorsal musculature or through the abdominal wall, coded wire tags inserted into the snout, chemical marking of otoliths through immersion in oxytetracycline hydrochloride (OTC), and most recently genetic identification (Jenkins et al. 1997, 2002, 2004, 2005; Renshaw 2003; Robbins et al. 2008).

In their review of genetic techniques and application to fisheries research, Jones et al. (2010) indicated that microsatellite markers were the primary choice for parentage assignment. Several researchers have reported using these nuclear DNA markers to discriminate released hatchery fish from their wild cohorts by matching hatchery offspring to broodstock with a low probability of mismatch (Ferguson and Danzmann 1998; Perez-Enriquez and Taniguchi 1999; MacDonald et al. 2004). Microsatellites provide sufficient resolution for identifying hatchery-produced red drum collected from an estuary and for conducting parentage analyses to determine whether individual fish are progeny of broodstock used in the stocking program (Liu and Cordes 2004; Tringali 2006; Karlsson et al. 2008; Tringali et al. 2008). The use of nonlethal genetic tools from small fin clips is an attractive alternative to sacrificing fish to extract otoliths.

The use of genetic tools for stocking evaluation was demonstrated by red drum stock enhancement work conducted in South Carolina by Jenkins et al. (2004), during which approximately 600,000 age-0 juveniles were released annually between 1995 and 1998 in the Colleton River estuary. All fish released during the experiment were marked prior to release by immersion in OTC (Jenkins et al. 2002). Fish collected from the wild were evaluated for both OTC and genetic marks. Renshaw (2003) demonstrated that genetic analyses could be used as a substitute for OTC marking; in blind tests, fish identified as being of hatchery origin by OTC marks were also correctly assigned by analyses of microsatellites. While both methods indicated that the fish of hatchery origin made up a substantial component (~19%) of each year-class in the target estuary, due to errors in reading OTC marks there was some disagreement as to which fish were stocked. In addition, the lack of baseline CPUE data for the stocked area meant that it was not possible to demonstrate whether stocking supplemented the existing population or displaced wild fish. For a stocking program to be effectively evaluated, such a metric must be documented. Thus, a new multiyear study was initiated in 1999 within the Charleston Harbor estuary to utilize genetic marks as the primary tracking tool. As reported by Jenkins et al. (2004) and Smith et al. (2004), the results of stocking OTC-marked fish suggest that stocked age-0 juveniles could make a substantial contribution to wild populations. The longevity of OTC marks and inherent variability in reader identification of the marks, however, make the use of genetic identification with microsatellites preferable to other methods for ascertaining contribution levels (Denson and Smith 2008).

This paper summarizes the evaluation of stocking red drum in two rivers within the Charleston Harbor estuary. The estuary was randomly sampled monthly over two decades, allowing population trends before, during, and after stocking to be evaluated. The research examined the contribution of stocked red drum juveniles (15–60 mm total length [TL]) on the localized population 1 year after release, when they recruit to fishery-independent sampling. Microsatellite analysis of individuals collected from fisheries-independent sampling was used to assess contribution. Hatchery genotypes were determined from captive broodstock, subsamples of juveniles retained from each release group, and samples collected in the wild. The efficacy of stocking age-0 red drum juveniles for enhancement of local populations and for further elucidating the factors that may be controlling red drum recruitment was also examined.

METHODS

Broodstock collection and spawning.—Red drum broodstock were acquired from the wild and produced hatchery year-classes during four consecutive years (1999–2002). In spring and summer 1999, 11 broodstock were collected from the local offshore adult population by SCDNR staff via bottom longline. Passive integrated transponder tags were implanted into the fish; the fish were sexed, measured, weighed, and returned to shore in live tanks. Upon arrival at the hatchery, fish were transferred to 3.8-m-diameter tanks. Each tank was equipped with a biological bead filter (Armant Aquaculture, Vacherie, Louisiana) and ultraviolet sterilizer (Aqua Ultraviolet, Temecula, California). Six fish (three males and three females) were placed in one tank, and five fish (two males and three females) were placed in another tank. A fin clip was removed from each fish for genetic analysis and stored in a solution of 1% sarcosyl, 6-M urea, 20-mM sodium phosphate, and 1-mM EDTA buffered at pH 6.8.

In 2000, an additional 14 red drum adults were collected and divided into tanks at sex ratios similar to that of the 1999 collection. In 2001, two tanks of fish captured during the previous years were recombined to provide a different genetic makeup; the fish were reconditioned and spawned to produce juveniles for the fall 2001 stocking efforts. These fish were subsequently released, and nine new fish were obtained from the wild to produce the 2002 hatchery year-class.

Red drum were conditioned and spawned by using compressed photoperiod and temperature cycles to simulate natural seasonal fluctuations. Control of spawning allowed us to prepare the nursery ponds at the Waddell Mariculture Center (WMC)
several weeks prior to stocking with larvae to optimize nursery rearing conditions. In addition to larvae produced from broodstock held at SCDNR, a private hatchery (Southland Fisheries, Hollywood, South Carolina) provided larvae from broodstock collected in South Carolina waters during 1999 for the first three stocking events.

During each year, 2–3-d-old red drum larvae were stocked at a density of 1.4 million larvae/ha in fertilized saltwater ponds (see Jenkins et al. 2004) and were harvested approximately 30 d after stocking. During harvest, fish were group-weighed to estimate the total biomass harvested from each pond. In addition, at least three subsamples from each pond were group-weighed and individually counted to estimate the number of fish per unit weight. In addition, 25 fish/pond were individually weighed and measured to document the size-frequency distribution.

Control groups of red drum were retained from every release group to document posthandling survival. Genetic samples were also collected from a subsample of 50 fish from each production unit. Fish were maintained in recirculating seawater systems for 30 d.

Stocking sites.—The two locations chosen for stocking were the Ashley and Wando rivers, which converge in Charleston Harbor. Sample sites in the two rivers and the harbor were partitioned into three separate strata in a statewide random fishery sampling survey (Figure 1). Beginning in 1990, each stratum was routinely sampled for numerous species by using standardized gear (trammel nets) throughout a 2-m tidal amplitude. Red drum were only available to the gear when the water was less than 1 m in depth and receded from the smooth cordgrass Spartina alterniflora marsh. Red drum first recruit to the sampling gear in early summer; therefore, age-1 data included only red drum less than 400 mm TL that were collected between July and December of each year. From 1992 to 1998, mean red drum CPUE for the Ashley River ranged from 0.3 to 1.4 fish/net set, while the Charleston Harbor stratum ranged from 1.2 to 5.3 fish/net set and the Wando River ranged from 1.2 to 8.4 fish/net set. These data suggested that the Ashley River usually had a smaller population of age-1 red drum than the Wando River.

The specific stocking locations within the study tributaries were previously identified by agency researchers as “good” nursery habitat, characterized by small creeks adjacent to high-marsh, mud flats, and oyster reefs where red drum had been previously captured (Figure 1). The fish were transported to boat landings adjacent to stocking sites in a 2,275-L, oxygenated hauling trailer and were acclimated for approximately 1 h before being removed from the trailer and placed in a boat-mounted tank equipped with compressed oxygen and high-pressure air stones. Fish were then transported to the stocking locations, where they were dispersed throughout the marsh during flood tide at or close to the time of high water.

Each river was stocked during three sequential years to allow for replication of treatments. In 1999, approximately 617,000 red drum juveniles (19–104 mm TL) from seven ponds were released into the Ashley River on six separate occasions between October 13 and November 30 (Table 1). Significant differences (Kruskal–Wallis analysis of variance: $H = 319.741$, df = 6, $P < 0.001$) in the size of fish originating from the different ponds dictated that the reported mean was weighted. Four of the release groups (574,191 fish) were made up of red drum that measured 19–47 mm TL (mean = 28.2 mm TL). The remaining
37,117 fish were significantly larger (mean = 63.0 mm TL). The mean size of stocked fish for the entire release group in 1999 was 31.0 mm TL. In 2000, the Ashley River was stocked with 604,884 juveniles (16–46 mm TL) on four stocking trips between September 29 and November 8, and the Wando River was stocked with 513,920 juveniles (16–37 mm TL) during two trips on November 9 and 14. In 2001, the Ashley River was stocked with 721,417 juveniles (17–28 mm TL) between October 18 and November 28. In 2002, the Ashley River was stocked with 344,949 juveniles (19–104 mm TL) during two trips on November 9, while the Wando River was stocked with 621,962 fish (13–36 mm TL) during three trips between September 11 and 26 and was the only river stocked in the Charleston Harbor estuary during that year.

Genetic methods.—Genomic DNA was extracted from red drum fin clips by using the SprintPrep Plasmid Purification system (Agencourt Bioscience) according to the manufacturer’s directions and was stored in 10-mM tris and 0.1-mM EDTA. All tissue samples were genotyped at eight microsatellite loci in three multiplexed panels (Table 2). Amplification of DNA was performed in 20-µL reactions containing approximately 10 ng of DNA, 0.2 mM of each deoxynucleotide triphosphate, 0.3 µM of forward and reverse primers, 1× HotMaster Taq buffer, 3.5 mM of MgCl₂, and 0.1 units of HotMaster Taq DNA polymerase (Eppendorf). Thermal cycling conditions included 2 min at 94°C; 18 cycles of 30 s at 94°C, 30 s at annealing temperature (decreasing from 55°C to 43°C with a 1.5°C decrease every two cycles), and 40 s at 65°C; 17 cycles of 30 s at 94°C, 30 s at 43°C, and 40 s at 65°C; and one cycle of 60 min at 65°C. The amplified products were separated on a CEQ 8000 automated sequencer (Beckman Coulter) along with a fluorescently labeled, 400-base-pair size standard. Genotypes were scored using the Beckman Coulter CEQ 8000 fragment analysis software. Two readers independently scored all data, and differences were reconciled in conference. All broodstock red drum were genotyped at least twice.

Expected allele and genotype frequencies were estimated by using Levene’s (1949) correction. The effective number of alleles (Aₑ) was calculated according to Kimura and Crow (1964) as

\[ Aₑ = 1/(1 - Hₑ), \]

where \( Hₑ \) is the expected heterozygosity. The significance of any deviation from Hardy–Weinberg equilibrium was

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank accession number</th>
<th>Forward (F) and reverse (R) primer sequence</th>
<th>Repeat motif</th>
<th>Multiplex group</th>
<th>Alleles (n)</th>
<th>Aₑ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soc014</td>
<td>AF183146</td>
<td>(F) GTATGTATTAAGGCACAAGGTG (R) GATTTGCTGCTGGACAGACTG</td>
<td>(GT)₂₁</td>
<td>1</td>
<td>16</td>
<td>5.2</td>
</tr>
<tr>
<td>Soc017</td>
<td>AF183148</td>
<td>(F) CCCCCGTCTACGTGACAGTATG (R) ATAGCTGCGCATCATTCGGTTG</td>
<td>(GT)₁₄</td>
<td>1</td>
<td>16</td>
<td>6.5</td>
</tr>
<tr>
<td>Soc243</td>
<td>AF073283</td>
<td>(F) GACGGGGATGCTCATGTG</td>
<td>(CCT)₉</td>
<td>2</td>
<td>7</td>
<td>3.5</td>
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<tr>
<td>Soc083</td>
<td>AF073269</td>
<td>(F) TGCTGTAATGAAAAACGACGTGTC</td>
<td>(GT)₁₉</td>
<td>2</td>
<td>17</td>
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</tr>
<tr>
<td>Cne612</td>
<td>—</td>
<td>(F) CAAGTTCAGGTGAGTAGTGGAG (R) AGGAACTCTGCACAAATCAA</td>
<td>(GT)₂₀</td>
<td>2</td>
<td>10</td>
<td>2.5</td>
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<tr>
<td>Soc029</td>
<td>AF183148</td>
<td>(F) GCGGATAGTACGGAAAAATTACATGG (R) GAT TTCTCTCTGACTGGGAGT</td>
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<tr>
<td>Soc129</td>
<td>AF073275</td>
<td>(F) GCGGCTGCAACACAAGAAATT (R) TGCAGCGGGAAACAGAACG</td>
<td>(ATCT)₄</td>
<td>3</td>
<td>20</td>
<td>11.4</td>
</tr>
<tr>
<td>Soc060</td>
<td>AF073267</td>
<td>(F) TCTATGGAGCCTGTAAGTTATTG (R) CAAGGAGGAGTGGGGAATGACAA</td>
<td>(GAG)₉</td>
<td>3</td>
<td>5</td>
<td>2.6</td>
</tr>
</tbody>
</table>
evaluated with sequential Bonferroni corrections (Rice 1989) as determined by an unbiased approximation of Fisher’s exact test using a Markov-chain randomization method implemented in GENEPOP version 3.3 (Guo and Thompson 1992; Raymond and Rousset 1995a, 1995b). Larvae were evaluated for adherence to expected Mendelian proportions by using a chi-square test. Linkage disequilibrium was tested between all pairs of loci in all wild year-class samples, again by using a Markov-chain-based randomization method as implemented in GENEPOP.

In 1999, red drum broodstock and hatchery juveniles were sampled at WMC, while only hatchery juveniles were sampled at the Southland Fisheries hatchery. For 2000–2002, progeny were produced only at the WMC and all broodstock genotypes and hatchery juvenile genotypes were available. For the progeny of the 1999–2002 year-classes, exclusion analysis implemented in PROBMX version 1.3 (Danzmann 1997) was used for parentage assignment. Broodstock genotypic data were compared with genotypic data from wild and hatchery samples. Probabilities of compatibility by chance (PCCs) were calculated using the genotypic data from wild and hatchery samples. Probabilities of assignment were genetically similar releases were combined. The reference groups then consisted of six genetically dissimilar populations: wild fish and the release groups that were held in tanks D1, D2–D3, D4–D5, C3, and C4. Individuals that did not match WMC broodstock by exclusion analysis were designated as “stocked” or “wild” if they were assigned by maximum likelihood analysis to any combination of Southland Fisheries reference populations or to the wild reference population, respectively, with a probability of at least 90%. Any individual that did not meet these criteria was designated unknown. The validation test was conducted on a subset (WMC-released and wild) of age-1 fish belonging to the 1999 year-class. Out of 105 samples, 98 assignments were in agreement among the two methods (93.3% concordance). In all cases, the GENECLASS2 method was more conservative (i.e., identified fish as wild when identified by PROBMX as cultured). Therefore, although the assumptions associated with mixed-stock analysis (GENECLASS2) algorithms are not usually met in stocking scenarios, our confidence level in this case appears sound as we find high concordance among exclusion (PROBMX) and mixed-stock analysis methods as well as zero false-positive identifications. In a worst-case scenario, we would underestimate the contribution of Southland Fisheries-released fish by 6.7%.

Sampling.—The SCDNR’s stratified random sampling efforts began in 1990, and each stratum was sampled monthly by using trammel nets deployed from the stern of a specially designed Florida net boat (Tremblay). Locations were randomly selected from 30 sampling locations in each stratum that could be effectively sampled with the gear type. These sites were typically along marsh banks with gradual slopes, near oyster reefs, mud flats, on either side of creek mouths, and near structure. Locations with drop-offs, fast-moving water, and submerged stumps or structure were avoided. The nets were 137 m long, consisting of 2.4-m-deep monofilament in three panels (two outside panels: 10-cm stretch; one internal panel: 5-cm stretch). The nets were deployed in an arc next to the bank while the boat was moving at approximately 28 km/h to trap the fish between the bank and net. After the net was set, the boat was driven through the sampling area and the fish were driven into the net. Red drum recruit to this sampling gear at 10 months of age, when they are approximately 250 mm TL. The CPUE data collected for each month were used to determine year-class strength and regional variability within each year-class. Red drum CPUE within a stratum was compared across years to evaluate changes between the years of stocking and the years prior to stocking. Additionally, within-year red drum CPUE was compared across strata to evaluate potential changes in relationships of CPUE among strata during the stocking experiment. Analyses of CPUE were conducted for each stratum and year (1999–2002) by using a general linear model. The CPUE data were log(x + 1) transformed prior to analysis, and Tukey’s multiple comparison tests were used to identify individual differences between strata. Due to the schooling behavior of subadult red drum, the variation...
RESULTS

Genotypes were evaluated for departure from Hardy–Weinberg equilibrium at each locus and for linkage disequilibrium at each pair of loci. No significant differences were detected between observed and expected allele frequencies after Bonferroni correction. A \( \chi^2 \) test of Mendelian inheritance for all loci by using offspring (\( n = 39 \)) from two known 2001 parental crosses confirmed that no loci significantly departed from expected Mendelian inheritance (\( P = 0.06–0.88, \chi^2 = 1.16–13.50, df = 2–7 \)). Post hoc power estimations ranged from 0.66 to 0.81 for these analyses (G*Power version 3.1; Faul et al. 2009).

The genetic analyses determined that 88.9% of the age-1 red drum captured in the Ashley River during 2000 were fish that had been stocked in 1999 (Table 3). In addition, 30.8% of the fish in the adjacent Charleston Harbor stratum had been stocked in the Ashley River and 14.6% of the fish captured in the Wando River were also stocked. Fish were also released in the Wando River during 2000, yet of fish sampled in the Wando River during 2001, only 13.5% were identified as those stocked in the Ashley River or Wando River. The samples collected in the Charleston Harbor stratum showed that even with almost 1 million fish stocked into two of its tributaries, only 6.7% of sampled age-1 red drum were stocked fish. In the Ashley River, fish that had been stocked in 2000 comprised 35.6% of the collected samples. The fish stocked in 2001 contributed 28.4% of the sampled fish in the Ashley River during 2002, while only 2.0% of those captured in the Wando River and 1.6% of those captured in the Charleston Harbor stratum were stocked fish. Finally, after stocking about 620,000 small juveniles in the Wando River during 2002, no stocked red drum were detected in any samples from the three strata the following year.

Arnott et al. (2010) evaluated wild red drum CPUE from the SCDNR trammel-net survey from 1991 to 2007 and provided a template-standardized analysis across multiple strata, including the Ashley River, Charleston Harbor, and Wando River. Their results illustrated the synchrony of age-1 red drum abundance between strata in each wild year-class (regional variability between year-classes along the South Carolina coast occurred in all estuaries simultaneously) and support the supposition that strong and weak year-classes of wild fish occur (Figure 2; see Arnott et al. 2010 for complete description). It is among these identified annual year-class strengths that we overlay results from our stocking experiments for evaluation of their relationship to recruitment patterns.

The 1999 year-class contributed 51.2% of stocked fish to the population in the combined Charleston Harbor estuary (Table 3) during a very weak natural recruitment year (Figure 2). The 2000–2002 year-classes of wild red drum appeared to increase in strength, and 2002 was one of the strongest year-classes reported from SCDNR sampling. Contribution of stocked red drum showed an inverse relationship to wild red drum year-class strength (Figure 3). The high variability in contribution between stocking years of similar-sized red drum suggests that interpretation on a year-class-specific basis might be necessary to fully understand the effects of stocking.

