Journal of Great Lakes Research xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Identification of ruffe larvae (*Gymnocephalus cernua*) in the St. Louis River, Lake Superior: Clarification and guidance regarding morphological descriptions

Greg S. Peterson [a,*], Julie E. Lietz [a,b]

[a] Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Office of Research and Development, 6201 Congdon Blvd., Duluth, MN 55804, USA
[b] ORISE (Oak Ridge Institute for Science and Education), USA

ARTICLE INFO

Article history:
Received 27 June 2016
Accepted 14 October 2016
Available online xxxx

Keywords:
Ruffe
Fish larvae
Identification
St. Louis River
Great Lakes

ABSTRACT

Non-native ruffe (*Gymnocephalus cernua*; family Percidae) were first detected in the Laurentian Great Lakes in 1986, and are not included in regional larval fish keys which were published several years prior to their discovery. In addition, subsequent scientific literature has inconsistently described ruffe larvae. As a result, identification of larval ruffe remains challenging. We used traditional morphology paired with DNA technology to develop diagnostics for ruffe larvae collected in the lower St. Louis River, and compared them to similar species. We found that ruffe <6 mm total length phenotypically resemble centrarchids, like black crappie, bluegill, and pumpkinseed, but have myomere counts that are intermediate between values for both common percid and centrarchid species. We suggest that developmental and pigment patterns as well as morphometrics can be used to distinguish ruffe from similar species at this size. At larger sizes, ruffe increasingly resemble other percids such as yellow perch, but can be distinguished using myomere counts and morphological features. The findings presented here clarify conflicting descriptions in the scientific literature, and provide additional data to support more confident morphological identification of larval ruffe.

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Introduction

Fish larvae are collected in a variety of research and monitoring activities, including species composition studies, identification of nursery areas and dispersal patterns (Allen and Barker, 1990; King, 2004; Robinson et al., 1998; Schluderman et al., 2012), and habitat management (Humphries et al., 2002). However, taxonomic identification of field-collected fish larvae remains challenging because taxonomic keys for larval fish often lack descriptions for various stages of development, or for entire species. This is especially true for recently established non-native species. For example, ruffe (*Gymnocephalus cernua*; family Percidae) were introduced to the Laurentian Great Lakes in 1986 (Pratt, 1988), but are not included in the Great Lakes larval fish key (Auer, 1982a), which was published prior to their detection.

Ruffe larvae have been described in subsequent peer-reviewed literature, but aspects of these descriptions are inconsistent and even contradictory. In particular, myomere counts, a key meristic and diagnostic used in taxonomic identification, are inconsistently described in the scientific literature (French and Edsall, 1992; Simon and Vondruska, 1991). Descriptions of larval pigment patterns and morphometrics are also inconsistent among the different literature describing larval ruffe. Like meristics, these features can be useful for distinguishing and identifying larval fishes (Bani et al., 2015; Kendall et al., 1984), but unlike meristics, often change with developmental stage. Further, pigment and morphometrics may vary as a result of environmental conditions (Blaxter, 1988; Fuiman et al., 1998; Sfakianakis et al., 2011), as well as handling and preservation (Theilacker, 1980). Consequently, field-collected specimens may differ phenotypically from laboratory-reared specimens (Blaxter, 1984) used to generate morphological and taxonomic descriptions of ruffe larvae. All of these discrepancies can contribute to error when identifying field-collected ruffe larvae.

