American Turtle SAFE Objective 3 Health and Welfare: Turtle Confiscations

- 3.1 Initial management of confiscated native U.S. turtles
- 3.2 Housing, Husbandry, and Biosecurity (short-term up to 3 weeks)
- 3.3 Initial and Comprehensive Health Assessments
- 3.4 Decision tree for determining if turtle(s) are releasable
- 3.5 Euthanasia considerations specific for time of confiscation
- 3.6 Standardized forms

Health and welfare are the paramount concerns regarding the outcome and future of confiscated turtles, indeed of any animal held in human care. It is important to note that health is not merely the absence of disease, but a state of complete well-being that encompasses mental and physical aspects.

Special consideration must be made with respect to confiscated turtles because of the many unknowns that typify these cases. There is a spectrum of potential conditions, ranging from good health to moribund, that confiscated turtles exhibit at time of confiscation. In all cases animals should be initially guarantined away from other living specimens for proper assessment, diagnosis and pending treatments. Although animals could appear healthy, and depending on the source may be, it is critical to take the most conservative approach to reduce the risk of spreading pathogens. Animals confiscated in large numbers are usually highly stressed and frequently exhibit signs of dehydration, starvation, hypo/hyperthermia, morbidity, disease, etc. Thus, immediate supportive care (i.e., fluids, food, appropriate temperature, lighting) is often the most pressing consideration. Information gained during investigations and at the time of confiscation are critical components of the health assessment process. The goal of this Strategic Objective is to develop the procedures, protocols, and best management practices needed to provide biosecurity, the necessary level of supportive care, assess overall health status, screen for infectious diseases and establish species-appropriate husbandry practices within the context of Law Enforcement needs during and beyond the Chain of Custody period. The intent of these procedures is to result in an animal that can return to functioning independent of human care and potentially be released to the wild, and the needs to meet this intent will vary depending upon the specific conditions of the turtles at the time of and subsequent to confiscation.

With respect to any animals intend for release to the wild, it is important to understand both the risk that released turtles may pose to the wild population as well as what disease risks the released turtles themselves may face from that environment. This objective will address this through the framework of a comprehensive, interactive database that maps the distribution of known pathogens in the native wild populations across the landscape (initially focused on North America).

3.1 Initial management of confiscated native U.S. turtles

- A. Confiscation holding location/transportation
- B. Biosecurity Considerations
- C. Initial triage
- D. Secondary triage

The duration that turtles have been held in captivity prior to confiscation is often unknown. While confiscated turtles might be in good condition and require standard care, illegally collected turtles are often held in crowded, unsanitary conditions at inappropriate temperatures while awaiting distribution. In the immediate post-confiscation period, we seek to hold the turtles in a safe, secure location, provide them with appropriate environmental conditions, and ensure that they are hydrated while awaiting definitive long-term management plans.

3.1.A. Confiscation holding location/transportation

1. Select a holding location that is free of non-native species, if possible, particularly avoiding proximity to non-native reptiles and pet reptiles. Even brief proximity to non-native species or pet reptiles greatly complicates the prospect of repatriation, possibly making repatriation impossible. If one must use a facility that maintains non-native species, a careful plan must be made to maintain strict biosecurity and isolation of the confiscated specimens, ideally in a separate building using separate personnel. Transmission of fatal infectious diseases has occurred among turtles housed in the same room, even when held in separate enclosures.

2. Transport the turtles from the confiscation site to the temporary holding location. Turtles can be packed in plastic totes, bins, buckets, kiddie pools, concrete mixing tubs, cardboard boxes, etc. Boxes used for shipping fruits and vegetables are often available from grocery stores and are useful for transport. If possible, line the containers with absorbent material such as paper towels, newspaper, towels, etc. During transport turtles often urinate and defecate, and the absorbent material helps to reduce contamination of other turtles and transport vehicles. Turtles are good climbers, so ideally secure a lid on the containers. If containers are airtight, drill some air holes in the sides or top. Try to minimize crowding if possible. A single "layer" of turtles is ideal. Turtles that are packed two or more layers deep often compress, traumatize, urinate, and defecate on each other, causing eye, skin, muscle, and shell injuries. If turtles must be packed in layers, place the larger specimens on the bottom and smaller specimens on top and try to reduce the duration of this situation. Studies have shown that transport is a physiologic stressor for turtles, so develop a plan to minimize the duration of transport as much as possible. Avoid direct sunlight as this can cause turtles to rapidly overheat.

3.1.B Biosecurity Considerations

Biosecurity at time of confiscation is of utmost importance, especially if confiscated turtles were housed with other species.

• Be sure to wear gloves and change gloves between handling individual(s) or groups.