In 1999, seven different groups of red drum were stocked in the Ashley River. Significantly larger-sized red drum were stocked earlier in the season, and smaller-sized red drum were stocked later in the season. Releases were pooled into two groups (release batches 1–3 and 4–7) as the fish produced from batches

<table>
<thead>
<tr>
<th>Year-class</th>
<th>Site</th>
<th>Stocking density (fish/ha)</th>
<th>Effort (net sets)</th>
<th>CPUE ± SD</th>
<th>Number sampled</th>
<th>Hatchery fish percentage</th>
<th>Total annual hatchery contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Ashley</td>
<td>630</td>
<td>41</td>
<td>1.73 ± 3.73</td>
<td>54 (34)</td>
<td>88.9 (82.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wando</td>
<td>0</td>
<td>32</td>
<td>1.00 ± 2.42</td>
<td>41 (38)</td>
<td>14.6 (7.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harbor</td>
<td>0</td>
<td>38</td>
<td>0.74 ± 1.03</td>
<td>26 (20)</td>
<td>30.8 (10.0)</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>Ashley</td>
<td>618</td>
<td>47</td>
<td>2.72 ± 7.97</td>
<td>59</td>
<td>35.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wando</td>
<td>184</td>
<td>33</td>
<td>3.97 ± 7.43</td>
<td>89</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harbor</td>
<td>0</td>
<td>50</td>
<td>3.68 ± 4.33</td>
<td>45</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>Ashley</td>
<td>737</td>
<td>51</td>
<td>1.04 ± 1.64</td>
<td>94</td>
<td>28.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wando</td>
<td>124</td>
<td>35</td>
<td>5.20 ± 8.46</td>
<td>49</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harbor</td>
<td>0</td>
<td>46</td>
<td>4.02 ± 6.87</td>
<td>64</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>Ashley</td>
<td>0</td>
<td>50</td>
<td>1.10 ± 2.44</td>
<td>17</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wando</td>
<td>232</td>
<td>30</td>
<td>5.47 ± 10.36</td>
<td>47</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Harbor</td>
<td>0</td>
<td>47</td>
<td>6.91 ± 14.88</td>
<td>37</td>
<td>0.0</td>
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</tr>
</tbody>
</table>
FIGURE 2. Long-term trends in standardized age-1 red drum catch per unit effort (CPUE) from 1988 to 2007 based on trammel-net surveys in seven independent strata along the South Carolina coast. Standardization protocols were developed based on the 1990–2007 year-classes; the mean CPUE of each stratum was standardized to a mean of 0 (shown here as a dashed line) and an SD of 1 (reprinted in part from Arnott et al. 2010, with permission).

1–3 had a larger mean size (61–64 mm TL) and were released earlier in the season (mid-October–early November) than the fish produced in batches 4–7, which had a mean of 22–27 mm TL and were released throughout November. The first release group comprised 6.1% of the fish released in 1999, yet 46.8% of those subsequently identified as stocked red drum from the 1999 year-class were genetically identified as being from this group (Table 4). Their relative survival (stocking success) was almost 10 times higher than that of the smaller-sized red drum stocked later in the season. These data support the concept of higher contribution from larger stocked fish. It is, however, impossible to determine which fish in a broad size distribution survived. In addition, for these stocking events we did not determine the effects of release timing in relation to available food resources or predator concentrations. Although we endeavored to produce similar-sized red drum across stocking events, variations occurred due to weather conditions and optimum tide for stocking. All fish were, however, reared on natural zooplankton blooms in ambient outdoor ponds during the normal spawning season and were stocked synchronously with wild fish of the same size at age. Even if we had excluded the larger-sized fish from our analyses, the patterns of contribution and relationships to natural recruitment among years would have remained the same (i.e., contribution in the Ashley River changes from 88.9% to 82.4%; Table 3). Additionally, we detected no differences in distribution patterns within the estuary among the

<table>
<thead>
<tr>
<th>Release batch</th>
<th>Number stocked</th>
<th>Mean (range) TL at release (mm)</th>
<th>Release date</th>
<th>Percentage of total number released</th>
<th>Number captured at age 1</th>
<th>Percentage of total hatchery fish captured</th>
<th>Stocking success ($\times 10^{-5}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16,064</td>
<td>61.4 (52–84)</td>
<td>Oct 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13,703</td>
<td>64.1 (40–104)</td>
<td>Oct 27</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7,350</td>
<td>64.2 (31–86)</td>
<td>Nov 3</td>
<td>6.1</td>
<td>29</td>
<td>46.8</td>
<td>78.1</td>
</tr>
<tr>
<td>4</td>
<td>97,821</td>
<td>38.9 (29–47)</td>
<td>Nov 3</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>156,112</td>
<td>26.8 (24–32)</td>
<td>Nov 15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>141,946</td>
<td>22.0 (19–26)</td>
<td>Nov 19</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>7</td>
<td>178,312</td>
<td>27.5 (20–40)</td>
<td>Nov 30</td>
<td>93.9</td>
<td>33</td>
<td>53.2</td>
<td>5.7</td>
</tr>
</tbody>
</table>
FIGURE 3. Relationship of year-class catch per unit effort (CPUE) and stocking contribution of age-1 red drum sampled in the three strata of the Charleston Harbor estuary (Wando = Wando River). Age-0 red drum were stocked in the estuary from 1999 to 2002.

size-groups (28 smaller red drum versus 20 larger red drum were recaptured in the Ashley River; three fish of both size-groups were recaptured in the Wando River; two smaller fish versus six larger fish were recaptured in Charleston Harbor). Therefore, without replication of the size-at-release and timing-of-release results, and given that all red drum were produced and released in phase with the natural spawning season of wild red drum, we refer to the combined 1999 year-class data for the remaining discussion.

The Ashley River, Charleston Harbor, and Wando River strata were sampled monthly during 2000–2003. Effort in each stratum differed by the number of available sites, distance between sites, and speed at which the tide changed. Each year, 41–51 net sets were made in the Ashley River stratum, 38–50 net sets were made in the Charleston Harbor stratum, and 30–35 net sets were made in the Wando River stratum (Table 3). Red drum from the 1999 year-class began to recruit to the sampling gear in July 2000. The CPUE of age-1 red drum in the Ashley River was 1.73 fish/net set, which was not significantly lower than that for the two adjacent strata ($P = 0.256, F = 1.38, df = 2$; Table 3; Figure 4), representing the first time since sampling began that the CPUE in the Ashley River trended as high as the Wando River and Charleston Harbor strata. In contrast, during 2000 the red drum CPUE was significantly different among the strata ($P = 0.011, F = 4.65, df = 2$); the Ashley River CPUE was significantly lower than the CPUE in Charleston Harbor (2000–2002) and the Wando River. The trend continued for the 2002 year-class, in which significantly fewer ($P = 0.000, F = 8.67, df = 2$) red drum were captured in the Ashley River than in the other two strata. Comparisons of relative year-class strength within strata for 1999–2002 showed that in the Ashley River, year-class strength for 1999 was not significantly lower ($P = 0.759, F = 0.39, df = 3$) than that for 2000–2002 due to the
high contribution (88.9%) of stocked red drum during a weak year-class. The CPUE for the 1999 year-class was, however, significantly lower than that for 2000–2002 in both Charleston Harbor \((P = 0.002, F = 5.35, \text{df} = 3)\) and the Wando River \((P = 0.002, F = 5.35, \text{df} = 3)\). These trends suggest that stocking red drum during years of low natural recruitment can have a substantial impact on the population, while stocking similar numbers during years of high natural recruitment can have a much smaller effect (Figure 5).

**DISCUSSION**

**Contribution and Natural Recruitment**

Stock enhancement of red drum has been occurring since the early 1980s in the southeastern United States. The three states that currently stock red drum (i.e., South Carolina, Texas, and Florida) typically release fish into diverse receiving waters, at varying densities, and using different methodologies, all of which affect their contribution to the population. Jenkins et al. (2004) reported high stocking contributions of red drum in a South Carolina estuary, although this study was limited by its low number of sampling sites and their proximity to stocking sites, resulting in higher estimates of contribution in these areas. In the present study, monthly sampling sites were chosen at random from 90 sites throughout the 21,872-ha Charleston Harbor estuary. Based on the random sampling design, overall contribution to the wild population in South Carolina from the 1999 year-class was 51.2%. In contrast, Karlsson et al. (2008) reported that in Texas during 2004, 1.1 million red drum juveniles (29–40 mm TL) were stocked into Galveston Bay and that subsequent genetic evaluation indicated a 9.35% contribution of stocked red drum to the wild population. Therefore, their study represents a stocking of approximately 1.5 times as many similarly sized juvenile red drum as were stocked in our study during 1999, and the estuary they used for stocking is approximately seven times larger (141,676 ha) than the Charleston Harbor estuary. It is conceivable that had fish been stocked into Galveston Bay at higher densities, contributions similar to those reported here would have been observed. Karlsson et al. (2008) also reported that dispersion of red drum was nonrandom and that hatchery fish were collected in greater numbers in close proximity to release sites despite stocking over a much larger area than was used in either South Carolina study.

In Florida, Tringali et al. (2008) documented a 2.8% contribution of stocked red drum from collections made throughout Tampa Bay and its neighboring waters (though sample collection was not random) after a release of 2,386,879 red drum juveniles of three size-classes. Tringali et al. (2008) also reported higher contributions of red drum captured closer to release sites, perhaps due to optimum habitat available at these locations for specific life stages. In 1999, we found that red drum stocked throughout the Ashley River were more densely aggregated in this stratum but had also moved into the adjacent strata and made substantial contributions within months of being stocked. If adequate habitat and resources are available close to release sites, juvenile red drum appear to have no need to disperse. Furthermore, their strong schooling behavior provides a successful strategy for survival, increasing the likelihood that higher concentrations of stocked fish will be observed closest to stocking sites, as was shown in each of the studies.

The studies by Jenkins et al. (2004), Karlsson et al. (2008), and Tringali et al. (2008) for South Carolina, Texas, and Florida, respectively, lacked a means to contextually evaluate the contribution of stocked red drum to a wild population. In these studies, red drum contribution or stocking success was linked to size of fish at release, season of release, proximity to release sites, habitat suitability, sample design, and a suite of other factors. We propose that in order to truly understand treatment effects, contribution must also be considered relative to natural population variability.

Fisheries-independent sampling in the Ashley River yielded relatively high CPUE values for the 1999 year-class. During poor recruitment years (1999) as well as during strong year-classes, the addition of small juveniles at higher densities appears to strongly influence fishery-independent indices of abundance in systems with historically small populations of red drum, such as the Ashley River. In 1999, the CPUE in the Ashley River was not significantly different from that in the other strata, in contrast to 2000–2002. The CPUE increased in the Ashley River to a level higher than previously measured during a poor wild year-class (1999), suggesting that stocking had an additive effect on the overall population rather than displacing wild fish through competition for limited resources. Additionally, these data suggest that resources are available for juvenile fish survival in the estuary even in year-classes that may be otherwise identified as below average. During 2000, CPUE increased in

![FIGURE 5. Summary of the number of wild and stocked red drum that were sampled from each of the three strata of the Charleston Harbor estuary for the 1999–2002 year-classes (shaded area of each bar = wild fish; hatched area = stocked fish).](image-url)
the Ashley River although the contribution of stocked fish to the total catch decreased, suggesting that the population had increased beyond historic averages and that the stocked fish constituted a much smaller proportion of the total population. In the Wando River, a system with typically high CPUE, during strong year-classes (2000–2001) the stocked fish constituted only 13% of sampled fish and this proportion decreased to 2.0% in the year after stocking. One potential explanation for these observations is that in 1999, the estuary may have lacked the environmental conditions, food availability, or both (Munro and Bell 1997) that were necessary to sustain wild red drum larvae, yet the trophic resources necessary for survival of juveniles (>25 mm TL) were sufficient. In the Ashley River during 1999, the unusually high CPUE, which included a large proportion (88.9%) of stocked red drum, demonstrated that the system had the capacity to support a larger population of juvenile red drum and that stocking would be a desirable management option if the goal is to increase local red drum availability for recreational anglers. Similar to these findings, previous stocking with small red drum juveniles as reported by Jenkins et al. (2004) indicated that this small juvenile life stage bypassed the limiting factors that establish year-class strength and recruitment into the recreational fishery; however, without long-term fishery-independent sampling, it was not possible for the authors to determine the effects of stocking in the context of relative intensity of wild year-class strength (Smith et al. 2003; Jenkins et al. 2004, 2005). From the present study, it is clear that as wild year-class strength increased the effect of stocking diminished, likely due to competition for trophic resources or habitat. If stocked fish had a competitive advantage over wild cohorts, however, we would expect that a large proportion of individuals collected in 2002 would also have been identified as stocked fish. In contrast, during 2002 no stocked fish were identified from fishery-independent sampling efforts, suggesting that any displacement of wild fish by stocked fish was not occurring. One hypothesis for the lack of stocked fish from the 2002 year-class was the presence of a strong wild 2002 year-class that followed two strong year-classes (2000 and 2001). It is therefore plausible that density-dependent factors (i.e., out-competition by the more numerous wild recruits or age-1–3 red drum) contributed to failure of the 2002 stocking.

Red drum exhibit a protracted spawning season that lasts from August to September, and different recruitment pulses of larvae are produced during this period. The timing of these pulses during the 2–3-month spawning window may have significant effects on ultimate year-class strength due to potential peaks in food availability as reflected in the size and health of the juvenile red drum entering the winter period, which subsequently impacts winter survival and year-class abundance (Scharf 2000; Stewart and Scharf 2008). Houser and Allen (1996) monitored zooplankton, which are primary foods for larval red drum (Daniel 1987), from May through October 1991 in North Inlet, South Carolina. Peaks of copepods, fiddler crab _Uca_ spp. zoeae, larval grass shrimp _Palaemonetes_ spp., and larval goby _Gobiosoma_ spp. were documented to occur during late July and August, a time that coincides with the peak red drum spawning season. Matlock et al. (1987), Scharf (2000), and Arnott et al. (2010) found intermittent occurrence of strong red drum year-classes across estuaries and suggested that factors determining abundance and overall distribution might vary over a large spatial scale that may affect life stages differentially. Those authors suggested that this could be due to egg or larval dispersal from spawning adults, the subsequent movement of juveniles, or environmental conditions that affect estuaries similarly along the coast. It is therefore plausible that the annual variation in wild year-class strength reported by others and in the present study (i.e., for 1999–2002) is a major factor affecting interpretation of stocking efficacy and must be incorporated into stock enhancement programs.

**Genetic Identification**

Criticisms of marine stock enhancement efforts have for many years largely centered on a lack of adequate technology to identify the effects of stocking in largely open systems. These limitations have been partly addressed through (1) the development of mariculture techniques that can produce fish for stocking that match the life history stage present in the wild and (2) the incorporation of long-term tags to enable quantitative monitoring (Shelbourne 1964; Blankenship and Leber 1995; Blaxter 2000; Chan et al. 2003). Our study utilizes microsatellite genotyping as a means of identifying stocked fish, an approach that has recently become a cornerstone standard tool for red drum stock stock enhancement research (Renshaw 2003; Jenkins et al. 2004, 2005; Gold et al. 2008; Karlsson et al. 2008; Tringali et al. 2008).

Smith et al. (2003) evaluated the same samples collected in this study, but they used OTC marks as indicators of stocking; the OTC method showed only a 78% contribution of stocked fish to the Ashley River in 1999, a 12% difference from our findings. In addition, their study indicated that the contribution from 1999 red drum stocking was 15% for Charleston Harbor (present study: 30%) and 12% for the Wando River (present study: 15%). These differences point to the underestimation of stocked fish contributions by using a qualitative technique rather than a quantitative technique. Denson and Smith (2008) demonstrated that in laboratory trials, mark readability degrades over time and is compromised by the cutting process and otolith morphological changes. Robbins et al. (2008) evaluated a sample of red drum from the 2000 and 2001 year-classes and also found discrepancies for OTC-marked fish that were processed by using both techniques. Additional stocked fish were identified with the genetic marks, and hatchery fish that were sampled from the wild were identified at a probability of 99.99%. In addition to utilizing a nonlethal sample technique, the use of these markers can allow the determination of which broodstock animals yielded which offspring, which parental crosses were most prolific for each spawning event, and which release groups provided the greatest contribution to the overall population.
In this study, multiple broodstock groups were used; therefore, we were able to determine the effective contribution from two different spawning batches of red drum stocked during two different months at two different sizes, similar to what has been reported in evaluating the effectiveness of two hatcheries releasing fish at different times of the year in Texas (Karlsson et al. 2008). The current stock enhancement studies at the SCDNR rely on multiple broodstock groups to increase hatchery effective population size and to provide unique genetic combinations of broodstock that permit progeny to be used in different stocking treatments. By using multiple broodstock groups, more complex stocking studies can be designed to evaluate critical stocking size at release across a wider range of life history stages, including larval fish.

Conclusions and Management Implications

The results of stocking 600,000 juveniles/year (~25 mm TL) in the Ashley River during 1999–2001 indicated that these stocked fish did not displace wild fish but rather had an additive effect on overall red drum abundance, supporting the hypothesis that trophic resources are not necessarily limiting for the postlarval age-0 life stage. Variable year-class strength of wild red drum could be driven by the prevailing environmental conditions during early life stages or by unsuccessful larval ingress. If a lack of egg production or a lack of larval transport is the key limiting factor in a given year, then direct stocking of larvae into the estuary appears to be capable of bypassing these recruitment bottlenecks. Future research should replicate these efforts to evaluate the effect of year-class strength on stocking contribution and should focus specifically on determining the factors limiting natural recruitment and linking these factors with measurable environmental conditions.

As managers consider the use of red drum juveniles to augment populations, the life stage that needs to be increased should be considered. Red drum are long-lived fish (40+ years), and the use of microsatellite genotypes as permanent markers will allow an evaluation of the contribution of stocked fish that recruit to the spatially separate adult population. Although our research demonstrates a contribution to the year-classes at age 1, determining the contribution of stocked red drum to the adult spawning population would be an appropriate next step.

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Assessment of a New Gear to Sample Walleye Eggs

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Assessment of a New Gear to Sample Walleye Eggs

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Abstract
A new gear for sampling walleye Sander vitreus eggs, egg sampling disks, is described, and the ability of these disks to estimate walleye egg density on mud, cobble, and rip-rap substrates is demonstrated. Each egg sampling disk was a concave steel disk covered with outdoor carpet. The ability of the disks to estimate walleye egg density and total egg deposition on a defined area was tested at Sherman Reservoir, Nebraska. The average cost of each egg sampling disk was US$8.00. An array of 10 disks could be processed (retrieved, complete the egg recovery procedure, and redeployed) in approximately 1 h. The egg sampling disks were an appropriate gear for estimating egg deposition on mud, cobble, and rip-rap substrate and could also be useful for estimating the total egg deposition of walleyes and other demersally spawning fish.

Effective walleye Sander vitreus management often includes knowledge of important spawning areas. Walleyes prefer to spawn on cobble/gravel substrate (Johnson 1961; Pitlo 1989; Lowie et al. 2001; Fouest and Haynes 2007), which is often absent in reservoirs (Katt 2009). When cobble is absent, walleyes will spawn on other substrates (Johnson 1961), including sand (Niemuth et al. 1959; Dustin and Jacobson 2003), silt or soft muck (Roseman et al. 2002; Dustin and Jacobson 2003; Fouest and Haynes 2007), flooded terrestrial and aquatic vegetation (Priegel 1970; Holzer and Von Ruden 1984; Roseman et al. 2002; Dustin and Jacobson 2003), and flooded rip-rap (Grinstead 1971; Weber and Imler 1974; Micheletz 1984).

Existing gears for sampling walleye eggs (as well as gears for sampling other demersally spawning fish) are limited to assessing egg deposition in specific environments or on a single substrate. In lotic waters, kick nets, anchored plankton nets, and surber samplers (Corbett and Powles 1986; Pitlo 1989; Dustin and Jacobson 2003; Fouest and Haynes 2007; Chalupnicki et al. 2010) can be used to sample eggs because the water current carries the suspended eggs into the net; however, in lentic waters, the lack of current limits the usefulness of such gears. Dip nets (Grinstead 1971; Fouest and Haynes 2007) can be used in both lotic and lentic waters for presence/absence sampling, but cannot be used to derive an egg density. Passive egg traps (Kelder and Farrell 2009) and samplers made of window screen (Micheletz 1984) are vulnerable to damage during severe weather and would fill with sediment when used on mud substrate. Cement blocks covered with furnace filter (Manny et al. 2007; Thompson 2009) are less prone to severe weather, but the accumulation of sediment when used on mud substrate could reduce the egg capture efficiency and make finding the eggs in the filter material difficult. Newburg (1975) used egg baskets, in which a hole was dug into the substrate where the basket was placed and the fill material was placed into the basket. The egg baskets are time consuming to deploy/retrieve (Perkins and Krueger 1994), require diver assistance, and would not work on rip-rap due to the large size of the boulders. Several studies have used a pump or suction device (Manz 1964; Grinstead 1971; Pitlo 1989; Fielder 2002; Roseman et al. 2002) to sample...
eggs from the substrate. Egg deposition estimates using these devices may be inaccurate on some substrates (such as rip-rap) because substrate irregularities make the area difficult to sample (Perkins and Krueger 1994). It is also unlikely these devices would work on rip-rap, because eggs would fall into the large interstitial spaces between boulders, or on mud, because of the large volume of sediment suctioned into the sample (Manz 1964).