Recent advances in DNA barcoding and sequencing technologies allow for identification and confirmation of field collected fish larvae (Ko et al., 2013). The combination of morphological and genetic analysis can be used to confidently describe and develop diagnostics for field-collected larval fish. As part of an invasive species early detection study, we identified fish larvae collected from the lower St. Louis River (SLR), which included the Duluth-Superior Harbor, in 2012 and 2013. Here we present a description of ruffe larvae from those samples based on genetically-confirmed individuals. We quantify meristics and other morphological characteristics for ruffe larvae and compare them with similar species found in the lower SLR. These data are used to clarify inconsistencies among published descriptions and to develop

* Corresponding author at: 6201 Congdon Blvd., Duluth, MN 55804, USA.
E-mail addresses: peterson.greg@epa.gov (G.S. Peterson), JElizBarker@gmail.com (J.E. Lietz).

http://dx.doi.org/10.1016/j.jglr.2016.10.005
0380-1330/Published by Elsevier B.V. on behalf of International Association for Great Lakes Research.

diagnostic traits to improve stage-based ruffe larvae identification. These traits are compared to the family-level dichotomous keys for yolk sac and larval fish stages found in the Great Lakes larval fish key (Auer, 1982a) to determine if they would lead to a correct designation of Percidae.

Methods

In 2012 and 2013, we sampled fish larvae at over 350 sites by neuston net, Tucker trawl, larval beach seine, larval tow sled, or light trap. Samples were preserved in >90% ethanol to maintain DNA integrity. All fish larvae within each sample were individually identified to the lowest practical taxonomic level using Auer (1982a), French and Edsall (1992), Leslie et al. (2002), and Simon and Vondruska (1991). In all, over 2000 and 12,000 fish larvae were morphologically identified in 2012 and 2013, respectively.

For diagnostics development, individual ruffe ($n = 10$), black crappie (*Pomoxis nigromaculatus*, $n = 10$), pumpkinseed (*Lepomis gibbosus*, $n = 9$), and johnny darter (*Etheostoma nigrum*, $n = 7$) were selected and removed from various samples. These species were chosen because their morphological characteristics are most similar to ruffe. All individuals compared were of similar size and all were subject to DNA analysis to verify morphological identification. The number of larvae removed from samples was minimal to maintain bulk sample integrity required for other aspects of the study. DNA was extracted from fish larvae tissue samples using a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), following manufacturer's guidelines. PCR using primers dgLC01490 and dgHC02198 (Folmer et al., 1994) amplified the 658 bp barcode region of the mitochondrial cytochrome *c* oxidase subunit 1 gene (CO1). The PCR product of each specimen was sequenced at USEPA Cincinnati, OH using Sanger Sequencing with BigDye v3.1 in an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, California). DNA barcode results for each individual were queried against the sequences of known reference material in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert, 2007) and to sequences we generated from fin clips of adult fish. A DNA barcode match to assign a species level identification to an individual was defined as >99% similarity to a unique reference species.

Prior to DNA analysis, fish larvae were photo-documented (Nikon SMZ-U microscope with Nikon digital sight DS-5M camera) and any observed patterns in pigmentation were noted. A suite of morphological measurements were made on digital images of individual larvae using NIS-Elements D4.30.01 (64-bit) software. Morphometrics included total length (TL)—anterior margin of snout (AS) to posterior margin of caudal fin (PC), pre-anal length—AS to posterior margin of vent (PV), head length—AS to origin of pectoral fin, swim bladder length—if present, maximum body depth—includes yolk sac if present, post-anal depth—behind (B) PV, head depth—B posterior margin of eye (PE), caudal peduncle depth, swim bladder depth—if present, maximum body width, and head width—BPE (Simon et al., 1987). In addition, we quantified each larva's pre-anal and post-anal myomeres. Each larva was staged as yolk sac, preflexion, flexion, or postflexion according to Kendall et al. (1984). Descriptions of larval ruffe focus on <6 mm TL yolk sac and preflexion stages, which made up over 95% of the approximately 800 ruffe captured among the five different gears used in our survey. These stages, therefore, represent a size likely to be collected in monitoring surveys. We also describe 10 to 20 mm TL (postflexion) ruffe larvae; however, our catches lacked ruffe between the sizes of 6 mm and 10 mm TL.

