BIOLOGICAL EVALUATION
FOR THE ENDANGERED SPECIES ACT
SECTION 7 CONSULTATION
FOR THE FLORIDA DEPARTMENT OF ENVIRONMENTAL PROTECTION'S
WATER QUALITY STANDARD REVISION FOR AMMONIA

Prepared by the Environmental Protection Agency Region 4
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Overview

The focus of this Biological Evaluation (BE) is the effects which may occur to threatened and endangered species and/or designated critical habitat from the revision to water quality standards (WQS) contained in the Florida Department of Environmental Protection's (FDEP) F.A.C. 62-302.530 Surface Water Quality Criteria (3) Ammonia (Total Ammonia Nitrogen) (Class I, Class III fresh water, and Class III-Limited fresh water). This provision was included as part of the Department's Triennial Review contained in Rules 62-4, 62-302, and 62-303. All of the Triennial Review revisions were considered and approved for adoption by the Florida Environmental Regulation Commission (ERC) at a public hearing on December 9, 2015. The Florida Department of Environmental Protection (FDEP) subsequently filed the amendments for adoption with the Florida Department of State on January 28, 2016. The rule amendments took effect on February 17, 2016. On June 14, 2016, the EPA received a letter from Frederick L. Aschauer, Jr., General Counsel of Florida Department of Environmental Protection to Ms. Heather McTeer Toney, Regional Administrator, U.S. EPA Region 4, dated June 7, 2016, certifying that the amendments were duly adopted pursuant to state law. The revisions were submitted to the U.S. Environmental Protection Agency (EPA) on June 15, 2016 for review, pursuant to section 303(c) of the Clean Water Act (CWA). This revision to the State's administrative code incorporates EPA's most recent CWA section 304(a) freshwater criteria recommendation for ammonia. This BE was prepared by the EPA to determine if the EPA's approval, pursuant to section 303(c) of the CWA, of the Department's water quality criteria for ammonia is likely to jeopardize the continued existence of, or adversely affect, federally listed endangered or threatened species, or result in the adverse modification of designated critical habitat of such species in fresh waters of the State of Florida in accordance with section 7(a)(2) of the Endangered Species Act (ESA).

Description of the Federal Action

Under section 303(c) of the CWA and 40 CFR § 131, States and authorized tribes have primary responsibility to develop and adopt WQS to protect their waters. As required by section 303(c) of the CWA and 40 CFR § 131, the EPA reviews new and revised WQS that have been adopted by States and authorized tribes. State and Tribal WQS are not considered effective under the CWA until approved by the EPA.

The Federal action being evaluated is the EPA approval of the revised WQS rule as it relates to the protection of aquatic life use as set forth in F.A.C. 62-302.530 Surface Water Quality Criteria (3) Ammonia (Total Ammonia Nitrogen) (Class I, Class III fresh water, and Class III-Limited fresh water).

Section 303(c)(3) of the CWA states in part: If the Administrator, within sixty days after the date of submission of the revised or new standard, determines that such standard meets the requirements of this Act, such standard shall thereafter be the water quality standard for the applicable waters of the State…

A. History of ESA Consultation for this CWA Action

Section 7(a)(2) of the ESA requires the EPA, in consultation with the FWS and/or the National Marine Fisheries Service (NMFS), to ensure that any action authorized by the agency is not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of designated critical habitat for such species. As provided in the Memorandum of Agreement between the EPA, the FWS, and the NMFS regarding enhanced coordination of CWA and ESA obligations, the EPA uses a BE to analyze whether a new or revised water quality standard may affect federally-listed species or designated critical habitat. This BE has been prepared to determine whether the EPA's approval of specific aspects of the Department's surface water quality standards may affect federally listed endangered or threatened species or the designated critical habitat of such species. However, if EPA determines that the action is likely to adversely affect listed species or critical habitat, then ESA formal consultation with the FWS is required to determine if the action in question is likely to jeopardize the continued existence of listed species or destroy or adversely modify designated critical habitat. if the EPA obtains concurrence on the "not likely to adversely affect" (NLAA) finding from the FWS

Early coordination began in August 2015 with a regional workgroup comprised of Fish and Wildlife Service and National Marine Fisheries Service (Services) and EPA Region 4 staff to discuss regional consultations on CWA 303(c) actions for EPA's 304(a) recommended criteria. The Department's proposed triennial review revisions were included in workgroup discussions. The workgroup also discussed how best to search for and assess new species data for consultation efforts. Tom Augspurger of the Raleigh Ecological Services Field Office provided a draft research strategy for workgroup review describing an approach by which EPA and the FWS could work together to determine the extent of the available information for use in this consultation. Following that approach for this consultation, EPA reviewed existing BEs and biological opinions that have been completed for ammonia consultations, conducted an Ecotox database retrieval, and reviewed data that have become available as of October 2012. EPA reviewed information that was provided by Tom Augspurger on March 9, 2016. The information was provided from a network of colleagues, researchers and scientists and included ammonia toxicity test data, literature and reports for aquatic species. This information is addressed below in the 'Analysis of New Data Section'. On March 7, 2016, the EPA initiated informal consultation with a request for technical assistance to obtain the official list of threatened and endangered species for the state of Florida. The EPA received the list of aquatic and aquatic dependent species from the Panama City Ecological Services FWS Conservation Office on April 21, 2016. This list was verified to be accurate as of August, 2016. Table 5 in the Summary of Species Present in Florida section below provides the list of threatened and endangered species in Florida under the jurisdiction of the FWS being considered for this action.

B. Overview of Water Quality Standards

The CWA provides the statutory basis for the water quality standards program and defines broad water quality goals. The purpose of the CWA is to restore and maintain the chemical, physical, and biological integrity of the nation's waters. 33 U.S.C.S. § 1251(a). Section 101(a)(2) of the CWA sets out a national goal that wherever attainable, waters achieve a level of quality that provides for the protection and propagation of fish, shellfish, and wildlife, and for recreation in and on the water ("fishable/swimmable"). 33 U.S.C.S. § 1251(a),

Section 303(c) of the CWA requires that all states adopt water quality standards and that the EPA review and approve or disapprove these standards. States first identify designated uses they wish to protect in state waters, such as drinking water supply, propagation of fish, or recreation. States then establish water quality criteria that protect those designated uses. Finally, state water quality standards must include an antidegradation policy and implementation procedures consistent with the EPA's policy to protect existing uses, high quality waters, and outstanding national resource waters. § 1313(c)(2)(A) (Supp. 1993); 40 C.F.R. § 131. States must review those standards every three years and revise the standards, as necessary. This public process, commonly referred to as the triennial review, allows for new technical and scientific data to be considered in order to update the standards. The regulatory requirements governing water quality standards are established at 40 CFR Part 131.

Any new or revised water quality standards must be submitted to EPA for review and EPA must approve or disapprove those standards. Upon approval by EPA, the state's standards become effective for CWA purposes. 40 C.F.R. § 131.21(c). If EPA disapproves the state standards, it must notify the state and specify the changes the state must adopt to meet CWA requirements. 33 U.S.C. § 1313(c)(3). If adequate revisions are not adopted by the state, EPA must propose federal water quality standards for the state and promulgate final standards unless, before promulgation, the state adopts standards that EPA determines to be in compliance with the CWA. 33 U.S.C. § 1313(c)(4).

Description of Provisions Considered by the EPA for the Endangered Species Act (ESA) Section 7 Consultation

Chapter 62-302.530 F.A.C. - Surface Water Quality Criteria (3) Ammonia

During their most recent triennial review, the Department adopted rules to revise the water quality criteria for un-ionized ammonia in fresh water. The revision is found at Chapter 62-302.530, F.A.C. Surface Water Quality Criteria (3) Ammonia (Total Ammonia Nitrogen) (Class I, Class III fresh water, and Class III-Limited fresh water).

The Department's criterion is a 30-day average Total Ammonia Nitrogen (TAN) value not to be exceeded, with no single value exceeding 2.5 times the value using the equation from the criteria document referenced above. For purposes of total ammonia nitrogen criterion calculations, pH is subject to the range of 6.5 to 9.0. The pH shall be set at 6.5 if measured pH is <6.5 and set at 9.0 if the measured pH is >9.0. The Department's 30-day chronic criterion is consistent with the EPA's 2013 30-day averaging period recommendation. The Department's 4-day chronic criteria is a single value not to exceed, rather than a 4-day average as recommended by the EPA. The Department's criteria are consistent with the EPA's recommendations of maximum ambient concentrations of ammonia protective of freshwater aquatic organisms from unacceptable chronic effects. The Department is utilizing a chronic criterion. This provision will serve additionally as an acute criterion. The approach to include chronic criteria only is considered more conservative than utilizing both acute and chronic criteria.

The specific language regarding the Department's adoption of ammonia criteria in 2016 is contained in Chapter 62-302.530, F.A.C. - Table: Surface Water Quality Criteria (3). The details of the provision are provided below.

Criteria for Surface Water Classifications		
Parameter	Units	Class I, Class III fresh water, and Class III-Limited fresh water
(3) Ammonia (Total Ammonia Nitrogen) (Class I, Class III fresh water, and Class III-Limited fresh water)	Milligrams/L as Total Ammonia Nitrogen (TAN = NH_4^+ + NH_3)	The 30-day average TAN value shall not exceed the average of the values calculated from the following equation, with no single value exceeding 2.5 times the value from the equation: $30-\text{day Average} = 0.8876 \times \left(\frac{0.0278}{1+10^{7.688-pH}} + \frac{1.1994}{1+10^{pH-7.688}}\right) \times \left(2.126 \times 10^{0.028\times(20-MAX(T,7))}\right)$ *T* and *pH* are defined as the paired temperature (°C) and pH associated with the TAN sample. For purposes of total ammonia nitrogen criterion calculations, pH is subject to the range of 6.5 to 9.0. The pH shall be set at 6.5 if measured pH is < 6.5 and set at 9.0 if the measured pH is > 9.0.

For comparison purposes, the table below includes EPA's 1999 and 2013 criteria recommendations for ammonia at various pH and temperature values.

Temp °C	pH 6.5		pH 7.0		pH 7.5		pH 8.0	
	1999	2013	1999	2013	1999	2013	1999	2013
20	4.68	2.1	4.15	1.9	3.06	1.4	1.71	0.78
25	3.4	1.5	3	1.4	2.2	1.0	1.25	0.56
30	2.5	1.1	2.18	0.99	1.61	0.73	0.897	0.41

EPA Clean Water Act Recommended 304(a) Ammonia Criteria Update History

Prior to the publication of EPA's 2013 304(a) recommended criteria, EPA published several updates to its original 1984 Ammonia Criteria for the protection of aquatic life in freshwater including revisions in 1992, 1998 and 1999. The 1999 revision reflected new research and data, including revised pH and temperature relationships for the acute and chronic criteria and the averaging period of the chronic criterion. The revisions resulted in the acute criterion for ammonia being dependent on pH and fish species, and the chronic criterion being dependent on pH and temperature. In addition, at lower temperatures, the dependency of chronic criterion was dependent on the presence or absence of early life stages of fish. In this criteria revision EPA recommended a 30-day averaging period for chronic criterion and included a not to exceed 4-day average concentration of 2.5 times the chronic criterion.