The limitations of these existing gears prompted the development of a new gear, called egg sampling disks. The objective of this study was to describe the egg sampling disks and demonstrate their ability to estimate egg deposition on substrates found in reservoirs (e.g., mud, cobble, and rip-rap, as defined by the modified Wentworth scale in Cummins [1962]; Katt et al. 2011). The egg sampling disks are compared with regard to the criteria of an ideal egg sampler (inexpensive, easy to process, not avoided by spawning fish, protective of eggs after capture, durable, and unimpaired by severe weather; Marsden et al. 1991) and some assumptions and potential biases are discussed.

METHODS

Each egg sampling disk (Figure 1) was an individual steel disk blade from an agricultural field disk. The disks were concave and ranged in diameter from 32 to 51 cm. After a 4.5-cm-diameter steel washer was welded upright in the center of the concave side of the disk, outdoor carpet was glued to the disk with a construction grade adhesive. Lead-core line ranging from 1 to 4 m in length was tied to the washer with a carabiner on the other end. An identification number was painted on the convex side of each disk. The area of each egg sampling disk was calculated based on its radius.

Laboratory tests were conducted to evaluate whether the percent of eggs recovered differed when two different people washed the disks or varied with disk size. The cumulative percent egg recovery following multiple egg recovery procedures was also estimated. Nine individual disks (34–42 cm diameter) were placed in individual 68-L tubs that were partially filled with water. One hundred viable walleye eggs were placed on each disk. The eggs were obtained from a hatchery, had been treated with bentonite clay to remove adhesiveness (mucked),

FIGURE 1. An individual egg sampling disk showing the position of the washer, outdoor carpet, and lead-core line.
FIGURE 2. An array of egg sampling disks deployed on (A) mud substrate and (B) cobble and rip-rap substrate. Panel (A) depicts a full array of disks deployed on mud substrate while panel (B) depicts one-third of an array of disks deployed on the cobble and rip-rap substrates.

and were water-hardened. The following day, each disk was removed from the tub and subjected to the established egg recovery procedure (described below) three separate times, with each recovery procedure being performed by a single person. The egg recovery procedure consisted of partially filling a tub (38–53 L) with water and placing an individual egg sampling disk in the tub. The entire disk surface was scrubbed by hand twice. The water was poured through a 500-µm pore-size sieve after the second scrubbing. The number of eggs recovered after each procedure from each disk was recorded. This test was conducted again with a different person completing the egg recovery procedures the following day.

The percent recovery of each person was calculated as the number of eggs recovered divided by the number of eggs still available for recovery after the first recovery procedure (n = 9 for each washer). Analysis of covariance (ANCOVA) with interactions was used to evaluate potential differences in percent egg recovery between each person and variation with disk size (α = 0.05). Cumulative percent egg recovery was estimated after the second and third recovery procedure to evaluate potential increases in percent egg recovery with additional effort.

Evaluation of the egg sampling disks ability to estimate wall-eye egg density and total walleye egg deposition took place at Sherman Reservoir (41.3033°N, 98.8824°W), Nebraska. Sherman Reservoir is an off-stream irrigation reservoir of the Middle Loup River. At conservation pool, the reservoir covers 1,151 ha and has a maximum depth of 11 m. Disks were deployed from March 18 through April 15, 2008, and from March 25 through April 22, 2009, to estimate egg density throughout the early spawn, peak spawn, and late spawn periods each season.

Egg sampling disks were deployed in arrays of 10 disks, with three arrays being randomly deployed on each of three substrates (mud, cobble, and rip-rap). Differences in site characteristics required use of two deployment methods. On the mud substrate (Figure 2a), two 2.4-m-tall t-posts were pounded into the reservoir substrate 3–5 m apart. Two u-bolts were attached to each t-post near the surface of the water, and five disks were attached to each post. On the cobble and rip-rap substrate (Figure 2b), three cement anchor weights were deployed 3–5 m apart. Foamcore line (1–3 m) was used to attach each anchor weight to a float. A 4.5–cm-diameter steel ring was placed around the foamcore line and three to four disks were attached to each ring. The egg sampling disks were retrieved weekly and each disk underwent the egg recovery procedure (as described above) to check for eggs.

To calculate the walleye egg density, the number of eggs recovered from an array of disks was adjusted based on the combined mean percent recovery from the laboratory test. The adjusted number of walleye eggs sampled in an array of disks was then divided by the total area of the egg sampling disks in the array (eggs/m²). This density was then divided by the number of spawn nights since the last disk check (eggs/m² per spawn night) to standardize sampling effort.

The total number of walleye eggs spawned (± SE) on a known area was derived by extrapolating the walleye egg density to the entire area of that substrate. For this study, walleye egg densities were extrapolated only on the cobble substrate because the area of cobble was known (1,295 m²; Katt et al. 2011) and remained the same with rising water levels. The areas of mud and rip-rap were not calculated by extrapolation because of complications with the rising water levels.

Since the egg sampling disks varied in diameter (32–51 cm), linear regression (α = 0.05) was used to determine whether disk size influenced the number of eggs sampled. To test this relationship, weekly egg catch data from individual disks at
sampling sites where all the disks sampled eggs during a week were used.

RESULTS

The mean percent egg recovery after the first egg recovery procedure did not differ between the two people ($F = 1.18$, $df = 1, 15, P = 0.28$) or vary with disk size ($F = 1.18$, $df = 1, 15, P = 0.30$). Therefore, percent egg recovery data were pooled within each recovery procedure to estimate a cumulative mean percent recovery. The cumulative mean number of eggs recovered ($\pm$ SE) after the first, second, and third egg recovery procedures was $66 \pm 2, 85 \pm 1$, and $91 \pm 1$ eggs, respectively.

The adjusted walleye egg densities ranged from 0 to 37 eggs/m² per spawn night on mud substrate, from 18 to 202 eggs/m² per spawn night on cobble substrate, and from 10 to 3,682 eggs/m² per spawn night on rip-rap (comparison of mean egg densities from different substrates can be found in Katt et al. 2011). The total number of walleye eggs spawned on the cobble substrate ($\pm$ SE) was estimated to be $2,389,275 \pm 505,050$ in 2008 and $1,414,140 \pm 145,040$ in 2009. Disk size did not influence the number of eggs sampled during either year of the study: 2008 ($F = 0.07$, $df = 73, P = 0.80$) and 2009 ($F = 1.04$, $df = 73, P = 0.31$).

DISCUSSION

According to Marsden et al. (1991), the ideal egg sampler would be inexpensive, easy to process, not avoided by spawning fish, protective of eggs after capture, durable, and unimpaired by severe weather. The egg sampling disks described here satisfy many of these criteria. The average cost of each disk was US$88.00, including the material and labor for constructing the disks. A four-person crew was able to process an array of disks (retrieve, conduct the egg recovery procedure, and redeploy) in approximately 1 h. Spawning walleyes did not appear to avoid the egg sampling disks because walleye eggs were sampled from each substrate type, but whether some walleyes avoided the disks because of the t-posts and foam core line used in the deployment is unknown. The egg sampling disks seemed to be protective of the eggs after capture as is evident from the need to use multiple recovery procedures to collect all the eggs from the disks. The outdoor carpet covering the disks also provided a surface for eggs to adhere to after fertilization and the concave shape of the disk may help retain eggs better than a flat disk.

The egg sampling disks were durable as most of the disks used during both spawning seasons. Some disks needed only minor repairs, such as replacing the outdoor carpet and repainting the identification numbers. Some disks were lost (6.3% of all disks used throughout the study) as a result of wave action caused by strong winds, which caused a disk to be displaced or rip-rap boulders to fall on a disk (3.7% of disks lost); rising water levels in the reservoir, which washed out a t-post (1.2%); angler disturbance (1.3%); and miscellaneous causes (0.1%). However, because multiple disks were deployed in an array, egg density could still be calculated when some of the disks in the array were lost or compromised.

While the egg sampling disks meet the criteria of an ideal egg sampler, the use of this gear does involve some underlying assumptions and potential bias. Egg loss from the disks during retrieval likely occurred, but the quantity of egg loss is unknown, which affects the accuracy of the density estimations. A similar proportion of eggs was assumed to be lost from each disk because all the disks were retrieved using the same method. This assumption was also made by Manny et al. (2007), using egg mats (cinder blocks covered with furnace filter). By making this assumption, egg densities from each substrate can be compared and should be unbiased. For the purpose of estimating total egg deposition on a defined area or substrate, efforts to estimate the percent egg loss during disk retrieval would be needed or acknowledged should be noted that the estimates made are conservative, such as those made in this study.

A concern with the egg sampling disks was that the use of different sizes of disks would affect the accuracy of the density estimations. However, in this study the size of the disk did not affect the number of eggs sampled in the field or the number of eggs recovered in the laboratory tests. The size difference of disks used was small, which made finding a relationship between egg recovery and disk size unlikely. Ideally, the same size of disks would be used, but the difficulty of finding enough disks of the same size was prohibitive.

An additional possible bias is incomplete egg recovery, which may influence the accuracy of the density estimates. Percent egg recovery did not differ between two people but laboratory tests to estimate egg recovery efficiency indicated that more eggs would be recovered in multiple egg recovery procedures. However, because egg recovery was incomplete even after three recovery procedures, the total number of eggs on each disk would still need to be extrapolated. Therefore, additional recovery procedures were determined to not be necessary.

The walleye eggs used in the laboratory tests came from a hatchery, where they were treated with bentonite clay (mucked) to remove the adhesiveness and water-hardened. Treating the eggs to remove the adhesiveness likely would not have influenced the results of the laboratory tests because walleye eggs naturally lose their adhesiveness once they are water-hardened (Pitlo 1989). Since walleye spawning typically occurs at night (Eschmeyer 1950) and processing of the disks did not begin until mid-morning, the eggs on the disks in the field were likely water-hardened and nonadhesive. Further, crews in the field did not observe clumps of eggs when processing the disks, an indication that recovered eggs were no longer adhesive. Therefore, the use of mucked (nonadhesive) eggs in the laboratory tests was an accurate representation of what was encountered in the field.

The estimated egg densities from each substrate and the total number of walleye eggs spawned on the cobble substrate are conservative estimates. Several challenges limit the preci-
sion of these estimates, including the unknown quantity of eggs lost during disk retrieval, incomplete egg recovery during the washing procedures, and high variability in the egg density data. Egg loss during disk retrieval and egg recovery percentages for individual people can be estimated and the densities adjusted based on these estimations. The egg densities presented here were adjusted based on the mean recovery percentage of two different people performing the laboratory test, but estimates of egg loss during retrieval were not made. The variability in the egg densities is somewhat problematic, but may be reduced by sampling only during the peak spawn or by using different disk arrays. Since densities of eggs were obtained throughout the entire spawning period (early spawn, peak spawn, and late spawn) were used, a wide range of egg densities was observed, which increased the variability in this study. This study used arrays of 10 disks as the sampling unit, but more arrays with fewer disks per array could be used to increase the number of sampling units.

The egg sampling disks were an appropriate gear to estimate walleye egg densities and compare densities among mud, cobble, and rip-rap substrate types. The egg sampling disks could also be useful for estimating the egg density of other demersal spawning species such as white bass Morone chrysops, lake sturgeon Acipenser fulvescens, and lake trout Salvelinus namaycush, among others. The disks have already been used for presence/absence sampling of walleye and white bass eggs at potential spawning sites in other Nebraska irrigation reservoirs (Martin 2008).

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REFERENCES


Comment: Rotenone Toxicity to Rainbow Trout and Several Mountain Stream Insects

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Comment: Rotenone Toxicity to Rainbow Trout and Several Mountain Stream Insects

The use of rotenone (and other piscicides) in fish management is of concern to many, particularly because of its impacts on nontarget aquatic species and associated communities. Despite its long use in fisheries, rotenone is poorly understood with regard to those impacts (Vinson et al. 2010). At present, the U.S. Environmental Protection Agency (USEPA 2008) has withdrawn its approval of rotenone for terrestrial, estuarine, and marine uses. The major reasons for the withdrawal are the weakness of the scientific data on the environmental effects of rotenone and suspected human health effects. It has also been withdrawn for terrestrial use by Canada (Health Canada 2008) and the European Union (2008). Rotenone is still allowed for use in freshwater habitats as a piscicide.

In a recent paper, Finlayson et al. (2010) drew several conclusions about the relative toxicities of two rotenone formulations under consideration for the latest project on Silver King Creek on the eastern slope of the Sierra Nevada (Carson-Iceberg Wilderness, Humboldt-Toiyabe National Forest), California. Current planning is controversial and contested. On September 6, 2011, the Federal Court for the Eastern District of California issued a permanent injunction on the project for violation of the Wilderness Act because of its negative effects on aquatic macroinvertebrates (Case 2:10-cv-01477-FCD-CMK, document 65). State and federal agencies have been removing Paiute cutthroat trout Oncorhynchus clarkii seleniris from the basin. The authors evaluated the lethal effect (dosage lethal to 50% of the test animals [LC50]) of Nusyn-Noxfish relative to that of CFT Legumine on rainbow trout Oncorhynchus mykiss and six species of stream insects (Ephemeroptera [mayflies]: Baetis tricaudatus, Rhithrogena morrisoni, Plecoptera [stoneflies]: Classenia subulosa, Oropetla barbara; Trichoptera [caddisflies]: Arctopsyche grandis, Hydropsyche tana/amblis). They extrapolated the findings to field data from a previous Nusyn-Noxfish poisoning of Silver King Creek. I question the findings and conclusions from this study based on the methods used, the statistical analysis, and the selection of field data for analysis. My comments follow the organization of Finlayson et al. (2010).

METHODS

Finlayson et al. (2010) exposed hatchery rainbow trout and six species of larval aquatic insects to a range of rotenone concentrations (based on dilutions of the two formulations) for 4 or 8 h. The active ingredients of Nusyn-Noxfish include 2.5% rotenone by weight, 2.5% piperonyl butoxide (a synergist), and an additional 2.5% other rotenoids derived from the plant used in rotenone extraction; CFT Legumine contains 5% rotenone and 5% other rotenoids.

Organism survival was to be judged 48 h after exposure in the replications of various rotenone concentrations and formulations. All tests were conducted in glass beakers under static test conditions with 5–10 replications of one individual per vessel per test concentration. The authors did not explicitly address whether the test vessels were aerated, only that they changed water to maintain “sufficient oxygen concentration,” among other factors. Some of the key test conditions are summarized in Table 1.

The general test conditions for judging the outcomes of toxicity testing can be assessed by ratios (loading factors) that use organism mass, test volume, and time. The authors reported loading factors defined in Rand and Petrocelli (1985). I assume they were referring to the chapter by Parrish (1985), who suggested that test conditions were adequate if loading factors were less than 0.5–0.8 g/L in static tests. Finlayson et al. (2010) reported the maximum loading they used. By my calculations, however, only one test condition had clearly satisfactory loading (that for mayflies [0.024 g/L]), two factors were questionable, and one was clearly outside the recommended level (Table 1). (No values could be calculated for caddisflies because average body mass was not reported, but the loading factor was somewhere between the others.) All tests were designed to last 48 h, so I also calculated the loading factor suggested by Sprague (1990) from his chapter on aquatic toxicology in the American Fisheries Society publication Methods for Fish Biology (Table 1). Sprague (1990) recommended incorporating time in the loading factor: test volume (L) divided by organism mass (g) times days (2 d, in this case, for the judgment of mortality). Based on this experience and review, Sprague (1990) recommended a loading factor greater than 2 if there were no aeration of test vessels or greater than 1 under other circumstances. Only the average
case for mayflies (20.8 L·g⁻¹·d⁻¹) satisfied this recommended loading factor for adequate water conditions. Caddisflies would not have met adequate loading if the individuals had averaged more than 0.15 g (a loading of 1) or 0.075 g (a loading of 2).

The study methods also incorporated bias in the sizes used in the rainbow trout assays (Table 1). Fish averaged 5.7 times larger (1.25 g) in the Nusyn-Noxfish tests than in the CFT Legumine tests (0.22 g). Differences in size, age, and other factors affect the level of toxicity for various chemicals (Sprague 1990). I am unaware of published studies that compare the effects of rainbow trout size on toxicity. It is well known that most other physiological responses are sensitive to fish size. Thus, the standard research design should take size into account by either randomizing sizes among tests, keeping size similar among tests, or using covariance analysis in some way. For one thing, using different fish sizes for each toxicant caused one group to have an even worse loading factor than the other—quite apart from whether size itself would change their sensitivity to the poisons. The effect of such a difference in rainbow trout sizes confounds the test and raises doubt about the conclusions from the results comparing the two formulations.

In addition to information on fish size, the authors should have provided information on larval insect stage or instar. The toxicity of pesticides to larval insects is also influenced by age (larval instar). As shown for midge larvae (Diptera: Chironomidae), the toxicity of a common pesticide (chlorpyrifos) nearly doubled over one difference in larval instar stage (Buchwalter et al. 2002). This finding means that comparison of the sensitivity of a toxin among or within species may depend on the instar in which exposure occurs. Without such information, the generalizations or extrapolations to field sensitivity by Finlayson et al. are misleading.

The overall test conditions for the invertebrates were more problematic than those for the rainbow trout. All of the insect species chosen for testing are flow-dependent organisms. That is, they require flow over their bodies to survive or function normally. In his classic work, Hynes (1970) reviewed this concept at some length. Without a current, these organisms shut down oxygen consumption even when the oxygen concentration in the water is maintained at high levels. All six of the insect species tested possess external gills. But they depend on water movement rather than static diffusion through external surfaces. In an attempt to compensate for the lack of flow, such species often move their bodies or body parts for short periods. Many observers of stream insects are familiar with the behavior of the stoneflies and mayflies tested. When removed from flow, stoneflies (e.g., *Calessenia*) soon begin to perform “push-ups” in an attempt to create a current, whereas many mayflies, particularly the *Rhithrogena* (family Heptageniidae), vibrate their abdominal gills (Hynes 1970; Eriksen et al. 1996). The net-spinning caddisflies (Hydropsychiidae) used in Finlayson et al. (2010) require a current for retreat construction and net deployment, and they are similarly affected in their oxygen consumption when current is restricted (Hynes 1970). As a consequence, the organisms in the test conditions (static tests lacking water current) used by Finlayson et al. were under continuous stress and would have impaired uptake of any chemical that requires passage through their external gills (e.g., rotenone). Subsequent estimates of mortality from poisons are probably biased and of uncertain relevance. Whether such unnatural conditions in a laboratory mean that toxicity is lower or higher in the field is speculative. One could just as well make the argument that because of the poor laboratory conditions and reduced oxygen uptake the organisms in the laboratory took up less toxicant (gills are the location for uptake) and hence survived better (longer) than they would have under field conditions. Thus, any conclusions about field survival are no more than speculation and have no place in the conclusions from the results. As concluded by Sprague (1985), “[I]t is therefore important that the investigator be familiar with the life history and general requirements of organisms used in testing.”

The three factors combined in the invertebrate tests—inadequate container loading in some tests, the lack of current, and unknown instar—are severe constraints. Finlayson et al. (2010) acknowledge (page 106) that the failure of some tests to last 48 h and the mortality of controls “may suggest test conditions less than optimal for these species.” I suggest that the basic conditions were inadequate for any conclusions.

**RESULTS**

Even if the test conditions had been adequate, the study failed to use statistical inference to make valid conclusions about the results. The authors reported LC50 means (geometric) and 95%
Confidence intervals (CIs) in statistical tests but apparently relied on inspection of the means for judging their significance. The tests on rainbow trout are instructive in this regard.

