AMPHIBIAN AND REPTILE DISEASES

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Investigating Amphibian and Reptile Mortalities: A Practical Guide For Wildlife Professionals

In the past 25 years, pathogens (e.g., *Batrachochytrium dendrobatids* [*Bd*], *B. salamandrivorans* [*Bsal*], *Ophidiomyces ophiodiicola*, and ranaviruses) have emerged in amphibian and reptile populations at varying spatial scales (Martel et al. 2013; Jacobson et al. 2014; Allender et al. 2015; Duffus et al. 2015). Over 2000 amphibian species have experienced population declines (Stuart et al. 2004) and over 100 are considered to be extinct as a result of chytridiomycosis (Barnosky et al. 2011). Populations of fire salamanders in the Netherlands have declined by 96% in a three-year time span, presumably due to direct mortality as a result of *Bsal* infection (Martel et al. 2013). Also, in Belgium, populations of fire salamanders have experienced similar large-scale declines associated with the emergence of *Bsal* (Stegen et al. 2017). Some rattlesnake species and populations have declined by 40% in some areas of the USA in association with the emergence of snake fungal disease (Clark et al. 2011; Allender et al. 2015). These discoveries have increased awareness within the herpetological community of the importance of investigating mortality events, ongoing population and pathogen monitoring, and research directed at better understanding disease dynamics in amphibians and reptiles. Furthermore, formal training in wildlife health and disease monitoring is not available at all institutions that train field biologists and variable levels of experience are common among wildlife professionals. Herein, we offer a basic guide for investigating mortality events involving amphibians and reptiles.

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Evaluating and Documenting the Site

When any mortality event of uncertain origin is discovered, the first steps include notifying the landowner, obtaining permission to enter the area, and to ensuring that the area is safe for further investigation. Also, appropriate state and conservation

Table 1. List of equipment to have on hand to sample a disease or die-off event. This kit can easily be put together in a plastic tool box or plastic container. *Instruments should ideally be metal to ensure they can be sanitized between uses. Disposable plastic instruments are available, but are not as robust and are not easily disinfected. **The container should be large enough to serve as a makeshift work surface.

Equipment	Number or concentration
Disinfectant (one of the following)	
– bleach	4% Solution
– Novalsan	1% Solution
– Virkon (powder)	1g/L
Disposable gloves	1 box
Ziplock or Whirl-Pak bags	100
1.5 or 2.0mL tubes	~150
70–100% ethanol	500mL
Sterile or distilled water	500mL
Scalpel (with multiple blades)*	2
Forceps*	2
Sharp scissors*	2
Ruler	1
Note pad	1
Pencil or ethanol-proof pen	1
Small container**	1
Small cutting board (plastic)	
Paper towels	1 roll
Lighter	1
Dry swabs with protective cover (individually wrapped swabs)	50
Garbage bags	3
Sharps disposal bin	1
Storage containers (e.g., 50mL tubes, 20mL scintillation vials)	25

TABLE 2. Recommendations for tissue sample collection from an animal for histology, bacteriology, and virology.

1) Sanitize your instruments by dipping them into 70–100% ethanol. If they are metal instruments, you can flame them (preferable) or rinse them in distilled or sterile water.

2) Carefully remove the entire lesion in a manner that leaves 2–3 mm of healthy tissue around the edges. Gently handle the sample only by the edges with forceps.

3) Cut the sample in half, preserve one half for histopathology and divide the other half for potential bacterial and/or virological testing.

   a) To fix the tissue sample properly for histopathology, it should be in 10x its volume of 10% buffered formalin and be left in the solution for at least 24 h before it is processed (trimmed).

   b) If buffered formalin is not on hand, samples can be preserved in 70–100% ethanol for 24–48 h. Samples should be transferred to 10% buffered formalin prior to histopathology processing or further storage.

4) Ensure that samples are properly labeled so that the geographic location, tissue of origin, and source animal can easily be identified.

