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science, education, policy, and environmental law

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RE: Application for Certification for the Ivanpah Solar Electric Generating System Docket No. 07-AFC-5: Comments on Staff Report for the Preliminary Staff Assessment December 2008 (07-AFC-5) CEC-700-2008-013-PSA – Ivanpah Solar Electric Generating System and the Draft Desert Tortoise Translocation/Relocation Plan

Dear Mr. Kessler,

The Center for Biological Diversity ("Center") is a non-profit environmental organization dedicated to the protection of native species and their habitats through science, policy, and environmental law. The Center has over 60,000 members throughout California and the western United States, including members that live nearby the vicinity of the proposed Ivanpah Solar Electric Generating System (ISEGS) and recreate there. On July 2, 2009, the Center was granted leave to intervene in this proceeding. The Center submits these comments regarding the December 2008 Staff Report for the Preliminary Staff Assessment ("PSA") and the Draft Desert Tortoise Translocation/Relocation Plan on behalf of our board, staff and members.

I. INTRODUCTION

The development of renewable energy is a critical component of efforts to reduce greenhouse gas emissions, avoid the worst consequences of global warming, and to assist California in meeting emission reductions set by AB 32 and Executive Order S-03-05. The Center strongly supports the development of renewable energy production, and the generation of electricity from solar power, in particular. However, like any project, proposed solar power projects should be thoughtfully planned to minimize impacts to the environment. In particular, renewable energy projects should avoid impacts to sensitive species and habitat, and should be sited in proximity to the areas of electricity end-use in order to reduce the need for extensive new transmission corridors and the efficiency loss associated with extended energy transmission. Only by maintaining the highest environmental standards with regard to local impacts, and effects on species and habitat, can renewable energy production be truly sustainable.

The current site proposed for this project in the Ivanpah Valley is relatively devoid of human disturbance except for a few dirt roads and the existing Southern California Edison (SCE) transmission lines and associated structures. We concur with the Preliminary Staff Assessment which states, “The ISEGS project would have major impacts to the biological resources of the Ivanpah Valley, significantly affecting many sensitive plant and wildlife species and eliminating a broad expanse of relatively undisturbed Mojave Desert habitat.” PSA at pg.1-9.

Significant impacts have been identified for a suite of species (PSA pg 5.2-1), including the federally and state listed threatened desert tortoise, and rare plants including Rusby’s desert-mallow, cave evening-primrose, Mojave milkweed, and desert pincushion, and negative impacts to numerous other rare plants and animals. Additionally, over 2000 ephemeral drainages covering 198 acres of waters of the state would be impacted by the ISEGS on the proposed site. The following comments address those issues:

II. COMMENTS ON THE DECEMBER 2008 PSA AND DRAFT TORTOISE RELOCATION PLAN

A. The Alternatives Analysis Outlined in the PSA Fails to Comply with CEQA or NEPA

Pursuant to CEQA, the “policy of the state” is that projects with significant environmental impacts may not be approved “if there are feasible alternatives or feasible mitigation measures available which would substantially lessen the significant environmental effects…” Pub. Res. Code § 21002; CEQA Guidelines § 15021(a)(2). A Project should not be approved if environmentally superior alternatives exist “even if these alternatives would impede to some degree the attainment of the project objectives, or would be more costly.” CEQA Guidelines §§ 15021(a)(2), 15126.6; Pub. Res. Code § 21002. The Project must be rejected if an alternative available for consideration would accomplish “most [not all] of the basic objectives of the project and could avoid or substantially lessen one or more of the significant effects.” CEQA Guidelines § 15126.6(c).

Accordingly, the environmental review documents must consider a range of alternatives that would achieve the basic objectives of the project while avoiding or substantially lessening significant environmental effects, and it is essential that the “EIR shall include sufficient information about each alternative to allow meaningful evaluation, analysis, and comparison with the proposed project.” CEQA Guidelines § 15126.6(d). Alternative sites must also be considered where relocating the project would substantially lessen the significant impacts of the project. Guidelines Section 15126.6(f)(2). *See Citizens of Goleta Valley v County of Santa Barbara* (1988) 197 Cal.App.3d 1167, 1178; *Save Round Valley Alliance v. County of Inyo* (2007) 157 Cal.App.4th 1437, 1456 (whether an alternative site may be feasible even where it requires a change in land use designation; to determine feasibility requires detailed analysis of the alternatives; and even if an alternative is less profitable than the project as proposed it may still be a feasible alternative).

NEPA similarly requires that a range of meaningful alternatives be explored in the environmental review process. 42 U.S.C. §§ 4332(C)(iii),(E). The agency must "study, develop, and describe appropriate alternatives to recommend courses of action in any proposal which involves unresolved conflicts concerning alternative uses of available resources." 42 U.S.C. § 4332(2)(E). This requirement applies whether the agency undertakes an environmental assessment ("EA") or an environmental impact statement ("EIS"). 42 U.S.C. § 4332(2)(E); *see* 40 C.F.R. §§ 1501.2(c), 1508.9(b). In addition, pursuant to the BLM's CDCA plan, impacts to wildlife from conflicting land uses should be avoided. CDCA Plan at 28. Impacts to BLM sensitive plant species, such as the Rusby's desert mallow, should also be avoided. CDCA Plan at 37. Avoidance can best be accomplished through alternative project siting and/or project design.

Most importantly in this instance, the EIR/EIS must look at alternative sites that could avoid impacts to desert tortoises and essential desert tortoise habitat, and which would avoid any need to move the tortoises off their native habitat – a so-called mitigation measure that in practice has proved to be a disaster for the species. The EIR/EIS should also fully explore other alternatives that would achieve the same level of renewable energy production—the basic objective of the project—but without the significant impacts of the proposed project.

While the PSA provides some review of alternative sites, we do not believe that the agency has as yet adequately explored alternative sites. This is evidenced by the fact that both alternative sites that were discussed in any detail—Siberia East Alternative and Broadwell Lake—would have similar impacts to biological resources as the proposed project. PSA at 7-2. Simply looking at sites with similar impacts as the proposed project does nothing to fulfill the agency's duty under CEQA. It strains credulity to believe that there are no other sites in California where the valid project objectives could be accomplished without lessening the significant impacts. Alternatives sites on previously disturbed lands, for example, should be explored including areas that were previously heavily disturbed for farming that has since been abandoned or reclaimed and un-reclaimed mining sites in the California desert. In addition, the Sierra Club recently proposed that an alternative site within the Ivanpah Valley closer to the highway be considered. To the extent that such an alternative site may limit some impacts it should also be explored as part of a meaningful range of alternatives. The agency is charged with considering alternatives to avoid and minimize impacts, it cannot lawfully fulfill this duty based on the limited alternatives analysis presented in the PSA.

In addition, by limiting the alternatives sites evaluated in detail in the PSA to sites on BLM lands with pending applications from this same project applicant (PSA at 7-9), the PSA fails to consider all feasible alternatives. Also of particular concern is the PSA's rejection of all alternatives sites on private lands that have previously been disturbed and which would therefore avoid many of the significant biological impacts of the proposed project. These alternatives were eliminated not because they are infeasible but because they would be "challenging." PSA at 7-65. This is unacceptable. The PSA analysis is deficient because it failed to meet the requirements of CEQA as outlined in *Preservation Action Council v City of San Jose* (2006) 141 Cal. App. 4th 1336. In *Preservation Action Council*, the Respondent lead agency relied heavily on the Real Parties' project objectives and the EIR rejected a smaller alternative that would have met all project objectives except for size, and would have been environmentally superior. *Id.* at 1355. The Court rejected the EIR finding that it did not meet the information requirements of CEQA because the inadequacies in the EIR's analysis "meant that the public and the City Council were not properly informed of the requisite facts that would permit them to evaluate the feasibility of this alternative." *Id*. The PSA draft provided to date is similarly deficient.

The PSA provides a basic description of the objectives of the project (PSA at 7-6 to 7-7), but it then unreasonably narrows the objectives used to consider the viability of alternatives and unreasonably includes timing of the environmental review as a basic objective of the project. PSA at 7-7. Given that the staff has stated that the applicant has to date failed to complete necessary studies and provide other information needed for the environmental review (*see, e.g.*, Status Report #7 filed March 4, 2009, at 1, 4-5; *see also* PSA at 5.9-11 (applicant has not provided information on water needs), the timing of the environmental review cannot fairly be used as a "basic objective" of the project such that it limits the consideration and evaluation of alternatives that would avoid significant impacts to environmental resources of California. Indeed, to the contrary, it appears from the available documents filed to date that the applicant has thus far been unable to provide the complete surveys and information regarding the impacts to the desert tortoise, which indicates that this site may be inappropriate for such a large-scale industrial development project. This further underscores the need for the agency to comprehensively explore a range of alternative sites that will avoid these and other significant impacts of the project.

Similarly, including "high solarity" and ground slope as basic objectives of the project (PSA at 7-7) provides an overly narrow view of the alternatives of the project. The basic objectives of the project are to provide 400-MW of renewable power in California. This goal can be met in a number of ways by feasible alternatives that would avoid impacts to the desert tortoise and intact habitat, rare plants, water resources, and waters of the state. While "high solarity" may be necessary for the type of large-scale solar thermal plant that the applicant prefers to build, the added costs and energy losses from transmission may make it more cost effective to locate a solar power generating facility closer to the point of use in the cities such as Los Angeles and San Diego which have significant "solarity" even if it is not the very highest amount. In evaluating this factor the agency should assess whether re-use of disturbed sites near existing population centers could both meet the project objectives and avoid many of the significant environmental impacts of the project including impacts to rare and endangered species. Given the economic set-backs in the past year, there are more and more large-scale industrial areas that are under-utilized in many parts of southern and central California. These industrial parks, malls and auto rows long ago replaced native habitat, they are connected to the power grid, and are readily accessible to workers. Converting these areas to solar centers is a feasible alternative that would have many societal benefits (including maintaining robust economic zones and avoiding urban blight) and would avoid nearly all of the environmental impacts of siting this project in intact desert tortoise habitat in the Mojave desert. Accordingly, the EIR should also explore the use of distributed smaller-scale solar as an alternative.

B. Additional Analysis is Needed to Assess All Impacts that Require Avoidance and Minimization

Even if the Project is eventually approved to go forward at the Ivanpah site, significant impacts must be avoided to the extent feasible and minimized. Some impacts that were not fully analyzed in the PSA that will need to be avoided or minimized and mitigated include growth-inducing impacts, fire risk, and habitat fragmentation.

Growth-Inducing Impacts: CEQA requires environmental analysis to consider the ways in which the proposed project could foster economic, housing, or population growth, whether directly or indirectly in the surrounding environment. Guidelines § 15126.2(d); *see also* 14 Cal. Code Regs § 15358(a)(1) ("Indirect or secondary effects may include growth-inducing effects and other effects related to induced changes in the pattern of land use, population density, or growth rate, and related effects on air and water and other natural systems, including ecosystems."). The Guidelines specifically require that the EIR should "discuss the charateristics of [] projects which may encourage and facilitate other activities that could significantly affect the environment, either individually or cumulatively." Guidelines § 15126.2(d). Growth-inducing impacts from the proposed project in the Ivanpah Valley include encouraging additional large-scale solar projects to be sited in this same area and making it more likely that additional solar development projects could be approved in this same area. For example, the placement of one industrial project with a new powerline connection, substations, and/or new access roads may make it more likely that a second or third project will be sited in this area. Siting multiple projects in this area could lead to complete collapse of the habitat values in this valley due to habitat loss and fragmentation. This would be a significant change to an area which now contains a significant amount of contiguous, high value, intact habitat for the desert tortoise and other species. The need for additional analysis of the impacts from multiple solar projects that have pending applications in this area is discussed further below in the section on cumulative impacts.

Fire Risk: Because the ISEGS project is a solar concentrating thermal power plant, which is comprised of fields of heliostat mirrors focusing solar energy on boilers located on centralized power towers, the superheated fluids that are essential for the operation of the project will be primarily sequestered in and adjacent to the power towers/generation site. The superheated liquids are a fire threat, particularly if flammable materials, like vegetation, are located nearby. With the majority of the site comprised of heliostat mirrors, which only reflect and focus the sun's ray on the power tower and do not contain any superheated liquids, there may be opportunities to conserve some of the existing on-site vegetation and habitat, which would avoid and minimize the impacts to the existing biological resources. In fact, solar farms are including habitat features into their project design http://www.optisolarfarms.ca/sarnia.htm. This same type of solar technology already installed and producing solar energy in Spain includes vegetation between the mirrors http://www.solarpaces.org/Tasks/Task1/PS10.HTM . Despite the problems of habitat fragmentation, vegetation islands provide stepping stones for wildlife across the site and are an improvement over complete vegetation removal.

Potential Minimization by Preservation of Vegetation: One of the maintenance issues with the heliostat mirrors is dust deposition resulting in decreased efficiency. Maintaining native vegetation will help to keep the fragile soils in place on the project site, reducing the amount of soil particles that could get airborne and be deposited on the mirrors. This will in turn reduce the need for groundwater pumping to provide water to wash the mirrors. Leaving vegetation in place provides the additional benefit of not increasing the particulate matter (PM) in the already compromised Mojave Desert Air Quality Management District.

We urge CEC staff to more fully explore the benefits of maintaining vegetation and habitat on site for this proposed project in the Final Staff Assessment.

C. Desert Tortoise: Analysis of Impacts is Inadequate and the Translocation Plan is Unsupportable

The desert tortoise is continuing to decline throughout its range (USFWS 2008) despite being under federal and state Endangered Species Acts protection as threatened. Prior to 2002, the project area was designated by BLM as Category 1 habitat for desert tortoise – the best desert tortoise habitat. The Northern and Eastern Mojave Plan (BLM 2002) changed that designation, not based on any site specific science, but on the establishment of Desert Wildlife Management Areas (DWMA's) elsewhere.

New science has become available since our scoping comments for the federal process (CBD scoping comment letter dated November 30, 2007). Murphy et al. (2007) undertook extensive genetic analysis across the range of the desert tortoise and identified genetically unique populations within the larger listed population. The desert tortoise located on the Ivanpah site represent a unique genetic group – the northeastern Mojave group. This localized area around the Ivanpah area is the only location of this unique genotype in California. Because these animals represent such a unique occurrence in California, adequate avoidance, minimization and mitigation must be applied to this project. The uniqueness of this population is also recognized both in the 1994 Desert Tortoise Recovery Plan (USFWS 1994) and the draft Revised Recovery Plan (USFWS 2008) as the North Eastern Mojave Recovery Unit and the Murphy et al. paper only confirms the uniqueness of this population.

Additionally, the Scientific Advisory Committee of the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office has recently concluded that "translocation is fraught with long-term uncertainties, notwithstanding recent research showing short-term successes, and should not be considered lightly as a management option. When considered, translocation should be part of a strategic population augmentation program, targeted toward depleted populations in areas containing "good" habitat. The SAC recognizes that quantitative measures of habitat quality relative to desert tortoise demographics or population status currently do not exist, and a specific measure of "depleted" (e.g., ratio of dead to live tortoises in surveys of the potential translocation area) was not identified. Augmentations may also be useful to increase less depleted populations if the goal is to obtain a better demographic structure for long-term population persistence. Therefore, any translocations should be accompanied by specific monitoring or research to study the effectiveness or success of the translocation relative to changes in land use, management, or environmental condition." (SAC 2009). Translocation should be used as a tool to augment populations within depleted recovery units, not as a mitigation strategy to allow for development in desert tortoise habitat.

The project fails to evaluate as an alternative or as an avoidance measure moving the project site from its proposed location to the proposed desert tortoise relocation areas, which are identified in the *Draft Desert Tortoise Translocation/Relocation Plan*. Note is made in translocation/relocation plan, that the area adjacent to Interstate 15 already has a lower population of desert tortoise, therefore impacts to the species would be reduced, the cost of implementation of any translocation/relocation that would need to be done, and tortoises that would have to be moved, would be moved into habitat that should be less affected by global climate change. Selecting a better site for project implementation that avoids, and minimizes the impacts to the environment is required under CEQA.

Draft Desert Tortoise Translocation/Relocation Plan for the Ivanpah Solar Electric Generating System: As noted in the PSA, at least 25 desert tortoises currently utilize the site. Translocation is proposed as the primary methodology for minimizing and mitigating impacts to these animals but the costs of such a project in terms of monetary outlays for the translocation, short and long term monitoring and surveys, are not discussed in the PSA. More importantly, the cost to the species of the translocation is not addressed in the PSA. For example, translocation is documented *at best* to have an 80% maximum success rate on small scale translocations (Dodd and Siegel 1991, Field et al. 2007). Indeed, the recent translocation project at Fort Irwin has already documented over 22% mortality *in the very first year* with 147 confirmed deaths out of 647 tortoises moved.

If translocation is approved for use as in this instance, the agency should carefully review the Desert Tortoise Recovery Plan (USFWS 1994) which provides seven recommendations for translocating desert tortoises. While the guidelines from the 1994 Recovery Plan are included in the Draft Translocation/Relocation Plan as Appendix B, several of these guidelines are not implemented within the Draft Translocation/Relocation Plan. Not only does the Draft Translocation/Relocation Plan fail to implement the recommendation in the Recovery Plan, it also fails to implement the Guidelines For Clearance And Translocation Of Desert Tortoise From The Ivanpah Solar Electric Generating System (ISEGS) Project dated 12/12/2008, which is U.S. Fish and Wildlife Service's guidelines included in Appendix A.

With regards to the Desert Tortoise Recovery Plan (1994) the project fails to include the following:

- Recommendation #2. The Draft Translocation/Relocation Plan fails to implement adequate monitoring to in fact, confirm that desert tortoise "establish home ranges and

- Recommendation #3. At least temporary fencing should be included in the relocation areas as well, due to the well documented fact that desert tortoises will try to return to their home range. Additionally, no provisions to deal with the fact that desert tortoises will end up along the new tortoise proof fences of the project site, trying to get back to their home territory, are included in the Draft Translocation/Relocation Plan. This behavior leaves them vulnerable to predation, and needs to be addressed.
- Recommendation #5. While FWS indicates that the density of desert tortoise should not exceed 39 animals/square kilometer, according to their guidelines, that is based on the Fort Irwin translocation, which has had a higher than normal death rate. Also, Fort Irwin is within a different Recovery Unit than the ISEGs project. Because significant numbers of carapaces were also identified on site, it may be more appropriate to determine the historic carrying capacity based on estimates of the carapaces and live animals. This number would more realistically reflect the recent carrying capacity of the landscape for desert tortoise. Additionally, in light of global climate change and the predicted warming of the desert, translocation zones should only be located at *higher* elevations, not lower parts of the Ivanpah Valley.
- Recommendation #6. The “latest available technology” to determine desert tortoise health for Upper Respiratory Tract Disease (URTD) is the Eliza test for the mycoplasmas. However, the Draft Translocation/Relocation Plan only proposes looking for symptomatic signs of infection. The Fort Irwin translocation required that all desert tortoise to be translocated be checked for exposure to disease, and those that tested positive were removed from the population in order to prevent disease spread. In addition, the host population was also tested for disease. In order to prevent an epidemic outbreak of disease, unhealthy animals should not be moved into healthy populations nor should healthy animals be moved into unhealthy populations, yet the Draft Translocation/Relocation Plan fails to acknowledge or test for this simple epidemiological issue. Translocation concentrates desert tortoise into higher densities, where diseases could be more problematic. Therefore identification of diseased animals and minimizing outbreaks of disease needs to be more fully addressed in the Draft Translocation/Relocation Plan.
- Recommendation #7. A two-year study should be undertaken on the host population, which the Draft Translocation/Relocation Plan fails to include.

On page 3 of the document, the applicant fails to incorporate the basic requirements put forth in Appendix A from U.S. Fish and Wildlife Service, which state that desert tortoise proof fencing will be necessary along I-15 and must be provided by the applicant. However in the Draft Translocation/Relocation Plan, the applicant suggests that CalTrans may be the entity putting in desert tortoise fencing. Relying on CalTrans' mitigation, presumably for the Joint Port of Entry project, fails to relieve the project applicant of their mitigation responsibilities for impacts to a federally and state listed threatened species. Additional adequate mitigation must be identified.

Under Transporation and Release, the Draft Translocation/Relocation Plan proposes that "Relocated tortoises would not be placed in existing occupied burrows" (at pg. 4). Desert tortoise generally have multiple burrows within their home ranges, which could all be considered to be "occupied", even if the animal is not always present (Luckenbach 1984). Greater clarification needs to be included on how the translocated tortoises will be kept from using on-site burrows, based on the fact that they will be moved into existing home ranges of other tortoises.

In that same section (at pg. 5), reporting is to be provided to the BLM. In addition, the U.S. Fish and Wildlife Service and the California Department of Fish and Game also need to have these reports submitted to them as the permitting agencies.

The Draft Translocation/Relocation Plan fails address all potential predators, and focuses almost exclusively on ravens. Ravens predate primarily on young tortoises, while the high level of mortality from the Fort Irwin translocation came from canids. A more comprehensive anti-predation strategy needs to be included.

In accordance with the guidelines provided by U.S. Fish and Wildlife Service (in Appendix A), "Brightsource must perform all clearance survey and translocation procedures for any portion of the project site during the spring (i.e., March-May) or fall (i.e., late August to early October) to avoid extreme temperatures". In other words tortoises should not be moved during the winter as proposed in the Draft Translocation/Relocation Plan on page 5.

Because translocation/relocation is still an experimental procedure, the proposed monitoring is inadequate to fully evaluate the even the short-term success of the desert tortoise translocation/relocation, and falls short of the guidelines that U.S. Fish and Wildlife Service provide in Appendix A. Three years of monitoring is inadequate to evaluate if the tortoises have successfully established home ranges, much less integrated into the social structure of the existing population. The goal as established in the Recovery Plan (1994) is to have the translocated/relocated tortoises integrated into the population *reproductively,* so that genetically, they continue to add to the diversity of the species. Three year of monitoring is wholly inadequate to evaluate if the tortoises have achieved this essential success criteria. We recommend that a minimum of fifteen years of monitoring of the translocated/relocated and host tortoises be required, or until unequivocal proof is acquired that shows reproductive success between the host and translocated/relocated populations (desert tortoise females can store sperm in excess of two years – Palmer et al. 1998)

Karl (2007) applied an intensive and thoughtful small-scale translocation effort, which included mapping all tortoise home ranges of both the translocated and host populations prior to translocation. Translocated tortoises were moved onto a site where their home ranges were mimicked as much as possible, including construction of burrows at appropriate locations, moving "neighborhoods" of tortoises in-tact so that the translocated animals would run into their known neighbors, fencing the translocation site, canid abatement, etc.

If habitat is left on site, as described in the avoidance and minimization section above, the EIR should also explore whether some tortoises could be left on site in the remaining habitat. Desert tortoises are known to successfully survive and reproduce in industrially altered landscapes (Lovich and Daniels 2000). Clearly, a comprehensive program on desert tortoise avoidance would also need to be developed and implemented if they were to be retained on-site, and that program needs to be included as part of the CEQA process.

In addition to the avoidance and minimization measures and translocation efforts, adequate mitigation at a rate of at least 5:1 to off-set the impacts to the desert tortoise is required, including acquisition of private lands in nearby desert tortoise habitat to be set aside as tortoise conservation areas. In order to accurately mitigate for the desert tortoise population that will be affected by the proposed project, the mitigation needs to occur within this same recovery unit, and as close to the proposed project site as possible. Additions to/expansions of the existing DWMA's and in other areas where a higher level of conservation for desert tortoise needs to put in place (ie. conservation as the highest priority), may also be a mechanism for required mitigation. Additions to/expansions of the Mojave National Preserve may also be appropriate.

D. Bighorn Sheep: Analysis of Impacts is Incomplete

Important native (i.e. not re-introduced) populations of desert bighorn sheep occur in the Clark, Mesquite and Spring mountains (Epps et al. 2004) adjacent to the ISEGS. Bighorn are a large and wide-ranging species that require connectivity across large landscapes in order to assure persistence. Existing anthropogenic barriers have already eliminated gene flow between certain populations (Epps et al. 2005). Elimination of sheep connectivity by ISEGS could lead to further isolation and inbreeding issues. Additional information on bighorn sheep movement corridors and the impact of development on them needs to be included. Avoidance of these areas needs to be included, or minimization and effective mitigation if the project actually could impact these important linkages.

Furthermore, no studies have been done on the effects that miles of mirrors may have on bighorn sheep movement or effects of their use of historical lambing areas. Data indicate that human caused disturbance negatively affects species fitness and population dynamics via the energetic and lost opportunity costs of risk avoidance (Frid and Dill 2002). More information about the potential impact from the installation and operation of mirrors on desert bighorn needs to be included.

Lastly, desert bighorn rely on springs and seeps, especially during the harsh summer months for their survival in the ranges adjacent to the proposed project site. Please refer to our water resources section pertaining to impacts to seeps and springs from the groundwater pumping proposed by the project, and please provide an analysis of the potential impacts to bighorn sheep.

E. Rare Plants: Analysis of Impacts and Avoidance Measures is Incomplete

As stated above, avoidance is the most preferred method to eliminate impacts to rare plants. Incorporation of design elements that would avoid rare plants should be fully investigated in the Final Staff Assessment, particularly for those species that are proposed to be significantly impacted including the Rusby's desert mallow (*Spheralcea rusbyi* var. *eremicola*), Cave evening primrose (*Oenothera cavernae*), Mojave milkweed (*Asclepias nyctaginifolia*), and Desert pincushion (*Coryphantha chlorantha*). Eleven additional rare plant species will also be impacted with 2-30% of their known populations proposed to be eliminated through project construction. The proposed site is also dense with cacti including approximately 6,400 barrel cactus.

Transplantation of rare plants has been documented to be mostly unsuccessful. Feidler (1991) found that mitigation-related transplantation, relocation and reintroduction projects involving endangered and threatened and rare plants was successful only 15% of the time. Reseeding of *Spheralcea rusbyi* var. *eremicola* (and other species) has occurred as part of on-site mitigation in the past (Hiatt et al. 1995) and met with at least short-term success (no monitoring was required by the project, but the authors voluntarily monitored for two years). Success for reestablishment was predicated numerous factors and were taxon specific.

Cacti have been successfully salvaged and replanted and that strategy where appropriate should be considered either on/off site.

If relocation is to be part of the mitigation effort, then a clear and concise relocation plan should be developed and included as supporting documentation in the Final Staff Assessment for public review. So many times these plans are proposed to be developed in the future, with no public input or review. We believe these plans should be included as part of the CEQA process and that their absence is a violation of CEQA. If plants are to be moved, requirements for interim monitoring during establishment (including triggers for adaptive management to meet the needs of plant survival) need to be put in place. Long-term monitoring for survivorship and successful reproduction and establishment also needs to be included as part of the mitigation requirements if relocation is a chosen strategy.

To assure conservation of the rare plants in addition to avoidance and minimization and mitigation presented above, seed collection and curation into a seed bank should be required, to preclude potential genetic loss of the species if the mitigation measures should fail.

If avoidance is not possible, then securing additional sites for conservation in perpetuity will be necessary. Focused surveys for locations outside of the project impact "footprint" must be done, and mechanisms must be put in place to secure those areas from future impacts. Actions such as eliminating grazing, establishing an extension to the DWMA in conjunction with higher levels of conservation, or an annexation into the Mojave National Preserve should all be reviewed.

F. Water Resources: Requires Additional Information and Analysis

Section 5.9 indicates that 76 to 149 AFY of water will be used to wash the mirrors and other site specific activities. Although no water will leave the site, additional information on the effects of groundwater pumping on nearby seeps and springs in the adjacent mountains. No data is presented that addresses the hydrological connection between these essential wildlife sustaining locations and the proposed project impacts.

Additionally, because of the substantial evaporation rate at the project site, please provide data on how much pumped ground water will actually be returned to the groundwater basin.

Waters of the State: The PSA indicates that 198.72 acres of Waters of the State, which include 1,973 ephemeral washes with a cumulative length of 291 miles of channels will need to be mitigated. Again we urge the CEC to look at avoidance and minimization of the impact through alternative siting.

If this site is ultimately approved for the project, because of the topography, water will still flow through the site. The project design must include measures to route water to minimize potential damage to the proposed infrastructure, while still allowing flow through to Ivanpah Lake, and supporting desert wash habitat. For example, the proposed configuration of the three solar sites should be revised to accommodate washes in the design stage.

As with the other sensitive resources, securing additional sites for conservation in perpetuity will be necessary, and may be accomplished in conjunction with sensitive species mitigations. Because the proposed project is relying on groundwater pumping as its water source, it is crucial to replicate the existing surface hydrology to enable groundwater replenishment, particularly with regards to the slow pace of groundwater recharge in the desert.

G. Cumulative Impacts are Not Fully Disclosed and Analyzed

Even before undertaking a fully adequate analysis of the cumulative impacts as outlined in the Cumulative Scenario, the PSA admits that there will be significant cumulative impacts from this project and concludes simply that there should be additional mitigation. PSA at 1-10. However, CEQA requires not only full disclosure of cumulative impacts but a full and fair effort on the part of the agency to first avoid such impacts, and then to ensure any remaining impacts are minimized and mitigated. Until the agency completes an adequate alternatives analysis, the staff conclusions that not all cumulative impacts can be mitigated are premature.

The cumulative impacts section needs to be updated to include more specific information regarding the Desert Xpress high-speed rail project. The Desert Xpress Draft Environmental Impact Report has been out for public review and presumably is moving towards finalization. One of the two proposed alternatives includes the ISEGS project site. Clearly there will be more impacts to the proposed desert tortoise relocation areas and potentially the translocation areas, if this alternative is selected.

Additionally, the cumulative impacts need to identify the impacts to desert tortoise by translocation and relocation efforts. As the other potential projects get implemented, it will push higher and higher numbers of desert tortoises into smaller and smaller areas. As proposed in the Draft Translocation/Relocation Plan, the translocation areas proposed for the ISEGs project will be effectively isolated if the Desert Xpress alternative is implemented. It will be surrounded by impermeable projects including the I-15 interstate to the east/south, the golf course to the north, the ISEGS to the north and west and the railway to the west, and therefore provides inadequate mitigation for the long-term survival of the species in this area. Additional development of other renewable energy projects in the northern part of the Ivanpah valley will also further isolate the existing population of resident, relocated and translocated desert tortoise in the northern and eastern recovery unit, even if the train proposal is not implemented in this area.

These same potential isolation issues due to the cumulative impacts of projects proposed in the Ivanpah Valley also need to be discussed for desert bighorn sheep. All of these cumulative impacts need to be included and analyzed in the final staff report.

III. CONCLUSION

For this and future proposed projects, mechanisms should be put in place that encourage solar facilities to be proposed and sited on disturbed lands instead of in fully ecologically functioning habitat such as is found in the Ivanpah Valley, which support a variety of rare and threatened species.

We hope and expect that the agency will carefully consider all meaningful alternatives and go beyond the admittedly "preliminary" information provided in the PSA. The agency should revisit these issues in detail and provide a full range alternatives as part of the Draft EIR for public review.

Thank you for the opportunity to submit these comments, and we look forward to participating in the project as the process moves forward. Please feel free to contact me for additional information at 535-654-5943 or at ianderson@biologicaldiversity.org

Best regards,

Ileene Anderson
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References (the following references are provided as attachments to this letter via email and on paper to the CEC and parties requesting mail service):

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Department of Interior

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BEFORE THE ENERGY RESOURCES CONSERVATION AND DEVELOPMENT COMMISSION OF THE STATE OF CALIFORNIA

1516 NINTH STREET, SACRAMENTO, CA 95814
1-800-822-6228 – WWW.ENERGY.CA.GOV

APPLICATION FOR CERTIFICATION
FOR THE *IVANPAH SOLAR ELECTRIC*
GENERATING SYSTEM

DOCKET NO. 07-AFC-5
PROOF OF SERVICE
(Revised 7/2/09)

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DECLARATION OF SERVICE

I, Lisa T. Belenky, declare that on July 8, 2009, I served and filed copies of the attached, CBD Comments on PSA and DT plan dated July 8, 2009. The original document, filed with the Docket Unit, is accompanied by a copy of the most recent Proof of Service list, located on the web page for this project at: **[www.energy.ca.gov/sitingcases/ivanpah]**.

The documents have been sent to both the other parties in this proceeding (as shown on the Proof of Service list) and to the Commission's Docket Unit, in the following manner:

(***Check all that Apply***)

FOR SERVICE TO ALL OTHER PARTIES:

✓ sent electronically to all email addresses on the Proof of Service list;

✓ by personal delivery or by depositing in the United States mail at ~~Sacramento~~ San Francisco, California with first-class postage thereon fully prepaid and addressed as provided on the Proof of Service list above to those addresses **NOT** marked "email preferred."

AND

FOR FILING WITH THE ENERGY COMMISSION:

✓ sending an original paper copy and one electronic copy, mailed and emailed respectively, to the address below (***preferred method***);

OR

______ depositing in the mail an original and 12 paper copies, as follows:

CALIFORNIA ENERGY COMMISSION
Attn: Docket No. 07-AFC-5
1516 Ninth Street, MS-4
Sacramento, CA 95814-5512
docket@energy.state.ca.us

I declare under penalty of perjury that the foregoing is true and correct.

Lisa T. Belenky

POINTS OF VIEW: A CONTROVERSY IN CONSERVATION BIOLOGY

EDITOR'S NOTE.—The following three papers constitute an essay by C. K. Dodd, Jr. and R. A. Seigel followed by two replies to the essay by, respectively, R. L. Burke and H. K. Reinert.

Herpetologica, 47(3), 1991, 336–350

RELOCATION, REPATRIATION, AND TRANSLOCATION OF AMPHIBIANS AND REPTILES: ARE THEY CONSERVATION STRATEGIES THAT WORK?

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ABSTRACT: Conservation strategies involving relocations, repatriations, and translocations (RRT) have been carried out, are underway, or are advocated for a number of endangered and threatened amphibians and reptiles. However, recent reviews of RRT projects involving birds and mammals suggest that the success rate is low and that the factors that lead to endangerment operate to impede effective RRT results. In this paper, we review available information on RRT projects involving amphibians and reptiles, examine the motives for advocating RRT strategies, and recommend biological and management criteria that should be considered prior to undertaking RRT projects. Most RRT projects involving amphibians and reptiles have not demonstrated success as conservation techniques and should not be advocated as if they are acceptable management and mitigation practices. We urge caution in accepting claims of success and urge colleagues to publish detailed methods and results of past and ongoing RRT projects.

Key words: Amphibians; Reptiles; Repatriation; Relocation; Translocation; Conservation; Management

THE concept of re-establishing populations of endangered or threatened species in areas where they have been extirpated has become extremely popular in recent years. For example, Griffith et al. (1989) reported that approximately 700 translocations or repatriations occurred each year, mainly in the United States and Canada. Variously termed "reintroductions", "translocations", and "repatriations", such programs have the laudable goal of reducing the probability of extinction by increasing the number of viable populations or increasing the number of individuals in small populations (Campbell, 1980; Scott and Carpenter, 1987). Repatriations into natural habitats are frequently combined with captive-breeding programs at zoological parks (Scott and Carpenter, 1987) and may spark wide public interest.

Despite the increasing popularity of repatriation programs as a conservation technique, serious questions have arisen about the theory behind such programs and their effectiveness (British Herpetological Society, 1983; Campbell, 1980; Conant, 1988; Griffith et al., 1989; Mlot, 1989; Scott and Carpenter, 1987; Tasse, 1989). In a comprehensive review of the success of repatriation and translocation programs for birds and mammals, Griffith et al. (1989) found an overall project success rate

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f 44%. They noted that success rates were pparently dependent on a variety of ecological factors, including the quality of the habitat where the release occurred, whether the individuals released were wild or captive-bred, and the feeding habits of adults.

There has been considerable recent interest in the conservation of reptiles and mphibians despite the fact that they lack he broad public appeal of birds and mamnals. In the United States, Puerto Rico, and the U.S. Virgin Islands, 11 species of amphibians and 29 species of reptiles are currently on the federal list of Endangered and Threatened Wildlife and Plants, with many other species protected by state and territorial regulations. Frequently, management, conservation, and recovery plans or endangered or threatened reptiles and mphibians involve repatriation, relocaion, or translocation (hereafter referred to as RRT) programs. Such programs often become highly visible and intertwined with local political concerns. For example, relocation programs for the gopher tortoise (*Gopherus polyphemus*) have been used as mitigation allowing development of uplands habitats throughout Florida. Given he extremely limited resources (both in ime and money) available for conservation programs for reptiles and amphibians, a detailed understanding of the effectiveness of repatriations or translocations is essential (Scott and Carpenter, 1987). However, we are unaware of any critical review of the success of repatriation or translocation programs for amphibians and reptiles. In this paper, we provide such a reiew.

Definitions

A wide variety of terms have been used to refer to programs where animals are released into areas where they have become extirpated or rare (British Herpetological Society, 1983; Conant, 1988; Griffith et al., 1989; Mlot, 1989; Scott and Carpenter, 1987; Tasse, 1989). For the urposes of this paper, we define the rease of individuals of a species into an area ormally or currently occupied by that species as a repatriation, whereas releases of individuals into geographic areas not historically occupied by that species are termed translocations. Relocation involves moving an animal or population of animals away from an area where they are immediately threatened (e.g., by development) to an area where they would be less prone to habitat loss; ideally, relocated animals should be moved to habitats where they historically occurred, but this is not always the case.

There is considerable confusion in the literature concerning what the term "success" means in the context of repatriation or translocation programs. Because the goal of any conservation program is the establishment (or enhancement) of a viable, self-sustaining population, we follow Griffith et al. (1989) in defining a repatriation, relocation, or translocation as successful only if evidence is presented that a self-sustaining population has been established. Hence, the presence of some breeding individuals does not, in our opinion, constitute evidence for success unless it can be shown that the population is at least stable. Because many endangered reptiles and amphibians have long life spans (e.g., sea turtles, tortoises), determining the success of a given release may be difficult and time-consuming. Nonetheless, we suggest that the burden of proof is on the investigator to show that a self-sustaining population exists before declaring success; to do otherwise would be to imply that the probability for extinction has been lowered for that species, when, in fact, this may not be true.

Our review is based on published references in the open literature, unpublished references (often in the form of reports to various resource management agencies), and personal communications solicited from colleagues. We recognize that we may have missed RRT programs whose results remain unpublished.

Discussion of RRT Programs

We documented RRT programs that had been carried out for 25 species of amphibians and reptiles (Table 1). We consider the RRT programs for *Chelonia mydas* separately, but combine RRT programs

TABLE 1.—Tabulation of actual and planned RRT projects involving amphibians and reptiles. U = unknown, E = eggs, L = larvae, J = juveniles, H = hatchlings, A = adults, N = not successful, C = casual observations. Reasons for relocation failure as follows: 1 = unknown, 2 = unsuitable habitat, 3 = unsuitable developmental conditions, 4 = human predation, 5 = animals moved away from release site, 6 = mongoose predation, 7 = poor release design.

Species	Location	Stage	Success	Reproduction	Follow-up	Reference
RRT projects completed or in progress						
Amphibians						
Salamanders						
Plethodontidae						
Plethodon idahoensis	Montana	A?	U(2, 7)		U	Anon (1990)
Salamandridae						
Triturus vittatus	USSR	J	U	Y	Y	Goncharov et al. (1989)
Frogs						
Bufonidae						
Bufo calamita	England	L, U	N(1)		U	Beebee (1983); Corbett (1989)
Peltophryne lemur	Puerto Rico	J, A	U		C	Miller (1985); Paine and Duval (1985); Paine et al. (1989); Paine (personal communication)
Pelobatidae						
Pelobates syriacus	USSR	L, J	U	Y	Y	Goncharov et al. (1989)
Reptiles						
Turtles						
Cheloniidae						
Caretta caretta	Virginia	E	N(1, 3)	N	C	Dodd (1988*a*)
Chelonia mydas	Caribbean Florida	H H	N(1) U	N U	N C	Carr (1984); Dodd (1982); Huff (1989); Parsons (1962)
Lepidochelys kempi	Texas	E	U	N	Y	Caillouet and Landry (1989)
Chelydridae						
Macroclemys temmincki	Georgia	H	U	U	U	Pritchard (1989)
Testudinidae						
Geochelone elephantopus	Galapagos Is.	J	U	Y	U	MacFarland et al. (1974); Bacon and Reynolds (1982); Snell (personal communication)
G. gigantea	Seychelles	A	U(4)	Y	Y	Stoddart et al. (1982); Samour et al. (1987); Spratt (1989)
Gopherus polyphemus	Southeast USA	A	U(1, 2, 4, 5)	Y	Y, N, U	Bard (1989); Burke (1987, 1989*a*,*b*); Diemer (1986, 1987, 1989); Dietlein and Smith (1979); Doonan (1986); FGFWFC (1989); Fucigna and Nickerson (1989); Godley (1989); Layne (1989); Lohoefener and Lohmeier (1986); Stout et al. (1989)
Xerobates agassizi	California	A	N(1, 5)		Y, C	Berry (1986); Cook (1983); Cook et al. (1978); St. Amant and Hoover (1980); Weber [illegible] (1979)

Stout et al. (1989)

Xerobates agassizi	California	A	N(1, 5)		Y, C	Berry (1986); Cook (1983); Cook et al. (1978); St. Amant and Hoover (1980), Weber et al (1979)

Species	Location	Stage	Success	Reproduction	Follow-up	Reference
Lizards						
Cordylidae						
Cordylus giganteus	South Africa	U	U		U	Jacobsen et al. (1990)
Iguanidae						
Conolophus subcristatus	Galapagos Is.	U	U	Y	U	Snell (personal communication)
Cyclura pinguis	Br. Virgin Is.	U	U		U	Lazell (1989)
Lacertidae						
Lacerta agilis	England	A	Y, N, U(1, 2)	Y	Y	Corbett (1988); Spellerberg (1988); Spellerberg and House (1982)
Teiidae						
Ameiva polops	US Virgin Is.	A	N(6)	N	C	Dodd (1978)
Snakes						
Colubridae						
Drymarchon corais	Southeast US	A, J	U(1)		U	Smith (1987); Speake et al. (1987)
Crocodiles						
Crocodylidae						
Alligator mississippiensis	Arkansas	U	Y	Y	C?	Joanen and McNeese (1990)
Crocodylus niloticus	Nigeria	A	U		N	Morgan-Davies (1980)
C. palustris	India	J	Y		Y?	Choudhury and Bustard (1982)
C. porosus	India	H	Y		Y	Choudhury and Chowdhury (1986)
Gavialidae						
Gavialis gangeticus	India	J, H	Y	Y	Y	deVos (1984); Choudhury and Chowdhury (1986); Rao (1990)
Planned or proposed RRT projects						
Amphibians						
Plethodontidae						USFWS (1982)
Batrachoseps aridus	California					
Bufonidae						USFWS (1990*a*)
Bufo hemiophrys	Wyoming					
Discoglossidae						Tonge (personal communication)
Alytes muletensis	Mallorca					
Reptiles						
Scincidae						Bloxam (1982); Tonge (personal communication)
Leiolopisma telfairi	Round Is.					
Teiidae						Tonge (personal communication)
Cnemidophorus vanzoi	St. Lucia					

for other species. Of these RRT projects, five (19%) were classified as successful, six (23%) were unsuccessful, and 15 (58%) could not be classified although in six instances reproduction occurred. Thus, the success rate for RRT programs for reptiles and amphibians is considerably lower than for birds and mammals (44%: Griffith et al., 1989). Moreover, the success rate for reptiles and amphibians varied phylogenetically; of the five successful programs, four involved crocodilians. If projects were considered individually rather than by species, especially for all gopher tortoise RRT's, the success rate would be lowered considerably. Although reproduction may have occurred, no RRT program has yet established a self-sustaining population of snakes, turtles, frogs, or salamanders.

We recognize that some of the cases marked as "unknown" could eventually prove to be successful, such as projects involving the Aldabra and Galapagos tortoises and Galapagos land iguana. We also note that some of the cases currently listed as successful are based on limited follow-up data, and long-term studies could show that initial optimism was premature. There are few published accounts dealing with the rationale, methodology, results, and criteria for success of conservation-related repatriation, relocation, or translocation projects (but see Stubbs, 1989).

Examples of RRT Projects

In the following section, we summarize data on several representative RRT activities. While space limitations preclude a detailed summary of each actual or proposed RRT project listed in Table 1, a summary can be obtained by contacting the authors.

Bufo houstonensis.—Conservation efforts for the Houston toad have involved extensive data collection on both natural populations and the husbandry of toads in captivity. The project was begun in 1978 by the Houston Zoo to identify remaining populations and to either supplement existing populations or to start new populations in protected areas using wild adults, naturally deposited eggs, or captive-reared juveniles and adults. Ten sites at Attwater Prairie Chicken National Wildlife Refuge (APCNWR) were chosen in 1982 for introduction, and tadpoles or juveniles w observed 6 wk after the 1982 and 1 releases. Detailed descriptions of husbandry, sites, release methods and numbers, and monitoring are contained in unpublished reports to the U.S. Fish and Wildlife Service (Quinn, 1980, 1981; Quinn and Ferguson, 1983; Quinn et al., 1984). However, despite careful laboratory and field techniques and the introduction of 0.5 milli individuals since 1982 (adults, juven recent metamorphs, tadpoles), not even new population of the Houston toad has been successfully established at APCNWR (H. Quinn, personal communication).

Lepidochelys kempi.—From 1978 through 1988, freshly deposited Kemp's ridley eggs (1000–3000/yr) were transported from Rancho Nuevo, Mexico, to Texas in an attempt to establish a ne nesting colony on protected Texas beach Eggs were incubated in sand at Padre land and hatchlings were allowed to enter the water at Padre Island National Seashore to allow for possible imprinting on environmental cues. Hatchlings were then shipped to a National Marine Fisheries Service rearing facility at Galveston for head-starting. More than 17,000 hatchlings were imprinted at Padre Island, ar >12,000 turtles have been released aft head-starting. Details of the project, i cluding rationale and objectives, methodology of transport, rearing, and release, numbers of turtles involved, and mortality and disease, have been outlined in a popular book (Phillips, 1989) and discussed by many papers in a symposium volume edited by Caillouet et al. (1989). The Padre Island phase of the Kemp's ridley project was terminated after the 1988 season.

Gopherus polyphemus.—The most nu merous and extensive relocations and translocations of any amphibian or reptile species involve the gopher tortoise in the southeastern United States. Although thousands of animals have been moved from one area to another, particularly within Florida, in efforts to mitigate development or mining of the tortoise's remaining habitat, few details are available nd these relate to only a few projects (Bard, 1989; Burke, 1987, 1989*b*; Diemer, 1986, 1987, 1989; Doonan, 1986; Fucigna and Nickerson, 1989; Stout et al., 1989). Additional animals have been released into populations from which they did not originate after use in tortoise races (e.g., Dietlein and Smith, 1979), although this practice now has ceased. Other efforts have sought to establish populations in areas that may be outside the historic range (e.g., in the Fall Line Hills of Alabama), in isolated locations at the limits of the species' range (e.g., in Tangipahoa Parish, Louisiana), or in reclaimed phosphate mines (Godley, 1989).

Diemer (1989) reviewed relocations of gopher tortoises that occurred in Florida prior to 1987. Details were provided on nine additional relocations at a 1987 symposium sponsored by the Florida Game and Fresh Water Fish Commission (Burke, 1989*b*; Fucigna and Nickerson, 1989; Godley, 1989; Layne, 1989; Stout et al., 1989). Four studies followed tortoises 2 yr or less. Each of the four short-term relocations involved moving a group of tortoises from one or more sites to one or more different sites. Generally about 50% of relocated tortoises remained within 0.5 km 1 yr after release.

Additional details are available from two studies reported at the 1987 symposium. Burke (1987, 1989*b*) reported that 35 of 85 relocated tortoises in south Florida remained 2 yr after relocation, an "apparently stable population". Although his study was of short duration, Burke (1989*b*) concluded that tortoises could be relocated "fairly successfully" and that his work did not support social factors as influencing success rate. In a central Florida relocation (Bard, 1989; Doonan, 1986), two of 12 radio-tagged tortoises could be accounted for after 41 mo while only three of 30 non radio-tagged animals were ever recaptured after release. Seven relocated tortoises were recaptured on 11 occasions compared with 144 captures of resident tortoises on 188 occasions.

Until 1990, moving tortoises from one area to another was accepted as a conflict mitigation measure, especially for Developments of Regional Impact (DRI's), by the State of Florida, particularly in the rapidly growing central and southern regions of the state. Between 75 and 100 relocations, involving thousands of tortoises, have occurred or been authorized (D. Wood and J. Diemer, personal communication). Details concerning these relocations are unknown.

Lacerta agilis.—After a severe fire on a nature reserve in 1976, surviving sand lizards were collected. In 1978, they were moved to an outdoor vivarium. In 1981, the vivarium held a breeding colony, the purpose of which was to furnish animals for eventual reintroduction to the burned area (Spellerberg and House, 1982). Lizards were released in 1981 and recolonized the burned area. By 1988, the heathland community had recovered and sand lizards were again prevalent (Spellerberg, 1988). Details concerning follow-up sampling or lizard numbers were not presented. Other relocations and translocations of this species have occurred throughout southeastern England (primarily Dorset), and more recently in northwestern areas, for at least 20 yr. However, little information appears in the literature concerning specific details. Four populations from releases 17 yr ago continue to survive: one survives after 13 yr, two survive after 5 yr, and only two have disappeared because of fire (Corbett, 1988). A population in the Inner Hebrides continues to survive 14 yr after establishment although this area is outside the known distribution and climatic requirements for the species (Corbett, 1988).

Crocodilians in India.—Relocation efforts in India have been summarized by de Vos (1984) and Choudhury and Chowdhury (1986), including discussions of objectives, criteria for relocation, problems, and the need for monitoring the release. However, specific data on individual reintroductions and the long-term status of introduced animals is unavailable.

More than 1000 muggers (*Crocodylus palustris*) have been reintroduced in 22 locations as of 1986. As of 1986, 1022 saltwater crocodiles (*C. porosus*) had been reintroduced in India in five locations

(Choudhury and Chowdhury, 1986). Reintroduction of both species is thought to be successful.

The reintroduction of gharials (*Gavialis gangeticus*) to areas where they had been eliminated or severely reduced is touted as a major conservation achievement in India. As of 1986, 1456 gharials had been released in eight locations (Choudhury and Chowdhury, 1986). Specific details are available only for the reintroduction at the National Chambal Sanctuary where monitoring has been conducted since 1975 (Rao, 1990). In 1988, 50 nests at 15 sites were reported, and the nesting population was estimated at 50 animals (Rao, 1990). A total of 1287 captive-raised gharial have been released in the Chambal River, and the total population estimate based on 1987–1988 surveys was 804.

WHY IS MOVING ANIMALS SO POPULAR?

Because the success rate of RRT movements for conservation-related purposes is not very high, the reasons for advocating such efforts as conservation strategies should be examined. We suggest the following reasons may help to explain the advocacy of RRT movements as conservation practices, and we recommend a change in attitudes concerning these practices.

Good publicity.—Moving animals from one area to another for what promoters describe as conservation-related purposes, particularly popular species such as sea turtles and tortoises, creates favorable media attention and publicity. Media attention in turn can be used to increase the public's awareness of problems facing the species and perhaps generate funding for other less public activities such as land acquisition and basic research. However, the "30-second spot" or short newspaper story may create a false positive image for the non-involved public, affected individuals (e.g., land developers or home owners), advocacy groups, and even land managers and agency administrators. The result is a belief that such movements are a proven conservation strategy that benefits the individual animal and species. Critical examinations of relocation results and co sequences are rarely part of mec coverage. From a cynical point of view positive public perception of the success of human-mediated animal movements may be desirable if alternatives are difficult to undertake or costly (see Political concerns below).

Some relocations are successful.—There have been successful conservation related RRT movements involving amphibians a reptiles (Table 1), for example, among crocodilians and for the sand lizard in Brit ain. Although there is not much information in the published literature, crocodilian biologists have exchanged unpublished information on relocation and reintroduction techniques through correspondence and attendance at the meetings of the Crocodile Specialist Group of the International Union for the Conservatio of Nature and Natural Resources. Lik wise, conservation groups in England ar closely situated to exchange information on sand lizard relocations. Exchange of information has undoubtedly facilitated the success of these efforts.

Perceived successes.—Perceived successes result from inadequate information presented to the general public, inappropriate extrapolation of results from on study to other taxa, and premature repor of success.

Some individuals and organizations (e.g., Tasse, 1989) have advocated RRT movements as a conservation strategy based on limited success in a few species: for example, the Arabian oryx repatriation or the rock wallaby translocation from Australia to Hawaii. We believe such advocacy is naive and ill-informed. If two species have similar biological requirements and evolutionary history, extrapolation of the results from one taxon to the other may be initially justified. However, we do not recommend the automatic acceptance of positive results on one species as a substitute for critical experimentation and long-term monitoring of the related species. The recent publication of critical examinations of movement-related management of a wide variety of birds and mammals should serve as a caution for even within-taxon extrapolation of results (Conant, 1988; Griffith et al., 1989).

Of greater concern to us, however, is the premature claim of "success" by researchers involved with RRT movements. For instance, we fail to understand how a 50–60% desertion rate by gopher tortoises relocated in south Florida, surrounded by urban development and monitored for only 2 yr or less, can be heralded as a success and proof that relocation works (Burke, 1989*b*). Such claims give credence to the perception that RRT movements are proven management strategies that can be used to mitigate questions of habitat loss. In turn, this perception undermines efforts to protect existing habitat and appears to provide an easy way out of difficult land use questions. Until long-term studies have demonstrated otherwise, human-mediated movements of amphibians and reptiles should not be taken as proven conservation strategies, but only as experimental strategies designed to fit specific needs. Researchers should temper their claims of success with a recognition of the need for long-term evaluation. If they do not, editors should.

Lack of information on failures.—We suspect one of the most likely reasons human-mediated movements of animals for conservation purposes are continually proposed is the lack of information on what has been attempted in the past. Information on criteria for RRT movements, techniques, and results are very difficult to obtain for most studies, even those claimed as "successes". Data on negative results are virtually impossible to find. Perhaps the reasons for failure of most RRT movements are unknown. However, we consider it essential that both positive and negative results be made available in accessible sources if mistakes are to be avoided in the future.

Political concerns.—Relocation has been advocated in areas where rapid development is occurring, particularly involving tortoises in south and central Florida. Moving animals rather than killing them during construction would seem to be a humane way of dealing with problems related to habitat loss. However, most relocated or translocated animals move off the relocation or translocation site, and long-term studies have yet to demonstrate the effectiveness of these techniques. *When* the animals die becomes more important than *if* they die. In addition, commensals and other less glamorous members of the threatened community often are not considered. Rather than creating within-habitat protected areas or dealing with the larger issues of habitat protection in rapidly growing areas, relocation allows an expedient answer to a crisis demanding immediate attention. As such, relocation and translocation efforts have become the "cost of doing business" rather than well thought out strategies for effective conservation.

Humane considerations.—Concern for the fate of individual animals has sparked interest in moving them from harm's way. Concern is shown generally for the larger and more charismatic or benign reptiles, particularly tortoises, although humane reasons are sometimes used as a justification for relocating crocodilians or smaller species. Relocating animals for humane considerations can be used to foster interest in nature and involve individuals, especially young persons and the elderly, in active participation in conservation issues and activities. However, animals relocated for humane reasons should be released in accordance with the same scientific principles that guide other relocations and translocations.

Self-interest.—We have received reports that a few consultants have promoted relocation not as a measure to mitigate habitat-related conflicts, but because they want to make a large profit from the relocation. Rumors exist of consultants charging clients exorbitant fees for relocations of tortoises in south Florida (G. Dalrymple, personal communication). While we believe that most consultants operate within professional and ethical guidelines, reasons for relocating amphibians and reptiles should not be based solely on the profit to be made from the relocation. Consultants should ensure that scientific principles guide the relocation and that provisions for the long-term survival of the relocated animals are in place prior to relocation.

RECOMMENDATIONS

In addition to the recommendations we have made in the preceding text, the topics discussed below should be addressed prior to advocating or undertaking RRT projects for conservation purposes. Lack of clearly defined objectives, methodology, measures of success, and provisions for long-term follow-up studies is an indication of a project likely to fail. In addition, we cannot over-emphasize the need to publish the results of RRT experiments in appropriate journals. The methodology and results of both successful and unsuccessful RRT experiments need to be presented in detail to ensure that future efforts benefit from past experience. Unfortunately, it is our experience that seemingly obvious questions often are not asked during the planning stages of RRT projects.

Know Causes of Decline

A sound recovery plan for any species should start with a detailed understanding of what caused the species to become endangered or threatened. Consequently, RRT programs should only be attempted if (a) the causes of the original decline are reasonably well understood, and (b) those problems have been eliminated. In several cases, an understanding of why the species became endangered or threatened was not apparent (e.g., *Bufo houstonensis, Peltophryne lemur*) or was ignored (e.g., *Ameiva polops*), and these RRT programs have not been successful.

Know Biological Constraints

Although intuitively obvious, the need for RRT projects to operate within the biological constraints imposed by the species must be re-emphasized. Several projects have failed, at least in part, because of lack of attention to the biological requirements of the species (Beebee, 1983; Berry, 1986; Dodd, 1988*a*). Biological constraints to conservation are those factors that set the limits within which human-mediated actions can be taken: i.e., they comprise animal's life history requirements. T include habitat, demographic, and bi physical components. Various authors have discussed the need to consider the biological and habitat requirements of herpetofaunal species in specific RRT projects (e.g., Bloxam, 1982; Berry, 1986; Diemer, 1989).

Habitat constraints.—We refer to habitat constraints as the physical characteristics, both macro and micro, that in ence a species' presence. These include sufficient space for feeding, reproduction, cover, and social interaction of all life stages; space to allow for a population sufficiently large so that environmental fluctuation and demographic stochasticity do not lead to extinction (Soulé, 1983); food of proper nutrient content and availability, especially for herbivores; habitats free fro adverse disturbance, especially from the related to human activity, roads, and predation or modification by introduced, feral, or domestic animals (especially dogs, cats, mongooses, pigs, and cattle); habitats designed to minimize "edge effects"; habitats without unnaturally large concentrations of natural predators, such as raccoons and ravens; and habitats free of toxic pollutants. Appropriate habitats should b available for all phases of the life cycle.

In addition to the size and disturbance factors above, the proper habitat must be available in sufficient quality. Factors to be considered include vegetative structure (e.g., important for gopher tortoises and many lizards), friable soils (for digging species), moisture requirements and access, access to dispersal agents (e.g., offshore currents for sea turtles), and acces to symbionts (e.g., bacteria to aid gut fermentation in herbivorous species).

For wide ranging species, corridors for dispersal or migration (Harris, 1988; Harris and Gallagher, 1989) should be factored into the selection of RRT sites. Active management should be planned for RRT release sites (Griffith et al., 1989), but we caution that single species management may have detrimental effects on other sensitive species and should generally be avoided.

Demographic constraints.—Population characteristics of both the released animals and animals already on-site, if any, must be considered prior to undertaking RRT projects. Factors include knowledge of both the age and size structure of affected animals, sex ratios, and social structure. Social structure must be considered in terms of mating system, spacing and movement patterns, and cannibalism.

Biophysical constraints.—As ectotherms, amphibians and reptiles have thermal requirements not common to endotherms. RRT projects should consider specialized biophysical requirements, especially to ensure the presence of undisturbed basking sites. Amphibians and reptiles also need a proper environment for egg development (temperature, moisture, gas exchange, waste excretion, pH, ion concentration). For species with environmental sex determination (ESD), sex ratios can be affected by the location of nest site and season of deposition (e.g., Mrosovsky et al., 1984; Mrosovsky and Provancha, 1989; Vogt and Bull, 1984). ESD thus affects existing and future population structure. Many reptiles have ESD (Deeming and Ferguson, 1988), especially those targeted for RRT projects (crocodilians, turtles).