In 2004, EPA published a federal register notice to inform the public of its intent to re-evaluate the freshwater ammonia criteria and requested new information on ammonia toxicity for all species including freshwater mussels. EPA also convened a mussel toxicity testing workshop in 2005. In 2009, EPA published a draft ammonia criteria document that included new data for acute and chronic datasets and also included the revised relationships and influence of pH and temperature on ammonia toxicity that were presented in the 1999 criteria document. The new 2009 acute dataset represented 67 genera, including 12 species of freshwater mussels, compared to only 34 genera in the 1999 document. Freshwater bivalve mollusks and snails were the predominant groups ranked as the more sensitive species. The four most acutely sensitive genera were all bivalves including clams and mussels. The 2009 chronic dataset also incorporated two new fish species and new data for three freshwater mussel species. The new species were two of

the most sensitive genera within the lowest 4 species used to calculate the criterion. The 2009 criteria recommendations were split, with a set of acute and chronic criteria values for waters with mussels present that reflects their greater sensitivity to ammonia, and a different set of criteria values for waters where mussels are absent. In the 2009 document, available data indicated that another freshwater mollusk taxon, the non-pulmonate (gill-bearing) snails, are also sensitive to the effects of ammonia. (See pages 62-64 for summary of all new acute data.)

The 2013 EPA recommended 304(a) ammonia criteria include additional data confirming the sensitivity of freshwater non-pulmonate snails. The 2013 document incorporated additional EPA-reviewed results from the latest literature search and included a reanalysis of previously considered data including laboratory toxicity tests that quantify the adverse effects of ammonia on freshwater aquatic life (amphibians, fishes, and macroinvertebrates). Particular attention was given to tests conducted with freshwater Unionid mussels and non-pulmonate snails, since such data were not available for many of these species previously and because these are sensitive mollusk species. While Unionid mussel species are not prevalent in some waters, such as in the arid west, non-pulmonate snails are broadly distributed across the U.S. Thus, considering that freshwater Unionid mussels are among the most sensitive genera in the dataset, and that all states have at least one freshwater Unionid mussel or bivalve mollusk and non-pulmonate snail species native or present in at least some of their waters, EPA recommended a single national acute and a single national chronic criterion be applied to all waters rather than different criteria based on the presence or absence of mussels.

For the 2013 304(a) recommended criteria, EPA conducted another comprehensive search and analysis of relevant new data specific for freshwater mussels to evaluate whether the existing pH-acute TAN toxicity relationship established in the 1999 update document similarly applies to this group of invertebrates. Based on the results of the literature review, EPA concluded that the pH and temperature relationships used to account for the influence of these factors were still applicable. EPA's final acute dataset for the 2013 304(a) recommended ammonia criteria includes acute toxicity data for 12 listed species and additional data on 54 species including new data for 21 mollusk (mussel or snail) species, the majority of which are sensitive in comparison to other dataset species. This information is included in Table 1 below.

Table 1 Listed species and mollusk species in EPA's Acute Dataset 2013

Common name (scientific name)	Listed Species	Mussel	Snail	Fish
Marsh ramshorn snail, *Planorbella trivolvis*			X	
Shortnose sturgeon, *Acipenser brevirostrum*	X			X
Atlantic salmon, *Salmo salar*	X			X
Dwarf wedgemussel, *Alasmidonta heterodon*	X	X		
Pink papershell, *Potamilus ohiensis*		X		
Coho salmon, *Oncorhynchus kisutch**	X			X
Rainbow trout, *Oncorhynchus mykiss**	X			X
Chinook salmon, *Oncorhynchus tshawytscha**	X			X
Topeka shiner, *Notropis topeka*	X			X
Great pond snail, *Lymnaea stagnalis*			X	
Mucket, *Actinonaias ligamentina*		X		
Pheasantshell, *Actinonaias pectorosa*		X		
Giant floater mussel, *Pyganodon grandis*		X		
Pagoda hornsnail, *Pleurocera uncialis*			X	
Pebblesnail, *Fluminicola* sp.			X	
Lost River sucker, *Deltistes luxatus*	X			X

Table 1 Listed species and mollusk species in EPA's Acute Dataset 2013				
Common name (scientific name)	**Listed Species**	**Mussel**	**Snail**	**Fish**
Atlantic pigtoe, *Fusconaia masoni*		X		
Pondshell mussel, *Utterbackia imbecillis*		X		
Pink mucket, *Lampsilis abrupta*	X	X		
Plain pocketbook, *Lampsilis cardium*		X		
Wavy-rayed lampmussel, *Lampsilis fasciola*		X		
Higgin's eye, *Lampsilis higginsii*	X	X		
Neosho mucket, *Lampsilis rafinesqueana*	X	X		
Fatmucket, *Lampsilis siliquoidea*		X		
Rainbow mussel, *Villosa iris*		X		
Oyster mussel, *Epioblasma capsaeformis*	X	X		
Green floater, *Lasmigona subviridis*		X		
Ellipse, *Venustaconcha ellipsiformis*		X		

The 2013 chronic dataset fulfills the eight minimum data requirements for developing a chronic criterion without having to use an acute to chronic ratio to develop the chronic criterion. Therefore, the chronic criterion is derived following the same genus level sensitivity distribution approach used to calculate the acute criterion. The EPA's *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (PB85-227049) list the following as the minimum requirements for acute criteria development: 1) the family *Salmonidae* in the class *Osteichthyes*, 2) a second family in the class *Osteichthyes*, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.), 3) a third family in the phylum *Chordata* (may be in the class *Osteichthyes* or may be an amphibian, etc.), 4) a planktonic crustacean (e.g., cladoceran, copepod, etc.), 5) a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.), 6) an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.), 7) a family in a phylum other than *Arthropoda* or *Chordata* (e.g., Rotifera, Annelida, Mollusca, etc.) and 8) a family in any order of insect or any phylum not already represented. The chronic dataset represents four invertebrates, five fish species including the sensitive salmonid (*Oncorhynchus*) and an insect genus. EPA's final chronic dataset in the 2013 ammonia document includes toxicity data for 3 listed species and additional data on 5 sensitive mollusk (mussel or snail) species the majority of which are sensitive in comparison to other dataset species. This information is included in Table 2 below.

Table 2 Listed species and mollusk species in EPA's Chronic Dataset 2013					
Common name (scientific name)	**Listed Species**	**Mussel**	**Snail**	**Fish**	**Other**
Lahontan cutthroat trout, *Oncorhynchus clarkii henshawi**	X			X	
Rainbow trout, *Oncorhynchus mykiss**	X			X	
Sockeye salmon, *Oncorhynchus nerka*	X			X	
Pebblesnail, *Fluminicola* sp			X		
Long fingernailclam, *Musculium transversum*		X			
Rainbow mussel, *Villosa iris*		X			
Fatmucket, *Lampsilis siliquoidea*		X			
Wavy-rayed lamp mussel, *Lampsilis fasciola*		X			

A. Derivation of Chronic Criteria

The chronic criterion may be determined by one of two methods. If all eight minimum data requirements are met with acceptable chronic test data (as is the case with ammonia), then the chronic criterion is derived using the same method used for the acute criterion. For the 2013 criteria document revision, additional chronic data were available for chronic effects on sensitive invertebrate genera, including Unionid mussels. Genus Mean Chronic Values (GMCVs) were

derived from available Species Mean Chronic Values (SMCVs) and were then rank-ordered from least to most sensitive, and the Final Chronic Value (FCV) was calculated based on regression analysis using the four most sensitive GMCVs (*Musculium*, *Lepomis*, *Villosa* and *Lampsilis*). The FCV directly serves as the basis for the chronic criterion because the endpoints measured represent very sensitive endpoints such as 20 percent effect concentrations (EC20) or no observed effect concentrations (NOEC). The information in Table 3 below identifies the chronic values used in EPA's chronic criteria derivation.

Table 3. Ranked Genus Mean Chronic Values

Rank	GMCV (mg TAN/L)	Species	SMCV (mg TAN/L)
16	73.74	Stonefly, *Pteronarcella badia*	73.74
15	53.75	Water flea, *Ceriodaphnia acanthina*	64.10
		Water flea, *Ceriodaphnia dubia*	45.08
14	41.46	Water flea, *Daphnia magna*	41.46
13	29.17	Amphipod, *Hyalella azteca*	29.17
12	21.36	Channel catfish, *Ictalurus punctatus*	21.36
11	20.38	Northern pike, *Esox lucius*	20.38
10	16.53	Common carp, *Cyprinus carpio*	16.53
9	12.02	Lahontan cutthroat trout, *Oncorhynchus clarkii henshawi (LS)**	25.83
		Rainbow trout, *Oncorhynchus mykiss (LS)*	6.663
		Sockeye salmon, *Oncorhynchus nerka (LS)*	10.09
8	11.62	White sucker, *Catostomus commersonii*	11.62
7	11.07	Smallmouth bass, *Micropterus dolomieu*	11.07
6	9.187	Fathead minnow, *Pimephales promelas*	9.187
5	7.828	Pebblesnail, *Fluminicola* sp.	7.828
4	7.547	Long fingernailclam, *Musculium transversum*	7.547
3	6.920	Green sunfish, *Lepomis cyanellus*	14.63
		Bluegill, *Lepomis macrochirus*	3.273
2	3.501	Rainbow mussel, *Villosa iris*	3.501
1	2.126	Fatmucket, *Lampsilis siliquoidea*	3.211
		Wavy-rayed lamp mussel, *Lampsilis fasciola*	1.408

EPA's 2013 national recommended chronic criterion is the 30-day average concentration of total ammonia nitrogen (in mg TAN/L) not to exceed, more than once every three years on the average, the chronic criterion magnitude (CCC) calculated using the following equation:

$$30-\text{day Average} = 0.8876 \times \left(\frac{0.0278}{1+10^{7.688-pH}} + \frac{1.1994}{1+10^{pH-7.688}}\right) \times \left(2.126 \times 10^{0.028\times(20-MAX(T,7))}\right)$$

The highest 4-day average within the 30-day averaging period should not be more than 2.5 times the CCC more than once in three years on average. The temperature and pH of the criteria are determined using the associated pH and temperatures of the particular water bodies. For these calculations EPA's recommendation is that pH be no lower than 6.5 as is shown in Table 6. *Temperature and pH-Dependent Values of the CCC (Chronic Criterion Magnitude)* in EPA's criteria document page 49. Due to the uncertainty regarding effects for some species, and the increasing scatter of data from the common regression line at lower pH, using pH of below 6.5 may prove less accurate in predicting protective ammonia concentrations. This is consistent with EPA's recommended aquatic life criteria for pH of 6.5 - 9 as published in Quality Criteria for Water 1986 (EPA 440/5-86-001).

