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Abstract

Reproduction by stocked Lake Trout Salvelinus namaycush is generally estimated as the relative abundance of fry, that is, catch per unit effort in emergent fry traps and in beam trawls, but these estimates have high variance due to spatially heterogeneous distributions of fry. We used calcein, which produces a fluorescent mark in calcified structures, to batch-mark fry and generate a mark–recapture estimate of fry abundance on a small, shallow spawning reef. Eggs collected from feral Lake Trout in Lake Champlain, Vermont were reared at ambient lake temperatures, and fry were marked 7 d after hatching. Fry were immersed in a salt solution for osmotic induction and then placed for 4 min in a calcein solution. After marking, 18,000 fry were released on a spawning reef, and 2,000 fry were maintained in the hatchery. Wild-caught fry and hatchery fry were checked for marks every 2–9 d. Mark clarity was highest in the mandible and tail rays. Marks may have faded, but they did not disappear: marks were visible in the mandible in 100% of hatchery fry after 68 d. An average of 37% of wild-caught fry had marks, yielding a Chapman population estimate (± SD) of 47,486 ± 2,301. The mark–recapture estimate was within the range of fry abundance estimated over 6 years based on egg density data and estimates of hatching success but was substantially higher than estimated for the same year-class. This work supports prior estimates of fry abundance and provides a potential method for assessing fry abundance on deep reefs and the success of fry stocking.

Population abundance estimates of fishes are largely based on catch-per-unit-effort (CPUE) to measure relative abundance over time or space. Actual population estimates are rare except when mark–recapture studies are possible, or when bodies of water can be fully sampled (such as with toxicants or draining). Lake Trout Salvelinus namaycush early life stage sampling is one such case: eggs can be sampled quantitatively to achieve estimates of egg density (Perkins and Krueger 1994a, 1994b), but fry sampling with emergent fry traps and with beam trawling for postemergent fry yield only relative CPUE data (e.g., Bronte et al. 1995; Marsden et al. 2005). An understanding of the relationship between CPUE and actual fry estimates would be valuable for evaluating mortality during egg incubation, comparing fry densities among different sites and lakes, estimating

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270
fry densities at sites that are too deep for fry traps (Janssen et al. 2007), and evaluating new sampling methods (e.g., Riley et al. 2011). Mark-recapture methods may provide an opportunity to convert CPUE into a more meaningful measure of population abundance.

Mark-recapture of newly hatched larval fishes requires using a noninvasive mark that will not cause high mortality. Ideally, the mark could be batch-applied by immersion, and the mark could be read externally without killing the fish, which would reduce processing time and costs. Calcein as a chemical fish mark offers two distinct advantages over other chemicals such as oxytetracycline (Brooks et al. 1994). Immersion time in the chemical is short (minutes rather than hours), which minimizes stress, and the mark is visible both externally (scales and fin rays) and internally (bones and otoliths) under fluorescent light, so that fish do not need to be killed for mark detection. The purposes of this study were to evaluate calcein as a mark for newly hatched Lake Trout fry and to use calcein in a mark–recapture study of fry emerging on a reef that has been historically sampled with emergent fry traps (Ellrott and Marsden 2005; Marsden et al. 2005; Riley et al. 2009).

METHODS

Eggs were collected from Lake Trout adults stocked as yearlings in Lake Champlain and were reared at ambient lake temperatures at the Ed Weed Fish Culture Station to time the emergence of hatchery fry to coincide with hatching of eggs in the lake. On March 29, 2007, five batches of approximately 4,000 newly hatched fry each (7 d posthatch) were marked. Osmotic induction (Mohler 2003) was used to enhance fry uptake of calcein. Each batch of fry was placed in a fine-mesh open bag and immersed for 3.5 min in an 18-L bucket containing a 50 g/L solution of NaCl to establish an osmotic potential. The bag of fry was then placed briefly on absorbent paper to remove excess salt solution and transferred to a 5 g/L solution of calcein (SE-MARK; Western Chemical, Inc., Ferndale, Washington) for 4–5 min. In each solution, fry in the bag were gently agitated to ensure complete exposure to the NaCl or calcein, taking care to avoid damage to the fry. The bag was transferred to a container of freshwater to rinse off excess calcein, after which the fry were returned to a rearing tank. Ten to 20 fry were removed from each batch for immediate examination of the mark. The rearing tank was uncovered, exposing it to indirect sunlight and to indirect fluorescent light inside the hatchery building.

