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DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

50 CFR Part 16

RIN 1018–BA77

[Docket No. FWS–HQ–FAC–2015–0005; FXFR13360900000–245–FF09F14000]

Injurious Wildlife Species; Listing Salamanders Due to Risk of Salamander Chytrid Fungus

AGENCY: Fish and Wildlife Service, Interior.

ACTION: Affirmation of interim rule as final; second interim rule and request for public comment.

SUMMARY: The U.S. Fish and Wildlife Service is affirming as final the 2016 interim rule that added all species of salamanders from 20 genera to the list of injurious amphibians. Under the injurious wildlife prohibitions of the Lacey Act, this final rule prohibits the importation into the United States and shipment between the continental United States, District of Columbia, Hawaii, Commonwealth of Puerto Rico, or any territory or possession of the United States of any live or dead specimen, including hybrids and parts, of those 20 genera of salamanders, except by permit for certain purposes or by Federal agencies solely for their own use. In addition to finalizing the listing of those 20 genera, we are publishing a new interim rule to add to the injurious amphibian list 16 genera that recent studies determined are also carriers of the fungus and to clarify some provisions from the final rule. This interim rule includes any live or dead specimen, hybrid, or parts of the 16 genera and opens a public comment period. We take these actions to protect U.S. ecosystems from the introduction, establishment, and spread of the lethal chytrid fungus *Batrachochytrium salamandrivorans*, which infects and is carried by salamanders, and which is not yet known to be found in the United States.

DATES: *Effective date:* The interim rule published at 81 FR 1534 on January 13, 2016, was effective January 28, 2016. This final rule affirming the January 13, 2016, interim rule and the interim rule set forth in this document are effective [INSERT DATE 15 DAYS AFTER DATE OF PUBLICATION IN THE FEDERAL REGISTER].

Comment submission: Interested persons are invited to submit written comments on the issues raised in the second interim rule as described below under *Information Requested* on or before [INSERT DATE 60 DAYS AFTER DATE OF PUBLICATION IN THE FEDERAL REGISTER].

ADDRESSES: *Comment submission:* You may submit comments by one of the following methods:

(1) Electronically: Go to the Federal eRulemaking Portal: *https://www.regulations.gov*. In the Search box, enter FWS–HQ–FAC–2015–0005, which is the docket number for this action. You may submit a comment by clicking on "Comment."

(2) By hard copy: Submit by U.S. mail to: Public Comments Processing, Attn: FWS–HQ–FAC–2015–0005, U.S. Fish and Wildlife Service, MS: PRB/3W, 5275 Leesburg Pike, Falls Church, VA 22041–3803.

We request that you send comments only by the methods described above. We will post all comments on *https://www.regulations.gov*. This generally means that we will post any personal information you provide us (see *Information Requested*, below, for more information).

Supplementary materials: Background documents related to this rulemaking action, including the final economic analysis for the affirmation of the 2016 interim rule, are available at *https://www.regulations.gov* in Docket No. FWS–HQ–FAC–2015–0005.

FOR FURTHER INFORMATION CONTACT: Kristen Sommers, Injurious Wildlife Listing Coordinator, U. S. Fish and Wildlife Service, Branch of Aquatic Invasive

Species; MS: FAC; 5275 Leesburg Pike; Falls Church, VA 22041–3803; 571–329–2214. Individuals in the United States who are deaf, deafblind, hard of hearing, or have a speech disability may dial 711 (TTY, TDD, or TeleBraille) to access telecommunications relay services. Individuals outside the United States should use the relay services offered within their country to make international calls to the point-of-contact in the United States.

SUPPLEMENTARY INFORMATION:

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I. Executive Summary

Why we need to publish a rule. We, the U.S. Fish and Wildlife Service (Service or FWS), are charged with administering 18 U.S.C. 42(a), as amended (commonly known as the injurious wildlife provision of the Lacey Act). Under this authority, the Secretary of the Interior may list by regulation those wild mammals, wild birds, fish, mollusks, crustaceans, amphibians, reptiles, and the offspring or eggs of any of the foregoing that

are injurious to human beings, to the interests of agriculture, horticulture, or forestry, or to the wildlife or wildlife resources of the United States.

We have determined that salamanders that can carry the fungus *Batrachochytrium salamandrivorans* (Bsal) are injurious to wildlife and wildlife resources of the United States. This determination was based on a review of the literature and an evaluation under the criteria for injuriousness by the Service. The purpose of listing these species as injurious wildlife is to prevent the introduction, establishment, and spread of Bsal in the wild in the United States. The fungus primarily affects salamanders, has lethal effects on many salamander species, and is not yet known to be found in ecosystems of the United States.

What this document does. This document serves two purposes: It finalizes a rulemaking action initiated with publication of an interim rule in 2016, and it promulgates a new and related interim rule.

On January 13, 2016, we published an interim rule that amended our regulations pertaining to injurious wildlife (81 FR 1534). That interim rule (hereafter referred to as "the 2016 interim rule") amended our regulations to add all species of salamanders from 20 genera, of which there were 201 species, to the list of injurious amphibians. Under the injurious wildlife prohibitions of the Lacey Act, the 2016 interim rule prohibited both importation into the United States and interstate transportation between States, the District of Columbia, the Commonwealth of Puerto Rico, or any territory or possession of the United States (the latter was clarified by a court decision in 2017) of any live or dead specimen, including parts, of these 20 genera of salamanders, except by permit for zoological, educational, medical, or scientific purposes (in accordance with permit conditions) or by Federal agencies without a permit solely for their own use.

A second interim rule is now needed because of recent studies documenting additional genera that also share the same traits that make them injurious as carriers of

Bsal. We are also revising some provisions from the 2016 interim rule in the final rule to make minor corrections to and improve clarity of the rule.

The basis for our action. Defensible scientific evidence indicates that we need to list a total of 36 genera of salamanders as injurious wildlife to protect U.S. ecosystems. Therefore, we hereby affirm the injurious wildlife listings in the 2016 interim rule of all species in the following 20 genera: *Chioglossa, Cynops, Euproctus, Hydromantes, Hynobius, Ichthyosaura, Lissotriton, Neurergus, Notophthalmus, Onychodactylus, Paramesotriton, Plethodon, Pleurodeles, Salamandra, Salamandrella, Salamandrina, Siren, Taricha, Triturus,* and *Tylototriton*. We also add new injurious wildlife listings of all species in the following 16 genera through the second interim rule: *Ambystoma, Andrias, Aneides, Aquiloeurycea, Calotriton, Chiropterotriton*, *Cryptobranchus*, *Desmognathus, Ensatina, Eurycea, Laotriton, Ommatotriton, Pachytriton, Proteus, Pseudobranchus,* and *Pseudotriton*.

The United States has the greatest diversity of salamanders in the world, salamanders are a vital part of native ecosystems, and numerous salamander populations are at risk of endangerment from Bsal. A risk assessment conducted by the U.S. Geological Survey (USGS) concluded that the potential for Bsal introduction into the United States is high, the United States has suitable conditions for Bsal survival, and the consequences of introduction into the United States are expected to be severe and occur across a wide geographic range within the United States. The most likely pathway of Bsal into the United States would be on the bodies of salamanders in the commercial salamander trade. Aside from our Bsal regulations, the ability and effectiveness of measures to prevent or control Bsal is currently low. Trade in wildlife occurs on a global scale, and amphibians are one of the most commonly traded animals.

Therefore, listing the genera as determined in this rulemaking action will help to reduce the likelihood that Bsal enters the United States and presents a threat to native salamander species.

II. Current Rulemaking Action

This document does the following:

- Affirms the current listing of 20 genera of salamanders as injurious species by the 2016 interim rule as described above and any species within those genera.
- Revises provisions in the preamble of the 2016 interim rule in response to a court decision that pertained to interstate transport of injurious wildlife as described below.
- Removes the 201 itemized species names from the list in 50 CFR 16.14. This itemized list of scientific and common names is unnecessary because the regulations in 50 CFR 16.14(a) state that the prohibitions pertain to the 20 genera "including but not limited to, the species listed in this paragraph." We provided the itemized list of species in the 2016 interim rule largely for the convenience of the public and our law enforcement staff, but the taxonomy of salamanders is evolving, and the list is not static. However, while many scientific and common names have changed, all of the listed species remain in their same genera.
- Clarifies prohibitions pertaining to hybrids and frozen specimens; clarifies what is not prohibited (including eggs or gametes; parts or tissues that have been chemically preserved, chemically treated, or heat treated so that the pathogen *Batrachochytrium salamandrivorans*, if present, is rendered non-viable; and molecular specimens consisting of only the nucleic acids from organisms).
- Adds 16 genera to the list in 50 CFR 16.14(a), as explained below, and solicits comments on these new genera.

III. Final Rule to the 2016 Interim Rule

A. Background

On January 13, 2016, under the authority of 18 U.S.C. 42(a)(1), as amended, we, the U.S. Fish and Wildlife Service, published an interim rule in the *Federal Register* (81 FR 1534) to add all species from 20 genera to the list of injurious amphibians and announced the availability of the draft economic analysis and the draft environmental assessment of the 2016 interim rule. The rule took effect on January 28, 2016, and revised the lists of injurious wildlife in part 16 of title 50 of the Code of Federal Regulations (CFR), specifically the list of injurious amphibians at 50 CFR 16.14. The 60-day public comment period closed on March 14, 2016. We solicited comments and supporting data to gain additional information. We also solicited peer review at the same time.

In this document, we present our consideration of the public comments and peer review received on the 2016 interim rule to inform our final determinations. We present a summary of the peer-review comments and the public comments and our responses to them in the "Summary of Comments Received on the 2016 Interim Rule" portion of the preamble to this final rule. The comments did not provide any substantive evidence that supported changing the genera in the interim rule. However, some comments did provide justification for modifying certain requirements stipulated in the 2016 interim rule.

The Service published an interim rule in 2016 instead of issuing a proposed rule for the listing under the Administrative Procedure Act (APA) (5 U.S.C. 551 *et seq*.). As explained in the 2016 interim rule, we had good cause to forgo notice and public comment on a proposed rule and instead take immediate action in the form of an interim rule to help prevent the fungus Bsal from being introduced, established, or spread in the United States for the reasons listed above. The fungus, lethal to many salamander species in the United States, is carried on the skin of salamanders and can be unintentionally imported by salamanders in trade. The 2016 interim listing of the 20 genera of salamanders has prohibited the importation of high-risk species under the injurious

wildlife prohibitions of the Lacey Act, and the fungus has remained absent from the United States.

In this document, a clarification from the 2016 interim rule reflects a court decision in 2017. Under the D.C. Circuit Court of Appeals decision in *United States Association of Reptile Keepers, Inc.* v. *Zinke,* 852 F.3d 1131 (D.C. Cir. 2017), import of injurious wildlife into the United States remains prohibited. In addition, transport of injurious wildlife between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42(a) (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States) remains prohibited. However, the court held that 18 U.S.C. 42(a) does not prohibit interstate movement between States within the continental United States. This means that transportation of injurious wildlife between the 49 States within the continental United States (the contiguous 48 States and Alaska) is not prohibited by the statute, unless that movement of the wildlife is restricted due to conditions associated with issued injurious wildlife permits. We note that transport from the lower 48 States to Alaska through Canada, or vice versa, remains prohibited, because that transport includes an import into the United States.

The language in 50 CFR 16.14(a) was, and still is, correct in that it does not prohibit interstate transport between States within the continental United States. The final economic analysis affirming the 2016 interim rule reflects the clarification of interstate transport authority between States within the continental United States since the court decision on April 7, 2017. However, injurious wildlife unlawfully imported into the United States or transported between the enumerated jurisdictions is still unlawful to transport within the continental United States. Under the Lacey Act amendments of 1981, 16 U.S.C. 3372(a)(1), it is unlawful for any person to import, export, transport, sell, receive, acquire, or purchase any wildlife transported in violation of any law of the

United States. This includes transport of any injurious wildlife imported into the United States or transported between the enumerated jurisdictions in violation of 18 U.S.C. 42.

B. Summary of Comments Received on the 2016 Interim Rule

The following comments were submitted during the peer and public comment period for the 2016 interim rule. Knowledge about Bsal has improved since then due to the many excellent studies by researchers and our own understanding of the disease. In the following responses to the comments under *Peer Review Summary* and *Public Comments Summary*, respectively, we have endeavored to answer the comments both as they related to the knowledge of Bsal, trade, and related issues at the time of the 2016 interim rule and as of the current knowledge as appropriate. The current knowledge from recent research affirms the 2016 interim rule, supports the second interim rule set forth in this document, and can be found below in *IV. Second Interim Rule*. In our responses to the comments, when we refer to "this rule," we mean that the information and the changes apply both to the final rule for the 2016 interim rule and the second interim rule.

Peer Review Comments and Our Responses

In accordance with peer review guidance of the Office of Management and Budget "Final Information Quality Bulletin for Peer Review," released December 16, 2004, we solicited expert opinion on information contained in the 2016 interim rule from three knowledgeable individuals selected from specialists in the relevant taxonomic group and ecologists with scientific expertise that includes familiarity with alien herpetological introductions and invasions, predictive tools for risk assessment, and invasion biology. We posted our peer review plan on the Service's Science Web site (*https://www.fws.gov/media/peer-review-plan-listing-salamanders-injurious-due-risk-salamander-chytrid-fungus*), explaining the peer review process and providing the public with an opportunity to comment on the peer review plan. No comments were received regarding the peer review plan. The Service solicited independent scientific reviewers

who submitted individual comments in written form. We avoided using individuals who had already expressed strong support for or opposition to the subject and individuals who were likely to experience personal gain or loss (such as financial or prestige) or otherwise could be perceived as having a conflict of interest as a result of the Service's decision. We received responses from three peer reviewers. A scientist with the USGS served as one of the peer reviewers.

We requested that the reviewers provide comments that were specific to the 2016 interim rule and the draft economic analysis. We reviewed all comments for substantive issues and any new information they provided. We consolidated their comments (without attribute) and our responses into key issues in this section. Some peer reviewer comments that called for technical changes or more minor corrections have not been noted, but we have made our best effort to correct those grammatical or biological errors and clarify certain ambiguous statements in the second interim rule and supporting documents. We prepared the second interim rule and second draft economic analysis to reflect peer reviewer comments and new scientific information where appropriate.

The comments we received indicated support for the 2016 interim rule and for the analysis that we conducted given the need to prevent harm to native species from Bsal. All three peer reviewers concluded that the data and analyses we used in the interim rule were appropriate and the conclusions we drew were logical and reasonable. All three peer reviewers provided additional insights (which we used to clarify points in the second interim rule) or references to recently published studies, which support the final rule. In general, the peer reviews supported the Service's conclusions and agreed that they were reasonable, though they did note that we generalized some of our findings, such as the average temperature of salamander habitats as an indicator of Bsal vulnerability with regards to salamanders nationwide. We have clarified these issues where practical in the second interim rule. The peer reviewers suggested that there was a need to expand our

discussion regarding possible treatment options, which we have added to the second interim rule. The peer reviewers also acknowledged that, while the rule is important, research questions remain that could shed light on ways to better prevent the introduction of Bsal into the United States.

General Comments

(PR1) *Comment*: In support of the 2016 interim rule, prevention provides an environmental and biodiversity benefit; the probability of introduction is at the very least reduced; and host species need not become established in the environment to transmit Bsal. If an owner houses multiple salamander species, transmission can occur in captivity to other species that may be able to establish a population in the wild. The commenter also agrees with the exclusion from the rule of tailless amphibians (frogs and toads), which were uniformly resistant in the tests by Martel et al. (2014).

Response: We concur with the comments. At the time of the 2016 interim rule publication, we lacked evidence of tailless amphibians as carriers, and this final rule simply affirms our findings on salamanders.

(PR2) *Comment:* The average temperature of salamander habitats as an indicator of Bsal vulnerability with regard to salamanders nationwide would be difficult to defend. The rule provides a very broad average, and extrapolating it weakens the point.

Response: We have clarified the rule under *Bsal Risk Assessments* in response to this comment to reflect more specifically the areas that the risk assessments identified as highest risk. We intended to demonstrate that most salamander species in the United States are not protected from Bsal by living outside of the Bsal optimal growth range or in areas beyond the threshold where Bsal can survive.

(PR3) *Comment:* If time allowed, a few simple tests to improve the scientific foundation of the interim rule could have strengthened the decision to include or exclude species.

Response: The Service is not a research agency, and we utilized available research when we found in 2016 that there was good cause to forgo notice and public comment on a proposed rule and instead take immediate action in the form of an interim rule to help prevent Bsal from being introduced, established, or spread in the United States. In this rule, we also utilize newly published or otherwise available research. In the years since the 2016 interim rule was published, many of the relevant studies affirmed our interim findings, while others support additional genera as documented in our second interim rule. None substantively contradicted our findings.

(PR4) *Comment:* Given the long time that Bsal has been around (150 years), the massive number of imported salamanders, and only recent characterization of Bsal, it may be possible that earlier Bsal was characterized as a related chytrid fungus, *Batrachochytrium dendrobatidis* (Bd).

Response: As of the completion of this rule, resource managers, scientists and other researchers have been utilizing the latest scientific techniques to actively look for Bsal in nonresearch captive populations and in wild populations in the United States. The USGS conducted a massive sampling effort of 11,189 samples from 594 sites in 223 counties within 35 U.S. States and 1 site in Mexico specifically for Bsal in wild populations (Waddle et al. 2020). The sites were chosen based on the species' susceptibility (including some frog species) and highest risk geographically. No Bsal was found. As of the completion of this rule, we are unaware of any positive Bsal detections in the wild. Testing of archived samples by other laboratories has been done, and no Bsal has been detected to our knowledge. The evidence is not conclusive that Bsal has never been in the wild in the United States, but there is no evidence that it has.

(PR5) *Comment:* When the rule repeats the information about invasive species, the point should be made that, even if a salamander found to be injurious could not establish a population in the wild, an infected salamander in captivity can still transmit

Bsal to native populations if that salamander escapes or if material touching it is disposed of improperly.

Response: We agree and have incorporated the suggested language into the second interim rule.

(PR6) *Comment:* The 2016 interim rule states that the main pathway for the global spread of Bsal is the international trade in salamanders and that the most likely pathway of a salamander that is a host to Bsal into the United States would include a pet store or online retailer. Since neither the United Kingdom or Germany Bsal outbreaks were related to a pet store or online retailer, the commenter recommends stating, "The most likely pathway of a salamander that is a host to Bsal into the United States would be the captive salamander commercial trade" to cover the diverse salamander trade.

Response: We have edited the second interim rule accordingly.

(PR7) *Comment:* Given that Bd was probably introduced by release of laboratory animals as well as pets, institutional use of listed salamanders should also be regulated to protect U.S. ecosystems from Bsal.

Response: This rule is intended to prevent the introduction of Bsal, whereas Bd was already widespread in the United States when that fungus was identified as the cause of major amphibian mortality. Importation of listed salamanders is regulated by this rule within the authorities under the injurious wildlife provisions of 18 U.S.C. 42. Any listed salamanders that are imported under a permit exception for zoological, educational, medical, or scientific purposes are required to observe sanitary procedures and double containment to prevent escape and are not allowed to be released. The Service may also establish additional permit conditions if deemed appropriate to ensure responsible use, maintenance, and containment of injurious wildlife specimens posing a risk of pathogen transfer and continued protection of the public interest and health, under 18 U.S.C. 42(a)(3) and the Service's permitting regulations in 50 CFR part 13 and part 16.

(PR8) *Comment:* What is the citation for the statements, "Based on scientific evidence, we know that the fungus is lethal to at least two salamander species native to the United States. Of the 190 native U.S. species, we find that at least 67 species are carriers and 20 are not carriers"?

Response: Martel et al. 2014 was the source for the first sentence, and we used a combination of sources for information about native species and the testing that was done. We stated our sources and findings for these statements in the relevant sections in this rule.

Vulnerability and Carrier Status of Native Species

(PR9) *Comment:* One of the considerations was that, even if a salamander listed by the 2016 interim rule could not establish a population in the wild, an infected salamander in captivity (or the water and soil in which it came into contact) can transmit Bsal to native populations. In addition to water and soil, how about if there is affected foliage or paper that was used in transit?

Response: The commenter is correct that a variety of materials could become contaminated with fungal spores if in contact with infected salamanders. However, it is not possible to provide a complete list of these potential fomites (materials, such as water, that can act as passive carriers and can transfer pathogens) in the text of the rule. Rather, we believe that listing the salamander species that may be carriers of Bsal as injurious wildlife, thereby prohibiting the importation of potentially infected individuals, reduces the risk for pathogen spread by any substrate.

(PR10) *Comment:* The considerations say that controlling Bsal is not practical. The peer reviewer recommends revising to note that, while there are control methods available for infected individual salamanders in captivity (Blooi et al. 2015a, 2015b), there are no practical control measures for free-ranging salamanders.

Response: We agree with the comment. In the second interim rule, we clarify that, while treatment options exist that may help reduce the threat posed by Bsal for imported and captive-held specimens, those options have not been standardized and their effectiveness remains uncertain for large-scale application.

Pathways

(PR11) *Comment:* The pathway by which Bsal spreads is unknown, except that water is involved. Thus, the States should be responsible for implementing measures on waterways that prevent the introduction of Bsal.

Response: We agree that the U.S. States should be strong partners in helping to prevent the introduction, establishment, and spread of Bsal. We conclude that the main pathway for the potential global spread of Bsal is the introduction into the United States through international trade in salamanders, and we are acting with this rule to reduce those risks.

(PR12) *Comment:* The pathway analysis, epidemiology of the disease, and investigation of the origins of the outbreak need more investigation. In addition, no laws or regulations exist to control the disposal of untreated water from captive salamander enclosures. Given the virulence of the disease, how did Bsal enter the European environment? Was it the result of open-system housing, such as outdoor pens or open-system water flow? Intentional release of pets seems an unlikely source since the course of the disease is rapid with signs of infection within 8 days.

Response: While it is possible that Bsal can be transmitted through other pathways and vectors, the comment does not provide any evidence that other pathways are more likely than international trade. Drawing on the evidence cited in this rule in the *Pathway Analysis*, we conclude that the primary potential pathway for the entry of Bsal into the United States is through the international trade in salamanders. Our analysis concludes that Bsal can survive on infected animals long enough for the pathogen to be

introduced into the environment and transmitted to species that are negatively impacted by Bsal.

(PR13) *Comment:* We suggest another pathway that should be addressed is that fishes, plants, and invertebrates may be co-cultured with newts. It is unknown if they can act as a fomite. For Bd, there is evidence for foliage and invertebrates as substrates. Amphibians can enter the United States as stowaways on agricultural and other imports. For example, the Cuban tree frog that invaded Florida hitchhiked in shipping crates coming from the Caribbean. It is also possible that Bsal could be transported in contaminated water that is entering the United States with imported fish for aquaculture or the pet trade.

Response: We concur with the comment. Other pathways are a concern; however, the Service concludes that the trade pathway in salamanders is the most significant means by which Bsal could be introduced into the United States. The final and second interim rules will be protective because a co-cultured salamander that has also been found to be a carrier would be prohibited from importation into the United States. The Service will also continue to seek opportunities to work with partners to minimize the risk from other pathways.

Species Additions

(PR14) *Comment:* Some of the Bd infections observed in species from *Ambystoma* may have been Bsal. California tiger salamanders (*Ambystoma californiense*) can survive chytrid infections that would make them likely carriers. Another peer reviewer stated that the rule states, “At least four [native species] are resistant to Bsal infection, of which one is expected to be a carrier because Bsal was able to invade the skin of that species long enough to move or transmit the fungus to other salamanders.” This is a reasonable assumption. The commenter makes the same assumption for *Ambystoma* based on their ability to be infected by Bd.

Response: The testing results available to the Service at the time of the 2016 interim rule provided no evidence that some species within *Ambystoma* are carriers of Bsal. However, subsequent research provides that evidence. Please see *IV. Second Interim Rule* below for that evidence.

Prohibition on Interstate Transportation

(PR15) *Comment:* Enforcing the interstate prohibition will be difficult. Also, it seems unnecessary if Bsal is not known to exist in the United States. While it is possible that Bsal may be present on a pet in the United States, the interstate transportation prohibition could prevent movement of that pet.

Response: As stated above under *A. Background* in *III. Final Rule to the 2016 Interim Rule*, the D.C. Circuit Court of Appeals held on April 7, 2017, that transportation of injurious wildlife between the 49 States within the continental United States (the contiguous 48 States and Alaska) is not prohibited by the Lacey Act, unless that movement of the wildlife is restricted due to conditions associated with issued injurious wildlife permits. The language in 50 CFR 16.14(a) was and still is correct in that it does not prohibit interstate transport between States within the continental United States. Transport of injurious wildlife between the enumerated jurisdictions set forth in the shipment clause of 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), codified in Federal regulations at 50 CFR 16.3, remains prohibited. The Service will continue to seek opportunities to encourage affected members of the public to take responsible actions related to listed species.

Prohibition on Preserved Specimens and Parts

(PR16) *Comment:* There is little risk of Bsal transmission from chemically preserved specimens. Even if contaminated with Bsal DNA, it is unlikely that the chytrid would be viable or lead to introduction of Bsal into the United States. However, the

prohibition should be maintained for live or frozen specimens, because it is unclear whether Bsal can survive freezing. Experimental studies are needed to elucidate the viability of Bsal after preservation and freezing.

Response: In response to comments we received explaining that preserved salamanders or their preserved parts pose a low risk of transmitting Bsal, the Service is removing chemically preserved specimens and body parts from the injurious wildlife listing as long as chemical preservation is adequate to render the fungus inviable. Frozen specimens remain regulated as parts of injurious wildlife as clarified in *A.*

Species Capable of Carrying Bsal

(PR17) *Comment:* The 2016 interim rule states in the section "Invasiveness and Transmission of Bsal" that the rough-skinned newt and the eastern newt are capable of carrying Bsal. What is the evidence for this?

Response: Martel et al. (2014) found that the eastern newt and rough-skinned newt were found to be lethally vulnerable to Bsal. Below in *E. Pathway Analysis*, *Introduction Pathways*, we added that Bsal can remain viable inside dead host tissue (Martel et al. 2013). We have concluded that lethally vulnerable salamanders are also carriers. More recent information affirms the newts as carriers (please see ***II.*** *Current Rulemaking Action* below for that evidence).

Invasiveness of Salamanders

(PR18) *Comment:* The rule states that Oriental fire-bellied newts (*Cynops orientalis*) and paddle-tailed newts (*Paramesotriton* (*Pachytriton) labiatus* or *brevipes*), which are native to China, have been found in the wild near an animal importer's facility in Florida. Because they were found outside of the facility does not necessarily mean that they are a breeding, invasive, reproducing population.

Response: We concur with the comment that being found outside of a facility does not necessarily mean that the species in question is actually invasive, although a

released salamander could persist long enough in the ecosystem to transmit Bsal if the salamander was exposed to viable spores.

(PR19) *Comment:* The rule states that Picco and Collins (2008) found that salamanders sold as bait were highly infected with both ranavirus and Bd, thereby increasing the likelihood of pathogen transmission into new areas of the United States through the act of fishing. Have declines from this pathway been documented? If not, the point is rather moot or at least weak.

Response: That comment refers to the section on invasiveness of salamanders. The Picco and Collins (2008) reference demonstrates that anglers routinely release salamanders into the areas where they fish, which serves as one pathway for salamanders being introduced into the environment, including nonnative habitats. This pathway may also serve as a vector for pathogens, including Bsal.

(PR20) *Comment:* The 2016 interim rule states that the four salamander genera most commonly imported into the United States from 2004 to 2014 were *Cynops*, *Paramesotriton*, *Triturus*, and *Pachytriton*. You should check Krysko et al. (2011) against the fire-bellied newt.

Response: Krysko et al. (2011) was cited by the USGS in its fact sheet for the Oriental fire-bellied newt in reporting nonindigenous occurrences, although none have been reported since 2010 (USGS Nonindigenous Aquatic Species (NAS) 2021 [CYOR]).

(PR21) *Comment:* In evaluating the potential to eradicate or manage established populations, the 2016 interim rule says that, while some introduced salamanders in the United States have been successfully controlled, others have not. However, evidence for control is sparse. There is a difference between a small population living in exceptional circumstances and an invasive species. In many cases, small populations of animals will persist but not spread. These are not invasive and should not be used as examples of the removal of invasives.

Response: Executive Order 13751 defines an invasive species as a nonnative organism whose introduction causes or is likely to cause economic or environmental harm or harm to human, animal, or plant health. Establishment and spread can increase the threat that a particular species causes, but establishment and spread are not in themselves mandatory criteria for defining a species as invasive or injurious. The analysis under *Potential To Eradicate or Manage Established Populations* in the 2016 interim rule was intended to show that there is a risk of harm from the introduction of Bsal even if a nonnative salamander population could be successfully controlled or eradicated in the environment.

(PR22) *Comment:* The 2016 interim rule states that the total risk of Bsal to native salamanders is high. It should probably take Bsal invasive risk into account.

Response: The total risk to native salamanders was based on a USGS risk assessment (Richgels et al. 2016). We took invasive risk into account in other sources. We discussed the issues related to invasion risk of Bsal under *Likelihood of Release or Escape*. To make our listing determination for salamanders, we drew upon the results of multiple independent risk assessments and our own analysis and found that Bsal is likely to be introduced into the United States if no additional risk mitigation steps were taken by the Service.

Bsal Infection

(P23) *Comment:* The 2016 interim rule states that Bsal can also be introduced into the environment through the improper disposal of contaminated water or other materials used to transport salamanders and that the fungus can likely persist in such materials independent of whether a salamander is present. Although the fungus can persist in the environment, it may not be at infectious levels.

Response: The number of fungal spores required to initiate a Bsal infection has not been well researched, and this number may vary among host species and with other

disease-related factors (environmental and Bsal-specific factors). The Service's analysis was based on whether the available evidence showed that a given genera was capable of carrying Bsal and introducing it into U.S. environments.

(PR24) *Comment:* The 2016 interim rule states that the discharge of untreated water used to house infected, captive animals could be a pathway for releasing infective zoospores into the environment and exposing native salamanders to Bsal. There is some evidence for Bd, such as the 30,000 zoospores detected after 10 hours in DiRenzo et al. (2014), but a more direct experiment occurred in Carey et al. (2006), where the solutions that had housed toadlets being tested were used to infect other toads. This is strong evidence and should be included.

Response: We concur with the comment and have added the reference to Carey et al. 2006 in the second interim rule.

Likelihood of Release or Escape

(PR25) *Comment:* An outbreak of Bsal in Germany was discovered in a captive salamander collection (Sabino-Pinto et al. 2015).

Response: The comment is correctly stated, and this point is now referenced in the second interim rule.

Ability To Prevent or Control the Spread of Pathogens or Parasites

(PR26) *Comment:* The two treatments from Blooi et al. 2015(a) should be changed from "in development" to "available." A sentence explaining that this treatment is limited in feasibility and applicability (that is, not all salamander species can tolerate the temperature treatment recommended) should be added.

Response: We concur with the commenter's statements as reflected in Blooi et al. 2015(b) and modified the second interim rule consistent with the comment.

(PR27) *Comment:* Control measures are available for Bsal-infected salamanders, but these would be more relevant for captive salamanders rather than free-ranging salamanders.

Response: We have edited the second interim rule accordingly by recognizing that control measures for Bsal-infected salamanders are more relevant for captive rather than free-ranging salamanders.

Impacts on Wildlife Resources or Ecosystems

(PR28) *Comment:* The 2016 interim rule states that, "[i]f rough-skinned newts were to experience severe declines from Bsal infection, a result could be significant additional inputs of carbon to the atmosphere as has been observed with other species. The commenter recommends modifying the sentence to read, "If rough-skinned newt populations were to experience severe declines from Bsal infection, atmospheric inputs of carbon may be altered, as has been observed with other species (Wyman 1998; Best and Welsh 2014)."

Response: We have edited the second interim rule consistent with the peer reviewer's comment.

(PR29) *Comment:* The 2016 interim rule noted that scientists and diagnostic laboratories are working to standardize laboratory protocols. What happens if they do?

Response: Standardized laboratory protocols are an important part of disease management, but the ability to validate, document, and enforce disease testing requirements is also necessary. Additionally, the capacity to implement quarantines and live-animal inspections may be required. Publication of the final rule does not preclude future regulatory action based on emerging science and increased capabilities.

Economic Analysis, Regulatory Flexibility Analysis, and Effect on Industry

(PR30) *Comment:* Alternative 3 was preferred over Alternative 4 in the draft economic analysis. It was not clear whether salamanders were excluded from Alternative

3 because they were not tested or whether all of the ones tested showed no infection. If they simply were not tested, Alternative 4 seems like the more responsible option given a precautionary approach since many salamander genera appear to be at risk and given that the difference in cost between Alternatives 3 and 4 seemed relatively small ($3.8 million versus $4 million). Moreover, untested genera may become substitutes when the genera under Alternative 3 are no longer available, which remains a problem if it is reasonable to expect some risks associated with the untested salamander genera.

Response: Under Alternative 3, we listed genera for which we have evidence that at least one species in a genus is a carrier of Bsal with no conclusive countervailing evidence that other species in that genus are not carriers. Alternative 3 does not include species from genera that have not been tested for Bsal vulnerability. Alternative 4 would include the listing of all salamander species. The expected increase in cost from Alternative 3 to Alternative 4 was not considered in our determination about the injuriousness of the species because the determination is based on defensible scientific evidence. The Service determined that there was unknown risk from genera where no species have yet been tested for Bsal and, therefore, could not list those genera at this time.

(PR31) *Comment:* It was not clear in Alternative 5 whether there would be administrative costs associated with health certificates and whether there is a probability of making a mistake.

Response: While most of the testing costs of administering a health-certificate program may not fall on the government or public, there would still be costs to the Service involved in staff time to set up the program and oversee it, as well as wildlife inspectors checking import shipments for the additional requirements. The draft economic analysis lumps the administrative costs with costs of testing, and both are mentioned as a concern in sections 4.1.1.5 and 5.0. A health-certificate program was not

our preferred alternative for a nationwide regulatory program by the Service at this time because of uncertainties with its effectiveness, including the effectiveness and sensitivity of current testing methods (including the return of false negatives); lack of validation and sufficient testing capacity; lack of standardized treatment methods; and lack of agency resources required to conduct inspections, interpret results, and issue health certificates.

(PR32) *Comment:* Has inflation been taken into account in the analyses of economic costs to adjust costs of today's dollar values?

Response: All prices in the draft economic analysis were updated for the final economic analysis to the 2021 price level Consumer Price Index for All Urban Consumers that was used for all indexing purposes (see section 3.1.2). Salamander retail price data was received in 2015 dollars during the course of the study. Tables labeled as 2021$, or 2015$, have either been adjusted for inflation or did not need adjustment. The original price level is the year for the citation unless otherwise noted. Tables without a price level or data origin year have been amended.

(PR33) *Comment:* The economic costs appear to reflect the maximum costs since it does not appear that alternative sales were considered. For example, if buyers cannot buy salamanders, would they buy other amphibians instead or would they simply buy nothing? Only the latter would result in the estimated costs. Similarly, the market for "local" salamanders may increase as a response.

Response: Section 3.1.2 of the analysis of economic costs explains three points. Estimated importation losses are stated as maximums due to the unknown effect on domestic breeding and consumer substitution. Domestic losses are also estimated at the maximum (loss of entire industry) due to the lack of data on transport between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (codified in Federal regulations at 50 CFR 16.3). We also added detail to this section to clarify why the losses

may range from zero to the maximums stated in each alternative analysis section later in the report.

(PR34) *Comment:* The pet industry will not be altered significantly by this rule, but Bsal would likely impact wild populations of salamanders. Perhaps with the exception of breeders, pet sales would probably shift to another animal with little loss of revenue.

Response: Exit from an industry or substituting a legal product is dependent on multiple factors as discussed in the report beginning in section 3.1.2 of the economic analysis. We added details and clarification to this section in the final economic analysis. Substitution can occur with other salamander species, an animal from another order, or another category of goods altogether.

(PR35) *Comment:* The draft economic analysis states under ES 1.1 Economic Analysis, "In addition, we used data from IMPLAN® (Minnesota IMPLAN Group, 2013) to estimate the direct effects of this rulemaking." MIG changed their name. They now go by IMPLAN Group LLC. In addition, what data year did you use?

