Occurrence of Two Non-indigenous Catostomid Fishes in the New River, Virginia

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Abstract - Two catostomid fishes, *Carpiodes cyprinus* (Quillback) and *Moxostoma collapsum* (Notchlip Redhorse), were recently discovered in the New River watershed (Ohio River basin) in Virginia. The New River fish fauna is naturally depauperate relative to surrounding watersheds, and it has been altered substantially due to non-indigenous species introductions. Notchlip Redhorse and Quillback are established in Claytor Lake and are dispersing into novel reaches of the mainstem New River. We suspect that these species became established following bait-bucket introductions or incidentally during game-fish stockings. Public education, policy changes, and stricter hatchery procedures are needed to minimize such occurrences of non-indigenous species introductions.

Introduction

The New River has a unique native fish community, with 9 endemic species and several notable absences, including large catostomid fishes common in the Ohio River basin (Jenkins and Burkhead 1994, Stauffer et al. 1995). *Hypentelium nigricans* (Lesueur) (Northern Hog Sucker) and *Catostomus commersonii* (Lacepéde) (White Sucker) are the only native catostomid fishes above Kanawha Falls (Easton and Orth 1994, Hocutt et al. 1978, Jenkins and Burkhead 1994, Masnik et al. 1978). Jenkins and Burkhead (1994) summarized records of additional sucker species within the New River drainage, including *Moxostoma cervinum* (Cope) (Blacktip Jumprock), *Moxostoma erythrurum* (Rafinesque) (Golden Redhorse), and *Thoburnia rhothoeca* (Thoburn) (Torrent Sucker), recognizing Torrent Sucker as probably native and both *Moxostoma* species as likely introductions.

The New River has a long history of non-indigenous species introductions. Jenkins and Burkhead (1994) noted that only 46 of the 89 fish taxa in the New River basin were native species. The Virginia Department of Game and Inland Fisheries collected *Carpiodes cyprinus* (Lesueur) (Quillback) and *Moxostoma collapsum* (Cope) (Notchlip Redhorse) in the New River of Virginia, beginning in 2006 and 2013, respectively (Tables 1, 2). Quillback were first collected during a gill-net survey on Claytor Lake, a hydropower reservoir on the mainstem New River (Fig. 1). Claytor Lake gill-net surveys in 2009 also yielded a single

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Quillback, and the species has been collected annually since 2011. Quillback have been reported as far upstream as Foster Falls, but not downstream of Claytor Lake. On 15 July 2013, a single Notchlip Redhorse was collected near Claytor Lake State Park during a youth-engagement event. Notchlip Redhorse are now becoming more common in Claytor Lake fish surveys. Like Quillback, Notchlip Redhorse have been collected as far upstream as Foster Falls. Unlike Quillback, Notchlip Redhorse have also been encountered downstream of Claytor Lake (10 April 2016, n = 1, TL = 626 mm). No young-of-year specimens have been collected to date. A specimen of each species from Foster Falls was deposited in

Table 1. Recorded *Carpiodes cyprinus* (Quillback) collections by Virginia Department of Game and Inland Fisheries. Sample sizes (n), mean total length (TL, mm) and standard deviation (SD) are presented. Fish collected via: BEF = boat electrofishing, and GN = gill net.

