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Predation by Captive Wild Brook Trout on Calcein-Marked versus Nonmarked Atlantic Salmon Fry

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Abstract.—Juvenile Atlantic salmon *Salmo salar* and other fish species marked with the fluorochrome dye calcein exhibit a green fluorescence in fin rays and other calcified structures under specific optical conditions. To determine whether brook trout *Salvelinus fontinalis* would preferentially prey upon calcein-marked individuals, we introduced calcein-marked and nonmarked Atlantic salmon fry simultaneously to captive wild brook trout in four controlled indoor raceway trials. Each trial consisted of 2 brook trout and 100 each of calcein-marked and nonmarked Atlantic salmon fry; no individuals were used in more than one trial. At the termination of each 3-d trial, predators were removed from raceways, and surviving Atlantic salmon fry were examined with a calcein detection device to tally numbers of marked and nonmarked individuals. In individual trials, 2 brook trout consumed between 20 and 99 Atlantic salmon fry over a 3-d period (10–49% of available prey). Replicated goodness-of-fit (*G*-statistic) analysis showed the number of calcein-marked and nonmarked Atlantic salmon fry eaten by captive wild brook trout did not fit the expected 1:1 ratio among all the trials ($P < 0.05$). However, pooled data from the four trials showed that the numbers of marked and nonmarked Atlantic salmon fry eaten by predators were nearly equal at 139 and 133 individuals, respectively. Correspondingly, *G*-statistic analysis of pooled data showed that numbers of marked and nonmarked Atlantic salmon fry eaten by brook trout did fit the expected 1:1 ratio. Overall, we found no conclusive evidence that calcein-marked Atlantic salmon fry were preferentially preyed upon by captive wild brook trout. Our experiment also demonstrates the utility of using calcein to mass mark Atlantic salmon fry as a means of performing evaluations that were not practical with previous tagging and marking methods.

The U.S. Fish and Wildlife Service (USFWS) and its state fishery agency partners in the northeastern United States rely largely on fry stocking in restoration programs for Atlantic salmon *Salmo salar*. Fry marking techniques are necessary to assess the effectiveness of such a management strategy. Since 1995, the USFWS Northeast Fishery Center, Lamar, Pennsylvania (NEFC), has experimented with immersing batches of nonfeeding Atlantic salmon fry in calcein solutions to produce a fluorescent mark in fin tissues (Mohler 1997). A nonlethal means of detecting calcein marks has also been devised at NEFC. The calcein detection device (patent applied for) processes white light via numerous glass filters and allows the operator to immediately discern marked and nonmarked fish by the presence or absence of a visible green fluorescence. The device permits rapid macroscopic examination of live or dead specimens and has been constructed as both a hand-held, battery-powered unit and a 110-V, benchtop unit. These devices have been used successfully to verify the presence of calcein marks in Atlantic salmon older than 2 years post-immersion (P. Farrell, USFWS, personal communication).

Calcein marks fluoresce a distinct green color visible to humans when viewed with the proper detection device. However, the sensory capabilities of fishes can differ dramatically from our own (Noakes and Baylis 1990); therefore, it is not known whether a typical fish predator such as brook trout *Salvelinus fontinalis* would preferentially select calcein-marked fry over nonmarked fry. Kunz (1987) reported that brown trout *Salmo trutta* and Atlantic salmon possess cones that are sensitive to ultraviolet (UV) radiation in the retina. Additionally, Hawryshyn and Beauchamp (1985) reported UV photosensitivity in goldfish *Carassius auratus*. When excited at the proper wavelength, calcein emits a visible fluorescence in the range of 500–530 nm. This is somewhat higher than UV wavelengths (260–400 nm), but it is prudent to investigate whether selective predation will occur prior to embarking on any fishery program that promotes use of calcein-marked fish. If one of the purposes of tagging and releasing fish is to evaluate survival, then tags that attract predators are counterproductive. Maynard et al. (1996) found that twice as many marked as unmarked age-0 steelhead *Oncorhynchus mykiss* were eaten by a visual predator during a study performed in circular tanks, but no marks employed in that study were fluorescent in nature. According to Phinney and Matthews (1969), fluorescent-pigment marking had no ill effect on the survival of age-0 coho