Response: We changed the name to "IMPLAN Group LLC" in the final economic analysis. We used study area data from 2013 for the economic analysis.

(PR36) *Comment:* Regarding the draft economic analysis under ES 1.4.5 Alternative 5, does the cost estimate of the loss of revenue to companies or individuals involved in the importation or interstate movement of any salamander species consider the cost of health-certificate examinations and inspections by veterinarians affiliated with the Department of Defense, zoos, and industry as well as private practitioners?

Response: Due to data limitations, the final economic analysis does not explicitly estimate the cost of health certificates. The analysis assumes that any additional costs for examinations and inspections would be absorbed by the importer or passed on to the consumer, but data limitations restrict the analysis from estimating whether the importer

or consumer would pay. Thus, we assume the estimated losses for all alternatives including Alternative 5 is the average sales price of a salamander. On average, we assume the estimated maximum loss (sales price) would include all testing costs.

(PR37) *Comment:* The commenter believes a job in IMPLAN is annualized. IMPLAN's definition is "A job in IMPLAN = the annual average of monthly jobs in that industry."

Response: We have changed the final economic analysis to reflect the above definition.

(PR38) *Comment:* The draft economic analysis, at ES 1.6 Conclusion, states that it is unclear how much testing, treatment, and the health certification processes would cost.

Response: It is correct that these costs are unknown and could not be estimated unless a compliance method is developed.

(PR39) *Comment:* The commenter found locating the tables and figures in the draft economic analysis to be challenging. For example, figure 1 is not shown for several pages after first being noted in 2.2 Salamander Market.

Response: Placement of tables and figures was determined by the progression of the analysis. As many numbers are referred to many times, they will not always be near all discussions. Table and figure numbers are given to allow the reader to find them.

(PR40) *Comment:* The draft economic analysis states in Table 3–Pet Stores Industry that the annual payroll for all is less than the annual payroll for small business. That does not seem right.

Response: We corrected the table in the final economic analysis.

(PR41) *Comment:* The draft economic analysis states in 2.3.5 U.S. Bred Salamanders, "Domestically bred salamanders would represent less than one percent of the United States salamander sales between 2012 through 2014 if this data depicts the

entire domestic supply." This is confusing because table 10 states that 76 percent of commercial salamanders are U.S. bred.

Response: The 1 percent refers to all salamanders, whereas the 76 percent refers to the species and genera listed in table 10. We amended table 10 in the final economic analysis to clarify this point.

(PR42) *Comment:* The draft economic analysis states under 3.1.1 Analysis of Economic Benefits, "Fewer outdoor recreationists could lead to a decrease in expenditures; to demonstrate we use $25,000. Implementing a fictional alternative, Alternative Y would reduce the probability of Bsal establishment to 10 percent from 80 percent. The expected costs in the current situation would be $20,000 ($25,000 × 0.8); with Alternative Y, the expected costs would be $2,500 ($25,000 × 0.1). Net avoided costs would be $17,500 ($20,000 - $2,500), one measure of the benefits of Alternative Y." The commenter finds this example to be confusing and suggests omitting.

Response: We deleted the example in the final economic analysis.

(PR43) *Comment:* In the Executive Summary of the draft economic analysis, you describe some of the potential costs of the regulation (for example, lost consumer surplus for pet owners). It seems in the discussion under 3.1.2 Analysis of Economic Costs that the costs in the cost/benefit sense are being conflated with lost revenue and the economic analysis. This is okay, but this section could be more clear.

Response: The Executive Summary indicates that consumer surplus cannot be estimated under the scope of this report and that an alternate methodology will be used. Sections 3.1.1–3.1.2 explain how the analysis uses the maximum sales as a proxy for the direct economic losses. No economic benefits are evaluated for the existence of a species in this report.

(PR44) *Comment:* The draft economic analysis states under 3.1.2.2 Small Business, "Estimates using the unique importers (average of 5 a year), or one breeder,

yield the maximum adverse impacts; no fewer entities would be impacted under the status quo. Applying these two methods brackets the impacts on importers and pet stores." It is unclear what "average of 5 a year" means.

Response: We changed "average of 5 a year" to "annual average" in the final economic analysis. The analysis was also updated to an annual average of six importers.

(PR45) *Comment:* In the draft economic analysis, the numbers in the sectors columns of tables 12–14 do not seem to correspond to anything. Could this column be omitted?

Response: The columns were deleted in the tables.

Public Comments and Our Responses

We reviewed all 280 comments we received during the public comment period for the 2016 interim rule (81 FR 1534, January 13, 2016). We received comments from Federal agencies, State agencies, commercial and trade organizations, conservation organizations, nongovernmental organizations, and private citizens. The comments provided a range of views on the proposed listing as follows: (1) Unequivocal support for the listing with no additional information included; (2) unequivocal support for the listing with additional information provided; (3) equivocal support for the listing with or without additional information included; (4) unequivocal opposition to the listing with no additional information included; and (5) unequivocal opposition to the listing with additional information included.

While all comments were reviewed and considered, several comments did not contain information that was new compared to other comments or included substantial information that required analysis. Comments included individual ideas, data, recommendations, or suggestions on the interim listing and the draft economic analysis. Some commenters addressed the 14 questions we posed in the 2016 interim rule. We consolidated comments and responses into key issues in this section. We edited some

comments for brevity or grammar while maintaining the intent. We combined comments that expressed similar perspectives.

Use of Scientific and Common Names

(1) *Comment:* The Service asked, for the species being listed in the 2016 interim rule, if the scientific and common names are the most appropriate ones accepted by the scientific community. Most of the herpetological community uses the Society for the Study of Amphibians and Reptiles joint societies-endorsed list (Crother 2012); both the Association of Fish and Wildlife Agencies (AFWA) and Partners in Amphibian and Reptile Conservation (PARC) use this nomenclature in our formal publications. However, some States use other nomenclature, while some others use older nomenclature simply due to the inability to update frequently.

Response: The comment identifies the disparate use of scientific and common names used among herpetological and management entities. We believe this approach supports our decision to remove the enumerated list of species within each genus in 50 CFR 16.14 for the second interim rule. Each species within each genus will therefore be included as injurious wildlife in the list of injurious amphibians.

(2) *Comment:* There are quite a few errors (some species listed twice under different Latin names) in the proposal.

Response: The commenter did not provide specific examples, so we cannot check this comment with additional references. The comment does support, however, our decision to remove the enumerated list of species within each genus in 50 CFR 16.14 for the second interim rule.

Listing of Preserved Specimens, Parts, and Eggs and Gametes

(3) *Comment:* Scientific specimens of salamanders that are desiccated or have been fixed or preserved in formalin or alcohol should be exempt from this rule because Bsal is no longer viable.

Response: We concur that preserved specimens do not pose a risk for pathogen transmission as long as chemical preservation is adequate to kill Bsal, and we have removed chemically preserved specimens from the reach of this final rule.

(4) *Comment:* What is included in the definition of "parts of salamanders" and why? Listing swabs makes testing for disease more difficult, which could adversely affect the intended effect of the rule. Please provide an exemption for tissue samples (including histological samples), molecular extractions, swabs, and other parts.

Response: Any item that contains cells or genetic material from a listed species is considered a "part" of the listed animal. This definition is not unique to the salamander rule but is consistent with standard regulatory definitions used by the Service. Specimens, such as skin swabs and tissue samples for microscopic analysis (histology), are included as "parts" in the rule consistent with the definition of "fish or wildlife" outlined in 50 CFR 10.12, which includes "any part, product, egg, or offspring thereof." Also, 50 CFR 10.12 states that "amphibians" means a member of the class, Amphibia, including, but not limited to, frogs, toads, and salamanders; including any part, product, egg, or offspring thereof, or the dead body or parts thereof (excluding fossils), whether or not included in a manufactured product or in a processed food product. Specimens such as swabs intended for culture or in transport or growth media will require permits. We may issue permits to facilitate all of the above-described activities. For purposes of this rule, eggs and gametes and purified extracted genetic material of salamanders are excluded from the prohibitions as "parts" because they are unable to cause pathogen transmission. However, swabs and histological samples that are preserved or fixed in appropriate concentrations of ethanol or formaldehyde-based solutions are also not injurious as long as chemical preservation is adequate to kill Bsal as described in current peer-reviewed literature. The appropriate concentration and minimum exposure time for a given chemical preservative or fixative to render any Bsal organisms non-viable varies with the

precise chemical formulation and should be utilized as described in association with such actions in the peer-reviewed literature. Please also refer to IV. Second Interim Rule.

Purpose of Listing As Injurious

(5) *Comment:* Several comments provided feedback on whether eggs and gametes should be included in this rule. As a comment noted, specimens require transport with some form of medium, such as water or plant materials, to remain viable, and that medium could harbor Bsal, thus constituting a threat by indirectly moving disease vectors with the eggs or gametes and increasing the risk of indirect Bsal transmission. Further, eggs at certain stages of development could contain keratinized tissues (for example, Xie and Yu (1992)), which could transmit the Bsal pathogen. However, other comments noted that if entire genera are excluded from the listing because they cannot be infected, then the relative risk from the transport of eggs is no greater.

Response: Our authority does not include the listing of the medium, such as water or plant materials, that the specimens are transported in. As noted in this rule, there is no evidence that salamander reproductive material also contains keratin that might harbor Bsal. Therefore, eggs and gametes are not listed by this rule.

Effect of Rule on Scientific Research

(6) *Comment:* The rule will have a negative impact on scientific research, especially on native taxa. The prohibition should not apply to scientific research, providing that the biologist in question is in possession of an approved permit from the State where the specimen(s) were originally collected.

Response: Permits from the Service for injurious listed species can be obtained for scientific, zoological, educational, and medical use for importation, shipment between the enumerated jurisdictions in 18 U.S.C. 42(a)(1) (codified in Federal regulations in 50 CFR 16.3), and transport for a previously permitted salamander. The statute does not cover

collection of native species or transport of injurious listed salamanders across State lines within the continental United States (see PR15).

Species Not on the List

(7) *Comment:* Several commenters advocated for adding various genera or listing at the family level, such as Salamandridae, while others advocated for listing all species.

Response: The salamander species listed by this final rule and the second interim rule are those found within a genus for which we have confirmation that at least one species in that genus is a carrier of Bsal, and there is no conclusive countervailing evidence suggesting that some species within the genus are not carriers. Although additional salamander species could be at risk from Bsal infection or could serve as a carrier, we are not listing species in those genera because they had not yet been tested. We considered listing more species based on the comments we received. However, the logic we used for listing at the genus level breaks down at the family level for one family. In the family Plethodontidae, the genus *Gyrinophilus* is not known to be a carrier, but the genera *Hydromantes* and *Plethodon* are carriers. As a result, we cannot list all species within Plethodontidae. We also cannot list a species without science-based documentation. We can list for the statutorily defined purposes under the statute codified at 18 U.S.C. 42(a); any other purpose is beyond the scope of this rulemaking. Please see *IV. Second Interim Rule* below for additional genera we have documented as injurious and are therefore listing.

(8) *Comment*: Some comments noted that while some salamander species appeared to be resistant to Bsal in infection experiments, it is unclear how strong this resistance will be outside of the optimal husbandry conditions found in laboratory settings.

Response: As part of the justification for listing, the Service acknowledges that salamander species known to be tolerant of Bsal infection under experimental conditions

may demonstrate more severe clinical disease when infection is combined with additional stressors in the wild, as has been found for other diseases, including those in amphibians (Wobeser 2007; Kerby et al. 2011; Kiesecker 2011). However, the Service needed evidence that a species was a carrier or likely to be a carrier before listing the genus as injurious.

(9) *Comment: Tylototriton podichthys* was recently described and should be added to the list (Phimmachak et al. 2015).

Response: All species in a genus are also listed as injurious even if they are not specifically identified in the rule. Because we identified *Tylototriton* as one of the genera listed in the 2016 interim rule and hereby affirmed, *T. podichthys* is one of the species listed as injurious. The comment supports our decision to remove the enumerated list of species within each genus in 50 CFR 16.14 for the second interim rule.

(10) *Comment:* The Service should establish an expedited process by which additional salamander species can be added to the list as new information becomes available.

Response: Rulemaking under 18 U.S.C. 42 is governed by the APA, under which we promulgated the 2016 interim rule and this final rule. The Service is adding new genera to the list with the second interim rule in this document.

Species Should Be Removed From the List

(11) *Comment:* Species from the genera *Cynops*, *Salamandra*, *Pleurodeles*, *Siren*, *Notophthalmus*, and *Triturus* should be removed. They are the most commonly kept species and listing will significantly affect those who raise, study, or keep animals from these species.

Response: Due to shared characteristics by species within a genus, other species within these genera are also likely to be carriers of Bsal. The Service found that species from the genera *Cynops*, *Salamandra*, *Pleurodeles*, *Siren*, *Notophthalmus*, and *Triturus*

can carry Bsal and, therefore, pose a substantive risk to native salamander populations. The listing of these species as injurious wildlife does not regulate possession, transport, breeding, or sale within the continental United States unless regulated under permit. Other Federal, State, Tribal, or Territorial laws may apply.

(12) *Comment:* No native species should be listed. Listing native species as injurious wildlife solely on the basis of their vulnerability or capacity to carry an absent foreign pathogen is concerning. Additionally, most of the animals tested that were lethally vulnerable were dead within about a month, as per Martel et al. (2014), and the odds of any of these animals being available for sale while carrying the disease are almost nonexistent.

Response: We listed native species in the 2016 interim rule partly because some native species that we concluded can be carriers of Bsal are raised outside the United States and imported into the country and partly because listing would prohibit transport of injurious salamanders between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (codified in Federal regulations at 50 CFR 16.3), in order to prevent introduction, establishment, and spread of the pathogen in U.S. ecosystems. Several native species of newts were already known to be highly susceptible to dying from Bsal. Not all species die immediately upon exposure to Bsal, and there is no evidence that lethally vulnerable species cannot survive long enough for Bsal to be transmitted within the United States if they are infected prior to their movement. At the time of the drafting of the 2016 interim rule, most of the research was being conducted on Asian and European species to find out where the fungus may have originated and why wild European salamanders were dying. After the 2016 interim rule published, many studies by U.S. researchers began to provide information for the conservation of native species in the event Bsal is introduced into the American environment. These studies demonstrate that many native salamanders are susceptible and can be Bsal carriers.

(13) *Comment:* Many of the listed species in some genera, such as *Plethodon*, *Taricha*, and *Notophthalmus*, have never been found to carry Bsal. These species should be delisted.

Response: New information confirms that species from the genera *Plethodon*, *Taricha*, and *Notophthalmus* can carry Bsal based on laboratory studies. As of the drafting of the second interim rule, all three species of *Notophthalmus* have been found to be lethally susceptible to Bsal (Gray et al. 2023), and two of the four *Taricha* species are carriers (Gray et al. 2023).

(14) *Comment:* The listing of the entire genus *Plethodon* is based on the Martel et al. (2014) study from a sample of two wild-caught *P. glutinous* imported to Europe. Under the circumstances, the evidence suggests that all species in the genus *Plethodon* should be removed from the list.

Response: We disagree with the comment. While Martel et al. (2014) classified the slimy salamander (*Plethodon glutinous*) as resistant to infection, the study also demonstrated by histology that Bsal could invade the skin of the slimy salamander, even though it apparently cleared the infection and did not show signs of clinical disease. Our examination of the supplementary data of Martel et al. (2014), including histology (microscopy) tests and subsequent discussions with the authors, indicates that there is sufficient evidence that Bsal was able to invade the skin of this species long enough to move or transmit the infection to other salamanders (Martel et al. 2014; A. Martel, University of Ghent, pers. comm. 2015; K. Lips, University of Maryland, pers. comm. 2015). Because we expect all species within a genus to respond in a similar way for Bsal carrier status, we conclude that all species of *Plethodon* are potential carriers. Since the 2016 interim rule published, additional studies have shown multiple species in the genus *Plethodon* can be carriers (DiRenzo et al. 2021); see *IV. Second Interim Rule*.

(15) *Comment:* Some species from the genus *Neurergus* have been bred over many generations and are in private collections (*N. crocatus*, *N. kaiseri*, and *N. strauchii*). *N. kaiseri*, which is listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I, has not been imported for years, and most of the animals in the United States are descendants of zoo colonies and hobbyist captive-bred animals. Because they are protected by other laws and not imported, they do not have any risk of transmitting Bsal and there is no need to list them.

Response: Regardless of protection level under other laws, these species are still injurious wildlife under 50 CFR 16.14 as part of the genus *Neurergus*. Their protection level under these other laws does not change the characteristics of the species that we find to be injurious to wildlife and the wildlife resources of the United States by reason of their potential to serve as vectors for the pathogen Bsal. Also, there is no way to confirm that captive-bred salamanders have not been exposed to Bsal through contact with other individuals. *Neurergus* has been confirmed to carry Bsal in a European collection (Fitzpatrick et al. 2018), and there is a chance a co-housed salamander of a different species could be imported into the United States.

Need for Rule

(16) *Comment*: The rule is unnecessary. The prohibition can be justified only if Bsal is found to be present in the United States. Other commenters stated that the rule is unnecessary because Bsal must be here already given the number of salamanders imported annually and their belief in the low likelihood of a captive salamander coming into contact with the wild populations and transmitting Bsal.

Response: Since the publication of the 2016 interim rule, Waddle et al. (2020) conducted a large-scale surveillance for Bsal across 594 counties in 35 States and 1 site in Mexico with 11,189 swab samples of wild salamanders and some frogs and toads, with no positive results for Bsal. The purpose of listing these species as injurious wildlife is to

prevent the introduction of the Bsal fungus in the wild in the United States. A species does not need to be already present in trade or in the environment to be listed as injurious wildlife. In fact, it is often difficult to achieve a prevention outcome once a species or pathogen occurs in the environment. To make the listing determination for salamanders, we drew upon the results of multiple independent risk assessments and our own analysis and found that Bsal is likely to be introduced into the United States if no additional risk mitigation steps are taken. Additional discussion on this topic can be found in *IV. Second Interim Rule* under the section *Likelihood of Release or Escape*.

(17) *Comment:* To list a native species of wildlife as injurious simply because it may act as a host to a rare but potentially devastating pathogen that has not been detected in the United States is an unmanageable proposition. Every native species of wildlife fits this criterion and would need to be listed as injurious for some rare pathogen detected in a very isolated outbreak on another continent, as has occurred with Bsal.

Response: The purpose of listing these species as injurious wildlife is to act preemptively to prevent the introduction, establishment, and spread of the Bsal fungus in the wild in the United States. The fungus affects many native salamanders, with lethal effects on many salamander species, and it is not yet known to be found in the wild in the United States. There is an existing pathway for the fungus to arrive by importation of salamanders, including species native to the United States that are raised in captivity outside of the United States and then imported back into the continental United States and the enumerated jurisdictions of the shipment clause. This regulatory action is being taken to prevent Bsal's arrival through the organisms-in-trade pathway. If we wait until the fungus arrives, it will likely be impossible to eradicate. We will continue to evaluate other species for possible risks and consider injurious wildlife actions as appropriate and authorized under 18 U.S.C. 42(a).

(18) *Comment:* Bsal can be treated and cured in captivity, so there is no reason to limit availability of the species in question.

Response: Voluntary actions, such as applying heat therapy as described in Blooi et al. (2015a) and Blooi et al. (2015b), may help reduce the threat posed by Bsal for specimens held in captivity. However, at this time it is not possible to determine the likelihood of success of such measures for all species or of achieving compliance with prophylactic treatment or treatment following the onset of symptoms. Therefore, it is unknown how effective treatment will be in preventing Bsal's introduction, establishment, and spread in the United States, and no Bsal control is known for salamanders in the wild.

(19) *Comment:* If a species that is not a carrier is similar in appearance with another species, neither species should be removed from the list unless both species are confirmed that they are not susceptible to or carriers of Bsal.

Response: The Service does not have the authority under the statute to list a species based solely on its similarity of appearance to another species. We list based on our determination of injuriousness.

(20) *Comment:* There is no case in the United States where salamanders, native or nonnative, have been proven as invasive or injurious. The 2016 interim rule does not substantiate injury by transplanted or exotic salamanders.

Response: The salamanders are listed because they are carriers of a fungus that makes them harmful to other salamanders, not because the salamanders are invasive. We concluded that even if the salamander species listed by this rule do not become established, some species capable of carrying Bsal and listed by this rule can survive long enough in the wild to transmit Bsal. Our findings are discussed in *Potential To Survive, Become Established, and Spread* in *IV. Second Interim Rule.*

Listing Purpose Is To Regulate Disease or Manage Native Species

(21) *Comment:* Listing salamanders as injurious is not an appropriate means to regulate an animal disease. The injurious wildlife provisions of 18 U.S.C. 42 pertain to animals and not diseases or pathogens. The focus of the 2016 interim rule is Bsal, a fungus that the Service possesses no authority to regulate as acknowledged in the interim rule. The law provides no provisions for testing, surveillance, or certification of health to allow for movement in trade.

Response: As we described in *Listing Species That Carry Pathogens* in the 2016 interim rule, the Service can list as injurious any member of the enumerated taxa that are hosts to or carriers of pathogens that cause the host or carrier to be harmful by its presence to one or more of the interests listed in the statute. We have previously listed species that serve as hosts to or carriers of pathogens, as in the case of fishes in the salmon family (Salmonidae) (32 FR 20655, December 21, 1967). We noted in the 2016 interim rule that there are concerns regarding the effectiveness and sensitivity of current testing methods (including the return of false negatives), lack of validation and sufficient testing capacity, and agency resources required to conduct inspections, interpret results, and issue health certificates. If these issues are resolved, it may be possible to establish a health certificate for salamanders that are free of Bsal. A health certificate was established for import of salmon under the authority of 18 U.S.C. 42. While the concerns remain, and therefore a Bsal health certification has not been established, this does not mean that there is no authority to establish a health certification if circumstances were to change. Appropriate conditions may also be included in injurious wildlife permits under the authority of and consistent with the purposes of 18 U.S.C. 42.

(22) *Comment:* Several commenters noted that, by definition, “pathogens” are injurious and are regulated under the authority of other agencies. The World Trade Organization and the United States Department of Agriculture (USDA) recognize the World Organisation for Animal Health [WOAH, formerly OIE] as the proper body to set

animal health standards. The WOAH helps develop and revise international standards for the safe trade of animals and animal products. The proper course to prevent the importation of salamanders carrying Bsal is to list the pathogen as a WOAH reportable disease, and instead of the 2016 interim rule, there should be a cooperative effort to respond to the disease threat as provided through the WOAH, World Trade Organization, and the National Aquaculture Health Plan and Standards (formerly called the National Aquatic Animal Health Plan) for the United States.

Response: The USDA and the Centers for Disease Control and Prevention have authority to regulate wildlife pathogens when those pathogens pose a risk to agriculture or human health, respectively. No such effects are currently known in the case of Bsal. The Service has authority to regulate the importation of certain species that pose a risk to wildlife and the wildlife resources of our country. This authority has been applied in the present case in response to a clear and immediate risk.

After the 2016 interim rule took effect, the WOAH did add Bsal as a reportable disease, but that action does not prevent importation. We work through such mechanisms as those provided by the WOAH and National Aquaculture Health Plan and Standards, and we support all efforts by the international community to participate in the global response to this pathogen. The Service, operating within its relevant regulatory authority to list injurious wildlife, took action through the 2016 interim rule due to the urgent need required to manage the threat Bsal poses to salamanders in the United States.

(23) *Comment:* This salamander rule not only prevents safe commerce, it eliminates any incentive for industry to pursue research into the detection and treatment of Bsal. Other comments expressed similar issues and asked whether it would be possible to make testing mandatory to allow interstate movement.

Response: While the Service acknowledges that some economic incentive may have been removed due to the prohibitions imposed by the injurious wildlife provisions

of the Lacey Act as a result of listing species of salamanders as injurious wildlife under this rule, many salamander genera were not listed due to insufficient evidence at the time as carriers, and they remain a possible threat. Furthermore, research for detection and treatment of Bsal has increased considerably in the United States since the rule took effect. Permits allowing importation can be obtained for zoological, educational, medical, and scientific use. This final rule explains that interstate transportation between States within the continental United States is not prohibited as of 2017; however, the injurious wildlife listing still prohibits import into the United States, and transport of injurious wildlife between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42(a)(1) (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), codified in Federal regulations at 50 CFR 16.3.

(24) *Comment:* The Lacey Act does not provide authority to list native species. The Lacey Act has been examined and critiqued over the last few decades within a variety of peer-reviewed and gray-literature publications (Dentler 1993, U.S. Congress 1993, Anderson 1995, Whalen 1998, Biber 1999, Jenkins et al. 2007, Alexander 2013). In no instance did these authors construe Lacey Act provisions to allow the listing of native animals as injurious. Notably, the U.S. Congress, Office of Technology Assessment, recommended in 1993: Congress could provide the Service with increased guidance on the purpose of this [injurious] list and the specific criteria for adding species to it.

Response: The provisions of 18 U.S.C. 42(a)(1) do not limit wildlife subject to the law to species not native to the United States. Under the law, the Service may list species that are indigenous to the United States if they cause injury to the interests enumerated in the law. The publications mentioned reflect the interpretations of the authors. Congress has also listed native species as injurious by statute, such as the Mariana fruit bat

(*Pteropus mariannus*), further demonstrating that the authority of 18 U.S.C. 42 is not limited to nonnative wildlife.

Additional Science and Data for Rule

(25) *Comment*: In the 2016 interim rule, the Service asked what species listed as threatened or endangered by one or more States would be affected by the introduction of Bsal. AFWA and several States indicated that several salamander species are of interest to them, though it is not yet evident how Bsal would affect all of these species. A number of State threatened and endangered or protected species (restricted or prohibited from take, possession, sale, or other activities) were provided during the public-comment period.

Response: We appreciate the additional information on State threatened and endangered species. While the Service concluded that some species identified by the States are not carriers, others are, such as species in the genus *Plethodon*. The carrier status of several species, at the time the public-comment period closed, had not yet been identified. However, more have been identified since then, including affirming the genus *Plethodon* in this final rule. This additional information helps provide additional justification for listing species that are capable of carrying Bsal, as Bsal presents a risk to wildlife and wildlife resources of the United States, including those identified by the States as in need of protection.

Pathways and Spread

(26) *Comment*: The Service asked the question, "Are there other pathways for Bsal into the United States that we should address? If so, what are they?" According to AFWA, a pathway of concern that appears to have little or no Federal regulatory authority or enforcement pertains to biological supply companies. Others include internet sales involving small shipments using couriers such as FedEx or UPS, traditional medicine or foreign food markets, and ceremonial uses of these species. AFWA is aware

of interstate shipments of some salamanders, though not necessarily the currently included species, for the purposes of the bait trade, but AFWA would like to see some exploration of whether there are imports for this purpose.

Another comment noted that, while the pet trade is an important pathway, salamanders may stow away in nursery stock, as was observed with northwestern salamanders (*Ambystoma gracile*) in Christmas trees (Rochford et al. 2015). In addition to terrestrial nursery stock, the aquatic plant and animal trade may also spread Bsal in shipment water.

Response: The Service's pathway analysis found that the main pathway for the global spread of Bsal is the international trade in salamanders, such as Martel et al. (2014) noted. While not explicitly discussed, that international trade could include the uses noted in the comment, whether intentional or as a hitchhiker. Biological supply and bait companies are commercial entities. These companies have always had to comply with import and export regulations under 50 CFR part 14. With this injurious listing, these commercial businesses will be subject to the same prohibitions as other entities. Likewise, animals unintentionally imported or transported between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42(a) (also set forth at 50 CFR 16.3) through nursery stock or other pathways would also be in violation of the injurious wildlife listing's prohibitions. As explained in *A. Background* in *Final Rule to the 2016 Interim Rule*, interstate transport between States within the continental United States is not prohibited by the current prohibitions of 18 U.S.C. 42(a).

(27) *Comment*: Bsal is known to persist in a moist environment for up to 7 weeks, even without an amphibian host. This ability creates an alarming pathway for the potential spread of Bsal into the United States through a variety of means not fully addressed by the 2016 interim rule. This unchecked pathway of Bsal into the United

States presents a major limitation in our ability to prevent introduction of this potentially devastating infectious wildlife disease.

Response: Materials that can transmit pathogens, such as water, represent a potential pathway. However, the Service does not have authority under the injurious wildlife listing provisions in 18 U.S.C. 42(a) to prohibit importations of water and fomites that may be infected with the Bsal pathogen. Listing the species that can carry Bsal is expected to limit the movement of such materials, but they do remain a concern. The Service will continue to explore opportunities to address this issue with partners and stakeholders.

(28) *Comment*: A comment suggested that it is premature to discount frogs and toads (anurans) and caecilians from getting Bsal.

Response: Under this final rule, we listed salamanders for which we had affirmation at the time of the rule drafting that they could carry Bsal into the United States, and subsequent evidence confirms the determination. We do know about positive Bsal test results for several species of anurans and will continue to monitor research on them and caecilians and on salamanders for which data is currently unavailable.

(29) *Comment:* The rule is unnecessary and will only hurt hobbyists. Hobbyists who keep salamanders may be tempted to release them into the wild if they cannot find alternatives and do not want to euthanize them. If so, the risk of Bsal being introduced into the wild might be increased.

Response: We believe this regulatory action will safeguard the health of wild salamanders and those kept in captivity. We have taken action with this rule to list salamanders that we find can carry Bsal. Pet owners will still be allowed to keep their salamanders and sell or give them away within the enumerated jurisdictions of 18 U.S.C. 42, also set forth at 50 CFR 16.3. In addition, many States have laws making it illegal to release certain animals into the wild, and injurious listed species cannot be released into

the wild under Federal law. Some States have amnesty programs that accept unwanted pets. The Service believes that the majority of pet owners and hobbyists would not intentionally release their animals into the wild; however, the pet trade was identified as the major vector for a potential Bsal invasion. To assist pet owners who might need to find homes for their animals, we posted information about responsible alternatives to releasing salamanders on our website when we published the 2016 interim rule. That updated information can be found at *https://www.fws.gov/node/266100*.

(30) *Comment:* The interstate prohibition will not help prevent the spread as the zoospores are most likely going to be spread through moving water. Also, many wildlife diseases are moved by wildlife themselves, including migratory birds. Without evidence of infected animals in the trade, it is inappropriate to indict an industry or to blockade any trade based on speculation. Additional studies are needed to determine sources and causes for outbreaks. Without further surveillance and supportive data, it cannot be substantiated that the international and interstate trade is the vector for spread of this disease.

Response: As we note in the final rule, the interstate prohibition has been clarified. In the 2016 interim rule, we did not indicate that the absolute cause of the spread of Bsal is the wildlife trade, although we concluded that the most likely pathway of Bsal into the United States is on the bodies of salamanders in the commercial salamander trade. We cited peer-reviewed journal articles that suggest the spread of Bsal has been human mediated due to the discontinuity of the global distribution of Bsal between Asia and Europe, and we cite the detection of the pathogen in imported captive exotics. Both of these pieces of information suggest the spread of Bsal has been human mediated. Other pathways for Bsal introduction are not expected to be as significant compared to the international-trade pathway. While the Service is concerned about contaminated water, Bsal is not yet known to be present in the United States. Listing is

intended to prevent the introduction, establishment, and spread of Bsal. Salamanders would have to come into contact with Bsal-contaminated water for the pathogen to be introduced. If no infected salamanders are here, they cannot transmit the pathogen to waters that can further spread the pathogen.

Research suggests that waterfowl can carry Bd on their toes, although Bd could not survive more than 60 minutes of desiccation on the scale tissue (Garmyn et al. 2012). As a result, while Bd could be transmitted from one habitat to another on short flights, transmission is unlikely to be an intercontinental threat. Given the similarities between Bd and Bsal, Bsal is not likely to be introduced to the United States through bird migrations.

Border Interstate Transportation

(31) *Comment:* The prohibition on importation will help to prevent the movement of Bsal into the United States provided that it is also prevented from entering Canada. If an infected salamander enters through Canada, Bsal could be transported via water and waterfowl into the United States, negating the prohibition's benefits.

Response: In 2017, after the 2016 interim rule was published, Canada passed a law prohibiting importation of all species of the order Caudata, alive or dead, and their gametes (ECCC 2017, 2018). Canada, Mexico, and the United States actively coordinate in wildlife conservation issues through the Canada/Mexico/U.S. Trilateral Committee of Wildlife and Ecosystem Conservation and Management meetings.

(32) *Comment:* The interstate prohibition will make it harder to acquire scarce animals. Prohibiting interstate movement will hurt honest hobbyists who are working hard to find or produce healthy captive-bred animals. The prohibition should apply only to wild-caught animals or importation only but allow for movement of captive-bred animals in the United States that have been tested and found to be free of Bsal, especially since Bsal has not been found in the United States.

Response: As explained under *A. Background* in *II. Final Rule to the 2016 Interim Rule*, the interstate prohibition has been clarified. Under 18 U.S.C. 42, the Service does not have the authority to selectively prohibit the importation of wild-caught or captive-bred animals for a species listed as injurious wildlife. Permits can be acquired for zoological, educational, medical, or scientific purposes.

Effect on Hobbyists

(33) *Comment:* Captive-breeding should be legal for private and hobbyist purposes.

Response: Captive-breeding is not prohibited by the injurious wildlife provisions of the Lacey Act as a result of listing species of salamanders as injurious wildlife under the rule.

(34) *Comment:* The rule will have a direct effect on both amphibian business owners and hobbyists as well as native ecosystems. The species that are listed are those most important to the hobby—animals that are easy to breed and that do well in captivity. The rule effectively transitions the hobby almost entirely away from captive-breeding. Captive-bred animals are healthier, less likely to carry diseases, more likely to thrive in captivity, and do not harm wild populations. Commercially wild-collecting animals can cause long-term damages to populations and has been known to play a role in disease transmission as collectors travel between areas and do not disinfect their equipment.

Response: The commenter states that captive-bred animals are healthier and less likely to carry diseases but does not provide evidence to support this statement. State wildlife agencies are responsible for regulating the collection of most wild salamanders, including injurious listed ones, and State authorities can be used to protect populations from overharvest.

(35) *Comment:* The science is wrong on the number of salamanders crossing State lines. The commenter knows one individual who sold 1,500 captive-bred tiger

salamanders last year outside their State. The interstate prohibition will cause a drop in the diversity of captive-bred species and related expertise in the country. This prohibition will severely limit many forms of research since expert American salamander keepers will be unable to maintain and share their experience through captive-breeding programs. Researchers will be limited largely to axolotls (*Ambystoma mexicanum*), which may not work for their needs. Even Martel et al. (2014) was largely dependent on captive-bred animals; in a few years, a similar study will be impossible from the United States.

Response: The rule will not end scientific endeavors that would benefit the injurious listed species. Additionally, as explained in this final rule, the prohibition on interstate movement between States within the continental United States has been clarified.

(36) *Comment:* The rule interferes with educational opportunities and exposes exhibitors, nature centers, wildlife rehabilitators, private citizen hobbyists, and commercial breeders to Federal prosecution and penalties under the Lacey Act.

Response: The rule is intended to protect native species, which will help ensure that the public maintains the opportunity to enjoy them in their wild habitats. Also, the injurious wildlife provisions of the Lacey Act do not prohibit ownership or breeding of injurious wildlife, unless unlawfully imported or transported between the enumerated jurisdictions or otherwise restricted due to conditions associated with issued permits. People and zoological institutions can still own salamanders where consistent with other Federal, State, and Tribal laws and regulations applicable to the species. The listing also will not prevent the continued use of these species for education, and prohibited activities may be authorized by permit for zoological, educational, medical, or scientific purposes (in accordance with permit conditions). Finally, as explained in the final rule, the interstate prohibition between States within the continental United States has been clarified.

Effect on Conservation Efforts

(37) Comment: Captive-breeding has been proven to be the most reliable way of ensuring the survival of endangered (or common) species. Furthermore, captive-breeding provides a backup gene pool for wild populations that may be drastically reduced from Bsal. Also, the listing would make it illegal to transport listed salamander species across State lines and would devastate conservation programs across the United States. The permitting process will keep many zoos and aquariums from participating in propagation efforts of salamander species on the list, many of which need help.

