*Herpetological Review*, 2017, 48(3), 564–568.


# Swabbing for *Batrachochytrium salamandrivorans* on Wild Rough-skinned Newts (*Taricha granulosa*) and Pet-Traded Amphibians on Southern Vancouver Island, British Columbia, Canada

*Batrachochytrium salamandrivorans* (*Bsal*) is a fungal pathogen that causes chytridiomycosis in salamanders and is similar to the more widely known *B. dendrobatidis* (*Bd*) that is believed to have been responsible for a global decline in over 200 amphibian species (Skerratt et al. 2007). *Bsal* is known to infect the skin of salamanders, causing skin lesions, anorexia, apathy, ataxia, and death (Martel et al. 2013). *Bsal* has been reported to have caused localized mortality of wild and captive salamander species in Europe (Spitzen-van der Sluijs et al. 2013; Martel et al. 2014; Sabino-Pinto et al. 2015). It has been hypothesized that *Bsal* was spread to Europe on human-transported salamanders originating in Asia, later spreading to wild populations (Martel et al. 2014). There is a concern that *Bsal* could impact native salamanders in North America through this introductive pathway (Gray et al. 2015; Stephen et al. 2015). In laboratory challenge experiments, Martel et al. (2014) reported Rough-skinned Newts (*Taricha granulosa*), a salamander endemic to the Pacific Northwest of North America, died within a few days of *Bsal* exposure. As a result of the risk of *Bsal* to native amphibians in North America, in 2015 the Pet Industry Joint Advisory Council (PIJAC) of Canada, promoted a voluntary moratorium on the importation of Fire-bellied Newts (*Cynops orientalis*) and Paddle-tailed Newts (*Pachytriton labiatus*) into Canada (PIJAC 2015); in 2016 the US Fish and Wildlife Service instated an interim prohibition on the importation of 20 genera of salamanders into the United States (US Fish and Wildlife Service 2016); and in May 2017 Canada instated a one-year import restriction on all salamander species within the order Caudata (Environment and Climate Change Canada 2017).

This study is the first field-based effort to test for the presence of *Bsal* on wild Rough-skinned Newts and pet-traded salamanders on southern Vancouver Island, British Columbia, Canada. Southern Vancouver Island was the chosen study region to test wild salamanders for two reasons. Firstly, it is part of the Rough-skinned Newt's native range, and secondly it has been indicated as a high vulnerability zone for *Bsal* in North America due to occurrence of known-susceptible species, suitable habitat conditions, and import trade centers (Yap et al. 2015).

From May–July 2016, nine water bodies, small lakes, or ponds with permanent water, were sampled on southern Vancouver Island for Rough-skinned Newts (Fig. 1). Sites were chosen from urban and rural areas, known to researchers to have contained Rough-skinned Newts in the past. To capture wild Rough-skinned Newts, minnow traps were deployed overnight and checked in the morning. Dip nets were also used at some sites. Captured Rough-skinned Newts were swabbed following the AmphibiaWeb "chytrid swabbing protocol" (Briggs NIH Research Group 2009) and then released. To avoid the spread of diseases from sample sites, the "British Columbia hygiene protocol for field staff working in aquatic environments" (Ecosystems Branch, British Columbia Ministry of Environment 2008) was followed.

In addition to testing wild Rough-skinned Newts for this study, researchers tested for the presence of *Bsal* on captive pet traded salamanders. The intention was to help determine if pet traded salamanders could be acting as vectors for *Bsal*. To locate pet-traded salamanders, over 30 pet stores were contacted across British Columbia by telephone and asked about their salamander inventory, and in some but not all cases, their anuran inventory. All but one pet store indicated that they did not have salamanders, but many indicated that they sold various species of anurans. Researchers did not further verify pet store information by visiting each store, due to cost and time limitations for this study.

By the end of the sampling period, 15 captive salamanders had been swabbed by researchers. One store in Victoria (Pet Store 1) was found to have 13 Fire-bellied Newts (*Cynops orientalis*) and 1 Axolotl (*Ambystoma mexicanum*), which were all swabbed (Table 1). One Barred Tiger Salamander (*Ambystoma mavortium)* was also opportunistically swabbed after being confiscated by the British Columbia Ministry of Environment from a pet store in the Lower Mainland, British Columbia (Pet Store 3; Table 1). Barred Tiger Salamanders are listed under Schedule A of the British Columbia *Wildlife Act* as "Wildlife" which are illegal to possess in captivity. While visiting Pet Store 1 (to initially swab salamanders) and Pet Store 2 in Gibsons, British Columbia (opportunistic visit by researcher while travelling), 15 captive anurans were additionally swabbed (Table 1).

