Harlequin Darter
Biological Status Review Report

October 27, 2017

FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION
620 South Meridian Street
Tallahassee, Florida 32399-1600

Biological Status Review Report
for the
Harlequin Darter
(*Etheostoma histrio*)
October 27, 2017

EXECUTIVE SUMMARY

The Florida Fish and Wildlife Conservation Commission (FWC) directed staff to evaluate all species listed as Threatened or Species of Special Concern in 2010 that had not undergone a status review in the past decade. The 2011 evaluation found that the Harlequin Darter did not meet any listing criteria. After considering reviewers' comments about insufficient data, staff reviewed the BRG findings and recommended that the Harlequin Darter be maintained as a Species of Special Concern until additional data could be collected. A Species Action Plan for the Harlequin Darter was developed in 2013 and the species was included in the Imperiled Species Management Plan, finalized in 2016. The ISMP identifies the need to re-assess all remaining Species of Special Concern by 2017. In 2017, FWC initiated the request to re-evaluate the harlequin darter.

Public information on the status of the Harlequin Darter was sought from May 10 to June 26, 2017. No information was received from the public during our information request period. The three member Biological Review Group (BRG) met August 2, 2017. Group members remained the same as the previous evaluation and were John R. Knight (FWC lead), William Tate (U. S. Fish and Wildlife Service), and Howard Jelks (U. S. Geological Survey) (Appendix 1). In accordance with rule 68A-27.0012, Florida Administrative Code (F.A.C.), the BRG was charged with evaluating the biological status of the Harlequin Darter using criteria included in definitions in 68A-27.001, F.A.C., and following the protocols in the *Guidelines for Application of the IUCN Red List Criteria at Regional Levels (Version 4.0)* and *Guidelines for Using the IUCN Red List Categories and Criteria (Version 13)*. Please visit http://myfwc.com/wildlifehabitats/imperiled/listing-process/ to view the listing process rule and the criteria found in the definitions.

The Harlequin Darter BRG evaluated the species based on historical and recently collected population demographic information. The new data addressed previous concerns raised about deficiency of population data for the species. The Harlequin Darter BRG concluded that the species did not meet any listing criteria. FWC staff recommends that the Harlequin Darter be removed as a Species of Special Concern from Rule 68A-27.005, F.A.C.

FWC staff gratefully acknowledges the assistance of the biological review group members and peer reviewers. Staff would also like to thank David Cook and Brad O'Hanlon for providing guidance with IUCN criteria and assistance in documenting the meeting.

BIOLOGICAL INFORMATION

Taxonomic Classification – This status report is for the Harlequin Darter, *Etheostoma histrio* (Jordan and Gilbert 1887), in Florida.

Life History References – Hubbs and Pigg (1972), Kuehne and Barbour (1983), Page (1983), Kuhajda and Warren (1989), Gilbert and Yerger (1992), Etnier and Starnes (1993), and Steinberg et al. (2000), Bass et al. (2004), Boschung and Mayden (2004).

Geographic Range and Distribution – Harlequin Darters are widely distributed in the lower Mississippi basin, predominantly below the fall line. Occasionally the species is distributed above the fall line (Ohio, Arkansas, Embarras, and Green rivers) (Kuehne and Barbour 1983). Specifically, Harlequin Darters range from Illinois, Indiana, and Kentucky, south to the Escambia River and west to Texas (Burr et al. 1996, Burskey and Simon 2008, Kuhajda and Warren 1989, Boschung and Mayden 2004, Etnier and Starnes 1993, Page 1983, Hubbs and Pigg 1972). In Florida, Harlequin Darters are only known from the Escambia River (Escambia/Santa Rosa counties). The species was first collected from Florida by Yerger and Suttkus (1962), and additional records were sporadic for the species from 1962 to 2009 (36 collections). However, from 2010 to 2017 an additional 139 collection locations were discovered (FWC Long Term Monitoring Database 2016, *unpublished data*). Harlequin Darters are found in both the mainstem Escambia River and its tributaries. Collection records from the mainstem range from the Escambia River at the Florida/Alabama state line, south to the White River section of the Escambia (17.5 km ESE of Milton Florida) (FWC Long Term Monitoring Database 2016, *unpublished data*). Harlequin Darters are known to occur in the following tributaries; Big Escambia, Little Escambia, Pine Barren, Canoe, and Mitchell creeks. The Harlequin Darter extent of occurrence is 547.8 km^2, and the area of occupancy was determined to be 120 km^2, based on a 2 km grid. The BRG determined that Harlequin Darter populations represent at least 12 locations (based on the IUCN definition of location).