Response: While captive-breeding is useful in many cases to ensure survival, it is less so when a novel, lethal pathogen is the cause. Listing the species as injurious in this rule will not affect legitimate conservation efforts that U.S. breeders can carry out for the species. The law allows for the issuance of permits authorizing otherwise prohibited movement or imports for scientific or zoological purposes, including non-commercial conservation breeding operations. The Service has provided information online to help people apply for a permit (see *Permitting Difficulties* below in this comment discussion for additional details). Finally, as explained in the final rule, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States.

(38) *Comment:* When scientists collect tissues or specimens for lab experiments, the animals are never released into the wild and therefore pose no threat to the spread of Bsal or any other pathogen. The Service's imposition of increased Federal permitting will inhibit scientists who are studying the biology of regulated species and may dissuade graduate students or other biologists from such work. This type of regulatory change can hinder conservation efforts before their need can even be evaluated.

Response: This listing should not adversely affect any valid conservation efforts. In general, all wildlife species must be declared at the time of importation (see 50 CFR

part 14), but most do not require special permits. Prior to this rule, only species of salamanders listed under the Endangered Species Act (ESA) or CITES required import permits under those wildlife laws implemented by the Service. For injurious wildlife, permits are not needed for interstate transport between the States within the continental United States (except into or out of the District of Columbia), and permits to allow import and transport between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42(a) may be granted for bona fide scientific purposes. This rule should have no significant effect on any conservation efforts that are currently being or will be carried out.

(39) *Comment:* One commenter has never owned a pet frog or salamander yet has educated more than 3,000,000 people about amphibians via online and printed educational materials and through live presentations and hikes to amphibian habitats to see local, native wild amphibians. The commenter states that truly inspirational amphibian experiences occur when humans come across wild amphibians, not captive amphibians.

Response: The Service encourages visitors to the Service's national wildlife refuges and other public lands to appreciate salamanders in their natural environments. The purpose of listing these salamander species as injurious wildlife is to prevent the introduction, establishment, and spread of Bsal in the wild in the United States to protect wildlife and wildlife resources, including native salamanders in the wild.

(40) *Comment:* The rule prevents the ability of salamander owners to further test their collections and, therefore, could unintentionally increase the spread of this disease rather than decrease it, if it arrives in this country. Another commenter noted that the current prohibition, especially on interstate movement, will discourage cooperation to get domestic collections tested for the disease.

Response: As explained in the final rule, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States. Treatment and testing that does not involve import into the United States, transport between the enumerated jurisdictions in 18 U.S.C. 42(a) (also set forth at 50 CFR 16.3), or injurious wildlife permits are not regulated by this rule.

(41) *Comment:* The rule does not list members of the *Ambystoma* genus, so this omission may increase the chances of legal and illegal collection of *Ambystoma*.

Response: Listing a species as injurious wildlife results in prohibitions on import into the United States and shipment between the enumerated jurisdictions in 18 U.S.C. 42(a), codified in Federal regulations at 50 CFR 16.3. Neither listing a species as injurious nor not listing it results in a prohibition on collection. It is the responsibility of a person who may be engaged in salamander collection to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to such activity. For example, some salamanders are federally protected from take (including, but not limited to, collection) under the Endangered Species Act, and other laws or regulations may otherwise prohibit or regulate collection of other salamanders in national wildlife refuges, national parks, or other Federal lands, or in accordance with State or Tribal laws. While it is possible that some people will switch to *Ambystoma* spp. in place of a listed species if they want to keep salamanders, they may currently do so in States where it is legal under State law. We are listing the genus *Ambystoma* with the second interim rule as a way to prevent the potential introduction of the fungus.

Permitting Difficulties

(42) *Comment:* Multiple commenters expressed concern that the listing would complicate research efforts or breeding programs for recovery efforts for some native

salamanders due to extended permit-application processing time and limited Federal resources to adequately address an increased number of applications.

Response: As explained in the final rule, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States. Fewer permit requests will be required because interstate transport between States within the continental States is not prohibited.

(43) *Comment:* The Service should consider adopting a cooperative agreement or memorandum of agreement to allow easier movement of prohibited species for certain purposes.

Response: Several commenters suggested memoranda of understanding (MOUs) or other mechanisms in lieu of permits. Those arrangements cannot be used to authorize import or transport between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), which are codified in Federal regulations at 50 CFR 16.3. Other interstate transport between States within the continental United States is not prohibited by 18 U.S.C. 42(a). The text of 18 U.S.C. 42(a)(3) requires that exceptions to otherwise prohibited activities with injurious wildlife be authorized by permit, and only if there has been a proper showing of responsibility and continued protection of the public interest and health. The regulations at 50 CFR 16.22 specifically provide that the Service may issue a permit authorizing the importation into or shipment between the continental United States. Thus, MOUs cannot be utilized for authorizing import or shipment between the enumerated jurisdictions. We have provided information online that helps people who are requesting a permit understand and navigate the process at *https://www.fws.gov/node/266100*. The Service is committed to processing permit applications as quickly as possible to minimize

any delay or disruption of legitimate activities. Permit applications can be found here: *https://fws.gov/service/3-200-42-import-acquisitiontransport-injurious-wildlife-under-lacey-act*.

(44) *Comment:* A commenter recommends that, to receive a permit to transport potentially infectious (non-inactivated) material, be it live or dead salamanders, parts of dead salamanders, or biological samples, one of the requirements should be proving absence of infection with Bsal. To acquire a permit, the sender or receiver or both would have to quarantine the salamanders or other material (and demonstrate that the quarantine measures are adequate to contain spread of the pathogen), sample a percentage of the total number of animals or biologic materials to be shipped, and submit those samples, such as skin swabs from live or dead non-fixed salamanders, to a diagnostic laboratory for PCR testing. Permit granting would depend upon confirmation of the negative status of the animals or biologic materials.

Response: While testing of specimens and live animals before moving them would be advisable, testing could not be a prerequisite for receiving a permit at this time because the details of reliable testing from all exporting countries have not been confirmed. And, as mentioned in *IV. Second Interim Rule* below, interstate transport between States within the continental United States is not prohibited under the current prohibitions of 18 U.S.C. 42(a) for the listed salamanders, making the requirement not necessary for many domestic shippers. We recommend that salamander transporters conduct best practices to reduce the risk of introducing, transporting, or spreading Bsal within the United States.

(45) *Comment:* The 2016 interim rule should be amended to allow accredited veterinary medical diagnostic laboratories to exchange, receive, and accept live or dead specimens, including parts of the 201 listed species, without the requirement of first obtaining a Federal permit. The first step in any Bsal response is to obtain an accurate

and confirmed diagnosis of Bsal. Requiring accredited labs to first obtain a permit is an unnecessary burden that slows the diagnostic process and any confirming diagnostic testing at different labs.

Response: We agree that the first step to any Bsal response is obtaining accurate diagnosis of Bsal. However, a permit is no longer necessary for shipment between States within the continental United States, as explained below in the preamble to this final rule.

(46) *Comment:* The double-containment requirements for transport and storage and uncertainties therein are concerning. More explicit guidance is requested regarding the double-containment requirements for transport, housing, or storage, or handling of animals, tissues, or other samples. Specifically, how does this requirement apply to species repatriation projects or State-approved releases of injurious listed salamanders back into the wild? Many States conduct health testing (in collaboration with diagnostic lab partners) and have established standards that must be met before repatriation is conducted. Such State-sponsored activities should be exempt. Another solution is to permit exemptions for double containment of fixed tissues, where the threat of Bsal transmission is removed by virtue of the fixative agent.

Response: The Service posted additional guidance on our website that includes further discussion about the "double escape-proof" containment for live animals and samples (*https://www.fws.gov/node/266100*). It is possible, however, in situations where live animals have been permitted and for which the "double escape-proof" containment requirements would apply, that repatriation would run counter to that requirement. This is the first time that native species that might be part of repatriation or recovery efforts have been listed as injurious. Because injurious wildlife must be carefully handled, all of the containment requirements must be met when salamanders are in captivity. However, the Service will work with people or institutions that are involved in State-approved

repatriation efforts to facilitate these efforts. Finally, we clarify in *A. Background* in *II. Final Rule to the 2016 Interim Rule* that preserved tissues are not considered injurious.

Other Impacts

(47) *Comment:* Collection of fishes for shipment cannot totally ensure that other species of "free riders," such as non-marketed amphibians, are not unintentionally included in the shipment process. Unintentionally including a single regulated amphibian, regardless of whether it is infected with Bsal, would subject the transporting farmer to severe civil and even criminal penalties. Notably, actual interstate transport of Bsal by some means not including a listed amphibian would not violate the rule.

Response: As explained in the final rule, interstate transport prohibitions have been clarified. We encourage anyone who transports live fishes to use best management practices that include transporting only the traded species and their uncontaminated media. Unintentional importation or transport between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), which are also set forth at 50 CFR 16.3, through nursery stock or other pathways would also be a violation of the prohibitions from listing.

Inaccurate or Incomplete Science

(48) *Comment:* The rule does not account for pressures that amphibians are already facing, such as habitat loss, rising temperatures, pesticide use, and siltation from agriculture. The Service's focus should be on the systematic degradation of the ecosystems in which the amphibians live and the capacity of the salamanders to fight the fungus.

Response: The Service noted in the rule that salamanders may demonstrate more severe clinical disease when infection is combined with additional stressors in the wild. The comment does not provide any evidence of how habitat loss, rising temperatures,

pesticide use, and siltation from agriculture diminishes the need for or benefits of the rule that may prevent salamander mortality. Native salamander species known to be negatively affected by Bsal infection under experimental conditions may demonstrate more severe clinical disease when infection is combined with additional stressors in the wild, as has been found for other diseases. Besides this rule, the Service is engaged in many other conservation measures designed to help improve and protect salamander habitats across the United States.

(49) *Comment:* Using a method of infecting a salamander from one genus with Bsal in a laboratory setting and then extrapolating results to all species within that genus is not in conformance with the framework of the World Organisation for Animal Health code or the National Aquatic Animal Health Plan (National Aquaculture Health Plan and Standards) for the United States and is contrary to the credible scientific findings of Martel et al. (2014). Therefore, the 2016 interim rule is arbitrary and capricious in violation of the APA. In addition, other aquatic diseases have shown laboratory infection, but the affected fish species are not included in regulatory lists.

Response: The WOAH has a different purpose than the injurious wildlife listing provisions of the Lacey Act, and the standards the WOAH uses are appropriate for their purposes. We followed the standards in 18 U.S.C. 42 and the APA. The issue of fishes that may carry diseases is beyond the scope of this rulemaking. Surrogate species are used elsewhere in the 2016 interim rule, such as for Bd for where information is lacking for Bsal and is common in scientific literature.

(50) *Comment:* The spread dynamics of Bd and Bsal are considerably different. Given that Bd is endemic to the United States, the estimated potential for Bsal distribution has been overestimated. This overestimation is confirmed by salamander import data, the lack of presence of Bsal in animals entering the United States, and its lack of presence in wild populations.

Response: The commenter states that Bd originated in the United States and is therefore endemic. We agree that Bd has occurred in the United States for many years and is currently ubiquitous throughout North America; however, we do not consider Bd endemic to the United States. For reasons identified in the 2016 interim rule and this second interim rule, we conclude that Bsal does pose a risk to native salamander populations. We have updated the research cited and still conclude that there is a risk of Bsal entering the country with salamanders, and that risk is greatly reduced by listing the genera in this rule.

(51) *Comment:* The 2016 interim rule reports that there is no accurate way to test for or eliminate Bsal in captivity. PCR-based testing has been well established for many years for the related Bd and has been effectively demonstrated for Bsal. Effective measures for clearing salamanders using heat alone or heat in conjunction with anti-fungal medications have also been published. The authors of both of those studies have reiterated in personal communication that the stated Service position in the rule justification is contradictory to the published data. Another comment noted that combined experience from members of Caudata.org in the captive maintenance and breeding of the species subject to this rule has shown that the temperatures required by these treatments are safe and will not harm the majority of salamanders of the Salamandridae, the family containing the bulk of the regulated species.

Response: While the comments do not provide any information on how the Service's finding is contradictory to the published data, the second interim rule clarifies these issues. We have revised the rule to note that testing and prophylactic treatments of imports of salamanders to manage Bsal are available but have uncertain effectiveness when applied as a nationwide regulatory tool by the Service.

(52) *Comment:* There is likely no Bsal in the United States, even with the huge numbers of salamanders that have recently been imported, because it gets too hot in the summer and too cold in the winter.

Response: As discussed under *Bsal Risk Assessment*, we found that there is a significant risk that Bsal can establish and spread in the United States. Some areas, such as south Florida, are likely to have low consequences from Bsal introduction, in part due to temperatures found in the region. The areas most likely to have consequences from Bsal introduction are the Pacific Coast and Appalachian Mountains (Richgels et al. 2016). Based on environmental suitability, areas of the United States most suited to Bsal growth (Blooi et al. 2015a), including the Southwest, Southeast (except south Florida as just noted), and Pacific regions, are also the areas of highest salamander diversity. The large land mass of the United States has a broad range of climates, many of which are similar to the other continents where Bsal is currently found.

(53) *Comment:* The Service did not publish the text of articles or the risk assessment it used for the 2016 interim rule.

Response: The file for the references used, as well as other supporting information used to develop the 2016 interim rule, was posted under "Supporting & Related Material" in *https://www.regulations.gov* (Docket No. FWS–HQ–FAC–2015–0005), and is available for public inspection as noted under **ADDRESSES** in the 2016 interim rule**.** Comments and materials we received, as well as citations for supporting documentation we used in preparing the interim rule, were available for public inspection. The texts of publications are often covered by copyright laws and those are therefore not posted.

(54) *Comment:* Species from the genera *Ambystoma* and *Gyrinophilus* were not listed because they were tested and proved resistant to Bsal. Why then were all *Plethodon* listed, since the one species tested (*P. glutinosus*) was also demonstrated to be resistant?

Response: We did not believe that there was enough evidence to list *Ambystoma* or *Gyrinophilus* at the time of the 2016 interim rule, but we found evidence to support listing *Plethodon*. Three native salamander species identified as resistant to Bsal infection included the spring salamander (*Gyrinophilus porphyriticus*), marbled salamander (*Ambystoma opacum*), and spotted salamander (*A. maculatum*) (Martel et al. 2014). At the time the public-comment period closed, there was no evidence that any species within these genera are carriers of Bsal. We discuss our reasoning for listing all *Plethodon* species in the second interim rule under *Vulnerability and Carrier Status*. In short, however, further histological analysis of the slimy salamander revealed that Bsal could invade the skin long enough to move or transmit the pathogen to other salamanders. No such evidence existed then or now for any species in the genus *Gyrinophilus*; therefore, we are not listing species from that genus. As explained in the second interim rule under *Vulnerability and Carrier Status of Native Species*, we have evidence now of carrier capability for *Ambystoma maculatum, A. mexicanum,* and *A. opacum* and are listing the genus.

(55) *Comment:* In Europe, where Bsal is believed to have been introduced by Asian imports, Bsal was found in populations of *Salamandra* and Alpine newts (*Ichthyosaura alpestris*) in the Netherlands and in Belgium. It has also been found in captive *Salamandra* in the United Kingdom and Germany, and possibly in wild German populations. Martel et al. (2014) shows that most lethally vulnerable species exposed to Bsal in the lab showed signs of infection within 8 days and were dead within 3 to 4 weeks. This means that non-resistant infected species in captive collections would have died during the comment period on the 2016 interim rule.

Response: The comment suggests that there are no specimens carrying Bsal at this time that might enter the United States and allow Bsal to be introduced, establish, and spread, but does not provide evidence that we can use in our analysis of the rule. Lethally

vulnerable specimens can still appear if the pathogen spreads, or if Bsal persists in tolerant or susceptible populations or carcasses.

(56) *Comment*: The 2016 interim rule states that surveys of anglers have indicated that they routinely release salamanders into the areas where they fish, which includes areas that are not part of the salamanders' native U.S. habitats, suggesting that animals are routinely moved long distances. No similar survey data exists for pet owners, so assuming the pet trade is the problem for releases is unfounded and targeting the pet trade simply because it is an easy target is unjust.

Response: Our statement relating to anglers was used to note that this invasion expansion pathway has been attributed to the use and subsequent release of salamanders used as fishing bait. Along with the other evidence we documented, we found that there is the potential for salamanders carrying Bsal to escape or be released into the wild where they can transmit the pathogen to native species. We provided evidence in the rule that we used to conclude that international trade is the main pathway for the global spread of Bsal.

Additional Science Needed

(57) *Comment:* Several areas would benefit from further investigation. For example, the origins of Bsal in wild salamanders needs to be better understood. It is important to continue and expand testing of salamanders in the wild and in trade in various locations. Additional testing of species within the same genus would be beneficial to guide field and collection surveillance.

Response: Since the 2016 interim rule was published, many studies have been published that address the commenter's concerns and are applicable to the rule, including a major surveillance of salamanders in the wild by the USGS. We have reviewed the studies, and they support our final and second interim rules. We agree that additional science will help address issues related to better understanding of this pathogen and

preventing its introduction into the United States, but we understand the need to take action now to list the species in the genera in this rule to prevent the introduction, spread, and establishment of Bsal.

Economic and Trade Data

(58) *Comment:* If the salamanders are already here, and Bsal is not, then that means that any salamanders traveling across State lines pose no risk. This law estimates that it will cause $3.8 million in damage to the U.S. economy, mostly in the small business sector ("Regulatory Flexibility Act," paragraph nine). Those numbers could be greatly lessened if interstate travel were allowed.

Response: As explained above in *III. Final Rule to the 2016 Interim Rule* in *A. Background* and *D. Required Determinations*, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States. Thus, the costs incurred are expected to be less than originally estimated in the 2016 draft economic analysis.

(59) *Comment:* Caudata.org conducted an online public survey from February 1 to March 12, 2016, to gather additional data of U.S. domestically bred animals. A total of 797 respondents to the survey reported shipping 25,649 domestically bred caudates across State lines in 1 year. Due to the low response rate relative to the number of U.S. registered members on Caudata.org (8 percent) and the short duration of the survey, this number likely represents a small fraction of the actual trade. It can be safely extrapolated that the Service has underestimated the trade in captive-bred newts and salamanders by at least two orders of magnitude. Caudata.org is uniquely situated at the interface of hobbyists, entrepreneurs, researchers, zoos, and aquariums. A summary of that data and some important numbers are presented here. Respondents to the survey possessed a total of 28,228 domestically bred salamanders or newts, the majority of which are subject to the rule. Respondents shipped on average 25,649 salamanders or newts over State lines

per year. This number is nearly two orders of magnitude greater than the "338" cited by the rule and represents just a small fraction of our members. The total yearly salamander- and newt-related revenue reported by our respondents was $207,528 for 2015.

The commenter further stated that Caudata.org has more than 10,000 unique registered U.S. members who have accessed their website in the past 5 years. Their total number of unique U.S. visitors (people who did not register for an account) in that time is orders of magnitude greater than this number. The commenter stated that, apparently, the Service did not perform due diligence in ascertaining the number of private U.S. citizens affected by this rule.

Response: While we did obtain data from the Pet Industry Joint Advisory Council (PIJAC; currently known as the Pet Advocacy Network) for the 2016 interim rule, we appreciate Caudata.org for supplying additional data. The survey does not indicate: (1) whether the salamanders are domestically bred; (2) the net importation for each State; or (3) what species they are. Since the survey data did not include information on species or whether they are transported between the listed jurisdictions, it is unknown if any of the revenue discussed would be lost due to prohibitions under the rule. Consequently, the data are not used in the final economic analysis for the 2016 interim rule. Furthermore, unlike the 2016 interim rule, the final rule clarifies that the current prohibition on interstate transport in 18 U.S.C. 42(a) does not apply to interstate transport between States within the continental United States.

(60) *Comment:* The economic figures provided by the Service are a gross understatement. Caudata.org has submitted the results of a survey on the numbers of animals sold across State lines, and just from their members, reported roughly $207,528 in income. Actual figures are probably much higher, given that this amount likely represents just a portion of the trade in the entire United States, and Caudata.org pertains only to captive-bred animals. In addition to the money spent purchasing animals, there's

also food, lighting, enclosures, plants, decorations, filters, shipping and packaging fees, and other costs associated with keeping salamanders. To house a pair of salamanders can cost $100 to $200 or more, with ongoing feeding costs. Overall, the U.S. salamander hobby probably represents well over $5 million to $10 million in economic activity each year.

Response: Regarding the Caudata.org data, see also response to Comment 59. The economic analysis addresses primary support services (such as food and shipping) and secondary economic impacts in Sections 2.3.2 and 3.1.3, respectively.

(61) *Comment:* The 2016 interim rule states that "a minimum of 338 domestically bred salamanders may be affected due to the interstate transportation prohibition." As an individual, the commenter has legally shipped 150-plus live specimens (eggs, larvae, and adults) in a single year and knows that many more people legally ship more specimens than that amount in the same period. The commenter has also received dozens of animals in a single year and knows that this occurrence is not unique. Many individuals will be affected by the listing.

Response: The minimum is based on available data from PIJAC and is stated as a minimum due to the expectation of the actual number being potentially larger. For this salamander breeding data, it is unclear which species are shipped and whether these specimens are shipped between listed jurisdictions. Unlike the 2016 interim rule, the final rule clarifies that the current prohibition on interstate transport in 18 U.S.C. 42(a) does not apply to interstate transport between States within the continental United States. Therefore, it is not incorporated into the final economic analysis.

(62) *Comment:* Many small businesses have commented that the prohibition on interstate transport will have a greater impact than the Service anticipates. In the 2016 interim regulatory flexibility analysis, the Service stated that it does not believe that the impact of prohibiting interstate transport will be significant. However, several small

breeders and hobbyists involved in selling salamanders in the United States have indicated a substantial domestic trade in salamanders. The U.S. Small Business Administration Office of Advocacy commented that a small business representative indicated that this number could be as high as 1,500 specimens shipped in a year for certain businesses. The difference between the limited information in the analysis and the information provided by commenters indicates that the analysis underestimates the effect of the prohibition of interstate transport.

Response: As explained above in *III. Final Rule to the 2016 Interim Rule* in *A. Background*, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States. Therefore, the interstate data provided are not incorporated into the final economic analysis and final regulatory flexibility analysis. As discussed in Comment 61, salamander breeding data that are submitted without specific details regarding species type are too general to be incorporated into the final economic analysis. It is possible that the domestic market is more robust than estimated. However, it is unclear whether any additional sales are related to species that are listed or not listed under the rule.

(63) *Comment:* Many breeders who produce these animals as their main source of income will lose significant income or go out of business without the ability to sell across State lines. For example, last year, a business owner produced more than 100 neotenic *Ichthyosaura alpestris*, and this year [2016] they will have to cull those eggs in light of the prohibition.

Response: The comment is incorrect that they will have to cull eggs, because eggs of listed salamander species are not considered injurious because they do not have the potential to serve as carriers of Bsal. Furthermore, as explained above in *III. Final Rule to the 2016 Interim Rule* in *A. Background*, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between

States within the continental United States after April 7, 2017. Therefore, the interstate data provided are not incorporated into the final economic analysis and final regulatory flexibility analysis.

(64) *Comment:* Many States also prohibit or limit sale by biological supply companies of certain native species, and the authority to regulate nonnative species may be either with the State fish and wildlife agency or the State's department of agriculture or shared in some instances. For example, commercial production of native salamanders is currently not legal in California, and the State's department of agriculture does not regulate or track production or sale of nonnative salamanders in the State. The only way to legally sell native salamanders in California is as a biological supply house with a permit to collect wild specimens for sale to scientific and educational facilities. Only one business is currently in possession of this permit, and it has not collected or sold salamanders.

Response: We appreciate the information.

(65) *Comment:* One commenter's company produces about 1,000 *Neurergus kaiseri*, 100 *N. crocatus*, 100 *N. strauchii*, and 200 *Ichthyosaura alpestris* a year.

Response: We appreciate the commenter supplying domestic breeding data. It has been incorporated as appropriate into the appendix to the final economic analysis.

(66) *Comment:* An international prohibition on trading gives small-time breeders within the United States an economic boost to supply the demand for these pets.

Response: We acknowledge that an international prohibition can have an indirect effect of reducing competition for domestic breeders in some markets. The rule was not implemented to provide an advantage to domestic breeders but rather to prevent Bsal introduction, establishment, and spread in the United States by salamander species that are carriers of the pathogen.

(67) *Comment:* The Service estimates that, without the 2016 interim rule, 217,000 salamanders would be imported each year. These imports will be prohibited if the 20 genera are listed under the Lacey Act as set forth in the 2016 interim rule. The Service further estimates that 338 domestically bred salamanders would be affected by the interstate transportation prohibition per year, resulting in impacts to domestic breeders of up to $23,000. These domestic production numbers do not pass a straight-face test; for the estimate to be accurate, each salamander would need to be worth an average of $68. In reality, salamanders typically sell for between $10 and $50, depending on the species. As several USARK members and others in the herpetoculture industry have reported to the Service in written comments, including trade numbers provided by Caudata.org, the actual number of domestically bred salamanders shipped across State lines is far higher than 338. The species listed in the 2016 interim rule comprise the overwhelming majority of those in the pet trade, so the economic effect of the listing will amount to nearly the full total of the industry's value.

Response: The *minimum* number for domestic production (338) and the corresponding prices for those salamanders were provided by PIJAC. The detailed data they provided is in table A1-2. Furthermore, after the 2016 interim rule was issued, as explained above in *A. Background* in *III. Final Rule to the 2016 Interim Rule*, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States.

Use of Categorical Exclusion

(68) *Comment*: The interim rule is not possible without the recently implemented categorical exclusion that bypasses the requirement to consider economic and social impacts under the National Environmental Policy Act (NEPA). The decision to use the categorical exclusion for the 2016 interim rule is flawed.

Response: We determined the categorical exclusion for injurious wildlife listing, located in the Department of the Interior Manual at 516 DM 8.5 C(9), applies to the action in accordance with the requirements of NEPA. The categorical exclusion does not bypass NEPA. We reviewed the rule under NEPA requirements and prepared an environmental action statement for the 2016 interim rule that was available for review (see "Supporting & Related Material" at *https://www.regulations.gov* under Docket Number FWS–HQ–FAC–2015–0005). Under NEPA, the human environment is interpreted comprehensively to include the natural and physical environment and the relationship of people with that environment (40 CFR 1508.14), and the economic or social effects are not intended by themselves to require preparation of an EIS (40 CFR 1508.14). We prepared a draft economic analysis and regulatory flexibility analysis separately as part of the required determinations under the APA for the 2016 interim rule and made them available for public comment. We determined that the regulations in that rule will not individually or cumulatively have a significant effect on the human environment.

Inaccurate Use of 18 U.S.C. 42(a)(1)

(69) *Comment*: The use of the Lacey Act in this manner opens the door for similar regulations of other animals, such as dogs, cats, fishes, horses, and chickens. The list of species is infinite, as would be the economic impact they could have.

Response: Under the authorities provided under the injurious wildlife provisions of the authorizing statute (18 U.S.C. 42), the Service can list only wild mammals, wild birds, fishes, mollusks, crustaceans, amphibians, and reptiles as injurious wildlife, meaning the Service cannot list domesticated species, thus eliminating the possibility to list domesticated dogs, domesticated cats, domesticated horses, and domesticated chickens.

(70) *Comment:* Congress has never interpreted the Lacey Act to apply to shipment between States within the continental United States.

Response: After the 2016 interim rule was issued, as explained above in *III. Final Rule to the 2016 Interim Rule, A. Background*, the current prohibition on interstate transport in 18 U.S.C. 42(a) has been clarified and does not apply to interstate transport between States within the continental United States. The final rule has been modified consistent with the prohibition in the shipment clause of 18 U.S.C. 42, which has been codified in Federal regulations at 50 CFR 16.3, for transport between the enumerated jurisdictions (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States).

(71) *Comment:* Although the pet trade is primarily regulated by USDA agencies, including the Animal and Plant Health Inspection Service, requirements for the movement of pets across State lines are generally reserved to individual States. Furthermore, courts have often found individual animals to be exempt from livestock regulations that would otherwise apply when those animals are characterized as household pets. Salamanders in the pet trade should similarly not be considered "wildlife" for purposes of Federal regulation of interstate transport. Other commenters also stated that the Service should defer this issue to another agency with additional resources for controlling importation (such as the USDA) because the Service has stated that an injurious listing under the Lacey Act is their only means of attempting to control Bsal.

Response: The provisions of 18 U.S.C. 42 make no distinction between pet salamanders and other salamanders. The purpose of listing these salamander species as injurious wildlife is to prevent the introduction, establishment, and spread of Bsal in the wild in the United States to protect wildlife and wildlife resources. The authority to take

action to list species as injurious wildlife under 18 U.S.C. 42 lies solely with the U.S. Department of the Interior.

(72) *Comment:* The regulations promulgated in the 2016 interim rule restrict not only international and interstate transport but any movement whatsoever of the listed genera. The regulatory language prohibits the importation, transportation, or acquisition of any live or dead specimen, including parts, but not eggs or gametes, of the genera. There is simply no authority in the Lacey Act to prohibit acquisition. Because the Lacey Act does not forbid acquisition of a listed animal, the interim regulation is beyond the law to the extent it purports to prohibit the same. The Service must amend the 2016 interim rule to clarify that the prohibitions do not apply to intrastate activities.

Response: Under the Lacey Act Amendments of 1981, 16 U.S.C. 3372(a)(1), it is unlawful among other things for any person to sell, receive, acquire, or purchase any wildlife transported in violation of any law of the United States. This includes acquiring any injurious wildlife imported into the United States or transported between the enumerated jurisdictions in violation of the shipment clause of 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), also set forth at 50 CFR 16.3. The conditions of injurious wildlife permits may also place limitations on subsequent sale or transfer of injurious wildlife under the permit without prior authorization from the Service. Those activities are in connection with transport of injurious wildlife between the listed jurisdictions in the shipment clause or import into the United States under 18 U.S.C. 42. It is the responsibility of a person who may be engaged in salamander acquisition to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to that activity.

The rule adding injurious salamanders to the lists of species does not change the scope of the prohibitions in 18 U.S.C. 42, 16 U.S.C. 3372, 50 CFR 16.3, or otherwise

found in 50 CFR part 16. The regulatory language referenced by the commenters (50 CFR 16.14) is identical to longstanding, existing language that appears at 50 CFR 16.11, 16.12, 16.13, and 16.15. Revision of the general regulations found at 50 CFR part 16 is beyond the scope of this rulemaking.

(73) *Comment:* The Lacey Act defines "transport" as "to move, convey, carry, or ship by any means, or to deliver or receive for the purpose of movement, conveyance, carriage, or shipment." Even if the law did authorize the Service to bar personal transport, which it does not, this definition would reach solely intrastate activities. Although the Service has agreed that States, not the Service, have the power to regulate ownership and sales within their borders, the commenter is concerned that the Service is laying the groundwork to involve itself in Federal regulation of wholly intrastate activities.

Response: The definition quoted by the commenter applies to the law codified at 16 U.S.C. 3371(j), also known as the Lacey Act Amendments of 1981. Consistent with this definition, Service regulations also provide a definition of transport found in 50 CFR 10.12. It is the responsibility of a person who may be engaged in salamander transportation to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to such activity. For further information see also response to Comment 72.

Interim Rule Is a Regulatory Taking

(74) *Comment:* The Service evaluated the 2016 interim rule and determined that it does not constitute taking. This conclusion is facially false—a restriction on interstate travel with a family pet not only is impermissible under the law, but most certainly denies the pet owner enjoyment and companionship (amounting to use) of that pet.

Response: Import and transport of injurious wildlife between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (and at 50 CFR 16.3) of any of the listed species is prohibited. The provisions of 18 U.S.C. 42(a) do not prohibit any person who owns one of the listed species at time of listing from continuing to possess the

species (such as listed salamanders) or engaging in transport and other activities within the enumerated jurisdictions of the shipment clause, as allowed under State, Tribal, or territorial law. Therefore, we concluded that the 2016 interim rule and this final rule do not constitute a regulatory taking. This action is consistent with all previous injurious wildlife listings that have affected listed species that members of the public might have owned at the time of listing. It is the responsibility of a person who may be engaged in salamander transportation to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to that activity.

Federalism Assessment Under Executive Order 13132

(75) *Comment:* Under Executive Order 13132, the 2016 interim rule requires a federalism assessment as the rule's provisions have significant federalism effects and will have several direct effects on States, which have primary jurisdiction over native wild animals not in captivity. The regulation of the movement of pets across State lines is reserved to individual States. Under Executive Order 13132, this interim rule does have sufficient federalism implications to warrant the preparation of a federalism assessment.

Response: A federalism assessment is not required. Executive Order 13132 says that policies that have federalism implications refer to regulations, legislative comments, or proposed legislation, and other policy statements or actions that have substantial direct effects on the States, on the relationship between the national government and the States, or on the distribution of power and responsibilities among the various levels of government. This rule does not limit the policymaking discretion of the States or preempt State law. The States are not restricted from also regulating the transport of listed salamanders or other activities related to such species within their State boundaries, such as sale or possession. The commenter did not provide evidence showing how the rule would be a substantial direct action impacting the States.

Law Enforcement Issues

(76) *Comment:* Salamanders will be smuggled into the country or sold through the black market once they are prohibited, as all contraband inevitably is, with no regard for fungal safety or often the health of the animals. One commenter noted that they have received messages from overseas asking them to illegally ship animals, with instructions for how to package and ship animals to demonstrate how easy it is to do so. The rule will not prevent Bsal from entering the United States.

*Respons*e: The injurious wildlife provisions of 18 U.S.C. 42 serve an important role in protecting humans, the interests of agriculture, horticulture, and forestry, and the wildlife or wildlife resources of the United States from injurious wildlife. The rule is intended to reduce opportunities for Bsal to spread disease to native species in the wild. As previously explained, the listing of salamander species that may be carriers of Bsal results in prohibitions on import and transport between the enumerated jurisdictions in the shipment clause, and violations of these prohibitions are subject to strict liability, 18 U.S.C. 42(b) (Whoever violates this section, or any regulation issued pursuant thereto, shall be fined under this title or imprisoned not more than six months, or both.). Additionally, pursuant to 18 U.S.C. 42(a)(1), all prohibited injurious wildlife imported or transported in violation of the Lacey Act "shall be promptly exported or destroyed at the expense of the importer or consignee." Where applicable, penalties may also be assessed under the Lacey Act Amendments of 1981, 16 U.S.C. 3371 *et seq*. Although we acknowledge that some unscrupulous dealers may take advantage of people or engage in illegal trade, the regulatory provisions we are promulgating play an important role in deterring and, as necessary, penalizing and remedying unlawful activity, in order to protect the interests under the Act. We strongly encourage compliance with the law, and we may take appropriate enforcement action against violations that may occur. However, our experience is that pet owners prefer to be responsible, law-abiding citizens and would

make informed decisions not to engage in import or transport contrary to the Lacey Act and thereby reduce the risk of spreading Bsal.

(77) *Comment:* The final rule will subject exhibitors, nature centers, wildlife rehabilitators, private citizen hobbyists, and commercial breeders to Federal prosecution and penalties from felonies under the Lacey Act.

Response: The interstate transport language in 18 U.S.C. 42 is clarified in *A. Background* in *III. Final Rule to the 2016 Interim Rule*. Prohibitions remain for importation and transportation between the enumerated jurisdictions of 18 U.S.C. 42, also listed in 50 CFR 16.3. Violations of the injurious wildlife listing prohibitions are a misdemeanor, not a felony.

(78) *Comment:* For salamander species not listed as injurious, the final rule should incorporate authority for the Service to collect Bsal samples from any shipment where dead animals are present upon importation. This is a noninvasive procedure, and these data are needed to help modify this rule in the future if additional Bsal carrier species are discovered. The presence of dead salamanders upon importation can be a smoking gun for the presence of Bsal (and other harmful pathogens).

Response: The recommended action is outside the scope of this final rule relating to the listing of injurious wildlife under 18 U.S.C. 42(a).

