Genetic Characterization of Intermixed Walleye Stocks in Claytor Lake and the Upper New River, Virginia

George C. Palmer

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B. R. Murphy, Co-Chair
E. M. Hallerman Co-Chair
R. V. Foutz
J. Williams

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Genetic characterization and intermixing of walleye stocks in Claytor Lake and the upper New River, Virginia

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(Abstract)

Historically, the New River may have contained a genetically unique, river-spawning population of walleye (*Stizostedion vitreum).* Additionally, a number of genetically different walleye stocks have been stocked in Claytor Lake, Virginia. The increasing importance of the walleye fishery led to interest in clarifying key unknowns regarding the biology of the New River walleye stock. A radio telemetry study and genetic evaluation of present-day stocks led to identification of three spawning areas and the characterization of the genetic stock structure of walleye in Claytor Lake and the upper New River.

Using radio telemetry to track movements of walleye, I determined that two resident walleye populations co-exist: one within Claytor Lake and the other in the New River above the lake. These populations establish spatially disjunct home ranges, remaining spatially separated throughout most of the year. Although there is no blockage to movement or migration, walleye within Claytor Lake generally spawn at Allisonia, while walleye within the upper New River spawn at Buck Dam. Some walleye from both populations spawn in other areas, such as Fosters Falls.

Using allozymes, microsatellite DNA, and mitochondrial DNA marker techniques to examine population structure of walleye, I determined that the walleye within Claytor Lake are a panmictic population. This is the result of years of stocking walleye from different genetic stocks and interbreeding among individuals. The genetic structure of walleye from the New River also shows the presence of more than one genetic stock. Within the New River population, there exists a genetic stock of walleye that is characterized by three mitochondrial DNA haplotypes (43, 44, and 45) that previously have not been seen. This may be indicative of a unique walleye stock that is native to the

New River and which has remained spatially or temporally segregated by spawning habits.

The co-existence of two different walleye populations in the Claytor Lake / upper New River system justifies different management strategies. I recommend that management of the walleye population in Claytor Lake focus on increasing the exploitation of this non-indigenous stock. Management of the upper New River walleye population should focus on conservation of the unique native stock through supportive breeding and/or strict harvest regulations.

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Introduction

Walleye, *Stizostedion vitreum* is a favored sport fish throughout its native and introduced range. The New River, part of the Ohio River drainage, lies within the geographic distribution of walleye in North America (Colby et al. 1979). The historical occurrence of walleye (*Stizostedion vitreum vitreum)* in the New River is uncertain. Hackney and Holbrook (1978) considered walleye to be native to the river and part of a southern stock commonly found throughout the Mississippi drainage and the southeastern portion of the United States. However, others believe that walleye were stocked in the New River beginning in 1921 (United States Fish Commission 1922, as cited in Jenkins and Burkhead 1994). Regardless of whether walleye are native, a population did reside in the New River before the Claytor Lake impoundment. Older citizens recall catching walleye in the New River since the early 1900s. This river-run stock, possibly an Ohio River drainage stock, was adapted to the river environment and declined in numbers due to loss of habitat and to blockage of spawning migration through dam construction (Hackney and Holbrook 1978).

The construction of the Claytor Lake Hydroelectric Dam on the New River in 1939 by Appalachian Power Company created a mainstem reservoir (Rosebery 1951). Claytor Lake has a riverine morphometry, with a 1,820-hectare surface area, widths between 0.29 and 0.95 kilometers, 161 kilometers of shoreline, a 15-meter average depth, and a maximum depth of 37.5 meters (Rosebery 1950; Boaze 1972; Kohler et al. 1980; Copeland 1999). The lake is mesotrophic to moderately eutrophic (Kohler et al. 1980), and dimictic with spring and fall turnovers (Boaze 1972). The lake has a mean retention time of 33 days and an annual water level fluctuation of 1.6 meters (Copeland 1999). Upon completion of the dam, the Virginia Department of Game and Inland Fisheries (VDGIF) initiated a stocking program in 1939 that successfully introduced northern-stock walleye into areas of the New River where the historical southern-stock of walleye possibly existed (Hackney and Holbrook 1978). The original southern population of walleye was self-sustaining and reported to grow to large sizes (Hackney and Holbrook 1978). After the Claytor Lake impoundment and the construction of three smaller dams above Claytor Lake, it was thought to be lost. However, Murphy (1981) found that

Claytor Lake probably contained some of this historical, river-spawning stock, albeit apparently mixed with lake-spawning, northern stocks.

The stocking history of walleye in Claytor Lake left the system with a mixed-origin walleye population (Murphy 1981). The first stocking phase began in 1939 and continued to 1946, totaling approximately 650,000 fingerlings (Kohler 1976). These fish were obtained from the Pennsylvania Fish Commission and were progeny of Lake Erie walleye stocks, making them the second genetic stock (in addition to the putative native genetic stock), in the New River (Murphy 1981). No further stockings of walleye took place in Claytor Lake for 28 years. Fish population assessments by Rosebery (1950) and Boaze (1972) showed walleye to be abundant in the lake. Walleye stockings resumed in 1974, when an annual VDGIF survey found no evidence of walleye reproduction (Murphy 1981). This second stocking phase, totaling approximately 274,000 fingerlings, occurred between 1974 and 1979, introducing a third genetic stock from Nebraska and Kansas, progeny of walleye from the Hudson Bay drainage in Minnesota (Murphy 1981). Fingerlings were stocked in alternate years between 1975 and 1979, during which time Murphy (1981) evaluated the genetic stock structure and recruitment of Claytor Lake walleye. He determined that there were at least two genetically distinct stocks of walleye in the lake, and that naturally reproducing populations contributed 33% of total recruitment during 1976-1979. The third phase of stocking, from 1980 through 1996, averaged 60,000 walleye fingerlings annually, and these fish came from a variety of places (Table 1-1) such as Ohio, New York, Kansas, Nebraska, Pennsylvania and Colorado (VDGIF stocking records). Several stockings from the same sources also took place in the upper New River above Claytor Lake in 1995 and 1996. Stocking was terminated in 1997.

Stocking history indicates that current walleye populations in Claytor Lake and the upper New River could represent four or more different genetic stocks of walleye. Recent VDGIF surveys have found several walleye of exceptional size (5 to 7 kilograms) in the upper New River above Claytor Lake. Over the past five years, the state size record has been broken three times, each by walleye caught in the upper New River (VDGIF records, 1999). This suggests that the putative native river stock, which historically included individuals of large size, may still exist. Spatial or temporal

Table 1-1. Stockings of walleye fingerlings into Claytor Lake and the upper New River.

Date	Number	State stock obtained
Claytor Lake		
1939-1946	650,000	Pennsylvania [1]
1974	40,000	Minnesota [2]
1975	65,000	Minnesota [2]
1977	114,000	Minnesota [2]
1979	55,000	Minnesota [2]
1980	67,590	Kansas
1981	55,125	Nebraska
1982	0	No Stocking
1983	75,000	New York [1]
1984	358,000	New York [1]
1985	24,163	New York [1]
1986	52,030	New York [1]
1987	24,412	New York [1]
1988	90,000	New York [1]
1989	0	No Stocking
1990	68,094	Colorado [3]
1991	67,000	Pennsylvania [1]
1992	67,000	Kansas or Colorado [3]
1993	70,670	Kansas and New York [1]
1994	47,771	New York [1]
1995	45,000	Pennsylvania [1]
1996	88,793	Pennsylvania [1]
New River		
1995	38,227	Kansas and Pennsylvania [1]
1996	93,980	Pennsylvania [1]

[1] Origin of walleye is Lake Erie.

[2] Origin of walleye is Kansas and Nebraska.

[3] Origin of walleye is uncertain.

separation of the native stock from the introduced stocks may have prevented or limited introgressive hybridization.

To investigate the possibility of different genetic stocks and the continuing existence of a historical river-stock walleye, a walleye radio telemetry and genetics study was initiated in summer 1997. This study focused on a 68-kilometer segment of the New River, beginning at the spillway of Buck Dam in Carroll County and continuing downstream to Claytor Lake Dam in Pulaski County, Virginia (Figure 1-1). This section features 35 kilometers of free-flowing river and 33 kilometers of the Claytor Lake reservoir. The purpose of this project was to assess the movements and genetic character of walleye stocks in Claytor Lake and the upper New River. The movements of walleyes were tracked through the use of radio telemetry. Individual movements were assessed to quantify the possible degree of migrational exchange among spawning walleye assemblages in Claytor Lake and upper New River. Data collected included characterizations of normal / home ranges, identification of spawning grounds and spawning ranges, and assessment of possible population interchanges between spawning grounds. Screening of collections of walleye using protein-level, microsatellite DNA and mitochondrial DNA genetic markers was carried out to test for the presence of one or more walleye stocks. The intent of this study was to improve the understanding of walleye biology in the Claytor Lake/New River ecosystem and to contribute to management of walleye fisheries throughout Virginia and the southeastern United States.

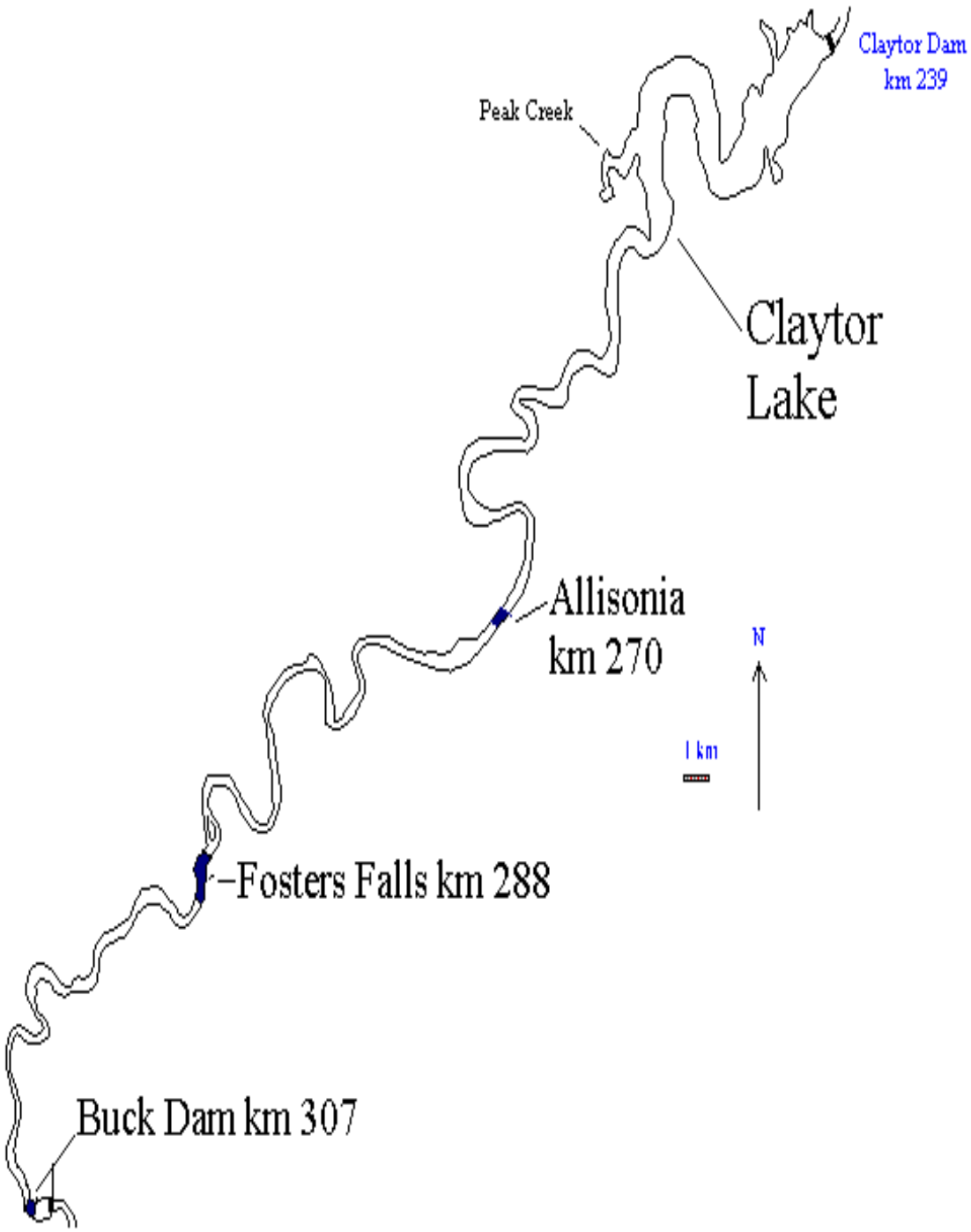

Figure 1-1. Walleye project study area on Claytor Lake and the upper New River, and walleye spawning locations in the New River above Claytor Lake.

Chapter I

Movements and Behavior of Walleye in Claytor Lake and the Upper New River, Virginia

Introduction

The effective management of walleye *Stizostedion vitreum* depends on a fisheries manager's ability to gather crucial information about the habitat needs and behavior of the fish in a given ecosystem. Information on movement patterns, habitat use, and spawning location will support better management decisions. Information gathered in previous walleye studies suggest that it is possible that more than one stock may exist in a given ecosystem, and that they can be genetically distinct (Terre et al. 1995; Jennings et al. 1996).

The upper New River in Southwestern Virginia may contain a unique river stock of walleye that is spatially or temporally segregated from stocked lake stock walleye in Claytor Lake. If stocks within the lake and river system are segregated, different management strategies may be required to sustain stock diversity. To advance better management of walleye stocks in Claytor Lake and the upper New River, radio telemetry was used to improve understanding of fish movements and behavior. The goals of this study were to gather evidence to determine whether the Claytor Lake / upper New River system contains resident lake and river stocks, where these stocks spawn, and whether the stocks mix.

Walleye Movements and Behavior

Walleye spawn in spring as water temperatures increase to approximately 6 - 12°C (Colby et al. 1979). Age at sexual maturity varies, with males and females becoming mature at 2-4 years and 3-6 years, respectively (Colby et al. 1979). The defining difference between lake- and river-stock walleye is the selection of lotic or lentic spawning habitat, respectively (Colby et al. 1979). Lake-stock walleye move to rocky reefs or gravel areas in reservoirs or lakes, while river-stock walleye migrate upstream to headwaters or riffle areas to spawn (Jennings et al. 1996). A telemetry study by Paragamian (1989) found that walleye leave overwintering pools as water temperatures reach approximately 7°C and move upstream to riffle areas to spawn. Schultz (1992)

noted that walleye in Dale Hollow Reservoir, Tennessee spawned in two different locations, the Obey River and the main basin of the reservoir. These stocks remained segregated during the spawning season (Schultz 1992). Because walleye tend to return to the same spawning grounds annually (Olson et al.1978; Paragamian 1989; Schultz 1992), it is possible for lake-stock walleye to remain genetically distinct from river stock walleye.

Following spawning, walleye establish feeding areas which they typically use throughout the warm months (Olson et al. 1978). Summer feeding areas may encompass large areas of lakes, reservoirs, or rivers. Tagging studies often show that walleye tagged in feeding areas are recaptured in the same vicinity after spawning (Forney 1963; Schoumacher 1965; Olson et al. 1978). It is unclear whether a summer feeding area can be considered a seasonal home range. A telemetry study on Center Hill Reservoir, Tennessee, found that walleye either used the entire reservoir or remained within a limited area depending on the individual (Ager 1976). Walleye in the Tombigbee Waterway, Alabama, moved frequently during early summer and held in areas with woody structure in late summer (Kingery and Muncy 1988). Paragamian (1989) found that walleye hold in pools and occasionally moved to other pools in summer. The use of summer feeding areas is highly variable, and walleye may not be restricted to one area. If adequate prey are available and environmental conditions are suitable, walleye may remain in a limited area for some time.

The onset of winter affects walleye in several ways. Cooler water reduces the metabolic rate and the demand for food. Walleye in lake systems move to deeper water to overwinter (Colby et al. 1979). Ager (1976) found that most walleye in Center Hill Reservoir, Tennessee moved from shallower water and flats to the channels of tributary arms or to the main reservoir channel. Schultz (1992) discovered two wintering grounds for walleye in Dale Hollow reservoir, one location in the main reservoir and one in the river feeding the reservoir. Paragamian (1989) noted that walleye in Ceder River, Iowa overwintered in deep pools and remained inactive. Because winter conditions and walleye behaviors vary among geographic locations, walleye activity in and around wintering grounds is probably correlated to length and severity of winter.

Walleye in Claytor Lake / upper New River

Claytor Lake walleye stocking by the Virginia Department of Game and Inland Fisheries (VDGIF) began in 1939 (Murphy 1983). Stocking continued annually until 1946, when sampling data indicated that a population had become established (Table 1-1). Supplemental stockings were made as needed beginning in 1974. A genetics-based study of walleye stock structure and recruitment in Claytor Lake (Murphy 1981, 1983) showed that supplemental stocking contributed 67% of year-class strength to walleye cohorts in the lake. The study also found that more than one genetic stock was present, possibly including a native New River stock. No further genetics study was conducted on walleye in Claytor Lake. Information concerning spawning movements, locations of spawning grounds, and home range of resident stocks was unavailable. Because the New River is at the southeastern edge of the native walleye range, walleye here may not exhibit behavior patterns observed in northern walleye tracked in previous studies.

Currently, there is a strong fishery for walleye in the New River, but a 1998 creel survey indicated that walleye angling constitutes less than one percent of the fishing effort in Claytor Lake (J. Copeland, VDGIF, personal communication). However, the 1998 creel survey excluded the Allisonia area of Claytor Lake where most of the walleye fishing effort occurs (J. Williams, VDGIF, personal communication). This river-reservoir ecosystem possibly could support both lake- and river-stock walleye, which may mandate different management strategies.

The goal of this research was to study the movements and behavior of walleye in Claytor Lake and the upper New River. The study objectives were: (1) to identify home ranges for walleye within the lake and river; (2) to locate spawning sites and infer whether distinct lake-spawning and river-spawning stocks co-exist; (3) to measure spawning ranges and movements of walleye during the spawn; and (4) to make comparisons between lake and river walleye spawning behaviors and ranges, and home range sizes. These data will support improved walleye management in Claytor Lake and upper New River.

Study Site

The study area was a 68-km segment of the New River in Virginia, beginning at the spillway of Buck Dam in Carroll County and continuing downstream to Claytor Lake

Dam in Pulaski County (Figure 1-1). This section features 35 km of free-flowing river and 33 km of Claytor Lake at full pool. Claytor Lake was formed for the production of hydroelectric power (Rosebery 1950). The lake is riverine, with 1,820 ha surface area, widths between 0.29 and 0.95 km, 161 km of shore line, 15 m average depth, and a maximum depth of 37.5 m (Rosebery 1950; Boaze 1972; Kohler et al.1980; Copeland 1999). The lake is mesotrophic to moderately eutrophic (Kohler et al. 1980) and dimictic, with spring and fall turnovers (Boaze 1972). The lake has a mean retention time of 33 d and annual water level fluctuation of approximately 1.6 m (Copeland 1999). The 35-km river section above the lake is free flowing and has common riverine features with interspersed pool, riffle and run habitats.

Methods

Twenty radio transmitters (Advanced Telemetry Systems, Isanti, Minnesota) in the 48 MHz frequency range (48.032 - 48.221), weighing approximately 19.0 g, 17 mm by 46 mm in length, with a signal of 40 pulses per minute (PPM) were used to track walleye in the upper New River. Twenty-three temperature-sensitive radio transmitters in the 49 MHz frequency range (49.341 – 49.651) with the same dimensions were used in Claytor Lake walleye. Radio transmitters in the lake were temperature sensitive in order to determine the depth of the fish. All radio transmitters had a 375-day half-life and a visible identification tag offering a reward for return of the transmitter if found or captured. Radio signals were received using a scannable radio receiver and directional loop antenna (Advanced Telemetry Systems, Isanti, Minnesota).

Walleye were captured using two methods. Experimental gill nets (61-m length with 38-, 51-, 64-, and 76-mm-bar-mesh) were set in Claytor Lake and retrieved at one-hour intervals. Nets were set during November 1997 and 1998 for initial transmitter implants. The same procedure was followed to re-implant transmitters that were lost or captured. Nets were set early in the morning hours. Collections from the spawning sites in spring 1998 and the sites from the upper New River in winter 1997 were made using a boat electrofishing unit with pulsed-DC output (Smith-Root, Vancouver WA). Collections from the spawning areas were made during the day, while other collections were made at night. Walleye weighing at least 0.91 kg were held briefly in a holding

tank to recover from collection prior to implantation. The size criterion of 0.91 kg was chosen because the transmitter should be no more than 2% of the fish's weight (Nielsen 1992).

After capture and acclimation, walleye were anesthetized with tricaine methosulfate (MS-222). Surgical procedures for transmitter implantation followed Hart and Summerfelt (1975), Winter (1983), and Nielsen (1992). Surgical skin staples (6.9 mm width x 3.9 mm length), were used to close the incision (Mortensen 1990). Following implantation, fish were held for approximately 0.5 h for observation before being released.

Radio transmitters were implanted in two phases. In the initial phase, 40 walleye were implanted with radio transmitters from November 1997 through April 1998. Sixteen were females and 24 were males, with an average weight of 1494 g ± 142.1 (SE) and an average length of 518 mm ± 9.3 (SE). Table 1-2 summarizes the locations of walleye radio implants. Following implant completion, signs were posted at access points along the study area to inform anglers of the project, and rewards were offered for the return of transmitters captured in tagged walleye.

The second phase of transmitter implants began in November 1998 and was completed in February 1999. Ten walleye were implanted during this phase. Seven radio tags were from angler-harvested fish and three were new transmitters. Six individuals were female and four were male, with an average weight of 1146 g ± 49.1 (SE) and an average total length of 518 mm ± 16.1 (SE) (Table 1-2).