Results and discussion

Meristics

Myomere counts of ruffe ranged from 13 to 15 pre-anal and 20 to 22 post-anal in our study (Table 1, Table 2). According to descriptions in the Great Lakes larval fish key (Auer, 1982b), these pre-anal counts are lower than common percids like yellow perch (*Perca flavescens*) and logperch (*Percina caprodes*) which are reported to have between 17 and 24 pre-anal myomeres, but overlap slightly with some Great Lakes species of darter (*Etheostoma* spp.), and johnny darter from the Ohio River drainage (15 preanal, 21 postanal; Simon, 2006). Myomere counts for ruffe also overlap with reported values for some common Great Lakes centrarchid species (Heang, 1982) including black crappie (10–14 pre-anal and 19–23 post-anal myomeres), and pumpkinseed (10–13 pre-anal and 16–22 post-anal). We observed pre-anal myomere counts of 11–12 and post-anal counts of 21–23 and 18–20, respectively, for black crappie and pumpkinseed (Table 1, Table 2). The percid species found in our samples that was most similar to ruffe with respect to myomere counts was johnny darter, which had 16–17 pre-anal and 20–22 post-anal myomeres (Table 1, Table 2). Thus, pre-anal myomere counts for ruffe are intermediate between centrarchids such as black crappie and pumpkinseed, and common percids such as johnny darter, yellow perch and logperch, the latter two we observed to have between 18 and 21 pre-anal (19–22 post-anal) myomeres.

Our myomere counts for ruffe overlapped the range reported by French and Edsall (1992) of 14–15 pre-anal and 22–24 post-anal myomeres for larvae hatched from laboratory-fertilized ruffe eggs collected from the St. Louis River. These ranges are also consistent with Slovakian and European literature describing ruffe larvae (Kovác, 1993; Urho, 1996), which report 13–16 pre-anal and 22–24 post-anal myomeres. However, our myomere counts differ from a description based on field-collected ruffe from the St. Louis River (Simon and Vondruska, 1991), which reports 17–20 pre-anal counts and 18–21 post-anal counts. While part of the variability in reported myomere counts may be due to differences in defining pre-anal versus post-anal myomeres (Auer, 1982a), the weight of evidence supports the conclusion that ruffe larvae have between 13 and 16 pre-anal and 20–24 post-anal myomeres. It is also important to recognize that in addition to natural variability, myomere counts are subject to observer interpretation and influenced by specimen condition.

Morphometrics

Means and ranges of ruffe morphometry in our study were generally similar to, and overlapping those of black crappie, pumpkinseed, and johnny darter (Table 1, Table 2). A notable exception, pre-anal length of yolk sac, ruffe fell generally between the ranges of the centrarchids and johnny darter. Mean pre-anal length for ruffe was 3.8% and 5.7% greater than pumpkinseed and black crappie respectively, and 5.4% smaller than johnny darter. At the preflexion stage, both pre-anal length and head length for ruffe were greater than that of the two centrarchids, and more similar to johnny darter. This stage based pattern in ruffe pre-anal length may indicate the start of an ontogenetic progression toward a more percid like phenotype which we observe in larger ruffe larvae at the postflexion stage. Overall, the increased proportion of pre-anal length relative to centrarchids is consistent with other percids in the Laurentian Great Lakes. Yellow perch and logperch had about equal pre-anal and post-anal myomere counts, with the anus located at approximately 50% or greater of the fish's total length (Auer, 1982b). Maximum body depth range for preflexion ruffe, however, is more similar to the centrarchids, and maximum body width is greater than all three species. Thus, ruffe does not consistently fit the yolk sac or preflexion morphometric profile of the other species. As a result, some stage based measures, most notably pre-anal length, may provide diagnostic support to help distinguish early life stage ruffe from pumpkinseed, black crappie and johnny darter.

Pigmentation

While pigment can be highly variable, we found some general patterns to aid morphological identifications. Similar to other percids, we

Please cite this article as: Peterson, G.S., Lietz, J.E., Identification of ruffe larvae (*Gymnocephalus cernua*) in the St. Louis River, Lake Superior: Clarification and guidance regarding morphological descriptions..., J. Great Lakes Res. (2016), http://dx.doi.org/10.1016/j.jglr.2016.10.005