(79) *Comment:* A Federal ban on interstate movement of salamanders is unenforceable given the Service's resource limitations. Many of the exotic caudate species listed as injurious are already widely distributed in private collections in virtually every State. There is no system in existence (or resources to create a system) to register or effectively monitor their numbers or locations.

Response: The Federal ban was lifted in 2017 as the result of a Federal court decision regarding the interpretation of the statute 18 U.S.C. 42(a). This final rule has been amended to address interstate transport as explained above in *III. Final Rule to the*

2016 Interim Rule in *A. Background* and *D. Required Determinations.* Whether a Federal ban on interstate movement is enforceable is beyond the scope of this rule.

(80) *Comment:* Regulation alone will not put a halt to the international and interstate traffic in species listed as injurious under the Lacey Act or under various State regulations. Accordingly, adequate law enforcement, especially at ports of entry, is critical to manage the ongoing, and possibly increased, volume of underground traffic in regulated wildlife. The commenter encourages building cooperative partnerships between State and Federal enforcement agencies to increase capacity and to capitalize on the specific expertise that the respective programs can bring to bear on this problem.

Response: We agree. The Service's Law Enforcement Office has a longstanding relationship with Customs and Border Protection and USDA inspectors regarding cooperation for enforcement on the borders. The Service's law enforcement officers at ports have and will continue to maintain strong working relationships with their State counterparts.

(81) *Comment:* The trade in species not listed by the rule needs to be monitored. The rule's prohibitions of the vast majority of commonly traded species may inadvertently create a new legal market for species not previously in demand by the salamander trade community. Those newly traded species could be carriers of Bsal.

Response: The Service collects information on all imported salamanders, listed or otherwise. This situation will not change with the listing of the species in this rule as injurious wildlife. This rule does not preclude the ability to take additional regulatory actions if new information emerges.

(82) *Comment:* There was a push to acquire species before the prohibition could go into effect after it was announced. Prior to the ban, some people would have only purchased captive-bred or long-time-in-captivity amphibians. Due to the prohibition, they stepped out of their comfort zone and purchased wild-caught salamanders.

Response: The comment supports the Service's decision to implement an effective date of 15 days after the date of publication for the 2016 interim rule and again for the second interim rule. We wanted to give shipments in transit or pending transit the time needed to complete the travel for the welfare of the live animals, but we did not want to encourage a rush to import over a longer period. Purchasing a wild-caught salamander listed under this rule is not prohibited under 18 U.S.C. 42, provided transport of the specimen occurs only within an enumerated jurisdiction of 18 U.S.C. 42 (also listed at 50 CFR 16.3) and complies with any permit condition for a specimen traded under an injurious wildlife permit. It is the responsibility of a person who may be engaged in salamander acquisition to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to that activity.

Alternatives to the 2016 Interim Rule

(83) *Comment:* Numerous commenters recommended health certification as an alternative to the injurious wildlife listing. For example, a commenter urged the Service to reconsider the listing of the 201 salamander species, and instead to employ models proven effective by USDA's Animal and Plant Health Inspection Service, such as utilization of certificates of veterinary inspection, pre- or post-import quarantine and treatment, import permits, and import restrictions based on risk assessments for given countries of origin. Another commenter urged the Service and other relevant agencies to work with its members to develop immediate measures to allow for preventive treatment and certification, without causing undue personal impacts.

Response: While we do work collaboratively with the USDA and nongovernmental organizations on many invasive-species issues, the authority to list species as injurious wildlife under 18 U.S.C. 42 lies with the U.S. Department of the Interior. Although some countries may have the necessary skills to prepare a health certificate that salamanders are free of Bsal, not all exporting nations may have the

necessary skills or resources. Scientists and diagnostic laboratories are also working to standardize laboratory protocols. Please see heading in the second interim rule on *Ability To Prevent or Control the Spread of Pathogens or Parasites* for more explanation. The Service will continue to seek opportunities to work with partners to ensure salamander conservation consistent with its mission but cannot commit to specific actions that do not fall under the scope of this rulemaking.

(84) *Comment:* Related to other comments about establishing a certification system, the Service should consider establishing a permit from which the proceeds would help manage certification, testing, and conservation efforts and, therefore, could both help fund the program and make it more scientifically accurate.

Response: While we are not establishing a certification system at this time under this rule, we acknowledge that the general statutory authority to charge fees for processing applications for permits and certificates is found in 31 U.S.C. 9701, which states that services provided by Federal agencies are to be "self-sustaining to the extent possible." Federal user-fee policy, as stated in Office of Management and Budget (OMB) Circular No. A-25 Revised, requires Federal agencies to recoup the costs of Federal activities that provide "special benefits" to identifiable recipients. Permits are special services, authorizing identifiable recipients to engage in activities not otherwise authorized for the general public. Please also see our response to Comment PR31.

(85) *Comment:* Chain pet stores should be prohibited from selling salamanders because it is too hard to regulate them. Only specialty pet places and breeders that have a permit should be allowed to sell salamanders.

Response: Regardless of the business size or type, as explained in the final rule, all import and transport of injurious wildlife between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42, codified in Federal regulations at 50 CFR 16.3, are prohibited for salamander species listed by this rule except by permit for authorized

purposes. Otherwise regulating the sale of salamanders is not within the scope of this rulemaking. It is the responsibility of a person who may be engaged in salamander sales to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to that activity.

(86) *Comment:* A mandatory holding period for salamanders should be considered for transport across State lines. Studies show that if an animal is infected, it will die within a very short period. Only animals that test negative for Bsal should be allowed to be shipped. All animals in quarantine should also be treated to prevent infection and spread of Bsal, once reliable protocols are developed, as they have been for Bd (Pessier and Mendelson 2010). Quarantine efforts would facilitate both prevention of introduction and compliance.

Response: Only lethally vulnerable species are expected to die in response to Bsal infection. Other species listed by this rule are also capable of carrying Bsal without lethal consequences and transmitting the fungus to native species. For these and other reasons discussed in this rule regarding certification and testing options, while research is ongoing, it is currently not feasible to establish such a system. The interstate prohibition has also been clarified as discussed in the second interim rule.

(87) *Comment:* Consider instead a CITES import ban of all species of salamanders and newts, under the notion that CITES exists to protect endangered species. There are 35 species of amphibians that would be at risk of being wiped out entirely if Bsal becomes introduced into the United States.

Response: CITES exists for a different purpose from the injurious wildlife provisions of the Lacey Act, and the purpose and use of CITES is beyond the scope of this rulemaking.

(88) *Comment:* The Service has not acknowledged nonregulatory approaches. The pet industry has taken voluntary action to halt the import of known carriers. For example,

PIJAC called for an immediate, temporary moratorium of Oriental fire-bellied newt and paddle-tailed newts on November 20, 2015.

Response: One of our alternatives (Alternative 1) involved taking no action on the Service's part. This is our status quo. We would not list any species of salamanders as injurious under this alternative. We did not select this option because of the significant risk that Bsal poses to native species and other wildlife resources in the United States. We expect that significantly greater financial and natural resources losses will be incurred by us and our partners in having to manage and respond to Bsal if the fungus establishes and spreads in the United States than by taking action now to prevent its introduction.

While we appreciate and support voluntary conservation efforts, we concluded that regulatory action was necessary to ensure compliance and protect native species. For example, the voluntary moratorium called for by PIJAC affected only two not-listed species, even though more have been identified as carriers (Martel et al. 2014). The species with the highest number of imports into the United States from 2004 to 2014 was the Oriental fire-bellied newt. This species comprised 54 percent of the total number of imported salamanders (USFWS OLE 2015). A review of LEMIS (Law Enforcement Management Information System) data in August 2016 shows that there were no shipments after November 20, 2015, for Oriental fire-bellied newts declared to the Service, except for 6 shipments totaling 539 live animals that occurred since the inception of the voluntary moratorium (all but 39 were before the rule took effect). This situation suggests that the rule is necessary because some importers, even if only a few, did not follow the voluntary moratorium and imported hundreds of specimens. However, since the 2016 interim rule took effect and as of the end of 2020, no *Pachytriton* spp. salamanders (not listed) have been imported, and we recognize and appreciate the role that the PIJAC moratorium likely played.

(89) *Comment:* Although Alternative 3 of the draft economic analysis, which declares 201 salamander species as injurious, is deemed most effective, the commenter suggests that Alternative 4, which declares all species of salamander as injurious, is necessary to fully prevent the spread of Bsal in the United States. Furthermore, the economic loss associated with Alternative 3 is estimated to be $10 million, while the economic loss associated with Alternative 4 is $10.7 million. The benefit of almost certainly preventing the spread of the fungus into the United States as provided by Alternative 4 far outweighs the marginal cost as compared to Alternative 3.

Response: The expected increase in cost from Alternative 3 to Alternative 4 was not considered in our determination about the injuriousness of the species. The Service determined that there was unknown risk from genera where no species have yet been tested for Bsal and, therefore, could not list those genera at this time.

(90) *Comment:* We need more citizen scientists to help with salamander conservation. Many knowledgeable hobbyists are available to assist if asked.

Response: We recognize that the public can play a critical role in conservation; however, this comment is outside the scope of this rulemaking.

(91) *Comment:* Put more funding into Bsal research to find a cure, treatments, and other ways of reducing the risk.

Response: We recognize the important contributions made by Bsal research; however, this comment is outside the scope of this rulemaking.

(92) *Comment:* As new evidence becomes available and while Bsal remains undetected in the United States, the commenter would like to see a proposed rule with a comment period for native U.S. species, rather than an interim final rule, before these new listings go into effect. For nonnative species, however, we would support other interim final rules to further reduce the chances of introduction via the importation pathway.

Response: This second interim rule is adding new nonnative and native species to the injurious list. See above in *III. Final Rule to the 2016 Interim Rule* in *A. Background*. Also, several native species are raised outside the United States and then imported into the country; this supports the Service's decision to implement a nearly immediate effective date of 15 days for all species listed under the rule. See also response to Comment 24.

(93) *Comment:* The costs to State fish and wildlife agencies to deal with pet salamanders that cannot be transported across State lines when the owner moves do not appear to have been evaluated and could place a significant burden on State agency staff that would be tasked with informing the public about the rules, working with rescues and zoos to provide rehoming opportunities, and law enforcement. The commenter would like to see a Service-administered education and outreach program that provides explicit instructions, and assistance, for pet owners to properly rehome or dispose of their salamanders. One commenter mentioned that the State of Florida has an Exotic Pet Amnesty Program in place that allows the public to surrender their regulated or unwanted exotic pets without penalty or cost. The commenter encourages continued Federal support of this program as an integral part of managing risks of nonnative introductions.

Response: The interstate prohibition was clarified by a court decision in 2017 as explained in the final rule, so the costs for transporting across State lines between States within the continental United States is not an issue now, unless regulated by other State or Federal laws. We share concerns about the irresponsible re-homing and disposal of pet salamanders into the wild and are working with partners, including the industry, to help ensure that release does not occur. The Service has been a partner with the State of Florida's Exotic Pet Amnesty Program and will continue to work with other partners to help encourage the public not to release animals that they own into the wild. The Service

does not have the funds necessary to implement a national amnesty and rehoming program.

(94) *Comment:* In the past, increased restrictions on species already in widespread possession (personal and commercial) have been accompanied by additional releases (such as walking catfishes, snakeheads). The commenter recommends consideration of regulatory approaches with the flexibility to accommodate existing ownership. Further Federal restrictions, without this "grandfathering" approach for current pet owners, may lead to an increase in the rates of release.

Response: The commenter offers no proof that releases have been caused by the new Federal regulation. The injurious wildlife provisions of the Lacey Act do not prohibit continued ownership of injurious wildlife that members of the public own at the time of listing. Under the injurious wildlife provisions of the Lacey Act, the Service is not authorized to grandfather in existing salamander owners as exempt from subsequent activities that are prohibited with injurious wildlife, including import or transport between the enumerated jurisdictions.

(95) *Comment:* Prohibit the use of amphibians as fishing bait. It has been shown that using animals, such as tiger salamanders, as fishing bait has led to species introductions (posing a major threat to California tiger salamanders) and the spread of disease, particularly Bd and ranaviruses. If Bsal ever enters the United States, it is far more likely to be spread through bait shops and fishermen than from hobbyists shipping to one another. Even if studies have shown tiger salamanders are unlikely to carry Bsal, the practice has already been shown to have spread other diseases, and other, more susceptible species may be used.

Response: The request is beyond the scope of this rulemaking. The Service, under the injurious wildlife provisions of 18 U.S.C. 42, is not authorized to prohibit amphibians for use as fishing bait, unless they are imported, transported between the enumerated

jurisdictions, or subject to injurious wildlife permits. We also note that the Service's State partners regulate fishing activities within their States and can, and often do, regulate use of amphibians for fishing bait.

(96) *Comment:* Include a clause that if a North American species is determined to be a carrier or lethally infected, it will immediately be included in the prohibition, and any species screened and determined to be insensitive and not capable of carrying Bsal will be removed from the list in a timely manner.

Response: The Service does not have authority to include or remove species on the injurious wildlife list without evidence regarding whether the wildlife is injurious to the interests protected under the Lacey Act. The determination of injuriousness is based on defensible scientific evidence. This rule does not preclude the ability to take additional regulatory actions if new information emerges. If a species is found to be incapable of carrying Bsal under all conditions, we may consider its removal from the injurious list by conducting an evaluation and promulgating a rule. Likewise, if a species is found to be a carrier of Bsal, we may consider its addition or the addition of its genus to the injurious list through this same regulatory process.

(97) *Comment:* Continue exploring a clean-trade program for future emerging infectious diseases. As indicated in previous correspondence with the Service, the commenter has consistently supported the concept of a clean-trade program for salamanders imported into the United States rather than restricting interstate movement of salamanders at this point. The commenter appreciates that the current situation makes executing such a program difficult, if not impossible; however, the commenter hopes that the Service will continue exploring options for such a program in the future for other emerging infectious diseases that are likely to impact U.S. wildlife species.

Response: The interstate prohibition has been clarified as explained in the final rule. Please also see heading in the second interim rule on *Ability To Prevent or Control*

the Spread of Pathogens or Parasites. The Service will consider other options as opportunities arise.

(98) *Comment:* The Service cites inadequate agency resources to conduct inspections and expenses associated with testing as additional reasons supporting its finding that there are not less restrictive means to prevent Bsal than those selected for the 2016 interim rule. To the extent that those expenses and hardships fall upon the owners of salamanders, a commenter would like to work with the Federal Government in developing safe, practical procedures. To the extent that those burdens fall upon the agency, the Service must not discriminate between regulation of salamanders in the pet trade and other species for which it has dedicated resources to developing satisfactory testing protocols.

Response: The Service welcomes and encourages engagement by a myriad of entities that can develop the science and help better manage wildlife pathogens entering, becoming established in, and spreading in the United States. No safe, effective alternatives have yet been presented to us. The fungus that affects the salamanders was discovered in 2013, much more recently than the pathogens infecting salmonids for which the Service has testing protocols. Much research needs to be done on the tremendous diversity of salamanders and their *in situ* environmental conditions to find an equitable, reliable, economical test as well as testing facilities in other countries.

Other Issues

(99) *Comment:* The Wildlife Society recommends the development of new comprehensive legislation to address the complexities of emerging wildlife diseases that encourages investment, increases professional capacity, focuses on collaborative prevention, and uses a multidisciplinary approach to better understand the interaction and transmission mechanisms of wildlife pathogens.

Response: The comment is outside the scope of this rulemaking.

(100) *Comment:* Since no method of pathogen control is likely to reduce risk of invasion by 100 percent, it is equally important to invest in proactive monitoring for Bsal emergence within the United States. In August 2015, the commenter launched a citizen science project on iNaturalist for people to report sightings of dead or diseased salamanders. The commenter would be happy to work together with the Service more quickly to identify and respond to potential sites of Bsal emergence. More information about the project "Saving Salamanders with Citizen Science" can be found at: *http://www.inaturalist.org/projects/saving-salamanders-with-citizen-science*.

Response: We shared this feedback with our National Wildlife Refuge System's Inventory and Monitoring Program. The Service is also helping the National Bsal Task Force and PARC to develop the protocols to monitor for Bsal's introduction and to allow for rapid response if it is identified in the United States.

(101) *Comment:* The commenter requests increased communication and education efforts around the Bsal rule. There is still a significant amount of confusion around the reasoning behind the scope of the action taken in the rule, including, but not limited to, why certain species were chosen and why interstate commerce was included. Addressing these concerns through a coordinated education and communication initiative would likely help garner further support for the implementation of the rule. Many groups, such as caudata.org, the National Bsal Task Force, and Partners in Amphibian and Reptile Conservation (PARC) would likely be able to play a role in helping to disseminate this information.

Response: The interstate prohibition has been clarified as explained in the final rule. The Service is a partner in PARC and a member of the Bsal Task Force and appreciates the need to better address the communication needs associated with the rule. We have also been providing additional information through our website to assist stakeholders in understanding the need for the rule and clarifying the permit process.

(102) *Comment:* The rule is being exploited by animal rights organizations who do not represent the majority of views of U.S. citizens. This rule was formulated in part due to a petition by the "Save the Frogs" organization.

Response: The Service received the petition from the Center for Biological Diversity and Save the Frogs! in mid-May 2015. However, we began discussions on what action to take in October 2014 and had already begun the regulatory process several months before we received the petition. Letters to the Service Director from such agencies as AFWA urged the Service to take action to prevent the fungus from entering the United States, and we took action as soon as we were able to make a determination based on defensible scientific evidence and comply with applicable rulemaking requirements to promulgate injurious wildlife listings under the Lacey Act within our available resources.

C. Affirmation of the 2016 Interim Rule

After careful consideration of the comments received and information presented, we are affirming our 2016 listing of the 20 genera of salamanders that the 2016 interim rule added to the lists of injurious wildlife in 50 CFR part 16 (81 FR 1534, January 13, 2016). All species in the 20 genera continue to be listed as injurious wildlife. The defensible scientific evidence continues to indicate that the importation of these genera poses significant risks of introducing Bsal into the United States, and none of the inputs received in response to the 2016 interim rule have changed this determination. Therefore, with this document, we affirm the addition of the following genera to 50 CFR 16.14: *Chioglossa, Cynops, Euproctus, Hydromantes, Hynobius, Ichthyosaura, Lissotriton, Neurergus, Notophthalmus, Onychodactylus, Paramesotriton, Plethodon, Pleurodeles, Salamandra, Salamandrella, Salamandrina, Siren, Taricha, Triturus,* and *Tylototriton.* Because we consider rulemaking on the 2016 interim rule to end with the publication of

this document affirming the 2016 interim rule, we are not soliciting comments regarding the genera listed in this final rule.

D. Required Determinations

We hereby affirm our responses to the following determinations required of the Federal rulemaking process as published in the January 13, 2016, interim rule (81 FR 1534):

- Executive Orders 12630, 12866, 12988, 13132, 13175, 13211, and 13563;
- Regulatory Flexibility Act and Congressional Review Act (5 U.S.C. 601 *et seq*. and 804(2)) (except a decrease in the economic effect on U.S. industries has occurred due to the clarification of the interstate transport prohibition);
- Unfunded Mandates Reform Act (2 U.S.C. 1501 *et seq*.);
- Paperwork Reduction Act of 1995 (44 U.S.C. 3501 *et seq*.);
- National Environmental Policy Act (42 U.S.C. 4321 *et seq*.); and
- Government-to-Government Relations with Native American Tribal Governments (59 FR 22951 and 512 DM 2).

IV. Second Interim Rule

Summary of Changes to the 2016 Interim Rule

For the injurious wildlife evaluation in this second interim rule, in addition to information used for the 2016 interim rule, we considered: (1) comments and new information from the public comment period for the 2016 interim rule, (2) comments on the 2016 interim rule from three peer reviewers, and (3) new information acquired by the Service after the 2016 interim rule published. This new information was used to update the science about Bsal and determine if any additional genera of salamanders should be added to the list according to the criteria laid out in the 2016 interim rule.

This second interim rule incorporates into 50 CFR 16.14 the clarifications and changes to the 2016 interim rule based on comments we received that are discussed

above in the final rule under *B. Summary of Comments Received on the 2016 Interim Rule*. This is because only one revision of 50 CFR 16.14 will be made from both rules and will include the clarifications from the final rule and the new genera and clarifications from this second interim rule.

We are clarifying, in response to public and peer-review comments, what is and is not injurious on a cellular or molecular level based upon chemical preservation or other methods that will kill the fungus. Unpreserved swabs are injurious; however, preserving swabs, such as by using 70 percent (or higher) ethanol for at least 1 minute (Van Rooij et al. 2017), renders the fungus unviable and, therefore, preserved swabs are not injurious and are excluded from the prohibitions. In addition, purified extracted genetic material of salamanders (salamander DNA or RNA) is unable to cause pathogen transmission; therefore, it is not injurious. Swabs collected for molecular biology applications should be preserved by using a higher ethanol concentration (95–99 percent), which is adequate for both the molecular preservation of DNA for testing and denaturing the proteins on the surface of fungi, rendering them unviable and thus not injurious (Marquina et al. 2021).

We provide evidence here that specimens that are chemically preserved to deactivate any live Bsal and purified extracted genetic material are not considered injurious. The Service has concluded that there is a low risk of transmission of Bsal to native species from eggs and gametes, preserved specimens, and purified extracted genetic material, which is consistent with the intent of what is not injurious in the 2016 interim rule. However, all other parts, such as unpreserved salamander tissues, fluids, and cells carried on swabs and on or in other media, will continue to be regulated under the listing. Specimens that are frozen are also included in the listing.

The Service reviewed research that has published since the 2016 interim rule took effect and is adding 16 genera of salamanders to the 20 already listed in 50 CFR 16.14. This action adds approximately 164 species in this second interim rule to the previously

listed 201 species. The genera are added under the same criteria that were used for the original 20 genera in the 2016 interim rule. However, the scientific community has made changes to salamander taxonomy within the 20 genera in the 2016 interim rule. Thus, the number of species that we identified in those genera increased from 201 to 262 species as of May 1, 2023. The combined total is 36 genera with approximately 426 species. We note that taxonomic changes within each genus may occur at any time for such reasons as new species discovered, subspecies elevated to full species, species split into two species, and other modifications resulting from genomic testing. All species subsequently scientifically added to the genera that we are listing are also heretofore considered listed species. For this reason, we are not enumerating all of the known salamander species in each of the 36 listed genera in 50 CFR 16.14.

The statute (18 U.S.C. 42(a)(1)) refers to "the offspring or eggs of any of the foregoing" as being injurious. Therefore, we are clarifying that hybrids of species in any listed genus, including offspring from a listed and a nonlisted parent, are injurious.

In response to the D.C. Circuit Court of Appeals Decision in *United States Association of Reptile Keepers, Inc.* v. *Zinke*, 852 F.3d 1131 (D.C. Cir. 2017), the prohibition on interstate transport in the 2016 interim rule has been modified. The D.C. Circuit Court of Appeals held that 18 U.S.C. 42(a) does not prohibit transport of injurious wildlife between States within the continental United States. Therefore, this interim rule clarifies that 50 CFR 16.3 does not prohibit interstate transport between States within the continental United States. This means that transportation of injurious wildlife between the 49 States within the continental United States (the contiguous 48 States and Alaska, provided no international borders are crossed) is not prohibited by the statute or injurious wildlife regulations, unless that movement of the wildlife is restricted due to conditions associated with issued injurious wildlife permits. This change took effect as of April 7, 2017. However, import of injurious wildlife into the United States remains prohibited. In

addition, transport of injurious wildlife between the listed jurisdictions in the shipment clause (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States) remains prohibited. As before, injurious species may not transit into or out of the District of Columbia. Also, injurious wildlife permits under 50 CFR 16.22 continue to be required to import injurious wildlife and to transport injurious wildlife between the listed jurisdictions for zoological, educational, medical, and scientific purposes; movements within the continental United States may be subject to conditions from an injurious wildlife permit.

Additionally, injurious wildlife unlawfully imported into the United States or transported between the enumerated jurisdictions is still unlawful to transport, including within the continental United States. Under the Lacey Act Amendments of 1981, 16 U.S.C. 3372(a)(1), it is unlawful for any person to import, export, transport, sell, receive, acquire, or purchase any wildlife transported in violation of any law of the United States. This includes transport of any injurious wildlife imported into the United States or transported between the enumerated jurisdictions in violation of 18 U.S.C. 42(a).

Need for an Interim Rule

Rulemaking under 18 U.S.C. 42 is governed by the Administrative Procedure Act (APA) (5 U.S.C. 551 *et seq.*). The process of issuing a proposed rule, providing the opportunity for public comment, and completing a final rule can take a significant amount of time to complete. During that time, the species proposed for listing are still allowed to be imported and transported, offering increased opportunities for introduction, establishment, and harm. Under section 553(b)(3)(B) of the APA, however, a proposed rule is not required when the agency for good cause finds (and incorporates the finding and a brief statement of reasons therefor in the rules issued) that notice and public procedure thereon are impracticable, unnecessary, or contrary to the public interest. There

is good cause to forgo notice and public comment on a proposed rule in this case and instead take immediate action in the form of an interim rule to help prevent the Bsal fungus from being introduced, established, or spread in the United States. Providing notice and public comment prior to implementing the injurious wildlife prohibitions would be contrary to the public interest because of the need to take immediate action due to the significant risk from Bsal. Not only could the fungus cause the devastation of some populations of native salamanders critical to ecosystem health, but it could also cause mortality if it spreads in the salamander pet trade. For these reasons, we find good cause in accordance with 5 U.S.C. 553(d)(3) to make the second interim rule effective 15 days after the date of publication in the *Federal Register*.

This second interim rule is the result of peer-reviewed, scientific information published since the publication of the 2016 interim rule. At the time the 2016 interim rule published, there was very little information on the newly described chytrid fungus species affecting salamanders (discovered in 2013). We used defensible scientific evidence to quickly stop the importation of the host species (salamanders) of the fungus. However, after the 2016 interim rule published, many research institutions realized the need for more research, both on the novel fungus and on the effect on and variety of host amphibians, to assess the validity of previous studies and determine other potential carriers. That body of research, done primarily in the United States, Europe, and Asia, has taken years to develop and put through the peer-review and journal-publication processes.

We have now compiled a more comprehensive picture of the fungus, including 16 more genera that we determined are injurious (using the same criteria as in the 2016 interim rule). We still have the opportunity to prevent the contagious lethal fungus from being introduced into the United States on salamanders in trade, hence the need for the second interim rule with the new high-risk species. Some of the new species are in the pet

trade, and a proposed rule would give the public the counterproductive opportunity to rush to import the proposed species prior to the regulation. We note that a shipment of 24 live fire salamanders (*Salamandra salamandra*) was imported into Los Angeles on January 26, 2016, which was 2 days before the listing took effect (USFWS OLE 2021), although we do not know that the shipment was intentionally shipped to preemptively avoid the 2016 interim rule's effective date. Fire salamanders are the species that brought this lethal fungus to the attention of scientists, and the shipment was exported from Germany, where the fungus had been detected in 2015 (Schultz et al. 2020), making the potential for Bsal introduction from this shipment a genuine threat. Fortunately, there is no evidence that those imported salamanders carried Bsal.

Purpose of Listing as Injurious

The purpose of listing the live specimens, dead specimens, hybrids, and parts (but not eggs, gametes, preserved specimens or parts (including tissue), and purified extracted genetic material) of 16 genera of salamanders as injurious wildlife is to prevent the accidental or intentional introduction of salamanders that are expected to serve as carriers of *Batrachochytrium salamandrivorans* (hereafter, Bsal), a fungus that poses a risk to native species of salamanders, into the United States. The genera are all from the order Caudata and are commonly referred to as salamanders and newts (hereafter, salamanders). If Bsal is introduced into wild populations of native salamanders, we expect it to cause significant harm to wildlife and the wildlife resources of the United States.

Under 18 U.S.C. 42(a), the Service, through the Secretary of the Interior, may prescribe by regulation any wild mammals, wild birds, fishes, mollusks, crustaceans, amphibians, reptiles, or the offspring or eggs of any of the foregoing found to be injurious to human beings, to the interests of agriculture, horticulture, forestry, or to wildlife or the wildlife resources of the United States. Salamanders are amphibians, and

the Service has the authority to list amphibians when we find that they are injurious to one or more of the statutory interests. We may list species before they are introduced into the United States and have the opportunity to harm interests of the United States as enumerated under 18 U.S.C. 42.

We have determined that salamanders that potentially carry Bsal are injurious to wildlife and wildlife resources of the United States. With this second interim rule, we are attempting to prevent the introduction and subsequent establishment and spread of the salamander chytrid fungus, Bsal, which is a pathogen capable of causing significant harm to native salamander species and their ecosystems. As described below under *Role of Salamanders in the Ecosystem*, the benefits that these native salamander species provide to ecosystems in ensuring ecosystem health and stability, and, in turn, the ecosystem services that benefit people, are significant.

As of the publication of the 2016 interim rule, Martel et al. (2014) and Cunningham et al. (2015) identified some of the salamander species that can carry Bsal and are at risk from infection. The researchers tested a limited number of the approximately 689 (currently 804) known species of salamanders that exist worldwide and found that not every species was negatively affected by the fungus, as determined by standard detection methods. However, the results clearly indicated a severe threat for many species of salamanders that will be negatively affected by this pathogen and others that could carry the fungus to the vulnerable species. Research showed that some tested species that are native to the United States were found to be lethally vulnerable to the fungus. Such an emerging infectious disease of fungal origin can cause a significant loss in biodiversity and ecosystem services (Fisher et al. 2012). Bsal research results and concerns about emerging infectious disease, as described by Spitzen-van der Sluijs et al. (2013), Martel et al. (2013), and Martel et al. (2014), generated a strong response from academia, industry groups, and conservation and other organizations who wrote to the

Service seeking quick and decisive action to ensure that Bsal does not have a similar impact on salamander populations that *Batrachochytrium dendrobatidis* (Bd) has had on frogs (see the 2016 interim rule). In early November 2014, the Service initiated a review to determine whether salamanders capable of carrying Bsal should be listed as injurious.

Martel et al. (2014, and others later) used several methods to determine vulnerability to Bsal-caused disease of some salamander species but do not have a category for the status as a carrier. While the vulnerability of native species is of great concern to the Service, the 2016 interim rule was primarily concerned with the ability of viable Bsal spores to remain on salamander species or their parts, thus introducing and spreading the fungus to the United States, causing chytridiomycosis disease outbreaks in native salamander populations. We reviewed the literature and based our criteria for determining carrier status of genera on whether a species was found, as determined by microscopic analysis of preserved tissue specimens (histology), qPCR (quantitative polymerase chain reaction), or other confirmatory approach to harbor viable spores.

We also looked at challenge studies, where a salamander that is free of Bsal as determined by initial pathogen-specific qPCR, is then inoculated with Bsal spores. A follow-up swab for qPCR is done at a specified period of days later to see if the spores caused disease according to field observations or histology or did not cause disease but was able to invade the skin of that species long enough to move or transmit the fungus to other salamanders, as confirmed by histology. We also looked at surveillance studies of swabs of wild and captive salamanders, where presence or absence of Bsal was determined by qPCR; negative results were not evidence for being classified as noncarrier whereas positive results were classified as carriers.

Regardless of the vulnerability of a species or the ability to manifest disease, if the species is a carrier, we consider that genus to be listable as injurious. However, if there is conclusive countervailing evidence that at least one species in that genus is not a

carrier, as shown by histology, then we do not list the genus. Case definition and diagnostic criteria are described in White et al. (2016).

The 2016 interim rule effectively reduced import volume of targeted species, but new research on species susceptibility suggests the list of regulated species was incomplete regarding Bsal reservoir species (Grear et al. 2021). Since the publication of the 2016 interim rule, additional research has provided additional evidence of the diversity of species and genera affected by Bsal or determined to be carriers (for example, Yuan et al. 2018, Carter et al. 2020, Barnhart et al. 2020, Gray et al. 2023). Based on the Service's genus-level carrier extrapolation from data obtained from the aforementioned publications, and because Bsal has not been found in natural environments in the United States (Waddle et al. 2020), the opportunity still exists to prevent the introduction of Bsal by adding new genera of salamanders to the injurious list. In 2017, following the 2016 interim rule and Canada's temporary import ban of all living or dead salamanders, eggs, sperm, tissue cultures, and embryos (made permanent in 2018; Environment and Climate Change (ECCC) 2018), we received a letter from the American Society of Ichthyologists and Herpetologists (ASIH 2017) requesting the Service prohibit all salamander imports into the United States. For reasons explained herein, we are not listing all salamanders, but we are adding more genera, which we suggested in the 2016 interim rule was a possibility. We specifically solicited comment on whether there is there any evidence suggesting that additional species are carriers of Bsal and should be listed by this rule, and if so, what species.

We reviewed Bsal risk in conjunction with those salamander species known or suspected to carry the fungus utilizing injurious wildlife evaluation criteria, described in more detail as part of this interim rule in G. *Factors That Contribute to Injuriousness of Salamanders.* These criteria were previously developed by the Service to evaluate whether a species qualifies as injurious under 18 U.S.C. 42. The resulting analyses form

the basis for the Service's regulatory decisions regarding injurious wildlife-species listings. This rule finds that Bsal is a significant threat to the wildlife and wildlife resources of the United States and lists 16 genera of salamanders that we have determined to be injurious because they are likely carriers of Bsal.

Multiple factors confirm that Bsal can be introduced, become established, and spread, thereby causing substantial damage and harm in the United States (Spitzen-van der Sluijs et al. 2013; Martel et al. 2014; Cunningham et al. 2015; Chytridcrisis 2015b). Specifically, these factors include: (1) the discovery of the newly emerging fungus Bsal in the Netherlands and the associated deleterious effects to native salamanders (*ibid*.); (2) its subsequent spread in the wild to Germany and Belgium (Spitzen-van der Sluijs et al. 2016) and Spain (Lastra González et al. 2019; Martel et al. 2020); (3) the appearance in captive collections in the United Kingdom, Germany, and Spain (Spitzen-van der Sluijs et al. 2016; Thumsová et al. 2021); and (4) laboratory research (numerous papers cited in this rule). The United States leads all other countries in the number of native salamander species; 9 of the 10 families of salamanders worldwide are found in the United States (AmphibiaWeb 2023a). Based on scientific evidence as of publication of the 2016 interim rule, we knew that the fungus is lethal to at least two salamander species native to the United States (eastern newt *Notophthalmus viridescens* in the Eastern States and rough-skinned newt *Taricha granulosa* along the Pacific coast).

Of the 221 native U.S. species known as of the preparation of this second interim rule (AmphibiaWeb 2023a), and including both rules in this document, we have determined that 13 genera with 164 species may be carriers, and 9 species are lethally vulnerable. Most of the remaining 10 genera (with 57 species) have not been scientifically tested, with a few that have had testing that was not conclusive; these may also be found to be carriers eventually. While the Service's greatest concerns are for species that are likely to die from Bsal, salamander species known to be tolerant of, or

susceptible to, Bsal infection under experimental conditions may also develop clinical disease or experience increased severity of disease in the wild. These species may be Bsal carriers and are concerning because their long lifespans increase their likelihood of spreading the fungal spores and serving as Bsal reservoirs (Gray et al. 2023). A disease reservoir may be defined as "a passive host or carrier that harbors pathogenic organisms without injury to itself and serves as a source from which other individuals can be infected" (in Laking et al. 2017). Nonlethal infection in salamanders may have other negative effects, such as slowing their growth (Barnhart et al. 2020). Bsal infections have been found to increase in severity as animals are exposed to additional stressors in the wild, including other amphibian diseases (Wobeser 2007; Kerby et al. 2011; Kiesecker 2011; Longo et al. 2019; McDonald et al. 2020).

Experience with the introduction of Bsal into the Netherlands and associated deleterious effects to native salamanders, along with laboratory research, confirm that Bsal can be introduced, become established, spread, and cause substantial and immediate harm in the United States (Spitzen-van der Sluijs et al. 2013; Martel et al. 2014; Cunningham et al. 2015; Chytridcrisis 2015b). The United States leads all other countries in salamander diversity (PARC 2014). Based on scientific evidence, we know that the fungus is lethal to at least nine salamander species native to the United States. While the Service's greatest concern will be for species that are lethally vulnerable to Bsal, salamander species known to be tolerant of or susceptible to Bsal infection under experimental conditions may also develop clinical disease or increased severity of disease, respectively, when infection is combined with additional stressors in the wild, as has been found for other diseases, including those in amphibians (Wobeser 2007; Kerby et al. 2011; Kiesecker 2011).