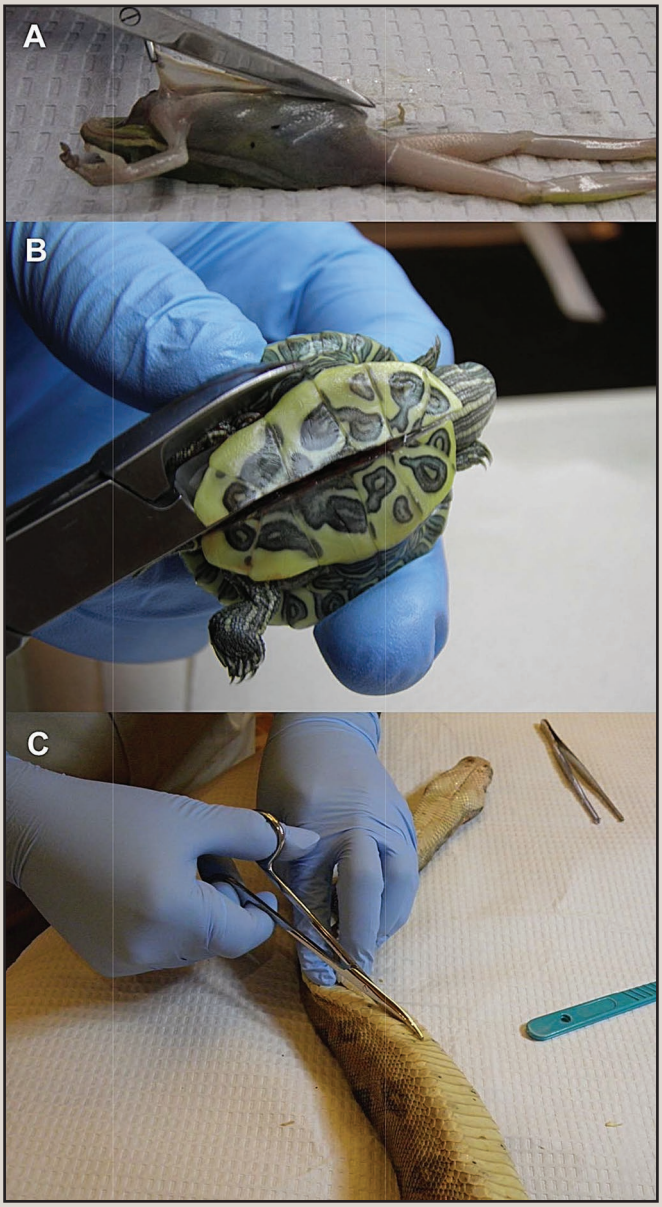

FIG. 1. Amphibian and reptile carcasses may be opened along the ventral midline. For larger turtles, the plastron will need to be removed and it is often easiest to remove it at the hinge area (for example by using a rongeur as in B).

agencies should be notified prior to sample collection. Instances when it may not be safe to immediately investigate may include if there is a suspicious odor (this may be indicative of an environmental toxin) or if there is physical evidence of imminent danger, such as a downed power line. In some cases, there may be illegal or private activities occurring on the lands, which result in a dangerous situation for trespassers. In these instances, it may be best not to enter the area, but rather seek help from appropriate sources, such as the local Public or Environmental Health Department or the relevant law enforcement agencies. Once it is deemed safe to enter the area and permissions are received (please note that permits for collection may also be required), begin to record the scene with photographic and written documentation, which should be done before anything is disturbed. Detailed records can assist with future epidemiological investigations of disease dynamics. Recording recent weather events and specific locations (e.g., with GPS or UTM coordinates) can assist with future research projects. Record all species involved and if possible, estimate the number of individuals of each species that are affected. Take many pictures of affected animals both *in situ* and at different angles. Suggested contents for a Mortality Event Response Kit are listed in Table 1. Personnel that can be helpful with recommendations for investigations include the state, territorial, or provincial agricultural and wildlife veterinarians, as well as wildlife health professionals from universities and diagnostic laboratories or the land jurisdiction where the event has occurred (e.g., federally administered land). Establishing working relationships with stakeholders ahead of mortalities can assist with communication and response when mortalities are observed. The amphibian pathogens, *Bd* and ranavirus, are listed as notifiable to the world organization for animal health (OIE) and diagnosis is required to be reported to OIE by many nations. For example, in the United States the Department of Agriculture reports to the OIE on an annual basis. For more information on notifiable diseases see the Aquatic Animal Health code at http://www.oie.int/international-standard-setting/aquatic-code/.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

The importance of PPE for wildlife mortality investigations include protection of people from zoonotic diseases (e.g., *Salmonella* spp.) while maximizing the information provided from the diagnostic laboratory (i.e., reducing the likelihood of contamination of the samples), and limiting the spread of pathogens to other animals. Gloves should be worn when investigating

