

**HIGHLIGHTS FROM THE NOVEMBER 2002 WORKSHOP ON
DESERT TORTOISE HEALTH AND DISEASE**

Final Revisions: March 2004

What We Know

What We Suspect

Research Priorities: What We Need to Know for Recovery

and

Management Priorities

prepared by

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STATEMENT OF GROUP FINDINGS

As in other wild populations, disease is only one of many factors causing declines in the tortoise populations in California and potentially elsewhere. Decreases in availability of nutritionally important and preferred foods for the tortoise have likely decreased their abilities and very possibly their immune responses for fighting off the diseases. Decreases in nutritional value of important forage plants have been caused by anthropogenic disturbances such as grazing, off-highway vehicle use, introduction of alien plants, and other ground-disturbing factors. There are certain annual plants that have Potassium Excretion Potential (or PEP) indices which tortoises appear to need for their dietary values. It may be possible to reseed areas with these plants to increase their numbers during wet years, which tortoises need to build up their reserves for use in dry years.

RESEARCH FINDINGS

IMPORTANT--Definitions:

1. *What we know:* This phrase is used to refer to published research findings only, primarily published as full manuscripts in peer-reviewed journals.
2. *What we suspect:* This phrase is used to refer to research findings that have not yet been published, and which are available only as data sets, in reports, or abstracts.
3. *What we don't know:* All material in this category was moved to research priorities as of August 11, 2003, in response to recommendations from several participants. The phrase, in our discussions, was used for a variety of situations, such as when no research findings are available or, more likely, the appropriate research experiments have not yet been undertaken.

WHAT WE KNOW AND WHAT WE SUSPECT ABOUT HEALTH AND DISEASE

What We Know About Healthy Desert Tortoises in General

1. The hematologic and biochemical values vary by:
 - sex (packed cell volume, hemoglobin concentrations, aspartate transaminase activity, cholesterol, triglyceride, calcium, and phosphorus concentrations)
 - site
 - season (reproductive cycle, hibernation)
 - rainfall
 - age
2. Year-to-year differences and long-term alterations in hematologic and biochemical values primarily reflect winter rainfall amounts in the Mojave Desert. These differences have not yet been demonstrated for the Colorado or Sonoran deserts.
3. Site differences are minimal, largely reflecting geographic differences in rainfall patterns.

4. Desert tortoises are capable of profound physiologic changes and toleration.
 - Up to 28-fold differences in field metabolic rates and 237-fold differences in water flux rates were recorded on a daily basis.
 - During high-rainfall years, field metabolic rates and water flux rates are higher than during drought.
 - Desert tortoises have a remarkable ability to respond behaviorally, reproductively, and physiologically to rainfall events and periods of drought.
5. Categorizing a tortoise as healthy cannot be done using overall appearance alone. Many diseases may be subclinical and some background lesions may be normal. Only by studying populations over time can we establish what is normal.
6. Otherwise healthy tortoises suffer increased chance of mortality if caused to urinate during handling or processing.

What We Know About Ill Tortoises in General, Regardless of Disease Type. There can be a fine line between ill and healthy tortoises, especially in early stages of diseases, when changes are in the initial stages. In general, as tools (new tests, new equipment) for assessing health improve, we will be able to improve and refine health evaluations.

1. Ill and healthy desert tortoises are physiologically and biochemically different.
2. Clinical signs of disease may be readily apparent, subtle, or subclinical.
3. Ill tortoises are likely to exhibit seasonal- and temperature-inappropriate behaviors, such as: lethargy; anorexia when forage is available; inactivity when temperatures are appropriate for basking, foraging, and walking; and remaining above ground during freezing temperatures in winter.
4. Tortoises with different diseases (e.g., respiratory tract, metabolic, infectious, and shell diseases) may have one or more clinical signs such as: a clear or purulent drainage from the eyes and nares; mucus in the eyes or on the lids; mild to severe edema of the palpebra and periocular area; eyes recessed in orbits; partially or completely occluded nares; staining around eyes, nares, and on the forelimbs from dried mucus embedded with soil and plant fibers; plaques on the tongue or roof of the mouth; and lesions on the shell and limbs from cutaneous dyskeratosis or other shell diseases; open mouth breathing or rasping breathes; and muscle atrophy.
5. Key physical, hematological and plasma biochemical indicators of starvation and dehydration-based ill health include, but are not limited to:
 - Low weight
 - Abnormal activities and behavior

