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Survival, Performance, and Resistance to *Myxobolus cerebralis* Infection of Lake Trout × Brook Trout Hybrids

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Abstract.—The survival, performance, and susceptibility to *Myxobolus cerebralis* infection of the two possible crosses between lake trout *Salvelinus namaycush* and brook trout *S. fontinalis* were compared over a 4-year period in Causey Reservoir, Utah, an oligotrophic reservoir. Splake were produced by crossing female lake trout with male brook trout and brookinaw by crossing female brook trout with male lake trout. In the hatchery, significantly more splake eggs survived from fertilization to hatching than did brookinaw eggs, but once the fish began feeding survival did not differ between the two hybrids. A total of 54,645 splake and 42,356 brookinaw were stocked in the reservoir, and over the course of the study, 92 splake and 69 brookinaw were recovered from spring and fall gill-net sets. Survival, average weight, average length, and condition factor did not significantly differ between the two hybrids for any of the 4 years. Differences in susceptibility to infection by *M. cerebralis* between the two hybrids were compared in the reservoir and in laboratory tests in which fish were exposed at either 5 or 10 weeks posthatch. No *M. cerebralis* myxospores were found in either hybrid during the first 2 years of reservoir study, after which the prevalence ranged from 7.5% to 25% for splake and from 0% to 25% for brookinaw. Infection prevalence did not significantly differ between the two hybrids for any of the sampling periods. In controlled exposure tests, prevalence of *M. cerebralis* did not differ significantly between trout exposed at 5 weeks posthatch (splake = 88.4%, brookinaw = 77.1%) or at 10 weeks (splake = 100%, brookinaw = 96.4%). Our results suggest that brookinaw and splake are similar in growth, survival, and resistance to the causative agent of whirling disease after the fish reach the free-swimming fry stage. For fishery programs that rely on wild lake trout for splake production, use of brookinaw could result in significant manpower savings without compromising poststocking survival.

Hybrids of female lake trout *Salvelinus namaycush* and male brook trout *S. fontinalis*, known as splake (Buss and Wright 1956), have become a popular sport fish (Martin and Baldwin 1960; Vladykov 1963; Spangler and Berst 1976) and continue to be part of fisheries management in many states. The reciprocal hybrid cross (female brook trout × male lake trout) produces the brookinaw (Sowards 1959). Annual splake production in some states relies on lake trout that are gillnetted from a wild source. Males typically outnumber the females on the spawning beds (Merriman 1935; Royce 1951), and fertile females are relatively scarce. To achieve annual production goals, collecting gravid females can take several weeks, often under hazardous conditions. If the reciprocal cross, using the more abundant lake trout male, were as viable as the splake, both in the wild and in the hatchery, significant reductions in labor could be possible. One objective of this study was to compare the performance of the two hybrids by monitoring growth, survival, and general health and condition in a reservoir. Hatchery performance was also monitored to determine if brookinaw were similar to splake.

The two hybrids may differ in their resistance to infection by *Myxobolus cerebralis*, the etiological agent of whirling disease. O'Grodnick (1979) noted differences in susceptibility among various salmonids tested, and lake trout appeared to be refractory to the disease. Brook trout were less susceptible to the disease than rainbow trout *Oncorhynchus mykiss* but more susceptible than lake trout (O'Grodnick 1979). By crossing lake trout with another salmonid, the former's inherent resistance to whirling disease may be passed on. Resistance to infection, functional sterility, and

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other characteristics of the hybrid could result in better survival in impoundments than is manifest in brook or lake trout. By testing the disease resistance of the two crosses, the results should indicate whether or not resistance is sex-linked and which hybrid might be more suitable for stocking in enzootic areas. Susceptibility testing in this study was conducted in both field and controlled laboratory exposures. The objective was to determine whether there are any differences in susceptibility to infection by *M. cerebralis* between splake and brookinaw and to learn more about how these differences might be inherited.

Methods

Field tests.—The field test was conducted in Causey Reservoir, Utah, where *M. cerebralis* was discovered and histologically confirmed in 1994 in rainbow trout and kokanee *O. nerka*. Negative test results the previous year indicated that the reservoir had been contaminated only recently. Causey Reservoir, at 1,735 m elevation, is within the Ogden River drainage in Weber County, Utah, and is classified as oligotrophic (but bordering on mesotrophy); it has a maximum surface area of 57 ha, maximum depth of 55 m, a mean depth of 20 m, a shoreline of 11.8 km, and a mean annual vertical fluctuation of about 19 m (Judd 1997). Water total hardness ranges from 157 to 173 mg/L as $CaCO_3$. Vertical profiles taken in early August in 1996, 1997, and 1998 indicated that temperatures ranged from 8–9°C at the bottom to 17–20°C at the surface. Dissolved oxygen ranged from 1.6–3.8 mg/L in the bottom meter of water to 6–12 mg/L in higher strata. The pH ranged from 7.7 to 8.1.