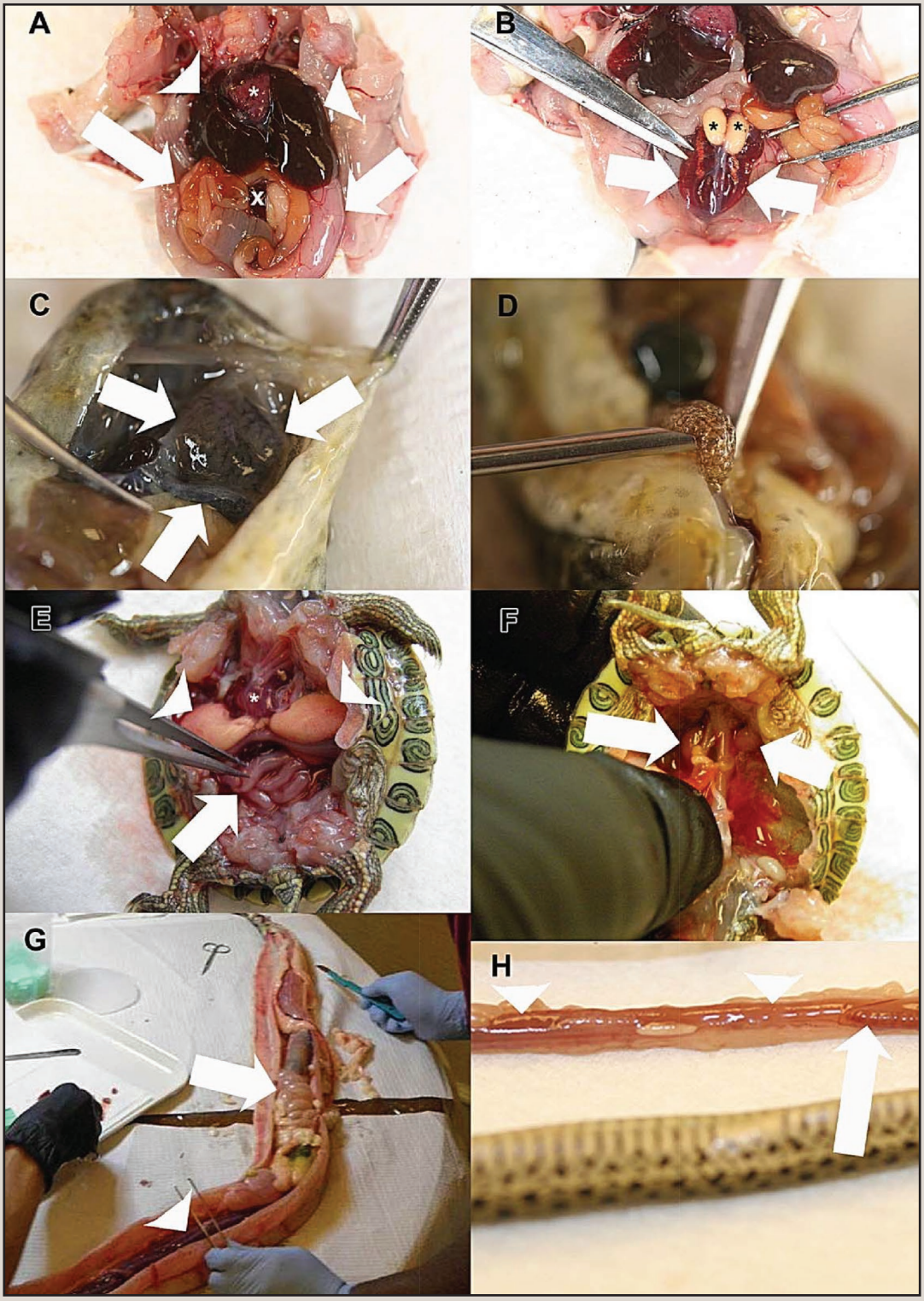

FIG. 2. Examples of normal anatomy in various species. A and B showing the heart (asterisk in A), liver (arrowheads), spleen (x), intestines (arrows in A), kidneys (arrows in B; often oval-shaped and along the vertebrae in amphibians), and testes (asterisks in B) of an anuran adult. C and D: Larvae will often have both gills (arrows in C; head of a tadpole opened along the midventral region of the body) and developing lungs (D; a developing lung externalized from body cavity where it extends along the dorsolateral aspect). E and F showing the heart (asterisk), liver (arrowheads; often bi-lobed; may be pale tan in young animals or animals that have not been eating well), intestines (arrow in E), kidneys (arrows in F; often bean-shaped) in turtles. G and H showing the elongated assemblage of organs in snakes; intestines (arrow), liver (arrowhead; is distant from the gallbladder, which is unique compared to other taxa), lungs (in many species of snakes, only one lung lobe is fully developed), and kidneys (H), showing the somewhat linear arrangement of the kidneys with one being more cranial (arrowheads) than the other (arrow).