- If turtles are clearly sick be sure to clean outside of the totes before transport of turtles in a vehicle (to reduce risk of transmission of potential pathogens).
- If sick turtles are transported in a vehicle, consider disinfecting the inside of the vehicle using appropriate disinfectant. UV and heat are also great disinfectants.
- Quarantine sick turtles from heathy turtles.
- Quarantine different species apart, if multiple are confiscated at one time.
- If confiscating multiple groups of turtles at one time from different locations, quarantine each group of turtles separately.

3.1.C. Initial triage (At time of confiscation)

Initial triage includes the very first observations of confiscated turtles and in an ideal situation will be conducted by a veterinarian. However, if a veterinarian is not available at time of confiscation, a few actions can be undertaken to optimize the outcome of confiscated turtles. Below, we highlight a few triage-based actions that should be undertaken immediately, at time of confiscation. Other considerations may include euthanasia, based on health-status of turtles, species, and other parameters (such as resources available); we do not cover these aspects here and are focused on the initial medical-based triage.

Be sure to keep turtles out of direct sunlight, wind, or other environmental conditions that may impact their ability to thermoregulate. Do not leave turtles in bins in direct sunlight. Provide adequate shade, even if temperatures are cool. Turtles can rapidly overheat in closed containers in direct sunlight.

Initial triage steps include (but are not limited to):

1. Determine if native or exotic species; aquatic or terrestrial.

2. Relocate the turtles to an environment with ambient temperature of 60-80 degrees F. Their body temperature is dependent on the environment, and proper body temperature is important for all of their vital functions. Most native species can tolerate colder temperatures if needed, but it is not ideal for confiscated specimens. Avoid extreme heat. If turtles are housed outdoors make sure they have access to shade.

3. Reduce crowding and reduce stocking density as much as possible by separating turtles into smaller groups, or if possible, distributing them into individual containers. Individuals do not need a large amount of space in the short-term. Similar containers that are described above for transport can be used for short-term holding. Plastic containers offer ease of cleaning and ease of water provision compared to wooden or cardboard containers. In some cases, turtles might be released into an outdoor pen or corral. Make sure that the pen is secure. Turtles are excellent diggers and climbers and they escape easily. They are also susceptible to predation by raccoons, foxes, etc. If heavily planted enclosures are used, be sure to acquire an accurate census. Turtles hide very well and can often be very difficult to locate. Avoid concrete enclosures if possible; it is very abrasive on their feet and shell.

4. Identify the species if not already done. If multiple species are present, if possible, separate them from each other.

5. As soon as possible, add shallow water to the containers such that it covers the bottom shell and feet, deep enough so that they can submerge their head to drink. Many turtles will drink readily, and this also helps to clean their skin, nostrils, mouth, and eyes of waste material, often helping to "unstick" unhealthy eyes, and flush out unhealthy sinuses. If maintained in group settings in outdoors pens, provide numerous shallow water bowls or trays that provide easy access for drinking (e.g. bowls used under flower pots).

6. If time allows, briefly examine each turtle, at least attempting to remove dead individuals, and also identifying animals that are sick and separating them from apparently healthy turtles. It is not always easy to tell if a turtle is dead. If in doubt, assume that it may be alive and maintain in same temporary, but separate, housing conditions as other turtles confiscated at same time. Dead turtles often have very sunken eyes, and rigor mortis may be noted. Look for swollen eyes, nasal discharge, skin and shell lesions, bleeding, etc. Look for turtles that feel too light (when picked up, healthy turtles should feel solid, roughly like a rock of similar size). If clearly dead, remove dead turtles and place them into a refrigerator or cooler containing ice. Do not freeze or discard them unless specifically told to do so by the director of the event. Separate sick turtles into individual containers, gather them into a location where they can be monitored closely, and provide an estimated number of sick turtles to the event director.

7. Check temperature of turtles. Turtles should feel room temperature. If cold, then slowly warm (~5F) per day until the turtle reaches a body temp around 78F (25.5C). If hot (>90F / 32CC), then slowly cool turtle(s) by misting with cool (air temp) water and monitoring. **Be sure to keep turtles out of direct sunlight, wind, or other environmental conditions that will impact their ability to thermoregulate.**

3.1.D. Secondary triage (24hrs to a few weeks post-confiscation, but before placed at longer term facility)

1. For subsequent days, most species can continue to be kept in shallow water, with water changed daily. Box turtles can be kept on paper substrate or bark mulch and provided with a shallow water bowl.

2. If time permits, provide each turtle with an opportunity for visual security. They often choose to be covered and hidden. Cereal boxes, milk cartons, large plastic bottles, halved flower pots, etc. can be used as shelters. A few inches of dry leaves may serve the same purpose.

3. If resources permit, the substrate and water should be discarded and the containers should be washed and disinfected daily. Warm water and liquid dish soap may be used to wash the enclosure, followed by disinfection with a dilute bleach solution (20 parts water to one part

household bleach). A contact time of one minute will kill most pathogens (Gray et al., 2017). Enclosures should then be thoroughly rinsed.