Population Status and Trend – A population status assessment was recently completed on Harlequin Darter from Big Escambia and Pine Barren Creeks (Harriger and Knight 2017). No quantitative population trend analysis is currently available for Harlequin Darters, although research is planned to conduct such an assessment in the near future. The species appears to be declining in some areas of its range, specifically in Alabama, Missouri, and Kentucky (Boschung and Mayden 2004, Kuehne and Barbour 1983). However, in certain areas within the Harlequin Darter range (Illinois, Indiana, and Florida), recent efforts indicate that it is far more abundant than once suspected (Fisher 2008, Henry et al. 2009, Harriger and Knight 2017). Harlequin Darters are generally uncommon in museum collections, due to the species' cryptic nature and most likely because the species' preferred habitats are difficult to sample (Boschung and Mayden 2004, Etnier and Starnes 1993, Fisher 2008, Henry et. al 2009). Recent FWC sampling (2010-2017) collected a total of 236 individuals from the mainstem Escambia River while conducting non-directed species sampling (FWC *unpublished data*). Species directed sampling from Pine Barren and Big Escambia Creeks collected 996 individuals (Harriger and Knight 2017).

Generation Length- Harlequin Darters reach sexual maturity at age one, and the maximum age observed is four years (Boschung and Mayden 2004, Kuhajda and Warren 1989). Therefore the estimated generation time is 2.5 years. Since the estimated time for 3 generations

is less than 10 years (the default minimum for IUCN assessment), a 10-year timeframe was used for this assessment.

Quantitative Analyses – There have been no population viability analyses (PVA) or other quantitative models conducted that include in their results a probability of extinction.

BIOLOGICAL STATUS ASSESSMENT

Threats – The Florida Harlequin Darter population is restricted to one watershed (Escambia), which makes the species susceptible to a catastrophic event within that watershed. Additional populations are available within the tributaries of the Escambia River, which could potentially re-populate a mainstem population if such an event were to occur. Harlequin Darters occur in the Escambia River (Conecuh River in Alabama), but that population is partially fragmented from a source population north of Point A dam (Point A Reservoir, Andalusia, Alabama).

Threats to Harlequin Darters include excessive snag and dead head log removal (a spawning site preference) and impounding lotic water bodies (Bass et al. 2004, Boschung and Mayden 2004). Steinberg et al. (2000) indicated that high turbidity and excessive sediment loads may affect reproductive success of the species. Additional threats to Harlequin Darters include oil and coal exploration in areas where such activities occur (Warren and Cicerello 1982).

Population Assessment – Findings from the BRG are included in a Biological Status Review information table. The Harlequin Darter BRG concluded from the biological assessment that the Harlequin Darter, *E. histrio,* did not meet any of the listing criteria. Two population estimates, using multi-census mark-recapture methods and visual survey techniques determined that there were >10,000 individuals from these two creeks alone (Harriger and Knight 2017, FWC *unpublished data*). Harriger and Knight (2017) estimated Harlequin Darter populations size from Big Escambia Creek was 5,121 (3,980-6,262 95% CI) and 5,461 (4,703-6,219 95% CI) from Pine Barren Creek. Additional unpublished FWC analysis estimated Harlequin Darter populations size from Big Escambia Creek was 7,221 (5,702-9,243 95% CI) and 8,805 (7,678-10,135 95% CI), respectively. Given that these two creeks represent only 2.4% (328 and 31 km^2) of the Escambia watershed area (1050 km^2), it is presumed that Harlequin Darter population size is substantially, if not an order of magnitude, greater than IUCN criteria for listing.

LISTING RECOMMENDATION

Based on the BRG findings and analysis of data from recent surveys, FWC staff recommends that the Harlequin Darter be removed from the Species of Special Concern list in Florida.