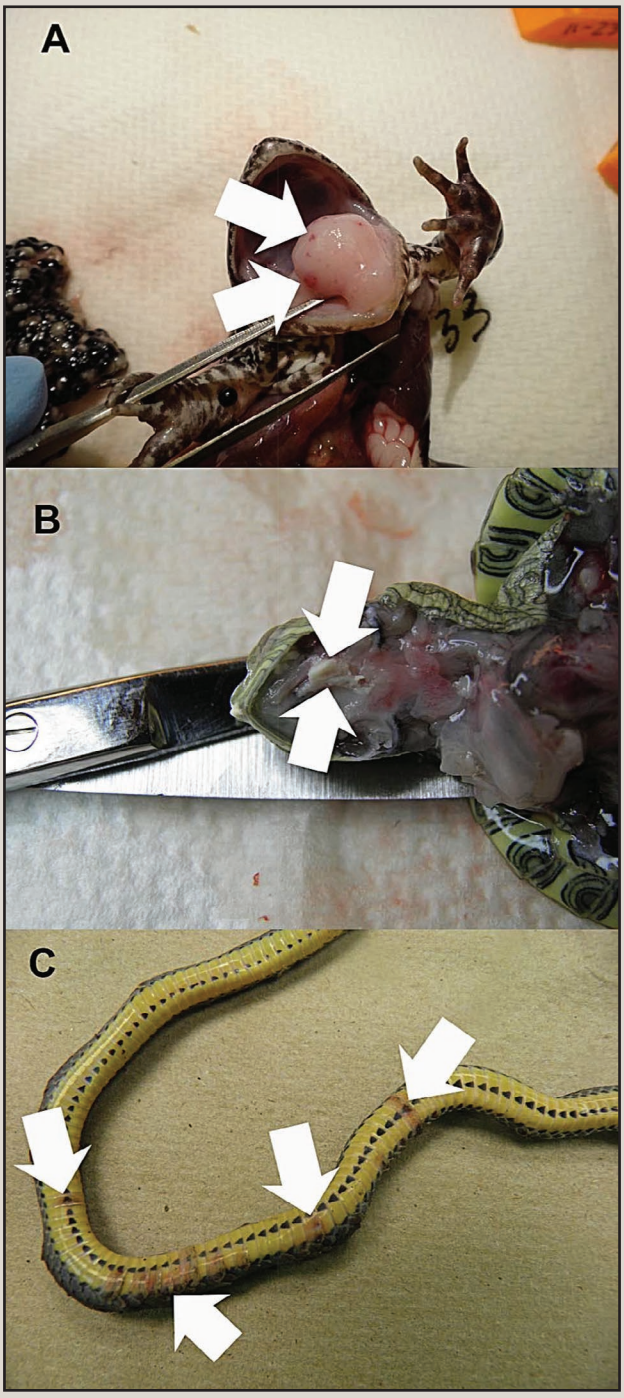

Fig. 3. Examples of lesions (arrows) that might be seen in morbidity and mortality events. A) Hemorrhages can be seen throughout the body and may vary in size and shape as in the tongue of this anuran. B) Areas of dead tissue (necrosis) often will present as tan friable plaques (as in the roof of the mouth of this turtle) or nodules and can be seen throughout the body. C) Areas of invasion by infectious agents often present as discolored areas, abscessed, or crusting (as in the skin of this snake) and can be seen throughout the body.

mortality events. Specific recommendations are available for use of gloves during amphibian handling (Cashins et al. 2008; Greer et al. 2009). If molecular testing (e.g., polymerase chain reaction) is to be performed, it is important to recognize that false-positive results can occur as a result of cross-contamination of genetic material (DNA). If the desired detection of pathogens is at the level of individual animals, then each individual needs to be handled with separate, single-use gloves, and maintained in separate clean containers (e.g., Ziploc or Whirl-Pak bag) because DNA can remain on multiuse equipment and cause contamination of samples. For example, if ten carcasses are collected and stored in the same container, the sample size of the animals potentially infected decreases from ten to one if only molecular testing is used. Additional recommendations for PPE use specific to field biologists and zoonotic pathogens are available (e.g., https://www.nps.gov/public_health/info/di/Field%20Guide%20NPS%20biologists.pdf) and these may vary with the geographic location and specific pathogens suspected.