The 68-km study area was divided into 0.25-river-kilometers (RKM) sections using previously measured river miles (American Electric Power Company). Walleye were tracked weekly, and locations were plotted in the field on a 7.5-minute topographic map. Walleye tracking began in November 1997 following initial implantation of Claytor Lake walleye. Tracking consisted primarily of daytime locations. Walleye were located by boat on Claytor Lake and by vehicle from the New River Trail, which paralleled the upper New River. These methods allowed the entire study site to be covered except for a 3-km section of the trail, which was blocked from access. Fish were located and locations were recorded by RKM to the nearest 0.25 kilometer. A global positioning system (GPS /Magellan) also was used to record fish locations in

Table 1-2. The two phases of walleye radio transmitter implantation, and the associated statistics. (Refer to figure 1 for tagging locations within the study site)

Phase	Season	Number of Walleye Implanted	Location Implanted
1	Fall 1997	10	Lower Claytor Lake
1	Fall 1997	10	Upper New River above Fosters Falls
1	Spring 1998	10	Allisonia
1	Spring 1998	8	Buck Dam
1	Spring 1998	2	Fosters Falls
2	Fall 1998	6	Lower Claytor Lake
2	Spring 1999	4	Upper New River above Fosters Falls

Claytor Lake. Depth of walleye in Claytor Lake was estimated by comparing water column temperature profiles to temperature-regulated pulse rates from radio transmitters. This was done only when the lake was thermally stratified. Water temperature was recorded weekly from both the lake and river. Tracking usually consisted of one day on Claytor Lake and one day on the New River Trail.

Data Analyses

Data collected from radio tracking were analyzed in terms of RKM location. Locations (RKM) for each walleye were plotted against location dates. Data from all telemetered walleye were examined individually. Walleye implanted with radio transmitters within Claytor Lake or at the Allisonia spawning site and maintaining a home range within the lake were considered resident Claytor Lake walleye for the purpose of this study. Walleye implanted with radio transmitters in the New River above the lake, and establishing home ranges within the river were considered resident New River walleye for the purpose of this study.

Movements to spawning areas were differentiated from normal home-range movements by identifying large distances moved in short time periods during the months of February, March, and April. These movements usually were associated with movements of other telemetered walleye. Total distances traveled to and from spawning grounds were calculated for each fish using its identified spawning site as the most distant location. Spawning sites were identified as the areas where telemetered walleye congregated once they left their normal home range areas. A pre-spawn starting point for each fish was calculated by taking an average of four RKM locations prior to spawning movement. A post-spawn ending point for each fish was calculated by taking an average of four RKM locations following the return from spawning. Total distance traveled to and from the spawning area was averaged to get the mean distance moved during spawning for each walleye. Total distances were compared between the lake and river walleye using the sample median of each group.

A modified probability level approach was used to identify the 95% utilization distribution of each individual fish within the study site. This distribution then was used as an estimate of home range (White and Garrott 1990). Location data collected in this

study were linear scale data; because the data were not normally distributed, a prediction interval (PI) was used to approximate the 95% home range. The interval was calculated as:

$$PI = (R/(N+1)) \quad (1)$$

where: PI is the percentage of the total range desired, in this case it is 0.95, N is the total number of locations for an individual fish, and R is what you are solving for, it is equal to the number of locations that encompasses 95% of the total number of locations. This statistic breaks the observations into spatial intervals. A fish's 95% home range is equal to the innermost 95% of the spatial intervals, in essence cutting off the outermost top and bottom 2.5% of the intervals. The return of a fish from the spawning ground was used as the identifier for the establishment of the home range. The home range included all the locations observed from the return from the spawn throughout the year until the walleye moved to spawn the following season. The data were ordered and Equation 1 was used to describe the home range. This 95% prediction range, hereafter referred to as the home range, eliminates observations or individual locations that appear to be outliers among the observed data. White and Garrott (1990) explain that home range is not all the area that an animal traverses, but rather the area where it normally moves. Points outside the normal location area, if infrequent, may be regarded as outliers. For my analysis, I did not consider spawning movements as part of the normal home range, but rather as a separate range unto itself.

Range sizes were contrasted among and between lake-tagged walleye and river-tagged walleye. Range size comparisons among lake walleye were examined using an exploratory data analysis technique, the box plot. Range sizes were compared to identify the existence of ranges that were significantly larger or smaller than the median range size of the group. Individual ranges were regarded as significantly different when they were ≥ 1.5 interquartile ranges (IQRs) outside of the inner 50% of the overall distribution. This is approximately equal to two standard deviations of the mean. The same process was used for comparing range sizes of river walleye. Comparison of the median range sizes between lake and river walleye was performed using a Mann-Whitney test.

Results

A total of 52 walleye were implanted with radio tags (Table 1-3). Anglers harvested nine walleye and five walleye died following surgery, thus leaving 38 telemetered walleye for tracking. The number of locations identified for each fish ranged from 30 to 90.

Walleye in Claytor Lake and the upper New River had different spawning and home range movement patterns (Figure 1-2). Individual walleye remained within certain areas of either the lake or the river throughout the summer, fall, and winter until the spring spawning season, at which time they migrated into the river or upriver to spawn. Telemetered walleye migrated to spawning areas beginning in mid-February when river surface water temperatures were 6-8°C. Most telemetered walleye congregated at one of three major spawning areas (refer to map in Figure 1-1) by mid-March. Male walleye generally reached the spawning areas one or two weeks prior to females. In 1998, males averaged reaching the spawning grounds on February 18, and females on March 8 (N = 6 and 6, respectively). In 1999, males averaged reaching the spawning grounds on February 27 and females on March 6 (N = 10 and 14, respectively). Walleye remained on or near the spawning sites until late April or early May when the water temperature in the river averaged 15-17 C. Walleye then returned to the areas previously occupied and reestablished home ranges.

Spawning

Telemetered walleye spawned in three different spawning locations (Figure 1-1), all located in the river. The Allisonia spawning site (RKM 270) is the first riffle section located above Claytor Lake. The area is approximately 100 m wide with cobbles creating riffles and washed gravel areas below. This site was heavily used by lake walleye: 83% of all walleye radio-tagged in Claytor Lake spawned here, and 63% of the tagged 1998 spawning fish returned to spawn again in 1999. The Fosters Falls spawning site (RKM 288) is 18 km upstream of Allisonia, below the largest set of rapids within the study site. Fosters Falls was the spawning site least used by telemetered walleye. However, when sampled, it contained the most spawn ready walleye of any site. Of the three telemetered walleye that spawned at Fosters Falls, one was from the lake and two from the river. The

Table 1-3. Walleye radio-tagged and associated statistics. An asterisk by the identification number in column 1 indicates a walleye that was harvested by anglers.

Walleye ID #	Location	Site Tagged	Date Tagged	Sex	Length (mm)	Weight (g)	Outcome
48.032	New River	Fosters Falls	2/13/98	F	507	1300	Died
48.042	New River	Austinville	1/28/98	F	742	4600	Tracked
48.052	New River	Austinville	1/25/98	M	572	1600	Tracked
48.061	New River	Austinville	1/31/98	M	510	1200	Tracked
48.071	New River	Austinville	2/17/98	M	530	960	Tracked
48.081	New River	Austinville	3/4/98	F	490	1000	Tracked
48.091	New River	Buck Dam	3/30/98	F	572	1200	Tracked
48.101	New River	Austinville	1/26/98	M	530	1680	Tracked
48.111	New River	Austinville	1/19/99	F	585	1500	Tracked
48.121	New River	Buck Dam	3/30/98	M	558	1200	Tracked
48.131	New River	Buck Dam	3/30/98	M	535	1200	Tracked
48.141	New River	Buck Dam	3/30/98	M	460	1200	Tracked
48.151	New River	Fosters Falls	3/4/98	F	755	4750	Died
48.161	New River	Buck Dam	3/30/98	F	621	1200	Tracked
48.171	New River	Buck Dam	3/30/98	M	475	1200	Tracked
48.181	New River	Austinville	12/30/98	M	542	1000	Tracked
48.191	New River	Buck Dam	3/30/98	M	518	1200	Tracked
48.201	New River	Austinville	2/17/99	F	595	1200	Tracked
48.211	New River	Austinville	1/27/98	M	543	1320	Tracked
48.221	New River	Buck Dam	3/30/98	M	508	1200	Tracked
49.341	Claytor Lake	Lower Lake	11/24/97	M	445	919	Tracked
49.351	Claytor Lake	Allisonia	2/25/98	F	655	4000	Tracked
49.361	Claytor Lake	Allisonia	2/25/98	M	493	1300	Tracked
49.371	Claytor Lake	Lower Lake	11/22/97	F	555	1200	Died
49.381	Claytor Lake	Lower Lake	11/20/97	M	510	1200	Tracked
49.391	Claytor Lake	Lower Lake	11/21/97	F	493	1200	Tracked
49.401	Claytor Lake	Lower Lake	11/23/98	M	470	950	Tracked
49.411	Claytor Lake	Allisonia	2/25/98	F	535	1700	Tracked
49.421	Claytor Lake	Lower Lake	11/19/97	M	484	1200	Tracked
49.431	Claytor Lake	Allisonia	2/25/98	F	471	1000	Died
49.441	Claytor Lake	Lower Lake	11/12/98	F	480	1300	Tracked
49.451	Claytor Lake	Lower Lake	11/19/97	M	461	1200	Died
49.461	Claytor Lake	Allisonia	2/25/98	F	594	2500	Tracked
49.471	Claytor Lake	Allisonia	2/25/98	M	471	1100	Tracked
49.481	Claytor Lake	Lower Lake	11/19/97	F	480	1200	Tracked
49.491	Claytor Lake	Allisonia	2/25/98	F	513	1500	Tracked
48.501	Claytor Lake	Fosters Falls	4/8/98	M	460	1350	Tracked
49.511	Claytor Lake	Lower Lake	11/23/98	F	475	1100	Tracked
49.521	Claytor Lake	Lower Lake	11/25/97	F	439	1200	Tracked
49.530	Claytor Lake	Lower Lake	11/26/97	F	460	1200	Tracked
49.611	Claytor Lake	Lower Lake	11/23/98	F	479	1000	Tracked
49.631	Claytor Lake	Lower Lake	11/23/98	F	489	1100	Tracked
49.651	Claytor Lake	Lower Lake	11/23/98	M	490	1100	Tracked
48.071*	New River	Fosters Falls	12/17/97	M	465	1200	Caught
48.111*	New River	Austinville	2/27/98	M	485	1000	Caught
48.161*	New River	Fosters Falls	3/4/98	F	515	1500	Caught
48.181*	New River	Austinville	1/31/98	M	466	1000	Caught
48.201*	New River	Austinville	1/24/98	M	490	1000	Caught
49.401*	Claytor Lake	Allisonia	2/25/98	M	465	1000	Caught
49.441*	Claytor Lake	Lower Lake	11/23/97	M	556	1200	Caught
49.501*	Claytor Lake	Allisonia	2/25/98	M	468	1000	Caught
49.511*	Claytor Lake	Allisonia	2/25/98	M	490	1400	Caught

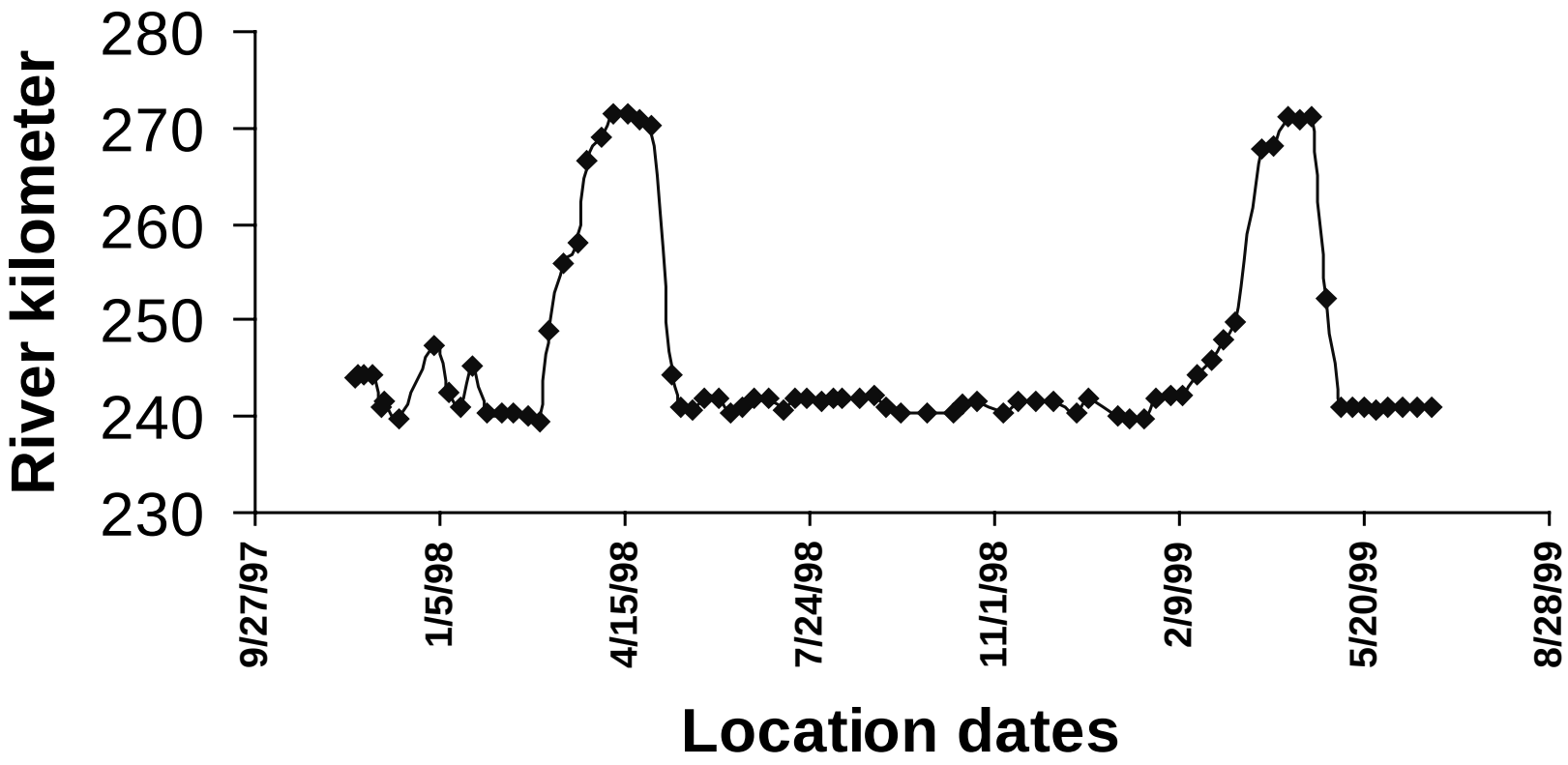

Figure 1-2. Location as a function of time for walleye number 49.521, showing the clear distinction between spawning movements and normal range movements exhibited by most walleye tracked in the study.

third spawning site was located below Buck Hydroelectric Dam (RKM 307), 37 km above Allisonia at the upstream end of the study site. The spawning area was located just below the spillway of the dam and in the riffle areas directly below. This site consists of clean gravel and cobble interspersed between riffles. Buck Dam was heavily used as a spawning site by river walleye; 85% of the walleye tagged in the river spawned in this section. In general, lake telemetered walleye typically spawned at Allisonia, while river telemetered walleye spawned at Buck Dam, although several walleye from each group spawned at sites different from the majority (Figure 1-3).

Distance traveled to spawn differed between lake- and river-telemetered walleye. In 1998 and 1999, the median spawning migration distances for lake-telemetered walleye were 24.7 and 21.1 km, respectively (Figures 1-4 & 1-5). During both years, most lake telemetered walleye that spawned at Allisonia migrated for 7-12 days to reach the site. However, several walleye completed the distance within a week. In 1998 and 1999, the median distance traveled to spawn for river-telemetered walleye was 9.6 and 4.0 km, respectively (Figures 1-6 & 1-7). River-telemetered walleye reached their spawning sites in 6-7 days. The difference between the 1998 and 1999 spawning migration distances for river walleye could be attributable to a low number of repeat spawners. Most river telemetered walleye that spawned in 1998 did not spawn in 1999. The 1999 river spawners were walleye that were tagged at Buck Dam in the 1998-spawning season.

Home Range

Post-spawn walleye generally returned to previously occupied areas. The home range was regarded as including all the area used by the walleye exclusive of the spawning movements. The sizes of areas used differed between fish. Lake-telemetered walleye established home ranges within the lake, and river-telemetered walleye established home ranges in the river, with no exceptions (N = 19 each).

The home range distribution of most telemetered walleye in Claytor Lake fell within the lower two-thirds of the lake, between RKM 239 and 259 (Figure 1-8). Four fish had home ranges extending beyond RKM 259, but these ranges did not extend past RKM 265. Two walleye had home ranges that overlapped both the lake and river, and

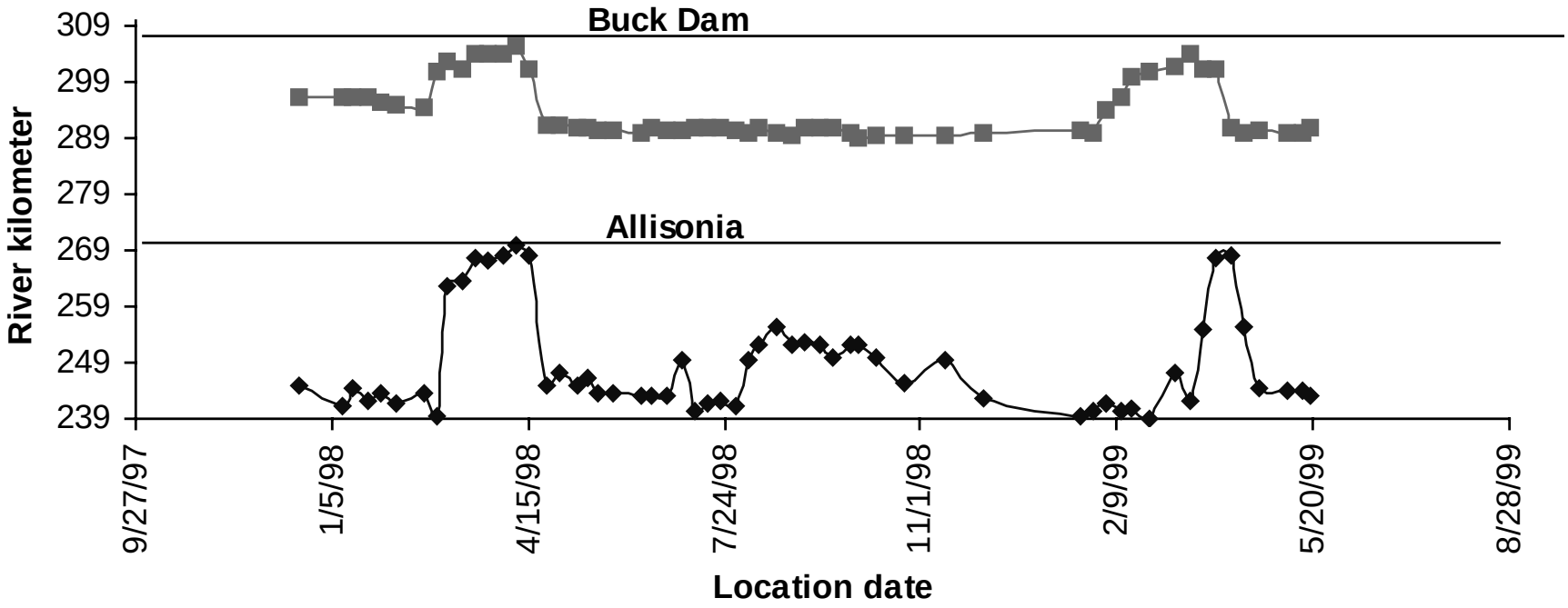

Figure 1-3. Location as a function of time for one lake and one river walleye; demonstrating the use of different spawning areas. The locations under the Allisonia line represent the lake walleye.

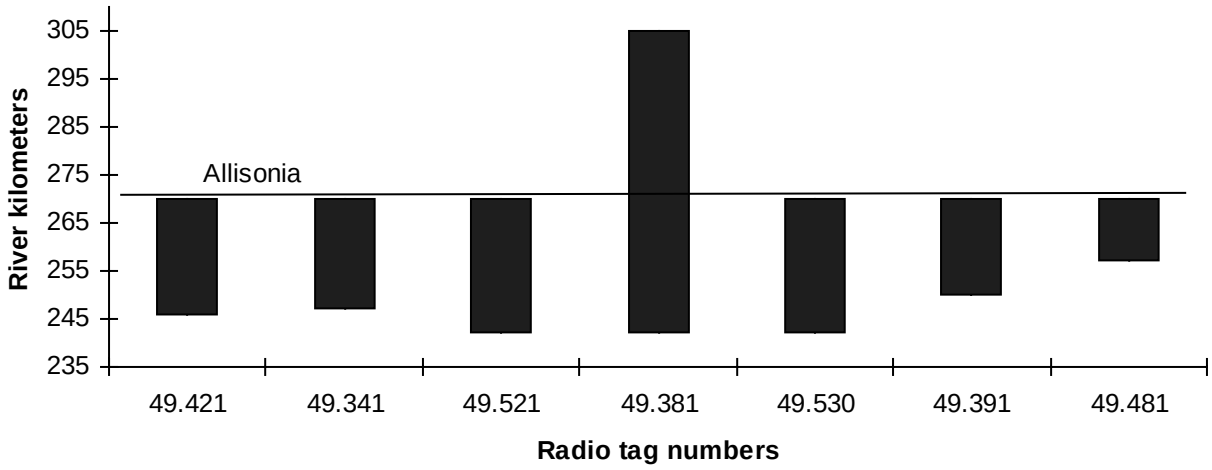

Figure 1-4. Radio tagged walleye from Claytor Lake that spawned in the 1998 spawning season and their associated spawning migration ranges.

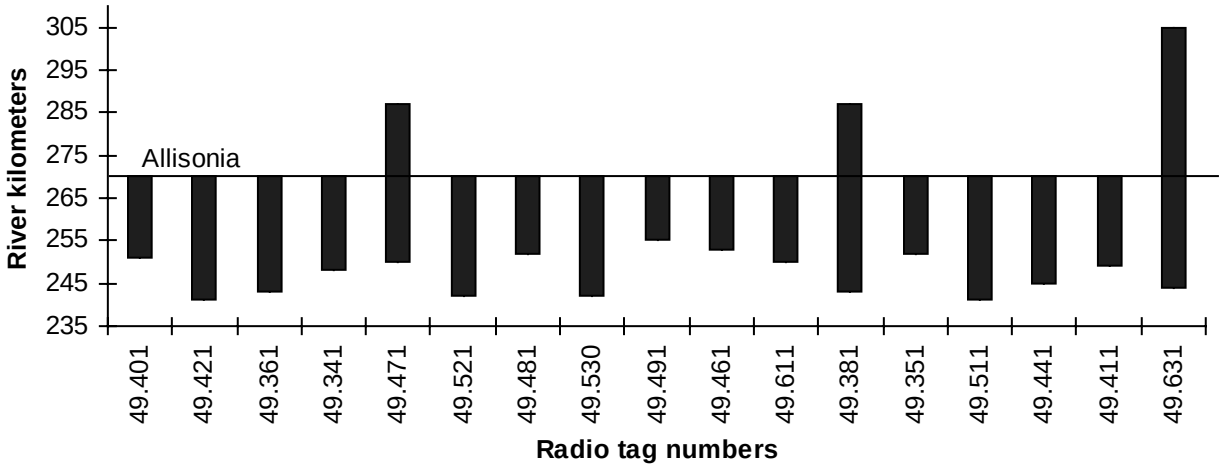

Figure 1-5. Radio tagged walleye from Claytor Lake that spawned in 1999 and their associated spawning migration ranges.