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salmon *Oncorhynchus kisutch* over a 6-month period in a relatively natural environment. In addition, Malone et al. (1999) found that visual implant fluorescent elastomer tags did not increase the susceptibility of blackeye gobies *Coryphopterus nicholsi* and bluebanded gobies *Lythrypnus dalli* to predation by kelp bass *Paralabrax clathratus*. However, in discussing the use of dyes, paints, and stains to mark intertidal fish, Moring (1990) stated that even though dye injection can be effective, virtually no information is available about how such marks might affect susceptibility to predation, and small pilot studies should be conducted to assure that the stain does not attract predators.

We selected brook trout as a predator due to their presence in most tributaries where Atlantic salmon fry are stocked for both the Connecticut River and Maine rivers restoration programs (J. McKeon and J. Marancik, USFWS, personal communication). A study by Henderson (2000) found that brook trout consumed more fry in both natural and artificial streams than did brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*. In addition, Legault and Lalancette (1987) reported predation of newly planted Atlantic salmon fry by brook trout in a stream in France.

Currently, calcein is not approved by the U.S. Food and Drug Administration for use in the United States on potential food fish; therefore, experimentation was performed in indoor raceways where escape of calcein-marked fish was prevented. We provide data concerning the efficacy of using calcein to batch-mark fish and document the first attempt to determine whether calcein-marked fish are more susceptible to predation. Our experiment also demonstrates the utility of using calcein to mass-mark Atlantic salmon fry as a means of performing evaluations that were not practical with existing tagging and marking methods.

Methods

In July and August 2000, we introduced calcein-marked and nonmarked Atlantic salmon fry simultaneously to captive wild brook trout as predators in four controlled indoor raceway trials to determine whether differential predation occurred upon the two treatment groups. Each trial consisted of 2 brook trout and 100 each of calcein-marked and nonmarked Atlantic salmon fry. Four preplanned trials took place under the conditions described below.

Experimental units.—Trials were conducted in indoor, light-gray concrete raceways. Each raceway was 8.4 m × 0.8 m with a water depth of 0.38 m. Four pieces of 20.3-cm-diameter, 30-cm-long PVC pipe, which had been cut in half longitudinally, were placed on the bottom of each raceway to provide some refuge for fry as well as cover for predators (Figure 1). Flow was approximately 57 L/min in all raceways and temperature was ambient (12–15°C). Raceways were screened on all sides with opaque black plastic sheeting to minimize human disturbance during trials. A small slit opening was made in the opaque plastic sheeting at the end of each raceway so periodic behavioral observations of predators and prey could be performed without eliciting fright reactions (Noakes and Baylis 1990). Black plastic screens with a mesh opening size of 2.5–5.0 cm were used to cover raceways to prevent escape of predators, while prey were contained within experimental units by a perforated aluminum screen at the end of each raceway. Containment sections on the downstream side of each raceway screen were examined three times daily for any fry that may have escaped the experiment. Lighting was natural photoperiod and consisted of windows at the head of each raceway with no auxiliary overhead lighting. A trial consisted of one raceway containing 2 pred-

