Final Report

Geographic variation and systematics of Kentucky and Tennesee populations of the *Notropis rubellus* complex (Teleostei: Cyprinidae)

Submitted to:

Research and Creative Productions Committee

Morehead State University

Morehead, Kentucky 40351

By

David J. Eisenhour

Department of Biological and Environmental Sciences

Morehead State University

Morehead, Kentucky 40351-1689

29 January 1999

Abstract

Samples of the Notropis rubellus complex were taken from 22 localities in Kentucky and Tennessee during the spring and summer of 1998. Based on nuptial coloration, scale and fin-ray counts, breeding tubercle patterns, and general body shape, two taxa were diagnosed and recognized as species in the study area. Notropis rubellus (Agassiz) occupies upland streams of the Ohio River basin upstream of the mouth of the Green River and the Cumberland River drainage above Cumberland Falls. This species has modally 25-26 circumferential scales, well developed tuberculation, and nuptial males have intense red widely distributed on the body, particularly on the dorsum of the head. Notropis micropteryx (Cope) occurs in upland streams of the Cumberland River drainage downstream of Cumberland Falls and the Tennessee River drainage. This species differs from populations of N. rubellus examined in having modally 24 circumferential scales, reduced tuberculation, and nuptial males with restricted distribution of red, primarily confined to the tip of snout, cleithrum, and bases of pectoral fins. Green River populations are intermediate in tuberculation, meristics, and some aspects of nuptial coloration. They are tentatively allocated to N. micropteryx based on shared morphometry and nuptial coloration with Tennessee-Cumberland N. micropteryx and zoogeographic evidence. Support for recognition of these taxa as evolutionary species includes zoogeographic evidence in addition to the array of morphological characters.

Introduction

The rosyface shiner, Notropis rubellus (Teleostei: Cyprinidae), is a small minnow (60-90 mm total length) widely distributed in eastern and central North America. This common species occupies fast currents in clear streams ranging in size from large creeks to medium rivers over substrates of gravel, boulders, and bedrock. Notropis rubellus has generally been placed in the subgenus Hydrophlox (Snelson 1968, Swift 1970) with eight additional species, although recent molecular evidence indicates it may belong in the subgenus Notropis (Mayden and Matson 1988). This species is a member of Notropis rubellus complex, which also includes N. suttkusi (Humphries and Cashner 1994) in Oklahoma and Arkansas.

The geographic variation of the Notropis rubellus complex has been poorly studied, with information available limited to just a few regional studies either west of the Mississippi River (Metcalf 1966, Humphries and Cashner 1994) or on the Atlantic Slope (Snelson 1968). Populations of the complex inhabiting the Tennessee and Cumberland River drainages have been recognized as a distinct species, Notropis micropteryx, (Cope 1868, Kuhne 1939, Snelson 1968), although most recent authors recognize these populations as a subspecies of N. rubellus (Burr and Warren 1986, Jenkins and Burkhead 1994). Because of the nebulous original description (Cope 1868) and lack of quantitative data collected from Tennessee and Cumberland River drainage populations, the validity of the nominal N. rubellus micropteryx was uncertain (Burr and Warren 1986, Etnier and Starnes 1993, Jenkins and Burkhead 1994). A study of allozymic and mitochondrial DNA variation of four populations of N. rubellus (including one of N. r. micropteryx) was unable to resolve the relationships among these populations (Dowling and Brown 1989). These and other researchers have recognized the need for additional detailed studies of geographic variation of the N. rubellus complex (Mayden et al. 1992, Humphries and Cashner 1994, Gilbert 1998). Clarification of the taxonomic confusion surrounding N. rubellus provides insight in the biodiversity, zoogeography, and evolution of the fish fauna of Kentucky and Tennessee.

Specimens of N. r. micropteryx are reported to differ from northern populations of N. rubellus in maximum size, snout shape, and coloration of nuptial males (Etnier and Starnes 1993, Jenkins and Burkhead 1994, D. Etnier, pers. comm.). Although some researchers have reported N. r. micropteryx confined to the Tennessee and Cumberland drainages (Snelson 1968, Dowling and Brown 1989), others have indicated the subspecies may also be present in the Green River drainage in Kentucky, but absent from the upper Cumberland River drainage (Burr and Warren 1986, Etnier and Starnes 1993). Hubbs and Lagler (1964) suggested that N. r. micropteryx may also be present in the Ozark highlands.

Examination of morphological variation among southeastern populations of the N. rubellus complex revealed striking differences in nuptial coloration, meristics, and morphometry between specimens from the Tennessee River drainage and Cumberland River drainage below Cumberland Falls and those from the upper Cumberland River and other Ohio River drainages. Here I present data on nuptial coloration, pigmentation, tuberculation, meristics, and morphometry of Tennessee and Kentucky populations of the N. rubellus complex. Additionally, I evaluate the systematics of these populations, elevate N. r. micropteryx to species status, and provide a zoogeographic hypothesis for the valid taxa.

Methods and Materials

Meristic and morphometric data were collected from 259 specimens of N. rubellus from 21 localities (Fig. 1; Appendix) in Kentucky and Tennessee. Data were collected following the methods of Hubbs and Lagler (1964). Multivariate analysis of the meristic characters was accomplished using principal component analysis (PCA). Principal components were factored from a correlation matrix of 11 non-transformed meristic variables using programs available in SAS (SAS Institute Inc., 1985).

Morphometric data analyzed included 29 variables taken from all specimens used in the meristic analysis. Truss-geometric protocol (Humphries et al. 1981, Strauss and Bookstein 1982, Bookstein et al. 1985) was used, in part, to archive body form and included 18 measurements distributed among three sagittal truss cells with an appended anterior triangle (Fig. 2).

Eleven additional measurements were included in the morphometric analysis (Fig. 2). Multivariate analysis of the morphometric variables were accomplished using sheared PCA (Humphries et al. 1981, Bookstein et al. 1985) to eliminate overall size effects. Principal components were factored from the covariance matrix of 29 log-transformed morphometric characters following recommendations of Bookstein et al. (1985). Males and females were subjected separately to sheared PCA because of meaningful sexual dimorphism in body proportions. Numerous additional specimens were examined to confirm identifications, determine ranges of taxa, and to make observations of qualitative characters, including pigmentation and tuberculation.

Specimens in nuptial condition were collected from 22 localities in Kentucky and Tennessee from 15 May to 10 July 1998 in order to assess geographic patterns of variation of chromatic colors and tuberculation. Field notes of coloration and color slides were taken from male and female specimens in life and recently formalin-killed. In addition, color notes taken during this study were compared to field notes of other researchers and available color photography of specimens of the N. rubellus complex.

Results

Nuptial coloration.---In all populations, males and females were in nuptial coloration from mid-May to early July. Maximum nuptial coloration occurred from late May to late June. During this period slight pressure on the abdomen of seined specimens released sexual products from both sexes. Nuptial coloration rapidly diminished in July; by mid-July nuptial coloration was largely absent. Populations of the N. rubellus complex from Kentucky and Tennessee exhibit one of three patterns of nuptial coloration.

Notropis rubellus from the Ohio River basin above the mouth of the Green River (Salt, Kentucky, Licking, Tygarts, and Kinniconick drainages) and the Cumberland River drainage above Cumberland Falls (herein referred to as "Upper Ohio N. rubellus") had the most intense chromatic coloration of the three groups. In life, large males from these areas had intense cherry to brick red present on the dorsal surface of the head from the tip of the snout to the occiput. Intense red was also present on the cleithrum, lips, and lower jaw, at the base of the dorsal fin as a poorly defined band, at the base of each of the pelvic fins as a small (1-2 mm) spot, and immediately posterior to the base of each of the pectoral fins as a large (3-7 mm) spot. After preservation in formalin, the cherry or brick red faded to a characteristic orange-red. Coloration was less intense on the cheek, lachrymal, operculum and base of the caudal fin, appearing as watery-red or strawberry. Immediately above a broad, silvery lateral stripe, a violet iridescent band was often present. Above these reflective areas, a diffuse brick-red wash was present on an olive iridescent background. This red wash intensified anteriorly, but was sharply set off at the occiput by the intense red of the dorsum of the head. Below the silvery lateral stripe, chromatic coloration was usually absent (except for those areas described above), but a few specimens had some scattered red chromatophores on the venter, creating a pale red or orange-red wash. These chromatophores were most concentrated on anterior lateral-line scales. One specimen from the Salt River drainage (collected 29 June) had intense red coloration on the lateral-line.

Females had similar patterns of chromatic coloration, except colors were not as intense. Typically, moderately intense red was present on the dorsum of the head and just posterior to the pectoral fin bases. Red coloration on the lachrymal, lips, lower jaw, cleithrum, operculum, cheek, dorsolateral and ventrolateral body, and bases of the pelvic and dorsal fins was diffuse or absent.