Date	Location	Coordinates	Gear	*n*	TL (SD)
1 Nov 2006	Claytor Lake	37°3'58.6"N, 80°36'32"W	GN	1	390
15 Dec 2009	Claytor Lake	37°2'49.5"N, 80°37'35.4"W	GN	1	475
26 Oct 2011	Claytor Lake	37°2'48.9"N, 80°39'56.3"W	GN	1	327
24 Oct 2012	Claytor Lake	37°1'42.9"N, 80°40'23.0"W	GN	1	445
25 Oct 2012	Claytor Lake	37°1'42.9"N, 80°40'23.0"W	GN	2	374 (23)
29 Nov 2012	Claytor Lake	37°2'49.8"N, 80°39'54.9"W	GN	1	395
29 Nov 2012	Claytor Lake	37°3'41.4"N, 80°36'54.8"W	GN	1	290
30 Oct 2013	Claytor Lake	37°1'43.1"N, 80°40'25"W	GN	2	446 (71)
30 Oct 2013	Claytor Lake	37°1'42.6"N, 80°40'22.8"W	GN	4	417 (9)
30 Oct 2013	Claytor Lake	37°3'42.4"N, 80°36'52.2"W	GN	1	408
29 Oct 2014	Claytor Lake	37°1'43.3"N, 80°40'24.5"W	GN	4	396 (81)
29 Oct 2014	Claytor Lake	37°2'49.9"N, 80°39'56.2"W	GN	1	374
29 Oct 2014	Claytor Lake	37°2'49.9"N, 80°37'35.0"W	GN	1	330
30 Oct 2014	Claytor Lake	37°2'49.9"N, 80°39'56.2"W	GN	1	440
30 Oct 2014	Claytor Lake	37°3'4.2"N, 80°40'27.4"W	GN	1	360
15 Oct 2015	Claytor Lake	37°3'15.2"N, 80°39'56.1"W	GN	1	356
21 Oct 2015	Claytor Lake	37°3'15.2"N, 80°39'56.1"W	GN	1	385
22 Oct 2015	Claytor Lake	37°2'49.9"N, 80°37'35.0"W	GN	1	509
22 Oct 2015	Claytor Lake	37°3'15.2"N, 80°39'56.1"W	GN	2	422 (32)
22 Oct 2015	Claytor Lake	37°3'15.9"N, 80°38'25.8"W	GN	2	413 (84)
25 May 2016	New River (Foster Falls)	36°53'50.7"N, 80°51'38.2"W	BEF	8	394 (19)
26 Oct 2016	Claytor Lake	37°1'42.3"N, 80°40'24.7"W	GN	2	361 (19)
26 Oct 2016	Claytor Lake	37°2'50.5"N, 80°39'54.2"W	GN	3	329 (14)
26 Oct 2016	Claytor Lake	37°3'15.2"N, 80°39'56.1"W	GN	1	329
26 Oct 2016	Claytor Lake	37°3'59.8"N, 80°36'33"W	GN	1	390
26 Oct 2016	Claytor Lake	37°3'46.3"N, 80°36' 52.4"W	GN	3	349 (18)
27 Oct 2016	Claytor Lake	37°3'5"N, 80°40'29.5"W	GN	1	355
27 Oct 2016	Claytor Lake	37°3'17.1"N, 80°39'55.8"W	GN	1	348
27 Oct 2016	Claytor Lake	37°1'42.3"N, 80°40'24.7"W	GN	2	390 (0)
27 Oct 2016	Claytor Lake	37°2'50.5"N, 80°39'54.2"W	GN	1	455
27 Oct 2016	Claytor Lake	37°2'50.7"N, 80°37'33.7"W	GN	2	373 (46)
2 Nov 2016	Claytor Lake	37°3'5.2"N, 80°40'29.2"W	GN	2	338 (7)
2 Nov 2016	Claytor Lake	37°2'50.7"N, 80°39'55.5"W	GN	1	320
2 Nov 2016	Claytor Lake	37°1'41.3"N, 80°40'21.8"W	GN	1	385
2 Nov 2016	Claytor Lake	37°3'58.7"N, 80°36'32.3"W	GN	3	345 (11)
2 Nov 2016	Claytor Lake	37°3'46"N, 80°36'51.8"W	GN	4	339 (13)

the Virginia Museum of Natural History (Catalog Numbers: *Carpiodes cyprinus* VMNH 151,000; *Moxostoma collapsum* VMNH 151,001).