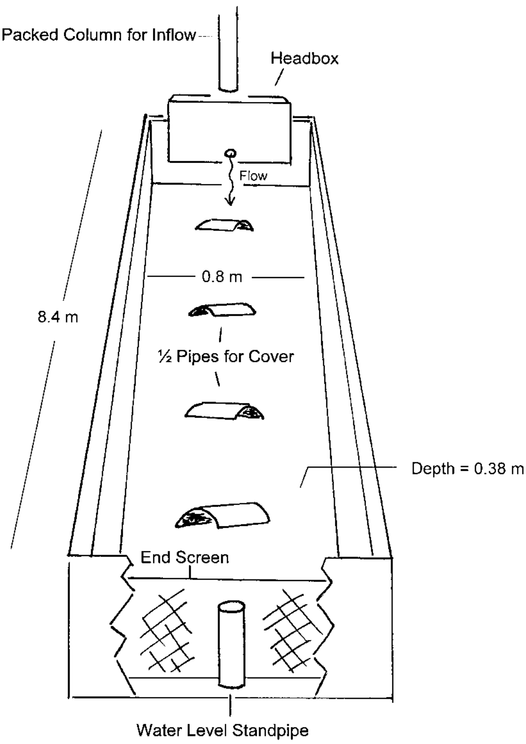

FIGURE 1.—Schematic drawing of the raceway setup used in predation trials.

ators and 200 prey (100 marked, 100 unmarked) for 3 consecutive days.

Predators.—Wild brook trout were collected from a local tributary by electrofishing, and two individuals were placed into each of four raceways as predators. Total lengths of predators ranged from 19 to 29 cm, and total weights ranged from 86 to 246 g. All predators were naive to any previous experimentation or exposure to Atlantic salmon fry and were acclimated to their respective raceways for 3 d prior to introduction of prey. At the termination of each 3-d trial, predators were removed and one from each trial was randomly selected for stomach content analysis to verify consumption of prey items. Stomach contents were scanned with the calcein detector to determine whether calcein marks could be seen in partially digested prey.

Prey.—Atlantic salmon fry were hatched at NEFC from eggs of Connecticut River domestic stock obtained from the USFWS, White River National Fish Hatchery in Bethel, Vermont. Marking procedures took place while alevins were in the yolk sac stage at a developmental index of about 85 (85% developed to the point of exogenous feeding) as determined by tracking temperature units during incubation (P. B. Gaston, USFWS, unpublished). Marking procedures began by placing groups of fry into plastic 10-cm-diameter strainers and immersing the strainers in a 5% NaCl bath. After 3.5 min in the NaCl bath, fry were quickly dipped into a freshwater bath to remove excess salt water. The strainers, still containing fry, were then immediately transferred either to a 1% calcein solution for 3.5 min (marked individuals) or to freshwater (nonmarked individuals). Finally, fry were released into culture tanks for continued rearing until trials began. Marked and nonmarked fish were reared separately to the age of 5 months posthatch, when trials began. Evaluation of calcein-marked fry with the detection device showed all fish were well marked at the onset of trials. Mean total lengths (SD) of marked and nonmarked fish were similar at 46.8 (3.6) and 46.5 (3.5) mm, as were mean weights at 0.84 (0.21) and 0.86 (0.20) g, respectively. After predators were acclimated to raceways for 3 d, 200 prey (100 marked and 100 nonmarked) were combined in a container and introduced at the head of each raceway with as little disturbance as possible. Three grams of the same feed used to rear the salmon (Fry Feed Kyowa C-700; Biokyowa, Inc., Chesterfield, Missouri) were added to each raceway daily. Numbers of eaten marked and nonmarked fish in each trial were derived by subtracting the number of survivors in each category from 100 (the number initially stocked) minus recovered mortalities.

Behavioral observations.—Behavorial observations were performed to construct a partial ethogram for both predators and prey. Observations consisted of three 5-min episodes per raceway daily (0800 hours, 1200 hours, and 1530 hours) and were accomplished by slowly lifting the viewing flap at the end of each raceway and documenting the number of times all observed behaviors of predators and prey occurred. Observations were limited to the downstream 2.5 m of each raceway, due to obstruction of view created by raceway cover screens. In all, 10 individual observations were performed for each trial except trial 4, for which only one observation was recorded.

Statistical analysis.—At the termination of each trial, predators were removed, and surviving fry were inspected for the presence or absence of a calcein mark by nonlethal examination with the detection device. Numbers of eaten marked and nonmarked fish were analyzed with replicated goodness-of-fit tests (G-statistic; Sokol and Rohlf 1981) at an alpha level of 0.05, to determine whether predation occurred on calcein-marked and nonmarked Atlantic salmon fry at the expected 1:1 ratio. This statistic allowed inspection of among-trial heterogeneity and its relative influence on an overall test of goodness of fit.