Unless habitat, demographic, and biophysical requirements of species are carefully considered, RRT success will be random and most likely to fail. We recommend that thorough knowledge of a species' life history requirements be a prerequisite to the adoption of RRT strategies. The lack of information on the life history of most amphibians and reptiles, especially in different geographic regions, emphasizes the need for basic research.

Population Genetics and Social Structure

Conservation biologists have recently focused considerable attention on the concept of the minimum viable population size (Samson, 1983; Samson et al., 1985; Shaffer, 1981; Shaffer and Samson, 1985): the minimum number of breeding individuals in a population needed to avoid possible deleterious effects of inbreeding and loss of genetic variability as the result of drift (Simberloff, 1988). Although the exact consequences of small population size remains unclear (Simberloff, 1988), a consideration of population genetic factors is considered to be essential to successful management (Frankel and Soulé, 1981; Lande, 1988).

The RRT programs that we reviewed, with the exception of the Puerto Rican crested toad project, did not give any consideration to population genetics when planning the repatriation or translocation. Even for *Peltophryne lemur*, studies on mitochondrial DNA began long after initial repatriation attempts. Although the exact numbers of individuals used in RRT programs often are not available, in several cases (e.g., many gopher tortoise programs), the number of individuals released is clearly much smaller that the 50–500 number frequently cited as the minimum necessary to sustain a viable breeding population (see Simberloff, 1988, for a review and critique of these numbers). In addition, because many newly-released individuals do not become part of the breeding population, the actual number of animals released may need to be much higher than the theoretical effective population size. If the planners of RRT programs rejected the idea of a minimum viable population size because of a sound theoretical argument, we would have little basis for criticism. However, to neglect the subject entirely suggests either ignorance of the consequences of small population size or wishful thinking that the project may "work out" despite the small number of individuals released.

In a similar manner, we suggest that more specific attention should be devoted to the social structure of the released group of animals based on specific information from natural populations. For example, if natural populations of a species have a characteristic sex ratio, then that sex ratio should be maintained among released animals because of its potential bearing on social interactions (e.g., dominance, hierarchies, harem formation, movements away from areas). Obviously, detailed information on the life history and population ecology of the managed species is required.

Disease Transmission

There are few studies on the effects of disease on natural populations of amphibians and reptiles. However, disease may be confined to localized populations and have serious consequences, at least on a short-term basis (e.g., Dodd, 1988*b*). Of more immediate concern is the potential for introducing disease to wild populations from either captive animals released into the wild or from moving diseased animals from one population to another.

For example, disease has proved catastrophic and led, in part, to federal protection for the desert tortoise in the western Mojave Desert (U.S. Fish and Wildlife Service, 1990*b*). The disease affects the upper respiratory tract, hence the name upper respiratory disease syndrome (URDS), and combined with nutritional problems and long-term environmental stress is nearly always fatal. Preliminary work suggests that the agent is a *Mycoplasma* (Jacobson and Gaskin, 1990) that is spread from individual to individual through direct contact. URDS is common in captive reptiles (Jacobson and Gaskin, 1990), and the locations of areas where the disease was first observed suggest that it may have been introduced to wild populations from released captives.

A similar URDS has been diagnosed in the population of *Gopherus polyphemus* on Sanibel Island, Florida, and more recently near Ft. Myers and along the Tamiami Trail. While it is premature to speculate whether the disease is identical with URDS in desert tortoises, preliminary data suggest that transmission is directly from one tortoise to another, and that the disease is highly contagious and often fatal (G. McLaughlin, personal communication). Captive tortoises are known to have been released on Sanibel Island, and it is possible that the disease was introduced by a released captive. The appearance of URDS in a wild population is cause for concern, because thousands of tortoises now are routinely relocated and translocated from one area to another within Florida.

Because of the threat of disease tra mission, we recommend that health che be adopted for animals scheduled to relocated or translocated prior to actu movement, particularly for groups such as tortoises that are known to be susceptible to contagious diseases. Release of long-term captives should always be discouraged. Health checks should include clinical evaluation using hematologic diagnosis (Rosskopf and Woerpel, 1982) by a veterinari familiar with herpetofaunal patholo Keeping animals in a pen or "half house" may increase the opportunity to observe disease problems prior to release, but may expose animals to other problems including disruption of social behavior and vandalism. Individuals from an area with known disease problems, such as Sanibel Island, should never be moved to areas where they could infect wild populations.

Need for Long-term Monitoring

There is a critical lack of information on the long-term success or failure of herpetofaunal-related RRT projects even when monitoring has been incorporated into management and conservation programs. Except for the study of gopher tortoises by Layne (1989), Aldabra tortoises in the Seychelles (Table 1), and the monitoring of crocodilian repatriation projec in India, details of reputed successes, suc as with sand lizards in Great Britain, are lacking.

For the other studies that we reviewed, data are either unavailable or the projects have not been monitored long enough to evaluate success or failure. We are especially critical of claims of relocation "successes" involving long-lived species where monitoring occurred for a relatively short time. For example, Burke (1989*a* claimed relocation had no effect on existing social structure of resident tortoises, and that tortoises could be successfully relocated (Burke, 1989*b*) despite data to the contrary on related species (Berry, 1986). He monitored relocated animals for only 2 yr at the end of which only 41% of the relocated tortoises remained on the release site. Monitoring a population of an animal for only 10% of the time it takes to reach

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sexual maturity hardly qualifies as enough time to measure long-term relocation "success." Likewise, we suggest that claims of success involving other tortoise relocations (e.g., Fucigna and Nickerson, 1989; Godley, 1989; Stout et al., 1989) are premature and tend to foster a false impression that relocation and translocation are proven management techniques.

Long-term monitoring of marked individuals will be required to establish the success or failure of RRT projects. What constitutes "long-term" will depend on the life-history characteristics of the species. For instance, a long-term monitoring program might continue 10–15 yr for a toad, but extend >20 yr for tortoises. Such long-term monitoring will establish not only the presence of released individuals but also the success or failure of reproduction. Long-term monitoring will ensure that release sites can maintain their integrity rather than become susceptible themselves to destruction or encroachment from "edge-effects".

We recommend that RRT projects involving amphibians and reptiles should not be attempted unless provisions are made for a biologically-based, long-term monitoring program. Considerations such as duration of monitoring that are based on non-biological priorities should not eclipse the need for evaluation within the biological constraints of the species. RRT movements should be considered experimental unless long-term studies document the feasibility of the movement on the same or a related species. Periodical evaluation is important. We caution our colleagues to exercise restraint when evaluating the "success" of such movements based on short-term monitoring and data collection.

SUMMARY

It is not our intention to belittle any of the biologists or RRT programs reviewed in this paper. We recognize that decision-making in conservation biology often is made by non-scientists or under crisis circumstances. Nonetheless, our review casts doubt on the effectiveness of RRT programs as a conservation strategy, at least for most species of amphibians and reptiles. Although RRT programs may work under certain circumstances, they should not be used unless all parties involved are prepared to make the necessary commitment for collecting baseline data, releasing animals under appropriate circumstances, providing for follow-up studies at periodic intervals, and publishing the methodology and results of the program regardless of whether the outcome is positive or negative. If such commitments cannot be made, other conservation strategies should be considered.

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RELOCATIONS, REPATRIATIONS, AND TRANSLOCATIONS OF AMPHIBIANS AND REPTILES: TAKING A BROADER VIEW

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THE review of "relocation, repatriation and translocation" (RRT's) of amphibians and reptiles by Dodd and Seigel (1991) provides a summary of the literature on the use of these techniques for conservation purposes. Their recommendations are generally sound, and apply not only to these conservation practices, but equally well to any of the myriad possible techniques used to help insure the preservation of a species. However, I believe that the evidence they use for support is weak, that their dissatisfaction with past efforts is only partially justified, and thus their conclusions extreme. Basically, the question that they attempt to answer is: given that conservation dollars are always limited, are RRT's cost effective and appropriate procedures for amphibian and reptile conservation programs? They find that these techniques have been successful in only a few cases, and thus they propose a rigid set of criteria to be addressed before any future attempts are begun. My comments on their work focus on two main points: whether amphibians and reptiles are generally poor candidates for RRT's, and how success should be determined.

REPTILES AND AMPHIBIANS AS RRT CANDIDATES

As Griffith et al. (1989) did for a much larger number of studies of birds and mammals, Dodd and Seigel reviewed RRT programs for 25 species of amphibians and reptiles and found that of the 11 projects that could be defined as successful or unsuccessful by their standards, five (45%) were successful. This is slightly higher than the success rate reported for 198 RRT's reviewed by Griffith et al. Even so, the use of this type of analysis is exceedingly crude, because it assumes that snakes, lizards, turtles, crocodilians, salamanders, and anurans have comparable potential for successful RRT. Certainly there is wide variation within each order as well as between them, and anyone considering an

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LETTER

Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep

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Abstract

The rapid expansion of road networks has reduced connectivity among populations of flora and fauna. The resulting isolation is assumed to increase population extinction rates, in part because of the loss of genetic diversity. However, there are few cases where loss of genetic diversity has been linked directly to roads or other barriers. We analysed the effects of such barriers on connectivity and genetic diversity of 27 populations of *Ovis canadensis nelsoni* (desert bighorn sheep). We used partial Mantel tests, multiple linear regression and coalescent simulations to infer changes in gene flow and diversity of nuclear and mitochondrial DNA markers. Our findings link a rapid reduction in genetic diversity (up to 15%) to as few as 40 years of anthropogenic isolation. Interstate highways, canals and developed areas, where present, have apparently eliminated gene flow. These results suggest that anthropogenic barriers constitute a severe threat to the persistence of naturally fragmented populations.

Keywords

Gene flow, genetic diversity, habitat fragmentation, metapopulation, *Ovis canadensis*, road.

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INTRODUCTION

As the level of connectivity among human populations continues to increase, natural populations of plants and animals are becoming increasingly isolated. Today the earth's surface is partitioned by an estimated *c.* 28 million km of highways (CIA 2003) that restrict the movement of many species (Trombulak & Frissell 2000; Underhill & Angold 2000). This loss of connectivity is suspected to impede exchange of individuals among populations, thereby accelerating the loss of genetic diversity because of genetic drift (Frankel & Soule 1981; Hedrick 2005). Reduced genetic diversity is likely to increase population extinction rates both in the short term (because of inbreeding, Saccheri *et al.* 1998; Westemeier *et al.* 1998; Coltman *et al.* 1999) and in the long term by reducing evolutionary potential, i.e. the ability of a population to adapt to future changes in biotic and abiotic factors such as climate change (Frankel & Soule 1981; Lande 1998; Fraser & Bernatchez 2001; Hedrick 2005). However, recently constructed barriers have rarely been found to affect genetic diversity in natural populations, particularly for long-lived, large-bodied species (e.g. Kyle & Strobeck 2003; Sumner *et al.* 2004). While roads have been shown to restrict gene flow for species with small body size or relatively low vagility such as amphibians (Reh & Seitz 1990) and beetles (Keller & Largiader 2003), there is growing concern that a much wider variety of taxa may be affected (e.g. Kramer-Schadt *et al.* 2004; Malo *et al.* 2004).

The objective of this study was to assess the effects of major highways and other recently constructed anthropogenic barriers upon genetic diversity in a metapopulation of *Ovis canadensis nelsoni* (desert bighorn sheep). In the desert regions of California, local populations of this long-lived, vagile mammal are often less than 50 individuals (Torres *et al.* 1994). Restricted largely to the steep, rocky mountain ranges that are scattered across the region, these populations are demographically independent and naturally fragmented by the intervening desert (Bleich *et al.* 1990). As resources are variable and local population extinctions common (Epps *et al.* 2004), some connectivity among populations is presumed essential to maintain the regional bighorn sheep metapopulation (Bleich *et al.* 1996). However, the southwest USA has been subject to an increasing degree of urbanization by humans, marked by widespread construction of interstate highways and water canals in this desert region over the last 40–70 years. Anecdotal evidence suggests that bighorn sheep rarely cross these continuously fenced barriers (Bleich *et al.* 1996). Thus it is likely that these barriers on the landscape have reduced connectivity among populations of desert bighorn sheep and possibly many other terrestrial species.

We examined putatively neutral genetic variation across desert bighorn sheep populations in southeastern California (Fig. 1) to assess whether human-made barriers have affected dispersal and genetic diversity to a significant degree. We also defined the geographical scale of current gene flow among these populations and considered the conservation implications of continuing anthropogenic fragmentation.

METHODS

The study area was comprised of the central Mojave, southern Mojave and Sonoran Desert regions of California. Habitat quality for desert bighorn sheep in these arid areas was strongly affected by the spatial and temporal variation in climate and population turnover is high (Epps *et al.* 2004). Apparent dispersal barriers erected in the 20th century include the Colorado River Aqueduct (constructed in the 1930s), urban development, the establishment of large mining operations in Lucerne Valley, the portion of State Highway 62 with four lanes and a concrete median barrier, and interstates 10, 15 and 40 (constructed in the 1960s) (Nystrom 2003). These barriers are largely continuous and have direct physical impediments to locomotion by bighorn sheep, including fences and steep concrete walls. Underground portions of the Colorado River Aqueduct (passing beneath several populations in the southeastern part of the study area) were not considered to be barriers. Major highways were by far the most common barriers between study populations.

We collected genetic samples across the study area during 2000–2003 from 27 populations with varying levels of anthropogenic isolation (Fig. 1). Estimated median population size for these populations was 38 individuals, range was 12–300 (Torres *et al.* 1994). Populations were defined as previously in a geographical information system (GIS) (Torres *et al.* 1994; Epps *et al.* 2004, 2005a), based upon the topographical features of the mountain ranges where they are found. We collected samples from all known populations within the focal study area, except five ranges containing individuals translocated from other populations in the region (Torres *et al.* 1994) (Fig. 1).

We used faecal pellets as the primary source of genetic material, obtained mostly during summer months when desert bighorn sheep congregate at water sources. We collected fresh pellets from observed bighorn sheep or selected the most recent-appearing pellets in the vicinity. Faecal samples were air-dried and stored in paper bags in a dry environment. We also obtained blood and tissue samples from bighorn sheep captured by the California Department of Fish and Game or killed by hunters during 2000–2004. We extracted genomic DNA from faecal samples using a modified DNA Stool Mini-Kit^{TM} (Qiagen, Valencia, CA, USA) protocol (Wehausen *et al.* 2004), and from blood and tissue samples using DNEasy Tissue Kits^{TM}

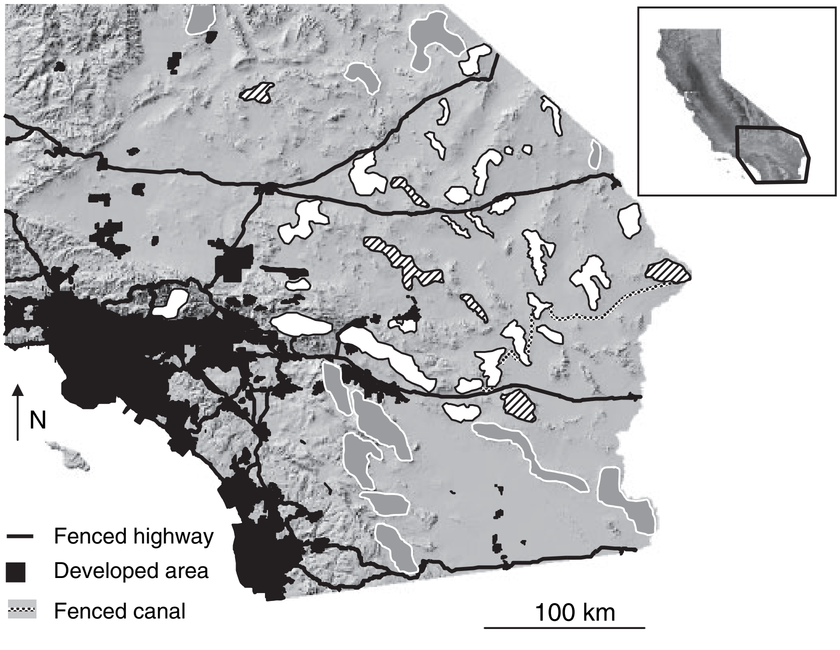

Figure 1 Topographical map of southern California with location and approximate size of the 27 desert bighorn sheep populations sampled (white polygons). Barriers, including canals, interstate highways, freeways, and urban areas, are represented in black or checkered (above-ground portions of the Colorado Aqueduct) patterns. Artificially translocated populations (cross-hatched) and other extant populations where sampling did not occur are also depicted (light grey polygons). Barriers outside the area of sampled populations are not fully represented.

(Qiagen). Before genotyping, we assessed extraction quality by amplifying a *c.* 200-bp fragment of nuclear DNA from the zinc-finger protein gene [Appendix S1(a)]. We visualized the amplification product on 2% agarose gels pre-stained with ethidium bromide; samples generating weak amplifications were not used in further analyses.

We genotyped 14 dinucleotide microsatellite loci for each DNA extraction [Appendix S1(b)]. We conducted a minimum of four replicate polymerase chain reactions (PCRs, Mullis *et al.* 1986) per faecal sample per locus to minimize genotyping errors resulting from degraded DNA (Taberlet *et al.* 1999), and conducted two replicate PCRs for blood and tissue samples. Alleles included in the final consensus genotypes were observed at least twice; if observed only once, an additional four replicates were conducted. We included two negative controls and two positive controls (samples with known genotypes) with every 96 PCR reactions as checks for contamination and to standardize genotypes among experiments.

We estimated the probability of an erroneous genotype because of allelic dropout (selective amplification of only one allele in a heterozygote because of low amounts of template DNA, Taberlet *et al.* 1999). We accomplished this by summing the observed number of allelic dropouts for each locus, and dividing this sum by the number of successful PCR reactions (i.e. the presence of an amplification product) for heterozygous individuals (allelic dropout could only be identified in the case of individuals determined to be heterozygous). Because we had a minimum criterion that each allele per sample per locus had to be observed at least twice (at least two successful replicate PCR reactions with identical results), we squared each per-locus dropout rate to estimate the probability of two dropouts in the same sample. We then summed these squared dropout rates over all loci, and added the average probability of a false allele over 14 loci (calculated from observed rates) to obtain our final estimated probability of a genotypic error per individual. While this method does not account for variability among samples (e.g. Miller *et al.* 2002), we assumed that pre-screening of extractions limited sample variability to a large degree.

We limited further data analyses to samples for which complete genotypes were obtained at all loci. We used the probability of identity (P_{ID}) to identify and eliminate duplicate genotypes resulting from the collection of more than one faecal sample from some individuals. DNA extractions from different faecal samples were inferred as originating from the same individual if the combined P_{ID} for a full-sib relationship was estimated at $< 10^{-2}$ using GIMLET (Valiere 2002), at the number of loci matching between a pair of different DNA extractions (which could be any number of loci less than the maximum of 14 employed in this study). This threshold level of P_{ID} was chosen because most population sizes were estimated at < 100 individuals (Torres *et al.* 1994); 10^{-3} was used for populations > 100. This analysis was undertaken in two steps; first within each population, and then subsequently for all populations combined and treated as a single panmictic population (after removal of all but one of each unique genotype in each population), to detect if any individuals were sampled in more than one population. We assessed the final data set obtained in this manner for any significant deviations from linkage disequilibrium and the expected Hardy–Weinberg genotype frequencies in each population using GENEPOP (Raymond & Rousset 1995).

We also assessed the diversity of mitochondrial DNA haplotypes in each population. Female bighorn sheep are less likely to move between mountain ranges (Festa-Bianchet 1991; Jorgenson *et al.* 1997); therefore maternally transmitted mitochondrial DNA provided an opportunity to assess female dispersal patterns. After identifying unique samples using the microsatellite data, we sequenced 515 nucleotides in the mitochondrial control region from each individual (except three samples that failed to amplify) [primers and protocols are described in Appendix S1(c)]. We sequenced all samples in both forward and reverse directions, editing and aligning them manually, to minimize sequence ambiguities. We used the number of unique haplotypes present in each population as a measure of female-mediated genetic diversity. To correct for variation in sample size, we subsampled each population 100 times using the minimum sample size and calculated the average number of unique haplotypes detected per population.

From the microsatellite data, we estimated the degree of genetic divergence among populations as F_{ST} (and thus Nm) for each population pair using GENEPOP (Raymond & Rousset 1995). F_{ST} rather than R_{ST} (Slatkin 1995) was used because F_{ST} is a more appropriate statistic for 'stepping stone' population models and systems where migration rate exceeds mutation rate (Hardy *et al.* 2003), as is most likely for these desert bighorn sheep populations given numerous observations of colonizations and dispersal between mountain ranges (e.g. Epps *et al.* 2005a,b). Furthermore, F_{ST} performs better when number of loci < 20 (Gaggiotti *et al.* 1999). We used allelic richness (the average number of alleles per locus or A) as our measure of genetic diversity in each population. We used FSTAT (Goudet 1995) to correct A for differences in sample size, as recommended by Leberg (2002). The smallest population sample size was employed as the global sample size.

To determine if human-made barriers (see below) had affected population genetic diversity, we used information theoretic model selection techniques (Burnham & Anderson 1998) to test multiple regression models incorporating either of two estimates of the degree of isolation for each population. We estimated isolation as (i) the harmonic mean of the geographical distance to the nearest three populations (e.g. Harrison & Ray 2002), which weights the mean towards the smallest distance, or (ii) the harmonic mean of the geographical distance to the nearest three populations, but with a 'barrier effect distance' added to the geographical distance between each population pair separated by a human-made barrier. These measures are referred as isolation$_{\text{distance}}$ and isolation$_{\text{distance+barriers}}$.

To quantify the above-mentioned barrier effect distance, we estimated the reduction in the relative gene flow parameter (*Nm*) caused by barriers among our study populations. The barrier effect distance was defined as the geographical distance yielding an equivalent decrease in the estimate of *Nm*. We first defined barriers as fenced highways, canals and areas of high-density urban development, and added them to the above employed GIS map. We then employed multiple regressions on all pairwise population comparisons to estimate the degree of correlation between geographical distance and *Nm* among populations that were (i) separated by human-made barriers and (ii) those that were not. Populations were considered as separated by human-made barriers if a straight line between the two closest edges of the population polygons intersected such a barrier. Connecting lines for all pairwise comparisons were generated in the GIS (Jenness 2004) and overlaid on the barrier map to determine which lines intersected barriers. Interpopulation geographical distances were estimated as the shortest distance between the edges of each population polygon (Jenness 2004).

Nm was estimated as [$F_{\text{ST}} = 1/(1 + 4\ Nm)$] (Wright 1921). The difference between the intercepts of the *y*-axis in the two regressions (denoted as ΔNm) was inferred to result from the effect of human barriers on the degree of genetic isolation (Fig. 2). Finally, we used the coefficient of the regression of population pairs without barriers (slope$_{\text{no barriers}}$) to estimate the barrier effect distance (in km) as log(barrier effect distance) = $\Delta Nm/\text{slope}_{\text{no barriers}}$.

After defining these two measures of population isolation (isolation$_{\text{distance}}$ and isolation$_{\text{distance+barriers}}$), we tested which measure explained the most variance in both *A* and mtDNA haplotype diversity. For both sets of genetic data, we used Akaike's Information Criterion with the small sample size correction (AIC$_c$) and Akaike weights (Burnham & Anderson 1998) to infer the best regression models. We estimated the overdispersion correction factor ($\hat{c}$) from the deviance of the most saturated model, as described by Lindsey (1999), to ensure that AIC$_c$ rather than the quasi-likelihood information criterion (QAIC$_c$) was most appropriate. We also tested whether other factors such as population polygon area and estimated current population size (which affects the rate of genetic drift) improved regression models.

We estimated the rate of reduction in genetic diversity (*A*) in those populations affected by human-made barriers by comparing the difference in the predicted level of genetic diversity with the existent barriers (obtained from the regression of *A* on isolation$_{\text{distance+barriers}}$ described above), and the predicted level of genetic diversity using the same equation but removing the barrier effect for each population. The resulting difference was then extrapolated over the average estimated age of the barriers.

We also analysed pairwise estimates of *Nm* using partial Mantel tests (Smouse *et al.* 1986; Manly 1991) to determine whether relative gene flow was affected by barriers, and at what spatial scale. We repeated this analysis using F_{ST} for comparison, although F_{ST} appeared to be subject to very high overdispersion in other analyses of this data set (not shown). *Nm* represents the amount of gene flow in an idealized Wright–Fisher island model that would yield the observed degree of genetic heterogeneity. Hence, *Nm* cannot be inferred to represent an estimate of the actual

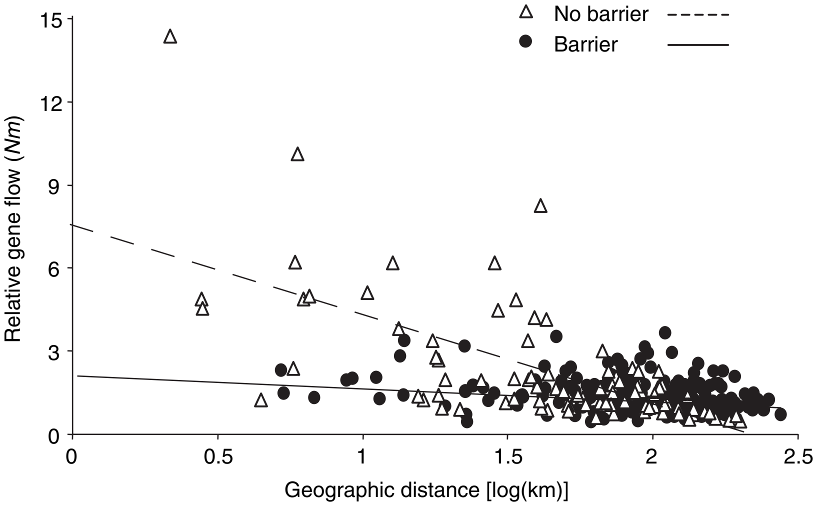

Figure 2 Pairwise population comparisons of migrants per generation (*Nm*, estimated from genetic distance F_{ST}) regressed on log(geographic distance in km). Comparisons are grouped by presence (dark circles) or absence (open triangles) of an intervening barrier. R^2 of pairs without barriers = 0.43, R^2 of pairs with barriers = 0.08. Regression lines are extended to cross the *y*-axis; difference in intercepts was used to calculate the 'barrier effect' (see text).

number of migrants (Whitlock & McCauley 1999). Under such a model Nm is correlated to F_{ST}, but in a nonlinear manner and thus we have used both metrics in our estimation. While our population is likely not in mutation-drift-migration equilibrium, Nm (and F_{ST}) can provide insights as to the relative levels of gene flow, especially when the rate of gene flow is larger than mutation (Slatkin 1993).

We employed partial Mantel tests over sequential geographical distance classes (0–15, 15–30, 30–60, 60–90, 90–120, 120–150 and > 150 km) (Legendre & Fortin 1989; Bjornstad *et al.* 1995; Dodd *et al.* 2002) to assess the partial correlation of barriers and geographical distance with Nm for each distance category. This allowed us to infer the spatial scale at which recent gene flow has occurred or has been disrupted by barriers. For a given distance category, interpopulation distances falling within that range were denoted as '1', all others as '0'. Similarly barriers were noted as '1' (present) or '0' (absent) for population comparisons within the given distance category. In this assessment, we excluded the Coxcomb Mountain population. Most of the bighorn in the Coxcomb Mountains were found to have immigrated from a nearby population, which was established by translocation from a distant population (Epps *et al.* 2005b). While the validity of estimates of type I error (here, falsely concluding that correlation of one independent matrix with the dependent matrix exists, because of correlation with a second independent matrix) in partial Mantel tests has been questioned (Raufaste & Rousset 2001; Rousset 2002), Castellano & Balletto (2002) argued that under even high levels of correlation between the independent matrices, partial Mantel tests closely approximate true type I error.

Finally, we employed the computer program SIMCOAL (Excoffier *et al.* 2000) to investigate if barriers could create a detectable increase in genetic distance between populations, given the time scale and data richness that apply to this study. Coalescent simulations were conducted under two different models, each simulating two adjacent populations 5 km apart. In the first model, we tested the effects of a recently constructed barrier by simulating two populations at mutation-drift-migration equilibrium except during the last seven generations (*c.* 42 years; Coltman *et al.* 2003), when Nm was set to zero. No such reduction in Nm was added to the second model. In each model 40 gene copies were sampled at each of 14 loci. SIMCOAL uses a pure stepwise migration model (in this case, without constraint on allele size), and requires the user to set migration rate m, effective population size N and mutation rate μ. SIMCOAL immediately multiplies these parameters to obtain Nm and θ, where $\theta = 4N\mu$. To obtain realistic values of Nm and θ for use in the model, we estimated $Nm = 6.2$ from the observed estimate of $F_{ST} = 0.039$ between a representative pair of mountain ranges, the Marble and South Bristol Mountains, that are separated by only 5 km with no intervening barrier. We estimated θ from the variance in allele size as $\theta = 2 \times$ (variance in allele size) (Wehrhahn 1975) for both of these mountain ranges ($\theta = 9.62$ and 8.32 respectively), and used the average of these values ($\theta = 8.97$) in our simulation. We also estimated θ from expected heterozygosity as $H_e = 1 - (1 + 2\theta)^{-1/2}$, giving an average of $\theta = 3.27$. For comparative purposes, we tested both of these measures of θ in our simulations, as well as $\theta = 1$. We varied values of Nm to include 2, 6.2 and 10. We calculated population pairwise F_{ST} between the two simulated populations for each simulation run using Arlequin (Schneider *et al.* 2000). For each parameter set, 1000 simulation runs from both models were compared to determine the average increase in F_{ST} because of barriers.

We compared this simulated average increase in F_{ST} because of barriers (for populations 5 km apart) to the observed increase in F_{ST} resulting from barriers for populations separated by this distance. We estimated the observed increase by regressing F_{ST} on log(geographic distance) for all population pairs with intervening barriers and for all population pairs without intervening barriers, and calculated the difference in the predicted F_{ST} values at 5 km using these two regression equations.

RESULTS

We obtained complete genotypes at all 14 microsatellite loci from 461 faecal and 47 blood or tissue samples. From our analyses of these 508 genotypes, we inferred that they represented a total of 397 individuals, yielding a mean sample size per population of 15 individuals (range 6–29, SD 5.9; Appendix S2). We identified 21 unique mtDNA haplotypes from 394 of these individuals; one haplotype had been previously described (GenBank no. AF076912, Boyce *et al.* 1999). New haplotype sequences were submitted to the GenBank database under the accession numbers AY903993–AY904012. Numbers of alleles and haplotypes per population, A, expected heterozygosity and other basic data are described in Appendix S2.

In the final microsatellite data set, we did not observe any case of allelic dropout among the consensus genotypes of the 111 samples that we identified as duplicates of previously sampled individuals. We found no evidence of linkage disequilibrium within populations after correcting for multiple comparisons.

The average rate of allelic dropout per locus per replicate for the faecal samples was estimated at 3.7%, while rate of occurrence of false alleles was estimated at 0.062%. Overall this yielded a final estimate of 0.022 genotypic errors per individual. Given an error rate of 0.022, in a sample set of *c.* 400 individuals typed at 14 loci, the expectation is approximately 10 single-locus errors in consensus genotypes.

Because this estimated error rate assumes that every sample is heterozygous at all loci, and that there were only two replicate PCR amplifications at each locus, this estimate of the genotype error rate is likely higher than the actual rate: most samples were successfully amplified three to four times. Assuming that genotype errors were randomly distributed with respect to population, this error rate was unlikely to bias our estimates of genetic diversity and divergence in a significant manner for the purposes of this study.

The 'barrier effect distance' was estimated at *c.* 40 km [$\Delta Nm = 5.05 = 3.177 \times \log$('barrier effect' in km)]. Genetic diversity was negatively correlated with both measures of population isolation (isolation$_{\text{distance}}$ and isolation$_{\text{distance+barriers}}$) (Fig. 3). However, using isolation$_{\text{distance+barriers}}$ significantly improved regression model fit for A (Table 1; Fig. 3), indicating that the presence of barriers reduced nuclear genetic diversity. The estimated decline in A for populations isolated by barriers from all three of the nearest populations was as high as 15%. Results for mtDNA haplotype diversity were more equivocal: although isolation$_{\text{distance+barriers}}$ had a better model fit than isolation$_{\text{distance+barriers}}$ as assessed by model F-statistic significance and R^2, and greater likelihood as assessed by AIC$_c$ weight, the difference was not enough to clearly indicate that isolation$_{\text{distance+barriers}}$ was the best model (Table 1). Fits of both models for mtDNA haplotype diversity were poor ($R^2 < 0.20$), suggesting that neither model was adequate. Genetic diversity (nuclear and mitochondrial) was not correlated with population area or current estimated population size (Table 1).

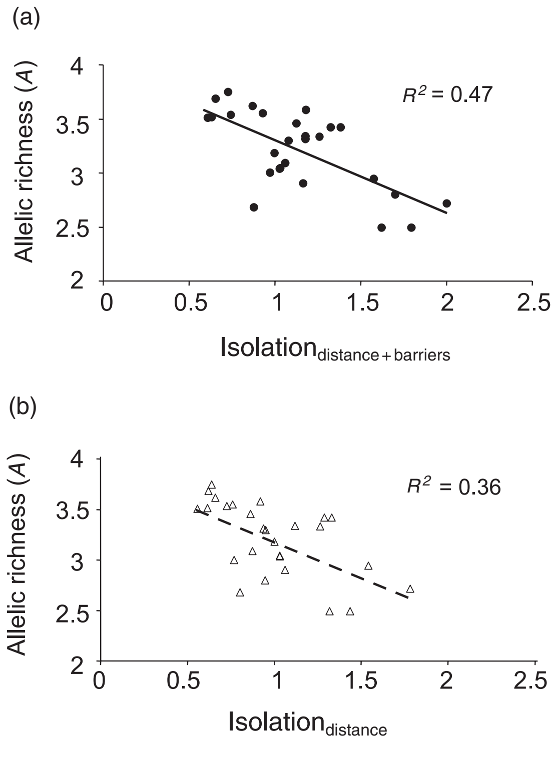

Figure 3 Regressions of allelic richness (A) on isolation as a function of distance and barriers (a) or distance alone (b). Isolation measures are based on log-transformed distances in km (see text).

The amount of gene flow among populations was strongly and negatively correlated with barriers at interpopulation distances of < 15 km (Mantel $r = -0.49$, $P = 0.0002$). When the effect of barriers was removed by partial correlation, Nm was strongly correlated among populations within 15 km (Mantel $r = 0.82$, $P = 0.0002$), weakly correlated among populations 15–30 km apart (Mantel $r = 0.16$, $P = 0.0448$), and not correlated among populations separated by greater distances. Plotting Nm as a function of distance also showed that Nm decreased sharply with distance for population pairs not separated by barriers (Fig. 2). Population pairs separated by barriers showed very low Nm values regardless of distance, suggesting that no exchange of individuals occurred across barriers (Fig. 2).

Partial correlations of pairwise F_{ST} values (genetic differentiation; Appendix S3) with barriers and distance showed a similar but weaker pattern. F_{ST} was positively correlated with the presence of barriers at interpopulation distances of < 15 km (Mantel $r = 0.168$, $P = 0.0220$) and 15–30 km (Mantel $r = 0.145$, $P = 0.0446$). F_{ST} was negatively correlated with the presence of populations within 15 km (Mantel $r = -0.444$, $P = 0.0002$), less strongly so at 15–30 km (Mantel $r = -0.174$, $P = 0.0264$), and not significantly correlated at greater distances. Because effects for both factors were detected in the first two distance classes, we also examined them across a 0–30-km distance class: F_{ST} was positively correlated with the presence of barriers (Mantel $r = 0.212$, $P = 0.0034$) and negatively correlated with the presence of populations within 30 km (Mantel $r = -0.441$, $P = 0.0002$).

Simulated datasets revealed that an increase in genetic distance (F_{ST}) because of barriers could be detected within the time frame of the age of the barriers in this study (*c.* 40 years). However, the increase in F_{ST} (0.012–0.018 depending on the parameter values used, Table 2) was not as large as the estimated increase in F_{ST} because of barriers for the actual study populations. The model of two simulated populations, 5 km apart with no intervening barrier, had an average F_{ST} ranging from 0.007 to 0.048 across the parameter set ($F_{\text{ST}} = 0.039$ between the study populations from which parameters were derived). Average F_{ST} between two simulated populations with a barrier present during the most recent seven generations increased for all parameter combinations; the increase did not appear to be greatly sensitive to the different values of Nm and θ

Table 1 Regression models of genetic diversity (corrected for sample size) as a function of human-made barriers, distance and other variables for $n = 27$ populations of desert bighorn sheep

Response variable	Model	*P*-value†	R^2‡	*k*§	ΔAIC_c	w_i¶
Allelic richness (corrected)	Log (isolation$_{\text{distance+barriers}}$)*	< 0.0001	0.47	3	0	0.88
	Log (isolation$_{\text{distance}}$)	0.0010	0.36	3	5.01	0.07
	Log (isolation$_{\text{distance}}$), population area	0.0031	0.38	4	6.96	0.03
	Log (isolation$_{\text{distance}}$), population size	0.0048	0.36	4	7.90	0.02
Number of mtDNA haplotypes (corrected)	Isolation$_{\text{distance+barriers}}$*	0.0388	0.16	3	0	0.63
	Isolation$_{\text{distance}}$*	0.0754	0.12	3	1.22	0.34
	Isolation$_{\text{distance}}$, population area, population size	0.3035	0.14	5	6.33	0.03

Model selection was performed using Akaike's Information Criterion (AIC)$_c$: models with lowest AIC$_c$ values are best fit, but models within two ΔAIC$_c$ units of the best model are considered equally explanatory. AIC weights (w_i) may be interpreted as the likelihood that the given model is the best of the candidate models (Burnham & Anderson 1998).

*Best-fit or competing model (within two AIC$_c$ units).

†Significance of model *F*-statistic.

‡Fit of linear regression model.

§Number of predictor variables + 2 for calculating AIC$_c$.

¶AIC$_c$ weight.

Table 2 Increases in average F_{ST} (with standard error) because of elimination of gene flow by a barrier for seven generations between two simulated populations (based on 1000 simulations)

θ	$Nm = 2$	$Nm = 6.2$	$Nm = 10$
1.00	0.015 (0.002)	0.012 (0.003)	0.013 (0.003)
3.27	0.018 (0.005)	0.015 (0.003)*	0.013 (0.003)
8.97	0.013 (0.004)	0.014 (0.002)†	0.012 (0.002)

**Nm* calculated from observed F_{ST}, θ estimated from observed heterozygosity.

†*Nm* calculated from observed F_{ST}, θ estimated from variance in allele size.

that we employed (Table 2). However, the relative increase was sensitive to *Nm* and θ, in that low values of *Nm* increased average F_{ST} values between populations but not the difference caused by barriers. Estimated F_{ST} between the actual study populations, 5 km apart with an intervening barrier, increased from 0.046 to 0.113. This estimated increase was based on the regression equations of F_{ST} on distance for population pairs without barriers [$F_{\text{ST}} = -0.029 + 0.108 \times \log(\text{geographic distance in km})$] and for population pairs with intervening barriers [$F_{\text{ST}} = 0.080 + 0.048 \times \log(\text{geographic distance in km})$].

DISCUSSION

Nuclear genetic diversity of desert bighorn sheep populations was negatively correlated with the presence of human-made barriers that blocked dispersal to nearby populations (Table 1; Fig. 3). This finding strongly suggests that these barriers have reduced genetic diversity for many of these populations. We estimate from our results that nuclear genetic diversity in populations completely isolated by human-made barriers has declined as much as 15% in the *c.* 40 years since most barriers were erected. This estimate implies that the rate of loss of genetic diversity in populations isolated by barriers was *c.* 0.4% per year; if this rate is constant, some populations may lose up to 40% of their pre-barrier genetic diversity in the next 60 years. Results for mtDNA markers were consistent with these findings, but did not clearly support the reduction of mitochondrial genetic diversity because of barriers. The low correlation of mtDNA diversity with either distance and barriers may reflect very low dispersal rates for female bighorn sheep, as suggested by Festa-Bianchet (1991) and Jorgenson *et al.* (1997). More probably, the ambiguous results for mtDNA may reflect the stochasiticity inherent in one genetic locus (as represented by the mtDNA genome) when compared with the results derived from 14 microsatellite loci.

We believe that genetic diversity declined so rapidly after isolation because N_e of each population was likely very small. Therefore, unless diversity was maintained by gene flow from other populations, genetic drift quickly eliminated diversity. Our analyses of gene flow based on regression and partial correlation of *Nm* and F_{ST} with barriers and distance showed that, where present, human-made barriers have essentially eliminated dispersal (Fig. 2). The suppression of migration by barriers was most detectable within the distances at which high relative gene flow was most detectable, in this case, at < 15 km. Populations < 15 km from other populations maintained higher genetic diversity unless a human-made barrier intervened.

Finally, genetic simulations demonstrated that barriers constructed only 40 years ago could create a detectable increase in genetic distance between populations, although the increase in genetic distance in the simulations was not as great as that observed. This discrepancy may have resulted from a variety of factors. For one, these simple simulations considered only two populations. Actual populations experienced gene flow from other nearby populations, and probably experienced strong fluctuations in population sizes (perhaps caused by strong environmental stochasticity), founder effects, and other demographic events not included in the simulations that may have increased genetic distances. Thus parameter estimates (based on equilibrium conditions) for these simulations may not have been correct, although simulations with varied parameter estimates showed similar increases in genetic distance because of barriers. Historical census data (Torres *et al.* 1994), tiny population sizes and frequent recent extinctions of populations of desert bighorn sheep in California (Epps *et al.* 2004) suggest that fluctuations and founder effects have been common in the decades since the barriers have been constructed. Such metapopulation dynamics may further explain why barriers had such a strong effect on genetic diversity and genetic distance in only *c.* 40 years; this question bears further investigation with more realistic models. However, the detectable differences that our simple simulations yielded support our inference that observed patterns of genetic diversity could be due to the effects of human erected barriers (i.e. occur over such short-time frame).

Because our analyses rely on correlation of the presence of barriers with decreased genetic diversity and increased genetic distance, we cannot exclude the possibility that the genetic structure apparently created by barriers is an artifact of historical genetic structure. However, no other biogeographical explanation for such structure is readily apparent. While it is possible that roads may be constructed preferentially in flat areas or valleys between mountain ranges, nearly all of the populations considered are topographically isolated by flat areas, regardless of the presence of barriers (Fig. 1). Distance thus appears to be the prevailing natural barrier in this system, as evidenced by the strong correlation of genetic diversity and gene flow with distance, and was included explicitly in this analysis. Non-equilibrium conditions may have also affected estimates of genetic distance and other analyses. Despite this, the large number of populations considered and the consistent relationships between genetic diversity, genetic distance and the presence of barriers suggest that these findings are robust.

Our analyses point to the conclusion that human-made barriers may greatly reduce stability of the system as a whole: populations are small and re-colonization of extinct habitat patches is critical for metapopulation persistence (Hanski & Gilpin 1997; Gonzalez *et al.* 1998). Extinction risk for many desert bighorn sheep populations in California is high, and may sharply increase in the coming century because of climate warming (Epps *et al.* 2004). If movement corridors from climatically stable refugia (high-elevation ranges in this case) to more ephemeral patches are severed, re-colonization or demographic 'rescue' will be unlikely to occur. Moreover, connectivity is critical to maintain genetic diversity over the whole metapopulation. Even though strong genetic drift may rapidly remove genetic diversity from individual populations in a functioning metapopulation, this loss can be off-set by gene flow from other populations. However, if barriers disrupt gene flow and recolonization, genetic diversity may be lost very rapidly from the system as a whole (given that the total number of populations in this instance is not large). Thus barriers can have severe consequences both for demographic and genetic processes in metapopulations and may increase the danger of metapopulation extinction.

We recommend that consideration be given to ways to mitigate existing human-made barriers, and that any future construction of major highways in desert bighorn habitat should be designed to minimize disruption of connectivity. Drainage tunnels under interstate highways already exist in some areas (e.g. under Interstate 40 between the Marble and Granite mountains); while presumably large enough to allow traversal by bighorn sheep, these tunnels are within the fenced interstate corridor. Underpasses and overpasses have been used successfully to aid dispersal of carnivores and ungulates (Foster & Humphrey 1995; Gloyne & Clevenger 2001). Changes in fencing could allow access to tunnels while still preventing livestock or wildlife from entering the highway corridor itself. Overpasses could be another, perhaps more effective means of reestablishing connectivity for bighorn sheep, although the cost of such structures could be very high.

As the human population continues to expand, the need to maintain connectivity of natural populations is even greater. Rapid development of highways and other barriers has reduced and fragmented habitat for many species, while global climate change is increasing local extinction rates and forcing latitudinal or elevational shifts in species' distributions (Walther *et al.* 2002). Species-specific solutions to restoring habitat connectivity both in previously fragmented landscapes and relative to future development must be implemented.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from http://www.Blackwell-Synergy.com:

Appendix S1 (a) Zinc-finger gene primers and protocol, (b) microsatellite analysis protocols and references, and (c) mitochondrial DNA sequencing protocols.

Appendix S2 Sample sizes for analyses of microsatellite genotypes and mtDNA sequences, and basic genetic and geographical statistics for the 27 populations of desert bighorn sheep used in this study.

Appendix S3 F_{ST} values for all sampled populations, estimated from 14 microsatellite loci using GENEPOP.

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Appendix 1-a Zinc-finger gene primers and protocol.

We used the following primers, designed by R. Ramey, to screen for and remove weakly-amplifying extractions: ZFYf2 5'-3' TTA CTG AAT CGC CAC CTT TTG GC and ZFYr1 5'-3' CTG CAG ACC TAT ATT CGC AGT ACT (annealing temperature 57°; same experimental conditions employed for microsatellite analyses in Wehausen et al. (2004)).

Appendix 1-b Microsatellite analysis protocols and references.

Experimental conditions and references for 11 of the 14 dinucleotide microsatellite loci used in this study were described previously (Wehausen *et al.* 2004); we used the additional loci OarFCB128 and OarFCB266 (Buchanan & Crawford 1993) (annealing temperature 57°) and D5S2 (Steffen *et al.* 1993) (annealing temperature 55°). Amplification products were visualized using an ABI Prism™ 377 (Applied Biosystem Inc., Foster City, USA); alleles were designated using GeneScan™ (version 3.7, Applied Biosystem Inc., Foster City, USA) and Genotyper™ (version 3.7 NT, Applied Biosystem Inc., Foster City, USA).

Appendix 1-c Mitochondrial DNA sequencing protocols.

For mtDNA sequencing, we used ABI Prism™ 377 and 3730 sequencers (Applied Biosystems, Inc., Foster City, USA) and the following primers designed by R. Ramey: L15712 5'-3' AAC CTC CCT AAG ACT CAA GG and BETH 5'-3' ATG GCC CTG

AAG AAA GAA CC. We used 20 µL PCR reactions with the following reaction conditions: 1x PCR Buffer I (Applied BioSystems Inc., Foster City, USA), 0.16 mM dNTPs, 10 µg bovine serum albumin (New England BioLabs, Beverly, USA), 1.9 mM $MgCl_2$, 400 nM each primer, 0.8 units of Amplitaq Gold DNA polymerase (Applied BioSystems Inc., Foster City, USA), and 1 µL of extracted DNA. We used an initial heating cycle of 94º C for 7 minutes 30 seconds, followed by 35 cycles of 94º C for 60 seconds, 61º C for 70 seconds, and 72º C for 90 seconds. We cleaned PCR reactions using 0.2 units of shrimp alkaline phospatase (USB, Cleveland, USA) and 2 units of Exo I (New England Biolabs, Beverly, USA) to clean 1 µL of amplified DNA. We cycle-sequenced with BigDye™ v3.1 (Applied Biosystem Inc., Foster City, USA) following standard protocols.

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Appendix 2 Sample sizes for analyses of microsatellite genotypes and mtDNA sequences (if different, noted parenthetically) and basic genetic and geographic statistics for the 27 populations of desert bighorn sheep used in this study. $Isolation_{\mathrm{DISTANCE}}$ is the harmonic mean of the distances to the nearest three populations; $Isolation_{\mathrm{DISTANCE+BARRIERS}}$ adds the "barrier effect distance" of 40 km to inter-population distances if a barrier intervened (see text).

Population	*n	†H_e	Total alleles	‡A	§N_{HAP}	¶N_{HAP} (corrected)	$Isolation_{\mathrm{DISTANCE}}$ (km)	$Isolation_{\mathrm{DISTANCE+BARRIERS}}$ (km)
Clark	12	0.614	57	3.52	2	1.92	4.1	4.3
Clipper	16	0.647	54	3.31	4	3.30	8.6	15.1
Coxcomb	7	0.622	51	3.46	3	2.86	7.3	13.5
Cushenbury	15	0.489	38	2.49	1	1	20.8	62.6
Chemehuevi	9	0.571	51	2.94	1	1	34.8	37.8
Eagle-Buzzard Spr.	17	0.653	61	3.68	1	1	4.2	4.5
Eagle-Lost Palms	14	0.627	62	3.75	3	2.31	4.4	5.3
Granite	21	0.627	66	3.62	6	3.52	4.6	7.5
Hackberry	13	0.637	49	3.18	1	1	10.0	10.0
Iron	11	0.537	43	2.68	2	1.51	6.4	7.6

Cady	12	0.591	53	3.34	4	3.28	13.2	15.1
Little San Bernardino	12	0.626	57	3.58	3	2.34	8.3	15.2
Marble	29 (28)	0.644	61	3.55	3	1.77	5.8	8.5
Newberry	15	0.496	37	2.49	2	1.93	27.2	42.0
Old Dad	25	0.561	51	3.04	3	2.75	10.7	10.7
Indian Spring	12	0.475	48	2.90	3	2.06	11.5	14.7
Orocopia	18	0.568	47	3.00	3	1.97	5.9	9.4
Old Woman	26	0.512	54	3.04	3	2.39	10.8	10.8
Piute Range	13	0.627	55	3.42	3	2.68	21.3	21.3
Providence	20	0.628	59	3.51	5	3.37	3.6	4.1
Queen	11	0.594	55	3.42	3	2.49	19.4	24.4
Riverside Granite	10 (8)	0.609	47	3.09	2	2.00	7.5	11.5
South Bristol	14	0.599	51	3.29	2	1.98	8.9	12.1

San Gorgonio	17	0.539	44	2.80	1	1	8.9	50.3
San Gabriel	6	0.549	38	2.71	1	1	60.6	101.8
Turtle	14	0.635	54	3.33	2	1.43	18.3	18.3
Wood	10	0.622	55	3.53	3	2.49	5.3	5.6

[*] number of individuals sampled per population

[†] expected heterozygosity

[‡] allelic richness corrected for variation in sample size

[§] number of mtDNA haplotypes detected

[¶] number of mtDNA haplotypes corrected for variation in sample size

Appendix 3 F_{ST} values for all sampled populations, estimated from 14 microsatellite loci using GENEPOP. All values were significantly different ($p<0.05$). Population names are abbreviated but are presented in the same order as in Appendix 2.

	CL	CO	CU	CV	EABZ	EALP	GR	HA	IR	KD	LS	MA	NE	OD	OE	OR	OW	PI	PR	QU	RG	SB	SG	SL	TU	WO
CK	0.13	0.14	0.29	0.16	0.10	0.11	0.09	0.11	0.21	0.15	0.13	0.14	0.25	0.15	0.20	0.15	0.18	0.09	0.12	0.13	0.12	0.15	0.21	0.15	0.14	0.10
CL		0.15	0.24	0.18	0.10	0.10	0.08	0.14	0.21	0.13	0.13	0.05	0.20	0.15	0.19	0.18	0.17	0.12	0.11	0.15	0.11	0.07	0.20	0.20	0.16	0.13
CO			0.27	0.16	0.11	0.07	0.07	0.10	0.16	0.10	0.10	0.14	0.26	0.06	0.20	0.19	0.12	0.11	0.10	0.09	0.10	0.15	0.16	0.22	0.16	0.06
CU				0.35	0.18	0.18	0.20	0.31	0.26	0.28	0.20	0.25	0.37	0.27	0.32	0.29	0.22	0.26	0.27	0.23	0.22	0.24	0.07	0.37	0.31	0.26
CV					0.21	0.21	0.12	0.15	0.29	0.18	0.24	0.18	0.33	0.18	0.24	0.25	0.22	0.16	0.19	0.22	0.20	0.19	0.27	0.26	0.22	0.14
EABZ						0.02	0.07	0.13	0.17	0.14	0.04	0.13	0.25	0.15	0.19	0.15	0.13	0.10	0.09	0.06	0.07	0.12	0.12	0.21	0.13	0.12
EALP							0.06	0.12	0.13	0.13	0.04	0.12	0.23	0.11	0.18	0.16	0.10	0.10	0.08	0.03	0.07	0.10	0.12	0.20	0.12	0.09
GR								0.06	0.17	0.11	0.08	0.10	0.18	0.09	0.13	0.17	0.12	0.08	0.05	0.11	0.08	0.11	0.13	0.17	0.13	0.05
HA									0.21	0.14	0.13	0.13	0.21	0.14	0.19	0.22	0.18	0.08	0.06	0.16	0.17	0.16	0.21	0.17	0.18	0.02
IR										0.24	0.18	0.21	0.32	0.19	0.29	0.29	0.05	0.19	0.18	0.14	0.17	0.23	0.19	0.21	0.21	0.19
KD											0.15	0.09	0.26	0.11	0.10	0.17	0.17	0.12	0.15	0.16	0.12	0.12	0.20	0.21	0.18	0.12
LS												0.14	0.24	0.16	0.21	0.17	0.13	0.12	0.12	0.05	0.11	0.14	0.15	0.22	0.13	0.13
MA													0.14	0.16	0.18	0.17	0.15	0.10	0.11	0.16	0.12	0.04	0.19	0.20	0.15	0.13
NE														0.27	0.35	0.30	0.26	0.15	0.19	0.25	0.28	0.19	0.27	0.32	0.25	0.24
OD															0.10	0.23	0.16	0.13	0.11	0.17	0.15	0.15	0.18	0.21	0.20	0.11
OE																0.26	0.20	0.19	0.18	0.22	0.21	0.20	0.24	0.24	0.26	0.17
OR																	0.23	0.20	0.22	0.13	0.15	0.16	0.24	0.25	0.20	0.22
OW																		0.14	0.15	0.10	0.11	0.17	0.16	0.18	0.15	0.16
PI																			0.07	0.13	0.12	0.12	0.16	0.17	0.09	0.05
PR																				0.13	0.15	0.14	0.17	0.18	0.15	0.05
QU																					0.09	0.14	0.16	0.21	0.15	0.15
RG																						0.13	0.16	0.22	0.13	0.13
SB																							0.20	0.23	0.18	0.14
SG																								0.27	0.21	0.18
SL																									0.22	0.18
TU																										0.16

Effects of Climate Change on Population Persistence of Desert-Dwelling Mountain Sheep in California

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Abstract: *Metapopulations may be very sensitive to global climate change, particularly if temperature and precipitation change rapidly. We present an analysis of the role of climate and other factors in determining metapopulation structure based on presence and absence data. We compared existing and historical population distributions of desert bighorn sheep (*Ovis canadensis*) to determine whether regional climate patterns were correlated with local extinction. To examine all mountain ranges known to hold or to have held desert bighorn populations in California and score for variables describing climate, metapopulation dynamics, human impacts, and other environmental factors, we used a geographic information system (GIS) and paper maps. We used logistic regression and hierarchical partitioning to assess the relationship among these variables and the current status of each population (extinct or extant). Parameters related to climate—elevation, precipitation, and presence of dependable springs—were strongly correlated with population persistence in the twentieth century. Populations inhabiting lower, drier mountain ranges were more likely to go extinct. The presence of domestic sheep grazing allotments was negatively correlated with population persistence. We used conditional extinction probabilities generated by the logistic-regression model to rank native, naturally recolonized, and reintroduced populations by vulnerability to extinction under several climate-change scenarios. Thus risk of extinction in metapopulations can be evaluated for global-climate-change scenarios even when few demographic data are available.*

Key Words: climate change, extinction, hierarchical partitioning, metapopulation, *Ovis canadensis*

Efectos del Cambio Climático sobre la Persistencia de la Población de Borrego Cimarrón en California

Resumen: *Las metapoblaciones pueden ser muy sensibles al cambio climático global, especialmente si la temperatura y precipitación cambian rápidamente. Presentamos un análisis del papel del clima y otros factores en la determinación de la estructura de la metapoblación con base en la presencia y ausencia de datos. Comparamos las distribución actual e histórica de la población de borrego cimarrón del desierto (*Ovis canadensis*) para probar si los patrones climáticos regionales estaban correlacionados con la extinción local. Utilizamos un Sistema de Información Geográfica (SIG) y mapas para examinar todas las cordilleras que tienen o tuvieron poblaciones de borregos en California y calificar variables que describen el clima, la dinámica metapoblacional, los impactos humanos y otros factores ambientales. Utilizamos regresión logística y partición jerárquica para evaluar la relación entre estas variables y el estado actual de cada población (extinta o existente). Los parámetros relacionados con el clima (elevación, precipitación y presencia de manantiales confiables) estuvieron poderosamente correlacionados con la persistencia de la población en el siglo veinte. Las poblaciones en cordilleras bajas y más secas tuvieron mayor probabilidad de extinción. El pastoreo de borregos*

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domésticos se correlacionó negativamente con la persistencia de la población. Utilizamos las probabilidades de extinción condicionales generadas por el modelo de regresión logística para clasificar a las poblaciones nativas, recolonizadas naturalmente y reintroducidas por su vulnerabilidad a la extinción bajo varios escenarios de cambio climático. Así, el riesgo de extinción en metapoblaciones puede ser evaluado para varios escenarios de cambio climático aun cuando sólo se disponga de pocos datos demográficos.

Palabras Clave: cambio climático, extinción, metapoblacione, *Ovis canadensis,*partición jerárquica

Introduction

Current climate-change scenarios predict an increase in global air temperature of 1.1–3.3° C over the next century (Houghton 1996; Field et al. 1999). Warmer temperatures during the last 30 years have affected the function and composition of ecological communities and the phenology and distribution of many species (Walther et al. 2002). Population declines and local and global species extinctions have also been linked to this warming trend (McCarty 2001).

As climate warms, vegetation communities shift in composition or distribution. High-elevation plant communities decrease in area, fragment, or vanish (Peters & Darling 1985). Species with fragmented distributions and low dispersal capability may be particularly vulnerable because dispersal to new sites may be limited (Walther et al. 2002). Therefore, species distributed in metapopulations (Levins 1969, 1970) may be at high risk. Climate change that decreases habitat quality or area may increase local extinctions and decrease the number of available habitat patches, conditions that can lead to extirpation of a metapopulation before all habitat becomes unsuitable (Hanski 1999). Moreover, environmental stochasticity or environmental change is usually regionally correlated, which reduces metapopulation size and persistence time (Levins 1969; Hanski 1999). We present an analysis of populations of desert mountain sheep (*Ovis canadensis nelsoni*, as defined by Wehausen & Ramey 1993, 2000) that demonstrates a simple and general way to analyze metapopulation response to climate-related environmental variation using relatively sparse data.