For this test, the lack of water current is not critical although loading problems remain. Nevertheless, the authors claimed that the toxicity of CFT Legumine (LC50 = 7.4 µg/L in 4 h, 5.3 µg/L in 8 h) was greater than that for synergized Nusyn-Noxfish (LC50 = 7.7 µg/L in 4 h, 6.2 µg/L in 8 h) and therefore that the synergist in piperonyl butoxide had little effect on the toxicity of rotenone for rainbow trout. But the 95% CIs of the means of the two formulations (their Table 2) overlapped for both the 4-h and 8-h tests. Use of confidence intervals acknowledges that the means have statistical properties to be considered when judging “difference.” Therefore, the true mean value could be anywhere in the CI range (with a probability used in the confidence interval). From these data alone, the authors should recognize that the actual means may not really be different. Again, Sprague (1990) recommended how to judge toxicity results, particularly when confidence intervals overlap. He provided a simple test statistic using average LC50 and confidence intervals called the f1,2 test:

\[ f_{1,2} = \exp(\sqrt{\log_{e}(f1)^2 + \log_{e}(f2)^2}), \]

where \( f_1 \) is the ratio of the upper 95% confidence limit to the average LC50 for substance 1 and \( f_2 \) is the analogous ratio for substance 2. A difference is significant if the test statistic is less than the ratio of the greater average LC50 divided by the lesser LC50.

Application of this statistical test shows that there was no significant difference (95% level) in the toxicity to rainbow trout of the two formulations at either 4 or 8 h of exposure; thus, toxicity was not greater for CFT Legumine than for Nusyn-Noxfish. The inference that the synergist in Nusyn-Noxfish had little effect on mortality remains; however, the bias caused by using larger fish (and poorer loading conditions) with Nusyn-Noxfish than with CFT Legumine renders even this conclusion risky. Marking (1977) reported much earlier that piperonyl butoxide caused more than additive toxicity with rotenone when used on rainbow trout.

Finlayson et al. (2010) also concluded that the results showed that the toxicity of Nusyn-Noxfish to aquatic invertebrates was generally greater because of the synergist (“may be twice as toxic” [page 107]) than the CFT Legumine formulation. Putting aside the issues of the test conditions, I examined the basis for this generalization. The study used six species of insects, two exposure periods, and two rotenone formulations, potentially yielding 24 data cells of average LC50 and 95% confidence intervals. Nine cells lacked data either entirely or for the confidence interval. Four other cells did not represent results for 48 h but were truncated at 24 h because of problems or early mortality. Of the remaining 11 cells, 5 had data only for one formulation for a given species and exposure time and could not be used to compare the relative toxicities of the formulations. Thus, six cells or three pairs of comparisons of relative rotenone formulation toxicity remained. Two of these comparisons were for the same stonefly species (O. barbara) at the two exposures; the last was for the mayfly R. morrisoni at 8 h. What may appear as a fairly robust study of species, exposures, and formulations, was in fact two independent tests of relative toxicity under highly questionable laboratory conditions. Nevertheless, whatever the results of subsequent statistical tests or comparisons, generalizations about toxicity are suspect because of poor methods.

Based on their findings, Finlayson et al. (2010) recommended that fisheries managers “use unsynergized formulations because the synergized formulation is less toxic to fish and more toxic to aquatic insects” (page 109). But the findings were untrue for rainbow trout (there was no difference) and unproven for aquatic insects.

There are additional questions concerning extrapolations from this toxicity study that pertain to the nature of rotenone formulations themselves. As noted above, the make-up and amount of active ingredients (all chemicals with pesticidal action) in the two formulations are different. As the authors pointed out, to use equal concentrations of rotenone twice as much Nusyn-Noxfish was used as CFT Legumine. In terms of all active ingredients, therefore, a test concentration of 10 µg rotenone/L made up from Nusyn-Noxfish would have 30 µg total active ingredients/L, compared with 20 µg/L for CFT Legumine. Other fish toxicants with different formulations (e.g., Synpren-Fish Toxicant, which contains 2.5% rotenone, 2.5% piperonyl butoxide, and 5.0% other cube resins) have total active ingredients that when doubled to equal the 10 µg rotenone/L concentration of CFT Legumine would contain 40 µg total active ingredients/L. Further, other synergized fish toxicants use synergists other than piperonyl butoxide (e.g., Pro-Noxfish uses sulfoxide as a synergist) that may have other properties. (The active ingredients in several common fish toxicants can be found in Finlayson et al. 2000.) Generalizing about toxicity for the variety of formulations and active ingredients is risky for fish and even more so for the enormous diversity of the invertebrate world.

**Field Application of Invertebrate Toxicity for Silver King Creek**

The authors used their laboratory data to compute toxic units (TU) of field exposure to rotenone (Finlayson et al. 2010; their Figure 2), where TU (from Rand and Petrocelli 1985) is the field rotenone concentration divided by the LC50 for a species. If the TU for the average LC50 (or confidence interval) of a species exceeded the field rotenone concentration, at least 50% of the population would be killed. Finlayson et al. used LC50 for the nine data cells at 8 h exposure that had any data, regardless of the differences in 24 or 48 h to observe mortality. They used a field rotenone exposure from 1991 to 1993 in Silver King Creek of 11 µg/L for 6–18 h, reportedly based on Trumbo et al. (2000a).

Trumbo et al. however, indicated that rotenone exposure in Silver King Creek (from Table 1 in Trumbo et al. 2000a)
averaged 22 h (range, 18.5–24 h) based on the only reported data from a downstream monitoring station. Furthermore, in each of the three years of rotenone application (1991–1993), two applications of Nusyn-Noxfish were made, separated by 1–2 d. In 1991, a third application was made 1 month later to the upper half of the basin when fish were found upstream from the earlier two applications, and the stream at its lower locations (but above the downstream boundary at Llewellyn Falls) “was heavily doused with rotenone from backpack sprayers” when caged sentinel fish escaped to the treatment area (Flint et al. 1998:22). It is apparently standard protocol, at least in California, that managers make two closely spaced, separate rotenone applications in a year. Two applications a year for 3 years (1994–1998) were also made in Silver Creek, California (Trumbo et al. 2000b), and two applications (separated by 2 weeks) in 2007 were made to the three major tributaries of Lake Davis, California (McMillin and Finlayson 2008). Plans for the latest poisoning of Silver King Creek called for application of a rotenone formulation twice a year for 2 or 3 years (USFWS 2010). (A recent environmental assessment of a similar project in Arizona states that a similar frequency of application is standard in that state; USBR 2007). The relevance of the toxic unit concept or toxicity data in general (e.g., Table 1 in Finlayson et al. 2010) to two or more closely spaced rotenone applications is unknown and unstudied.

The implication from the toxic unit analysis (Figure 2 in Finlayson et al. 2010) was that rainbow trout would certainly be killed (i.e., suffer more than 50% mortality) by either formulation despite the obviously limited sizes and ages of the test fish), whereas all of the aquatic insects (with the possible exception of the mayfly R. morrisoni, whose upper confidence interval exceeded the toxic unit threshold) would suffer less than 50% mortality. The authors concluded that some mortality would occur for both species of mayfly tested and that there would be little, if any, mortality for the caddisfly A. grandis or the stonefly O. barbara. They drew no conclusions about the caddisflies in the genus Hydropsyche spp. or the stonefly C. subulosa, possibly because they lacked data on Nusyn-Noxfish toxicity for those taxa. Nevertheless, they concluded that if the study were representative of toxicities and other mountain stream invertebrate species,

a rotenone treatment with a mean rotenone concentration of 11 µg/L with an exposure time of 6–18 h would result in complete mortality to trout and some mortality of invertebrate species, but many invertebrate species would survive (page 109).

In Silver King Creek (and elsewhere), however, multiple applications of rotenone were made each year, and the average exposure time was 22 h. None of the tests conducted by Finlayson et al. (2010) exceeded one-time exposures beyond 8 h. Recent planning for another poisoning of Silver King Creek downstream from the areas exposed in the 1990s also called for the application of CFT Legumine or Noxfish twice a year for 2–3 years at rotenone concentrations of 25–50 µg/L (USFWS 2010). Such a protocol is also standard procedure in Arizona (USBR 2007).

In the area of the project conducted in the 1990s, Silver King Creek had also been poisoned previously (USFWS 2010), first with Pro-Noxfish (50 µg rotenone/L plus the synergist sulfoxide) in 1964 and again in 1976 (first with antimycin and then twice more with Pro-Noxfish at 50 µg rotenone/L). Some stream sections have received as many as eight separate rotenone exposures over 29 years (USFWS 2010). Fish, and presumably aquatic invertebrates, develop resistance to rotenone when it is used repeatedly (Orciari 1979), as is well known for insects’ responses to other pesticides. Hence, one might expect increasing tolerance in those species that survive previous rotenone projects, which would make the response of the invertebrate community in Silver King Creek difficult to extrapolate to other locations.

Field Samples of Invertebrates from Silver King Creek

Finlayson et al. (2010) also analyzed some invertebrate bottom-sample data from the rotenone project on Silver King Creek in the 1990s. They compared data (their Table 3) using 14 invertebrate metrics for five poisoned stations and one unpoisoned (control) station from 1991 to 1996. Personnel collected samples before and after rotenone applications in each year from 1991 to 1993 and yearly thereafter. Thus, in this data set the samples from 1991 before poisoning represented the only “before” data (complete sample data from 1990 for all the stations have been lost, and the 1991 data are 15 and 27 years after earlier poisoning). From analysis of variance (ANOVA) the authors found significantly lower density (critical α = 0.05) in the poisoned stations than in the unpoisoned control for total invertebrate abundance and Coleoptera abundance. But none of the other 12 metrics of invertebrate assemblages were significantly different (Table 3 in Finlayson et al. 2010). On this basis, they argued that there was limited impact on the macroinvertebrate community from the poisoning.

The use of ANOVA in this way may be statistically valid in a general sense, but it is uninformative. Such an analysis maximizes the importance of variability and insensitivity: sequential collections during 6 years, and collections before poisoning and during poisoning years are not separated by effect. As noted by the authors, five poisoned stations and one control (with attendant differences in the total number of bottom samples) were compared. In addition to the variability from the treatments and time, there was confounding of variables in both space and time (see the discussion in Waters and Erman 1990), along with station variability from the wide range of stream sizes, elevations, past livestock grazing, grazing exclusion at some stations, stations without fish predators part of the time, habitat restoration efforts, and so forth. Without any attempt to account for this variability (say, by measuring the factors and using them as variables), the conventional method of increasing sample size by adding more stations or times may only add variability (Downes 2009).
FIGURE 1. Means and standard deviations of 14 metrics used by Finlayson et al. (2010) to compare macroinvertebrates collected from rotenone-poisoned (solid squares) and control (open squares) stations in Silver King Creek. The underlying data (yearly estimates) for poisoned stations (solid triangles) are shown for comparison; yearly estimates for a single control station lack a standard deviation.

The ANOVA analysis used by Finlayson et al. (2010) is also unlikely to detect differences because of the high degree of dependence of the standard deviation on the means. A logarithmic plot of the 14 metrics by mean versus standard deviation for control and poisoned stations is given in Figure 1. The underlying values from yearly estimates of total density and the total number of taxa for poisoned stations are also shown for comparison and have the same relationship. Yearly estimates of the single control station lack a standard deviation. This dependence violates a major assumption of the ANOVA model. Although the 14 metrics were each considered as different factors in the analysis by Finlayson et al., 5 of the metrics are subsets of the total abundance metric (i.e., the number of individuals in the various orders) and the remaining 8 metrics (taxa counts) are themselves subsets of the total number of taxa.

Analysis of variance is said to be fairly forgiving as to the violation of assumptions, and a significant difference from a test is probably valid even when the assumptions of the model are violated (Chittenden 2002). But strong departures from model assumptions mean that the lack of significant differences is a likely outcome that may not be true. For some time, invertebrate ecologists have recognized that abundance data from invertebrate samples are prone to this dependence of the standard deviation on the mean (Elliott 1977). Logarithmic transformation of the abundance data (Elliott 1977), other transformations (Allan 1984), or nonparametric methods are possible improvements to the analysis. In this case, however, transformation of the dependent variables would make little difference in the other sources of variability. As presented, the analysis by Finlayson et al. (2010) maximizes the likelihood of finding few differences in invertebrate communities from rotenone poisoning within the welter of unaccounted-for variation and weak statistical methods.

The data set could be analyzed to avoid some of the statistical problems. A hypothesis of the Finlayson et al. (2010) study is that for most of the 14 metrics there was no difference between control and poisoned stations. If these 14 metrics reflect their importance as measures of the effects of rotenone, then a reasonable hypothesis is that there is no difference in the ranks of the 14 metrics based on poisoned or unpoisoned stations. I tested such a ranking using the nonparametric Wilcoxon signed rank test. This null hypothesis was rejected ($P = 0.0088$), that is, there was probably a significant negative effect of the rotenone poisoning on the invertebrate community in Silver King Creek.

There was contention over the latest project to poison Silver King Creek (Finlayson et al. 2010:103), in large part because of the lack of species identification of the invertebrate samples. As Finlayson et al. (2010) and others (Sprague 1990) point out, organisms, particularly invertebrates, vary in their sensitivity to toxins (and possibly the other active ingredients and interactions of the chemicals in the formulations). Thus, one might expect attention to focus both on determining the species composition of the community and on whether or not the species composition is altered after poisoning. However, for the invertebrate collections made during the 1990s and earlier in Silver King Creek,
more than one-half of the individuals (53.7%) were identified only to family or a higher classification based primarily on larval specimens.

From 1984 to 1996, collections from the stations in Silver King Creek made by various state and federal agency personnel were sent to the former U.S. Forest Service National Aquatic Ecosystem Monitoring Center in Provo, Utah, for processing and identification. Annual reports of data analysis from the center noted the loss of many taxa that were present before poisoning began in 1991. From the original data sheets, I used the same list of taxa (family, genus, and some species level identification) for 1991 at each station as the base before poisoning. I compared this list with that of subsequent samples through 1996 for the five poisoned stations and one control (Figure 2). The percentage similarity of taxa composition, even at these levels of identification, was significantly lower in the poisoned stations (mean, 53.4%) than in the control (68.0%) (Mann–Whitney U-test, $P = 0.0004$). A large difference in similarity between the control (about 70% similarity to prepoisoned) and unpoisoned stations (about 58%) remained 2 and 3 years after the last poisoning in 1993 (Figure 2).

Further effects on specific taxa were apparent in the original data. In particular, the abundance of the cockroach-shaped stonefly *Yoroperla* (sometimes identified to *Y. brevis*), the most abundant (and easily identified larva) stonefly in Silver King Creek before poisoning began, sharply declined with each poisoning event until in the last 2 years of the study (2–3 years after the last application of Nusyn-Noxfish), scarcely any individuals were found in the poisoned stations (Figure 3). A nearly identical decline occurred in abundance of *Yoroperla* (Figure 3) after a similar poisoning project on nearby Silver Creek (Trumbo et al. 2000b). These results for Silver Creek (1994–1998) further strengthen the inference from Silver King Creek (1991–1996) because the two studies had little overlap in time, thus reducing possible climatic or hydrologic variation as a confounding factor. As might be expected, other taxa expanded in abundance, although the overall composition of the community and the relative abundances of various taxa differed from the preproject condition. The conclusion by Finlayson et al. (and, for that matter, Trumbo et al. 2000a, 2000b) that past poisoning caused few significant effects on the macroinvertebrate community of Silver King Creek is incorrect.
Conclusions

The study by Finlayson et al. (2010) had serious methodological problems in toxicity testing and analysis that render their conclusions suspect or incorrect. Similarly, their analysis of the invertebrate data collected from Silver King Creek failed to account for variability and violated test model assumptions and thereby clouded the impacts from poisoning. My analyses of their data show significant impacts from Nusyn-Noxfish poison on a variety of metrics and on certain taxa found in the benthic invertebrate community of Silver King Creek.

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A Method to Train Groups of Predator-Naive Fish to Recognize and Respond to Predators When Released into the Natural Environment

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Abstract

Hatcheries are effective at producing large numbers of fish for augmenting fisheries or conserving endangered populations, but the fish they produce are often predator-naive, resulting in high levels of predation mortality when the fish are first released into natural water bodies. Fish normally acquire recognition of novel stimuli as indicators of danger when injury-released chemical cues from conspecifics (a known indicator of an actively foraging predator) are presented simultaneously with a novel stimulus (e.g., predator odor, image). Thus, fish in wild populations quickly learn the sight and smell of their predators. Past research has demonstrated that predator-naive, hatchery-reared fish can be trained to recognize predators and that fish trained by this method have a significantly greater probability of surviving an encounter with a predator. To implement predator training in fishery management, predator recognition training must be feasible on a large scale in a way that does not place an undue financial or logistical burden on fisheries managers. Here, we demonstrate that groups of fish can be quickly and easily conditioned to recognize the odor of a novel predator and react to it with antipredator behavior. This simple method could improve the cost effectiveness of any stocking program, result in greater densities of managed stocks, and enhance the direct and indirect economic benefits of a fishery.

Effective antipredator behavior is a major determinant of cohort survival. The first step in any antipredator response is recognition of predation risk (Lima and Dill 1990; Smith 1992). Antipredator response to chemical cues released by injured conspecifics is a widespread phenomenon among aquatic animals, ranging from ciliates to amphibians (see Wisenden 2003 for a review) because these cues are reliably released in the context of predation. Recognition of predators and correlates of predation risk by fish is often mediated directly or indirectly by chemical cues released as signature body odors of predators and chemical alarm cues passively released by injured prey (Brown 2003; Ferrari et al. 2010).

A mechanism for acquired predator recognition that has been demonstrated many times in the literature is the coupling of chemical alarm cues from injured conspecifics with the sight or smell of a predator (Ferrari et al. 2010). In the parlance of comparative psychologists, chemical alarm cue is an unconditioned stimulus that elicits an innate response (the unconditioned response) without requiring prior experience (Suboski 1990). Once chemical alarm cues of conspecifics have been detected, the receiver is primed for releaser-induced recognition learning (Suboski 1990). In this type of learning, any novel stimulus (the conditioned stimulus) presented simultaneously with alarm cues becomes associated with predation risk. A remarkable aspect of this type of learning is that a single pairing is sufficient to confer near-permanent association of risk with the novel stimulus. In laboratory aquaria, releaser-induced recognition learning can be used to manipulate fish to associate predation risk with the sight or odor of predators (Chivers and Smith 1994), nonpredatory stimuli such as a goldfish Carassius auratus (Chivers and Smith 1994), or even a flashing red light (Yunker et al. 1999) or electronic tones (Wisenden et al. 2008). Behavioral response to indicators of predation risk commonly involves reduction in activity because predators detect prey by detecting motion (Lawrence and Smith 1989; Lima and Dill 1990). Reduced activity is often associated with reduction in foraging activity, movement to the bottom, an increase in shelter use and increase in shoal cohesion (Chivers and Smith 1998; Ferrari et al. 2010).

Suboski and Templeton (1989) and Olla and Davis (1989) were among the first to anticipate that this form of learning could be used as a potential management tool to prepare predator-naive
animals reared in captivity for release into the wild. Fisheries management practices have long used hatchery populations to augment fisheries, but a major limitation of stocking programs is the high mortality of stocked fish, particularly in the first days and weeks of introduction into natural water bodies (for recent examples see Berejikian et al. 1999; Karam et al. 2008; Kekäläinen et al. 2008; Sudo et al. 2008; Tomiyama et al. 2009; Christensen and Moore 2010; Ochiwada-Doyle et al. 2010; Thorstad et al. 2011).

Several studies have applied releaser-induced recognition learning to hatchery populations of economically significant fish species and have shown that game species do indeed acquire predator recognition this way (Brown and Smith 1998; Berejikian et al. 1999, 2003; Mirza and Chivers 2000; Wisenden et al. 2004; Hawkins et al. 2008), and that trained fish have a significantly higher probability of surviving encounters with predators than untrained fish (Olla and Davis 1989; Berejikian et al. 1999; Mirza and Chivers 2000).

Hatchery-reared Chinook salmon Oncorhynchus tshawytscha trained to recognize predacious cutthroat trout O. clarkii reduced activity and foraging behavior in response to cutthroat trout odor and subsequently showed a 9% increase in survival when released into the wild (Berejikian et al. 1999). Hatchery-reared brook trout Salvelinus fontinalis fingerlings trained to recognize the odor of chain pickerel Esox niger showed a decrease in activity, decrease in foraging activity, and increase in shelter use when presented with pickerel odor (Mirza and Chivers 2000). These behavioral changes in the trout translated into a 20% increase in survival in direct encounters with pickerel in laboratory tanks and a 5% increase in survival in staged encounters in large outdoor enclosures (Mirza and Chivers 2000).