In general, large individuals of both males and females had the most intense red coloration, but there was substantial variation among individuals of the same sex and size class. Because of this variation, chromatic coloration was not always a reliable indicator of sex. Little geographic variation within this group were present, except that chromatic coloration in both populations examined from the Kentucky River drainage was more violet-red than cherry or brick red. However, both of these populations were sampled when the streams were flooding and very turbid, indicating the possibility of environmental influence on nuptial coloration.

Notropis micropteryx from the Tennessee and Cumberland River drainages from below Cumberland Falls exhibited the least red coloration of the three groups. Pale red, pink, or red-orange coloration was confined to the tip of the snout and a small (2-4 mm) spot just posterior to the base of the pectoral fin in nuptial males. Chromatic coloration on the dorsum of the head was usually lacking, although a pale red wash was sometimes present. This red coloration, if present, was never set off sharply from the nape. General dorsolateral body coloration was gray or olive with a green or blue iridescent sheen. A pale red wash above or below the lateral stripe was occasionally present, but never as prominent as Upper Ohio N. rubellus. Red coloration in females was usually less intense or often absent, even in large specimens. However, very large females (55-62 mm SL) from Buck Creek (middle Cumberland drainage) were as intensely colored as smaller nuptial males in the same collection.

Nuptial males from the Green River system had red coloration on the dorsum of the head, snout, lips, lachrymal, cheek, operculum, and cleithrum. This red coloration was not as intense as Upper Ohio N. rubellus, but more intense than Tennessee-Cumberland N. micropteryx. Red-orange was also present at the bases of the caudal, dorsal, pelvic, and pectoral fins. Nuptial males in peak condition exhibited a red-orange wash over the body, most prominent behind the bases of the pectoral fin, fading dorsally and posteriorly. Red-orange chromatophores tended to be concentrated on the lateral-line in specimens not quite in peak condition. Red on the head and nape was about the same intensity, unlike the sharply set off red head of specimens of Upper Ohio N. rubellus. Background coloration was as in Tennessee-Cumberland N. micropteryx. Female coloration was similar, but less intense overall. Females generally had red or orange-red confined to the top of the head, snout, lips, and base of pectoral fins.

Meristics.---Meristic data are presented in Tables 1-9. Four variables (anal fin rays, pelvic fin rays, infraorbital pores, and preoperculomandibular pores) showed little geographic variation (Tables 1, 7-8). Duck and Lower Cumberland N. micropteryx had slightly fewer mean pectoral fin rays than other, more northeastern populations (Table 2). In general, mean scale counts of Tennessee and Cumberland N. micropteryx were lower than those of Upper Ohio N. rubellus, particularly for scales below the lateral line and circumferential scales (Tables 5, 9). Scale counts of Green River N. micropteryx typically were intermediate between those of N. rubellus and other N. micropteryx and often exhibited higher variance than other populations (e.g., Table 9).

Principal component analysis of the meristic data showed a similar pattern. Mean PC scores of Upper Ohio N. rubellus and Tennessee-Cumberland N. micropteryx were separated into two non-overlapping clusters, with most discrimination occurring along the PC 1 axis (Figure 3). However, plots of individual PC scores indicated considerable overlap between these groups along this axis (Figure 4). Loading values indicated Tennessee-Cumberland N. micropteryx had fewer scales than Upper Ohio N. rubellus (Table 10). Green River populations were intermediate in principal component space between Upper Ohio N. rubellus and Tennessee-Cumberland N. micropteryx.

Morphometrics.---Sheared PCA of a single collection of N. micropteryx from the Powell River (upper Tennessee River drainage) separated males and females into two nonoverlapping clusters, primarily along the sheared PC 2 axis (Fig. 5). Examination of loading values indicated males had longer pelvic and dorsal fins, a longer snout to occiput length, and a shorter origin of pelvic fin to origin of anal fin length (Table 11). In addition, male Tennessee-Cumberland N. *micropteryx* attained a shorter maximum standard length than females (51.5 mm vs. 61.5 mm).

Considerable overlap among populations of the N. *rubellus* complex was present in a sheared PCA when individuals were plotted, although some trends in shape variation were present (Fig. 4). In order to facilitate interpretation of shape differences, mean PC scores of populations were plotted and evaluated (Fig. 6). Most separation between Upper Ohio N. *rubellus* and N. *micropteryx* occurred along the sheared PC 3 axis, although the sheared PC 2 also was important for discriminating males of these taxa. Green River populations clustered with Tennessee-Cumberland N. *micropteryx* for both sexes. Examination of loading values indicated that Upper Ohio N. *rubellus* are more robust than N. *micropteryx* for both sexes, and that male Upper Ohio N. *rubellus* have shorter fins and a caudal peduncle than N. *micropteryx* (Table 12).

Specimens of N. *micropteryx* collected had a smaller mean SL than N. *rubellus* (males 41.4 mm vs. 47.0 mm; females 49.7 mm vs. 50.5 mm). However, N. *rubellus* attained only a slightly larger maximum size (males 53.5 SL mm vs. 51.5 mm SL; females 62.3 SL mm vs. 61.5 mm SL).

Tuberculation.---Although tuberculate males of Tennessee-Cumberland N. *micropteryx* were as small as 34 mm SL, Upper Ohio N. *rubellus* males were not tuberculate below 45 mm SL. Large males of Upper Ohio N. *rubellus* in peak breeding condition had large, recurved uniserial tubercles on pectoral fin rays 2-5 or 2-6. Tiny tubercles were usually present on all dorsal and anal fin rays, but sparse or absent on pelvic and caudal fin rays. Small, conical tubercles covered the dorsum of the head and snout. Smaller, less dense tubercles were present on the cheek, operculum, lachrymal, and lower jaw. The posterior edge of all scales except those on the belly had 4-6 small, conical tubercles on their posterior edge. These tubercles were all about equally sized and did not differ appreciably in size from the head tubercles.

Females of Upper Ohio N. rubellus differed from males in lacking fin tubercles and having reduced head and body tuberculation. Head tubercles in females were smaller, more rounded and less dense than those of males. Tubercles similar to those on the dorsum of the head were found on the posterior margin of dorsolateral scales. Tubercle size and density on body scales decreased ventrally and posteriorly.

Green River specimens did not differ appreciably from Upper Ohio N. rubellus in tuberculation. Tennessee-Cumberland N. micropteryx tuberculation patterns were similar to Upper Ohio N. rubellus, but tubercles were considerably smaller and more rounded. However, the reduced tuberculation of Tennessee-Cumberland N. micropteryx males may be due to the smaller size of N. micropteryx males (mean 41.4 mm SL) than N. rubellus males (mean 47.0 mm SL) sampled.

Pigmentation.---Populations of the N. rubellus complex exhibit little variation in pigmentation. Dorsolateral scales just below the dorsal fin tend to have melanophores more concentrated on the posterior half or two-thirds of the scale, leaving a depigmented anterior area in Tennessee-Cumberland N. micropteryx. In Upper Ohio N. rubellus, these scales usually had melanophores more evenly distributed over the entire scale, leaving no depigmented area. However, considerable variation occurs; pigmentation in some specimens is intermediate or like that of the other species. In general, pigmentation differences are most consistent in larger (> 45 mm SL) specimens for both species.

Discussion

Taxonomic considerations.---Populations of N. rubellus examined and N. micropteryx from the Tennessee and Cumberland River drainages differ in morphology. In addition to subtle differences in pigmentation, body shape, and nuptial male tuberculation, these species show consistent, modal differences in several scale counts. Both species occur allopatrically in the Cumberland River drainage. Near the contact area, N. micropteryx show patterns of tuberculation, morphometry, meristics, and nuptial male coloration similar to other Cumberland and Tennessee River drainage N. micropteryx. Likewise, Upper Cumberland N. rubellus are similar in morphology to Upper Ohio N. rubellus. This lack of clinal variation suggests no meaningful gene flow between N. micropteryx and N. rubellus in the Cumberland River drainage.

Green River populations are rather intermediate in scale counts and intensity of red nuptial coloration between Upper Ohio N. rubellus and Tennessee-Cumberland N. micropteryx. Data from limited observations indicate Green River populations share the lack of a distinctly red head and a more gracile body form with Tennessee-Cumberland N. micropteryx, but share well-developed body tuberculation with Upper Ohio N. rubellus. A phylogenetic analysis of allozymic data taken from these populations of the N. rubellus complex supports a monophyletic N. micropteryx, but indicates Green River populations are quite divergent from Tennessee-Cumberland N. micropteryx (R. Mayden, pers. comm.). Attribution of intermediate morphology to retained ancestral polymorphism or independent evolution is supported by zoogeographic analysis, discussed below. Alternatively, morphological intermediacy may be due to introgression of N. rubellus and N. micropteryx following the breakup of the preglacial Teays River system. Green River populations are tentatively allocated to N. micropteryx based on intrinsic morphological evidence and extrinsic zoogeographic evidence.