Desert mountain sheep, hereafter referred to as desert bighorn sheep, are desert-adapted ungulates with small population sizes, low dispersal rates (Geist 1971), and naturally fragmented distributions often characterized as metapopulations (Schwartz et al. 1986; Bleich et al. 1990; Bleich et al. 1996). Desert bighorn sheep inhabit numerous, but often small and isolated, desert mountain ranges throughout the Sonoran, Mojave, and Great Basin deserts of the southwestern United States. A few populations are also found in the more mesic Transverse and Peninsular mountain ranges of southwestern California. Most populations of desert bighorn sheep are small, with 41 of 56 extant populations in the state of California estimated at fewer than 100 individuals in 1993 (Torres et al. 1994).

Desert sheep are well adapted to xeric conditions (Hansen 1982), persisting as the climate of the southwestern United States has become increasingly arid since the end of the Wisconsonian glacial period (Van Devender & Spaulding 1979; Spaulding 1990). However, recent regional trends in warming and drying have been particularly severe. From 1901 to 1987, mean annual temperature in the deserts of the southwestern United States increased 0.12° C per decade (Lane et al. 1994). Annual precipitation decreased by roughly 20% over the last century in southeastern California, one of the largest such decreases in the United States (Ball et al. 1998). Because drought can cause increased mortality among desert bighorn sheep (Monson 1960), affect recruitment dynamics (Wehausen et al. 1987), and has perhaps led to population extinction in several cases (Weaver & Mensch 1971), the distribution of desert bighorn sheep may already have been affected by these climatic trends.

Biologists have attempted to estimate the presence and size of bighorn sheep populations within California since 1940, and in some cases earlier records exist (Torres et al. 1994; Wehausen 1999). Although imperfect, this data set presented an opportunity to examine the role of spatial and temporal climatic variation and other factors in the population persistence of desert bighorn sheep. Historic and current population sizes have been estimated variously from ground, waterhole, and helicopter surveys (Torres et al. 1994). The nature and quality of these inventories have varied, but partial population inventories were compiled in 1940, 1946–1948, 1957, 1970–1974, 1979–1985, 1994, and 2002 (Wehausen 1999). Significant population turnover was observed: about 30 of 80 populations of desert bighorn sheep have gone extinct in California during the last 60 years, with an estimated 4300 desert bighorn sheep remaining by 1993 (Torres et al. 1994). Desert bighorn sheep have been reestablished in seven mountain ranges by translocation (Torres et al. 1994) (Fig. 1). Three apparent natural recolonizations have been observed in recent years. It is possible that additional extinctions and subsequent recolonizations were undetected between survey periods.

Additional Causes of Population Extinction

Factors other than climate must be considered in any systematic analysis of turnover of bighorn sheep populations.

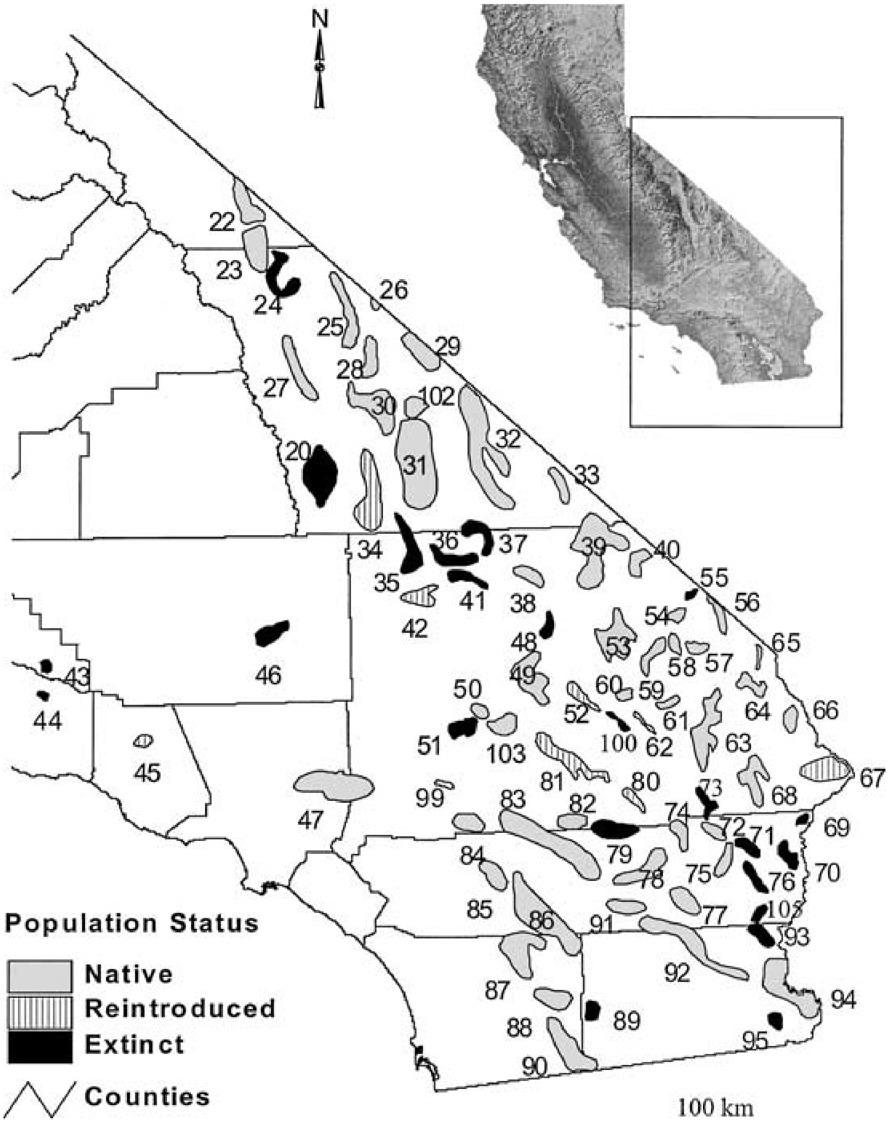

*Figure 1. Native, relocated, and extinct populations of desert bighorn sheep (*Ovis canadensis nelsoni*) in California (from Torres et al. 1994). Numbers are those assigned to the ranges of the populations demarcated by the polygons.*

Many of these factors are related to patterns of historical and current human use. Contact with livestock is detrimental to bighorn sheep because of competition for forage (Hansen 1982) and disease transmission, particularly from domestic sheep (Foreyt & Jessup 1982; Jessup 1985). Feral burros may contribute to the decline of bighorn populations by destroying water holes and competing for forage (Buechner 1960; Hanley & Brady 1977; Dunn & Douglas 1982), but there has been little quantification of subsequent reductions in bighorn numbers (Welles 1962; Jones 1980). Poaching and unregulated hunting have historically reduced populations (Buechner 1960), particularly in areas where mining occurred (Graham 1980), but poaching probably has had little impact in recent times (Weaver 1982). Nonetheless, development and general use of bighorn habitat by humans remains a concern in specific areas (Papouchis et al. 2001; Rubin et al. 2002).

Small population size has been considered an important but controversial predictor of population vulnerability of desert bighorn sheep, although the reasons for variation in population sizes have not been considered (Berger 1990; Krausman et al. 1993, 1996; Goodson 1994; Wehausen 1999). There is little argument, however, that population size is a potentially important factor in population persistence (Caughley 1994). Both Berger (1990) and Wehausen (1999) concurred that small populations of these unique ungulates were more vulnerable to extinction than large ones.

Hypotheses

We predicted that the probability of population extinction of desert bighorn sheep in California would be inversely correlated with climatic factors (temperature and precipitation) that increase annual nutrient availability and with the presence of predictable surface water, which helps desert bighorns survive periods of severe drought. Elevation was used as a surrogate for temperature because of the lack of detailed spatial data on temperature, although it is also correlated with precipitation. Furthermore, elevation can be easily measured in other systems where detailed spatial information on climate is not available. Although numerous human-made water sources have been made available to desert bighorn in California (Bleich & Pauli 1990), we limited analyses to natural water sources as a better reflection of water availability during most of the twentieth century.

We evaluated several other hypotheses concerning the distribution of desert bighorn sheep in California. In most metapopulation patch models, extinction probability decreases with increasing patch area (Hanski 1991, 1997). Extinction probability also decreases with increasing immigration, which, in turn, depends largely on interpatch distance (Levins 1969, 1970; Hanski 1991, 1997). Therefore, we tested the following hypotheses: (1) extinct populations inhabit ranges with smaller two-dimensional area than ranges with extant populations, and (2) extinct populations are more isolated from other mountain ranges containing bighorn habitat than are extant populations.

We evaluated additional plausible influences on desert bighorn sheep persistence to control for possible correlation with climate-related variables. These included geological variation, presence of domestic and feral livestock, and measures of human use of bighorn habitat. Finally, after exploring how regional climatic variation affected population extinction, we used the global-climate-change scenario described by Field et al. (1999) to simulate how the risk of extinction for remaining populations might change over the next century.

Methods

Spatial Analysis of Population Extinction

We scored 80 mountain ranges with extinct or extant populations of desert bighorn sheep in California for average annual precipitation, elevation, isolation, area, presence of dependable natural springs, geologic parent material, domestic sheep and cattle allotments, presence of feral horses and burros, deposits of precious metals, cities and towns, and vehicle access (Table 1). We converted all data from the geographic information system (GIS) to raster format in Universal Transverse Mercator (UTM) projection units and overlaid them on the population map (Torres et al. 1994). Data not based on the GIS were recorded only as presence or absence.

To determine which ranges to include, we updated a GIS map of desert bighorn sheep populations (Fig. 1) compiled by Torres et al. (1994) with population lists from Wehausen (1999). The rough population polygons (Torres et al. 1994) generally were based on the basal contours of each range and were usually defined as areas of contiguous mountainous terrain, separated from other populations by areas of flat desert or low relief. Because dispersal between mountain ranges is extremely limited (Ramey 1995), these populations function largely as independent demographic units. The Mule Mountains (Fig. 1, no. 105) were added to the list based on evidence of beds and fairly heavily used trails (R. Weaver, personal communication).

Elevation and precipitation scores were the map-grid cells with the highest value overlapping each population polygon. Bighorn sheep move easily within mountain ranges and thus can select the best conditions within the area; hence, the highest precipitation and elevation values probably best reflect the range of habitat available for use. We determined the presence of dependable springs by interviewing experts on desert water in bighorn sheep habitat in California (Table 1) and consulting reports on wildlife-accessible desert water sources (Weaver et al. 1968, 1969, 1972; Weaver & Mensch 1970*a*, 1970*b*, 1970*c*, 1971; Weaver & Hall 1971*a*, 1971*b*, 1972, Weaver 1972). For each mountain range, we compiled a list of springs that do not dry up even during extended drought.

We estimated isolation for each mountain range by taking the harmonic mean of the distance from the edge of each population polygon to the edge of the nearest three population polygons (Harrison & Ray 2002) (Table 1). Area was calculated directly from the GIS population polygons.

We scored geologic parent material because the geology of bighorn habitat in the Mojave and Sonoran deserts of California is diverse, and the type and quality of vegetation is often influenced by parent material (Barbour et al. 1980). Resulting variation in forage could affect the persistence of bighorn sheep populations. We examined the percent area of volcanic or granite parent material and the presence or absence of limestone in each range.

We used information from the California Desert Conservation Area (CDCA) plan (Bureau of Land Management 1980) to test whether population extinctions were more common when cattle or domestic sheep grazing allotments, feral burros and horses, and mineral deposits suitable for mining were present in bighorn sheep ranges since population inventories began around 1940. The presence of mineral deposits suitable for mining was the best available index of mining activity, thought to conflict with bighorn sheep as a result of poaching by miners in the earlier periods and habitat destruction (Buechner 1960). We interviewed range and wildlife biologists for the U.S. Forest Service, Bureau of Land Management, Deep Canyon Reserve, and San Diego Zoo (S. Loe, B. Brown, M. Frael, A. Muth, & E. Rubin, personal communications) to score mountain ranges not described in the CDCA plan (Fig. 1, nos. 43, 44, 45, 47, 84, 85, 86, 87, 88, 89, 90, 99). We also determined whether degree of road access and

Table 1. Parameters included in logistic-regression analyses of extinction of desert bighorn sheep populations[a] in California.

Parameter	*Descriptive statistic*	*Data type/description[b]*	*Source[b]*
Precipitation	maximum value in each population polygon	GIS/isohyets of average annual precipitation 1900–1960, 400+ ha resolution	UCLA/Teale Data Center[c]
Elevation	maximum	GIS/3-arc-second digital elevation models, 90 m resolution	UCLA/Teale Data Center
Dependable springs	presence/absence	interviews/experts on wildlife water sources in California	G. Sudmeier (SCBS); R. Weaver (CDFG, retired)
Granite	area (%)	GIS/"Geologic Map of the United States"	King & Beikman 1974
Volcanic rock	area (%)	GIS/"Geologic Map of the United States"	King & Beikman 1974
Limestone	presence or absence	paper/1:250,000 scale geologic maps of California	Jenkins 1958
Isolation	harmonic mean of distance to nearest 3 populations	GIS/"Status of Bighorn Sheep in California, 1994"	Torres et al. 1994
Area	polygon area	GIS/"Status of Bighorn Sheep in California, 1994"	Torres et al. 1994
Distance to towns/cities	minimum distance from sheep polygons	GIS/urban areas (1990 census)	UCLA/Teale Data Center
Mining	presence or absence of "economically viable mineral deposits"	paper/map 11, "Economic Mineral Resources"	BLM 1980
Road access (ordinal)	closed (1), approved roads (2), existing roads (3)	paper/map 10, "Motorized-Vehicle Access"	BLM 1980
Feral burros and horses	presence or absence	paper/map 8, "Wild Horse and Burro Management Area"	BLM 1980
Cattle	presence or absence of grazing allotments	paper/map 9, "Livestock Grazing Allotments"	BLM 1980
Domestic sheep	presence or absence of grazing allotments	paper/map 9, "Livestock Grazing Allotments"	BLM 1980

[a] *Population polygons were drawn by Torres et al. (1994) using the basal contour of each mountain range inhabited or formerly inhabited by desert bighorn sheep.*
[b] *Abbreviations: BLM, Bureau of Land Management; CDFG, California Department of Fish and Game; GIS, geographic information system; SCBS, Society for Conservation of Bighorn Sheep; UCLA, University of California, Los Angeles (mirror site of Teale Data Center, Web site: http://gisdb.cluster.ucla.edu:3080/)*
[c] *Stephen P. Teale Data Center, Web site: http://www.gis.ca.gov/*

minimum distance to towns and cities correlated with extinction (Table 1). We assumed that these measures provided an index of general human activity and that current vehicle access (closed or restricted to varying degrees) reflects historical patterns of use.

Logistic Regression and Model Selection

We combined all parameters in logistic-regression models using an approach similar to that of Sjögren-Gulve and Ray (1996). The response parameter categories were "extinct" or "native" population status. We treated all reestablished populations as extinct because reintroductions (translocations through direct human intervention) and natural recolonizations (Fig. 1, nos. 24, 73, 100) took place in ranges where population extinctions occurred previously. We calculated log-likelihood and chi-squared values using JMPstart (Sall & Lehman 1996).

We tested the univariate model for each parameter and determined that Pearson correlations between all parameters were <0.7, as recommended by Hosmer and Lemeshow (2000) (Table 2). Initially, we explored biologically relevant interaction terms between the variables but found little support for further testing.

We used $QAIC_c$, a modified version of Akaike's information criterion (AIC) (Burnham & Anderson 1998), for model selection. This statistic corrects for small sample size relative to the number of estimated parameters and for an overdispersion factor between 1 and 4. Overdispersion was estimated as 3.9 from the likelihood-ratio chi-squared value and degrees of freedom of the global model including all 14 parameters (Burnham & Anderson 1998) and may result from correlated environments among adjacent populations. We grouped parameters by category of hypothesis (climate, geology, metapopulation, domestic or feral livestock, and human use) into models and compared $QAIC_c$ values with univariate and global models. We then combined parameters with strong effects in additional models.

After identifying a series of competing best models, we used hierarchical partitioning to assess the independent and joint effects of each parameter in a single model with all parameters included in the best models (Chevan & Sutherland 1991). Hierarchical partitioning serves as an additional control for multicollinearity and uses a measure of model fit to separate the independent and joint contributions of each parameter by comparing the fit of all models containing a particular parameter to all corresponding

Table 2. Correlation of parameters in global logistic-regression model of desert bighorn sheep population extinction.

Variable	*Elev.*	*Spr.*	*Grn.*	*Volc.*	*Lim.*	*Iso.*	*Area*	*City*	*Mine*	*Road*	*Bro.*	*Ctl.*	*D.Shp.*
Precipitation	0.65*	0.33*	0.03	−0.21	0.20	0.34*	0.16	−0.36*	−0.05	−0.12	−0.18	0.23*	0.43*
Elevation (Elev.)	—	0.47*	0.07	−0.22	0.49*	0.06	0.35*	−0.09	0.18	−0.22	0.16	0.39*	0.21
Springs (Spr.)		—	−0.03	0.08	0.04	0.08	0.27*	−0.04	0.09	−0.26*	0.14	0.23*	0.21
Granite (Grn.)			—	−0.28*	0.01	−0.04	0.04	−0.22	−0.02	0.04	−0.24*	0.21	−0.02
Volcanic (Volc.)				—	−0.16	−0.06	−0.12	0.30*	−0.18	0.08	0.10	0.10	−0.08
Limestone (Lim.)					—	−0.07	0.18	0.03	0.29*	0.10	0.19	0.33*	0.03
Isolation (Iso.)						—	−0.05	−0.18	0.15	−0.13	−0.15	−0.09	0.66*
Area							—	−0.32*	0.21	−0.10	0.05	0.03	0.06
City distance (City)								—	0.23*	−0.01	0.30*	0.16	−0.17
Mining (Mine)									—	0.13	0.21	0.09	0.04
Road access (Road)										—	0.08	0.05	−0.08
Burros/horses (Bro.)											—	0.08	−0.08
Cattle (Ctl.)												—	−0.04
Domestic sheep (D.Shp.)													—

**Significant Pearson correlation,* $p < 0.05$.

models without that parameter. This allows greater confidence that the action of a parameter is not masked in the model by coaction with other parameters (Chevan & Sutherland 1991; Mac Nally 1996, 2000). We used the likelihood-ratio chi-squared statistic for each model as the measure of fit to be hierarchically partitioned (Chevan & Sutherland 1991; Mac Nally 1996). The likelihood chi-squared statistic compares the log-likelihood of the model to that of the reduced model with predictor variables removed (Sall & Lehman 1996). Larger values indicate a better fit.

We used parameters included in the competing best-fit models to evaluate extinction probabilities for all native, naturally recolonized, and reintroduced populations for the next 60 years: the mean and modal times of earliest population estimates for all desert bighorn sheep populations in California were 63 and 60 years, respectively. Logistic-regression models give the probability of transition between response variables for each observation (Sall & Lehman 1996) and therefore can be used to calculate the conditional extinction probabilities for each population with regard to factors considered in the model (Sjögren-Gulve & Ray 1996). To assess future risk to native and reintroduced populations, we calculated extinction probabilities (E) as

$$E_i = \frac{e^{(a+bx_i+cy_i+dz_i)}}{1+e^{(a+bx_i+cy_i+dz_i)}}, \tag{1}$$

where a is a constant, b, c, and d are the parameter estimates for the ith population, and x, y, and z are the corresponding predictor variables.

Using the Extinction Model to Evaluate Climate-Change Scenarios

We modeled how population extinction of desert bighorn sheep in California may change with decreasing precipitation (Ball et al. 1998) and increasing temperature (Field et al. 1999) over the next 60 years by using observed relationships with climate-related variables in the final best-fit extinction models. We used the same model chosen for hierarchical partitioning. A warming trend in climate results in a given average temperature occurring at a higher elevation, and organisms with minimum elevation requirements will be found at higher elevations (e.g., Grabherr et al. 1994). To simulate this, we regressed average maximum daily temperature on elevation from 21 weather stations throughout the study area against station elevation (over station history, usually from about 1940 to present) to calculate the adiabatic lapse rate, or rate at which temperature changes with elevation. Bighorn sheep are largely diurnal, and average daily maximum temperature better reflects the extreme temperatures encountered in daytime during foraging and watering. Further, elevation was most correlated with maximum temperature.

We converted 100-year estimates for future temperature change (Field et al. 1999) to 60-year estimates by assuming a linear rate of change. Using the adiabatic lapse rate, we translated the minimum and maximum predicted temperature changes into "losses" in elevation. We subtracted these elevation losses from each population's elevation score. To simulate a further decrease in precipitation, as was observed in the twentieth century in the study area (Ball et al. 1998), we decreased each precipitation score by 12% (60% of the observed 20% change). Finally, using the chosen best-fit extinction model, we used the modified precipitation and elevation scores to recalculate extinction risk for each population.

Results

Causes of Extinction

The AIC testing revealed three competing models within two ΔQAIC units of the best model, which contained

Table 3. Logistic-regression models of bighorn sheep population extinction in California, with log-likelihood values, number of parameters (*k*) including model parameters, intercept, and residual variance (Burnham & Anderson 1998:17), and QAIC$_c$ values.[a]

Rank	*Model*	*Log likelihood*	*k*	*QAIC$_c$*	*Δ QAIC$_c$*
1	precipitation, elevation, sheep	−29.67	5	25.87	0.00*
2	precipitation, springs, sheep	−30.56	5	26.33	0.46*
3	precipitation, elevation, springs, sheep[b] (climate + sheep)	−28.48	6	27.61	1.74*
4	elevation, springs, sheep	−33.12	5	27.63	1.75*
5	elevation	−46.79	3	30.08	2.46
6	water	−47.71	3	30.55	2.93
7	sheep	−48.11	3	30.75	3.14
8	area	−49.82	3	31.62	4.01
9	cattle	−49.99	3	31.71	4.09
10	limestone	−50.80	3	32.12	4.50
11	mining	−51.14	3	32.29	4.68
12	precipitation	−51.31	3	32.38	4.76
13	isolation	−51.59	3	32.52	4.90
14	volcanic	−52.44	3	32.95	5.33
15	burro	−52.50	3	32.98	5.37
16	granite	−52.79	3	33.13	5.51
17	city distance	−52.79	3	33.13	5.52
18	limestone, sheep	−45.43	4	31.61	5.73
19	road access	−50.27	4	34.06	6.45
20	isolation, area, sheep (metapopulation + sheep)	−42.74	5	32.52	6.64
21	isolation, area (metapopulation processes)	−48.55	4	33.19	7.31
22	burro, cattle, sheep (domestic, feral livestock)	−44.95	5	33.64	7.76
23	precipitation, elevation, springs (climate)	−45.00	5	33.67	7.79
24	city distance, roads, mining, sheep (human use + sheep)	−39.75	7	32.47	9.88
25	granite, volcanic, limestone, sheep (geology + sheep)	−44.98	6	36.00	10.12
26	granite, volcanic, limestone (geology)	−50.52	5	36.47	10.59
27	city distance, road access, mining (human use)	−47.51	6	37.28	11.40
28	global model (all parameters)	−23.39	17	55.75	29.87

[a] *Models are ranked by ΔQAIC$_c$ values (a modified version of Akaike's information criterion); competing models with ΔQAIC$_c$ values of <2 are marked with an asterisk.*

[b] *Model used for hierarchical partitioning and climate simulations. Coefficients are 11.497549 ± 0.55382193 (negative if dependable natural springs are present) ± 7.2903257 (positive if domestic sheep grazing is present) − 0.0161136 ∗ precipitation − 0.0015005 ∗ elevation (see Eq. 1 and Results).*

the parameters of maximum average annual precipitation, maximum elevation, and presence of domestic-sheep grazing allotments (Table 3). The three competing models included the presence of dependable springs, the presence of domestic-sheep allotments, and either maximum annual precipitation, maximum elevation, or both (Table 3). Extinction was negatively correlated with precipitation, elevation, and dependable springs but positively correlated with the presence of domestic-sheep grazing allotments. We chose to use model 3 (Table 3) for hierarchical partitioning and climate simulations because it included all the parameters in the three competing models.

Hierarchical Partitioning of Parameters

Hierarchical partitioning of model 3 (Table 3) revealed that all four parameters retained in the four best competing models had reasonably large independent effects (Table 4). The action of elevation was largely independent. The presence of dependable springs had the weakest independent effect on the model and a large, positive joint effect, indicating that its action in the model was due in part to a high correlation with precipitation and elevation (Table 2). The presence of domestic-sheep allotments had a large negative joint effect, as did precipitation (Table 4). A likely interpretation is that the presence of domestic-sheep allotments was strongly correlated with precipitation but acted in opposition to it with regard to extinction (Table 2): domestic sheep are primarily grazed in the wetter ranges that otherwise favor the persistence of bighorn sheep populations.

Modeling Climate Change

Elevation explained 94% of the variation in average maximum daily temperature at 21 weather stations throughout the study area ($p < 0.0001$) but only 58% of the variation in minimum daily temperature ($p < 0.0001$). Using the regression of maximum daily temperature on elevation, maximum daily temperature $= -0.0078 *$ (meters elevation) $+ 31.687$, we calculated the adiabatic lapse rate (rate at which temperature changes with elevation) as 7.8° C/1000 m of elevation. A mean global temperature increase of 0.7° C over the next 60 years (60% of a 1.1° C

Table 4. Hierarchical partitioning* of third-ranked extinction model of desert bighorn sheep populations in southeastern California, containing maximum annual precipitation, maximum elevation, presence of dependable natural springs, and presence of domestic sheep allotments.

	Precipitation	*Maximum elevation*	*Dependable springs*	*Domestic sheep*
Parameter alone	3.23	12.28	10.43	9.61
Independent effects	7.92	9.80	6.76	24.42
Total independent effects (%)	16	20	14	50
Joint effects	−4.69	2.48	3.67	−14.81
Total joint effects (%)	18	10	14	58

**Hierarchical partitioning uses likelihood ratio χ^2 statistics from logistic regression models as a measure of model fit, and using all possible combinations of models with any of the above four parameters assesses the independent contribution of each variable to model fit. Negative joint effects indicate that a variable acts in opposition to or "suppresses" other variables. The sum of the independent and joint effects for each parameter equals the χ^2 statistic of the univariate model for that parameter.*

increase over the next century) thus translates to a "loss" of 85 m elevation in our climate-change simulations. An increase of 2.0° C over the next 60 years translates to a loss of 254 m. We assumed no change in the availability of surface water and set all domestic-sheep allotment scores to "zero" because domestic-sheep allotments are generally no longer permitted on desert bighorn habitat (K. Allison, personal communication).

In the minimum temperature-change scenario of +0.7° C in the next 60 years (Field et al. 1999), average extinction probabilities of native populations increased only slightly, from 0.21 to 0.22. However, in the maximum temperature-change scenario of +2.0° C in the next 60 years, average risk of extinction increased substantially to 0.26. Extinction risk also increased drastically when precipitation was reduced, such that a 0.7° C increase combined with a 12% decrease in precipitation elevated extinction probabilities to levels observed with a 2.0° C increase with no change in precipitation. Average extinction risk increased from 0.21 (no change) to 0.30 when a 2.0° C increase was combined with a 12% precipitation decrease.

Discussion

Elements in the Final Model

Extinction of desert bighorn populations in California in the twentieth century did not occur randomly. Populations in mountain ranges of lower elevation were much more likely to become extinct, particularly at <1500 m (Fig. 2a). Populations in regions with the lowest annual precipitation, especially <200 mm annual precipitation, were also more likely to become extinct (Fig. 2b), as were populations without dependable springs and populations in which domestic-sheep grazing allotments formerly overlapped or abutted desert bighorn habitat. This suggests not only that desert bighorn sheep are vulnerable to climate warming but that climate warming has already affected their distribution in California.

Hierarchical partitioning established that elevation and precipitation each had relatively strong independent effects in the model, despite their high degree of colinearity (Table 4). We suggest that the correlation between low elevation and higher risk of extinction resulted largely from the highly predictive relationship between elevation and temperature. Lower mountain ranges experience higher temperatures, and, as a result, bighorn sheep could have a greater dependency on water sources or poorer nutrition, resulting in lower survival. Higher-elevation ranges have an extended growing season: spring growth starts first at the lower elevations, and green-up progresses up the elevation gradient. Therefore, taller

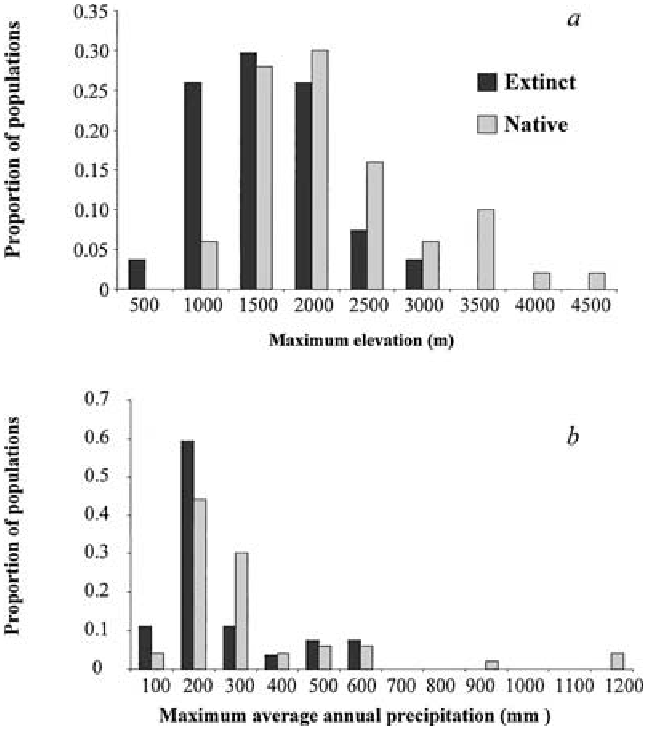

*Figure 2. Distribution of (a) maximum elevation and (b) maximum average precipitation values for ranges of native and extinct bighorn sheep (*Ovis canadensis nelsoni*).*

mountains have some green vegetation and thus better forage for much longer periods than low-elevation ranges (Wehausen 1980, 1992).

The relationship between extinction and precipitation probably results from the dynamics of water availability, soil moisture, and forage quality. Shrub cover in the Mojave Desert strongly correlates with mean annual precipitation (Beatley 1974). In arid regions even a slight decrease in moisture content, whether through increased temperature and increased evapotranspiration or through a decrease in precipitation, could have drastic effects on diet quality and therefore demography. Douglas and Leslie (1986) found that precipitation during gestation accounts for the largest proportion of variability in lamb survival. Wehausen et al. (1987) detected a positive relationship between winter precipitation and recruitment in the Santa Rosa Mountains of California (Fig. 1, no.86). Thus, precipitation apparently plays a large role in reproductive success. More explicit spatial data describing temperature and precipitation may further clarify these relationships.

The absence of dependable natural springs was also correlated with extinction, although this relationship was weaker than that of other model elements (Table 4). Nonetheless, bighorn sheep in many ranges make extensive use of springs and water holes, occur close to water during hot summer months (Andrew et al. 1997, 1999), and physiologically depend on ready access to water during summer (Turner & Weaver 1980).

Extinction of populations of desert bighorn sheep in California was not sensitive to patch size (two-dimensional area of the inhabited mountain ranges). This was surprising because patch size is often considered the most important predictor of population persistence (Hanski 1999; but see Fleishman et al. 2002), and this effect has been detected in Rocky Mountain bighorn sheep (*O. c. canadensis*) (Singer et al. 2001). The strong effect of patch size on persistence is thought to result from the expected correlation with population size if populations are strongly regulated by density dependence. If populations are regulated by environmental factors, however, one can expect a much weaker relationship between patch size and population size (Andrewartha & Birch 1954). Our findings that precipitation and elevation, but not patch size, were correlated with population extinction are consistent with strong environmental regulation of desert bighorn sheep populations.

Population isolation also did not affect extinction in our analysis. We measured isolation as the harmonic mean of the distance to the nearest three mountain ranges used at some time by bighorn sheep, but when adjacent populations were extinct, distances to the nearest inhabited patches may have been much greater. We could not use these distances as a measure because the timing of extinctions are poorly known. However, populations of desert bighorn sheep are generally demographically independent because of low female dispersal rates (Ramey 1995; Boyce et al. 1999). Although extinctions may appear regionally clustered (Fig. 1), this is probably a result of autocorrelated environmental factors such as precipitation.

Higher risk of extinction in lower, drier ranges was detected despite significant correlation of higher precipitation and higher elevation with sheep and cattle grazing and proximity to cities (Table 2). However, elements not retained in final models may still be important to consider during management on a case-by-case basis. Our conditional "extinction probabilities" (Fig. 3) are related solely to variables included in the final best-fit models. Thus, our models provide potentially biased estimates of extinction probabilities that are not all-inclusive.

Evaluating How Climate Change May Influence Population Extinction

Although crude, these climate-change simulations demonstrate that global warming could have serious consequences for desert bighorn sheep, particularly if coupled with decreases in precipitation. Other scenarios can be evaluated with these models as climate projections change. Absolute extinction probabilities should always be viewed with caution (Beissinger & Westphal 1998), but they provide a way to compare the vulnerability of populations to specific threats.

Changes in precipitation patterns, which are more difficult to predict than changes in temperature (Field et al. 1999), could balance or amplify the effects of changes in temperature because precipitation may be more limiting than temperature in these ecosystems (e.g., Wehausen et al. 1987). Careful analyses of how precipitation and temperature affect the growing season of forage plants, and thus diet quality among sheep, may improve future models of the population persistence of wild sheep. Climate warming may have more complicated or more detrimental effects when competition, predation, and disease affect desert bighorn sheep.

Our results have important implications for management actions. For future reintroductions of desert bighorn sheep, managers should consider expected precipitation and elevation within the mountain range of consideration. We do not advocate abandoning all efforts in mountain ranges that are at high risk: some may serve as valuable "stepping stones" for gene flow or demographic "rescue" (Bleich et al. 1990), and the extinction model may not be correct for all locations at all times. However, knowledge of climate-based risk of extinction may allow managers to focus further efforts on locations with the highest probability of success. Understanding which populations are under the most climate-related stress could also be critically important in coming decades (Fig. 3). Because of regionally correlated environmental conditions, whole regions of populations and subsequent opportunities for gene flow or recolonization may be lost (Fig. 3).

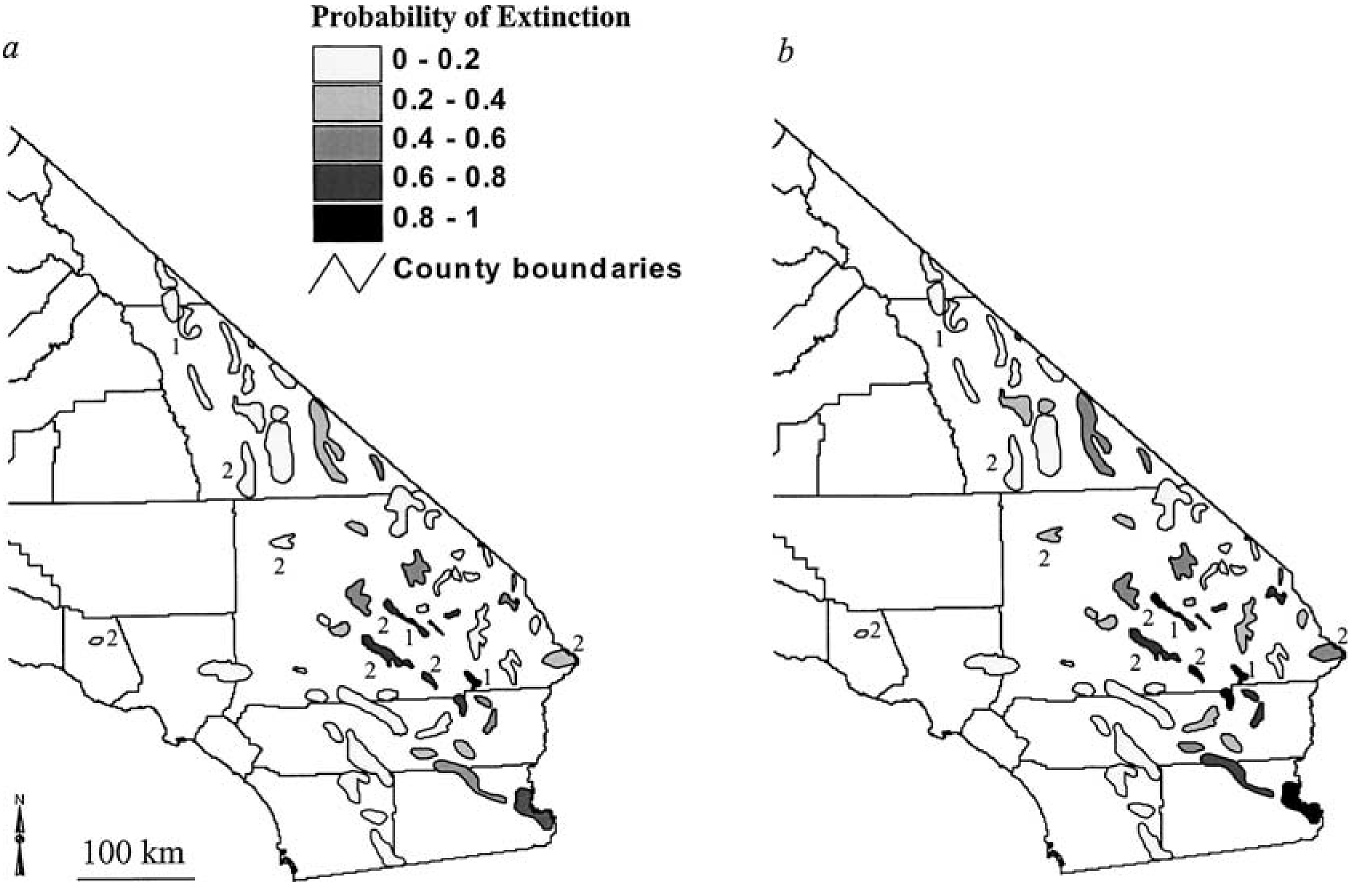

*Figure 3. Conditional extinction probabilities for unnumbered, native desert bighorn sheep (*Ovis canadensis nelsoni*); 1, desert bighorn sheep naturally recolonized since 1994; and 2, reintroduced populations of desert bighorn sheep for the next 60 years; assuming (a) no further climate change and no interactions with domestic sheep or (b) the maximum predicted increase in global temperature (+2.0° C), a 12% decrease in precipitation, no change in surface-water availability, and no interactions with domestic sheep.*

Heightened monitoring of population size, condition, and water availability, with appropriate action, may be necessary to conserve populations of desert bighorn sheep in the future.

The changes in the distribution of desert bighorn sheep observed in the twentieth century are consistent with directional climate change, but we cannot rule out the action of climatic stochasticity. It may be that the distribution of the desert bighorn in California has fluctuated for centuries, with expansion into areas of poorer habitat during cooler and wetter periods and retreat during times of increased drought frequency and intensity. Although the relationships between local climate and extinction are clear, whether current trends are the result of long-term climate change is not.

Using presence and absence data, we demonstrated that population extinctions of desert bighorn sheep in the twentieth century are consistent with a range contraction to areas of higher elevation and greater precipitation. Updated and more detailed climate scenarios can be explored through the relationships with extinction risk, elevation, and precipitation described here. This approach demonstrates that simple population viability analysis can sometimes be conducted even when detailed demographic data are absent. A similar approach might be used in systems where no prior population surveys existed, if suitable criteria for identifying empty habitat patches existed, in a variation of the incidence-function approach used by Hanski (1999) to parameterize metapopulation models.

Many species, particularly those in arid or montane regions, may have already suffered some effects of global climate warming. Elevational shifts in distribution consistent with climate change have been detected in Edith's checkerspot butterfly in the Sierra Nevada of California (Parmesan 1996), montane trees (Fisher 1997), and species in the cloud forests of Costa Rica (Pounds et al. 1999). Sparse data on population size and distribution may have hampered our ability to detect these changes elsewhere. Desert bighorn sheep may serve as a model to help us understand how similar systems may react to the coming changes.

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Return to the wild: Translocation as a tool in conservation of the Desert Tortoise (*Gopherus agassizii*) ☆

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ABSTRACT

Translocation could be used as a tool in conservation of the threatened Mojave Desert Tortoise (*Gopherus agassizii*) by moving individuals from harm's way and into areas where they could contribute to conservation of the species. Numerous factors may affect the success of translocations, including the conditions experienced by tortoises in holding facilities while awaiting translocation. The tortoises available for our translocation study had been provided supplemental water during their years spent in a captive holding facility, potentially inducing carelessness in water conservation. In addition to generally investigating the efficacy of translocation, we compared the effects of continuing with the effects of ceasing the holding facility's water supplementation regimen. After exposure to one of the two water regimens, all tortoises were given the opportunity to hydrate immediately prior to release. We examined behavior, body mass, carapace length, movement, and mortality of tortoises for two activity seasons following release to the wild. Water supplementation was correlated with high rates of carapace growth and distant movements by males after release. Lengthy movements following translocation may be problematic for conservation planning, but this should be evaluated in light of the goals and circumstances of each translocation project. Although the mortality rate was 21.4% in 1997, data suggest that drought conditions at the site rather than the translocation itself negatively affected the tortoises. None of the tortoises died during their second season at the site. Our results indicate that translocation should be considered a useful tool in conservation of the Desert Tortoise.

1. Introduction

Translocations of animals or intentional releases to the wild as attempts to establish, reestablish, or augment populations (Griffith et al., 1989) have been used with a number of species with varying levels of success. One review of translocation programs for reptiles and amphibians reported that only 19% were successful (Dodd and Seigel, 1991). Success rates may be higher, however, when programs of indeterminate success are eliminated from the calculation (Burke, 1991).

☆ Information on obtaining unpublished reports and documents cited in this paper may be requested by contacting the US Fish and Wildlife Service's Desert Tortoise Recovery Office in Reno, NV, USA.

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Definitions of success are variable and determining ultimate success can require lengthy studies (Fischer and Lindenmayer, 2000; Seigel and Dodd, 2002). Translocation may be a useful tool in conservation of some species, yet well designed studies are necessary to properly evaluate its efficacy.

The Mojave population of the Desert Tortoise (*Gopherus agassizii*) that occurs north and west of the Colorado River in the United States is protected as a threatened species under the federal Endangered Species Act (USFWS, 1990). The recovery plan associated with this federal listing included guidelines for experimental translocations (USFWS, 1994). In Las Vegas, Nevada, many Desert Tortoises were maintained in captivity at the Desert Tortoise Conservation Center (DTCC) after their removal from land undergoing urban development. We viewed experimental translocations as opportunities to test whether tortoises otherwise destined for lifetimes in captivity could be used to contribute to the recovery of the species. Some biologists have cautioned against releasing formerly captive animals because they may represent sources of disease, stress, and/or unplanned gene flow to wild tortoise populations (Berry, 1972, 1975; St. Amant and Hoover, 1978; Berry, 1986; Bury et al., 1988; Dodd and Seigel, 1991; Jacobson et al., 1991). Previous translocation studies suggested that formerly captive Desert Tortoises may not be competent in foraging or finding suitable shelter in the wild and short-term survival rates ranged from 0% to 100% for various cohorts (Berry, 1974; Cook et al., 1978; Cook, 1983). These studies did not provide sufficient evidence to support or contest the efficacy of translocation as a tool in conservation of the Desert Tortoise.

Captive and free-ranging Desert Tortoises differ considerably in their access to and use of water. Infrequent and unpredictable rainfall in the Mojave Desert allows wild tortoises few opportunities to drink, whereas tortoises at the DTCC receive provisions of water daily throughout their active seasons. Tortoises at the DTCC anticipate activation of the sprinklers and drink frequently (Ruby et al., 1994; Charles LaBar, personal communication). In addition, captive tortoises may not drink after rainstorms (Minnich, 1977) and some frequently void dilute urine (Robert Espinoza, personal communication). Retention of bladder water is important in that it can be reabsorbed for regulation of bodily solute levels (Dantzler and Schmidt-Nielson, 1966; Minnich, 1977) and hydration of dry plant matter in the gut (Peterson, 1996b). Captive tortoises conditioned to plentiful drinking water and no need to be conservative in retaining bladder water may experience functional drought conditions upon release to the wild. Although Desert Tortoises are able to cope with temporary imbalances in water budget (Nagy and Medica, 1986; Peterson, 1996a), tolerate high plasma osmolalities (Dantzler and Schmidt-Nielson, 1966; Minnich, 1977; Peterson, 1996a), and have low rates of water loss (Schmidt-Nielsen and Bentley, 1966; Naegle, 1976; Tracy, 1982; Nagy and Medica, 1986; Peterson, 1996a), mortality or morbidity caused by dehydration can be prevalent in drought years. During a drought in 1990, eight of nine deaths among a sample of 22 tortoises monitored in California were attributed to dehydration and related starvation (Peterson, 1994). We were concerned that the tortoises at the DTCC may have become too negligent about water conservation to do well in the wild, and we were interested in testing the effects of discontinuing water supplementation prior to release. In this study, we generally investigated the efficacy of translocation and tested the hypothesis that ending the supplementation of water in the fall prior to the spring release would increase initial success in translocation as measured through changes in body mass, changes in carapace length, behavior, movements, and mortality of translocated tortoises. This initial period began at time of release in spring and went up to the second period of winter inactivity following release. We refer to the periods of activity between hibernation events as activity seasons, thus from release to first hibernation is the first season and from end of first hibernation through beginning of second hibernation is the second season in the wild.

2. Materials and methods

2.1. Study subjects

We used 32 adult Desert Tortoises that had been maintained in outdoor pens at the DTCC for 7 yr and 10 juveniles that had been at the DTCC for 2 yr. All experimental tortoises were classified as negative on ELISA tests for antibodies to *Mycoplasma* spp. This reduced the chances of translocating tortoises infected with the pathogen *Mycoplasma agassizii*, which has been implicated as a cause of Upper Respiratory Tract Disease (URTD) (Brown et al., 1994). At the DTCC, tortoises received water daily throughout their active seasons until they entered hibernacula in fall 1996. Tortoises were removed from their pens on 25 and 26 March 1997, before many individuals had emerged from hibernacula and prior to the time that water was provisioned for that season. Adult experimental tortoises were 200–274 mm in carapace length with body masses of 1308–3401 g. Juvenile carapace lengths were 125–165 mm and body masses 334–603 g. On 27 March, the experimental tortoises were given the opportunity to drink for 30 min. After their body masses were recorded (Acculab Z6000 electronic balance), tortoises were placed in burrows inside randomly assigned experimental pens. Four males, four females, and two or three juveniles were released into each pen. Minimum time spent in the pens under experimental conditions was 27 days with some tortoises remaining in pens to up to 57 days. Each tortoise was fitted with a radio transmitter (AVM models G3, SB2, or SB2-RL for adults; SM1-H for juveniles) and was marked by notching the marginal scutes (Cagle, 1939) and by attaching a small numbered tag (of paper) to the carapace with epoxy. Transmitter attachment added <5% to the body mass of any animal.

2.2. Experimental pens

Tortoises were housed in four pens (15.2 m × 15.2 m) as the precondition before translocation. The pens had fiberglass walls (0.8 m) and water sprinklers. Two pens received water daily from 07:45 to 08:00 h (local time) beginning 28 March 1997. Three terracotta saucers were placed beneath the sprinkler's spray to collect water for the tortoises to drink. Tortoises from these pens are referred to as water-supplemented (WS). Two pens received no water, and those tortoises are referred to as not supplemented (NS). In each pen, three artificial burrows were constructed of polyvinyl chloride (PVC) pipes (1.22 m long and 38.1 cm diameter) cut in half lengthwise and buried at a downward angle in the soil. Two additional pieces of PVC pipe (30 cm diameter), cut in the same fashion as above, were laid on the ground as additional cover sites. The pens had comparable numbers of native shrubs. Tortoises ate dried alfalfa and slightly moistened iguana chow pellets (Zeigler Bros. Inc. 20% protein, 1/8 in. pellets, no. 53-6406-18-39) in keeping with the DTCC's feeding schedule.

2.3. *Translocation site*

The 90 km^2 translocation site, hereafter referred to as the Large-Scale Translocation Study (LSTS) site, was located in southern Nevada (WGS 84 Zone 11: 647,000 m E 3,953,000 m N). The north (bordered by Nevada Highway 161), south, and east (bordered by Interstate Highway 15) sides of the site had tortoise-proof fencing, and the unfenced western border was formed by the Spring Mountains. The resident, wild tortoise density was approximately 15–20 tortoises/km^2 (USFWS, unpublished) in a Mojave Desert scrub plant community dominated by the creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*) association (Turner, 1982). Climate of the site was typical for the northeastern Mojave Desert with approximately 97 mm of annual precipitation (occurring in summer and winter) and temperatures ranging from the mean January minimum of −0.1 °C to the mean July maximum of 40.1 °C (Rowlands, 1995).

The release area was located approximately 32 km southwest of the DTCC. Tortoise density in the release area was likely depressed due to mortality by motor vehicles on Interstate Highway 15 prior to installation of fencing for this translocation project (Hoff and Marlow, 2002). We dug 13 burrows (0.3 m long, spaced 19–49 m apart) with a power auger and shovels in the central-eastern section of the LSTS site. We did not plan to release more than 6 tortoises a day (limited by observer availability), yet wanted enough burrows available in the event that some tortoises occupied these burrows subsequent to their days of release. Burrows were labeled with metal tags, and their Universal Transverse Mercator (UTM) coordinates were measured using a Global Positioning System (GPS) unit. These burrows served as the starting points of released tortoises.

An automated weather station and four rain gauges on site measured rainfall. Additional rainfall data were obtained from the Jean Airport (7 km from release area, <0.5 km from northeastern border of LSTS site) and McCarran International Airport (approximately 45 km northeast of the LSTS site).

2.4. *Release*

Tortoises were placed in plastic tubs and transported by truck to the LSTS site (48 km by road). So that water supplementation regimen (to address potential careless voiding of bladder water after release) would be a variable, rather than time since last drink, all tortoises were given access to about 3 cm of water in their tubs for 20 min prior to release. Body masses before and after this procedure as well as observations of drinking and/or voiding were recorded. Tortoises were released by placing them headfirst into burrows. Twenty-eight tortoises were released from 23 April to 23 May 1997. Releases took place between 08:00 and 09:57 h, when air temperatures ranged from 21.5 to 30.0 °C. Six females, eight males, and one juvenile from the WS group were released, while seven females, five males, and one juvenile from the NS group were released (Table 1). High ambient temperatures prevented releases 6–19 May and prohibited release of the remaining 14 tortoises.

Each tortoise's behavior was observed for approximately 4 h on the days of release. Observers recorded items ingested and marked the paths traveled by the tortoises with flagging, so that the actual distances moved by tortoises could be calculated.

2.5. *Body mass and carapace length*

Body masses were measured using a Pesola spring scale in 1997 and an Ohaus electronic balance (model CT 6000) in 1998. Straight-line carapace lengths were measured with slide calipers (Haglof Inc., Sweden). Body mass and carapace length were recorded on day of release, 15 days after release, and once a month thereafter.

2.6. *Animal movements*

Tortoises were located up to twice weekly using a handheld receiver (Telonics) and antenna through July in 1997, except when radio signals were lost temporarily. Tortoises were tracked once each month from August 1997 to April 1998 and once each week from May 1998 to November 1998. Data recorded each time a tortoise was located included UTM coordinates, descriptive location, behavior, and condition of the animal.

2.7. *Analyses*

Data were checked for homogeneity of variance using Brown–Forsythe tests and for normality using Kolmogorov–Smirnov tests. Analyses of covariance were used to analyze change in body mass of WS and NS tortoises while in experimental pens and on the day of release with body mass at time of placement into experimental pens as the covariate. Repeated measures analyses of variance (ANOVA) were used to analyze changes in body mass after day of release, with sex and treatment as factors, and tortoise movements, with sex by treatment group as a factor. Home range sizes were calculated and mapped in ArcView™ (ESRI, Redlands, CA, USA) with the animal movement extension (Hooge and Eichenlaub, 1997) using the minimum convex polygon method. Mean home range sizes were compared using ANOVA with sex by treatment group as a factor, followed by a comparison between the sexes. Mean rates of changes in carapace length were compared using ANOVA for tortoises that survived for the length of the study with year, sex, and treatment as factors. Rates of mortality for the sex by treatment groups were compared using Pearson's Chi-square and Fisher's Exact tests. Software used for calculations included StatView™ v.4.51 and

Table 1 – Summary of Desert Tortoises translocated and their changes in body mass on day of release before and after the opportunity to drink

Tortoise #	Sex	Experimental group	Date released (1997)	% Change in body mass	Observed to drink	Excreted urine or feces
L1002	F	NS	23 April	16.67	Yes	–
L1003	F	NS	23 April	0.00	No	–
L1001	J	NS	23 April	0.00	Yes	Feces
L1005	F	WS	23 April	0.00	No	Feces
L1004	J	WS	23 April	0.00	Yes	Feces
L1006	M	WS	23 April	0.00	No	Feces
L1025	F	NS	29 April	7.14	Yes	–
L1026	M	NS	29 April	25.00	Yes	Urine (very little)
L1024	M	WS	29 April	−3.26	Yes	Feces
L1023	M	WS	29 April	0.00	No	–
L1222	F	NS	05 May	14.66	Yes	–
L1223	M	NS	05 May	0.00	No	–
L1226	M	NS	05 May	26.56	Yes	–
L1224	F	WS	05 May	−0.06	No	Feces
L1225	F	WS	05 May	−2.13	No	Feces
L1294	F	NS	20 May	9.46	Yes	Urine
L1296	M	NS	20 May	5.63	Yes	–
L1297	M	NS	20 May	23.81	Yes	Urine
L1295	F	WS	20 May	1.89	Yes	–
L1299	F	WS	20 May	−4.74	No	Urine
L1298	M	WS	20 May	0.00	–	Feces
L1346	F	NS	21 May	15.00	Yes	–
L1347	F	NS	21 May	13.81	Yes	–
L1349	F	WS	21 May	1.19	No	–
L1348	M	WS	21 May	0.00	No	–
L1367	M	WS	22 May	2.27	Yes	Feces
L1368	M	WS	22 May	0.00	No	–
L1363	M	WS	23 May	1.38	No	–

No datum was recorded as to whether L1298 was seen drinking. Excretion of urine or feces occurred between the measurements of body mass. F = female, J = juvenile (undetermined sex), M = male, WS = water-supplemented, NS = not supplemented.

SuperANOVA™ v.1.11 (Abacus Concepts Inc., Berkeley, CA, USA).

3. Results

3.1. Behavioral observations

On the days of release, all tortoises exited their initial burrows within 30 min and ate during the observation period. Tortoises primarily ate dry plants of the following species: *Schismus barbatus*, *Bromus madritensis* (*rubens*), *Plantago* sp., and *Erioneuron pulchellum*. *Chamaesyce albomarginata* and *Baileya multiradiata* were eaten green. Eight of 13 NS tortoises and 7 of 15 WS tortoises ate cacti (*Opuntia basilaris* and *Opuntia ramosissima*).

Half of the tortoises, seven from both the WS and NS groups, were observed digging on the days of release. Most of these animals did not construct complete burrows during the observation period. One male NS tortoise (L1296) successfully completed a burrow in a sandy wash in less than 1.2 h.

Only two tortoises showed obvious signs of stress on their day of release. A NS female (L1222) began frothing at the mouth at 12:45 h on 5 May 1997 and immediately started digging beneath a creosote bush. During the next hour of observation, she stopped frothing, walked to a previous location, began to froth again, and dug beneath another creosote bush where she stopped frothing and remained for the last hour of observation. A WS male (L1298) began frothing at 11:40 h on 20 May 1997, but details of his behavior are unknown. No tortoises showed signs of heat stress during observation periods after the day of release.

3.2. Body mass

Adult WS tortoises gained 14.2% (SD = 7.7) while NS tortoises lost 2.4% (SD = 4.4) ($F_{1,23} = 31.7$, $p = 0.0001$; regression coefficient = −0.012, $p = 0.0200$) of their body masses while in experimental pens before translocation. Natural drinking opportunities during the treatment period were non-existent to extremely limited as no precipitation was recorded at the DTCC in March and May and 1 mm was recorded in April. After access to water on the days of release, adult NS tortoises increased body mass by 13.2% (SD = 9.1), while WS tortoises lost 0.25% (SD = 1.9) ($F_{1,23} = 27.0$, $p = 0.0001$) (Table 1). Many WS tortoises voided feces or urine in the tubs of water. The NS tortoises gained more body mass during the opportunity to drink than they had lost while in the experimental pens (paired *t*-test: $t_{11} = -4.741$, $p = 0.0006$).

Most adult tortoises (24 of 26) lost body mass following their release into the LSTS site until rainfall began in July

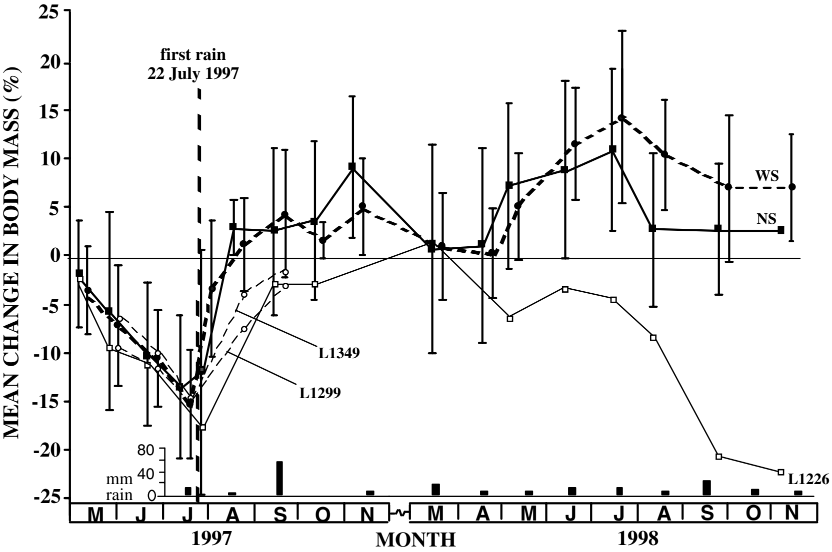

Fig. 1 – Mean change (%) in body mass (±1 SD) of WS and NS adult *G. agassizii* following release at the LSTS site. L1299, L1349, and L1226 were tortoises that showed signs of respiratory disease for >1 month and were not included in the group means.

1997. A single tortoise (L1367) voided small amounts of clear urine on three occasions (November and December 1997, January 1998) when handled. Changes in body mass were compared both by date (18 time periods), such that rain events would be reflected in changes in body mass of all tortoises during those time periods, and by number of days since release (four time periods). There were no significant relationships between initial body masses (potential covariate) and changes in body mass when examined by day since release or by date. Three tortoises (L1226, L1299, L1349) had visible signs of respiratory disease for extended periods of time and their changes in body mass were not included in the comparisons. Changes in body mass did not differ across repeated measures by date for the sexes ($F_{1,18} = 0.229$, $p = 0.6378$), treatments ($F_{1,18} = 0.123$, $p = 0.7300$), or the sex by treatment interaction ($F_{1,18} = 0.552$, $p = 0.4670$). In addition, WS and NS tortoises did not differ within time periods examined ($F_{16,142} = 1.009$, $p = 0.4507$) (Fig. 1). When controlling for number of days since release, groups of males and females with and without supplemental water (sex by treatment interaction) did not have different changes in body mass across all days ($F_{1,18} = 0.379$, $p = 0.5458$) or within the time periods ($F_{3,39} = 0.510$, $p = 0.6777$) (Fig. 2). Throughout 1998, groups that had been with or without supplemental water were heavier on average than they were on the days that they were released (Figs. 1 and 2).