Page 31 of the criteria document addresses chronic toxicity to freshwater animals. Ammonia chronic toxicity data are available for 21 species of freshwater organisms: ten invertebrate species (mussels, clam, snail, cladocerans, daphnid, and insect) and 11 fish species, including three Federally-listed salmonid species. The chronic dataset includes data for three freshwater Unionid mussel species, one freshwater non-pulmonate snail species, and two fish species. It also includes an estimate of chronic effects for the Phylum Annelida, to meet the data requirement of a species in "a family in any order of insect or any phylum not already represented," as described below. Each chronic test was reviewed to determine acceptability consistent with ASTM standards. Acceptable 28-day survival tests using juvenile freshwater mussels and juvenile freshwater snails and growth tests using juvenile freshwater snails were evaluated for inclusion in the derivation of the chronic aquatic life criterion when the test concentration caused a reduction in survival or growth of 20 percent or more of these types of organisms at those life stages. Based on evaluation of the individual studies (Wang et al. 2007a; Wang et al. 2011), growth data for juvenile mussels was not used in the derivation of the chronic criterion due to uncertainty in method of measurement for the growth endpoint. Further discussion on this subject is addressed below and includes reference to the Effects Characterization discussion starting on page 52 of the criteria document.

All chronic data in individual studies were analyzed using regression analysis to demonstrate the presence of a concentration-effect relationship within the test. For those studies that demonstrated a concentration-effect relationship, EPA used regression analysis to estimate the EC20. (See page 32 of criteria document.) Page 11 of the criteria document identifies the chronic measures of effect including EC20, NOEC, LOEC, and maximum acceptable toxicant concentration (MATC) used to estimate a low level of effect observed in chronic datasets that are available for ammonia. EC20 is the concentration of a chemical that is estimated to result in a 20 percent effect in a chronic endpoint (e.g., growth, reproduction, and survival) of the test organisms. The NOEC is the highest test concentration at which none of the observed effects are statistically different from the control. The LOEC is the lowest test concentration at which observed effects are found to be statistically different from the control. The MATC is the calculated geometric mean of the NOEC and LOEC. For life-cycle (LC) and partial life-cycle (PLC) tests, the toxicological variables used in regression analyses were survival, embryo production, and embryo hatchability.

Regression analysis was used, both to demonstrate that a concentration-effect relationship was present, and to estimate chronic values at a consistent level of effect. Estimates of effect concentrations can generally be made with precision for a 50 percent reduction in response (EC50), but at low percent reductions such precision is decreased. A major reduction, such as 50 percent, is not consistent with the intent of establishing chronic criteria to protect the population from long-term effects. In contrast, a concentration that causes a low level of reduction in response, such as an EC5 or EC10, is rarely statistically significantly different from the control treatment. EPA selected EC20 values to be used to estimate a low level of effect that would be statistically different from control effects, yet not so severe as to be expected to cause chronic impacts at the population level (see U.S. EPA 1999). For calculation of the chronic criterion, the EC20 point estimate was selected for use over a NOEC or LOEC as the measure of effect to use, as NOECs and LOECs are highly dependent on test concentrations selected. Furthermore, point estimates provide additional information that is difficult to determine using NOEC and LOEC

effect measures, such as a measure of effect level across the range of tested concentrations, and the confidence intervals around those measures of effect. The typical assessment endpoints for aquatic life criteria are based on unacceptable effects on growth, reproduction, or survival of the assessed taxa. These measures of effect on toxicological endpoints of consequence to populations are provided by results from the acute and chronic toxicity tests with aquatic plants and animals. The toxicity values (i.e., measures of effect expressed as genus means) are used in the genus sensitivity distribution of the aquatic community to derive the aquatic life criteria. Endpoints used in this assessment are listed on page 13 of the criteria document.

Sixteen GMCVs are presented on page 39 of the criteria document. The four lowest values were used to calculate the FCV. EPA calculated the chronic criterion based on the fifth percentile of the GMCVs. The four most sensitive species are predominantly mollusks although Lepomis species (bluegill and green sunfish) comprise the third most sensitive GMCV. Figure 4 on page 33 of the criteria document shows the GMCVs ranked according to sensitivity and shows the 2013 chronic criteria magnitude as well as the 1999 criterion value for comparative purposes.

Page 34 of the criteria document contains the section *Summaries of the studies used in the chronic criteria determination* under the *Effects Analyses for Freshwater Organisms* section (page 21). The information in the criteria document includes a species-by-species discussion of freshwater chronic data used in deriving the chronic criterion magnitude for ammonia. SMCVs were used when data were available for only one species. When data for more than one species in a taxon were available, GMCVs were calculated from the SMCVs. All of the CVs (EC20 values), SMCVs, and GMCVs derived are tabulated and included (Appendix B of the criteria document). For some of the new chronic data, authors reported EC20 values on the basis of TAN. In such cases these reported CVs were normalized to pH 7 and 20°C (temperature normalization for invertebrates only), and utilized for the analysis.

For the Unionid mussel *Lampsilis fasciola* (Wavy-rayed lampmussel), EPA's criteria document states that Wang et al. (2007a) published results of the effect of ammonia on survival and growth of 2-month old juvenile freshwater Unionid mussels. The 28-day juvenile test was part of a series of studies designed to refine the methods for conducting acute and chronic toxicity tests with early life stages of freshwater mussels. Dissolved oxygen was maintained above 7.0 mg/L during the 28-day test. Survival in the control treatment and lowest ammonia test concentration (0.13 mg TAN/L) were 100 and 83 percent, respectively. Survival decreased to 30 percent at 1.02 mg TAN/L, and zero at 1.98 mg TAN/L. There was no concentration-response relationship for either length at 28 days or change in length after 28 days. The survival EC20 for this freshwater Unionid mussel species was calculated to be 1.408 mg TAN/L adjusted to pH 7 and 20°C (Appendix B) using EPA's TRAP (EPA's Statistical Program: Toxicity Relationship Analysis Program Version 1.21) model. Appendix G of the criteria document entitled *Results of the Regression Analyses of New Chronic Data for Unionid Mussels* addresses results from these Unionid mussel tests in detail and provides the figures generated using EPA's TRAP program that was used to calculate EC20s for the most recent chronic ammonia toxicity studies conducted with Unionid mussels. Appendix G explains that the decision not to use the growth data from these tests was based on the uncertainty in the test methods for assessing the growth endpoint and the need for additional research that is expected to improve feeding environments in toxicity tests in order to be able to conduct longer-term exposures (e.g., 90 d), and to compare growth

effect to potential reproductive effect in partial life-cycle exposure. (Wang et al. 2011). In addition to the uncertainties discussed above, the growth response during the Unionid mussel chronic tests showed a high degree of variability, and the test methods for assessing growth, based on substrate or water-only exposures are currently being evaluated. The figures for juvenile mussels including Fatmucket, Wavy-rayed lampmussel, and rainbow mussel on pages 150-153 depict the growth response of juvenile Fatmucket in the 28-day tests reported in Wang et al. (2011).

For the Unionid mussel *Lampsilis siliquoidea* (Fatmucket), EPA's criteria document (page 35) states that Wang et al. (2011) evaluated the influence of substrate on the sensitivity of two-month-old juvenile mussels to ammonia in 28-day water-only exposure and substrate exposure. The methods used were similar to those in an earlier study (Wang et al. 2007a) except for how the organisms were exposed. The survival response between the water-only and substrate treatments was similar with a reported LOEC of 0.53 mg TAN/L in the water-only and 0.88 mg TAN/L for the substrate treatment at the test pH 8.25 and temperature 20°C. Mean control survival in both the water-only and substrate treatments was 95% at the end of the 28-day exposures, which met acceptability requirements. Dry weight measurements of the mussels increased by 165% in the water-only exposure compared to 590% increase in the substrate exposure suggesting that the presence of the substrate increased food availability, as noted by the authors.

Using TRAP threshold sigmoid regression of the survival response results in an EC20 of 0.5957 mg TAN/L for the water-only and EC20 0.8988 mg TAN/L for the substrate exposure at the test pH and temperature or, adjusted to pH 7 and 20°C, chronic values equivalent to 2.128 and 3.211 mg TAN/L, respectively (Appendix B). Based on the apparent improved health of the test organisms in the substrate exposures, and the lack of any significant alteration of water chemistry in the exposure, the SMCV 3.211 mg TAN/L, based on survival of juvenile Fatmucket from the substrate exposures was used to calculate the CCC rather than the water-only exposure. The geometric mean of SMCVs for Fatmucket and Wavy-rayed lamp mussel of 3.211 and 1.408 mg TAN/L, respectively, results in a GMCV of 2.126 mg TAN/L for the genus *Lampsilis*. See Table 4 *Ranked Genus Mean Chronic Values*.

For the *Villosa iris* (rainbow mussel) (page 36), the effect of ammonia on survival and growth of this freshwater Unionid mussel species was also reported in the study by Wang et al. (2007a). Juvenile (2-month-old) rainbow mussels were tested via a 28-day test under similar conditions as described above. Survival was ≥ 98 percent up to the 0.81 mg TAN/L exposure, but fell to 15 percent at 1.67 mg TAN/L and zero percent at 3.45 and 7.56 mg TAN/L. EPA's TRAP was used to generate a chronic value for this species based on survival resulting in EC20 of 1.063 mg TAN/L at the test temperature of 20°C and pH of 8.2 (Appendix G *Results of the Regression Analyses of New Chronic Data for Unionid Mussels*), or 3.501 mg TAN/L adjusted to pH 7.0. Wang et al. (2007a) elected to exclude length estimates for concentrations above those where significant survival effects were measured (or in this case, 1.67 mg TAN/L). As a result, growth data are available for only three effect concentrations, even though there was 15% survival at the 1.67 mg TAN/L treatment level. Due to the uncertainties in the limited growth data for this test the growth data was not used in the calculation of the GMCV. The SMCV and GMCV for this

freshwater Unionid mussel species is 3.501 mg TAN/L when adjusted to pH 7 and 20°C (Appendix B Chronic Toxicity of Ammonia to Aquatic Animals).