Zoogeography.---The distributions of the three forms identified in this study (Tennessee-Cumberland N. micropteryx, Green River N. micropteryx, and Upper Ohio N. rubellus) can largely be attributed to the drainage history of streams of the Eastern Highlands region. As reviewed by Burr and Page

(1986), Burr and Warren (1986), Starnes and Etnier (1986), and Mayden (1988), the Green River drainage has been independent of the Tennessee and Cumberland River drainages and the Upper Ohio basin since the Pliocene. During this period, the Cumberland and lower Tennessee rivers flowed independently of the Old Ohio River and joined it in western Tennessee. Upper and middle portions of the Tennessee River were independent of the lower Tennessee River, draining southward into the Mobile Basin or the lower Mississippi River. Most of the present upper Ohio River tributaries, including the Big Sandy, Licking, Kentucky, and possibly the Salt rivers, was drained by the ancestral Teays River, which flowed westward across Indiana and Illinois to the upper Mississippi River. Glacial advances during the Pleistocene eliminated the Teays River and diverted its tributaries southward, into the present Ohio River. Gradient advantages created during glacial melting episodes allowed the lower, middle, and upper portions of the Tennessee River to become integrated.

Allocation of Green River populations to N. micropteryx is supported by zoogeographic evidence. Green River fishes show greater zoogeographic similarities with Tennesee and Cumberland fauna than with Upper Ohio fauna (Mayden 1988). In addition to N. micropteryx, four other taxa, Nocomis effusus, Notropis leuciodus, Phenacobius uranops, and the Noturus elegans complex are shared exclusively by the Green, Cumberland, and Tennessee River drainages. Two other taxa, Campostoma oligolepis and the Etheostoma stigmaeum complex, are shared exclusively by these drainages in the Eastern Highlands. These species provide evidence of stream capture and ensuing faunal exchange between the Cumberland and Green-Barren River drainages (Burr and Page 1986, Burr and Warren 1986, Starnes and Etnier 1986). In contrast, faunal exchange of upland fishes between the Green River drainage and the upper Ohio River basin has apparently been limited. The Salt River drainage populations of Fundulus catenatus may have occurred during a stream capture event with the upper Green River (Branson and Batch 1971, Burr and Page 1986). Percina stictogaster is only fish shared exclusively by the upper Green and Kentucky River drainages. However, the disjunct populations are deeply divergent in mtDNA haplotypes (Strange and Burr 1997), and the zoogeographic history of these populations is unclear.

The divergent morphology of Green River N. micropteryx is likely due to the lengthy isolation of the Tennessee and Cumberland River drainages and the Green River drainage. Extrinsic and intrinsic drainage isolation and extraordinary habitat biodiversity has resulted in extreme endemism (over 60 fish species, Etnier and Starnes 1993) in the Tennessee and Cumberland River drainages (Starnes and Etnier 1986, Etnier and Starnes 1993). In contrast with the proximate Wabash, White, Salt, Kentucky, and Licking River drainages, which lack endemics, the Green River has eight endemics, Thoburnia atripinnis, Noturus elegans, Etheostoma barrenense, Etheostoma bellum, Etheostoma barbouri, Etheostoma rafinesquei, an undescribed species in the Etheostoma stigmaeum complex (Layman 1994), and an undescribed species in the Etheostoma spectabile complex (Ceas and Page 1997). The pattern of divergence between Green N. micropteryx and Tennessee-Cumberland N. micropteryx is congruent with patterns of allozymic divergence within Notropis leuciodus, a cyprinid with a distinctively similar distribution (Mayden and Matson 1992).

The present Salt River has not been unequivocally linked with either the Old Ohio River or the Teays River (Burr and Warren 1986). Analyses of drainage relationships based on faunal composition are hampered by recent stream capture events in the region (Burr and Page 1986, Mayden 1988). The presence of Notropis rubellus in the Salt River drainage is suggestive of a pre-Pleistocene connection with the Teays River, although dispersal into this system may have occurred during the relatively recent breakup of the Teays drainage.

Populations of N. rubellus above Cumberland Falls likely originated from stream capture between the Upper Cumberland and the Kentucky and/or Big Sandy River drainages. Transfer of several fishes, including Ericymba buccata, Etheostoma baileyi, Etheostoma nigrum, Etheostoma sagitta, by stream capture between the Upper Cumberland and Kentucky River systems is well-documented (Kuehne and Bailey 1961, Burr and Warren 1986, Starnes and Etnier 1986, Strange 1998). The lack of N. rubellus in the Tennessee River and the Cumberland River below Cumberland Falls supports entrance of N. rubellus into the upper Cumberland River system from the upper Kentucky River system. This appears to be the most common direction of transfer for fishes in the Upper Cumberland River drainage (Starnes and Etnier 1986).

Summary.---The lack of identifiable introgression in specimens from the Cumberland River drainage indicates N. rubellus and N. micropteryx are maintaining their identities, a requirement of the evolutionary species concept (Wiley 1981). The apparent intermediacy of Green River populations is not interpreted as introgression, but rather independent evolution resulting from attenuated isolation of the Green River drainage. Morphological data support recognition of two evolutionary species of the N. rubellus complex in Kentucky and Tennessee. Their names, diagnostic traits, and ranges in Kentucky and Tennessee are listed below.

Notropis micropteryx (Cope 1868)

Highland Shiner

Diagnosis.---A member of the Notropis rubellus complex as diagnosed by Humphries and Cashner (1994) and distinguished from N. micropteryx by the following combination of characters: red pigmentation poorly developed, mostly confined to the tip of the snout, bases of pectoral fins, cleithrum, sometimes on dorsum of head; red coloration on head and nape not distinctly different in intensity; scales below the lateral line modally three; scales around the caudal peduncle 12-13; circumferential scales modally 24; body more gracile; body tubercles poorly developed and rounded.

Range.---Upland streams of the Cumberland River drainage below Cumberland Falls and the Tennessee River drainage (Fig. 1). Populations of the Notropis rubellus complex occurring in the Green River drainage are tentatively allocated to N. micropteryx based on preliminary data collected during this study.

Notropis rubellus (Agassiz 1850)

Rosyface Shiner

Diagnosis.---A member of the Notropis rubellus complex as diagnosed by Humphries and Cashner (1994) and distinguished by the following combination of characters: red pigmentation well developed, often intensely expressed on the snout, dorsum of head, cleithrum, bases of pectoral, pelvic, dorsal, and caudal fins and along the lateral line; red coloration much more intense on dorsum of head than on nape; scales below the lateral line modally four; scales around the caudal peduncle modally 13-14; circumferential scales modally 25-26; body more robust; body tubercles well developed and conical.

Range.---In Kentucky and Tennessee, this species occurs in the Cumberland River drainage above Cumberland Falls and in upland streams of the Ohio River basin above the mouth of the Green River (Fig. 1). These tributaries of the Ohio River include the Salt, Kentucky, Kinniconick, Tygarts, Little Sandy, and Big Sandy River drainages. The range likely extends throughout most of the remainder of the Ohio River basin and the Great Lakes basin, except for populations in the upper New River in Virginia and West Virginia, which may represent an undescribed species (R. Mayden, pers. comm.).

Further study.---This research provides a working hypothesis of the systematics and distribution of N. micropteryx, but additional data are needed to confirm the results of this preliminary study. Additional field collections are needed to confirm color observations, particularly for N.

micropteryx. The small sample sizes used in the meristic and morphometric analyses need to be supplemented with additional observations from populations proximal to the contact area (i.e., Green and Cumberland River drainages). The Notropis rubellus complex is unique among members of the subgenus Notropis in possessing the intense red nuptial coloration. Clarification of the evolution of nuptial coloration of the N. rubellus complex will require examination of breeding adults from other populations of the complex and outgroups.

Acknowledgements

I am most indebted to Lynn V. Eisenhour, who assisted in all field collections, photography, and analysis of the data. This study was inspired by field collections of N. rubellus and discussions with David A. Etnier. Partial funding was provided by a research grant from Morehead State University. Collecting permits were provided by Kentucky Department of Fish and Wildlife and Tennessee Wildlife Resources Agency.

Literature Cited

Agassiz, L. 1850. Lake Superior: its physical character, vegetation and animals, compared with those of other similar regions. By Louis Agassiz. With a narrative of the tour, by J. Elliott Cabot. And contributions by other scientific gentlemen. Gould, Kendall, and Lincoln, Boston, Massachusetts.

Bookstein, F. L., B. Chernoff, R. L. Elder, J. M. Humphries, Jr., G. R. Smith, and R. E. Strauss. 1985. Morphometrics in evolutionary biology. Acad. Nat. Sci., Philadelphia Special Publ. 15:1-277.