3.3. *Carapace length*

Tortoises that survived the length of the study grew much more slowly in 1997 (0.001 mm/day, SD = 0.009) than they did in 1998 (0.026 mm/day, SD = 0.022) ($F_{1,26} = 12.696$, $p = 0.0014$). No significant effects were produced by sex ($F_{1,26} = 2.834$, $p = 0.1043$), treatment ($F_{1,26} = 0.437$, $p = 0.5143$), or any of the interactions. When data from 1997 and 1998 were pooled and a single rate of change in carapace length for each tortoise was calculated for the length of the study, adult WS tortoises grew significantly faster overall (0.014 mm/day, SD = 0.006), than did NS tortoises (0.007 mm/day, SD = 0.006) ($F_{1,15} = 6.230$, $p = 0.0247$). The data on five tortoises ended in September 1997, so we examined changes in carapace length for all tortoises through the end of August 1997. Interestingly, the tortoises shrank during this period by an average of 0.0145 mm/day (SD = 0.0195). Only the two juveniles and two of the adults had positive growth rates during this time.

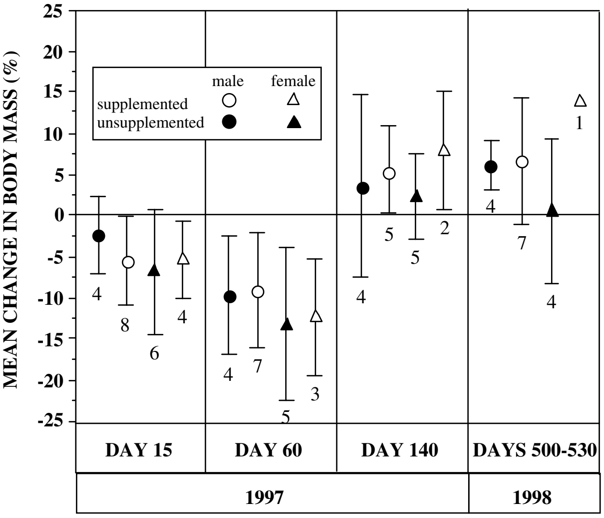

Fig. 2 – Mean change (%) in body mass (±1 SD) of adult *G. agassizii* from day of release. Sample sizes are given below each mean. Day 15 occurred from 7 May to 6 June, day 60 from 21 June to 20 July, day 140 from 9 September to 8 October, and days 500–530 from 29 September to 5 October. In 1998 measurements were recorded monthly, rather than for specific days since release.

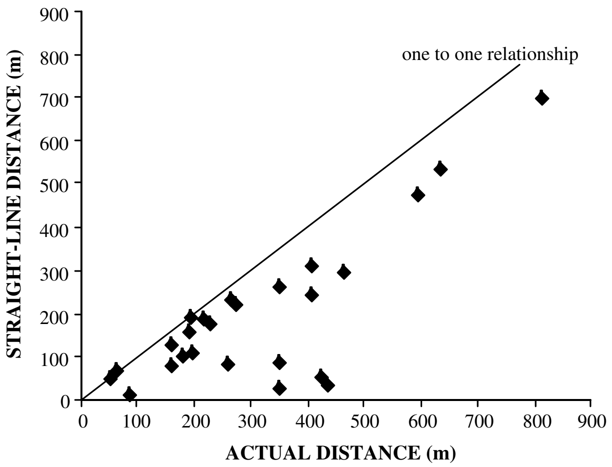

Fig. 3 – Straight-line and actual distances moved from initial burrow on day of release. The line represents a one to one relationship between the two metrics of distance moved. Tortoises with points close to the line traveled in nearly straight lines from their initial burrows. See text for discussion of tortoises that deviated from the line.

3.4. *Movement*

All but two tortoises moved away from their burrows on the days of their release. Movement patterns varied from nearly straight-line travel for many of the animals to meandering travel within the area of release (Fig. 3). There were no differences in actual or straight-line distances moved from initial burrows on days of release for the sexes (actual: $F_{1,19} = 0.010$, $p = 0.9225$; straight-line: $F_{1,19} = 0.206$, $p = 0.6551$), the treatments (actual: $F_{1,19} = 1.483$, $p = 0.2382$; straight-line: $F_{1,19} = 0.621$, $p = 0.4403$) or the sex by treatment interaction (actual: $F_{1,19} = 0.455$, $p = 0.5079$; straight-line $F_{1,19} = 0.326$, $p = 0.5750$). The amounts of time that the tortoises were observed moving were used as covariates (actual: regression coefficient = 0.773, $p = 0.0649$; straight-line: regression coefficient = 0.392, $p = 0.5917$).

The straight-line distances moved in 1997 by the groups were compared for six time periods. WS females were not included in the analysis because only one tortoise was not lost to mortality or transmitter failure at some point during the six time periods examined. The sex by treatment groups did differ ($F_{2,12} = 5.86$, $p = 0.0168$). Male WS tortoises moved significantly farther from the area of release than did NS males (Scheffe's S $p = 0.0172$) (Fig. 4a). Most of the movement away from the points of release occurred during the first 2 weeks following release. The tortoises did not show tendencies to orient northward toward the DTCC and the Las Vegas Valley (Fig. 5).

Total distances moved in 1997 also were compared by adding together the straight-line segments among locations (for the same periods of time that distance from point of release was calculated). Again WS females were not included in the overall analysis and the sex by treatment groups were different ($F_{2,12} = 4.48$, $p = 0.0352$) with WS males moving farther in total distance than NS males (Scheffe's S $p = 0.0383$). Approximately 20 weeks after release (one of the six time periods examined), total distance moved averaged 5845 m (SD = 2633) for WS males, 1872 m (SD = 1738) for WS females, 1781 (SD = 784) for NS males, and 3182 m (SD = 1950) for NS females. Total distances moved for animals with data points in the last period examined were not correlated with the number of relocation events ($R^2 = 0.052$, $F_{1,13} = 0.719$, $p = 0.4118$).

In their second season after release, tortoises remained much closer to their hibernacula than they had to their release burrows. The mean distance from hibernacula to areas of activity from May through September 1998 (11 time periods examined) was 275 m (95% CI ± 29.18) for all tortoises with no differences among the sex by treatment groups ($F_{2,11} = 0.370$, $p = 0.6991$) (Fig. 4b). Two WS males (L1298 and L1363) had movement patterns unlike those of the other tortoises (Fig. 4b). Their outlying points, as well as data from the single WS female were not included in the comparison.

Total distances moved in 1998 also were compared. Tortoises were located 21–38 times after emergence from hibernacula in 1998. The total distances moved and the number of relocation events between emergence from hibernacula and return to hibernacula were not correlated ($R^2 = 0.0004$, $F_{1,16} = 0.007$, $p = 0.9346$). Total distances moved did not differ for the sex by treatment groups ($F_{2,13} = 2.264$, $p = 0.1433$). Adult tortoises moved 5160 m (SD = 1633) in total distance during 1998.

3.4.1. *Use of burrows*

Tortoises used burrows as shelter sites during the study with no differences in the number of burrows used among the sex and treatment groups (sex: $F_{1,14} = 0.012$, $p = 0.9161$; treatment: $F_{1,14} = 0.933$, $p = 0.3506$; interaction: $F_{1,14} = 0.012$, $p = 0.9161$). Individuals tracked continuously through the end of 1997 used an average of six burrows (SD = 1.9, range = 3–10), and tortoises used eight burrows (SD = 2.6, range = 5–13) in 1998. On average, tortoises continued to use only one (SD = 0.87, range 0–3) burrow in 1998 that they first used in 1997.

Two tortoises returned to their initial human-made burrows. A WS female (L1295) was found in her initial burrow on the morning of 21 May 1997, 1 day after her release. The previous day this tortoise moved 439 m during the 3.5 h observation period (129 m straight-line distance). On 8 June 1998, a NS male (L1297) was found in the burrow within which it had been released on 20 May 1997. This tortoise was found up to 291 m from this burrow for all prior locations.

Many tortoises used their 1997–1998 hibernacula as shelter sites in 1998. Eleven of the 18 tortoises for which hibernacula were known returned to hibernacula after emergence. Two tortoises used the same burrows as both their 1997–1998 and 1998–1999 hibernacula.

3.4.2. *Home range*

Home ranges were calculated for adults in 1998 (Fig. 6), except for the two males (L1298 and L1363) that moved long distances in September 1998. Home range sizes did not differ for the sex by treatment groups ($F_{2,11} = 3.433$, $p = 0.0694$; single WS female not included) and males were not affected by treatment ($F_{1,8} = 1.225$, $p = 0.3006$). Because males and females typically have different home range sizes, data from treatment groups were combined and sexes were compared. The mean size of home ranges for male tortoises, 25.5 ha

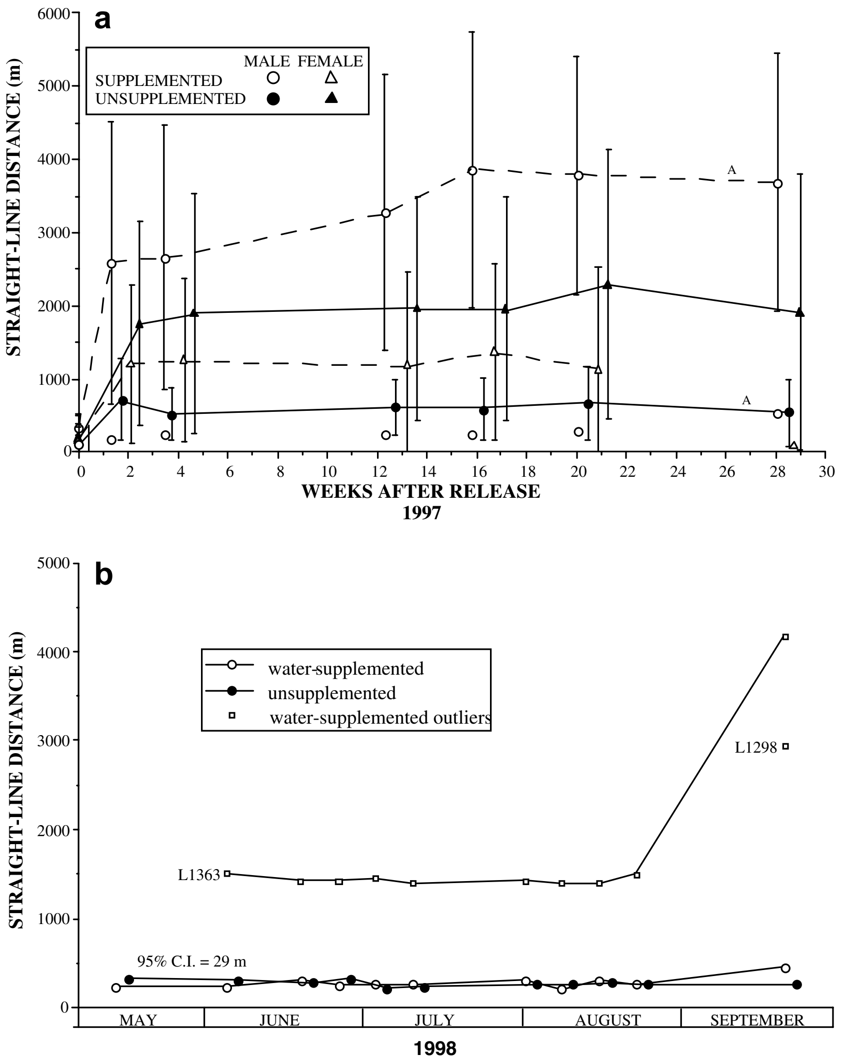

Fig. 4 – (a) Mean straight-line distances of *G. agassizii* from their initial burrows in 1997. Error bars represent one standard deviation. WS males moved significantly farther than did NS males (A). The open circles without connecting lines represent an outlier of the WS males (L1023). The open triangle without a connecting line represents a single WS female (L1005). (b) Mean straight-line distances from hibernacula of WS and NS adult *G. agassizii* in 1998. Two tortoises (L1363, L1298) had points that were outliers of the WS tortoises. WS and NS tortoises did not differ across all time periods $F_{1,15} = 0.053$, $p = 0.8209$, or within time periods $F_{10,139} = 1.310$, $p = 0.2309$.

(SD = 15.1, range = 9.94–62.73), was larger than that of females, 8.9 ha (SD = 1.9, range = 6.89–11.14) ($F_{1,13} = 5.804$, $p = 0.0315$). Tortoises located more times did not have larger home range sizes than those located fewer times (regression coefficient = −0.016, $p = 0.1914$).

3.5. *Mortality*

All six tortoise deaths occurred in 1997 (Appendix) giving a mortality rate of 21.4% (10.7% unknown outcome, 67.9% known survival) for tortoises from release to hibernation in 1997. Mortality rates were not significantly different for the main effects of sex (chi square = 3.467, df = 1, Fisher's Exact $p = 0.1602$), water treatment (chi square = 0.5159, df = 1, Fisher's Exact $p = 0.6546$), or among the sex by treatment groups (chi square = 4.573, df = 3, chi squared $p = 0.2059$).

The only adult male that died (L1348) had been supplemented with water. This animal had traveled as far as 1241 m from its initial burrow during the 48 days that it lived at the LSTS site. The tortoise had wet nares, a possible sign of disease, 1 week before its death. On 7 July 1997, the tortoise's intact carcass was found 1185 m from its initial burrow, and it had used four other burrows. There was no evidence that predation was the cause of death.

Two NS females died. L1002 was never found using a burrow between its release and death. This tortoise traveled long distances following release, and 21 days after release (13 May) its intact carcass was found overturned 4195 m from the initial burrow. L1025's carcass was found 166 days after release (11 October). The carcass was found soon after death at a location 5399 m from its initial burrow. The condition of the carcass and manner in which it was slightly buried and

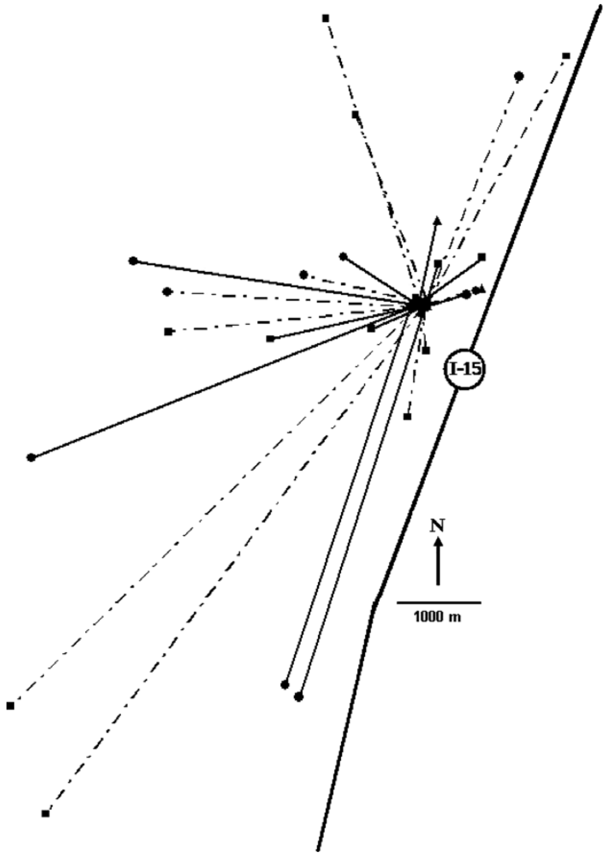

Fig. 5 – Straight lines from initial burrows to last known locations of all tortoises in 1997. Solid lines are NS, broken lines are WS, circles are females, squares are males, and triangles are juveniles.

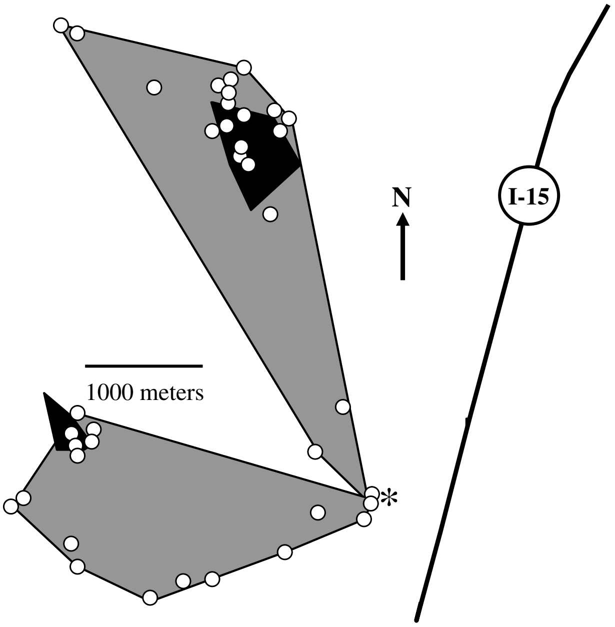

Fig. 6 – Movements by WS male L1006 (upper) and NS female L1222 (lower). 1997 locations (white dots) and minimum convex polygon home ranges for 1997 (grey) and 1998 (black) to show inappropriateness of calculating home ranges for first-season translocatees. * marks the release area. Interstate Highway 15 runs along the eastern border.

covered with grasses was consistent with predation by either bobcat (*Lynx rufus*) or mountain lion (*Felis concolor*).

Three WS females died. Females L1299 and L1225 were found with wet nares before their deaths and were preyed upon or scavenged. L1299's radio signal was last heard 117 days after release (13 September) and its carcass was located 18 February 1998. L1225 only used a single burrow up to its death 57 days after release (30 June). L1295 was found using one burrow until 91 days after release (18 August) when it was found dead in its second burrow. The burrow was located in a small wash and had collapsed, encasing the carcass in soil and cobble. The circumstances suggest that this tortoise remained in the burrow during a rainstorm and did not dig itself out when the burrow collapsed.

4. Discussion

4.1. *Behavioral observations*

Although the tortoises had spent years in captivity at the DTCC, upon release they were capable of finding appropriate food items, digging burrows, and generally using resources as necessary for survival in the wild. A previous translocation study raised concerns that released captives may have diminished ability to forage, find shelter sites, respond appropriately to environmental conditions, and avoid predators because all 5 tortoises died after translocation (Berry, 1974).

Overheating was shown to be a problem for tortoises in another translocation study with 6 of 7 deaths attributed to lethal body temperatures, three of which occurred on days of release (13 June and 2 July 1977) and three within 2 weeks of release (Cook et al., 1978; Cook, 1983). Tortoises that approach lethal body temperatures often produce large amounts of foaming saliva, which spread to the head and neck (McGinnis and Voigt, 1971). Two of our tortoises (L1222, L1298) were moving around the area of release and frothing at the mouth while all other tortoises released on those days were in shaded locations either at rest or digging beneath shrubs. During the observation periods both tortoises rested briefly in shade although they did not use shade competently as temperatures increased during the day. Both tortoises survived the length of the study, indicating that inappropriate thermoregulatory behaviors were likely limited a short period of time immediately following translocation. Problems associated with overheating would likely be minimized by conducting translocations in early to mid-spring, rather than late spring to summer, and by releasing tortoises such that on their first day they have several hours to move about when ambient temperatures are not likely to be problematic.

4.2. *Body mass*

Fluctuations in body mass of the Desert Tortoise largely are caused by changes in state of hydration (Minnich, 1977; Peterson, 1996a). When water is available, Desert Tortoises commonly drink 11–28% of their body mass (Minnich, 1977; Nagy and Medica, 1986), and in some cases, Desert Tortoises have been observed to increase body mass up to 43% after drinking (Miller, 1932).

Our concern that daily water supplementation in captivity could cause negligence in bladder water retention after release and our prediction that WS tortoises would lose more body mass after release than would NS tortoises were not supported by our data. Indeed, all tortoises lost similar amounts of body mass after release during the dry period preceding the first rain (22 July 1997). As expected, all tortoises gained body mass when rain provided drinking water. The two groups of tortoises continued to have similar fluctuations in body mass for the duration of the study.

Evaporative water loss is low in Desert Tortoises, but highly active animals lose more water through evaporation than do less active animals (Tracy, 1992). Desert Tortoises in California, at Ivanpah Valley (IV) and the Desert Tortoise Natural Area (DTNA), had a mean net water loss rate of <2 mL kg^{-1} day^{-1} (0.083 mg g^{-1} h^{-1}) during a severe drought; the typical rate was 1 mL kg^{-1} day^{-1} (0.042 mg g^{-1} h^{-1}) (Peterson, 1996a). Based on Peterson's observed rates, the tortoises at the LSTS site are predicted to lose 1.5–3.0% of their starting body masses after 15 days, but the actual body mass lost by LSTS tortoises was 5.3%. The LSTS tortoises are predicted (from Peterson's data) to drop 6.0–12.0% of their starting body masses after 60 days. The 10.8% (SD = 5.7) lost by LSTS tortoises is within the predicted range.

4.3. *Carapace length*

The fifth wettest year on record for southern Nevada (as measured in the Las Vegas Valley, approximately 45 km northwest of the LSTS site) occurred in 1998, with wettest ever February (73 mm) and tenth wettest March (26 mm) (Gorelow, 2005). February and March 1997 had below normal rainfall with 5 mm and 0 mm respectively. Late winter and early spring rains allow for germination and growth of the annual plants that make up much of the tortoise's diet (Oftedal, 2002). Tortoises translocated to the LSTS site grew about 25 times faster in carapace length during 1998 than they did during 1997. Shell growth positively correlates with rainfall (Medica et al., 1975; Nagy and Medica, 1986) and likely is dependent on nitrogen provided by green plants (Peterson, 1996b). Additionally, with drinking water available, tortoises can increase consumption of forage without elevating plasma solute concentrations to dangerous levels. The observed shrinking of carapace length from the time of release until the end of August 1997 helps to account for the large difference in growth rates for 1997 and 1998. During a tortoise's lifetime there are likely many periods when no growth or shrinking occurs. Adults and juveniles may experience no growth or shrinking during drought, yet in productive seasons juveniles may rapidly approach the size of more slowly growing older tortoises. Decrease in carapace length during drought was noted for two juvenile tortoises in another study (Berry et al., 2002) and shrinking has been measured in marine iguanas in times without food (Wikelski and Thom, 2000).

Carapace growth was marginally greater for tortoises that were supplemented with water although the small difference in growth rate was only detectable when the data from 1997 and 1998 were combined. The increase in size was not great enough to expect increased reproductive capabilities or decreased vulnerability to certain predators.

4.4. *Movement*

Familiarity with surroundings likely influenced the reduced movements made by tortoises in 1998 compared to those in 1997. We translocated a cohort of tortoises to the LSTS site in spring 1998 as part of another experiment. These tortoises moved an average of 1579 m (SD = 1071) from their initial burrows that year whereas tortoises released the year before moved only 275 m from their 1998 start points (hibernacula). The two cohorts were very similar vis-à-vis their movements in their first year after release suggesting that reduction of movement by tortoises in their second year was not simply caused by break of the drought, but by familiarity with the area.

The concept of home range was described and defined by Burt (1943) as "that area traversed by the individual in its normal activities of food gathering, mating, and caring for young." Occasional movements to points outside of the area typically used should not be included in the home range and home ranges should not be calculated for animals that are wandering (Burt, 1943). The movement patterns of tortoises during their first season at the translocation site clearly were not consistent with the definition. Calculations of home ranges appeared to be appropriate for most animals in their second season after release. Desert Tortoises do make lengthy journeys outside of their normal activity areas to exploit resources such as calcium rich soils (Marlow and Tollestrup, 1982). Three tortoises that made long distance movements in 1998 did not return to their previous areas of activity, so home range calculations were inappropriate. Home range sizes of female (8.9 ha, range 6.9–11.1) and male (25.5 ha, range 9.9–62.7 ha) tortoises during their second activity season at the LSTS site were comparable to the home range sizes of native wild Desert Tortoises in a nearby valley in a non-drought year (females 5.9–11.2 ha, males 7.7–49.0 ha) (O'Connor et al., 1994). The characteristic home range sizes and the short distances moved from hibernacula provide evidence that second-year translocatees were similar to native wild tortoises from other studies.

Fidelity to the release site shown by some tortoises during their first and second seasons after release could, in part, be predicted by examining the patterns of movement on days of release. Six tortoises deviated greatly from straight-line travel and/or moved small straight-line distances from their initial burrows (Fig. 3). Two of these animals were frothing from the mouth. These two tortoises may have meandered because they became overheated, or the meandering may have been due to unfamiliarity with the surrounding area and misuse of shade resources. The other four tortoises (L1295, L1297, L1346, and L1005) were closer to their initial burrows ($\leqslant$110 m) at their last known locations in 1997 than were the other tortoises. The four tortoises represented each of the sex by treatment groups except for the WS males, who were already making long, linear movements away from their initial burrows. Three of the four tortoises survived through 1998 and were closest to their initial burrows at their last locations in 1998 as well (139–415 m) (Table 2). All other tortoises moved greater straight-line distances from their initial burrows of release and/or tended to move in nearly straight-lines from their

Table 2 – Straight-line distances moved by tortoises

Tortoise #	Sex	Experimental group	1997 Straight-line (m)	1998 Straight-line (m)	Final straight-line (m)
L1002	F	NS	4195 (21)*	–	–
L1003	F	NS	4314 (239)	65	4262
L1025	F	NS	3483 (139)*	–	–
L1222	F	NS	2433 (227)	290	2706
L1294	F	NS	349 (2)*	–	–
L1346	F	NS	67 (210)	349	415
L1347	F	NS	836 (211)	174	833
L1001	J	NS	886 (120)*	–	2322
L1026	M	NS	1332 (233)	158	1491
L1223	M	NS	467 (195)	343	721
L1226	M	NS	685 (226)	943	596
L1296	M	NS	404 (211)	260	660
L1297	M	NS	110 (211)	244	220
L1005	F	WS	23 (238)	144	139
L1224	F	WS	2103 (132)*	–	–
L1225	F	WS	1049 (57)*	–	–
L1295	F	WS	92 (91)*	–	–
L1299	F	WS	2591 (117)*	–	–
L1349	F	WS	422 (117)*	–	–
L1004	J	WS	483 (239)	60	477
L1006	M	WS	3206 (238)	95	3399
L1023	M	WS	527 (232)	0	527
L1024	M	WS	2118 (233)	0	2118
L1298	M	WS	2893 (211)	2910	5802
L1348	M	WS	1185 (48)*	–	–
L1363	M	WS	5429 (208)	3777	6126
L1367	M	WS	6245 (210)	771	6975
L1368	M	WS	2080 (209)	789	1725

1997 straight-line = point of release to last known 1997 location, 1998 straight-line = 1997 hibernacula to 1998 hibernacula, Final straight-line = point of release 1997 to 1998 hibernacula, F = female, J = juvenile (undetermined sex), M = male, NS = not supplemented, WS = water-supplemented. The numbers of days after release corresponding to each tortoise's last location in 1997 is in parentheses. Asterisks indicate tortoises lost in 1997.

burrows of release. These tortoises ended up 404–6245 m from their initial burrows in 1997. Some tortoises traveled long distances away from the release area in nearly straight-lines and others started traveling in straight-lines, but switched directions after the observation periods on days of release and remained near to the release area. The tendency for some tortoises to travel in straight-lines for long distances after translocation has been described previously (Berry, 1974). In that study, only translocatees that were originally captured in the wild tended to travel far and/or in straight-lines from points of release. Translocatees that were former captives stayed within a few hundred meters of their points of release and did not venture more than 100 m from burrows that they established (Berry, 1974). A recent study of the Gopher Tortoise (*Gopherus polyphemus*), which inhabits the southeastern United States, suggests that fidelity to the release area increases with increased time spent in temporary outdoor enclosures at the site (Tuberville et al., 2005). While there is currently more contiguous habitat remaining for Desert Tortoises than for Gopher Tortoises, there could be situations where reducing movements away from the release area would be desirable and achievable through various methods. Although eliminating water supplementation prior to release did reduce the dispersal of males in our study, it did not appear to affect females similarly.

Homing attempts, especially for short distance translocations, have been shown to be problematic for various species including the Gila Monster (*Heloderma suspectum*), which shares its distribution with the Desert Tortoise (Sullivan et al., 2004). In one study, translocated Desert Tortoises of captive origin showed little to no tendency to orient toward home, while 9 of 12 tortoises of wild origin did orient toward home (Berry, 1974). Eastern Box Turtles (*Terrapene c. carolina*) moved 32–131 km did show a weak, yet variable tendency to home (Cook, 2004). The tortoises in our study did not tend to travel toward home, possibly due to the distance between the LSTS site and their former homes.

4.5. *Mortality*

One might expect that traveling long distances in unfamiliar surroundings would increase translocated tortoises' chances of mortality. Desert Tortoises have good spatial memories and will reuse shelter sites and other resources in locations that are familiar to them (Berry, 1974; Marlow and Tollestrup, 1982). Although the WS males traveled long distances from the release area before reducing their movements, only one WS male died.

The mortality rates of females and males were not significantly different for the LSTS tortoises, however, given the small sample sizes and extremely low male mortality as

Table 3 – Numbers of translocated tortoises lost at the LSTS site

Sex	Experimental group	Total released	Partial carcasses	Intact carcasses	Lost radio signals, no carcass
F	NS	7	1	1	1
F	WS	6	2	1	2
M	NS	5	0	0	0
M	WS	8	0	1	0
J	NS	1	0	0	0
J	WS	1	0	0	0

Partial carcasses had evidence of predation or scavenging. F = female, M = male, J = juvenile (undetermined sex), NS = not supplemented prior to release, WS = water-supplemented prior to release.

compared to females, this question should be addressed with a larger sample size. In a previous study in which translocated and resident tortoises were monitored in plots of irrigated and unirrigated desert habitat, female translocated tortoises were reported to have a higher mortality rate than resident females, resident males, or translocated males (SAIC, 1993). We recalculated mortality rates for tortoises in unirrigated plots from the first two seasons after release by requiring recovery of a carcass for a tortoise to be considered dead. This new analysis of the data indicates that the translocated females had a mortality rate of 20.0% in two activity seasons, while resident females, resident males, and translocated males experienced no mortality (SAIC, 1993).

In times such as drought when predators (e.g. coyotes, kit foxes, bobcats) have fewer mammalian prey available, they will increase take of less preferred prey including tortoises (Woodbury and Hardy, 1948; Berry, 1974). During droughts, coyotes apparently killed most of the tortoises in one study at the DTNA (Peterson, 1994) and 21–28% of the marked wild population in a study near Ridgecrest, California were killed by canids. Predation was the suspected cause of death of most wild resident and first-season translocated tortoises in a study concurrent with ours that took place approximately 30 km to the north at Bird Spring Valley (BSV), Nevada (Nussear, 2004). Although half of the carcasses in our study showed signs of having been eaten, it should not be assumed that predation was the cause of death in all cases (Table 3). It is possible that the tortoises died of other causes and were quickly scavenged, or tortoises may have become dehabilitated and therefore susceptible to predation. Many times the cause of death of released animals is reported to be predation without dehabilitation considered as the ultimate cause (Soderquist, 1994). Two of the three animals whose carcasses were eaten had damp nares, a possible sign of disease, before death.

Wild tortoises were not equipped with telemetry radios at the LSTS site, so a proper experimental control with which to compare the mortality rate of translocatees did not exist. Tortoises translocated to BSV in the spring of 1997 had a total mortality rate of 11.7% (7 of 60 released) that year, while residents at BSV had a mortality rate of 15.1% (8 of 53) that same year (Nussear, 2004). The mortality rates of translocated and resident animals at BSV were not significantly different (chi square = 2.563E−4, df = 1, Fisher's Exact $p > 0.9999$). The mortality rate of 21.4% (6 of 28 released) at the LSTS site was not different from the 11.7% calculated for tortoises translocated to BSV (chi square = 1.445, df = 1, Fisher's Exact $p = 0.3327$). The data from BSV and previous studies suggest that all tortoises at the LSTS site, regardless of translocated or resident status, likely were negatively impacted by drought conditions at the site in 1997. Additionally, a cohort of tortoises that we released at the LSTS site for another experiment in the spring of 1998 had a 2.5% (1 of 40) mortality rate that year, further suggesting that the translocation itself did not strongly influence mortality rates, while drought did.

4.6. *Conservation implications*

The translocation of tortoises to the LSTS site in spring of 1997 occurred at the end of a period with little rainfall. Annual vegetation was sparse and dry, and there was no rainwater for tortoises to drink until late July 1997. Because the conditions at the LSTS site were harsh, the ability of tortoises to adjust to life in the wild could be examined under adverse conditions. Despite harsh conditions, most of our translocated tortoises quickly became adept at life in the wild. Although initial mortality rates may be lower when translocations occur in years with plentiful rainfall, translocations during dry years may be acceptable because drought conditions likely affect mortality of resident and translocated tortoises similarly. It may be beneficial, however, to release tortoises with unknown histories (e.g. unknown access to sufficient food and water in years prior to translocation) in non-drought years. At small translocation sites or when goals include increased density in particular portions of the site, travel by male tortoises may be reduced by not providing supplemental water from the end of last captive hibernation up to release in spring. We conclude that, regardless of water supplementation regimen, initial success in our translocation demonstrates high potential for longer-term successes. We strongly suggest that translocation be considered a valid tool available for conservation of the Desert Tortoise. Although translocated tortoises fared well during their initial adjustment period, long-term survival and productivity of these animals will be subject to the same factors that continue to dwindle populations of the Desert Tortoise across its range. If we are able to effectively abate the myriad of threats that lessen the likelihood of this species' persistence, translocation of tortoises to appropriate areas will be essential to bolster decimated populations toward a sustainable existence.

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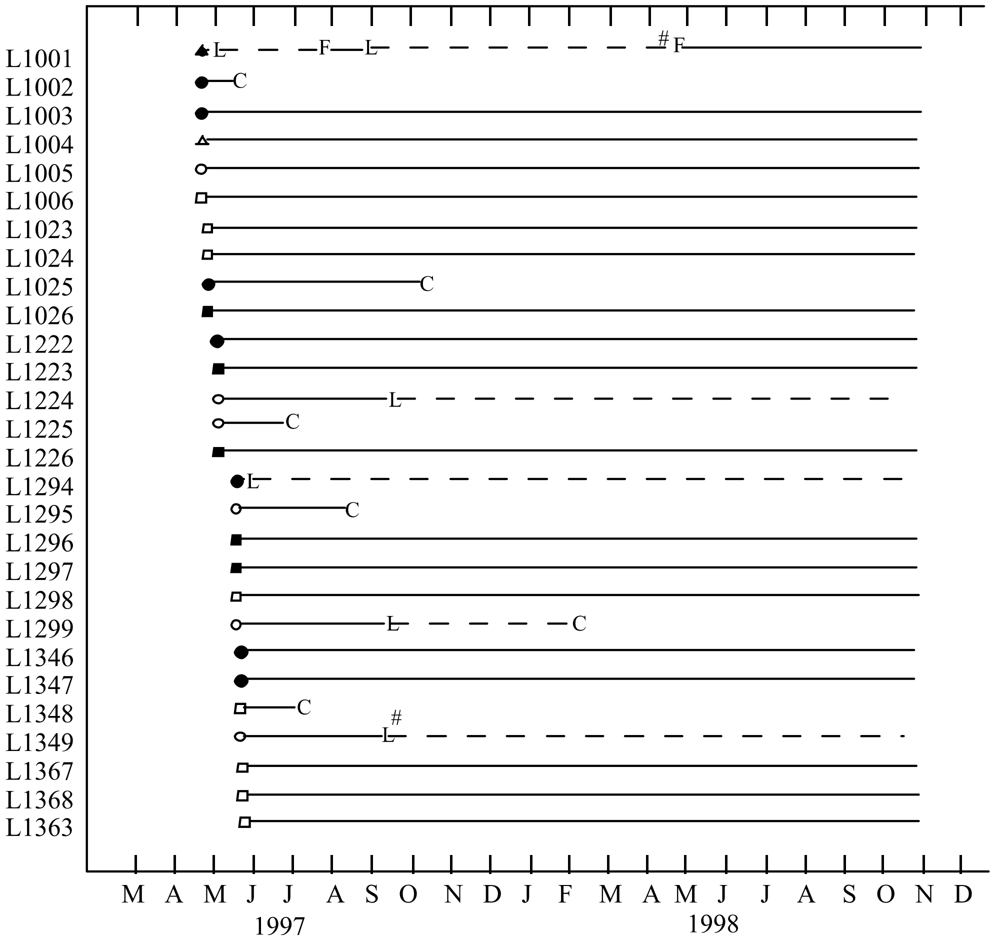
Appendix

Release histories of 28 *G. agassizii* at the LSTS site. Release dates are indicated by open shapes for the WS tortoises and by filled shapes for the NS tortoises, with circles for females, squares for males, and triangles for juveniles (unknown sex). L = lost radio signal; C = carcass found; F = live tortoise found; # = known transmitter failure. Solid lines indicate that a tortoise was monitored continuously and dashed lines indicate that a tortoise was lost.



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Synthesis

Human-caused Disturbance Stimuli as a Form of Predation Risk

Alejandro Frid[1] and Lawrence Dill

ABSTRACT. A growing number of studies quantify the impact of nonlethal human disturbance on the behavior and reproductive success of animals. Athough many are well designed and analytically sophisticated, most lack a theoretical framework for making predictions and for understanding why particular responses occur. Behavioral ecologists have recently begun to fill this theoretical vacuum by applying economic models of antipredator behavior to disturbance studies. In this emerging paradigm, predation and nonlethal disturbance stimuli create similar trade-offs between avoiding perceived risk and other fitness-enhancing activities, such as feeding, parental care, or mating. A vast literature supports the hypothesis that antipredator behavior has a cost to other activities, and that this trade-off is optimized when investment in antipredator behavior tracks short-term changes in predation risk. Prey have evolved antipredator responses to generalized threatening stimuli, such as loud noises and rapidly approaching objects. Thus, when encountering disturbance stimuli ranging from the dramatic, low-flying helicopter to the quiet wildlife photographer, animal responses are likely to follow the same economic principles used by prey encountering predators. Some authors have argued that, similar to predation risk, disturbance stimuli can indirectly affect fitness and population dynamics via the energetic and lost opportunity costs of risk avoidance. We elaborate on this argument by discussing why, from an evolutionary perspective, disturbance stimuli should be analogous to predation risk. We then consider disturbance effects on the behavior of individuals—vigilance, fleeing, habitat selection, mating displays, and parental investment—as well as indirect effects on populations and communities. A wider application of predation risk theory to disturbance studies should increase the generality of predictions and make mitigation more effective without over-regulating human activities.

INTRODUCTION

More than 30 years ago, Walther (1969) published an experiment in which he assumed that animals perceive human disturbance similarly to predation risk. Walther approached Thomson's gazelles (*Gazella thomsoni*) with his car to test whether flight initiation distance (the distance between the predator and prey at which prey begin to flee) depended on age, sex, and social status. He also studied gazelles fleeing from wild dogs (*Lycaon pictus*) and other predators, and appeared satisfied that the variables affecting responses to a car would have been similar had the stimuli been actual predators (Walther 1969). Walther's experiment was stimulated by the work of Hediger (1934, cited in Walther 1969), who three decades earlier had approached African ungulates with a car to determine interspecific differences in flight initiation distance.

Since these pioneering studies, research on disturbance has begun to embrace the principle that nonlethal disturbance stimuli caused by humans are analogous to predation risk. The notion works because responses both to predation risk (Lima and Dill 1990, Lima 1998) and to disturbance stimuli (e.g., Gutzwiller et al. 1994, Steidl and Anthony 2000) divert time and energy from other fitness-enhancing activities such as feeding, parental care, or mating displays. (In the context of our argument, *disturbance* denotes a deviation in an animal's behavior from patterns occurring without human influences. We use the term *disturbance stimulus* for a human-related presence or object [e.g., birdwatcher, motorized vehicle] or sound [e.g., seismic blast] that creates a *disturbance.*) Animals optimize these trade-offs when their investment in antipredator behavior tracks short-term changes in predation risk (Lima and Dill 1990, Lima 1998). For example, woodchucks (*Marmota monax*) decrease their flight initiation distance when thry are closer to a refuge burrow (Bonenfant and Kramer 1996); and gray squirrels (*Sciuris carolinensis*) show the same response when nearer to a refuge tree (Dill and Houtman 1989). Individuals near a refuge that tolerate closer approaches by potential predators avoid

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fleeing costs (abandoning a feeding site and expending energy on locomotion) that do little to increase safety (see Ydenberg and Dill 1986). The plasticity of flight initiation distance is based on the same economic principle—optimization of trade-offs—that drives antipredator behavior in general (Lima and Dill 1990, Lima 1998).

When encountering disturbance stimuli, ranging from the dramatic, low-flying helicopter to the quiet wildlife photographer, an animal's response should follow the same economic principles used by prey encountering predators (Berger et al. 1983, Madsen 1994, Gill et al. 1996, 2001, Gill and Sutherland 2000). We call this verbal model the risk-disturbance hypothesis. It predicts that responses by disturbed animals track short-term changes in factors characterizing disturbance stimuli, with responses being stronger when perceived risk is greater. The level of perceived risk may result from a combination of factors that characterize disturbance stimuli, along with factors related to natural predation risk (e.g., Frid 2001*a,* Papouchis et al. 2001).

Although earlier work (notably, Berger et al. 1983, Madsen 1994) used predation risk as an analogy for understanding human disturbance of wildlife, Gill and Sutherland (2000) explicitly argued that disturbance stimuli and predation risk indirectly affect survival and reproduction through trade-offs between perceived risk and energy intake. Their models predict how density-dependent processes interact with food distribution and disturbance stimuli to determine habitat shifts and population dynamics (Gill et al. 1996, 2001, Gill and Sutherland 2000).

Here we develop the risk-disturbance hypothesis further. We discuss why, from an evolutionary perspective, disturbance stimuli should be analogous to predation risk. Most disturbance studies focus on responses directly related to energy trade-offs: fleeing, vigilance, and habitat selection. We use examples from that rich literature to assess some predictions of the risk-disturbance hypothesis. We also use a predation risk framework to explore four areas in which disturbance effects are less studied: mate acquisition, parental investment, population dynamics, and interactions at the community level. Finally, we evaluate when and how disturbance studies might increase their conservation value by applying the risk-disturbance hypothesis.

ARE DISTURBANCE STIMULI REALLY ANALOGOUS TO PREDATION RISK?

A devil's advocate might argue that disturbance stimuli are not analogous to predation risk because prey have evolved predator-specific antipredator behaviors (e.g., Walther 1969, Ghalambor and Martin 2000), and many disturbance stimuli (e.g., aircraft) are too recent for animal responses to reflect adaptive programming. However, prey have evolved antipredator responses to generalized threatening stimuli, such as loud noises and rapidly approaching objects (e.g., Dill 1974*a, b*). Prey respond when such stimuli cross a threshold, even when the specific source is new to the prey's evolutionary history (e.g., introduced predators or motorized vehicles). The zebra danio (*Brachydanio rerio*), a small fish, provided one extreme example when exposed to real predators (largemouth bass: *Micropterus salmoides*), a predator-shaped model, and a 'cinematographic' predator (a film of a black dot increasing in size, simulating an approaching object). In all three cases, danios fled when the angle subtended by the predator at the prey's eye reached a threshold rate of change (see Appendix 1). This threshold 'loom' rate depended on the size and speed of the approaching 'predator', and responses were qualitatively similar for the different 'predator' types. In other words, danios appeared to decide the timing of fleeing by relating the loom rate to a margin of safety, regardless of whether the predator was real, a model, or a film (Dill 1974*a, b*).

A devil's advocate might also argue that predation risk and disturbance stimuli are not analogous because disturbed animals do not necessarily risk direct mortality. The counter-argument is that it is irrelevant if disturbance stimuli are nonlethal because predation risk differs from predation itself. Although death is the outcome of predation, the outcome of predation risk is a decision made by prey to compromise the rate of resource acquisition or other activities to reduce the probability of death (Abrams 1993, Hugie and Dill 1994, review in Lima 1998). Specifically, predation risk results from the interaction of factors that affect attack and capture probabilities, mainly (1) the structure of the environment, including the distribution of vegetation cover where predators might hide and of refuges where prey might escape, (2) social factors, including the prey's group size and position in the group, (3) the distribution and abundance of predators, and (4) the behavior of predators (whether they are searching for and selecting a given prey type). Because antipredator behavior responds to changes in these factors rather than to predation rate per se, it is reasonable to expect responses to nonlethal disturbance stimuli to follow the same decision rules as responses to predation risk.

Does habituation invalidate this premise? Shouldn't prey that behave optimally recognize that nonlethal stimuli do not warrant the costs of antipredator behavior? Animals rarely have perfect information, and generally are expected to maximize fitness by overestimating rather than underestimating risk. Overestimation costs, such as lost feeding opportunities, have milder fitness consequences than the cost of underestimating danger, which might be immediate death (Bouskila and Blumstein 1992). Thus, habituation to disturbance stimuli, although it generally occurs to some extent, often is partial (e.g., Burger and Gochfeld 1981, 1990, Steidl and Anthony 2000) or negligible (e.g., Berger et al. 1983, Bleich et al. 1994). It is unlikely to disprove the risk-disturbance hypothesis in most situations, even for animals that provide archetypal examples of habituation, such as urban corvids (Ward and Low 1997). Furthermore, there are instances in which antipredator-type responses to nonlethal stimuli should become stronger with repeated exposure to such stimuli. For example, prey learn to associate the low loom rate of an approaching predator that is still far away with the faster loom rate that occurs once the predator is closer. Thus, flight initiation distance or vigilance might increase with repeated exposure to a nonlethal stimulus if the latter is sufficiently aversive (Dill 1974b).

A final issue to consider is that human hunters have represented a real threat to some species over evolutionary time. Thus, in some cases such as people on foot approaching large vertebrates, disturbance stimuli and true predatory stimuli may be indistinguishable from the animal's perspective.

TRADE-OFFS DIRECTLY RELATED TO ENERGY GAIN

We next use the rich literature dealing with disturbance effects on fleeing, vigilance, and habitat shifts to assess some predictions of the risk-disturbance hypothesis. Our assessment is not a quantitative test based on a random sample of the literature, which could be biased by failure to publish negative results. Rather, it is based on selected references and is meant to demonstrate the explanatory value of the hypothesis and to stimulate future studies explicitly designed to test its predictions. We have not included all examples known to us, but only enough to illustrate our arguments.

Fleeing

Prey that have detected a potential predator should make optimal fleeing decisions that balance the benefits of reducing capture probability against the costs of abandoning a resource patch and expending energy on locomotion (Ydenberg and Dill 1986). Fleeing probability should increase when the predator approaches more directly, because a direct approach may convey detection and intent to capture (Burger and Gochfeld 1981, 1990, Cooper 1997, 1998). Although the angle of approach is two-dimensional in some systems (e.g., terrestrial animals on flat ground), in others it has horizontal and vertical components. For instance, an aerial predator may change the approach angle by shifting its elevation relative to prey (rather than lateral distance). The same principle applies to aircraft disturbance, or when ground disturbance stimuli approach animals on landscapes with a vertical dimension (e.g., mountain slopes, trees). The angle of approach is a geometric correlate of the nearest distance between the trajectory of the approaching stimulus and the animal being approached. Thus, greater distances (lateral and vertical) correspond to larger angles and less direct approaches. Angles are more difficult to measure than distances, and most field studies quantify the latter.

Fleeing probability or flight initiation distance should increase when predators are larger or approach faster because prey will experience the loom rate that triggers flight at greater distances (Dill 1974*a*). Flight initiation distance increases as distance to a refuge becomes greater because risk of capture increases (Ydenberg and Dill 1986, Dill and Houtman 1989, Bonenfant and Kramer 1996). Prey may also respond to predator group size. For instance, Thomson's gazelles had smaller flight initiation distances when approached by single hyenas (*Crocuta crocuta*) than when approached by packs (Walther 1969). Flight initiation distance and fleeing probability also increase when prey are at a site that is poor in resources (e.g., little food), because the benefits of clinging to a resource patch are less likely to outweigh the risk of remaining (see data on waterstriders, *Gerris remiges*,, in Ydenberg and Dill 1986).

Predictions of the risk-disturbance hypothesis stimulated by studies of fleeing responses to predators include the following. Fleeing probability and flight initiation distance increase when disturbance stimuli (A) approach more directly, (B) approach more quickly, and (C) are larger in size. They also increase when (D) distance from refuge is greater, (E) group size of the disturbers is greater, and (F) the costs of fleeing are lower. Lower fleeing costs might arise when the resource patch is poor (i.e., less is lost by leaving), rich resources are evenly distributed and easy to locate elsewhere, or environmental conditions (temperature, snow depth) are mild at the time of disturbance, thereby not increasing locomotion costs (see Ydenberg and Dill 1986). We did not consider obstructive cover because it represents a constraint to early detection rather than an influence on the decision to withhold fleeing. Also, we did not consider the effects of group size of disturbed animals, which are difficult to predict due to the opposing effects of dilution and additional sensory organs (Ydenberg and Dill 1986).

Reviewed studies were consistent with Prediction A. Fleeing probability increased for Dall's sheep *Ovis dalli dalli* (Frid 2001*a, b*), ringed seals *Phoca hispida* (Born et al. 1999), and Pacific Brant *Branta bernicla nigricans,* and Canada Geese *B. canadensis* (Ward et al. 1999) as a helicopter or fixed-wing aircraft approached more directly. Bald eagles (*Haliaeetus leucocephalus*) perched on trees alongside a river were more likely to flee from a paddle raft when perches were closer to the river or lower in height (Steidl and Anthony 1996). Table 1 summarizes these and additional examples.

Table 1. Literature examples for assessing predictions concerning fleeing responses. Predictions were that fleeing probability or flight initiation distance increase when disturbance stimuli (A) approach more directly, (B) approach more quickly, and (C) are larger in size. They also increase when (D) distance from refuge is greater, (E) group size of disturbers is greater, and (F) fleeing costs are lower (see *Fleeing* for details).

Study	Species	Stimuli	Predictions		
			Supported	Rejected	Not tested or controls lacking
Born et al. (1999)	ringed seal	helicopter and fixed-wing aircraft	A, F		B, C, D, E
Burger (1998)	Common Tern	motorized watercraft	A, B		C, D, E, F
Burger and Gochfeld (1998)	Louisiana and Little Blue Herons	people on foot	A, E		B, C, D, F
	other waterbird spp.		A	E	B, C, D, F
Delaney et al. (1999)	Mexican Spotted Owl	helicopter	A, F		B, C, D, E
Frid (2001*a,b*)	Dall's sheep	helicopter, fixed-wing aircraft	A, D		B, C, E, F
Hamr (1988)	chamois	hikers, skiers	E, F		A, C, B, D
Lafferty (2001)	Snowy Plover	people on foot	A	B, E	C, D, F
Papouchis et al. (2001)	bighorn sheep	hikers, bikers, vehicles	A, D	E	B, C, F
Steidl and Anthony (1996)	Bald Eagle	paddle raft	A*	A*	B, C, D, E, F
Swarthout and Steidl (2001)	Mexican Spotted Owl	single hiker	A		B, C, D, E, F
Ward et al. (1999)	Pacific Brant and Canada Goose	helicopter and fixed-wing aircraft	A		B, C, D, E, F

*The prediction was supported for fleeing probability, but not flight initiation distance.

Flight initiation distance has been found to increase as lateral distance from the disturbance stimulus becomes greater (e.g., Steidl and Anthony 1996, Frid 2001*a.* These results apparently reject prediction A, but geometric correlations could confound interpretation. Flight initiation distance cannot be smaller than the nearest distance between the animal and the trajectory of the stimulus. Thus, if animals flee when the stimulus is nearest to them, flight initiation distance during indirect approaches will always be larger than during direct approaches. Future analyses need to consider potential artifacts that could arise from this geometric correlation.

Support for Prediction B was inconsistent (Table 1). Supporting the prediction, more Common Terns (*Sterna hirundo*) fled their nests when motorized watercraft approached more quickly (Burger 1998). Contradicting the expectation that earlier fleeing is triggered by a higher velocity (Dill 1974*a,* joggers were less disturbing to Western Snowy Plovers (*Charadrius alexandrinus nivosus*) than were people walking, even after distance to the stimulus was controlled (Lafferty 2001). Future work should analyze whether, under certain conditions, slower disturbance stimuli are analogous to a stalking predator and are perceived as more damgerous than a faster stimulus.

We could not evaluate Prediction C. Although comparisons exist between the effects of aircraft of different sizes, noise level is an important covariate (e.g., Ward et al. 1999) that probably confounds size effects.

The two reviewed studies with relevant data supported Prediction D (Table 1). During indirect approaches by helicopters, Dall's sheep far from rocky slopes were much more likely to flee than were sheep on rocky slopes (Frid 2001*a,*), which provide a refuge from cursorial predators (e.g., Berger 1991). Distance from a refuge probably affects how sheep perceive risk from the generalized stimulus of a large object approaching rapidly, rather than from a perceived aerial predator per se. Ground disturbance had similar effects: bighorn sheep (*Ovis canadensis*) disturbed by hikers, bikers, or road traffic had stronger fleeing responses when farther from rocky slopes (Papouchis et al. 2001).

Support for Prediction E was inconsistent (Table 1). Little Blue Heron (*Egretta caerulea*) and Louisiana Heron (*E. tricolor*) were more likely to flee when birdwatchers were in larger groups. Other species of waterbirds, however, did not respond to disturber group size (Burger and Gochfeld 1998). Flight initiation distance and distances fled by chamois (*Rupicapra rupicapra*) approached by hikers increased with hiker group size (Hamr 1988). Disturber group size of hikers, mountain bikers, and vehicles did not influence fleeing responses by bighorn sheep, however (Papouchis et al. 2001).

Several studies were consistent with Prediction F (Table 1). Ringed seals disturbed by aircraft were more likely to abandon a haul-out site on the ice pack and dive into the sea when the thermal benefits of staying hauled out were lower due to higher wind chill (Born et al. 1999). Mexican Spotted Owls (*Strix occidentalis lucida*) did not flee from helicopters when attending young at their nest, but fled readily during the postfledging period (Delaney et al. 1999). Chamois were more reluctant to flee when deep snow entailed a high cost to locomotion (Hamr 1988).

Vigilance and related activity shifts

A vast literature indicates that prey are more vigilant when the perceived risk of predation is greater. This response increases the chances that the prey will avoid capture, but at the cost of reducing time spent foraging or engaged in other activities. Antipredator vigilance responds not only to the mere presence or absence of predators, but also to factors affecting the level of perceived risk, including group size, distance from a refuge, and obstructive cover (see reviews in Elgar 1989, Lima 1998).

Studies of antipredator vigilance, as well as our discussion on angle of approach from the previous section, stimulated the following predictions. More time is spent vigilant, or less time is spent foraging or resting, when disturbance stimuli (A) approach more directly, (B) remain at closer distances, (C) have a greater group size (e.g., more ecotourists), and (D) occur concurrently with a greater level of natural predation risk (e.g., animals are in smaller groups, closer to obstructive cover, farther from refuge).

The reviewed studies were consistent with Prediction A (Table 2). For instance, vigilance responses or disruptions of resting and foraging by bighorn sheep (Stockwell et al. 1991), Dall's sheep (Frid 2001*b,*) began earlier and lasted longer when aircraft approached more directly.

Table 2. Literature examples for assessing predictions concerning vigilance responses. Predictions were: more time is spent vigilant or less time is spent foraging or resting when disturbance stimuli (A) approach more directly, (B) remain at closer distances, (C) have a greater group size (e.g., more ecotourists), and (D) occur concurrently with a greater level of natural predation risk (see the *Vigilance* section for details).

Study	Species	Stimuli	Predictions		
			Supported	Rejected	Not tested or controls lacking
Burger and Gochfeld (1998)	several waterbird spp.	people on foot	C		A, B, D
Duchesne et al. (2000)	woodland caribou	people on skis or snow shoes	C		A, B, D
Frid (2001*b*)	Dall's sheep	fixed-wing aircraft	A		B, C, D
Galicia and Baldassarre (1997)	American Flamingo	motorboat	C	B	A, D
Papouchis et al. (2001)	bighorn sheep	cars, bikes	C		A, B, D
Stockwell et al. (1991)	bighorn sheep	helicopter	A		B, C, D
Ward and Low (1997)	American Crow	urban pedestrians and traffic	B, D		A, C

Support for Prediction B was inconsistent (Table 2). Crows (*Corvus brachyrhynchos*) in urban environments were more vigilant and foraged less efficiently where disturbance stimuli were nearer (Ward and Low 1997), but American Flamingos (*Phoenicopterus ruber ruber*) were more vigilant when motorized tourboats were farther away (Galicia and Baldassarre 1997).

Several studies were consistent with Prediction C (Table 2). For instance,larger groups of people on foot had stronger effects on vigilance increases by both birds and ungulates (Burger and Gochfeld 1998, Duchesne et al. 2000).

Consistent with Prediction D, vigilance by crows following urban disturbance stimuli was affected by distance to obstructive cover and flock size (both positive relationships). Vigilance was also inversely related to distance to protective cover (Ward and Low 1997).

Habitat selection

Habitat choice is the outcome of decisions that balance the trade-off between predation risk and resource richness. Theoretically, animals should select habitats that minimize the ratio of mortality risk to net energy intake (Gilliam and Fraser 1987, reviews in Lima and Dill 1990, Lima 1998). Consistent with theory, individuals of taxa as diverse as fish (e.g., Gilliam and Fraser 1987), ungulates (e.g., Edwards 1983, Berger 1991), and small mammals (Morris and Davidson 2000) spend less time in places where richer resources are associated with greater danger. Whether animals under risk shift their habitats depends on the relative costs and benefits of leaving their current site and going elsewhere. Such decisions may be constrained by the species' perceptual range, the distance from which individuals can perceive key landscape elements (Lima and Zollner 1996, Zollner and Lima 1997).

Gill et al. (2001) use a predation risk framework to argue that animals that do not shift habitats when exposed to disturbance stimuli might have no suitable alternative habitats. Thus, animals are still impacted by being forced to remain at disturbed sites where the increased energetic costs of antipredator behavior reduce effective habitat quality.

Predictions regarding habitat selection, which have been proposed previously by disturbance studies grounded in predation risk theory, include the following. (A) Long-term, intense disturbance stimuli will cause habitat shifts (or observed avoidance of disturbed habitats when pre-treatment data are not available) at the cost of reduced access to resources. A different form of the prediction is that animals will access resources in habitats previously affected by disturbance stimuli once the latter are removed (Madsen 1994, 1998, Gill et al. 1996, Gill and Sutherland 2000). (B) Habitat shifts will not occur if alternative habitats are too distant or of low quality, such that the net benefits of shifting habitats do not outweigh the costs of remaining at disturbed sites (Gill et al. 2001).

Almost all of the reviewed studies strongly supported Prediction A, implying that alternative habitats were available in those systems (Gill et al. 2001). The pygmy marmoset (*Cebuella pygmaea*), a neotropical primate, shifted to the upper canopy in areas disturbed by ecotourists on foot and motorboats, but used the lower strata (which it normally prefers) in less disturbed areas (de la Torre et al. 2000). Available habitat and access to food were substantially reduced for disturbed Pink-footed Geese (*Anser brachyrhynchus;* Gill et al. 1996). For woodland caribou (*Rangifer tarandus caribou*) during late winter, functional habitat loss caused by avoidance of roads and other developments amounted to 48% of a 6000-km^2 study area (Dyer et al. 2001). Bottlenose dolphins (*Tursiops truncatus*) avoided important foraging areas when motorboat traffic was high (Allen and Read 2000). Table 3 summarizes these and additional examples. Such habitat shifts probably increase the strength of density-dependent processes (e.g., intraspecific competition) that affect demography (Gill and Sutherland 2000).