SUMMARY OF THE INDEPENDENT REVIEW

Independent scientific review of the biological assessment was sought from 4 scientists and received from 3 scientists. All 3 agreed that the darter did not meet the criteria for listing as a Threatened species. One reviewer noted that the generation length of 2.5 years may have been the life expectancy of

the species; the Biological Review Group used the definition of generation length found in the *Guidelines for Using the IUCN Redlist Categories and Criteria, version 13* (March 2017), "Generation length is the average age of parents of the current cohort (i.e., newborn individuals in the population). Generation length therefore reflects the turnover rate of breeding individuals in a population. Generation length is greater than the age at first breeding and less than the age of the oldest breeding individual, except in taxa that only breed once." Staff note that a shorter generation length would still lead to a 10 year window of review, and the conclusions drawn would be unchanged. One reviewer noted that long term data are absent; staff concur and the need for continued monitoring at levels appropriate to assess trend and address management needs will be incorporated into the revision of the Species Action Plan for the Harlequin Darter. Another reviewer noted that the Florida population is at the edge of the range of the species and may possess unique genetics. Staff concurs that this is a possibility, and several research needs will be included in the revised SAP.

The complete scientific reviews are provided in Appendix 3. Staff of the FWC gratefully acknowledge the assistance of the members of the Biological Review Group and of the Independent Reviewers.

LITERATURE CITED

Barrett, Mark. 2017. GIS analysis prepared for the Harlequin Darter Biological Status Review. Unpublished report. Florida Fish and Wildlife Conservation Commission. Tallahassee, FL.

Bass, G., T. Hoehn, J. Couch and K. McDonald. 2004. Florida Imperiled Fish Species Investigation. Final Report to the U. S. Fish and Wildlife Service. Federal Grant R-3. Florida Fish and Wildlife Conservation Commission, Holt, Florida. 59 pp.

Boschung, H.T., and R. L. Mayden. 2004. Fishes of Alabama. Smithsonian Institution Press, Washington D.C. 736 pp.

Burr, B.M, K.M. Cook, D.J. Eisenhour, K.R. Piller, W.J. Poly, R.W. Sauer, C.A. Taylor, E.R. Atwood, and G.L. Seegert. 1996. Selected Illinois Fishes in Jeopardy: New Records and Status Evaluations. Transactions of the Illinois State Academy of Science. 89(3): 169-196.

Burskey, J.L. and T.P. Simon. 2008. Distribution of Fishes of the Muscataatuck National Wildlife Refuge, Indiana. Proceedings of the Indiana Academy of Science. 117(1). 63-70

Dorazio, R.M. 2017. Population analyses for the Harlequin Darter prepared for the Harlequin Darter Biological Status Review. Unpublished report. US Geological Survey. Gainesville, Florida. USA.

Etnier, D.A., and W.C. Starnes. 1993. The Fishes of Tennessee. The University of Tennessee Press. Knoxville TN. 681 pp.

Fisher, B.E. 2008. Current Status and Distribution of Indiana's Seven Endangered Darter Species (Percidae). Proceedings of the Indiana Academy of Science. 117(2): 167-192

Gilbert, C.R., and R.W. Yerger. 1992. Harlequin Darter, *Etheostoma histrio*. Pp. 84-87 in C.R. Gilbert (ed.). Rare and Endangered Biota of Florida. Vol. II Fishes. University Press of Florida, Gainesville, FL.

Harriger, K.M. and J.R. Knight. 2017. Population Estimate for Harlequin Darters *Etheostoma histrio* in the Escambia River Watershed, Florida. Florida Fish and Wildlife Research Institute- Freshwater Fisheries Research Annual Report. Florida Fish and Wildlife Conservation Commission.

Henry, D.B., L.J. Picher, and C.E. Beachum. 2009. Status Surveys and Management Implications of the Harlequin Darter and Eastern Sand Darter in Southeastern Illinois. Illinois State Wildlife Grant Number T-37-P-1. 47 pp.

Hubbs, C., and J. Pigg. 1972. Habitat preferences of the Harlequin Darter, *Etheostoma histrio*, in Texas and Oklahoma. Copeia. 1972: 193-194.