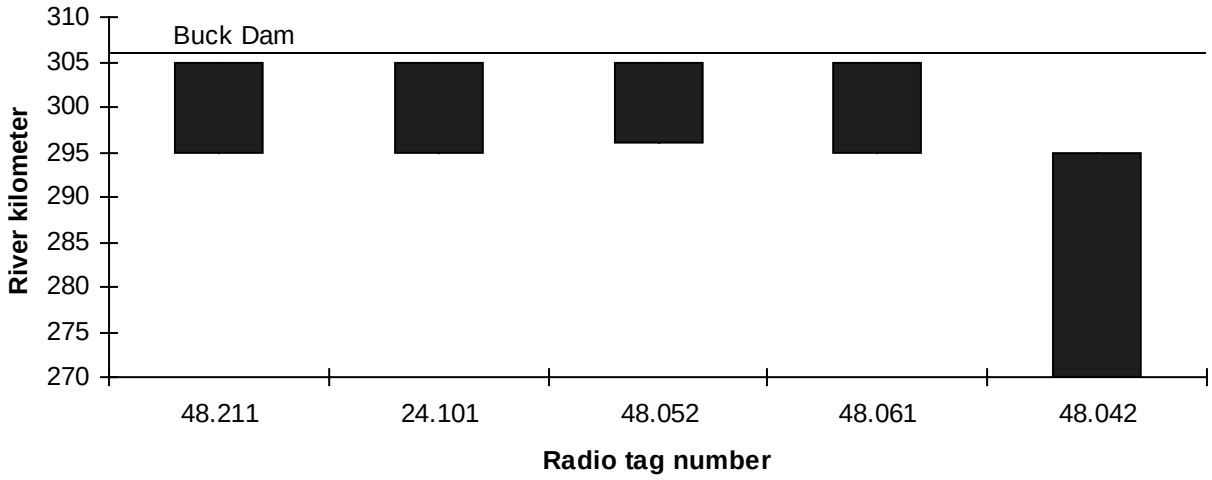

Figure 1-6. Radio tagged walleye from the upper New River that spawned in the 1998 spawning season and their associated spawning migration ranges.

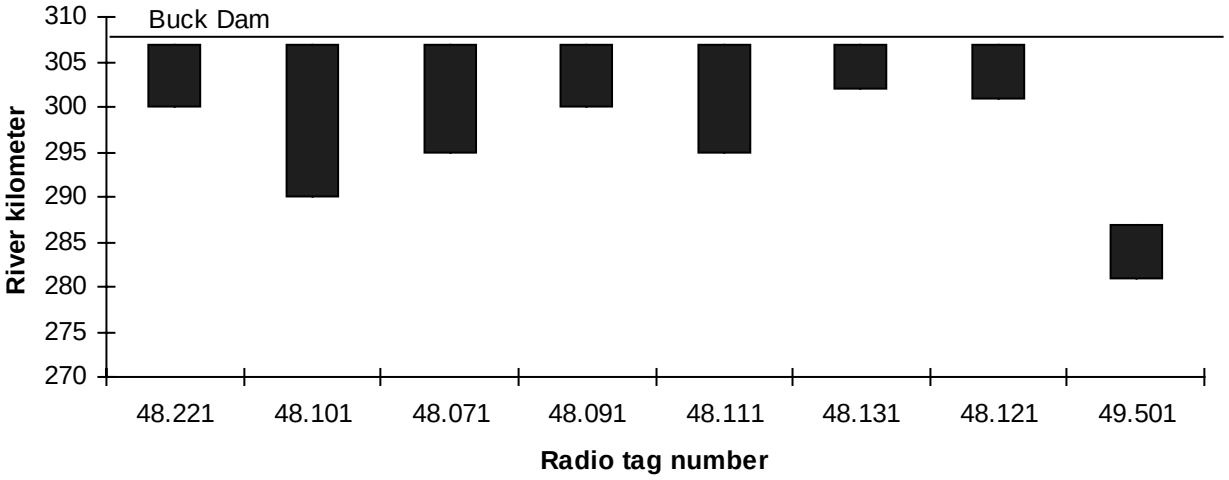

Figure 1-7. Radio tagged walleye in the upper New River that spawned in 1999 and their associated spawning migration ranges.

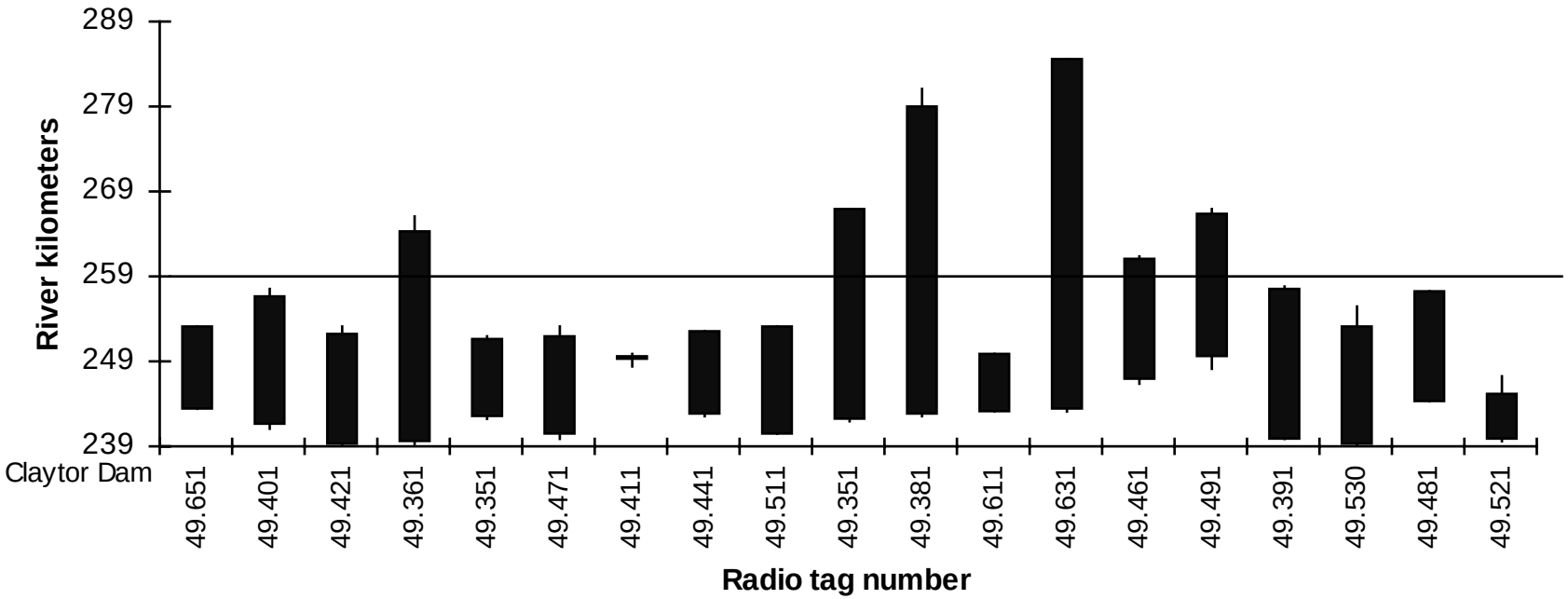

Figure 1-8. Home ranges of walleye in Claytor Lake. The horizontal line at RKM 259 is a reference point to show that the majority of these home ranges fall within the lower two-thirds of the lake.

both these fish spawned at Buck Dam (Figure 1-9). However, the majority of the time these fished were tracked, they remained in the lake area. The median 95% home range size for lake-telemetered walleye was 13 RKM. The sizes ranged from 0.25 to 41.2 RKM. Two walleye had ranges that were considered to be outliers relative to other ranges (Figure 1-10). These were the two fish whose home ranges extended into the river. Most walleye moved frequently within their normal home range area. It was not uncommon to find walleye two to five RKM from previous weekly locations. Two lake-telemetered walleye exhibited very narrow movement patterns, remaining in almost the same location weekly (Figure 1-9). There was no difference among male and female walleye home range sizes in Claytor Lake (Wilcoxon-Mann Whitney, $T = 0.52$, $p = 0.61$, $df = 17$). The majority of lake-telemetered walleye transversed large areas of Claytor Lake.

The home ranges of all but one river-telemetered walleye were located in the upper 18 km of the study site above Fosters Falls (Figure 1-1 & 1-11). One walleye maintained its home range within a six-RKM area below Fosters Falls; this individual was the only river-telemetered walleye implanted below Fosters Falls. All other walleye maintained home ranges within large pools in the river. Thirteen of 19 radio tagged walleye congregated in two pools. The remaining five walleye remained in smaller pools dispersed throughout the river above Fosters Falls. The habitat of these pools consisted largely of cobble interspersed among boulders along with undercut banks. Some pools had wood debris piles. River-telemetered walleye moved very little within the pools where they resided. River walleye never were observed traveling down river into the lake area below the Fosters Falls location, with but the one exception.

The median home range for radio telemetered walleye in the upper New River was 4.7 RKM. The sizes ranged from 1.8 to 9.5 RKM. A comparison of the ranges identified one outlier (Figure 1-11). There was no difference among male and female walleye home range sizes (Wilcoxon-Mann Whitney, $T = -0.13$, $p = 0.90$, $df = 17$). When flows were low during the summer, walleye moved into deeper areas of pools. No other location changes were observed. River walleye had smaller home range sizes than lake-telemetered walleye; Mann-Whitney test result was significant ($W = 218$, $p = .00001$).

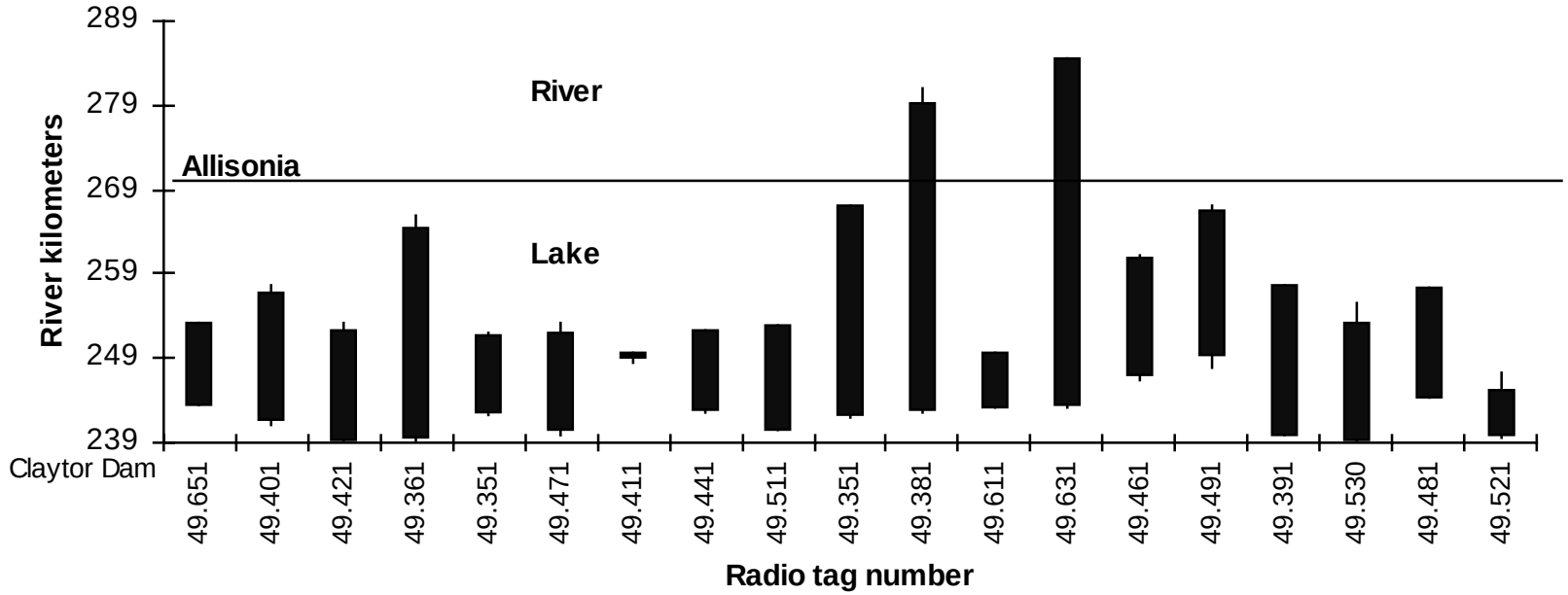

Figure 1-9. Home ranges for all radio-tagged walleye in Claytor Lake. The dark bar is the 95% prediction interval for each fish. Kilometer 239 is Claytor Lake Dam and kilometer 270 is Allisonia.

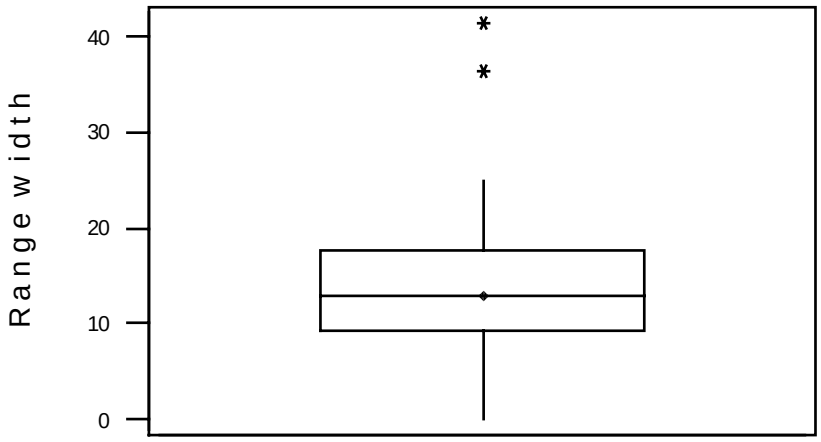

Figure 1-10. Range analysis for lake-telemetered walleye, showing the distribution of home range sizes and two outliers, which fall more than 1.5 inner quartile range distance away from the median range.

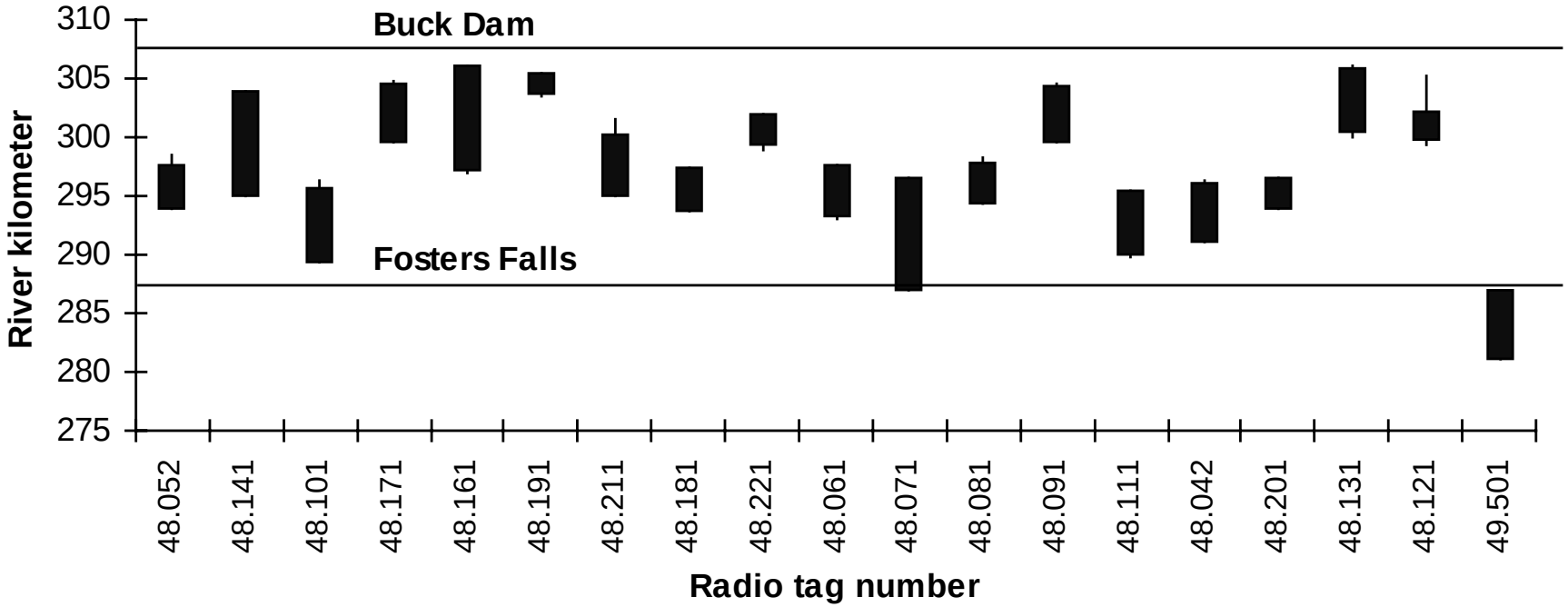

Figure 1-11. Home ranges of radio tagged river walleye. The dark bar indicates the 95% prediction interval for each fish. The two lines indicate locations of Fosters Falls and Buck Dam.

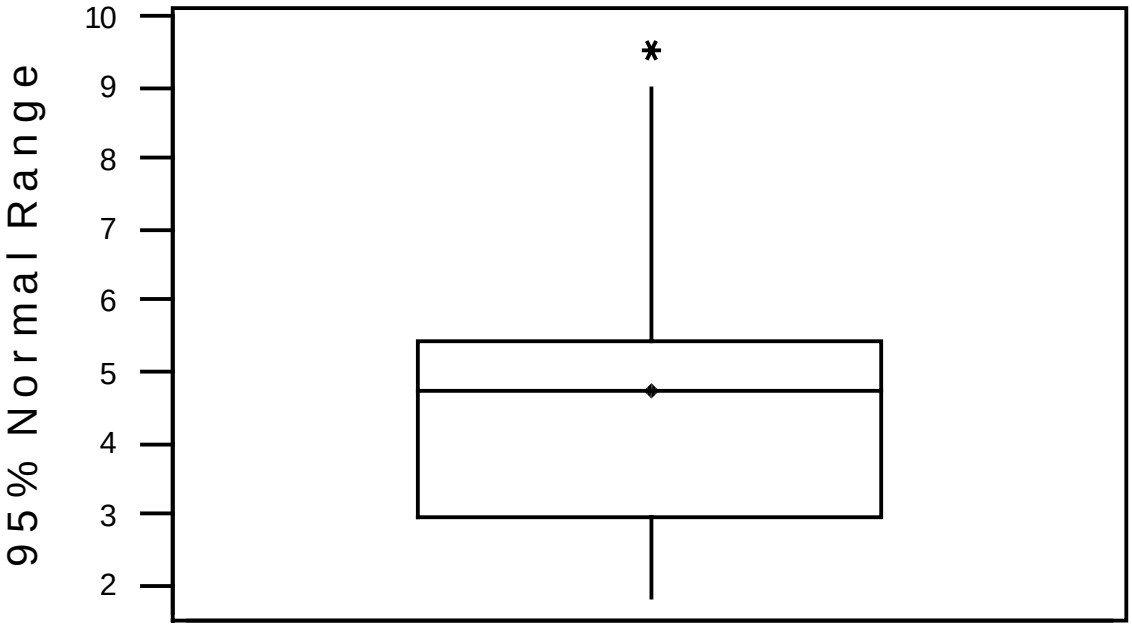

Figure 1-12. Box plot of the 95% normal range size distribution of walleye in the upper New River. There is one outlier among the range sizes indicated by the star in the graph. The outlier falls at least 1.5 inner quartile range distance away from the distribution median.

Discussion

Spawning

Previous studies of walleye in both lentic and lotic environments have shown that walleye within the same system may use different spawning sites (Olson and Scidmore 1962; Crowe 1962; Olson et al. 1978; Parks and Kraai 1991; Jennings et al. 1996). Two explanations have been offered regarding choice of spawning site by walleye. Some studies suggest that choice of spawning site is influenced by natal imprinting or is a heritable trait that influences walleye to home to preferred spawning areas (Olson and Scidmore 1962; Crowe 1962; Jennings et. al 1996). The second explanation is that site selection is a learned behavior; walleye would follow other walleyes to spawning sites and repeat these movements in consecutive years (Olson et al. 1978). Olson et al. (1978) found walleye imprinting unlikely because wind and river currents move eggs before the fry develop. Olson and Scidmore (1978), and Olson et al. (1962) believed that if walleye did imprint it was weak.

Walleye choice of spawning sites in the present study could be explained by either of the two mechanisms presented above. Walleye in Claytor Lake and the New River come from a variety of locations across the United States and represent both lake and river stocks. Claytor Lake contains spawning habitat suitable for lake stock walleye but no evidence of such walleye spawning has been previously documented (Murphy 1981). Lake spawning was not observed in this study, but it was thought possible and the study was designed to consider this factor. However, all lake-tagged walleye spawned in areas of the New River above Claytor Lake. It is possible that lake spawning does occur at low frequency, such that it was not identified in this study. If supplementally stocked walleye spawn, these fish could be choosing spawning areas through adult-learned behavior. These fish could be influenced by the walleye spawning at Allisonia.

The majority of river-telemetered walleye spawned at Buck Dam. The selection of this spawning area also may result from natal imprinting or learned behavior, as discussed above. However, spawning at the suitable site farthest upstream could result from dam blockage. The area of the river above the lake was stocked with walleye only in 1995 and 1996 (VDGIF, unpublished data). Some walleye in this section may be

naturally reproduced. Naturally reproduced walleye may have a natal imprint to spawn at Buck Dam even though there are other suitable spawning sites available. Stocked walleye, therefore, may be learning from naturally reproduced walleye where to spawn. This could explain the large concentrations of walleye seen at the Buck Dam site.