Results

Predation

Because no fry were seen on the downstream side of perforated containment screens during scheduled observation periods, we assumed that all fry unaccounted for at the end of the study were consumed by predators during the 3-d trial period. The G-statistic for total heterogeneity of the experiment (G_T) showed that the proportion of marked and nonmarked fish eaten by the predators did not fit the expected 1:1 ratio ($G_T = 11.9$, $P < 0.05$; Table 1). Significant heterogeneity among the results from the individual replicates existed (G_H; Table 1), and this accounted for the significant deviation observed in the test for total heterogeneity. In particular, more marked fish were preyed upon in trial 1, and more nonmarked fish were preyed upon in trial 4. These two competing results effectively nullified each other, such that the G-statistic for the pooled data (G_P; Table 1) showed that predation on marked and nonmarked fry did not deviate from the expected 1:1 ratio. In trials

TABLE 1.—Numbers of calcein-marked and nonmarked Atlantic salmon fry eaten during 3-d predation trials and results of replicated goodness-of-fit (G-statistic) analysis to determine whether calcein-marked and nonmarked Atlantic salmon fry were preyed upon by captive wild brook trout in a 1:1 ratio. The following abbreviations are used: G_H = heterogeneity of the replicates; G_P = heterogeneity of the pooled data; G_T = total heterogeneity; G_I = heterogeneity of individual trials; and G_C = critical G-values. Asterisks (*) denote data that do not fit the expected 1:1 ratio and are considered heterogeneous ($P < 0.05$). Numbers of uneaten mortalities are in parentheses.

Trial	Fry eaten					
	Marked	Nonmarked	G_I	G_C	df	P-value
1	48 (1)	29 (2)	4.737	3.841	1	0.03*
2	13 (3)	7 (5)	1.828	3.841	1	0.18
3	50 (0)	49 (1)	0.010	3.841	1	0.92
4	28 (1)	48 (5)	5.326	3.841	1	0.02*
			G_P			
Pooled	139 (5)	133 (13)	0.132	3.841	1	0.72
			G_H			
			11.768	7.815	3	0.01*
			G_T			
			11.901	9.488	4	0.02*

2 and 3, about equal numbers of marked and nonmarked fish were preyed upon. Thus, given the lack of any clear and consistent directionality in the replicated results, we conclude there is no evidence to suggest that marked fish are preyed upon more heavily than are nonmarked fish.

Out of 200 fry available to 2 predators per raceway, predation rates ranged from 20 to 99 individuals per raceway over a 3-d period, which represented 10–49% of available prey (Figure 2). Uneaten mortalities recovered from raceways totaled 5 marked and 13 nonmarked fry (Table 1). Marked and nonmarked fry removed from brook trout stomachs could be differentiated with the calcein detector even though they were partially digested. The greatest number of fry recovered from the stomach of a predator was 10.

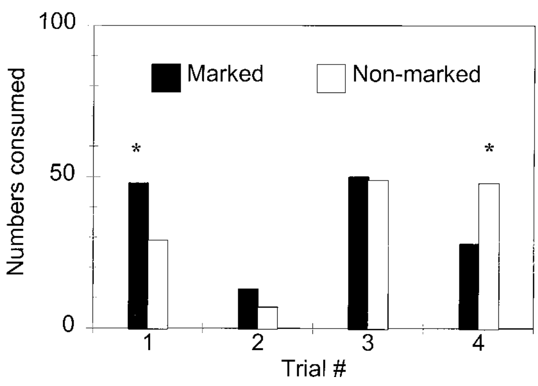

FIGURE 2.—Numbers of calcein-marked and nonmarked Atlantic salmon fry consumed by captive wild brook trout during four 3-d trials. A total of 200 fry (100 marked and 100 nonmarked) were available to predators during each trial. Asterisks indicate that predation by brook trout on marked versus nonmarked fry did not conform to the expected 1:1 ratio ($P < 0.05$).