In contradiction to Prediction A, woodland caribou shifted habitats in response to wolves (*Canis lupus*), but not in response to people on skis and snowshoes (they did suffer costs to foraging and resting: Duchesne et al. 2000). Alternative habitat may have been of low quality and not worth shifting to without a very high risk of wolf predation. Unfortunately, Prediction B was not tested.

Consistent with Prediction B, diving ducks (mainly *Aythya* spp.) at a bay on Lake Erie fled from motorboats and promptly returned to the bay during spring, but during fall often redistributed into the adjacent and very large main lake. Spring was correlated with harsher conditions and partial ice cover on Lake Erie, which probably reduced the benefits of shifting habitats.

In contrast, the lake was suitable alternative habitat during fall, when habitat shifts did occur (Knapton et al. 2000). Similarly, Florida manatees (*Trichechus manatus latirostris*) during winter continued to use a bay with thermal springs (an essential resource for avoiding hypothermia) and did not shift into colder waters outside the bay, regardless of very high levels of boat traffic. At a smaller spatial scale and consistent with Prediction A, they selected areas within the bay with the least boat traffic (Buckingham et al. 1999).

ACQUIRING MATES

Individuals of many taxa signal to attract mates or warn off intruders to their territories, but such advertisement can attract predators (see reviews in Lima and Dill 1990, Magnhagen 1991, Lima 1998). Male Tungara frogs (*Physalaemus pustulosus*) are a classic example because their chorusing attracts both potential mates and predatory bats (*Trachops cirrhosus*). Frog responses to a model bat flying above their pond ranged from silencing mating calls but remaining on the pond's surface, to abandoning the site by diving (stronger responses corresponded to more direct approaches). The cost of safety was postponed access to mates (Ryan 1985).

Disturbance stimuli may cause similar trade-offs. Passerine birds sing to defend territories or attract mates; Gutzwiller et al. (1994) reported that some species (but not all) reduced their singing activity when humans walked through or near their territories. Relative to areas with little disturbance, pygmy marmosets in areas used intensely by ecotourists (on foot and in motorboats) had a lower frequency of trills, a display that serves to maintain contact with social groups and that might affect mate acquisition (de la Torre et al. 2000).

Table 3. Literature examples for assessing predictions concerning habitat shifts. Predictions (in shortened form; see text for more detail) were (A) long-term, intense disturbance stimuli will cause habitat shifts at the cost of reduced access to resources, but (B) habitat shifts will not occur if alternative habitats are unavailable or unsuitable. Unless both treatments are addressed, support for Prediction A makes Prediction B inapplicable. (See *Habitat selection*.)

Study	Species	Stimuli	Predictions		
			Supported	Rejected	Not tested or controls lacking
Allen and Read (2000)	bottlenose dolphin	motorboats	A		
Buckingham et al. (1999)	Florida manatee	motor and paddle boats	A, B		
de la Torre et al. (2000)	pygmy marmoset	people on foot and boats	A		
Duchesne et al. (2000)	woodland caribou	people on skis or snow-shoes		A	B
Dyer et al. (2001)	woodland caribou	roads, other linear developments	A		
Gill et al. (1996)	Pink-footed Geese	roads, vehicular traffic, related activities	A		
Knapton et al. (2000)	diving ducks	motorboats	A, B		
Lafferty (2001)	Snowy Plover	people on foot	A		
Lord et al. (1997)	New Zealand Dotterel[a]	people on foot	A		
Mace et al. (1996)	grizzly bear	roads, vehicular traffic, related activities	A		
Madsen (1998)	waterfowl (quarry and non-quarry species)	hunting activities	A		
Nellemann and Cameron (1998)	barren-ground caribou	road density and associated activities	A		
Nellemann et al. (2000, 2001), Vistness and Nellemann (2001)	reindeer[b]	road traffic; centers of human activity	A		
Papouchis et al. (2001)	Bighorn sheep	road traffic	A		

[a] *Charadrius obscurus aquilonius.*
[b] *Rangifer t. tarandus.*

For some lekking species, such as the Great Snipe (*Gallinago media*), both disturbance stimuli and predation risk can cause males to flee from a lek. These males must then choose between returning to the lek at the risk of re-encountering the predator, or losing access to females by continuing to hide. Males with a high mating probability are more likely to risk returning sooner (Kålås et al. 1995). Yet consider the likely case of an ecotourist who, after disrupting a lek, remains in place determined for another photograph, forcing lek members to hide longer and precluding matings for that period.

The potential impact of ecotourism on the reproductive success of lekking ungulates was discussed almost 30 years ago by McTaggart-Cowan (1974). Walther (1969) also warned that female Thomson's gazelles, whose flight initiation distance from a car is greater than that of males, would be unable to access male territories found within areas frequented by ecotourists. Although data are lacking, it is likely that human divers intent on photographing or viewing reef fishes often disrupt mating by displacing fish from their territories.

PARENTAL INVESTMENT

Predation risk can impact reproduction via decisions about parental investment. When predators threaten both offspring and the parent, should the parent defend the young and face potential death, or should it flee and expect future reproduction to outweigh the loss of current offspring? The decision depends on the parent's residual reproductive value. Parents of iteroparous species that produce few young per year but have long reproductive life-spans should be more likely to save themselves and abandon their current offspring, thus maintaining options for future reproduction (reviews in Clutton-Brock and Godfray 1991, Magnhagen 1991).

Disturbance stimuli may create similar trade-offs. If parents chose to desert young, however, offspring mortality may result from physical factors (e.g., cold temperatures) or facilitation of real predators, and not directly from the disturbance stimuli that threatened the parent. For example, during helicopter disturbance, a Dall's sheep lamb straying behind its fleeing mother fell prey to a Golden Eagle (*Aquila chrysaetos;* Nette et al. 1984). Many bird studies found that nestling predation increased when parents disturbed by humans abandoned the nest (e.g., Tremblay and Ellison 1979, Anderson 1988, Piatt et al. 1990). Further support is found in the lower survival rates of mountain goat (*Oreamnos americanus*) kids separated from their mother during disturbance events (caused by helicopter overflights or all-terrain vehicles) or during encounters with grizzly bears (*Ursus arctos;* Côté and Beaudoin 1997).

When a predator threatens offspring but not the parent, the parent may face a trade-off between energy gain and offspring protection. For Seychelles Warblers (*Foudia sechellarum*), nest guarding by males brings a seven-fold reduction in the rate of egg losses to predation, but time spent nest guarding is time not spent provisioning young or self-feeding. Such trade-offs may not only cause current offspring to starve, but also impact the parents' body condition and future reproduction (Komdeur and Kats 1999).

Parental investment theory should guide predictions of when disturbance stimuli will cause parent—offspring separation, thereby indirectly increasing predation rates on young (see Ghalambor and Martin 2000). Consider female bears (*U. arctos, U. maritimus,* and *U. americanus*) with vulnerable offspring hibernating inside dens. Costs of den abandonment can be high (one study reports a 10-fold increase in cub mortality) and a variety of disturbance stimuli, including seismic blasts, may cause den abandonment. Responses of individual mothers, however, are variable (review in Linnell et al. 2000). Could the probability of den abandonment be predicted in terms of the mother's residual reproductive value?

Although Clark and Ydenberg (1990) provide a framework for quantitative predictions, some qualitative predictions derived from parental investment theory are as follows: (A) When disturbance stimuli are very strong (e.g., direct approaches by helicopters, nearby seismic blasts) and vulnerable young cannot flee, parents with high residual reproductive value will be more likely to desert their young than parents that have already fulfilled most of their reproductive potential. (B) When young are not abandoned and disturbed parents increase their vigilance, hiding, or protection of young, parents with low residual reproductive value will compromise provisioning less than self-feeding, while parents with high residual reproductive value will do the opposite.

We did not find studies that could evaluate these predictions beyond indicating that disturbance does alter provisioning and self-feeding rates. Bald Eagles disturbed by campers increased the time spent protecting nestlings; consequently, the amount of prey consumed during provisioning and self-feeding dropped by almost one-third (Steidl and Anthony 2000). Mexican Spotted Owls decreased prey delivery rates after disturbance from helicopters and chainsaw-related noise, and the effect was stronger when stimuli approached more closely (Delaney et al. 1999). European Oystercatchers (*Haematopus ostralegus*) decreased the time spent incubating eggs, self-feeding, and provisioning young when disturbed by people on foot (Verhulst et al. 2001). Testing the effects of residual reproductive value is more difficult and would require studies of marked individuals of known ages.

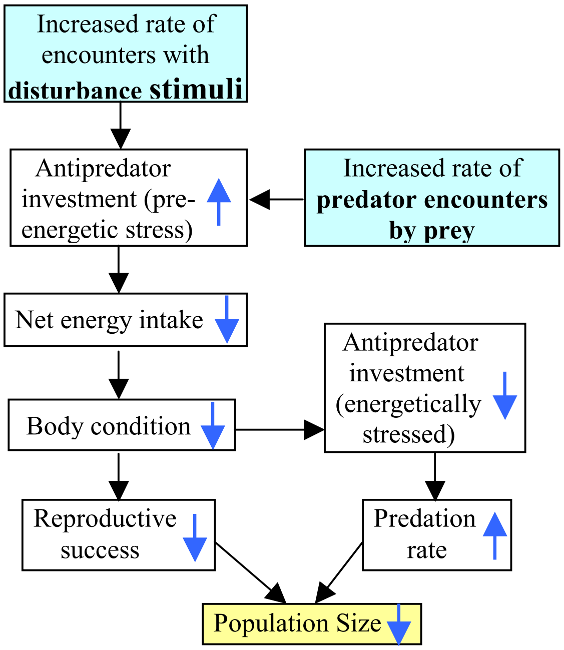
INDIRECT EFFECTS ON POPULATIONS

High levels of predation risk may indirectly affect survival and reproduction by causing prey to divert a large proportion of time and energy away from resource acquisition, so that body condition deteriorates and survival and reproductive success are reduced (Hik 1995, review in Lima 1998, Morris and Davidson 2000). Furthermore, as proposed by the Predation-Sensitive Foraging Hypothesis (Sinclair and Arcese 1995), animals in poor condition may experience greater predation rates when trying to avoid starvation by searching for additional food in dangerous habitats (McNamara and Houston 1987, Hik 1995, Sinclair and Arcese 1995, Sweitzer 1996).

Although only predation causes direct mortality, both predation risk and disturbance stimuli potentially affect population dynamics indirectly by altering an individual's investment in antipredator behavior (reviews in Lima 1998, Gill and Sutherland 2000). If the response includes shifting habitats, then animals displaced from disturbed sites may experience greater intraspecific competition when forced to spend more time in suboptimal habitats, or when crowding into the small areas of good habitat that remain undisturbed (Gill and Sutherland 2000). In some systems, such redistribution in response to disturbance stimuli might also enhance the hunting success of real predators (Kilgo et al. 1998; but see Brown et al. 1999 and next section).

As outlined in Fig. 1, the risk-disturbance hypothesis predicts that long-term and intense disturbance stimuli can cause population declines via reduced body condition and consequent reductions in reproductive success, particularly during periods of high environmental stress (White 1983, Madsen 1994). Reduced body condition caused by high disturbance levels could also contribute to increased predation rates (Fig. 1; see Sinclair and Arcese 1995).

Fig. 1. Conceptual model outlining the behavioral mechanisms by which increased rates of human disturbance or of predator encounters by prey could cause population size to decline. Downward-facing arrows inside boxes indicate a negative response and upward-facing arrows indicate a positive response.



Observations of Pink-footed Geese are consistent with this prediction. Individuals in undisturbed areas gained body mass and had a breeding success of 46%. In contrast, individuals in areas where farmers consistently scared them off their fields did not gain mass and had a breeding success of 17% (Madsen 1994).

Ungulate studies provide further evidence of indirect disturbance effects on populations. The reproductive success of mule deer (*Odocoileus hemionus*) experimentally disturbed by an all-terrain vehicle (Yarmoloy et al. 1988) and of caribou disturbed by low-elevation jet flights (Harrington and Veitch 1992) decreased as disturbance rates increased. For elk (*Cervus elaphus*) disturbed experimentally by people on foot, the ratio of young to mothers was inversely related to disturbance rate (Phillips and Alldredge 2000). These studies did not address the underlying behavioral mechanisms, but were qualitatively consistent with energetic models of the behavioral responses and reproduction of caribou disturbed by seismic exploration blasts (Bradshaw et al. 1998) and low-elevation military jet-fights (Luick et al. 1996). Correlational evidence corroborates the experimental studies. Body mass and population size of barren ground caribou (*R. tarandus*) decreased as rates of military jet overflights increased (Maier 1996). The reproductive success of mountain goats correlated negatively with the cumulative number of seismic exploration lines placed two years prior to the birthing season (each kilometer of seismic line represented 6—8 km of helicopter flying and 22 person-days of ground work; Joslin 1986).

A study of grizzly bears foraging on army cutworm moths (*Euxoa auxiliaris*) provides additional support (White et al. 1999). When disturbed by hikers, bears reduced their energy intake by an average of 12 kcal/min (50.2 x 10^3kJ/min), and spent energy fleeing or acting aggressively toward hikers. The body condition and reproductive success of bears are likely to deteriorate if such reductions of net energy gain are long-term and cumulative (White et al. 1999). Similar examples exist for Snow Geese (*Chen caerulescens atlantica;* Bélanger and Bédard 1990).

Proper tests of reproductive impacts often will be difficult for large vertebrates, because it is often logistically not feasible to reach the large sample sizes required for adequate statistical power (e.g., Delaney et al. 1999).

INDIRECT EFFECTS ON COMMUNITIES

Facilitating predation by natural predators on vulnerable young is one way in which disturbance stimuli might negatively impact one trophic level while having a positive effect on another (see *Parental Investment*). Other community-level effects derive from predation risk affecting the distribution of foragers and, consequently, the foragers' influence on the density of their own prey. For example, when animals spend less time foraging in risky places, they consume a lower proportion of available resources in the area (e.g., Milinski 1985, Morris and Davidson 2000), which could have cascading effects at lower trophic levels (e.g., Chase 1998). Similarly, herbivores may consume a lower proportion of the plant biomass available in the vicinity of a disturbance center (e.g., roads; Gill et al. 1996, Nellemann et al. 2001, Vistness and Nellemann 2001). Thus, human activities can indirectly affect plant community structure by influencing the distribution and intensity of herbivory.

Community effects could also become manifest when 'fear' depletes a prey patch. For example, when two predators share a common prey, the hunting activities of Predator A might increase prey wariness (e.g., forcing it to spend more time in a refuge, increasing aggregation), thereby lowering the foraging success of Predator B (Crowder et al. 1997, Sih et al. 1998, Brown et al. 1999). In the case of disturbance, an analogous situation might arise when fish schools dive deeper to evade motorized vessels (e.g., Gerlotto and Fréon 1992), forcing aquatic predators that breathe air (e.g., cetaceans and seabirds) to increase their foraging costs through deeper dives.

Kilgo et al. (1998) hypothesize that human disturbance might facilitate predation by Florida panthers (*Felis concolor coryi*) on white-tailed deer (*Odocoileus virginianus*). The deer respond to hunting activities by shifting away from roads and open habitats and by increasing their nocturnal activity, which might increase their encounter rates with panthers (Kilgo et al. 1998). However, the predation rate might not necessarily increase with encounter rate (see Lima and Dill 1990, Abrams 1993). At least until the risk of starvation becomes high (Sinclair and Arcese 1995; see Fig. 1), deer can invoke an enhanced state of alertness (e.g., higher vigilance levels, larger and tighter groups) that might counteract increases in encounter rates. It is plausible that deer hunting activities could, in fact, decrease the hunting success of panthers (see Brown et al. 1999).

WHY INVOKE THE RISK-DISTURBANCE HYPOTHESIS?

Hundreds of useful and analytically sophisticated studies already address the effects of disturbance stimuli on animals without invoking the risk-disturbance hypothesis, or indeed any other theoretical framework, and many of them test predictions similar to those discussed here. So what is gained by formalizing and applying the risk-disturbance hypothesis? Although in some cases little might be gained, we argue that the hypothesis often will enhance a study's design and conservation value.

Some predictions regarding fleeing and vigilance are intuitive, and many studies address them at some level, although usually without formalizing them (Tables 1 and 2). Two types of predictions, however, are unlikely to be made a priori without a predation risk framework. First, there appear to be few studies that test how factors related to natural predation risk combine with the characteristics of disturbance stimuli (Prediction D in Tables 1 and 2). Although such predictions may not be relevant to all systems, not testing them will limit the explanatory power of some analyses. Second, it also appears rare for disturbance studies to consider how fleeing responses are affected by resource distribution or by environmental factors that affect locomotion costs (Prediction F of Table 1); none of the studies we reviewed did so a priori. Not considering this prediction may result in a large proportion of unexplained variability and may limit interpretation of the energetic costs of a given disturbance (see Ydenberg and Dill 1986).

In the case of habitat selection, the risk-disturbance hypothesis is not necessary for the intuitive prediction that disturbed animals will shift habitats when aversive disturbance is long term. A predation risk framework is more likely to provide an explicit focus for quantifying lost access to resources, but still is not essential (Table 3). The main issue arises when lack of habitat shifts is interpreted as no disturbance impact. As Gill et al. (2001) argued, there is a danger here of compromising the conservation implications of a study; animals that do not shift habitats simply may have no alternative place worth going to. If forced to remain in the disturbed habitat, their activity budgets probably will be disrupted.

In the case of mate acquisition and parental investment, studies not grounded on predation risk and life-history theory are unlikely to explain mechanisms behind interspecific differences in how disturbance stimuli affect mating displays, or why there is individual variability in how disturbance stimuli affects parental care. Not surprisingly, we found few data to assess our predictions.

Several studies did not need predation risk theory to make notable advances toward predicting and quantifying disturbance effects on population dynamics. We suggest that the risk-disturbance hypothesis, however, would increase the scope of models by ensuring that underlying mechanisms are considered a priori. In particular, none of the reviewed studies considered risk of starvation and predation-sensitive foraging (see Sinclair and Arcese 1995). In the case of community dynamics, the territory is wide open for disturbance studies, and a predation risk framework would be fruitful for guiding predictions. For instance, the model of Gill et al. (1996) could easily be modified to consider the perspective of the plant community, rather than of the herbivore. It was not surprising that we did not come across studies addressing how disturbance increases the baseline level of wariness of a prey species, and therefore indirectly reduces the hunting success of a predator that may be more tolerant of disturbance (see Brown et al. 1999).

At a very practical level, many disturbance studies begin by measuring myriad environmental and biological variables, and go through complex exploratory statistics to reduce the number of factors that would fit a parsimonious model. Clearly, a theoretical framework would focus predictions from the outset, streamline fieldwork and analyses, and increase the generality of results. We suggest that this approach would save time and money for conservation agencies because general predictions would shorten the path toward effective mitigation measures that do not over-regulate human activities.

Although we generally found that literature examples were consistent with predictions of the risk-disturbance hypothesis, some inconsistencies did arise, indicating that much stimulating work lies ahead for refining the concepts. We hope that our discussion stimulates a wider application of predation risk theory, as well as explicit tests of its predictions.

Responses to this article can be read online at: *http://www.consecol.org/Journal/vol6/iss1/art11/responses/index.html.*

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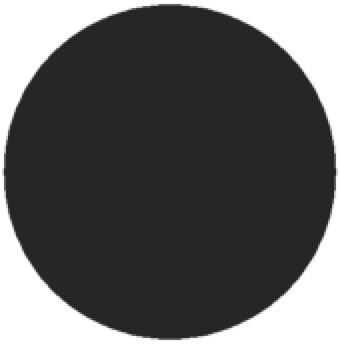
Appendix 1. An example of a generalized fright stimulus. Many animals initiate flight when the rate of change of angle subtended by an approaching object ("loom") exceeds some threshold. To repeat the animation, please refresh your browser or click the image. (For pdf version readers: Go to http://www.consecol.org/Journal/vol6/iss1/art11/appendix1.html to view this animated image)



Reseeding Four Sensitive Plant Species in California and Nevada

H.D. Hiatt
T.E. Olson
J.C. Fisher, Jr.

Abstract–The Kern River Gas Transmission pipeline was constructed in 1991 to supply natural gas to be used in the thermally enhanced recovery of crude oil in Kern County, California, as well as to distribute natural gas in Utah, Nevada, and southern California. Populations of nine sensitive plant species were observed during surveys conducted prior to construction of the pipeline. Mitigation measures for this project included avoidance of identified populations, and reseeding of Rusby's desert mallow (*Sphaeralcea rusbyi* ssp. *eremicola*), Parish's phacelia (*Phacelia parishii*), rosy twotone beardtongue (*Penstemon bicolor* ssp. *roseus*) and yellow twotone beardtongue (*Penstemon bicolor* ssp. *bicolor*). Revegetation success varied within species. The number of Parish's phacelia plants in 1992 that germinated in adjacent seeded and unseeded plots was 706 and 10, respectively. In 1993, the number of plants increased to 2,702 in the seeded plot and 245 plants in the non-seeded plot. During an inventory in 1992, 216 Rusby's desert mallow plants were observed in 73 of 128 standard seeded plots (57 percent), and 300 rosy twotone beardtongue plants were found in the 11 seeded plots.

The Kern River Gas Transmission pipeline (KRGT) was constructed in 1991 to deliver new supplies of natural gas to be used in the thermally enhanced recovery of heavy crude oil in Kern County, California, and also to distribute natural gas in Utah, Nevada, and southern California. The pipeline route extended from a point near Opal, Wyoming, through Utah and Nevada to Daggett, California. Total pipeline length was 596 miles, which included 119 miles in Nevada and 95 miles in California.

The Final Environmental Impact Report/Environmental Impact Statement (Chambers Group 1987) identified potential habitat for several rare plant species along the proposed pipeline route. Additional information regarding rare plants in Nevada and California was acquired during 1989 and 1990 field surveys (Dames & Moore 1990a, b). Those surveys followed a mitigation plan developed by Dames & Moore (1990c). Potential species of special concern included federal candidate species, state-listed species and species on the Northern Nevada Native Plant Society (NNNPS) and California Native Plant Society (CNPS) lists of rare plants (United States Fish and Wildlife Service 1990; NNNPS 1989; Smith and Berg 1988). One sensitive plant species was found in April 1991, after construction began, and further mitigation measures were developed.

The objective of this paper is to document implementation of mitigation measures regarding reseeding of four taxa of sensitive plants along the Nevada and California portions of the Kern River pipeline route and the subsequent reestablishment of these sensitive populations.

Sensitive Plant Surveys and Mitigation Measures

During sensitive plant surveys conducted in 1989 and 1990 along the Kern River pipeline route in Nevada and California, populations of nine sensitive species were observed within the 200-foot wide survey corridor (Table 1). The pipeline was generally constructed in a 75-foot wide disturbance zone located within the survey corridor. These populations were described and mapped (Dames & Moore 1990a, b). Additional surveys were conducted in May 1991 to locate *Phacelia parishii*.

Mitigation measures for this project to facilitate reestablishment of sensitive plants included avoidance, minimization of disturbance to the extent practicable, salvage of topsoil, use of an imprinter during reclamation, and reseeding. Seedbank material, including seeds of sensitive plants saved along with the topsoil, assisted in reestablishment of rare plants. Similarly, the use of an imprinter aided in the retention of precipitation, which assisted the revegetation of both common and sensitive plants.

Active revegetation measures were evaluated for their appropriateness in reestablishing sensitive plants. The methods evaluated for potential use included reseeding of sensitive species, and transplanting bearclaw poppy (*Arctomecon californica*). Mormon needle grass (*Stipa arida*) and scaly cloak fern (*Cheilanthes cochisensis*) plants were located at or adjacent to the 200-foot wide corridor, and avoidance of plants was considered more appropriate than reseeding. The same consideration applied for the annual species three corner milk vetch (*Astragalus geyeri* var. *triquetrus*) and sticky buckwheat (*Eriogonum viscidulum*) which were observed outside of the standard 75-foot-wide disturbance zone and work spaces. In anticipation of possible transplantation efforts, a permit to collect 50 bearclaw poppy plants was obtained from the Nevada Division of Forestry. However, because reseeding and transplanting

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Hermi D. Hiatt, Biologist, Dames & Moore, Las Vegas, Nevada; Thomas E. Olson, Biologist, Dames & Moore, Santa Barbara, California; Jack C. Fisher, Jr., deceased.

Table 1—Sensitive plant species observed along the Kern River pipeline route, Nevada and California, 1989 to 1991.

Taxon name	Common name	Protection status
Sphaeralcea rusbyi ssp. *eremicola*	Rusby's desert mallow	C2, CNPS 1B
Phacelia parishii	Parish's phacelia	C2, CNPS 1B*
Penstemon bicolor ssp. *roseus*	Rosy twotone beardtongue	C2
Penstemon bicolor ssp. *bicolor*	Yellow twotone beardtongue	C2
Stipa arida	Mormon needle grass	CNPS 2
Cheilanthes cochisensis	Scaly cloak fern	CNPS 2
Astragalus geyeri var. *triquetrus*	Three corner milkvetch	C2, Nevada CE, NNNPS T
Eriogonum viscidulum	Sticky buckwheat	C2, Nevada CE, NNNPS T
Arctomecon californica	Bearclaw poppy	C2, Nevada CE, NNNPS T

*Currently reclassified to CNPS 2; CNPS = California Native Plant Society; NNNPS = Northern Nevada Native Plant Society.

of this species has not been successful in the past (Knight 1990), those methods were not implemented. The remaining four sensitive plant taxa were located within the pipeline zone and could not be avoided during construction of the pipeline. These species were Rusby's desert mallow (*Sphaeralcea rusbyi* sp. *eremicola*), Parish's phacelia (*Phacelia parishii*), rosy twotone beardtongue (*Penstemon bicolor* ssp. *roseus*) and yellow twotone beardtongue (*Penstemon bicolor* ssp. *bicolor*). Reseeding of these species appeared to be appropriate and the methods are described below.

Reseeding Methodology

Seed Collection and Storage

Reseeding efforts were accomplished for Rusby's desert mallow, Parish's phacelia, rosy twotone beardtongue, and yellow twotone beardtongue. Ripe seeds of Rusby's desert mallow were collected on 20-21 June, 1991. This species is located near Keany Pass on the east and west sides of the Clark Mountains, San Bernardino County, California, approximately 10 miles west of the Nevada/California border and Interstate 15. Seed collections for this species were segregated as east and west side samples to maintain local gene pools. Seed material for Parish's phacelia was collected on 21 May, 1991. Seeds were gathered from a larger population located less than one mile north of the population that intercepted the pipeline near the Manix Trail, approximately 12 miles northeast of Yermo, California. Collections of seeds of the two subspecies of twotone beardtongue were accomplished during July 1990, within and adjacent to locations along the proposed pipeline construction zone in which the subspecies were observed during the initial plant surveys. Rosy twotone beardtongue seeds were collected near Apex, Clark County, Nevada, at the intersection of the pipeline and State Highway 93. Yellow twotone beardtongue seeds were collected in a wash about 0.5 mile northeast of Wilson Tank in the Bird Spring Range, approximately 8 miles north of Goodsprings, Nevada.

Seeds of all taxa were air dried and stored at constant temperature (approximately 65 to 70 °F). Seed material of Rusby's desert mallow and Parish's phacelia was not cleaned and contained capsules, small leaves and some stems, but seeds of the two subspecies of twotone beardtongue were cleaned by removing most of the extraneous plant matter.

Seed viability of the four taxa was tested by either the Ransom Seed Laboratory in Carpinteria, California, or the Colorado Seed Laboratory at Colorado State University in Fort Collins, Colorado. The following tests were performed to obtain the percentage of total live seed. Germination tests resulted in 1 to 2 percent germination within 21 days for Rusby's desert mallow seeds, and 0 to 6 percent within 14 days for Parish's phacelia seeds. The remaining seeds were treated with gibberellic acid and 5 to 50 percent of the seeds germinated (percent hard seed, as shown in Table 2). Ungerminated Parish's phacelia seeds were then evaluated for viability with tetrazolium. The two subspecies of twotone beardtongue were only tested with tetrazolium, resulting in 83 to 87 percent total live seed (Table 2).

Reseeding Rusby's Desert Mallow

Rusby's desert mallow, a perennial herb, was reseeded in late October and early November, 1991. Seventy-two reseeding plots (designated as E-1 through E-72) were placed in the disturbance zone on the east side of Keany Pass. Plots E-1 to E-8 were 6-foot diameter circles, and the remaining sites were 8 feet in diameter. Seven or eight plots each were placed within 10 of 11 drainages in that portion of the right-of-way. For the purpose of placing the sample plots, a drainage was defined as the area from hilltop to hilltop. West of Keany Pass, 52 plots were placed within approximately a one-mile stretch of pipeline (plots W-1 to W-52), and 5 plots were placed in the area of a disjunct population (plots W-53 to W-57) approximately 2.25 miles west of Keany Pass. Sample plots W-1 to W-20 were 8 feet in diameter, and the remaining plots each covered a 4-foot diameter circle. The 5 plots in the area of the disjunct population were placed on a west-facing slope; the other 52 sample plots were placed within 11 drainages on both east- and west-facing slopes. Each plot was marked with rebar and metal tags.

Because of pipe repair, the seed material in sample plots E-5 and E-8 was salvaged subsequent to reseeding. Topsoil and seed material were removed from those two sample sites in November 1991, and new plots were established 2 weeks

Table 2—Results of germination tests for seeds collected along the Kern River pipeline route in Nevada and California, 1991.

Species	Sample number	% Germination	% Hard seed	% Tetrazolium	% Total live seed
Rusby's desert mallow (east side)	#1	1	44	—	45
	#2	2	50	—	53
Rusby's desert mallow (west side)	#1	1	9	—	10
	#2	1	5	—	6
Parish's phacelia	#1	3	39	17	59
	#2	6	17	51	74
	#3	0	22	55	77
Rosy twotone beardtongue	#1	—	—	85	85
Yellow twotone beardtongue	#1	—	—	83	83
	#2			87	87

later. The soil was deposited within a new 4-foot diameter circular area in the vicinity of the old plot sites.

Each plot was raked prior to reseeding to loosen compacted soils. Seed material (0.5 ounce) was broadcast by hand in each sample plot and the ground was raked again to cover seeds with a small amount of soil. Seed material was estimated to contain approximately 1,190 Rusby's desert mallow seeds per 0.5 ounce. Thirty-six ounces of material were broadcast on the east side of Keany Pass, and 29 ounces on the west side. Overall, it was estimated that approximately 155,000 seeds were dispersed in the Clark Mountain area.

Reseeding Parish's Phacelia

Before reseeding the annual Parish's phacelia, special preparation of the reseeding plot was implemented. This species generally grows on desert alkaline flats, specifically in desiccation cracks of thick clay accumulations. A reseeding plot of 270 by 30 feet was chosen on the spoilside of the pipeline within the 75-foot wide disturbance zone. The plot was sprayed with 4,000 gallons of water on 3 December, 1991. Then the site was allowed to dry and crack. An equally large unwatered control plot was chosen on the workside of the pipeline within the disturbance zone. After 2 weeks, the soil had dried and cracked and the habitat of the reseeding plot approached nearly natural conditions. Transects were walked width-wise, and the seed material was evenly broadcast in 25 subsamples. Seeds were distributed for an additional 10 feet beyond the disturbed area, to compensate for depletion of plants during seed collection. It was estimated that approximately 1,300,000 Parish's phacelia seeds were broadcast during reseeding. The plot was marked with rebar and metal tags. To reduce motor vehicle travel through the plot, orange wooden stakes were placed across the width of the plot.

Reseeding Twotone Beardtongue

Reseeding of the two perennial subspecies of twotone beardtongue was accomplished in early November 1991. Reseeding areas were selected according to the general location of collection to maintain local gene pools. In addition, existing populations were located near the pipeline before reseeding within the disturbance zone.

Rosy twotone beardtongue was reseeded near the intersection of the pipeline and Highway 93. Five sites were chosen within the 0.5-mile stretch west of the highway. Seeds were dispersed within 11 three-foot diameter subplots. At Site 1, which is located at the greatest distance from the highway, 1 subplot was established at the southern edge of the pipeline disturbance. Site 2 was established in a prominent wash approximately 0.4 miles from the highway. Three subplots were placed across the disturbance zone, with additional plots east of the wash. One subplot each was placed at the northern edge of the pipeline disturbance at Sites 3 and 4, located approximately 2,000 and 1,800 feet from the highway, respectively. Site 5 consisted of 2 subplots at the northern edge of the disturbance zone and a broad wash adjacent to Highway 93. Approximately 30,600 rosy twotone beardtongue seeds were broadcast in the 11 subplots.

Yellow twotone beardtongue was reseeded in a small wash dissecting the disturbance zone approximately 0.5 miles northeast of Wilson Tank in the Bird Spring Range. An approximately 115-by-53-foot plot was established and marked at the corners. The equivalent of 45 subplots of 9 to 16 square feet was established within the large plot. Individual subplots were raked and a small amount of seed was spread within the subplot. The subplots were then raked to cover the seed. Approximately 17,400 seeds were broadcast.

Reseeding Results

Reseeding success was evaluated by inventorying plant establishment the first season after reseeding activities. One species was evaluated for a second season. The survey methods and the results of reestablishment of each species are described below.

Rusby's Desert Mallow

The 57 plots located on the west side of the Clark Mountains were inventoried on 21 June and 9 August, 1992.

Table 3—Results of follow-up surveys of Rusby's desert mallow, Clark Mountains, San Bernardino County, California, June, August 1992.

	Side of Keany Pass			
	Standard plot		Expanded plot	
	East	*West*	*East*	*West*
Plots surveyed	71	57	71	57
Total plants observed	69	147	84	237
Plants/Plot	1.0	2.6	1.2	4.5
Frequency (%)	46	70	46	72
Density (plants/100 ft^2)	2.0	3.8	—	—

Seventy-one of 72 plots on the east side of the Clark Mountains were inventoried on 20 June, 1992. Locations of these plots were identified by markers that had been installed during reseeding. The boundaries of the plots were determined by observing the rake marks that were still discernible, and by measuring from the center stake. Plot E-5 was not found during the inventory.

Many Rusby's desert mallow plants were observed, sometimes in greater densities in areas adjacent to the reseeded plots. It was speculated that seeds were transported outside of the reseeded plots by wind and rain erosion of the seedbed. As a result, counts were made in the original reseeded plot as well as in an expanded plot of approximately 40 feet in diameter.

The total number of plants counted in the original plots was 69 plants on the east side of the Clark Mountains and 147 plants on the west side (Table 3). The east-side plots contained an average of 1.0 plants, and 33 of the 71 plots contained at least 1 plant. The average number of plants per plot of the west side was 2.6 plants. Growth of at least 1 plant occurred in 40 of 57 plots. The average densities of plants in the east side and west side plots were 2.0 per 100 square feet and 3.8 per 100 square feet, respectively.

In the expanded plots, 84 plants were counted on the east side of the Clark Mountains and 237 plants on the west side. The average number of plants per plot was 1.2 (east) and 4.5 (west), respectively. The number of plots that contained at least 1 plant was almost the same as in the original plots, as shown in Table 3. Frequency of the original and expanded plots was 46 percent in the east side plots, and 70 and 72 percent on the west side.

Parish's Phacelia

Reestablishment of Parish's phacelia was evaluated on 18 April, 1992, and during April 1993. In addition to the reseeded plot, two additional plots were inventoried as control sites. One control site was located within the disturbance zone of the pipeline; it had not been reseeded in 1991. The other control plot was located in an undisturbed area outside the disturbance zone. Each plot measured 270 by 30 feet. The number of Parish's phacelia plants observed in each plot was counted. The first year after reseeding, 706 Parish's phacelia had germinated in the reseeded plot, and only 2 plants in the control plot within the disturbance zone (Table 4). In the adjacent non-reseeded plot, a total of 10 plants was found. In 1993, approximately 2,702 plants were found in the reseeded plot, which represented a density of 33.4 per 100 square feet. In the non-reseeding plots in and adjacent to the disturbance zone, 245 plants and 1,014 plants were counted, respectively.

Rosy Twotone Beardtongue

The 5 reseeding sites were inventoried on 6 October, 1992. Locations of the plots were identified by markers that had been installed during reseeding activities. Although many of the sites had been disturbed by off-road vehicle traffic, 10 of the 11 subplots contained small plants, ranging from 3 to 68 per subplot (Table 5), with a total of 300 plants. In the following year, no counts were made. However, cursory observation indicated that plants were flowering and covered the entire surface of one three-foot diameter subplot. Other subplots were less prolific.

Yellow Twotone Beardtongue

During the October 1992 inventory, the reseeding plot and surrounding area contained a moderate to dense cover of species of a wild horse seed mix, including *Penstemon* sp. seedlings. Subsequent to the sensitive species reseeding effort in 1991, the pipeline disturbance zone in that area was reseeded with a different seed mixture. The second reseeding effort was completed at the request of the Bureau of Land Management with the objective of enhancing the wild horse habitat in that area. The yellow twotone beardtongue reseeding area was included in the second reseeding effort. The wild horse seed mix contained Palmer's

Table 4—Results of follow-up surveys of Parish's phacelia, San Bernardino County, California, April 1992 and April 1993.

	Within disturbance zone		Adjacent to disturbance zone
	Reseeded	Non-reseeded	Non-reseeded
1992			
Number of plants	706	2	10
Density (plants/100 ft^2)	8.7	<0.1	0.1
1993			
Number of Plants	2,702	245	1,014
Density (plants/100 ft^2)	33.4	3.0	12.5

Table 5—Results of follow-up surveys of rosy twotone beardtongue, Clark County, Nevada, October 1992.

Site	Number of subplots	Number of plants observed/subplot
1	1	45
2	6	0-45
3	1	55
4	1	68
5	2	3-26
Total	11	300

penstemon (*Penstemon palmeri*). Yellow twotone beardtongue could not be distinguished from Palmer's penstemon at this morphological stage, and reseeding efforts were not determined.

Discussion and Conclusions

Review of this project indicated successful mitigation for impacts to sensitive plant species. Avoidance and minimization of disturbance zones is still considered the preferred method, but active revegetation in the form of reseeding can be an effective mitigation alternative for some species if avoidance is not possible.

Early planning is important for successful reestablishment of sensitive plant species. Several points need to be considered. These are: site analysis of habitat prior to disturbance; biotic and physical requirements of each taxon; optimal time for seed collection; specific site preparation to create an environment favorable for reestablishment; and developing methods of monitoring.

Reseeding of Rusby's desert mallow required no specific seedbed preparation. This species appears to be adapted to disturbed areas; many of the seeds collected came from plants found on old powerline spur roads. Other factors, however, may have influenced the difference in reestablishment between the east and west populations (2.0 plants versus 3.8 plants per 100 square feet, respectively). Specifically, the one-time seed collection was apparently suboptimal. Seeds appeared to be at a late stage of seed dispersal; the west side population was further advanced than the east side population. In addition, the east side material contained 50 percent hard seeds compared to 10 percent in the west side population. Since afterripening may break dormancy over time (Young and Young 1986), a second year of monitoring may have been beneficial. Also, seed collections should have occurred over a period of time to obtain high quality seeds.

Some species require special methods for seedbed preparation. For instance, the disturbance zone was graded and leveled at the end of construction. Parish's phacelia specifically grows in desiccation cracks of thick clay accumulations, and re-creating this microenvironment was critical for reestablishment. Because rain is unpredictable in desert environments, application of 4,000 gallons of water was essential for re-creating desiccation cracks. During the second year after reseeding, natural seed dispersal and subsequent rains to create desiccation cracks in the control plots apparently increased seed germination in both the reseeding and control plots.

Frequently, provisions are not made for monitoring the success of reestablishment projects, for both sensitive and common species. Hall (1987) reported that 7 of 15 mitigation projects failed because of lack of maintenance and monitoring. No monitoring was required for this project, neither of the sensitive plant taxa nor of the imprinting success and natural revegetation of the common species. Monitoring the reseeding results of these four taxa was only due to a voluntary effort of the authors. Valuable information could be gained from revegetation projects, if monitoring the success rate would be a condition of project approvals by the responsible governmental agencies.

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HYUNDAI MOTOR AMERICA MOJAVE PROVING GROUNDS DESERT TORTOISE TRANSLOCATION STUDY

2006 ANNUAL SUMMARY

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BACKGROUND

The Habitat Conservation Plan (HCP) for the Hyundai Motor America (Hyundai) Mojave Proving Grounds Project (Project)[1] detailed the methods and requirements for a translocation program on desert tortoises (*Gopherus agassizii*) removed from the Hyundai Project site[2]. The program included both translocation of tortoises from the Hyundai Project site and a follow-up study on specific effects of translocation.

The primary goals of translocating tortoises from the Hyundai site were to prevent the mortality of tortoises that lived on the site, to maintain the integrity of the population as much as possible, and to maintain breeding individuals in the population. Secondarily, it was hoped that translocation would facilitate the repopulation of another nearby area that had experienced tortoise density declines resulting from drought and disease, and were thereby well under carrying capacity in a normal forage year.

The primary objectives of the translocation study were to address four primary questions:

1 – What is the effect of translocation on survival?
2 – What is the effect of translocation on health status, especially (a) exposure to *Mycoplasma. agassizii* and other pathogens, (b) disease expression, and (c) condition indices?
3 – Is fencing a translocation site a reasonable procedure for site repatriation of areas that are depauperate due to stochastic climatic events or other factors that have not reduced the habitat quality at the translocation site?
4 – How are activity levels affected by translocation?

Each question was further segregated into effects relating to gender, age/size, variation in forage levels, rehydration, activity levels, and time since translocation.

Desert tortoises were removed from the proving grounds and translocated to one of two translocation sites in April of 2004 and 2005 (see attached summaries for the details of each translocation effort). The sites were fenced with tortoise-proof fencing that would removed after at least 18 months to investigate the repatriation objective

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[1] Sapphos Environmental, Inc. 2004. Environmental Assessment/Habitat Conservation Plan for issuance of an engangered species Section 10(A)1(B) Permit for the incidental take of the desert tortoise (*Gopherus agassizii*). January 6, 2004.

[2] Karl, A. E. 2003. Hyundai Motor America Mojave Test Track Site. Desert tortoise translocation program. Appendix A of Sapphos Environmental, Inc,. 2004, Environmental Assessment/Habitat Conservation Plan for issuance of an endangered species Section 10(A)1(B) Permit for the incidental take of the desert tortoise (*Gopherus agassizii*).

of the project. Studies began on the translocated tortoises prior to their translocation, in October 2003, and have been continuous since.

This report summarizes activities in 2006. Data analyses are preliminary and ultimately will be incorporated into comprehensive analyses for each subject area. Such analyses are continually underway for several of the multi-year activities and will become available as results reach a logical threshold.

SITE DESCRIPTION

Based on requirements of the HCP, two translocation sites were established. Site choice was based on habitat quality, carrying capacity considerations, size and proximity to other protected (or likely to be protected) lands, proximity to the Hyundai Project site (i.e., same tortoise population), and ability to protect the site in perpetuity. (See Karl [2003][1] for discussions of these considerations).

The two translocation sites are approximately 30 km northeast of the Hyundai Proving Grounds and adjacent to previously existing lands in the California Department of Fish and Game Ecological Reserve (CDFG ER; Figure 1). They have subsequently become part of that reserve as part of Hyundai Project compensation. The translocation sites are also adjacent to the expansion boundary of another neighboring reserve, the Desert Tortoise Research Natural Area (DTRNA), and the Fremont-Kramer Desert Wildlife Management Area (DWMA).

The westernmost translocation site ("Translocation S12") is one square mile and occupies Section 12 in Township 31S and Range 39E. The eastern translocation site ("Translocation SE") is 1.25 square miles and encompasses Section 17 and the southeastern quarter of Section 18 in Township 31S and Range 40E. Both sites were fenced with wire mesh field fencing prior to translocating tortoises there, in order to preclude entry by sheep and recreationists. Three-foot-wide, tortoise-proof hardware cloth was attached to the lower portion of the fence, with two feet extending above the ground surface and the remaining foot buried, to temporarily keep translocated tortoises in the translocation site.

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The sites have inherently medium quality habitat, based on shrub and annual species present, vegetation density, topography, soils, and substrates. The shrub community is dominated by creosote bush (*Larrea tridentata*), white bursage (*Ambrosia dumosa*), and goldenhead (*Acamptopappus sphaerocephalus*), and snake head (*Ericameria cooperi*), with subdominant cheesebush (*Ambrosia salsola*), winterfat (*Krachenninnikovia lanata*), Mojave aster (*Xylorhiza tortifolia*), stipa (*Stipa speciosa*), and wolfberry (*Lycium cooperi*). The topography is flat to very gently undulating and soils are soft loamy sands. Sheep grazing during the historic past has decreased the habitat quality somewhat by reducing the diversity of shrubs and potentially promoting the dominance of two exotic annuals, split grass (*Schismus*

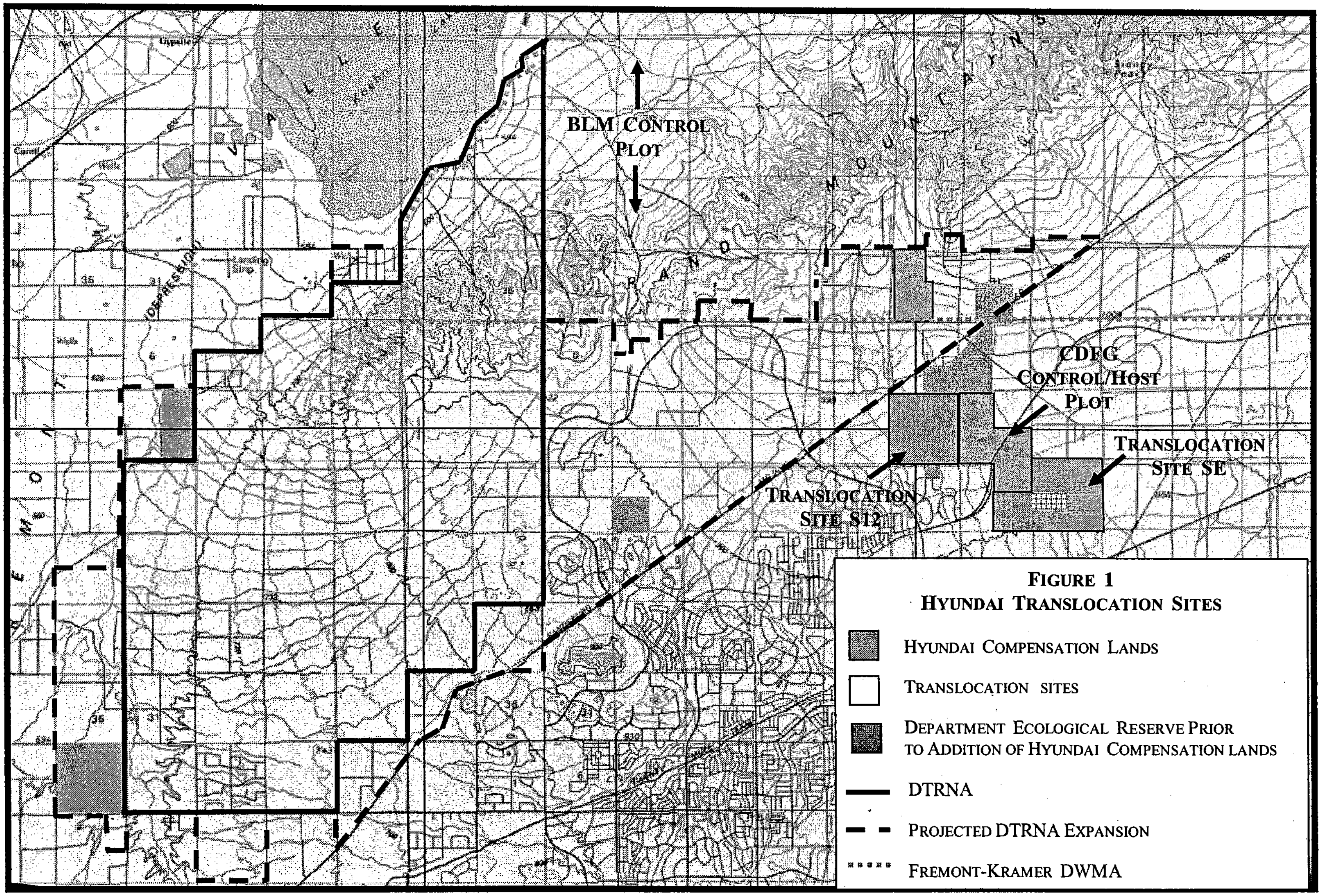

FIGURE 1
HYUNDAI TRANSLOCATION SITES

arabicus) and filaree (*Erodium cicutarium*). There are, however, a number of native species present that are consumed by tortoises (in addition to consuming the split grass and filaree). Onsite disturbances consist of a few well-developed off-highway-vehicle (OHV) trails (motorcycle and all-terrain vehicle) and scattered tracks and faint trails. There are no OHV "play" areas of concentrated damage, although there are a couple of old sheep bedding areas on Translocation SE.

METHODS

Study Cohorts

Hyundai tortoises were translocated to the two translocated sites in April 2004 (Translocation S12) and April 2005 (Translocation SE), because of staggered site availability. (One tortoises, H15, was left outside the Hyundai Project site because she was seropositive for exposure to *Mycoplasma agassizii*.) Both sites were searched for host tortoises prior to releasing translocated tortoises there. However, host tortoises were only found on Translocation SE; none was found in Translocation S12. In March and April 2004, a control site ("BLM Control") was established north of the Rand Mountains (Figure 1) and the site was searched for resident tortoises. A secondary area abutting and between the two translocation sites, and affected both by the initial tortoise-proof fencing as well as the ultimate influx of new tortoises following removal of that fencing ("CDFG Control/Host"), was also searched for resident tortoises. In March and April 2005, the Translocation SE site was searched for host tortoises prior to translocating the remaining Hyundai tortoises there. In summary, the study cohorts consist of:

- ◊ Two translocation cohorts:
    - Translocation S12 - 15 tortoises
    - Translocation SE - 12 tortoises
- ◊ Translocation SE Host Tortoises- 9 tortoises
- ◊ CDFG Control/Host Tortoises - 6 tortoises
- ◊ BLM Control Tortoises - 22 tortoises
- ◊ Hyundai Site (Outside Fence) Tortoises – 1 tortoise

Table 1 describes the size and gender composition of each cohort.

General Methods

At the time of capture, all study tortoises (translocatees, host, and control tortoises) were weighed, measured, photographed, sexed, and described. To facilitate future identification, each was permanently marked (notched) with a unique number, including a distinctive notch to distinguish these tortoises from those used in other nearby studies (e.g., DTRNA trend plots), and secondarily marked with a small epoxy

Table 1
Hyundai Desert Tortoise Translocation Project
Initial Composition of All Study Cohorts, by Site

Tortoise Number	Gender	MCL[1]
Translocated Tortoises-Translocation S12		
H 04	Male	299.5
H 07	Male	272
H 08	Female	280
H 14	Male	313.5
H 16	Female	241
H 18	Male	286
H 21	Female	244
H 23	Female	254
H 24	Female	243
H 25	Female	251
H 26	Female	227
H 28	Male	288
H 31	Female	248
H 34	Male	261
H120	Male	267
Translocated Tortoises-Translocation SE		
H 05	Male	282
H 06	Male	299
H 17	Female	260
H 19	Female	247
H 22	Female	255*
H 27	Female	276
H 29	Male	277
H 32	Male	234
H 35	Male	285
H 36	Male	278
H 37	Female	239
H 40	Female	290
Hyundai Site (Outside Fence)		
H 15	Female	248

Tortoise Number	Gender	MCL[1]
BLM Control		
H 101	Female	235
H 102	Female	222
H 103	Male	299
H 104	Male	217
H 105	Male	180
H 106	Female	243.5
H 107	Female	238
H 108	Male	263.5
H 109	Female	244
H 110	Male	184.5
H 111	Female	249
H 112	Male	283
H 113	Female	198
H 114	Male	290
H 115	Female	221
H 116	Male	258
H 117	Male	251
H 118	Male	268
H 119	Male	264
H 121	Male	273
H 130	Female	241.5
H 407	Male	230
CDFG Control/Host		
H 201	Male	246
H 202	Male	285
H 203	Male	296
H 204	Female	255
H 205	Male	254+
H 212[2]	Male	209
Translocation SE Host		
H 206	Female	259
H 207	Male	277
H 209	Male	297
H 210	Female	235
H 211	Female	247
H 213	Male	256
H 214	Female	245
H 215	Female	224
H 217	Male	303

1. Maximum Carapace Length at initial capture
2. H212 moved several kilometers away from the study site, so was removed from the Study cohort.

number on the fourth costal. Holohil R1-2B transmitters (24mm wide by 11 mm thick; 14.9 g) were attached to each tortoise, fitted to insure safety to the individual and lack of interference with growth and behaviors (Figure 2). Transmitters are scheduled to last 18or 24 months and are changed prior to scheduled battery life or sooner, if they exhibit symptoms of malfunctioning.

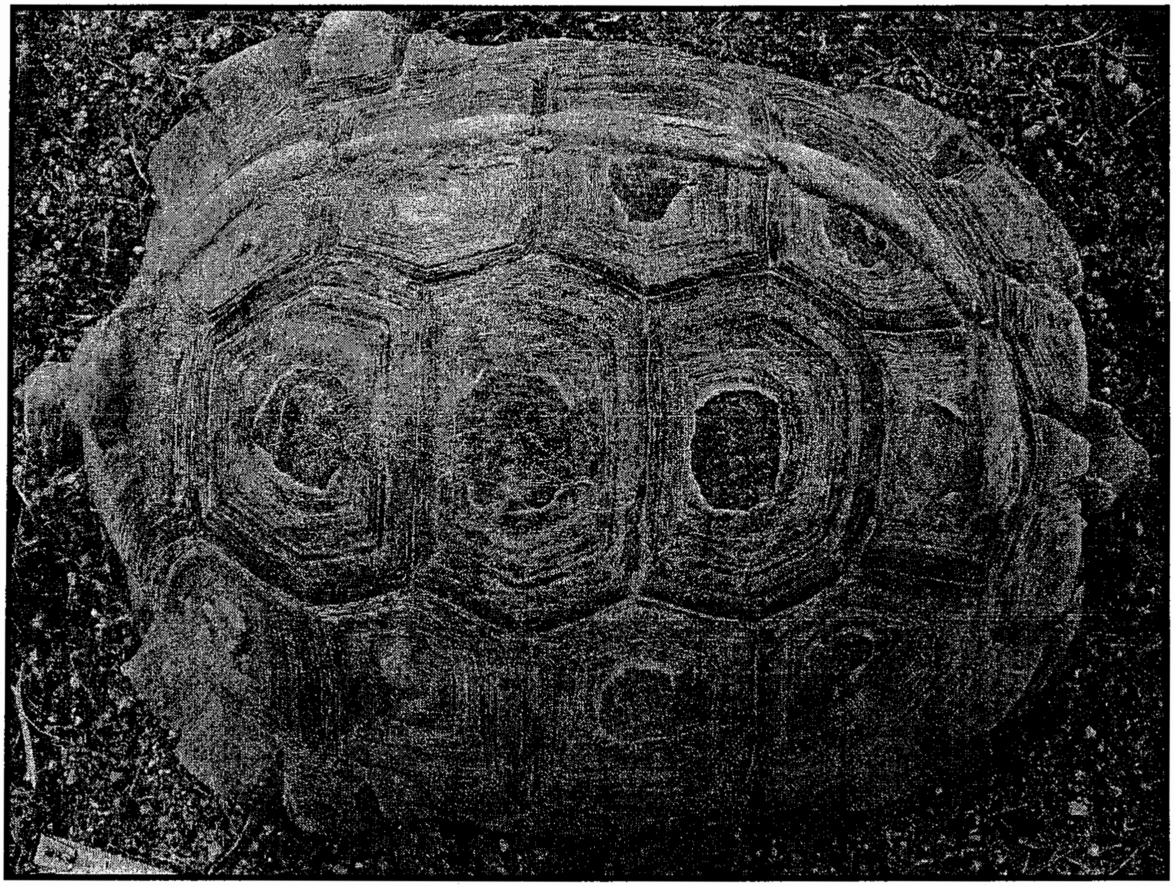

Figure 2. Standard transmitter placement on male Hyundai study tortoises (here, H203). Note the data logger attached to the pygal scute on the left side of the photo.

Survival and general health are monitored through body condition indices (mass to volume ratios[3]), clinical signs, serology and cultures. Condition indices are measured three times during each year: (1) when tortoises exit from hibernation (late March),; (2) following the

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[3] Volume is calculated as half the volume of a spheroid, or $\frac{1}{2}(4/3 \cdot \pi \cdot r^3)$. For a tortoise, this translates into $\frac{1}{2}$ $[4/3 \cdot \pi \cdot (\text{length}/2) \cdot (\text{width}/2) \cdot \text{height}]$.

spring activity period and after nesting (July); and (3) immediately prior to hibernation (late October). All tortoises are examined for clinical signs of disease while measuring condition indices. Serum and nasal samples were taken for all study animals at initial capture and at translocation, and are collected annually to test for the presence of antibodies to *Mycoplasma agassizii* (ELISA test) or *M. agassizii* infection (PCR culture), respectively.

Activity patterns (i.e. increased aboveground activity levels), which may affect body temperatures and body condition and ultimately health and survival, are monitored by temperature data loggers (HOBO© TidBits [(www.onsetcomp.com)]), which continuously collect data, every ten minutes. These have been mounted on all males in the study cohorts and also in sample burrows. (Only males can carry the data loggers without interference with righting or copulatory behaviors because the data loggers' tall profile necessitates that the unit be attached to the pygal scute. See Figure 2.)

Translocated tortoises are located on a sufficiently intense schedule to collect the necessary health data, download data loggers, change transmitters, identify faulty transmitters and other equipment, and monitor coarse-grained use areas. In general, this includes locations every ten days during the height of the spring activity period (April), twice-monthly locations during the remainder of the spring activity period (May through June) and fall activity period (October), and once a month in all other months. The exception to this schedule was immediately following translocation. All tortoises were watched for at least one full day immediately following release to observe behaviors and insure that no tortoise exhibited behavior that could compromise survival.

Vegetation data have been collected annually for comparison among all sites, including the original capture site (Hyundai Proving Grounds). Comparisons include plant community characterization (density, frequency, species composition, and percent cover) as well as forage cover and biomass. Specific methods will be presented during a separate report on vegetation results.

Weather is monitored continuously using an HOBO© Weather Station (www.onsetcomp.com) on the Translocation S12 site. Precipitation, wind speed, relative humidity, air temperature, surface temperature, and barometric pressure are recorded hourly. A rain gauge is also maintained on the Hyundai Project site to record precipitation there.

Year 2006

In July 2005, the tortoise-proof fabric at the Translocation S12 site was removed. Because of the possibility of extreme tortoise movements after release, especially following the July 2005 monsoons, translocated tortoises in the Translocation S12 cohort were located three times a week until early September, at which point there was some confidence that they would not be lost due to extreme movements. (Control tortoises were located weekly for comparability of movement.) Rates of locating tortoises remained elevated over normal autumn rates until hibernation, but only at approximately every ten days rather than three times a week.

RESULTS FOR YEAR 2006

Survival

No tortoises died in 2006. To date, only two tortoises in the study group have died, Translocated Male H120 and Host SE Female H215. There was no obvious cause of death for Tortoise H120, who was a relatively old male, judging from shell wear. The female, who died approximately June 1, 2005, apparently died of exposure due to being overturned on a hard surface, where she was unable to right herself prior to overheating.