Three lake- and two river-telemetered walleye displayed unusual spawning migration patterns. The lake-tagged walleye migrated either an additional 18 RKM above Allisonia to Fosters Falls or an additional 37 RKM above Allisonia to Buck Dam to spawn. The river-tagged walleye migrated downstream, up to an additional 20 RKM, to spawn at Fosters Falls or Allisonia. These fish may be naturally reproduced walleye that are homing to their natal sites.

Home Range

Based on this telemetry study, I believe that the Claytor Lake / upper New River system contains resident river and resident lake walleye populations. These populations generally remain separated during the year, but can mix during the spawn. There is no barrier to movement between these populations.

The habitat use of walleye within this study site differed from that found in other studies. Lake-telemetered walleye often were observed in the main channel of the lake. These walleye usually were suspended in open water or on rock shelves near main channel drop-offs. Only a few walleye were found to remain in coves for brief periods of time (i.e., < 3 weeks). This is contrary to the telemetry results of other walleye studies in southern reservoirs. In two different studies in Kentucky, walleye were found mostly in coves on reservoirs, and almost always associated with wood structure or standing timber (Williams 1997; Wilson 1997). Parks and Kraai (1991) found telemetered walleye in Meredith Reservoir, Texas to be located frequently along brushy or rocky shorelines. Ager (1976) reported that walleyes were located in the main channel of Center Hill Reservoir during the early spring and early autumn, however they moved to major tributary channels and headwaters of tributary embayments in winter and summer. In contrast, Claytor Lake is narrow and steep-sided. The shoreline is not as dendritic as most other southern reservoirs that have walleye populations. Coves and shoreline have limited wood cover. This could explain why Claytor Lake walleye generally were found

in the main channel and not in coves. The lake is riverine in character, and walleye located near rock ledges or shelves were generally within 10 meters of the main channel. The lake has a large prey base of gizzard shad and alewife (Copeland 1999), and most lake walleye probably rely on this food source.

Other than spawning migrations, there was no identifiable seasonal trend in walleye locations. Walleye did not shift to other areas of the lake or change habitat use patterns. During the months of June-September, walleye in Claytor Lake suspended above the thermocline in 11 to 14 meters of water. When it was overcast, walleye were found closer to the surface. Walleye also were found near the surface when tracking at night, although this was done infrequently.

Habitat selection by telemetered walleye in the upper New River, other than during spawning migration, showed no seasonal shift. Studies of walleye in river systems showed walleye movements differ seasonally. Paragamian (1989), Kingery and Muncy (1988) and Smith et al (1952) demonstrated a wide range of seasonal movements of walleye. In the Tombigbee River, Kingery and Muncy (1988) found walleye moved 4-43 km from the tagging site. Paragamian (1989) reported extensive walleye movement in Ceder River, Iowa. However, walleye in the upper New River above Claytor Lake tended to be sedentary. This could be a result of the locally suitable environment and available food sources, or limited suitable areas to which to move. Stomach contents of New River walleye in this study indicated that river walleye ate minnows, darters, log perch and sunfishes, indicating that these fish are opportunistic predators. If the pools which river walleye inhabit contained adequate prey, walleye would not need to move to other areas in the river seasonally.

River walleye tended to have smaller home ranges than lake walleye in this study. I hypothesize that walleye within the lake are nomadic. This itinerant habit may be correlated with the movements of alewife schools. Walleye in reservoirs commonly demonstrate nomadic behavior (Ager 1976; Holt et. al 1977; and Williams 1997). The small home range size of river walleye could be a result of microhabitat and prey distributions in the river habitat.

Home range sizes for walleye in the present study cannot be accurately compared to home ranges of walleye in other studies. Studies estimating walleye home ranges in

lentic systems have used different methods for measuring range size, (i.e. grid-square or polygon methods). Such methods calculate home ranges in surface area. In this study, we characterized ranges linearly due to the riverine nature of the study area.

Management Implications

To better manage walleye in Claytor Lake / upper New River, I recommend that the data gathered in this study be applied to the current walleye management efforts. This system, if managed correctly, may be able to support a stronger walleye fishery.

Fishing pressure on walleye focuses on the river stock for most of the year and on both the lake and river stock during the spawning season. Intensive angling pressure occurs at all three spawning sites February - May when walleye are congregated. During the 1998 season, anglers caught 30% of the telemetered walleye. In the 1999 season, another 16% were caught. During this time, angling is focused primarily on walleye, and many anglers harvest heavily throughout the upper river. This is not the case on Claytor Lake. During the study, only one telemetered walleye was caught in the lake proper. Data from a 1998-99 creel survey on Claytor Lake, (J. Copeland, VDGIF, personal communication) showed that walleye fishing constituted less than one percent of total fishing effort and that harvest was very low. This however, is not an accurate picture of walleye fishing effort and harvest throughout the entire study site because the creel survey on Claytor Lake did not include the Allisonia area or the upper river. The nature of the Claytor Lake / upper New River walleye fishery tends to make the lake stock vulnerable to fishing only during the spawning season, while the river stock is vulnerable year-round. Because of this trend, different regulations restricting the harvest of walleye during February - May could be justifiable. A size minimum limit or smaller creel limit may reduce the impact of harvest by anglers. Reports of trophy walleye catches in the upper New River by anglers and popular magazines (e.g., Walleye Insider, March 1999), has caused an increased interest in the fishery. If fishing pressure increases even more in the future, the current creel limit of eight fish / day may not be restrictive enough to sustain the population. However, if supplemental stocking of walleye is reinitiated in both the lake and river, the current limit harvest regulation regime may be adequate; population dynamics modeling is needed to anticipate likely outcomes.

Previous walleye stockings have come from areas outside the New River drainage. If stocking done in the future, I recommend collecting putative native walleye broodstock (chapter 2) from spawning sites identified in this study. Progeny from these fish then would be used to continue walleye stocking into the Claytor Lake / upper New River system. If walleye home to spawning areas and if this is a genetically determined trait, collecting brood stock from local New River spawning areas and re-stocking with progeny from the putative native stock may result in larger spawning assemblages and improved spawning success of walleye in the future. Collecting broodstock within the system and planting of young also may help sustain any native or unique walleye stock that may exist in this system. Such river-stock walleye may be better adapted to the Claytor Lake / upper New River system and may exhibit higher recruitment than other stocks.

Information on home ranges can be used to direct angler efforts towards the Claytor Lake walleye fishery that is currently under-utilized (J. Copeland, VDGIF, personal communication), to develop effective stocking protocols, and to quantify the amount of suitable habitat available. Walleye in Claytor Lake have home ranges that are near 13 RKM in length and generally were observed in the lower two-thirds of the lake. Walleye in the lake suspend in open water and on rock shelves making them hard to catch and sample correctly. With this information, VDGIF fisheries managers can educate anglers about walleye habits, to help anglers target the walleye. Range information also can be used to increase the success of both sampling and management efforts.

Home range information also can be useful in making stocking decisions. Knowing that walleye in the river have a limited range and remain mostly in pool habitat can lead to determination of suitable available habitat. The available habitat will influence the survival of walleye within the river. Stocking numbers can be increased or decreased and location optimized based on this habitat and range information.

Chapter II

Genetic Analysis of Walleye Stocks in Claytor Lake and the Upper New River

Introduction

Walleye (*Stizostedion vitreum)* is a highly valued sportfish species. The native range of walleye is thought to reach throughout most of Canada into the eastern half of the United States, narrowing as it reaches south towards the Gulf of Mexico (Colby et. al 1979). The New River in southwestern Virginia is located on the eastern edge of the native range of walleye distribution; whether walleye are native to the New River is debated (Jenkins and Burkhead 1994).

The New River originates in North Carolina, flows north across Virginia, and northwest through West Virginia. It meets the Gauley River and forms the Kanawha River, which flows into the Ohio River (Jenkins and Burkhead 1994). The New River is the oldest major stream in the United States. The New River, formerly the Teays River, flowed directly into the Gulf of Mexico until the advance of northern glaciers buried the lower two-thirds of its course (Jenkins and Burkhead 1994). The southeastern, upstream portion of the river could have provided a glacial refugium for walleye. Subsequent migration from downstream was blocked by Kanawha Falls in West Virginia. Hence, native walleye stocks in the refugium would have remained segregated from other stocks, thereby preserving any genetically unique characters.

Walleye have been stocked outside of their native range throughout most of the United States (Hackney and Holbrook 1978). The stocking of walleye from different geographic origins has resulted in many ecosystems containing mixtures of native and introduced walleye stocks. This could lead to loss of any genetically unique, local walleye stocks through introgressive hybridization.

Genetically distinct stocks of walleye have been mixed in Claytor Lake and the upper New River, Virginia as a direct result of stocking. The first known walleye stocking was conducted in 1920 by the US Fish Commission (Jenkins and Burkhead 1994). The Virginia Department of Game and Inland Fisheries began a walleye stocking program in 1939 following the completion of Claytor Lake Dam that introduced several

different genetic stocks of walleye (Murphy et. al 1983). Showing that at least some of these stockings were successful, results of a survey of isozyme markers (Murphy et. al 1983) were consistent with the existence of multiple walleye stocks in Claytor Lake / the upper New River. All stocking was suspended in 1997.

Among mixed stocks in the New River system there may be a genetically unique native walleye stock, a stock whose integrity is threatened by the addition of genetically different walleye stocks. In the past few years, several large (5-7 kg) walleye have been found in the upper New River above Claytor Lake. These observations sparked interest as to whether these large fish may be part of a native stock of walleye. Walleye are known to grow to large sizes in the southern areas of the walleye range (Hackney and Holbrook 1978). This study was undertaken to characterize the genetic population structure of walleye in Claytor Lake and the upper New River. The results of the study could be used to improve the management plan for the walleye fishery within the ecosystem.

Study Site

The study area was a 68-km segment of the New River in Virginia, beginning at the spillway of Buck Dam in Carroll County and continuing downstream to Claytor Lake Dam in Pulaski County, Virginia (Figure 2-1). This section includes 35 km of free-flowing river and 33 km of Claytor Lake reservoir. Claytor Lake has a riverine shape, with a 1,820-ha surface area, widths between 0.29 and 0.95 km, 161 km of shoreline, a 15-m average depth, and a maximum depth of 37.5 m (Copeland 1999; Kohler et al. 1980; Boaze 1972; Rosebery 1950). The lake is mesotrophic to moderately eutrophic (Kohler et al. 1980), and dimictic with spring and fall turnovers (Boaze 1972). The lake has a mean retention time of 33 d and annual water level fluctuation of 1.6 m (Copeland 1999).

Methods

Collection

Walleye were collected at five locations within the study site (Figure 2-1). Claytor Lake was sampled October-December 1997-98 using gill nets, and approximately

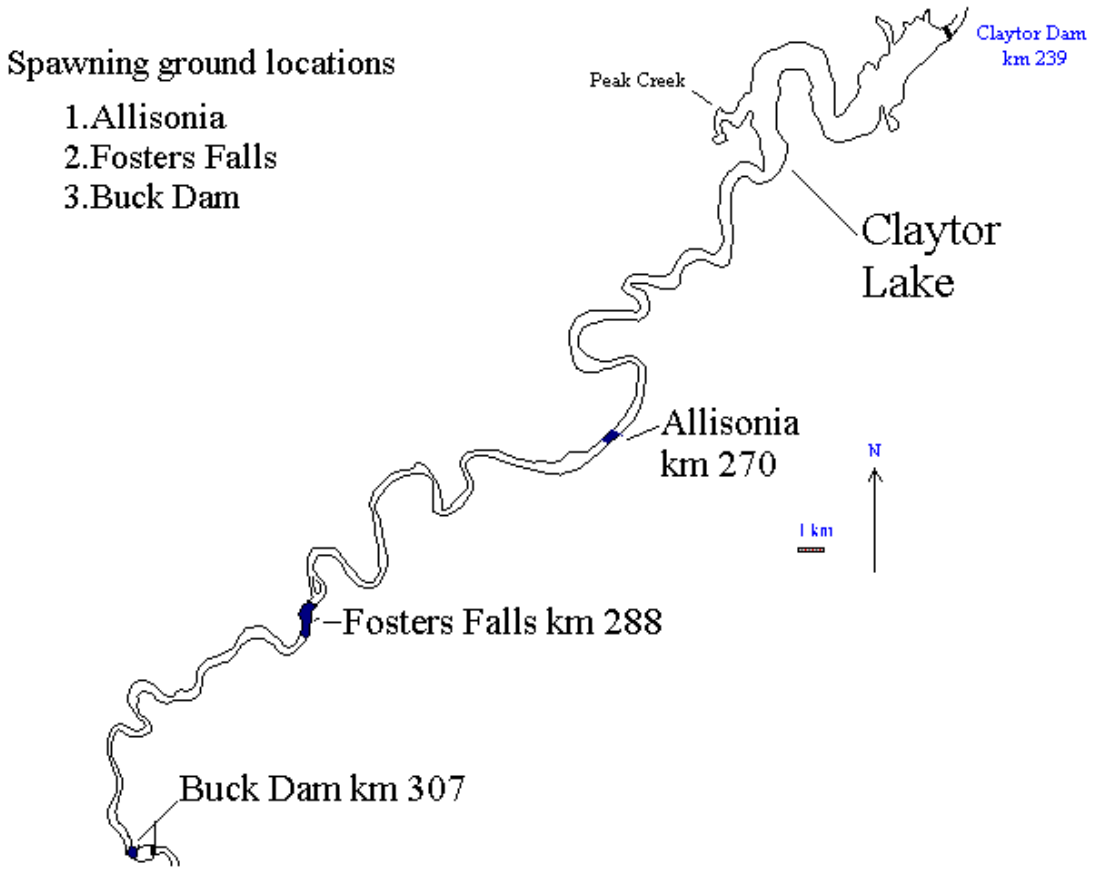

Figure 2-1. Walleye sampling sites and spawning ground locations on the Claytor Lake / upper New River study area.

200 walleye were taken. All data used is recorded in appendix 3. Three collections were made at spawning locations within the river: Allisonia, Fosters Falls, and Buck Dam. Spawning assemblages were sampled by electrofishing during the 1997-99 spring spawning seasons; approximately 60-65 spawning adult walleye were taken from each spawning location (Table 2-1). The fifth collection site was in the New River at Austinville, between the Fosters Falls and Buck Dam spawning sites, where 40 walleye of various ages were taken (Figure 2-1). Fish were packed in ice and taken to the laboratory. Samples of liver, white skeletal muscle, eye, and fin tissues were taken from each walleye and stored at -60^{o}C.

Allozyme screening and statistical testing

Allozymes were screened using samples of liver and white skeletal muscle. I performed the analysis following the methods of Murphy (1983) and Terre (1985). I surveyed 379 walleye using three different protein systems known to be polymorphic in walleye (Murphy 1983, 1990; Terre 1985). Allele frequencies at the three protein encoding loci were determined, malate dehydrogenase (sMDH-B*), isocitrate dehydrogenase (IDHP-A*) and muscle myoglobin (MYO-A*). No modifications were made to the published methods, other than adjusting the time for starch gel electrophoresis to 1800 volt-hrs. Histochemical staining procedures followed those of Harris and Hopkinson (1976). Locus and allele designations followed the conventions of Shaklee et al. (1990). All stained gels were photographed with a digital camera as a permanent record.

I examined allozyme frequencies for three different groupings of data among the walleye collected: (1) the entire study site, including all walleye collected, (2) lake versus river collections, as two different populations, and (3) three different spawning sites, as different populations. Chi-square goodness-of-fit tests were calculated to assess departures of observed genotype frequencies from those expected under Hardy-Weinberg equilibrium. Tests pooling data at all three loci were made for all three groupings using a contingency chi-square analysis. BIOSYS-1 (version 1.7, Swofford and Selander 1989) was used to perform the tests. For all three data groupings tested, the null hypothesis was

that the genotype frequencies conformed to Hardy-Weinberg equilibrium. Test results were regarded as significant at an alpha level ≤ 0.05.

Table 2-1. Sampling seasons, sample size, collection site and allele frequencies for protein systems.

Season / Site	IDHP-A* (1.1.1.42)	MDH-B* (1.1.1.37)	MYO-A*
Spring 1997			
Allisonia	1=.481	1=.058	1=.365
N=26	2=.519	2=.827	2=.635
		3=.115	
Fosters Falls			
N=0			
Buck Dam	1=.231	1=.154	1=.846
N=13	2=.769	2=.557	2=.154
		3=.269	
Austinville	1=.764	1=.000	1=.794
N=17	2=.235	2=.529	2=.206
		3=.471	
Fall 1997			
Claytor Lake	1=.476	1=.065	1=.468
N=65	2=.524	2=.701	2=.532
		3=.234	
Spring 1998			
Allisonia	1=.393	1=.107	1=.464
N=14	2=.607	2=.786	2=.536
		3=.107	
Fosters Falls	1=.270	1=.176	1=.730
N=37	2=.730	2=.338	2=.270
		3=.486	
Buck Dam	1=.417	1=.083	1=.889
N=18	2=.583	2=.389	2=.111
		3=.528	
Austinville	1=.278	1=.056	1=.889
N=18	2=.722	2=.638	2=.111
		3=.306	
Fall 1998			
Claytor Lake	1=.457	1=.005	1=.378
N=96	2=.543	2=.798	2=.622
		3=.197	
Spring 1999			
Allisonia	1=.600	1=.000	1=.400
N=25	2=.400	2=.680	2=.600
		3=.320	
Fosters Falls	1=.288	1=.000	1=.692
N=26	2=.712	2=.423	2=.308
		3=.577	
Buck Dam	1=.097	1=.000	1=.778
N=29	2=.903	2=.111	2=.222
		3=.889	

Microsatellite DNA screening and statistical tests

Microsatellite DNA was screened on material isolated from fin clips. Microsatellite DNA variability was examined using polymerase chain reaction (PCR) and six different microsatellite DNA primer pairs developed for walleye (Borer et al. 1999). The laboratory group of Dr. Anne Kapuscinski at the University of Minnesota provided pre-publication primer sequence information. I extracted the DNA from fin tissue of 244 walleye using the Puregene DNA isolation kit (Gentra, Minneapolis, MN). DNA was amplified using the following PCR protocol. One µl of DNA (100 ng), 2.5 µl 10x $MgCl_2$-free PCR buffer + 1.5 µl, 25 mM, $MgCl_2$ (ProMega), 0.5 µl of 25 mM dNTPs, 0.2 µl *Taq* polymerase (ProMega), and 1.5 µl each forward and reverse primers were brought to a final volume of 25 µl with sterile ddH_2O. The cycling conditions for PCR consisted of denaturation at 95° C for 5 min, followed by 10 cycles (30 sec at 94°C, 30 sec at 55°C and 30 sec at 72° C), 29 cycles (30 sec 89° C, 30 sec at 55°C and 30 sec at 72°C), a final step of 10 min at 72°C, and cooling to room temperature. The PCR products were subjected to electrophoresis through 7 % TBE non-denaturing polyacrylamide midi-gels (Hoefer SE 600, Amersham Pharmacia Biotech inc., San Francisco, CA). Gels were silver-stained using a protocol provided by Kerry-Ann Naish, University of Swansea, Swansea, Wales (Appendix 1). All gels were scored visually for allelic variation. A molecular weight ladder was used as a standard for scoring on every gel (10-bp ladder, Life Technologies, Baltimore, MD). Gels were photographed using a digital camera as a permanent record.

Microsatellite DNA data analyses were performed using four software programs. Groupings of data among collection sites followed those for allozymes described above, with measures of genetic diversity estimated for both individuals and groups. Average expected heterozygosity, average number of alleles, total numbers of alleles, number of unique alleles, average variance in number of repeats, and average range in number of repeats were estimated using the program Microsat (version 1.5, Minch et al 1995). Deviations from Hardy-Weinberg equilibrium were tested for each microsatellite locus, and population subdivision was assessed using the program Arlequin (version 1.1, Schneider et al. 1997). Measures of phylogenetic relatedness were made among individuals and groups. The program Microsat was used to estimate pairwise genetic

distances using the kinship coefficient (Dkf) and the proportion-of shared-alleles (Dps) metrics (Bowcock et al. 1994). Nei's standard distance metric (G_{ST}, Nei 1987) was used to quantify relatedness among groups. The phylogenetic analysis was performed using data from all individuals (N = 244). Phylogenetic tree diagrams were constructed from the Dkf, Dps, and Gst distance matrices using the *neighbor* function of the program Phylip (version 3.5c, Felsenstein 1993), and trees were drawn using the program Treeview (version 1.5, Page 1998).

Mitochondrial DNA screening and statistical testing

Samples of liver from 45 walleyes, 15 from each of three spawning sites, were analyzed for variation in mitochondrial DNA. Mitochondrial DNA variation was observed and analyzed by Dr. Neil Billington and Dr. Ed Heist at Southern Illinois University following the procedures outlined in Billington and Hebert (1987). The mtDNA data were analyzed using haplotype frequencies and genetic distances. The chi-square metric was used to test the null hypothesis that haplotype frequencies were independent of spawning site. For this purpose, haplotypes were grouped into two classes; (1) haplotype 43, the most common variant in this study, and (2) all other haplotypes.

Results

Allozymes

Allozyme frequencies at three polymorphic loci were screened successfully on 379 walleye (Table 2-1). Mean sample size per locus, mean number of alleles per locus, and mean heterozygosity are summarized in Table 2-2 for the three data groupings: (1) entire site, (2) lake versus river, and (3) spawning sites. Combined data for the Claytor Lake and Allisonia collections, representing walleye that constitute lake fish in the lake-versus-river grouping (2), exhibited the highest mean heterozygosity (0.41 ± 0.062) of any group tested. In the spawning grouping analysis, the Buck Dam spawning site exhibited the lowest mean heterozygosity (0.17 ± 0.029).

Results of tests of departure of observed allozyme frequencies from those expected under Hardy-Weinberg equilibrium for all three population groupings are summarized in Table 2-3. All three loci in grouping (1), the entire site, exhibited

Table 2-2. Genetic variability statistics for Claytor Lake and upper New River walleye populations (standard errors in parentheses).

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic	Mean heterozygosity Direct-count	Mean heterozygosity Hardy-Weinberg expected
Entire study site combined	379.6 (0.6)	2.3 (0.3)	100.0	.347 (0.032)	.495 (0.011)
Lake + Allisonia	226.0 (0.0)	2.3 (0.3)	100.0	.410 (0.062)	.459 (0.034)
River sites combined	153 (0.0)	2.3 (0.3)	100.0	.255 (0.020)	.428 (0.064)
Allisonia site	65.3 (0.3)	2.3 (0.3)	100.0	.378 (0.068)	.461 (0.033)
Fosters Falls	63.0 (0.0)	2.3 (0.3)	100.0	.302 (0.033)	.465 (0.058)
Buck Dam	60.0 (0.0)	2.3 (0.3)	100.0	.167 (0.029)	.375 (0.063)

Table 2-3. Hardy-Weinberg analyses for allozyme variation for given geographic groupings of the data.