Notchlip Redhorse was formerly considered a geographic race of Silver Redhorse, but more recently was elevated as its sister species (Marcy et al. 2005). A phylogenetic study recommended retention of Notchlip Redhorse as a species, given a lack of evidence to synonymize with *M. anisurum* (Rafinesque) (Silver Redhorse) (Harris et al. 2002). Presently, specimens from the Mississippi River basin and north are Silver Redhorse, whereas Atlantic-slope specimens from the Roanoke River, VA, south to the Altamaha River, GA, are Notchlip Redhorse (Page and Burr 2011). Published works, however, lack diagnostic characteristics to differentiate the species because few states have both Notchlip and Silver Redhorse, and many ichthyological references are outdated.

Field-site Description

The New River (Kanawha River watershed, Ohio River basin) flows from North Carolina through Virginia and forms the Kanawha River at its confluence with the Gauley River near Gauley Bridge, WV. Our specimens were collected from Claytor Lake, a human-made impoundment on the river (Fig. 1).

Methods

We studied from a sample of 14 unidentified *Moxostoma* specimens collected from Claytor Lake (New River, Pulaski County, VA) in 2016 using experimental gill nets. We identified Notchlip Redhorse and Quillback using a combination of anatomical and molecular traits. We identified Quillback based on lower-lip morphology (absence of nipple-like projection) and lateral-line scale counts (≥37 scales) of specimens, as reported by Etnier and Starnes (1993). After initial

Table 2. *Moxostoma collapsum* (Notchlip Redhorse) collections or specimens reported to Virginia Department of Game and Inland Fisheries. Sample sizes (*n*), mean total length (TL, mm) and standard deviation (SD) are presented. Fish collected via: BEF = boat electrofishing, GN = gill net, and AN = anglers. NM indicates no measurement for length was taken.

Date	Location	Coordinates	Gear	*n*	TL (SD)
15 July 2013	Claytor Lake	37°3'9.37"N, 80°37'27.61"W	BEF	1	NM
29 Oct 2014	Claytor Lake	37°1'43.3"N, 80°40'24.5"W	GN	1	297
16 Dec 2014	Claytor Lake	37°1'43.3"N, 80°40'24.5"W	GN	1	420
22 Oct 2015	Claytor Lake	37°2'50.5"N, 80°39'54.2"W	GN	1	448
10 Apr 2016	New River (Whitethorne)	37°11'26.0"N, 80°34'30.2"W	AN	1	626
25 May 2016	New River (Foster Falls)	36°53'50.7"N, 80°51'38.2"W	BEF	12	338 (65)
26 Oct 2016	Claytor Lake	37°02'50.5"N, 80°39'54.2"W	GN	1	400
26 Oct 2016	Claytor Lake	37°03'15.2"N, 80°39'56.1"W	GN	1	445
27 Oct 2016	Claytor Lake	37°3'17.1"N, 80°39'55.8"W	GN	1	400
27 Oct 2016	Claytor Lake	37°2'50.5"N, 80°39'54.2"W	GN	2	410 (7)
27 Oct 2016	Claytor Lake	37°2'50.7"N, 80°37'33.7"W	GN	1	415
2 Nov 2016	Claytor Lake	37°03'05.2"N, 80°40'29.2"W	GN	1	413
2 Nov 2016	Claytor Lake	37°03'58.7"N, 80°36'32.3"W	GN	1	377

examination of *Moxostoma* specimens, we concluded that the specimens were either Silver Redhorse or Notchlip Redhorse, based on lip morphology (semipapillose) and dorsal-ray counts (14–16; Page and Burr 2011). We incorporated DNA