Jordan and Gilbert *in* Gilbert. 1887. Description of new and little known etheostomids. Proceedings of the U.S. National Museum. 10:47-64.

Kuehne, R.A., and R.W. Barbour. 1983. The American Darters. University Press of Kentucky, Lexington, KY. 177 pp.

Kuhajda, B.R, and M.L. Warren, Jr. 1989. Life history aspects of the Harlequin Darter, *Etheostoma histrio*, in western Kentucky. ASB Bulletin. 36(2):66-67.

Page, L.M. 1983. Handbook of Darters. T.F.H. Publishers, Neptune City, NJ.

Schueller, P. 2017. Population analyses for the Harlequin Darter prepared for the Harlequin Darter Biological Status Review. Unpublished report. Florida Fish and Wildlife Conservation Commission. Tallahassee, FL.

Steinberg, R., L.M. Page, and J.C. Porterfield. 2000. The spawning behavior of the Harlequin Darter, *Etheostoma histrio* (Osteichthyes: Percidae). Ichthyological Exploration of Freshwaters 11 (2):141-148.

Warren, M.L., Jr and R.R. Cicerello. 1982. New records, distribution, and status of ten rare fishes in the Tradewater and lower Green Rivers, Kentucky. Southeastern Fishes Council Proceedings. 3(4):1-8.

Yerger, R.W. and R.D. Suttkus. 1962. Records of freshwater fishes in Florida. Tulane Studies. Zoology 9(5):322-330.

Biological Status Review Information
Findings

Species/taxon:	Harlequin Darter (Etheostoma histrio)
Date:	08/02/17
Assessors:	John Knight (FWC Lead), Howard Jelks (USGS)
	William (Bill) Tate (USFWS)
Generation length:	2.5 years

Criterion/Listing Measure	Data/Information	Data Type*	Criterion Met?	References
*Data Types - observed (O), estimated (E), inferred (I), suspected (S), or projected (P). Criterion met - yes (Y) or no (N).				
(A) Population Size Reduction, ANY of				
(a)1. An observed, estimated, inferred or suspected population size reduction of at least 50% over the last 10 years or 3 generations, whichever is longer, where the causes of the reduction are clearly reversible and understood and ceased[1]	Population data are available, however we do not have 10 years of data. Harvest of essential habitat (submerged woody stems) has not equaled 50% in 10 years.	I	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)
(a)2. An observed, estimated, inferred or suspected population size reduction of at least 30% over the last 10 years or 3 generations, whichever is longer, where the reduction or its causes may not have ceased or may not be understood or may not be reversible[1]	Recent data are available, but no trends are determinable from historical data. Have not lost 30% of essential habitat (submerged woody stems).	I	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)
(a)3. A population size reduction of at least 30% projected or suspected to be met within the next 10 years or 3 generations, whichever is longer (up to a maximum of 100 years) [1]	90%+ of the riparian zone protected on Escambia river, maybe some level of timber harvest (Northwest Florida Water MD). Future harvest levels will probably remain stable (i.e., not 30% increase in 10 years).	S	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)
(a)4. An observed, estimated, inferred, projected or suspected population size reduction of at least 30% over any 10 year or 3 generation period, whichever is longer (up to a maximum of 100 years in the future), where the time period must include both the past and the future, and where the reduction or its causes may not have ceased or may not be understood or may not be reversible.[1]	BRG suspects no change in deadhead logging (=habitat removal) from the past 10 years and through the next 10 years. Population appears to be increasing/stable.	S	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)

[1] based on (and specifying) any of the following: (a) direct observation; (b) an index of abundance appropriate to the taxon; (c) a decline in area of occupancy, extent of occurrence and/or quality of habitat; (d) actual or potential levels of exploitation; (e) the effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