To date, predator recognition training has not been adopted by fisheries managers because, in part, the logistics of implementation were not the focus of earlier research. Past studies trained fish one to three at a time, which is not practical on a commercial scale. For predator recognition training to be feasible to implement, a method for training groups of hatchery fish needs to be developed.

In this study, we tested the efficacy of a predator recognition training paradigm specifically adapted to existing practices of fisheries workers. We aimed to develop a training method that does not add cost (equipment, personnel, time) and yet confers the benefits of predator recognition by stocked fish. We emulated the process of transporting fish in a holding tank (200 L) from the point of rearing (hatchery or rearing pond) to the destination natural water body. Training took place in the holding tank over 2 h, estimated to be the typical transportation time. The fish were then “released” into test aquaria where they were tested for recognition and response to predator odor. We used fathead minnows Pimephales promelas as our test organism because they are well suited for laboratory study and have been the study organism for many previous studies on predator recognition learning (Ferrari et al. 2010). The phenomenon of predator recognition learning occurs broadly across all fish taxa; therefore, results gained from fathead minnows apply broadly to economically important game species.

METHODS

Laboratory-reared fathead minnows were obtained at 30 d of age from the Environmental Protection Agency laboratories in Duluth, Minnesota, and maintained in the aquatic research facility at Minnesota State University Moorhead (MSUM) on a diet of commercial flake food and a photoperiod of 12 h light : 12 h dark.

Preparation of test cues.—Chemical alarm cues were prepared from fathead minnows obtained from a local bait dealer. Four fathead minnows were killed by cervical dislocation (MSUM Institutional Animal Care and Use Committee protocol 10-R-T-BIOL-010-N-Y-C) and measured (mean ± SE total length [TL] = 74.25 ± 3.03 mm). The skin was carefully filleted from each side of the body, laid flat on a piece of wet glass to measure skin area, then transferred to a beaker of deionized water resting on a bed of crushed ice. A total of 29.02 cm² of skin was harvested, homogenized with a hand blender for 30 s, diluted to a final volume of 100 mL with deionized water, aliquoted into two 50-mL tubes, and frozen at −20°C until needed.

Predator odor was tank water from a 185-L aquarium containing two West African saddled bichirs Polypterus endlicheri (TLs = 23.4 and 24.9 cm) maintained on a diet of commercial pellets. Selection of the predator species was made arbitrarily because predator recognition training is open to any stimulus. An exotic species such as a bichir is certain to be novel to the test minnows used in this experiment.

Conditioning protocol.—The experimental group was housed in a 265-L tub filled with about 200 L of dechlorinated tap water filtered with a large sponge filter. The tub contained 30 fathead minnows that were all 110-d-old (TL = 33.9 ± 0.8 mm [mean ± SE]). Fish in the experimental tub were conditioned with 100 mL of thawed minnow skin extract and 18.95 L of thawed predator cue and left for 2 h. The control group of 36 fathead minnows (TL = 32.4 ± 0.7 mm [mean ± SE]) were in an identical tub, and all were 103-d-old. The control tub received nothing. The size of fish did not differ between the groups (t18 = 1.35, P = 0.194).

Experimental set up.—After the 2-h conditioning period, 15 fish from each tub were arbitrarily selected and assigned to 30 separate test aquaria containing fresh dechlorinated tap water. One minnow was placed in each tank. Treatment assignments alternated from one tank to the next to control for any effect of location in the testing room. Test tanks were all-glass, 38-L aquaria with a sponge filter and a thin layer of naturally colored gravel. A length of airline tubing was wedged into the lift tube of the sponge filter to serve as a stimulus injection tube through which test stimuli could be introduced surreptitiously. Grid lines (5-cm × 5-cm cells) were drawn on the small pane of each test tank that was used for scoring fish behavior. Pieces of
Experimental protocol. — After a 24-h acclimation period, fish were tested for a behavioral response to predator odor. A 60-mL syringe was used to withdraw 60 mL of tank water through the stimulus injection tube. This water was discarded because it served only to rinse the tube of any residue. A second 60 mL was withdrawn into the syringe and retained for later use to flush test stimuli from the injection tube. A 5-min prestimulus observation period recorded activity and vertical distribution. Activity was scored as the total number of grid lines crossed over the 5-min observation period. Vertical distribution was recorded as the grid row occupied by the fish at 10-s intervals over the 5-min observation period. The row nearest the surface was row 1, the row at the tank bottom was row 5. The scores from 30 observations over the 5-min period were averaged into one mean score of vertical distribution per trial. After the prestimulus observation period, 120 mL of predator odor followed by the 60 mL of previously retained tank water was introduced through the injection line into the turbulent upflow of air and water of the sponge filter. The air and water currents served to quickly disperse the predator odor throughout the test tank. A second 5-min observation period (the poststimulus period) began immediately after cue injection. Activity and vertical distribution were recorded as before. Common antipredator responses in fishes are a reduction in activity and movement toward the bottom (Lawrence and Smith 1989; Ferrari et al. 2010).

Data were analyzed using a mixed-model analysis of covariance (ANCOVA; using PASW release 18.0 [2009], type III sums of squares, alpha set at 0.05) using poststimulus behavior as the response variable, conditioning treatment (present or absent) as a factorial predictor, and prestimulus behavior as a continuous predictor (covariate).

RESULTS

The activity of fish before presentation of the predator odor was a good predictor of activity after presentation of predator odor for control fish that were not conditioned with predator odor and skin conspecific skin extract (Figure 1). However, poststimulus activity of conditioned fish was not related to prestimulus activity levels because conditioned fish significantly reduced activity in the poststimulus observation period (Figure 1). The treatment differences resulted in a significant interaction term between prestimulus activity and the conditioning treatment (ANCOVA: conditioning $F_{1,26} = 0.786, P = 0.384$; preactivity $F_{1,26} = 8.571, P = 0.007$; conditioning $\times$ preactivity $F_{1,26} = 8.454, P = 0.007$).

In response to predator odor, most conditioned fish reduced vertical distribution but a few sought refuge at the surface, resulting in no overall net effect of the conditioning treatment when compared to unconditioned fish (ANCOVA: conditioning $F_{1,26} = 0.447, P = 0.510$; preactivity $F_{1,26} = 10.850, P = 0.003$; conditioning $\times$ preactivity $F_{1,26} = 0.457, P = 0.505$; Figure 2).

DISCUSSION

We demonstrated that a group of predator-naive fish can be trained to recognize and fear a novel predator with the addition of chemical cues of injured conspecifics and odor cues of a novel predator. The training method used is cost effective and
easily implemented with existing management practices. Chemical cues can be used in place of the direct experience with predators that fish in natural water bodies use to acquire predator recognition (Olla and Davis 1989; Chivers and Smith 1995). Naive fish from a hatchery or rearing pond loaded into a holding tank on a delivery vehicle can be trained by adding a small volume of conspecific skin extract and predator odor to the holding tank. By the time the truck reaches the destination lake, the fish will be trained to recognize the novel odor as an indicator of danger. Several studies show that behavioral responses such as reduced activity allow trained fish to be more likely than untrained fish to survive encounters with predators (Mathis and Smith 1993; Berejikian et al. 1999; Mirza and Chivers 2000). Now that we have demonstrated the effectiveness of group training, the next step is to begin large-scale experimental implementation of this management tool.

The aim of this experiment was to test the efficacy of group training using a convenient model species, the fathead minnow. The phenomenon of predator recognition is not specific to minnows, to fish generally (Wisenden 2003; Ferrari et al. 2010), or even to aquatic ecosystems (e.g., Griffin et al. 2000). This training method is the final piece of the process required to clear the way for large-scale implementation of this “head-start” management strategy on fish of commercial and conservation importance (Crane and Mathis 2011).

Fish in this study responded to predator odor with reduced activity but not with any detectable change in vertical distribution. This is not an unusual occurrence in that activity is a more-sensitive metric of antipredator response than others such as vertical distribution, shelter seeking, etc. Berejikian et al. (1999) noted that conditioned Chinook salmon fingerlings reduced activity in response to the odor of cutthroat trout but did not exhibit any other behavioral responses (foraging and vertical distribution remained unchanged). Nevertheless, when released conditioned cutthroat trout reared in complex hatchery habitat show a 20% increase in survival over unconditioned trout from similar hatchery habitat (Berejikian et al. 1999).

In this study, we conditioned fish to recognize and respond to the odor of one novel species. We used laboratory-reared minnows as test subjects and the odor of an exotic predator as the test odorant, thus ensuring that the test subjects had no previous experience or evolved response to the odor of this predator. In cases where prey species have preexisting innate recognition of predator odor, this technique of predator recognition imparts strengthened response to predator odor (Berejikian et al. 2003). In complex ecosystems with multiple predators, a complex bouquet of predator odors may be necessary to confer adequate life skills training to naive fish (Darwish et al. 2005). In some instances, one predator odor may be generalizable to a multiple-predator species if predators are phylogenetically related (Ferrari et al. 2007) or specific predator training timed with predators active at a particular time or season of release (Pearsons 2010).

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First Report of Abundant Rudd Populations in North America

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MANAGEMENT BRIEF

First Report of Abundant Rudd Populations in North America

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Abstract

The Rudd Scardinius erythrophthalmus was first introduced into U.S. waters about a century ago, and the species’ popularity as a baitfish in the 1980s has facilitated its spread to at least 21 states and the province of Ontario. Several established populations have been identified, but low abundances have led to little research and management attention. Rudds comprised 48.7% of the 14,130 fish captured in spring trap-netting surveys of Buffalo Harbor (northeastern Lake Erie) and the upper Niagara River during 2007–2008. Rudd was the most abundant species sampled, being captured at 11 of 12 locations and comprising 23.6% of the total catch in Buffalo Harbor and 70.3% of the catch in the upper Niagara River. Documented presences and absences in historical reports indicate that rudd became established in these waters between 1986 and 1991. Research is needed to understand the effects of rudds on native aquatic resources, especially nearshore macrophyte assemblages and the fish they support.

The distributions of numerous aquatic species have expanded well beyond their native ranges due to human actions such as intentional releases, the removal or bypassing of natural barriers through the construction of shipping channels, and unintentional introductions via bait bucket transfers and ballast water exchanges (Mills et al. 1993). Introductions of nonnative fishes such as the sea lamprey Petromyzon marinus and round goby Neogobius melanostomus have had serious and well-documented negative effects on native communities (Hansen 1999; Janssen and Jude 2001). The rudd Scardinius erythrophthalmus is a European minnow that now has a nearly global longitudinal distribution due to human translocations; the rudd ranges from New Zealand to the Midwestern USA and is established on five continents (Nico et al. 2010; Froese and Pauly 2011).

The rudd was intentionally introduced into the USA during the late 19th or early 20th century (Burkhead and Williams 1991; Mills et al. 1993), but for several decades its distribution remained limited to the northeastern USA and Oconomowoc Lake, Wisconsin, where it was intentionally introduced (Cahn 1927). The popularity of the rudd as a baitfish in the 1980s facilitated its spread to the waters of at least 21 states, including the St. Lawrence River, New York, a boundary water between the USA and Canada (Klindt 1990, 1991; Crossman et al. 1992; Nico et al. 2010). Although populations of rudds became established in several North American waters during the past century, their low abundances resulted in little attention from fisheries biologists. For example, the rudd is still considered rare in Oconomowoc Lake (Susan Byler, Wisconsin Department of Natural Resources, personal communication), despite its having been introduced in 1916 (Cahn 1927). Similarly, rudds are present but considered rare in the Ohio waters of Lake Erie (Travis Hartman, Ohio Department of Natural Resources, personal communication), the New York waters of Lake Erie outside of Buffalo Harbor (Donald Einhouse, New York State Department of Environmental Conservation, personal communication), and the St. Lawrence River (J. M. Farrell, unpublished data).

The rudd is omnivorous, and larger individuals (>150 mm) often consume aquatic macrophytes (Lake et al. 2002; Hicks 2003), exploiting food resources that are not typically utilized by native fishes in the north temperate regions of North America. Rudds typically grow to approximately 350 mm in length and 1,800 g in weight in their native range (Crossman et al. 1992).
The largest rudd on record was 617 mm long and weighed 3,623 g (Šprem et al. 2010). Such large, macrophyte-consuming fish are not common in North American freshwater ecosystems, so an abundant rudd population could negatively affect aquatic communities by altering macrophyte assemblages and accelerating internal nutrient loading (eutrophication) by remobilizing the nutrients stored in sediments and macrophytes (Hansson et al. 1987; van Donk and Gulati 1995; Vanni 2002; Hicks 2003; Nurminen et al. 2003). Additional threats to aquatic communities include (1) juvenile rudds competing with native fishes for benthic invertebrate prey (Crossman et al. 1992; Hicks 2003), (2) maintaining parasites and pathogens and spreading them to native fishes (Popović et al. 2001), and (3) hybridizing with golden shiner *Notemigonus crysoleucas* (Burkhead and Williams 1991), thereby causing genomic extinction (i.e., the loss of local evolutionary lineages; Allendorf and Luikart 2007).

This report is the first to document the presence of abundant rudd populations in North America, specifically in Buffalo Harbor (northeastern Lake Erie) and the upper Niagara River. The presence of rudds in these waters is especially notable because several habitat restoration projects are under way or planned for these areas (USFERC 2007), some of which include the restoration of aquatic macrophytes. Herbivory by abundant rudd populations may nullify or reduce the benefits of these macrophyte restoration efforts.

**METHODS**

A spring trap-net survey was conducted in Buffalo Harbor and the upper Niagara River during 2007–2008 to capture spawning muskellunge *Esox masquinongy* for research and quantifying fish assemblages at nearshore sites. Oneida (1.8–2.4 m high with 6.1-m wings, 15.2–121.9-m leaders, and 2.54-cm bar measure mesh) and hoop style trap-nets (0.9–1.2 m high with 6.1-m wings, 15.2–30.5-m leads, and 2.54-cm bar measure mesh) were deployed at 11 sites from 14 May to 8 June 2007 and at 7 sites from 15 May to 11 June 2008 (some sites were sampled in both years; Figure 1). The mesh size of our nets was probably too large to capture small, young (e.g., age-1 and age-2) rudds. The sites sampled were typically 1–2.75 m deep at the trap end and adjacent to deeper water. They tended to be associated with fine sediment substrates with filamentous algae but undeveloped aquatic macrophytes during the netting period; aquatic macrophytes were common at the trap-net sites later in the season. Water temperatures ranged from 9.1°C to 21.3°C during our trap-net surveys in 2007 and from 10.6°C to 21.1°C.
TABLE 1. Composition of the fish species captured in trap nets in Buffalo Harbor (BH) and the upper Niagara River (UNR) during spring 2007–2008.

<table>
<thead>
<tr>
<th>Species</th>
<th>2007</th>
<th></th>
<th></th>
<th>2008</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BH</td>
<td>UNR</td>
<td>BH</td>
<td>UNR</td>
<td>BH</td>
<td>UNR</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Rudd Scardinius erythropthalmus</td>
<td>819</td>
<td>23.2</td>
<td>1,247</td>
<td>64.8</td>
<td>719</td>
<td>24.1</td>
</tr>
<tr>
<td>Brown bullhead Ameiurus nebulosus</td>
<td>486</td>
<td>13.8</td>
<td>158</td>
<td>8.2</td>
<td>523</td>
<td>17.5</td>
</tr>
<tr>
<td>Rock bass Ambloplites rupestris</td>
<td>779</td>
<td>22.1</td>
<td>220</td>
<td>11.4</td>
<td>501</td>
<td>16.8</td>
</tr>
<tr>
<td>Smallsnout bass Micropterus dolomieu</td>
<td>669</td>
<td>19.0</td>
<td>21</td>
<td>1.1</td>
<td>599</td>
<td>20.0</td>
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<tr>
<td>Common carp Cyprinus carpio</td>
<td>58</td>
<td>1.6</td>
<td>42</td>
<td>2.2</td>
<td>42</td>
<td>1.4</td>
</tr>
<tr>
<td>Northern pike Esox lucius</td>
<td>57</td>
<td>1.6</td>
<td>13</td>
<td>0.7</td>
<td>55</td>
<td>1.8</td>
</tr>
<tr>
<td>Pumpkinseed Lepomis gibbosus</td>
<td>131</td>
<td>3.7</td>
<td>37</td>
<td>1.9</td>
<td>170</td>
<td>5.7</td>
</tr>
<tr>
<td>Yellow perch Perca flavescens</td>
<td>173</td>
<td>4.9</td>
<td>30</td>
<td>1.6</td>
<td>55</td>
<td>1.8</td>
</tr>
<tr>
<td>Bluegill Lepomis macrochirus</td>
<td>74</td>
<td>2.1</td>
<td>30</td>
<td>1.6</td>
<td>121</td>
<td>4.0</td>
</tr>
<tr>
<td>Quillback Carpiodes cyprinus</td>
<td>94</td>
<td>2.7</td>
<td>28</td>
<td>0.9</td>
<td>35</td>
<td>0.6</td>
</tr>
<tr>
<td>Redhorse Moxostoma spp.</td>
<td>16</td>
<td>0.5</td>
<td>22</td>
<td>1.1</td>
<td>43</td>
<td>1.4</td>
</tr>
<tr>
<td>Largemouth bass Micropterus salmoides</td>
<td>31</td>
<td>0.9</td>
<td>18</td>
<td>0.9</td>
<td>50</td>
<td>1.7</td>
</tr>
<tr>
<td>White sucker Catostomus commersonii</td>
<td>60</td>
<td>1.7</td>
<td>10</td>
<td>0.5</td>
<td>21</td>
<td>0.7</td>
</tr>
<tr>
<td>Bowfin Amia calva</td>
<td>1</td>
<td>0.0</td>
<td>31</td>
<td>1.6</td>
<td>35</td>
<td>0.6</td>
</tr>
<tr>
<td>White perch Morone americana</td>
<td>10</td>
<td>0.3</td>
<td>9</td>
<td>0.5</td>
<td>23</td>
<td>0.8</td>
</tr>
<tr>
<td>Common shiner Luxilus cornutus</td>
<td>6</td>
<td>0.2</td>
<td>22</td>
<td>1.1</td>
<td>14</td>
<td>0.2</td>
</tr>
<tr>
<td>Goldfish Carassius auratus</td>
<td>19</td>
<td>0.5</td>
<td>1</td>
<td>0.1</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>Black crappie Pomoxis nigromaculatus</td>
<td>11</td>
<td>0.3</td>
<td>1</td>
<td>0.1</td>
<td>9</td>
<td>0.3</td>
</tr>
<tr>
<td>Freshwater drum Aplodinotus grunniens</td>
<td>14</td>
<td>0.4</td>
<td>1</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Longnose gar Lepisosteus osseus</td>
<td>5</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Muskellunge Esox masquinongy</td>
<td>3</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Channel catfish Ictalurus punctatus</td>
<td>3</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>Gizzard shad Dorosoma cepedianum</td>
<td>4</td>
<td>0.1</td>
<td>2</td>
<td>0.1</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Goldfish × common carp</td>
<td>2</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>Rainbow trout Oncorhynchus mykiss</td>
<td>1</td>
<td>0.0</td>
<td>1</td>
<td>0.1</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td>Round goby Neogobius melanostomus</td>
<td>4</td>
<td>0.2</td>
<td>1</td>
<td>0.0</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Walleye Sander vitreus</td>
<td>4</td>
<td>0.1</td>
<td>3</td>
<td>0.1</td>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>White bass Morone chrysops</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Northern hog sucker Hypentelium nigrican</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Muskellunge × northern pike</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td>Green sunfish Lepomis cyanellus</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.1</td>
<td>1</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3,530</strong></td>
<td><strong>1,925</strong></td>
<td><strong>2,989</strong></td>
<td><strong>5,686</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Nonnative species.

in 2008 and were often below the 18°C preferred by spawning rudds (Hicks 2003; Tarkan 2006). Each net was emptied daily or every other day, and captured fish were identified to species and enumerated. In 2009, two hoop-style trap nets were deployed for 1–2 d each month during May–August to capture rudds for a diet analysis. Only data on the total length of rudds is reported here because this effort was spatially and temporally limited and not all fish were enumerated.