In the United States, Bsal has either not been introduced, has been introduced but has failed to become established, or is present but has not been positively detected.

Although we do not have any conclusive evidence showing that introductions have occurred, history from other pathogens similar to Bsal, such as Bd, suggests that the fungus is likely to spread quickly throughout the United States if it is not prevented from being introduced. Moreover, efforts to control or eradicate introduced or established invasive species and manage the costs they incur to society are generally less effective and more expensive and difficult than efforts that prevent establishment (Leung et al. 2002; Finnoff et al. 2007). Prevention of invasive species is typically the most cost-effective measure to avoid the damage that such species cause (Leung et al. 2002; Lodge et al. 2006; Keller and Springborn 2014). As noted in the *2016–2018 National Invasive Species Management Plan*, preventing the introduction of potentially harmful organisms is not only the first line of defense for minimizing the spread and impact of invasive species, it is also the most cost-effective strategy; science-based risk analyses are used to inform regulations that can prohibit the entry of certain nonnative organisms at jurisdictional borders (National Invasive Species Council 2016). Invasive species prevention is a priority of the Department of the Interior (2021).

If Bsal has unknowingly been introduced but failed to establish in the United States for unknown reasons, it is still important to act now because additional introductions increase the likelihood of establishment and harm. As more salamanders that can carry Bsal are imported into the United States, the probability increases that one or more of those salamanders, through a phenomenon called propagule pressure or "introduction effort," described in Lockwood et al. (2005) as a measure of the number of nonnative individuals released into a region, will give Bsal the opportunity to establish in the United States and spread. The 2016 interim rule significantly reduced the number of salamanders being imported by about 95 percent (average per year for the period 2016–2020) from the 6 years before publication of the 2016 interim rule.

The salamander species listed by this second interim rule follow the same criteria as for the 20 genera in the final rule to the 2016 interim rule and are those found within genera for which we have evidence that at least one species in that genus is a carrier of Bsal with no countervailing conclusive evidence that other species in that genus are not carriers. We describe our rationale for this course of action below under *Vulnerability and Carrier Status*. Our decision making for the final rule to the 2016 interim rule and the second interim rule included the following considerations: All 20 genera of salamanders in the final rule to the 2016 interim rule, plus any new species identified within the genera listed by this second interim rule, are found to be injurious because suitable climate exists in parts of the United States to support Bsal; even if a salamander listed by this second interim rule could not establish a population in the wild, an infected captive salamander (or the water and soil in which it came into contact) released into the environment can transmit Bsal to native populations; Bsal is capable of causing extensive damage to wildlife and wildlife resources, including federally endangered and threatened species; eradicating Bsal would be extremely difficult once introduced and established; and controlling Bsal in wild salamanders is not practical.

Listing the salamanders as injurious will help keep Bsal out of the United States by preventing the importation of salamanders capable of carrying the fungus and serving as the vector of introduction into U.S. ecosystems, thereby causing injurious effects consistent with 18 U.S.C. 42. Bsal is not known to be present in U.S. ecosystems (Waddle et al. 2020). Given the expected consequences that the introduction of Bsal would have to wildlife and wildlife resources of the United States, we are listing species that we have determined to be injurious. This second interim rule lists some species that are currently in U.S. trade as well as some that are not. We have the authority to list species as injurious even if they are not currently in trade or known to exist in the United States. This regulation is not a ban on possessing or selling any of the species. The import

and transport between the enumerated jurisdictions in the shipment clause in 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), codified in Federal regulations at 50 CFR 16.3, of any of the listed species is prohibited. The provisions of 18 U.S.C. 42(a) do not prohibit any person who owns one of the listed species at the time of listing from continuing to possess the salamander or engaging in transport and other activities within the enumerated jurisdictions, unless such movement of the wildlife is restricted due to conditions associated with issued injurious wildlife permits. Those activities may be regulated by other Federal, State, Tribal, or territorial law. It is the responsibility of a person who may be engaged in transport or use of injurious wildlife to be aware of any Federal, State, Tribal, or territorial law or regulation that applies to that activity.

The salamander species listed by this second interim rule are those, in addition to the species listed in the final rule to the 2016 interim rule, that are found within genera for which we have evidence that at least one species in that genus is a carrier of Bsal with no conclusive countervailing evidence that other species in that genus are not carriers. We describe our rationale for this course of action below under *Vulnerability and Carrier Status*. Our decision making also included the following considerations:

- All 16 genera of salamanders, plus any new species identified within the genera listed by this second interim rule, are found to be injurious because suitable climate exists in parts of the United States to support Bsal;
- Even if a salamander listed by this second interim rule could not establish a population in the wild, a carrier salamander that was released from captivity (or the water and soil in which it came into contact) can transmit Bsal spores to native populations;
- Bsal is capable of causing extensive injury to wildlife, including federally endangered and threatened salamander species;

- No method is known to eradicate Bsal in the environment once it is introduced and established; and
- Controlling Bsal is not practical in free-ranging salamanders carrying the fungus.

We clarify what is considered a salamander part that is not injurious and that would not need an injurious wildlife permit (partially adapted from WOAH 2021a):

- Heat-sterilized hermetically sealed amphibian products, that is, a heat treatment at 121 °C for at least 3.6 minutes (or any time or temperature equivalent that has been demonstrated to inactivate Bsal) (WOAH 2021a);
- cooked amphibian products that have been subjected to heat treatment at 100 °C for at least 1 minute (or any time or temperature equivalent that has been demonstrated to inactivate Bsal) (WOAH 2021a);
- pasteurized amphibian products that have been subjected to heat treatment at 90 °C for at least 10 minutes (or any time or temperature equivalent that has been demonstrated to inactivate Bsal) (WOAH 2021a);
- mechanically dried amphibian products and skin leather (that is, a heat treatment at 100 °C for at least 30 minutes or any time or temperature equivalent that has been demonstrated to inactivate Bsal) (WOAH 2021a); and
- chemical treatment of amphibian skin leather that inactivates Bsal (Van Rooij et al. 2017).

The above conditions apply to all salamanders listed as injurious in 50 CFR 16.14 due to the risk of carrying Bsal. Also not considered injurious, and therefore exempt, are eggs, gametes, chemically preserved specimens or parts (including tissues), and molecular specimens consisting of only the nucleic acids (DNA or RNA) from organisms. The appropriate concentration and minimum exposure time for a given chemical preservative or fixative to render any Bsal organisms non-viable varies with the precise chemical formulation and should be utilized as described in association with such

actions in the peer-reviewed literature. For example, Bsal is killed when exposed to 70 percent ethanol for at least 60 seconds (Van Rooij et al. 2017). However, parts that are otherwise preserved by air-drying or at a temperature and time that does not meet the above criteria or at a cold temperature (such as freezing) are considered injurious because Bsal is not inactivated by those methods. Purchase, sale, and other activities with the listed salamanders strictly within the boundaries of the enumerated jurisdictions within the shipment clause are not regulated under 18 U.S.C. 42.

This second interim rule takes effect on the date specified above in **DATES**, but we are providing the public with a period of time to comment on the listing and associated documents. The resulting final rule will contain responses to comments received on the second interim rule, state the final decision, and provide the justification for that decision.

Listing Species That Carry Pathogens

Pathogens are such agents as viruses, bacteria, and fungi that cause disease in animals and plants. The Service does not have the direct authority under 18 U.S.C. 42(a)(1) to list pathogens as injurious. We also cannot list or regulate fomites (materials, such as water, that can act as passive carriers and transfer pathogens). However, we can list wild mammals, wild birds, fishes, mollusks, crustaceans, amphibians, or reptiles that are hosts to or carriers of pathogens and that can be injurious if the likelihood, scope, and severity of effects significantly affect one or more of the interests listed in the statute. Even if the host species cannot establish populations in the wild, the host can present significant risk if the pathogen the host is carrying can infect wildlife or wildlife resources or affect human beings or the interests of agriculture, horticulture, or forestry in the United States. Among other impacts, diseases caused by introduced pathogens reduce biodiversity (the variety of different types of life in a region) and have been implicated in the local extinction of many animal taxa (Daszak et al. 2000).

Listing and Evaluation Process

The regulations contained in part 16 of title 50 of the Code of Federal Regulations (CFR) implement 18 U.S.C. 42(a)(1) and include the names of species determined by the Service or by Congress to be injurious. Under the terms of the statute, the Secretary of the Interior may prescribe by regulation those wild mammals, wild birds, fishes, mollusks, crustaceans, amphibians, reptiles, and the offspring or eggs of any of the foregoing that are injurious to humans, to the interests of agriculture, horticulture, or forestry, or to the wildlife or wildlife resources of the United States. The lists of injurious wildlife species are found at 50 CFR 16.11–16.15, with § 16.14 being for amphibians. Under these regulations, species are added to the lists of injurious wildlife to protect statutorily enumerated interests from potential and known negative effects. Most species listed have the capacity to establish populations in the wild, spread, and cause harm. However, a species can be listed based solely on its capacity to cause harm. For example, uneviscerated dead salmonids without a health certificate are not capable of establishing in the United States, but they are injurious because the pathogens they may carry are harmful.

Under 18 U.S.C. 42, the Service can list species that are nonnative and those that are indigenous to the United States. In the case of an indigenous species, for example, the Service may find that it is injurious because its transport and release outside the species' range may cause harm to human beings, agricultural or forestry interests, or natural systems. Furthermore, a species does not have to be currently imported or present in the wild in the United States for the Service to list it as injurious. For species not yet imported into the United States, the objective of listing is to prevent that species' importation and likely introduction and possible establishment and spread in the wild, thereby preventing injurious effects consistent with the purposes of 18 U.S.C. 42.

In response to the D.C. Circuit Court of Appeals Decision in *United States Association of Reptile Keepers, Inc.* v. *Zinke*, 852 F.3d 1131 (D.C. Cir. 2017), the prohibition on interstate transport in the 2016 interim rule has been modified. Because of the court's decision, injurious wildlife listings, including those listed by Congress through statutes (fruit bats (genus *Pteropus*), mongoose, zebra mussel, brown tree snake, bighead carp, quagga mussel), no longer result in a statutory prohibition on interstate transport of injurious wildlife between States within the continental United States. This means that transportation of injurious wildlife between the 49 States within the continental United States (the contiguous 48 States and Alaska) is not prohibited by 18 U.S.C. 42(a) (codified in Federal regulations at 50 CFR 16.3), unless that movement of the wildlife is restricted due to conditions associated with issued injurious wildlife permits. Thus, an injurious wildlife permit is generally not required to transport injurious species across State lines of any of the 49 continental States. However, a permit is still required for the movement of an injurious animal that was previously permitted for import or for the progeny of an individual that was permitted for import.

Import of injurious wildlife into the United States remains prohibited. In addition, transport of injurious wildlife between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42 (the continental United States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any territory or possession of the United States), codified in Federal regulations at 50 CFR 16.3, remains prohibited. These prohibited activities may be undertaken by permit for zoological, educational, medical, or scientific purposes (in accordance with permit regulations at 50 CFR 16.22), or by Federal agencies without a permit solely for their own use, upon filing a written declaration with the District Director of Customs and the U.S. Fish and Wildlife Service inspector at the port of entry. Interstate transport between States within the continental United States is not prohibited under the current prohibitions of 18 U.S.C. 42(a), and 18 U.S.C. 42(a) does

not prohibit intrastate transport (transport within a State or territory) or possession of injurious species. However, injurious wildlife unlawfully imported into the United States or transported between the enumerated jurisdictions is unlawful to transport within the continental United States, or to transport within a State or Territory, under the Lacey Act Amendments of 1981, 16 U.S.C. 3372(a)(1). It is the responsibility of a person who may be engaged in transport or use of injurious wildlife to be aware of any Federal, State, Tribal, or Territorial law or regulation that applies to that activity.

The Service prepares a listing rule by first assessing the relevant aspects of the biology of the species, such as its habitat, diet, reproductive capacity, climate, predatory capacity, and threats to its survival. This assessment is used to develop the next step, which is to evaluate whether any of these aspects contribute to the species being invasive or otherwise harmful.

The Service uses one or more of the injurious wildlife listing criteria identified below to evaluate whether a species qualifies as injurious under 18 U.S.C. 42. The results of the analysis using these criteria serve as a general basis for the Service's regulatory decisions regarding injurious wildlife species listings. Biologists and risk managers within the Service who are knowledgeable about a species that is being evaluated assess both the factors that contribute to and the factors and measures that reduce or remove the likelihood of injuriousness.

(1) Factors that contribute to injuriousness:

- The likelihood of release or escape;
- Potential to survive, become established, and spread;
- Impacts on wildlife resources or ecosystems through hybridization and competition for food and habitats, habitat degradation and destruction, predation, and pathogen transfer;
- Impacts to threatened and endangered species and their habitats;

- Impacts to human beings, forestry, horticulture, and agriculture; and
- Wildlife or habitat damages that may occur from control measures.

(2) Measures that reduce the likelihood of the species being considered as injurious:

- Ability to prevent escape and establishment;
- Potential to eradicate or manage established populations (for example, making organisms sterile);
- Ability to rehabilitate disturbed ecosystems;
- Ability to prevent or control the spread of pathogens or parasites; and
- Any potential ecological benefits to introduction.

For this second interim rule, we provide a general summary of the biology of salamanders and of the fungus, followed by the evaluation for both as injurious. For injuriousness of the salamanders, we focused on the third bullet above "Impacts on wildlife resources or ecosystems through * * * pathogen transfer." The issue in this rule is not about a given salamander species or genus being invasive but rather the role of salamanders in introducing the Bsal fungus into the United States and the scope and severity of effects caused by salamanders that are carriers of Bsal on the wildlife or wildlife resources of the United States.

The Service obtains an extensive amount of amphibian import data from our Office of Law Enforcement's (OLE) Law Enforcement Management Information System (LEMIS). LEMIS is an electronic database utilized by all Service law enforcement officers, including the Service's conservation officers, wildlife inspectors, refuge officers, and special agents. LEMIS serves as the portal in which all Service wildlife violations are documented and intelligence is gathered and shared between law enforcement offices across the country. LEMIS also serves as the conduit for all declared imports and exports of wildlife and wildlife products and the database of all such wildlife trade data in the United States, both legal and illegal. The database provided us with information for this

rule on what species were imported; quantity; countries of origin; ports of import; whether imported as live, dead, eggs, parts, or other; purpose for importing; and other relevant variables for the years 2010 to 2020 (USFWS OLE 2021).

We evaluated Bsal and the salamander species that carry this fungus using the injurious wildlife evaluation criteria, described in more detail as part of this second interim rule in G. *Factors That Contribute to Injuriousness of Salamanders,* which the Service developed to evaluate whether a species qualifies as injurious under the Act. The resulting analysis serves as a basis for the Service's regulatory decision regarding injurious wildlife species listings. This second interim rule finds that Bsal is a significant threat to the wildlife and wildlife resources of the United States and lists 16 genera of salamanders that we have determined to be injurious because they are likely carriers of Bsal and may introduce the fungus into the United States.

A. Species Information for Salamanders

Salamander Nomenclature and Taxonomy

The Service does not have a uniform policy for taxonomically identifying amphibians. In this interim rule, we use taxonomic nomenclature as described by AmphibiaWeb (*http://amphibiaweb.org*) with some comparison to the Integrated Taxonomic Information System (ITIS) (*http://www.itis.gov*). The system used by AmphibiaWeb represents one of the most widely accepted salamander taxonomic systems in the scientific community because it relies on criteria including, but not limited to, monophyly (common descent from a single ancestor), stability, expertise of scientists, and general acceptance by the amphibian conservation community and is frequently updated. As a Federal resource for taxonomic information, the Service also uses ITIS as an agency resource. As of May 1, 2023, AmphibiaWeb (2023b) reported 804 species of all salamanders in 68 genera and 10 families, and ITIS reported 738 species in 70 genera and 9 families.

In this rule, when we refer to salamanders, we include animals from the order Caudata commonly referred to as salamanders and newts. The nomenclature and taxonomy of salamander species that we are regulating should be provided as accurately as possible to the public so that the public and law enforcement officers know what is being regulated. However, the science is evolving, making consistency even from the 2016 interim rule difficult. The classification remained relatively unchanged from the 1960s until the 1990s, when advances in DNA sequencing enabled researchers to examine species relationships more closely (Petranka 1998). Furthermore, dozens of amphibian species from remote regions of the world are discovered every year (AmphibiaWeb 2021). This is generally why the number of species listed increased within the 20 genera in the final rule to the 2016 interim rule. For these reasons, we are not including the names of the species within each listed genus in 50 CFR 16.14. As long as the species is within a listed genus, it is covered as an injurious species, as in the final rule.

Salamander Biology

Salamanders belong to the class Amphibia, a group of cold-blooded vertebrate animals comprising frogs and toads (order Anura), salamanders and newts (order Caudata), and caecilians (order Gymnophiona). The word “amphibian” is derived from the fact that most of the species spend part of their lives in water and part on land. Frogs and toads have legs but no tails as adults, and caecilians have tails but no legs. Morphologically, salamanders are generally characterized by their relatively large, vertically flattened tails, two front and two hind legs that are approximately the same size (Petranka 1998), and skin with glands that can be either rough or smooth (Stebbins and Cohen 1997). Exceptions include Sirenidae, which have two small forelimbs and no hindlimbs, and Amphiumidae, which have four vestigial limbs. Adult salamanders range in length from around 4 centimeters (1.5 inches) to over 1.5 meters (5 feet) (Stebbins and

Cohen 1997). Another distinction between anurans (the frogs and toads) and the caudata (salamanders and newts) is that the anurans have internal gills as larvae and salamanders have external gills as larvae.

Salamanders can live for many years, but documented lifespans vary. Larger salamanders tend to live longer than smaller ones, and, with proper care, salamanders in captivity frequently live longer than those in the wild (Duellman and Trueb 1986). Records for captive animals range from 5 years for most plethodontids (lungless salamanders) to 55 years for the Japanese giant salamander (*Andrias japonicus*) (Duellman and Trueb 1986). The Olm or blind cave salamander (*Proteus anguinus*), which lives in caves in southern Europe, has been documented living for at least 48 years in the wild, with an estimated lifespan of more than 100 years (Live Science 2015).

Salamanders are carnivorous and eat a wide variety of prey, depending on habitat and the stage of their life cycle. Terrestrial adult salamanders eat earthworms, insect eggs, and other small invertebrates, while aquatic salamanders eat all of these in addition to small fish, aquatic insects, and other amphibians. Some salamander larvae can also be omnivorous and eat plants and animals.

Many salamanders have unique structural features, including costal grooves (grooves on the sides of the body that increase skin surface area for water absorption and transport) and nasolabial grooves (vertical slits between the nostril and upper lip used for sensing chemical stimuli in the environment) that can be used to differentiate between salamander species (Petranka 1998). Important features for identifying salamanders include head shape and size, fin shape and color, gill morphology, color patterns, number of toes, size, body shape, tooth patterns, and number of costal grooves. Some species appear similar to each other, and similarity of appearance within some families, such as Salamandridae, can make it difficult to differentiate between species, requiring close inspection of small physical characteristics.

Salamanders occupy a wide range of habitats, including streams, trees, land (including forests, grasslands, and rocky slopes), underground, and caves. Salamanders are cryptic (difficult to find) partly because they occupy moist, cool places, such as underneath logs and between rock crevices on land or under rocks and logs in the water.

Salamander courtship between males and females is regulated by chemicals that are released from specialized glands in the skin. Most salamanders reproduce by laying eggs in water with two exceptions: most members of family Plethodontidae lay their eggs on land, and the European species known as the alpine salamander (*Salamandra atra*) gives birth to live young (Stebbins and Cohen 1997). Eggs are surrounded by a protective jelly or membrane that keeps them from drying out. Almost all species of salamanders breed during specific seasons, and the length of time between mating and egg-laying varies considerably between species (Petranka 1998). Species that lay aquatic eggs place them in either streams or ponds, and species that lay their eggs on land choose hidden places, such as underground burrows, decaying logs, and moist rock crevices (Petranka 1998), where the young typically undergo direct development, whereby metamorphosis occurs in the egg and fully formed salamanders emerge from the eggs.

The majority of the species in Ambystomatidae (mole salamanders) spend most of the year underground in rodent burrows and emerge only on rainy nights to mate and feed. Ambystomatid salamanders are famous for the migration of large numbers of individuals to breeding ponds. One example of a species that spends most of its life on land but that moves to aquatic areas to breed is the California tiger salamander (*Ambystoma californiense*). During winter rains, this species migrates across land to aquatic pools, such as cattle tanks and ephemeral pools, to breed. At the breeding pools, individuals come in contact with each other, even though they may not come in contact with each other during most of the rest of their lives on land (Barry and Shaffer 1994). However, the related axolotl (*A. mexicanum*) is unlike other salamanders by being

neotenic (they do not undergo metamorphosis). Furthermore, some ambystomatids retain their larval morphology as reproductive adults until certain environmental cues trigger metamorphosis into terrestrial adult morphology.

Habitat Conditions and Native Range of U.S. Salamanders

With more native salamander species than any other country in the world, the United States is a salamander diversity hotspot (PARC 2015). Salamanders are widespread in the United States (Caudata Culture 2015a; U.S. National Park Service 2015). Areas of particularly high salamander diversity include the Eastern United States, with large numbers of plethodontid salamanders in the southern Appalachian Mountains (Richgels et al. 2016).

Salamanders in the United States occupy a wide range of habitats, including streams, trees, land (including forests, grasslands, and rocky slopes), underground, and caves. These locations are most conducive to the relatively cool, moist conditions under which both salamanders and Bsal thrive (Duellman and Trueb 1986; Piotrowski et al. 2004; Blooi et al. 2015a). Central and North American salamanders as a group are active at average temperatures of 11 °C (52 °F) to 20 °C (68 °F) (Duellman and Trueb 1986), fully encompassing the optimum temperature for Bsal growth as described below under *Climate Tolerance*. Salamanders require some amount of constant moisture for physiological function, such as osmoregulation (controlling body fluid, water, and salt balance) or for cutaneous respiration, as in the lungless family Plethodontidae, or for temperature regulation (Duellman and Trueb 1986).

Twenty species of U.S. salamanders from seven genera (*Ambystoma*, *Batrachoseps*, *Cryptobranchus*, *Eurycea*, *Necturus*, *Phaeognathus*, *Plethodon*) are currently listed as endangered or threatened under the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq.*) (ESA). The specific vulnerability and carrier status

of these species to Bsal is described below in *Vulnerability and Carrier Status of Threatened and Endangered Species.*

Of the 221 salamander species in 23 genera in 9 families native to the United States, we expect that at least 164 species in 13 genera and in 5 families are capable of being carriers of Bsal: Ambystomatidae, Cryptobranchidae, Salamandridae, Sirenidae, and Plethodontidae. In North America, species in the family Salamandridae occur on the west coast of the United States and Canada, from southern California to southeastern Alaska, as well as much of the eastern half of the United States and extreme southeastern Canada (Caudata Culture 2015a). Members of the family Sirenidae occur throughout the southeastern Atlantic and Gulf of Mexico coastal plains and the Mississippi River Valley (Leja 2005) (lesser siren, *Siren intermedia*) and in the Atlantic coastal plains from south Florida to Virginia (greater siren, *Siren lacertina*) (Hendricks 2005). The distribution of salamanders of the family Plethodontidae in the western hemisphere is from southern Canada to Bolivia and Brazil, except for members of the genus *Hydromantes*, which occur in California (AmphibiaWeb 2023a, Caudata Culture 2015a). Ambystomatidae has only one genus, and the 32 species are widely distributed in North America from southern Canada south to Mexico City. Cryptobranchidae is represented by one species in North America (eastern hellbender *Cryptobranchus alleganienesis*).

Role of Salamanders in the Ecosystem

Salamanders play important roles in ecosystem function and as indicators of ecosystem health and stability (Davic and Welsh 2004). For example, salamanders of family Plethodontidae have life-history characteristics that make them highly effective at controlling invertebrates that would otherwise consume the leaf litter, thus releasing carbon to the environment (Best and Welsh 2014).

In forests, salamanders are also among the most abundant vertebrates. Despite the relatively small size of most salamanders compared to most other native vertebrates, this

sheer abundance contributes to a significant amount of biomass in the ecosystem, and, therefore, salamanders make significant contributions to nutrient cycling and transport (Burton and Likens 1975). For example, Ambystomatid salamanders can make significant contributions to energy and nutrient transport in forest ecosystems (Regester et al. 2006) and in pond ecosystems (Holomuzki et al. 1994). Many terrestrial salamanders consume arthropods (insects and related invertebrates) that feed on leaf litter, and the invertebrates' feeding process causes the release of carbon dioxide from the ground into the atmosphere. With fewer salamanders and more litter-consumers, more carbon is released from the soil, contributing to an excess of carbon dioxide in the atmosphere. Salamander populations help reduce carbon emissions from leaf litter decomposition, which has implications for the global carbon cycle (Wyman 1998; Best and Welsh 2014; North American Bsal Task Force 2020, Laking et al. 2021). This process is known as carbon sequestration, which is the storage of carbon dioxide to slow or reverse atmospheric carbon dioxide pollution and to mitigate or reverse climate change. Salamanders that live underground also contribute to soil dynamics by creating, modifying, and otherwise regulating the systems of underground burrows in which they live (Davic and Welsh 2004). Finally, salamanders are important prey species themselves and provide energy sources for higher predators (Davic and Welsh 2004), including fishes, reptiles, birds, and mammals.

In vernal pond communities, *Ambystoma* species are the top predators and, therefore, control the abundance of aquatic invertebrates and other amphibians (Petranka 1998). The high numbers of many amphibians, including salamanders, in some ecosystems also provide a substantial source of prey for other vertebrates in the ecosystem (Harper et al. 2008; Davic and Welsh 2004); therefore, other native species that prey on salamanders can also be affected by disease-related declines.

B. Species Information for *Batrachochytrium salamandrivorans*

General Information About Bsal

Bsal is a fungus in the phylum Chytridiomycota and the order Rhizophydiales. It was identified in 2013 after reports of a dramatic mortality event of fire salamanders in Europe (Martel et al. 2013). In drawing some of our conclusions about the effects of Bsal on U.S. wildlife and wildlife resources for the 2016 interim rule, the Service used *Batrachochytrium dendrobatidis* (Bd) as a surrogate (similar substitute) species because little was known about the emerging disease caused by Bsal. Considerably more was known about Bd due to its discovery and description more than 15 years earlier (Berger et al. 1998, Longcore et al. 1999). Bd is found on every continent that supports amphibians, while Bsal is known to be only in Europe and Asia. Bd has resulted in the serious decline and extinction of more than 200 species of amphibians worldwide and has posed the greatest threat to biodiversity of any known pathogen (Martel et al. 2013). The severe effects that Bd, also a fungal pathogen species closely related to Bsal, has had on amphibian populations raised additional alarm about the expected consequences of a Bsal introduction and the need to take immediate action under an interim rule.

Two scientific risk assessments of Bsal used Bd in determining the risk of Bsal based on transmission, spread, and population-level effects (Stephen et al. 2015; Richgels et al. 2016). Gray et al. (2015) found that both fungi infect the epidermal cells of the amphibian skin, and the clinical signs for both include excessive skin shedding, lethargy, anorexia, abnormal posture, and death; however, the lesions produced by Bd are mainly epidermal hyperplasia and hyperkeratosis (rarely ulcerations), and those produced by Bsal are mainly skin ulcerations and destruction of the epidermis. Similarities and differences between the two fungal pathogens and the diseases they cause are discussed in Farrar et al. (2017), Longo et al. (2019), Rebollar et al. (2020), and Rollins and Le Sage (2021).

Since the 2016 interim rule was published, scientists have been studying many aspects of Bsal, and this rule reflects new research. Relevant studies confirm or expand on our previous information, with many adding new insight to the fungus and disease, and some providing documentation to support adding new genera as carriers.

Until Bsal was discovered, the fungal disease chytridiomycosis was thought to be caused by a single species of pathogenic fungus, Bd, which was the only species from that phylum known to parasitize vertebrate hosts (Longcore 1999; Johnson and Speare 2003). Bd has been implicated in the decline and extinction of amphibian species at the global scale (Berger et al. 1998; Daszak et al. 2003; Lips et al. 2006; Walker et al. 2008; Vredenburg et al. 2010; Cheng et al. 2011). Bd has been found on every continent except Antarctica, and it is known to have affected more than 500 species of amphibians, including all orders of amphibians (frogs, salamanders, and caecilians) worldwide (Chytridcrisis 2015a; Fisher et al. 2009; Olson et al. 2013).

Bsal came to the attention of the scientific community in 2013 when Spitzen van der Sluijs et al. (2013) observed a 96 percent decline in fire salamanders in the Netherlands but was "unable to attribute this to any known cause of amphibian decline, such as Bd, ranavirus or habitat degradation." Martel et al. (2013) subsequently identified the cause of the salamander decline in the Netherlands as a newly described species of fungus now known as Bsal. Their work confirmed that Bsal is closely related to Bd and is also capable of causing chytridiomycosis; both are in the genus *Batrachochytrium*. Analysis of a broad range of representative chytrid fungi show that Bsal represents a previously undescribed species that shares early evolutionary origins with the pathogenic fungus Bd (Martel et al. 2013).

The natural amphibian hosts of Bsal remain unknown, but as of publication of the 2016 interim rule, Bsal had been found only in salamanders and appeared capable of causing lethal chytridiomycosis only in salamanders (Martel et al. 2014). Subsequently,

several species of anurans have been found to be carriers, such as the midwife toad (a frog) *Alytes obstetricans* (Stegen et al. 2017) and small-webbed firebelly toad *Bombina microdeladigitora* (Nguyen et al. 2017).

How the Fungus Affects Salamanders

The "salamandrivorans" in *Batrachochytrium salamandrivorans* means "salamander eating" and figuratively describes the effects of the fungus on salamanders. Bsal infects primarily the skin of salamanders, but deeper tissues or internal organs may be affected (McDonald et al. 2020). The skin of post-embryonic salamanders has a layer of keratin (Seifert et al. 2019) covered by a mucosal microbiome of beneficial biota that normally protect them from harmful microbes entering the body (Bletz et al. 2018; Rebollar et al. 2020), such as a fungus.

The cells of the fungus (thalli) embed themselves in the skin cells of the salamander, thereby causing erosive lesions. Lesions consist of sores on the skin that erode and ulcerate, with secondary bacterial infection likely occurring after the sores appear (Martel et al. 2013; Gray et al. 2015; Bletz et al. 2018), although many of the salamanders reported at the beginning of the European Bsal outbreak seemed to lack obvious external lesions (Spitzen-van der Sluijs et al. 2013). The loss of epidermal integrity from the lesions impairs the skin's ability to maintain fluid and electrolyte balance and also perforates the barrier that protects the animal from pathogens and compromises their line of defense against disease (Gray et al. 2015; Rebollar et al. 2020). Experimental infections of fire salamanders in the laboratory caused death 12 to 18 days after exposure, with the same clinical signs and pathological lesions found in the European outbreak (Martel et al. 2013) and in another experimental infection around 3 weeks after a high dose exposure (Stegen et al. 2017). Martel et al. (2013) found that infected fire salamanders developed shallow skin lesions and deep ulcerations all over the body, became anorexic and apathetic, and suffered from neurological signs including a

loss of voluntary movement and muscle coordination. Death occurred within 7 days of clinical signs first appearing in species with lethal vulnerability. Death is generally preceded by a brief episode of abnormal body posture and behavior (Gray et al. 2015).

Some species succumb quickly to Bsal after infection, while others seem to tolerate it and eventually clear the infection (Martel et al. 2014). However, long-term effects on tolerant species are not known. Several studies suggest negative long-term effects. For example, the long-term proliferation of the fungus within the keratinized limb tissue of the olm (*Proteus anguinus*) may coincide with a more subtle cost associated with increased energy expenditure, impaired locomotion, or increased vulnerability of the limbs to secondary infection (Li et al. 2020). Another study detected potential sublethal reductions of growth caused by Bsal exposure in juvenile spotted salamanders soon after metamorphosis, although not in older juveniles (Barnhart et al. 2020). The initial exposure to Bsal may have created a stress response that helped activate the immune system; this activation dissipated after the threat dissipated but may come at a cost because juvenile salamanders with higher corticosterone release rates immediately after exposure to Bsal had lower growth compared to control group salamanders 30 days post-exposure.

The outcomes of coinfections by Bd and Bsal on salamanders have been studied because they both affect the skin. Bd and Bsal are the only known Chytridiomycota to have adapted to colonize vertebrates, yet Bd infects all three orders of Amphibia (especially Anura), while Bsal is currently known to infect primarily the order Caudata (Farrar et al. 2017). If Bsal enters the Western Hemisphere where Bd is already widespread, coinfections could occur, and some research suggests the results could be more serious to the Bsal-naïve salamanders than Bd infection alone (Longo et al. 2019; McDonald et al. 2020). Longo et al. (2019) and McDonald et al. (2020) studied coinfection in eastern newts (*Notophthalmus viridescens*), a widespread native

salamander. Longo et al. (2019) tested newts to see if prior exposure to Bd provided immune protection from Bsal or instead reduced the protection. They found that newts can clear Bd alone; resistance is specific to Bd and does not prevent Bsal infection; simultaneous coinfections were the most lethal, even at reduced dosages; host mortality from Bsal can be much slower than previously found; and some wild newts may have innate Bsal immunity from prior exposure to Bd, but other factors may be involved. McDonald et al. (2020) found that Bd and Bsal coinfection reduced the host's immune response more than with Bsal alone. Thus, if Bsal enters a newt population where Bd already exists, the Bsal infection may be compounded by the Bd infection.

The Service has no direct evidence that Bsal affects reproductive tissue, such as eggs or gametes. Since Bsal attaches to and utilizes keratin-containing substrate for growth, and eggs and gametes do not contain keratin, we have no evidence that eggs and gametes will carry Bsal (L. Sprague, USFWS, pers. comm. 2021). Thus, we do not believe that salamander reproductive material can serve as a vector for Bsal introduction into the United States.

Thermal Tolerance

Temperature has a significant effect on the growth and disease development of Bsal in salamanders (Martel et al. 2014; Carter et al. 2021). Bsal appears to prefer an in vitro temperature range for growth and infection of 10–15 °C (50–59 °F) (Martel et al. 2013; Blooi et al. 2015a; Stephen et al. 2015; Thomas et al. 2019). Bsal has shown some spore growth in temperatures as low as 5 °C (41 °F) and dies at 25 °C (77 °F) and above (Martel et al. 2013). However, the majority of Bsal-infected salamanders in natural Vietnamese ponds were in water temperatures of 20–25 °C (68–77 °F) and as high as 26.43 °C (79.6 °F) (Laking et al. 2017). In a laboratory study, salamanders were most easily infected by Bsal at temperatures of 15 °C (59 °F) and 20 °C (68 °F), while Bsal growth was inhibited at 25 °C (77 °F) (Blooi et al. 2015a). The same temperature

response was also observed for Bsal raised in culture (Blooi et al. 2015a). Grear et al. 2021 used 15 °C (59 °F) as the thermal optimum for Bsal growth and evaluated the impact of a 27 °C (81 °F) thermal maximum to the resulting risk. Carter et al. (2021) found that adult and juvenile *Notophthalmus viridescens* died faster due to Bsal chytridiomycosis at 14 °C (57 °F) than at 6 °C (43 °F) and 22 °C (72 °F).

These experimental data suggest that salamanders living at cooler temperatures are more at risk to infection by Bsal. Animals that survive at temperatures above the optimal range for fungal growth are likely to be at reduced risk to infection. However, the average temperature range of North and Central American salamander species is from 11 °C (52 °F) to 20 °C (68 °F) (Duellman and Trueb 1986; the citation does not separate North and Central American data), so salamanders regularly reaching 25 °C (77 °F) in the natural environment is uncommon. Bales et al. (2015) noted that the native salamander species, and by extension ecosystems, most at risk from a Bsal introduction would likely be those that occupy similar thermal ranges as the European fire salamander (Bales et al. 2015). Richgels et al. (2016) also cited research that Bd is capable of infecting amphibians along a larger temperature profile than originally predicted, though it is unknown whether this is the case for Bsal.

