Differences in sexual maturity and fall emigration between diploid and triploid brook trout (*Salvelinus fontinalis*) in an Adirondack lake

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Abstract: High levels of emigration coincident with maturity and spawning have been reported from brook trout (*Salvelinus fontinalis*) populations in Adirondack lakes. These lakes typically had few spawning areas and required stocking to maintain populations. We compared diploid and triploid brook trout to identify differences in gonadal development and emigration. Age 1+ and 2+ diploid and triploid brook trout held in captivity were examined internally for gonadal development. More diploid trout were mature than triploid fish ($p < 0.01$). Of triploid brook trout that matured, all were males. Yearling diploid and triploid brook trout were also stocked into a lake that had an outlet but no spawning areas. During the fall spawning season, only mature yearling triploid males, diploid males, and diploid females were caught in an outlet trap. No triploid females were caught. A greater proportion of diploids emigrated than triploids ($p < 0.01$). Triploidy in females arrested emigration by preventing sexual maturation. Triploid male brook trout should not be stocked because they can pose a reproductive risk to wild brook trout downstream from lakes. Stocking triploid females could reduce fall emigration and thus reduce the loss of catchable brook trout from Adirondack lakes with outlets and little spawning habitat.

Résumé : Des degrés élevés d'émigration coïncidant avec la maturité et le frai ont été signalés dans le cas de population d'ombles de fontaine (*Salvelinus fontinalis*) vivant dans les lacs des Adirondacks. De manière typique, ces lacs étaient pauvres en aires de frai et devaient être ensemencés pour assurer le maintien des populations. Nous avons comparé des ombles de fontaine diploïdes et triploïdes pour étudier les différences au niveau du développement des gonades et de l'émigration. Des ombles diploïdes et triploïdes d'âge 1+ et 2+ gardés en captivité ont fait l'objet d'un examen interne pour vérifier le développement des gonades. Les individus matures étaient plus nombreux chez les diploïdes que chez les triploïdes ($p < 0,01$). Les individus triploïdes qui sont parvenus à maturité étaient tous des mâles. Des ombles de fontaine diploïdes et triploïdes de l'année ont été ensemencés dans un lac qui possédait un émissaire, mais qui était dépourvu d'aires de frai. Au cours de la saison de frai, seuls des mâles triploïdes de l'année mature, des mâles diploïdes, des femelles diploïdes ont été capturés dans l'émissaire. Aucune femelle triploïde n'a été capturée. Chez les émigrants, on a constaté une plus forte proportion de diploïdes que de triploïdes ($p < 0,01$). La triploïdie chez les femelles a enrayé l'émigration en empêchant la maturation sexuelle. On ne devrait pas ensemencer d'ombles de fontaine mâles triploïdes parce qu'ils peuvent constituer un risque reproductif pour les ombles de fontaine sauvages vivant en aval des lacs. L'ensemencement de femelles triploïdes pourrait réduire l'émigration d'automne et, ainsi, réduire la perte d'ombles exploitables dans les lacs des Adirondacks possédant des émissaires, mais peu d'habitats de frai.
[Traduit par la Rédaction]

Introduction

Large-scale fall emigration of mature brook trout (*Salvelinus fontinalis*), coincident with spawning season, was reported from stocked lakes with outlets in the Adirondack region of New York State (Josephson and Youngs 1996). The stocked lakes had few spawning areas, and brook trout probably had emigrated in search of suitable spawning sites. Fall emigration was associated with sexual maturity and represented 37–69% potential losses from the stocked populations. Thus, the trout fisheries in these lakes could have been severely affected. If maturity could be suppressed or eliminated in the brook trout used for stocking, losses from fall emigration likely would be reduced and fisheries improved. Extensive evidence suggests that triploidy reduces or prevents maturation in brook trout (Allen and Stanley 1978) and other salmonids (Thorgaard and Gall 1979; Lincoln and Scott 1984; Benfey et al. 1989; Benfey 1991). Therefore, triploid brook trout rather than diploids could be stocked to reduce fall emigration losses and enhance put–grow–take fisheries in similar lakes.

The purpose of this study was to compare the fall emigration of diploid and triploid brook trout stocked into a small lake that had an outlet but no spawning areas. The objectives were to determine (*i*) if triploidy reduced gonadal development

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Table 1. Sex and maturity of diploid and triploid yearling brook trout stocked fall of 1993 into Mountain Pond, New York.

			Female		Male	
Ploidy	Number stocked	Unknown (immature)	Ripe	Green	Ripe	Green
2*n*	110	35	0	26	45	4
3*n*	95	58	0	0	0	37

Note: Maturity was assessed by external examination.

or otherwise inhibited sexual maturation and (*ii*) whether triploid trout emigrated less than diploid trout.