RESULTS

A total of 14,130 fish were captured in trap nets during 2007–2008, 6,887 (48.7%) of which were rudds (Table 1). The rudd was the most abundant species sampled, being captured at 11 of 12 sites (Figure 1) and comprising 23.6% of the total catch in Buffalo Harbor and 70.3% in the upper Niagara River. Other nonnative fishes (7 species) comprised 2.8% of the total catch in Buffalo Harbor and 4.7% in the upper Niagara River, whereas native species (23 species) comprised 73.6% of the total catch in Buffalo Harbor but only 25.0% in the upper Niagara River. Captured rudds were not measured in 2007–2008, but we estimated that their total lengths ranged from 200 to 450 mm and noted the presence of several length modes indicative of age structure. The rudds captured in trap nets in 2009 from the upper Niagara River averaged 349 mm in total length (range, 138–445 mm; n = 190). The rudds captured during 2007–2008 also appeared
to be well conditioned and sexually mature (we only attempted to extrude gametes from a subsample of these rudds, but both eggs and milt were observed).

**DISCUSSION**

Rudds were first collected in the system in a 1991 biological survey of the Buffalo River (a tributary to Buffalo Harbor; Mikol et al. 1993) but were notably absent from several reports that documented the presence of nonnative fishes in Buffalo Harbor and the Niagara River in 1928–1986 (State of New York Conservation Department 1929; Goodyear et al. 1982; Makarewicz et al. 1982; Spotila 1986). Therefore, it appears that the rudd became established in Buffalo Harbor and the upper Niagara River after 1986 but before 1991. This timeline is consistent with the rudd becoming popular as a baitfish in the 1980s, thereby implicating bait bucket transfers as the most likely source of introduction.

Buffalo Harbor and the upper Niagara River appear to contain favorable environmental conditions for rudd reproduction and survival, based on the abundances and multiple length modes that we observed. In addition, young-of-the-year rudds were captured in other surveys of the upper Niagara River, indicating that they are reproducing in this water (Kapuscinski 2011). Nurminen et al. (2003) captured rudds from Lake Hidenvesi, Finland, that were up to age 15 but that on average were smaller (mean total length, 150 mm at age 5 and 204 mm at age 9) than the rudds we captured. Quantitative analyses of the reproductive dynamics and age and size structures of the rudd populations in Buffalo Harbor and the Niagara River will help resource managers understand the invasion histories of these systems and make predictions about future range expansions.

The abundant rudd population in the upper Niagara River challenges the existing paradigm of optimal rudd habitat and requires us to expand our concept of the waters vulnerable to invasion. Most studies have focused on lake populations, and the rudd is typically described as a littoral species that prefers lentic habitats (Johansson 1987; Lake et al. 2002) and spawns on aquatic macrophytes in shallow, nearshore waters (Orr 1966; Kennedy and Fitzmaurice 1974). There are few references to rudds occupying slow-flowing lotic habitats (Cadwallader 1978; Zerunian et al. 1986). Hicks (2003) did report the presence of rudds in the tailrace of the Karapiro Dam, Waikato River, New Zealand. Cadwallader (1978), who surveyed 25 New Zealand waters, captured recently hatched rudds in shallow, nearshore areas of 17 lentic waters and only one gently flowing reach of a reservoir near the mouth of a stream. The discharge of the Niagara River averages about 5,550 m³/s (Harrison and Hadley 1978), and the velocity at nearshore vegetated areas averages about 0.04 m/s (K. L. Kapuscinski, unpublished data). The rudd’s ability to thrive in a lotic habitat such as the Niagara River requires us to gain a better understanding of the environmental conditions that affect its population dynamics.

The rudd’s relatively large body size and ability to obtain nutrients from algae, macrophytes, and detritus, which is considered an adaptation to avoid competition within its native range (Johansson 1987), will probably facilitate its spread into the Great Lakes by buffering both top-down and bottom-up controls (as described for gizzard shad *Dorosoma cepedianum* by Stein et al. 1995). Furthermore, the macrophyte-consuming rudd will establish novel trophic links and possibly contaminant pathways in the ecosystems it invades (as reported for round goby by Kwon et al. 2006). Perhaps most importantly, however, the rudd may cause shifts in aquatic vegetation communities (van Donk and Gulati 1995; van Donk and Otte 1996; Hicks 2003) that many native fishes rely upon for spawning, nursery, and foraging habitat. The numerous threats that the rudd poses to native aquatic communities should be of concern to resource managers, especially because the populations documented here have direct access to critical habitats that support economically and ecologically important fish communities in the Great Lakes basin. Three rudds were captured by commercial fishermen in Long Point Bay, Lake Erie, in 2009 (Gislason et al. 2010), and although the origin of these rudds is unknown, they may have been immigrants from the Buffalo Harbor or Niagara River populations. Comprehensive surveys that employ multiple gears (e.g., electrofishing, Gill nets, seines, and trap nets) to sample the overall fish community are needed to evaluate the abundance of rudds relative to other fishes in the Buffalo Harbor, Niagara River, and other waters where rudds may be of concern. Research focused on elucidating the effects of rudd herbivory on nearshore macrophyte assemblages and the fishes they support will be especially important for guiding habitat restoration projects in the Great Lakes basin.

**ACKNOWLEDGMENTS**

Many New York State Department of Environmental Conservation and State University of New York, College of Environmental Science and Forestry personnel provided assistance in the field and logistical and administrative support. The authors are especially grateful to Mike Clancy, Charles Curry, Brad Gruber, Robin Holevinski, Paul McKeown, and Jon Sztukowski. The authors also thank the Ontario Ministry of Natural Resources, especially Alastair Mathers, for permitting surveys in Ontario waters. Gillian AvRuskin assisted with figure creation. Derek Crane, Geoffrey Eckerlin, and two anonymous reviewers provided constructive comments on a draft of this paper. This work was funded by a grant from the Niagara River Greenway Ecological Fund Standing Committee and a Federal Aid in Sport Fish Restoration Grant administered by the New York State Department of Environmental Conservation.

**REFERENCES**


Contrasting Maturation and Growth of Northern Rock Sole in the Eastern Bering Sea and Gulf of Alaska for the Purpose of Stock Management

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 MANAGEMENT BRIEF

Contrasting Maturation and Growth of Northern Rock Sole in the Eastern Bering Sea and Gulf of Alaska for the Purpose of Stock Management

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Abstract

The primary purpose of this study was to provide commercial fishery managers with the age- and length-at-maturity information about northern rock sole *Lepidopsetta polyxystra* needed for them to set a sustainable overfishing limit and evaluate the precision of the two predictors of maturity. The estimated length at which 50% of eastern Bering Sea female northern rock sole matured (*L*50) was 309 mm, which was significantly smaller than that for Gulf of Alaska females. We determined that the differences in *L*50 between populations were probably the result of differences in the rate of female growth. Growth was significantly faster in the Gulf of Alaska than in the eastern Bering Sea during 1996 and 1999. However, by 2007 the growth rates were similar between these areas. The variability in growth was correlated with seawater temperature. There were also differences in the age at which 50% of the females matured (*A*50) between the populations in the eastern Bering Sea (9 years) and the Gulf of Alaska (7 years). In contrast, within the eastern Bering Sea, females maintained a similar *A*50 over several years, which indicates that age is the most reliable predictor of maturity for northern rock sole.

The northern rock sole *Lepidopsetta polyxystra* is the third most numerous groundfish species in the eastern Bering Sea, the current population and biomass being over $10^9$ individuals and over $2 \times 10^6$ metric tons (mt; Acuna and Lauth 2008). Northern rock sole distribution overlaps widely with its congener, rock sole *L. bilineata*, in the eastern Aleutian Islands and southern portion of the eastern Bering Sea, the Gulf of Alaska, and down the west coast of North America to Puget Sound (Stark and Somerton 2002). Northern rock sole is predominant in the southern Bering Sea and Aleutian Islands, and rock sole predominates in the Gulf of Alaska. Northern rock sole also overlaps with the ricecake sole (also known as the dusky sole) *L. mochigarei* in the western extent of its distribution off the Kuril Islands of the western North Pacific. Northern rock soles are most abundant in the southeastern Bering Sea (Fadeev 1965) where it is widely distributed in depths to 200 m but concentrated off the Pribilof Islands and in Bristol Bay (Acuna and Lauth 2008). In contrast, 80% of all the Gulf of Alaska northern rock soles is found in depths less than 100 m in the western and central areas (von Szalay et al. 2010).

Northern rock sole has high commercial value, primarily from the roe and fillet products (Wilderbuer and Nichol 2007). Sustainable management of northern rock sole is dependent upon having realistic estimates of the female reproductive potential for each stock. Typically the most reliable correlates of female maturity are age and body length. Previously, management of eastern Bering Sea northern rock soles relied on age at maturity estimates that were based on macroscopic anatomical maturity classifications collected by fishery observers during the 1993 and 1994 eastern Bering Sea rock sole roe fishery. To improve the precision of the maturity estimates, this study was initiated as part of the Bering Sea northern rock sole stock assessment conducted by the National Marine Fisheries Service’s Alaska Fisheries Science Center (AFSC) Resource Assessment and Conservation Engineering Division. The maturity estimates are used with the AFSC survey estimates of population recruitment to estimate the spawning biomass and spawner–recruit relationship, which are used to calculate the population’s maximum sustainable yield (MSY), the fishery mortality rate that would give MSY (*F*MSY), and the overfishing limit (Wilderbuer and Nichol 2007). This study employed histological maturity classification methods to determine female maturity, which are known to be less biased than the anatomical maturity classification methods (Hunter et al. 1992) previously...
used for eastern Bering Sea northern rock soles. Histological maturity assessments were previously used by Stark and Somerton (2002) to define the maturity of the Gulf of Alaska northern rock sole population and its congener the rock sole.

**METHODS**

*Sample collection.*—Observers of the AFSC Fisheries Monitoring and Analysis Division collected 209 northern rock soles taken in the eastern Bering Sea and delivered by commercial bottom trawl vessels to seafood processing plants in Dutch Harbor, Alaska, during February and March 2006. The observers were specifically trained in the taxonomic identification of rock soles. The species were differentiated using blind side coloration and gill raker and supraorbital pore counts (Orr and Matarrese 2000). All specimens were measured for total length (cm) and selected using length-stratified samples of three to seven females from each centimeter category. The specimen measurement scale was designed so that each 10 cm unit of measure spans the range from −5 to +5 mm total body length (L; note L was measured in centimeters and converted to millimeters for purposes of analysis).

Ovary tissue was excised from each specimen, placed in a labeled histology cassette, which was submerged in a bottle containing a solution of 10% formalin. The maturity collection included specimens as small as 21 cm. This length range was considered fully representative, based on a 1999 Gulf of Alaska northern rock sole maturity collection (Stark and Somerton 2002), which included specimens as small as 19 cm but found no mature females smaller than 30 cm in length.

Northern rock sole growth determinations were made from unpublished AFSC bottom trawl survey age data, stratified by length and area, taken from the eastern Bering Sea and Gulf of Alaska bottoms trawl surveys of 1996, 1999, and 2007. These survey collections were designed to provide the most representative growth data available and allow for valid comparisons between years and areas. The AFSC surveys used standardized fishing methods, including standardized station patterns to assess the condition of groundfish stocks across the eastern Bering Sea. The full bottom depth distribution of northern rock soles was trawl-sampled, and for each North Pacific Fishery Management Council statistical area, specimens were selected using random stratified sampling of two to six specimens from each 1-cm category of total body length distributed. These collections included northern rock sole specimens with body lengths as small as 5 cm, as effected by a standard 0.89-cm-mesh cod end liner in the survey trawl.

*Maturity assessment.*—In the laboratory the cassette of ovary tissues were then removed from the 10% formalin preservative, dehydrated through graded ethanol, cleared, and embedded in Paraplast blocks. The embedded ovaries were cut into sections 0.003−0.005 mm thick, mounted on a glass slide with a cover slip, stained with hemotoxin, and counterstained with eosin. The cover slip was then sealed onto the slide. The stains were used to unambiguously delineate important structures within the oocytes and ovary. Eosin was used to give a bright pink coloration to all yolk residing within all oocytes contained within the ovary tissue. The eosin also stained fully developed outer cell membranes (chorion) found in all oocytes; maturity stages ranged from cortical alveoi to hydrated. The hemotoxin stain gave a distinct violet coloration to noneosinophilic structures of the cell to make them clearly discernable. All specimens were assessed according to a five-stage scale of ovary maturity based on cell structure criteria (Table 1). Females were classified according to the most advanced stage of histological development that occurred in the ovary. Mature females included those individuals that had any of the yolk-filled oocyte development stages (i.e., vitellogenesis, advanced yolk, and hydrated). Mature females also included those with postovulatory follicles. All the mature females were those expected to spawn that year. Spawning females were those with maturity stage classifications of either hydrated or postovulatory follicle.

**Statistical methods.**—All analysis utilized S-Plus software (version 2000 Professional release3, MathSoft Inc., Cambridge, Massachusetts). Maturity was estimated as a function of L by fitting a logistic function to the binary maturity data with generalized linear modeling (Venables 1997). The between-month and between-area differences were tested by fitting the model of maturity as a function of L, with and without terms distinguishing month and area, and then judging the significance of the terms via analysis of deviance (Venables 1997). Length at 50% maturity (L50) was estimated for each month and area by evaluating the fitted model of maturity at 50% maturity and

<table>
<thead>
<tr>
<th>Development stage</th>
<th>Maturity category</th>
<th>Cellular structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perinucleus</td>
<td>Immature</td>
<td>Oocyte with multiple nuclei bordering the nucleus; chorion absent.</td>
</tr>
<tr>
<td>Cortical alveoli</td>
<td>Immature</td>
<td>Oocyte with cortical alveoli adjacent to developing chorion.</td>
</tr>
<tr>
<td>Vitellogenesis</td>
<td>Mature</td>
<td>Oocyte predominantly contains yolk spheres.</td>
</tr>
<tr>
<td>Advanced yolk</td>
<td>Mature</td>
<td>Oocyte filled with fused yolk; no individual yolk spheres.</td>
</tr>
<tr>
<td>Hydrated</td>
<td>Mature and spawning</td>
<td>Oocyte expanded by hydration, located in lumen of ovary.</td>
</tr>
<tr>
<td>Postovulatory follicles</td>
<td>Mature and spawning</td>
<td>Postovulatory follicles present.</td>
</tr>
</tbody>
</table>
algebraically solving for length. The variance of $L_{50}$ was estimated using bootstrapping (Efron and Tibshirani 1993) based on 1,000 resamplings, with replacement, of the maturity and length data. Differences in the $L_{50}$ by month and area were tested with a Z-test (Sokal 1969). The same procedures were used to compare maturity as a function of age and to compare length results from this study with northern data from a Gulf of Alaska study by Stark and Somerton (2002). Based on their study, February was assumed to be the most representative period for sampling because all females that were expected to spawn that year would unambiguously appear to be mature and few would have completed spawning. The March collection provided the means to test whether smaller and younger females matured later in the year than larger females.

Length at age was described by the von Bertalanffy growth function, fitted using nonlinear least squares (Venables 1997). To evaluate differences in northern rock sole growth between areas and years the von Bertalanffy model of growth was fitted to the length-at-age data with terms distinguishing area and year and retested for the combined areas and years. The goodness of fit to the data was tested comparing the separate-category and combined-category models via the likelihood ratio test (Kimura 1980). Otoliths were aged by personnel of the AFSC Age and Growth Program with standard validated methods (Fargo and Chilton 1987; Kimura and Anderl 2005; Kimura et al. 2007), which includes sectioning transversely through the otolith nucleus and heating the otolith to increase the contrast between the growth (transparent) and opaque layers. The growth layers are considered to be the annuli, which are enumerated via a dissecting microscope.

RESULTS
Age and Length at Maturity
The collection included females ranging in length from 210 mm to 490 mm; ages ranged from 4 to 23 years. We found no differences between the February and March collections for maturity at age ($P = 0.89$) and length ($P = 0.73$); consequently the data were pooled to increase sample size to 162 for age and 209 for length. Fifty percent of female northern rock soles in the eastern Bering Sea reached maturity at the age ($A_{50}$) of approximately 9 years (Figure 1; Table 2), which was significantly ($P < 0.001$) older than for Gulf of Alaska females ($A_{50} = 7$ years; Stark and Somerton 2002). The youngest mature eastern Bering Sea female collected was also the smallest mature female, at 240 mm and age 6. Half the eastern Bering Sea females matured ($L_{50}$) at 309 mm in the eastern Bering Sea (Figure 2; Table 3), which was significantly ($P = 0.003$) smaller than Gulf of Alaska females (328 mm; Stark and Somerton 2002). The proportion of mature eastern Bering Sea female northern rock soles was similar during February and March 2006, although the rate of spawning increased from less than 5% in February to approximately 30% in March (Figure 3). This result suggests that the spawning season of eastern Bering Sea northern rock soles began just before the February collection period and peaked during the spring.

FIGURE 1. Maturity estimated as a function of age for female northern rock soles collected in February and March 2006 ($n = 162$) in the eastern Bering Sea, as depicted by fitting a logistic function to the maturity data. The mean age at 50% maturity (8.9 years) is indicated by solid vertical line; the 95% confidence interval is represented by the dotted lines.
TABLE 2. Female northern rock sole age-at-maturity results based on ovary histology samples collected from the eastern Bering Sea by the Alaska Fisheries Science Center during February and March 2006. The parameters of the logistic equation fit to the data are \( n \) (sample size), \( B \) (slope of the line) and its variance, \( A \) (y intercept) and its variance, the covariance of \( A \) and \( B \) (the product of the SDs of \( B \) and \( A \) and the coefficient of correlation between them), and \( A_{50} \) (the age at which 50% of females were expected to reach sexual maturity) and its variance.

<table>
<thead>
<tr>
<th>Sampling variable</th>
<th>Sampling statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>162</td>
</tr>
<tr>
<td>( B )</td>
<td>0.74466</td>
</tr>
<tr>
<td>( A )</td>
<td>-6.63200</td>
</tr>
<tr>
<td>Variance of ( B )</td>
<td>0.00107</td>
</tr>
<tr>
<td>Variance of ( A )</td>
<td>5.48510</td>
</tr>
<tr>
<td>Covariance of ( B, A )</td>
<td>-0.00385</td>
</tr>
<tr>
<td>( A_{50} ) (years)</td>
<td>8.90603</td>
</tr>
<tr>
<td>Variance of ( A_{50} )</td>
<td>0.19481</td>
</tr>
</tbody>
</table>

Growth

The precision of rock sole age determinations was tested by AFSC Age and Growth Program personnel (Kimura and An-dler 2005). The average reader and tester agreement for these years averaged 70% (CV = 3). To determine if area differences in length at maturity were consistent with growth, I made the first growth comparison between regions for northern rock sole (Figure 4; Table 4). The results indicate that female growth rates were significantly \((P < 0.001)\) faster in the Gulf of Alaska than in the eastern Bering Seas during 1996 and 1999. However, by 2007 the rate of female growth had increased in the eastern Bering Sea to a rate similar to that of the Gulf of Alaska population \((P = 0.69)\).

DISCUSSION

Length at Maturity and Growth

My study is the first to determine both the age and length at maturity of female northern rock soles in eastern Bering Sea, and the histological maturity classification methods used improved accuracy of the estimates. The results from the February collection were corroborated by the results from the March collection and suggested that by February all females that would mature for the annual maturation cycle had matured.