Appendix I *Qualitative Weight-of-Evidence Test data* addresses the additional 28-day Toxicity Test data for Freshwater Mussels where Wang et al. (2007a) attempted to determine the effect of ammonia on growth of 2-month old juvenile rainbow mussel, Fatmucket, and Wavy-rayed lampmussel. The 28-day tests were conducted following the same methods (see ASTM 2006). The mean length of juvenile rainbow mussel and Fatmucket exposed to the lowest ammonia concentrations tested was reduced by 13 and 12 percent compared to mean length of control animals, respectively, but increased by 7 percent for the Wavy-rayed lampmussel. There was no consistent effect of ammonia, however, on either length at 28 days or change in length after 28 days for Fatmucket and Wavy-rayed lampmussel at test concentrations where survival was unaffected; only the 28-day test with rainbow mussel exhibited such a concentration response for length and change in length. For the reasons explained above contained in the *Summaries of studies used in chronic criterion determination* section of the criteria document, the growth endpoint was not used from these tests to derive the chronic criterion, and instead, the reported IC25 (inhibition concentration) estimated for these tests are included in *Other Chronic Ammonia Toxicity Data* (Appendix C of the criteria document). The reported growth IC25 for juvenile rainbow mussel, Fatmucket, and Wavy-rayed lampmussel from their respective 28-day tests were 0.73, 0.44, and 0.57 mg TAN/L at test pH of 8.2 and temperature 20°C. These values, when adjusted to pH 7 and 20°C, are 2.406, 1.450 and 1.878 mg TAN/L, respectively and are shown in Appendix C of the criteria document.

In Table 4 of the criteria document, genus mean chronic values are presented for the species utilized in EPA's chronic criteria derivation. The lowest GMCV in this criteria update is for an invertebrate species (*Lampsilis sp.*). The lowest genus mean value is 2.126 mg TAN/L for the two species' chronic values of *Lampsilis siliquoidea* (3.211) and *Lampsilis fasciola* (1.408) average. (See Table 4 page 39 of the criteria document). The 2013 final chronic criteria or CCC (rounded to 4 significant figures) is 1.887 mg TAN/L at pH 7 and 20°C. This value is 11.2 percent lower than the lowest GMCV of 2.126. However, this value is above the species mean chronic value for *Lampsilis fasciola* of 1.408 in EPA's chronic dataset. Therefore, if a species in this genus is present at a site and there is information to indicate that the species may be sensitive at concentrations below the final chronic criteria, then it may be appropriate to consider the option to derive a site-specific criterion magnitude as described on page 47 of the criteria document.

Page 51 of the criteria document addresses protection of downstream waters. EPA regulations at 40 CFR 131.10(b) provide that "[i]n designating uses of a water body and the appropriate criteria for those uses, the state shall take into consideration the water quality standards of downstream waters and ensure that its water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters." In cases where downstream waters are characterized by higher pH and/or temperature, or contain species that are more sensitive to ammonia, then a more stringent ammonia criteria than that required to protect in-stream uses (at the upstream point) may be necessary in order to ensure that water quality standards provide for the attainment and maintenance of the water quality standards of downstream waters.

Page 52 of the criteria document addresses considerations for site-specific criteria derivation. The 1999 chronic criterion (CCC) magnitude was based on the effects on fish early life stages, whereas based on the new data, the 2013 CCC magnitude is based on the effects on sensitive invertebrate genera, including Unionid mussels. When mussels are present, the 2013 CCC magnitude is protective of fish early life stages regardless of temperature. Appendix N of the criteria document contains additional information on developing site-specific criteria for ammonia for sites where certain mussel species, early life stages of fish or other site specific conditions may warrant the development of site specific criteria.

The *Effects Characterization* section on page 52 of the criteria document characterizes the potential effects of ammonia on aquatic life considering available test data and describes additional lines of evidence not used directly in the criteria calculations, but which support the 2013 aquatic life criteria values. This section provides a summary of the uncertainties and assumptions, as well as providing explanations for decisions regarding data acceptability and usage in the effects assessment. In addition, this section describes the substantive differences between the 1999 ammonia ambient water quality criteria (AWQC) and the 2013 update resulting from the incorporation of the latest scientific knowledge. The tables in Appendix A and B of the criteria document include the new acute and chronic ammonia toxicity data for freshwater mussels in the Family Unionidae and reflect the latest science informing the determinations regarding acceptable test conditions and associated data for glochidia and juvenile mussels.

Page 56 of the criteria document addresses freshwater chronic toxicity data for *Lampsilis siliquoidea* (Fatmucket) including the use of 28-day juvenile Unionid mussel data. EPA decided that growth data from 28-day tests with juvenile Unionid mussels presented in the Wang et al. studies from 2007 and 2011 would not be used in calculating the 2013 chronic criterion. The decision not to use the growth data was based on the uncertainty in the test methods for assessing the growth endpoint and the need, as stated by the authors, for additional research "to optimize feeding conditions, to conduct longer-term exposures (e.g., 90 d), and to compare growth effect to potential reproductive effect in partial life-cycle exposure" (Wang et al. 2011). The growth endpoint showed a high degree of variability, and the test methods for assessing growth, based on substrate or water-only exposures, are currently being evaluated. For these reasons, the survival data for 28-day juvenile mussels were used in the calculation of the CCC and not the growth data. Appendix G *Results of the Regression Analyses of New Chronic Data for Unionid Mussels* provides the TRAP EC20s for survival for rainbow mussel and both *Lampsilis* species, and a comparison to the growth of Fatmucket mussel from 28-day tests reported by Wang et al. (2007a, 2011), which shows the additional uncertainty in the concentration-response relationship based on growth.

Page 62 of the criteria document addresses the protection of endangered species. The dataset for ammonia is particularly extensive and includes data representing species that are Federally-listed as threatened or endangered by the U.S. Fish and Wildlife Service and/or NOAA National Marine Fisheries Service. Summaries of significant chronic data are provided below describing the data for the listed species and demonstrating that the 2013 ammonia criteria update is considered protective of these species, based on best available scientific data.

As a result of differences in the data used in the chronic criteria derivations, the 2013 CCC is 1.9 at pH 7 and temperature of 20°C recommended for waters with mussels present. Based on a new study by Wang et al. (2011) described in the Effects Analysis section under Summaries of Studies Used in Chronic Criterion Determination (page 34 of the criteria document) the lowest GMCV for the mussel genus *Lampsilis* increased from 1.154 mg TAN/L in the 2009 draft AWQC to 2.216 mg TAN/L in the 2013 AWQC. As a result, compared to the four lowest GMCVs in the 2009 draft CCC, the four lowest GMCVs in the 2013 CCC have a smaller range of variation in values (2.216 to 7.547 mg TAN/L) which decreases the uncertainty of the 5th percentile GMCV estimation. Also in the 2009 draft CCC, there were only 13 GMCVs in the dataset used to derive the CCC while in the 2013 CCC, there are 16 GMCVs used to derive the CCC. This is based upon the addition of the GMCVs for *Hyalella azteca*, the insect *Pteronarcella badia*, and salmonids (*Oncorhynchus spp*.). The new GMCVs affect the chronic species sensitivity distribution and the cumulative probability (P) decreases as a function of the increased number of GMCVs and results in an increase in the final chronic value/chronic criterion.

Page 68 of the criteria document shows the comparison of statistical approaches that were used to develop the chronic criterion. In the 2013 ammonia criteria update, the CCC is based on a 20 percent reduction in survival, growth, or reproduction, which is a risk management decision made by EPA in 1999 and also retained for the 2013 document. When an EC20 was not provided in a study, the EPA's TRAP program was used to estimate the EC20 for *Lampsilis siliquoidea* of 3.211 and *Lampsilis fasciola* of 1.408. These two chronic values resulted in the GMCV of 2.126 for *Lampsilis*. The final chronic criteria of 1.9 mg TAN/L was calculated from the genus mean chronic values shown in Table 3 above using the standard fifth percentile procedure from *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (PB85-227049). An alternative chronic measure of effect that is commonly used is the MATC expressed mathematically as the geometric mean of the NOEC and LOEC. In the case of the current ammonia dataset, using MATCs to derive the chronic criteria would result in an FCV of 1.972 and CCC of 2.0 mg TAN/L. This comparison demonstrates that, for the current ammonia chronic dataset, the use of TRAP to estimate EC20 values does not result in a significant difference from the MATC, another statistical approach frequently used to develop chronic effects assessments and criteria.

Description of the Geographic Area That May Be Affected by the Action

The action area for this BE includes all fresh waters within the State of Florida to which these criteria apply.

Summary of Species Present in Florida

The EPA received a list of aquatic and aquatic dependent species from the Panama City, Florida Ecological Services FWS Conservation Office on April 21, 2016 which was verified to be accurate as of August 2016. M Table 4 below provides the list of threatened and endangered species in Florida under the jurisdiction of the FWS that are being considered for this action. The name and status of each is presented including critical habitat status, type of habitat and the

EPA's final ESA section 7 determinations of no effect, may affect, not likely to adversely affect (NLAA) with either discountable or insignificant effects.

Table 4. Florida Species Considered							
Status	**Animal Species/Listing Name**	**Group**	**Designated Critical Habitat**	**Aquatic**	**Aquatic Dependent**	**Terrestrial**	**ESA Determination**
T	Purple bankclimber (*Elliptoideus sloatianus*)	Mussel	Yes	X			May affect, NLAA discountable
E	Choctaw bean (*Villosa choctawensis*)		Yes	X			May affect, NLAA - discountable
E	Round ebonyshell (*Fusconaia rotulata*)		Yes	X			May affect, NLAA - discountable
E	Southern Kidneyshell (*Ptychobranchus jonesi*)		Yes		X		May affect, NLAA - discountable
E	Gulf Moccasinshell (*Medionidus penicillatus*)		Yes	X			May affect, NLAA - discountable
E	Ochlockonee moccasinshell (*Medionidus simpsonianus*)		Yes	X			May affect, NLAA - discountable
T	Fuzzy pigtoe (*Pleurobema strodeanum*)		Yes	X			May affect, NLAA - discountable
T	Narrow pigtoe (*Fusconaia escambia*)		Yes	X			May affect, NLAA - discountable
E	Oval pigtoe (*Pleurobema pyriforme*)		Yes	X			May affect, NLAA - discountable
T	Tapered pigtoe (*Fusconaia burkei*)		Yes	X			May affect, NLAA - discountable
T	Southern sandshell (*Hamiota australis*)		Yes	X			May affect, NLAA - discountable
E	Shinyrayed pocketbook (*Lampsilis subangulata*)		Yes	X			May affect, NLAA – insignificant
T	Chipola slabshell (*Elliptio chipolaensis*)		Yes	X			May affect, NLAA - discountable
E	Fat threeridge (*Amblema neislerii*)		Yes	X			May affect, NLAA - discountable
T	Suwanee moccasinshell (*Medionidus walkeri*)		No	X			May affect, NLAA - discountable
E	West Indian Manatee (*Trichechus manatus*)	Mammal	Yes	X			May affect, NLAA - discountable
E	Florida bonneted bat (*Eumops floridanus*)		No		X		May affect, NLAA - discountable
E	Indiana bat (*Myotis sodalis*)		No		X		May affect, NLAA - discountable
T	Frosted flatwoods salamander (*Ambystoma cingulatum*)	Amphibian	Yes			X	No effect
E	Reticulated flatwoods salamander (*Ambystoma bishopi*)		Yes			X	No effect
C	Striped newt (*Notophthalmus perstriatus*)		No			X	No effect
T	Atlantic salt marsh snake (*Nerodia clarkii taeniata*)	Reptile	No		X		May affect, NLAA - discountable
T	American Crocodile (*Crocodylus acutus*)		Yes	X			May affect, NLAA - discountable
T	Audubon's crested caracara (*Polyborus plancus audubonii*)	Bird	No			X	No effect
E	Everglade snail kite (*Rostrhamus sociabilis plumbeus*)		Yes		X		May affect, NLAA - discountable