Ecology and Habitat Preferences

The chytrid fungus Bd can live outside of a host and requires water to disperse because it reproduces asexually by forming motile zoospores; preliminary studies of Bsal indicate that similar modes of survival and transmission are highly likely (Longcore 1999; Martel et al. 2013). As the threat assessment by Stephen et al. (2015) noted, “Bd is known to remain viable for several days to weeks in water (Johnson and Speare 2013) and moist organic matter (Johnson and Speare 2003), even in the absence of nutrients. It is likely that Bsal can also survive in moist environments, independent of an amphibian host.” Stegen et al. (2017) states that Bsal adopts a dual transmission strategy, with

environmentally resistant nonmotile spores in addition to the motile spores identified in Bd. Bsal retains its virulence in water and soil as well as in anurans and less susceptible salamander species that function as a reservoir of infectious pathogens. The combined characteristics of the disease ecology suggest that Bsal is able to rapidly extirpate highly susceptible salamander populations across Europe. Stegen et al. (2017) also found that infected fire salamanders were shown to contaminate the forest soil and Bsal DNA could be detected even after 200 days. Actual transmission through contaminated forest soil was demonstrated up to 48 hours after the soil had been in contact with an infected animal. Encysted Bsal spores were shown to remain infectious in pond water for at least 31 days. Together, the presence of a resistant spore with the ability to persist environmentally and to transmit through contaminated water and soil, combined with the occurrence of long-term-infected and pathogen-shedding amphibian hosts, creates the potential for extensive environmental reservoirs and hampers any effort to eradicate Bsal from an infected ecosystem.

Environmental Conditions Needed To Survive

The transmission and ecology of Bsal in the wild is likely to be similar to Bd based on the close taxonomic relationship between the species, their structural similarities, and their comparable pathophysiology (Martel et al. 2013; Stephen et al. (2015). Disease transmission is the means by which communicable pathogenic microorganisms, such as fungi, are spread from one organism to another. Johnson and Speare (2003) reported that Bd can survive in tap water and deionized water for up to 3 and 4 weeks, respectively, and up to 7 weeks in lake water. Bsal is also likely to survive in moist environments independent of an amphibian host; for example, Stegen et al. (2017) found that encysted spores can survive and remain infectious for at least 31 days in filtered pond water. While we do not have information on the response of Bsal to desiccation, Bd is highly impacted by drying and can survive desiccation for no more

than 1 hour in the laboratory (Garmyn et al. 2012); Bsal would likely respond in a similar way. Bsal appears to be adapted to lower preferential temperatures compared to Bd, with optimal growth between 10 ºC and 15 ºC, and Bsal spore death occurring at temperatures greater than 25 ºC (Martel et al. 2013). These findings support the hypothesis that the pathogen coevolved with salamanders in the part of the world from which it is endemic, most likely in Asia (Martel et al. 2014; Laking et al. 2017).

C. Population-Level and Ecosystem-Level Effects of Bsal

Population-Level Effects

Several pathogens, including Bsal, Bd, ranaviruses, and aquatic oomycetes (water molds), have caused significant population-level declines in a range of amphibian species, and disease is thought to be a major driver of global amphibian decline (Bosch et al. 2001; Daszak et al. 2003; Martel et al. 2013). Disease poses a greater risk to small, isolated populations as well as those with decreased genetic diversity (Smith et al. 2008). Within the United States, diseases have been cited as contributing factors in the listing or need for recovery of several native amphibian species under the ESA. Examples include Bd in the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) (76 FR 61956, October 6, 2011), an undiagnosed disease in Sonora tiger salamanders (*Ambystoma tigrinum stebbinsi*) (62 FR 665, January 6, 1997), and Bd in the mountain yellow-legged frog (*Rana muscosa*) (82 FR 24256, April 29, 2014; Vredenburg et al. 2010).

As noted above in *General Information About Bsal*, Bsal is the most recently discovered pathogen associated with population-level amphibian declines, including a 96 percent reduction in Dutch populations of the European fire salamander in the period 2010–2013 (Spitzen-van der Sluijs et al. 2013; Martel et al. 2013). Due to the overall sensitivity of amphibian populations to disease; a history of adverse, population-level effects in native amphibians; a direct association between Bsal and the decline of at least one European salamander population; and the adverse effects of some native salamanders

to Bsal under experimental conditions, we conclude that the introduction of Bsal into the United States would cause significant, adverse, population-level effects in a number of native species.

Ecosystem-Level Effects

The preferred temperature range of Bsal can help predict those ecosystems that are at greatest risk should Bsal be introduced into the United States (Stephen et al. 2015). The native salamander species, and by extension ecosystems, most at risk from a Bsal introduction would likely be those that occupy similar thermal ranges as the European fire salamander (Bales et al. 2015).

Salamanders are important parts of the ecosystems in which they occur. Salamanders are often the most abundant vertebrates in terrestrial forest and riparian (the banks of watercourses) ecosystems, where they may compose a total biomass greater than or equal to birds or small mammals (Davic and Welsh 2004). This means that, despite their small size, the total weight of all salamanders in a given area may be more than the combined total weight of all birds or all small mammals. Because of their abundance under normal circumstances, salamanders are important prey species themselves and are energy sources for higher predators (Davic and Welsh 2004), including fishes, reptiles, birds, and mammals.

Salamanders may be the dominant predator in headwater streams and ephemeral waterbodies where fish are absent (Davic and Welsh 2004). Within some food webs, salamanders are considered keystone predators due to their control of invertebrate prey populations and their resulting regulation of detritus decomposition and nutrient cycling (Davic and Welsh 2004). By definition, keystone species are those that occupy niches that affect ecosystems and have little functional overlap with other species (Davic and Welsh 2004). Therefore, loss of these keystone species would result in significant ecosystem-level change.

In addition to their roles in food webs and nutrient cycling, salamanders participate in a number of interspecific (between species) ecological relationships. Salamander species interact with one another through competition and predation to control the composition of their assemblages (taxonomically related species that occur within the same geographic community) (Davic and Welsh 2004; Fauth et al. 1996). Frequently, a single species is dominant within a given assemblage, particularly in terrestrial habitats, but which species dominates varies by location and ecosystem (Davic and Welsh 2004). We expect that ecosystems where the dominant salamander species is susceptible to lethal or sublethal Bsal infection would be at risk from an introduction of this pathogen.

Salamanders also interact with invertebrate species in other ecologically important ways. Semi-aquatic salamander species can move mollusks and shrimp eggs between waterbodies during their migrations, allowing these invertebrates to inhabit new areas (Davic and Welsh 2004). For example, a native species of salamander, the mudpuppy (*Necturus maculosus*), is a required host for developing stages of the salamander mussel (*Simpsonaias ambigua*), a native, freshwater mollusk (Davic and Welsh 2004; Gangloff and Folkerts 2006). We conclude that invertebrate species that depend on salamanders for aspects of their life cycle or ecology are likely to be adversely affected if their host species declines in response to a Bsal introduction.

D. Invasiveness of Salamanders and Bsal

Invasiveness of Salamanders

Executive Order 13751 defines an "invasive species" as a nonnative organism with regard to a particular ecosystem whose introduction causes or is likely to cause economic or environmental harm, or harm to human, animal, or plant health. Two key components of invasiveness are introduction into nonnative ecosystems and causing harm.

Globally, 90 percent of salamander introductions have occurred through intentional releases (Tingley et al. 2010). As of 2010, salamanders comprised 22 percent of all recorded amphibian introductions, with the highest number of salamander introductions (15) from the family Salamandridae, followed by salamanders from the families Ambystomatidae (4), Cryptobranchidae (2), and Proteidae (2) (Tingley et al. 2010). Nonnative salamander introductions have been documented in the United States. The USGS's Nonindigenous Aquatic Species database has U.S. records for 17 salamander species that have been observed outside their native range (USGS 2023). Of those, 14 are native to the United States but were discovered outside of their native ranges, and 3 (Japanese fire-bellied newt *Cynops pyrrhogaster,* Oriental fire-bellied newt *Cynops orientalis,* and paddle-tailed newt *Paramesotriton (Pachytriton) labiatus*) are species native to the eastern hemisphere. In Florida, the Oriental fire-bellied newt and paddle-tailed newt (family Salamandridae), which are native to China, have been found in the wild near an animal importer's facility, either as the result of intentional releases or escapes from enclosures (Krysko et al. 2011), although none have been reported since 2010 (USGS NAS 2021 [CYOR, PALA]). While these two species apparently did not establish invasive populations, their presence in the wild demonstrates a possibility that escaped individuals can persist long enough in the wild to transmit Bsal to native populations.

Other invasions have been attributed to the use and subsequent release of salamanders used as fishing bait. Surveys of anglers have indicated that they routinely release salamanders into the areas where they fish, which includes areas that are not part of the salamander's native habitats, suggesting that animals are routinely moved long distances (Picco and Collins 2008). Furthermore, Picco and Collins (2008) found that salamanders sold as bait were highly infected with both ranavirus and Bd, thereby

increasing the likelihood of disease transmission into new areas of the United States through the act of fishing.

Invasiveness and Transmission of Bsal

As noted above under *General Information About Bsal*, Europe has been experiencing a severe decline in wild fire salamander populations in the Netherlands (Spitzen-van der Sluijs et al. 2013). This decline is so significant that fire salamander populations are facing local extinction in the Netherlands. A sharp decline in numbers has been observed since 2010, despite the species being listed as endangered on the Netherlands Red List, and at population levels that were thought to be stable. This enigmatic decline was not attributed to any known cause of amphibian decline, such as chytridiomycosis due to Bd, ranavirus, or habitat degradation. In late 2013, Bsal was isolated from infected fire salamanders in the Netherlands (Martel et al. 2013).

Martel et al. (2014) later established the highly pathogenic nature of this new chytrid fungus. Molecular testing found Bsal in specimens collected from the wild (though none from North America) and even in an archival (museum) sample that was 150 years old (Martel et al. 2014). Alpine newts (*Ichthyosaura alpestris*) and smooth newts (*Lissotriton vulgaris*) in the wild are also now known to be infected (Spitzen-van der Sluijs et al. 2016), as are palmate newts (*L. helveticus;* Lastra González et al. 2019).

A wide variety of salamanders are negatively affected by the pathogen, but 10 species of frogs and toads and the 1 caecilian species did not appear to be (Martel et al. 2014). More recently, two anuran species have been shown to be carriers of Bsal. Small-webbed fire-bellied toads from wild populations in Vietnam and from individuals from that region imported into Germany tested positive for Bsal by qPCR swabs (Nguyen et al. 2017), and the midwife toad was capable of infecting fire salamanders for several weeks after experimental inoculation (Stegen et al. 2017). However, we are listing only caudate genera with this rule, and anurans would need to be considered in separate rulemaking.

The pathogenic nature of the fungus and its ability to infect a wide variety of salamanders, as described below in *Vulnerability and Carrier Status*, definitively demonstrate an invasive threat to salamanders in the United States.

Emerging infectious diseases that can cause mass mortality are especially worrisome because they can cause extinction and subsequent loss of biodiversity relatively rapidly. The only *in situ* example of the spread of Bsal is with fire salamanders in Europe. Using this example, Schmidt et al. (2017) developed a model to explore the effects of the recently emerged Bsal. They showed that disease outbreaks can occur at very low host densities (one female per hectare (2.5 acres)) in the wild and that this is much lower than host densities in the wild. Therefore, all naturally occurring populations are at risk, and the model predicts a rapid collapse of the host population. Experiments have shown that Bd can be transferred from one species to another when an uninfected species comes into contact with an infected species (active carrier) or infected fomites (Carey et al. 2006) (passive carrier). Bsal can similarly be transmitted from one species to another (Martel et al. 2014; Stegen et al. 2017; Homan et al. 2018). Bd has contributed to the decline of at least 501 amphibian species worldwide (6.5 percent of described amphibian species), which is the largest documented loss of global biodiversity attributable to a pathogen (Scheele et al. 2019). Bsal is expected to have a similar effect, although perhaps not as much on anurans.

Salamanders that breed in ponds and temporary wetlands are often explosive breeders, meaning that hundreds to multiple thousands of individuals will congregate at the same time (Gill 1978), creating dense numbers of individuals and increasing opportunities for the pathogen to spread. After breeding, the adults then return by land to their original habitats, potentially carrying the pathogen to new locations. Pathogens are also likely to be transmitted by salamander species that travel long distances for breeding and dispersal migrations, such as those that exhibit a metapopulation structure (Bancroft

et al. 2011). A metapopulation is a group of discrete breeding populations of the same species (Gill 1978). For example, within salamander metapopulations, California tiger salamanders have been documented traveling up to 1.2 miles (1.9 kilometers) from upland habitat to aquatic breeding sites (USFWS 2000), and newts travel many kilometers to breeding sites (Gill 1978).

Salamander species that have abundant populations with widespread distributions can also contribute to the spread of Bsal because of the increased likelihood that they will come in close contact with other salamanders that could then become infected. Salamanders that can carry Bsal from one place to another are more likely to do so if they have a broad range where they will come in contact with other members of the same species (for abundant distributions) or other species (for widespread distributions). Species with broad distributions are adapted to a wide range of environmental conditions that are more likely to overlap with habitat suitable for Bsal as well as habitat suitable for that species, providing increased opportunities for Bsal to spread.

For example, the rough-skinned newt has a wide range along the West Coast from Alaska to California, and the eastern newt is found widely across the Eastern United States and Canada, occurring in 34 States (AmphibiaWeb 2023a). Both species have had lethal responses with laboratory infections of Bsal (Martel et al. 2014; Carter et al. 2021; Gray et al. 2023), and both are capable of carrying Bsal. In addition to its broad range, the eastern newt also migrates long distances; this species will frequently travel many kilometers to migrate to new ponds (Gill 1978), further increasing the risk of this species spreading Bsal. The eastern newt's widespread distribution, high dispersal ability, high susceptibility, and juxtaposition with a high diversity of other salamander species position the species to significantly contribute to the spread of Bsal and the decline of salamander populations in the Eastern United States (Malagon et al. 2020).

E. Pathway Analysis

Introduction Pathways

The main pathway for the global spread of Bsal is the international trade in salamanders (Martel et al. 2014; Yuan et al. 2018). The introduction of Bsal into mainland Europe is linked with the commercial trade of Asian salamanders (*Cynops* spp.) from East Asia, particularly Thailand, Vietnam, Japan (Martel et al. 2014), and China (Yuan et al. 2018). Combined, species from the genus *Cynops* were by far the most commonly imported into the United States from 2004 to 2014 (USFWS OLE 2015), with *Cynops orientalis* alone comprising 54 percent of the salamander imports. Since the 2016 interim rule went into effect, *Cynops* spp. imports have been restricted to those with approved permits from the Service. As described above in *How the Fungus Affects Salamanders,* there is no evidence that eggs and gametes are vectors. However, salamanders that have been identified as carriers, whether live or dead (except if chemically or heat preserved), appear capable of transmitting Bsal through contact with their skin (Gray et al. 2015; Van Rooij et al. 2015; Carter et al. 2020), which contains keratin (Seifert et al. 2019). We are also concerned that any infected and lethally vulnerable salamanders may die in transport and continue post-mortem to carry Bsal into the United States, or that salamanders may knowingly be imported dead. Bsal can remain viable inside dead host tissue (Martel et al. 2013), although it is unclear how long a dead host remains infectious (WOAH 2021b). One study found that viable Bsal loads on carcasses of eastern newts did not decline in 72 hours after euthanasia, and carcasses were capable of transmitting Bsal to susceptible hosts for at least 72 hours after death; the infections that developed in the susceptible animals caused nearly 100 percent mortality in cohousing treatments that allowed for contact (Carter et al. in review). Therefore, we expect unpreserved, dead salamanders and body parts, except for purified extracted genetic material, to be a pathway for introduction.

Individual amphibians in trade are often transported in containers with many other individuals of the same species or with many other species that can all be from different sources. These conditions are highly conducive to pathogen transmission and dispersal. Pathogens can transfer from host to host in crowded conditions, and crowded conditions create stress on animals that can reduce amphibian hosts' natural ability to resist infections (Rowley et al. 2007, Rachowicz et al. 2005, Rollins-Smith et al. 2011). Bsal can also be introduced into the environment through the improper disposal of contaminated water or other materials used to transport salamanders. As described above under *Environmental Conditions Needed To Survive*, the fungus can likely persist in those materials independent of the presence of a salamander. Water and fomites have served as a transmission medium and passive vector, respectively, to introduce other similar pathogens into the environment. For example, Bd has been found in water used to transport amphibians that were traded in Hong Kong (Kolby et al. 2014). As the authors noted, "[T]he abundance of aquatic amphibian species traded by Hong Kong * * *, prolonged environmental persistence of infectious * * * Bd particles, and employment of trade activities that neither disinfect water nor safely dispose of deceased animals creates an ideal pathway for disease transmission to native Hong Kong amphibians." While experiments of fomite transmission for Bsal have not been conducted yet to our knowledge, Bd has been shown capable of infecting boreal toads (*Bufo boreas*) if an uninfected individual comes in contact with water where an infected individual has shed spores, even if the infected individual is no longer present (Carey et al. 2006). Similarly, encysted Bsal spores have been shown to remain infectious in pond water for at least 31 days and capable of adhering to salamander skin and the feet of waterfowl (Stegen et al. 2017).

Disinfecting of containers and substrate is always advisable when transporting amphibians. However, Van Rooij et al. (2017) found that the cell wall of the

zoosporangium and the encysted spores within it provide a double barrier against the action of the disinfectants. This may explain (partially) why higher disinfectant concentrations or a longer contact time are necessary to achieve full fungal killing of Bsal, compared to those necessary for inhibition of Bd. Also, the clustering of multiple Bsal zoosporangia may protect centrally located sporangia from the full impact of a given disinfectant.

Drawing on this evidence, the primary pathway for the entry of salamanders that are carriers of Bsal into the United States is through the international commercial wildlife trade. Overall, 99.9 percent of live salamander importation into the United States is for commercial purposes (USFWS OLE 2021). From 2010 to 2015, live salamanders were imported through 20 ports of entry into the United States; the 3 ports of entry with the largest numbers of imported salamanders were Los Angeles (54.6 percent), Tampa (34.8 percent), and New York (6.8 percent). From 2016 to 2020, live salamanders were imported through 8 ports of entry, with the top 4 from Miami (63.3 percent), Boston (11 percent), Newark (10.5 percent), and New York (9.2 percent). After import, many of the salamanders are transported to animal wholesalers, who then transport the salamanders to pet retailers.

The most likely pathway of a salamander that is a host to Bsal into the United States would be through captive salamander commercial trade. We make that conclusion based on Martel et al. (2014), who noted that, given the discontinuity of the global distribution of Bsal, introduction from Asia into Europe must have been human mediated. Cunningham (2015), Sabino-Pinto et al. (2015), and Grear et al. (2021) also implicated trade as a key factor in the spread of Bsal. The United States is more isolated than European countries from other countries where salamanders could migrate independently, but there is an active trade in salamanders into the United States (USFWS OLE 2021), as discussed more in the following section (and would be more so without the second

interim rule and final rules). People can purchase salamanders from pet stores or online retailers and keep them in captivity. Amphibians and reptiles kept as pets may eventually be released by their owners into the wild either intentionally or accidentally (Kraus 2009, Krysko et al. 2011). For example, owners may no longer be able to care for their captives or an animal may escape its enclosure. Sick captive salamanders are often released instead of being euthanized; for example, around 200 Japanese fire-bellied newts were released in the Miami, Florida, area in 1964 when they became sick and unsuitable for sale (USGS NAS 2021 [CYPY]). They died, but fortunately, this was before highly contagious amphibian pathogens were known to be in the United States.

In addition to the risk from a release of an infected pet salamander into the wild, the water that is used to house an infected pet in captivity could feasibly contain Bsal zoospores. As a result, the discharge of untreated water used to house infected, captive animals could be a pathway for releasing infective zoospores into the environment and exposing native salamanders to Bsal (Stephen et al. 2015). A reduction in the transport of salamanders in trade would reduce the potential for contaminated water to carry spores to other areas.

International Trade in Salamanders

Trade in wildlife occurs on a global scale, and amphibians are some of the most commonly traded animals (Smith et al. 2009). More than 52,149,000 documented amphibians were imported into the United States from 2004 to 2014, based on the Service's LEMIS data (USFWS OLE 2015), and 37,344,000 were imported from 2010 to 2020 (USFWS OLE 2021). Salamanders comprised 2,504,590 (4.8 percent) of the total imports of amphibians (USFWS OLE 2015) and 892,190 (2.4 percent) from 2010 to 2020 (USFWS OLE 2021). The 2004 to 2014 LEMIS dataset should be considered as a conservative estimate because many import records identified the animal being imported only as a member of the Class Amphibia (rather than identifying it to species or genus

level). In addition, incorrect salamander identifications to genus and species level appear to have commonly occurred in reporting to LEMIS (USFWS OLE 2015). LEMIS data for 2004 to 2014 shows that 65 percent of imported salamanders came from captive sources, and 35 percent were from wild sources (USFWS OLE 2015); for 2016 to 2020, 46 percent came from captive sources, and 54 percent were wild caught (USFWS OLE 2021). The LEMIS data recorded only 83 percent of declared salamander imports at the species level, whereas 17 percent were recorded only to the genus level (USFWS OLE 2015); for 2010 to 2020, it was 95.5 percent to species and 6.5 percent to genus respectively (USFWS OLE 2021).

The four salamander genera most commonly imported into the United States from 2004 to 2014 were *Cynops*, *Paramesotriton*, *Triturus*, and *Pachytriton* (USFWS OLE 2015). *Cynops, Triturus,* and *Paramesotriton* are three genera that are known to serve as carriers for Bsal (Martel et al. 2014). Of the 20 genera listed by the 2016 interim rule, 18 had been traded (live or parts) over the 11 pre-interim rule years, and they comprised 95 percent of imported salamanders. From 2016 through 2020, live imports of the top four salamander genera were *Paramesotriton* (524), *Cynops* (500), *Bolitoglossa* (191), and *Pleurodeles* (179); all but the *Bolitoglossa* were under injurious wildlife permits.

The species with the highest number of imports into the United States from 2004 to 2014 was the Oriental fire-bellied newt. This species comprised 54 percent of the total number of imported salamanders (USFWS OLE 2015). Twelve species of salamanders that are native to the United States were also imported into the United States from other countries from 2004 through 2014 (USFWS OLE 2015). From 2016 through 2020, 16 species of native salamanders were imported. Live eggs from the spotted salamander (*Ambystoma maculatum*), axolotl, and Japanese fire-bellied newt were imported between 2016 and 2020.

F. Risk Assessments of Bsal

Bsal Risk Assessments

Three Bsal risk assessments were used to help determine the risk associated with Bsal introduction into North America for the 2016 interim rule. Richgels et al. 2016 and Yap et al. (2015) conducted risk assessments for the United States that helped determine the level of risk associated with Bsal introduction. Stephen et al. (2015) also conducted a Bsal risk assessment for Canada that showed Canada is also at risk.

Richgels et al. (2016) concluded that the potential for Bsal introduction into the United States is high, the United States has suitable conditions for Bsal survival, and the consequences of introduction into the United States are expected to be severe and occur across a wide range of the United States. To evaluate the potential for Bsal introduction, the assessment combined information on the number of individual salamanders imported at each port of entry and the number of pet-supply establishments by county. Based on this evaluation, Bsal introduction potential was highest in central and southern Florida, southern California, and near New York City, New York (Richgels et al. 2016).

As noted in Richgels et al. (2016), the areas of highest potential for Bsal introduction are not necessarily the same as the areas of greatest risk for impacts to wildlife and wildlife resources. To determine the consequences of Bsal introduction into the United States, including from the areas where the introduction potential was highest, Richgels et al. (2016) evaluated environmental suitability, spatial data on imports and pet trade activity, species richness, and predicted species susceptibility. Overall, the total risk of Bsal to native salamanders is high. While not all areas of the United States are at risk from Bsal, based on both likely introduction and resultant consequences, the risk of Bsal is highest for the Pacific coast, southern Appalachian Mountains, and mid-Atlantic regions (Richgels et al. 2016). Some areas, such as south Florida and parts of the West, are likely to have low consequences from Bsal introduction. The areas most likely to have consequences from Bsal introduction are the Pacific Coast and Appalachian

Mountains (Richgels et al. 2016). Based on environmental suitability, areas of the United States most suited to Bsal growth (Blooi et al. 2015a), including the Southwest, Southeast, and Pacific regions, are also the areas of highest salamander diversity (Richgels et al. 2016).

In the United States, Yap et al. (2015) identified the Southeastern (southern end of the Appalachian Mountains and neighboring southeast region) and Western United States (Pacific Northwest and the Sierra Nevada) as zones of high risk. Yap et al. (2015) identified a narrower total risk in the United States over a smaller area for Bsal compared to Richgels et al. (2016). For example, Yap et al. (2015) identified south Florida as low risk of Bsal vulnerability, while Richgels et al. (2016) found that there was some risk, if not the highest, of Bsal to native salamanders in Florida. Richgels et al. (2016) noted differences in the methods used between the two papers as the reason. For example, Richgels et al. (2016) uses the thermal range of Bsal rather than the native Asian host distribution applied in Yap et al. (2015), which Richgels et al. (2016) noted may not be vulnerable to infection across the entire range of those species. The model in Richgels et al. (2016) took environmental suitability into account but also used species diversity, proximity to ports of entry, and areas of high pet-trade activity to predict total risk. This approach may over- or underestimate risk for some areas.

Some salamander species may be protected from Bsal by temperatures in their regions that are outside of the Bsal optimal growth range (Richgels et al. 2016) (see *Climate Tolerance* section). However, the average temperature preferences of salamanders from Central and North America (Duellman and Trueb 1986), which range from -2.0 °C (28.4 °F) to 30.0 °C (86.0 °F), suggest that most salamander species, including those within the United States, are active near the thermal growth optimum for Bsal (Blooi et al. 2015a). As a result, most salamander species in the United States are

not protected from Bsal by living outside of the Bsal optimal growth range or in areas beyond the threshold where Bsal can survive.

Most U.S. salamander species are also dependent upon forests, a habitat type dominated by relatively cool, moist conditions, for the majority of their life cycle (Davic and Welsh 2004). It is possible that cool seasons or microclimate selection by salamanders could facilitate disease outbreaks in areas where the average temperatures are outside the preferred range of the fungus.

A fourth risk assessment was added for this second interim rule, led by the USGS (Grear et al. 2021). They used post-interim rule outcomes and new information to update the Richgels et al. (2016) assessment. Grear et al. (2021) evaluated the effects of the 2016 interim rule on the introduction of Bsal into the United States and reviewed new information on species susceptibility to reevaluate the risk of Bsal to the United States. Since no comprehensive surveillance for Bsal was available prior to the 2016 interim rule, they also used the results from Waddle et al. (2020) of their large-scale surveillance for Bsal across 594 counties in 35 States and 1 site in Mexico, with 11,189 swab samples of wild salamanders and some anurans. The surveillance sites were strategically chosen for highest risk based on species susceptibility and geography. The surveillance did not detect any Bsal, which was as hoped, but the surveillance plan they developed can continue to be used for early detection of Bsal at high-risk locations.

Grear et al. (2021) also found that the 2016 interim rule reduced the importation of listed genera by several orders of magnitude, which concurs with our results. They noted several additional genera of salamanders and several of anurans (particularly *Bombina* spp.) that could be carriers of Bsal but were not listed in 2016; the additional salamander genera are included for listing in this interim rule, while the anurans are not (see *Impacts on Wildlife Resources or Ecosystems* for explanation why). Among other variables, Grear et al. (2021) used updated information about the thermal tolerance of

Bsal that included water temperatures associated with detection of Bsal in its presumed native geographic and host ranges. They noted that the change and spatial variation in risk scores from considering a higher maximum-temperature threshold had no discernible net effect on the consequence score. Therefore, they chose not to use the temperature threshold in recalculating the consequence and total-risk estimation; instead, they focused their risk comparisons on import regulations, host-species range, and surveillance. The study looked at the change in ports of import between pre- and post-interim rule and calculated the risk change for those regions. They concluded that, while the import regulations mitigated some of the import risk, overall risk is still driven by the potential of release from undetected Bsal circulating in captive amphibians in the United States and the consequences of that release in high salamander-biodiversity areas; however, little information is known on movement of captive host species and possible undetected Bsal in U.S. captives.

Vulnerability and Carrier Status

The urgent need to prevent Bsal introduction risks with the 2016 interim rule was raised by evidence presented by Martel et al. (2014), who tested Bsal on 35 species from all 3 orders of amphibians: frogs, salamanders, and caecilians. Martel et al. (2014) further screened 5,391 specimens collected from 4 continents for evidence of Bsal infection. Martel et al. (2014) defined a "resistant" salamander as one that either was not infected or developed a short-term infection without clinical signs following exposure to Bsal; a "tolerant" salamander is one that maintains a more prolonged infection with no signs of disease; a "susceptible" salamander becomes infected and has clinical signs of disease with the possibility of subsequent recovery; and a salamander that responds in a "lethal" manner to Bsal dies as a result of infection. According to Martel et al. (2014), resistant salamanders are not a risk for transmitting Bsal. However, based on the available scientific data, we concluded that resistant species with evidence of short-term or

transient infection, as well as those reported to have tolerant, susceptible, or lethal responses to Bsal, are carriers capable of transmitting Bsal to other salamanders and introducing the fungus into the United States.

The Service considered a species to be a "noncarrier" when Martel et al. (2014) classified the species as "resistant" and no histologic or field surveillance data was found to suggest that short-term Bsal infection could occur; "noncarriers" were considered incapable of transmitting Bsal to other salamanders or introducing the fungus into the United States. If Martel et al. (2014) classified the species as resistant with no histology (or qPCR) to verify, its carrier status was inconclusive. We use this same definition in this second interim rule.

We also found, and still maintain, that the likelihood of species within the same genus as being carriers can be drawn from a comparison to Bd, which, as described above under *General Information About Bsal*, is a close relative of Bsal. As noted earlier, the two risk assessments of Bsal used Bd in determining the risk of Bsal based on transmission, spread, and population-level effects (Richgels et al. 2016; Stephen et al. 2015). Considerably more was known about Bd than Bsal due to its discovery and description more than 15 years earlier (Berger et al. 1998; Longcore et al. 1999), while Bsal was discovered in 2013 (Martel et al. 2013). Bd has caused amphibian declines and extinctions worldwide (Skerratt et al. 2007). Bd affects species in patterns (Skerratt et al. 2007), and more closely related species have similar outcomes for Bd at the family level (Smith et al. 2009; Bancroft et al. 2011).

Amphibians experiencing the most severe declines are grouped by relatedness, which is likely due to the shared evolutionary histories of closely related species with a similar response to chytridiomycosis (Corey and Waite 2008). The USDA uses a similar approach. Closely related species are considered more likely to have similar traits and are used in risk assessments to determine threats from a target species of interest; a potential

pest is regarded as a threat when other species in a genus pose a similar threat (Wapshere 1974; Gilbert et al. 2012). The European Union's study on the feasibility of a movement ban of traded salamanders concluded that, due to the complexity of the taxonomy as well as the lack of evidence related to all the species, a movement ban at the level of taxonomic order is likely to be more effective and more feasible than a species-specific ban (EFSA et al. 2017).

Many salamanders exhibited a strong, adverse response to experimental Bsal infection; many species from outside of the native range of the fungus (Asia) exhibited lethal vulnerability. For the 2016 interim rule, our review of Martel et al. (2014) and follow-up communication (A. Martel, University of Maryland, pers. comm. 2015) categorized 25 species from 19 genera as carriers of Bsal. Additional communications (Chytridcrisis 2015b; Cunningham et al. 2015; P. Nanjappa, Association of Fish and Wildlife Agencies, pers. comm. 2015) identified another two species from two separate genera as carriers: the pygmy marbled newt (*Triturus pygmaeus*) and the golden striped salamander (*Chioglossa lusitanica*). Because Martel et al. (2014) had previously identified members of the *Triturus* genus as carriers, it was already accounted for within the 19 genera. The addition of this species brought the total number of known carrier species to 26. In addition to *Triturus, Chioglossa* was identified as another genus capable of serving as a Bsal carrier by Chytridcrisis (2015b), Cunningham et al. (2015), and P. Nanjappa (Association of Fish and Wildlife Agencies, pers. comm. 2015). As a result, the total number of genera known to serve as carriers of Bsal was 20 genera, and these were *Chioglossa*, *Cynops*, *Euproctus*, *Hydromantes*, *Hynobius*, *Ichthyosaura*, *Lissotriton*, *Neurergus*, *Notophthalmus*, *Onychodactylus, Paramesotriton*, *Plethodon*, *Pleurodeles*, *Salamandra*, *Salamandrella*, *Salamandrina*, *Siren*, *Taricha*, *Triturus*, and *Tylototriton*.

Further studies since the 2016 interim rule have included species from some of the same genera listed in 2016, which we reviewed to see if they were consistent with

earlier conclusions. Carrier status is further supported for *Chioglossa* (*C. longipes* and *C. lusitanica* in Bosch et al. 2021); *Lissotriton* (*L. boscai*, Fitzpatrick et al. 2018, Bosch et al. 2021; *L. lusitanica*, Lastra González et al. 2019), *Triturus* (*T. dobrogicus*, *T. ivanbureschi*, *T. karelinii*, and *T. marmoratus*, Fitzpatrick et al. 2018; *T. marmoratus*, *T. pygmaeus*, Bosch et al. 2021), *Neurergus* (Fitzpatrick et al. 2018), *Notophthalmus* (Fitzpatrick et al. 2018; Gray et al. 2023), *Plethodon* (*P. cinereus*, *P. cylindraceus*, *P. glutinosus*, *P. montanus*, and *P. shermani*, DiRenzo et al. 2021), *Salamandra* (*S. atra*, Fitzpatrick et al. 2018), *Siren* (*S. lacertina*, Gray et al. 2023), and *Taricha* (*T. granulosa*, *T. torosa*, Gray et al. 2023). Studies for *Chioglossa* (Fitzpatrick et al. 2018), and *Plethodon* (Perreira and Woodley 2021) showed no conclusive countervailing evidence.

Using the same criteria as in the 2016 interim rule, this second interim rule adds the following 16 genera: *Ambystoma*, *Andrias*, *Aneides*, *Aquiloeurycea*, *Calotriton*, *Chiropterotriton*, *Cryptobranchus*, *Desmognathus, Ensatina*, *Eurycea*, *Laotriton, Ommatotriton, Pachytriton*, *Proteus*, *Pseudobranchus,* and *Pseudotriton*. This increases the total number of species listed by approximately 164.

In conducting our analysis, the Service initially focused on identifying species for listing as injurious that scientific evidence demonstrates are capable of carrying Bsal. As we described above, we find that, due to shared characteristics by species within a genus, other species within these genera are also highly likely to be carriers of Bsal, even if not every species in the genus has been tested to verify that it is a carrier of Bsal. This conclusion is because closely phylogenetically related species, such as those found within the same genus, share common traits. Our analysis found no conclusive evidence to the contrary that suggested that some species within such genera are not carriers.

We have focused our findings on taxa of salamanders and their genera that we determined to be capable of carrying Bsal. We included genera identified as resistant by Martel et al. (2014) because carrier status was inconclusive in that study when histology

was not done, and there are other studies supporting carrier status; these are *Ambystoma*, *Hynobius*, *Lissotriton*, and *Plethodon* (see *Vulnerability and Carrier Status of Native Species* below). Based on our analysis of their data and other studies, we have no evidence for the salamander genera we are not listing of being capable of introducing Bsal to the United States or otherwise transmitting Bsal to native populations. In addition, we are not listing genera where there was no data as of the drafting of this second interim rule because we do not have a basis for doing so, even though the Service recognizes that it is possible that untested genera may also be capable of carrying Bsal.