I estimated that female northern rock soles of the eastern Bering Sea reached \( L_{50} \) at 309 mm, which was significantly smaller than the Gulf of Alaska \( L_{50} \) estimate of 328 mm. The smallest mature females found in the eastern Bering Sea were 240 mm, compared with 300 mm for the Gulf of Alaska collection. The smaller female \( L_{50} \) in the eastern Bering Sea was

FIGURE 2. Maturity estimated as a function of total length for female northern rock soles collected in February and March 2006 \((n = 209)\) in the eastern Bering Sea, as depicted by fitting a logistic function to the maturity data. The mean length at 50% maturity (309 mm) is indicated by solid vertical lines; the 95% confidence interval represented by dotted lines.
probably due to significant differences in the rate of growth between the two populations. The female northern rock sole population in the Gulf of Alaska had approximately twice the rate of growth as the Bering Sea population, during 1996 and 1999. However, by 2007 the rate of growth of the eastern Bering Sea female population had become similar to the growth rate of the Gulf of Alaska population. In contrast, the Gulf of Alaska female population maintained a similar rate of growth during 1996, 1999, and 2007. Growth rates of eastern Bering Sea northern rock sole, and two other flatfish species were directly affected by summer seawater temperatures on the shelf (Matta et al. 2010). Summer is the feeding and growing season for these species, and they have the same feeding grounds every year. The otolith annuli growth correlated chronologically with summertime bottom temperatures for northern rock sole ($R^2 = 0.34$), yellowfin sole *Limanda aspera* ($R^2 = 0.81$), and Alaska plaice *Pleuronectes quadrituberculatus* ($R^2 = 0.61$), based on fish aged as old as 23 years.

Extreme average summer sea bottom temperatures occurred in 1999 (0.83°C) and 2003 (3.87°C), which produced narrow growth annuli within the otoliths of all three flatfish species. In contrast, broad annuli growth occurred during years that had average bottom temperatures of 2.3°C. Colder bottom temperatures correlated with slower growth in northern rock sole, and this was confirmed for northern rock sole reared under laboratory conditions (Hurst and Abookire 2006). The summer

### TABLE 4. Female northern rock sole length at age as described by the von Bertalanffy growth equation for females in the Bering Sea and Gulf of Alaska during 1996, 1999, and 2007, based on summer Alaska Fisheries Science Center groundfish surveys. Components include $n$ (the sample size) and equation parameters $L_\infty$ (length at maximum age), $k$ (the estimated growth increment), and $t_0$ (the theoretical age when fish length is 0).

<table>
<thead>
<tr>
<th>Sampling variable</th>
<th>Bering Sea</th>
<th>Gulf of Alaska</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>280</td>
<td>298</td>
</tr>
<tr>
<td>$L_\infty$ (mm)</td>
<td>488.807</td>
<td>571.613</td>
</tr>
<tr>
<td>var($L_\infty$)</td>
<td>193.4323</td>
<td>1022.7257</td>
</tr>
<tr>
<td>$k$</td>
<td>0.1096</td>
<td>0.0710</td>
</tr>
<tr>
<td>var($k$)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>$t_0$</td>
<td>0.2563</td>
<td>0.0982</td>
</tr>
<tr>
<td>var($t_0$)</td>
<td>0.0329</td>
<td>0.0873</td>
</tr>
<tr>
<td>cov($L_\infty$, $k$)</td>
<td>-0.1039</td>
<td>-0.2548</td>
</tr>
<tr>
<td>cov($L_\infty$, $t_0$)</td>
<td>-1.8848</td>
<td>-7.9425</td>
</tr>
<tr>
<td>cov($k$, $t_0$)</td>
<td>0.0012</td>
<td>0.0022</td>
</tr>
</tbody>
</table>
growing period bottom temperatures and spring plankton bloom directly correlate with the extent of the annual winter sea ice sheet formation and the timing and extent of the sea ice melts on the Bering Sea shelf (Stabeno et al. 2001). Sea ice that extends into the shelf during March or April indicates stratification of the water column, which produces an early and intense plankton bloom at the ice edge. This type of plankton bloom can produce up to 65% of the annual primary productivity over the shelf (Niebauer et al. 1990). In years that have strong mixing of the seawater column during March and April, there is no ice formation, and the plankton bloom is delayed until May or June and is attenuated. Water temperatures are expected to undergo more frequent fluctuations and rise an average of 2°C by the year 2050 in the eastern Bering Sea (Hollowed et al. 2009). The change would further affect northern rock sole growth through changes in food supply and body metabolism and, consequently, the size at which females mature in the future. In contrast, females of the Gulf of Alaska northern rock sole population may continue to have a lower interannual variability in growth and mean length at maturity than the eastern Bering Sea population because of a more moderate temperature regime. Compared with the Bering Sea, the Gulf of Alaska is more insulated from arctic temperatures because of its lower latitude and greater circulation of seawater (Stabeno et al. 2001). Consequently, the Gulf of Alaska is not subject to an annual accumulation of sea ice, which can alter the timing and abundance of primary and secondary production and, thereby, northern rock sole metabolism and growth.

**Age at Maturity**

Although female northern rock soles in the eastern Bering Sea had very different growth rates over years, the age at which females matured was similar between years. Based on my study, the eastern Bering Sea population reached $A_{50}$ at an estimated age of 9 years. The $A_{50}$ estimate was comparable to the previous age estimate of 9–10 years used by eastern Bering Sea stock managers (Wilderbuer and Nichol 2007), that was based on a 1994 collection. Similarities in the estimated female age at maturity in the eastern Bering Sea between 1994 and 2006 suggests that age is a reliable predictor of maturity for northern rock soles, at least for the eastern Bering Sea population. As a consequence of maturing at a significantly older age (2 years older) than Gulf of Alaska females, the eastern Bering Sea population was more dependent upon older females to sustain the population. Females of both populations have the same potential life span (Figure 4). Compared with the younger maturing Gulf of Alaska females, delayed maturation in the eastern Bering Sea means that these females would probably have fewer years available for spawning, potentially decreasing the individual reproductive production from each female.

The results suggest the spawning season begins before February and peaks during the spring for the eastern Bering Sea population, which is consistent with observations by Shubnikov and Lisovenko (1964) in the southeastern Bering Sea and in the Gulf of Alaska (Stark and Somerton 2002). The depth and location of spawning is not well known for the eastern Bering Sea population. In the western Bering Sea northern rock sole spawned in depths less than 200 m (Pertseva-Ostroumova 1961), and in the Gulf of Alaska spawning occurred in depths less than 65 m (Stark and Somerton 2002). The spawning grounds were located within bays, near shore, and on the inner portions of banks in areas that had gravel and a hard sandy bottom. This type of substrate is essential for the attachment of the rock sole’s demersal adhesive eggs.

Results of my study provide fishery managers with the necessary maturity estimates that allow them to refine the overfishing and allowable catch limits of northern rock sole in the eastern

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**FIGURE 4.** Total length at age of female northern rock soles from collections made in the eastern Bering Sea (solid triangles, solid line) and Gulf of Alaska (open squares, dashed line) during areawide groundfish assessment surveys conducted by the Alaska Fisheries Science Center. Sample sizes by year—1996, 1999, and 2007, respectively—were 281, 298, and 263 for the Bering Sea and 142, 227, and 261 for the Gulf of Alaska.
Bering Sea. I determined that age-based maturity estimates would be much more reliable than length-based estimates and provides managers with a much more accurate estimate of the age at which females mature. Consequently managers can now produce more precise estimates of the spawning biomass and spawning-recruit relationship. As a result, managers will have more precise estimates of the maximum sustainable yield, fishery mortality rate, and overfishing limits than was previously possible using other methods.

ACKNOWLEDGMENTS

The following Alaska Fisheries Science Center personnel made this study possible: T. Wilderbuer, A. Hollowed, and R. Nelson. Collections were made by A. Baattista, K. Hardin, and L. Kerr. Age determinations were made by B. Matta and D. Anderl. Reviews were made by D. Somerton, G. Duker, J. Lee, and M. Wilkins. This manuscript was improved by suggestions from two anonymous reviewers, editor C. Griswold and the associate editor.

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Evaluation of a Gastric Lavage Method for Sturgeons

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Evaluation of a Gastric Lavage Method for Sturgeons

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Abstract.—Because of their threatened status, sturgeons (Acipenseridae) can no longer be sacrificed for stomach content analysis. We tested a nonlethal method of gastric lavage on Siberian sturgeon Acipenser baeri. The efficiency and harmlessness of the method were tested with four different volumes of food (10, 20, 30, and 40 cm³), each composed of brown shrimp Crangon crangon, Chironomidae, earthworm Lumbriscus terrestris, and sand goby Pomatoschistus minutus. The Siberian sturgeons were force-fed before the gastric lavage was performed. Some prey were recovered from all the sturgeons, and the average food item recovery rate from stomach contents was 67.5%; recovery of brown shrimp and sand goby (78.2%) was greater than that of vermiciform items (51.4%). The volume of food had no significant influence on the prey recovery rate. No mortality resulted from the gastric lavage. However, the method is not totally benign because the fish that had undergone gastric lavage averaged significantly greater weight loss than the control fish during the 60-d follow-up period.

Because many sturgeon (Acipenseridae) species have a threatened status, the study of their feeding habits must employ methods that are as safe and efficient as possible. The combination of these criteria explains partly why data on sturgeon diet are scarce. Diet studies of acipenserids had involved the analyses of stomach contents recovered from corpses, such as with the Gulf sturgeon Acipenser oxyrinchus desotoi (Mason and Clugston 1993) and the shortnose sturgeon A. brevirostrum (Dadswell 1979; Carlson and Simpson 1987). Since then, gastric lavage procedures have been tested on the Atlantic sturgeon A. oxyrinchus and shortnose sturgeon to determine harmlessness; however, lavage efficiency by type of prey contained in the stomach has not been studied (Haley 1998). In lake sturgeon A. fulvescens, gastric lavage using water reflux has been tested for efficiency and shown no influence due to the type of prey contained in the stomach (Nilo 1996). However, Sprague et al. (1993) showed that water at too high a pressure could lead to internal injuries or even death by rupturing the swim bladder. Because of that, techniques using a syringe connected to a flexible tube have been proposed and tested (Meehan and Miller 1978; Haley 1998).

We chose a pulsed gastric lavage method (Foster 1977) inspired by those that use a pressurized water reservoir (Light et al. 1983; Nilo 1996), which has the advantage of providing a continuous supply of water. The aim of this work was to test the efficiency of this method in recovering various types of prey from Siberian sturgeon A. baeri and to determine any resulting damage, such as death or weight loss.

Methods

The tests were performed from January to March 1999 on farmed Siberian sturgeons at the Cemagref experimental station (St-Seurin-sur-l’Isle, France). Four test groups of five individuals were formed (N = 20, lavage set), and one group of 15 individuals was kept as a control. All groups were of similar fork length (FL; 78–105 cm). Each lavaged sturgeon was force-fed a mixture of five different types of prey: earthworms Lumbriscus terrestris, chironomid larvae, sand goby Pomatoschistus minutus, brown shrimp Crangon crangon, and sand goby Pomatoschistus minutus.

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Received January 17, 2001; accepted January 3, 2002
Table 1.—Number of prey used for the different volumes force-fed to Siberian sturgeon.

<table>
<thead>
<tr>
<th>Set</th>
<th>Number of sturgeon</th>
<th>Force-fed volume (cm³)</th>
<th>Chironomids</th>
<th>Earthworms</th>
<th>Small shrimp</th>
<th>Large shrimp</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavage</td>
<td>5</td>
<td>10</td>
<td>40</td>
<td>1</td>
<td>9</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Lavage</td>
<td>5</td>
<td>20</td>
<td>50</td>
<td>2</td>
<td>14</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Lavage</td>
<td>5</td>
<td>30</td>
<td>60</td>
<td>4</td>
<td>21</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Lavage</td>
<td>5</td>
<td>40</td>
<td>60</td>
<td>5</td>
<td>28</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

chistus minutus (1–3 cm total length), and small (cephalothorax <1 cm) and large (cephalothorax >1 cm) brown shrimp Crangon crangon (Table 1). Quantities of the five prey types in the mixture to be force-fed were known. Volumes of prey varied and were based on previous measurement of stomach volumes of several sturgeons of the same length range. Force-feeding took about 1 min, during which a flexible tube (12 mm outside diameter [OD], 20 cm long) filled with an exact volume of prey items was inserted about 5 cm into the digestive tract and purged with a piston.

The materials used for gastric lavage were modeled on those used by Nilo (1996). The system was composed of a preset-pressure garden sprayer with a 7-L tank (maximum pressure of $2 \times 10^5$ Pa in the tank at the beginning of the lavage); a 500-μm-mesh sieve for collecting the stomach contents; a 6-mm OD flexible tube for injecting the water; a 12-mm OD flexible tube for flushing out the water (plus the food bolus) into which the plastic 6-mm-OD flexible tube was inserted (Figure 1). Throughout the operations, which lasted less than 2 min, a fish was held dorsal side down in an improvised cradle, and its head and gills were continuously hosed with freshwater. Both tubes were inserted into the forward part of the digestive tract. The large tube was inserted about 5 cm into the tract, and the water injection tube was inserted as far as the first stomach loop, approximately 20 cm (Figure 2). The injection tube was gently manipulated in a to-and-fro motion to dislodge and carry...
Figure 2.—Digestive tract of Siberian sturgeon (119 cm fork length), showing placement of the small water inflow tube (6 mm outside diameter [O.D]) and the large outflow tube (12 mm O.D) used in gastric lavage (see Figure 1).
the prey from the stomach. The length of the injection tube was obtained by preliminary tests on five sacrificed Siberian sturgeons.

The control fish were not given a gastric lavage and only underwent simulated force-feeding (i.e., introducing the tube and pushing the piston but without prey). Fish were weighed immediately after the gastric lavage ($W_0$) and at 30 ($W_{30}$) and 60 ($W_60$) d after lavage. The mean relative change in weight ($R_t$) during these periods was calculated as $R_t = (W_{30} or 60 - W_0)/W_0$. The standard error of this ratio was estimated according to Cochran (1977). Mortality was monitored over the 60-d test period, at the end of which all the sturgeons (55) were sacrificed so that their digestive tracts could be examined. Five more individuals used for preliminary tests were sacrificed immediately after gastric lavage and force-feeding and lavaging to assess more immediate injuries.

We used the Kruskall–Wallis $H$-test to test whether recovery rate (efficiency of the method) differed among prey types. We examined the weight variation within (Wilcoxon matched-pairs test) and between (Mann–Whitney $U$-test) the sets to test whether or not the method harmed the fish. All the statistics were performed using SYSTAT software (SPSS 1998).

## Results

### Efficiency of the Method

Some prey were recovered for all Siberian sturgeons tested ($N = 20$), and the average prey recovery rate was 67.5% over all the samples (Table 2). The type of prey was found to have a significant influence on the recovery ($P < 0.001$), with a significantly lower recovery rate ($P < 0.001$) for group A (earthworms and chironomids; 51.4%) than for group B (large and small shrimp and fish; 78.2%). The volume of prey used had no significant influence on the recovery rate ($P = 0.97$), regardless of the prey type ($0.07 < P < 0.90$).

### Impact on the Sturgeons

No mortality was observed during the 60-d following lavage. No traces of injury were observed in the forward part of the digestive tract in the set of fish sacrificed immediately after gastric lavage ($N = 5$). A significant loss of weight occurred in both the lavaged ($P < 0.001$) and the control sets ($P = 0.001$) after 60 d (Table 3). No significant difference in weight change was noted between the lavaged and control sets at either 30 d ($P = 0.395$) or 60 d ($P = 0.188$) postlavage. However, a significant difference in weight change between the lavaged (an average loss of 7.97%) and control sets (an average loss of 5.84%) was found when the 30-d and 60-d data were combined ($P = 0.008$).

### Discussion

Overall, the stomach content recovery rate is satisfactory using this technique because we recovered items from 100% of the fish tested, which is higher than results for lake sturgeons (96%) by Nilo (1996) using a similar method or for Atlantic sturgeon (91%) by Haley (1998) using a syringe. Regardless of the volume fed, all prey types were recovered. However, the variation in the recovery rate among prey types may lead to a distorted pic-

### Table 2.—Mean gastric lavage recovery rate for prey types and volume of prey force-fed to Siberian sturgeon. Group A = chironomids + earthworms; Group B = small and large shrimp + fish.

<table>
<thead>
<tr>
<th>Force-fed volume (cm$^3$)</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chironomids</td>
<td>Earthworms</td>
<td>Mean</td>
<td>Small shrimp</td>
</tr>
<tr>
<td>10</td>
<td>62.0</td>
<td>20.0</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>54.7</td>
<td>80.0</td>
<td>67.4</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>45.4</td>
<td>45.0</td>
<td>45.2</td>
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</tr>
<tr>
<td>40</td>
<td>28.3</td>
<td>76.0</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>47.6</td>
<td>55.3</td>
<td>51.4</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3.—Average (standard deviation) of Siberian sturgeon weight and relative weight loss rate, $R_t$, during the 60-d period following gastric lavage ($t_0$, $t_{30}$, and $t_{60}$ correspond to the 1st, 30th, and 60th days of observation).

<table>
<thead>
<tr>
<th>Set</th>
<th>$N$</th>
<th>$t_0$</th>
<th>$t_{30}$</th>
<th>$t_{60}$</th>
<th>$R_{0-30}$</th>
<th>$R_{30-60}$</th>
<th>$R_{60}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavage</td>
<td>20</td>
<td>6,145 (1,135)</td>
<td>5,670 (1,303)</td>
<td>5,655 (1,296)</td>
<td>-7.73 (1.81)</td>
<td>-0.26 (0.06)</td>
<td>-7.97 (1.86)</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>6,160 (1,494)</td>
<td>5,827 (1,419)</td>
<td>5,800 (1,454)</td>
<td>-5.41 (1.48)</td>
<td>-0.46 (0.13)</td>
<td>-5.84 (1.60)</td>
</tr>
</tbody>
</table>
ture of the actual diet of Siberian sturgeons examined by this method. That is, we recovered approximately 50% of the vermiform prey and 75% of the larger prey; so variation in prey-type recovery is likely when using this method on fish in their natural environment. Foster (1977) noted that certain methods (suction pumping and regurgitation) gave significantly different results according to the prey type, whereas other methods (pulsed or reflux gastric lavage) did not result in such variations. Nilo (1996) did not observe such biases, but Haley (1998) suggested a need to examine this possibility.

Using a pressurized water reservoir of several liters enables rapid gastric lavage compared with other methods, such as that used by Haley (1998), in which the reservoir is a 60-mL syringe. Similarly, using natural prey in tests provides better simulation of natural conditions than does use of artificial food (i.e., Haley 1998). Our method limits handling time and associated stress and allows for the rapid release of wild fish near their capture site. The use of double tubing reduces problems linked with potential contractions of the pharynx or esophagus that could limit the recovery of the food bolus. Moreover, the insertion of the larger tube far enough into the digestive tract could prevent the introduction of the small tube (injection tube) into the swim bladder.

In preliminary tests conducted before our study herein reported, we found that at 2 hr or more after sturgeons were force-fed food, very few of the originally injected prey items were recovered, which indicates that only the items recently ingested are recovered with our technique. Having access only to the forward part of the digestive tract may be a handicap, compared with other methods used on other sturgeon species (Haley 1998). On the other hand, accessing the recently ingested fraction of the food bolus provides the advantage of enabling the identification of the local feeding ground, which could be useful for habitat studies.

No mortality was observed over the 60 d following gastric lavage, which supports findings of similar studies (Meehan and Miller 1978; Gaudin et al. 1981; Light et al. 1983; Hartleb and Moring 1995). Occasional weight losses similar to those we noted in the lavaged set are regularly observed in Siberian sturgeon broodstock during the winter at the biological station (P. Williot, Cemagref, personal communication). However, the slight but significant difference we observed in weight change between the lavaged and control sets indicates that this method is not totally benign and may induce some long-term modifications in the fish’s feeding behavior. We would consequently recommend using it only once on a particular animal, even if recaptured beyond an interval of several months.

The results we achieved with this method encouraged us to use it on juvenile European sturgeon {Acipenser sturio} captured in the Gironde estuary in France (N = 77; 56–103 cm FL); prey were recovered in about 98% of the cases (Brosse et al. 2000). The analysis of the contents shows that European sturgeons feed mainly on small polychaetes (about 3–5 cm long) and, to a lesser degree, brown shrimp (2.5 cm maximum length) and sand goby (3 cm TL).