4. Provide a light cycle of 12-16 hours per day. Lights should be left on continuously (i.e. do not leave lights on overnight). Failure to provide at least partial darkness at night may lead to physiological stress that exacerbates other medical problems.

5. It is not essential to provide food during the first few days, but it can be offered if resources permit. Earthworms are a favorite food for most species. Brightly colored, fragrant fruits such as strawberries, raspberries, and melon may also be accepted.

6. Develop a plan for the long-term quarantine period in collaboration with the event director. Consider options for non-AZA SAFE species or non-T&E or non-SGCN species, especially in light of available funding and resources. Such species which are of lower conservation concern may need to be transferred to another facility and, in worst case scenarios, euthanized.

7.) Individualized care can now be provided to turtles based on available resources. Examples include more thorough health assessments, more involved treatments for injuries, and evaluation via radiographs for gravid females.

3.2 Best Management Practices (BMPs) for housing, husbandry, and biosecurity

Long-term housing of confiscated turtles will occur at AZA facilities or at another facility with a dedicated area for such turtles. In the meantime, a few key aspects for shorter term housing should be covered.

Aquatic turtles must have access to water and a dry area for basking. An appropriate housing situation can be obtained in a large tote (or kiddy pool) set at an angle (one end raised) and non-skid material on the bottom. Water can be added such that the lower end of the tote is fully submerged while still maintaining a dry area on the elevated end of the tote. This will provide the turtle(s) with a dry area for basking. The water should be changed every 2-3 days, or when it becomes fouled with food and/or feces. This would be similar to a traditional 'dump and fill' set up.

Terrestrial turtles should be kept dry, with a basking area and also a shaded area. A tote similar to what is used for aquatic turtles can be used, along with a similar non-skid rubber mat. A basking area can be created using a UV bulb at the appropriate height from one end of the tote; there should be adequate space such that the turtle(s) can get out of the basking light. Water and food can be provided in small bowls. Water bowls should be large enough that turtles can rest in them but shallow enough that they can exit without difficulty.

For both terrestrial and aquatic turtles, it is imperative that the turtles cannot crawl over the side of the tote or pool.

Both terrestrial and aquatic turtles will require UV lights and, if the environment is cold, heat lamps. Note that if the turtles are in these temporary housing situations for only a few weeks, UV lights directed at the basking area(s) may not be necessary. Temperatures should be maintained between 75-85F (23-29C), with a slight decrease in temperature at night.

Food should be offered 1-2 times per week during this initial period. For aquatic and terrestrial turtles, earthworms are an easy food item to provide and are generally recognized as food by most turtles. Commercially available floating turtle pellets can also be offered to aquatic turtles but individual turtles may not be conditioned to eating them if they have to been exposed to this food source before. For terrestrial turtles, a variety of fruits and vegetables can be offered with brightly colored ones (melons, strawberries, tomatoes) being the most likely to be accepted initially.

3.3 Health Assessments/Pathogen and Disease screening

A. Initial and Comprehensive Health Assessment (Best Management Practices, BMPs)

B. Pathogen/Disease screening BMPs

C. Decision tree for determining if turtle(s) are releasable based on health considerations,

- and if so what must happen to determine this
- D. Diagnostic labs: List of agreed upon labs and methodology for testing
- E. Training materials
- F. Sample kits
- G. Sample costs
- H. Novel approaches (i.e., microbiomes)

3.3.A. Initial and Comprehensive Health Assessment (BMPs)

Initial Triage:

While confiscations of small numbers of animals can allow for more individualized attention, most confiscations deal with large numbers of animals, and in those cases population level health assessment, diagnostics, and medical care are required. An initial triage is generally necessary for health assessments and treatments. Upon stabilization of the situation, more comprehensive exams can be performed.

Initial triage, as outlined above, should consist of brief exams to determine which animals are deceased (ideally confirmed by a veterinarian and Doppler), which are obviously clinically ill and in need of priority treatment, and which are relatively healthy. Necropsies and disease screening should be performed on at least a portion of the deceased animals to help inform care of the living turtles. We recommend that at least 3 animals and no more than 5% of the total number confiscated that are clinically ill or apparently deceased be submitted for complete diagnostic workup (necropsy, histopathology, pathogen testing).

Supportive care for the living animals should consist of separating them by species and by degree of medical care needed, provision of an appropriate ambient temperature/thermal gradient, obtaining body weights, providing fluid support (by soaking and/or parenteral), nutritional support, and chemotherapeutics or other treatments for ill turtles (traumatic injuries, metabolic problems, infections, etc.).