The parental lake trout for the hybrids were gillnetted from Fish Lake in Sevier County, Utah; the brook trout were from captive broodstock at Egan State Fish Hatchery in Bicknell, Utah. The eggs of both hybrids were incubated at 4–5°C to the eyed egg stage at a facility on Twin Creeks at Fish Lake. The eyed eggs were shipped to the Mammoth Creek Hatchery, Hatch, Utah, and subsequently reared until stocking. Mortality from hatching to initial feeding was expressed as a percentage of the number of dead alevins (mortalities and cripples that were culled) divided by the initial number of fertilized eggs. Survival from initial feeding to stocking was calculated from the ratio of the number stocked divided by the hatchery mortality plus the number stocked. The survival of the two hybrids was compared using the Mann–Whitney *U*-test. Growth of the two hybrids in the hatchery was determined from monthly sample counts that we used to calculate specific growth rates (SGR) for the period of hatchery residence by using the formula of Busacker et al. (1990):

$$\text{SGR} = 100 \times [\log_e(\text{final weight}) - \log_e(\text{initial weight})] \div \text{days of growth.}$$

Feed conversion ratios were calculated as the total weight of fish feed fed divided by the total gain in fish weight. Alevin mortality, percent hatch, juvenile survival, and feed conversion ratios for the two hybrids were compared using Student's *t*-test. In July 1996, total length and weight of 150 fish from each hybrid were measured to determine the variability (coefficient of variation = 100 · SD/mean) in size among the two groups.

TABLE 1.—Number and size of splake and brookinaw stocked into Causey Reservoir, Utah, from 1995 to 1998.

Stocking date	Species	Number of fish	Mean weight (g)	Mean length (mm)
3 Aug 1995	Splake	15,066	9.3	104
	Brookinaw	15,134	9.1	103
16 Jul 1996	Splake	13,804	11.2	111
	Brookinaw	13,746	7.7	94
10 Jul 1997	Splake	14,658	11.3	112
	Brookinaw	0		
8 Jul 1998	Splake	25,775	11.7	116
	Brookinaw	13,476	14.5	117

The reservoir was stocked annually in July with splake and brookinaw from 1995 to 1998 (Table 1). Each year, brookinaw were marked by removal of the adipose fin 1–2 weeks before stocking to differentiate them from the splake upon recapture. In 1997, no brookinaw were stocked because of overripe eggs of brook trout females in the previous fall that resulted in total loss. However, splake were still stocked in 1997, but a ventral fin was clipped to differentiate this year-class from the others. Splake stocked in 1997 were not included in the survival analysis, but the 13 splake with a ventral clip recovered in 1998 were tested for *M. cerebralis*. Other sport fish present in the reservoir included kokanee, cutthroat trout *O. clarki*, rainbow trout, and brown trout *Salmo trutta*, all of which reproduce in the tributaries except the rainbow trout. Rainbow trout stocking was discontinued in 1994.

Four experimental gill nets (1.8 m deep by 36.7 m long composed of five 7.3-m panels with square mesh sizes of 1.9, 2.5, 3.8, 5.1, and 6.3 cm) were

set overnight (1 night) in the spring and fall of every year of the study. Total length and weight were recorded for all fish captured. In addition, except for fall 1996, up to 20 splake and 20 brookinaw were examined according to the necropsy-based Health and Condition Profile (HCP) system (Goede and Barton 1990). Samples from the head tissues were also collected individually to determine the prevalence of *M. cerebralis* using the pepsin–trypsin digest method (Thoesen 1994). The saw was rinsed and brushed with a strong chlorine bleach solution between individuals to prevent cross-contamination.