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	Degrees of freedom	*P*-value
			Entire Site			
IDHP-A*	1-1	75	57.68	13.98	1	<0.001
	1-2	146	180.65			
	2-2	158	140.68			
MDH-B*	1-1	11	0.98	185.32	3	<0.001
	1-2	12	23.66			
	1-3	5	13.38			
	2-2	178	138.62			
	2-3	90	157.12			
	3-3	82	44.25			
MYO-A*	1-1	142	119.27	22.41	1	<0.001
	1-2	142	187.46			
	2-2	96	73.27			
			Lake & Allisonia sites verses all upper New River sites			
Lake & Allisonia population						
IDHP-A*	1-1	56	51.48	1.45	1	.229
	1-2	104	113.03			
	2-2	66	61.48			
MDHP-B*	1-1	6	0.302	123.58	3	<0.001
	1-2	3	12.78			
	1-3	2	3.62			
	2-2	138	127.03			
	2-3	60	72.16			
	3-3	17	10.11			
MYO-A*	1-1	38	37.74	0.005	1	.943
	1-2	109	109.52			
	2-2	79	78.74			
River population						
IDHP-A*	1-1	19	10.36	13.14	1	<0.001
	1-2	42	59.28			
	2-2	92	83.36			
MDHP-B*	1-1	5	0.78	71.88	3	<0.001
	1-2	9	8.58			
	1-3	3	11.90			
	2-2	40	23.02			
	2-3	30	64.38			
	3-3	66	44.36			
MYO-A*	1-1	103	93.24	21.38	1	<0.001
	1-2	33	52.50			
	2-2	17	7.25			

Table 2-3. Continued

Locus	Genotype	Observed frequency	Expected frequency	Chi-square	Degrees of freedom	*P*-value
			Allele frequencies for spawning sites			
Allisonia						
IDHP-A*	1-1	18	16.63	.46	1	.496
	1-2	30	32.75			
	2-2	17	15.62			
MDHP-B*	1-1	2	0.12	40.64	3	<0.001
	1-2	1	4.53			
	1-3	1	1.24			
	2-2	42	37.03			
	2-3	14	20.40			
	3-3	6	2.68			
MYO-A*	1-1	12	10.28	.79	1	.373
	1-2	28	31.44			
	2-2	25	23.28			
Fosters Falls						
IDHP-A*	1-1	10	4.76	10.91	1	.001
	1-2	15	25.48			
	2-2	38	32.76			
MDHP-B*	1-1	3	0.62	21.06	3	<0.001
	1-2	4	4.89			
	1-3	3	6.86			
	2-2	14	8.64			
	2-3	15	24.82			
	3-3	24	17.16			
MYO-A*	1-1	35	32.04	3.36	1	.067
	1-2	20	25.92			
	2-2	8	5.04			
Buck Dam						
IDHP-A*	1-1	7	2.95	9.13	1	.003
	1-2	13	21.10			
	2-2	40	35.95			
MDHP-B*	1-1	2	.0176	61.19	3	<0.001
	1-2	3	2.06			
	1-3	0	4.59			
	2-2	14	5.00			
	2-3	4	22.94			
	3-3	37	25.24			
MYO-A*	1-1	45	41.60	10.29	1	.001
	1-2	10	16.81			
	2-2	5	1.60			

significant departure ($\underline{P} < 0.001$) from genotype frequencies expected under Hardy-Weinberg equilibrium. In grouping (2), lake versus river, genotypes at two of the three loci in the lake population showed no departure ($\underline{P} = 0.23$ and 0.94) from Hardy-Weinberg equilibrium. Genotype frequencies in the river population in grouping (2) showed significant departures from Hardy-Weinberg equilibrium at all three loci ($\underline{P} < 0.001$). In grouping (3), spawning sites, genotype frequencies at two of three loci in the Allisonia collection fit the Hardy-Weinberg equilibrium ($\underline{P} = 0.37$ and 0.49), and at one did not ($\underline{P} < 0.001$). In the Fosters Falls collection, genotype frequencies at two of the three loci failed to fit, and at one did fit Hardy-Weinberg expectation ($\underline{P} = 0.001$, <0.001 and 0.067 respectively). In the Buck Dam collection, genotype frequencies at all three loci did not fit the Hardy-Weinberg equilibrium ($\underline{P} < 0.001$, 0.001, and 0.003).

Microsatellite DNA

Characterization of microsatellite DNA variation in walleye from Claytor Lake and the upper New River was completed on 244 individuals from four collection sites: Claytor Lake, Allisonia, Fosters Falls and Buck Dam. A total of 61 different alleles were identified at six different microsatellite loci. The number of alleles ranged from five to fifteen per locus (Table 2-4). Allele frequencies differed among the four groups; the Buck Dam collection showed high frequencies of one particular allele of each locus, while other populations showed more even distributions of allele frequencies. The Buck Dam collection exhibited the lowest level of genetic diversity among the groups (Table 2-5), measured as: average number of alleles (7.5 vs. 9.0), total number of alleles (36 vs. 40-42), number of unique alleles (0 vs.0-3), average range in the number of repeated microsatellite motifs (8.15 vs. 10.16-10.83) and observed heterozygosity (0.49 vs. 0.56-0.72). Given the observed allele frequencies, the expected heterozygosities for the Claytor Lake, Allisonia and Fosters Falls groups were approximately 0.80, and the expected heterozygosity for the Buck Dam group was 0.65.

Phylogenetic distance relationships were estimated from pairwise genetic distances using the Dkf and Dps metrics (Bowcock et al. 1994) for all individuals and for the four groups. The G_{ST} distance (Nei 1987) was estimated for the four groupings only. The individual genetic distance analysis depicted several notable features (Figure 2-2).

Table 2-4. Numbers of occurrences of alleles (left) and allele frequencies (right) at six microsatellite loci among walleye from four collection sites in the Claytor Lake / upper New River system, Virginia.

Locus *SVI-17*		**Population**	**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**		**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**
Total numbers of alleles	10	**Number of individuals**	67	55	59	58	**Number of alleles**	134	110	118	116
Alleles	95	**Count**	7				**Frequency**	0.052	0.00	0.00	0.00
	99		2	2	32	32		0.015	0.02	0.27	0.28
	101		3	4	26	67		0.022	0.04	0.22	0.58
	103		45	28	37	12		0.336	0.25	0.31	0.10
	105		22	16	4	3		0.164	0.15	0.03	0.03
	107			5	1			0.000	0.05	0.01	0.00
	109		25	27	14	1		0.187	0.25	0.12	0.01
	111		30	19	2	1		0.224	0.17	0.02	0.01
	113			9	2			0.000	0.08	0.02	0.00
Locus *SVI-18*		**Population**	**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**		**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**
Total numbers of alleles	5	**Number of individuals**	65	57	60	58	**Number of alleles**	130	114	120	116
Alleles	118	**Count**	43	33	22	17	**Frequency**	0.33	0.29	0.18	0.15
	120		22	8	31	40		0.17	0.07	0.26	0.34
	122		35	35	32	22		0.27	0.31	0.27	0.19
	124		30	34	20	9		0.23	0.30	0.17	0.08
	126		4	4	15	30		0.03	0.04	0.13	0.26
Locus *SVI-26*		**Population**	**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**		**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**
Total numbers of alleles	13	**Number of individuals**	59.5	42.5	58	57	**Number of alleles**	119	85	116	114
Alleles	152	**Count**	15	10	4	12	**Frequency**	0.13	0.12	0.03	0.11
	154		8	14	12	24		0.07	0.16	0.10	0.21
	156		3	11	10	1		0.03	0.13	0.09	0.01
	158		12	4	28	63		0.10	0.05	0.24	0.55
	160		20	14	19	8		0.17	0.16	0.16	0.07
	162		3	3	15			0.03	0.04	0.13	0.00
	165		21	13	10	1		0.18	0.15	0.09	0.01
	167		14	12	3	2		0.12	0.14	0.03	0.02
	169		7	2	8	2		0.06	0.02	0.07	0.02
	171		5		3	1		0.04	0.00	0.03	0.01
	181		4					0.03	0.00	0.00	0.00
	183		2	1	2			0.02	0.01	0.02	0.00
	185		3	1	2			0.03	0.01	0.02	0.00

Table 2-4. Continued.

Locus *SVI-33*		Population	Claytor Lake	Allisonia	Fosters Falls	Buck Dam		Claytor Lake	Allisonia	Fosters Falls	Buck Dam
Total numbers of alleles	10	**Number of individuals**	64	55	59	59	**Number of alleles**	128	110	118	118
Alleles	78	**Count**		5	24	45	**Frequency**	0.00	0.05	0.20	0.38
	84		2	6	11	22		0.02	0.05	0.09	0.19
	86		17	31	42	37		0.13	0.28	0.36	0.31
	90		6	13	3	2		0.05	0.12	0.03	0.02
	92		8	3	1			0.06	0.03	0.01	0.00
	96		18	2	4	4		0.14	0.02	0.03	0.03
	98		31	18	18	4		0.24	0.16	0.15	0.03
	100		25	22	9	2		0.20	0.20	0.08	0.02
	102		4	1		1		0.03	0.01	0.00	0.01
	104		17	9	6	1		0.13	0.08	0.05	0.01
Locus *SVI-4*		**Population**	**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**		**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**
Total numbers of alleles	8	**Number of individuals**	66.5	56	59	59	**Number of alleles**	133	112	118	118
Alleles	100	**Count**		6	3		**Frequency**	0.00	0.05	0.03	0.00
	104		1	23	5			0.01	0.21	0.04	0.00
	106		12	16	10	7		0.09	0.14	0.08	0.06
	108		30	17	53	62		0.23	0.15	0.45	0.53
	110		43	34	27	23		0.32	0.30	0.23	0.19
	112		29	7	13	21		0.22	0.06	0.11	0.18
	114		15	8	6	5		0.11	0.07	0.05	0.04
	116		3	1	1			0.02	0.01	0.01	0.00
Locus *SVI-6*		**Population**	**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**		**Claytor Lake**	**Allisonia**	**Fosters Falls**	**Buck Dam**
Total numbers of alleles	15	**Number of individuals**	53	43.5	50.5	56	**Number of alleles**	106	87	101	112
Alleles	130	**Count**	2	1	2		**Frequency**	0.02	0.01	0.02	0.00
	136		15	1	4	1		0.14	0.01	0.04	0.01
	138		4					0.04	0.00	0.00	0.00
	140			3	19	3		0.00	0.03	0.19	0.03
	142		41	45	19	7		0.39	0.52	0.19	0.06
	144		10	11	6	1		0.09	0.13	0.06	0.01
	146		4	2	1	1		0.04	0.02	0.01	0.01
	148		10	12	6	3		0.09	0.14	0.06	0.03
	150		7	5	7	12		0.07	0.06	0.07	0.11
	152		2	1				0.02	0.01	0.00	0.00
	154		5	1	29	2		0.05	0.01	0.29	0.02
	158		3	3	4	73		0.03	0.03	0.04	0.65
	161		2		1	8		0.02	0.00	0.01	0.07
	163				3	1		0.00	0.00	0.03	0.01
	165		1	2				0.01	0.02	0.00	0.00

Table 2-5. Genetic variability metrics across six different microsatellite loci among groupings of walleye in Claytor Lake and the upper New River.

Population	Subpopulation	N	Expected het.	Observed het.	Average # of alleles	Total # of alleles	Unique alleles	Average variance in # of repeats	Average range in # of repeats
Claytor Lake		125	0.80	0.68	9.83	44	5	6.87	11.16
	Lake	68	0.8	0.72	9.00	42	3	6.71	10.67
	Allisonia	57	0.79	0.63	9.00	40	0	6.38	10.83
New River		118	0.76	0.52	9.16	41	1	6.84	10.67
	Fosters Falls	60	0.79	0.56	9.00	41	0	7.54	10.16
	Buck Dam	59	0.65	0.49	7.5	36	0	4.16	8.16

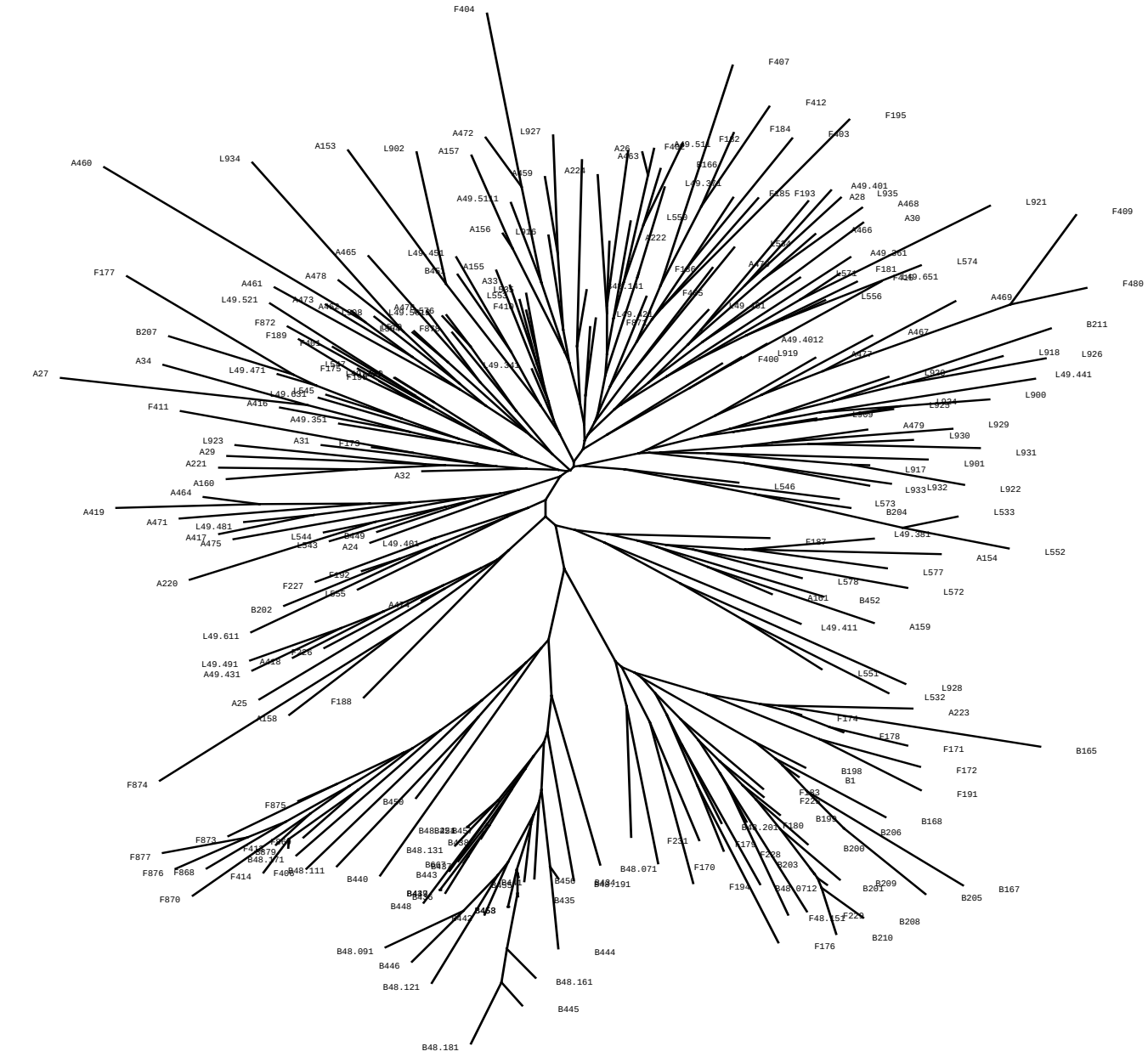

Figure 2-2. Genetic distances among individual walleye in the Claytor Lake / upper New River system, as estimated using data from six microsatellite loci using the kinship coefficient (Dkf) distance metric.

There were two major clusters observed. The first cluster included all of the walleye collected in Claytor Lake and Allisonia, mixed with several fish from the Buck Dam and Fosters Falls (Figure 2-2, upper 3/5 of the figure). The first cluster showed no obvious subgrouping. The second cluster included only walleye collected at Buck Dam and Fosters Falls, with the exception of one Allisonia fish (Figure 2-2, lower 2/3 of the figure). The second clusters showed a separation into two subgroups; both subgroups included both Fosters Falls and Buck Dam walleye. Both the Dkf and the Dps distance measures appeared similar in cluster pattern.

The greatest genetic distance between the groups Claytor Lake, Allisonia, Fosters Falls and Buck Dam consistently was observed between the Buck Dam group on the one hand, and the Claytor Lake and Allisonia groups on the other, for all three distance estimators (Table 2-6). The smallest genetic distance was between the Claytor Lake and Allisonia groups for two of the three estimators. The inferred phylogenetic associations among the groups are shown in Figure 2-4. Bootstrap values of 70 or greater were considered significant. All three distance -measurements - Dkf, Dps and G_{ST} - identified the most-related groups to be the Claytor Lake and the Allisonia groups, with these then linked to the Fosters Falls group. All three bootstrap values were 100 (Figure 2-3). The next closest relationship was between the Fosters Falls and Buck Dam collections, with the Dkf, Dps and G_{ST} bootstrap values being 71, 84 and 82, respectively. This pattern of differentiation among the four groups using the F_{ST} estimator (Table 2-7; Michalakis and Excoffier 1996, Weir and Cockerham 1984) showed results similar to the phylogenetic distance measures. The Buck Dam group was the most distant from the Claytor Lake and Allisonia groups, followed by the Fosters Falls group from the Claytor Lake and Allisonia groups. All these measures, (Dps, Dkf, G_{ST} and F_{ST}) examine the genetic relativeness, measures by distance, of the common alleles shared between the identified groups. From analysis of the groups within the study site, Claytor Lake and Allisonia shared the most in common making them the least genetically distant, followed by Fosters Falls and then Buck Dam.

Hardy-Weinberg analysis of genotype frequencies among groups showed significant departures from homogeneity (X^2, $\underline{P} > 0.0001$). This result was strongly influenced by the high number of alleles observed at each locus. A much larger sample

Table 2-6. Matrices of genetic distance metrics (kinship coefficient, Dkf; proportioned shared alleles, Dps; and Nei's genetic distance, G_{ST}) among the four groupings of walleye in Claytor Lake and the upper New River, VA.

		Claytor Lake	Allisonia	Fosters Falls
Dkf	Claytor Lake			
	Allisonia	0.02		
	Fosters Falls	0.05	0.06	
	Buck Dam	0.16	0.17	0.08
Dps	Claytor Lake			
	Allisonia	0.24		
	Fosters Falls	0.38	0.38	
	Buck Dam	0.60	0.60	0.37
G_{ST}	Claytor Lake			
	Allisonia	0.07		
	Fosters Falls	0.24	0.26	
	Buck Dam	0.55	0.58	0.24

Table 2-7. Matrix of F_{ST} values among groupings of walleye in Claytor Lake and the upper New River, Va.

		Claytor Lake	Allisonia	Fosters Falls
F_{ST}	Claytor Lake			
	Allisonia	0.02		
	Fosters Falls	0.06	0.05	
	Buck Dam	0.15	0.15	0.04

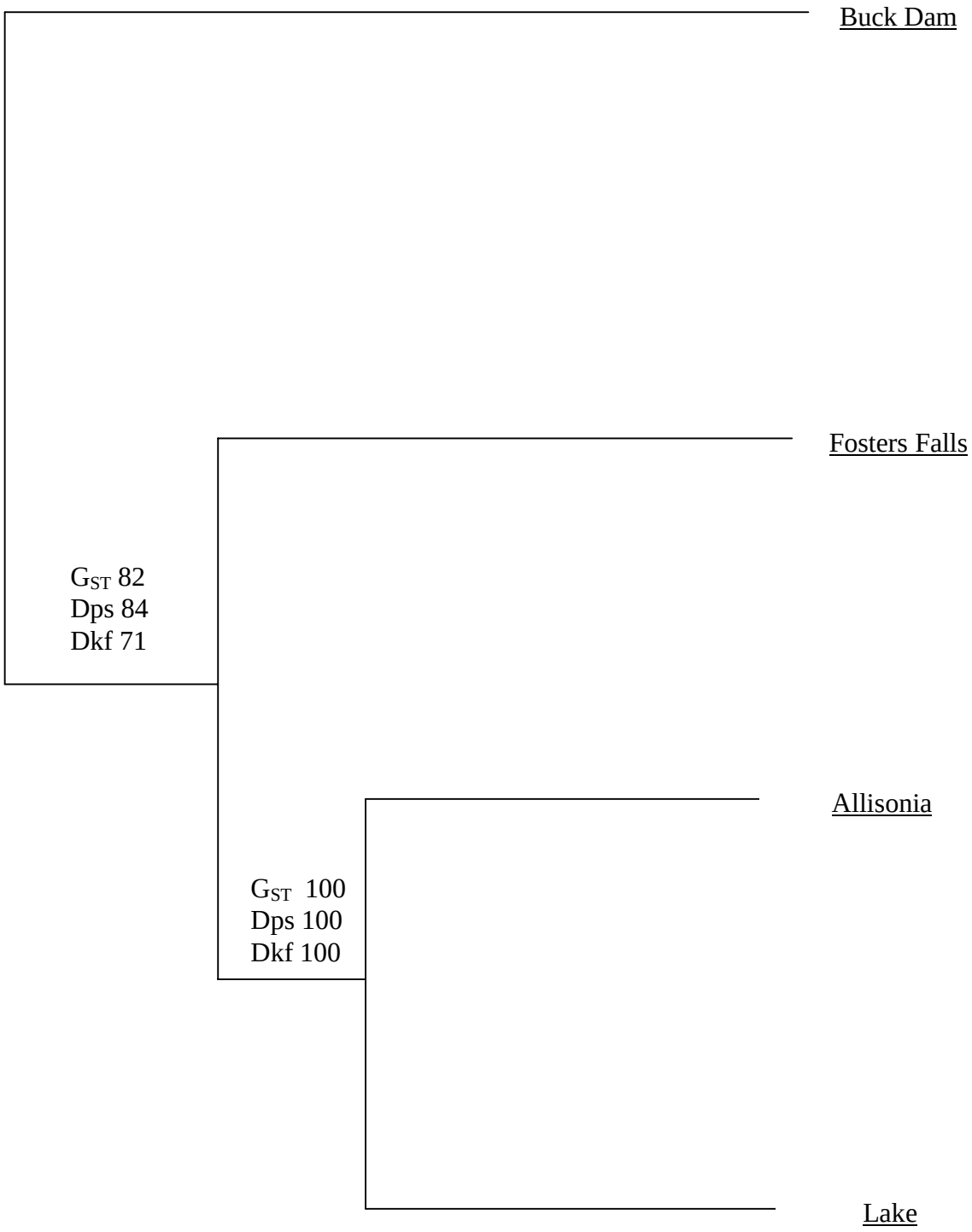

Figure 2-3. Genetic distances between groups of walleye in Claytor Lake and the upper New river. Numbers following the different distance measures equal the bootstrap values for the distance measure.

size would be needed to obtain a robust picture of the relation of this population to Hardy-Weinberg equilibrium.

mtDNA

Forty-five walleye (15 collected from each of the three spawning sites) in 1998 were analyzed for mtDNA variation. Among 39 walleye successfully analyzed, six different haplotypes were identified (Table 2-8). Three of the haplotypes observed - 1, 4, and 10 - have been described previously in other walleye populations (Billington et al. 1992). The remaining three haplotypes - 43, 44, and 45 – had not been described before this study. Observation of unique genetic material suggests a unique genetic stock of walleye in this system. Haplotype 43 was the most frequent haplotype among walleye at the Fosters Falls (90%) and Buck Dam (74%) spawning sites, while all six haplotypes were found at the Allisonia site (Figure 2-4). A chi-square test for departure of haplotype frequencies from random distribution among spawning sites was significant ($X^2 = 18.63$, df = 2, and $\underline{P} < 0.0001$).