After the initial triage, more individualized medical care and more complete physical exams may be possible. Any external parasites (i.e. leeches, ticks, etc.) should be collected, placed into alcohol (70% ethanol), or frozen for later identification or ancillary testing (PCR, etc.). Depending on the numbers of animals, clinicians can consider the following tiered approach to diagnostic testing:

| | PCV/TS/WBC estimate | Full CBC ¹ and cytology for hemoparasites | Chem panel | FTA ² card for hemoparasite PCR | Genetic analysis | Choanal/ Cloacal ³ swab for pathogen screen | Fecal direct and float |
|--------------------------------------|------------------------|--|---------------|--|---------------------|--|------------------------------|
| Triage of ill animals | х | | | | | x | |
| Complete workup of ill animals | | x | x | | | lf not already done | x |
| Prelease screen (ideal) | | x | x | x | x | x | x |

1. Blood collection not to exceed 0.8% of body weight. Hematological analysis including CBC, packed cell volume (PCV), total solids (TS), estimated or calculated white blood cell (WBC) count, and differential blood cell counts can be performed using whole blood according to published methods (Boers et al., 2019; Rapheal et al. 2019; Sheldon et al. 2016; Sykes et al. 2008). Slides can be made of whole blood, dried and fixed immediately (within 24hrs) and stained at a later time. Slide review is also valuable for hemoparasite assessment.

2. Blood spots can also be placed onto filter papers (FTA, and 903 protein-saver; Whatman Inc.) and air-dried and placed into plastic bags containing desiccant for long-term storage. These samples can be used for ancillary or additional diagnostic and genetic testing, and health assessments.

3. A single combined choanal and cloacal (ch/cl) swab should be collected from each turtle, or duplicate combined ch/cl swabs in separate tubes if pooling samples* and placed in a sterile 2mL screwcap tube. Microswabs should be cotton swabs with plastic shafts. Metal shafts or wooden shafts, as well as foam tips, should be avoided. A fresh pair of disposable gloves should be used when taking a sample for PCR analysis, however if gloves are limited or the number of turtles to be assessed precludes use of separate gloves for each individual care should be taken using proper technique to minimize the risk of cross contamination of samples. If samples cannot be stored cold or frozen shortly after being collected, the sample should be placed in

RNA*later*[™] to preserve the nucleic acid until sample can be stored in a freezer. Samples should be stored frozen and shipped on ice packs until further testing can take place. It is worth noting that if turtles are small and/or an adequate blood sample is not feasible, then these swabs may also be used for genetic testing of host.

*Pooling swabs: Pooling samples may make it logistically easier and more cost effective for large numbers of confiscated turtles. To save on testing cost, swab samples can be pooled up to 5 microswabs per tube (i.e. 2mL screw cap tube). If any positive pooled samples need to be traced back to individual animals we suggest taking a duplicate swab sample in RNA later for each animal at the time of sampling so that the individual swab can be retested at a later time if necessary, or in case any pooled samples come back positive and the animal has to be traced. If animals are housed together and do not need to be traced back to an individual, then duplicates do not need to be taken. Please contact the lab to discuss alternative pooling options.

Procedure for storing samples in RNA later

RNA later[®] preserves both DNA and RNA in swab samples (choana/cloacal, nasal, and ocular).

- Place swab in 500ul RNAlater[®] Solution (provided). If pooling swabs ensure the amount of RNA later covers the swabs completely.
- When not transporting samples, they should be archived at -20°C or -80°C.
- Ship samples as soon as possible at the coolest temperature possible.

Use of RNAlater[®] Solution with fresh tissue

- Do not freeze tissues before immersion in RNAlater[®] Solution.
- Before immersion in RNAlater[®] Solution, cut large tissue samples to ≤ 0.5 cm in any single dimension.
- Place the fresh tissue in 5–10 volumes of RNAlater[®] Solution and mix well
- When not transporting samples, they should be archived at -20° C or -80° C.
- Ship samples as soon as possible at the coolest temperature possible.

If using RNA later for fecal samples, RNA later should be added to the feces at a 1:1 ratio to ensure efficient penetration and preservation of the nucleic acid. Follow the sample protocol as above.

RNA later preserves nucleic acid (particularly RNA) for up to 1 day at 37°C, 7 days at 15–25°C, or 4 weeks at 2–8°C, allowing transportation, storage, and shipping of samples without ice or dry ice.