Because of difficulty in differentiating the year-classes after stocking, growth comparisons were restricted to the first cohort ($N = 21$ brookinaw and 22 splake). Survival for each year of the study was analyzed using chi-square analysis to compare the recovery ratios (cumulative net recovery numbers over the course of the study to number stocked) of splake and brookinaw. A power analysis of the chi-square survival model was made by calculating the noncentrality parameter, multiplying it by the sample size (total fish stocked plus recovered) and deriving the power estimate from a published table (Agresti 1990). We used chi-square analysis, including Fisher's Exact test and maximum likelihood estimation, to test for frequency differences between the hybrids in the categorical variables of the HCP (e.g., kidney, eye, spleen, gill, and fat and bile indices) and the prevalence of *M. cerebralis*. For continuous variables of the HCP (e.g., length, weight, condition factor) and SGR, the Kolmogorov–Smirnov test was used to test the assumption of normality; the Mann–Whitney *U*-test was used for nonnormally distributed data, and Student's *t*-test was used for normally distributed data. The HCP data were analyzed separately for each sampling date.

Laboratory exposures to M. cerebralis.—We used three coolers (47 × 27 cm) to expose splake and brookinaw at either 5 or 10 weeks after hatching to 1,000 triactinomyxons (tams) per fish in 8.0 L of hatchery well water for 2 h. The number of fish per cooler in the first exposure varied from 10 to 13 and was limited by the number of available tams. Control fish (2 tanks of 7–11 fish each) were also transported in the coolers and aerated for 2 h but were not exposed to tams. After exposure, fish were randomly assigned to one of the three plastic tanks used for each hybrid type; two tanks for each hybrid type held the controls. The tanks were supplied with water from a spring-fed pond that ranged in temperature from 6.5°C to 9.0°C for the 5-week-old group and from 5.5°C to 13.5°C for the 10-week-old group. Fish were fed a commercial diet daily by automatic feeders. Tanks were cleaned three times a week, and mortality and temperature were also monitored at those times.

In the second exposure (10-week group), the number of brookinaw were limited (40/cooler versus 60/cooler for splake). Exposures were standardized at a rate of 1,000 tams/fish. Three treatment replicates and two control replicates were randomly assigned to tanks.

In addition to the two hybrids, 60 lake trout and 60 brook trout fry were exposed at 75 d (10.5 weeks) after hatching to the same exposure dose and duration. These fish were from the same lots used to produce the hybrids and were produced on the same day as the hybrids. Tank space was limited, so only one replicate tank was used for each species. Fish were cared for as noted for the hybrids.

Hybrids from both age groups and parent species were sampled 5 weeks after exposure for analysis by the single-round modification (Schisler et al. 2001) of the polymerase chain reaction assay developed by Andree et al. (1998). Severity of infection was rated by the intensity of the DNA banding pattern (Schisler et al. 2001): negative (–), weak positive (w+), positive (+), strong positive (++), and very strong positive (+++). The intensity is a subjective visual estimate but has been weakly correlated with myxospore burden (Schisler et al. 2001). Samples sizes in the 10-week-old group were 12 for controls and 16–20 for fish exposed to tams.

In each tank, a varying number of fish from the 10-week-old group were retained until total incubation time was 20 weeks. Deformities, monitored on four dates during those 20 weeks, were categorized as cranial (highly sloped head), spinal-caudal (lordosis or kyphosis), shortened opercle, silver discoloration, or mandibular. With the exception of silver discoloration, these deformities have been reported as clinical signs of *M. cerebralis* infection (Markiw 1992a). Any whirling behavior was noted during the thrice-weekly cleaning and mortality assessment. After 20 weeks, fish heads were sampled individually for analysis by the pepsin–trypsin digest method (Thoesen 1994). Myxospore density was estimated by counting the number of myxospores in 100 microscope fields at 400×. Total grams of tissue were not recorded, so meaningful myxospore burden data could not be compared between the hybrids.

Student's *t*-test was used to compare mortality

TABLE 2.—Comparison of survival and hatchery performance (specific growth rate [SGR] and feed conversion ratio [FCR]) of splake and brookinaw. Survival to hatching and sac-fry mortality (including cripples) were calculated as percentages of green eggs. Observed mortality from initial feeding to stocking (M_{i-s}) was calculated as a percentage of the sum of observed mortality and the total number stocked. Data for splake are the means of three lots within each year. An asterisk indicates a significant difference between the hybrids (*t*-test, $P < 0.05$).