Sample Collection

Once the area is photographed, samples can be collected. Samples might include water, soil, carcasses, or tissue samples from live animals (e.g., blood, swabs), depending on the specific objectives of the investigation. When collecting animals, always remember that "fresh is best." Collection of a subset of the freshest carcasses may yield the highest quality results from the diagnostic laboratory. Euthanasia of moribund animals could be considered with euthanasia guidelines available from the American Veterinary Association (https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) or an institutional or agency-specific animal care committee. Members of the American Association of Amphibian and Reptile Veterinarians can be contacted for assistance with humane methods for euthanasia of amphibians and reptiles, particularly in remote locations (http://arav.org). As a rough guideline for events involving numerous animals, 5–10 animals (preferably of each species) should be collected and shipped overnight on ice packs to a diagnostic laboratory for full necropsy; however, it is best to contact the laboratory to find out how many animals are preferred for diagnostic purposes. Dry ice is generally not necessary to submit carcasses or samples to a laboratory and wet (i.e., cubed) ice should be avoided at all costs as it melts in transit and can damage the quality of the specimen. It is also recommended to freeze a small number (e.g., 3–5) of carcasses, if available, for future investigation. If animals are < 10 g, a subset of whole carcasses can be placed in a fixative (e.g., tadpoles, small frogs, small snakes, hatchling turtles; or sections of tissues for large animals, see post-mortem examination below), such as 10% neutral buffered formalin or 70–90% ethanol. For carcasses that are deemed too large to ship or if shipping overnight is not an option (e.g., due to physical location), a necropsy can be performed in the field or at a specified field site and tissue samples collected. Photographs of the necropsies can be helpful for diagnosticians assisting with the investigation.

Post-Mortem Examination

Examination of a dead animal with collection of tissues for testing (i.e., post-mortem investigation or necropsy) can be performed by a field biologist, and is an option when it is not possible to ship animals to a laboratory within 24 h of death or if larger animals are involved. Regardless of the species, there are general guidelines to follow for all post-mortem investigations. It is important to maintain proper biosafety and biosecurity features, such as the use of disposable gloves, disinfection of equipment between animals, and maintenance of detailed

photographic and written records. It is best to use one set of instruments for opening the body cavity and one for internal organs as a number of commensal microorganisms present on the skin surface can interfere with certain ancillary test results; however, if this is not possible, instruments can be sterilized between opening the body cavity and dissecting internal tissues. Most necropsies can be performed with scissors, forceps, and a disposable scalpel; however, larger animals may require larger, sharp knives, kitchen or poultry shears, pruning shears or reciprocating or Stryker saws (e.g., for removal of the plastron in turtles). Finally, dissecting scopes or magnifying lamps can assist with necropsy of very small specimens, such as tadpoles.

To begin the necropsy, first weigh and measure the animal, and examine the exterior of the animal for any ectoparasites, abrasions, hemorrhage, swellings, or other abnormalities. Open the mouth and carefully examine the oral cavity. If there is any discharge from the nose, mouth and/or cloaca, these regions should be swabbed for microbial (e.g., bacteria, viruses) culture and molecular testing. This can be done by gently rolling the swab between your thumb and index finger (of a gloved hand) so that the discharge covers the surface of the swab. The swab should then be placed into a dry tube (for molecular testing) or the appropriate medium (for microbial culture) without touching any other surface. If swabs are not available, the discharge can be collected into a clean plastic, sealable bag or small sealable tube and frozen. Whenever possible, lesions (defined as abnormalities in the tissue; both external and internal) should be collected for histological (microscopic) evaluation by a trained professional to attempt to morphologically identify etiologic (causative) agents and determine the pathological process (e.g., inflammation vs neoplasia; bacterial vs viral vs parasitic). Samples for histological evaluations are best limited to less than 1-cm thickness and are placed in 10% neutral buffered formalin (or 70–90% ethanol) at a ratio of 10–20 parts fixative: 1 part tissue. See Table 2 for the recommended procedure for sample collection.