Discussion

Years of stocking walleye in Claytor Lake and the upper New River created a genetically mixed walleye fishery. I tested the null hypothesis that walleye within the Claytor Lake / upper New River system represented one panmictic stock. Analysis of the molecular genetic marker data supports the alternative hypothesis that more than one genetic stock exists, but suggest some degree of genetic mixing. The data also suggested that a unique genetic stock of walleye has persisted among the mixed genetic stocks.

The alternative hypothesis of more than one genetic stock was supported by the results of all three genetic techniques used in this study. Data from the entire system at all nine allozyme and microsatellite DNA loci examined provided evidence that the population was not panmictic in structure. The mtDNA data also showed evidence that the population was made up of multiple walleye stocks. Haplotypes 1, 4, and 10 typify walleye stocks throughout most of the species range (Billington et al. 1992). Appendix 2 shows the distribution of these three common haplotypes throughout the native walleye range distribution. Generally, walleyes with haplotype 1 are from the

Table 2-8. Walleye collection sites, sex, and mitochondrial DNA haplotypes.

Site	Sex	Haplotype
Allisonia	M	1
Allisonia	M	4
Allisonia	M	4
Allisonia	M	4
Allisonia	M	4
Allisonia	M	4
Allisonia	M	4
Buck Dam	M	4
Buck Dam	M	4
Allisonia	M	10
Allisonia	F	43
Buck Dam	F	43
Buck Dam	M	43
Buck Dam	F	43
Buck Dam	M	43
Buck Dam	M	43
Buck Dam	M	43
Buck Dam	M	43
Buck Dam	M	43
Buck Dam	M	43
Buck Dam	M	43
Foster Falls	F	43
Foster Falls	M	43
Foster Falls	F	43
Foster Falls	F	43
Foster Falls	F	43
Foster Falls	M	43
Foster Falls	M	43
Foster Falls	M	43
Foster Falls	F	43
Foster Falls	M	43
Allisonia	M	44
Allisonia	M	44
Allisonia	M	44
Allisonia	M	44
Buck Dam	F	44
Buck Dam	M	44
Foster Falls	F	44
Allisonia	M	45

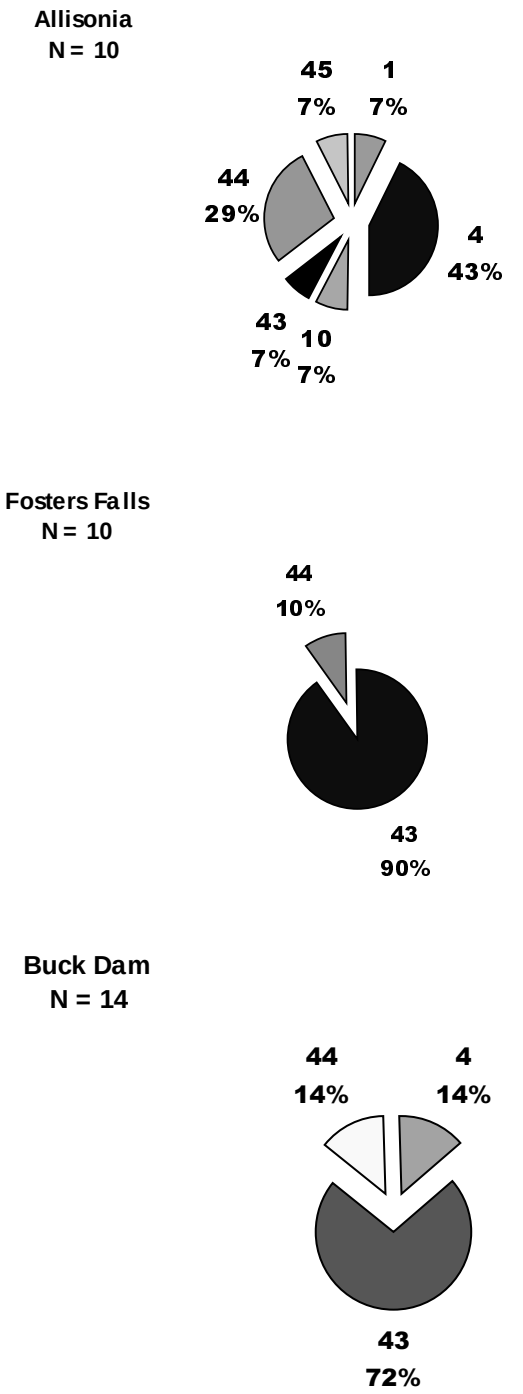

Figure 2-4. Numbers and percentages of walleye mtDNA haplotypes that were observed in collections from each spawning site.

eastern Great Lakes region of the native walleye range (Billington et al. 1992). Walleye with haplotype 10 are from the west of the Lake Superior region of the range and walleye with haplotype 4 are from the central region of the native walleye range (Billington et al. 1992). These common haplotypes represent walleye that probably have been stocked into the Claytor Lake / upper New River system. However, haplotypes 43, 44, and 45 were unique to the New River system. The observed haplotypes suggests that a mixture of non-native and native stocks are present within the system.

Separation of the study site into two collection groups (lake and river) showed that the lake group, which included all the samples collected from within Claytor Lake and at Allisonia, genetically fit the hypothesis of one panmictic genetic stock. Phylogenetic distances estimated between the Claytor Lake and Allisonia collections indicated that they were more closely related to each other than to those from sites in the upper river. This finding is not surprising, given that the Allisonia area is the interface between Claytor Lake and the upper New River, and was the primary spawning site for Claytor Lake walleye. The Allisonia spawning site showed the presence of all six mtDNA haplotypes observed in the study. This is most likely a result of mixed walleye stockings in the past plus the possible presence of fish of native haplotypes. This evidence leads to the inference that the Claytor Lake and Allisonia sites contain one well-mixed walleye population.

The river group, comprising walleye from all collection sites in the upper New River, showed evidence of the presence of more than one distinct genetic stock. Analysis of allozyme frequencies indicated that this finding held for data from the river as a whole and also when data from the river was broken out into spawning sites (Fosters Falls and Buck Dam). The genetic distance measures, estimated from microsatellite DNA data, consistently showed the Fosters Falls and Buck Dam groups to be separated from the Allisonia and Claytor Lake groups. This evidence points to the conclusion that the walleye stock in the New River is unlike that in Claytor Lake. The mtDNA results showed a previously unknown haplotype at high frequencies in collections from the river. This is taken as evidence that walleye in Claytor Lake and Allisonia largely resemble stocked northern stock walleye, while the river population may include a high percentage of a unique, putatively native walleye stock. The New River could have been a glacial

refugium for walleye during the last ice age. Walleye in this refugium, remaining separated from immigration, would have been subject to a unique evolutionary history. Over time, the segregated New River stock would become genetically differentiated from other walleye stocks. Although these walleye may have been reduced in numbers as humans impacted the New River, the gene pool could have persisted among the stocked walleye that have been added to the system. Spawning habits may temporally, spatially, or behaviorally separate this stock from introduced stocks.

The genetically unique stock of walleye, characterized by the previously unknown mtDNA haplotypes 43, 44, and 45 was found primarily at the Fosters Falls and Buck Dam sites. The mitochondrial DNA haplotypes themselves are highly divergent from known haplotypes, and is most closely related to a haplotype 38 that is known from the Rock Castle River in Kentucky (N. Billington, Shippensburg University, personal communication). Both the New River and the Rock Castle River are tributaries to the Ohio River. I hypothesize that the haplotypes 43, 44, and 45, and haplotype 38 characterize an ancestral strain that existed in the Teays River before the last Ice Age; the Pleistocene, thought to have ended approximately 10,000 years ago (Cooney et al. 1990). Glaciers would have separated the ancestral populations and the two groups became genetically divergent over time, both from each other and especially from other walleye stocks. Further investigation of this hypothesis by examination of other regional populations is warranted.

The haplotype-43-bearing walleye from the upper New River also exhibited a common allele at two microsatellite loci. At the *SVI-17* locus, the 99/99-homozygous genotype was observed in 94% of all the haplotype-43 walleye. This concordance was not seen for any of the other mtDNA haplotypes identified. The *SVI-33* locus also showed a unique allele, 78, in 77% of the haplotype-43 walleye. These two alleles had frequencies of 25% and 28%, respectively, in the river walleye population. The concordance of nuclear allele frequencies with mitochondrial DNA haplotype frequencies supports the hypothesis of co-existence of distinct walleye stocks in the Claytor Lake / upper New River ecosystem. Based on the frequencies of microsatellite genotypes that could have the unique mtDNA haplotype 43, it could be estimated that as few as one in four walleye from the upper river exhibit the diagnostic characters of the unique genetic

stock. Further investigation of the unique walleye stock with newly established microsatellite DNA primer pairs (W. Eldridge, University of Minnesota, personal communication) might reveal loci that increase the concordance of mtDNA with microsatellite DNA markers.

This putatively unique walleye stock may be unique to the New River or may represent a southern walleye stock (Hackney and Holbrook 1978) that was thought to reside in southern rivers. When data from a panel of samples of microsatellite DNA from the upper New River walleye was compared to samples of some walleye in Minnesota, the New River samples were very different (W. Eldridge, University of Minnesota, personal communication). Further comparisons between the putatively unique walleye in the New River and other walleye populations needs to be performed to support a more informed conclusion.

Conclusions

From spring 1997 through fall 1999, I used radio telemetry and molecular genetic techniques to investigate hypotheses concerning the walleye population in the Claytor Lake / the upper New River system. These hypotheses dealt with the population genetic structure, movement, spawning, and habitat use of walleye in the river-reservoir system. The goal of the study was to gather information that could be used to better manage the walleye fishery. I found that at least two genetic stocks coexist, one putatively representing a unique genetic stock of walleye. I recommend adoption of practices to conserve the unique genetic stock and implementation of different management strategies for both stocks.

Of the two stocks, one primarily inhabits the lake and the other the river. There is some degree of genetic differentiation between the two stocks. The lake walleye appear to be a genetically panmictic population. In contrast, departures from Hardy-Weinberg genotype frequencies suggest the co-existence of more than one stock in the river. Telemetry data suggest that the lake stock resides in the lake through most of the year, but spawns in the river at Allisonia. The river stock resides in the river and spawns mostly below Buck Dam. However, there are some individuals from both stocks that spawn in other areas, such as Fosters Falls. The two stocks overlap in spawning areas, but the amount of genetic mixing between the stocks is uncertain. Concordance among mitochondrial and nuclear DNA markers suggests that mixing is not so great as to alter the genetic integrity of the putative native stock. Hence, my data set indicates that a unique genetic stock of walleye persists in the river.

Management Implications

Genetic analysis of walleye stocks in Claytor Lake and the upper New River revealed information that can be applied directly to fisheries management. Prior to this study, little was known about the genetic structure of the walleye population in this system. A history of stocking of non-native walleye left the system with mixed genetic

stocks. However, this study has demonstrated the persistence of a genetically unique, putatively native walleye stock in the New River above Claytor Lake.

Because these stocks are genetically distinct, they should each be subject to a targeted management plan. Management should direct more fishing pressure towards the underutilized lake fishery rather than towards the relatively well exploited river fishery. Management of the river walleye fishery should focus on the conservation of the unique walleye stock. I recommend that enhancement of this unique walleye stock become the priority for walleye management in this system. If walleye stocking is going to occur, then only the unique genetic stock found in the New River should be stocked into the system. Stocking exclusively the river stock and determining and enforcing a well-estimated total allowable catch could enhance the demographics of the upper New River walleye stock.

If walleye stocking is going to occur, hatchery-based enhancements of the unique, putatively native walleye stock will depend on the identification of prospective broodstock expressing molecular genetic variants characteristic of the stock. The unique walleye stock is characterized by a high frequency of individuals bearing the unique mitochondrial DNA haplotypes 43, 44, and 45. There also is a high frequency of the 99 and 101 alleles at the microsatellite DNA locus *SVI-17* and of the 78 allele at the *SVI-33* locus. Hence, native New River walleye can be distinguished from other walleye of non-native stocks for purposes of choosing broodstock for a hatchery-based enhancement program.

Genetic marker-based selection in choice of hatchery broodfish is not uncommon and easily can be adapted to the proposed walleye-stocking program for the New River. I recommend that if stocking is going to occur, that walleye be collected from the Fosters Falls and Buck Dam spawning sites during the peak spawning run. Both males and females should be marked with physical identification tags, such as anchor or dart tags, and the numbers recorded. A small piece of fin (approximately 20 mg, a 1-cm square) will be removed from each fish, and placed in a scale envelope marked with the number of the fish. Following tagging, walleye should be held and the genetic analysis of each fish carried out. Alternatively, should it be necessary to strip walleye for immediate fertilization, egg lots could be held separately until genetic characterization of each

family is complete. The microsatellite DNA locus *SVI-17* should be screened to identify walleye that bear only the 99 or 101 alleles. The *SVI-33* locus also should be screened for presence of the 78 allele. Both of these alleles are highly correlated with the haplotypes characteristic of the unique walleye stock. Microsatellite data from this study showed that of walleye from Fosters Falls and Buck Dam, the occurrence of these alleles was approximately one in two. Hence, approximately 50 of every 100 walleye collected could be used. Once walleye with the unique genetic markers have been identified, they could be used as broodfish for hatchery production.

As many native walleye as practical need to be used in the mating process so that stocking of a limited number of genotypes does not actually reduce the genetically effective population size of the targeted stock (Ryman and Laike 1991). That is, stocking many offspring of a limited number of spawners actually can reduce the genetic diversity of the stocked population. The upper New River and Claytor Lake then could be stocked with the offspring of the unique walleye stock. The newly-stocked walleye all will be of the unique genetic stock, thereby elevating the frequency of the putatively native genotypes in the walleye population system-wide. Broodstock or families not found appropriate for stocking the New River could be used for stocking systems in the Commonwealth not containing native walleye stocks.

Walleye have not been stocked in the Claytor Lake / upper New River system since 1996. If stocking is reinstated with the unique walleye stock, biologists will be able to assess the contribution of natural walleye reproduction in the system. A stocking program in which walleye are stocked every other year can make this possible. By aging walleye collected every year, biologists will be able to estimate the contribution to natural reproduction from walleye that are from the years when no stockings occurred. I also recommend a re-evaluation of the population genetic structure of walleye within five years of restocking the native stock in order to assess the allele frequency of the unique genetic markers within the population. This will help determine if the population genetic structure is being driven toward the native stock. The positive impact from implementation of these recommendations is that the unique, putatively native walleye stock can be used as broodstock to build up the walleye population in the New River system and drive it towards its original genetic character. The VDGIF no longer will rely

on other states such as Pennsylvania and New York to obtain walleye fry for stocking, thereby limiting the influx of different walleye stocks into aquatic ecosystems within the Commonwealth. This will help preserve the unique, locally adapted character of walleye native to the New River. The very existence of a genetic stock unique to the New River warrants its conservation, because it is unique, locally adapted, and genetically unlike any other stock identified before. The genetic techniques used in this study can be applied to manage and enhance the current walleye fishery. Further genetic surveys of walleye populations throughout rivers in southwest Virginia are needed to expand the understanding of walleye biology and population genetics.

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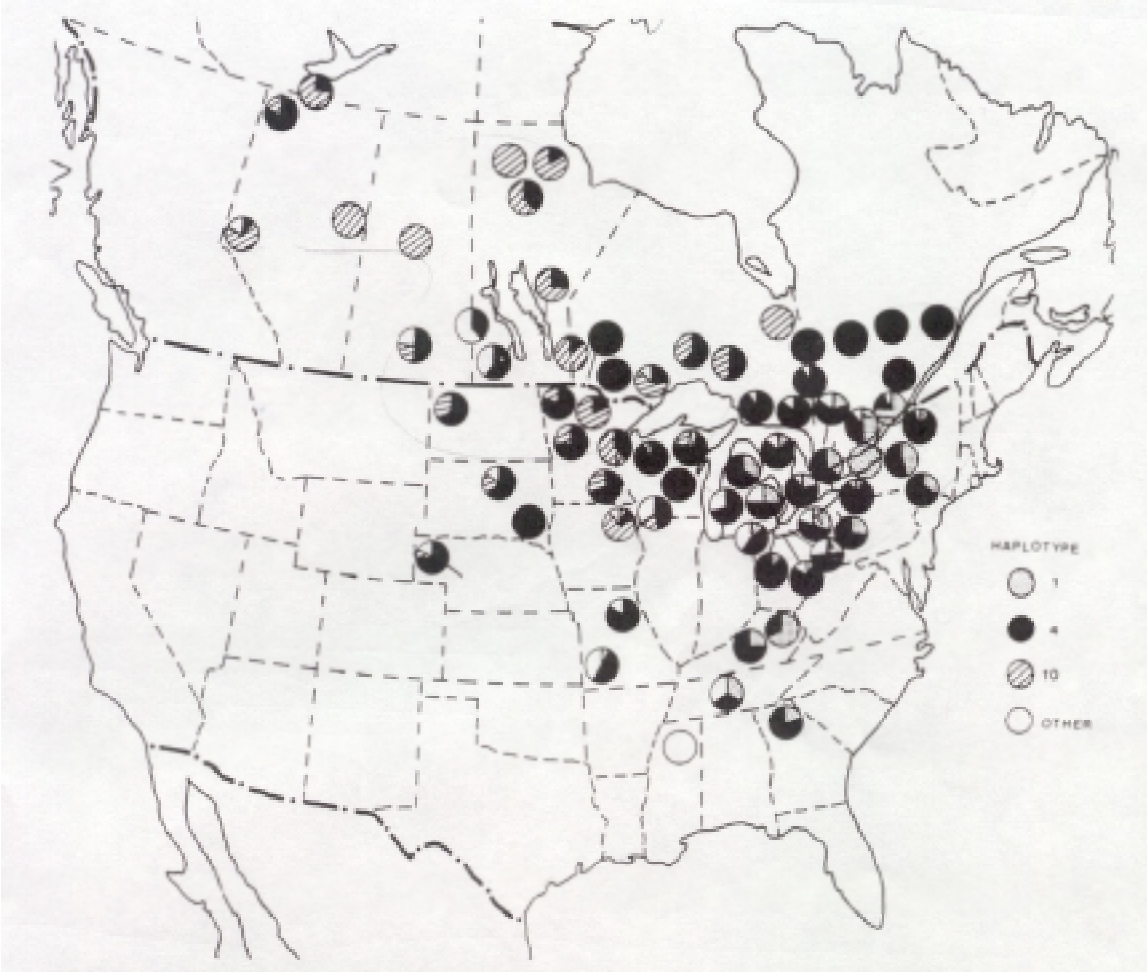
Appendix 1. Silver- staining protocol used for observing microsatellite DNA amplification products.

Step	Time	Ingredient	Amount per gel	Final concentration
Fixative solution	5 minutes	Water	125 ml	--
		Ethanol	14 ml	10%
		Acetic acid	0.7 ml	0.5%
Impregnating solution	8 minutes	Water	112.5 ml	--
		1% Silver nitrate	12.5 ml	0.1 %
Rinse	3 minutes	Water		
Developer	10 minutes	1.5% Sodium hydroxide	125 ml	1.5 %
		1% Sodium borohydride	1.3 ml	0.01%
		Formaldehyde	0.5 ml	0.15%
Storage solution	--	Water	125 ml	--
		Ethanol	14 ml	10%
		Acetic acid	0.7 ml	0.5%



Appendix 2. Distribution of walleye mtDNA haplotypes (1, 4, 10, and all others) throughout North America. Figure from Billington et al. (1992).

Appendix 3. Data collected on all walleye used in the allozyme, microsatellite DNA, and mtDNA portion of this study. Table A presents allozyme and mtDNA data, and Table B presents microsatellite DNA data.

Table A. Data is arranged as follows: month and year each walleye was collected, water body (CL = Claytor Lake, NEW = upper New River) and area within the study site that the walleye were collected (All = Allisonia, R52 = route 52, IVH = Ivanhoe, AUS = Austinville, B/FF = Below Fosters Falls, Lower = lower Claytor Lake) log number, total length in mm, weight in g, year class of each fish determined by aging otoliths, sex, genotype for the MYO protein system, genotype for the MDH-C protein system, genotype for the IDH-C protein system, and mtDNA haplotype.