3.3.B. Pathogen/Disease screening Best Management Practices

Infectious diseases are an important contributor to population declines in many species of chelonians (Todd et al. 2010). For example, Mycoplasma, Intranuclear coccidia (TINC), and

viruses such as Herpesvirus, Adenovirus and Ranavirus are relevant pathogens to screen for that are known to be associated with disease in chelonians and represent a risk when new animals are introduced. *Mycoplasma* spp infection, which can cause upper respiratory tract disease, is an important cause of morbidity and mortality and has been implicated in the decline of populations of desert (*Gopherus agasizzi*) and gopher (*G. polyphemus*) tortoises (Jacobson et al. 2007; Seigel et al. 2003; Berry KH, 1997), as well as causing morbidity and mortality in a number of box turtle species (Palmer et al., 2016). Outbreaks of bacterial septicemia have been implicated in population declines of flattened musk turtles (*Sternotherus depressus*) (Dodd et al.1988; Fonnesbeck and Dodd.2003). Fatal ranavirus infections have been documented in free ranging gopher tortoises and eastern box turtles (*Terrapene carolina carolina*) (Westhouse et al. 1996; Johnson et al. 2008). Herpesviruses are also important pathogens to monitor for in terrestrial and freshwater chelonians and are known to cause respiratory and fatal disease in a number of turtle species (Ossiboff et al. 2015; Frye et al. 1977; Cox et al. 1980; Jacobson et al.1982; Sim et al. 2015; Jungwirth et al. 2014; Pettan-Brewer et al. 1996).

Obtaining data at both the individual and population levels and identifying the presence or absence of potential pathogens are important for maintaining biosecurity in a facility that houses animals and when considering risk versus benefit of translocation programs (Wedland et al. 2009; Seimon et al. 2017). Screening for the following pathogens is recommended as best practices for maintaining biosecurity, preventing pathogen spillover, and for decisions related to the animal welfare and management of these animals. All animals that enter a quarantine facility should be screened soon after arrival, a second test should be conducted before leaving the facility, and when available compared to the results of the intended release population.

Animals should be screened for fecal and blood parasites. Fecal culture is recommended if there is a clinical indication where culturing and analysis would be beneficial. In addition, combined ch/cl swabs should be collected for PCR testing of the following pathogens:

- Herpesvirus
- Ranavirus
- Mycoplasma
- Adenovirus
- Intranuclear coccidia (TINC) (prioritize for tortoises)

Other pathogens may be screened for as needed, based on clinical signs (for example, shell lesions may warrant screening for *Emydomyces testovarans*).

3.3.C. Diagnostic labs

WCS Molecular Laboratory Wildlife Health Center Zoological Health Program Wildlife Conservation Society - Bronx Zoo 2300 Southern Blvd. Bronx NY, 10460 Primary Contact: Dr. Tracie Seimon, tseimon@wcs.org

Institute for Conservation Medicine Saint Louis Zoo One Government Drive St. Louis, MO 63110 Primary Contact: Kathleen Apakupakul, <u>apakupakul@stlzoo.org</u>

Wildlife Epidemiology Laboratory University of Illinois College of Veterinary Medicine 3846 Vet Med Basic Sciences Bldg., Urbana, IL 61802 Primary Contact: Matt Allender, <u>mcallend@illinois.edu</u>

3.3.D. Training materials – To be addressed at later time

3.3.E. Molecular Sample kits:

Each facility should have the following materials available on hand for sample collection of choanal/cloacal, nasal, ocular swabs, feces, blood or tissue for molecular analysis:

- Disposable gloves
- Lab marking pen
- RNA later [®] (RNA stabilization reagent), Thermo Fisher Scientific: Cat. No. AM7021
- 2.0mL PCR grade screwcap microtubes; Fisher Scientific: Sarstedt Cat. No. 72.694.006 or equivalent.
- Sterile culture swabs with plastic shaft (Equivalent to Advantage Bundling/Medical Wire Co. catalog number **MW113**).
- FTA, and 903 protein-saver cards for preservation of blood spots; Whatman Inc. (if blood pathogens are suspected or a genetic sample is needed).

Shipping samples:

Samples should be shipped to one of the participating labs (3.4.D) on ice packs in an insulated foam box by overnight delivery service (i.e. FedEx, etc.).

3.3.F. Cost of Molecular pathogen DNA testing:

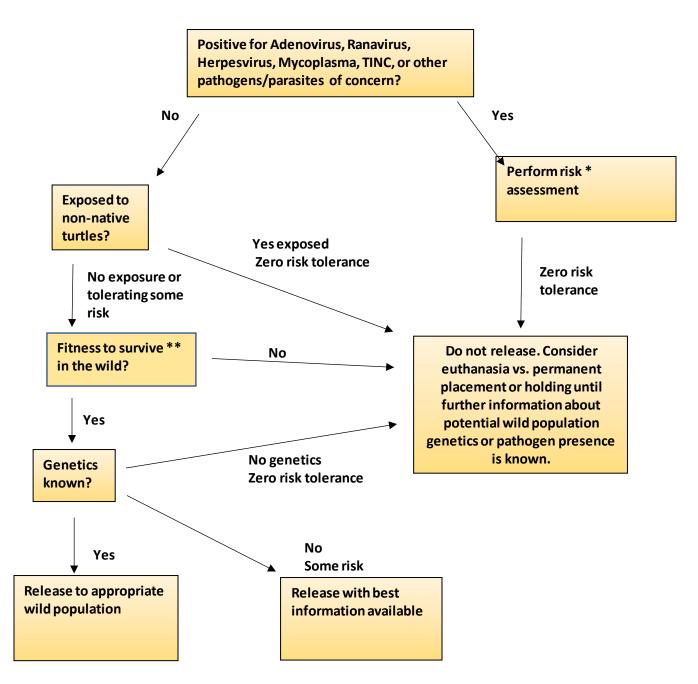
The cost of DNA/RNA tests will depend on the lab and test being run and can range from \$50/test to as much as \$225/test if a pathogen panel is run. The cost may or may not include