After the external evaluation is completed, the body cavity is opened by making an incision along the ventral midline from the oral cavity to the anus (Fig. 1). For turtles, the plastron will need to be removed. This is accomplished most easily by first separating the plastron from the carapace at the "hinges" (i.e., where the plastron meets the carapace) using a coping or hack saw or if available a reciprocating saw, and then carefully dissecting it away from the underlying soft tissues. Once the body cavity is exposed, any free fluid within the body cavity should be noted and the quantity, color, and consistency (e.g., liquid, stringy, gelatinous) should be recorded. Once any fluid is removed and the body wall fully reflected (i.e., cut edges turned outward to expose the body cavity), the tissues within the coelomic cavity can be examined. It is recommended that examiners familiarize themselves with normal amphibian and reptile anatomy prior to performing necropsy examinations to be able to recognize abnormalities. Various anatomic charts are available (e.g., Whitaker and Wright 2001; Drivers and Mader 2005). Small organs, such as adrenal glands, thyroid glands, and in some cases spleen (e.g., in tadpoles) and gonads (e.g., in young animals) can be very difficult to locate once organs are removed. To locate them, gently reflect the more superficial organs to view deeper organs (Fig. 2). Keep in mind that organs might be in slightly different locations or look different in different species. For example, snakes are long and narrow, thus their organs are often long and narrow, and paired organs (e.g., kidneys, gonads) might be placed such that one lies much more cranial (i.e., towards the head) than the other (Fig. 2). Examine all organs for lesions, including muscles and bone. Once all organs are located, you can begin to collect samples. Samples of all lesions should be collected fresh (i.e., kept cool, not frozen or fixed) for microbial testing (typically for culture), frozen for molecular testing, and fixed in ethanol or formalin for histology (microscopic examination; Table 2). If lesions are too few or small to collect for all three storage methods, it is often best to fix them, as histology and molecular testing are

FIG. 4. Examples of packaging samples for shipment. Photos A and B show proper packaging using plastic containers with screw top lids and shipping within a styrofoam cooler in a tertiary cardboard box. Contents of the package are included in a packing slip. Absorbent material (paper towel) and cushioning have been added. Photos C and D show types of packaging that should be avoided. Glass jars with lightly twisted lids can easily break or leak in shipment. All shipments should have three layers of packaging with absorbent material to handle any leakage and provide cushioning. Contents should be labeled as per the requirements of the commercial carrier and at minimum labeled as diagnostic specimens. A packing slip with a list of all samples included in the shipment should be included and it is best to place it in a sealable plastic bag in case of any leakage. Photo E shows an appropriate biohazard label for a Category B biological specimen, which includes all diagnostic specimens.

TABLE 3. Organ Sampling Checklist (adapted from Pessier and Pinkerton 2003).

Organ samples to be collected
Skin
Skeletal muscle
Lung/gill
Liver
Stomach
Spleen
Kidney
Ovary/testis
Urinary bladder
Toe (optional for *Bd* screening/histology)
Tadpole mouth parts (optional for *Bd* screening/histology)
Brain/skull/eye
Oral cavity (tongue and any lesions)
Samples from ALL lesions

possible in some situations. Ethanol interferes less with molecular testing than formalin. In general, fixation tends to decrease the reliability of molecular tests, as a result of denaturation of DNA, but technological advances have improved our abilities to detect genetic material from tissues that were previously fixed in formalin and embedded in paraffin wax. Importantly, if a delay of > 48 h prior to receipt by the diagnostic laboratory is anticipated, the tissues or carcasses should be frozen. It is always best to check with a diagnostic laboratory regarding type of sample and storage prior to collection. Contact information for USA diagnostic laboratories with experience dealing with reptiles and amphibians can be found at www.salamanderfungus.org/resources/labs. Recommended samples to collect for histological evaluation are summarized in Table 3. It is important to remember that some changes occur microscopically that cannot be appreciated by the naked eye. The diagnostician receiving the samples will be able to synthesize the most information if a comprehensive list of the included tissue samples is provided. A good rule-of-thumb is to first collect the small organs (e.g., adrenal glands, immature or inactive gonads, spleen) that may be more difficult to locate prior to proceeding with sampling for the remaining organs in a methodical fashion (e.g., starting at the anterior end of the animal and moving caudally). To avoid fecal contamination of samples that could interfere with some diagnostic tests, it is best to process the gastrointestinal tract last. Most organs have a consistent color throughout, if they are mottled with multiple colors (e.g., red and tan), blotchy (e.g., large irregular red areas) or spotted (e.g., red pinpoint areas or white spots), this may suggest that they are abnormal (Fig. 3). It is important to remember that autolysis and prior freezing can alter the coloration of tissues. Document, by photography and written descriptions, of anything that appears abnormal. Gently remove organs as needed, leaving the gastrointestinal tract for last, as previously noted. Prior to removal, the gastrointestinal tract may be reflected for viewing and removing underlying urinary and reproductive tracts and adrenal glands.