Health Analyses

Laboratory Results. Titer levels of antibodies identify exposure to *M. agassizii*. No tortoises translocated from Hyundai have seroconverted (i.e., became positive for exposure to *M. agassizii*). Four tortoises have changed titers over the study period: BLM Control tortoises H106, H112 and H119, and the tortoise remaining outside the Proving Grounds, H15. H106 had a titer level of <32 and considered negative for exposure to *M. agassizii* at capture in April of 2004[4]. In April of 2005, this tortoise had a titer level of 32 and was suspect for exposure. In July 2006, the titer was once again <32. H112 had a titer level of 128 at capture in April 2004. In 2005, the titer level was 64 and the tortoise was still considered positive for exposure to *M. agassizii*. In July 2006, the titer level was 32 and the tortoise was considered suspect for exposure. Tortoise H119 was consistently negative through two samplings (April and October) in 2004, with a titer level of <32. In July 2005 and in subsequent samples, the titer level was 32, suggesting a possible exposure to *M. agassizii*.

Tortoise H15, a female from the Hyundai Project site that moved outside the fence prior to the translocation effort and has been monitored as a study animal where she remains, has been continually seropositive since her capture in October 2003. Her titer was 128 from 2003 through 2005. In 2006, the level was 64. Clinical signs have been variable on this tortoise but generally have been confined to swollen eyelids, especially the palpebral. She has never exhibited a nasal discharge or other secretions that are consistent with mycoplasmosis, Other inconsistent signs that may be considered clinical indicators of mycoplasmosis have included occasionally moist nares, moist eyes, or dirt in the nares. However, these conditions are not uncommonly observed in seronegative tortoises and are most likely a response to living in a subterranean, dirt burrow.

Cultures have been consistently negative for all tortoises, even those with positive or suspect titer levels. This is not considered unusual because of the difficulties of culturing the microorganism (Lori Wendland, DVM, University of Florida Mycoplasma lab, pers. comm.).

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[4] The University of Florida Mycoplasma lab rates tortoises as positive, suspect, or negative for exposure, based on titer levels. A titer of 32 is the threshold.

Table 2
Hyundai Desert Tortoise Translocation Project
Comparative Laboratory Results for *Mycoplasma agassizii* Exposure and/or Infection From Initial Capture and Year 2006

Tortoise	Condition at Initial Capture			Condition in 2006		
	Titer	ELISA Result	PCR Result	Titer	ELISA Result	PCR Result[1]
Translocated Tortoises-Translocation S12						
H 04	<32	Negative	Negative	<32	Negative[2]	Negative
H 07	<32	Negative	Negative	<32	Negative	Negative
H 08	<32	Negative	Negative	<32	Negative	---[1]
H 14	<32	Negative	Negative	<32	Negative	---[1]
H 16	<32	Negative	Negative	<32	Negative	---[1]
H 18	<32	Negative	Negative	<32	Negative	Negative
H 21	<32	Negative	Negative	<32	Negative	---[1]
H 23	<32	Negative	Negative	<32	Negative	---[1]
H 24	<32	Negative	Negative	<32	Negative[2]	---[1]
H 25	<32	Negative	Negative	<32	Negative[2]	---[1]
H 26	<32	Negative	Negative	<32	Negative	---[1]
H 28	<32	Negative	Negative	<32	Negative[2]	Negative
H 31	<32	Negative	Negative	<32	Negative	Negative
H 34	<32	Negative	Negative	<32	Negative	Negative
H 120	<32	Negative	Negative	<32	Negative[3]	Negative
Translocated Tortoises-Translocation SE						
H 05	<32	Negative	Negative	<32	Negative	---[1]
H 06	<32	Negative	Negative	<32	Negative	Negative
H 17	<32	Negative	Negative	<32	Negative	---[1]
H 19	<32	Negative	Negative	<32	Negative	Negative
H 22	<32	Negative	Negative	<32	Negative	Negative
H 27	<32	Negative	Negative	<32	Negative	---[1]
H 29	<32	Negative	Negative	<32	Negative	Negative
H 32	<32	Negative	Negative	<32	Negative	Negative
H 35	<32	Negative	Negative	---[5]	---[5]	---[1]
H 36	<32	Negative	Negative	<32	Negative	Negative
H 37	<32	Negative	Negative	<32	Negative	---[1]
H 40	<32	Negative	Negative	<32	Negative	---[1]
Translocation SE Host						
H 206	<32	Negative	Negative	<32	Negative	Negative
H 207	<32	Negative	Negative	<32	Negative	Negative
H 209	<32	Negative	Negative	<32	Negative	Negative
H 210	<32	Negative	Negative	<32	Negative	Negative
H 211	<32	Negative	Negative	<32	Negative	Negative
H 213	<32	Negative	Negative	<32	Negative	Negative
H 214	<32	Negative	Negative	<32	Negative	Negative
H 215	<32	Negative	Negative	<32	Negative[3]	Negative

H 217	<32	Negative	--- (1)	<32	Negative	---[1]
BLM Control						
H 101	<32	Negative	Negative	Tortoise is temporarily lost		
H 102	<32	Negative	Negative	<32	Negative	Negative
H 103	<32	Negative	Negative	<32	Negative	Negative
H 104	<32	Negative	Negative	<32	Negative	Negative
H 105	<32	Negative	Negative	<32	Negative	Negative
H 106	<32	Negative	Negative	<32	Negative	Negative
H 107	<32	Negative	Negative	<32	Negative	Negative
H 108	<32	Negative	Negative	<32	Negative	Negative
H 109	<32	Negative	Negative	<32	Negative	Negative
H 110	<32	Negative	Negative	<32	Negative	Negative
H 111	<32	Negative	Negative	<32	Negative	Negative
H 112	128	**POSITIVE**	Negative	32	**SUSPECT**	Negative
H 113	<32	Negative	Negative	<32	Negative	Negative
H 114	<32	Negative	Negative	<32	Negative	Negative
H 115	<32	Negative	Negative	<32	Negative	Negative
H 116	<32	Negative	Negative	<32	Negative	Negative
H 117	<32	Negative	Negative	<32	Negative	Negative
H 118	<32	Negative	Negative	<32	Negative	Negative
H 119	<32	Negative	Negative	32	**SUSPECT**	Negative
H 121	<32	Negative	Negative	<32	Negative	Negative
H 130	<32	Negative	Negative	<32	Negative	Negative
H 407	<32	Negative	Negative	<32	Negative	Negative
CDFG Control/Host						
H 201	<32	Negative	Negative	<32	Negative	---[4]
H 202	<32	Negative	Negative	Tortoise is temporarily lost		---[4]
H 203	<32	Negative	Negative	<32	Negative	Negative
H 204	<32	Negative	Negative	---[5]	---[5]	Negative
H 205	<32	Negative	Negative	---[5]	---[5]	Negative
Hyundai Site (Outside)						
H 15	128	**POSITIVE**	Negative	64	**POSITIVE**	Negative

1 PCR cultures not yet completed by University of Florida for Year 2006. Year 2005 shown if available. (Some 2005 results have not been supplied by the University of Florida yet.).

2 Unable to extract tortoise on any sampling occasion in 2006. Results shown are from July or October 2005.

3 Tortoise died in 2005. Results shown are from 2005

4. Tortoise lost in 2005 due to early transmitter failure. (Note: AVM Instruments transmitters were used on a portion of the study group initially, but due to untimely transmitter failure and poor operation, they were all replaced with Holohil transmitters.. Some tortoises wearing AVMs were temporarily lost, but re-found through extensive and repeated searches.)

5. ELISA results currently unavailable

Hence, although a negative result does not signal absence, a positive result is definitive for presence of *M. agassizii*.

Condition Indices. Condtion indices for translocated tortoises were similar to both control and host tortoises (Table 3), for both genders, and there is no apparent loss of body mass at one or two years post-translocation that is due to translocation. Females in 2006 emerged from hibernation in slightly better condition than males (although not significant: $P_{1,49} = 0.28$), but following the spring oviposition period, had significantly lower condition indices ($P_{1,49} < 0.001$). These lower condition indices remained into hibernation ($P_{1,49} = 0.009$).

Table 3
Hyundai Desert Tortoise Translocation Project
Comparative Condition Indices ($g/m^3 * 10^{-3}$) for Translocated, Control, and Host Tortoises in Year 2006

Cohort	April		July		October	
	Female	Male	Female	Male	Female	Male
Translocated Tortoises **(both sites)**	1.146	1.145	0.942	1.049	0.818	0.980
BLM Control	1.157	1.114	0.946	1.051	0.880	0.931
Host Tortoises **(CDFG, Translocation SE)**	1.158	1.122	0.931	1.064	0.947	0.987

Tortoise Movement Following Fence Removal

The tortoise fence was detached from the perimeter fence on the first translocation site (Translocation S12) and removed during July 2006. This was 27 months after tortoises had been translocated to that site. It was also prior to the autumn period of high tortoise activity. During the period between fence removal and hibernation, only three tortoises moved off the site. Two of these, Female H24 and Male H04 moved less than 100 meters off the translocation site and then moved back onto the site. H24 finally hibernated less than 50 meters off the site. Female H21 remained on the site until October, when she moved approximately 40 meters off site. She ultimately hibernated approximately 220 meters north of the site. The remaining 12 translocated tortoises remained on the translocation site.

Autumn 2006 followed late June precipitation (1.8 mm) and was accompanied by early October precipitation (2.4 mm), with the resultant germination of several forage species. In addition to normal elevated testosterone levels in the fall, these environmental conditions promoted activity and thereby heightened the opportunity for tortoises to leave the site of translocation. The result that only two tortoises moved a very short distance off the site suggests that, at least in the short term, repatriation of the site is a success. The remaining two years of the study will identify further movement patterns and provide more information on the value of this repatriation technique for re-populating depauperate areas.

CONTINUED STUDIES

The tortoise fence from the second translocation site (Translocation SE) was removed during Winter 2006/7, 22 months following translocation. Tortoises will emerge from hibernation without the constraints of a border fence. Intensive monitoring has begun to help insure that tortoises will not be lost should they move substantial distances. Based on the lack of movement away from the translocation site for the first set of tortoises released (Translocation S12), the second release occurred both earlier and preceding spring, which is generally a period of maximum foraging, as well as nesting, for tortoises. Releasing the translocatees during different seasons and following different time periods since translocation will provide an opportunity to examine repatriation success under different conditions.

The remaining aspects of the Translocation Study are ongoing.

ATTACHMENTS

1. **Initial summary of tortoise translocation from the Hyundai facility**

2. **Initial summary of 2005 tortoise translocation from the Hyundai Proving Grounds**

MEMORANDUM

To: Steve Juarez, Judy Hohman, Nicholas Browning
From: Alice Karl
Date: April 18, 2004

Re: Initial summary of tortoise translocation from the Hyundai facility

This memorandum provides a brief summary of the initial tortoise translocation from the Hyundai facility. A more detailed account, with pictures, will follow when I have access to software for downloading the films and some of the behavioral data have been analyzed.

On April 10 and 11, fifteen tortoises from the Hyundai test track facility were translocated to the one square mile translocation site (Section 12 in Township 31S and Range 39E). This removed all of the tortoises that were currently inside the site boundary (with the exception of one injured animal and one recently found clinically ill animal), plus several that were typically traveling on and off the site and had been observed pacing the fences near the site border. (Note: Because the border of the site is not yet fenced and a substantial amount of disturbance has occurred at the site, many of the currently transmittered 26 tortoises had moved off the site shortly after exiting from hibernation this spring.)

All tortoises were weighed and assessed for clinical signs at the time of translocation. None had clinical signs. Three of the translocated tortoises, captured after serology tests were run last October, have not yet been tested for *Mycoplasma agassizii* exposure. (This was foreseen and is consistent with the translocation plan and HCP.) Serology testing on all animals in the study, including control, translocated, resident, and remaining Hyundai site tortoises, will occur again in approximately one week. (It takes approximately 6-8 weeks after exposure to the pathogen for a tortoise to mount a sufficient titer level for serology tests, so any positive test for exposure to *M. agassizii* will not be the result of translocation.)

Artificial burrows were constructed for all of the tortoises prior to translocation. All tortoises were captured in the late afternoon and released at their burrows at night, when the animals were inactive and largely asleep. Eight of the tortoises were penned with temporary tortoise-proof fencing in approximately 15-foot diameter pens; the remaining seven tortoises were released without pens. Fences were removed for the penned animals at Day 3.

Tortoises were moved in relatively the same geographic configuration as they originally occurred at the Hyundai site, such that a tortoise moving east, for instance, would meet the same tortoise it would have met on the Hyundai site. Two male-female pairs of tortoises were moved together because they were either captured together in the same burrow at the time of translocation or had spent the winter in the same burrow.

Behavioral assessments will follow at a later date, but briefly, tortoises ate, copulated, and have individually either remained at their artificial burrows, occupied other tortoise's artificial burrows, or begun to construct their own burrows; they have moved various distances and directions. Tortoises, while a generalist species, are individual specialists, and their behaviors on this project are no exception to this pattern.

MEMORANDUM

To: Steve Juarez, Judy Hohman, Nicholas Browning
From: Alice Karl
Date: May 8, 2005

Re: Initial summary of 2005 tortoise translocation from the Hyundai Proving Grounds

This memorandum provides a summary of the translocation of the remaining seronegative tortoises originally found on the Hyundai facility. The new translocation site is 1.25 mi^2 and encompasses Section 17 and the southeastern quarter of Section 18 in Township 31S and Range 40E. It abuts the Department Ecological Preserve on the latter's southeastern boundary. The site has been entirely fenced with hog wire (a.k.a. "field fence") to deter intrusion by off-highway-vehicle recreationists. Attached to the lower portion of the fence is ¼ -inch mesh hardware cloth, buried and extending two feet above the ground surface, as a tortoise-proof barrier.

On April 11 and 12, following completion of the tortoise-proof fence around the new translocation site and a search of the entire site for resident tortoises, the twelve remaining seronegative tortoises from the Hyundai test track facility were translocated to the new translocation site. Tortoises were collected in the late afternoon and released at night because this coincides with the time of the day when they are inactive. All were relaxed and remained in their burrows at the time of release.

An approximately 1.5-meter long artificial burrow was constructed for each tortoise prior to translocation. No tortoises were penned at their translocation burrow, as they were in the first translocation effort in 2004. This was based on the observations in 2004 that penned, translocated tortoises spent a substantial amount of time pacing the fence and there was no evidence that penned tortoises had greater burrow fidelity than un-penned tortoises.

Tortoises were moved in relatively the same geographic configuration as they originally occurred at the Hyundai site, such that a tortoise moving east, for instance, could encounter the same tortoise it would have met on the Hyundai site.

All tortoises were watched for at least one full day immediately following release to observe behaviors and insure that no tortoise exhibited behavior that could compromise survival. In fact, the tortoises appeared "relaxed" to all observers and all tortoises spent substantial amounts of time foraging (especially on the abundant *Lotus humistratus*) as well as frequently seeking shade and/or constructing pallets for resting. Two tortoises encountering the boundary fence walked the fence prior to seeking shade. All translocated tortoises continued to be located approximately weekly during April, following this initial observation period. Subsequent locations will be consistent with the translocation plan (i.e., increased locations during high-activity periods; decreased locations during periods when tortoises could be expected to travel shorter distances).

All tortoises were weighed and assessed for clinical signs at the time of translocation. Each had been weighed previously for comparative condition indices to control (un-translocated) tortoises. Serology testing had already been completed in October 2004 on all but one of the translocated tortoises. The remaining tortoise was tested within two weeks of release, however.[5] The eight resident tortoises at the translocation site were also tested at this time. All were seronegative and none had clinical signs.

It should be noted that most tortoises had been foraging on the abundant forage this year since early March. Ample forage still remained at the translocation site at the time of translocation, although it was beginning to senesce. Ambient temperatures remained cool for most of April, so it was generally unnecessary for translocated tortoises to seek burrows for thermal relief.

In accordance with the translocation plan, vegetation data have been collected for comparison between the original capture site (Hyundai Proving Grounds), the translocation sites, and the control site. Comparisons include site characterization (density, frequency, species composition, and percent cover) as well as forage biomass. This, in combination with condition indices (i.e., mass to volume ratios), and activity levels (from data loggers) are currently being analyzed.

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[5] It takes approximately 6-8 weeks after exposure to the pathogen for a tortoise to mount a sufficient titer level for serology tests, so any positive test for exposure to *M. agassizii* will not be the result of translocation.

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Environmental Characteristics of Desert Tortoise (*Gopherus agassizii*) Burrow Locations in an Altered Industrial Landscape

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ABSTRACT. – **In the Colorado Desert of California, the western distributional limit of the desert tortoise (*Gopherus agassizii*) occurs in the Whitewater Hills of the southeastern San Bernardino Mountains. Much of the area has been developed for wind energy generation and tortoises often live in association with altered industrial landscapes. Natural habitat in the area was characterized by a sharp transition zone of plant associations including representatives of the Colorado and Mojave Deserts, coastal, and montane ecosystems. We examined the environmental factors associated with the locations of desert tortoise burrows at a site developed for wind energy generation. Measurements were taken at the opening of burrows, including elevation, slope, aspect, and distance to various natural and anthropogenic features of the landscape. We compared this data set with identical measurements for random points that lacked burrows in the same landscape. The analysis demonstrated that desert tortoises within the study area did not randomly select their burrow sites. Desert tortoise burrows were located closer to roads and concrete foundations associated with wind energy turbines and transformers than were random points. The results challenge the paradigm that desert tortoises are negatively affected by all forms of anthropogenic disturbance and suggest that with proper planning, some forms of development in the desert are compatible with conservation of sensitive species.**

KEY WORDS. – **Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; burrows; wind energy generation; habitat selection; Mojave Desert; Colorado Desert; California; USA**

Habitat use by animals is influenced by several factors that can have a dramatic influence on an individual's fitness. Selection of specific habitats can facilitate access to important resources such as food, water, mates and brood/nest sites, provide protection from predators and harsh environmental conditions, and limit competition with con- and hetero-specifics. When specific habitats are selected by animals, they are used disproportionately to their availability. Major assumptions of habitat selection are that animals select habitats that maximize their ecological requirements and fitness, and that high quality habitats are selected more than low quality habitats (Rosenzweig, 1981; Manly et al., 1993). In comparison with transient occupancy of habitat, location of nests, burrows, and other structures used by animals for longer periods of time represent a relatively long-term, and potentially costly, commitment to a particular microhabitat (Hansell, 1993). Consequently, the location of these structures has significant physiological and life-history consequences (Fig. 1).

The desert tortoise (*Gopherus agassizii*) is federally protected as a threatened species throughout about half of its range in the United States, which includes portions of California, Nevada, Arizona, and Utah (Fish and Wildlife Service, 1994). In California, as much as 98% of the annual activity cycle of the desert tortoise is spent underground in burrows or other shelter sites that it usually constructs (Nagy and Medica, 1986). Burrows are used for thermoregulation (McGinnis and Voigt, 1971; Zimmerman et al., 1994), hibernation (Bailey et al., 1995; Rautenstrauch et al., 1998), nesting sites (Turner et al., 1986), and as foci for social interactions (Bulova, 1994, 1997). The location of burrows can also provide protection from flooding and fire. Burrows provide a special microhabitat where the humidity is higher and the temperature is lower and more constant than the environment on the surface. Thus, using burrows helps reduce evaporative water loss rates and provides protection from thermal extremes. Under adverse surface conditions, desert tortoises may stay in burrows for weeks or months at a time (Ernst et al., 1994).

Most research on the desert tortoise has focused on areas far removed from human population centers, although much of the habitat occupied by desert tortoises has been affected by humans to some extent (Lovich and Bainbridge, 1999), sometimes severely. Although human activities have been invoked as causes of population declines in the species (Fish and Wildlife Service, 1994; but see Corn, 1994, and Bury and Corn, 1995), few data are available to evaluate these impacts critically. The purpose of this study was to examine the environmental characteristics of desert tortoise burrow locations in an industrial landscape developed for wind energy generation near Palm Springs, California. Two questions were asked at the beginning of the study: (1) do desert tortoises randomly locate burrows in the study area? and, (2) if burrow locations are not random, do desert tortoises avoid constructing burrows in proximity to industrial activities?

METHODS AND MATERIALS

Site Description. — The study site was located on land administered by the Bureau of Land Management (BLM) in the Whitewater Hills of the southeastern San Bernardino Mountains in western Riverside County, California. The area, known locally as the Mesa wind park (Mesa), was developed for wind energy generation starting in the 1980s. Wind energy turbines and their associated infrastructure were the most conspicuous elements of the landscape with about 460 turbines, 51 electrical transformers, and an extensive network of unpaved roads in place at the time of the study (Fig. 2). Concrete foundations were associated with each turbine and electrical transformer. In addition, the area was grazed by cattle in most years as part of the Whitewater Grazing Allotment administered by the BLM. A vigorous breeding population of desert tortoises occupies the site (Lovich et al., 1999).

The study site was characterized by a mixture of plant communities representing several ecosystems. Sitting at the interface between coastally influenced plant associations and the desert, Mesa had exceptional perennial plant diversity. North-facing slopes and the western edge of the study area were dominated by chaparral and coastal sage scrub plant species (Schoenherr, 1992) including chamise (*Adenostoma fasciculatum*) and California sage brush (*Artemisia californica*). Other cismontane species (*sensu* Schoenherr, 1992) included California juniper (*Juniperus californica*), condalia (*Condalia parryi*), and isolated oaks (*Quercus* spp.). South-facing slopes and the eastern edge of the study area were characterized by typical Mojave Desert (Vasek and Barbour, 1977) and Colorado Desert (a subdivision of the Sonoran Desert, Burk, 1977) plants, including creosote bush (*Larrea tridentata*), burrobush (*Ambrosia dumosa*), honey mesquite (*Prosopis* spp.), cholla (*Opuntia* spp.), bladder pod (*Isomeris arborea*), linear-leaved goldenbush (*Haplopappus linearifolius*), encelia (*Encelia farinosa*), and cheesebush (*Hymenoclea salsola*). A signature species of the Colorado Desert that occurred on site was teddy-bear cholla (*Opuntia bigelovii*). Another species, spiny hopsage (*Grayia spinosa*), a common plant of the Mojave Desert, but rare in the Colorado Desert except for the Whitewater Hills (Jaeger, 1940), was relatively abundant. The study site was mountainous with elevations at desert tortoise capture locations ranging from about 660 m in the valleys to over 880 m on the peaks and ridges. The topography at the northern boundary of the study site limited the distribution of tortoises, which usually occur below 1500 m (Germano et al., 1994), as elevation rises sharply to over 3500 m.

Figure 1. The location of a desert tortoise burrow can have dramatic consequences for its occupant. This photograph shows the carcass of a desert tortoise that died of third-degree burns in its burrow during a wildfire at the study site. The shallow burrow was located under a dense thicket of *Grayia spinosa* that ultimately became the funeral pyre for the animal. If the burrow would have been located in the open, or if it had been deeper, the occupant may have survived. Many of the tortoises at the site bear the scars of encounters with fire. Photo by JEL.

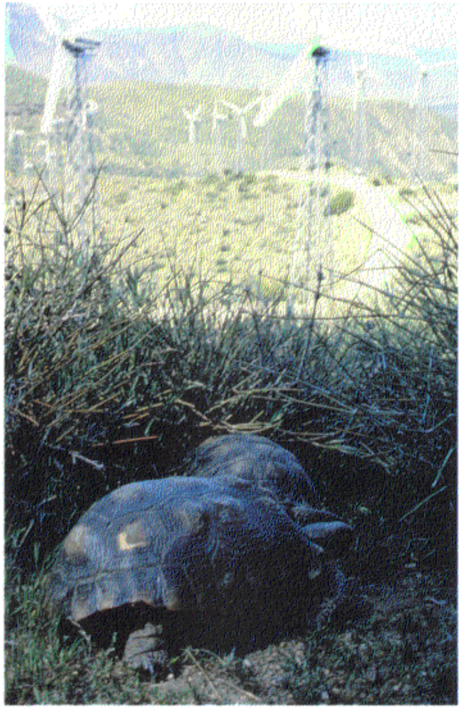

Figure 2. Partial view of study site showing wind energy turbines and desert tortoises (female in foreground, male in background) as they were found. The female bears the remnants of an old tag that was epoxied to her shell by a previous researcher for purposes of individual identification. Photo by JEL.

The study site was at the westernmost edge of the distribution of the desert tortoise in the Colorado Desert (Luckenbach, 1982; Patterson, 1982), where the steep terrain and unique plant associations are atypical of desert tortoise habitat elsewhere in the Colorado Desert (Fish and Wildlife Service, 1994). The coastally influenced climate resulted in greater rainfall than tortoise habitat immediately to the east, due to a rain-shadow effect, and this generally promoted high production of winter annual plants (Lovich et al., 1999) on which desert tortoises feed.

Methodology. — We collected data during 1995 and 1996, although anecdotal observations continued through 1998 during the course of our separate research on the reproductive ecology of desert tortoises at the site (Lovich et al., 1999). Burrows were located during systematic searches

Table 1. Desert tortoise (*Gopherus agassizii*) burrow attributes reported in the literature. Aspect refers to the predominant orientation of the entrance of the burrows.

Aspect	Slope	Cover Association	Region	Reference
North-northeast	—	72% under shrubs	southern Nevada	Burge, 1978
West-southeast	—	79% under shrubs	California deserts	Berry and Turner, 1986
North	—	—	southern Nevada	Bulova, 1994
South	44.1º	40% under shrubs	Arizona	Bailey et al., 1995
—	—	68% under perennial plants	California	Duda, 1998
Southwest	17.7º	41% under shrubs	Mesa, California	This study

of the wind park conducted by walking transects through areas bounded by unpaved roads or rows of turbines. Only burrows that were approximately shell-depth or more (thus excluding shallow excavations or pallets according to the definition of Burge, 1978), and known or appearing to be actively used, were included in the statistical analyses. All burrows but one, occupied by a juvenile, were typical of the size used by adult tortoises (18–37 cm carapace length, Ernst et al., 1994). The following variables were quantified for each burrow: SLOPE (in degrees), ASPECT (compass direction in degrees of the predominant facing slope), and ELEVATION. In addition, distances were measured from the opening of each burrow to various natural and anthropogenic features in the landscape, including the following variables: ROAD (unpaved roads, as no paved roads are located at the site), PAD (concrete foundations for turbines and electrical transformers), LARREA (creosote bush, *Larrea tridentata*), ENCELIA (brittlebush, *Encelia farinosa*), CACTUS (several cactus species of the genus *Opuntia*), YUCCA (*Yucca* spp.), and ROCK (rock outcrops or rockpiles). These variables were selected because they were prominent features of the landscape. Distance variables were measured using a flexible tape. All plants were alive at the time the burrow was constructed, although many were dead at the time of measurement due to the effects of a major fire in 1995. The importance of using fine scale habitat characteristics to infer ecologically meaningful patterns in desert tortoise burrow distribution has previously been demonstrated by Baxter (1988). Computer-generated random points were used to locate sites that did not have burrows and the same variables were measured. The statistical analysis included 32 desert tortoise burrows and 32 random points.

Following Zar (1984), data were transformed for statistical analyses using the natural logarithm of (x+1), unless indicated otherwise, to meet the assumption of normality. Multivariate Analysis of Variance (MANOVA) was used to assess the overall significance of differences between all variables measured (except ASPECT) for burrows and random plots. This technique is superior to using multiple univariate ANOVAs because it uses correlations among characters rather than ignoring them (Willig et al., 1986). Principal components analysis (PCA) was then used on transformed variables, normalized to have a mean of zero and a standard deviation of one, as a data reduction technique to identify orthogonal factors and the variables that loaded highly in each. Separate MANOVAs were calculated for variables that loaded highly in each factor. Following identification of multivariate significance, a two-group discriminant function analysis (DFA) was conducted using the influential variables selected with PCA. The classification accuracy of the function was assessed by tabulating actual locations (burrows and random points) vs. locations predicted by the function.

Because ASPECT is a circular scale variable, it was analyzed separately using Oriana© software for circular statistics. Other statistical procedures were executed using SYSTAT (Wilkinson et al., 1992). Levels of statistical significance were set at an alpha of 0.05.

RESULTS

Of the 32 burrows analyzed, 13 (41%) were located under shrubs, including *Larrea, Ambrosia, Ephedra* sp., *Hymenoclea*, and *Grayia* (Table 1). One of the burrows included in our statistical analysis was located under the concrete pad of an electrical transformer (Fig. 3a). Subsequent observations made after our study concluded, but not included in our statistical analysis, demonstrated that this was not an unusual burrow location and that burrows were commonly associated with anthropogenic features in the landscape (Figs. 3b-f).

There were few significant differences among the variables measured, with YUCCA being the single exception (Table 2). However, the results of a MANOVA using log-

Table 2. Means, standard deviations (in parentheses), and ranges for variables measured at desert tortoise burrows and random points. The probability for ASPECT is based on Watson's F-test for two circular means. Probabilities for other variables are based on univariate F-tests (ANOVA) comparing the means of log-transformed data for burrows and random points. All measurements are in meters, except for slope and aspect, which are in degrees.

	SLOPE	ASPECT	ROAD	PAD	LARREA	ENCELIA	CACTUS	YUCCA	ROCK	ELEVATION
Burrows	17.7	188.3	22.1	49.7	16.8	5.1	4.8	31.7	10.3	770.3
	(10.2)	(66.5)	(22.6)	(77.1)	(25.4)	(6.0)	(4.6)	(30.1)	(16.0)	(32.3)
	0–45	26–340	0–101	0–343	0–100	0.2–24.4	0–25	3.3–102	1.7–92.4	699.8–844.3
Random	15.9	193.1	33.9	66.2	23.7	6.5	7.8	16.1	14.7	775.1
	(10.7)	(97.3)	(41.5)	(59.2)	(45.4)	(5.9)	(9.7)	(18.4)	(14.1)	(29.0)
	2–40	88–360	0–145.8	0.5–188.8	0–233	0.2–24	0.7–51.1	1–90	1.2–47.7	719.3–850.4
Probability	0.62	0.84	0.96	0.14	0.61	0.21	0.12	0.04	0.18	0.53

Figure 3. Desert tortoise burrows at the study site were frequently associated with human disturbances in the environment. All photos by JEL unless noted otherwise. **(a)** This female, visible in the center of the photograph, constructed her burrow under the concrete pad of an electrical transformer and shared it with a packrat (*Neotoma* spp.). Her frequent use of the burrow (spanning about 2 years) was shown by scratches on her carapace caused by passing under the concrete lip of the foundation. Photo by Claude Kirby. **(b)** Another female used a burrow under a different electrical transformer pad. The entrance is the wide area shown on the right side of the pad. **(c)** Yet another female constructed her burrow in the road cut next to a turbine. She deposited a clutch of eggs in the apron of her burrow in 1997. **(d)** Close-up of a juvenile desert tortoise (6.9 cm carapace length) at the entrance to the burrow shown in Fig. 3e. **(e)** A juvenile desert tortoise constructed a burrow under a piece of waste concrete next to a turbine. A lens cap (lower center of the photograph) is shown above the entrance to the burrow. **(f)** Desert tortoise burrows at the study site are frequently constructed in cut banks along roads. Note the burrow under the white marker pole.

transformed data for all variables except ASPECT, revealed significant differences among the variables characterizing burrows and random points (Wilk's Lambda = 0.730; df = 9, 54; p = 0.035). The mean vector for burrow ASPECT was southerly (188.3º) but it was not significantly different from the mean vector (193.1º) for random points (Watson's F-test, F = 0.04, p = 0.84, df = 62; Table 2, Fig. 4).

PCA revealed four principal components with eigenvalues greater than unity that together explained 74.7% of the total variance. The first principal component was related to anthropogenic features in the landscape as shown by high loadings for ROAD and PAD. The other principal components were related to distance to various plant species (Table 3). A simplified MANOVA using the highest loading vari-

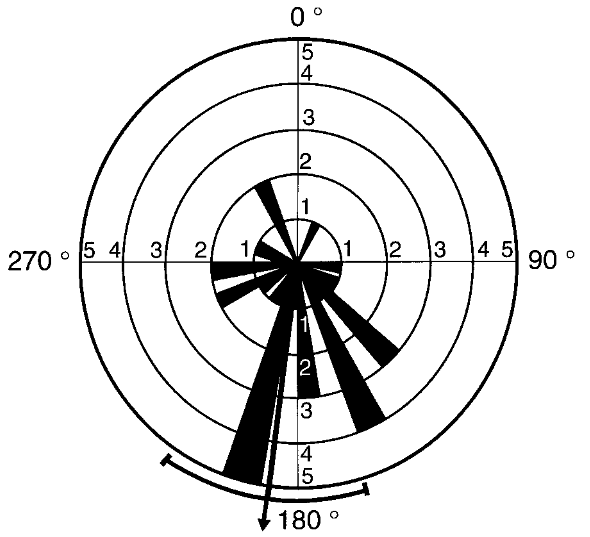

Figure 4. Plot of vectors showing aspect of slopes for locations with desert tortoise burrows. Bar width is 10°. Frequency is shown by the radius of wedge. The mean vector (188.3°) and the 95% confidence interval are shown.

ables in each principal component (PAD, LARREA, YUCCA, ENCELIA) was not significant (Wilk's Lambda = 0.870; df = 4, 59; *p* = 0.080). Because the first principal component was related to anthropogenic features, it seemed logical to include ROAD as another variable in MANOVA. Although ROAD and PAD were correlated as shown by their high loadings and same sign (Table 3), there were many occasions when the nearest road to a burrow or random point was not the road next to the nearest pad (which always had service road access). MANOVA using an expanded model including ROAD, PAD, LARREA, YUCCA, and ENCELIA approached significance (Wilk's Lambda = 0.839; df = 5, 58; *p* = 0.064).

The DFA on PAD, LARREA, YUCCA, and ENCELIA achieved an overall classification accuracy of 67.2% with most misclassifications occurring for burrows (Table 4). An expanded model including ROAD did not change the results appreciably, but a full model incorporating all variables, with the exception of ASPECT, achieved 71.9% classification accuracy (Table 5). The discriminant scores for burrows were significantly different than those for random points as shown by a two-tailed Student's t-test (t = -4.788, df = 62, *p* < 0.001). A summary of habitat relationships based on discriminant scores is depicted in Fig. 5.

Table 3. Unrotated principal component loadings for principal components with eigenvalues greater than one. Variance explained by each component is shown in parentheses.

	Principal Component			
Variable	I (30.717)	II (18.619)	III (13.354)	IV (12.106)
PAD	0.844	0.058	-0.280	0.011
ROAD	0.799	-0.336	-0.116	-0.008
ELEVATION	-0.724	-0.505	0.011	0.087
SLOPE	0.565	-0.449	0.443	0.108
LARREA	-0.476	-0.610	-0.043	0.452
CACTUS	-0.281	0.592	0.146	0.521
YUCCA	0.304	0.262	0.785	0.282
ENCELIA	0.244	0.272	-0.523	0.611
ROCK	-0.334	0.487	0.013	-0.377

DISCUSSION

The results of our analysis demonstrate that desert tortoise burrow sites were not randomly located as shown by the results of MANOVA of log-transformed variables. This was not unexpected in that other investigators have demonstrated the preference of desert tortoises for certain environmental attributes. Baxter (1988) studied desert tortoise burrow locations near Twentynine Palms, California, approximately 50 km from our study site. He found that at the landscape level, burrow distribution was not statistically different from random. However, the abundance of burrows differed across six plant assemblages reflecting both the non-randomness of the plant assemblages in the landscape, and the preference of desert tortoises for certain assemblages, particularly along ecotones. At the same site (near Twentynine Palms), Duda (1998) found that tortoise burrow locations were statistically different from both random and Poisson distributions, with the data further suggesting that the underlying distributions were clumped.

Hibernation burrows of desert tortoises in the Sonoran Desert of Arizona are often associated with vegetation (dead or alive) and packrat (*Neotoma albigula*) nests (Bailey et al., 1995). Most hibernation burrows examined were located on steep (>45°) south-facing slopes in soils composed of silt, silt with loose gravel, diatomite and/or diatomaceous marl, or layers of well-lithified volcanic ash.

The characteristics of desert tortoise burrow sites in southern Nevada were studied by Burge (1978). Most burrows faced east, northeast, or north, and 72% were located under shrubs. Shrubs were utilized disproportionately to their abundance. For example, 37.7% of the burrows located under shrubs were found under *Acacia greggii* despite the low density of that shrub at the study site. According to Burge, the shade provided by *A. greggii* may have been the reason for its disproportionate use. Similarly, burrows were

Table 4. Classification accuracy of discriminant function analysis for variables PAD, LARREA, ENCELIA, and YUCCA. Row totals are in parentheses.

	Predicted Group		
Actual Group	Burrows	Random Points	Total
Burrows	20 (62.5%)	12 (37.5%)	32
Random points	9 (28.1%)	23 (71.9%)	32
Total	29	35	64

Table 5. Classification accuracy of discriminant function analysis for variables ROAD, PAD, LARREA, ENCELIA, YUCCA, SLOPE, CACTUS, ROCK, and ELEV. Row totals are in parentheses.

	Predicted Group		
Actual Group	Burrows	Random Points	Total
Burrows	22 (68.8%)	10 (31.2%)	32
Random points	8 (25.0%)	24 (75.0%)	32
Total	30	34	64

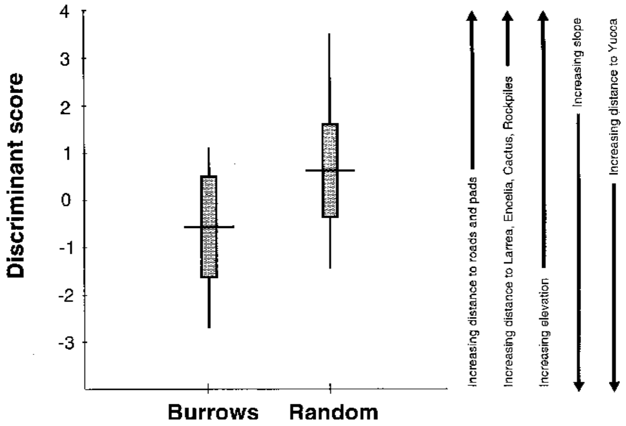

Figure 5. Plot of discriminant score statistics based on a full model incorporating log-transformed ROAD, PAD, LARREA, ENCELIA, YUCCA, SLOPE, CACTUS, ROCK, and ELEV. Means are shown with one standard deviation and ranges for burrows and random points. Arrows show direction of correlation among variables and discriminant scores. The means are significantly different at $p < 0.001$.

frequently located under *Yucca schidigera*, although this plant was numerically under-represented at the study site. Burge suggested that *Yucca* provided roof structure and possibly insulation for burrows. In sharp contrast, the results for our study showed that tortoise burrows were located farther from *Yucca* than were random points, a phenomenon for which we have no explanation.

More recently, Wilson et al. (1999) quantified the physical and microhabitat characteristics of burrows used by juvenile desert tortoises in a field enclosure located in the western Mojave Desert of California. The majority of burrows were located well under large shrub canopies, especially the two species *Larrea tridentata* and *Lycium pallidum*, than were located under the canopy margin or in the open. The mean angle of burrow orientation was 71°. The authors hypothesized that placement of burrows well under large shrubs conferred better protection from predators and/or provided more favorable microclimates for juvenile desert tortoises than burrows located under smaller shrubs or in the open.

Our analysis shows that the principal component explaining the greatest variance in burrow site attributes at Mesa was related to the proximity of anthropogenic features in the landscape. The question that needs to be addressed is: what factors encouraged adult desert tortoises at our study site to locate their burrows in close proximity to roads and turbines? This question is especially pertinent in recognition of well-established evidence showing that roads have generally negative consequences for wildlife due to: (1) mortality of animals along roadways (Rosen and Lowe, 1994; Boarman and Sazaki, 1996), (2) habitat fragmentation and restriction of movements and gene flow, and (3) increased access to remote areas for illegal collection and vandalism of plants and animals (Boarman and Sazaki, 1996). However, none of the roads at our study site are paved and the combination of light traffic (public access is strongly restricted) and generally slow vehicle speeds minimize direct mortality.

Desert tortoises may construct burrows along the elevated berms of unpaved roads because the topography mimics that formed along the banks of desert washes, a preferred site for burrow construction (Luckenbach, 1982). Of the 207 burrows observed by Burge (1978) in large washes, 151 were located in banks with the remainder in the channel bed. She also noted that the elevated dirt berms along roads served as burrow sites for a small portion of her sample. Because desert tortoises appear to prefer the steeply eroded banks of washes for burrow sites in some areas, they may not discriminate between natural banks and the elevated berms associated with most unpaved roads in the desert.

Another explanation for why tortoise burrows at Mesa tend to be located closer to roads than are random points stems from the fact that plant productivity in the desert is often greater along roadsides. "Edge-enhancement" of perennial shrubs along the margin of roads is substantiated by past research in the Mojave Desert showing that plants along roadsides are denser, larger, more vigorous, and support greater numbers of foliage arthropods than those away from roadsides (Vasek et al., 1975; Lightfoot and Whitford, 1991). Primary productivity, as measured by standing crop, increased about 17 times on the basis of vegetated area alone, and 6 times when the area of the bare, paved road surface was included as part of the calculated area. Unpaved roads showed increases of 6 and 3 times, respectively, in each category (Johnson et al., 1975). The increase in vigor has been shown to attract herbivorous insects (Lightfoot and Whitford, 1991), so it is conceivable that the herbivorous desert tortoise selects burrows in close proximity to high densities of food plants as well. In Florida, gopher tortoise (*Gopherus polyphemus*) densities are positively correlated with the percent herbaceous cover, an indicator of food resources (Breininger et al., 1994).

Baxter (1988) found that high density plant ecotones were important determinants of desert tortoise abundance near Twentynine Palms, California, an area that is relatively close to our study site. The distribution of burrows observed by Baxter led him to conclude that desert tortoises are "edge" species. Again, desert tortoises may not discriminate between natural edges and those formed by roads. Similarly, Garner and Landers (1981) observed that roadsides and the edges of fields were common burrowing sites for *G. polyphemus* in Georgia. They also noted that vegetation in those areas generally contained more minerals than food plants on natural sand ridges.

Terrestrial desert chelonians sometimes include roads in their movement patterns. Nieuwolt (1996) observed that some individuals of *Terrapene ornata luteola* used roads to make most of their movements and that distances moved on roads were significantly greater than distances moved off-road. No explanation was offered for the observed difference but it seems logical that roads offer less impediments to terrestrial turtle movement than natural areas and thus facilitate faster transit rates. Desert tortoises sometime use washes and trails as "natural highways" according to Baxter (1988), and it is conceivable that unpaved roads would be used in a similar fashion.

While the scenario above might explain why desert tortoises construct their burrows next to roads it does not necessarily explain why so many locate their burrows under concrete pads. Desert tortoises often construct their burrows under caliche overhangs exposed in the banks of washes (Germano et al., 1994). Caliche overhangs are "hardpan" soil horizons of calcium carbonate crust that form in some desert areas. These layers cement the gravels and cobbles in the soil together, forming a matrix almost as hard as concrete. According to maps presented in a soil survey of Mesa (Soil Conservation Service, 1980), the Chuckwalla Series of soil is noncalcareous throughout and caliche layers do not occur at the locale. Desert tortoises at Mesa may take advantage of the concrete electrical transformer pads as a kind of "artificial caliche," and benefit from the roof stability that they confer. Alternatively, tortoises may associate with concrete because of its thermal inertia relative to soil. On several occasions we have observed desert tortoises "basking" on the pads on overcast mornings when the concrete was notably warmer to the touch than the surrounding soil surface.

The non-random distribution of burrows at Mesa demonstrates the importance of fine-scale habitat characteristics in modeling desert tortoise burrow locations. Some of the unexplained variation in our DFA is probably due to the fact that one tortoise may use more than one burrow over a short period of time. Burge (1978) observed tortoises using 12–25 cover sites per year, and Bulova (1994) found that desert tortoises in southern Nevada used 3–18 burrows during a five month study. At nearby Twentynine Palms, California, Duda et al. (1999) determined that the average number of burrows used per year ranged from 3.1–6.9, and differed among drought and wet years. Better models might be generated by considering individual variation in burrow use. Another source of unexplained variation may be the importance of larger-scale landscape features, as has been demonstrated for *G. polyphemus* burrow orientation by McCoy et al. (1993).

By now it is nearly axiomatic among conservation biologists working in the Mojave Desert that virtually any human alteration of habitat is deleterious to desert tortoise populations (Fish and Wildlife Service, 1994). The cumulative impacts of human activities on ecological patterns and processes in the California deserts are well documented (Lovich and Bainbridge, 1999), but still poorly understood in terms of the exact consequences to wildlife and the habitat on which they depend. While few would argue that outright habitat destruction is anathema to conservation of virtually all wild species, insufficient credible data are available to test the hypothesis that other forms of habitat alteration, or human presence, contributed to the purported decline of the desert tortoise.

While the potentially harmonious situation between desert tortoises and turbines at Mesa is more a result of serendipity than design, the results of our study suggest that certain forms of development may be compatible with conservation of species such as the desert tortoise. Our analysis suggests that the desert tortoise is more adaptable to certain anthropogenic changes in the environment than the above axiom suggests.

Most of the wind energy operations in the area prohibit or greatly restrict access by the public with locked gates, no trespassing signs, and barbed wire fences. This eliminates or greatly minimizes negative impacts associated with vandalism, illegal collection of plants and animals, off-highway vehicle use, and other human impacts (Fish and Wildlife Service, 1994; Brooks, 1999; Lovich and Bainbridge, 1999). In effect, the areas become preserves if they are large enough to meet the needs of the species living therein.

It is important to note that neutral or positive effects of wind energy development to charismatic or politically important species may not be shared by other species or their habitat. For example, wind energy development may cause increased avian mortality (Byrne, 1983; Musters et al., 1996) and increased erosion in hilly terrain (Wilshire and Prose, 1987). Therefore, we are not advocating the proliferation of wind energy development in habitats occupied by either the desert tortoise or other protected species, but rather suggest that by recognizing and planning for the needs of wildlife, the negative impacts of development can be lessened or perhaps even ameliorated.

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Ecology and Management of the Desert Tortoise (*Gopherus agassizii*) in California

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Abstract

The distribution and abundance of the desert tortoise (*Gopherus agassizii*) in California were determined from 137 surveys, each a square transect 1.6 km (1 mi) per side. Search was made for tortoises, burrows, scats, and other sign, and information was compiled for vegetation, soil type, and elevation. An index of relative density was combined with the broader habitat assessments to develop a map of the relative abundance of the tortoise in California. A distribution map is provided and a matrix delineates the range of environmental factors that affect the distribution and abundance of the tortoise in California. A review of the ecology of the species clarifies factors important to its survival. Land ownership patterns were identified and correlated with tortoise distribution to identify critical management areas. Information on the present and future status of the tortoise in California is discussed. Tortoise populations apparently are adversely affected by collection, roadkill, grazing, off-road vehicle use, and other human impact. Conservation efforts and management policies are reviewed and management needs listed.

A pronounced and steady decline in populations of the desert tortoise (*Gopherus agassizii*) in several parts of the southwestern United States has been noted for several years. Detrimental factors include encroachment by urbanization (Leach and Fisk 1969), collection by the pet trade and by individuals (G. R. Stewart, personal communication; Bury and Marlow 1973), highway mortality (Berry 1972, 1974*a*, 1975), overgrazing (Woodbury and Hardy 1948), maliciousness (Bury and Marlow 1973), and, most recently, off-road vehicle use (Bury et al. 1977; Luckenbach and Bury, in press). With increasing demands being made on the public lands of the California deserts, there was an apparent need for a better understanding of the status, distribution, habitat relations, and potential for management of the desert tortoise.

Objectives of this research were to: (1) assess the overall demographic status of the desert tortoise in California and, in particular, locate areas of high tortoise density; (2) study the natural history of desert tortoises and factors adversely affecting them; (3) evaluate tortoise habitat in terms of human disturbance; (4) determine ownership patterns of lands having tortoise populations; and (5) propose management programs to ensure the future of the tortoise and its desert home in California.

Materials and Methods

Procedures

To determine the abundance and status of the desert tortoise in California, I used a transect sampling procedure. I would drive or walk to a locality, stop and walk on a compass bearing for 1.6 km (1 mi), turn 90°, and continue to walk until a square transect 1.6 km on a side had been covered. I made 137 such transect surveys (a total of 880 km of walking observations) and made other casual surveys during related fieldwork. Surveys were conducted between June and October 1973, and in November 1974 and January 1975. Extensive observations were also made on habitat and other biota during the surveys. Between June and October 1973, I logged more than 14,000 km on the road and hiked 880 km. One helicopter and two fixed-wing flights supplemented the ground survey and provided additional data for mapping the extent of potential tortoise habitat.

Since the fieldwork was conducted during the late summer and early fall of 1973 and in the winter of 1974–75, peak above-ground activity was not encountered. Therefore, signs of tortoise rather than absolute tortoise numbers became important measures of tortoise abundance. Signs used were: (1) tortoise burrows, (2) scats, (3) shells, and (4) tracks. These measures of relative density were standardized by comparing the frequencies of these indicators from areas of known density with those from areas of unknown density.

The criteria used to determine the presence of desert tortoises were:

Burrow counts.—The relations between burrow numbers and tortoise numbers may vary greatly with geographic location (Auffenberg 1969) but is relatively consistent for some areas (see Auffenberg and Franz, this volume). Woodbury and Hardy (1948) report a ratio of 4:1 for summer burrows and winter dens. In California, a distinction between burrows and dens as defined by Woodbury and Hardy (1948) is difficult to make. A tortoise may use one burrow continuously for several weeks, then move and begin to use another (Marlow 1974). Alternatively, a tortoise may use several burrows within its home range. Further, some desert tortoises in California may spend the winter dormancy period in only a shallow burrow. I did not differentiate between summer burrows and winter dens—all were scored as burrows

In making burrow counts, I noted the size and shape of the burrow as well as activity indicators such as plastron-slide marks, the amount of debris at the mouth of the burrow, lack of spider webs or the crescent shape of existent webbing, and tracks. I used these criteria to determine whether a burrow was occupied and to estimate the approximate size of the animal using it. A flashlight or reflective mirror was often used to light the interior of burrows. Probing with a meter stick sometimes caused tortoises to stir and, occasionally, pounding on the ground at the entrance caused a tortoise to emerge. Because signs of burrow use are often lacking during the fall and winter, it is almost impossible to determine whether a burrow is occupied unless the animal is in sight. Locating burrows is crucial in the censusing process and careful, concentrated search not only of open ground but also around bushes is necessary to find all the burrows along a transect.

Tortoise burrows are distinctly crescent-shaped and slant downward at a slight angle. If the burrow entrance was small and crescent-shaped, but suddenly tapered to a rounded hole, it was assumed to be the burrow of a kangaroo rat (*Dipodomys* sp.). Ground squirrels (*Spermophilus* and *Ammospermophilus*) also construct round holes. Burrows constructed by juvenile tortoises are small and may resemble rodent or lizard burrows. Illumination of the burrow interior usually is needed for accurate identification of the animal that built it. The size of a tortoise burrow is related to the size of the animal using it. For example, an adult male with a carapace length of 25.1 cm (examined near Daggett, San Bernardino County, on 23 August 1973) occupied a burrow 38.5 cm wide and 17.5 cm high.

Scats.—The presence of scats confirms that a tortoise once used an area, and the size of the scat is an indicator of the size of the tortoise. A medium-sized female (129 mm carapace length) deposited scats that averaged 16.3 mm long (N=13). Tortoise scats are distinctive (Fig. 1), and with practice can be distinguished from the fecal material of any other desert vertebrate. They are cylindrical, often tapered to a point at one end, and composed largely of vegetable matter; some contain large amounts of sand and grit or consist entirely of sand. Recently deposited scats have a shiny, blackish coating; with exposure, this coating wears off and the scat disintegrates rapidly. Scats deposited in early spring disintegrate readily because they are composed of flowering stalks and other succulent portions of annuals. Summer food consists largely of the dry portions of annuals, especially grasses, and scats containing these persist for longer intervals. Predator scats were also examined to determine presence of tortoise remains.

Tortoise shells.—The presence of tortoise shells also indicates that tortoises live (or formerly lived) in an area. However, since shells may be transported by carnivores and humans, their presence in a particular locality does not necessarily mean that the tortoise succumbed at that site.

The deterioration of the shell gives some indication of time since the animal's death (Berry 1974*a*, 1975). The horny scutes may adhere tightly to the bony skeleton from 6 months to 1 year; over a period of 1-2 years, they gradually dry, lighten in color, and peel. The underlying bony structure of the shell then begins to disintegrate a process that may take from 2 to 5 years.

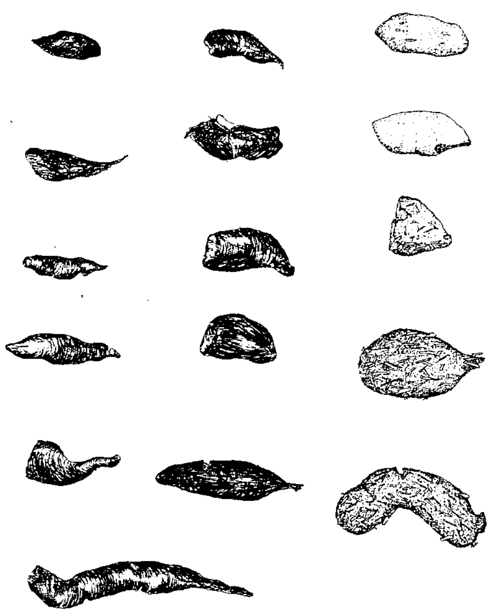

Fig. 1. Examples of desert tortoise scats from a series collected near Hinkley, San Bernardino County, California. In the right column, the top three consist of large amounts of sand and gravel, and the bottom two are relatively old scats that show weathering (from Luckenbach 1976).

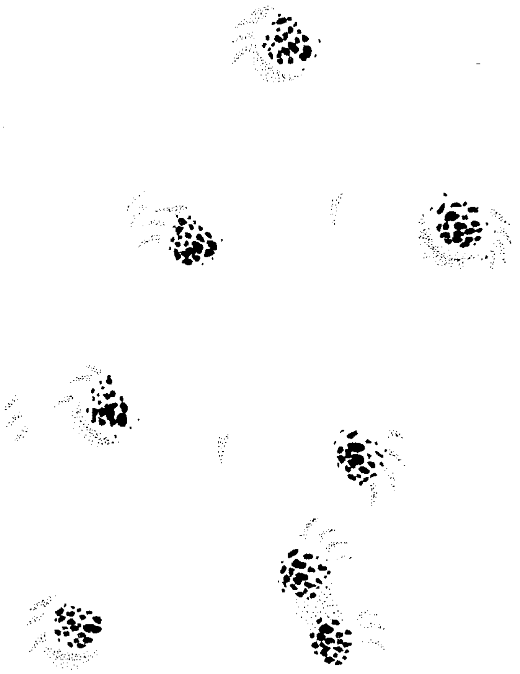

Fig. 2. Desert tortoise tracks made in aeolian sand. Tail drag between tracks probably indicates a male (from Luckenbach 1976).

Tortoise tracks—The use of tortoise tracks in estimating population size is of limited value. Tracks may be visible only in areas of sand or soft soil and may reflect only periods of above-ground activity. Careful tracking of individual tortoises may yield data on home range size, burrow usage, feeding habits, and reproductive state. Copulating animals may often leave a small, round depression as well as signs of recent urination. If tracks lead to a certain plant, close examination may reveal whether the plant has been eaten.

Figure 2 shows tracks made in aeolian sand near Hinkley, San Bernardino County. Note that the main track is left by the hind foot pad while the forelimbs left only a small depression; note also the tail drag, indicative of males.

Estimation of Population Density

Population density (individuals per unit area) is measured in many ways (for reviews see Southwood 1966; Lamotte and Bourliere 1969; and Seber 1973). The ideal is to measure absolute density (the total number of individuals within a defined area or community) by: (a) counting total population, (b) harvesting, and (c) estimating from survey techniques such as mark-recapture indices or intensive quadrat sampling. The method selected depends on the nature of the animal species and the length of time that can be devoted to the study.

Because of time constraints and the large size of the census area, I measured the relative density of tortoise populations. Determining relative density depends on collecting or measuring samples that represent a relatively constant but unknown relation to the population size. Although such an estimate is not a reliable measure of absolute density, it is a useful index for comparing populations.

Population estimates for the Desert Tortoise Reserve, Kern County, California, are given by Marlow (1974 and personal communication), and I determined the density of tortoises for an area north of Hinkley, San Bernardino County. Transect samples from these two areas yielded a baseline of known absolute density with which to

Table 1. *Comparison of survey data from the Desert Tortoise Reserve and Hinkley Site and from 137 transect surveys. N = number of transects assigned to a density category. Transects are listed in the Appendix. Values are* $\bar{x} \pm$ *S.D. (Range).*

		No. of tortoises		No. of burrows		Scats	Relative density[a] (km^2)
	N	Adult	Juv.	Active	Inactive		
Desert Tortoise Reserve	5	22.8±13.0 (8-43)	1.4±1.9 (0-4)	44.8±9.2 (36-60)	41.2±4.8 (36-48)	19.6±3.0 (16-24)	358
Hinkley	5	14.4 ± 3.4 (9-18)	0.8±0.8 (0-2)	26.0±7.6 (16-36)	18.8±4.4 (12-24)	22.8±9.6 (12-36)	217
High density	33	4.4 ± 3.0 (0-10)	0.8±1.0 (0-3)	30.7±7.8 (18-47)	28.3±6.0 (17-37)	11.9±7.7 (0-32)	157
Medium density	69	3.0 ± 2.8 (0-11)	0.7±1.0 (0-4)	13.2±5.6 (3-29)	8.8±5.2 (0-21)	6.0±5.1 (0-20)	79
Low density	35	1.0 ± 1.2 (0-4)	0.2±0.4 (0-1)	4.6±4.9 (0-19)	2.8±3.4 (0-11)	1.1±2.2 (0-7)	27

[a] Relative density is determined by the formula:
No. Ad. + No. Juv. + Active Burrows ÷ 2 = X (No. per 13 ha); X ÷ 0.13 = No. per km^2.

compare transect surveys (Table 1). The number of tortoises encountered during each transect was extrapolated to density estimates per square kilometer. The ratio of active burrows to tortoises on the Desert Tortoise Reserve is about 2:1 (R. W. Marlow, personal communication). Assuming a ratio of 2 burrows per tortoise, I estimated tortoise numbers by burrow counts and used this ratio throughout my census.

The survey areas were transects 6.4 km long and covered a width of 20 m (10 m on each side of the path). Burrows were often difficult to detect beyond this limit since they were easily obscured by bushes. The total area covered on each line transect (20 m x 6.4 km) was 13 ha. I extrapolated the counts from each survey to densities per square kilometer. These estimates should be recognized as only approximations of population size.

Data from ground and air surveys (1973 and 1974-75) were combined to develop density patterns over the California desert. Distinctions are made for high, medium, and low population levels. High population densities are considered to be more than 155 tortoises/km^2, medium to be 40-155/km^2, and low to be less than 40/km^2. Density patterns are based on a combination of factors: evidence of tortoise presence from surveys, habitat condition, and degree of habitat disturbance.

Extent of Survey

The survey encompassed the Mojave Desert and Colorado Desert of California. The principal geographic and land features of this region are provided in Fig. 3; my field survey sites are shown in Fig. 4.