Swabs from wild Rough-skinned Newts and from captive pet-store salamanders and anurans were sent to the BC Ministry of Agriculture's Animal Health Centre in Abbotsford, British Columbia. Swabs from Rough-skinned Newts were tested for *Bsal*, and the swabs from pet store salamanders and anurans were tested for both *Bsal* and *Bd* using quantitative polymerase chain reaction (qPCR) analyses (*Bsal*: Blooi et al. 2013; *Bd*: Boyle et al. 2004). Samples were run as single reactions and both *Bd* PCR and *Bsal* PCR were run independently. Exogenous internal controls, negative extraction controls, no-template controls, and positive controls were used for both *Bd* and *Bsal* PCRs. Previously, the BC Ministry of Agriculture's Animal Health laboratory had participated in a ring test for *Bd* and *Bsal*, organized by the *Bsal* Task Force Diagnostic Working Group (H. Snyman, pers, comm., Animal Health Centre, British Columbia Ministry of

**PURNIMA GOVINDARAJULU**
*British Columbia Ministry of Environment, Victoria, BC V8T 5J9, Canada; e-mail: Purnima.Govindarajulu@gov.bc.ca*
**ELYSE MATTHEWS**
*British Columbia Ministry of Environment, Victoria, BC V8T 5J9, Canada; e-mail: Elyse.Matthews@gov.bc.ca*
**KRISTIINA OVASKA**
*Habitat Acquisition Trust, Victoria, BC V8W 1E4, Canada e-mail: ke.ovaska@gmail.com*

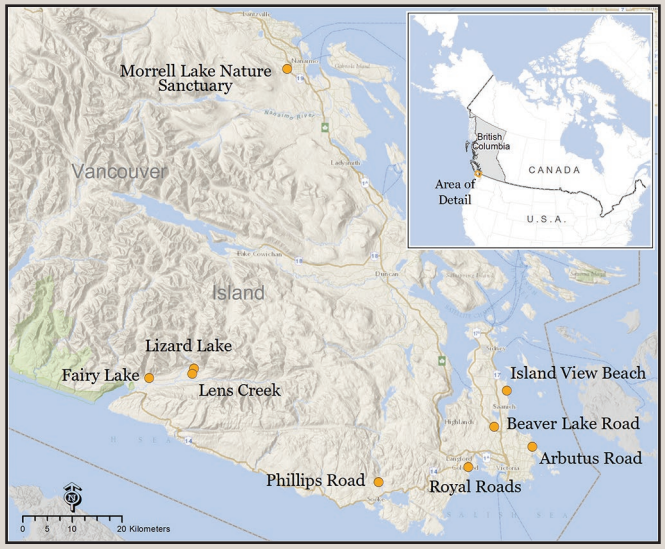


FIG. 1. Sample sites for *Batrachochytrium salamandrivorans* on wild Rough-skinned Newts (*Taricha granulosa*) on southern Vancouver Island, British Columbia, Canada.

Agriculture, November 2016; Bsal Task Force 2016). This ring test was intended to test for standardization of laboratory methods and results across North American laboratories in Canada and the US.

*Bsal* was not detected by qPCR analyses of swabs from the 82 wild Rough-skinned Newts and 15 captive salamanders. Additionally, *Bd* was not detected from samples of the captive salamanders tested (wild salamanders were not tested). From samples of the 15 captive anurans, *Bsal* was not detected (Table 1), however, *Bd* was detected from one captive Ornate Pacman Frog (*Ceratophrys ornata*) (Table 1).

In our preliminary surveys, we found no evidence that *Bsal* had infected wild Rough-skinned Newts on southern Vancouver Island. However, it is recommended that future studies increase the sample size and number of sites to allow for greater confidence. In addition, expanding sampling across a wider area of the Rough-skinned Newt's native range would be desirable, including the Lower Fraser Valley where newt populations are embedded within populated and highly modified landscapes.