- Elevated levels of one or more plasma biochemical values such as blood urea nitrogen (BUN), uric acid, total bilirubin, osmolality, sodium, and chloride concentrations and hypocalcemia.
 - Abnormalities in packed cell volumes and heterophil counts
6. Abnormalities in hematologic and plasma biochemical values, coupled with clinical signs of disease and other laboratory tests such as the ELISA test for *Mycoplasma agassizii*, can be useful in predicting health status and the potential for mortality. Such data are especially valuable when gathered seasonally and consecutively.

Hematologic and plasma biochemical values are only one part of the health assessment and need to be placed in the context of all the clinical findings that can be used. As new tests are developed, then they should be incorporated into the evaluation process.

7. Azotemia (increased BUN) was the most common abnormal hematologic and plasma biochemical value. It can be an indicator of the hydration status of the tortoise. When severe or persistent, it was often associated with underlying urolithiasis or bladder infection.
8. The severe associated problems of positive *Mycoplasma* cultures, oral lesions, dehydration, and moderate to severe shell disease in the Goffs tortoises between 1990-1995 may have signaled the high mortality rate in the nearby population between 1994 and 2000.
9. Ticks were significantly more likely to be observed on tortoises that had oral lesions (typical of herpesvirus) in the previous year.

What We Know About Mycoplasmosis

1. Many biologists/ecologists equate the phrase and acronym, Upper Respiratory Tract Disease (URTD) with mycoplasmosis. The two are not necessarily the same, and we need to correct the erroneous use of URTD. Different pathogens can cause similar clinical signs, all of which result in the appearance of URTD. Differentiation of the particular disease type and pathogen needs to be undertaken at the microscopic level. For example, mycoplasmosis is one of the URTDs, herpesvirus has been identified in animals with clinical signs consistent with URTD, and an iridovirus was identified in a gopher tortoise with URTD.
2. Mycoplasmosis as a URTD was recognized in captive tortoises before it was observed in the wild.
3. Desert tortoises with clinical signs of URTD were first observed in wild populations of desert tortoises at the Desert Tortoise Research Natural Area in California in 1988. Wild desert tortoises with clinical signs of URTD were seen on the Beaver Dam Slope, Utah, in the 1970s.
4. Captive desert tortoises in California, Nevada, and Utah were observed with clinical signs of URTD in the 1970s and very possibly much earlier. The percentage of captive desert tortoises with positive ELISA tests and cultures that live in desert cities (Las Vegas, Barstow,

Ridgecrest, Joshua Tree, etc.) is higher than in wild tortoise populations, especially populations in areas remote from desert urban settings. Near Tucson, Arizona, populations have up to 84% positive ELISA tests, while tortoises from “remote” populations have tested negative with the ELISA test.