On April 3, approximately 18,000 of the marked fry were placed into four plastic bags, each containing approximately 16 L of water and supplemental oxygen. The bags were taken immediately to a spawning site less than 1 km from the hatchery; this site, consisting of the cobble base of an emergent breakwall, has been intensively sampled for eggs and fry since 2000 (Ellrott and Marsden 2004; Marsden et al. 2005). A diver opened each bag underwater, adjacent to the substrate, at a depth of 3–4 m, and swam over the entire area of spawning substrate while allowing fry to disperse. Fry were observed to move rapidly downward into the substrate. A fifth bag, containing approximately 2,000 fry, was transported to the lake with the other bags but then was returned to the hatchery to assess mortality due to handling and for periodic examination of mark retention during development.

After the fry were released, 10 emergent fry traps (Marsden et al. 1988, 2005) were deployed over the area of substrate where the fry had been released. The traps are rigid metal mesh pyramids, 51 cm square, open at the base, with a capture bottle at the top; an inverted funnel in the capture bottle prevents most fry from exiting the trap. Five more traps were added on April 22 (19 d postrelease). Traps were checked for fry every 2 to 7 d until June 5 (63 d postrelease); all fry collected were frozen immediately in a small volume of water until they could be evaluated for the presence of a mark. Usually, high numbers of fry (>75) in a few traps resulted in the death of a few fry. The 2,000 marked fry that had been returned to the hatchery were held until June 5, when all fry had completed yolk sac adsorption and begun feeding. A small sample of fry (21–41 fry) was collected from the hatchery every 5 to 9 d, including most of the days on which fry traps were checked; these fry were also frozen until examination for marks.

In the laboratory, all fry were thawed and examined using a SE-MARK detector (Western Chemical, Inc.); the detector emits a 490-nm-wavelength light that causes calcein to reflect yellow-green fluorescence when present. Fry were examined at 3× magnification in low ambient light conditions to enhance mark visibility. Marks were initially viewed by three individuals to evaluate location and brightness categories of the marks. Subsequently, a single individual recorded visibility of the mark in all fry as absent, faint, or bright in the mandible, caudal peduncle, tail rays, and fin rays.

We used a Chapman mark–recapture estimator (Chapman 1951; cited by Robson and Regier 1964) to estimate size of the Lake Trout fry population on the spawning reef. For purposes of the estimate, only fry with a detectable mark in the mandible or tail rays (see Results) were considered to be recaptures. Fry population estimates were compared with estimates derived from egg collections in 2002, 2003, 2005, 2006, 2011, and 2012 (Marsden et al. 2005; Riley et al. 2011; J. E. Marsden unpublished data). Egg densities per square meter were extrapolated to the area of the reef (53 m²; Marsden et al. 2005) and then multiplied by estimates of egg survival made in 2002 and 2003 (Marsden et al. 2005). Egg survival estimates ranged from 1.1% to 18.2%; for reasons explained below, we used the higher estimate in our calculations.

RESULTS

Fry did not show signs of distress during or after marking with calcein; mortality of marked fry in the hatchery through emergence was less than 5% and did not differ from mortality of unmarked fry from the same feral broodstock reared in the same
TABLE 1. Number and percent of hatchery Lake Trout fry \( (N = 262) \) in which calcein marks were observed in four parts of the body. Fins = rays in any of the paired or medial fins other than tail. Not all mark locations could be scored for some fish due to physical damage to the specimens during thawing.

<table>
<thead>
<tr>
<th>Mark intensity</th>
<th>Mandible</th>
<th>Caudal peduncle</th>
<th>Tail rays</th>
<th>Fin rays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bright</td>
<td>157</td>
<td>29</td>
<td>72</td>
<td>4</td>
</tr>
<tr>
<td>Faint</td>
<td>97</td>
<td>128</td>
<td>149</td>
<td>55</td>
</tr>
<tr>
<td>No mark</td>
<td>6</td>
<td>105</td>
<td>32</td>
<td>200</td>
</tr>
<tr>
<td>% bright</td>
<td>60.4</td>
<td>11.0</td>
<td>28.5</td>
<td>1.5</td>
</tr>
<tr>
<td>% faint</td>
<td>37.3</td>
<td>48.9</td>
<td>58.9</td>
<td>21.2</td>
</tr>
<tr>
<td>% no mark</td>
<td>2.3</td>
<td>40.1</td>
<td>12.7</td>
<td>77.2</td>
</tr>
</tbody>
</table>