Coverage of the California deserts varied. In the northwest Mojave Desert, extensive surveys were made of areas along State Highways 58, 14, and 395 and along dirt roads in the El Paso and Rainbow Basin Mountains and in the Harper and Water Valleys. Limited reconnaissance was made along the eastern flanks of the Sierra Nevada to determine elevational limits of tortoises in the foothills. Searles Valley was poorly surveyed, and Panamint Valley was not surveyed at all. Because of the large number of privately owned land parcels and housing developments, the area of Antelope Valley and Victorville was surveyed only briefly. In the central Mojave Desert, a careful survey was made in the area north of Hinkley and in the area southeast of Barstow—the Stoddard Valley; the Ord, Rodman, and Newberry Mountains; and the eastern end of Lucerne Valley. The alluvial flanks of both the Cady and Bristol Mountains were also thoroughly checked. A reconnaissance was done in the Yucca-Twentynine

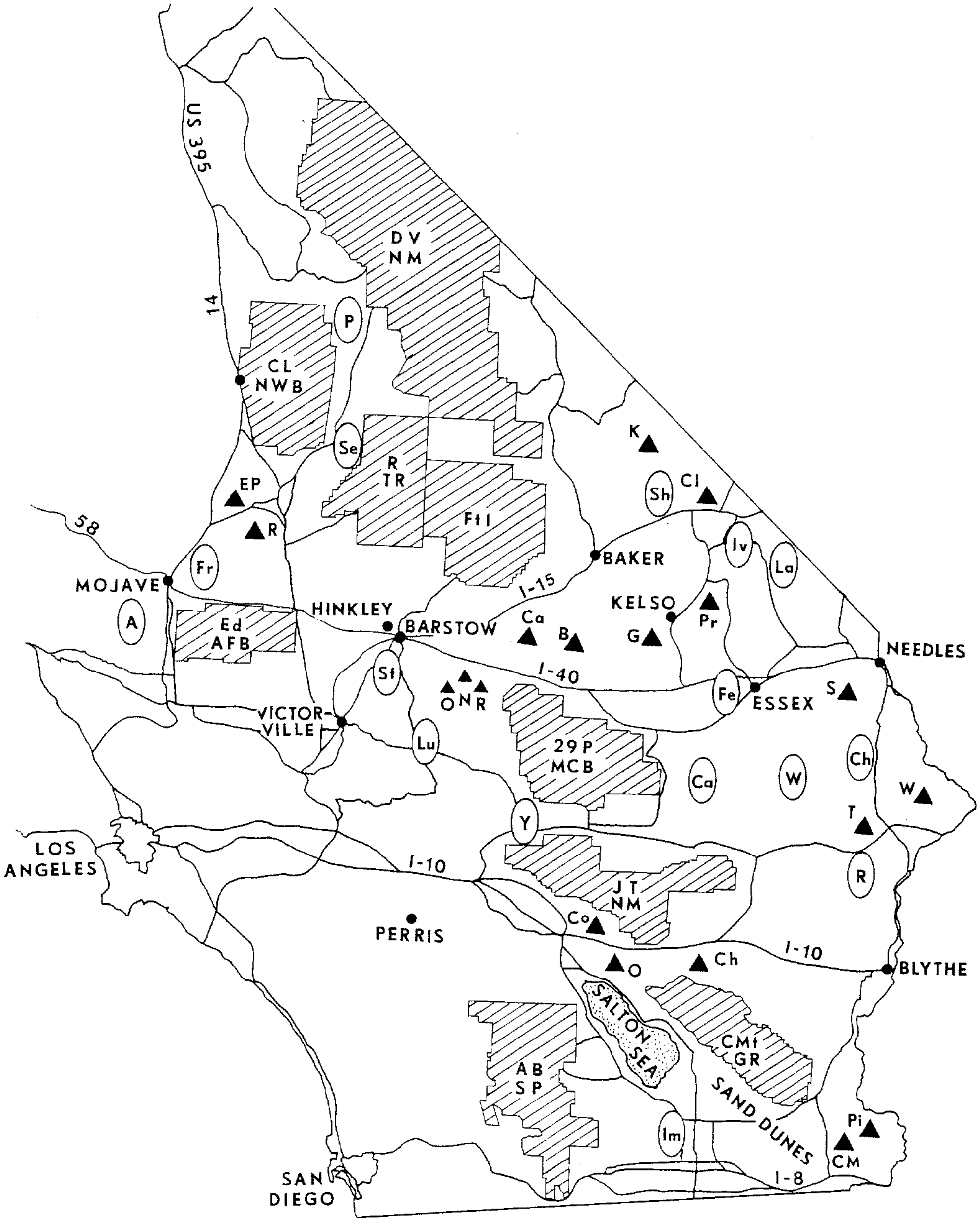

Fig. 3. Major geographic features of the California Desert that are mentioned in the text.

Abbreviations of military and park lands are: Death Valley National Monument; Joshua Tree National Monument; Anza Borrego State Park; China Lake Naval Weapons Base; Randsburg Test Range; Fort Irwin; Edwards Air Force Base; Twentynine Palms Marine Corps Base; Chocolate Mountains Gunnery Range.

Major mountain ranges are indicated by triangles: B—Bristol; Ca—Cady; Ch—Chuckwalla; Cl—Clark; Co—Cottonwood; CM—Cargo Muchacho; EP—El Paso; G—Granite; K—Kingston; O—Orocopia; Pi—Picacho; Pr—Providence; R—Rand; S—Sacramento; T—Turtle; W—Whipple.

Major valleys are enclosed in ellipses: A—Antelope; Ca—Cadiz; Ch—Chemehuevi; Fe—Fenner; Fr—Fremont; Im—Imperial; Iv—Ivanpah; La—Lanfair; Lu—Lucerne; P—Panamint; R—Rice; Se—Searles; Sh—Shadow; St—Stoddard; W—Ward; Y—Yucca.

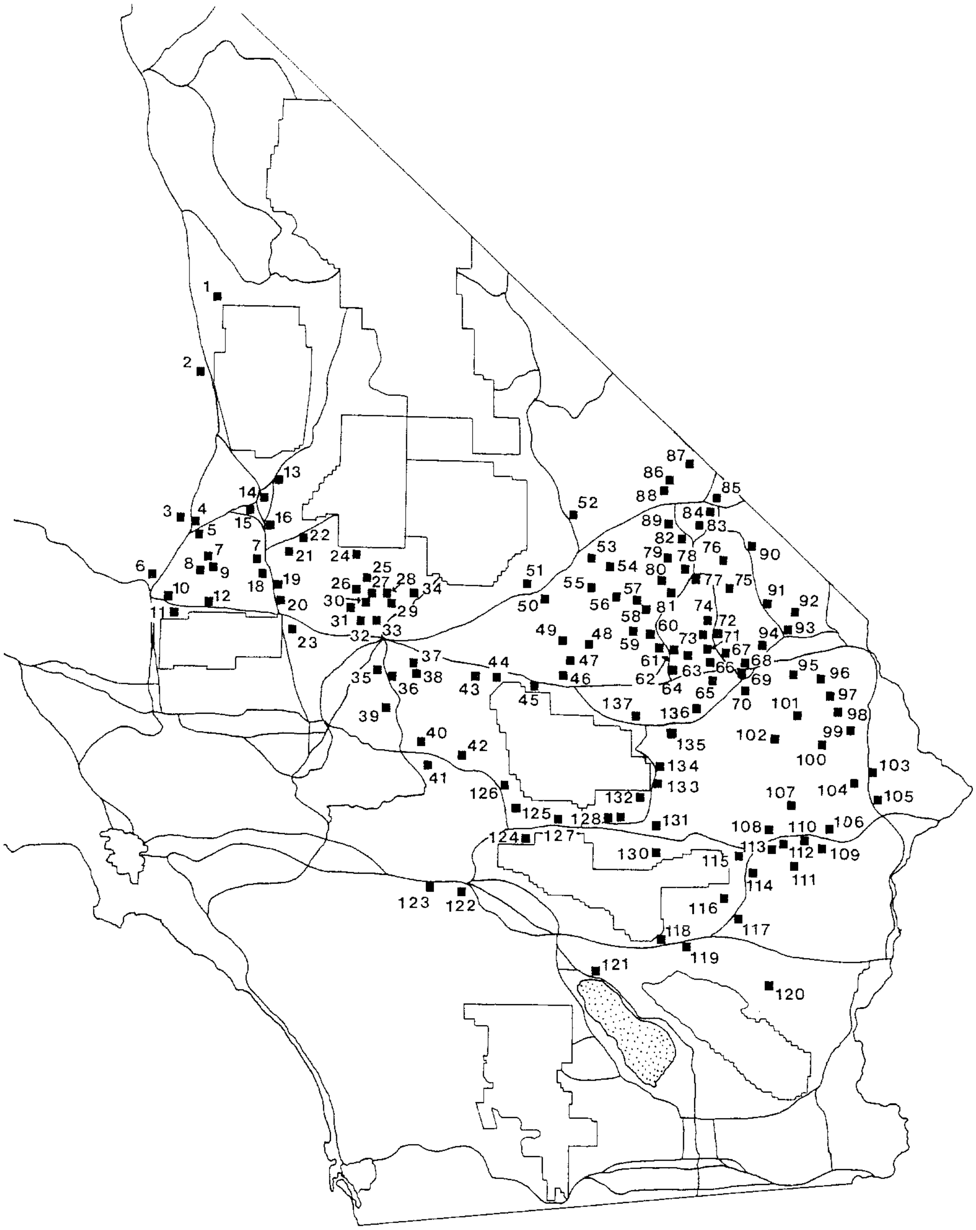

Fig. 4. Field survey sites in California. Numbers refer to transects listed in the Appendix.

Palms area and in the Mojave Valley. Joshua Tree National Monument was excluded from this study because captives have been repeatedly introduced, and animals there are already protected. In the eastern Mojave Desert, the area south of Interstate 15 (in particular, the Cima-Providence Mountains-Essex region) was surveyed intensively. The valley north of Baker, the upper half of Shadow Valley, and Mesquite Valley were surveyed to a lesser extent. The area north of the Avawatz and Shadow Mountains and the Kingston Range was visited briefly. South of the Providence Mountains, field surveys were concentrated in the Fenner, Ward, and Chemehuevi Valleys. The area adjacent to the Colorado River, south of Needles, was not surveyed. In the Colorado Desert, the survey was confined to areas east of the Salton-Imperial Valley trough. Palen Valley was the region most thoroughly surveyed. Most of the transects and field reconnaissance work in this area was conducted during August and September 1973, when extreme heat made fieldwork difficult. The Colorado-Sonoran portion of the California deserts thus received only cursory coverage, and the Cargo Muchacho and Picacho Mountains were not surveyed.

China Lake Naval Ordnance Test Station, Fort Irwin, Edwards Air Force Base, Twentynine Palms Marine Corps Training Center, and the Chocolate Mountains bombing range were excluded from this study for logistic reasons.

Land ownership patterns of the desert lands surveyed were also determined. Basic information was obtained from the Bureau of Land Management's (BLM) Resource and Recreation maps (Nos. 8-11, 16; scale 1:250,000; available from the Bureau of Land Management, 2800 Cottage Way, Rm. E-2841, Sacramento, California 95825) and from county records.

Distribution of *Gopherus agassizii* in California

During the surveys, vegetation, soil type, and elevation were recorded. Tortoise presence was then correlated with these environmental factors to provide an environmental matrix for the desert tortoise (Fig. 5).

Precipitation was estimated from elevation and the nature of the plant community. Estimation of the annual bloom potential was subjective, although the criteria were based in part on the amount of remnant annual growth plus elevation and soil type. Perennial diversity was derived by comparison of the number of different perennial species present in survey areas. Vegetation, soil, and elevation in relation to tortoise distribution and abundance are discussed in the following sections.

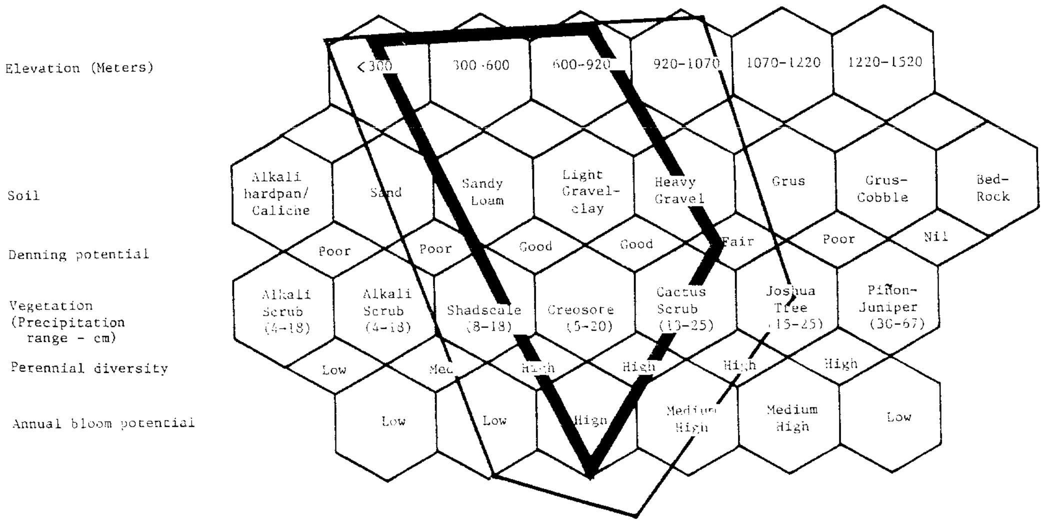

Fig. 5. Environmental matrix for the Desert tortoise in California. Thick line indicates the preferred range, thin line the estimated potential range.

Vegetation

Discussion of the distribution of tortoises in California in relation to vegetation necessitates assessment of the vegetational communities of the California desert. Recent reviews of the desert vegetation of California can be found in Johnson (1976) and Barbour and Major (1977). Following the community structure of Munz and Keck (1959), four scrub communities (alkali, creosote, shadscale, and sagebrush) and two woodland communities (Joshua tree and pinon-juniper) are recognized in the California deserts.

Creosote bush (*Larrea tridentata*) is the dominant plant and forms the major community on well-drained sandy flats, bajadas, and upland alluvial slopes throughout both the Mojave and Colorado-Sonoran Deserts. Commonly, *Larrea* forms associations with other perennials. Beatley (1969) recognized six *Larrea*-dominated associations in southern Nevada. I have discerned seven such associations in the Providence Mountains region in the eastern Mojave Desert of California. The creosote-burroweed association (*Larrea tridentata-Ambrosia dumosa*) is the most characteristic and constitutes as much as 70% of the Mojave Desert (Shreve 1942).

Low-lying areas of bolsons and valleys characterized by saline soils display alkali scrub communities. The important components consist of halophytic species such as saltbush (*Atriplex* sp.) and succulent chenopods of the genera *Allenrolfea, Salicornia, Suaeda,* and *Sarcobatus.*

At slightly higher (1,200-1,800 m) and sometimes at drier sites than those where creosote scrub predominate, vegetation usually consists of low shrubs broadly classified as shadscale scrub. In the northern Mojave, where contact with the Great Basin Desert occurs, shadscale scrub composed chiefly of shadscale (*Atriplex confertifolia*) and sagebrush (*Artemisia spinescens*) is distinct from creosote scrub and sagebrush scrub. Over most of the Mojave Desert, shadscale scrub is dominated by blackbrush (*Coleogyne ramosissima*) in conjunction with various species of yucca (*Yucca* spp.), Mormon tea (*Ephedra* spp.), and cholla (*Opuntia* spp.).

Sagebrush scrub is the typical Great Basin association and is dominated by the Great Basin sage (*Artemisia tridentata*) and bitterbrush (*Purshia tridentata*). It is common at higher and colder sites than are typical of the Mojave Desert. Sagebrush scrub is largely restricted to the eastern highland areas in the California Desert.

The most conspicuous plant of the Mojave Desert is the endemic Joshua tree (*Yucca brevifolia*), which forms a characteristic woodland on well-drained soils in areas of moderate rainfall (more than 24 cm per year). Joshua trees form overstories with many scrub associations; at higher elevations, they grade into juniper (*Juniperus californica* or *J. osteosperma*) or pinon woodland (*Pinus monophylla*).

Vegetational patterns are more diverse than can be described in a single community framework. Ecotones, gradations, and combinations of communities are common. Creosote can form associations with any one of the other scrub or woodland communities. Other communities may be limited in area but locally important. Although not recognized as a separate community, cactus scrub is important in both the Mojave and Colorado-Sonoran Deserts. In the Mojave, it most commonly consists of a jumping cholla (*Opuntia bigelovii*) and burroweed (*Ambrosia dumosa*) association. It is present in the Sacramento and Woods Mountains, and in Ward Valley, San Bernardino County. In the Colorado-Sonoran Desert, cholla (*Opuntia*), cacti (*Echinocactus, Echinocereus*), and other stem succulents comprise the cactus scrub.

Margins of arroyos often support distinctive plant associations. I discerned at least six wash associations in the region of the Providence Mountains. In the Colorado Sonoran Desert, wash vegetation often forms dense thickets that can be considered a wash woodland community.

Thorne (1976) lists four other groups as separate recognizable vegetative communities: desert rock plants, desert dune sand, semisucculent scrub, and desert microphyll woodland.

In the California deserts, *G. agassizii* mostly frequents four communities: creosote scrub, cactus scrub, shadscale scrub, and Joshua tree woodland (Fig. 5). Sometimes tortoises occur in alkali scrub.

Soils, Topography, and Flora

Soil type is an important limiting factor for tortoises. Probably no type is preferred, but the soil must be friable enough for the digging of burrows and firm enough so that burrows will not collapse. In the areas of high tortoise population density, soil type varies from gravelly flats in Stoddard Valley to sandy soil with some clay content in the Fremont Valley, and to fine, windblown sand and stabilized dunes near Hinkley and in the Pinto Basin of Joshua Tree National

Monument. Tortoises, however, are absent or scarce in most other sandy areas such as the Algodones and Kelso Dunes.

Creosote scrub occurs on a range of soils from cobble or desert pavement to sand. However, it is often excluded from fine-textured basin soils of high salinity and low oxygen content (Lunt et al. 1973). These soil characteristics negatively affect the growth of annual vegetation and thus ultimately lower the carrying capacity of alkali scrub for tortoise populations.

Desert tortoises may be found in rocky areas. In the Mojave Desert, cactus scrub is often best developed on rocky substrate such as dissected rocky benches; tortoises frequent such areas. In the Colorado Sonoran Desert, cactus scrub seems to occur on south-facing slopes or fine-grained soils. Tortoises are less common here than in adjacent creosote scrub or wash woodland areas. The upper parts of bajadas and alluvial fans are generally too rocky for burrow construction.

Pinyon-juniper woodland is found in the western Mojave Desert above 1,200 m on the flanks of the Sierra Nevada and San Bernardino Mountains or on isolated ranges. Below this rim, Joshua tree woodland occurs in areas of moderate rainfall or intermediate elevation (760-1,200 m). Soils of this Joshua tree woodland vary from granitic gravels along the Sierra Nevada piedmont to sandy soils in the Antelope, Yucca, Fremont, and Harper Valleys. Below 1,000 m on well-drained alluvial soils, the vegetation is dominated by creosote scrub. The spring aspect is one of green shrubs and showy spring flowers; the late summer aspect is of dull-colored shrubs and scattered dry bunchgrass (*Oryzopsis hymenoides, Hilaria rigida, Schismus barbatus, Stipa speciosa*). Poorly drained soils often have caliche layers and are characterized by the presence of shadscale scrub. Playa and alkali flats display elements of alkali scrub or are devoid of vegetation.

In the western Mojave Desert, the greatest density of free-living desert tortoises found thus far occurs in creosote scrub in the Fremont Valley (Fig. 6), an area of relatively uniform creosote habitat with light gravel to sandy soil. Tortoises are found also in Joshua tree woodland, but the shrub story in most of the western Mojave is a dense

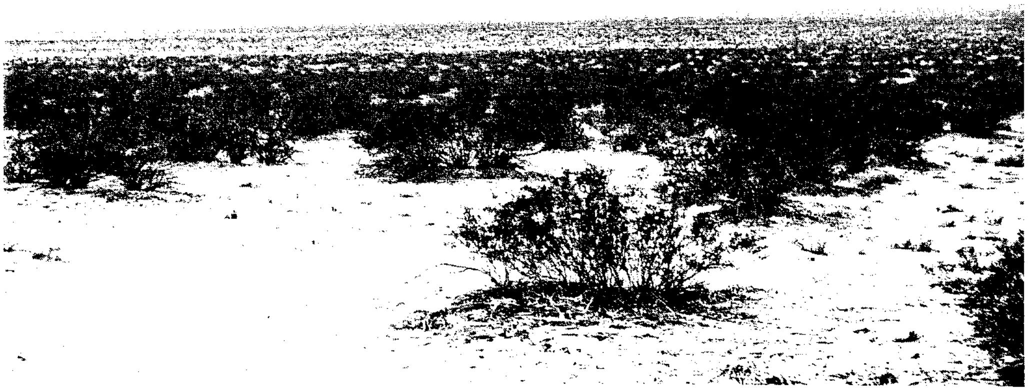

Fig. 6. Area of high tortoise population density in western Mojave Desert. Creosote-burroweed association in the Desert Tortoise Reserve, Kern County, California.

Fig. 7. High tortoise density area north of Hinkley, San Bernardino County, California. Joshua tree and creosote-burroweed associations. Soils here are sandy.

creosote association. Tortoises are also found in moderate numbers in areas of alkali scrub habitat. Around Koehn Dry Lake, tortoises were found in an area of stabilized sand hummocks with mesquite (*Prosopis juliflora*) and saltbush (*Atriplex* spp.). In the area south and west of Fremont Peak and towards Kramer Junction and near Hinkley (Fig. 7), an area with extensive stands of saltbush (*Atriplex confertifolia* and *A. polycarpa*) in mostly sandy soils, tortoises are common but less so than on adjacent creosote shrub habitats.

Topography of the eastern Mojave Desert is varied, with elevations above 2,100 m in the Providence Mountains and Kingston Range, and more than 2,400 m in the Clark Mountains. Physiographically, this area is an extension of the Basin and Range Province; the basins are seldom lower than 760 m, and the area is typified by great relief. Several vegetational communities may be encountered within short distances. Like the western Mojave, most of the eastern Mojave Desert is considerably higher than the Colorado Desert.

Climatic diversity is high in the eastern Mojave Desert, and much of the area adjacent to the Colorado River often experiences late summer thunderstorm activity. These storms are generated by easterly airflow from the south Atlantic Ocean, which crosses the Gulf of Mexico and continues across Texas, New Mexico, and Arizona. Partly dissipated cells of low air pressure linger over the Colorado River and are "revitalized" by moisture-laden convective air. Periodic strengthening of easterly airflow patterns causes these cells to move westward, and convectively- and orographically-induced precipitation results. Such thunderstorms may be intense, though brief and highly localized; flash flooding frequently accompanies them. Some eastern Mojave areas receive two peaks of annual precipitation—the normal winter peak and variable summer rains. This bimodality of rainfall resembles that of the Arizona-Sonoran Desert. Low-lying areas immediately west of the Colorado River from about Needles south to the vicinity of Blythe can be considered as a portion of the Arizona-Sonoran Desert. This bimodal rainfall pattern has resulted in diverse vegetational communities. Annual plants respond with two flowering peaks, one in the spring and the other in the fall.

Biotically, the Providence-New York-Granite Mountains represent a meeting of four deserts of the Southwest: the Great Basin, Mojave, Colorado-Sonoran, and Arizona-Sonoran Deserts. This confluence is reflected by the great diversity of the regional flora and fauna. Piñon-juniper woodland with Great Basin sage (*Artemisia tridentata*) are found between 1,070 and 2,100 m. Two piñon pines (*Pinus edulis* and *P. monophylla*) occur together in the Ivanpah Range. At mid-elevations, elements of the Arizona-Sonoran Desert finger into the Providence Mountains and other ranges bordering the Colorado River. The northernmost limit of the Colorado-Sonoran Desert is found here. Ocotillo (*Fouquieria splendens*) and ironwood (*Olneya tesota*) are found in washes at lower elevations. Typical Mojavean vegetation is dominant on level areas at lower elevations. The finest stands of Joshua trees in the Southwest occur in the Lanfair Valley and Cima Dome area; these trees are the short-leafed variety (*Yucca brevifolia* var. *Jaegeriana*).

Rocky areas at elevations from 900 to 1,200 m are dominated by succulent vegetation. Chollas in Woods Mountains and in the upper Ward Valley (Camino Valley) develop into large gardens. Barrel cactus (*Echinocactus acanthodes*) is limited to an altitudinal belt of 900-1,500 m. Mojave yucca (*Yucca schidigera*) is also distinctive in this belt, although it also extends to lower elevations. Below 850 m, the predominant vegetation is creosote scrub. Along the south flank of the Providence Mountains, creosote scrub yields to a mixed buckwheat scrub community, and washes are numerous. Alkali scrub is typical around the numerous dry lake beds of the area.

The large Dumont and Kelso dune systems and a minor crescent system south of Soda Lake bed are adjacent to Providence Mountains. Other areas of aeolian sand deposition include southern Cadiz Valley, the Dale area, lower Ward Valley, and the Rice Valley.

Desert tortoises in the eastern Mojave Desert occur predominantly in creosote scrub, cactus scrub, and Joshua tree woodland and, sparsely, in shadscale scrub. On the lower slopes of bajadas, tortoises are commonly found in wash habitats.

The Colorado-Sonoran Desert is warmer than the Mojave and has basins lower in elevation than those of the Mojave. Yearly precipitation here is also bimodal. Dominant plant cover consists of the *Larrea-Ambrosia* association in conjunction with brittlebush (*Encelia farinosa*) and ocotillo. Bushes are widely spaced, and the aspect is desolate. Creosote scrub is best developed on coarse, well-drained soils with low salinity, whereas alkali shrub occurs in heavier soils and in salt-laden areas. Development of arborescent species is pronounced in the Colorado-Sonoran Desert, resulting in varied wash associations, and detrital fan aprons are incised by numerous sandy washes. The bases of the Cottonwood, Eagle, and Chocolate Mountains are etched by dendritic arroyos, where the vegetative cover is primarily an arborescent wash woodland. Smoke tree (*Dalea spinosa*), Palo verde (*Cercidium floridum*), ironwood, and desert willow (*Chilopsis linearis*) are common in these washes.

Between Yuma, Arizona, and the Imperial Valley of California is a north-south series of large sand dunes — the Algodones Dunes. Creosote bushes and the shrubby buckwheat (*Eriogonum deserticola*) attain large size on the lower slopes of these dunes. East and north of the Salton Sea are isolated oases of fan palm (*Washingtonia filifera*). Similarly, isolated stands of saguaros (*Cereus giganteus*) occur on the eastern end of the Little Chuckwalla and Whipple Mountains.

Tortoises are mostly uncommon in the Colorado-Sonoran Desert. Where they occur, vegetation consists of creosote scrub or wash woodland associations. In the Palen and Chuckwalla Valleys and on the Palo Verde Mesa, tortoises occur in creosote scrub and occasionally in cactus scrub.

On the western and eastern flanks of the Chocolate and Cottonwood Mountains, tortoises are found in association with wash habitats. No tortoises were found in the sandy areas of the Algodones Dunes or in the dense creosote scrub of the East Mesa (east of the Imperial Valley). Recently, Dimmitt (1977) found few tortoises in the Algodones region but located moderately dense populations (active burrows 54-93/km^2) in washes and bajadas of the Cottonwood Mountains and Chuckwalla Bench (northeast of the Salton Sea).

The abundance and diversity of perennial vegetation apparently indicate the overall potential of a habitat to support tortoise populations. Diverse and lush shrub growth is only possible where rainfall is sufficient. Rainfall also governs the potential for blooms of the ephemeral plants (annuals and some herbaceous perennials) which are the prime forage for tortoises. Biomass of ephemerals is apparently critical as the food supply for California tortoises.

Elevation

Although chiefly an inhabitant of the Lower Sonoran Life Zone, the desert tortoise occasionally ranges into the Upper Sonoran. Tortoises are known from elevations ranging from below sea level in Death Valley to 2,256 m in the Clark Mountains (Johnson et al. 1948). The Clark Mountain record was a shell that may have been carried to this altitude. Recently, two live individuals were found in Wildrose Canyon, Death Valley National Monument, at elevations of 1,280 and 2,225 m (P. Sanchez, personal communication). I have found tortoise burrows at 1,463 m in Cedar Canyon in the Providence Mountains and at 1,158 m near the Woods Mountains. Tortoises seldom range above 915 m along the flank of the Sierra Nevada or San Bernardino Mountains and only infrequently occur above 1,000 m throughout the western Mojave Desert.

Tortoise burrows were found in the Kelso Valley, Kern County (Kelso Valley is only a few kilometers from the Kern River drainage). Given suitable environmental conditions, a tortoise could follow the dry bed of the Kern River into the San Joaquin Valley. Such movement may have been responsible for its presence during the Pleistocene at McKittrick in the San Joaquin Valley (Miller 1932).

Distributional Limits

The distribution of *G. agassizii* in California shown in Fig. 8 is based on animals encountered in my field surveys and on museum records. The large number of pets released in California complicated the determination of the natural range. For example, the tortoise pictured by Carr (1952) from Perris, Riverside County, was probably a pet (J. St. Amant, personal communication).

Numerous tortoise breeders defend their practice on the basis that they release a portion of the young that are hatched. The Antelope Valley is near the Los Angeles metropolitan area and has long been a release site for liberated pets. Some 300 were released in Saddlebag Butte State Park in the Antelope Valley in 1975–76. Between 1971 and 1972, 65 tortoises were released in the Vallecito Mountains within Anza Borrego Desert State Park, which is an area outside the natural range of *G. agassizii*. Some reproduction of these animals has been reported (M. Getty, personal communication).

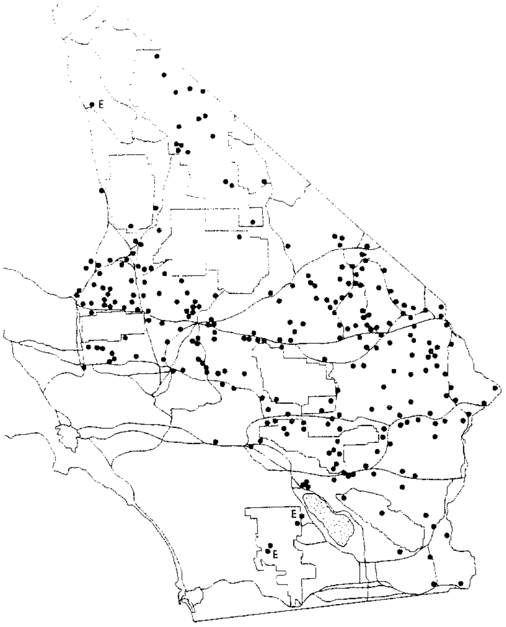

Fig. 8. Distribution of *Gopherus agassizii* in California. E = Extralimital localities.

Results of Field Survey

Western Mojave Desert

The highest known population density of desert tortoises in California is in the Fremont Valley (Table 1). Tortoise population densities determined by R. W. Marlow (personal communication) on plots adjacent to the Desert Tortoise Reserve north of California City ranged from 347 to 540/km^2. This is an area of diverse perennial scrublands, relatively high rainfall (15 cm/year or more), and excellent ephemeral blooms. Soils are broadly red deserts, predominately sandy loams. Similar population densities appear to continue in a belt from the Fremont Valley and just north of State Highway 58 along the southern flanks of the Rand Mountains to the southern portion of Fremont Peak, then numbers decrease north of Harper Lake and south of the Rainbow Basin–Opal Mountain area (Fig. 9). Vegetation in this area is predominately shrubs and scattered stands of Joshua trees.

Areas of wind-blown sand are found east of the town of Mojave, to the northeast of Harper Lake, and at the east end of Koehn Lake. Although subject to deflation, these sand areas support relatively high tortoise populations. Some tortoises occur in the Pinto Basin (Joshua Tree National Monument) and Dale Dry Lake in the central Mojave Desert. Elsewhere in the Mojave and Colorado Deserts, sandy areas seem to lack viable tortoise populations.

The second highest known density of tortoise populations is in an area north of Hinkley. This area is largely wind-blown and stabilized sand on top of tertiary lava flows, with scattered Joshua trees and numerous washes. Estimates made from mark-recapture data compiled by D. and N. Shade (of Twentynine Palms) and from my surveys indicate a population of 116-193/km². The Barstow Unified School District's Desert Research Station (5 km north of Hinkley) has a known population of about 77/km² on a more rocky substrate (L. Hunter, personal communication).

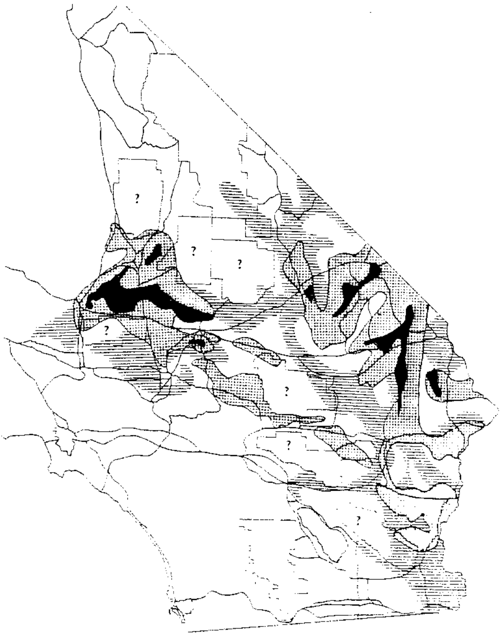

Fig. 9. Relative densities of *Gopherus agassizii* in California. Dark areas indicate high densities; cross-hatching, medium densities; horizontal lines, low densities; and question marks, unknown areas (mostly on military bases). See text for explanation.

In the past, high densities of *G. agassizii* undoubtedly extended from the Fremont Valley into the Antelope Valley. These valleys are similar in that they receive large amounts of winter rainfall, have diverse perennial vegetation (Antelope Valley is mostly Joshua tree woodland), and are noted for exceptional spring ephemeral blooms. Homesteading, subdivisions, and other human pressures have greatly reduced the tortoise population in the Antelope Valley. Although populations survive in small parcels of habitat, they are apparently declining. If proposals for a giant jet airport in the Antelope Valley materialize, the impact on the remaining desert environment in this area could be catastrophic. East of the town of Mojave, improvement of Highway 58 into an interstate highway necessitated a relocation of some tortoises, but the project had only limited success. The relocation effort demonstrated some homing abilities in desert tortoises (Berry 1974*a,b*).

Other known high-density populations occur west of Hinkley Valley, in the Stoddard and Upper Lucerne valleys, and in the vicinity of Fry Mountain. Isolated populations occur near Helendale, Victorville, and Mojave Valley; all are areas rapidly being developed for housing tracts. Natural habitat is poor in the Cady and Bristol Mountains. Resident populations have been all but eliminated from Lucerne Valley and the area of Yucca Valley and Twentynine Palms, Victorville, and portions of the Antelope Valley.

Eastern Mojave Desert

Tortoise habitat in the eastern Mojave Desert often was found in areas of extensive desert pavement with washes and a vegetational aspect of widely scattered cacti and creosote shrubs on the flats with smoke trees and mesquites in the washes.

Tortoise population densities were high in the Ivanpah Valley, on the periphery of Cima Dome and into Shadow Valley, along the tributary washes of Kelso Wash, and along alluvial slopes southeast of Baker. Banks and berms of washes are preferred places for burrows; such denning along washes, however, frequently results in significant mortality during flash floods. In July 1971, I followed the activity of tortoises in a wash north of Essex. Fifteen adult tortoises along 0.2 km of wash were drowned by flash flooding resulting from a downpour over uplands several kilometers distant. Subsequent reconnaissance of the area revealed that all burrows had been destroyed except one,

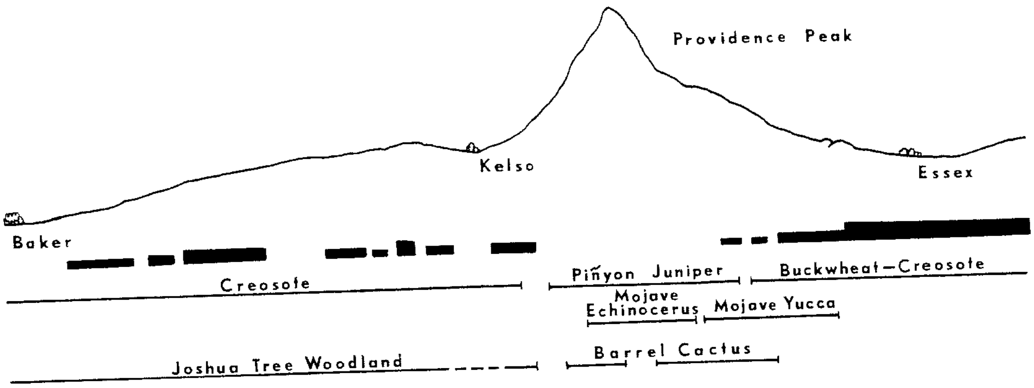

Fig. 10. Ecological transect of the Providence Mountains, San Bernardino County. Thickness of bars indicates relative tortoise densities encountered.

which contained three adult females. All the tortoises had been using shallow burrows in the banks of the wash.

A generalized transect across the Providence Mountains (Fig. 10) depicts the occurrence of tortoises in relation to elevation and vegetation in this region. The northern flank of the Providence Mountains supports higher tortoise population densities than do the southern flanks. The alluvium of the northern flank is sandy, whereas the southern flanks are covered by rocky aprons. The sandy areas around the nearby Kelso Dunes are surprisingly depauperate of tortoises. Considering the high-density areas near Hinkley (in the western Mojave) in similar habitat, this is an anomalous situation. A partial explanation may be the removal of tortoises by humans, because the area has long been a popular recreational site with easy access. Some mining activity in the area now threatens to expand to other portions of the dunes. However, even in areas of the dunes that have received little abuse, population densities of tortoises are low.

A similarly anomalous situation occurs in the Joshua tree woodlands in Lanfair Valley and on Cima Dome. Here the soils are composed of grus (derived from weathered granites) and can be excavated easily. Vegetation is diverse, and precipitation amounts to about 25 cm a year. Yet tortoise population densities appear to be lower than those at lower elevations in creosote scrub. Summer temperatures in Joshua tree woodland range from 2° to 3°C cooler than lower scrub areas, but whether this temperature difference is a limiting factor for tortoises is not known. Cattle grazing, which is concentrated in the higher regions (Joshua tree woodland), may affect tortoises. A comparative study of plots in low creosote scrub and in high desert should clarify these questions.

The highest tortoise densities I found in the eastern Mojave Desert were in the Fenner Valley, upper Ward Valley (Camino Valley), and portions of the Chemehuevi Valley (Figs. 3 & 9). All have friable substrates and are at relatively low elevations. Fenner Valley has sandy soils but displays little diversity of perennial scrub. Tortoises in this valley mostly frequent washes. In the other valleys substrates are either sandy or grus-like, and the diversity of the perennial scrub is great. The west side of the upper Ward Valley has a good admixture of perennial shrubs and is an area of extensive spring annual and fall grass development. The east side, along the alluvial apron of the Sacramento Mountains, is rocky and exhibits little scrub diversity. Interfluves are well-varnished pavements. Similar areas are found throughout the Chemehuevi Valley. Scattered in the Chemehuevi Valley, however, are pockets of friable sandy loams with good scrub growth that support high tortoise population densities.

Communications with local residents and the presence of abandoned burrows and shells indicate that some areas formerly supported large tortoise populations: the sandy area northeast of Twentynine Palms, the lower Ward Valley, and Rice Valley. Twentynine Palms has grown rapidly since the homesteading activities of the 1950's. Numerous "jackrabbit homesteads" dot the landscape. Roads, brush removal, off-road vehicle (ORV) use, and other human pressures apparently have drastically reduced the number of tortoises in this area. Rice Valley was the location of General Patton's World War II field maneuvers. More than 100,000 men were stationed there during the war years and extensive armored vehicle maneuvers were conducted, probably reducing tortoise numbers during this period. Tank and other vehicle tracks can still be found in Rice Valley, indicating the duration of ORV damage. North of Essex a similar military training center existed during the war years; however, since this was an infantry division, the overall impact was far less than that resulting from armored vehicle use in Rice Valley. Mining developments at Iron Mountain perpetuate human pressure in the lower Ward Valley.

During 1965, extensive war games were conducted over most of the eastern Mojave Desert in operation "Desert Strike." Thousands of men and hundreds of vehicles were involved. Tank pits, trash, and unexploded ordnance are still prominent in the landscape, and the activities of the military personnel no doubt had a serious and detrimental effect on the natural environment. Essex area residents reported that many dead tortoises were found after these maneuvers. Remarkably, in the western Fenner Valley, tortoises survived both the World War II maneuvers and the "Desert Strike" and have maintained healthy populations.

Construction and maintenance of railroad, highway, aqueduct, gas-line, and power-line corridors have repeatedly brought humans into contact with tortoises. Although cattle ranching is largely confined to higher elevations, it presents competition to tortoises in the Fenner and Granite Mountains. Feral burros are numerous in the Providence and Whipple Mountains (California DFG 1972). I found several tortoise burrows crushed by burro activities in the Woods Mountains. Burros compete with tortoises for forage, and during spring ephemeral blooms, burros forage in the Fenner and other valleys, denuding areas of vital tortoise food.

Children who lived in the small towns along Highways 66 and 95 once commonly collected tortoises to sell to tourists. Railroad section hands also transported tortoises found in the desert to stations in Needles and points east for promotional purposes or for sale to train passengers. Camp (1916) said of tortoises that "old ones are a favorite delicacy among Indians and Mexican section-hands who live with their families along the railroad lines." Most such encampments have now been abandoned. Further, the small towns along old Highway 66 became ghost towns after nearby Interstate 40 was completed in 1973. Pressure from local inhabitants has thus decreased because the human population of the area has recently declined.

The elevation of much of the eastern Mojave Desert is high and the terrain rocky or steep; few tortoises occupy such areas. Lake beds are essentially devoid of vegetation and tortoises. Most tortoises in the eastern Mojave occur in low-lying areas where there are scattered shrubs.

Colorado-Sonoran Desert

Tortoises apparently do not occur naturally west of the Salton Sea; this may be due to repeated Pleistocene flooding of the Salton Sea, a remnant of pluvial Lake Cahuilla. Tortoises are seldom seen in the low portions of the Salton Sink and are of only sporadic occurrence in the Orocopia and Chuckwalla Mountains. Populations occur at the Chuckwalla Bench and Cottonwood Mountain (Dimmitt 1977). Records from near Yuma, Arizona, are old, and the present status of tortoises in the southeastern corner of California is not well known. I located some tortoise populations in the Palen and Chuckwalla Valleys, and along the eastern and western flanks of the Chocolate Mountains. However, tortoise population densities in the Colorado-Sonoran Desert appear to be lower than those found in the Mojave Desert.

Ecology

Little is known about the ecology of the desert tortoise. Most of the information derives from a study of a Utah population by Woodbury and Hardy (1948). This study and other information on the natural history of the desert tortoise have been summarized by Pope (1939), Carr (1952), Stebbins (1954), and Ernst and Barbour (1972). A comprehensive bibliography for the genus *Gopherus* was given by Douglass (1975, 1977). Recently, Auffenberg and Franz (1978) listed selected pertinent literature for the species. Several ecological studies of this species by both university and government scientists are now in progress.

The Woodbury and Hardy (1948) study was of a desert tortoise population at the extreme northern limit of the range in southwestern Utah. Their findings portray behavioral adaptations of the animal in a continental climatic regime and are not broadly applicable to other populations. Attempts to apply the data known about the Utah population to other populations has led to misunderstanding and, in some cases, to mismanagement of tortoise populations. At the western margin of the tortoise range, an intensive ecological study is now being completed by R. W. Marlow for his doctoral dissertation; the study population, near California City in the western Mojave Desert, occurs in an area of relatively high rainfall, which may be responsible for higher densities here than elsewhere in the range. There is no "typical" tortoise population because of the great variation among local populations.

The following discussion summarizes the behavior and natural history of the desert tortoise, principally for California populations.

Activities

Daily activity patterns vary according to season. During early spring (March-April), tortoises usually emerge from their burrows in late morning. Once emerged and warmed, tortoises may be found on the surface throughout the remaining daylight hours, foraging or engaging in courtship. During summer, activity bouts become bimodal because daytime heat causes a cessation of aboveground activities from about 1000 to 1900 h. This pattern grades into a unimodal late morning to sunset pattern again in the fall (September-November). The desert tortoise is diurnal except, perhaps, during rare rainstorms that may trigger nocturnal emergence.

The spring emergence of desert tortoises is associated with the earliest prolonged warm period. In southern California under normal climatic conditions, this occurs in late February to early March, but the exact timing varies with geographic locality. Tortoises are voluntarily active at a wide range of body temperatures—19.8° to 38.3°C (Brattstrom 1965; McGinnis and Voight 1971). Thus, any warm day in midwinter can cause some animals to come out of dormancy for short periods of basking. A rise in tortoise activity coincides with increasing ambient temperatures and daily insolation. Peak activity occurs in the spring months when temperatures are mild and food is abundant in the form of newly sprouted ephemeral plants and their blossoms. Berry (1975) found that adult and juvenile activity patterns differ in that the time spent above ground by juveniles predominantly occurs during the peak of the spring ephemeral plant season. Adults and subadults may be active year-round, except for the winter dormancy period. In California, inactivity usually extends from October to February but depends on geographic location and climatic conditions. Certain Mexican populations may be active above ground all through the year (Auffenberg 1969), and certain California populations may be similarly active in mild years. Depending on the occurrence of late summer thunderstorms, a secondary peak of activity may take place in the fall. Although hatchlings emerge in the fall, they are seldom encountered then. Most young dig their own burrows or retreat into larger existing burrows.

Home ranges of adults varied from 0.4-4 ha in Utah (Woodbury and Hardy 1948) to 10-14 ha near the Desert Tortoise Reserve in the western Mojave Desert, California (Marlow 1974). Berry (1974a) reported that near China Lake, California, males have home ranges of 1.2-2.6 km^2, and females have slightly smaller activity areas. Similarly, Burge and Bradley (1976) reported that the mean home range of males might be somewhat larger than that of females. In southern Nevada, Burge (1977) found that the mean home range of 9 male tortoises was 32.3 ± 6 ha (range 11-65) and of 8 females, 14.8 ± 2.6 ha (6.4-27). Auffenberg (1969) indicated that some Mexican populations are nomadic. Hatchlings and juveniles restrict their activities to small home ranges usually associated with 1 or 2 burrows, and the radius of the juvenile home range may be 50 m or less (Berry 1975).

Tortoise Burrows

Burrows function primarily as thermoregulatory aids (Woodbury and Hardy 1948). They are warmer in winter and cooler in summer than the surrounding environment and usually have a higher and more constant humidity. The entrances may be plugged to increase the thermoregulatory advantage and to aid in water conservation. Burrows also provide protection from predators.

Burrowing habits of desert tortoises vary greatly in different geographic localities. Since temperatures in Mexico are generally mild in winter, a simple pallet (a scrape or depression, often under a bush) or a single shallow hollow apparently suffices for nocturnal shelter (Auffenberg 1969). However, Bury et al. (1978) found that some desert tortoises dig shallow burrows up to 1 m long for winter retreats on Tiburón Island, Sonora, Mexico. In northern and central Arizona, winter temperatures force winter dormancy, and hibernacula consist of burrows in the sides of arroyos that are deep enough to allow the posterior portion of the tortoise shell to be flush with the arroyo wall (Auffenberg 1969).

In southern Nevada, Burge (1978) defined four types of cover sites: den, burrow, pallet; and nonburrow. The average density of repeatedly-used cover sites (pallets and burrows) was 3.5/ha. Of 783 burrows and pallets, most (85%) were in soil with varying amounts of gravel; 72% were located under shrubs, and 26% were dug into banks or beds of washes. Individual tortoises (fitted with radio transmitters) used 12-25 cover sites each year, and most were used repeatedly. Other aspects of burrow design and use are presented in her paper.

At the extreme northern limit of the tortoise range in Utah, two distinct denning behaviors are evident—summer burrows and winter dens. Winter dens are the hibernacula and are usually horizontal tunnels 2-5 m long, but may be as long as 10 m (Woodbury and Hardy 1940, 1948). Winter denning sites are mostly in banks of washes, but they may also be located on flats or hill slopes. Auffenberg (1969) notes that these hibernacula are usually situated on south-facing slopes and are used communally. Emergence from the hibernacula is usually followed by migration to spring and summer foraging areas (Woodbury and Hardy 1948). Summer burrows are dug or old ones are cleaned out and used for nocturnal and diurnal shelter; they are shallow hollows, dome-shaped, up to 1 m long, and usually sunk at a downward angle. Most summer burrows are situated on flats and interwash areas, often under bushes, but they may also be found in the sides of washes. Animals return from foraging areas to dens in the fall. The ratio of summer burrows to winter dens in Utah averaged about 4:1 (Woodbury and Hardy 1948).

In most of California, there appear to be permanent deep burrows as well as shallow temporary burrows or pallets. The permanent burrow form that is found in most of southern California is about 1 m deep and sunk downward at an angle between 10 and 30° (Fig. 11). Marlow (1974) found that burrow depth near the Desert Tortoise Reserve seldom was greater than 3 m; the average was about 1 m. Deep labyrinth burrows similar to those found in Utah (Woodbury and Hardy 1948) are uncommon in California, but I found some near Hinkley in the western Mojave Desert (Fig. 12). A shallow burrow is usually constructed to be just deep enough to cover the tortoise (Fig. 11) and may occasionally be employed for periods of dormancy.

Fig. 11. Typical tortoise burrow under a creosote bush. Desert Tortoise Reserve, Kern County, California.

Adult tortoises in the Desert Tortoise Reserve usually have one to three permanent burrows and two to three shallow shelters or pallets (R. W. Marlow, personal communication). Burrow size is directly related to size of the individual tortoise. The location of each burrow and distance to the burrow apparently is known by each animal, since some tortoises on long forays return to the same burrow at the termination of activity.

Often during the summer, California desert tortoises may simply seek shelter of a bush and scrape a small depression (pallet) beneath it for shelter; this behavior resembles that of *Gopherus berlandieri* in Texas (Auffenberg and Weaver 1969) and of Mexican populations of *G. agassizii* (Auffenberg 1969). Berry (1972) has suggested that an adult tortoise may accrue a thermal advantage by spending the summer or spring night above ground in the open. At night, surface soil and air temperatures drop to 20-24° C. The body temperature of a tortoise in the open would be similar. Air temperatures in a burrow 1-1.2 m deep may remain between 30 and 34° C. Thus a tortoise that spent the night in the open under a bush or in some other

Fig. 12. Large deep burrows in side of wash. Near Hinkley, San Bernardino County, California.

type of pallet could start the morning with a body temperature 1-6°C cooler than one that spent the night in a burrow. Since a longer time is required to reach the critical maximum temperature (Brattstrom 1965; McGinnis and Voight 1971), a longer period of activity may be possible in the morning. However, Cowles and Bogert (1944) showed that when desert reptiles become active in the morning, they can quickly increase their body temperatures to the preferred level. Further, Bury (1972) has shown that in the emydid turtle (*Clemmys marmorata*) the increase in body temperature from the time of emergence (about 18°C) to a preferred level (30-32°C) takes only about 30 min. Thus, a tortoise emerging from a burrow and one that spent the night above ground would probably reach the preferred body temperature at about the same time and would behaviorally maintain that level throughout the day. Tortoises may remain on the surface at night because of cooler ambient temperatures, which would maintain body temperatures at lower levels for the night. Such a preference for low temperatures may be important to reduce the rate of metabolism.

Commensals

A number of animal species have been noted in a commensal relation with tortoise burrows, especially those of *Gopherus polyphemus* (Carr 1952; Brode 1959; Blair and Kilby 1936; Young and Goff 1939). Existence of commensals within *G. agassizii* burrows has generally not been acknowledged or has been deemed unimportant (Grant 1936). Since underground shelter in the desert is limited, tortoise burrows represent places for many animals to escape predation and extremes of heat, cold, and dryness. Some species are found repeatedly in association with tortoises. Woodbury and Hardy (1948) commented that a large number of animals used *G. agassizii* burrows in Utah, and Burge (1978) noted use of burrows by other species in Nevada.

In the Stoddard Valley of western San Bernardino County, I found three burrow complexes occupied by tortoises and Burrowing owls (*Athene cunicularia*). Each complex consisted of 6 to 11 burrow openings, of which 3 or 4 were used by tortoises and the remainder by the owls (Fig. 13).

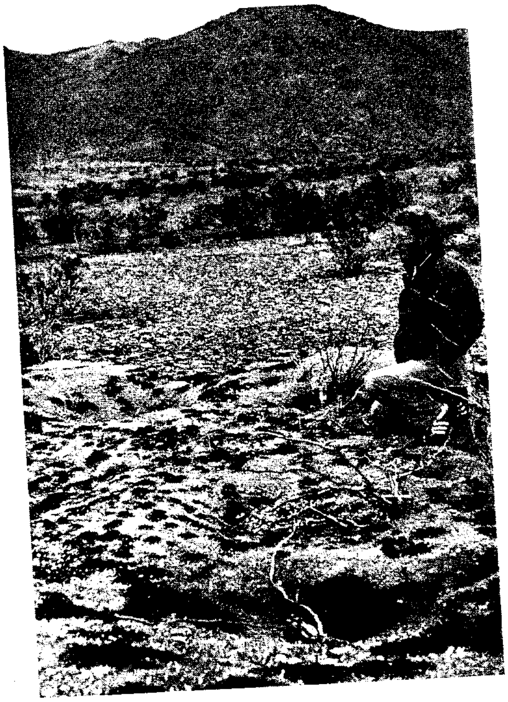

Fig. 13. Complex burrow system south of Barstow, San Bernardino County, California. Burrows are occupied by tortoises, burrowing owls, and antelope ground squirrels.

Ground squirrels used some of the burrow complexes. I have flushed Poorwills (*Phalaenoptilus nuttallii*) from other tortoise burrows during the summer; the burrows may serve as hibernacula for Poorwills during the winter months. I observed Black-tailed jackrabbits (*Lepus californicus*) using tortoise burrows or pallets for resting and for escaping from summer heat. Rattlesnakes frequently occur in tortoise burrows in the Fremont Valley, notably the Mojave rattlesnakes (*Crotalus scutulatus*). Sidewinders (*C. cerastes*) also frequent tortoise burrows, but they usually use shallow pallets. Black widow spiders (*Latrodectus mactans*) build orbs inside larger tortoise burrows. Animals known to be commensals of the desert tortoise are listed in Table 2; no obligates, however, have yet been noted.

Diet

The desert tortoise is herbivorous. The most important foods of California tortoises apparently are desert annuals, plants that often have a life span of less than 30 days. Both flowers and vegetative portions are eaten, although during the peak of flowering I have observed that flowers are preferred. On 7 July 1973, I watched an adult tortoise at Hinkley forage exclusively on annuals for 3 h. I have observed tortoises eating the plants that are listed in Table 3.

Peak tortoise activity usually coincides with the abbreviated period of annual bloom. During the spring bloom, tortoises apparently consume enough annual forage to sustain them through the summer aestivation and winter dormancy periods.

Table 2. *Commensals of the desert tortoise and its burrows. Sources: 1, Utah (Woodbury and Hardy 1948); 2, Nevada (Burge 1978); and 3, California (this study).*

Common name	Scientific name	Source
Ticks	Acarina	1,2
Black-widow spider	*Lactrodectus mactans*	2,3
Tarantula	*Aphonopelma* sp.	2
Silverfish	Thysanura	1,2
Roaches	Orthoptera	1,2
Ant lions	Neuroptera: Myrmeleontidae	1
Ground beetles	Coleoptera: Tenebrionidae	1-3
Tarantula hawk	*Pepsis* sp.	2
Desert spiny lizard	*Sceloporus magister*	1
Zebra-tailed lizard	*Callisaurus draconoides*	3
Desert iguana	*Dipsosaurus dorsalis*	3
Side-blotched lizard	*Uta stansburiana*	3
Whiptail lizard	*Cnemidophorus tigris*	3
Banded gecko	*Coleonyx variegatus*	1
Coachwhip	*Masticophis flagellum*	1,2
Gopher snake	*Pituophis melanoleucus*	1
Spotted night snake	*Hypsiglena torquata*	1
Western rattlesnake	*Crotalus viridis*	1
Mojave green rattlesnake	*Crotalus scutulatus*	3
Sidewinder	*Crotalus cerastes*	1-3
Poorwill	*Phalaenoptilus nuttallii*	3
Burrowing owl	*Athene cunicularia*	2,3
Pocket mouse	*Perognathus* sp.	2,3
Canyon mouse	*Peromyscus crinitus*	1
White-footed mouse	*Peromyscus* sp.	2
Kangaroo rat	*Dipodomys merriami*	2,3
Desert woodrat	*Neotoma lepida*	1-3
Antelope ground squirrel	*Ammospermophilus leucurus*	2,3
Desert cottontail	*Sylvilagus auduboni*	1,3
Black-tailed jackrabbit	*Lepus californicus*	1-3
Kit fox	*Vulpes macrotis*	2,3

Table 3. *Some plant foods of the desert tortoise in California.*

Common name	Scientific name
Broadflowered gilia	*Gilia latiflora*
Gilia	*Gilia* sp.
Brown-eyed primrose	*Oenothera clavaeformis*
Primrose	*Oenothera* sp.
Dapple-pod locoweed	*Astragalus lentiginosus*
White mallow	*Malvastrum exile*
Yellow peppergrass	*Lepidium flavum*
Lacy phacelia	*Phacelia tanacetifolia*
Phacelia	*Phacelia* sp.
Tansy mustard	*Descurainia pinnata*
Checker fiddleneck	*Amsinckia tessellata*
Ghost flower	*Mohavea confertiflora*
Verbena	*Abronia* sp.
Plicate coldenia	*Coldenia plicata*
Yellow comet	*Mentzelia affinis*
Blazing star	*Mentzelia albicaulis*
Desert star	*Monoptilon bellioides*
Pincushion flower	*Chaenactis Fremontii*
Wild daisy	*Erigeron* sp.
Coreopsis	*Coreopsis Bigelovii*
Eriophyllum	*Eriophyllum Wallacei*
Paperflower	*Psilostrophe Cooperi*
Desert dandelion	*Malacothrix glabrata*
Yellow saucers	*Malacothrix sonchoides*
Desert marigold	*Baileya* sp.
Desert chicory	*Rafinesquia* sp.
Glyptopleura	*Glyptopleura setulosa*
Creosote	*Larrea tridentata* (seeds)
Ricegrass	*Oryzopsis hymenoides*
Schismus grass	*Schismus* sp.
Galleta grass	*Hilaria rigida*
Brome grass	*Bromus rubens*
Storksbill	*Erodium cicutarium*

Forage must also be sufficient to allow the female to accumulate energy reserves for egg production. In dry springs, tortoises may rely on fat reserves accumulated during the previous spring. This phenomenon has been observed in other reptiles (Hahn and Tinkle 1965). Berry (1974*b*) reported that egg laying by the large herbivorous lizard *Sauromalus obesus* ceased in drought years. Because tortoises are large herbivorous reptiles, they might be expected to respond to reduced forage during drought conditions similarly by foregoing egg laying. Such a response would be typical of animals characteristically large-bodied, long-lived, and slow to mature, and that have low recruitment rates.