Table 4. Florida Species Considered							
Status	**Animal Species/Listing Name**	**Group**	**Designated Critical Habitat**	**Aquatic**	**Aquatic Dependent**	**Terrestrial**	**ESA Determination**
T	Red knot (*Calidris canutus rufa*)	Bird	No		X		May affect, NLAA - discountable
T	Piping plover (*Charadrius melodus*)		Yes		X		May affect, NLAA - discountable
T	Florida scrub-jay (*Aphelocoma coerulescens*)		No			X	No effect
E	Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*)		Yes			X	No effect
E	Florida grasshopper sparrow (*Ammodramus savannarum floridanus*)		No			X	No effect
T	Wood Stork (*Mycteria americana*)		No		X		May affect, NLAA - discountable
T	Roseate tern (*Sterna dougallii dougallii*)		No		X		May affect, NLAA - discountable
E	Kirtland's warbler *(Setophaga kirtlandii)*		No			X	No effect
E	Bachman's Warbler (*Vermivora bachmanii*)		No			X	No effect
E	Red-cockaded woodpecker (*Picoides borealis*)		No			X	No effect
E	Ivory-billed woodpecker (*Compephilus principalis*)		No		X		May affect, NLAA - discountable
E	Whooping crane (*Grus americana*)		No		X		May affect, NLAA - discountable
T	Squirrel chimney cave shrimp (*Palaemonetes cummingi*)	Crustacean	No	X			May affect, NLAA - discountable
T	Okaloosa darter (*Etheostoma okaloosae*)	Fish	No	X			May affect, NLAA - discountable
T	Gulf Sturgeon (*Acipenser oxyrinchus desotoi)*		Yes	X			May affect, NLAA - discountable

Species of Interest for the ESA Consultation

According to the Endangered Species Consultation Handbook, in order for the EPA to determine that a proposed action is a may affect, but NLAA action for threatened and endangered species or designated critical habitat, all of the effects of that action must be expected to be discountable, insignificant, or completely beneficial. Also, according to the Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria Methods Manual, there are three different types of species: "aquatic" which have at least one of their life stages spent as a water-breathing organism or plants that are submerged or emergent; "aquatic dependent" which are not water-breathing organisms, but a meaningful amount of their diet includes aquatic organisms; and "terrestrial" which have only limited amount of exposure to "waters of the U.S." Therefore, the majority of listed species listed above will not be the primary focus of this consultation as the EPA actions will have either no effect or a may affect, NLAA-discountable effect on these species.

No Effect Determination

No effect determinations: For species in Table 5 that are identified as not aquatic dependent or primarily terrestrial species including the Audubon's crested caracara, Frosted flatwoods salamander, Reticulated flatwoods salamander, Florida scrub-jay, Cape Sable seaside sparrow, Florida grasshopper sparrow, Kirtland's warbler, Bachman's Warbler, Red-cockaded woodpecker and Striped newt, the EPA expects that, based on the fact that these species are primarily terrestrial and/or are not aquatic dependent, the freshwater ammonia criteria will have no effect on these species.

May affect, NLAA-discountable Effects Determination

May affect, NLAA-discountable effects determinations:
The Florida bonneted bat, Indiana bat, Everglade snail kite, Red knot, Piping plover, Atlantic salt marsh snake, Wood Stork, Roseate tern, Ivory-billed woodpecker, and Whooping crane are categorized as aquatic dependent species. The main reasoning for this determination is that the level of interaction for these species in freshwater aquatic environments is not expected to adversely affect the species. Exposure to the proposed chronic criteria concentrations is considered extremely unlikely due to lack of prolonged exposure from water or diet. Due to the very limited dietary exposure through consumption of prey species and water, and the non-bioaccumulative nature of the parameter being considered.. The proposed FDEP chronic criteria concentrations which include duration and frequency will protect species from chronic effects. Also, it is expected that there is likely to be a lack of exposure through prey species. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on these species will be may affect, NLAA-discountable.

The American Crocodile lives primarily in coastal areas of parts of Mexico, Central and South America, the Caribbean, and at the northern end of South Florida. They live in brackish or saltwater areas, and can be found in ponds, creeks, coves and mangroves. They are occasionally encountered inland in freshwater canals and other areas of the southeastern Florida coast due to the widespread manmade canal system. The primary threat to the species is habitat alteration and human disturbance. The proposed FDEP chronic criteria concentrations which include duration and frequency will protect the species from chronic effects. Also, it is expected that there is likely to be a lack of exposure through the crocodile's prey species. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on this species will be may affect, NLAA-discountable.

The West Indian Manatee habitat includes fresh, brackish and marine waters in Florida, Greater Antilles (large islands of the Caribbean Sea), Central and South America. They swim in large slow-moving rivers, river mouths, and shallow coastal areas where there are coves and bays. During the winter season, manatees flock around warm springs and power plants that discharge warm water. During the summer season some manatees can travel as far north as Virginia and Maryland when waters are warm. The greatest threats to manatee survival in Florida are collisions with boats and other watercraft and habitat loss. Residential development has reduced the area and number of natural springs that provide warm water habitat. Other threats include flood gates and canal locks that can crush or drown manatees and toxins associated with red tide blooms. The proposed FDEP chronic criteria concentrations which include duration and frequency will protect the species from chronic effects. Also, it is expected that there is likely to

be a lack of exposure through diet of contaminated food items. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on this species will be may affect, NLAA-discountable.

The Squirrel chimney cave shrimp exists in one sinkhole named Squirrel Chimney near Gainesville, Florida and has not been documented in any other location. It is possible that this species is extinct. Because of its limited habitat to one sinkhole, major threats include changes in current water quality in the sinkhole, population growth, urban development in the area surrounding the sinkhole and nearby cave/sink systems in the area and possibly predation. The proposed FDEP chronic criteria concentrations which include duration and frequency will protect the species from chronic effects. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on this species will be may affect, NLAA-discountable.

The Okaloosa darter is a small darter, with adults reaching a size of about two inches. Its habitat is known to be around the edges of clear flowing streams that have ground water inflow. More than 98.7% of the habitat of the darter is located on Eglin Air Force Base in northwest Florida. (Florida Fish and Wildlife Conservation Commission) The darter habitat consists of several stream systems totaling over 200 stream miles. This degraded habitat has been managed and improved by Eglin Air Force Base personnel and the U.S. Fish and Wildlife Service for over 15 years. The additional habitat of the darter not on the air force base is in the areas of Niceville and Valparaiso where habitat preservation and improvement is challenging. Threats to the darters include stream degradation in the form of erosion and siltation and competition of the introduced brown darter. The proposed FDEP chronic criteria concentrations which include duration and frequency will protect the species from chronic effects. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on this species will be may affect, NLAA-discountable.

The Gulf Sturgeon is an anadromous fish that occupies both saltwater and freshwater during portions of its life. The Gulf Sturgeon is currently under the jurisdiction of the FWS. The sturgeon swims into freshwater rivers to spawn starting in February through April and then returns to saltwater in September and continuing through November. The sturgeon returns each year to the same stream where they were hatched. For the winter months the Gulf Sturgeon live in the Gulf of Mexico where they consume most of their diet. They do not eat very much when they are migrating up and down the freshwater rivers where they spawn. According to the FWS management plan, threats to the sturgeon include dams and other water control structures, river and coastal dredging, groundwater withdrawal, lowering of river flows and poor water quality. According to presentations at EPA's invited expert meeting (see Table 5 below) in September 2015, the sturgeon species do not appear to be the most sensitive species in regards to ammonia. The most sensitive species are freshwater mussels (*Unionidae* Family) rather than either freshwater fish or amphibians.

Table 5. Presentations from EPA's Invited Expert Meeting on Revising U.S. EPA's Guidelines for Deriving Aquatic Life Criteria, September 14-16, 2015
Derivation and application of taxon-specific criteria: Additional resolution in WQC recommendations (Tom Augspurger, USFWS, Raleigh, NC; Chris Mebane, USGS, Boise, ID; Ning Wang, John Besser, Chris Ingersoll, USGS, Columbia, MO; and Sandy Raimondo, USEPA, Gulf Breeze, FL)

Minimum data requirement for developing water quality criteria: Use of toxicity data from under-represented organisms (Ning Wang, Chris Ingersoll, John Besser, Chris Ivey, James Kunz, Bill Brumbaugh (USGS, Columbia, MO); Tom Augspurger (USFWS, Raleigh, NC); Ed Hammer, Candice Bauer (USEPA, Chicago, IL); Sandy Raimondo (USEPA, Gulf Breeze, FL); Chris Mebane (USGS, Boise, ID); and David Soucek (Illinois Natural History Survey, Champaign, IL)

The proposed FDEP chronic criteria concentrations which include duration and frequency will protect the species from chronic effects. Based upon the information presented, the EPA has found that any effects that the freshwater ammonia criteria may have on this species will be may affect, NLAA-discountable.

Based on the above information, the EPA will focus the rest of this BE on the consideration of possible effects to the fifteen mussel species and designated critical habitat. The listing status and identified critical habitat designations are shown in Table 6 below. These species are defined as aquatic and have increased exposure to freshwater. The analyses and conclusions are discussed in detail below.

Table 6. Listed Mussel species in Florida

Status	Animal Species/Listing Name	Critical Habitat Designated
T	Bankclimber, purple (*Elliptoideus sloatianus*)	YES
E	Bean, Choctaw (*Villosa choctawensis*)	YES
E	Ebonyshell, round (*Fusconaia rotulata*)	YES
E	Kidneyshell, southern (*Ptychobranchus jonesi*)	YES
E	Moccasinshell, Gulf (*Medionidus penicillatus*)	YES
E	Moccasinshell, Ochlockonee (*Medionidus simpsonianus*)	YES
T	Pigtoe, fuzzy (*Pleurobema strodeanum*)	YES
T	Pigtoe, narrow (*Fusconaia escambia*)	YES
E	Pigtoe, oval (*Pleurobema pyriforme*)	YES
T	Pigtoe, tapered (*Fusconaia burkei*)	YES
E	Pocketbook, shinyrayed (*Lampsilis subangulata*)	YES
T	Sandshell, Southern (*Hamiota australis*)	YES
T	Slabshell, Chipola (*Elliptio chipolaensis*)	YES
E	Threeridge, fat (*Amblema neislerii*)	YES
T	Suwanee moccasinshell (*Medionidus walkeri*)	NO

Life History and Designated Critical Habitat Summaries

Please refer to Attachment A *Life History and Critical Habitat Designations for Freshwater Mussels* for complete species information on the fifteen freshwater mussel species identified above.