MM	YY	WATER	SITE	LOG	TL	WT	SEX	YC	MYO	MDH-C	IDH-1	mtDNA
3	97	CL	ALL	1	550	1688	M	92	12	2 3	22	
3	97	CL	ALL	2	502	1131	F	94	12	2 2	11	
3	97	CL	ALL	3	447	920	M	94	12	2 2	22	
3	97	CL	ALL	4	456	769	M	94	12	2 2	12	
3	97	NEW	R52	5	350	335	M	95	11	3 3	11	
3	97	NEW	R52	6	397	600	M	95	12	2 3	12	
3	97	CL	ALL	7	405	787	M	95	22	1 2	11	
3	97	CL	ALL	8	397	530	M	95	22	2 2	12	
3	97	CL	ALL	9	438	729	M	94	12	2 2	12	
3	97	CL	ALL	10	470	940	M	94	22	2 2	12	
3	97	CL	ALL	11	544	1650	M	87	12	2 2	11	
3	97	NEW	IVH	12	392	530	M	94	22	11	12	
3	97	NEW	IVH	13	343	309	M	95	11	22	12	
3	97	NEW	IVH	14	515	1206	M	90	11	3 3	12	
3	97	NEW	IVH	15	449	757	F	94	12	2 3	11	
3	97	NEW	IVH	16	445	786	F	94	12	2 2	11	
3	97	NEW	IVH	17	526	1405	M	90	11	3 3	11	

3	97	NEW	IVH	*	864	7300	F	*	.	.	.	
3	97	CL	ALL	18	663	2520	F	92	22	2 3	12	
3	97	CL	ALL	19	530	1727	F	94	12	2 2	11	
3	97	CL	ALL	20	500	1122	M	92	22	2 2	12	
3	97	CL	ALL	21	500	1155	F	94	22	2 2	12	
3	97	CL	ALL	22	406	736	M	95	11	1 1	22	
3	97	CL	ALL	23	540	1513	F	94	12	2 3	22	
3	97	CL	ALL	24	565	1827	M	92	12	2 3	11	
3	97	CL	ALL	25	553	1723	M	92	11	2 2	12	
3	97	CL	ALL	26	485	1001	F	94	12	2 2	22	
3	97	CL	ALL	27	546	1471	M	92	22	2 2	12	
3	97	CL	ALL	28	520	1336	M	92	11	2 3	22	
3	97	CL	ALL	29	435	699	M	94	22	2 2	12	
3	97	CL	ALL	30	490	1082	M	93	11	2 2	11	
3	97	CL	ALL	31	544	1578	M	92	22	2 2	11	
3	97	CL	ALL	32	515	1276	M	92	12	2 2	12	
3	97	CL	ALL	33	492	1230	M	94	22	2 3	12	
3	97	CL	ALL	34	482	926	F	94	22	2 2	12	
3	97	NEW	AUS	35	368	624	.	95	11	2 3	12	
3	97	NEW	AUS	36	406	567	.	94	22	2 2	12	
3	97	NEW	AUS	37	349	425	.	95	11	2 2	11	
3	97	NEW	AUS	38	419	680	.	95	11	2 2	11	
3	97	NEW	AUS	39	762	4500	M	86	11	2 2	11	
3	97	NEW	IVH	40	310	245	F	95	11	2 2	11	
3	97	NEW	IVH	41	334	293	M	95	11	2 2	11	

3	97	NEW	IVH	42	365	342	M	95	11	12	11	
3	97	NEW	IVH	43	347	282	M	95	11	12	22	
3	97	NEW	IVH	44	337	308	M	95	11	2 2	12	
3	97	NEW	IVH	45	355	325	M	95	11	2 2	11	
3	97	NEW	IVH	46	510	1116	M	90	11	3 3	11	
4	97	CL	PC	47	491	1169	M	92	22	2 2	12	
4	97	NEW	AUS	48	559	1723	.	86	11	3 3	11	
4	97	NEW	AUS	49	*	1428	.	90	11	3 3	11	
4	97	NEW	AUS	50	546	1592	.	90	11	3 3	12	
4	97	NEW	AUS	51	457	816	.	90	11	2 2	11	
4	97	NEW	AUS	52	717	3220	.	90	11	3 3	12	
4	97	NEW	AUS	53	419	680	.	94	22	2 3	12	
3	97	NEW	AUS	54	457	953	.	94	22	2 3	12	
10	97	CL	Lower	61	346	619	M	96	11	1 2	12	
10	97	CL	Lower	62	404	565	F	96	12	1 1	12	
10	97	CL	Lower	63	357	460	M	96	12		22	
10	97	CL	Lower	64	336	369	M	96	11	2 2	12	
10	97	CL	Lower	65	368	472	M	96	12	1 1	12	
10	97	CL	Lower	66	346	366	M	96	12	1 1	11	
10	97	CL	Lower	67	396	576	M	96	12	2 2	12	
10	97	CL	Lower	68	412	671	M	96	12	2 2	22	
10	97	CL	Lower	69	376	531	M	96	12	2 2	11	
10	97	CL	Lower	70	412	663	M	96	22	2 2	12	
10	97	CL	Lower	71	380	519	M	96	12	2 2	12	
10	97	CL	Lower	72	380	502	M	96	12	1 2	12	

10	97	CL	Lower	73	369	460	M	96	12	2 2	12	
10	97	CL	Lower	74	323	326	M	96	12	2 3	12	
10	97	CL	Lower	75	360	443	M	96	12	2 2	11	
10	97	CL	Lower	76	393	610	M	96	12	3 3	12	
11	97	CL	Lower	77	410	583	M	96	12	2 3	11	
11	97	CL	Lower	78	437	860	M	96	12	2 2	12	
11	97	CL	Lower	79	389	566	F	96	22	2 3	11	
11	97	CL	Lower	80	391	566	F	96	12	2 3	12	
11	97	CL	Lower	81	391	640	M	96	11	2 3	12	
11	97	CL	Lower	82	374	534	M	96	22	2 3	12	
11	97	CL	Lower	83	400	623	M	96	22	2 2	22	
11	97	CL	Lower	84	487	1116	F	95	22	2 3	12	
11	97	CL	Lower	85	405	666	M	96	22	2 2	11	
11	97	CL	Lower	86	398	576	F	96	11	2 3	12	
11	97	CL	Lower	87	406	666	M	96	12	3 3	22	
11	97	CL	Lower	88	280	561	M	96	11	3 3	11	
11	97	CL	Lower	89	445	645	M	96	11	2 2	11	
11	97	CL	Lower	90	395	632	M	96	12	2 2	22	
11	97	CL	Lower	91	399	610	F	96	11	3 3	11	
11	97	CL	Lower	92	391	525	M	96	22	2 2	12	
11	97	CL	Lower	93	380	530	F	96	12	2 3	12	
11	97	CL	Lower	94	381	503	F	96	12	23	11	
11	97	CL	Lower	95	430	768	F	96	12	2 2	11	
11	97	CL	Lower	96	535	1671	M	90	22	2 2	12	
11	97	CL	Lower	97	492	1128	F	95	12	3 3	11	

11	97	CL	Lower	98	536	1480	F	94	11	2 2	12	
11	97	CL	Lower	99	455	949	M	95	22	2 2	11	
11	97	CL	Lower	100	521	1472	F	94	12	2 2	12	
11	97	CL	Lower	101	425	709	F	96	11	3 3	22	
11	97	CL	Lower	102	394	570	F	96	12	2 2	22	
11	97	CL	Lower	103	381	479	M	96	22	2 2	12	
11	97	CL	Lower	104	385	580	M	96	11	2 2	12	
11	97	CL	Lower	105	368	518	M	96	11	2 2	12	
11	97	CL	Lower	106	416	712	F	96	12	2 2	11	
11	97	CL	Lower	107	393	624	M	96	22	2 2	11	
11	97	CL	Lower	108	525	1574	M	94	22	2 2	12	
12	97	CL	Lower	119	373	494	M	96	12	2 3	12	
12	97	CL	Lower	120	383	560	M	96	11	2 3	22	
12	97	CL	Lower	121	392	585	M	96	12	2 3	22	
12	97	CL	Lower	122	390	664	M	96	12	2 3	12	
12	97	CL	Lower	123	425	797	F	96	12	2 2	12	
12	97	CL	Lower	124	520	1277	F	94	22	2 3	12	
12	97	CL	Lower	125	513	1344	M	94	12	2 2	11	
12	97	CL	Lower	126	392	524	M	96	22	2 2	12	
12	97	CL	Lower	127	416	642	M	96	12	2 3	12	
12	97	CL	Lower	128	368	471	F	96	12	2 2	22	
12	97	CL	Lower	129	397	640	M	96	12	2 2	22	
12	97	CL	Lower	130	373	527	M	96	22	2 3	22	
12	97	CL	Lower	131	406	712	M	96	12	2 2	11	
12	97	CL	Lower	132	400	647	M	96	12	2 2	12	

12	97	CL	Lower	133	540	1580	F	94	12	2 2	22	
12	97	NEW	B/FF	135	411	693	M	.	11	2 2	12	
12	97	NEW	B/FF	136	352	370	F	.	11	2 2	11	
12	97	NEW	B/FF	137	320	261	M	.	11	3 3	11	
1	98	NEW	AUS	138	271	160	F	96	11	2 3	11	
1	98	NEW	AUS	139	462	864	F	95	11	2 3	12	
1	98	NEW	AUS	140	269	142	F	96	11	2 3	11	
1	98	NEW	AUS	141	255	121	F	96	11	2 3	12	
1	98	NEW	AUS	142	339	319	M	96	11	2 3	11	
1	98	NEW	AUS	143	289	184	F	96	11	2 3	11	
1	98	NEW	AUS	144	448	840	F	96	11	2 3	22	
1	98	NEW	AUS	145	288	182	F	96	11	2 2	12	
1	98	NEW	AUS	146	32 in	7200	F	87	11	2 2	12	
1	98	NEW	AUS	147	442	776	F	96	11	2 2	12	
1	98	NEW	AUS	148	271	157	F	96	11	1 2	11	
1	98	NEW	AUS	149	347	333	F	96	22	1 2	12	
1	98	NEW	AUS	150	380	475	M	96	11	2 2	11	
1	98	NEW	AUS	151	403	559	M	94	12	2 3	22	
1	98	NEW	AUS	152	437	773	M	94	12	2 2	11	
2	98	NEW	ALL	153	456	880	M	95	22	2 2	12	4
2	98	NEW	ALL	154	386	555	M	96	22	2 2	11	4
2	98	NEW	ALL	155	425	759	M	96	11	2 2	12	4
2	98	NEW	ALL	156	495	1100	M	94	12	2 2	12	4
2	98	NEW	ALL	157	428	686	M	95	22	2 2	11	4
2	98	NEW	ALL	158	418	702	M	96	22	2 2	22	44

2	98	NEW	ALL	159	350	414	M	96	12	2 2	22	44
2	98	NEW	ALL	160	357	402	M	96	12	2 2	22	44
2	98	NEW	ALL	161	443	906	M	96	12	2 2	11	45
3	98	NEW	B/FF	162	270	165	F	96	11	2 3	11	43
3	98	NEW	B/FF	163	341	325	M	96	11	2 2	11	43
3	98	NEW	B/FF	164	320	290	F	96	11	3 3	11	43
3	98	NEW	IVH	165	464	940	F	95	11	3 3	12	43
3	98	NEW	IVH	166	325	330	F	96	11	2 3	11	44
3	98	NEW	IVH	167	415	630	M	95	11	3 3	11	43
3	98	NEW	IVH	168	325	302	F	96	11	3 3	11	43
3	98	NEW	B/FF	169	487	1100	F	95	12	2 3	22	44
3	98	NEW	B/FF	170	325	274	M	96	11	3 3	11	
3	98	NEW	B/FF	171	421	671	M	95	11	3 3	11	
3	98	NEW	B/FF	172	326	318	M	96	11	2 3	11	
3	98	NEW	B/FF	173	464	1040	M	94	12	1 1	22	
3	98	NEW	B/FF	174	249	372	M	96	11	2 2	11	
3	98	NEW	B/FF	175	487	1100	F	95	12	2 3	11	43
3	98	NEW	B/FF	176	416	660	M	95	11	2 3	11	
3	98	NEW	B/FF	177	351	379	M	96	11	2 3	22	
3	98	NEW	B/FF	178	342	330	M	96	11	3 3	11	
3	98	NEW	B/FF	179	298	210	F	96	11	3 3	12	43
3	98	NEW	B/FF	180	339	310	M	96	11	3 3	11	
3	98	NEW	B/FF	181	398	618	M	96	12	2 2	12	
3	98	NEW	B/FF	182	479	1091	F	95	12	2 3	12	
3	98	NEW	B/FF	183	338	331	M	96	11	2 3	11	

3	98	NEW	B/FF	184	442	1091	F	95	12	1 3	11	
3	98	NEW	B/FF	185	385	541	M	96	12	1 2	22	
3	98	NEW	B/FF	186	482	1090	F	95	12	1 3	11	
3	98	NEW	B/FF	187	346	378	M	96	12	1 1	22	
3	98	NEW	B/FF	188	340	378	M	96	11	3 3	22	
3	98	NEW	B/FF	189	361	390	M	96	12	2 3	12	
3	98	NEW	B/FF	190	395	640	M	96	12	2 2	12	
3	98	NEW	B/FF	191	340	324	M	96	11	3 3	11	
3	98	NEW	B/FF	192	370	324	M	96	12	1 1	12	
3	98	NEW	B/FF	193	331	339	M	96	22	1 2	11	
3	98	NEW	B/FF	194	348	368	M	96	11	1 2	11	
3	98	NEW	B/FF	195	480	1090	F	95	22	1 2	11	
3	98	CL	Lower	196	413	700	F	98	22	2 2	12	
3	98	CL	Lower	197	420	760	F	98	12	1 1	11	
3	98	NEW	IVH	198	406	535	M	98	11	1 1	11	43
3	98	NEW	IVH	199	422	619	M	98	11	3 3	12	43
3	98	NEW	IVH	200	422	628	M	98	11	3 3	11	43
3	98	NEW	IVH	201	384	442	M	98	11	3 3	11	43
3	98	NEW	IVH	202	452	838	M	98	12	2 2	22	4
3	98	NEW	IVH	203	444	696	M	98	11	1 2	12	43
3	98	NEW	IVH	204	410	548	M	98	12	2 2	11	4
3	98	NEW	IVH	205	411	656	M	98	11	2 2	22	43
3	98	NEW	IVH	206	428	642	M	98	11	2 2	22	43
3	98	NEW	IVH	207	369	454	M	98	12	2 3	12	44
3	98	NEW	IVH	208	351	360	M	98	11	2 3	12	

3	98	NEW	IVH	209	320	256	M	98	11	3 3	12	
3	98	NEW	IVH	210	307	216	M	98	11	3 3	12	
3	98	NEW	IVH	211	509	>1200	F	98	12	2 2	22	
3	98	NEW	ALL	220	571	>1200	M	98	12	2 2	11	1
3	98	NEW	ALL	221	520	>1200	M	98	11	2 2	11	10
3	98	NEW	ALL	222	383	483	M	98	12	1 1	22	4
3	98	NEW	ALL	223	347	371	F	98	12	3 3	11	43
3	98	NEW	ALL	224	360	396	M	98	11	1 3	12	44
3	98	NEW	B/FF	225	550	>1200	M	98	12	3 3	11	43
3	98	NEW	B/FF	226	497	>1200	F	98	12	1 3	12	
3	98	NEW	B/FF	227	480	909	F	98	12	2 2	12	
3	98	NEW	B/FF	228	413	565	M	98	12	3 3	11	43
3	98	NEW	B/FF	229	332	276	M	98	11	2 2	11	43
3	98	NEW	B/FF	230	320	250	F	98	11	3 3	11	43
3	98	NEW	B/FF	231	310	235	M	98	11	3 3	11	43
10	98	CL	Lower	800	445	846	F	98	22	2 2	22	
10	98	CL	Lower	801	431	848	F	98	22	2 2	11	
10	98	CL	Lower	802	451	1200	M	98	11	2 2	11	
10	98	CL	Lower	803	485	1190	F	98	11	2 2	12	
10	98	CL	Lower	804	443	808	M	98	22	2 2	12	
10	98	CL	Lower	805	444	896	F	98	22	2 3	11	
10	98	CL	Lower	806	442	1257	F	98	12	2 3	22	
10	98	CL	Lower	807	410	727	M	98	22	2 2	11	
10	98	CL	Lower	808	445	1290	M	98	12	2 3	12	
10	98	CL	Lower	809	472	1253	F	98	22	2 2	12	

10	98	CL	Lower	810	460	1060	F	98	11	2 2	22	
10	98	CL	Lower	811	460	1138	M	98	11	2 2	11	
10	98	CL	Lower	812	445	814	M	98	22	2 3	12	
10	98	CL	Lower	813	490	1308	F	98	12	2 2	22	
10	98	CL	Lower	814	441	762	F	98	12	2 2	22	
10	98	CL	Lower	815	430	912	M	98	11	2 2	11	
10	98	CL	Lower	816	439	1280	M	98	22	2 2	11	
10	98	CL	Lower	817	475	1188	F	98	22	2 2	12	
10	98	CL	Lower	818	465	912	M	98	12	2 3	22	
10	98	CL	Lower	819	442	741	F	98	12	3 3	11	
10	98	CL	Lower	820	426	743	M	98	22	2 2	22	
10	98	CL	Lower	821	445	835	F	98	12	2 2	11	
10	98	CL	Lower	822	475	1080	M	98	12	2 2	22	
10	98	CL	Lower	823	446	1110	F	98	22	2 2	22	
10	98	CL	Lower	824	439	875	M	98	12	2 2	12	
10	98	CL	Lower	825	407	604	M	98	12	2 3	11	
10	98	CL	Lower	826	441	1082	M	98	12	2 2	12	
10	98	CL	Lower	827	480	919	F	98	22	2 2	12	
10	98	CL	Lower	828	474	1035	F	98	12	2 2	12	
11	98	CL	Lower	829	507	1450	F	98	11	2 3	22	
11	98	CL	Lower	830	444	800	M	98	12	2 2	11	
11	98	CL	Lower	831	555	1500	F	98	22	2 3	22	
11	98	CL	Lower	833	455	999	M	98	22	2 2	12	
11	98	CL	Lower	834	422	720	M	98	12	2 3	11	
11	98	CL	Lower	835	473	110	M	98	12	2 2	12	

11	98	CL	Lower	836	444	852	M	98	12	2 3	12	
11	98	CL	Lower	837	462	900	F	98	11	2 2	11	
11	98	CL	Lower	838	453	905	F	98	22	2 3	12	
11	98	CL	Lower	839	455	786	F	98	11	2 2	12	
11	98	CL	Lower	840	451	1000	F	98	22	2 3	12	
11	98	CL	Lower	841	425	830	M	98	22	2 2	11	
11	98	CL	Lower	842	453	1089	F	98	12	2 3	12	
11	98	CL	Lower	843	471	1100	F	98	12	2 2	11	
11	98	CL	Lower	844	432	759	M	98	22	2 3	11	
11	98	CL	Lower	845	433	700	M	98	12	2 2	11	
11	98	CL	Lower	846	456	1000	M	98	22	2 2	11	
11	98	CL	Lower	847	453	985	M	98	12	2 3	11	
11	98	CL	Lower	848	462	1100	M	98	12	2 2	12	
11	98	CL	Lower	849	502	1300	F	98	12	2 3	12	
11	98	CL	Lower	850	437	700	M	98	12	2 2	22	
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11	98	CL	Lower	852	462	1087	F	98	22	2 2	12	
11	98	CL	Lower	853	445	960	M	98	22	2 2	12	
11	98	CL	Lower	859	464	1000	M	98	12	2 2	12	
11	98	CL	Lower	860	469	1095	M	98	12	2 2	12	
11	98	CL	Lower	861	467	950	M	98	22	2 2	12	
11	98	CL	Lower	862	424	800	M	98	22	2 2	11	
11	98	CL	Lower	863	446	900	M	98	22	2 2	12	
12	98	CL	Lower	900	490	1150	F	98	22	2 2	11	
12	98	CL	Lower	901	508	1300	F	98	22	2 2	22	

12	98	CL	Lower	902	513	1450	F	98	12	1 3	12	
12	98	CL	Lower	903	476	1100	M	98	11	2 2	11	
12	98	CL	Lower	904	422	650	M	98	12	2 3	22	
12	98	CL	Lower	905	464	950	M	98	12	2 3	12	
12	98	CL	Lower	906	433	750	M	98	12	2 3	12	
12	98	CL	Lower	907	455	900	M	98	22	2 2	12	
12	98	CL	Lower	908	470	1000	F	98	12	2 3	12	
12	98	CL	Lower	909	479	1000	F	98	12	2 3	22	
12	98	CL	Lower	910	481	1100	M	98	12	3 3	11	
12	98	CL	Lower	911	509	1150	F	98	12	3 3	11	
12	98	CL	Lower	912	495	1150	F	98	11	2 2	11	
12	98	CL	Lower	913	424	800	M	98	22	2 2	12	
12	98	CL	Lower	914	411	650	M	98	12	2 3	12	
12	98	CL	Lower	915	483	950	M	98	11	2 2	11	
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12	98	CL	Lower	919	459	950	M	98	12	2 2	11	
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12	98	CL	Lower	925	456	900	M	98	22	2 3	12	
12	98	CL	Lower	926	463	900	M	98	22	2 3	11	
12	98	CL	Lower	927	507	1300	F	98	12	2 2	22	

12	98	CL	Lower	928	508	1150	F	98	11	2 3	22	
12	98	CL	Lower	929	442	800	M	98	12	2 2	22	
12	98	CL	Lower	930	446	850	M	98	12	2 2	22	
12	98	CL	Lower	931	454	950	F	98	11	2 2	12	
12	98	CL	Lower	932	481	1100	M	98	12	2 3	22	
12	98	CL	Lower	933	472	1050	M	98	22	2 2	12	
12	98	CL	Lower	934	530	1750	F	98	22	2 2	22	
12	98	CL	Lower	935	457	1000	F	98	22	2 2	22	
12	98	CL	Lower	936	437	850	M	98	22	2 2	12	
12	98	CL	Lower	937	490	1150	M	98	12	3 3	11	
3	99	New	B/FF	869	433	709	M	99	11	33	11	
3	99	New	B/FF	870	401	578	F	99	11	33	11	
3	99	New	B/FF	871	511	1200	M	99	12	23	22	
3	99	New	B/FF	872	483	1040	M	99	11	22	12	
3	99	New	B/FF	873	432	691	M	99	11	33	11	
3	99	New	B/FF	874	393	522	M	99	11	33	11	
3	99	New	B/FF	875	433	721	M	99	11	33	12	
3	99	New	B/FF	876	420	639	M	99	11	33	11	
3	99	New	B/FF	877	389	496	M	99	11	33	11	
3	99	New	B/FF	878	430	760	M	99	11	23	12	
2	99	New	Ivh	879	838	15 lbs	F	99	11	33	11	
3	99	New	B/FF	400	546	1600	F	99	22	22	22	
3	99	New	B/FF	401	453	856	M	99	22	23	12	
3	99	New	B/FF	402	562	1900	F	99	22	33	12	
3	99	New	B/FF	403	542	2000	F	99	12	22	22	