*Bsal* was not detected in any of the captive salamanders sampled. However, the small sample size (N = 15) and small study region precludes conclusions about *Bsal* infections of pet-traded salamanders on Vancouver Island and the Lower Mainland of British Columbia. Only a small number of pet-traded salamanders were found during the study period, suggesting that there are currently few salamanders in the regional pet trade. In future studies, in-store visual inspections of taxa for sale, in addition to phone interviews, would help verify the presence or absence of salamanders in stores.

Without the ability to compare previous salamander numbers in the pet trade prior to this study, it is difficult to conclude with confidence that the low numbers observed were a result of the Pet Industry Joint Advisory Council's moratorium on the importation into Canada and/or the US Fish and Wildlife Service's prohibition on the importation of salamanders into the US. Since PIJAC's voluntary moratorium and the US prohibition, it is possible that salamander wholesalers stopped importing salamanders to comply with recommendations, or they have not been able to order them from suppliers in the United States that have been undergoing an importation ban. One importer in Vancouver, British Columbia was interviewed by researchers over the phone, and stated that their business had stopped ordering salamanders after receiving a call from PIJAC about the voluntary moratorium. Prior to this call from PIJAC, the wholesaler had been ordering 100–200 salamanders on a bi-weekly basis to supply retailers across British Columbia (Vancouver wholesaler, pers. comm., 2016). This is an indication of the application of PIJAC's past moratorium, and the importance of working with wholesalers and industry representatives in the future to address concerns about *Bsal*.

While this importer's account is important to understand the impacts of PIJAC's moratorium and the US prohibition, a larger survey, directed towards large retail and independent pet stores, wholesalers, online and private traders, and hobbyists, is warranted. It would be valuable for future studies to gather information across the spectrum of stakeholders, to get a sense of their knowledge and experiences with PIJAC's moratorium, the US import prohibitions, and the recent Canadian importation restrictions. It is important to capture such variation when making conclusions on the impacts of these moratoriums and prohibitions.

Even though this study was intended to test for *Bsal*, opportunistic testing of captive, pet store amphibians for *Bd* was also possible. As a result of this testing, we found that *Bd* was detected in one of 15 swabbed anurans. In British Columbia, chytridiomycosis caused by *Bd* is a reportable disease. Unfortunately, the infected anuran had been sold before analyses of the samples were completed; therefore, a second test and proceeding quarantine measures were not taken. The detection of *Bd* on this individual indicates that *Bd* of unknown and potentially virulent strains could be transferred by the pet trade into British Columbia. Introduced American Bullfrogs swabbed in the wild, have been found to be carriers of strains of *Bd* on southern Vancouver Island (Leblanc 2012). It is possible that if other non-native anurans are introduced, they could also be carriers of strains of non-native chytrid fungi.

*Bsal* was not detected in this study on swabbed captive anurans. Recently however, Iwanowicz et al. (2017) reported laboratory detections of *Bsal* from a Rough-skinned Newt and American Bullfrog (*Lithobates catesbeianus*) in Oregon, USA, but the result could not be confirmed with additional testing, which supported either a contaminated sample or detection of a *Bsal* -like organism. *Bsal* infection of an anuran (Midwife Toad, *Alytes obstetricans*) has recently been reported in Europe (Stegen et al. 2017), raising concerns for anuran reservoirs transmitting the pathogen. These findings support the need for further *Bsal* research and monitoring, especially in the northwest of North America where potentially susceptible species occur.

Based on the findings from this study and recent studies, future testing of pet-traded salamanders and anurans is recommended to determine the likelihood of them acting as vectors for non-native chytrid fungi (*Bsal* and *Bd*) into British Columbia. It is further suggested, that future studies indicate the *Bsal* or *Bd* pathogen load of infected amphibians and the specific strain of infection.