Effects of the Action on Species of Interest for ESA Consultation

This section of the BE sets out the basis for the EPA's conclusion that the Department's revised ammonia water quality criterion is not likely to jeopardize the continued existence of any endangered or threatened species including the listed Unionid mussels or result in the destruction

or adverse modification of designated critical habitat. The EPA has determined that this action will have "may affect, NLAA-insignificant" impacts on the mussel species listed above. These conclusions presume any effects are small relative to the size of the impact and should never reach the scale where "take" to the listed species occurs.

Representation of Endangered and Threatened Mussel Species in EPA's Criteria Document

Table 7 below identifies the listed mussels in Florida and how they are represented in EPA's 304(a) recommended ammonia criteria document.

Table 7 Florida's listed mussels as represented in EPA's database

Genus	Listed Species in Florida	Family represented in acute dataset	Genus represented in acute dataset	Family represented in chronic dataset	Genus represented in chronic dataset
Villosa	Choctaw bean (*Villosa choctawensis*)	X	X (1)	X	X (1)
Fusconaia	Round ebonyshell (*Fusconaia rotulata*)	X	X (1)	X	
	Narrow pigtoe (*Fusconaia escambia*)	X	X	X	
	Tapered pigtoe (*Fusconaia burkei*)	X	X	X	
Medionidus	Gulf moccasinshell (*Medionidus penicillatus*)	X		X	
	Ochlockonee moccasinshell (*Medionidus simpsonianus*)	X		X	
	Suwanee moccasinshell (*Medionidus walkeri*)	X		X	
Pleurobema	Fuzzy pigtoe (*Pleurobema strodeanum*)	X		X	
	Oval pigtoe (*Pleurobema pyriforme*)	X		X	
Lampsilis	Shinyrayed pocketbook (*Lampsilis subangulata*)	X	X (6)	X	X (2)
Hamiota	Southern sandshell (*Hamiota australis*)	X		X	
Elliptio	Chipola slabshell (*Elliptio chipolaensis*)	X		X	
Amblema	Fat threeridge (*Amblema neislerii*)	X		X	
Elliptoideus	Purple bankclimber (*Elliptoideus sloatianus*)	X		X	
Ptychobranchus	Southern kidneyshell (*Ptychobranchus jonesi*)	X		X	

Note: the values in parenthesis indicate the number of species in the genus in the national dataset

Research Strategy and Consideration of New Data

As stated above informal discussions began in August 2015 with a regional workgroup comprised of Fish and Wildlife Service and National Marine Fisheries Service (Services) and EPA Region 4 staff to discuss regional consultations on CWA 303(c) actions for EPA's 304(a) recommended criteria, and how to search for and assess new species data for consultation efforts. Tom Augspurger of the Raleigh Ecological Services Field Office provided a draft research strategy describing an approach by which EPA and the FWS can work together to determine the extent of the available information for use in this consultation. Following that approach for this consultation, EPA reviewed existing BEs and biological opinions that have been completed for ammonia consultations. This information was primarily focused on cold water species' protection for salmonids. This information is not relevant for Florida's ammonia criteria where cold water species are less sensitive than the freshwater mussel species in the Unionid family. The EPA completed a search for data that became available after October 2012 including information provided by Tom Augspurger on March 9, 2016. The information from various studies and papers provided by Tom Augspurger is outlined in Attachment B. In addition, the EPA completed a search for new ammonia data utilizing EPA's ECOTOXicology database (ECOTOX). ECOTOX is a source for locating single chemical toxicity data for aquatic life,

terrestrial plants and wildlife. ECOTOX was created and is maintained by the U.S. EPA, Office of Research and Development and the National Health and Environmental Effects Research Laboratory's Mid-Continent Ecology Division. ECOTOX integrates three previously independent databases - AQUIRE, PHYTOTOX, and TERRETOX - into a unique system which includes toxicity data derived predominately from the peer-reviewed literature, for aquatic life, terrestrial plants, and terrestrial wildlife. The ECOTOX search provided data on single freshwater fish which was considerably less sensitive than any freshwater mussel species.

Summary of EPA Analysis and Conclusions

Acute analysis

The Department's ammonia criteria contained at Chapter 62-302.530 (3), F.A.C. is based upon the 2013 EPA recommended 304(a) criteria for chronic criteria. The Department applies the chronic value as an acute value for WQS purposes. The approach to include chronic criteria only is considered more conservative than utilizing both acute and chronic criteria. Therefore, this consultation focuses on chronic criteria. Please refer to the chronic analysis section below.

Chronic analysis

The Department's chronic criteria as outlined in detail on pages 5-6 above are consistent with EPA's 2013 30-day averaging period chronic criteria recommendation. The Department's 4-day chronic criteria is more conservative than EPA's 4-day average recommendation by including this value as a single value not to exceed, rather than a 4-day average value not to exceed. These criteria are consistent with the EPA's maximum ambient concentrations of ammonia that are intended to protect freshwater aquatic organisms from unacceptable chronic effects.

Endangered Species Act Section 7 Effects Determination

May affect, NLAA-discountable Effects Determination

Based upon information outlined above including the toxicity data used in the development of the chronic criteria, the criteria development procedures for deriving the final chronic value and the information included in Attachment B, the EPA has determined that the Department's water quality criteria for total ammonia at Chapter 62-302.530 (3), F.A.C. are not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of their critical habitat. The EPA believes the Department's chronic criteria concentrations for ammonia which include and frequency and duration will protect the fourteen mussels species identified in the list below from chronic effects. Therefore, the EPA's determination is that this action will have "may effect, NLAA-discountable" effects on these freshwater mussel species of concern identified in the list below. As discussed in the Endangered Species Act Section 7 Effects Determination Guidance March 2014, a "may effect, NLAA-discountable" effect determination is appropriate if the action is determined to be infrequent or intermittent. Discountable effects are those that are extremely unlikely to occur and, based on best judgment, cannot be meaningfully measured, detected, or evaluated.

Choctaw bean (Villosa choctawensis)
Round ebonyshell (Fusconaia rotulata)
Narrow pigtoe (Fusconaia escambia)
Tapered pigtoe (Fusconaia burkei)
Gulf moccasinshell (Medionidus penicillatus)
Ochlockonee moccasinshell (Medionidus simpsonianus)
Suwanee moccasinshell (Medionidus walkeri)
Fuzzy pigtoe (Pleurobema strodeanum)
Oval pigtoe (Pleurobema pyriforme)
Southern sandshell (Hamiota australis)
Chipola slabshell (Elliptio chipolaensis)
Fat threeridge (Amblema neislerii)
Purple bankclimber (Elliptoideus sloatianus)
Southern kidneyshell (Ptychobranchus jonesi)

May affect, NLAA-insignificant Effects Determination

As discussed in the Endangered Species Act Section 7 Effects Determination Guidance March 2014, an insignificant effects determination is appropriate if the size or severity of the action or associated effects of the action are determined as undetectable, not measurable, or so minor that they cannot be meaningfully evaluated. Insignificant is the appropriate effect conclusion when conceivable effects may happen, but will not rise to the level of creating an adverse chronic effect. In other words, the ESA-listed species may be affected, but not harmed or harassed and the effect would not be expected to exist at such a level where "take" would occur.

Based upon information outlined above including the toxicity data used in the development of the chronic criteria, the criteria development procedures for deriving the final chronic value and the information included in Attachment B, the EPA has determined that the Department's water quality criteria for total ammonia at Chapter 62-302.530 (3), F.A.C. are not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of their critical habitat. The EPA believes the Department's chronic criteria for ammonia at the limited duration and limited frequency of exposure from the pollutant of concern will protect against chronic effects to the mussel species identified below. Therefore, the EPA's determination is that this action will have "may effect, NLAA-insignificant" effects on the one freshwater mussel species of concern identified below.

Shinyrayed pocketbook (Lampsilis subangulata)

EPA's chronic criteria document explains how EPA's final chronic criterion may not be protective of the *Lampsilis* genus including the listed *Lampsilis subangulata*. In Table 4 of the criteria document, genus mean chronic values are presented for the species utilized in EPA's chronic criteria derivation. The lowest GMCV in this criteria update is for an invertebrate species of the *Lampsilis* genus. The lowest genus mean value is 2.126 mg TAN/L for the average of the two species' chronic values of *Lampsilis siliquoidea* (3.211) and *Lampsilis fasciola* (1.408). (See Table 4 page 39 of the criteria document.) The 2013 final chronic criteria or CCC (rounded to 4 significant figures) is 1.887 mg TAN/L at pH 7 and 20°C. This value is 11.2 percent lower than

the lowest GMCV of 2.126. However, this value is above the species mean chronic value for *Lampsilis fasciola* of 1.408 in EPA's chronic dataset. As described in this document on page 13, if there is information to indicate that a species in this genus is present at a particular site and there is information to indicate that the species may be sensitive at concentrations below the final chronic criteria, then it may be appropriate to consider the option to develop a site specific criteria for this species. In this case if there is information for the Shinyrayed pocketbook that indicates that it may be sensitive to chronic effects at concentrations below the final chronic criterion, a specific criterion to protect the sites where this species occurs may be necessary.

Presumed Beneficial Effects

Because the revised ammonia criteria is more stringent than the current un-ionized ammonia criteria it may be assumed that there are beneficial effects to all Florida freshwater species including listed mussels.

Attachment A

Life History and Critical Habitat Designations for Freshwater Mussels

Life History information for the Gulf moccasinshell, Ochlockonee moccasinshell, Oval pigtoe, Shinyrayed pocketbook, Chipola slabshell, Fat threeridge and Purple bankclimber was summarized from the U.S. Fish and Wildlife Service Fact Sheet for Endangered and Threatened Mussels in the Apalachicola-Chattahoochee-Flint Basin (Appendix B), the online Florida Wildlife Conservation Guide (Appendix C), and the NatureServe Explorer website (explorer.natureserve.org). The information for the Round Ebonyshell, Southern Kidneyshell, and Choctaw Bean, Tapered Pigtoe, Narrow Pigtoe, Southern Sandshell, and Fuzzy Pigtoe was taken from the U.S. Fish and Wildlife Service federal register final rule (Docket No. FWS-R4-ES-2011-0050: 4500030113) dated 10/10/2012 which became effective 11/9/2012 (Appendix D). The information for the Suwannee moccasinshell was taken from the U.S. Fish and Wildlife Service federal register proposed rule (Document Citation 80 FR 60335) dated 10/06/2015.