We have determined that all species are injurious in the 16 genera where at least one species has been conclusively identified as a carrier of Bsal and there is no conclusive countervailing evidence suggesting that some species within the genus are not carriers. Where one species has been identified as a carrier, we expect that the other species in that genus are also carriers. This finding includes as injurious the intrageneric hybrids (crosses of species found within the same genus), intergeneric hybrids of species in two listed genera, and intergeneric hybrids from a listed and an unlisted genus.

For this second interim rule, we maintain that, due to shared characteristics by species within a genus, other species within these genera are also likely to be carriers of Bsal if one species has been identified as a carrier, even if not every species in the genus has been tested to verify that it is a carrier of Bsal. Our updated review found no conclusive countervailing evidence that species differed within a genus with respect to their ability to act as carriers. Thus, we expect all species in a genus to respond similarly as carriers or noncarriers to Bsal. Therefore, based on existing scientific evidence, and as described in more detail below, we are listing all species in the 16 genera, including all species, that we now conclude constitute a threat to introducing and spreading Bsal in the United States because those species can carry the fungus and transmit it to other species that would be negatively impacted.

Vulnerability and Carrier Status of Native Species

Including both the final rule to the 2016 interim rule and this second interim rule, we conclude that approximately 426 salamander species from around the world are carriers of Bsal (36 genera in 7 families). The United States currently has approximately 221 species of native salamanders in 23 genera (AmphibiaWeb 2023a), and this second interim rule includes 164 of those species (13 genera in 5 families) that we have determined are carriers of Bsal. Of the remaining 57 native species, we find that either they are not carriers or the vulnerability and carrier status is unknown.

Of the 190 native U.S. salamander species as of the 2016 interim rule, carrier status had not been assessed in 103 species from 16 genera. The untested genera were *Amphiuma*, *Aneides, Batrachoseps, Cryptobranchus*, *Desmognathus, Dicamptodon*, *Ensatina, Eurycea*, *Hemidactylium, Necturus, Phaeognathus, Pseudobranchus, Pseudotriton, Rhyacotriton, Stereochilus,* and *Urspelerpes*.

Since the 2016 interim rule went into effect, we have evidence that eight more native genera, not previously tested, support listing with this second interim rule: *Ambystoma*, *Aneides*, *Cryptobranchus*, *Desmognathus*, *Ensatina, Eurycea*, *Pseudobranchus*, and *Pseudotriton*. We previously considered *Ambystoma* as resistant because Martel et al. (2014) had done so for two species, and Bsal was not detected during testing. However, Martel et al. did not perform histology on the *Ambystoma* subjects because they did not die, so it was undetermined if the individuals harbored encysted zoospores.

Since the 2016 interim rule, initial or additional testing has been done on *Ambystoma* spp. (Fitzpatrick et al. 2018; Sabino-Pinto et al. 2018; Barnhart et al. 2020; Gray et al. 2023); *Aneides aeneus* (Gray et al. 2023); *Cryptobranchus* (Gray et al. 2023); *Desmognathus ocoee* (Gray et al. 2023); *Ensatina eschscholtzii* (Gray et al. 2023); *Eurycea lucifuga* and *E. wilderae* (Carter et al. 2020), *E. wilderae* (DiRenzo et al. 2021),

and *E. bislineata, E. lucifuga* and *E. wilderae* (Gray et al. 2023); *Pseudobranchus striatus* (Gray et al. 2023); and *Pseudotriton ruber* (Carter et al. 2020) that provides support for carrier status of these native genera. There is no conclusive countervailing evidence. Five more native species were found to be lethally affected by Bsal (*Eurycea bislineata*, *E. wilderae, Pseudotriton ruber*, *Ensatina eschscholtzii*, *Aneides aeneus*). Gray et al. (2023) tested only native North American species specifically to access the conservation risk to U.S. species.

Based on the gradient responses from resisting infection to lethal response among the genera Martel et al. (2014) and others tested experimentally, other genera could be at risk from Bsal infection or could serve as carriers. However, we are not listing species in those genera because the genera had not yet been tested or confirmed as carriers by the drafting of this second interim rule.

Controlled Bsal experiments have proliferated since the discovery of the fungus. A study by Kumar et al. (2020) shows variation in experimental methodologies could thwart knowledge advancement by introducing confounding factors that make comparisons difficult among studies. They tested whether passage duration of Bsal culture (the number of times the fungus was transferred from its culture into fresh culture media), exposure method of the host to Bsal (water bath versus skin inoculation), Bsal culturing method (liquid versus plated), host husbandry conditions (aquatic versus terrestrial), and skin-swabbing frequency influenced diseased-induced mortality in a susceptible host species, the eastern newt. They found that disease-induced mortality was faster for eastern newts when exposed to a low passage isolate (a "young" Bsal isolate that had been passed into fresh culture media only 20 times), when newts were housed in terrestrial environments, and if exposure to zoospores occurred in a water bath. They did not detect differences in disease-induced mortality between culturing methods or swabbing frequencies. Their results illustrate the need to standardize methods among

Bsal experiments, but they do not discount the results of the studies used to determine our results.

Vulnerability and Carrier Status of Threatened and Endangered Species

As of the drafting of this interim rule, 20 native species of salamanders in 6 genera are threatened or endangered under the ESA. As of the drafting of the 2016 interim rule, none of the salamander species listed as endangered or threatened under the ESA in the United States had been specifically tested for Bsal vulnerability under laboratory conditions. Bsal had not been detected in their wild populations (Martel et al. 2014, Bales et al. 2015). Since publication of the 2016 interim rule, several species have been laboratory-tested.

One species with two federally endangered subspecies (eastern hellbender *Cryptobranchus a. alleghaniensis* and Ozark hellbender *C. a. bishopi*) has been laboratory tested and is considered a carrier in the second interim rule in this document (Gray et al. 2023). Notably, *Cryptobranchus* has only one species in the genus, so if the species is extirpated by Bsal, so is the genus.

As we describe above in *Vulnerability and Carrier Status*, while the Service did find evidence that shows some species within a genus may vary in their specific vulnerability, the carrier status of tested species can be extrapolated to related species, including those that are listed as endangered or threatened, candidates for ESA listing, and under review.

Of the other new genera that include native species that we have identified as carriers, the following 12 species are federally listed as endangered or threatened: 5 species of *Ambystoma* (California tiger salamander (*A. californiense*), frosted flatwoods salamander (*A. cingulatum*), reticulated flatwoods salamander (*A. bishopi*), Sonoran tiger salamander (*A. mavortium stebbinsi*), Santa Cruz long-toed salamander (*A. macrodactylum*)); and 7 species of *Eurycea* (Austin blind salamander (*E. waterlooensis*),

Barton Springs salamander (*E. sosorum*), Georgetown salamander (*E. naufragia*), Jollyville Plateau salamander (*E. tonkawae*), Salado salamander (*E. chisholmensis*), San Marcos salamander (*E. nana*), and Texas blind salamander (*E. rathbuni*)). Notably, *Ambystoma* is the only genus in the family Ambystomatidae, so if the genus is extirpated by Bsal, so is the family.

No information is available regarding the effect of Bsal or carrier status of the remaining four ESA-listed species native to the United States: the desert slender salamander (*Batrachoseps aridus*), the Alabama waterdog or black warrior waterdog (*Necturus alabamensis*), Neuse River waterdog (*N. lewisi*), and Red Hills salamander (*Phaeognathus hubrichti*). Three *Plethodon* species from the 2016 interim rule are federally listed as endangered or threatened: Shenandoah salamander (*P. shenandoah*), Cheat Mountain salamander (*P. nettingi*), and Jemez Mountains salamander (*P. neomexicanus*) (USFWS 2023). There were no candidate species of salamanders as of the drafting of this second interim rule. Three *Plethodon* species identified as carriers in the 2016 interim rule remained federally listed.

G. Factors That Contribute to Injuriousness of Salamanders

Likelihood of Release or Escape (of Salamanders)

In general, there is widespread concern over the increasing spread of pathogens moved through the wildlife trade (for example, Chinchio et al. 2020; IPBES 2020). Substantial evidence shows that Bd has spread extensively throughout the world through the amphibian trade (Fisher and Garner 2007; Schloegel et al. 2009; Schloegel et al. 2012; Galindo-Bustos 2014; Kolby 2014; Kolby et al. 2014). Similar mechanisms of transmission and persistence in the closely related Bsal pathogen, along with detection of Bsal in captive salamanders imported by the pet trade into Great Britain, indicate that global movement of Bsal, similar to that of Bd, is not only possible but is already occurring (Cunningham 2015). Bsal was also found in a private pet collection in

Germany, where it killed over half of a collection of approximately 200 salamanders in the genus *Salamandra* (Sabino-Pinto et al. 2015). Although the origin of Bsal in the German collection was unknown, it is probable that Bsal is also present in other private or professional collections across Germany and possibly also in other European countries (Sabino-Pinto et al. 2015). Amphibian trade fairs in Spain, where the largest fairs in southern Europe take place, as well as in private collections in Spain, had positive test results for Bd and Ranavirus (Thumsová et al. 2021) and are known to house and co-house sick amphibians. These collections may serve as a reservoir of Bsal within the wildlife trade or as sources of Bsal release into the environment.

Considering the occurrence of Bsal in the global pet trade, the risk to North American native species, and the number of salamanders that are imported into and transported throughout the United States through trade, Bsal is likely to be introduced into and spread throughout native salamander populations in the United States unless immediate action is taken to limit the importation of salamanders that are likely to carry Bsal. The 2016 interim rule has limited importation, and this second interim rule is intended to further reduce risk.

Infected salamanders can transmit Bsal to other species even if the introduced salamander fails to establish a population. Evidence indicates that at least some of the salamanders capable of carrying Bsal can escape or be released and introduce Bsal into the environment. As described earlier, evidence exists for release of salamanders into the wild in the United States (Picco and Collins 2008; USGS 2015a, b, c, d, e, f). As noted above in *Invasiveness of Salamanders*, the USGS's Nonindigenous Aquatic Species database (USGS 2023) has records for 17 salamander species that have been observed in the environment outside their native range. Of those, 14 are native to the United States and were discovered outside of their native ranges, and 3 are species not native to the United States. These findings mean that salamanders have been shown to exist, even if

temporarily, outside their native range in the environment. Thus, they are capable of transmitting Bsal into nonindigenous ecosystems. Infected native species that are imported and escape or are released into native habitats would also be capable of carrying Bsal into native salamander ecosystems where Bsal has not previously been found.

Infectious Bsal zoospores can also be released into the environment if water or other materials used to house infected salamanders enter the environment due to improper disinfection and disposal methods. The water and materials become passive carriers to introduce the fungus into the environment if not decontaminated or disposed of properly. As described above under *Environmental Conditions Needed To Survive,* Bsal can survive in filtered pond water for at least 31 days (Stegen et al. 2017). Bd is similarly known to remain viable for weeks in water and moist organic matter and is capable of being transmitted to uninfected specimens through such means. Given our assumption that Bd can serve as a surrogate for predicting Bsal's effects in salamanders at the population level, and since Bd does not require an amphibian host to remain viable, we expect that Bsal can also persist outside salamanders (as long as it has sufficient water or moist soil and conducive temperature). Since the effects of desiccation or the viability of encysted Bsal spores in deceased hosts have not been thoroughly investigated, we also expect that Bsal can be transmitted on unpreserved dead salamanders or body parts and tissues.

As discussed above in *Introduction Pathways*, there is evidence that Bd has escaped into the environment through untreated wastewater, increasing the likelihood that Bsal could also escape if brought in via contaminated water or improperly disposed of materials. While standards for the treatment and prevention of Bd exist, in part due to recognition of its status as an internationally notifiable disease under the World Organisation for Animal Health (WOAH), the effectiveness and widespread application of those standards are uncertain given that international protocols for responding to Bd do

not exist and the need to improve international mechanisms to respond to disease-related threats to biodiversity (Voyles et al. 2014).

Given the number of specimens that have been imported into the United States and Canada, it is not known why Bsal has not yet been found in these countries (Muletz et al. 2014; Bales et al. 2015; Stephen et al. 2015; Richgels et al. 2016). A comparison of Bd, which has spread in the United States, to Bsal yields some insights. Based on genetic analyses and examination of historical specimens, Bd may have originated from different places, including Japan, South Africa, or South America (Farrer et al. 2011; Rodriguez et al. 2014). In contrast, Bsal may have originated only from Asia, giving it fewer pathways to the United States (Martel et al. 2014; Laking et al. 2017). Importation of salamanders into the United States has also declined in recent years, suggesting that the propagule pressure may also be a factor by limiting the number of times in which Bsal could possibly be introduced into the environment through trade (Lockwood et al. 2005; USFWS OLE 2015). Bd may have spread more quickly than Bsal because of its ability to infect frogs, whereas research so far has found only a few frog species that may carry Bsal (see *Impacts on Wildlife Resources or Ecosystems* below). Based on LEMIS data, frogs are traded in higher volumes than salamanders, increasing the probability of trade of a Bd-infected individual over a Bsal-infected individual. The USGS Nonindigenous Aquatic Species database also provides evidence for this higher level of trade, in that greater numbers of frogs are reported than salamanders. In addition, many frogs in trade, such as *Rana catesbeiana* (bullfrogs), are adaptable to a wide variety of environments and can easily become invasive once released in a watershed, as bullfrogs have become in the American West (Jennings and Hayes 1994; Rosen and Schwalbe 1995; Funk et al. 2011; Sepulveda et al. 2015).

Taken together with the other data we reviewed, this evidence suggests that Bsal is less likely to enter the United States than Bd. However, without action, the pathways

for introduction and escape of Bsal are a significant and imminent threat. Listing salamanders that can carry Bsal as injurious wildlife to prohibit their importation targets those pathways, thereby minimizing opportunities for Bsal to be introduced, become established, and spread in the United States.

Potential To Survive, Become Established, and Spread

Even if a salamander species does not become established, there is evidence that it may be capable of carrying Bsal long enough in the wild to transmit Bsal. The USGS Nonindigenous Aquatic Species database has records of 17 species and populations that have been observed in the United States outside of their native range (USGS 2023). Of those, 14 are native and have established populations in the United States outside of their native U.S. range: Eastern tiger salamander (*Ambystoma tigrinum*), northwestern salamander (A. *gracile*), blotched tiger salamander (*Ambystoma mavortium melanostictum*), long-toed salamander (*Ambystoma macrodactylum*), three-toed amphiuma (*Amphiuma tridactylum*), California slender salamander (*Batrachoseps attenuatus*), seal salamander (*Desmognathus monticola*), Santeetlah dusky salamander (*Desmognathus santeetlah*), black-bellied salamander (*Desmognathus quadramaculatus*), mudpuppy, eastern newt, red-spotted newt (*Notophthalmus viridescens viridescens*), large-blotched ensatina (*Ensatina eschscholtzii klauberi*), lesser siren (*Siren intermedia*), and rough-skinned newt (*Taricha granulosa*). The three species from outside the United States are the Japanese fire-bellied newt (*Cynops pyrrhogaster*), Oriental fire-bellied newt (*Cynops orientalis*), and paddle-tailed newt (*Paramesotriton (Pachytriton*) *labiatus*), none of which are known to have become established. No foreign terrestrial salamander species have been detected in USGS surveillance for Bsal (M. Adams, USGS, pers. comm. 2021). As discussed earlier under *Introduction Pathways* and *Environmental Conditions Needed To Survive*, Bsal is expected to be able to survive outside of salamander hosts for several weeks given suitable conditions in water. If a

salamander comes in contact with Bsal and then transmits it during a time when salamanders congregate, such as during breeding as described above under *Salamander Biology,* the potential for Bsal to survive, establish, and spread through animals or animal parts (except for purified, extracted genetic material, eggs, and gametes) is significant. As we describe above under *How the Fungus Affects Salamanders*, Bsal can be transmitted on unpreserved dead tissue where keratin is present, particularly skin, but we do not find that Bsal can be transmitted through reproductive tissue, including eggs and gametes. There is no evidence to suggest that Bsal can survive in purified, extracted genetic material from salamanders; in chemically preserved specimens, tissues, samples, or swabs; or in salamander eggs and gametes; hence, these parts are not covered by the listing.

As Richgels et al. (2016) noted, "Given the large number of suspected *Bsal* carriers imported into the USA each year (*Cynops* spp. and *Paramesotriton* spp., more than 100,000 [per year]), *Bsal* is likely to be introduced if no additional risk mitigation steps are taken. Though precise estimates for the invasion process (proportion of imported individuals infected, frequency of release of captive individuals, and contact of released animals with native amphibians) do not exist for *Bsal*, the establishment of invasive amphibians common in US amphibian trade * * * and the patterns of global *Bd* spread * * * suggest these processes are also likely for *Bsal*." The Service finds that the capacity of infected salamanders in trade to potentially infect wild salamanders, together with the capacity of Bsal to survive for an extended period independent of an amphibian host, suggests that Bsal has a high likelihood of surviving, becoming established, and spreading once it is introduced into a new area.

As we noted above in *Purpose of Listing as Injurious*, even if a salamander found to be injurious could not establish a population in the wild, an infected or carrier

salamander from captivity can still transmit Bsal to native populations if that salamander escapes or if material touching it is improperly disposed.

Impacts on Wildlife Resources or Ecosystems

If Bsal is introduced into the United States, we expect the species with lethal vulnerability would be at greatest risk. However, disease outbreaks can result from a combination of biotic and abiotic factors, including species vulnerability, exposure, host behavior, host immunity, co-infections, and environmental conditions (Wobeser 2007). Therefore, the vulnerability of individuals under laboratory conditions is an incomplete predictor of disease effects (Wobeser 2007). Native salamander species known to be tolerant of Bsal infection under experimental conditions may demonstrate more severe clinical disease when infection is combined with additional stressors in the wild, as has been found for other diseases, including those in amphibians (Wobeser 2007; Kerby et al. 2011; Kiesecker 2011). For example, Bodinof et al. (2011) noted that Bd may be found more frequently in hellbenders that are immunocompromised or that Bd infection increases the adverse effects of other co-infections. Considering these cumulative factors, as well as the lack of testing for the majority of native salamander species, our assessment of risk in native species is likely conservative.

Bsal can severely affect wildlife resources. At least nine native species are lethally vulnerable to Bsal, and at least one is tolerant to Bsal infection. At least 164 native species may act as carriers or sources of infection for other species. While not all species have been tested for their response to Bsal, based on the high rates of infection that have been observed, the fungus may have significant negative effects on additional species.

As described above in *Ecosystem-Level Effects*, salamanders are important parts of the ecosystems in which they occur. They are often the most abundant vertebrates in their ecosystems, and, as a vital part of the food web, they are both important prey for and

predators of many species (Holomuzki et al. 1994; Regester et al. 2006). In some places, they are considered keystone species that help control some invertebrate populations and affect cycling of nutrients in an ecosystem, contributing significantly to overall ecosystem health. For example, by consuming arthropods that would otherwise release carbon dioxide into the atmosphere by decomposing leaf litter in forests, salamanders slow carbon emissions from leaf litter decomposition, which has implications for the global carbon cycle (Best and Welsh 2014). As described earlier, invertebrate species that depend on salamanders for aspects of their life cycle or ecology are likely to be adversely affected if their host species declines in response to a Bsal introduction. Loss of these keystone species would result in significant ecosystem-level change.

Salamanders constitute much of the vertebrate biomass of forests, and they play an important role in ecosystems as insect consumers, shapers of the landscape, and climate mediators (Burton and Likens 1975; Davic and Welsh 2004; Wyman 1998; Best and Welsh 2014). If native U.S. salamander species do experience declines from Bsal infection as the fire salamander experienced in the Netherlands (Spitzen-van der Sluijs et al. 2013), we expect detrimental ecological effects. Nine native salamanders are documented as lethally vulnerable.

The eastern newt, one of the lethally vulnerable species (Martel et al. 2014; Gray et al. 2023), is one of the most widespread salamander species in North America (Roe and Grayson 2008, Martel et al. 2014). As top predators in pond ecosystems, eastern newts regulate frog tadpole abundance and, therefore, affect the amount and type of nutrients available in the ponds, keeping them in ecological balance (Morin et al. 1983; Morin 1995). If eastern newt populations decline because of Bsal infection in the wild, imbalances could result in ponds and ecosystems throughout the Eastern United States. Eastern newts also travel long distances between aquatic and terrestrial habitats (Roe and

Grayson 2008), so if the species was to be eliminated from an area, the amount of nutrients available in upland areas would also be affected.

The rough-skinned newt is another native U.S. species known to be lethally vulnerable to Bsal (Martel et al. 2014; Gray et al. 2023) and is geographically widespread along the Pacific coast of North America from Santa Cruz, California, to southeastern Alaska (AmphibiaWeb 2023a). The rough-skinned newt plays an important role in ecosystems through its consumption of invertebrates that break down leaf litter and release carbon into the atmosphere (Davic and Welsh 2004). If rough-skinned newt populations do experience severe declines from Bsal infection, atmospheric inputs of carbon may be altered, as has been observed with other species (Wyman 1998; Best and Welsh 2014).

The green salamander (*Aneides aeneus*) was found by challenge tests to be lethally vulnerable when all 10 salamanders in the study became infected and 5 died as a result (Gray et al. 2023). This species is State-listed as endangered in Indiana, Ohio, Maryland, and Mississippi and threatened in Pennsylvania (AmphibiaWeb 2023a); it is found in a narrow range from southwestern Pennsylvania southwest to Alabama. Of 15 Blue Ridge two-lined salamanders (*Eurycea wilderae*) that were challenge-tested, all became infected and 10 of those died (Gray et al. 2023; see also mortality in Carter et al. 2020; DiRenzo et al. 2021), and 6 of 9 infected northern two-lined salamanders (of 9 *Eurycea bislineata* challenged; Gray et al. 2023). Red salamanders (*Pseudotriton ruber*), widely found from New York State to Alabama and Florida, had 100 percent mortality (Carter et al. 2019; Gray et al. 2023). The Ensatina salamander (*Ensatina eschscholtzii*) is the only species in its genus, although it has seven subspecies, all found on the West Coast; two subspecies were documented as having lethal results (Gray et al. 2023).

Other taxa besides salamanders may also be negatively affected by Bsal. Several species of anurans have been found to carry Bsal (midwife toad *Alytes obstetricans*,

Stegen et al. 2017; fire-bellied toad *Bombina microdeladigitora*, Nguyen et al. 2017; Cuban treefrog *Osteopilus septentrionalis*, Towe et al. 2021; Gray et al. 2023). However, little is known about the negative effects of the disease on the anurans. None of these species is native to the United States, but all are imported in trade. Therefore, there is a risk of spreading Bsal to native frogs and toads, the susceptibility of which we do not know, and also a risk of spreading to salamanders. As explained above, we are not adding any frogs or toads to the list of injurious wildlife because they are in a different order (Anura), and we did not include the possibility of adding the order Anura in the 2016 interim rule, which would give the public a chance to comment.

As Richgels et al. (2016) noted, some parts of the United States may reach temperatures above the thermal range of Bsal on a seasonal basis. However, wildlife and habitats would suffer losses if local populations of salamanders affected by Bsal prior to temperatures rising as part of the regular seasonal cycle suffered declines (and possible extirpation) and were unable to return to pre-infection levels in those ecosystems.

Gray et al. (2023) estimated mean infectious and lethal doses for the North American species they tested with sufficient infection and mortality data and derived an amplification potential. Species with high amplification potential may contribute disproportionately to transmission events because they are easy to infect, less likely to die quickly from infection, and likely to be more infectious due to greater pathogen loads on their skin. Species that were susceptible to infection but did not die from Bsal are likely to be carriers. Considering all the variables, there is an immense potential for amphibian communities in North America to harbor carrier species that serve as reservoirs, amplification species that disproportionally transmit Bsal, and Bsal-susceptible species that are at high risk of population decline and extirpation.

For the above reasons, we conclude that the negative impact to wildlife resources or ecosystems is expected to be high if Bsal is introduced into U.S. ecosystems.

Impacts to Threatened and Endangered Species and Their Habitats

As of publication of the 2016 interim rule, none of the salamander species listed as endangered or threatened under the ESA in the United States had been specifically tested for Bsal vulnerability under laboratory conditions; Bsal had not been detected in their wild populations (Martel et al. 2014, Bales et al. 2015). As of the final rule to the 2016 interim rule in this document, only the eastern hellbender (of which two subspecies are federally endangered) has been tested (Gray et al. 2023) and is considered a carrier. Of the genera that include native species that we have identified as carriers, 20 salamander species are federally listed as threatened or endangered and 2 salamanders are candidates or proposed for listing. Because not all species have been tested, it is possible that the fungus will negatively affect other ESA-protected species.

Impacts to Human Beings, Forestry, Horticulture, and Agriculture

We do not expect direct effects to forestry, horticulture, or agriculture. Trees and other plants are also not affected. Bsal does not appear to infect humans or other animals except for salamanders and a few anurans. Indirectly, the introduction or establishment of Bsal would have negative effects on humans primarily from the loss of native wildlife biodiversity. These losses would affect the aesthetic, recreational, and economic values currently provided by native wildlife and healthy ecosystems. However, other indirect links to human health may occur. Many salamander species prey on mosquito larvae, and if the salamander numbers decline (such as from Bsal), the population of mosquitoes is likely to increase. Insect repellants used in surface waters for mosquito control have been linked to salamander larvae mortality and deformities, thus reducing predation on mosquito larvae, also leading to increased numbers of mosquitoes (Almeida et al. 2018). Similarly, a correlation has been made for Bd causing declines of mosquito-eating frogs, which then led to increased numbers of malaria-carrying mosquitoes (Springborn et al. 2022). Educational values would also be diminished through the loss of biodiversity and

ecosystem health. However, we are not listing the species because of the indirect impacts to humans, forestry, horticulture, or agriculture, but rather due to their impacts to wildlife and wildlife resources.

Wildlife or Habitat Damages That May Occur From Control Measures

Richgels et al. (2016) stated, "[T]here are few known viable treatment or management options for responding to the introduction of Bsal * * * Strategies focused on prevention or reduction of introduction events remain the best control option for emerging diseases." As discussed below in *Ability To Prevent or Control the Spread of Pathogens or Parasites,* current control strategies appear to focus on treating salamanders in a controlled laboratory setting. We are not aware of control measures that are effective in treating infected free-ranging salamanders over a large-scale area that could eliminate Bsal without killing the salamanders themselves, have low side effects, or do not require significant resources to implement. In addition, the life history of salamanders makes it highly unlikely that all individuals, including those that are infected, could be captured and treated. Many species are long-lived and inhabit areas that may be hard to reach. Furthermore, the effects on other wildlife of chemically treating an area, if such a treatment becomes available to eradicate infected salamanders and if capturing and treating individually is not practical, is unknown but is likely to be severe.

H. Measures That Reduce or Remove Injuriousness of Salamanders

Ability To Prevent Escape and Establishment

As discussed below in *Ability To Prevent or Control the Spread of Pathogens or Parasites,* the ability and effectiveness of measures to prevent or control Bsal is currently low. While less certain, we also expect the ability to prevent escape and establishment is also low. Nonregulatory actions, such as implementing voluntary Best Management Practices or individual State action, are possible. The Service, for example, is working with partners on such efforts as Habitattitude™, a national campaign that encourages

responsible consumer actions with respect to pet ownership. Such actions include finding alternatives to releasing pets into the environment. In November 2015, PIJAC (currently known as the Pet Advocacy Network) asked its member entities to voluntarily ban their importation of paddle-tailed newts and Oriental fire-bellied newts to prevent the unintentional introduction of Bsal (PIJAC 2015). Voluntary actions, such as applying heat and antifungal medication therapy as described in Blooi et al. (2015a) and Blooi et al. (2015b), may help reduce the threat posed by Bsal for specimens held in captivity. However, at this time it is not possible to determine the likelihood of success of such measures in preventing the introduction, establishment, and spread of Bsal in the United States.

As described above under *Invasiveness of Salamanders* and *General Information About Bsal,* nonnative salamanders have escaped into the United States, and Bd, a related fungus, has also escaped and established in the United States. While treatment options exist that may help reduce the threat posed by Bsal for imported and captive-held specimens, those options have not been standardized and their effectiveness remains uncertain for large-scale regulatory purposes. Treatment options for free-ranging specimens are not practical at this time. Therefore, we expect the likelihood of the ability to prevent escape and establishment of Bsal through infected salamanders to be low. Although voluntary actions are vital to help minimize the threat of invasive species, the Service is highly concerned about the extensive damage that introduction of Bsal would do to our Nation's natural resources. Thus, we concluded that we cannot rely on voluntary actions alone to address the severity of the threat that Bsal poses and that other measures to prevent escape and establishment are not sufficient to ensure Bsal is not successfully introduced.

Therefore, we find that we cannot rely on these approaches to prevent escape and establishment of Bsal and that our current capacity to prevent escape and establishment is low.

Potential To Eradicate or Manage Established Populations

While some introduced salamanders in the United States have been successfully controlled, such as the lesser siren (which was eliminated from a backyard pond outside its native U.S. range), others, such as the three-toed amphiuma, have not been as successfully controlled. However, evidence for control is sparse. Given the high rates of infection among salamanders tested by Martel et al. (2014), and the lack of control measures for Bsal that could be employed outside of a controlled facility, it is likely that Bsal would persist once introduced into the environment given appropriate environmental conditions, especially if a tolerant or susceptible salamander established a population and continued to spread Bsal.

Ability To Rehabilitate Disturbed Ecosystems

Bsal infection can lead to the loss of keystone species in the ecosystem. The ability to rehabilitate disturbed ecosystems is expected to be low. We considered whether the Service's National Fish Hatchery System (NFHS) could be used to maintain salamanders in refugia while areas are treated, assuming the salamanders could be treated for the fungus. However, it is impractical to equip NFHS facilities to be able to rapidly protect numerous salamander populations and maintain them for an extended time, such as might be required due to the introduction of Bsal. Although, as described in the next section, a few options exist to treat individual salamanders, none have been identified that can be used to clear Bsal from a widespread area. Consequently, we expect that, once Bsal has been introduced, it will persist and spread with little opportunity for widespread disinfection of ecosystems.

Studies have also questioned the effectiveness of captive-breeding programs to address such threats as infectious disease to amphibians, including salamanders (Harding et al. 2015). However, a recent study showed enhanced resistance following a second exposure of Bsal in eastern newts, both for increased survival and decreased Bsal loads by QPCR (L. Rollins-Smith, Vanderbilt University, pers. comm. 2021). Also, another study found a second higher Bsal dose led to decreases in Bsal infection intensity over time as compared to salamanders exposed only once to a lower dose that sustained infections over time (DiRenzo et al. 2021). Since that study was not designed specifically to study the effects of immune priming, these results are indicative but not proven. Therefore, it may be possible to stimulate an immune response in captive salamander populations that would allow them to be reintroduced into ecosystems where Bsal may still exist; however, this response has not been demonstrated for Bd, and research is needed in a broader array of conditions and species and to determine how resistance or immunity works.

Therefore, the ability to rehabilitate disturbed ecosystems is expected to be low because the Service would be unable to ensure that all salamander populations expected to be affected by Bsal could be treated and protected in the wild.

Ability To Prevent or Control the Spread of Pathogens or Parasites

The ability and effectiveness of nonregulatory measures to prevent or control Bsal on a widespread scale from live specimens is currently low. The risk is compounded beyond the effect of a more common type of injurious listing (where the species cause harm by being invasive) by having two separate variables that can each spread—the fungus and the host species (salamanders). Few options can ensure potentially infected salamanders do not carry Bsal, and none exist on a broad scale.

Blooi et al. (2015a) has shown that treating salamanders infected with Bsal by exposing them “to 25 °C [77 °F] for 10 days resulted in complete clearance of infection

and clinically cured all experimentally infected animals. This treatment protocol was validated in naturally infected wild fire salamanders." The authors found that temperature treatment could be an effective option given the host salamander's thermal tolerance. However, the treatment does have some shortcomings. Not all salamander species can tolerate the thermal regime required, and the researchers noted that there is a "narrow margin between the temperature able to limit [Bsal] and the upper thermal limit most urodelans tolerate." Blooi et al. (2015a) also noted that there is some uncertainty as to whether the method is completely effective. Evidence of Bsal was found after thermal treatment, although it is possible that the evidence consisted of dead cells only. While thermal treatment is promising, the paper's introduction noted that it was intended to "help to develop treatment protocols" and, therefore, is not intended to serve as the standalone standard treatment. As the treatment has not been standardized as a protocol for use at the landscape scale for salamanders in the wild or throughout trade, its ability to prevent introduction or control the spread of Bsal is low or uncertain.

In the 2016 interim rule and corresponding economic analysis, one of the five alternatives that we considered was requiring a health certificate upon import stating that the animal being moved is free of Bsal, in lieu of or in addition to listing. During our evaluation for the 2016 interim rule, we considered whether it was practical for an exporting foreign nation to provide a health certificate stating that a possible carrier of Bsal has been tested and found to be free of the fungus or treated with antifungal drugs and thermal procedures to ensure that any Bsal that the salamanders might be carrying has been killed. We acknowledged that these testing and treatment methods existed and may be effective under certain circumstances. Requiring a health certificate would help ensure that Bsal does not escape from an exporting nation by being carried on an infected salamander. However, considering information from the public comments and other more recent information, we have significant concerns about this requirement's feasibility for

large-scale regulatory use for exporting countries given the effectiveness and sensitivity of current testing methods (including the return of false negatives), lack of validation and sufficient testing capacity, lack of standardized treatment methods, and agency resources required to conduct inspections, interpret results, and issue health certificates. The cost of testing could also be prohibitive for some exporters, since the cost of testing may be per animal. In the United States, qPCR testing can run around $25 to $65 per salamander, and the salamander may wholesale for only $5.

In May 2017, the WOAH listed infection with Bsal as an emerging disease in its Aquatic Animal Health Code; now WOAH considers Bsal as a disease of amphibians, with a chapter in the Aquatic Animal Health Code (WOAH 2021a) and a chapter in the manual (WOAH 2021b). The WOAH is the intergovernmental organization responsible for improving animal health worldwide. The WOAH chapter on Bsal provides recommendations that "may include" 13 named species of Asian, European, and North American newts and salamanders (WOAH 2021b); those species were a portion of the species covered by our 2016 interim rule. The recommendations include that the consignment be accompanied by an international aquatic animal health certificate issued by the Competent Authority of the exporting country.

That recommendation came after the 2016 interim rule took effect. The 2016 draft economic analysis did not explain the costs of health certification because it was unclear how much testing, treatment, and the health-certification processes would cost. Because the details for these recommendations were not available for regulatory consideration for the 2016 interim rule's public and peer comments and because much of those details are still not clear, we are not adding a health certification to this second interim rule. However, we believe there are valuable recommendations in the WOAH chapter 8.2 (WOAH 2021a) that we support for the public to voluntarily help prevent or control the

spread of Bsal. They include, but are not limited to (summarized from WOAH 2021a; details found there):

- quarantine;
- treating or disposing of shipment water, equipment, containers, and packaging in a biosecure manner; and
- treating effluent and waste materials (fomites) to inactivate Bsal.

The European Union, Switzerland, and United Kingdom have each implemented Bsal-specific health-certification requirements, and their regulations are similar to each other's. Imports to these countries are prohibited unless they are from WOAH member countries and accompanied by a health certificate. The European Union's implementing decision lays out testing and quarantine protocols along with providing a model health certificate. The United Kingdom costs for Bsal quarantining starts at £250 ($340) per consignment monitored and £40 ($54) for testing where required, and these could be untenable for most importers. Although some countries may have the necessary expertise to certify that salamanders are free of Bsal, not all exporting nations may have the necessary skills or resources, nor do we know which ones do. At some point in the future we may be able to propose health-certification criteria that are reliable and tenable, with the costs borne by the trade.

Scientists and diagnostic laboratories are also working to standardize laboratory protocols, but there are currently no standardized sampling, testing, and screening protocols in the United States, and we do not know the standards, if any, in the various countries from which salamanders are currently being or may be exported. Assay sensitivity can vary between laboratories. A wide variety of laboratory equipment, reagents, techniques, protocols, and personnel experience is available, thus contributing to non-standardized techniques that can lead to variable or inconsistent results. Each laboratory has different equipment and uses different reagents (L. Sprague, USFWS,

pers. comm., 2021). The North American Bsal Task Force recommends corroborative assays or further testing to validate a positive PCR or qPCR result, although WOAH does not state this; they also recommend quarantining under various scenarios (North American Bsal Task Force 2022). Considering the lack of amphibian quarantine facilities at ports of import and the unknown standards of testing for certification, we conclude it is currently not sufficient to rely on methods similar to those of the European Union and United Kingdom for preventing the introduction and establishment of Bsal. We need more information on this, which we requested below in *K. Information Requested* in question *(10)*.