Behavioral Observations

Predators and prey were seen during 60% and 90%, respectively, of the scheduled observation periods, with the most frequently observed behavior of each group best described as ''stationary finning.'' The least observed behaviors for predators were lunge-attacks at prey and surface swirls. Both predator and prey were observed utilizing the half-pipes, presumably for cover. Aggression between prey individuals was a fairly common behavior and was characterized by one fish darting at and displacing another individual. Prey were observed fleeing from the sudden approach of a predator on numerous occasions.

Discussion

An animal is said to exhibit a preference when it consistently chooses one alternative from those available to it (Dawkins 1969). However, the selectivity of prey by a predator does not occur only in the presence of several types of prey but may also occur among prey of the same species as a result of one or more individual peculiarities (Ivlev 1961). Additionally, preference is an expression of the ease of capture and detectability of the prey by the predator (Paloheimo 1979). We found no consistent selection for marked or nonmarked Atlantic salmon fry by brook trout in our study. Results of individual trials were mixed, since trial-1 brook trout showed a significant preference for calcein-marked fry, while in trial 4 the predators showed a preference for nonmarked fry (Table 1; Figure 2). No obvious explanation for these patterns was found, but Paloheimo (1979) provides a possible solution with the rationale behind his index of food-type preference by a predator: where clumping or clustering of prey occurs, the index may be distorted, showing either more or less preference to the particular prey. We observed prey clustering during our behavioral observations but can only assume that marked and nonmarked salmon were evenly distributed within those clusters. If uneven distributions of prey types occurred within some clusters of salmon fry in trials 1 and 4, this may explain the contradictory results of those trials.

Noakes and Baylis (1990) stated that preferences measured in the laboratory must always be treated with reservation unless and until their ecological relevance is known, that is, other physical and environmental factors might influence these fish in nature. The concrete raceways used in our trials were dissimilar to a natural stream in many ways. However, because detection of the calcein mark is a light-induced optical phenomenon, we designed the experiment so that only natural lighting was available. No light intensity measurements were taken in the study, but during daylight hours sufficient natural illumination was available to easily view predators and prey via the slit openings previously described. Our experimental design also provided high encounter rates by using prey densities exceeding those which normally occur in the wild. Furnished with ample prey, predators had the opportunity to be selective in capturing marked or nonmarked salmon fry. Use of this experimental design is supported by the work of Ivlev (1961), who states that equal opportunities available to the predator, accompanied by a decrease in the predator's need for food, result in an increased electivity for preferred forms and a decrease in those avoided. Therefore, limiting predator–prey encounters by decreasing numbers of prey to some low level may serve to conceal any real preference. This was demonstrated by Werner and Hall (1974) with bluegill *Lepomis macrochirus*, where various size-classes of *Daphnia magna* were used as prey at various densities. Results showed bluegills were nonselective and took daphnids ''as encountered'' at low prey densities, but at high prey densities they consistently showed a strong selection preference for larger daphnids.

Our behavioral observations showed that prey attempted to flee predators, predators performed lunge attacks on prey, and both utilized available cover to some extent, similar to what would be expected in the wild. In one trial, two predators consumed 99 salmon fry over a 3-d period (Figure 2). Since our raceways contained only limited cover consisting of four pieces of half-pipe and no microhabitats of detritus, rocks, gravel, and so forth, such as found in a natural stream, these reported predation rates (given similar prey density) may be somewhat inflated over what would naturally occur but showed that brook trout can consume relatively large numbers of Atlantic salmon fry.

Contradictory results in trials 1 and 4 lend some doubt as to whether brook trout showed a preference for marked or nonmarked prey, but in three out of four trials we showed that brook trout did not preferentially select calcein-marked Atlantic salmon fry over nonmarked fry. This was also reflected by the results of the *G*-test on pooled data from the four trials, which showed that brook trout preyed upon marked and nonmarked individuals at a 1:1 ratio. Therefore, we conclude that there is no evidence to suggest that calcein-marked Atlantic salmon fry were the preferred prey of captive wild brook trout in our study.

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