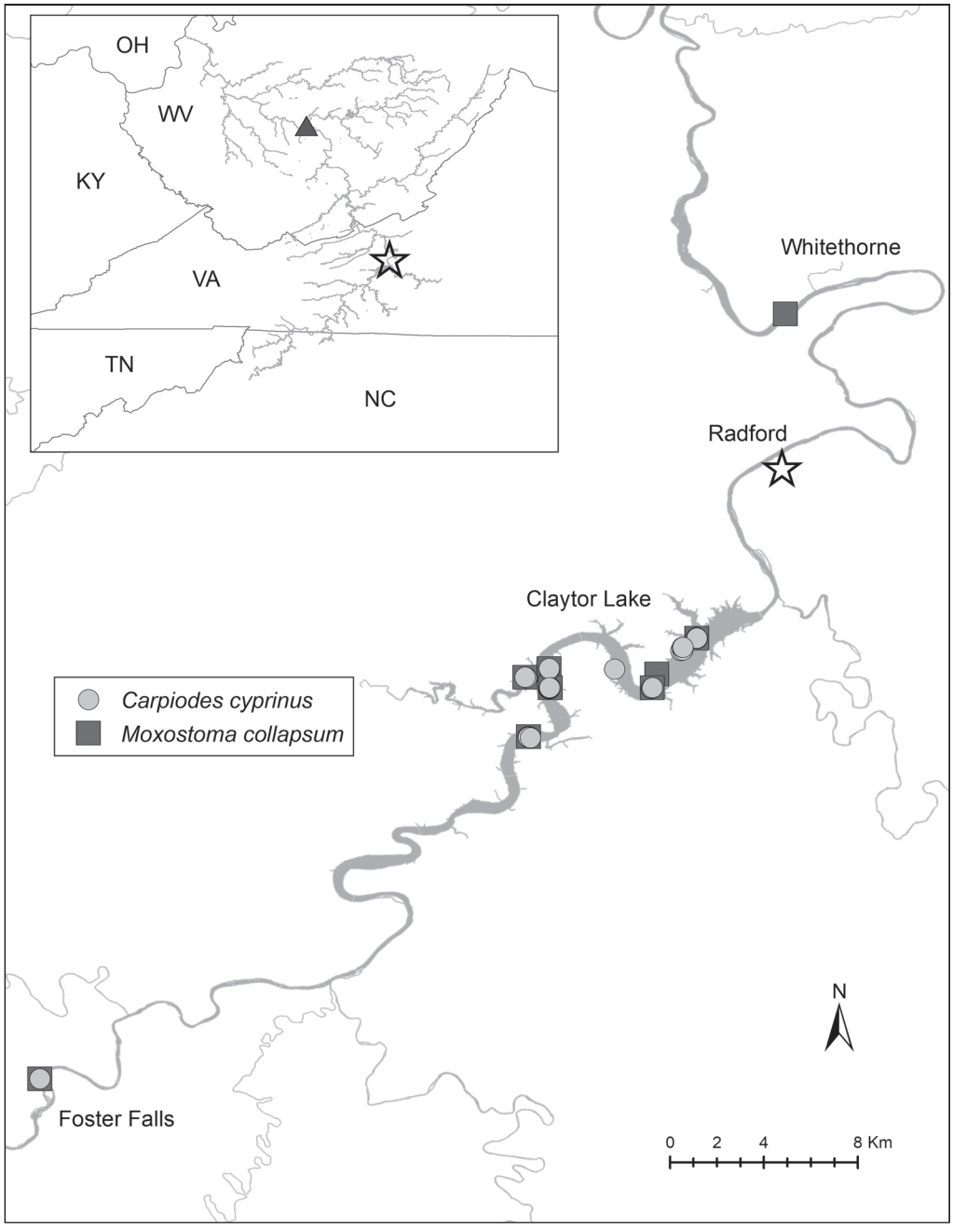

Figure 1. Occurrence of *Carpiodes cyprinus* (Quillback) and *Moxostoma collapsum* (Notchlip Redhorse) within the New River watershed in relation to the city of Radford, VA (denoted by the star). Location of the Kanawha River watershed within the region represented in the inset with Kanawha Falls denoted by a black triangle.

markers in the further identification of the *Moxostoma* specimens because distinguishing between these 2 species on a morphological basis can be challenging.