(B) Geographic Range, EITHER				
(b)1. Extent of occurrence < 20,000 km^2 (7,722 mi^2) OR	547.8 km^2	O	Y	FWRI/FWC GIS Data (Barrett 2017), FWC (2016 Long term Monitoring Dataset)
(b)2. Area of occupancy < 2,000 km^2 (772 mi^2)	2km grid system = 120 km^2 / 0.2km grid system = 4.04 km^2	O	Y	FWRI/FWC GIS Data (Barrett 2017), FWC (2016 Long term Monitoring Dataset)
AND at least 2 of the following:				
a. Severely fragmented or exist in ≤ 10 locations	12 locations determined by using known and probable catastrophic event locations (road crossings and chemical plants). Locations are not substantially disconnected by lack of woody habitat.	I	N	Knight Pers Comm (2017), Harriger and Knight (2017), Harriger unpublished analyses (2017), FWC (2016 Long Term Monitoring)
b. Continuing decline, observed, inferred or projected in any of the following: (i) extent of occurrence; (ii) area of occupancy; (iii) area, extent, and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals	Have long term data that does not show a decline, if anything, data has an increasing trend. No decrease in subpopulations or locations. We know of more mature individuals range wide. No change in extent of occurrence. Think sampling effort is confounded within spatial extent variables (AOO). May rerun analyses for better estimation.	O/I/S	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)
c. Extreme fluctuations in any of the following: (i) extent of occurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals	No evidence of extreme fluctuations. When present, only collected in low #s. Database is improving to see what fluctuations may occur. Can infer that there is not a 10 fold increase/decrease based on FWC LTM data	O	N	Knight Pers Comm (2017), Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long Term Monitoring)

(C) Population Size and Trend				
Population size estimate to number fewer than 10,000 mature individuals AND EITHER	> 10,000 mature individuals (3 estimates). Estimates just for big Escambia and pine barrens creek. Survey area roughly 10km.	E	N	Harriger and Knight (2017), unpublished analysis from Dorazio (2017) and Scheuller (2017)r , FWC (2016 Long Term Monitoring)
(c)1. An estimated continuing decline of at least 10% in 10 years or 3 generations, whichever is longer (up to a maximum of 100 years in the future) OR				
(c)2. A continuing decline, observed, projected, or inferred in numbers of mature individuals AND at least one of the following:				
a. Population structure in the form of EITHER				
(i) No subpopulation estimated to contain more than 1000 mature individuals; OR				
(ii) All mature individuals are in one subpopulation				
b. Extreme fluctuations in number of mature individuals				
(D) Population Very Small or Restricted, EITHER				
(d)1. Population estimated to number fewer than 1,000 mature individuals; OR	> 10,000 mature individuals (3 estimates). Estimates just for big Escambia and pine barrens creek. Survey area roughly 10km.	E	N	Harriger and Knight (2017), unpublished analyses from Dorazio (2017) & Schueller (2017)
(d)2. Population with a very restricted area of occupancy (typically less than 20 km^2 [8 mi^2]) or number of locations (typically 5 or fewer) such that it is prone to the effects of human activities or stochastic events within a short time period in an uncertain future	# locations > 5. AOO = 88km2 based on 2km sampling grid. These locations are not prone to human events.	O/I/S	N	Harriger and Knight (2017), unpublished analyses by Scheuller (2017) and Dorazio (2017), FWC (2016 Long term Monitoring Data), John Knight Pers Comm (2017)
(E) Quantitative Analyses				
e1. Showing the probability of extinction in the wild is at least 10% within 100 years	No data available for PVA, all evidence does not point towards extinction.	S	N	

Initial Finding (Meets at least one of the criteria OR Does not meet any of the criteria)	Reason (which criteria are met)
Does not meet any criteria.	N/A
Is species/taxon endemic to Florida? (Y/N)	N

If Yes, your initial finding is your final finding. Copy the initial finding and reason to the final finding space below. If No, complete the regional assessment sheet and copy the final finding from that sheet to the space below.