The lungs can be difficult to find in some animals if they are deflated. You can locate them by gently probing along both sides of the heart. In snakes, the lungs can be especially difficult to find, as they can be translucent. Furthermore, often only the right lung is well developed in snakes, with the left lung ranging from nearly as developed as the right (e.g., boids) to being nearly absent (e.g., colubrids). In snakes, the lungs may start cranial to the heart (e.g., most venomous species), but generally extend caudally to the cranial edge of the liver. Snake lungs extend caudally (i.e., towards the tail) as air sacs. In amphibians, the lungs are often pigmented and may extend very far caudally (e.g., to the kidneys) or even fill the body cavity when fully expanded. Be aware that some species (e.g., members of Plethodontidae) do not have lungs and instead may rely on gills for oxygen exchange. Sampling of gills both fresh (i.e., not frozen) and in fixative is essential for a comprehensive diagnostic work up in these species. In turtles, the lungs are situated lateral and dorsal to the heart and can be difficult to locate when deflated. The lungs of turtles are like thin-walled sponges, in squamates and amphibians, lungs are generally thin-walled sacs with a honeycomb appearance on the internal surface. Although aquatic larval amphibians have gills, they may also have developing (primordial) lungs. In general, the gills of salamanders are external and those of anurans are internal (Fig. 2). Keep in mind that the skin is an important respiratory organ for most amphibians and should be handled delicately and be sampled appropriately. Often (especially in snakes and amphibians) the lungs contain parasites that are visible to the naked eye. Although many parasites are normal "fauna" of many wild animals and may not be associated with clinical disease, it is important to document parasitic findings. The recommended technique for collection of ecto- and endoparasites is fixation in 70% ethanol.

The kidneys can vary greatly in shape and location (Fig. 2). In some lizards and turtles, the kidneys are round to spherical and very caudally placed such that the floor of the pelvis must be removed to find them. In some chelonian and amphibian species, the kidneys are long and narrow and located dorsally along the midline. In larvae and hatchlings, the kidneys often are hard to distinguish from developing gonads and are collected as a unit. Not all species have urinary bladders and this varies widely. When present, it is generally a thin tan sac located ventral to the rectum. The urinary bladder should be opened and examined, as it may contain bladder stones (often termed calculi), which can be sampled for further analysis if present.

Organ morphology may vary by species. For example, the liver is long and narrow in snakes, but somewhat dome-shaped and slightly multilobulated in amphibians. Additionally, some turtles have a thin lobe of liver (left lobe) that overlies the stomach and another (right lobe) that is much larger and more typical of livers in other species. Similarly, the spleen may be spherical in some species, but thin and tongue-shaped in others.

Lastly, the gastrointestinal (GI) tract can be removed by transecting the esophagus and large intestine as close to the respective opening as possible. Gently cut the mesentery (thin membranous and transparent lining that holds the internal organs in place) to linearize the gastrointestinal tract. This allows for sampling of different portions of the GI tract (e.g., esophagus, stomach, duodenum, jejunum, ileum, large intestine, and cloaca). Whenever possible, open the entire length of the gastrointestinal tract to expose the contents and internal surfaces (this may not be possible with small specimens, such as tadpoles). Gastrointestinal contents can be collected and submitted for parasite evaluation if desired, but ideally should be evaluated within 24 h for optimum results. Examine the gastrointestinal tract for any regions that appear ulcerated or different from the rest of the gastrointestinal tract. Normal gastrointestinal mucosa should be white-tan,

smooth, and slightly shiny in appearance. Take representative samples of each section (e.g., tongue, esophagus, stomach if present, upper intestine, middle intestine, and caudal intestine) for histology. If possible, collect samples near landmarks (areas that are common in all animals) or in areas where lesions are suspected. For example, the cranial portion of the intestine can often be collected with pancreas. In some species, the mid intestine can be collected with spleen. This allows the pathologist to know the anatomical location of the tissue when it is examined under the microscope. Be aware that tadpoles do not have stomachs and the intestines fill most of the body cavity.

After the completion of the examination of the coelomic cavity, the brain, eyes, bone marrow, and any other structures (e.g., endolymphatic sacs, scent glands, and gills) can be collected. For small specimens, the head might be collected whole into a fixative or cut in half, with one half frozen and the other placed into fixative. For larger specimens, the skin should be removed from the top of the skull and the skull cap carefully removed with a scissors, shears, or saw (i.e., use the tool that is necessary based on the size and density of the skull). Once the brain is exposed, it can be carefully removed, examined and sectioned. It often is not necessary or is too difficult to remove eyes, and they might simply be submitted with the skull (or a portion of it).