Cohort-year hybrid	Total green eggs	Hatch (%)	Sac-fry mortality (%)	M_{i-s} (%)	SGR (%/d)	FCR
			1994–1995			
Splake	291,452	65.3	13.5	17.6	2.48	1.13
Brookinaw	369,020	32.0	8.8	42.1	2.66	1.47
			1995–1996			
Splake	308,797	64.4	13.9	4.0	2.63	1.08
Brookinaw	158,388	42.4	8.2	8.0	3.30	1.43
			1996–1997			
Splake	283,951	66.4	7.8	5.6	2.82	1.18
Brookinaw	0					
			1997–1998			
Splake	332,026	57.7	8.8	9.3	2.63	1.17
Brookinaw	143,446	21.0	5.5	12.9	2.62	1.03
			Average			
Splake		63.4*	11	9.1	2.64	1.14
Brookinaw		31.8	7.5	21.0	2.86	1.31

rates between the two hybrids for 5- and 10-week-old groups exposed to *M. cerebralis*. Mortality resulting from human error was not included in the analysis. Each class of deformity was analyzed separately for each sampling date, comparing exposed versus unexposed for each hybrid using the percentage within each tank (arcsine-transformed) with a *t*-test. Deformity percentages between the two hybrids were similarly compared using data for exposed fish only. Differences in infection prevalence between the hybrids were compared with a *t*-test of the arcsine-transformed percentages for each tank and by chi-square analysis of the combined data from all tanks.

Results

Field Tests

Hatchery performance.—Hatching rates were significantly higher for splake (63.4%) than brookinaw (31.8%; $t = 5.84$, df = 5, $P = 0.002$; Table 2). Alevin mortality did not significantly differ between splake (11.0%) and brookinaw (7.5%; $t = 1.87$, df = 5, $P = 0.12$). Similarly, mortality from initial feeding to stocking did not differ significantly between splake (9.1% ± 6.8 [mean ± SD]) and brookinaw (21.0 ± 18.5%; $t = -1.09$, df = 13, $P = 0.38$). Mean feed conversion ratios did not differ significantly between splake (1.14 ± 0.15) and brookinaw (1.31 ± 0.24; $t = -1.54$, df = 13, $P = 0.15$).

Health and condition profiles of fish before stocking indicated generally healthy fish. A small percentage of opercular deformities occurred in splake (22% in 1995; 8% in 1996; 7% in 1997) and brookinaw (5% in 1995; 0% in 1996). Caudal deformities were noted for brookinaw in 1996 (16.2%, $N = 80$). The coefficient of variation was similar between the two hybrids for length (14.1% for splake and 15.1% for brookinaw) and weight (44.8% for brookinaw and 42.5% for splake).

Reservoir tests.—We recovered 92 splake (plus 13 with ventral fin clips) and 69 brookinaw over the course of the study from a total of 54,645 splake and 42,356 brookinaw stocked. Cumulative survival did not significantly differ between splake (0.19%) and brookinaw (0.16%) for any of the 4 years (Table 3). Power analysis indicated a strong test (power = 1.00). The two hybrids did not significantly differ in average length, weight, or condition factor when analyzed separately by date (Table 3), except for the spring 1998 sample in which brookinaw were significantly longer ($P = 0.003$) and heavier ($P = 0.003$) than splake. In June 1999, condition factor was slightly, but significantly ($P = 0.014$), higher for brookinaw (0.875) than splake (0.819). Specific growth rates did not sig-

TABLE 3.—Mean values of health and condition profile variables of splake and brookinaw sampled from Causey Reservoir, Utah, from 1996 to 1999. The cumulative survival (cumulative total of recovered fish divided by the cumulative number of fish stocked times 100) is given for each hybrid. Fulton's condition factor, K_{TL}, is defined as $(W \times 10^5)/L^3$, where W is weight in grams and L is total length (TL) in millimeters. Asterisks indicate a significant difference between the two hybrids; $P < 0.05$*, $P < 0.01$**. Empty cells in the statistics summary indicate either that the two hybrids had identical results or that Fisher's exact test was used.