Final Finding (Meets at least one of the criteria OR Does not meet any of the criteria)	Reason (which criteria are met)
Does not meet criteria.	N/A

Biological Status Review Information
Regional Assessment

Species/taxon:	Harlequin Darter (Etheostoma histrio)
Date:	8/2/17
Assessors:	John Knight (FWC Lead)
	Howard Jelks (USGS)
	William (Bill) Tate (USFWS)

Initial finding	
2a. Is the species/taxon a non-breeding visitor? (Y/N/DK). If 2a is YES, go to line 18. If 2a is NO or DO NOT KNOW, go to line 11.	N
2b. Does the Florida population experience any significant immigration of propagules capable of reproducing in Florida? (Y/N/DK). If 2b is YES, go to line 12. If 2b is NO or DO NOT KNOW, go to line 17.	Y
2c. Is the immigration expected to decrease? (Y/N/DK). If 2c is YES or DO NOT KNOW, go to line 13. If 2c is NO go to line 16.	N
2d. Is the regional population a sink? (Y/N/DK). If 2d is YES, go to line 14. If 2d is NO or DO NOT KNOW, go to line 15.	
If 2d is YES - Upgrade from initial finding (more imperiled)	
If 2d is NO or DO NOT KNOW - No change from initial finding	
If 2c is NO or DO NOT KNOW- Downgrade from initial finding (less imperiled)	No Change
If 2b is NO or DO NOT KNOW - No change from initial finding	
2e. Are the conditions outside Florida deteriorating? (Y/N/DK). If 2e is YES or DO NOT KNOW, go to line 24. If 2e is NO go to line 19.	
2f. Are the conditions within Florida deteriorating? (Y/N/DK). If 2f is YES or DO NOT KNOW, go to line 23. If 2f is NO, go to line 20.	
2g. Can the breeding population rescue the Florida population should it decline? (Y/N/DK). If 2g is YES, go to line 21. If 2g is NO or DO NOT KNOW, go to line 22.	
If 2g is YES - Downgrade from initial finding (less imperiled)	

If 2g is NO or DO NOT KNOW - No change from initial finding	
If 2f is YES or DO NOT KNOW - No change from initial finding	
If 2e is YES or DO NOT KNOW - No change from initial finding	
Final finding	No Change

Additional notes - over 1200 animals collected in last 7 years. Age at maturity 1, 4-5 year life span

A(a)1: Substantial wood harvesting in Escambia river. FWC can create guides for removal of logs to maintain critical darter habitat. Massive log jam in Escambia resulted in removal of every stick from logjam (more than was expected). It is possible to equate wood to populations. New study supports that HEDA are correlated to wood availability in streams.

A(a)2: John Knight has access to DEP permits to show removal of logs / critical habitat. Can track down information as needed.

A(a)3: What's valuable to deadhead loggers is old trees with minerals. Recent trees are not as valuable, still provide habitat, not under as much of a risk from harvest. John Knight: Pulling old and new timber, does this create a lack of demand? New wood is not the same quality or value. Can assume that logging will not greatly expand. DEP may not allow such an increase. FWC gets input on permitting process. References: John has seen emails and related information is under his sphere of influence. Political whim may be involved...

A(a)4: There may be some change in habitat, but logs will lodge in other places. Habitat relocation versus habitat loss. Hard to know how much wood accumulation would be in streams if not for man. Community reports are available for citation, however does not go into wood/habitat availability in streams.

B(b)1: IUCN uses MCP for linear system. Will never hit threshold in Florida.

B(b)2: 2km grid system used

B(b)2a: Data may be sampling artefact because you can only sample a portion of a stream every year. Each tributary and node on tributary constitutes a location. Locations identified are Big Escambia, Little Escambia, Pine Barren Creek, Mitchell Creek, McDavid Creek, Canoe Creek. Escambia mainstem # locations is unclear. Have fish kill data from chemical spills, 100,000+ kgs in 50's so may be at least 2 locations in mainstem. Braided channel area (White River, Delaney River) may be unique locations according to threats. Mainstream of the Escambia has 2 bridge locations Quintette Road and SR 4, plus bridge crossing north of FL border in Alabama (location starts at FL state line). Definition of location hinges on catastrophic event (toxic event) which is likely to occur from roadway or chemical plant (formerly Monsanto). A total of 12 locations were identified.

B(b)2b: Mature individuals were only animals captured. Appears to be a decline in #s based on sample frame. May need to re run analyses in 10 year blocks. Current results show "where sampling has occurred". Sampling regime is conflicted with #'s. Need to correlate sample areas with time to be able to determine any decline. Recalculate area of occupancy, experts think that this will not change. MCPs skew data, especially if one sample is found in an extreme reach of river, especially if sampling is not frequent. Experts don't believe there is a decline, although data analysis suggests that one may exist... The data is only as good as sampling efforts allow. Samples from 2007 may be outliers. Functionally AOO would need to be sampled every 5-10 years to determine any change. This may be revisited after a more relevant data analysis is performed.