4. *Mycoplasma agassizii* is a bacterium and was the first pathogen isolated from desert tortoises demonstrated experimentally to be a cause of URTD.
5. *Mycoplasma agassizii* is horizontally transmitted, most likely through direct contact between tortoises.
6. *Mycoplasma agassizii* has acute and chronic stages and is associated with high rates of mortality in some wild tortoise populations. Some captive tortoises with URTD, supplemented by good nutrition and water, can survive for years.
7. Tortoises that are antibody-positive for *Mycoplasma agassizii* using the ELISA test may or may not have clinical signs of the disease. The same situation may occur with tortoises that are culture positive for *Mycoplasma agassizii*. The disease can be silent with clinical signs manifesting occasionally and with differing levels of severity.
8. More than one species of *Mycoplasma* has been identified in desert tortoises with clinical signs of URTD. A second species of *Mycoplasma*, soon to be described as *Mycoplasma testudinium* (formerly called, temporarily, *cheloniae*), has been found in wild desert tortoises from the Desert Tortoise Research Natural Area, Lucerne Valley, and Joshua Tree National Park in California. There may be multiple strains, and/or species each with different levels of virulence.
9. Several tortoises salvaged for necropsies have had either *Mycoplasma agassizii* or *M. testudinium* (new species, formerly temporarily named *cheloniae*). These tortoises were salvaged from throughout the Mojave Desert, with one only one tortoise coming from the Colorado Desert (Pinto Basin, Joshua Tree National Park).
10. The ELISA test for *Mycoplasma agassizii* is reliable for detecting antibodies to this species but is not necessarily reliable for detecting *M. testudinium* (new species, formerly temporarily named *cheloniae*).
11. Four other strains or species of mycoplasmas have been detected in the gopher tortoise (*Gopherus polyphemus*) and may be present in some wild or captive populations of desert tortoises.
12. Relatively few correlative studies have been done where both ELISA positive and ELISA negative tortoises have been necropsied to determine presence or absence of specific lesions.

What We Suspect About Mycoplasmosis

1. *Mycoplasma* may not last for a long time outside the body, so is unlikely to persist in burrows or the environment for very long.
2. *Mycoplasma testudinum* (new species, formerly temporarily named *cheloniae*) is likely to be pathogenic and cause clinical signs associated with URTD.
3. Escape and release of ill captive tortoises may be an important source of pathogenic mycoplasma in wild populations.
4. We suspect that wild tortoises under poor environmental conditions may be more susceptible to mycoplasmosis/URTD.
5. Clinical signs of mycoplasmosis have been seen in tortoises from the Beaver Dam Slope and Red Cliffs Reserve in Utah. We suspect that mycoplasmosis may be responsible for the high level of recently observed mortalities, but tests and necropsies are essential to confirm the cause(s).

What We Know About Herpesvirus Infections:

1. Herpesvirus infections have been reported in captive desert tortoises (four) and have been associated with illness and mortality.
2. Herpesvirus infections have been reported in other species of turtles and tortoises.
3. Transmission studies have been undertaken for the European *Testudo* and have demonstrated that herpesvirus is a pathogen for these tortoises. The herpesvirus ELISA test has been validated for European species of *Testudo* but not for *Gopherus agassizii* or other species of *Gopherus*.
4. Clinical herpesvirus infections can be rapid and progressive, resulting in large die-offs in other species of vertebrate animals.

What We Suspect About Herpesvirus Infections:

1. Herpesvirus is a potential threat to desert tortoise populations.
2. Both wild and captive desert tortoises have tested positive to the ELISA test for herpesvirus in the Mojave and Sonoran deserts, a test that was developed for European species of *Testudo*.
3. Oral lesions similar to those seen with herpesvirus infection were observed in desert tortoises at Goffs.

4. The frequency of tortoises with herpesvirus positive ELISA tests increased considerably in samples of desert tortoises between 2001 and 2002 in California.