Branson, B. A., and D. L. Batch. 1971. Stream capture in Kentucky indicated by distributional records of Fundulus catenatus and Etheostoma spectabile. Amer. Midl. Nat. 86:496-500.

Burr, B. M., and L. M. Page. 1986. Zoogeography of fishes of the lower Ohio-upper Mississippi basin, p. 287-324. In: C. H. Hocutt and E. O. Wiley (eds.). The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Burr, B. M, and M. L. Warren, Jr. 1986. A distributional atlas of Kentucky fishes. Kentucky Nature Preserves Comm. Sci. and Tech. Series 4.

Ceas, P. A., and L. M. Page. 1997. Systematic studies of the Etheostoma spectabile complex (Percidae; subgenus Oligocephalus), with descriptions of four new species. Copeia 1997:496-522.

Cope, E. D. 1868. On the distribution of fresh-water fishes in the Allegheny region of southwestern Virginia. J. Acad. Nat. Sci., Philadelphia, Ser. 2, 6:207-247.

Dowling, T. E., and W. M. Brown 1989. Allozymes, mitochondrial DNA, and levels of phylogenetic resolution among four minnow species (Notropis: Cyprinidae). Syst. Zool. 38:126-143.

Etnier, D. A., and W. C. Starnes. 1993. The fishes of Tennessee. The Univ. of Tennessee Press, Knoxville.

Gilbert, C. R. 1998. Type catalogue of recent and fossil North American freshwater fishes: Families Cyprinidae, Catostomidae, Ictaluridae, Centrarchidae, and Elassomatidae. Florida Mus. Nat. Hist. Special Publ. 1.

Hubbs, C. L., and K. F. Lagler. 1964. Fishes of the Great Lakes region. Univ. Michigan Press, Ann Arbor.

Humphries, J. M., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder, and S. L. Poss. 1981. Multivariate discrimination by shape in relation to size. Syst. Zool. 30:291-308.

Humphries, J. M., and R. C. Cashner. 1994. Notropis suttkusi, a new cyprinid from the Ouachita uplands of Oklahoma and Arkansas, with comments on the status of Ozarkian populations of N. rubellus. Copeia 1994:82-90.

Jenkins, R. E., and N. M. Burkhead 1994. Freshwater fishes of Virginia. Amer. Fish. Soc. Bethesda, Maryland.

Kuhne, E. R. 1939. A guide to the fishes of Tennessee and the mid-South. Div. Fish and Game, Tennessee Dept. Cons., Nashville.

Kuehne, R. A. and R. M. Bailey. 1961. Stream capture and the distribution of the percid fish Etheostoma sagitta, with geologic and taxonomic considerations. Copeia 1961:1-8.

Layman, S. R. 1994. Phylogenetic systematics and biogeography of darters of the subgenus Doration (Percidae: Etheostoma). Unpubl. Ph.D. Dissertation, Univ. Alabama, Tuscaloosa.

Mayden, R. L. 1988. Vicariance biogeography, parsimony, and evolution in North American freshwater fishes. Syst. Zool. 37:329-355.

Mayden, R. L., B. M. Burr, and R. R. Miller. 1992. The native freshwater fishes of North America, p. 827-863. In: R. L. Mayden (ed.). Systematics, historical ecology, and North American freshwater fishes. Stanford Univ. Press, California.

Mayden, R. L., and R. H. Matson. 1988. Preliminary analysis of systematic placement of Notropis rubellus (Cyprinidae). Abst. ASB Bull. 35:1988.

Mayden, R. L., and R. H. Matson. 1992. Systematics and biogeography of the Tennessee shiner, Notropis leuciodus (Cope) (Teleostei: Cyprinidae). Copeia 1992:954-968.

Metcalf, A. L. 1966. Fishes of the Kansas River system in relation to zoogeography of the Great Plains. Univ. Kansas Publ., Mus. Nat. Hist. 17:23-189.

Snelson, F. F., Jr. 1968. Systematics of the cyprinid fish Notropis amoenus, with comments on the subgenus Notropis. Copeia 1968:776-802.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 edition. SAS Institute Inc., Cary, North Carolina.

Starnes, W. C., and D. A. Etnier. 1986. Drainage evolution and fish biogeography of the Tennessee and Cumberland River drainage realm, p.

325-362. In: C. H. Hocutt and E. O. Wiley (eds.). The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.

Strange, R. M. 1998. Mitochondrial DNA variation in johnny darters (Pisces: Percidae) from eastern Kentucky supports stream capture for the origin of upper Cumberland River fishes. Amer. Midl. Nat. 140:96-102.

Strauss, R. E., and F. L. Bookstein. 1982. The truss: body form reconstruction in morphometrics. Syst. Zool. 31:113-135.

Strange, R. M., and B. M. Burr. 1997. Intraspecific phylogeography of North American highland fishes: A test of the Pleistocene vicariance hypothesis. Evolution 51:885-897.

Swift, C. C. 1970. A revision of the eastern North American cyprinid fishes of the Notropis texanus species group (subgenus Alburnops), with a definition of the subgenus Hydrophlox, and materials for a revision of the subgenus Alburnops. Unpubl. Ph.D. Dissertation, Florida State Univ.

Wiley, E. O. 1981. Phylogenetics. The theory and practice of phylogenetics. Wiley, New York.

Table 1. Frequency distribution of anal and pelvic fin rays of selected Kentucky and Tennessee populations of the Notropis rubellus complex.

	Anal fin rays						Pelvic fin rays					
Population	9	10	11	n	mean	SD	7	8	9	n	mean	SD
Notropis rubellus												
Tygarts/Licking	2	22	5	29	10.10	0.49	1	28		29	7.97	0.19
Kentucky	6	34	7	47	10.02	0.53	2	44	1	47	7.98	0.25
Salt	2	22	1	25	9.96	0.35	1	24		25	7.96	0.20
Upper Cumberland	2	24	2	28	10.00	0.38		26	2	28	8.07	0.26
Notropis micropteryx?												
Green	3	22	3	28	10.00	0.47		27	1	28	8.04	0.19
Notropis micropteryx												
Middle Cumberland	10	22	3	35	9.80	0.58		35		35	8.00	0.00
Lower Cumberland	2	16	1	19	9.95	0.40		18	1	19	8.05	0.23
Duck		14	3	17	10.18	0.39		16	1	17	8.06	0.24
Upper Tennessee	3	26	2	31	9.97	0.41	1	30		31	7.97	0.18

Table 2. Frequency distribution of pectoral fin rays of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	11	12	13	14	15	n	mean	SD
Notropis rubellus								
Tygarts/Licking	1	4	19	5		29	12.97	0.68
Kentucky		13	29	5		47	12.83	0.60
Salt		5	19	1		25	12.84	0.47
Upper Cumberland		2	17	9		28	13.25	0.59
Notropis micropteryx?								
Green		1	20	7		28	13.21	0.50
Notropis micropteryx								
Middle Cumberland		10	20	5		35	12.86	0.65
Lower Cumberland			9	8	2	19	13.63	0.68
Duck			4	12	1	17	13.82	0.53
Upper Tennessee		4	20	7		31	13.10	0.60

Table 3. Frequency distribution of predorsal scale row of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	17	18	19	20	21	n	mean	SD
Notropis rubellus								
Tygarts/Licking		10	12	5	2	29	18.97	0.91
Kentucky	2	18	19	7	1	47	18.72	0.85
Salt	3	5	11	6		25	18.80	0.96
Upper Cumblerland	1	9	9	7	2	28	19.00	1.02
Notropis micropteryx?								
Green	3	13	10	2		28	18.39	0.79
Notropis micropteryx								
Middle Cumberland	4	13	10	8		35	18.63	0.97
Lower Cumberland	1	9	7	2		19	18.53	0.77
Duck	1	8	8			17	18.41	0.62
Upper Tennessee		14	16	1		31	18.58	0.56

Table 4. Frequency distribution of lateral-line scales of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	36	37	38	39	40	n	mean	SD
Notropis rubellus								
Tygarts/Licking	1	7	11	6	4	29	38.17	1.07
Kentucky		4	16	21	6	47	38.62	0.82
Salt		7	9	7	2	25	38.16	0.94
Upper Cumberland		4	10	9	5	28	38.54	0.96
Notropis micropteryx?								
Green	1	8	11	7	1	28	37.96	0.92
Notropis micropteryx								
Middle Cumberland		7	17	10	1	35	38.14	0.77
Lower Cumberland		9	9	1		19	37.58	0.61
Duck		4	8	5		17	38.06	0.75
Upper Tennessee	6	9	10	6		31	37.52	1.03

Table 5. Frequency distribution of scales above lateral line and scales below lateral line of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

	Scales above lateral line						Scales below lateral line					
Population	5	6	7	n	mean	SD	3	4	5	n	mean	SD
Notropis rubellus												
Tygarts/Licking		28	1	29	6.03	0.19	2	26	1	29	3.97	0.33
Kentucky	1	43	3	47	6.04	0.29	2	42	3	47	4.02	0.33
Salt		24	1	25	6.04	0.20		25		25	4.00	0.00
Upper Cumberland		23	5	28	6.18	0.39	2	25	1	28	3.96	0.33
Notropis micropteryx?												
Green	2	26		28	5.93	0.26	5	22	1	28	3.86	0.45
Notropis micropteryx?												
Middle Cumberland	4	30	1	35	5.91	0.37	18	17		35	3.49	0.51
Lower Cumberland	4	15		19	5.79	0.42	12	7		19	3.37	0.50
Duck	4	13		17	5.76	0.44	13	4		17	3.24	0.44
Upper Tennessee	6	25		31	5.81	0.40	18	13		31	3.42	0.50

David J. Eisenhour

29 January 1999

Carol Morella, Chair, Research and Creative Productions Committee
901 Ginger Hall
Morehead State University
Morehead, KY 40351

Dr. Morella,

Enclosed is a final report of a University-funded research for 1998. Presently, there are no publications associated with this research. I plan to present a paper on this research in the fall and again in the following spring. Additional research is required before this can be submitted to a major journal. There was no equipment or library materials purchased with the grant (e.g., "Form E" not needed).