DNA sequencing for any positives, so it is best to contact the lab in advance to check on pricing, turn-around time, pathogen tests available, and their ability to perform testing on pooled samples.

Pending adequate funding, the number of turtles screened for pathogens should be based off the desired confidence level that at least one positive turtle would be detected assuming a suspected prevalence of the pathogen in question and a given population size that the confiscated turtles are being integrated into. An example that can be used to help guide this process is a table taken from Samuel et al. 2003 (page 40, adapted from Roe and Cannon, 1982*), Gray et al., 2017 (Tables 1 and 2), or Raphael et al., 2019. Often prevalence levels for pathogens in turtle populations will be unknown, or data is insufficient, and this will need to be estimated based on literature review and available working knowledge of the target population. Once the number of turtles that needs to be screened for a pathogen is determined, additional cost savings can be made by pooling samples. Up to 5 turtles per pooled sample can be combined to reduce the overall testing cost. However, any positives from a pooled sample may warrant individual testing.

3.4 Decision tree for determining if turtle(s) are releasable with respect to health, fitness, and genetics

The decision on whether to release confiscated turtles back to the wild depends on a risk assessment of the potential health threats that are posed by release to the wild population as well as consideration of the individual's fitness and genetic background. The following decision tree is for turtles that appear healthy on physical exam, are considered fit enough to thrive in the wild, do not demonstrate significant signs of disease on bloodwork (if performed) or other ancillary testing, and that have minimal to no endoparasites; if endoparasites are present they are assessed as not posing a risk to wild populations. Additional considerations include the infectious disease and endoparasite status of the wild population and a good genetic match with the wild population (see genetic section of this document).



* If an animal is apparently healthy but tests positive for a disease of potential concern, further analysis is advisable. Ideally the pathogen is better characterized and sequenced as appropriate. The pathogen in the confiscated animal should be compared to that found in the wild metapopulation where the potential release would occur. If the pathogen has already been detected in the wild population and does not appear to induce significant clinical disease in the wild population, the animal should be considered for release. If the pathogen has not previously been detected in the wild, then release into that area should undergo additional evaluation and risk assessment to prevent introduction of the detected pathogen into any potentially naïve population.

** An individual is considered "Fit" if it has been through comprehensive veterinary screening and quarantine and shows no signs of infectious diseases or disability that would adversely affect it from surviving independently, is in good body condition and strength, and is considered able to forage and avoid predators after release. Other factors to be considered when evaluating fitness for release into the wild include whether the individual was originally taken from its natural wild habitat or was a captive born individual as well as the length of time in captivity (IUCN (2019). *Guidelines for the Management of Confiscated, Live Organisms*. Gland, Switzerland: IUCN. iv + 38pp. Timing of release will also be dependent upon selection of appropriate season to allow abundant food resources and habitat choice prior to brumation.

3.5 Euthanasia considerations specific for time of confiscation

Euthanasia of any species included in the AZA SAFE American Turtles Program should be considered only when absolutely necessary, recommended by the attending AZA veterinary clinician, and for humane reasons. Furthermore, federal and state partners should be involved in the decision process, given the T&E and SCGN status of these species. Specific considerations for determining to euthanize confiscated turtles include:

- 1. Humane welfare and quality of life as well as release potential should be top considerations when considering euthanasia for confiscated turtles.
- 2. Accepted guidelines of euthanasia methods for reptiles must be followed (i.e., <u>American</u> <u>Veterinary Medical Association Guidelines for Euthanasia of Animals: 2020 Edition</u> (<u>AVMA 2020</u>).
- 3. If sick turtles will hinder the successful treatment, housing, recovery, or release of other turtles in the confiscated group due to facility funding and operational capacity, then euthanasia of sick turtles may be considered and warranted. In these instances, overall herd health should be considered.
- 4. Euthanasia may be an acceptable option for species that are not T&E or of conservation importance (SGCN), or are considered an invasive species. Coordination with state and federal partners is necessary in these cases.
 - a. If an AZA facility is not in support of euthanizing such species, consideration should be given to place these groups in a rehabilitation facility.