What We Know About Shell Diseases:

1. Shell diseases have been seen in most Arizona populations and are common in tortoise populations in the eastern Mojave and Colorado deserts of California but less so in the western Mojave Desert. Shell disease occurs in all sizes and ages of desert tortoises.
2. The cause(s) of shell disease have not been determined. No evidence has yet been found to indicate a bacterial or viral origin.
3. More than one type of shell disease exists. The most commonly described shell disease in desert tortoises is cutaneous dyskeratosis.
4. Shell disease (cutaneous dyskeratosis) was significantly more severe with increasing tortoise age at sites in California (e.g., Goffs, Ivanpah, Desert Tortoise Natural Area, and Chuckwalla Bench). This finding suggests a chronic, cumulative problem.
5. Hyperglobulinemia, positive *M. agassizii* cultures, and oral lesions were significantly associated with shell disease at Goffs and suggest chronic infection or antigenic stimulation may be factors in pathogenesis of cutaneous dyskeratosis.
6. At the three sites (Desert Tortoise Research Natural Area, Ivanpah Valley, Goffs) in the health profile research program in California, there was a significant increase in numbers of tortoises with moderate to severe plastron disease and active carapace lesions between 1990 and 1995.
7. Shell diseases (e.g., cutaneous dyskeratosis) reflect metabolic and physiological changes that involve far more than the shell.
8. Protein composition of scutes may be altered in tortoises with certain systemic illnesses.
9. Positive nasal cultures for *Mycoplasma agassizii* had relatively high positive predictive values for tortoises with moderate to severe shell disease. However, it is not known whether this is a cause or effect.

What We Suspect About Shell Diseases:

1. The location and histologic appearance of lesions seen in tortoises with cutaneous dyskeratosis are suggestive of either a deficiency disease or toxicosis or both.

2. The flaking and loss of scute laminae and thinning of bone observed in tortoises with cutaneous dyskeratosis may render the tortoise more vulnerable to other diseases such as fungal infections and multicentric visceral inflammation. Such a disease may inhibit or slow growth rates, and the thin shell may make the tortoise more vulnerable to predators.

What We Know About Elemental Toxicity:

1. Tortoises ill with mycoplasmosis at the Desert Tortoise Research Natural Area had mercury levels in the liver that were 11 times higher than in healthy control tortoises from Ivanpah Valley.

What We Suspect About Elemental Toxicity:

1. Necropsy data indicate that a wide range of potentially toxic elements are found in elevated levels in desert tortoise tissues in California.
2. Based on necropsy data and using analyses of kidney, liver, and scute tissues: ill tortoises with a variety of diseases (infectious diseases, URTD, urolithiasis, metabolic disease, shell diseases, etc.) have statistically significantly higher levels of a variety of potentially toxic elements than do healthy tortoises.
3. No one single element or group of known or potentially toxic elements is found at elevated levels in the tissues of ill and dying tortoises. Instead, various such elements can be found both at the same and different sites. Arsenic is one of several elements found in ill tortoises at elevated levels.
4. Elemental toxicity may compromise the immune system of tortoises or otherwise detrimentally affect physiological functions, rendering them more susceptible to disease.

What We Know About Tortoise Nutrition and Its Relationship to Disease

1. Nutritional physiology: Many desert plants contain more potassium than tortoises can excrete in fluid urine based on the water content of these plants.
2. Tortoises produce urate salts from protein breakdown to excrete excess potassium, but at the cost of diverting protein nitrogen from other uses.
3. Under captive conditions, tortoises are able to discriminate between potassium levels and avoid high potassium foods.
4. During high rainfall years, healthy adult tortoises gain in lean mass (which is largely water and protein), but in years of no or low rainfall female tortoises lose lean mass and apparently in negative nitrogen balance (net loss of body protein).

5. In years of high winter/spring rainfall, both the diversity and biomass of annuals and herbaceous perennials increases, providing tortoises greater opportunity for foraging choices.
6. Although tortoises sample a wide range of plants, in high rainfall years they may feed predominantly from a few species, even if these are scarce.
7. These targeted plant species in high rainfall years are mostly of high quality, as measured by the Potassium Excretion Potential (PEP) index, resulting in an ingested diet of high quality.