Thank you for the grant. It helped to offset some of the research costs associated with this pilot study.

Sincerely,

David J. Eisenhour

David J. Eisenhour

Table 6. Frequency distribution of caudal peduncle scales of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	12	13	14	15	n	mean	SD
Notropis rubellus							
Tygarts/Licking	3	9	17		29	13.48	0.69
Kentucky	12	13	21	1	47	13.23	0.87
Salt	3	13	9		25	13.24	0.66
Upper Cumberland	2	7	19		28	13.61	0.63
Notropis micropteryx?							
Green	12	8	8		28	12.86	0.85
Notropis micropteryx							
Middle Cumberland	11	12	12		35	13.03	0.82
Lower Cumberland	11	6	2		19	12.53	0.70
Duck	7	8	2		17	12.71	0.61
Upper Tennessee	13	14	4		31	12.71	0.69

Table 7. Frequency distribution of infraorbital pores of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	8	9	10	11	n	mean	SD
Notropis rubellus							
Tygarts/Licking	9	13	7		29	8.93	0.75
Kentucky	10	18	16	3	47	9.26	0.87
Salt		15	6	4	25	9.56	0.77
Upper Cumberland	7	12	7	2	28	9.14	0.89
Notropis micropteryx?							
Green	4	16	5	3	28	9.25	0.84
Notropis micropteryx							
Middle Cumberland	5	22	8		35	9.10	0.61
Lower Cumberland	1	10	6	2	19	9.47	0.77
Duck	3	11	3		17	9.00	0.61
Upper Tennessee	4	19	7	1	31	9.16	0.69

Table 8. Frequency distribution of preoperculomandibular pores of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	8	9	10	11	12	13	14	n	mean	SD
Notropis rubellus										
Tygarts/Licking			10	11	7	1		29	10.97	0.87
Kentucky	1	4	17	15	7	-	3	47	10.74	1.26
Salt		1	9	13	2			25	10.64	0.70
Upper Cumberland			4	13	10	-	1	28	11.32	0.86
Notropis micropteryx?										
Green		1	4	18	4	1		28	11.00	0.77
Notropis micropteryx										
Middle Cumberland		1	9	18	6	1		35	10.91	0.82
Lower Cumberland			5	11	2	1		19	10.95	0.78
Duck			7	5	4	1		17	10.94	0.97
Upper Tennessee		3	14	8	5	1		31	10.58	0.99

Table 9. Frequency distribution of circumferential scales of selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

Population	23	24	25	26	27	n	mean	SD
Notropis rubellus								
Tygarts/Licking			10	10	9	29	25.96	0.82
Kentucky		5	12	15	15	47	25.85	1.00
Salt		2	16	7		25	25.20	0.58
Upper Cumberland		5	10	12	1	28	25.32	0.82
Notropis micropteryx?								
Green	3	2	4	12	7	28	25.64	1.25
Notropis micropteryx								
Middle Cumberland	4	21	10			35	24.17	0.62
Lower Cumberland	8	11				19	23.58	0.51
Duck	8	9				17	23.52	0.51
Upper Tennessee	9	18	3	1		31	23.87	0.72

Table 10. Principle component loadings for 11 meristic variables for 259 selected Kentucky and Tennessee populations of the Notropis rubellus complex.

Variable	PC 1	PC 2
Anal rays	0.046	0.121
Pelvic rays	-0.102	0.544
Pectoral rays	-0.184	0.636
Predorsal scales	0.289	0.361
Lateral-line scales	0.287.	0.206
Scales above the lateral line	0.381	-0.030
Scales below the lateral line	0.473	-0.077
Caudal peduncle scales	0.412	0.217
Infraorbital pores	0.054	-0.204
Preoperculomandibular pores	0.078	-0.124
Circumferential scales	0.491	-0.035
Eigenvalue	2.321	1.351
Proportion of variance	0.211	0.123

Table 11. Sheared principle component loadings for 29 morphometric variables for 10 female and 13 male Notropis micropteryx from the Powell River, Claiborne County, Tennessee.

Measurement	sheared PC 2	sheared PC 3
Standard length	-0.114	-0.076
Head length	0.001	-0.034
Orbit length	0.016	0.031
Jaw length	0.041	0.080
Snout length	0.081	-0.034
Head width	0.023	0.166
Body width	-0.012	-0.026
Pectoral fin length	-0.070	0.094
Pelvic fin length	0.555	-0.202
Dorsal fin length	0.413	-0.177
Anal fin length	0.206	0.045
Snout to occiput	0.265	-0.037
Snout to pectoral fin origin	0.040	0.013
Occiput to dorsal fin origin	0.101	-0.008
Dorsal fin base length	-0.196	-0.052
Pectoral fin origin to pelvic fin origin	0.149	0.570
Pelvic fin origin to anal fin origin	-0.347	-0.302
Dorsal fin end to dorsal origin of caudal fin	-0.185	0.262
Anal fin origin to ventral origin of caudal fin	-0.016	-0.133
Occiput to pelvic fin origin	-0.079	-0.160
Pectoral fin origin to dorsal fin origin	-0.217	-0.152
Dorsal fin origin to anal fin origin	-0.204	-0.064
Pelvic fin origin to dorsal fin end	0.008	0.145

Table 11. (cont.)

Measurement	sheared PC 2	sheared PC 3
Dorsal fin end to ventral origin of caudal Fin	-0.097	0.270
Anal fin origin to dorsal origin of caudal fin	-0.109	-0.229
Occiput to pectoral fin origin	0.019	-0.280
Dorsal fin origin to pelvic fin origin	0.036	0.104
Dorsal fin end to anal fin origin	-0.085	0.242
Caudal peduncle depth	-0.090	0.140

Table 12. Sheared principle component loadings for 29 morphometric variables for 130 females and 129 males from selected Kentucky and Tennessee populations of the *Notropis rubellus* complex.

	Females		Males	
Measurement	sheared PC 2	sheared PC 3	sheared PC 2	sheared PC 3
Standard length	-0.064	-0.121	-0.043	0.101
Head length	0.039	0.076	0.084	-0.059
Orbit length	0.127	0.023	0.255	0.147
Jaw length	0.047	0.006	0.120	-0.022
Snout length	0.077	0.034	0.072	-0.222
Head width	-0.049	0.205	0.026	-0.234
Body width	-0.228	0.543	-0.247	-0.409
Pectoral fin length	0.342	-0.132	0.337	0.094
Pelvic fin length	0.467	0.016	0.455	-0.025
Dorsal fin length	0.279	0.019	0.299	0.060
Anal fin length	0.340	0.009	0.281	-0.106
Snout to occiput	0.104	-0.056	0.079	-0.008
Snout to pectoral fin origin	0.130	0.067	0.094	0.010
Occiput to dorsal fin origin	-0.119	-0.235	0.005	0.038
Dorsal fin base length	0.197	-0.122	0.090	-0.058
Pectoral fin origin to pelvic fin origin	-0.109	-0.007	-0.050	0.045
Pelvic fin origin to anal fin origin	-0.260	-0.288	-0.153	0.101
Dorsal fin end to dorsal origin of caudal fin	-0.054	-0.138	-0.109	0.402
Anal fin origin to ventral origin of caudal fin	-0.112	-0.277	-0.141	0.331

Table 12. (cont.)