The designated critical habitat information for each freshwater mussel can be found in the following federal register notices: 1) *Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for Five Endangered and Two Threatened Mussels in Four Northeast Gulf of Mexico Drainages; Final Rule Thursday 11/15/2007 50 CFR Part 17*, and 2) *Endangered and Threatened Wildlife and Plants; Determination of Endangered Species Status for the Alabama Pearlshell, Round Ebonyshell, Southern Kidneyshell, and Choctaw Bean, and Threatened Species Status for the Tapered Pigtoe, Narrow Pigtoe, Southern Sandshell, Fuzzy Pigtoe, and Designation of Critical Habitat; Final Rule Wednesday 10/10/2012 50 CFR Part 17*. These federal register notices are included as Attachments C and D.

Within each federal register notice the delineation of critical habitat for each mussel species is identified in the Unit maps including individual stream name(s), state(s) and estimated linear map scale in miles or kilometers. An estimatation of stream length in miles and kilometers is identified in the federal register notices. Below are the 2 lists from the federal register notices identifying the Unit maps, stream names, state(s) and freshwater mussel common names.

November 15, 2007 50 CFR Part 17 Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for Five Endangered and Two Threatened Mussels in Four Northeast Gulf of Mexico Drainages; Final Rule

Unit 1: Econfina Creek, Florida
- Gulf Moccasinshell
- Oval Pigtoe

Unit 2: Chipola River, AL and FL
- Fat Threeridge
- Shinyrayed Pocketbook
- Gulf Moccasinshell
- Oval Pigtoe
- Chipola Slabshell

Unit 3: AL

Unit 4: GA
Unit 5: northern and southern, GA
Unit 6: eastern and western, GA
Unit 7: western and eastern, GA
Unit 8: Apalachicola River, FL
- Fat Threeridge
- Purple Bankclimber

Unit 9: Upper Ochlockonee River, GA and FL
- Shinyrayed Pocketbook
- Ochlockonee Moccasinshell
- Oval Pigtoe
- Purple Bankclimber

Unit 10: Lower Ochlockonee River, FL
- Purple Bankclimber

Unit 11: Santa Fe River and New River, FL
- Oval Pigtoe

October 10, 2012 50 CFR Part 17 Endangered and Threatened Wildlife and Plants; Determination of Endangered Species Status for the Alabama Pearlshell, Round Ebonyshell, Southern Kidneyshell, and Choctaw Bean, and Threatened Species Status for the Tapered Pigtoe, Narrow Pigtoe, Southern Sandshell, and Fuzzy Pigtoe, and Designation of Critical Habitat; Final Rule

Unit AP1 and AP2: AL
Unit GCM1 (South): Lower Escambia River Drainage, AL and FL
- Round Ebonyshell
- Southern Sandshell
- Southern Kidneyshell
- Choctaw Bean
- Narrow Pigtoe
- Fuzzy Pigtoe

Unit GCM1 (North): Lower Escambia River Drainage, AL and FL
- Round Ebonyshell
- Southern Sandshell
- Southern Kidneyshell
- Choctaw Bean
- Narrow Pigtoe
- Fuzzy Pigtoe

Unit GCM2: AL
Unit GCM3: AL
Unit GCM4: AL
Unit GCM5: Yellow River Drainage, AL and FL
- Southern Sandshell
- Choctaw Bean
- Narrow Pigtoe

- Fuzzy Pigtoe

Unit GCM6 (South): Choctawhatchee River and Lower Pea River Drainage, AL and FL
- Southern Sandshell
- Southern Kidneyshell
- Choctaw Bean
- Tapered Pigtoe
- Fuzzy Pigtoe

Unit GCM6 (Central): Choctawhatchee River and Lower Pea River Drainage, AL and FL
- Southern Sandshell
- Southern Kidneyshell
- Choctaw Bean
- Tapered Pigtoe
- Fuzzy Pigtoe

Unit GCM6 (North): Choctawhatchee River and Lower Pea River Drainage, AL and FL
- Southern Sandshell
- Southern Kidneyshell
- Choctaw Bean
- Tapered Pigtoe
- Fuzzy Pigtoe

Unit GCM7: AL

Purple bankclimber (*Elliptoideus sloatianus*)

Status: Threatened

Location: This species is known from the Apalachicola and Ochlockonee Rivers in Florida. It appears to be very rare or no longer exists in the Chipola and Chattahoochee Rivers.

Habitat and Diet: Habitat includes small to large rivers with slow to moderate current, and substrate of sand, sometimes mixed with mud or gravel. It is less prevalent or absent in tributary streams or impoundments. Mussels are filter feeder and filter their food from the water. The diet of this species primarily consists of plankton, organic matter and detritus. Little is known about the life history of this mussel. Reproductive females have been found from February to April in water with a temperatures from 8.1-15°C. It is believed that males release sperm into the water and females receive the sperm through a siphon. Eggs are fertilized in the female's shell and the larvae (glochidia) are released into the water. The larvae attach to the gills or fins of a host fish to develop. The mosquito fish (*Gambusia holbrooki)* and the blackbanded darter (*Etheostoma*

edwini) are thought to be the host fish. When the larvae metamorphose into juvenile mussels they release from the fish and settle in their primary habitat.

Threats: Principal causes of decline include impoundments, channelization, pollution, and sedimentation that have altered or eliminated habitats that are essential to the long-term viability of this riverine mussel population. Other threats include overcollection, habitat alteration including loss of shoal habitat, thermal alterations, daily discharge fluctuations, bank sloughing, seasonal oxygen deficiencies, cold water releases, turbulence, turbidity and high silt loads, altered host fish distribution, metals, nutrients, ammonia (runoff from poultry and animal feedlots and aquaculture ponds), industrial/municipal effluent, herbicides/pesticides, urban stormwater runoff, sedimentation from agricultural, silvicultural, and roadway activities, clearing of riparian vegetation, gravel mining, livestock grazing, construction of highways, infrastructure, water withdrawals and introduced species (Asiatic clam, zebra mussel, black carp).

Gulf Moccasinshell (*Medionidus penicillatus*)

Status: Endangered

Location: This species is known from the Chipola River and Econfina Creek in Florida. It may exist in the Apalachicola, Chattahoochee, Choctawhatchee and Yellow Rivers

Habitat and Diet: Habitat is medium-sized creeks to large rivers with sand, muddy sand, and gravel substrates and slow to moderate currents and is less prevalent in backwater areas with no current. Mussels are filter feeders and filter their food from the water. The diet of this species primarily consists of plankton, organic matter and detritus. There is limited information about the life history of many mussel species. It is believed that males release sperm into the water and females receive the sperm through a siphon. Eggs are fertilized in the female's shell and the larvae (glochidia) are released into the water. The larvae attach to the gills or fins of a host fish to develop. Gulf moccasinshell glochidia are released in early to late spring. Gravid females were discovered to exist in March, April, September, and November indicating that this species is a parent-overwintering with summer release of glochidia. Primary host fish include *Etheostoma edwini* (brown darter), *Percina nigrofasciata* (blackbanded darter), *Gambusia holbrooki* (eastern mosquitofish), *Poecilia reticulata* (guppy).

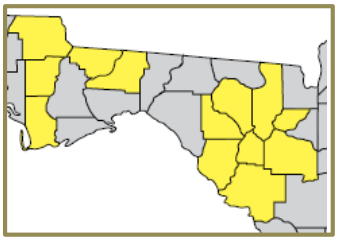
Threats: Threats include development, impoundments of fresh waterways, sedimentation of habitat, habitat fragmentation of actual mussel populations and separation of mussels from host fish. River dredging impacts and destroys freshwater mussel populations on river floors. The invasive Asian clam (*Corbicula fluminea*) out-competes the Gulf moccasinshell for resources in

its habitat. Pesticide and chemical pollution also pose threats to mussels since they are filter feeders and may ingest chemicals directly from their habitat.

Oval pigtoe (*Pleurobema pyriforme*)

Status: Endangered

Location: This species is known from the Chipola, Ochlockonee, and Suwannee river systems and Ecofina Creek.



Habitat and Diet: The oval pigtoe inhabits small rivers and medium sized creeks with slow to moderate current and silty sandy and sand-gravel mix substrates.

The oval pigtoe is a filter feeder. Little is known about the life history of the oval pigtoe. Reproductive females have been found between the months of March and July. It is believed that males release sperm into the water and females receive the sperm through a siphon. Eggs are fertilized in the female's shell and the glochidia (larvae) release into the water. The larvae attach to the gills or fins of a host fish to develop. The sailfin shiner (*Pteronotropis hypselopterus*) and eastern mosquitofish (*Gambusia holbrooki*) are the primary host fish for the oval pigtoe. The time of development depends on water temperature and the species of host fish. When the larvae metamorphose into juvenile mussels, they release from the fish and settle in their primary habitat stream substrate.

Threats: Freshwater mussels face a host of threats due to an increased human population and development. The main threat to fresh water mussels is the impoundment of waterways which modify habitat, cause habitat fragmentation and separate mussels from host fish. Siltation of rivers and streams causes reductions in population. Invasive species such as the Asian clam (*Corbicula fluminea*), out-compete the oval pigtoe for resources in its habitat. Pesticide and chemical pollution pose threats to mussels, since they are filter feeders and may ingest chemicals directly from their habitat.

Shinyrayed pocketbook (*Lampsilis subangulata*)

Status: Endangered

Location: This species is known from the Apalachicola, Chipola and Ochlockonee Rivers in Florida.

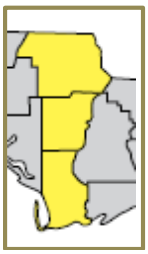
Habitat and Diet: The Shinyrayed pocketbook is a filter feeder and filters food from the water, such as plankton and detritus (dead organic matter). Males release sperm in rivers with low to moderate currents and the females will receive the sperm through a siphon. Eggs are fertilized in the female's shell and the glochidia (larvae) release into the water when mature. Glochidia are released from late May through mid-July when the water temperature ranges from 68°F-74.3°F (20-23.5°C). To attract host fish, the shinyrayed pocketbook produces a long rope of glochidia (larvae) called a superconglutinate. Its resemblance to a fish attracts the host fish to eat the superconglutinate, which allows the glochidia to attach to the fish's gills. The largemouth bass (*Micropterus salmoides*) and spotted bass (*Micropterus punctulatus*) are the primary hosts for the shinyrayed pocketbook. When the larvae metamorphose into juvenile mussels they release from the fish and settle into their stream habitat.

