

Amphibian Mortality Information Sheet

This sheet has been produced (for non-pathologists) by the Declining Amphibian Populations Task Force (DAPTF) to provide simple guidelines for dealing with dead and diseased amphibians that are found during a suspected disease outbreak or amphibian mortality event.

Introduction

The optimum specimen for diagnostic tests is a still-living, diseased amphibian. Dead specimens are less informative because of rapid decomposition, but may still provide some information. Generally, it is desirable to submit normal animals together with sick specimens in order to provide a comparative control. Amphibians and fish of sympatric species may also be useful, and these should be collected where possible along with sick and "normal" animals of affected species.

Sources of Diagnostic Assistance

In many countries, diagnostic laboratories are located within a veterinary college or university animal sciences department. Commercial laboratories, chemical and pharmaceutical companies, and governmental research facilities may also have staff veterinary pathologists. Most veterinarians will know the location of their nearest diagnostic laboratory.

Collecting from an Amphibian Casualty Site

In order to minimize any potential spreading of disease to other areas, it is important that the guidelines on working with amphibians produced by the DAPTF (see the DAPTF Fieldwork Code of Practice) are followed whenever investigating, or collecting from, an amphibian casualty site.

Sick and dead amphibians should be collected immediately and submitted to a diagnostic laboratory as soon as possible. Before submitting ANY specimens, it is crucial to identify and consult a suitable laboratory which is willing to receive them. Bear in mind, too, that pathological investigation can be expensive, and a source of funding may be needed in order to cover sufficient testing for any worthwhile result. Live specimens should be delivered in person or sent by express mail or courier. Dead amphibians should be divided into two groups, one group should be promptly frozen (ideally to -70°C ; -94.0°F), but the temperature of a normal household freezer (-20°C ; -4.0°F) is sufficient for most diagnostic investigations) and the second group should be placed in a fixative solution (e.g. 70% ethanol or neutral buffered 10% formalin). The coelomic cavity must be opened (via a single midline cut through the skin and muscle of the ventral body wall) before carcasses are placed in fixative. Also, to ensure thorough fixation, specimens should be immersed in approximately 20 times the volume of fixative to tissue volume. If a suitable diagnostic laboratory cannot be located at the time of the incident, representative samples of carcasses should be saved fixed and frozen (as above) until such a laboratory can be found. If unknown or cryptic species are collected, a number of

specimens should be retained for species identification and preserved by both the above methods.

Many mass mortality events occur quickly, and sufficient specimens for pathological analysis should be collected immediately, as carcasses or sick animals might not be found on a return visit to the site. Submission of as many life stages (egg, tadpole, metamorph, adult) as possible is desirable, along with control specimens such as apparently healthy animals of the affected species and any sympatric amphibian or fish species. Again, check that the laboratory is willing to receive apparently "normal" specimens. It may also be illegal to submit live or control animals for investigation without the specific request of a veterinarian or appropriate licenses.

Sample Size

The number of submitted animals may be the limiting factor in determining which diagnostic tests can be performed. Many amphibians are very small, and may prove daunting to pathologists. Generally, the smaller the amphibian, the greater the number of animals required to perform a thorough diagnostic investigation. A sample size of 60 fish is recommended to assure 95% confidence that all serious diseases are detected in commercial hatchery operations. Although this number of tadpoles, frogs, salamanders or caecilians is not suggested here, the figure is cited as an example of the sample required in order to have confidence that important infectious diseases are detected.

The Casualty Site

It should be ascertained at any casualty site whether any other vertebrate classes have apparently suffered any mortality. If this is the case, the event may have been caused by a toxic chemical or a predator. The appropriate authorities should be informed if any listed endangered species are involved. Accurate records of all aspects of the casualties, specimens collected and location(s) of their collection should be kept, and each specimen should be carefully and individually labeled with similar information. This is especially important if endangered species are involved and/or criminal proceedings are likely to follow against a possible polluter.

Sample Records

1. Date and time of day.
2. Location: river; metres of river, pond, pond area, nearby roads, landmarks and towns, map reference if possible.
3. Name of observer(s).
4. Estimated time when deaths began.
5. Water quality characteristics such as color, odor, temperature and (if possible) dissolved oxygen concentration, pH, conductivity, osmolarity, nitrates and salinity.
6. Condition of each species and life-stage at the site: e.g. numbers of live, moribund, dead, scavenged and decaying eggs, larvae, metamorphs and adults (with common, scientific and/or local species names as appropriate).
7. Physical examinations of each affected animal: e.g. discoloration or other changes, abnormal postures abnormal swimming patterns, amount of mucus on the skin and any lesions or other abnormal features.
8. Other animals at the site: e.g. numbers of dead fish, abnormal fish behavior, snails out of the water, crustaceans or fish attempting to leave the water and discolored vegetation.
9. Weather conditions on the day and the previous day and night including temperature, cloud cover, precipitation and wind speed and direction.
10. Names of persons notified or contacted, with dates and times.

Containers and Transportation

Live animals of any stage should be placed in a large, solid container holding 1-2 times as much air as water. Water-tight, durable plastic bags may be used as an alternative. Separate containers should be used for control animals, different species, different stages, sick animals, healthy animals and animals from different sites. For terrestrial amphibians, use a solid container with some air holes and well-moistened, unbleached paper towels. Animals from cool or high-altitude environments may be transported with the use of ice packs, but the animal should not be in direct contact with the ice. Containers of live animals should be packed for transport into cardboard boxes lined with non-toxic, insulating material. Frozen and refrigerated specimens should not be transported in the same container, and specimens preserved in fixative should never be frozen. Transportation to a diagnostic laboratory should occur as soon as possible, and preferably within 24 hours of collection.

Remember, if in any doubt, contact a local veterinarian.

This information sheet has been produced with the assistance of Andrew Cunningham and is based on guideline originally set out by D. Earl Green and John E. Cooper.

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For further information on the DAPTF or details of local DAPTF Working Groups who may be able to assist with investigation of mortality event, please contact John Wilkinson at this address or tel +44 0 1908 652274; fax +44 0 1908 654167; e-mail DAPTF@open.ac.uk.

Occurrences of mortality in the UK should be reported to: The Frog Mortality Project, Froglife, Triton House, Bramfield, Halesworth, Suffolk IP19 9AE.

Malformed amphibians in the USA and Canada can be reported to the North American Reporting Center for Amphibian Malformations at <http://frogweb.nbi.gov/narcam/>