Measurement	Males sheared PC 2	Males sheared PC 3	Females sheared PC 2	Females sheared PC 3
Occiput to pelvic fin origin	-0.071	0.016	-0.037	-0.017
Pectoral fin origin to dorsal fin origin	-0.218	-0.176	-0.095	-0.111
Dorsal fin origin to anal fin origin	-0.140	-0.214	-0.163	0.015
Pelvic fin origin to dorsal fin end	-0.143	-0.081	-0.232	-0.139
Dorsal fin end to ventral origin of caudal fin	-0.167	-0.136	-0.177	0.414
Anal fin origin to dorsal origin of caudal fin	-0.058	-0.165	-0.042	0.200
Occiput to pectoral fin origin	0.143	0.363	0.042	-0.333
Dorsal fin origin to Pelvic fin origin	-0.189	0.137	-0.282	-0.110
Dorsal fin end to anal fin origin	-0.162	0.287	-0.250	-0.093
Caudal peduncle depth	-0.078	0.065	-0.060	-0.053

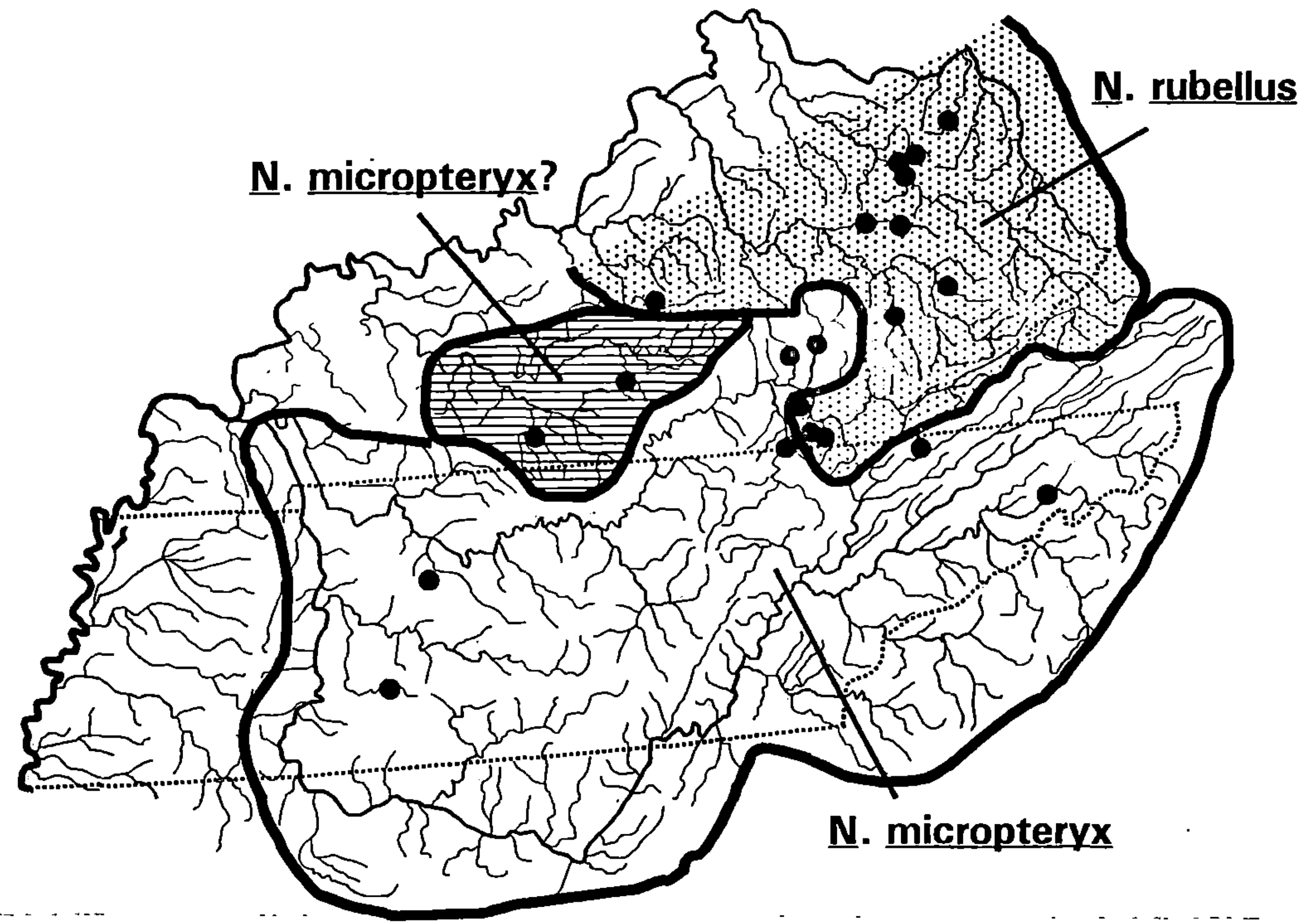

Figure 1. Distribution of the Notropis rubellus complex in Kentucky and Tennessee. Location of specimens used for meristic and morphometric analyses are shown by solid circles. The range of Notropis rubellus extends northward off the map.

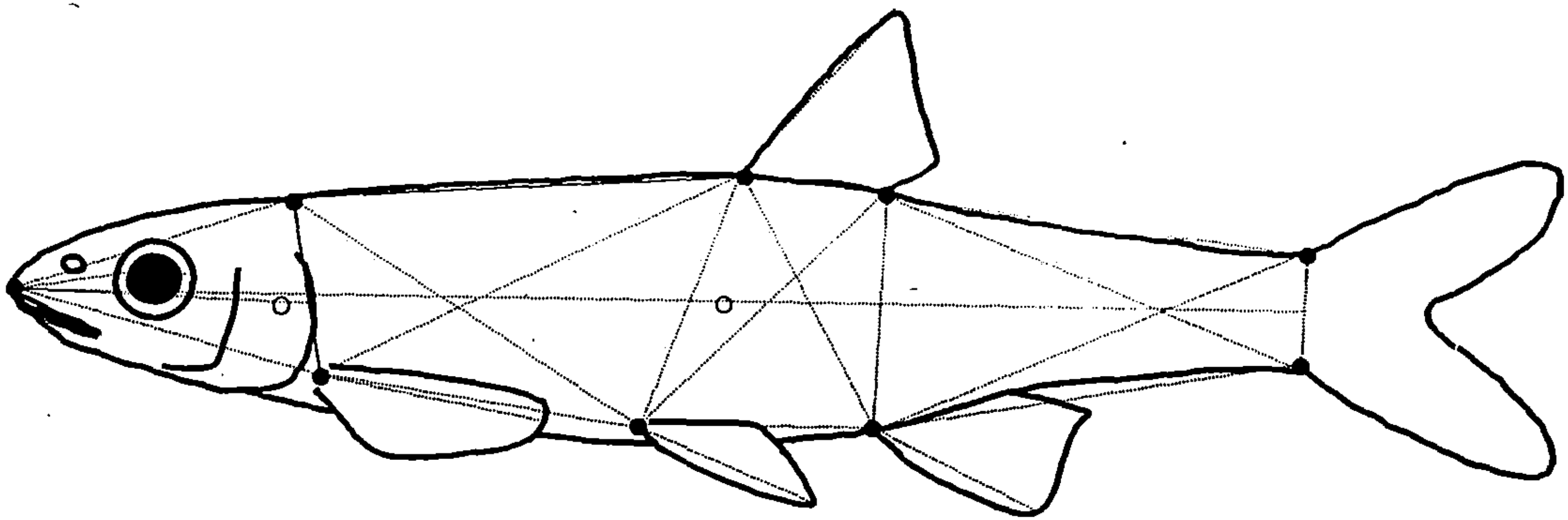

Figure 2. Diagramatic representation of 29 measurements used in sheared principal component analysis for the <u>Notropis</u> <u>rubellus</u> complex.