Materials and methods

Strains, ploidy manipulation, and stocking

Triploid and diploid brook trout were produced at the Little Moose Field Station of the Adirondack Fishery Research Program, located near Old Forge, New York. Temiscamie (female) × domestic (male) interstrain crosses were used in this study. The domestic strain matures at ages 0 or 1. This strain originated from several thousand Rome strain fingerlings that were transferred from the state fish hatchery in Rome, N.Y., in 1979 and held at the Little Moose Field Station for several generations (see Rome strain description in Perkins et al. 1993). The Temiscamie strain matures at ages 1–3 and originated from gametes collected from 20–30 adults captured in the fall each year in 1965 and 1967 from the Temiscamie River, Quebec. Adults captured were presumed to be upstream migrants from a population in Lake Albanel 128 km downstream. This strain was subsequently held and propagated in Adirondack ponds in New York (Van Offelen et al. 1993).

Triploid trout were created in early November 1991 by heat shocking diploid zygotes 10 min after fertilization for 10 min at 28°C (Scheerer and Thorgaard 1983). Each ploidy lot was created from the same collection of gametes from 30 adults (1:1 sex ratio); five males were used to fertilize the eggs from each female. Trout were marked with jaw tags and blood samples were drawn from each fish in June 1993. The blood samples were tested by flow cytometric analysis of erythrocytes to determine the ploidy of each individual (Allen 1983). Blood drawn from the caudal peduncle was dispensed (1 μL) into 300 μL of a stain solution of 0.05 mg/mL propidium iodide and 0.1% sodium citrate. Analysis of the samples was performed on an Epics Profile flow cytometer (Epics Division of Coulter Corporation, Hialeah, Fla.). Diploid brook trout blood was used as a comparative standard to presumed triploids. Based on the results of this analysis, diploid trout were discarded to create a 100% triploid lot. Diploid and triploid trout were reared under identical conditions until stocking in October 1993.

One-hundred-ten yearling diploid and 95 yearling triploid brook trout, from the 1992 year-class, were stocked into Mountain Pond on 1 October 1993 (mean diploid length 194 mm, range 145–235 mm; mean triploid length 184 mm, range 155–226 mm). Mountain Pond (43°42′N, 75°52′W) is a 3.4-ha lake with an outlet, located in the southwest region of the Adirondack Park, New York State. Three days prior to stocking, maturity of the fish was assessed by external examination. In addition, approximately 60 diploid and triploid trout were not stocked but retained in the hatchery for later internal assessment of maturity and gonadal development.

Assessment of maturity and gonadal development

External assessment of maturity was based on body shape, coloration, and gamete ripeness. Each fish stocked and those that emigrated were classified as immature, green, ripe, or spent. Fish that did not appear ready to spawn and showed no bright coloration were classified as immature. Green fish were those that had body cavities rounded with gametes (females) or were brightly colored (males) but that did not extrude gametes. Fish that extruded gametes when gently squeezed were categorized as ripe. Spent fish had flaccid body cavities that appeared as if they had been distended with gametes but were now empty.

Internal assessment of gonadal development was performed over two spawning seasons (1993 and 1994) on fish not stocked but held in the hatchery. Twenty-one diploids and 20 triploids from the 1992 year-class (age 1+) were frozen on 4 October 1993 and dissected 3 days later. Twenty-six diploid and 28 triploid age 2+ fish from the 1992 year-class were dissected on 7 November 1994. Based on dissection, gonads of fish were classified as either undeveloped, fully developed, or partially developed. Undeveloped gonads were small, showed no maturation or development, appeared stringy, and sex was indeterminable. Fully developed gonads were developed to the point of ripeness or near ripeness, and sex was easily recognized. Eggs in these fish were large and testes were white and long. Sex of gonads classified as partially developed was recognizable, but eggs were small and testes were gray and shorter than fully developed gonads. Gonadosomatic indices (Vladykov 1956) were calculated from the dissected fish to compare gonadal development among diploids and triploids. The gonadosomatic index (GSI) was calculated as

$$\mathrm{GSI} = \frac{\text{gonad weight (both halves) (g)} \times 100}{\text{body weight (g)}}$$

Outlet trapping

Emigration of the stocked brook trout from Mountain Pond was monitored with an inclined screen fish trap (Wolf 1951) constructed across the outlet, located 100 m downstream from the lake. After stocking, the trap was tended once or twice weekly for a 2.5-month period, from 1 October to 15 December, 1993. Tag numbers were recorded from fish caught to determine their ploidy, and each fish was examined externally to assess maturity. Fish caught in the trap were given a lower caudal fin clip and released back into the lake. No additional data were recorded from trout recaptured at later dates.