B(b)2c: No evidence of any boom or bust cycles. No knowledge of 10 fold increase or decrease at any location, population is stable. Numbers may fluctuate based on sampling techniques (eg: targeted HEDA surveys vs general electrofishing).

C: 3 population estimates. All with means > 10k animals. Lots of streams have not been sampled, very conservative estimates

E: Estimated #s of individuals, subpopulations, no projected decline, no evidence of extreme loss of habitat. No reason to suspect a decline that would lead to extinction.

Regional Assessment Comments: Probably less than 10% recruitment is crossing state boundary. Immigration/emigration is potentially equal. Is there a difference of genetic structure in AL/FL fish (future research need).

APPENDIX 1. Brief biographies of the Harlequin Darter Biological Review Group members

John Knight II received his B.S. in Fisheries from the University of Georgia and Master's degree from Auburn University. John worked for the University of Georgia, Institute of Ecology and Georgia Museum of Natural History, where he primarily conducted research and monitoring on Federally Endangered and Threatened fish species. In 2005 he accepted a position with FWC's Fish and Wildlife Research Institute (FWRI). For the past twelve years, he has worked primarily on developing monitoring strategies/techniques to effectively characterize fish communities from streams and rivers in Florida. He is currently the Principal Investigator on multiple threatened and endangered species projects (both state and federally listed). These projects include research on Gulf Sturgeon, Crystal Darters, and Saltmarsh Topminnows. Additional duties while working for FWRI include biological comments on the effects of development on state and federally listed species, consultation for scientific permit applications, assist USFWS with sampling for federally listed fish and mussel species, and work on numerous interagency technical committees and partnerships within the state of Florida. John served on the original Biological Review Group for the Harlequin Darter

Howard Jelks has diverse experience from wetland plants, aquatic invertebrates, fish, and wading birds. He has studied these organisms from the estuaries of Apalachicola Bay, freshwater marshes of the Everglades, and streams of the Piedmont and Coastal Plain. Although he specializes in fishes, he classifies himself as a general naturalist/ecologist. How the dynamic environment structures biotic communities is his broad interest. Imperiled freshwater fishes are his specialty, but nonindigenous and marine taxa are also studied. For the past 22 years, he has been a leader of Okaloosa darter monitoring and recovery planning at Eglin Air Force Base in northwest Florida. He has developed skills in sampling design, database development, geographical information system and statistical analyses. Howard currently is on the graduate committee of Kate Harriger who is researching Harlequin Darters in the Escambia River system. Howard served on the original Biological Review Group for the Harlequin Darter.

Bill Tate is the lead biologist for the US Fish and Wildlife Service office at Eglin Air Force Base. This office is responsible for supporting a variety of natural resource conservation programs on the 485,000 acre Eglin reservation. A fish biologist by training, Bill has been primarily focused on protecting the Okaloosa darter, a darter species managed successfully enough for the last two decades that it qualified for down-listing from federally endangered to threatened in 2011. Having spent the more than 15 years as a fishery biologist in Florida, his expertise extends to all North Florida darters and many other benthic (therefore cryptic) freshwater species and habitats. Bill served on the original Biological Review Group for the Harlequin Darter.

APPENDIX 2: Summary of letters and emails received during the solicitation of information from the public period of May 10 to June 26, 2017

No information was received from the public.

Appendix 3. Peer Reviews of Independent Scientists

LOYOLA UNIVERSITY NEW ORLEANS
DEPARTMENT OF BIOLOGICAL SCIENCES

Melissa Tucker
Assistant Section Leader/Protected Species Coordinator
Species Conservation Planning Section
Florida Fish and Wildlife Conservation Commission
620 S. Meridian Street
Mail Station 2A
Tallahassee, FL 32399

October 22, 2017

Dear Melissa:

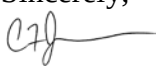
This letter constitutes my peer review of the Harlequin Darter Biological Status Review prepared by John R. Knight (FWC), William Tate (USFWS), and Howard Jelks (USGS).