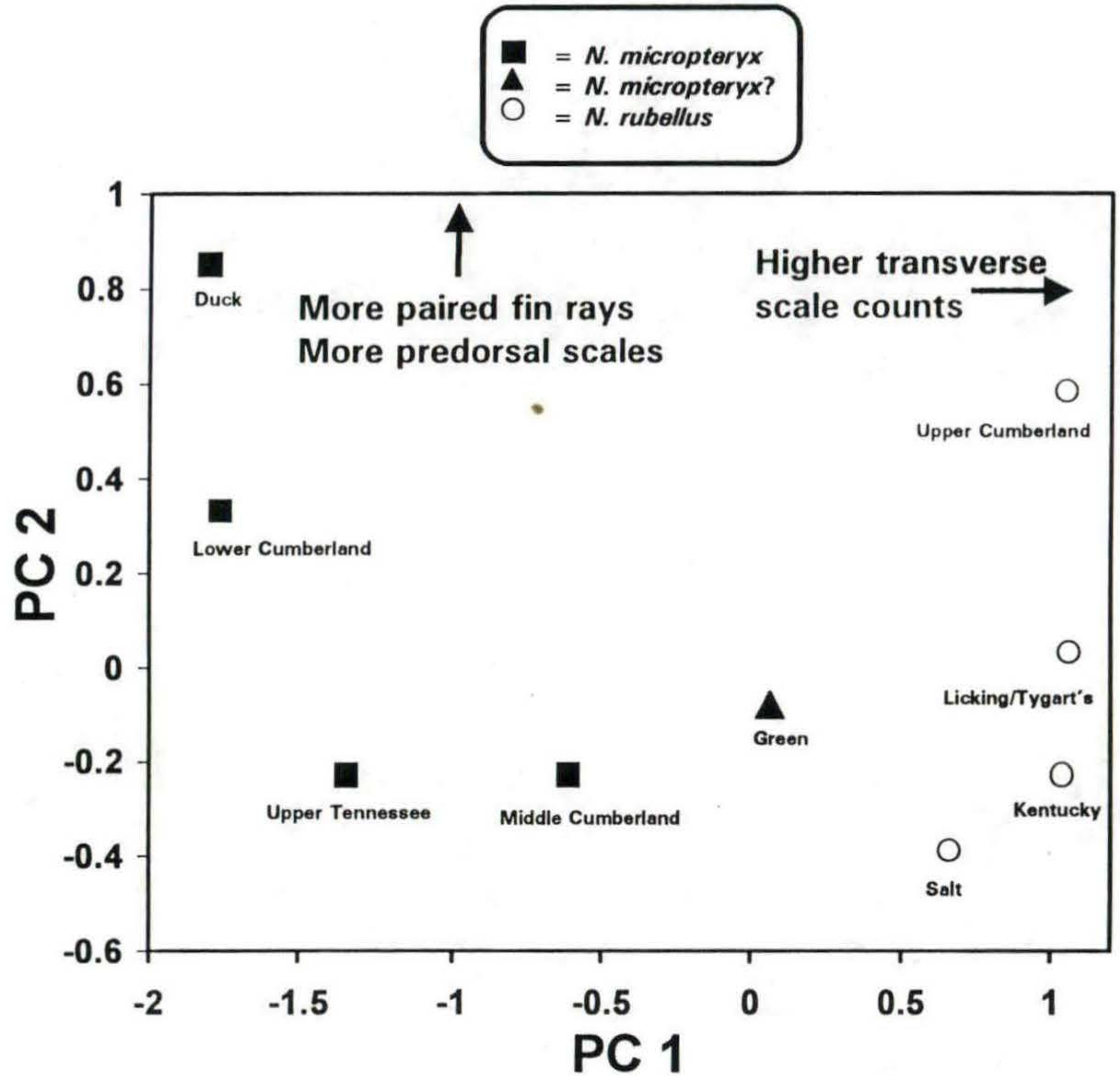

Figure 3. Mean meristic scores on PC axes 1 and 2 for 259 specimens of the Notropis rubellus complex from Kentucky and Tennessee.

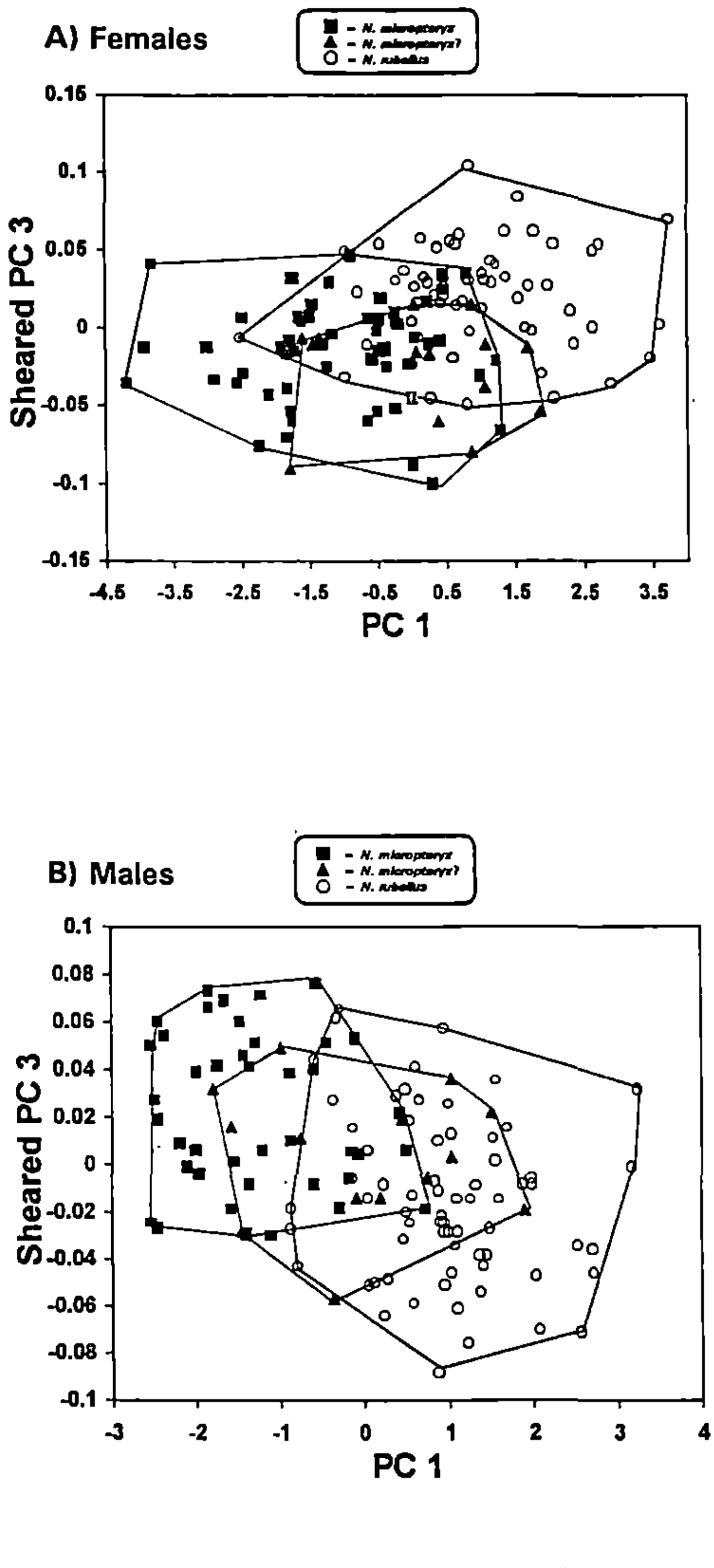

Figure 4. Mean scores on meristic axis PC 1 and morphometric axis sheared PC 3 for specimens of the Notropis rubellus complex from Kentucky and Tennessee. A) 130 females. B) 129 males.