Statistical procedures

Binomial proportion tests (π; decision level = 0.01) were used to compare the ploidy and maturity proportions of fish kept in the hatchery and of those that were stocked and subsequently emigrated. The null hypotheses for these tests were the proportion of diploid fish that matured was equal to the proportion of triploid fish that matured (just prior to stocking), the proportion of diploid fish that emigrated was equal to the proportion of triploid fish that emigrated, and the proportion of mature diploid fish that emigrated was equal to the proportion of mature triploid fish that emigrated. These tests presumed that the sex ratio between ploidy lots was similar and that mortality was similar between diploid and triploid trout for the 2.5-month period after stocking. Chi-square tests of independence (χ^2 ; decision level = 0.01) were used to determine whether maturity and emigration, and ploidy and emigration, were independent. Two sample *t*-tests (unequal variance) were conducted to compare GSI values between ploidies of the same gender and age.

Results

Diploid versus triploid maturity

Maturity of the brook trout to be stocked, externally assessed 3 days prior to release, revealed that the proportion of the diploid lot already mature (0.68; green or ripe) was higher than that of the triploid lot (0.39) (π, $p < 0.001$; Table 1). All triploid males were classified as green, and most displayed external coloration typical of ripe diploid males. No triploid females, either green or ripe, were identified by external examination; however, green diploid females were observed. A larger number of triploids ($n = 58$) than diploids ($n = 35$)

Table 2. Gonadal development and mean GSI of diploid and triploid age 1 and 2 brook trout reared in the hatchery and dissected in October 1993 and in November 1994.

Ploidy	Sex	Age	Gonadal development	Mean GSI	Sample size
October 1993					
Diploid	Unknown (immature)	1	Undeveloped	na	1
Diploid	Male	1	Fully developed	2.96	9
Diploid	Female	1	Fully developed	10.6	5
Diploid	Female	1	Partially developed	0.53	6
Triploid	Unknown (immature)	1	Undeveloped	na	11
Triploid	Male	1	Partially developed	2.04	9
November 1994					
Diploid	Female	2	Fully developed	14.0	9
Diploid	Male	2	Fully developed	2.58	17
Triploid	Female	2	Partially developed	0.09	10
Triploid	Male	2	Partially developed	0.53	18

Note: Maturity was assessed by internal examination. GSI was not available (na) for immature fish (sex undetermined) because gonadal tissue was not distinct.

Table 3. Maturity level of female and male yearling brook trout that had emigrated from Mountain Pond and were caught in the outlet trap during the fall 1993.

		Female				Male			
	Immature	Green	Ripe	Spent	Total	Green	Ripe	Spent	Total
N_{EM}[a]	0	0	9	3	12	9	25	6	40
Percent[b]	0	0	17	6	23	17	48	12	77

[a] N_{EM}, number of emigrants caught.
[b] Percentage of total emigrants.

were identified as immature (i.e., sex could not be determined).

Differences in maturity, related to sex and ploidy, were observed based on internal examination of brook trout from the two lots retained in the hatchery and not stocked (Table 2). Among age 1+ fish examined in 1993, only 1 diploid fish of 21 had undeveloped gonads in comparison with 11 of 20 triploid fish examined. All yearling male and female fish ($n = 14$) with fully developed gonads were diploids. Nine triploid males and six diploid females had partially developed gonads. All triploids were classified as either male or immature; no triploid females were identified. All diploid males were ripe, and all triploid males were green. The next year (1994) as age 2+ fish, all 26 diploid fish had fully developed gonads and the gonads of all 28 triploid fish were partially developed. All age 2+ triploid females had partially developed but small ovaries.

Diploid GSI measurements were always greater than triploid GSI measurements in same-sex comparisons of age 1 and 2 trout held in the hatchery (Table 2). Yearling diploid male GSI values were different from triploid male GSI values (*t*-test, $p \cong 0.00$). Similar differences were observed the next year between GSI values for diploid and triploid age 2 males (*t*-test, $p < 0.001$) and between diploid and triploid age 2 females (*t*-test, $p < 0.001$).

Emigration

Fall emigration and maturity of brook trout were dependent (χ^2, $p < 0.01$; Table 3). Only mature brook trout were caught in the outlet trap in the fall of 1993. Most fish (77%) that emigrated were males. Ploidy and emigration of trout were also dependent (χ^2, $p < 0.01$; Table 4). Based on the total number of each lot stocked, a greater percentage of diploid trout (32%) emigrated from Mountain Pond than triploids (17%; Table 4; π, $p < 0.001$). However, when only the number of mature fish stocked within each lot was considered, the percentage of diploid (47%) and triploid (43%) trout that emigrated was not statistically different (π, $p > 0.05$; Table 4).