Acknowledgments

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References


Standard Weight (Wₚ) Equation and Length Categories for Shovelnose Sturgeon

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Standard Weight (W_r) Equation and Length Categories for Shovelnose Sturgeon

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Kansas State University, 205 Leasure Hall, Manhattan, Kansas 66506, USA

Abstract.—Weight–length data were compiled from 32 populations of shovelnose sturgeon Scaphirhynchus platorynchus (N = 11,820) from nine states within the geographic distribution of the species. We used the regression-line-percentile technique, which provides a 75th-percentile standard, to develop the standard weight (W_r) equation. The proposed equation in metric units is \( \log_{10} W_r = -6.287 + 3.330 \log_{10} FL \); \( W_r \) is weight in grams and FL is fork length in millimeters. The equivalent equation in English units is \( \log_{10} W_r = -4.266 + 3.330 \log_{10} FL \); \( W_r \) is weight in pounds and FL is fork length in inches. These equations are proposed for use with shovelnose sturgeon between 120 mm (5 in) and 1,050 mm (41 in). Values for relative weight (W_s) calculated with the \( W_r \) equation did not consistently increase or decrease with increasing fish length, indicating absence of length bias. We propose the following length categories for calculation of proportional stock density (PSD) and relative stock densities (RSDs): stock, 250 mm (10 in); quality, 380 mm (15 in); preferred, 510 mm (20 in); memorable, 640 mm (25 in); and trophy, 810 mm (32 in). We found significant relations between size structure indices and mean population \( W_s \). Additionally, we found significant differences among incremental \( W_s \) values. We believe the \( W_r \) equation and length category designations will be useful tools for managing shovelnose sturgeon populations.

Relative weight \( W_r = 100 \cdot W/W_s \), where \( W \) is the observed weight and \( W_s \) is the length-specific standard weight value for the species; Wege and Anderson (1978) is commonly used as a management tool to assess the physiological well-being of fishes (i.e., condition). Relative weight is easier to interpret than the traditional Fulton condition factor \( K \), because unlike \( K \), \( W_r \) neither increases with increasing fish length nor varies by species. Thus, \( W_s \) is useful when comparing within and among fish populations.

Stock density indices are also useful management tools to assess fish populations. Proportional stock density [PSD = (number of fish greater than or equal to quality length/number of fish greater than or equal to stock length) · 100] and relative stock density [RSD = (number of fish greater than or equal to a specified length/number of fish greater than or equal to stock length) · 100] are commonly used to assess size structure. However, before PSD or RSD can be calculated for a fish population, species-specific length categories must be defined. See Gabelhouse (1984a) for methods to determine stock, quality, preferred, memorable, and trophy lengths.

Despite the widespread use of \( W_s \) and stock density indices with warmwater sportfish, \( W_r \) equations and length categories are virtually nonexistent for large-river, nongame fishes. Therefore, the objectives of this study were to (1) develop a \( W_r \) equation for shovelnose sturgeon Scaphirhynchus platatorynchus based on the regression-line-percentile (RLP) technique, (2) evaluate the consistency of \( W_r \) across various lengths, (3) evaluate \( W_r \) distributions of individuals and populations, (4) determine length categories (i.e., stock, quality, preferred, memorable, and trophy lengths), and (5) assess the relations between \( W_s \) and size structure for shovelnose sturgeon populations.

Methods

Weight–length data for shovelnose sturgeon were solicited from biologists within the geographical distribution of the species (Lee et al. 1980). Populations represented by less than 30 individuals were omitted from all analyses. All data were evaluated as fork lengths (FL). One data set submitted as total lengths (TL) was converted to fork length with an equation developed from data sets that included both FL and TL (FL (mm) = (TL – 43.14)/1.02; \( r = 0.99, P = 0.0001 \)). Additionally, all weights and lengths were converted to grams and millimeters.

The RLP technique was used to develop the \( W_r \) equation for shovelnose sturgeon (Murphy et al. 1991). The minimum length for weight precision was determined by plotting the variance-to-mean ratio by 1-cm length-groups, as suggested by Murphy et al. (1990). Minimum length was set where the variance-to-mean ratio exceeded approximate-
ly 0.01 (Neumann and Murphy 1991). Minimum sample size for the application of the RLP technique was determined by methods described in Brown and Murphy (1996). Independence of \( W_r \) across length-classes for each study population was evaluated by assessing the number of significant positive and negative slopes of \( W_r \) regressed on fish length. The total number of significant positive and negative slopes were compared with a binomial test to detect any length-related bias in the \( W_r \) equation (Mehta and Patel 1995).

Length categories were determined by methods described by Gabelhouse (1984a). He suggested that minimum stock, quality, preferred, memorable, and trophy lengths be determined from lengths encompassed by 20–26%, 36–41%, 45–55%, 59–64%, and 74–80% of world record length, respectively. We used the largest fish in our data set (1,052 mm FL; 8,164 g) as the world record length because the largest shovelnose sturgeon recorded by the National Freshwater Fishing Hall of Fame weighed 2,155 g.

Several authors have noted seasonal variations in stock density indices (Carlile et al. 1984; Serns 1985; Willis et al. 1993). However, nearly all data sets represented summer or early fall sampling, which made separation by season impractical. Similarly, size-related biases can result from gear selectivity (Hamley 1975; Reynolds and Simpson 1978). Relations between \( W_r \) and size structure were determined for those populations known to be sampled with gill or trammel nets from riverine habitats. Mean \( W_r \) was plotted as a function of PSD and as a function of incremental \( W_r \) and RSDs (i.e., stock to quality (S–Q), quality to preferred (Q–P), preferred to memorable (P–M), and memorable to trophy length (M–T)). The relationships were analyzed with correlation techniques. Differences of \( W_r \) among length categories were tested with analysis of variance (ANOVA; SAS 1989). A probability level of 0.05 was used to reject the null hypothesis in all statistical tests.

Results and Discussion

Weight–length regressions were developed for 32 shovelnose sturgeon populations from nine states (\( N = 11,820 \); Table 1). We found that 30 populations were required to develop a \( W_r \) equation with the RLP technique (sample variance of slopes = 0.0018; Michael L. Brown, South Dakota State University, personal communication). Shovelnose sturgeon 120 mm and longer were included in the weight–length regressions because the variance-to-mean ratio was below 0.01 for all fish used. All weight–length regressions were significant (\( P \leq 0.0001 \)), and all but three correlation coefficients were 0.93 or higher (Table 1).

The following \( W_r \) equation for shovelnose sturgeon was calculated with the 75th-percentile RLP technique:

\[
\log_{10} W_s = -6.287 + 3.330 \log_{10} FL;
\]

where \( W_s \) is standard weight in grams and FL is in millimeters. The equivalent in English units is

\[
\log_{10} W_s = -4.266 + 3.330 \log_{10} FL;
\]

where \( W_s \) is standard weight in pounds and FL is in inches. These equations are proposed for use with shovelnose sturgeon from 120 mm (5 in) to 1,050 mm (41 in).

Although \( W_r \) may vary among lengths in a given population, there should be no consistent pattern of increasing or decreasing \( W_r \) values for a series of populations. When \( W_r \) was regressed on fish length for 32 populations, 20 populations had significant slopes (\( P \leq 0.05 \)). The number of positive slopes (11 slopes) was not significantly different than the number of negative slopes (9 slopes; \( P = 0.41 \)), indicating no length bias associated with the \( W_r \) equation.

Forty-three percent of the populations had mean \( W_r \) values within the suggested benchmark target range of 95–100 (Wege and Anderson 1978). Although this is similar to those percentages reported for white bass \( Morone chrysops \) (37%), palmetto bass (a hybrid of female striped bass \( Morone saxatilis \) × male white bass; 32%; Brown and Murphy 1991), and white crappie \( Pomoxis annularis \) (Neumann and Murphy 1991), 85% of these populations were from Montana. Sixty percent of the Montana populations had mean population \( W_r \) values between 95 and 105. Conversely, 66% of the shovelnose sturgeon populations from states other than Montana had mean population \( W_r \) values between 80 and 90. These data suggest that universal target values may be inappropriate for shovelnose sturgeon.

Numerous researchers have described differences in \( W_r \) values associated with lentic and lotic populations (e.g., Fisher et al. 1996); however, we are unaware of any studies identifying variation in \( W_r \) along a longitudinal scale in lotic ecosystems. The variability between lentic and lotic systems has prompted the development of differing target ranges for \( W_r \). For example, Fisher et al. (1996) found that \( W_r \) values for burbot \( Lota lota \) were consistently lower in lotic ecosystems than in len-
TABLE 1.—Sample size (N; fish ≥ 120 mm), intercept (a), slope (b), and correlation coefficient (r) for regressions of log10 weight (g) on log10 fork length (mm). Mean relative weight (W) values for shovelnose sturgeon length categories (stock to quality, S–Q; quality to preferred, Q–P; preferred to memorable, P–M; and memorable to trophy, M–T) are provided. Numbers in parentheses below the mean are sample size and SD of mean. All regressions were significant at \( P \leq 0.0001 \).

<table>
<thead>
<tr>
<th>State and location</th>
<th>N</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>S–Q</th>
<th>Q–P</th>
<th>P–M</th>
<th>M–T</th>
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<tr>
<td>Illinois, Missouri</td>
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<td></td>
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<td></td>
</tr>
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<td>30</td>
<td>-5.392</td>
<td>2.941</td>
<td>0.91</td>
<td>122</td>
<td>86</td>
<td>(15, 32.17)</td>
<td>(15, 21.38)</td>
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<td>72</td>
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<td>96</td>
<td>99</td>
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<td>Mississippi River, Pool 13</td>
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<td>75</td>
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<td>-5.793</td>
<td>3.119</td>
<td>0.99</td>
<td>91</td>
<td>83</td>
<td>(5, 11.07)</td>
<td>(29, 5.94)</td>
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<td>Kansas River, Ogden</td>
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<td>-6.619</td>
<td>3.426</td>
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<td>79</td>
<td>82</td>
<td>(14, 20.44)</td>
<td>(206, 6.81)</td>
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<td>Kansas, Missouri</td>
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<td>Missouri River, St. Joseph</td>
<td>47</td>
<td>-5.972</td>
<td>3.174</td>
<td>0.99</td>
<td>78</td>
<td>80</td>
<td>(5, 7.29)</td>
<td>(21, 11.93)</td>
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<td>81</td>
<td>81</td>
<td>(27, 10.59)</td>
<td>(23, 6.52)</td>
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<tr>
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<td>2.824</td>
<td>0.96</td>
<td>151</td>
<td>106</td>
<td>(8, 23.87)</td>
<td>(27, 10.70)</td>
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<td></td>
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<tr>
<td>Big Muddy River</td>
<td>357</td>
<td>-6.206</td>
<td>3.294</td>
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<td>95</td>
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<td>(212, 11.02)</td>
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<td>-6.432</td>
<td>3.380</td>
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<td>96</td>
<td>96</td>
<td>(15, 21.38)</td>
<td>(206, 6.81)</td>
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<td>3.267</td>
<td>0.95</td>
<td>96</td>
<td>105</td>
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<td>(5, 10.66)</td>
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<td>Missouri River, Whiteside</td>
<td>69</td>
<td>-6.246</td>
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<td>126</td>
<td>103</td>
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<td>Missouri River, Stafford Ferry</td>
<td>441</td>
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<td>95</td>
<td>(47, 13.16)</td>
<td>(51, 12.79)</td>
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<td>Missouri River, Robinson Bridge</td>
<td>1,922</td>
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<td>3.394</td>
<td>0.97</td>
<td>105</td>
<td>92</td>
<td>(16, 14.54)</td>
<td>(256, 11.04)</td>
</tr>
<tr>
<td>Missouri River, Fort Peck</td>
<td>732</td>
<td>-6.290</td>
<td>3.317</td>
<td>0.96</td>
<td>91</td>
<td>93</td>
<td>(11, 7.66)</td>
<td>(599, 14.45)</td>
</tr>
<tr>
<td>Missouri River, Milk River confluence</td>
<td>168</td>
<td>-5.874</td>
<td>3.159</td>
<td>0.96</td>
<td>93</td>
<td>88</td>
<td>(454, 9.00)</td>
<td>(1,004, 11.19)</td>
</tr>
<tr>
<td>Missouri River, Wolf Point</td>
<td>112</td>
<td>-6.084</td>
<td>3.229</td>
<td>0.97</td>
<td>89</td>
<td>84</td>
<td>(12, 5.81)</td>
<td>(46, 7.95)</td>
</tr>
<tr>
<td>Missouri River, Red Water River confluence</td>
<td>58</td>
<td>-6.041</td>
<td>3.229</td>
<td>0.98</td>
<td>82</td>
<td>89</td>
<td>(25, 9.28)</td>
<td>(14, 8.63)</td>
</tr>
<tr>
<td>Missouri and Yellowstone River confluence</td>
<td>782</td>
<td>-6.449</td>
<td>3.382</td>
<td>0.98</td>
<td>90</td>
<td>90</td>
<td>a</td>
<td>(590, 12.33)</td>
</tr>
<tr>
<td>Powder River</td>
<td>70</td>
<td>-7.540</td>
<td>3.768</td>
<td>0.95</td>
<td>96</td>
<td>101</td>
<td>a</td>
<td>(599, 14.45)</td>
</tr>
<tr>
<td>Tongue River</td>
<td>272</td>
<td>-6.706</td>
<td>3.474</td>
<td>0.93</td>
<td>96</td>
<td>100</td>
<td>a</td>
<td>(599, 14.45)</td>
</tr>
<tr>
<td>Yellowstone River, Highway 23 bridge</td>
<td>133</td>
<td>-6.736</td>
<td>3.478</td>
<td>0.98</td>
<td>110</td>
<td>90</td>
<td>a</td>
<td>(178, 10.59)</td>
</tr>
<tr>
<td>Yellowstone River, Forsyth</td>
<td>187</td>
<td>-6.988</td>
<td>3.574</td>
<td>0.97</td>
<td>96</td>
<td>98</td>
<td>(21, 11.99)</td>
<td>(48, 11.81)</td>
</tr>
<tr>
<td>Yellowstone River, Miles City</td>
<td>60</td>
<td>-8.211</td>
<td>4.000</td>
<td>0.97</td>
<td>93</td>
<td>96</td>
<td>(12, 7.05)</td>
<td>(101, 11.73)</td>
</tr>
<tr>
<td>Yellowstone River, Powder River confluence</td>
<td>588</td>
<td>-7.189</td>
<td>3.643</td>
<td>0.96</td>
<td>91</td>
<td>93</td>
<td>(8, 10.11)</td>
<td>(36, 9.85)</td>
</tr>
<tr>
<td>Yellowstone River, Glendive</td>
<td>853</td>
<td>-6.821</td>
<td>3.509</td>
<td>0.98</td>
<td>89</td>
<td>93</td>
<td>(37, 9.35)</td>
<td>(400, 11.07)</td>
</tr>
</tbody>
</table>

Mean \( W \) values for length categories:

- \( S–Q \)
- \( Q–P \)
- \( P–M \)
- \( M–T \)
Table 1.—Continued.

<table>
<thead>
<tr>
<th>State and location</th>
<th>N</th>
<th>a</th>
<th>b</th>
<th>r</th>
<th>S-Q</th>
<th>Q-P</th>
<th>P-M</th>
<th>M-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellowstone River, above Intake</td>
<td>565</td>
<td>-6.483</td>
<td>3.399</td>
<td>0.97</td>
<td>(6, 18.86)</td>
<td>(60, 11.25)</td>
<td>(334, 10.39)</td>
<td></td>
</tr>
<tr>
<td>Yellowstone River, below Intake</td>
<td>1,158</td>
<td>-6.349</td>
<td>3.337</td>
<td>0.99</td>
<td>(29, 18.44)</td>
<td>(95, 10.86)</td>
<td>(337, 8.27)</td>
<td>(582, 9.67)</td>
</tr>
<tr>
<td>Nebraska Platte River</td>
<td>117</td>
<td>-6.001</td>
<td>3.203</td>
<td>0.94</td>
<td>(13, 12.09)</td>
<td>(97, 7.99)</td>
<td>(7, 3.83)</td>
<td></td>
</tr>
<tr>
<td>North Dakota Lake Oahe</td>
<td>91</td>
<td>-4.579</td>
<td>2.669</td>
<td>0.88</td>
<td>(38, 9.41)</td>
<td>(53, 8.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Sakakawea</td>
<td>45</td>
<td>-5.826</td>
<td>3.134</td>
<td>0.98</td>
<td>(15, 21.00)</td>
<td>(23, 23.17)</td>
<td>(12, 6.21)</td>
<td>(21, 11.19)</td>
</tr>
<tr>
<td>Wisconsin Chippewa River</td>
<td>1,085</td>
<td>-4.572</td>
<td>3.381</td>
<td>0.85</td>
<td>81</td>
<td>89</td>
<td>83</td>
<td></td>
</tr>
</tbody>
</table>

*a* Represents less than five individuals.

**Figure 1.**—Relationship between mean population relative weight of memorable length to trophy length ($W_{M-T}$) and relative stock density of memorable length to trophy length (RSD M–T) for shovelnose sturgeon.

Ecosystems. Consequently, they suggested target values of 95–105 for lentic populations and 75–85 for lotic ecosystems. Kruse and Hubert (1997) found similar relations with cutthroat trout *Oncorhynchus clarki* but elected to develop separate equations for lotic and lentic populations rather than propose different target values. Similarly, Willis et al. (1991) identified a broad distribution in body condition of yellow perch *Perca flavescens* and suggested that the variability in condition reinforces the concept that universal target values may be inappropriate in many situations. Therefore, we suggest target values of 95–105 for Montana populations and 80–90 for other populations of shovelnose sturgeon.

We recommend the following length categories be used when calculating stock density indices for shovelnose sturgeon: stock, 250 mm (10 in); quality, 380 mm (15 in); preferred, 510 mm (20 in); memorable, 640 mm (25 in); and trophy, 810 mm (32 in). All populations had PSD values greater than 79, except for a Mississippi River population (Table 1). The number of populations with high PSD values is not surprising because shovelnose sturgeon are typically collected with large-mesh (250-mm-bar-measure) nets. The primary gears used to sample shovelnose sturgeon for this study were either gill or trammel nets. Thus, stock-length to quality-length shovelnose sturgeon were less likely to be sampled.

Stock density indices are used to quantify size structure of a fish population. It is debatable whether such indices reflect growth, mortality, and recruitment. However, Willis (1989) and Guy and Willis (1995) documented relations between PSD and growth. Gabelhouse (1984a) suggested that low PSD values may reflect poor habitat, overharvest, and reduced food supply. The relation between PSD and $W_r$ against growth are probably one of cause and effect (Willis 1989; Gabelhouse 1991; Guy and Willis 1995). We were unable to...
Relations between mean relative weight ($W_r$) by length category—stock to quality (S–Q), quality to preferred (Q–P), preferred to memorable (P–M), and memorable to trophy (M–T)—for shovelnose sturgeon. Bars represent ±SE. Data points without a letter in common represent significant differences ($\alpha = 0.05$) among length categories.

Muscle growth data for shovelnose sturgeon used in the development of the $W_r$ equation. Thus, we used relations between $W_r$ and PSD to evaluate the utility of these management tools for large-river species.

Mean population $W_r$ values were positively correlated ($r = 0.47$, $P = 0.033$) with PSD with a curvilinear regression model. Thus, populations with higher PSD values had higher mean condition values. Similar results have been reported for other species, such as northern pike ($Esox lucius$) (Willis and Scalet 1989), crappies ($Pomoxis$ spp.) (Gabelhouse 1984b; Guy and Willis 1995), and brook trout ($Salvelinus fontinalis$) (Johnson et al. 1992).

We also plotted relations between incremental RSD and $W_r$. The best relationship occurred between $W_r$ of M–T and RSD of M–T ($r = 0.68$, $P = 0.0001$; Figure 1). In addition, we found differences among incremental $W_r$ values. Mean $W_r$ generally decreased as fish attained greater lengths. Stock-length to quality-length shovelnose sturgeon had significantly greater $W_r$ values than P–M and M–T fish ($P \leq 0.05$; Figure 2). These data illustrate the importance of size-specific $W_r$ analyses.

Although the relation between PSD and $W_r$ probably will vary with growth, we surmise that these indices will be useful in assessing shovelnose sturgeon size structure and condition. At best, these indices will provide information on growth, mortality, and recruitment of shovelnose sturgeon in large-river ecosystems. We encourage fisheries biologists to develop $W_r$ equations and size structure categories for large-river species so that these tools will be available for condition and size structure assessment.

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References