What We Suspect About Tortoise Nutrition and Its Relationships to Disease

1. The negative nitrogen balance of tortoises in years of no or low rainfall represents a nutritional stress.
2. Hatchling tortoises exhibit high mortality once exposed to *Mycoplasma*, even if initially on a high plane of nutrition.
3. Periodic access to high PEP plants is essential to long-term nitrogen balance, reproductive performance and juvenile growth.
4. Removal of high PEP plants by grazing livestock may adversely affect the PEP quality of the diet eaten by tortoises in spring.
5. What is the role of nutrition in the epidemiology of URTD?
6. Is cutaneous dyskeratosis a consequence of nutritional deficiency?

RESEARCH PRIORITIES: WHAT WE NEED TO KNOW

NOTE: We have included all the items previously listed as “What we need to know about...”.

Handling and Protocols

1. Develop range-wide protocols for use of radiotransmitters. Standardize all aspects of transmitter use, including epoxy type and color, application sites (location on carapace), maximum battery size, etc. The standardization should include the appropriateness of different techniques by habitat type, size, sex, and age. The effects of transmitters on survivorship should be determined.

Basic Research on Immunology

1. The immune system of desert tortoises needs to be defined.
2. The effects of seasonal variation on immune responses need to be determined.
3. The effects of stress—nutritional, environmental, and research-induced (handling)--on immune function should be investigated.
4. The question of whether gender influences immunity against infectious agents should be investigated.
5. How do nutritional and environmental stress, handling, gender, and other factors influence immunity against infection agents?
6. Do markers of immune function exist, and can the markers be used to evaluate the status of a tortoise population?

General Information on Healthy and Ill Tortoises: What We Need To Know About Ill Tortoises In General

1. We need to identify “threshold values” for certain laboratory tests that alert biologists or veterinarians to disease, even if they are not specific for a particular disease. These values will differ by season. The most likely analytes will be BUN, bile acids, heterophil counts, and possibly aspartate aminotransferase(AST).

Concurrently, advances are being made in validating certain methods (e.g., white cell counts) and in automated equipment for various tests. As technology improves, it will be important to use the latest and best available equipment.

2. The cause(s) of oral lesions needs to be determined histopathologically and by electron microscopy.

3. We need to better understand what occurs with the liver when tortoises are ill or stop eating or both: What is normal versus abnormal hepatic function? What factors are causing or contributing to atrophy, lipidosis, hemosiderosis? Are these changes reversible? Are there ways of supplementing diets that will help prevent or reverse the changes? How do liver changes contribute to mortality?
4. We need to better appreciate, recognize, and evaluate specific clinical signs of disease in tortoises with URTD, shell diseases, herpesvirus, metabolic disease, and with two or more of these diseases. Although clinical signs are fairly well described, interpretation can be difficult when more than one disease is present.
5. We need to better define and understand the nature of the defect(s) present in shell disease.
6. We need more salvage of ill, dying, and recently dead tortoises to determine causes of illness and death in individuals and at the population level. More effort needs to be focused on viruses as potential pathogens contributing to ill health.
7. New technologies, such as a rapid, in-field diagnostic test to determine threshold values for laboratory analytes that alert biologists/veterinarians to ill health (e.g., BUN, bile acids, heterophil counts, and AST) need to be developed. There are currently in existence some non-invasive blood analyzers that can detect blood chemicals, such as glucose, directly through the skin without the need for drawing blood, and which can measure them continuously. We need to apply these new technologies for use in tortoises where possible.
8. After shell disease(s) are better defined, a rapid diagnostic test to assess shell disease would be useful—a test that does not require a biopsy, perhaps a chemical that could be placed on the shell with a color change indicating the degree of integrity or abnormality in the keratin.
9. Research should be conducted on effects of nutritional supplementation for tortoises ill with the different diseases in experimental settings. Can disease processes be reversed with good nutrition and water?
10. Research needs to be conducted on the susceptibility of tortoises on different diets to infection/disease under experimental conditions to determine the role of diets in general health and disease.