Harlequin Darters are widely distributed in the lower Mississippi basin and are limited to the Escambia River and its tributaries in Florida. This species has a strong preference for submerged logs and woody debris and these habitats are not amenable to quantitative sampling using traditional methods such as seines and electrofishing. Historical data on the occupancy and abundance of Harlequin Darters based on these methods therefore are not reliable. The 2011 FWC evaluation of the status of Harlequin Darters recognized the shortcomings and limitations of previous work on Harlequin Darters and wisely recommended that a visual assessment be carried out to obtain more accurate estimates of the abundance of this species. Harlequin Darters are an excellent candidate for visual census because of their use of relatively shallow streams, their sedentary behavior, and their strong preference for logs and woody debris. Karen Harriger and John Knight conducted the visual survey and provided the census data that the Biological Review Group (BRG) needed to accurately assess the conservation status of Harlequin Darters in Florida. The methods, assumptions, and conclusions of both the Harriger and Knight study and the subsequent status review by the BRG appear to be sound and based on accepted conservation criteria. It is not likely that Harlequin Darters are imperiled in Florida given an estimated population size of greater than 10,000 individuals from portions of two tributaries that represent just 2.4% of the Escambia River drainage.

An important caveat to the above conclusion is that there are no long-term data to determine if the abundance of Harlequin Darters is increasing, stable, or declining. I recommend that FWC periodically reassess abundance of Harlequin Darters in the Escambia River basin in order to verify that Florida populations of this species are stable or increasing. The environment is undergoing rapid change due to habitat loss, overharvesting, invasive species, pollution, and climate change associated with overconsumption of natural resources by an ever-increasing human population. Increased demand for submerged logs or changes in land use could have strong negative effects on Harlequin Darters in Florida.

In summary, I agree with the conclusion of the Harlequin Darter Status Review that this species does not require special conservation status in Florida.

Sincerely,



Dr. Frank Jordan
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This is an independent review by Bernard Kuhajda of the Biological Status Review Report for the Harlequin Darter (*Etheostoma histrio*) by John Knight. Howard Jelks, and William Tate. This review is at the request of the Florida Fish and Wildlife Conservation Commission.

The biological review group (BRG) for the Harlequin Darter concluded the species did not meet any listing and that the species be removed from the Species of Special Concern from Rule 68A-27.005, F.A.C.

The appropriate literature has been cited, threats to the species have been addressed, and the data deficiencies from the 2011 Biological Status Review Report have been filled in with several surveys and population estimates for two tributaries to the Escambia River. I agree with the BRG that the species does not meet any listing criteria.

The one aspect of the Florida population of Harlequin Darters that was not addressed by the BRG is the potential for a population at the edge of a species' range to possess unique genetics relative to populations nearer the center of the range. The potential for unique genetics in the Escambia River population is even great because, based on replicated biogeographic patterns for other freshwater fish species, the Escambia River is isolated from the Mobile Basin, with no gene flow between freshwater fishes occupying these two drainages. Therefore the Escambia River population of Harlequin Darters has likely been isolated from other populations in the Mobile Basin and further west for a very long time, increasing the chances that there are unique genetics for the species in this river system. No current range-wide genetic assessment of Harlequin Darters has been investigated. This should be a research need for this species.

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10 August 2017

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Assistant Section Leader/Protected Species Coordinator
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Dr. Ms. Tucker:

The biological information and data analyses in the Biological Status Review on the Harlequin Darter, dated 25 August 2017, are complete and mostly accurate. I have only one question: Given sexual maturity at one year of age, which is characteristic of most if not all species of darters, I don't understand how the "estimated generation time is 2.5 years." A new generation appears annually. I think the authors may have intended to say that the life expectancy is 2.5 years. Regardless of our different interpretations of generation time, I agree with the assumptions and conclusions of the report.

Based on this report as well as my experiences and interpretations of information, the Escambia River drainage is in very good condition overall, even outstanding in several areas, and the Harlequin Darter seems to be doing well.

Thank you for the opportunity to review this report.

Sincerely,

Larry M Page

Director, iDigBio
Curator of Fishes