Workable approaches for regulating imports and testing protocols for *Bsal* and other amphibian pathogens are required to prevent their introduction through the pet trade into wild populations and requires collaboration by researchers, policy makers, and pet trade industry. Along with swabbing of wild and captive amphibians, we recommend exploring

TABLE 1. *Batrachochytrium salamandrivorans* (*Bsal*) sampling locations and dates in British Columbia, Canada. Wild Rough-Skinned Newts (*Taricha granulosa*) were sampled for *Bsal* testing on southern Vancouver Island. Sampling was conducted at three pet stores of southern Vancouver Island and the Lower Mainland where various species of captive salamanders and anurans were tested for *Bsal* and *B. dendrobatidis* (*Bd*). *Bsal* was not detected, and *Bd* was detected only from the sample of the Ornate Pacman Frog at Pet Store 1.

| Location | Coordinates | Species common name | Species scientific name | Date swabbed | Number of individuals | Life stage | Result *Bsal* | Result *Bd* |
|---|---|---|---|---|---|---|---|---|
| Beaver Lake Rd, Central Saanich | 48.5074°, -123.4044° | Rough-skinned Newt | *Taricha granulosa* | 06-May-16 | 2 | Adult | Not Detected | N/A |
| Beaver Lake Rd, Central Saanich | 48.5076°, -123.4046° | Rough-skinned Newt | *Taricha granulosa* | 08-May-16 | 3 | Adult | Not Detected | N/A |
| Lizard Lake, Port Renfrew | 48.6070°, -124.2250° | Rough-skinned Newt | *Taricha granulosa* | 31-May-16 | 7 | Adult | Not Detected | N/A |
| Lizard Lake, Port Renfrew | 48.6070°, -124.2250° | Rough-skinned Newt | *Taricha granulosa* | 01-Jun-16 | 8 | Adult | Not Detected | N/A |
| Fairy Lake, Port Renfrew | 48.5850°, -124.3499° | Rough-skinned Newt | *Taricha granulosa* | 02-Jun-16 | 3 | Adult | Not Detected | N/A |
| Lens Creek Rd, Port Renfrew | 48.5973°, -124.2288° | Rough-skinned Newt | *Taricha granulosa* | 02-Jun-16 | 14 | Adult | Not Detected | N/A |
| Fairy Lake, Port Renfrew | 48.5839°, -124.3500° | Rough-skinned Newt | *Taricha granulosa* | 03-Jun-16 | 3 | Adult | Not Detected | N/A |
| Beaver Lake Rd, Central Saanich | 48.5074°, -123.4044° | Rough-skinned Newt | *Taricha granulosa* | 17-Jun-16 | 4 | Adult | Not Detected | N/A |
| Island View Beach, Central Saanich | 48.5720°, -123.4002° | Rough-skinned Newt | *Taricha granulosa* | 29-Jun-16 | 1 | Adult | Not Detected | N/A |
| Arbutus Rd, Victoria | 48.4701°, -123.2984° | Rough-skinned Newt | *Taricha granulosa* | 30-Jun-16 | 1 | Adult | Not Detected | N/A |
| Morrell Lake Nature Sanctuary, Nanaimo | 49.1526°, -123.9879° | Rough-skinned Newt | *Taricha granulosa* | 28 & 29-Jun-16 & 2-July-16 | 25 | Adult | Not Detected | N/A |
| Royal Roads, Victoria | 48.2372°, -123.7372° | Rough-skinned Newt | *Taricha granulosa* | 14-Jul-16 | 2 | Adult | Not Detected | N/A |
| Royal Roads, Victoria | 48.4312°, -123.4799° | Rough-skinned Newt | *Taricha granulosa* | 14-Jul-16 | 8 | Adult | Not Detected | N/A |
| Philips Rd, Sooke | 48.4055°, -123.7171° | Rough-skinned Newt | *Taricha granulosa* | 29-Jul-16 | 1 | Adult | Not Detected | N/A |
| Pet Store 1, Victoria BC | N/A | Axolotl | *Ambystoma mexicanum* | 24-Jun-16 | 1 | Adult | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Chinese Fire-bellied Newt | *Cynops orientalis* | 24-Jun-16 | 1 | Adult | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Oriental Fire-bellied Toad | *Bombina orientalis* | 24-Jun-16 | 1 | Adult | Not Detected | Not Detected |

TABLE 1. Continued.