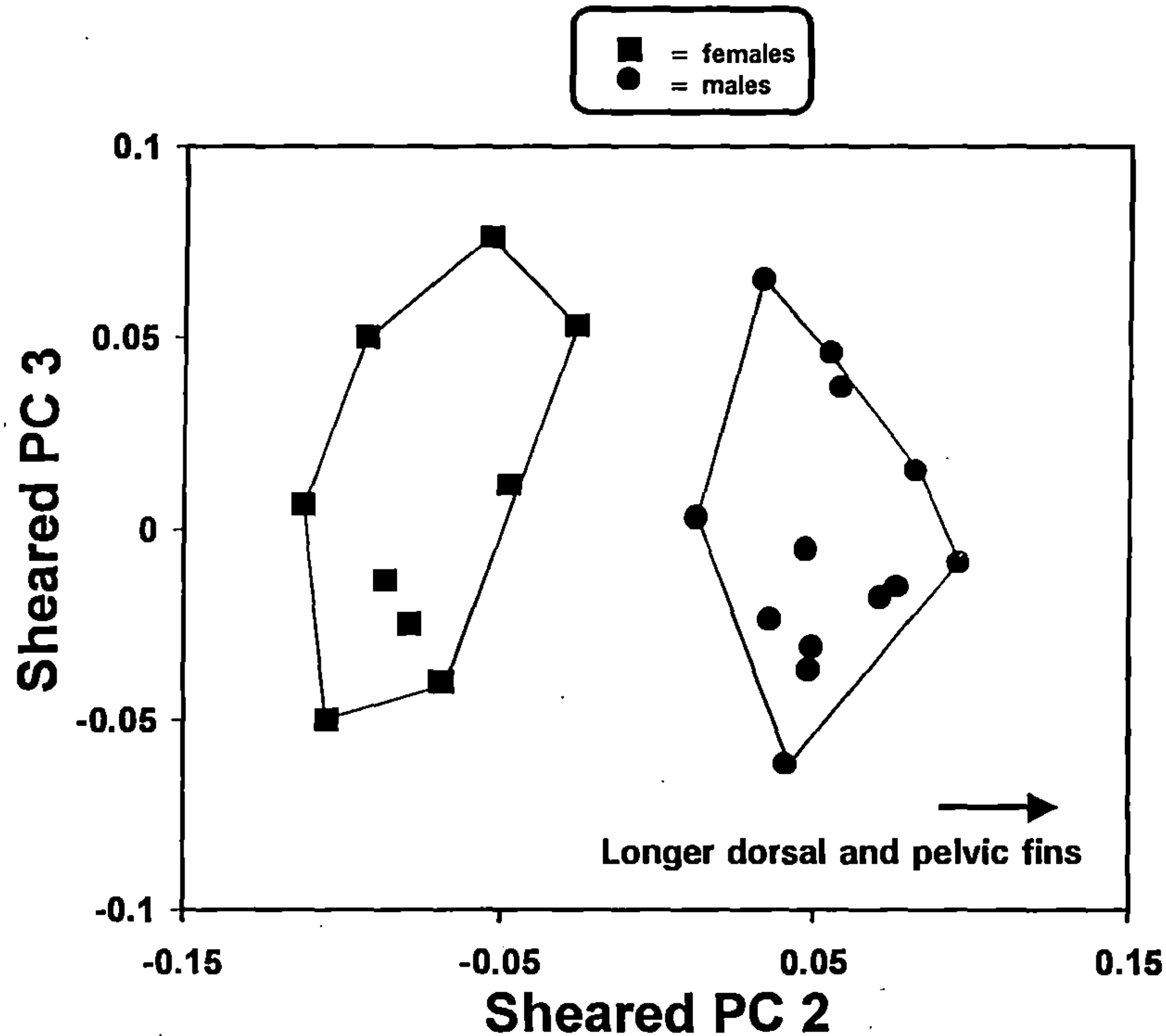

Figure 5. Morphometric scores on sheared PC axes 2 and 3 for 10 female and 13 male Notropis micropteryx from the Powell River, Claiborne County, Tennessee.

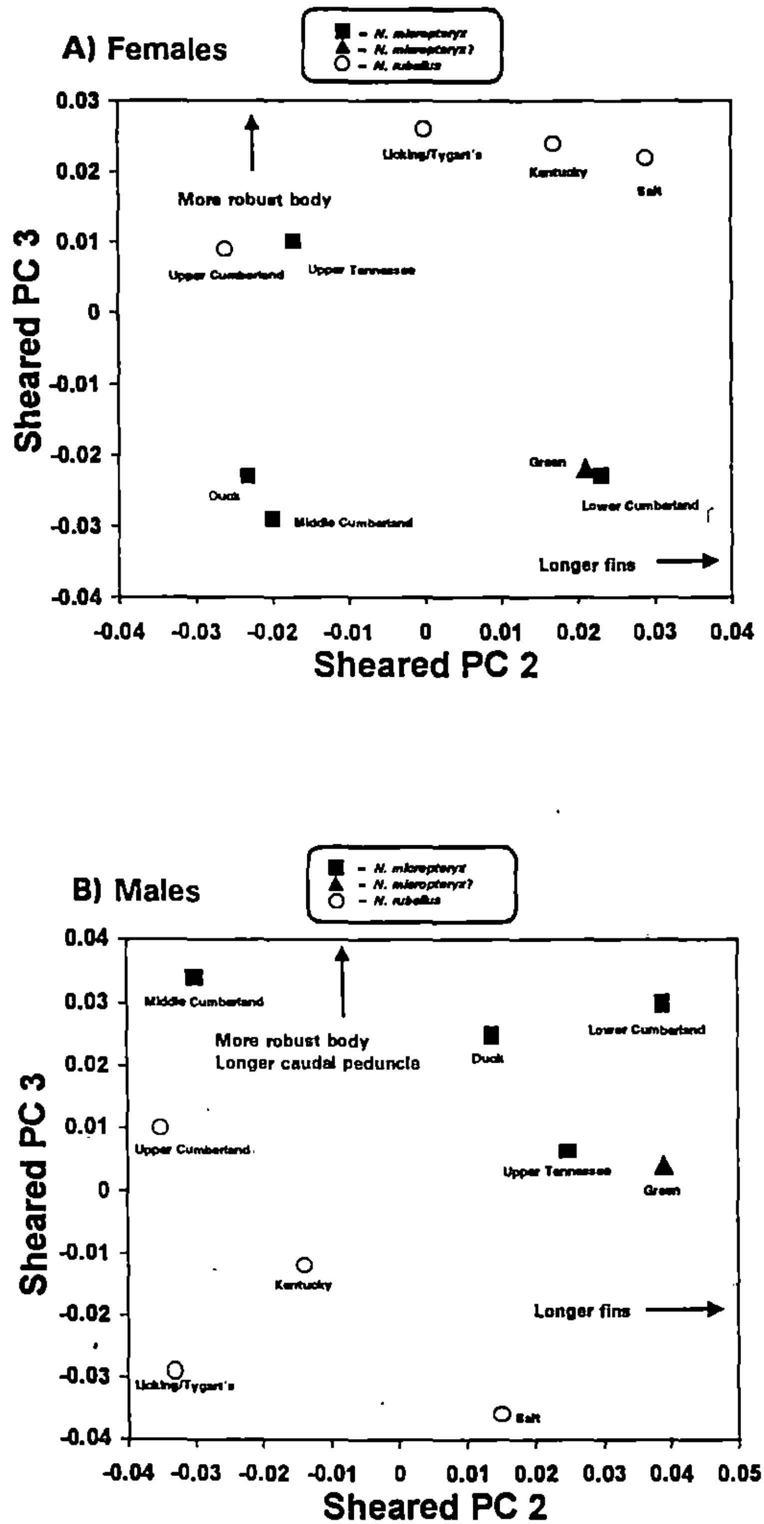

Figure 6. Mean morphometric scores on sheared PC axes 2 and 3 for specimens of the Notropis rubellus complex from Kentucky and Tennessee. A) 130 females. B) 129 males.

Appendix. Specimens of the Notropis rubellus complex used in the meristic and morphometric analyses. Parenthetical numbers refer to number of specimens examined. MOSU = Morehead State University Collection of Fishes.

Tygarts/Licking.

Kentucky: Licking R. at KY 826, Bath Co., MOSU 512 (9) 13 July 1984; Tygarts Cr. at KY 182, Carter Co., MOSU 1069 (5) 15 May 1998; North Fork Triplett Cr. on Bull Fork Road, Rowan Co., MOSU 1078 (10) 20 May 1998; Triplett Cr. at Morehead City Park, Rowan Co., MOSU 1121 (5) 20 June 1998.

Kentucky.

Kentucky: Troublesome Cr. near Haddux, Breathitt Co., MOSU 751 (10) South Fork Kentucky R. at Bishop Bend School Road, Owsley Co., MOSU 1114 (10) 23 June 1998; 1 June 1995; Red R. at Bowen, Powell Co., MOSU 609 (15) 18 July 1987; Red R. 3.2 km E of KY 77-KY 715 intersection, Wolfe Co., MOSU 1085 (12) 22 May 1998.

Salt.

Kentucky: Rolling Fork of Salt River at New Market Mill Road ford, Marion Co., MOSU 1173 (25) 29 June 1998.

Upper Cumberland.

Kentucky: Marsh Cr. at KY 92, McCreary Co., MOSU 750 (5) June 1996; Indian Cr. at KY 700 bridge, McCreary Co., MOSU 1112 (5) 24 June 1998; Jellico Cr. at Old Jellico Creek Road, Whitley Co., MOSU 749 (3) 19 June 1996, MOSU 1177 (15) 24 June 1998.

Middle Cumberland.

Kentucky: Big South Fork Cumberland R. 1.1 km upstream of Troublesome Cr. mouth, McCreary Co., MOSU 1096 (2) 25 May 1998; Rockcastle R. at I-75, Rockcastle Co., MOSU 1107 (23) 3 June 1998; Buck Cr. at KY 3268-Old Mt.

Vernon Road intersection, Pulaski Co., MOSU 1131 (5) 25 June 1998, MOSU 1185 (5) 9 July 1998.

Lower Cumberland.

Tennessee: Turnbull Cr. at mouth of Sullivan Br., 3 km W of Kingston Springs, Cheatham Co., MOSU 1164 (19) 30 June 1998.

Duck.

Tennessee: Buffalo R. at mouth of Grinder's Cr., Lewis Co., MOSU 1182 (17) 30 June 1998.

Upper Tennessee.

Tennessee: Powell R., 7.1 rd. km E of US25E on Powell River Road, Claiborne Co., MOSU 1205 (23) 9 July 1998; Nolichucky R. at Davy Crockett State Park, Greene Co., MOSU 1218 (8) 10 July 1998.

Green.

Kentucky: South Fork Little Barren R. 1 mi. NW of Beechville, Metcalfe Co., MOSU 1140 (5) 29 June 1998; Middle Fork Drake's Cr. at Duncan Road ford, 1 km NE of Drake, Warren Co., MOSU 1157 (23) 1 July 1998.