Table 4. Ploidy of brook trout that emigrated as yearlings from Mountain Pond during the fall 1993 and caught in an outlet trap.

			Emigrants			
Ploidy	No. stocked (N_s)	No. mature at stocking (N_m)	No. (N_{EM})	Percent male	Percent of total stocked[a]	Percent of total mature at stocking[b]
2*n*	110	75 (68%)	36	67	33	48
3*n*	95	37 (39%)	16	100	17	43

[a] Percent of total stocked = N_{EM}/N_s.
[b] Percent of total mature at stocking = N_{EM}/N_m.

Discussion

Comparisons of diploid and triploid brook trout of the same sex and age demonstrated that differences in maturity and gonadal development were due to ploidy. Gonads in age 1 and 2 diploid brook trout were significantly larger than those in triploid fish. After this study was concluded, triploid brook trout from the next year-class (1993) were held until age 3+, and no mature triploid females were observed, which is consistent with observations by others (e.g., Boulanger 1991). Triploidy seems to prevent maturation in female brook trout at least through age 3.

Triploid brook trout were less likely to emigrate in the fall than diploid trout. However, when only mature fish were considered, ploidy had little effect on emigration tendency. Mature triploids (and only males matured) emigrated at approximately the same proportion as mature diploids (both males and females matured) after being stocked into Mountain Pond. Because no mature triploid females were observed, most of the immature triploids were probably females. The absence of emigration by immature triploids (probably females) supported the idea that suppression of maturity through triploidy would reduce fall emigration by brook trout.

Triploid male brook trout displayed the same secondary sexual traits and had similar but smaller testes at ages 1 and 2 as diploid males. Likewise, Thorgaard and Gall (1979) reported that triploid male rainbow trout (*Oncorhynchus mykiss*) had partially developed testes similar to diploid males in appearance. Testes of triploid rainbow trout were grayer, firmer, and slightly smaller than testes of diploids, and triploids had a lower GSI (Lincoln and Scott 1984). Benfey et al. (1989) reported similar differences in gonadal development for male diploid and triploid rainbow trout and pink salmon (*Oncorhynchus gorbuscha*). Absence of ripe triploid males in our study was probably due to an absence of sperm ducts in these fish, and thus, gametes could not be extruded (Boulanger 1991). In rainbow trout, the presence of a third set of chromosomes resulted in abnormal (aneuploid) sperm, if sperm was formed at all (Benfey et al. 1986). Despite the differences in gonadal development between diploid and triploid males, males of both ploidies exhibited similar emigration from the lake.

No female triploid brook trout were identified in this study by external characteristics at spawning time; however, females which had small, undeveloped, stringy ovaries were identified by dissection. Similar undeveloped ovaries were observed in triploid female rainbow trout (Lincoln and Scott 1984; Thorgaard and Gall 1979). In a comparative study of female diploid and triploid rainbow trout and pink salmon, the triploids did not mature, and a lack of oogenesis was common, perhaps as a result of abnormal endocrine function (Benfey et al. 1989). These studies explain the absence of externally identifiable triploid females observed in our study. Further, the differences in gonadal development we observed between diploid and triploid females were also associated with behavioral differences. Emigration from the lake was exhibited by yearling diploid females but not by yearling triploid immature fish presumed to be mostly females. In 1994 when the fish in this study were age 2+, no water flowed in the outlet of Mountain Pond until late in the spawning season (3 November) owing to a drought. However, six fish of the 1992 year-class were caught in the outlet trap, and all were diploids. Emigration of age 2+ triploid females also seems unlikely based on the lack of fully developed gonads observed in the age 2+ triploid fish held in the hatchery (Table 4).

Based on the results of our study, stocking triploid female brook trout could reduce fall emigration of age 1 and 2 fish from lakes with outlets. Many lakes in the Adirondack region have little or no natural reproduction of brook trout, and thus, their fisheries are dependent on stocking. Stocking of mixed-sex triploid lots of brook trout, male and female, as an approach to reduce fall emigration, however, is undesirable in two aspects. First, some male triploid brook trout did mature and emigrate from Mountain Pond, which compromised the effectiveness of the approach. Second, mature triploid male brook trout will likely exhibit spawning behavior and, therefore, could compete for females and disrupt normal spawning of wild fish (Boulanger 1991). Because triploid males will emigrate, stocking of these fish could be detrimental to wild populations that may exist downstream from lake outlets. To minimize these problems, only female triploid brook trout should be used for stocking when using ploidy as an approach to control emigration. Ultimately, this approach may be viable if all female triploid lines can be produced. All-female lots of brook trout can be produced by various sex reversal strategies (e.g., Galbreath et al. 1994).

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