Some treatment options also exist, such as treatment with antifungal medications that can be applied on animals that do not tolerate 25 °C (77 °F) (A. Martel, University of Ghent, pers. comm. 2015; Blooi et al. 2015b). It may be possible to treat amphibians in the wild for Bd with antifungals by capturing individuals and soaking them in a bath of the chemical, then releasing them back into the environment. As Hardy et al. (2015) showed for Cascades frogs (*Rana cascadae*), this process does not seem to be as effective as desired given possible side effects, but it may delay the eventual outcome of an outbreak enough to help individuals persist in the population. However, this process left unanswered questions about its applicability for salamanders and whether reinfection from fomites could still occur. Blooi et al. (2015b) identified a method for treating infected fire salamanders for Bsal with a combination of antifungals and temperature control that successfully cleared the fungus. However, such treatment worked only for controlled settings, such as those found in a laboratory or conservation facility, and side effects are unknown.

It is impractical to treat widespread areas in the natural environment given the likely cost, personnel, and time needed to locate and treat all salamanders in the wild. Additionally, without a standardized process it is impractical to set required protocols

that would apply to all specimens in trade. The possibility also exists for unknown and unintended consequences from such large-scale treatments, such as possible side effects from the widespread use of antifungal drugs. While promising, the treatment has not been standardized for use in a widespread manner for any salamander species. As we have noted above under *Environmental Conditions Needed To Survive*, Bsal is likely capable of persisting in the environment without a host by transmission to infected materials. Even if all individuals of a population could be successfully treated, the threat of reintroduction from environmental contamination would still exist.

Even without the capacity to treat animals, research has shown that it is possible to identify whether a salamander is infected with Bsal, which would allow animals to be screened prior to importation. Blooi et al. (2013) presented a method of sampling salamanders and testing them to determine whether they carry Bsal. However, if certification occurs prior to importation, the process would require trained technicians in the country of origin, specialized equipment, and certainty that the salamanders would not become infected following certification. The results of those tests must then be interpreted by qualified health professionals and documented through a health-certification process. We cannot rule out the possibility of false negative tests or falsified documentation that could allow Bsal to be introduced into the United States. Post-import health certification by the Service at ports of entry would require holding salamanders at least 1 day for processing, testing, and diagnosis. Quarantine facilities would be needed while samples are processed and the health of the salamanders is certified. In addition, wildlife inspectors at ports of entry would need to be trained in testing and diagnosis procedures.

In comparison, the certification process for listed salmonid species is standardized and salmonids are easier to test. For example, the tests for regulated salmonids are non-molecular, validated, require pathogen culture, and are, therefore, more straightforward.

There are also only 12 countries (Australia, Canada, Chile, Denmark, Finland, Iceland, Isle of Man, New Zealand, Northern Ireland, Norway, Scotland, and Wales) that have officials certified to sign health certificates to export salmonids (generally as eggs) to the United States. Generally, only a few countries export to the United States in a given year. For example, in 2019, the Service received requests to import salmonid eggs from Canada, Denmark, Finland, and Iceland. The Service concluded that there is currently insufficient certainty that a certification program utilizing the method described by Blooi et al. (2013) would be effective in preventing the introduction or spread of Bsal in the United States. However, we may consider a certification system in the future and have posed a question below for public comment.

Given the expected severity of consequences of Bsal introduction, imported salamanders that could be carriers may need to be treated, even with a health-certification process, which is not practical at this time for implementation by the Service as a broad-scale regulatory tool. The studies that have been conducted have not been standardized or agreed to as a suitable diagnostic or treatment effort on a large scale for treating all of the specimens that would potentially be imported. Not all species will tolerate treatment, and reliable diagnostic capacity is needed to verify that animals do not carry Bsal following treatment. If an outbreak occurs, it would not be practical to locate and treat all free-ranging individuals in the wild in U.S. ecosystems. While antifungal agents could be applied to all animals, either in the laboratory or perhaps applied over a large geographic area, we are concerned about side effects on the animals being treated or nontarget species. We are also concerned about possible negative environmental effects if a chemical was widely applied (Gyllenhammar et al. 2009; Hasselberg et al. 2008).

Researchers are also looking into the composition of the skin microbiome of various salamander species to gauge if it is possible to determine natural resistance to Bsal. For example, Bletz et al. (2018) found that European fire salamanders maintain

complex skin microbiotas that have some Bsal-inhibiting properties, but the bacterial numbers are too low to protect sufficiently against Bsal. Currently, we do not know if skin microbiota can be enhanced to inhibit Bsal sufficiently because of the complexities involved.

In contrast to live specimens, salamanders that are chemically preserved with common scientific and museum collection protocols present no risk of introducing or transmitting Bsal. Tissue samples fixed in 10 percent formalin or embedded in paraffin (usually both in sequence) after routine histological processing (or both), including those from amphibians known to, or suspected of, carrying Bsal, contain only nonviable material and, thus, are not considered injurious (M. Forzán, Bsal Task Force, pers. comm. 2021). In addition, experimental trials have demonstrated that Bsal is killed when exposed to 70 percent ethanol for at least 60 seconds (Van Rooij et al. 2017). Therefore, skin swabs preserved in 70 percent ethanol are not considered injurious and may be used for PCR testing since this chemical does not damage DNA quality. However, freezing a salamander as a way to kill Bsal spores is not a proven method to kill Bsal to our knowledge. For Bd, analysis of some sample results suggest that the freeze-shock treatment reduced the probability that Bd will grow, but all the Bd strains had at least a portion of samples that grew and produced zoospores following a freeze shock of -12 °C (10.4 °F) for 24 hours (Voyles et al. 2017). Since we know of no evidence that unpreserved dead salamanders, including species that may be frozen, are not capable of carrying Bsal, they are considered injurious.

In conclusion, various methods for mitigating the spread of Bsal by salamanders have been studied. We reviewed studies on thermal treatments, health certifications, anti-fungal medications, and resistance by skin microbiota, and none individually or in combination are sufficiently effective, safe, and broadly applicable to negate the need to list salamanders as injurious.

Any Potential Ecological Benefits to Introduction

No known benefits would result from Bsal or salamanders carrying Bsal occurring in the United States. The risks to native wildlife and wildlife resources greatly outweigh any unlikely benefits. Moreover, we are aware of no other potential ecological benefits for the introduction of Bsal or of Bsal-infected or Bsal-carrier salamanders into the United States.

I. Summary and Conclusion for Interim Rule

Overall, there is a high risk to the wildlife and wildlife resources of the United States from salamanders that are capable of carrying Bsal. The United States harbors 221 species of salamanders, more than any other country. Of the 23 native genera, 13 genera were found to be vulnerable to or carriers of Bsal as of the drafting of this second interim rule. We find that the fungus is lethal to at least 9 native salamander species in 6 genera and that 164 native species are considered carriers of Bsal. As of the drafting of this document, the vulnerability to disease and carrier status of 10 genera have not been tested or do not have conclusive results as carriers, many of which may have species vulnerable to this potentially deadly fungus. Under wild conditions, the disease may stress species to a point below the lethal threshold, and if these species are stressed by other factors, Bsal could cause cumulative harm to additional species. The benefits that these native salamander species provide to ecosystems, and in turn the ecosystem services that benefit people, are significant. The Service concludes that preventing Bsal from infecting native salamanders will prevent harmful effects to the wildlife and wildlife resources of the United States and merits listing of salamanders capable of carrying Bsal as injurious.

Salamanders capable of carrying Bsal have the potential to escape and spread Bsal into the environment. Species capable of carrying Bsal can survive long enough in the wild to transmit the fungus or can transmit it to other carriers while in transit. Bsal can also be introduced and infect native salamanders by people improperly disposing of

material that comes in contact with infected salamanders and can persist long enough in the environment without a host to represent a threat.

Substantive evidence exists that all species within a genus where at least one species has been identified as a carrier of Bsal can also be a threat. Our review found no conclusive countervailing evidence. We find that, due to shared characteristics by species within a genus, other species within these genera are also highly likely to be carriers of Bsal, even if not every species in the genus has been tested to verify that it is a carrier of Bsal. For these same reasons, hybrids of species found in a genus that has at least one carrier species are also expected to be carriers.

The main pathway for the global spread of Bsal is the international trade in salamanders. The most likely pathway of Bsal into the United States would be from salamanders being imported into the United States for commercial trade, including native species that are propagated outside the United States and subsequently imported into the United States. Listing salamanders that can carry Bsal as injurious wildlife will significantly confine this pathway and limit the capacity of Bsal to be introduced, become established, and spread in the United States.

The current capacity to prevent escape and establishment is low. Rehabilitation of disturbed ecosystems is expected to be low, if not impossible. The ability and effectiveness of measures to prevent or control established Bsal is currently low. There are no known benefits of Bsal.

The Service is listing live or dead specimens, hybrids, including parts, as injurious, but not eggs, gametes, preserved specimens or parts (including tissue), or purified extracted genetic material, where “preserved” means the preservation techniques kill the pathogen and thereby prevent transmission of Bsal. We find the risk of transmission of Bsal to other salamanders is high from both live and unpreserved dead specimens. Any salamanders that are infected and lethally vulnerable may die in transport

and continue to carry Bsal into the United States. The risk is also high from improper disposal of materials that might be contaminated by those live or unpreserved dead specimens. Dead specimens, including those that are or were frozen, are considered injurious. Although under the authority of 18 U.S.C. 42 we cannot list contaminated materials (fomites) as injurious, by listing the carriers of Bsal, we seek to prevent the introduction of those materials. Conversely, material that is not injurious includes purified extracted genetic material because it carries a low risk of infection by Bsal; however, other tissue samples, such as skin swabs, are listed unless they have been chemically preserved to deactivate any live Bsal. Swabs that are preserved by exposing to 70 percent ethanol for at least 60 seconds are not considered injurious. The Service is not listing specimens that are chemically preserved. We conclude the risk of infection from such specimens is low.

The Service is not adding eggs or gametes to the listing because there is no evidence that Bsal affects salamander reproductive tissue, such as eggs or gametes. The Service is not listing genera that we concluded are not carriers of Bsal because we do not have direct evidence that they are capable of introducing Bsal to the United States or otherwise transmitting it to native populations. We are also not listing genera where there is no data, even though it is possible that untested genera may also be capable of carrying Bsal.

For the reasons stated, the Service finds the 16 genera comprising approximately 164 species of salamanders to be injurious to the wildlife and wildlife resources of the United States. The potential for Bsal introduction into the United States is high, the United States has suitable conditions for Bsal survival, and the consequences of introduction into the United States are expected to be significant and occur across a wide range of the United States. By listing species that can carry Bsal, we are taking preemptive action to help ensure the fungus does not enter the United States and infect

native salamander populations and cause severe individual mortality, population declines, and ecosystem and economic harm.

J. Required Determinations

Regulatory Planning and Review

Executive Order 12866 (E.O. 12866), as reaffirmed by E.O. 13563 and E.O. 14094, provides that the Office of Information and Regulatory Affairs (OIRA) in the Office of Management and Budget (OMB) will review all significant rules. OIRA has determined that this rulemaking action is not significant.

Executive Order 14094 reaffirms the principles of E.O. 12866 and E.O 13563 and states that regulatory analysis should facilitate agency efforts to develop regulations that serve the public interest, advance statutory objectives, and are consistent with E.O. 12866 and E.O. 13563. Regulatory analysis, as practicable and appropriate, shall recognize distributive impacts and equity, to the extent permitted by law. E.O. 13563 emphasizes further that regulations must be based on the best available science and that the rulemaking process must allow for public participation and an open exchange of ideas. We have developed this rule in a manner consistent with these requirements.

The economic analysis for the second interim rule is provided in this section of the preamble. We are presenting the alternatives considered to identify whether there is a more effective option that can achieve the desired goals of the rule. The Service considered three alternatives for the economic analysis: Alternative 1: No action; Alternative 2: listing all species in 16 genera in which there is at least one confirmed carrier and all species in the genus are likely to be a carrier; and Alternative 3: listing all salamanders. We eliminated two alternatives that were considered for the 2016 interim rule. One was "listing species that were identified by Martel et al. (2014) and other scientific sources to be carriers of Bsal." This alternative was eliminated because new research provided evidence that this narrow approach could allow potential carriers to be

imported. The other alternative was "requiring a health certificate stating that the animal being moved is free of Bsal, in lieu of or in addition to listing." This alternative was eliminated because we do not have enough information to develop a reliable health certificate system at this time.

To establish the baseline, analysis of current market conditions for imports and domestically bred salamanders is necessary. However, available U.S. salamander market data are minimal. The analysis uses two data sources to estimate the imported salamander industry: the Service's LEMIS (USFWS OLE 2021) for data on the number of imported salamanders (live, dead specimens, hybrids, or parts) and the data submitted during the comment period for the 2016 interim rule by the Pet Industry Joint Advisory Council (PIJAC 2016) on live salamander pricing (updated to 2021 dollars). Due to limited data availability, we cannot estimate domestically bred salamander sales that are transported between the enumerated jurisdictions. We expect impacts to domestically bred salamanders to be minimal because, as determined by the 2017 court decision discussed above in this document, none of the alternatives prohibit interstate transport between States within the continental United States. We are requesting public comment on the number and sales of salamanders (by species) that are domestically bred and the percentage that are transported between the enumerated jurisdictions (see *Information Requested* below).

We establish the baseline as the years 2017 to 2019. This baseline accounts for the changes in imports and sales that resulted in January 2016 from the first interim rule (after the rule published, only 20 salamanders were imported in 2016); the April 2017 court decision that overturned the prohibition on interstate transport between States within the continental United States; and the outlier 2020 data due to the pandemic. The second interim rule prohibits the importation of live or dead specimens, hybrids, including parts, as injurious, but not eggs, gametes, preserved specimens or parts

(including tissue), unless an exemption is issued for scientific purposes. From 2017 to 2019, 11 genera of live salamanders (of which 5 genera are herein being listed; table 1), and 16 genera of salamander specimens (that is, dead salamanders or parts; of which 6 genera are herein being listed; table 2) were imported with no discernible trend. Live imports of the genera being listed herein are minimal, totaling 373 salamanders and approximately $18,000 (table 1). From 2017 to 2019, approximately 1,000 salamander specimens were imported (table 2), of which 25 percent would qualify as injurious under the second interim rule. The values for live salamanders with scientific purposes and for salamander specimens are unknown. No other salamanders (dead or parts) were imported during the baseline period, except for eggs, which would not be affected by this rule.

Table 1—Total Number of All Live Imported Salamanders, 2017–2019 (2021$)
(Data from USFWS OLE 2021)

Genus*	Purpose	Live Salamanders	Estimated Sales
Ambystoma	Commercial	**90**	**$8,000**
	Scientific	**13**	**
Amphiuma	Commercial	1	<$1,000
Andrias	Zoos	**4**	**$3,000**
Bolitoglossa	Commercial	5	<$1,000
Cynops	Personal	1	**
Desmognathus	Commercial	**119**	**$3,000**
Eurycea	Commercial	**137**	**$3,000**
Necturus	Commercial	163	$4,000
Pleurodeles	Scientific	108	**
Pseudotriton	Commercial	**10**	**<$1,000**
Salamandra	Scientific	100	**
Total all genera		751	$24,000**
Total new genera		**373**	**$18,000****

*Genera in bold are listed under the second interim rule. *Amphiuma*, *Cynops*, *Pleurodeles*, and *Salamandra* are listed under the final rule to the 2016 interim rule.
**The value of live salamanders for scientific purposes is unavailable, and estimating this value is beyond the scope of this analysis.

Table 2—Total Number of All Imported Salamander Specimens[1], 2017–2019
(Data from USFWS OLE 2021)

Genus[2]	Purpose	Salamander Specimens[1]
Ambystoma	Scientific	200
*Amphiuma**	Commercial	200
*Bolitoglossa**	Scientific	99
	Commercial	80
Cryptobranchus	Traveling Exhibit	1
Desmognathus	Scientific	24
Eurycea	Scientific	9
Laotriton	Scientific	2

*Necturus**	Commercial	6
Notophthalmus	Scientific	9
*Nototriton**	Scientific	6
*Oedipina**	Scientific	2
Paramesotriton	Scientific	4
Plethodon	Scientific	6
Salamandra	Scientific	187
Triturus	Scientific	11
Tylototriton	Scientific	111
TOTAL		**957**

[1]Specimens are animals that are preserved for scientific or museum use; however, it is unknown whether these specimens would meet the preservation standards and be exempt from listing.

[2]Genera in bold are listed under the second interim rule. Genera with * are not listed under either rule.

Alternative 1 is the no action alternative and is the status quo. We would not list additional species of salamanders as injurious. Retail sales of imported salamanders would continue; there would be no prohibition on transportation between the enumerated jurisdictions; and imports would continue. Salamander and ancillary industries would not incur any additional costs unless Bsal is introduced in the United States.

Alternative 1 would not reduce the risk of introducing Bsal to the United States, and any benefits that accrue under Alternative 2 (this second interim rule) would not accrue under Alternative 1. Under Alternative 1, Bsal would continue to pose risk to native species and other wildlife resources in the United States. Furthermore, Alternative 1 does not meet the purpose of the listing, which is to prevent the introduction, establishment, and spread of Bsal in the wild in the United States. Therefore, we expect that greater financial and natural resources losses would be incurred due to managing and responding to Bsal if the fungus establishes and spreads in the United States compared to taking action now to prevent and minimize its introduction.

Alternative 2 (second interim rule) lists all species in 16 genera for which there is at least one confirmed carrier and all species in that genus are likely to be a carrier. From 2017 through 2019, live individuals imported from genera that would be listed under Alternative 2 were in *Ambystoma, Desmognathus, Eurycea,* and *Pseudotriton*, and

specimens (dead individuals or parts) were in *Ambystoma, Cryptobranchus, Desmognathus, Eurycea,* and *Laotriton*. Under this alternative, live commercial imports totaled about 370 salamanders and $14,000, which represented approximately 47 percent of all live salamander imports and 77 percent of sales. Live imports for scientific and zoological purposes totaled 17 salamanders and represented 8 percent of scientific imports. All specimens under this alternative (235 specimens) were imported for scientific purposes, and importers would be eligible to apply for a permit. Under Alternative 2, imports of these genera would discontinue unless the importer is approved for a scientific permit.

In the long term, the second interim rule is expected to benefit the economy. Efforts to control or eradicate invasive species (in this case, an invasive pathogen on a host wildlife species) and manage the costs they incur to society, once they have become established, are generally recognized as being less effective and more expensive than efforts to prevent potentially invasive species from establishing in the first place (Cuthbert et al. 2022). Emerging pathogens are currently underrepresented in databases of the cost of invasives, so adding them would greatly increase the estimated costs in the framework of biological invasions (Diagne et al. 2021). As a result, sectors of the economy that will not need to expend resources to control or manage injurious wildlife will be expected to gain from a timely listing process.

Alternative 3 proposes listing all 804 species of salamanders in the world. Although some species may or may not serve as carriers of Bsal, this alternative takes immediate action against those genera for which current scientific research and analysis has provided evidence are carriers of Bsal, along with other genera that may eventually be found to be carriers of Bsal. Under Alternative 3, all salamander imports would be prohibited (tables 1 and 2). This alternative would have the largest impact on salamander imports and the highest probability of preventing the introduction of Bsal in the wild. We

did not select this option because we do not have enough evidence at this time that all genera could be carriers. However, evidence could be established in the future, or another reason could surface, such as the appearance of a hypervirulent variant of the fungus.

We considered other alternatives that we rejected because we do not have sufficient information at this time that they could be effectively implemented to prevent introduction, establishment, and spread of Bsal from salamanders. For example, we do not have the capacity to establish and enforce a quarantine system or confidence in its effectiveness at preventing Bsal. We noted in the 2016 interim rule that, absent concerns regarding the effectiveness and sensitivity of current testing methods (including the return of false negatives), the lack of validation and sufficient testing capacity, and agency resources required to conduct inspections, interpret results, and issue health certificates, it may be possible to establish a health certification for salamanders that are free of Bsal. These concerns remain, and no such health certification has been established. However, this situation does preclude us from establishing health certification in the future if circumstances change. Appropriate conditions may also be included in injurious wildlife permits under the authority of and consistent with the purposes of 18 U.S.C. 42.

We also considered encouraging partners to take nonregulatory action, such as voluntary best management practices or individual State action. The Service will pursue such actions as we move forward, and we are working with partners on such efforts as Habitattitude™, which encourages responsible consumer behaviors with respect to pet ownership. Voluntary actions, such as applying heat therapy as described in Blooi et al. (2015a) and Blooi et al. (2015b), may help reduce the threat posed by Bsal, but standardization and widespread application of the methods remain as challenges. Although voluntary actions are vital to help minimize the threat of invasive species, the Service is highly concerned about the extensive damage that introduction of Bsal would

do to our Nation's natural resources and concluded that we cannot rely on voluntary actions alone in this instance to address the severity of the threat that Bsal poses.

Regulatory Flexibility Act

Under the Regulatory Flexibility Act (as amended by the Small Business Regulatory Enforcement Fairness Act (SBREFA) of 1996), whenever a Federal agency is required to publish a notice of rulemaking for any proposed or final rule, it must prepare and make available for public comment a regulatory flexibility analysis that describes the effect of the rule on small entities (i.e., small businesses, small organizations, and small government jurisdictions) (5 U.S.C. 601 *et seq.*). However, no regulatory flexibility analysis is required if the head of an agency certifies that the rule would not have a significant economic impact on a substantial number of small entities. Thus, for a regulatory flexibility analysis to be required, impacts must exceed a threshold for "significant impact" and a threshold for a "substantial number of small entities." See 5 U.S.C. 605(b). SBREFA amended the Regulatory Flexibility Act to require Federal agencies to provide a statement of the factual basis for certifying that a rule would not have a significant economic impact on a substantial number of small entities.

The U.S. Small Business Administration defines a small business as one with annual revenue or employment that meets or is below an established size standard for industries described in the North American Industry Classification System (NAICS). To assess the effects of the rule on small entities, we focus on (1) entities that import animals or animal parts and hybrids of listed genera and (2) entities with sales of animals, animal parts, and hybrids that are transported between the enumerated jurisdictions listed in 18 U.S.C. 42(a)(1) and 50 CFR 16.3. Small entities affected by the rule are represented by categories and standards from the NAICS. The NAICS categories pertaining to this rule are those entities with:

(1) receipts less than $32.0 million for “Pet and Pet Supplies Stores” (NAICS 459910);

(2) receipts less than $2.75 million for “All Other Animal Production” (NAICS 112990);

(3) receipts less than $34.0 million for “Zoos and Botanical Gardens” (NAICS 712130);

(4) receipts less than $34.5 million for “Colleges, Universities and Professional Schools” (NAICS 611310); and

(5) fewer than 1,000 employees for “Research and Development in the Physical, Engineering, and Life Sciences” (NAICS 541715).

Under the second interim rule, we expect the effect on entities that import the 16 genera to be small. From 2017 to 2019, seven businesses imported live salamanders from some of those genera, which represented 0.1 percent of all pet and pet-supplies establishments and less than 0.1 percent of all other animal-production establishments. Three businesses imported the listed specimens for scientific purposes, which represented less than 0.1 percent of all universities and research facilities (USFWS OLE 2021). We expect the effect on entities that sell the 16 genera between the enumerated jurisdictions to be small as well, because the interim rule does not prohibit interstate transport between the 49 States in the continental United States. Furthermore, pet stores outside the 49 States in the continental United States represent less than 1 percent of all stores and less than 1 percent of total pet store sales (USCB 2017).

Therefore, we certify that this interim rule will not have a significant economic effect on a substantial number of small entities as defined under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*). An initial regulatory flexibility analysis is not required. Accordingly, a small entity compliance guide is not required.

The second interim rule makes no changes in the compliance requirements of any business. The Service is unaware of any duplicative, overlapping, or conflicting Federal rules. Several States implement similar acts that are more restrictive than the Federal law.

Congressional Review Act

The interim rule is not a major rule under 5 U.S.C. 804(2), the Congressional Review Act. This rule:

a. Would not have an annual effect on the economy of $100 million or more. The rule listing 16 genera of salamanders, including approximately 164 species, would prohibit an estimated 125 live salamanders imported per year and prohibit the transport of domestically bred individuals between the enumerated jurisdictions. In addition, businesses would also face the risk of fines if caught transporting these salamanders or their parts between the enumerated jurisdictions in the shipment clause of 18 U.S.C. 42(a)(1), which is codified in Federal regulations at 50 CFR 16.3. The penalty for violation of this law is not more than 6 months in prison and not more than a $5,000 fine for an individual and not more than a $10,000 fine for an organization.

b. Would not cause a major increase in costs or prices for consumers, individual industries, Federal, State, or local government agencies, or geographic regions.

c. Would not have significant adverse effects on competition, employment, investment, productivity, innovation, or the ability of United States-based enterprises to compete with foreign-based enterprises.

Unfunded Mandates Reform Act (2 U.S.C. 1501 et seq.)

In accordance with the Unfunded Mandates Reform Act (2 U.S.C. 1501), the Service makes the following findings:

a. This rule would not produce a Federal mandate. In general, a Federal mandate is a provision in legislation, statute, or regulation that would impose an enforceable duty upon State, local, or Tribal governments, or the private sector.

b. The rule would not have a significant or unique effect on State, local, or Tribal governments or the private sector. A statement containing the information required by the Unfunded Mandates Reform Act (2 U.S.C. 1531 *et seq.*) is not required.

Takings

In accordance with Executive Order 12630 (Government Actions and Interference with Constitutionally Protected Private Property Rights), the rule does not have significant takings implications. A takings implication assessment is not required. This rule would not impose significant requirements or limitations on private property use. While import and transport between the enumerated jurisdictions of any of the listed species is prohibited, 18 U.S.C. 42(a) does not prohibit any person who owns one of the listed species at the time of listing from continuing to possess the salamander or engaging in intrastate transport and other activities within their State or territory, as allowed under State, Tribal, or territorial law.

Federalism

In accordance with Executive Order 13132 (Federalism), this interim rule does not have significant federalism effects. A federalism assessment is not required. This rule would not have any direct effects on States, on the relationship between the Federal Government and the States, or on the distribution of power and responsibilities among the various levels of government. Therefore, in accordance with Executive Order 13132, we determine that this rule does not have sufficient federalism implications to warrant the preparation of a federalism assessment.

Civil Justice Reform

In accordance with Executive Order 12988, the Office of the Solicitor has determined that the interim rule does not unduly burden the judicial system and meets the requirements of sections 3(a) and 3(b)(2) of the Executive order. The interim rule has been reviewed to eliminate drafting errors and ambiguity, was written to minimize

litigation, provides a clear legal standard for affected conduct rather than a general standard, and promotes simplification and burden reduction.

Paperwork Reduction Act of 1995 (44 U.S.C. 3501 et seq.)

This rule does not contain any new collections of information that require approval by the Office of Management and Budget (OMB) under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501 *et seq.*). OMB has previously approved the information collection requirements associated with filing declarations and the importation of injurious wildlife and assigned the following OMB Control Numbers:

- 1018–0012, "Declaration for Importation or Exportation of Fish or Wildlife, 50 CFR 14" (expires 03/31/2024, and in accordance with 5 CFR 1320.10, an agency may continue to conduct or sponsor this collection of information while the submission is pending at OMB), and
- 1018–0078, "Injurious Wildlife; Importation Certification for Live Fish and Fish Eggs (50 CFR 16)" (expires 01/31/2024, and in accordance with 5 CFR 1320.10, an agency may continue to conduct or sponsor this collection of information while the submission is pending at OMB).

An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number.

National Environmental Policy Act

We have reviewed this rule in accordance with the criteria of the National Environmental Policy Act (NEPA) and our Departmental Manual in 516 DM. This rule does not constitute a major Federal action significantly affecting the quality of the human environment. Under Department of the Interior agency policy and procedures, this rule is covered by a categorical exclusion (516 DM 8.5 C(9)), and preparation of a detailed statement under NEPA is not required because it adds species to the list of injurious wildlife under 50 CFR subchapter B, part 16, which prohibits the importation into the

United States and shipment between some jurisdictions of wildlife found to be injurious (for further information on the categorical exclusion, see 80 FR 66554, October 29, 2015). The categorical exclusion states, "The adding of species to the list of injurious wildlife regulated under the Lacey Act (18 U.S.C. section 42, as amended) as implemented under 50 CFR subchapter B, part 16, which prohibits the importation into the United States * * * of wildlife found to be injurious." We have also determined that the rule does not involve any of the extraordinary circumstances listed in 43 CFR 46.215 that would require further analysis under NEPA.

Government-to-Government Relationship with Tribes

In accordance with the President's memorandum of April 29, 1994, "Government-to-Government Relations with Native American Tribal Governments" (59 FR 22951), Executive Order 13175, and the Department of the Interior's manual at 512 DM 2, we readily acknowledge our responsibility to communicate meaningfully with recognized Federal Tribes on a government-to-government basis. In accordance with Secretarial Order 3206 of June 5, 1997 (American Indian Tribal Rights, Federal-Tribal Trust Responsibilities, and the Endangered Species Act), we readily acknowledge our responsibilities to work directly with Tribes in developing programs for healthy ecosystems, to acknowledge that Tribal lands are not subject to the same controls as Federal public lands, to remain sensitive to Indian culture, and to make information available to Tribes. For this interim rule, we sent a letter to the leaders of the almost 580 federally recognized Tribes providing some background and asking for their comments. We received none. We have evaluated potential effects on federally recognized Indian Tribes and have determined that there are no potential effects. This rule involves the importation of salamanders and shipment of salamanders between the enumerated jurisdictions of the shipment clause of 18 U.S.C. 42, also set forth in 50 CFR 16.3. We are unaware of such movement in these species by Tribes.

Effects on Energy

Executive Order 13211 requires agencies to prepare statements of energy effects when undertaking certain actions. This rule is not expected to affect energy supplies, distribution, and use. Therefore, this action is a not a significant energy action and no statement of energy effects is required.

Clarity of Rule

We are required by Executive Orders 12866 and 12988 and by the Presidential Memorandum of June 1, 1998, to write all rules in plain language. This means that each rule we publish must:

a. Be logically organized;

b. Use the active voice to address readers directly;

c. Use clear language rather than jargon;

d. Be divided into short sections and sentences; and

e. Use lists and tables wherever possible.

If you feel that we have not met these requirements, send us comments by one of the methods listed in **ADDRESSES**. To help us revise the rule, your comments should be as specific as possible. For example, you should tell us the numbers of the sections or paragraphs that are unclearly written, which sections or sentences are too long, and the sections where you feel lists or tables would be useful.

K. Information Requested

We are soliciting public comments and supporting data for this second interim rule to add 16 new genera to the current list of 20 genera of salamanders that are listed as injurious amphibians under 18 U.S.C. 42, including comments and supporting data on the economic information as described above in the Required Determinations. As stated above in this document, we are not soliciting comments regarding the listing of the

genera that were listed in the 2016 interim rule. We will review the public comments for the preparation of a second final rule.

You may submit your comments and materials concerning this second interim rule by one of the methods listed in **ADDRESSES**. We will not accept comments sent by email or fax or to an address not listed in **ADDRESSES**. We will post your entire comment—including your personal identifying information—on *https://www.regulations.gov*. If your written comments provide personal identifying information, you may request at the top of your document that we withhold this information from public review. However, we cannot guarantee that we will be able to do so.

Comments and materials we receive, as well as supporting documentation we used in preparing this second interim rule, will be available for public inspection on *https://www.regulations.gov* under Docket No. FWS–HQ–FAC–2015–0005, or by appointment, during normal business hours at the Service's office in Falls Church, VA (see **FOR FURTHER INFORMATION CONTACT**).

We are soliciting public comments and supporting data to gain additional information, and we specifically seek comment on the following questions:

(1) How many of the new genera listed by this interim rule are currently in domestic production for wholesale or retail sale, and in how many and which States?

(2) How many businesses sell salamanders from the genera listed by this interim rule, and how many businesses transport these listed genera between enumerated jurisdictions?

(3) How many businesses breed salamanders of one or more of the genera listed by this interim rule?

(4) What species listed as threatened or endangered by one or more States would be affected by the introduction of Bsal?

(5) What provisions in this interim rule should the Service have considered with regard to: (a) the impact of the provision(s) (including any benefits and costs), if any, and (b) the alternatives, if any, that the Service should consider, as well as the costs and benefits of those alternatives, paying specific attention to the effect of the rule on small entities?

(6) How could this interim rule be modified to reduce costs or burdens for some or all entities, including small entities, consistent with the Service's requirements? For example, we seek comment on the distinct benefits and costs, both quantitative and qualitative, of (a) the prohibitions on importation and (b) the prohibitions on transport between enumerated jurisdictions of the genera listed by this rule. What are the costs and benefits of the modifications?

(7) Is there any evidence suggesting that Bsal has been introduced into the United States or may have already established?

(8) Is there evidence suggesting that any of the genera listed by this interim rule are not carriers of Bsal? If so, which ones?

(9) Is there evidence suggesting that additional salamander genera are carriers of Bsal and should be listed as injurious? If so, which ones?

(10) Could a reliable health certificate within the Service's authority be developed that would allow Bsal-free salamander imports? Are there treatments that would ensure salamanders imported into the United States are reliably free of Bsal, and how could compliance be monitored?

(11) Are there other means of preserving or treating salamander specimens, parts, or products that are not identified in this rule and that are proven adequate to render Bsal non-viable?

(12) Should the Service add eggs or other reproductive material of listed salamanders to the list of injurious wildlife because they may also carry Bsal?

(13) What are relevant Federal, State, or local rules that may duplicate, overlap, or conflict with this interim rule?

We will also submit the rule for peer review concurrent with public comments. In conducting peer review, we will follow guidance from the Office of Management and Budget "Final Information Quality Bulletin for Peer Review" (OMB 2004) and the Service's own guidance.

References Cited

A complete list of all references used in this rulemaking is available at *https://www.regulations.gov* under Docket No. FWS–HQ–FAC–2015–0005.

Authors

The primary authors of this rule are the staff members of the U.S. Fish and Wildlife Service's Branch of Aquatic Invasive Species.

List of Subjects in 50 CFR Part 16

Animal diseases, Imports, Reporting and recordkeeping requirements, Transportation, Wildlife.

Regulation Promulgation

For the reasons discussed in the preamble, the U.S. Fish and Wildlife Service amends part 16, subchapter B of chapter I, title 50 of the Code of Federal Regulations, as set forth below:

PART 16—INJURIOUS WILDLIFE

1. The authority citation for part 16 continues to read as follows:

AUTHORITY: 18 U.S.C. 42.

2. Revise § 16.14(a) to read as follows:

§ 16.14 Importation of live or dead amphibians or their eggs.

(a) The importation, transportation, or acquisition of any live or dead specimen or hybrid, including parts (except for eggs or gametes; parts or tissues that have been

chemically preserved, chemically treated, or heat treated so that the pathogen *Batrachochytrium salamandrivorans*, if present, is rendered non-viable; and molecular specimens consisting of only the nucleic acids from organisms), of all species in the genera *Ambystoma, Andrias, Aneides, Aquiloeurycea, Calotriton, Chioglossa, Chiropterotriton, Cryptobranchus, Cynops, Desmognathus, Ensatina, Euproctus, Eurycea, Hydromantes, Hynobius, Ichthyosaura, Laotriton, Lissotriton, Neurergus, Notophthalmus, Ommatotriton, Onychodactylus, Pachytriton, Paramesotriton, Plethodon, Pleurodeles, Proteus, Pseudobranchus, Pseudotriton, Salamandra, Salamandrella, Salamandrina, Siren, Taricha, Triturus,* and *Tylototriton* is prohibited except as provided under the terms and conditions set forth at § 16.22 of this part.

* * * * *

Shannon Estenoz,

Assistant Secretary for Fish and Wildlife and Parks.

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