Whenever possible, remember to collect three sets of tissues for all organs and lesions. One set in fixative for histology, one set frozen for molecular testing, and one set fresh (i.e. not frozen or fixed) for pathogen retrieval (e.g., bacterial or fungal culture, virus isolation) (Table 2). A reminder that the latter set (i.e., for pathogen retrieval) can be frozen (or eliminated) if there will be a long delay in submitting for testing. After all samples are collected, the remains of the animal can then be placed into a labeled, sealable container for storage (either frozen or in fixative). Make sure that all of the samples you have taken are individually labeled and identifiable to the individual from which they were collected. Banking tissues or carcasses from a mortality event is recommended. As new techniques are developed and new pathogens are potentially discovered, having an organized repository to go back to can be an invaluable resource to gather more data. Thus, permanent storage of at least a subset of specimens should be considered and might consist of frozen, fixed, or paraffin-embedded tissues. In general, freeze-thaw cycles should be minimized. The temperature recommended for storage may depend on available equipment, space, and anticipated ancillary testing. Fresh or flash frozen (-80°C) samples are preferred for bacterial culture or virus isolation. Molecular testing and toxicology can be performed on samples stored at -20°C.

Shipment

Consult with appropriate carriers for guidelines on shipment of biological materials. In general, diagnostic samples are considered Category B biological specimens (UN 3373) by the Department of Transportation (http://www.un3373.com/category-biological-substances/) in the USA. Samples should be appropriately packaged (e.g., double-bagged and in sealed containers). Consult with your local carrier regarding recommendations for transport of potentially hazardous materials, such as ethanol or formalin. It is recommended to provide additional seals of parafilm on containers and to place the primary container in a secondary container (e.g., sealed Ziplock bag containing absorbent material, such as paper towel) to prevent leakage of formalin onto other samples. Use a thick, hard-sided cooler for shipping as well as solid ice packs ("blue ice"). The use of "wet ice" (ice cubes or bagged ice) is strongly discouraged as the ice will melt and the samples will become wet and potentially ruined. Liquid nitrogen or dry ice is generally not necessary for shipment of diagnostic specimens. Avoid glass tubes or containers as they can easily be broken during shipment. Consultation with the diagnostic lab that you are shipping to ahead of time can assist with avoidance of problems as a result of inadequate packaging (Fig. 4). Additionally, samples often need to be shipped and submitted to the laboratory with the appropriate submission forms, which can often be obtained from the laboratory prior to shipment. The sender should confirm that the samples can be received to ensure that there are no delays in delivery that could degrade samples.

It is important to note that permits may be required to ship certain samples and the receiving laboratory may require a permit for receipt of samples.

Biosecurity

There is increasing evidence that transmission of pathogens can be facilitated by movements of humans and other anthropogenic-related factors (Eskew and Todd 2013). Unintentional anthropogenic transmission of pathogens in this manner should be avoided due to unknown conservation implications of the introduction of a pathogen into a susceptible population (e.g., white-nose syndrome in bats; Lorch et al. 2016). Any instruments and surfaces used for mortality investigation, as well as, waders, boots, and other equipment should be thoroughly disinfected between sites. Recommendations for disinfection of field materials can be found at http://www.separc.org/products/diseases-and-parasites-of-herpetofauna. Use of site-specific materials and an appropriate facility for mortality investigations with easily disinfected surfaces should be considered. Surfaces also need to be cleaned (with soap and water) and organic material removed prior to disinfection, as many disinfectants (e.g., bleach) will not work in the presence of organic matter. Disinfectants require adequate contact time for optimum effectiveness that is often pathogen-specific. Consideration of aquatic portions of the life cycle of specific pathogens (e.g., chytrid fungi) is necessary as they may remain viable and can be transferred to potentially naive sites in water. Recommendations for water treatment, quarantines, treatment of animals in captivity, and recommendations for translocations can be found at: http://www.amphibianark.org/pdf/Amphibian_Disease_Manual.pdf.

Conclusion

As novel pathogens emerge and amphibian and reptile populations decline, it is becoming increasingly important to conduct methodical investigations of mortality events to tease out potential causative relationships between disease and population declines. Additionally, maintaining repositories of tissues and specimens (e.g., biobanks) by wildlife health groups and natural history collections can be invaluable in retrospective analyses when novel pathogens are discovered. The information provided from archived samples can provide a timeline and geographical map for emergence and spread of a particular pathogen (e.g., Martel et al. 2014). This information is essential for evidence-based decisions by wildlife managers for potential mitigation of the negative impacts of diseases on populations. Use of appropriate biosafety and biosecurity procedures is also necessary for prevention of zoonotic diseases and prevention of anthropogenic pathogen spread.

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