Infectious Diseases: Mycoplasmosis

1. We do not know if antibodies to *Mycoplasma agassizii* or *M. testudinum* (new species, formerly temporarily named *cheloniae*), occur in equal concentrations in lymph and in plasma. This finding is of major concern for seroepidemiological studies and is of high priority. This may need to be tested with necropsy cases.
2. The stability of antibodies in plasma needs to be determined at different temperatures, to ensure protocols for shipment and handling in the laboratory are appropriate.

3. The effectiveness of using the existing ELISA test for *M. agassizii* to determine presence of antibodies to *M. testudinum* (new species, formerly temporarily named *cheloniae*) needs to be determined. We suspect that these two species of *Mycoplasma* will not cross-react and that a second ELISA test will be necessary.
4. The pathogenesis and mode(s) of transmission for *M. testudinum* (new species, formerly temporarily named *cheloniae*) need to be determined.
5. Research to determine if vertical transmission occurs in *Mycoplasma agassizii* and *M. testudinum* (new species, formerly temporarily named *cheloniae*) is critical for many reasons, including translocation and semi-wild breeding programs. Sample sizes from preliminary studies were too limited to provide conclusive results; furthermore, the experimental research protocols were not designed to address the subject in depth. If vertical transmission occurs, we need to know the frequency of occurrence. Since the frequency of vertical transmission may be low ($\leq 5\%$), it is important to use large sample sizes in experiments.
6. We need to determine if tortoises can have both *Mycoplasma agassizii* and *M. testudinum* (new species, formerly temporarily named *cheloniae*) simultaneously, and if so, the impact on health and mortality rates of the host (*Gopherus agassizii*).
7. The presence of other strains/species of mycoplasma, such as those found in the gopher tortoise in Florida, needs to be determined through much more thorough sampling of tortoises with nasal lavages/cultures in CA, NV, AZ, and UT. Such sampling has been minimal in NV, AZ, and UT.
8. We need to know whether nutritional status contributes to susceptibility and response to mycoplasmosis and whether specific management actions can mitigate these effects.
9. We need to know what factors might affect differences in susceptibility to, morbidity from and mortality-related to mycoplasmosis/URTD in Mojave and Sonoran tortoise.
10. We need to know the role of environmental conditions (availability of food and water, anthropogenic stressors) on susceptibility of tortoises to URTD.
11. The recovery capabilities of captive tortoises with clinical signs and laboratory confirmation of URTD should be assessed, particularly if provided an improved diet. Are ill tortoises able to recover simply by diet and without antibiotic and other interventions? How is reproduction affected by disease? Long-term studies need to be conducted.

Infectious Diseases: Herpesvirus. Many of the subjects mentioned above for mycoplasmosis are applicable to research on herpesvirus infections.

1. Isolates of herpesvirus from desert tortoises must be obtained and the pathogenesis and modes of transmission need to be determined. The existing ELISA test needs to be validated, that is, determine if it is actually measuring levels of antibodies in the desert tortoises similar to what has been found in European species of tortoises.
2. We do not know the effect of herpesvirus on health of wild desert tortoises.
3. We need to determine whether herpesvirus and mycoplasmosis can interact in desert tortoises and the combined effects of both these diseases on health and mortality rates.
4. We do not know how long herpesvirus can exist in the wild and under different kinds of conditions, e.g., fomites in burrows.
5. We need to determine whether herpesvirus is transmitted horizontally and/or vertically.
6. Range-wide and local epidemiological studies are essential, coupled with collection of data on clinical signs of disease and mortality.
7. We need to know about the latency of the disease and latency-associated markers.
8. We do not know whether infected tortoises ever clear the infection. It is possible, as with other herpesviruses in other animals, that the tortoises will be infected for life.