hatchery for stocking. Calcein immersion produced externally visible marks on Lake Trout fry; the proportion of fry examined that had a mark in a given location did not decline over the 68-day observation period, indicating that the marks did not fade to nonrecognition over that period. Marks were most readily seen in the mandible of Lake Trout fry: 97% of fry were observed to have a faint (37%) or bright (60%) mark on the mandible (Table 1; Figure 1). Eighty-seven percent of fry had a mark visible in the tail rays, 60% in the caudal peduncle, and 23% in the fin rays. The majority of marks in locations other than the mandible were faint (21–57%, depending on location); only 1.5–28% of fry had bright marks in these other locations. Bright marks were unmistakable; faint marks were less obvious, and care was taken to avoid recording reflections of the fluorescent light sources from wet fish that resembled faint marks.

A total of 699 wild fry were collected in fry traps. Of these, 643 were examined for marks; the remaining fry were dead when captured and were slightly decomposed, so that the tail rays were not intact. On the basis of the hatchery fry results, and to ensure certainty in counting marked fry, we examined only the mandible and tail rays. Fifteen fry (2.3%) had a bright mark in the mandible and seven (1%) had a bright mark in the tail rays. Faint marks were observed in the mandible of 257 (40%) fry and in the tail rays of 165 (26%) fry. If a bright mark was seen in the tail, there was also a bright mark in the mandible; one of the fry with a bright mark in the mandible had no mark in the tail rays, but the remainder had bright (7) or faint (7) mark in the tail rays.

The proportion of wild-caught fry that had a mark was reasonably consistent throughout the capture period, averaging 37%, except on April 22 and 24 (19 and 21 d postrelease), when 88% and 56% of fry had marks, respectively (Figure 2). The population of fry on the spawning reef estimated using all the fry with any mark intensity in either location was 45,949 \( \pm 2,198 \) (\( \pm SD \)) (Figure 3). Fry population estimates from 2001 to 2012 generated from egg densities and an estimated 18.2% survival...
FIGURE 2. Percentage of Lake Trout fry collected on the Grand Isle breakwall, Lake Champlain, with a faint or bright calcein mark in either the mandible or tail rays, 15–68 d after marking and release on April 3.

to hatch ranged from 18,336 ± 12,337 to 92,823 ± 15,993 fry; the estimate for 2007 was 18,366 ± 12,337 (Figure 3).

DISCUSSION

We used calcein marking in a novel application to estimate population size of preemergent lake trout fry on a spawning reef in Lake Champlain. Calcein provides a low-stress mark procedure for very young fish, easy to apply and easy to read. Marking success was high, and mark reading was straightforward. Although we did not need to keep marked fish alive, marks could certainly have been read without killing the fry. Overall, marks were seen most often in the mandible and least often in the fin rays. The high proportion of faint marks we saw is probably a consequence of fry not all having equal or sufficient exposure to the calcein solution. This problem should be readily resolved by marking smaller batches of fry, or by using a broad, shallow tray for immersion, for greater individual exposure. Fading may also have been a consequence of freezing and storing the fry; although we were generally advised that this process would not affect the marks, the effect of freezing should be quantitatively evaluated.

The Chapman estimate of fry abundance on the spawning reef in 2007 fell within the range of annual estimates from 2001 to 2012; it was, however, more than twice as high as the estimate generated for the 2007 year-class from egg collections in 2006 (Figure 3). The assumptions of Chapman mark–recapture estimation were largely met. Mortality due to starvation does not occur while fry are feeding from their yolk sac. The population of fry on the reef is closed (no emigration, immigration, or births); fry reside within the spawning reef until emergence. Loss due to predation probably occurred, but it is reasonable to assume predation was equal among marked and unmarked fry, as was found for Atlantic salmon by Mohler et al. (2002). Marked fry were distributed widely around the reef to maximize mixing with unmarked fry. The ratio of marked to unmarked fry remained fairly constant during the 7 weeks of fry trapping, except on April 22 and 24, when the ratio was higher. If marked and unmarked fry had different susceptibility to capture, as a consequence of disorientation or other altered behavior due to handling and release, we would expect fry to have acclimatized to the reef by the time traps were first checked over 2 weeks later. Marked fry in the hatchery did not exhibit unusual mortality. Recalculating the Chapman estimate based only on collections after May 1 (28 d postrelease), when the proportion of marked fry had stabilized, yielded an estimate of 48,891 ± 2,440, only 6% larger than the original estimate.