| Location | Coordinates | Species common name | Species scientific name | Date swabbed | Number of individuals | Life stage | Result *Bsal* | Result *Bd* |
|---|---|---|---|---|---|---|---|---|
| Pet Store 1, Victoria BC | N/A | Azoreus Blue Poison Dart Frog | *Dendrobates tinctorius* | 08-Jul-16 | 3 | Juvenile | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Oriental Fire-bellied Toad | *Bombina orientalis* | 08-Jul-16 | 5 | Adult | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Green Tree Frog | *Hyla cinerea* | 08-Jul-16 | 2 | Adult | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Chinese Fire-bellied Newt | *Cynops orientalis* | 24-Aug-16 | 12 | Adult | Not Detected | Not Detected |
| Pet Store 1, Victoria BC | N/A | Ornate Pacman Frog | *Ceratophrys ornata* | 23-Aug-16 | 1 | Adult | Not Detected | Detected |
| Pet Store 1, Victoria BC | N/A | Amazon Milk Frog | *Trachycephalus resinifictrix* | 23-Aug-16 | 1 | Adult | Not Detected | Not Detected |
| Pet Store 2, Gibsons BC | N/A | Oriental Fire-bellied Toad | *Bombina orientalis* | 19-Aug-16 | 2 | Adult | Not Detected | Not Detected |
| Pet Store 3, North Vancouver BC | N/A | Barred Tiger Salamander | *Ambystoma mavortium* | 07-Sep-16 | 1 | Adult | Not Detected | Not Detected |

eDNA sampling of amphibian pathogens from water samples (Kirshtein et al. 2007; Chestnut et al. 2014; Iwanowicz et al. 2017) as this technique would potentially allow for increased sampling efficiency in pet-store shipments and across large areas in the field. The importance of developing effective eDNA protocols for *Bsal* and other amphibian disease organisms is thus important in moving forward. We also see strategic monitoring of populations of Rough-skinned Newts and other amphibian species deemed susceptible to *Bsal* across their range as a priority to ensure that problems are detected early on, when preventative measures are still possible.

*Acknowledgments.*—This study was supported by funding from the British Columbia Ministry of Environment and from Habitat Acquisition Trust (Victoria, British Columbia) through an anonymous donation. A "Fish and Wildlife Management Branch Animal Care Application" and a British Columbia "Wildlife Act" General Permit (MRNA16-231158) were approved prior to undertaking this study.

## LITERATURE CITED

BLOOI, M., F. PASMANS, J. E. LONGCORE, A. SPITZEN-VAN DER SLUIJS, F. VERCAMMEN, AND A. MARTELL. 2013. Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. J. Clin. Microbiol. 12:4173–4177.

BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. MORGAN, AND A. D. HYATT. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples usingreal-time Taqman PCR assay. Dis. Aquat. Org. 2:141–148.

BRIGGS NIH RESEARCH GROUP. 2009. Chytrid swab protocol. AmphibiaWeb. http://amphibiaweb.org/chytrid/swab_protocol.html [Accessed May 2016].

BSAL TASK FORCE. 2016. Bsal Task Force Annual Report. Salamanderfungus.org. [Accessed May 2016].

CHESTNUT, T., C. ANDERSON, R. POPA, A. R. BLAUSTEIN, M. VOYTEK, D. H. OLSON, AND J. KIRSHTEIN. 2014. Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. PLOS ONE 9(9): e106790.

ECOSYSTEMS BRANCH, BRITISH COLUMBIA MINISTRY OF ENVIRONMENT. 2008. Hygiene protocol for field staff working in aquatic environments. http://www.cwhc-rcsf.ca/docs/miscellaneous/BC-Disinfection-Protocol-AquaticFieldResearchers-2008.pdf [Accessed May 2016].

ENVIRONMENT AND CLIMATE CHANGE CANADA. 2017. Environment and Climate Change Canada (ECCC)'s import restrictions on salamanders: Customs Notice 17-17, Ottawa, May 26, 2017. Canada Border Services Agency, Canada. http://cbsa-asfc.gc.ca/publications/cn-ad/cn17-17-eng.html [Accessed 12 July 2017].

GRAY, M. J., J. P. LEWIS, P. NANJAPPA, B. KLOCKE, F. PASMANS, A. MARTEL, C. STEPHEN, G. PARRA OLEA, S. A. SMITH, A. SACERDOTE-VELAT, M. CHRISTMAN, J. WILLIAMS, AND D. H. OLSON. 2015. *Batrachochytrium salamandrivorans*: the North American response and a call for action. PLoS Pathogens 11(12):e1005251.

IWANOWICZ, D. D., W. B. SCHILL, D. H. OLSON, M. J. ADAMS, C. DENSMORE, R. S. CORNMAN, C. ADAMS, C. FIGIEL, JR., C. W. ANDERSON, A. R. BLAUSTEIN, AND T. CHESTNUT. 2017. Potential concerns with analytical methods used for the detection of *Batrachochytrium salamandrivorans* from archived DNA of amphibian swab samples, Oregon, USA. Herpetol. Rev. 48:352–355.