Variable	Sample date					
	5 Jun 1996	27 Jun 1997	30 Oct 1997	3 Jun 1998	20 Oct 1998	29 Jun 1999
Splake						
Cumulative survival (%)	0.073	0.080		0.166		0.192
Fat index	0.67	1.00	1.63	1.88	1.25	2.75
Thymus index	0.00*	1.00	0.25	0.71	0.75	0.55
Bile index	0.5	3.00	1.63	1.47	2.50	1.40
Pseudobranch (% of normal)	100	100	87.5	94.1	100	80
Length (mm)	246	184	263	231**	258	244
Weight (g)	115.1	49.0	160.6	104.9**	211.5	123.6
K_{TL}	0.733	0.787	0.787	0.815	0.771	0.819*
N	6	1	8	17	4	20
Brookinaw						
Cumulative survival (%)	0.046	0.093		0.093		0.163
Fat index	1.00	1.4	1.67	1.50	2.50	2.65
Thymus index	1.00*	0.40	0.44	0.00	1.00	0.45
Bile index	1.50	2.75	1.89	2.50	3.00	1.90
Pseudobranch (% of normal)	100	100	100	100	100	100
Length (mm)	220	230	296	286**	324	249
Weight (g)	77.2	107.0	224.8	186.3**	305.5	138.8
K_{TL}	0.723	0.826	0.852	0.774	0.844	0.875*
N	2	5	9	4	2	20
Statistics: G^2, df						
Cumulative survival (%)	0.914, 1	0.319, 1		0.730, 1		1.145, 1
Fat index			1.640, 3	2.540, 2	4.866, 2	0.672, 3
Thymus index				5.174, 2	1.046, 2	0.659, 2
Bile index			3.420, 3	5.189, 3		4.830, 3
Pseudobranch (% of normal)						
Statistics: t, df						
Length (mm)	1.109, 6	−0.951, 4	−1.432, 15	−3.378, 19	−0.734, 4	−0.582, 38
Weight (g)	1.109, 6	−0.988, 4	−1.367, 15	−3.388, 19	−0.388, 4	−1.081, 38
K_{TL}	0.253, 6	−0.569, 4	−1.587, 15	1.785, 19	−0.754, 4	−2.579, 38

nificantly differ between the two hybrids in the first cohort at the end of the first summer of growth (splake, 3.25; brookinaw, 3.22; $t = 0.28$, df = 2, $P = 0.80$), after 219 d (splake, 1.36; brookinaw, 1.32; $t = 0.88$, df = 3, $P = 0.44$), or after 306 d (splake, 1.07; brookinaw, 1.04; $t = 0.88$, df = 6, $P = 0.412$). Phenotypically, the splake and brookinaw were indistinguishable.

The health and condition profile data generally indicated healthy fish for both hybrids. Spleen, kidney, eye, and liver tissues were all normal, except for an occassional coffee-colored liver among both hybrids (9 of 133). A few fish had pale gills, probably symptomatic of remaining in the gill nets too long, but most were normal. A few splake had swollen pseudobranchs ($N = 6$), but the frequency did not differ significantly from the brookinaw when analyzed separately by date (Table 3). Fin condition, opercular deformities, and bile index did not significantly differ between the hybrids for any sampling period. The fat index did not differ between the two hybrids in any sampling period (Table 3) but increased over the course of the study ($r = 0.85$, df = 11, $P < 0.001$). A similar trend in condition factor was not significant for splake ($r = 0.76$, df = 5, $P = 0.082$) or brookinaw ($r = 0.75$, df = 5, $P = 0.084$). Similarly, bile and thymus indices did not increase over time for either splake ($r = 0.28$, $P = 0.25$; and $r = 0.45$, $P = 0.36$, respectively; df = 5), or brookinaw ($r = 0.39$, $P = 0.45$; and $r = 0.27$, $P = 0.60$, respectively; df = 5; Table 3). The thymus index was significantly higher for brookinaw (1.0) than splake (0.0) in the first sample in the spring of 1996 but did not differ between the two in subsequent samples (Table 3).

TABLE 4.—Comparison of the prevalence of *Myxobolus cerebralis* between splake and brookinaw recovered from gill nets in Causey Reservoir, Utah, from 1995 to 1999. *N* is the number of fish sampled on that date.

Sampling date	Splake		Brookinaw	
	Prevalence (%)	*N*	Prevalence (%)	*N*
7 Nov 1995	0.0	2	0.0	3
10 Mar 1996	0.0	3	0.0	2
5 Jun 1996	0.0	6	0.0	2
23 Oct 1996	0.0	11	0.0	15
27 Jun 1997	0.0	1	0.0	5
30 Oct 1997	0.0	8	0.0	9
3 Jun 1998	17.6	17	25.0	4
20 Oct 1998	25.0	4	0.0	2
29 Jun 1999	7.5	53	0.0	27

No *M. cerebralis* myxospores were found in either hybrid until the spring of 1998. After that, the infection prevalence ranged from 7.5% to 25% for splake and from 0% to 25% for brookinaw (Table 4). The infection prevalence did not significantly differ between the two hybrids for any of the sampling periods. Prevalence of infection for the other species sampled was determined for the last three sampling dates; a total of four rainbow trout were recovered in this period, one of which was infected. Only three kokanee were recovered in the same samples, two of which were infected. Prevalence of *M. cerebralis* in wild cutthroat trout was 66.7, 50.0, and 43.8% for 3, 8, and 16 fish sampled in spring 1998, fall 1998, and spring 1999, respectively. Brown trout sampled on the same dates had infection rates of 25.0, 25.0, and 0.0% (N = 4, 4, and 7). No deformities consistent with the clinical signs of whirling disease were observed in any of the fish sampled.