3.6 Standardized forms:

| AZA SAFE America | in Turi | tle Intake Record | | | | |
|---------------------------------------|-----------|-------------------------|----------------|------------|----------|--|
| | | | Date of intake | | | |
| Animal ID | | | Intake site | | | |
| Species | | Expert verified? \Box | LE case ID | | | |
| Male □ Female □ | l Unk | nown 🗆 | PIT tag | | | |
| Photos obtained \Box | | Distinct markings | | | | |
| Weight 🛛 g | j □ kg | Carapace length (| (cm) | □ straight | □ curved | |
| Source information (| if availa | ble) | | | | |
| Date of collection | | | | | | |
| Location | | | | | | |
| Transport/handling | | | | | | |
| notes | | | | | | |
| | | Initial veterinary | avam | | | |
| Date performed | Performed | | | | | |
| □ Turtle has no noted health concerns | | | | | | |
| Health concerns identified | | | | | | |
| Treatments initiated | : | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| Dethe see severe in se | | | | | | |

Pathogen screening performed:

| Venipuncture | 🗆 Oral swa | b [| ⊐ Cloacal swa | ab E | J Fecal | parasite | screen |
|----------------|------------|------|-----------------|------|---------|----------|--------|
| □ Ectoparasite | Sample | Banł | king location _ | | | | |

Animal movement/transitions

| | Facility | Date of intake | Date of |
|--------------------|----------|----------------|-----------|
| | | | departure |
| Triage (α Q1) | | | |
| Phase 1 quarantine | | | |
| (Q1) | | | |
| Phase 2 quarantine | | | |
| (Q2) | | | |
| Phase 3 holding | | | |

Final disposition: Died Euthanasia

Disposition date _____

Permanent placement – Facility ______

Release/repatriation – Location _____

| | Date |
|---|--|
| AZA SAFE American Turtle Health | Assessment site |
| Assessment | Performed by |
| | |
| Animal ID | PIT tag |
| Species Male D Fema | ale 🗆 Unknown 🗆 |
| Weight \Box g \Box kg Carapace length | h (cm) 🗆 straight 🛛 curved |
| Physical exam | |
| Mentation: \Box BAR \Box QAR \Box LethargioHeart rateRespiratory rate | c □ Obtunded □ Unresponsive e quality |
| Temperature $\square^{\circ}C \square^{\circ}F \square$ Cloac | al 🗆 Surface |
| Body condition: WNL Thin Emaciat | ted Score: |
| Dehydration: None Mild Moderate | □ Severe |
| Eyes: OD | Stain: 🗆 Pos 🗖 Neg |
| OS | Stain: □ Pos □ Neg |
| Nares Oral cavity Muo | cus membrane color |
| Skin condition/wounds | |
| Extremities Carapace and plastron | |
| Coelom | |
| Vent □ | Prolapse |
| Venipuncture Site Quantity PCV: TS: | D Banked for genetics |
| □ CBC □ Blood gasses/iStat □ Che | emistry: Lab or analyzer |
| □ Filter paper | |
| Medications/treatments | |
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References:

Berry K. H. 1997. Demographic consequences of disease in two desert tortoise populations in California, USA. in Proceedings: conservation, restoration, and management of tortoises and turtles—an international conference. ed Van Ebbema J. (Wildlife Conservation Society Turtle Recovery Program and the New York Turtle and Tortoise Society, New York, N.Y), pp 91–99.

Boers, KL, Allender, MC, Novak, LJ, Palmer J, Adamovicz L, and Deem SL. 2019. Assessment of hematologic and corticosterone response in free-living eastern box turtles (*Terrapene carolina carolina*) at capture and after handling. Zoo Biology. 1–10. <u>https://doi.org/10.1002/zoo.21518</u>.

Cox WR, Rapley WA, Barker IK. 1980. Herpesvirus-like infection in a painted turtle (Chrysemys picta). J Wildl Dis 16: 445–449. PMID: 6251285

Dodd Jr CK. 1988. Disease and population declines in the flattened musk turtle Sternotherus depressus. Am Midl Nat 119:394-401.

Frye FL, Oshiro LS, Dutra FR, Carney JD. 1977. Herpesvirus-like infection in two Pacific pond turtles. J Am Vet Med Assoc 171: 882–884. PMID: 200595

Fonnesbeck CJ, Dodd Jr CK. 2003. Estimation of flattened musk turtle (*Sternotherus depressus*) survival, recapture, and recovery rate during and after a disease outbreak. J Herpetol 37:602–607.

Gray MJ, Duffus AJ, Haman KH, Harris RN, Allender MC, Thompson TA, Christman MR, Sacerdote-Velat A, Sprague LA, Williams JM, Miller DL. 2017. Pathogen Surveillance in Herpetofaunal Populations: Guidance on Study Design, Sample Collection, Biosecurity, and Intervention Strategies. Herpetological Review 48(2): 334-351.