The degree to which the remaining assumption, that marks were not lost, was met is not entirely certain. Calcein has the disadvantage that the marks may fade in direct and artificial sunlight (Bashey 2004; Honeyfield et al. 2008; Elle et al. 2010; Hill and Quesada 2010). Mark loss in these studies primarily occurred in fish held in shallow water, such as hatchery tanks and raceways. We did not see a reduction in the proportion of marked fry in the hatchery over the 68 d of the study; however, fry were exposed only to filtered sunlight and dim fluorescent light. Lake trout fry in the wild are unlikely to be exposed to direct sunlight for long periods, as lake trout are photophobic until ages 4 to 6 weeks, remaining in dark, interstitial spaces during daylight hours (Baird and Krueger 2000). Soon after emergence they descend to deeper water, where sunlight is filtered, and continue to occupy deeper water into the fall (Bronte et al. 1995). Loss of mark intensity is also related to growth, as the marks become more diffuse with increasing size of marked bones and fin rays (Negus and Tureson 2004). Negus and Tureson did not see mark intensity fade until after 12 months, when rainbow trout had reached smolt size; because we examined fry within 9 weeks of marking, mark intensity was unlikely to have faded due to growth hin that period. Nevertheless, we did observe a larger proportion of fry with faint marks relative to bright marks in the wild-caught fry than in hatchery fry: the proportion of total fry caught with bright marks each day declined steadily from 40% (mandible) and 33% (tail rays) to zero by May 21. These data suggest that marks may have faded over the period of fry emergence. However, the proportion of total fry captured that had a mark did not change after May 8, suggesting that marks did not disappear. If marks faded to the point that they could not be recognized, our data would have overestimated fry abundance.

Estimates of fry abundance from egg density data could also be flawed. Density of eggs in individual egg bags is highly variable (Riley et al. 2011), as seen particularly in 2002 (Figure 3). Marsden et al. (2005) estimated egg survival by trapping eggs in egg bags that were covered after spawning and then counting the hatched fry in spring. This method yielded egg survival estimates from Grand Isle, Lake Champlain, of 1.1% and 18.2% in 2001–02 and 2002–03, respectively, and 1.8% at Whallon Bay in Lake Champlain. The lowest of these estimates is probably recognized, our data would have overestimated fry abundance.

Additional density estimates at Grand Isle contained so many eggs that close proximity probably caused fungus-related mortality (Ellrott and Marsden 2004). For fry densities in 2007 to be equivalent to our mark–recapture population estimate, hatching success would need to approach 50%. However, this may not be unrealistic. Once eggs are entrained into interstices, the mortality from predation by interstitial species—sculpins (Cottus spp.) and crayfish (Orconectes spp.)—and other sources further reduce hatching success. Densities of both species on Lake Trout spawning reefs in Lake Champlain are low compared with sites in the Great Lakes (Marsden et al. 2005). Perkins and Krueger (1994b) determined that egg loss due to mortality and dislodgment overwinter in uncovered egg bags was 1.5–5.4%, compared with 8.4–15.6% in covered bags and 14.7–39% in incubators. Egg seedling experiments in egg bags showed that egg retention is higher at Grand Isle than at sites in Lake Michigan (Claramunt et al. 2005; Fitzsimons et al. 2007). Modeling of overwinter egg mortality under a range of predator density
MARK–RECAPTURE OF LAKE TROUT FRY

scenarios suggests that 50% survival is highly probable, given the predator densities at the Grand Isle site (J. W. Riley, unpublished data). The fact that the mark–recapture estimates were within the range of independent estimates of the fry populations at this site, extrapolated from egg densities, lends support to the probability that this estimate is robust.

In summary, this study confirms the usefulness of calcein for batch-marking very early stages of salmonid fry and its facility for readily observing marks in live or dead fry. Marking effectiveness can be improved by ensuring better exposure of all fry to the dye bath. Future studies should examine the extent to which marks may have faded in the wild. Despite these limitations, our estimates of fry population confirm that prior estimates derived from egg density data and estimates of survival to hatch are robust. Mark–recapture estimates would be of particular value for assessing fry production on deep reefs, such as the Mid-Lake Reef Complex in Lake Michigan, where fry collection is limited to sampling by remotely operated vehicles, and density estimates are infeasible (Janssen et al. 2007; Riley et al. 2011). Calcein would also be valuable for assessing the strategy of stocking Lake Trout at the early fry stage, by enabling assessment of survival within at least the first year of life.

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