Laboratory Exposures to M. cerebralis

Prevalence of *M. cerebralis* did not significantly differ between splake (88.4%) and brookinaw (77.1%) exposed at 5 weeks posthatch (G^2 = 1.75, df = 1, P = 0.19). The infection rate for splake exposed at 10 weeks posthatch (100%, N = 60) also did not significantly differ from brookinaw (96.4%, N = 56; G^2 = 2.80, df = 1, P = 0.094). Chi-square analysis of PCR results using the severity categories indicated no susceptibility differences between hybrids for either age-group (data pooled across replicates; G^2 = 1.78, 4.67; df = 2, 3; P = 0.41, 0.20; Table 5). No controls were infected in either age-group. Brook trout and lake trout were both 100% infected (Table 5).

Mortality for the 5-week-old group was negligible (only one brookinaw died) and did not differ significantly between splake (0.0 ± 0.0%) and brookinaw (1.0 ± 2.2%; t = −1.00, df = 4, P = 0.37). For the 10-week-old group, mortality over the 20-week period was significantly higher for brookinaw (35.0%) exposed to triactinomyxons than for splake (6.1%; t = −4.54, df = 9, P = 0.001). However, mortality rates for control brookinaw were also higher (30%) than for splake controls (10.6%), indicating that exposure to triactinomyxons was not the reason for the difference in mortality.

TABLE 5.—Percentage of splake and brookinaw by category of *Myxobolus cerebralis* infection severity, as determined by polymerase chain reaction assay (5- and 10-week-old groups) or pepsin–trypsin digest (20-week-old group) methods. Each hybrid was exposed to 1,000 triactinomyxons per fish for 2 h at either 5 or 10 weeks of age and sampled 5 or 20 weeks after exposure.

Infection classification and number sampled (*N*)	Percentage by infection category			
	Splake	Brookinaw	Brook trout	Lake trout
Exposed at 5 weeks old, sampled at 5 weeks				
Negative	11.6	22.9		
Weak positive	11.6	11.4		
Positive	76.8	65.7		
Strong positive	0.0	0.0		
N	43	35		
Exposed at 10 weeks old, sampled at 5 weeks				
Negative	0.0	3.6	0.0	0.0
Weak positive	10.0	7.1	10.0	5.0
Positive	90.0	87.5	90.0	95.0
Strong positive	0.0	1.8	0.0	0.0
N	60	56	20	20
Exposed at 10 weeks old, sampled at 20 weeks				
Negative	57.4	50.0	0.0	86.4
Positive	42.6	50.0	100.0	13.6
N	54	10	36	22

The percentages of cranial deformities on the four sampling dates were not significantly different between splake exposed to triactinomyxons and unexposed splake or between exposed and unexposed brookinaw (Table 6). There were no significant differences between exposed and unexposed fish in any of the other deformity categories either. Deformity rates similarly did not significantly differ between exposed splake and brookinaw for any of the deformity categories and sampling dates.

Whirling behavior was noted for both splake and brookinaw in a few of the tanks. Whirling behavior was not observed among lake trout but was seen among brook trout (2 of 36). Whirling was inconsistent, appearing at some times but not at others. It first appeared at 22 d postexposure in brookinaw

TABLE 6.—Deformity percentages (N = two to three tanks of 3–39 surviving fish in each) for various categories of deformities for splake and brookinaw either exposed to *Myxobolus cerebralis* (WD+) or not exposed (controls) and sampled on four dates postexposure in 2000. Also shown are the t-statistics comparing (1) exposed and unexposed deformity rates and (2) exposed splake and exposed brookinaw ($P > 0.05$ for all tests).