Shell Diseases

1. We need to determine causes and contributors to cutaneous dyskeratosis and other shell diseases and how the diseases affect different organ systems in the body.
2. As part of research on shell diseases, compare carapace to plastron (physiological). Conduct laboratory investigations into anatomy/physiology/environmental factors (e.g., moisture, humidity) to try and determine why cutaneous dyskeratosis more significantly affects the plastron than the carapace.
3. We need to know the epidemiology of the disease in populations throughout the geographic range, and how the disease may contribute to elevated mortality rates.
4. We need to know if shell diseases are affected by nutrition and how.
5. We need to know relationships between drought, precipitation levels, humidity and moisture in cover sites and shell diseases.
6. We need to know if elemental toxicity is a contributing factor. The relationships to elemental toxicity may be determined through feeding experiments with captive hatchling tortoises.

7. We need to know if alkaloid poisoning (from alien mustards, for example) plays a role.
8. The potential for recovery of tortoises with shell diseases needs to be determined through controlled, experimental, nutritional treatments.

Elemental Toxicity

1. Research on effects of potential toxicants and contaminants on the desert tortoise needs to be continued. Research needs to be correlative as well as experimental in nature.
2. Elevated levels of some potentially toxic elements are positively correlated with illness in some tortoises. We need to know the sources of the elements and pathways in the environment on a local and regional basis.
3. We need to know the concentrations of elements that are likely to have short-term and long-term deleterious effects on tortoise health, especially if elements are in combination. We need to know if age-related or gender-related sensitivities and susceptibilities exist.
4. We need to know the relationships between nutritional status in the short- and long-term and elemental toxicity, including cumulative effects.

Nutrition

1. Initial observations and a preliminary study indicate that juvenile and adult tortoises on a low plane of nutrition are more likely to exhibit signs of UR TD after exposure to *Mycoplasma*. This work needs to be confirmed by a controlled, prospective study.
2. Research needs to be conducted on a local and regional scale to determine if tortoises face a chronic shortage of PEP plants, and if so, how can the chronic shortage be altered constructively by habitat management? Is, for example, a deficiency in PEP plants caused in part by the invasion of alien plant species?
3. Research on the availability of nitrogen in the diet is important. Do tortoise populations suffer from chronic nitrogen insufficiency, and if so, is this due to changes in diet in the past century? What were the sources of nitrogen in the past versus today?
4. Are outbreaks of UR TD in wild populations correlated to chronic nutritional stress, and if so, how do we measure this?
5. What is the role of nutrition in the epidemiology of UR TD, herpesvirus, and shell diseases?
6. Is cutaneous dyskeratosis a consequence of nutritional deficiency?

Translocation

1. Assess existing Large Scale Translocation Site Population at Jean, Nevada. Conduct detailed health assessments (following Berry and Christopher); gather data using blood samples for *Mycoplasma* and herpesvirus and conduct nasal lavages for cultures; record data on shell disease(s).

Other Related Subjects

1. Assess genetic variability, including genetic variability in immune systems. Little is known about the genetic variability between and within tortoise populations. Such knowledge is essential in planning for head-starting, translocations, and possible captive rearing.
2. Conduct research on stressors, specifically how to measure stress and its consequences. At this time, published data are available on effects of environmental changes (seasonal changes, drought, high precipitation levels), but not necessarily on effects of anthropogenic disturbances.
3. Investigate effects of obscurants use by the military including the use of chemicals / smoke to provide realistic training. What effects do these chemicals have on the respiratory tract of tortoises (as well as skin, plants in the area, soil, etc.)

RECOMMENDATIONS OF PRIORITIES FOR MANAGEMENT ACTIONS:

NOTE that Management priorities included, but were not limited to:

1. Protection of habitat and reduction of ground disturbing activities are critical, because undisturbed habitats help to keep key forage plants healthy and intact; replenish seed banks; and reduce dust which can spread toxicants and have negative effects on mucous membranes. Protection also includes fencing.
2. Eliminate sheep and cattle grazing in desert tortoise habitat, particularly in years of high rainfall. Oftedal (et al) determined that tortoises need high PEP plants to survive, even if these plants are only available in good years to give tortoises chance to fuel up during wet years. Livestock also utilize most of these same plants. If grazing cannot be eliminated, remove livestock earlier in spring, summer, and fall during germination and sprouting, growth, flowering, and fruiting of PEP plants.
4. Focus revegetation efforts on high PEP plant species. Certain plants (mostly, if not all, annuals) contain a higher PEP index (potassium excretion potential). These plants should be a focus of revegetation efforts in critical habitats. Revegetation efforts need to be accompanied by small research programs to determine if they will work and are worth the effort.