Jacobson ER, Gaskin JM, Wahlquist H. 1982. Herpesvirus-like infection in map turtles. J Am Vet Med Assoc 181: 1322–1324. PMID: 6294034

Jacobson ER. 2007. Bacterial diseases of reptiles. In: Infectious diseases and pathology of reptiles, Jacobson ER, editor. Taylor and Francis Group, Boca Raton, Florida, pp. 470-472.

Johnson, AJ et al. 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. J Wildl Dis. 44(4):851-63.

Jungwirth N, Bodewes R, Osterhaus ADME, Baumgärtner W, Wohlsein P. 2014. First report of a new alphaherpesvirus in a freshwater turtle (Pseudemys concinna concinna) kept in Germany. Vet Microbiol 170: 403–407. doi: 10.1016/j.vetmic.2014.02.029 PMID: 24667062

Ossiboff RJ, Raphael BL, Ammazzalorso AD, Seimon TA, Newton AL, Chang TY, Zarate B, Whitlock AL, McAloose D. 2015. Three novel herpesviruses of endangered *Clemmys* and

Glyptemys turtles. PLoS One. 10(4):1–10.

Palmer JL, Blake S, Wellehan JF, Childress A, Deem SL. 2016. First reported clinical *Mycoplasma* sp. infections in free-living three-toed box turtles (*Terrapene carolina triunguis*) in Missouri. J. Wildl. Dis. 52: 378-382.

Pettan-Brewer KC, Drew ML, Ramsay E, Mohr FC, Lowenstine LJ. 1996. Herpesvirus particles associ- ated with oral and respiratory lesions in a California desert tortoise (Gopherus agassizii). J Wildl Dis 32: 521–526. PMID: 8827680

Raphael BL, Macey SK, Platt K, Platt SG, Seimon TA, Ossiboff RJ, Horne BD, Gamarra, AL Barrera MG, Lwin T, Soe MM, Aung SH, New SS, Khaing LL, New SS, and Aung SH. 2019. Health Screening of Burmese Star Tortoises (Geochelone platynota) Prior to Introduction to the Wild. Chelonian Conservation and Biology. https://doi.org/10.2744/CCB-1353.1

Samuel MD, Joly DO, Wild MA, Wright SD, Otis DL, Werge RW, and Miller MW. Surveillance strategies for detecting chronic wasting disease in free ranging deer and elk. Workshop Proceedings. Madison, WI: US Geological Survey National Wildlife Health Center, 2003: pp. 40–41.

Seigel RA, Smith RB, Seigel NA. 2003. Swine flu or 1918 pandemic? Upper respiratory tract disease and the sudden mortality of gopher tortoises (*Gopherus polyphemus*) on a protected habitat in Florida. J Herpetol 37:137–144.

Seimon TA, Horne BD, Tomaszewicz A, Pruvot M, Som S, In S, Sokha C, Platt S, Toledo P, McAloose D, Calle P. 2017. Disease screening in southern river terrapins (Batagur affinis edwardmolli) in Cambodia. J. Zoo and Wildlife Med. Dec;48 (4):1242-1246. doi: 10.1638/1042-7260-48.4.1242

Sheldon JD, Stacy NI, Blake S, Carbrera F, and Deem SL. 2016. Comparison of total leukocyte quantification methods in free-living galapagos tortoises (Chelonoidis Spp). J Zoo and Wildl Med 47(1):196-205.

Sim RR, Norton TM, Bronson E, Allender MC, Stedman N, Wellehan JFX. 2015. Identification of a novel herpesvirus in captive Eastern box turtles (Terrapene carolina carolina). Vet Microbiol 175: 218– 223. doi: 10.1016/j.vetmic.2014.11.029 PMID: 25575878

Sykes JM, Klaphake E. 2008. Reptile Hematology in: Vet Clin North Am Exot Anim Pract 11:481-500.

Todd BD, Willson JD, Gibbon JW. 2010. The global status of reptiles and causes of their decline. In: Sparling, D.W., Linder, G., Bishop, C.A., Krest, S. (Eds.), Ecotoxicology of Amphibians and Reptiles, second ed. CRC Press, Boca Raton, USA. Wedland L, Balbach H, Brown M, Berish JD, Littell R, Clark M. 2009. Handbook on Gopher Tortoise (Gopherus Polyphemus). USArmy Corps of Engineers, Construction Engineering Research Laboratory, ERDC/CERK TR009-1; 32-37.

Westhouse RA et al. 1996. Respiratory and pharyngo-esophageal iridovirus infection in a gopher tortoise (*Gopherus polyphemus*). JWD 32:682-686.