Comparison	Deformity percentages and t-statistics					
	Cranial	Spinal	Short opercle	Silver discoloration	Mandibular deformity	Blacktail
Sampled 11 May at 86 d postexposure						
Splake						
Control	0.0	0.0	0.0	0.8	2.3	0.0
WD+	0.0	0.0	5.4	0.9	0.0	0.0
t (df = 4)			−0.614	−0.138	1.769	
Brookinaw						
Control	0.0	0.0	0.0	0.0	0.0	0.0
WD+	6.7	0.0	0.0	0.0	0.0	0.0
t (df = 3)	−0.775					
Splake versus brookinaw (both WD+)						
t (df = 4)	−1.000		1.579	1.000		
Sampled 2 June at 108 d postexposure						
Splake						
Control	0.0	0.0	3.1	1.6	2.3	0.0
WD+	0.0	2.8	5.1	0.0	0.0	0.0
t (df = 4)		−1.679	0.587	1.000	1.769	
Brookinaw						
Control	0.0	0.0	0.0	0.0	0.0	0.0
WD+	5.7	0.0	0.0	0.0	0.0	0.0
t (df = 3)	−0.775					
Splake versus brookinaw (both WD+)						
t (df = 4)	−1.00	1.679	1.680			
Sampled 22 June at 123 d postexposure						
Splake						
Control	0.9	1.9	4.1	1.8	4.3	0.0
WD+	0.0	2.7	2.8	1.4	0.0	0.0
t (df = 3)	0.775	−0.279	0.383	0.256	4.491	
Brookinaw						
Control	0.0	0.0	0.0	0.0	0.0	0.0
WD +	17.7	0.0	0.0	0.0	0.0	0.0
t (df = 3)	−1.426					
Splake versus brookinaw (both WD+)						
t (df = 3)	−1.421	1.342	1.342	1.342		
Sampled 7 July at 143 d postexposure						
Splake						
Control	0.9	0.0	2.7	2.7	3.6	0.0
WD+	2.8	0.0	2.8	2.8	1.4	0.0
t (df = 3)	−0.626		−0.034	−0.017	1.506	
Brookinaw						
Control	0.0	0.0	0.0	0.0	0.0	0.0
WD+	8.3	0.0	0.0	0.0	0.0	0.0
t (df = 3)	−0.775					
Splake versus brookinaw (both WD+)						
t (df = 3)	−0.513		1.000	1.342	1.342	

(1 of 32) and at 111 d postexposure in splake (1 of 39 in each of two tanks). One of the brookinaw that was first observed whirling on day 127 was dead the following day. Silver discoloration was observed mainly in the caudal peduncle region and may or may not have been related to whirling disease. The discoloration was not noticeable during the fin-clipping of the thousands of hatchery fish, yet controls in this study also had the anomaly. The cause for the silver discoloration remains unknown. Brook trout had clinical signs of whirling disease, including blacktail (5.4%, N = 37) and highly-sloped heads (5.4%) beginning 128 d after exposure. Lake trout also had cranial deformities (9.5%, N = 21) that appeared at 128 d but no blacktail deformities.

After 20 weeks incubation, *M. cerebralis* prevalence (pepsin–trypsin method) did not differ significantly between splake (42.6%, $N = 54$) and brookinaw (50.0%, $N = 10$; pooled data for exposed fish; $G^2 = 0.18$, df = 1, $P = 0.66$). Prevalence of *M. cerebralis* differed significantly among exposed splake, lake trout, brook trout, and brookinaw ($G^2 = 68.9$, df = 3, $P < 0.001$). Prevalence of *M. cerebralis* among lake trout was only 13.6% ($N = 22$) compared with 100% for brook trout ($N = 36$). Brookinaw were significantly lower in prevalence than brook trout (Fisher's Exact test, $P < 0.001$) and nearly significantly higher than lake trout (Fisher's Exact test, $P = 0.072$). Splake infection rates were significantly higher than lake trout (Fisher's Exact test, $P = 0.018$) and significantly lower than brook trout (Fisher's Exact test, $P < 0.001$).

Discussion

Early hatchery performance of splake and brookinaw has varied widely in the literature, and the higher egg survival we observed for splake than for brookinaw has been observed by others. Buss and Wright (1956) reported that fewer than 1% of brookinaw eggs survived to hatching and only 0.7% survived to initial feeding; splake had higher survival, with 28% of the initial number of eggs surviving to initial feeding. Stenton (1952) reported splake survival of 96% to hatching and 75% from egg to initial feeding for the pooled eggs of two females. Seguin (1957) noted that brookinaw had lower early hatchery survival (5.6% hatched in one lot, 57.3% survived 2.4 months in another lot) than splake (82% hatched in one lot, 94.5% survived to 2.4 months in another). However, Carlander (1969) reported that survival rates of these two hybrids to the free-swimming stage were similar (75–79%). Sowards (1959) noted 73% hatch for brookinaw (single pair mating) and 38.5% hatch for splake; the egg and fry survival of splake in this study was less than that generally observed by Wagner (1996) for either lake trout or brook trout parent. There are some problems associated with the brookinaw in early hatchery rearing, and given a choice between the hybrids, splake are superior overall because of this. However in our study, the differences were minor once the fish started feeding. These early losses could easily be compensated by fertilizing additional brook trout eggs.