KIRSHTEIN, J. D., C. W. ANDERSON, J. S. WOOD., J. E. LONGCORE., AND M. A. VOYTEK. 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Dis. Aquat. Org. 77:11–15.

LEBLANC, J. 2012. *Batrachochytrium dendrobatidis*, the causal agent of an emerging infectious disease. Ph.D Thesis, University of Victoria, Victoria, British Columbia, Canada.

MARTEL, A., M. BLOOI, C. ADRIAENSEN, P. V. ROOIJ, W. BEUKEMA, M. C. FISHER, R. A. FARRER, B. R. SCHMIDT, U. TOBLER, K. GOKA, K. R. LIPS, C. MULETZ, K. R. ZAMUDIO, J. BOSCH, S. LOTTERS, E. WOMBWELL, T. W. J. GARNER, A. A. CUNNINGHAM, A. SPITZEN-VAN DER SLUIJS, S. SALVIDIO, R. DUCATELLE, K. NISHIKAWA, T. T. NGUYEN, J. E. KOLBY, L. VAN BOCXLAER, F. BOSSUYT, AND F. PASMANS. 2014. Recent introduction of a chytrid fungus endangers western Palearctic salamanders. Science 346(6209):630–631.

———, A. SPITZEN-VAN DER SLUIJS, M. BLOOI, W. BERT, R. DUCATELLE, M.C. FISHER, A. WOELTJES, W. BOSMAN, K. CHIERS, F. BOSSUYT, AND F. PASMANS. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Nat. Acad. Sci. USA 110(38):15325–15329.

PIJAC. 2015. Immediate Temporary Moratorium Recommended on Importation of Firebelly and Paddletail Newts. Pet Industry Joint Advisory Council. http://pijaccanada.blogspot.ca/2015/12/moratorium-recommended-on-importation.html [Accessed May 2016].

SABINO-PINTO, J., M. BLETZ, R. HENDRIX, R. G. BINA PERL, A. MARTEL, F. PASMANS, S. LÖTTERS, F. MUTSCHMANN, D. S. SCHMELLER, B. R. SCHMIDT, M. VEITH, N. WAGNER, M. VENCES, AND S. STEINFARTZ. 2015. First detection of the emerging fungal pathogen *Batrachochytrium salamandrivorans* in Germany. Amphibia-Reptila 36(4):411–416.

SKERRATT, L. F., L. BERGER, R. SPEARE, S. CASHINS, K. R. MACDONALD, A. D. PHILLOTT, H. B HINES, AND N. KENYON. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

SPITZEN-VAN DER SLUIJS, A. M., F. SPIKMANS, W. BOSMAN, M. MARNIX DE ZEEUW, T. VAN DER MEIJ, E. GOVERSE, F. KIK, M. PASMANS, AND A. MARTEL. 2013. Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. Amphibia-Reptila 34(2):233–239.

STEGEN, G., F. PASMANS, B. R. SCHMIDT, L. O. ROUFFAER, S. VAN PRAET, M. SCHAUB, S. CANESSA, A. LAUDELOUT, T. KINET, C. ADRIAENSEN, F. HAESEBROUCK, W. BERT, F. BOSSUYT, AND A. MARTEL. 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature 544:353–356.

STEPHEN, C., M. J. FORZAN, T. REDFORD, AND M. ZIMMER. 2015. *Batrachochytrium salamandrivorans*. Threat Assessmentt of Salamander Chytrid Disease. Technical report, Canadian Wildlife Health Cooperative. 30 pp. http://www.cwhc-csf.ca/technical_reports.php [Accessed May 2016].

US FISH AND WILDLIFE SERVICE. 2016. Salamander Species Listed as Injurious Wildlife Under 50 CFR 16.14 Due to Risk of Salamander Chytrid Fungus, Effective January 28, 2016. https://www.fws.gov/injuriouswildlife/pdf_files/List-of-Salamander-Species.pdf; [Accessed May 2016].

YAP, T. A., M. S. KOO, R. F. AMBROSE, D. B. WAKE, AND V. T. VREDENBURG. 2015. Averting a North American biodiversity crisis. Science 349:481–482.