We used standard DNA barcoding techniques to identify the species of these unknown *Moxostoma* individuals. We used a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) to extract DNA from a pectoral fin-clip taken from each specimen. We employed a cocktail of universal primers to isolate and amplify DNA at the *COI-3* region (Ivanova et al. 2007). We used the forward primers VF2_t1 and FishF2_v1 (Ward et al. 2005) and the reverse primers FishR2_t1 and FR1d_t1 (Ivanova et al. 2007, Ward et al. 2005). Each polymerase chain reaction (PCR) consisted of a total volume of 22 µL and included 14.9 µL of H_20, 2 µL of 5X Colorless GoTaq® Flexi Buffer (Promega Corporation, Madison, WI), 2 µL of 25X $MgCl_2$, 0.4 µL of dNTP (2.5 mM), 0.4 µL of each primer (10 uM), 0.1 µL of GoTaq® Flexi DNA Polymerase (5u/µL, Promega Corporation,) and 1 µL of DNA template. Thermocycling conditions for PCR followed the protocol for the *COI-3* region described by Ivanova et al. (2007). We visualized PCR products on a 2% agarose gel to verify amplification and amplicon length.

We carried out full Sanger sequencing at the Virginia Biocomplexity Institute (Blacksburg, VA). We prepared samples for DNA sequencing using forward primer VF2_t1 and reverse primer FishR2_t1. We assembled and trimmed forward and reverse sequences using Geneious 10.0.9 software (Geneious, Auckland, New Zealand). We then queried consensus sequences using the basic local alignment search tool (BLAST, Altschul et al. 1990) against the GenBank database for comparison against archived sequences. Comparisons of Claytor Lake *Moxostoma* with archived sequences yielded 99–100% similarity with Roanoke River Notchlip Redhorse and 98–99% similarity with Silver Redhorse. Archived sequences of Roanoke River Notchlip Redhorse yielded highest total and maximum similarity scores in our comparison. Consequently, we concluded that these *Moxostoma* within the Virginia portion of the New River drainage are Notchlip Redhorse.

Discussion

We can only speculate on the sources of these non-indigenous fishes, but bait-bucket and "hitchhiker" introduction mechanisms seem plausible. The explanation of bait-bucket introductions is reasonable because the New River provides a popular *Micropterus dolomieu* Lacepéde (Smallmouth Bass) fishery, as well as a robust *Esox masquinongy* Mitchill (Muskellunge) population. Claytor Lake also supports a *Morone saxatilis* (Walbaum) (Striped Bass) fishery. Further, introductions of 3 catostomid species to the Yadkin River basin were explained as likely bait-bucket transfers (Tracy et al. 2013). Therefore, bait-bucket introductions of Notchlip Redhorse and Quillback are possible when anglers use live bait collected from other river systems. However, non-indigenous suckers could have been introduced through game-fish stocking efforts in Claytor Lake. Notchlip Redhorse and Quillback could have entered the New River via Claytor Lake stocking as hitchhikers because fish have been stocked from in-state and out-of-state sources. Regardless of the introduction mechanism, understanding

any ecological impacts of these introduced species in the New River is important for native fish conservation.

Non-indigenous species introductions represent a major threat to biodiversity worldwide (Moyle and Light 1996). The life histories of these species indicate that they would not likely generate ecological impacts to native fishes due to predation, unless egg predation occurs. Notchlip Redhorse and Quillback both feed on aquatic invertebrates, while Quillback also consume algae, plant material, and detritus (Boschung and Mayden 2004, Rohde et al. 2009, Stauffer et al. 2016). However, competitive interactions and interference during spawning are possible. Both species make spawning movements (Coughlan et al. 2007, Parker and Franzin 1991) and may occupy spawning habitats suitable for other species upstream of Claytor Lake. As with any biological invasion, these non-indigenous fishes generate concern for native New River fishes. Further monitoring is needed to understand their influence on native suckers and species with similar niches.

Presently, prohibition of sucker introductions is difficult to enforce because fishing with live bait is permitted and members of the family Catostomidae can be difficult to distinguish. To prevent future introductions, managers and scientists must educate the public on problems associated with non-indigenous species and responsible bait-use practices. Education and policy represent the best tactics to limit non-indigenous species transfers (Litvak and Mandrak 1993, Rahel 2004). In addition, fisheries managers should consider using hatchery and stocking procedures that prevent accidental stocking of undesired species.

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