Our results suggest that brookinaw and splake have similar rates of growth and survival. Brookinaw growth rates and poststocking survival have not been previously documented. Budd (1957) noted average fork lengths of 353 mm for splake 1 year after stocking in Lake Huron, which was greater than the rate we observed (296 mm total length) or that noted for Mill Meadow Reservoir, Utah (288 mm; E. Wagner, unpublished data). Growth rates of splake in Algonquin Park lakes was higher than brook trout and lake trout, especially after age 2 (Martin and Baldwin 1960). Berst and Spangler (1970) also noted faster growth rates for splake than lake trout in Lake Huron, splake reaching 0.9 kg by fall of their second year after stocking.

Survival did not differ between the two hybrids after stocking, based on our gill-net recovery data. Other research has indicated that splake survival is quite variable among lakes. Splake recovery from angling and gillnetting in selected Ontario lakes ranged from less than 0.5% to 30% (Fraser 1972). Splake recoveries were lowest in waters where other salmonids, especially lake or brook trout, were present (Fraser 1972). However, Martin and Baldwin (1960) reported higher angler recovery in mixed plantings of lake trout, brook trout, and splake in Ontario lakes than for splake-only plantings. Their reported angler recovery rate was generally less than 2% of the splake stocked, although recovery for one lake in Algonquin Park was high, where anglers harvested 65% in 1956 and 1957 and experimental gill nets took an additional 10%. Angler harvest was not monitored in our study; however, angling was noted during our visits in spring, fall, and winter and was indicated by the lack of older hybrids in the gill nets and by the presence of a Boy Scout camp on the north arm of the reservoir.

Our results suggest that splake and brookinaw are susceptible to infection by *M. cerebralis*, unlike the results in previous literature suggesting that lake trout and splake were refractory to whirling disease (O'Grodnick 1979; Hoffman 1990; Markiw 1992a). In the study by O'Grodnick (1979), lake trout were exposed for only 3 d in an infected stream. In our study, both hybrids in Causey Reservoir were infected with *M. cerebralis*, which suggests both hybrids are susceptible to infection. We also observed a 100% infection rate in lake trout fry, which indicated that this species is not immune to infection either. However, the infection rate for lake trout was dramatically reduced 20 weeks after exposure (13.6% by pepsin–trypsin digest) compared with the rate at 5 weeks (100% by PCR). Some of the difference may be explained by differences in assay methods, but the 100% infection

rate in brook trout by pepsin–trypsin techniques supports the hypothesis that lake trout possess some innate ability to fight infection by this parasite. Although being susceptible to *M. cerebralis* infection, some of the lake trout in our study appeared to be able to reduce the infection over time. Controlled studies are needed to explore this further. We observed a small percentage of lake trout with cranial deformities that developed within 128 d of exposure. Hoffman and Putz (1969) reported typical symptoms of the disease developing in lake trout, although the dose of tams was unknown. So, given that both lake trout and brook trout are susceptible to the parasite, their hybrids are also probably susceptible to infection.

Although infection rates did not differ between the two hybrids, the level of infection was much lower in both hybrids than it was for cutthroat trout in the reservoir. However, the hybrids were stocked and the cutthroat trout were naturally produced, so a direct comparison of susceptibility among the three groups is not possible. Nonetheless, using species of greater disease resistance such as splake or brookinaw could reduce the total myxospore production within a body of water, which could diminish the impact of whirling disease by reducing numbers of the infective stage (triactinomyxons). Triactinomyxon numbers correlate with higher mortality rates, earlier development of clinical signs, higher numbers of myxospores produced, and more severe histopathological impacts (Markiw 1992b; E. K. N. Ryce and coworkers, Cooperative Fisheries Research Unit, Montana State University, personal communication).

Other than early hatchery survival, brookinaw did not differ from splake in many traits, including poststocking survival, growth, and resistance to infection by *M. cerebralis*. Given the difficulties in producing splake with wild lake trout brood, brookinaw appear to be a viable alternative for fisheries managers.

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