3	99	New	B/FF	404	551	1800	F	99	22	23	22	
3	99	New	B/FF	405	510	1500	F	99	11	22	12	
3	99	New	B/FF	406	538	1800	M	99	11	33	11	
3	99	New	B/FF	407	470	872	M	99	12	22	11	
3	99	New	B/FF	408	555	1800	F	99	11	33	11	
3	99	New	B/FF	409	460	922	M	99	11	33	11	
3	99	New	B/FF	410	451	865	M	99	11	22	11	
3	99	New	B/FF	411	480	1190	F	99	22	33	11	
3	99	New	B/FF	412	439	990	M	99	11	33	11	
3	99	New	B/FF	413	357	390	M	99	12	23	11	
3	99	New	B/FF	414	466	970	M	99	22	22	11	
3	99	New	B/FF	415	431	700	M	99	11	22	12	
3	99	New	ALL	416	503	1525	F	99	11	22	12	
3	99	New	ALL	417	391	552	M	99	22	22	12	
3	99	New	ALL	418	526	1152	M	99	12	33	11	
3	99	New	ALL	419	444	942	F	99	12	22	22	
3	99	New	IVH	434	461	822	M	99	22	33	11	
3	99	New	IVH	435	350	352	M	99	11	33	12	
3	99	New	IVH	436	442	643	M	99	11	33	11	
3	99	New	IVH	437	386	452	M	99	22	33	11	
3	99	New	IVH	438	400	468	M	99	12	33	11	
3	99	New	IVH	439	380	391	M	99	11	33	11	
3	99	New	IVH	440	387	420	M	99	11	33	11	
3	99	New	IVH	441	547	1170	F	99	11	33	11	
3	99	New	IVH	442	425	601	M	99	11	33	11	

3	99	New	IVH	443	501	910	M	99	11	33	11	
3	99	New	IVH	444	433	643	M	99	11	33	11	
3	99	New	IVH	445	381	492	M	99	11	33	11	
3	99	New	IVH	446	382	423	M	99	11	33	11	
3	99	New	IVH	447	402	470	M	99	11	33	11	
3	99	New	IVH	448	384	463	M	99	12	33	11	
3	99	New	IVH	449	421	570	M	99	22	22	22	
3	99	New	IVH	450	552	1525	M	99	11	33	11	
3	99	New	IVH	451	560	1822	F	99	12	22	12	
3	99	New	IVH	452	471	862	M	99	22	22	22	
3	99	New	IVH	453	452	720	M	99	11	33	11	
3	99	New	IVH	454	405	531	M	99	11	33	11	
3	99	New	IVH	455	449	361	M	99	11	33	11	
3	99	New	IVH	456	452	351	M	99	11	33	11	
3	99	New	IVH	457	497	491	M	99	11	33	11	
3	99	New	IVH	458	460	834	M	99	11	33	11	
3	99	New	ALL	459	376	400	M	99	12	23	11	
3	99	New	ALL	460	563	1870	M	99	22	22	12	
3	99	New	ALL	461	513	1540	M	99	12	23	12	
3	99	New	ALL	462	521	1500	M	99	22	22	12	
3	99	New	ALL	463	503	1352	M	99	11	22	22	
3	99	New	ALL	464	471	1100	M	99	12	23	11	
3	99	New	ALL	465	440	810	M	99	11	23	12	
3	99	New	ALL	466	435	751	M	99	22	22	22	
3	99	New	ALL	467	478	1080	M	99	11	22	11	

3	99	New	ALL	468	423	692	M	99	22	23	12	
3	99	New	ALL	469	496	1200	M	99	22	33	12	
3	99	New	ALL	470	523	1410	M	99	11	33	12	
3	99	New	ALL	471	407	680	M	99	12	23	22	
3	99	New	ALL	472	457	1058	M	99	22	22	12	
3	99	New	ALL	473	418	651	M	99	12	23	12	
3	99	New	ALL	474	447	940	M	99	12	22	22	
3	99	New	ALL	475	461	883	M	99	12	22	22	
3	99	New	ALL	476	413	1410	M	99	22	33	22	
3	99	New	ALL	477	406	500	M	99	12	22	12	
3	99	New	ALL	478	459	936	M	99	22	23	11	
3	99	New	ALL	479	468	1011	M	99	22	22	22	
3	99	New	IVH	667	523	1275	F	99	12	33	11	

Appendix 3, Table B. Microsatellite DNA data collected on the 244 walleye screened in the study. The letter before the ID # represents the site where the fish was collected (A= Allisonia, B= Buck Dam, F= Fosters Falls, and L= Claytor Lake). Data for each fish takes up two rows, one for each allele scored at the six different microsatellite loci examined.

ID #	SVI-18	SVI-4	SVI-17	SVI-6	SVI-26	SVI-33
A153	120	110	103	142	156	86
	120	110	103	142	156	98
A154	124	110	103	?	160	96
	124	116	103	?	165	?
A155	122	108	103	142	152	98
	126	110	103	?	158	104
A156	122	106	107	142	160	86
	122	110	109	142	167	104
A157	122	106	107	142	?	86
	122	106	109	142	?	100
A158	124	112	105	142	156	86
	126	114	111	144	156	86
A159	118	112	103	?	156	86
	124	112	103	?	156	100
A160	118	110	99	140	152	78
	118	106	105	142	152	86
A161	122	112	103	?	?	86
	124	112	111	?	?	86
A220	122	108	99	150	154	86
	120	108	99	161	158	90
A221	118	110	103	146	?	86
	118	106	105	146	?	78
A222	118	108	103	?	?	90
	124	114	103	?	?	92
A223	118	110	99	?	160	78
	118	110	99	?	160	86
A224	118	106	103	142	160	86
	124	110	103	142	160	86
A24	118	108	109	148	156	86
	122	110	111	158	167	90
A25	120	106	109	142	154	86
	120	110	111	150	154	86
A26	122	112	103	142	160	86
	122	114	103	142	160	98
A27	118	106	103	144	156	84
	118	106	103	?	156	86
A28	124	108	103	142	165	86
	124	108	111	150	?	90
A29	118	112	103	142	154	78
	118	114	107	144	154	86
A30	122	108	111	140	152	90
	124	110	111	165	165	90
A31	118	106	109	142	152	86

	118	108	113	150	169	98
A32	118	106	103	142	152	86
	122	110	109	150	154	90
A33	118	108	?	?	158	98
	122	110	?	?	165	?
A34	118	106	105	144	?	86
	118	108	105	144	?	98
A416	118	110	105	144	162	86
	124	114	113	144	167	98
A417	122	104	109	142	?	98
	122	110	109	148	?	98
A418	124	104	105	?	?	84
	124	110	111	?	?	86
A419	122	104	109	142	165	100
	122	104	109	148	167	102
A459	122	108	105	142	160	90
	122	110	109	142	160	100
A460	124	104	109	158	152	100
	124	104	113	158	152	104
A461	118	104	109	142	156	100
	122	100	111	148	156	100
A462	118	104	109	?	?	100
	124	110	111	?	?	100
A463	122	110	111	142	154	90
	124	110	113	142	154	92
A464	122	104	109	142	?	78
	122	104	111	148	?	84
A465	118	104	107	142	167	98
	124	110	109	142	167	98
A466	124	?	107	142	165	90
	124	?	109	148	167	98
A467	118	104	105	142	183	100
	124	100	113	142	185	104
A468	124	104	109	142	165	98
	124	104	111	148	165	100
A469	118	100	105	?	?	84
	124	100	111	?	?	104
A470	122	108	105	142	152	86
	124	110	113	148	154	86
A471	122	104	109	165	165	92
	122	110	113	?	167	100
A472	122	104	103	142	154	100
	122	110	113	148	154	100
A473	118	106	109	140	152	100
	124	110	113	148	165	100
A474	120	104	109	?	?	86
	124	110	111	?	?	98
A475	118	104	105	144	158	90
	122	104	111	152	165	98
A476	118	104	105	142	167	90
	124	110	109	142	169	100
A477	118	104	105	136	154	98

	120	100	109	142	160	104
A478	118	104	111	?	?	84
	124	110	111	?	?	100
A479	120	104	105	142	167	100
	122	108	109	144	167	104
A49.351	124	108	103	144	154	86
	118	110	109	?	160	96
A49.361	124	108	99	142	162	98
	126	108	109	148	162	100
A49.401	124	100	101	142	165	100
	124	114	103	154	165	104
A49.4012	118	106	?	142	?	?
	120	108	?	142	?	?
A49.431	124	108	109	130	154	84
	126	110	111	?	154	86
A49.471	?	?	?	?	?	100
	?	?	?	?	?	104
A49.511	122	106	103	142	160	86
	122	114	103	142	160	98
A49.5111	122	106	101	142	?	100
	122	114	103	148	?	104
B1	120	108	101	158	152	78
	126	110	101	158	154	86
B165	126	110	99	158	160	78
	126	112	99	158	160	78
B167	118	112	103	140	152	86
	118	112	111	142	152	98
B168	120	108	99	158	152	84
	120	110	99	158	152	86
B198	118	108	99	158	152	78
	122	110	99	158	152	86
B199	118	108	99	158	158	78
	122	110	99	158	158	84
B200	118	108	99	158	158	78
	124	108	99	158	158	86
B201	120	108	99	158	158	78
	120	110	99	158	158	86
B202	122	114	103	142	165	96
	124	114	103	144	169	98
B203	120	108	99	158	158	78
	120	110	99	158	158	86
B204	118	110	?	142	?	96
	118	112	?	150	?	102
B205	118	108	99	158	152	78
B205	120	108	99	158	152	78
B206	118	108	99	158	152	78
	120	108	99	158	158	84
B207	118	112	103	?	158	86
	120	112	103	?	158	96
B208	120	108	99	158	158	78
	120	110	99	158	158	78
B209	120	108	99	158	158	78

	122	108	99	158	158	84
B210	120	108	99	158	158	78
	120	108	99	158	158	86
B211	120	112	105	136	158	78
	120	112	105	142	160	96
B434	126	108	101	150	154	78
	126	110	101	161	158	86
B436	120	108	101	158	158	78
	126	112	101	158	158	86
B437	120	108	101	158	158	78
	122	112	101	158	158	86
B438	120	108	101	158	158	78
	122	110	101	158	158	86
B439	120	108	101	158	158	86
	126	110	101	158	158	86
B440	124	108	101	150	160	84
	126	112	101	150	167	84
B441	120	108	101	150	158	84
	126	112	101	158	158	86
B442	120	108	101	150	154	78
	120	112	101	161	158	84
B443	122	108	101	158	158	84
	122	110	101	158	158	86
B444	126	108	101	150	158	78
	126	112	101	161	158	86
B445	126	108	101	158	154	78
	126	112	101	158	158	78
B446	120	108	101	158	158	78
	120	112	101	161	158	78
B447	120	108	101	158	158	86
	126	110	101	158	158	86
B448	122	108	101	150	158	84
	122	108	101	161	158	86
B449	118	110	105	142	?	90
	122	110	109	148	?	98
B450	122	108	101	158	158	78
	124	108	103	158	158	86
B451	120	110	103	142	154	98
	124	114	103	148	158	100
B452	122	108	103	140	169	84
	124	112	103	146	171	90
B453	120	108	101	158	154	78
	126	112	101	158	158	84
B454	120	106	101	158	154	78
	118	106	101	158	154	86
B455	120	106	101	?	158	78
	126	108	101	?	167	84
B456	120	108	101	158	154	78
	126	112	101	158	158	86
B457	120	108	101	158	154	78
	122	112	101	158	158	86
B458	126	112	101	158	154	84

	120	108	101	158	158	78
B48.071	122	110	101	158	154	84
	122	112	101	161	154	86
B48.0712	118	108	99	150	152	86
	118	108	99	163	158	86
B48.081	?	?	?	?	?	?
	?	?	?	?	?	?
B48.091	120	106	101	158	154	78
	120	108	101	158	158	78
B48.111	122	108	101	158	160	78
	124	108	101	158	160	84
B48.121	120	108	101	150	158	78
	120	110	101	150	158	78
B48.131	122	108	101	158	156	84
	126	106	101	158	158	86
B48.141	122	108	103	142	154	100
	124	110	111	142	158	104
B48.161	126	108	101	158	154	78
	126	108	101	158	158	84
B48.171	118	108	101	148	154	78
	126	108	101	161	154	84
B48.181	126	106	101	158	152	78
	126	108	101	158	154	78
B48.191	118	110	101	158	154	78
	126	110	101	158	158	86
B48.201	120	108	99	150	158	84
	118	114	99	161	158	86
B48.221	118	106	101	158	154	84
	126	108	101	158	158	86
B667	122	108	101	158	154	78
	122	112	101	158	158	86
B879	118	108	101	154	154	84
	120	108	101	154	154	86
F170	118	106	99	154	154	?
	120	112	99	163	160	?
F171	120	108	99	154	160	78
	120	110	99	154	160	86
F172	126	108	99	154	160	78
	126	110	99	154	167	84
F173	118	106	101	142	162	86
	122	108	109	142	165	98
F174	118	108	99	?	160	78
	120	110	99	?	160	86
F175	118	108	103	?	160	86
	118	110	103	?	165	98
F176	120	104	99	150	152	78
	120	106	99	154	152	86
F177	120	112	103	?	152	86
	120	112	103	?	154	92
F178	120	108	99	?	160	86
	126	110	99	?	160	86
F179	120	108	99	140	160	78

	122	108	99	148	169	86
F180	120	108	99	?	154	78
	120	110	99	?	158	86
F181	124	108	105	142	154	86
	124	108	109	150	160	104
F182	118	114	103	136	160	86
	122	114	103	140	160	98
F183	120	110	99	150	158	78
	122	112	99	163	158	86
F184	122	104	103	140	162	78
	122	108	103	140	165	86
F185	118	110	101	140	162	98
	122	112	103	140	162	98
F186	124	?	103	142	165	86
	122	108	103	?	165	98
F187	120	110	101	130	167	86
	124	112	103	140	171	98
F188	124	110	109	130	158	?
	126	116	109	144	160	?
F189	118	110	103	140	160	86
	118	110	103	148	165	98
F190	118	108	?	142	165	86
	118	110	?	146	169	98
F191	126	108	99	154	158	78
	126	110	99	154	158	86
F192	124	112	103	142	160	86
	122	114	103	144	169	98
F193	118	106	103	140	160	86
	122	112	103	140	169	96
F194	122	108	99	148	160	84
	122	110	99	150	169	84
F195	126	106	103	140	165	?
	126	110	103	140	165	?
F225	124	108	99	158	158	78
	120	110	99	163	158	84
F226	124	106	109	142	162	86
	124	110	111	144	162	98
F227	122	108	103	144	154	86
	120	114	109	158	154	98
F228	120	108	99	150	152	86
	120	114	99	161	158	98
F229	120	108	99	136	158	78
	122	108	99	?	158	84
F231	124	108	99	150	154	86
	120	112	99	?	160	96
F400	118	108	103	?	165	90
	122	114	109	?	169	104
F401	118	108	103	142	158	100
	124	110	103	142	162	100
F402	122	106	103	142	162	86
	124	108	103	142	162	86
F403	124	108	103	140	162	86

	124	110	103	140	162	98
F404	122	?	103	154	154	100
	122	?	109	154	154	100
F405	122	106	103	140	158	86
	124	110	109	142	171	96
F406	118	108	101	154	158	100
	124	108	101	154	158	100
F407	122	106	109	140	183	96
	122	108	109	140	185	104
F409	120	100	105	158	158	86
	126	100	113	158	158	90
F410	118	104	103	142	?	98
	122	110	113	142	?	104
F411	118	106	101	142	162	78
	118	112	101	142	171	86
F412	122	104	101	140	162	100
	122	108	103	140	162	104
F413	122	108	101	148	?	78
	120	108	101	154	?	78
F414	118	108	101	154	154	78
	120	108	101	154	154	84
F415	124	108	103	142	183	98
	124	108	107	144	185	104
F48.151	120	110	99	136	158	78
	126	110	99	?	158	86
F480	120	100	105	?	158	78
	126	104	105	?	158	86
F868	122	108	101	154	156	86
	126	108	101	154	156	86
F869	120	108	101	154	154	78
	120	110	101	154	158	84
F870	120	108	101	148	156	78
	126	108	101	148	156	84
F871	122	108	103	?	165	86
	124	110	109	?	169	100
F872	118	108	103	136	158	98
	118	110	103	144	158	100
F873	122	108	101	154	158	78
	122	108	101	154	158	86
F874	120	108	109	154	156	86
	126	108	111	154	156	86
F875	120	108	101	154	158	78
	122	112	101	154	158	84
F876	120	108	101	154	156	78
	126	108	101	154	156	84
F877	122	108	101	154	156	78
	122	108	101	154	156	84
F878	118	108	103	142	162	86
	124	112	109	142	169	90
L49.341	118	108	?	142	?	98
	122	110	?	150	?	100
L49.371	122	106	103	148	165	86

	124	108	103	150	167	86
L49.381	124	110	103	136	158	86
	124	112	103	?	160	98
L49.391	118	108	103	148	?	96
	120	110	109	165	?	104
L49.401	122	108	103	?	154	98
	124	110	109	144	154	86
L49.411	122	106	103	150	156	84
	122	112	111	158	160	98
L49.421	122	108	103	142	?	86
	124	114	109	144	?	90
L49.421	122	108	?	?	?	96
	124	110	?	?	?	100
L49.441	120	112	101	154	183	90
	120	114	103	161	185	100
L49.451	120	106	103	142	154	100
	124	110	103	150	165	102
L49.461	122	106	101	142	154	98
	124	108	103	158	165	104
L49.471	120	110	103	144	152	86
	118	112	103	?	158	98
L49.481	122	110	109	?	158	98
	122	116	111	?	167	98
L49.491	124	106	109	130	?	86
	124	110	111	?	?	96
L49.5011	?	?	?	163	160	?
	?	?	?	161	162	?
L49.5012	118	110	103	142	162	90
	124	112	109	150	?	98
L49.521	118	108	103	136	160	100
	118	110	103	142	160	100
L49.611	124	114	103	142	?	98
	126	114	109	142	?	98
L49.631	118	110	103	144	158	84
	118	112	109	150	167	86
L49.651	120	108	109	142	165	92
	122	108	109	142	165	100
L532	?	108	111	146	160	86
	?	110	111	?	169	96
L533	118	114	95	142	181	86
	120	116	95	154	185	96
L534	122	108	103	142	152	86
	122	108	109	148	158	96
L535	118	104	103	142	165	96
	122	110	101	142	169	98
L543	118	110	109	144	152	96
	122	?	111	150	158	98
L544	122	110	103	?	152	?
	122	112	109	?	158	?
L545	118	110	103	148	152	86
	122	112	103	152	158	96
L546	118	108	103	142	165	86

	122	112	111	144	169	96
L547	118	110	103	142	165	96
	118	108	103	144	169	98
L550	118	106	103	142	160	96
	122	108	103	148	160	98
L551	124	112	103	146	160	86
	126	114	111	146	167	96
L552	118	112	95	142	181	96
	120	114	95	142	185	102
L553	118	108	103	142	165	96
	122	110	109	?	165	98
L554	118	108	109	142	160	86
	124	110	111	142	160	104
L555	118	112	99	130	160	98
	122	114	109	142	167	104
L556	118	108	103	?	152	98
	124	108	103	?	160	104
L571	124	106	109	?	152	90
	124	110	111	?	158	98
L572	124	112	103	142	152	96
	124	112	109	154	158	96
L573	118	110	111	142	169	98
	122	112	111	?	171	102
L574	124	108	103	?	152	90
	124	108	103	?	158	98
L576	118	110	103	?	?	90
	124	112	109	?	?	98
L577	124	112	103	?	167	96
	124	114	103	?	171	98
L578	122	112	103	?	162	96
	124	112	111	?	169	98
L579	118	108	109	?	?	?
	124	110	111	?	?	?
L580	118	108	103	?	?	?
	120	110	109	?	?	?
L900	120	108	103	136	165	100
	120	114	109	138	171	100
L901	122	106	105	136	181	?
	124	114	111	142	181	?
L902	118	110	95	142	156	98
	120	110	111	158	156	98
L908	118	106	109	142	165	100
	118	110	111	144	167	104
L909	118	110	105	136	165	98
	120	112	111	148	167	100
L916	122	110	105	136	158	86
	122	112	111	142	165	100
L917	118	110	105	136	?	104
	122	108	111	154	?	104
L918	120	?	95	142	154	92
	120	?	105	148	160	100
L919	120	108	109	142	165	100

	122	110	111	142	171	104
L920	118	112	105	142	171	98
	120	114	105	148	183	100
L921	124	108	105	?	160	92
	124	108	105	?	169	100
L922	118	108	105	136	154	104
	122	116	111	148	154	104
L923	118	108	105	142	154	86
	118	110	105	148	154	98
L924	118	108	105	136	165	92
	120	110	105	142	165	100
L925	118	106	105	136	165	100
	120	110	111	142	165	100
L926	120	106	99	142	?	92
	120	110	105	142	?	92
L927	122	110	105	136	160	100
	122	112	111	142	160	100
L928	120	112	95	146	160	98
	124	114	111	152	160	98
L929	118	112	105	136	167	104
	122	114	109	144	167	104
L930	118	110	105	138	167	100
	122	112	111	144	167	100
L931	122	110	105	138	167	92
	126	110	111	144	167	104
L932	122	110	105	136	152	92
	126	112	111	142	152	104
L933	118	110	103	136	152	100
	118	112	105	142	152	104
L934	118	110	111	136	152	102
	118	106	111	138	152	104
L935	124	110	103	154	165	86
	124	112	103	161	165	100

VITA

George Caleb Palmer was born in Bogota, Columbia, South America on January 21, 1967. He was raised in Richmond and Charlottesville, Virginia. He attended Albemarle High School and graduated in 1986. In 1986, he joined the United States Marine Corps Reserve and was attached to Helicopter Marine and Maintenance – 774, stationed in Norfolk, Virginia. He was honorably discharged in 1993 at the rank of Sergeant. He received as A.S. degree in general studies from Piedmont Virginia Community College in 1994. He received a B.S. in Fisheries Science from Virginia Polytechnic Institute and State University in 1996. In 1997, he became a candidate for the M.S. degree in Fisheries and Wildlife Science, at Virginia Polytechnic Institute and State University.

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George C. Palmer