Threats: Main threats to freshwater mussels are due to an increased human population, development and impounds to waterways. This causes habitat change through sediment build up in the river which covers the mussels. Impoundments cause habitat fragmentation, which separates mussels from other mussel populations and also from host fish. River dredging, invasive species (*Corbicula fluminea*), and pesticide and other water pollution pose threats to mussels since they are filter feeders and are exposed to chemicals directly from their habitat.

Chipola slabshell (*Elliptio chipolaensis*)

Status: Threatened

Location: This species is known from the Chipola River system.



Habitat and Diet: The Chipola slabshell is a filter feeder and the diet primarily consists of plankton and detritus. Due to its rarity, little is known about the life history of the Chipola slabshell. It is believed that males release sperm in the water and the female receives the sperm through its siphon. Eggs are fertilized in the female's shell and the larvae release into the water. The larvae attach to the gills or fins of a host fish to develop. When the larvae metamorphose into juvenile mussels, they release from the fish and settle in their primary habitat.

Threats: Freshwater mussel threats include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Fat threeridge (*Amblema neislerii*)

Status: Endangered

Location: This species is known from the Apalachicola and lower Chipola Rivers in Florida.

Habitat and Diet: The Fat threeridge inhabits slow to moderate current rivers with sand, gravel, and rocky rubble floors. The Fat threeridge is a filter feeder and the diet primarily consists of plankton and detritus. Little is known about the life history of the Fat threeridge. It is believed that males release sperm in the water and the females receive the sperm through a siphon. Eggs are fertilized in the female's shell and the larvae release into the water. The larvae attach to the gills or fins of a host fish to develop. The preferred host fish are different types of minnows and different species in the sunfish family. When the larvae metamorphose into juvenile mussels, they release from the fish and settle in their primary habitat.

Threats: Freshwater mussel threats include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Ochlockonee moccasinshell (*Medionidus simpsonianus*)

Status: Endangered

Location: The species is known from the Ochlockonee River drainage.

Habitat and Diet: The species inhabits large creeks and mid-sized rivers of moderate current that contain a sandy, gravel floor. The mussel is a filter feeder that primarily eats plankton and

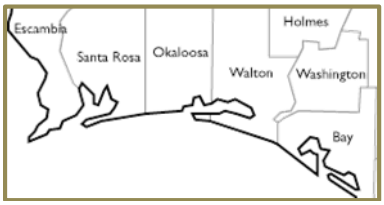
detritus. Due to its extreme rarity, the life history of the Ochlockonee moccasinshell is not known. It is believed that males release sperm in the water and the females receive the sperm through a siphon. Eggs are fertilized in the female's shell and the larvae release into the water. The larvae attach to the gills or fins of a host fish to develop. When the larvae metamorphose into juvenile mussels they release from the fish and settle in their stream habitat.

Threats: Threats to freshwater mussels include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Choctaw bean (*Villosa choctawensis*)

Status: Endangered

Location: The species is known from the Escambia River, Yellow and Choctawhatchee Rivers.



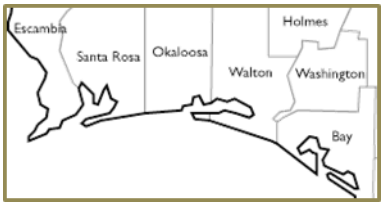
Habitat and Diet: It is found in medium creeks to medium rivers in stable substrates of silty sand to sandy clay with moderate current. The Choctaw bean is a filter feeder and the diet primarily consists of plankton and detritus. Very little is known about the habitat requirements or life history of the Choctaw bean. It is believed to be a long-term brooder, with females gravid from late summer or autumn to the following summer. Its fish host is currently unknown

Threats: Threats to freshwater mussels include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Round ebonyshell (*Fusconaia rotulata*)

Status: Endangered

Location: The species is known from the Conecuh-Escambia River drainage.



Habitat and Diet: It occurs in small to medium rivers, typically in stable substrates of sand, small gravel, or sandy mud in slow to moderate current. The Round ebonyshell is a filter feeder and the

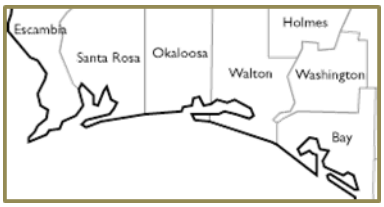
diet primarily consists of plankton and detritus. Very little is known about the habitat requirements or life history of the Round ebonyshell. It is believed to be a short-term brooder, and gravid females have been observed in the spring and summer. The fish host or hosts for the Round ebonyshell are currently unknown.

Threats: Freshwater mussel threats include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Southern Kidneyshell (*Ptychobranchus jonesi*)

Status: Endangered

Location: The species is known from the Escambia, Choctawhatchee and Yellow River drainages.



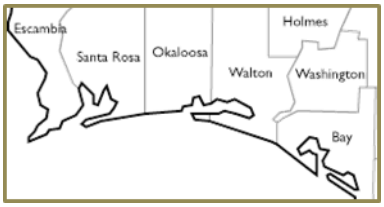
Habitat and Diet: It is typically found in medium creeks to small rivers in firm sand substrates with slow to moderate current. Very little is known about the habitat requirements or life history of the Southern kidneyshell. This species is considered a filter feeder and its diet primarily consists of plankton and detritus. A recent status survey in the Choctawhatchee basin in Alabama found its preferred habitat to be stable substrates near bedrock outcroppings. The Southern kidneyshell is believed to be a long-term brooder, with females gravid from autumn to the following spring or summer. Preliminary reproductive studies found that females release their glochidia in small conglutinates that are bulbous at one end and tapered at the other. Host fish for the Southern kidneyshell are currently unknown; however, darters serve as primary glochidial hosts to other members of the genus *Ptychobranchus*.

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Fuzzy pigtoe (*Pleurobema strodeanum*)

Status: Threatened

Location: The Fuzzy pigtoe mussel is known from the Escambia, Yellow, and Choctawhatchee River drainages in Alabama and Florida



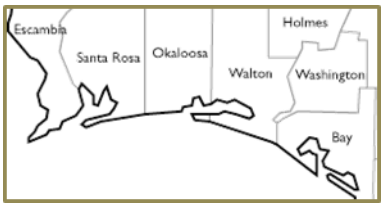
Habitat and Diet: The Fuzzy pigtoe is found in medium creeks to medium rivers in stable substrates of sand and silty sand with slow to moderate current. This species is considered a filter feeder and its diet primarily consists of plankton and detritus. The reproductive biology of the Fuzzy pigtoe indicates that it is a short-term brooder, with females gravid from mid-March to May. The Blacktail shiner (*Cyprinella venusta*) was found to serve as a host for Fuzzy pigtoe glochidia in the preliminary study trial.

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Narrow pigtoe (*Fusconaia escambia*)

Status: Threatened

Location: The mussel is known from the Escambia and Yellow River drainages in Florida.



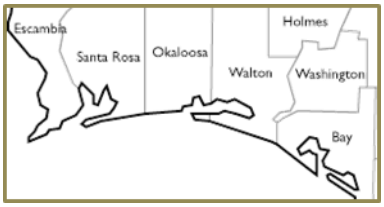
Habitat and Diet: It is found in medium creeks to medium rivers, in stable substrates of sand, sand and gravel, or silty sand, with slow to moderate current. This species is considered a filter feeder and its diet primarily consists of plankton and detritus. The species is known to tolerate a small reservoir environment. Little is known about the habitat requirements or life history of the Narrow pigtoe. It is believed to be a short-term brooder, with females gravid during spring and summer. The host fish for the Narrow pigtoe is currently unknown.

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Tapered pigtoe (*Fusconaia burkei*)

Status: Threatened

Location: The mussel is known from the Choctawhatachee River drainage.



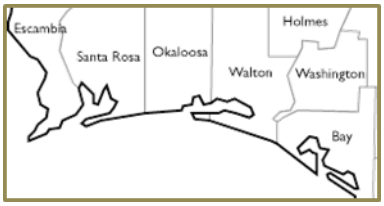
Habitat and Diet: The Tapered pigtoe is found in medium creeks to medium rivers in stable substrates of sand, small gravel, or sandy mud, with slow to moderate current. This species is considered a filter feeder and its diet primarily consists of plankton and detritus. The reproductive biology indicates that it is a short-term brooder, with females gravid from mid-March to May. The Blacktail shiner (*Cyprinella venusta*) was found to serve as a host for Tapered pigtoe glochidia in the preliminary host trial.

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Southern sandshell (*Hamiota australis*)

Status: Threatened

Location: The mussel is known from the Yellow and Choctawhatachee River drainages.



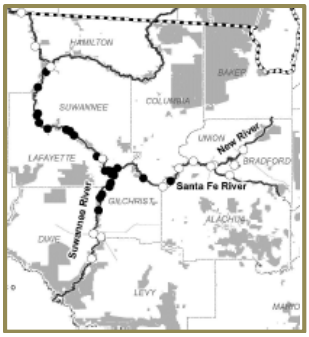
Habitat and Diet: The Southern sandshell is typically found in small creeks and rivers in stable substrates of sand or mixtures of sand and fine gravel, with slow to moderate current. This species is considered a filter feeder and its diet primarily consists of plankton and detritus. It is a long-term brooder, and females are gravid from late summer or autumn to the following spring. The Southern sandshell is one of only four species that produce a superconglutinate to attract a host. The superconglutinate mimics the shape, coloration, and movement of a fish and is produced by the female mussel to hold the larval mussels from one year's reproductive effort. The fish host for the Southern sandshell has not been identified, however, it likely to use predatory sunfishes such as basses, like other species within the *Hamiota* genus.

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.

Suwannee moccasinshell (*Medionidus walkeri*)

Status: Threatened

Location: The mussel is known from the Suwannee River, Santa Fe River and Withlacoochee River.



Habitat and Diet: The mussel typically inhabits larger streams where it is found in substrates of muddy sand or sand with some gravel, and in areas with slow to moderate current.

Diet: Adult freshwater mussels obtain food items both from the water column and from the sediments. They filter feed by taking water in through the incurrent siphon and across four gills that are specialized for respiration and food collection. They can also move sediment material into the shell by using cilia (hair-like structures) on the foot or through currents created by cilia. Juvenile mussels typically burrow completely beneath the substrate surface for the first several months of their life. During this time, they feed primarily with their ciliated foot which they sweep through the sediment to extract material, until the structures for filter feeding are more fully developed. Mussels feed on a variety of microscopic food particles that include algae, diatoms, bacteria, and fine detritus (disintegrated organic debris).

Threats: Freshwater mussel include urbanization and increased human population, impoundments, habitat fragmentation, separation of mussels from their own populations and host fish, river dredging, invasive species, and pesticide and other water pollution.