5. Identify monitoring sites for toxicants and pesticides. Evaluate sites before surface disturbance is allowed, where possible. Set up monitoring stations to monitor such sites as dry lake beds, mining areas, and roads.
6. Continue assessment of impact of desert tortoise habitats by invasive plant species.
7. Prohibit/curtail the release of captives. Release of captives contributes to the spread of new infectious diseases because captives are significantly more likely to have one or more infectious diseases, some of which have not yet been described or identified, than wild tortoises.
8. Develop an Emergency Response Team and Funds for when population declines and outbreaks of diseases are first seen, such as at the Red Cliffs Reserve. Funds would be used for salvage of tortoises and determination of disease type(s); isolation of either healthy or infected populations; fencing to protect healthy populations; and other urgent needs.
9. Increase/Focus Law Enforcement efforts in specific areas. Reportedly and historically, captive releases occur more frequently at the Desert Tortoise Natural Area, Joshua Tree National Park and near desert towns. Poaching tends to occur more frequently in certain areas, as does mortality caused by firearms. Increasing and/or focusing law enforcement operations in these areas will likely have a better benefit based on effort.
10. Train more biologists to conduct full health assessments (Berry and Christopher, 2001). The training needs to include drawing blood and conducting the nasal lavages for cultures.
11. Salvage more ill and dying tortoises from all populations throughout the geographic range in the United States for determination of causes and contributors to death. Obtain range-wide salvage permits, possibly segregated by state, for this work. Researchers may need more flexibility from Fish and Wildlife Service in dealing with salvage. At present, in California, only Dr. Kristin Berry and sub-permittees are able to conduct salvage.
12. Empanel a group to discuss potential translocation sites for the proposed expansion of the National Training Center (NTC), Fort Irwin. Animals currently living in the proposed NTC expansion area will likely be translocated. A panel to discuss where they could / should be moved needs to be created. Participants from other sciences need to participate, so this suggestion was tabled. A very broad-based and long-term study (> 5 years) needs to be conducted as part of the translocation.
13. Existing protocols for handling tortoises need to be revised periodically to incorporate new and better methods to reduce stress. For example, existing protocols need to incorporate methods to reduce voiding of bladders. While some scientists/Pis/field staff are already doing this, protocols are not necessarily being followed by all parties.

14. Incidental take permits need to be improved to include tracking of data on health, disease, and mortalities. Incidental take permits should include costs of necropsies for tortoises killed during and related to the project.
15. Continue research on study plots to track trends on health and diseases, mortality, and status of populations.
16. Priorities need to be established for management of tortoises at the Nevada Desert Tortoise Conservation Center. Priorities include, in order:
 - (a) Adoption of healthy animals only. The tortoises need to be comprehensively tested for the presence of URTD, herpes, and other infectious and non-infectious diseases using every test available, including ELISA and PCR tests and cultures.
 - (b) Healthy animals that cannot be adopted and sick or injured animals ineligible for adoption should be considered for research experiments (i.e., transmission studies, challenge studies, toxicological studies, nursery-hatchery experiments. In many cases, animals to be used in such experiments will have various health requirements that must be met.
 - (3) Euthanasia should be considered as a last resort for unadoptable tortoises when resources (i.e., space, funding) are limited or exhausted. Our use of the word unadoptable here includes both healthy and unhealthy animals.

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