Grasses are mostly secondary food items and are probably used only to maintain summer activity. Dried grasses and dried annuals are normally the only food available during the late summer (July-September). In some areas, late summer thunderstorms can initiate a secondary germination of desert plants and thus induce localized secondary peaks of tortoise activity. Such late summer rains cause the appearance of "6-week grasses" such as foxtail chess (*Bromus rubens*) and chinch weed (*Pectis papposa*). Although Woodbury and Hardy (1948) seem to have overemphasized the role of grasses as a food source, all their observations on feeding behavior were made from September to November and in January, when the only plant material available was grasses.

Food habits of tortoises in northern Arizona and southern Utah were investigated by scat analysis by Hansen et al. (1976). They found that three species of grasses (*Aristida, Tridens,* and *Bromus*) accounted for 61% of the diet. However, since the collection of scats was not identified by season, the samples may have been biased for certain food items (such as grass parts) that produce lasting scats. Further, they found little regional variation in diet.

Since forage availability influences activity patterns, Mexican and southern Arizona populations may be active year-round in response to warmer winter temperatures and the availability of year-round forage (Auffenberg 1969). Some geographic variability in feeding patterns is apparent in California tortoises. In the northern Mojave Desert, schismus grass (*Schismus* sp.) has not been reported as eaten by tortoises (Berry 1972, 1974*b*, 1975); yet in the eastern and central Mojave, I have observed that *Schismus* is commonly eaten, particularly during late summer months.

With the exception of prickly pear pads (*Opuntia basilaris*), I have never observed a desert tortoise feeding on the vegetative portions of desert perennials. Their avoidance of these plants is probably related to the high salt content. In this respect, tortoises are similar to chuckwallas (*Sauromalus obesus*), which apparently eat only annuals, grasses, or the flowers of shrubby perennials (Berry 1974*b*), and die if they are force-fed leaves from certain perennial shrubs (Nagy 1972, 1973).

In a Nevada population studied by Burge and Bradley (1976), tortoises were observed eating seven different species in the shrub layer. The shrub species most frequently used were desert mallow (*Sphaeralcea ambigua*) and ratany (*Krameria parvifolia*). Tortoises also ate the terminal growth buds of pencil cholla (*Opuntia ramosissima*) and, seasonally, prickly pear pads. The most important dietary item was common plantain (*Plantago insularis*), which was eaten throughout the year. Hansen et al. (1976) noted that although food of Utah and Arizona tortoises consisted of a variety of grasses, sedges (*Carex* sp.), and forbs and shrubs, generally only the succulent portions such as leaves and flowering parts were eaten. They also found sand, bird feathers, mammal hairs, snake and lizard skin, and arthropods in some scats.

Desert tortoises in captivity eat a wide variety of foodstuffs including meat (Nichols 1953; Ernst and Barbour 1972). I examined about 200 scats in the field during 1973, of which only two contained any animal matter, and both were beetles (one *Cryptoglassa* sp. and one unidentified tenebrionid that could have been ingested accidentally).

Both captive and wild animals have been observed eating small amounts of sand. Frequency of sand ingestion appears to increase towards the end of summer and in early fall. Scats excreted at this time may be composed almost entirely of sand. I have also found sand in the large intestine of several autopsied specimens. Geophagy has been reported from a number of other chelonians (Sokol 1971; Kramer 1973). The function of soil in the diet of tortoises is not known, but it may be an aid in digestion, and such scats may serve as territorial or individual markers.

When tortoises forage, they wander from plant to plant, stopping briefly to put their noses to the ground or the plant. Head bobbing often accompanies this exploration (Eglis 1962), and olfaction apparently is involved (Weaver 1970). Both males and females have well-developed integumentary glands on their chins; these tend to be larger in males. Although I have spent much time observing tortoise activity and have never knowingly witnessed scent-marking, I believe it to be a distinct possibility. Such marking could play a role in foraging or in delineating home range. Its function need not be territorial; it could be valuable simply as an aid to locate the tortoise's activity space, and it may be used in sex recognition. Weaver (1970) reported that sniffing by *G. berlandieri* occurred in nearly all the combat and courtship encounters he observed. Chin gland secretions are different in each *Gopherus* species (Rose et al. 1969; Rose 1970).

A special posture is used in thermoregulation and, presumably, to aid in digestion by increasing the body temperature. The tortoise lies spread-eagled in the sun or under a bush, with limbs and neck extended and limp. Sleeping individuals were frequently seen lying in this manner after active foraging.

Water Metabolism

Standing water is seldom encountered by tortoises. Desert springs are usually located in steep, rocky terrain that is often inaccessible to tortoises. However, free-living tortoises in desert flatlands may drink from shallow depressions, some of which are dug and expanded by tortoises (Nagy and Medica 1977; Medica et al. *in press*). Home range familiarity allows the animals to locate rainfall puddles in these catchments and natural depressions. Specific boulders with depressions where water persists after rains are well known and widely used. On three occasions, I observed individuals licking moisture from rocks. Wild individuals may come to puddles left on asphalt roadways following rains. Where groundwater is close to the surface, the density of tortoises increases. For example, tortoises were abundant along the Mojave River before agricultural pursuits changed the habitat (Stebbins 1954).

Tortoises may subsist on water derived from food and metabolic pathways (Stebbins 1954; Auffenberg 1969). Leopold (1961) reported that water is stored in two "sacs" under the carapace. These sacs constitute a bilobed urinary bladder, and may contain large amounts of urine.

Dantzler and Schmidt-Nielsen (1966) demonstrated that the desert tortoise is capable of withstanding considerable dehydration and can tolerate large increases of ion concentrations in its blood plasma. They also found that the kidney remains functional even during mild dehydration and that nitrogenous wastes are stored in the bladder; these walls are more permeable to water in the desert tortoise than are those of freshwater turtles. Wastes are precipitated in the bladder as semisolid urates. When water is available, urine is not reabsorbed by the bladder but is excreted as dilute urine. But tortoises apparently are also able to go for months without discharging urine from the bladder. Approximately equal amounts of urea and uric acid were excreted by the *G. agassizii* examined by Dantzler and Schmidt-Nielsen (1966).

Evaporation from the integument constitutes a major source of water loss from desert tortoises, with respiratory loss secondary in importance. However, both types of water loss are much less than that of turtles found in damper climates. Thus, cutaneous water loss in *G. agassizii* at 23°C is 1.5 mg/cm^2 per day, compared with 5.3 mg in the box turtle, *Terrapene carolina* (Schmidt-Nielsen and Bentley 1966). Water conservation also occurs in the egg shell, which is resistant to water loss (Stebbins 1954).

Minnich (1976, 1977) measured water turnover rates in a desert tortoise population in the Mojave Desert. He found that the rates during most of the summer of 1970 were exceedingly low (0.36 mL/100 g per day) and only slightly greater than rates of water metabolic production (0.31 mL/per day). During the summer period, osmotic pressure of the bladder urine increased steadily until it equalled that of the plasma, suggesting that as the tortoise dehydrates it reabsorbs water from the bladder. After one rainfall of 1.2 cm, tortoises drank rainwater (an average of 14.4 mL/100 g of body weight), gained weight, and produced a dilute urine that was stored in the bladder.

Urine and large amounts of semisolid urates are frequently voided during handling. Patterson (1971) reported that tortoise urine could pucker the mouth of a kit fox. Thus, urination may serve as a predator defense. Handling often causes urination and may represent a severe water loss to the tortoise, particularly to juveniles. Proper handling techniques can eliminate this danger. Folding the tail over the cloaca by placing a finger between the carapace and plastron often prevents a tortoise from urinating.

Reproduction

Mating starts with spring emergence of the tortoises and may continue until the fall dormancy period. I observed mating in August near Hinkley in the western Mojave Desert, and Berry (1975) noted mating as late as October at China Lake. Peak breeding activity, however, is in spring (March-June). Males often approach one another and fights ensue. When courting, a male approaches a female with head and neck extended and head bobbing. The male then proceeds to bite and nip the female's head and forelimbs or the edge of the carapace. Ramming and circling by the male often occurs. The male mounts from the rear and, by standing on the tips of his front claws, is able to bring his shell into a nearly vertical position. Urination may occur at this time, but its significance, if any, is not known. Much grunting, stretching of limbs, and rhythmic humping is typical of male activity (Weaver 1970). Tortoises may be surprisingly vocal during mating. Campbell and Evans (1967) reported two types of sounds—a grunt and a drawn-out moan. Patterson (1973) recorded numerous vocalizations, some of which sound like baying hounds.

Nesting occurs mainly from May through July. Captive individuals have nested as late as October and often have two or three clutches per year (Stewart 1954; Miller 1955). Some wild individuals may also have multiple clutches, and late nesting may explain the overwintering of eggs reported by Grant (1936).

Tortoises dig nests in sandy or friable soil by using the hind feet or by first using front legs, then hind legs. When both are used, the female first digs a broad, shallow hole with the front legs and then backs into this hole and digs with her rear feet (Booth 1958; Edell 1970). Measurements of a nest given by Nichols (1953) were about 23 cm (9 in.) in diameter at the top, 18 cm (7 in.) in diameter at the bottom, and 15 cm (6 in.) deep. Occasionally, eggs are deposited singly and at random sites (Miller 1932). The nesting cavity may be dug in the mouth of a permanent burrow.

Observations of nest construction by captive tortoises suggests that the depth of nests varies with the length of the female as well as with the hardness of the soil. Females apparently construct nest sites that resemble undisturbed ground and usually urinate in the nest before or after filling it.

After nesting, females appear to be less active above ground than males. Males remain above ground longer than females and apparently remain sexually active throughout the summer. Of 124 individuals I examined in the field during August 1973, 80% were adult males.

Eggs vary from elliptical to nearly spherical, with a dull, chalky color and rough texture. Of 19 eggs that I measured, the average dimensions were 47.9 mm long and 39.0 mm in maximum width (37.6 mm in minimum width). Average egg weight was 33.6 g; similar weights were reported by Grant (1936) and Miller (1932).

Clutch size varies from 2 to 14 eggs; 5 or 6 is the typical number (Grant 1936; Ernst and Barbour 1972). Clutch size is related to the size of the female, with larger females generally having larger clutches—a phenomenon also found in other species of reptiles (Fitch 1970).

In the wild, incubation apparently varies from 90 to 120 days. An incubation period of 118 days was noted by Grant (1936) for eggs of animals in captivity. Artificially incubated eggs usually hatch in 80-90 days (Lampkin 1966; Shade 1972). For tortoises in captivity, an 80% hatching rate is considered high; hatching success of 60% or less is more common. Failure of some artificially incubated clutches may be due to high constant temperatures maintained in incubators. At hatching, the yolk sac remains attached in the center of the plastron, but it is rapidly absorbed. The yolk sac is about one-third the size of the hatchling tortoise and greatly impedes locomotion for the first few hours of life.

Hatching generally occurs from August to October; some eggs apparently overwinter, and hatchlings appear in the following spring. Little food is available at the time of most hatching in late summer, and hatchlings spend little time on the surface. Hatchlings dig their own small burrows, or use an existing larger burrow. Dormancy shortly follows, probably before the hatchling has eaten or taken a drink. My observations of hatching in captivity suggest that food is ignored between the time of emergence and the beginning of dormancy.

Growth and Maturation

Hatchlings are nearly as wide as they are long. The shell is soft and remains soft for 5 to 10 years; during this time, the hatchlings are very susceptible to predation. The shell of the young is generally dull yellow and the edges of the scutes are brown, a cryptic color pattern that merges well with the late summer aspect of the desert. Hatchlings are pugnacious and will butt at anything that moves.

Sexual maturity of the desert tortoise in the wild apparently is reached at a carapace length of 230-265 mm, between 15 and 20 years of age (Woodbury and Hardy 1948). Berry (1975) reported that females may mature at a carapace length of 215-220 mm. With regular feeding for two-thirds of the year, tortoises in captivity may reach maturity at 12 to 13 years of age (G. R. Stewart, quoted in Berry 1975). Growth rates of captive tortoises are given by Patterson and Brattstrom (1972). Accelerated growth rates and early maturity ascribed to year-round activity and continuous high-quality nutrition have been reported for captive animals (Jackson et al. 1976). Medica et al. (1975) reported that the growth rate in tortoises in Nevada was related to environmental conditions; growth was greatest following winters of high precipitation. Immature animals (less than 200 mm in plastron length) increased from 1.8 to 12.3 mm per year ($\bar{x}$ = 9 mm) over a 5-year period. Under natural conditions, the feeding period lasts only from 6 weeks to 3 months in good forage years, which occur on an average of once in 5 years. Thus, growth and maturity are often delayed in the wild.

Population Structure

At China Lake, California, a tortoise population was composed of 2% hatchlings, 8% juveniles (1-10 years old), 31% subadults, and 59% adults, when a carapace length of 215-220 mm was used as the criterion for the adult age class (Berry 1975, 1976). The sex ratio was 1.78:1 in favor of females. Similarly in the Fremont Valley, 2% of a marked population were hatchlings, 56% were juveniles and subadults, and 42% were adult (Marlow 1974). Burge and Bradley (1976) found similar percentages (55% adults, 44% juveniles and subadults, 1% hatchlings) in a tortoise population in Nye County, Nevada.

The Utah tortoise population on Beaver Dam slope reported by Woodbury and Hardy (1948) consisted of 90% adults. In adults, the sex ratio was 1.51:1 in favor of females. Since 1945 this Utah population has experienced a drastic decline, from 59.5 individuals/km² to 10.4/km² and a reversal in sex ratio (2.33:1) in favor of males (Coombs 1977).

Natural Mortality

It is common for 50% of a clutch from desert tortoises in captivity to be infertile (Lampkin 1966), and similar losses may occur in the wild. Surviving juveniles apparently maximize their chances of reaching maturity by spending little time above ground. Juveniles emerge in the spring to bask and forage, but they return to their dens when the ephemeral annual plants wither. Thus, juveniles may spend less then two months of the year above ground. Adults are potentially active on the surface nine months each year in California, albeit at varying levels of activity.

Of the 281 animals (90% adults) that were marked in Utah over a 10-year period (Woodbury and Hardy 1948), the remains of only 30 animals were found and the mortality estimated at 1% per year. Berry (1972, 1975) reported similar rates of less than 5% mortality per year in a tortoise population in the western Mojave Desert. The rate for these long-lived animals was low, as expected.

The coyote (*Canis latrans*) is common throughout the desert and is a major predator of tortoises. Coyotes are persistent and chew on the limbs and carapaces of tortoises for extended periods, and they often return to pursue a tortoise left earlier (Berry 1972). Coyotes may also dig tortoises out of their burrows, excavating behind the burrow entrance (Berry 1972). During field surveys (see Appendix), I encountered large numbers of tortoise carcasses, for many of which coyotes were apparently responsible. When tortoise remains are found, however, it is not always possible to distinguish the results of active predation from those of scavenging. It is not uncommon to find tortoises with scarred or cracked shells and damaged or amputated limbs (or both), which are likely indications of attempted predation by carnivores. Overall, the coyote may be an inefficient predator on adult tortoises because so much energy is expended in an attempted kill.

Badgers (*Taxidea taxus*) are more common in the desert than is generally recognized and can easily open an adult tortoise. Bobcats (*Lynx rufus*), skunks (*Spilogale putorius* and *Mephitis mephitis*), and kit foxes (*Vulpes macrotis*) may prey on both eggs and tortoises. However, since most of these animals are nocturnal, they encounter tortoises infrequently. These carnivores could dig out smaller tortoises. Robert Mallette of the California Department of Fish and Game (personal communication) has found hatchling tortoises to be a major food item of a pair of Golden eagles (*Aquila chrysaetas*) during the nesting season in the Mojave Desert. Other avian predators on small tortoises probably include Red-tailed hawk (*Buteo jamaicensis*), Holarctic raven (*Corvus corax*), Burrowing owl (*Athene cunicularia*), and roadrunners (*Geococcyx californianus*). Coachwhip snakes (*Masticophis flagellum*) are capable of taking both eggs and juvenile tortoises, and the Gila monster (*Heloderma suspectum*) is a known predator also (Hensley 1950).

One of the most important limiting biotic factors on desert tortoise populations is probably predation. Predation on the small-sized juveniles would be expected to be high and remains are usually not found. This loss, combined with the possible low fertility and with predation on eggs, probably accounts for the small percentages of hatchlings and small tortoises found in the wild.

Tortoise populations today may be expanding (or have expanded) as a result of predator control programs in the southwestern deserts. Most such programs have recently been discontinued, and predators are again increasing. Consequently, tortoise populations may be approaching a new equilibrium with expanding predator populations.

Flash flooding during the summer months occasionally causes the loss of animals denning in washes. I have observed such mortalities in both the Fenner and Fremont Valleys; flooding can wipe out local populations.

Overturned tortoises probably result from fighting or predation. A tortoise in this position is rarely helpless; only if the substrate is hard or if the animal is weak or deformed will it be unlikely to right itself. If a tortoise remains overturned in direct sunlight for any length of time, however, it becomes vulnerable to overheating. When its body temperatures exceeds 39.5°C, a tortoise suffers thermal stress and may die if not quickly removed to a cooler environment.

The adobe tick (*Ornithodorus turicata*) is known to parasitize *G. agassizii* (Harbison 1937; Ryckman and Kohls 1962). I have found infestations to be common in the Hinkley population, although individuals did not appear to be weakened by the ticks. The effects of tick infestation are not known. Occasionally a bone disease occurs in the desert tortoise which causes loss of scales and scutes (Miller 1932; Frye 1973); the disease is generally fatal.

Woodbury and Hardy (1948) reported that occasional grass fires kill tortoises in Utah. Such fires, however, are rare in the California desert.

It is my conclusion that the most significant cause of mortality today is human activity. The same conclusion was reached by Auffenberg and Weaver (1969) in their studies of *G. berlandieri* in southeast Texas. This cause of mortality is discussed below.

Conservation

Human Impact

Historical Depredation

Human pressures have been a factor affecting desert tortoise populations for thousands of years. Although the Mohave Indians specifically avoided the use of turtles as food, neighboring Chemehuevi and other Piutes ate them (Kroeber 1925). Tortoises were used for food, bowls, scrapers, rattles, decorations, trade and barter items, and as pets. However, such pressure on tortoise populations was probably minimal because native Indian populations were small and seminomadic. Many more tourists may be in the desert on one winter weekend now than there were resident Indians historically.

Collection and Removal

Until recently, the most important human activity affecting the distribution and abundance of desert tortoises has been direct removal, despite the longstanding law that prohibits harming, collecting, or removing tortoises from areas in California. In the spring, when most people visit the desert, tortoises are above ground in large numbers and are easily captured because they are slow-moving and diurnal.

In the spring of 1973, temperatures in the Mojave Desert were mild, and publicity about the flower displays attracted large numbers of tourists to the desert. The following autumn, many *G. agassizii* were brought to the Alexander Lindsey Junior Museum of Walnut Creek near San Francisco. During October, 45 tortoises were presented to the museum. Evidently, many local residents had visited the desert during the spring of 1973, and many had brought tortoises back with them. Acquistion of captive tortoises by this means is a seasonal event at most of the Bay Area nature centers and museums, but the numbers of individuals involved during 1973 were unusually high.

A survey by the International Turtle and Tortoise Society reported a minimum number of 23,000 turtles and 6,500 tortoises kept by its members (Anon. 1971); no geographical breakdown is given, but the Society's California chapter is the largest. Further, 75% of the above totals were species native to the members' area. Because there are only two native chelonians in California (the other is the Western pond turtle), the desert tortoise comprised a significant percentage of all such pets. *Gopherus agassizii* is a common pet throughout the United States and, formerly, could be bought in department and pet stores. If one of every 100 families in Los Angeles County had a pet tortoise, there would be 20,000 tortoises in Los Angeles. The densities of tortoises in some metropolitan counties (Los Angeles, Orange, Riverside, Contra Costa) probably are comparable to densities found throughout most of the California desert. There is also a sizeable backyard accumulation of tortoises in Bakersfield, California (R. Marlow, personal communication). Similarly, Keasey (1971), writing of Tucson, Arizona, said, "Because of their hardiness and reproductivity [sic] in captivity, the population of backyard tortoises in this desert city is only a little short of phenomenal."

Since the study by Woodbury and Hardy (1948), the Utah population has declined from a known population of about 300 to only 40 individuals. The location of the site is well known and is visited several times each year by field trip groups from numerous colleges and universities. Furthermore, these animals are occasionally collected, although the desert tortoise is now protected in Utah. Representatives from schools as far away as the Atlantic seaboard stop by annually on collection trips (Coombs 1974). Coombs (1974, 1977, and personal communication) stated that this type of collecting pressure has been one factor in reducing tortoise numbers on the Beaver Dam slope in Utah.

Illegal trafficking for commercial purposes is also a major cause of depletion. Although few people are involved in commercial traffic, the total number of tortoises is probably similar for commercial and private removal. Captured specimens of desert tortoises were once distributed worldwide. Improved enforcement apparently is curtailing such removal to some extent. One arrest was made of a California dealer in 1970, a case involving the illegal collection of 290 animals (Bury and Marlow 1973). Chambers of Commerce of desert communities have repeatedly used tortoises for promotional purposes in the past.

Railroad section workers once sold many tortoises to train passengers. Railroads also collected them for promotion and display at their stations, such as in Needles and Kelso. These activities have ceased.

In California, pets are frequently liberated, occasionally on a large scale. Before 1969, the California Department of Fish and Game (DFG) was releasing about 50 tortoises per year (Stewart 1973), some of which were *G. berlandieri.* In 1973, the DFG released 259, for which they kept records (J. St. Amant, memorandum, 16 April 1973). Between 1969 and 1972, G. R. Stewart and his students released 248 tortoises (personal communication). Mortality of these released animals is presumed to have been high. Kristin Berry (personal communication) suggests that mortality is related, in part, to the length of time tortoises are retained in captivity. Further, Stewart (1973) notes that though many tortoises consume native foods while in captivity, they fail to develop and use natural defense reactions. At first, released tortoises are not mobile and appear to wait, expecting to be fed. Potential dangers of liberating pets include the introduction of diseases (such as influenza, bone disease, or other epizootics), gene pool mixing, disruption of social structures, and introduction of exotic species. Most release programs have been stopped.

I strongly advise against the release of captive tortoises to the wild, except for special instances when the animal is unaccustomed to captivity (recently caught) and can be returned to the exact site of capture within a few months. The California DFG has a program to rehabilitate tortoises for possible reintroduction to depleted areas (St. Amant 1977); it can also authorize a permit for keeping tortoises that have been in captivity for long periods. If an animal is no longer desired as a pet, the assistance of the California DFG (or a comparable agency in other states) should be requested. The California Turtle and Tortoise Club also accepts tortoises for their adoption program (Lewis 1977).

Berlandier's tortoise occurs in Texas and northern Mexico, where it is protected. However, many *G. berlandieri* have been imported into California for the pet trade, mostly from northeast Mexico through New Mexico to avoid Texas laws (Brame and Peerson 1969). Auffenberg and Weaver (1969) reported that 4,000 *G. berlandieri* were collected for one shipment. Glenn R. Stewart (personal communication, 1974) mentioned one shipment of 8,000 animals transported in two vans and estimated that some 40,000 *G. berlandieri* were being imported to California each year. The California DFG attempts to regulate trafficking in *G. berlandieri* and *G. agassizii;* the sale of both species is now illegal in California. Many shipments have been confiscated, but most animals have been released later. Differences between *G. berlandieri* and *G. agassizii* present a problem of recognition for nonspecialists; frequently *G. berlandieri* is sold as "Gopherus" or "desert tortoise." In 1970, G. R. Stewart (personal communication) found the remains of a *G. berlandieri* near Palm Desert, Riverside County; other specimens have also been observed in the desert by his students. About 20% of the tortoises turned into the Alexander Lindsey Junior Museum in Walnut Creek, California, are Berlandier's tortoise, which indicates how common they were on the commercial market.

Impact of Traffic and Off-road Vehicles

Decreases in tortoise populations due to direct contact with humans will increase as the desert becomes more popular and accessible for recreation. During movements and foraging, tortoises cross roads where they may be collected by people seeking a pet or killed by passing vehicles (Fig. 14).

Fig. 14. Two adult tortoises killed by vehicles on a road near California City, California (Photo by R. W. Marlow).

Nicholson (1978) found that vehicular traffic on paved roads has a detrimental effect upon tortoise populations within about 1 km of the road, and that such roads may be a major factor contributing to the reduction of tortoise populations.

Human activity in the desert has a second major impact on tortoise populations. Nearly everyone who now visits the desert comes to drive over it. Every sort of off-road vehicle (ORV) is used: dune buggies, motorcycles, mini-bikes, motorized tricycles, all-terrain vehicles, sail planes, and four-wheel drive (4WD) vehicles. There has been a great increase in visitation in recent years. Recreational use of the California Desert in 1958 was estimated by the BLM (1968) to be 4.9 million visitor-use days (defined as one person spending 12 h). The total in 1973 was 13 million and the trend continues (Carter 1974). This massive increase in the influx of visitors increases the chances for direct removal of tortoises by collecting. About 1 million motorcycles are owned by Californians for off-road use; dune buggies number about 200,000, and 4WD's number about 500,000. Organized ORV events are held almost weekly through the fall, winter, and spring, attracting thousands of participants. Although the BLM recognized the need to regulate their use, control of ORVs has been minimal. Not until 1972 did the BLM begin to require that special land-use permits be obtained by organizers of competitive events. Between September 1972 and September 1973, 151 such events were held in California deserts, involving more than 67,000 participants (Carter 1974), and many more were held without permits. Some ORV users often travel by themselves.

Off-road vehicle use on the California desert has already had significant effect on the distribution and abundance of tortoises. In 1974–75, Bury et al. (1977) found 18 tortoises on 8 unused areas (2 ha each) but only 5 tortoises on 8 ORV-used sites. In 1976–77, we censused two 25-ha sites of similar terrain near Barstow, San Bernardino County; 34 tortoises were found in the control area but only 15 in the ORV-used area (Bury 1978; Bury and Luckenbach, unpublished data). The estimated tortoise biomass was 3.4 kg/ha in the control area and 0.5 kg/ha in the ORV-used area. In the control area, 171 burrows (51% actively used) were found versus 62 burrows (35% actively used) in the ORV area. Adult tortoises apparently were removed or killed in the ORV area. Off-road vehicles also collapse tortoise burrows. In general assessments, Berry (1973) and Bury et al. (1977) concluded that a pronounced detrimental effect on the desert ecosystem results from ORV use. Such effects are widespread and enduring, if not permanent (Stebbins 1974; Luckenbach 1975).

Growth of annuals and herbaceous perennials is severely reduced by ORV activity (Luckenbach and Bury, *in press*), and the basic energy fixation and transfer systems of the desert are disrupted or destroyed by vehicular activity. For example, insects and arthropods are important in pollinating desert plants as well as in conditioning desert soils; they, in turn, are food for lizards, snakes, rodents, birds, and carnivores. Because tortoises are herbivorous, they respond immediately to disruption of the food web.

Impact of Grazing

Grazing has been suspected as a factor detrimental to tortoise populations (Bury and Marlow 1973). Marlow (1974) pointed out that sheep eat many of the same plants as tortoises but that sheep are better competitors for food resources by virtue of their larger size, more efficient dentition, and greater mobility. Because they are trailed in large flocks, sheep can consume nearly all of the spring forage in an area. Marlow (1974) states that he counted 23 pallets and 4 tortoise burrows trampled by a flock of sheep in a 10-ha area near the Desert Tortoise Reserve. In this same vicinity, Busack and Bury (1974) reported that sheep grazing had a negative effect on the lizard populations, probably because of loss of cover, reduction in food sources, disturbances of social structure, and casualties; these factors probably also affect tortoises. Young tortoises have been found crushed by the hooves of livestock. Inferences about the potential and actual impacts of grazing on tortoise populations are further discussed by Berry (1978).

Evidence shows that sheep and cattle grazing have detrimentally affected the Beaver Dam Slope population of desert tortoises in Utah (Woodbury and Hardy 1948; Hardy 1976; Coombs 1977; Berry 1978). Livestock appear to compete with tortoises for many food plants and to cause deterioration of the general condition of the range inhabited by tortoises (by reducing productivity, decreasing available perennials and forbs, and trampling).

Management

Legal Protection

The desert tortoise is legally protected in California; a 1961 statute makes it unlawful to "sell, purchase, needlessly harm, or take the desert tortoise or to shoot any projectile" at it (Leach and Fisk 1969). This statute was further amended in

August 1972 to include prohibition of transportation and possession of all species of *Gopherus* in California (Bury and Stewart 1973). This amendment provides for a permit system whereby owners who can demonstrate legal acquisition may possess tortoises. Permits can also be issued for possession for educational, scientific, and zoological purposes. In 1972, the desert tortoise was declared the state reptile. On 1 March 1972, the California DFG amended its wildlife regulations to include a "zero bag limit" for all species of the genus *Gopherus*. These recent laws prohibit the importation of all North American tortoises (*Gopherus*) into California. The laws are enforced, but manpower is inadequate for proper enforcement. Furthermore, these laws are not widely known. Not only are few tortoise owners aware of their legal obligation to register their pets, some confusion exists as to just how registration should be done and what constitutes a "legal" animal. A solution to these problems is being sought (St. Amant 1977).

Land Ownership

Ownership of lands in the California desert is divided among many agencies and organizations, but Federal ownership predominates. Six military reservations (Edwards Air Force Base, Fort Irwin, U.S. Naval Ordnance Test Station at China Lake, Randsburg Wash Test Range, Twentynine Palms Marine Corps Training Center, Chocolate Mountains Gunnery Range) under the Department of Defense comprise about 1.2 million ha; other agencies in the Department of Interior (National Park Service, Bureau of Reclamation, and the Bureau of Indian Affairs) administer about 0.81 million ha. Private and state ownership account for about 0.45 million ha. The Southern Pacific Land Company is the largest single private owner; its ownership stems from the Public Railway Act of 1862 which granted to the company odd-numbered sections, 32 km on each side of the planned railroad route. Once surveyed, nearly half of the granted lands were sold in the first 20 years to help finance construction, but most of the desert holdings were deemed worthless and remained unsold; Southern Pacific retains ownership and apparently has no immediate plans for development. The State of California is involved with the desert in a variety of capacities, including highway rights-of-way, irrigation districts, school districts, state parks, and fish and game regulation. Private business interests include mines, real estate, agriculture, grazing lands, ranches, and tourist facilities.

Cabins and rural retreats in the form of small tracts (mostly smaller than 2 ha) are the basis for some private ownership patterns in the desert. Between 1959 and 1969, about 20,000 ha were sold under the Federal Small Tract Act for small-acreage recreational homesites; the largest concentration of these lies in the western Mojave Desert from just east of Twentynine Palms to Victorville, with smaller concentrations in the Antelope Valley and in the Barstow and Ridgecrest regions. As a result of these and other land sales of the 1950's and early 1960's, an estimated 120,000 unimproved small parcels are now privately owned in the California Desert. Tortoises are found throughout the low-lying areas in these regions of "jackrabbit homesteads."

Also, mining claims number more than 250,000 (BLM 1969). Mining activities, however, occupy only a small percentage of the land area and most are concentrated in upland areas that are less frequented by tortoises.

The largest portion of the California desert is administered by the BLM, which manages about 4.45 million ha. These lands were primarily designated as grazing and mining leases or held without designated uses. Such lands are now being classified under broader multiple-use designations (BLM 1980), but recreational use and energy development are foremost. Such planning places the future of many tortoise populations in jeopardy because nearly all major concentrations and prime habitat of desert tortoises in California are located on BLM Natural Resource lands.

A major conservation effort is under way to establish a Desert Tortoise Reserve near California City in the western Mojave Desert. This proposed reserve contains the highest known densities of the tortoise. The BLM is attempting to set aside 98 km^2 of land for its natural values, principally for protection of the desert tortoise. A Desert Tortoise Preserve Committee has been instrumental in promoting the reserve (Forgey 1977) and works with its own funds. The Nature Conservancy has also assisted by purchasing a few critical parcels of private land. The proposed reserve has been fenced to deter unauthorized grazing and impact by ORVs, and a nature center and trail system for the reserve are now being considered.

Energy leases and transmission corridor easements on natural resource lands have been granted to various power and utility companies. Some of these projects consider the best possible planning alternatives with respect to tortoise populations (e.g., Stevens 1976). Other projects have opened access to desert lands through road construction along corridor routes for transmission lines or underground pipelines.

The Department of Defense lands in the California desert probably support sizeable tortoise populations (Berry 1976). These facilities have served as reserves because of their restricted use patterns and controlled access. Conversely, some attrition has probably occurred during the various training exercises.

Desert Tortoise Council

In 1975, a Desert Tortoise Council was established through the volunteer efforts of representatives from local, State, and Federal agencies, and from utility companies, colleges, and universities, as well as private citizens and civic groups. Its major aims are to provide a forum for discussion of the problems of tortoise management and to coordinate efforts relating to the survival of the tortoise throughout its range. Among the activities of the Council are efforts to define tortoise respiratory diseases, the release of rehabilitated captive animals into the wild, and several education projects to focus attention on the plight of the tortoise. The Executive Committee of the Council now meets several times each year and annual symposiums are held. Work on the management and biology of the tortoise is published in the *Proceedings of the Desert Tortoise Council.*

Recommendations

My field studies have identified several prime tortoise areas (Table 4); existing reserves for the desert tortoise in California are given in Table 5. Only in the Desert Tortoise Reserve has a substantial tortoise population been identified for protection.

The ancestors of the desert witnessed the large-scale Pleistocene extinctions of many animal species, but they adapted to the increasing aridity of the Southwest. The tortoise survived this period to become the largest native herbivore on most of the Southwestern flatland desert. The desert tortoise is well adapted and resistant to the climatic and biological demands of an arid region, but its future survival is in jeopardy because of human activities. Tortoise populations have already suffered significant losses from collection and removal, grazing, roadkill, military activity, and vandalism, and more recently by rapidly increasing ORV use. Its habitat is extremely vulnerable to disruption by ORVs.

Although the desert tortoise has adequate legal protection in California, its habitat does not. The major high-density areas are largely on BLM-administered public lands. The future of the desert tortoise can best be assured by proper management of these lands for their wildlife values.

Specific management needs of the desert tortoise identified by the present study include the following:

- Prohibit or restrict use of ORV activities in areas of high tortoise abundance;
- Investigate the impacts of grazing, especially the short-term, concentrated trailing of sheep;
- Coordinate efforts of the Department of Defense in California to identify areas critical to unique wildlife and plant species on their lands, and to adopt suitable management programs within the framework of their research and training priorities; and
- Coordinate similar efforts concerning tortoise populations in other states and in northwestern Mexico.

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Table 4. *Prime desert tortoise areas in California.*

Locality	Habitat	Human disturbance	Ownership	ORV Classification[a]
1. Upper Ward – Chemehuevi Valleys	Creosote scrub with ocotillos. Cholla gardens and other succulents; wash.	Area of "Desert Strike" maneuvers; unexploded ordnance, some trash, tracks (heavy in NE). N-S power line; E-W Gas and power lines and access roads.	Most bajadas of Ward Valley are consolidated BLM Natural Resource Lands. Most of Chemehuevi Valley is BLM lands, but northern part is mixed ownership.	Valleys: existing roads and trails. Old Womans area and perimeter of Turtle Mtn.: designated roads and trails. Small closure by I-40. North of 40: designated road and trails.
2. Fenner and Piute Valleys	Creosote scrub, cholla gardens, and other succulents; wash.	Area of "Desert Strike" maneuvers: ordnance, fox holes, trash, airport, old barracks; range cattle; roads; power and gaslines.	Mining claims. Railroad checkerboarding, private lands, mining claims, and grazing leases.	Western end of Fenner Valley: existing roads and trails. The remainder: designated roads and trails.
3. Water Valley	Creosote scrub, Joshua Tree woodland.	Power and gaslines; roads; sheep use seasonally; old mines.	Railroad checkerboarding on western end. Some consolidated BLM land on east end. Private lands.	Eastern end: designated roads and trails. Western section: some ORV event design sites.
4. Fremont Valley	Creosote scrub, Joshua Tree woodland.	Subdivisions; powerlines; encroaching irrigated agriculture; numerous roads; sheep grazing seasonally; ORV activity.	Almost wholly in private sector with exception of tortoise preserve.	Unclassified because of private ownership. Tortoise Reserve is closed.
5. Stoddard Valley	Creosote scrub, Mojave yucca, and other succulents at higher elevations.	Power and gas line roads; mining activity in past. Range cattle, sheep grazing seasonally; camping and ORV activity.	Mostly consolidated BLM lands; grazing leases, some patented mining claims.	Special design and existing roads and trails. Competitive Events Areas.
6. Ivanpah Valley	Creosote, shadscale and alkali, sink scrub.	Power and gasline roads; Union Pacific railroad; range cattle.	Largely BLM lands with some school district sections. Some private lands near Cima. Grazing leases.	Designated roads and trails.
7. E. Kramer Hills W. Hinkley Valley	Creosote scrub, scattered Joshua Trees.	Power and gas line roads; subdivisions; and small private tract plots; sheep grazing seasonally; ORV activity.	East of Hwy 395, well-consolidated BLM lands. Mostly private holdings along Mojave flood plain and in Hinkley Valley.	Kramer Hills is a special design area. An ORV Competitive Events Area is planned.

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[a] Refers to classifications in the California Desert ORV Recreation Management Plan (1 November 1973).

Table 5. *Areas serving as reserves for the desert tortoise in California.*

Name	Facility	Size (ha)	Comment
Joshua Tree	National Monument	18,200	Moderate numbers of native and released animals.
Death Valley	National Monument	810,000	Low population densities.
Picacho	State Park	1,975	Status of tortoises, if present, unknown.
Providence Mtns.	State Recreation Area	2,120	Small numbers at lower elevations.
Saddlebag Butte	State Park	1,160	Abundant: frequent release site.
Redrock Canyon	State Park	800	Resident populations severely reduced.
Wildflower and Wildlife Sanctuaries	Los Angeles County Park	860	Eight separate sanctuaries--mostly in creosote scrub in Antelope Valley. Tortoises locally abundant.
Desert Tortoise Reserve	BLM and private land	8,500	Highest known densities in Calif. Education and scientific use area.

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Appendix

Pooled data from 5 surveys conducted on the Desert Tortoise Reserve, 5 on the Hinkley Study Site, 33 from high-density areas, 69 from medium-density regions, and 35 from low-density areas. Total values are averages plus or minus one standard deviation; ranges are given in parentheses—i.e., $\bar{x}$ ± SD (range).

Standardization Transects

Site	Transect	Tortoises		Burrows		Scats
		Adult	Juvenile	Active	Inactive	
Desert Tortoise Reserve (N=5)	A	26	0	40	48	18
	B	8	0	60	36	21
	C	17	0	36	40	16
	D	20	4	46	44	24
	E	43	3	42	38	19
		22.8±13.0	1.4±1.9	44.8±9.2	41.2±4.8	19.6±3.0
		(8-43)	(0-4)	(36-60)	(36-48)	(16-24)
Hinkley Study Site (N=5)	A	18	0	36	24	20
	B	9	2	30	19	29
	C	14	0	16	12	12
	D	15	1	26	21	36
	E	16	1	22	18	17
		14.4±3.4	0.8±0.8	26±7.6	18.8±4.4	22.8±9.6
		(9-18)	(0-2)	(16-36)	(12-24)	(12-36)

High Density (N=33)

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
5	0	0	18	22	7
7	4	0	27	31	12
8	7	2	35	26	15
9	6	1	20	33	9
17	5	1	36	24	32
18	6	0	19	27	7
21	2	2	31	35	0
26	8	3	39	26	9
27	0	0	25	19	2
29	1	0	32	29	11
30	10	3	31	36	22
31	3	2	21	28	14
32	6	1	36	27	3
54	3	1	19	24	10
58	6	1	43	31	0
63	4	0	19	26	4
66	2	0	24	32	23
67	3	0	31	41	25
68	8	2	37	29	17
69	4	0	22	30	13
70	0	0	41	34	17
71	2	0	29	19	5
72	7	1	36	26	11
78	6	0	28	17	19
80	0	0	28	18	7
81	9	2	30	27	3

High Density (N=33) continued

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
82	4	0	27	36	9
91	3	1	39	37	22
94	2	0	29	19	14
96	6	0	37	28	6
97	10	0	47	36	11
98	7	2	39	29	14
101	2	0	40	32	21
	4.4±3.0 (0-10)	0.8±1.0 (0-3)	30.7±7.8 (18-47)	28.3±6.0 (17-37)	11.9±7.7 (0-32)

Medium Density (N=69)

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
4	2	0	14	7	3
6	7	0	17	10	0
10	0	0	12	14	5
11	5	1	9	7	1
12	6	1	13	13	4
13	1	0	8	21	9
14	3	0	14	6	14
15	6	2	10	3	2
16	2	0	16	11	2
19	1	1	9	6	9
20	9	3	27	13	11
22	0	0	9	3	11
23	8	0	11	7	0
24	0	0	14	5	1
25	5	2	16	0	0
28	2	0	6	3	6
35	11	2	29	14	17
36	4	0	27	19	0
38	6	2	15	7	13
40	7	1	18	9	7
42	4	0	20	11	1
44	0	0	8	3	10
48	2	1	10	7	9
52	1	0	9	4	2
53	2	0	14	9	0
55	0	0	4	1	7
56	9	3	18	17	3
57	3	1	16	2	20
59	2	0	15	7	17
60	4	0	10	8	7

Medium Density (N=69) continued

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
61	3	0	9	7	9
62	0	0	7	9	15
64	1	0	3	0	4
65	2	1	5	1	3
73	0	1	7	4	9
74	4	0	9	13	3
75	2	0	12	3	8
76	2	2	17	9	0
77	1	0	8	6	2
79	7	1	13	10	12
83	11	0	28	17	13
84	0	0	16	11	17
85	4	0	21	14	7
88	3	1	15	13	9
89	0	0	9	5	3
90	5	3	12	3	2
92	2	1	7	0	2
93	0	1	18	4	7
95	1	0	15	8	0
99	2	0	7	12	11
102	0	0	11	8	3
103	1	0	17	13	10
104	3	2	7	10	7
105	0	0	11	9	5
106	4	1	14	16	12
107	4	0	10	17	5
108	1	2	9	3	2
109	0	0	10	14	3
110	1	0	18	16	2
112	4	0	17	12	2
113	8	4	22	12	7
114	2	3	5	11	0
125	2	1	14	9	7
126	4	2	17	7	11
128	0	0	22	14	3
129	5	1	13	3	0
130	0	2	11	16	2
131	5	1	21	5	9
136	3	0	18	17	0
	3.0±2.8 (0-11)	0.7±1.0 (0-4)	13.2±5.6 (3-29)	8.8±5.1 (0-21)	6.0±5.1 (0-20)

Low Density (N=35)

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
1	0	0	0	0	0
2	0	0	0	1	0

Low Density (N=35) continued

Transect	Tortoises		Burrows		Scats
	Adult	Juvenile	Active	Inactive	
3	0	0	0	3	0
33	1	0	3	2	0
34	1	0	5	2	0
37	3	1	8	5	0
39	4	0	9	11	7
41	0	0	0	2	0
43	2	0	0	1	3
45	0	0	3	1	0
46	2	1	13	2	2
47	3	1	17	9	7
49	2	0	19	11	5
50	4	0	9	2	1
51	1	0	3	0	0
86	0	0	4	2	0
87	0	0	1	2	0
100	3	1	6	1	7
111	0	0	2	2	0
115	1	0	7	9	2
116	1	0	4	3	0
117	0	0	2	0	0
118	1	0	8	10	4
119	1	1	4	3	0
120	2	0	9	5	0
121	0	0	0	0	0
122	0	0	0	0	0
123	0	0	0	0	0
124	0	0	0	2	0
127	1	1	9	1	0
132	2	0	6	4	1
133	1	0	4	1	0
134	0	0	0	0	0
135	0	0	0	0	0
137	0	0	0	0	0
	1.0±1.2 (0-4)	0.2±0.4 (0-1)	4.6±5.0 (0-19)	2.8±3.3 (0-11)	1.1±2.2 (0-7)

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A Genetic Assessment of the Recovery Units for the Mojave Population of the Desert Tortoise, *Gopherus agassizii*

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Abstract. – In the 1994 Recovery Plan for the Mojave population of the desert tortoise, *Gopherus agassizii*, the US Fish and Wildlife Service established 6 recovery units by using the best available data on habitat use, behavior, morphology, and genetics. To further assess the validity of the recovery units, we analyzed genetic data by using mitochondrial deoxyribonucleic acid (mtDNA) sequences and nuclear DNA microsatellites. In total, 125 desert tortoises were sampled for mtDNA and 628 for microsatellites from 31 study sites, representing all recovery units and desert regions throughout the Mojave Desert in California and Utah, and the Colorado Desert of California. The mtDNA revealed a great divergence between the Mojave populations west of the Colorado River and those occurring east of the river in the Sonoran Desert of Arizona. Some divergence also occurred between northern and southern populations within the Mojave population. The microsatellites indicated a low frequency of private alleles and a significant correlation between genetic and geographic distance among 31 sample sites, which was consistent with an isolation-by-distance population structure. Regional genetic differentiation was complementary to the recovery units in the Recovery Plan. Most allelic frequencies in the recovery units differed. An assignment test correctly placed most individuals to their recovery unit of origin. Of the 6 recovery units, the Northeastern and the Upper Virgin River units showed the greatest differentiation; these units may have been relatively more isolated than other areas and should be managed accordingly. The Western Mojave Recovery Unit, by using the new genetic data, was redefined along regional boundaries into the Western Mojave, Central Mojave, and Southern Mojave recovery units. Large-scale translocations of tortoises and habitat disturbance throughout the 20th century may have contributed to the observed patterns of regional similarity.

Key Words. – Reptilia; Testudines; Testudinidae; *Gopherus agassizii*; tortoise; conservation genetics; distinctive population segment; evolutionary significant unit; management units; microsatellites; mitochondrial DNA; Mojave Desert; USA

The desert tortoise (*Gopherus agassizii*) is a widespread species (or possible species complex) occurring in the southwestern United States and northwestern Mexico (Fritts and Jennings 1994; Berry et al. 2002; Stebbins 2003). The US Fish and Wildlife Service (USFWS) federally listed the species as threatened under the Endangered Species Act, as amended, in the northern one third of its geographic range, specifically, populations living north and west of the Colorado River in the Mojave and Colorado deserts (USFWS 1990; Fig. 1). The listing occurred primarily because of population declines and habitat loss and deterioration, which were attributed to human activities. In recognition of the distinctiveness of the threatened populations, the USFWS developed the *Desert Tortoise (Mojave Population) Recovery Plan* (referred to herein as *Recovery Plan*) (USFWS 1994) and designated 26,087 km^2 of critical habitat (Berry 1997). About 83% of the critical habitat is on land managed by government agencies.

The federal listing of the desert tortoise as a threatened species brought about a redirection of government efforts to recover the species within its 4 southwestern states (California, Arizona, Nevada, and Utah). Several government agencies prepared new long-term management plans or amended older land-use plans to support recovery efforts (Berry 1997), a process that required more than 16 years. The extent of landscape affected by these efforts was significant and included parts of the Mojave Desert and the Colorado Desert (also called western Sonoran Desert). For convenience, the USFWS termed the populations within critical habitat as the "Mojave" population, when in fact they occur in both the Mojave and Colorado deserts. Herein, we follow this terminology. For populations in the Sonoran Desert of Arizona, we use "Sonoran" populations.

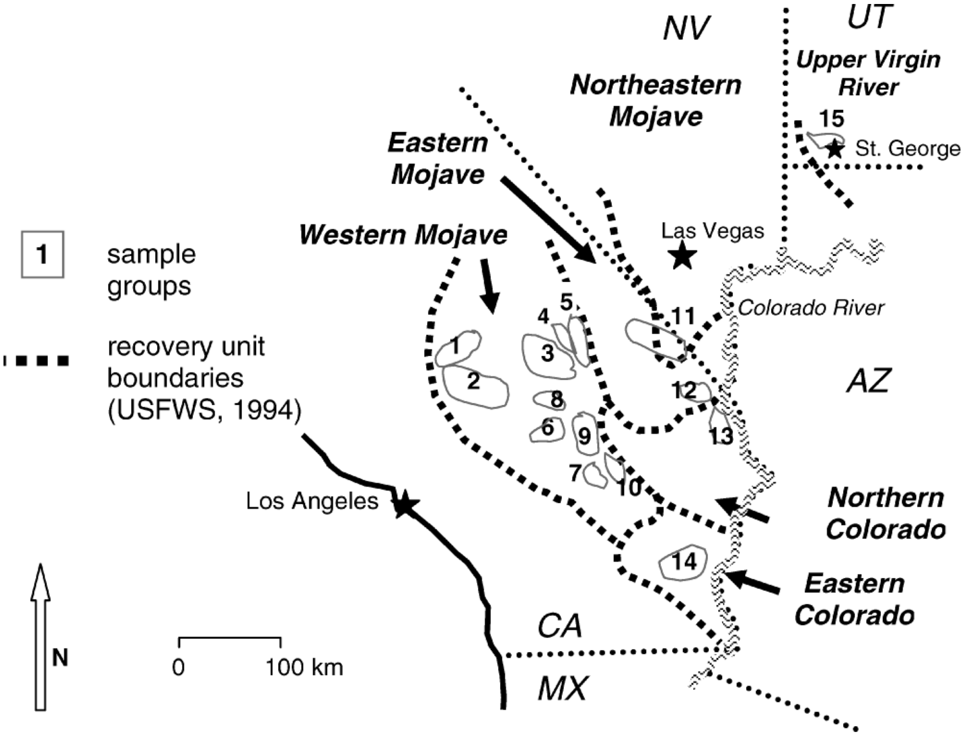

Figure 1. Sample groups and recovery unit boundaries for *Gopherus agassizii* as described in the *Desert Tortoise (Mojave Population) Recovery Plan* (USFWS 1994) and sample sites for this study. Because of their geographic proximity, 3 tortoises from the Eastern Mojave Recovery Unit were combined with 57 tortoises from the Northeastern Mojave Recovery Unit to form sample group 11.

Desert tortoises exhibit substantial differences in morphology (Weinstein and Berry 1987; Germano 1993), physiology (Turner et al. 1986; Wallis et al. 1999; Averill-Murray 2002; Averill-Murray et al. 2002a, 2002b; McLuckie and Fridell 2002), behavior (e.g., Woodbury and Hardy 1948; Burge 1977; Averill-Murray et al. 2002b; Jennings 2002), and genetics (Lamb et al. 1989; Lamb and Lydeard 1994; McLuckie et al. 1999; Lamb and McLuckie 2002) throughout the geographic range in the United States. This variation occurs within and between the Mojave and Sonoran populations.

The authors of the *Recovery Plan* recommended protection of 6 evolutionarily significant units (ESUs) or distinct population segments (DPSs) in 6 "recovery units" (Ryder 1986; Waples 1991, 1998; US Department of the Interior and US Department of Commerce 1996). They noted that the ESUs (or DPSs) consisted of "populations or groups of populations that show significant differentiation in genetics, morphology, ecology or behavior . . . and thus are important components of the evolutionary legacy of *Gopherus agassizii*" (USFWS 1994). They stated that the conservation of all ESUs would help to ensure that "the dynamic process of evolution [in this species] will not be unduly constrained in the future [Waples 1991]" (USFWS 1994). It is important to note that the authors used the phrases ESUs, DPSs, and recovery units synonymously, and their intent was to draw on multiple criteria to delineate units (after Waples 1991, and similar to Crandall et al. 2000). The USFWS also recommended that concepts in the *Recovery Plan* be subjected to hypothesis-testing. In the case of genetics, the limited available mitochondrial deoxyribonucleic acid (mtDNA) data suggested that *G. agassizii* might be composed of more than 1 species, with the Colorado River acting as a boundary in the northern part of the geographic range (Lamb et al. 1989; summarized in Berry et al. 2002).

Since the *Recovery Plan* (USFWS 1994) was published, the fields of population and conservation genetics have advanced rapidly. Numerous new, powerful techniques are now available for processing, statistically analyzing, and interpreting genetic samples (e.g., DeSalle and Amato 2004; Pearse and Crandall 2004; Manel et al. 2005; Allendorf and Luikart 2007). In 1996, the federal government further clarified the Endangered Species policy on DPSs for vertebrates (US Department of the Interior and US Department of Commerce 1996). The academic dialog on the definitions and applicabilities of ESUs, DPSs, and other related concepts, such as management units (MUs), Canadian designatable units (DUs), and adaptive evolutionary conservation has continued to be rigorous and brisk (Crandall et al. 2000; Fraser and Bernatchez 2001; Pearman 2001; Moritz 2002; Green 2005). However, distinct infraspecific populations of American vertebrates, except for salmonid fishes, can currently only receive legal protection as DPSs, not as ESUs.

A factor complicating the genetic study of desert tortoise populations has been human-mediated translocation. The tortoise has received much well-intended attention by governmental agencies and concerned citizens since the 1930s (California Code of Regulations 2007). Thousands of tortoises have been taken into captivity and then released. Still others have been translocated from one area to another in the desert. Commercial harvesting and interstate transportation have been significant.

Our objectives are to contribute to recovery efforts for this species by: 1) characterizing genetic differences in the Mojave populations to determine whether the existing 6 recovery units are genetically distinguishable and, if so, to what extent; 2) evaluating the potential effects of numerous releases and translocations of tortoises on genetic structure; and 3) placing the genetic data in the context of ecological and behavioral differences in desert tortoises to support the conservation of ecological and evolutionary processes.

METHODS

Sample Collection

We salvaged blood from desert tortoises used in research projects on health, disease, and physiology, and through collaboration with other scientists (Henen et al. 1997; Brown et al. 1999; Christopher et al. 1999, 2003; Edwards 2003). Desert tortoises were captured by hand in the field by following federal and state protocols (Averill-Murray 2000; Berry and Christopher 2001). Samples were collected from tortoises ($n = 628$) at 31 study sites that occur within the geographic range where the tortoise is federally listed (USFWS 1990) (Table 1; Fig. 1). We did not include sites from Nevada or the Beaver Dam Slope, Utah. Study sites were in remote areas as well as < 2 km from towns or human habitation. We also obtained mtDNA sequences from 4 *G. agassizii* from the Sonoran Desert of Arizona (Edwards et al. 2003), 1 sample of the bolson tortoise (*Gopherus flavomarginatus*) from a private collection, and 1 sample of the Texas tortoise (*Gopherus berlandieri*) from the Department of Animal Care and Technologies at Arizona State University, Tempe (J. Badman).

About 1 ml whole blood was collected via brachial, jugular, or subcarapacial venipuncture, and the samples were stored on ice or dry ice in (ethylenediamine tetraacetic acid [EDTA]), lithium heparin, or 95% ethanol. Most samples (from health and disease studies) were centrifuged first, the plasma was removed, and the red blood cells were retained and frozen for DNA extraction.

Molecular Techniques

Molecular procedures were conducted at the Genomic Analysis and Technology Core, University of Arizona. Genomic DNA was isolated from blood by overnight lysis with proteinase K at 55°C, followed by a phenol/chloroform extraction and isopropanol/sodium acetate precipitation (Goldberg et al. 2003). The DNA was resuspended in low TE (10 mM Tris-pH 8.0, 0.1 mM EDTA) and diluted to a 5 ng/μL working stock for polymerase chain reaction (PCR) amplifications.

MtDNA Sequencing. — We amplified an ca.1500–base-pair (bp) portion of the nicotinamide adenine dinucleotide dehydrogenase subunit (ND)3, arginine transfer RNA (tRNA) ND4L, and part of the ND4 genes by using primers Nap2 and New Gly (Arévalo et al. 1994; Britten et al. 1997; Edwards 2003). PCR followed Edwards (2003), and the PCR products were purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and were sequenced on an ABI Prism 3700 DNA Analyzer (PE Biosystems, Foster, CA). Internal primers were designed by using Oligo Primer Analysis Software 6.68 (Molecular Biology Insights, Inc, Cascade, CO): Nap2IN 5’AGGCGGTCAATAATGC-TAATC3’ and NewGIN 5’TAATAAAACCAGACAAT-GAAAAAC3’. These primers amplified an 1109-bp portion of ND3/ND4, which was aligned and evaluated by using Sequence Navigator 1.0.1 (Applied Biosystems, Inc, Foster, CA).

Nuclear DNA Assessment. — Data gathering was carried out on an ABI Prism 3730 DNA Analyzer (PE Biosystems). All samples were tested for 16 microsatellite loci (Table 2). The loci were PCR amplified in 6 separate multiplex reactions by using 5’ fluorescently labeled forward primers. We sequenced selected products for all loci to verify repeat motifs. Repeat motifs were identified by using Sequence Navigator 1.0.1 (Applied Biosystems, Inc). Reliably scored, variable loci were used for analysis.

Analysis

Grouping of Samples. — Sample sizes from each of the 31 study sites ranged from 3 to 74 (Table 1). Study sites were assigned to 1 of 15 sample groups based on location, proximity to nearby sites ($\leq$ 60 km), potential topographic or geographic barriers to movement of tortoises, region of the desert, recovery unit as described in the *Recovery Plan* (Fig. 1), and the need to maintain a minimum sample size for statistical analyses. Thus, the 15 sample groups contained 18–83 tortoises (Table 1). Sample group 11 combined individuals from Ivanpah, California ($n = 57$), which belong to the Northeastern Mojave Recovery Unit, with 3 tortoises from Shadow Valley in the Eastern Mojave Recovery Unit owing to close geographic proximity of the localities. We assigned groups to regions of the Mojave and Colorado deserts by using boundaries similar to those described in Rowlands et al. (1982), and the boundary between the Mojave and Colorado deserts as described in Jaeger (1957), Benson and Darrow (1981), Rowlands et al. (1982), and Turner et al. (1995). For boundaries delineating the northern and eastern regions within the Colorado Desert, we followed the *Recovery Plan* (USFWS 1994; Rowlands 1995a, 1995b).

MtDNA. — We selected 125 tortoises representing all recovery units, including 47 samples from the Northeast-

Table 1. Desert tortoise study sites and sample groupings representing 8 regions for the Mojave population.

Desert region/recovery unit	Study site	No. samples	Group	No. samples in group
Western Mojave	Desert Tortoise Research Natural Area	58	1	62
	Fremont-Valley	4	1	
	Hinkley	12	2	83
	Kramer	3	2	
	Edwards Air Force Base	57	2	
	Fremont-Kramer	11	2	
"Central Mojave"	Superior-Cronese	10	3	19
	Fort Irwin (Goldstone)	9	3	
	Fort Irwin (Tiefort)	31	4	31
	Fort Irwin (Soda Mtns.)	33	5	47
	Fort Irwin (Eastgate 2)	14	5	
"Southern Mojave"	Lucerne Valley	12	6	26
	Ord-Rodman	14	6	
	MCAGCC[a] (Emerson)	9	7	71
	MCAGCC (Sand Hill)	62	7	
	Daggett	74	8	74
	MCAGCC (Lavic Lake)	8	9	27
	MCAGCC (Maumee Mine)	7	9	
	MCAGCC (Sunshine Peak)	12	9	
	MCAGCC (Bullion)	16	10	19
	MCAGCC (Lava)	3	10	
Northeastern Mojave	Ivanpah	34	11	60
	Ivanpah (site 14)	23	11	
	Shadow Valley[b]	3	11	
Eastern Mojave	Fenner	4	12	31
	Goffs	27	12	
Northern Colorado	Chemhuevi	7	13	18
	Upper Ward Valley	11	13	
Eastern Colorado	Chuckwalla	18	14	37
	Chocolate Mtns.	19	14	
Upper Virgin River	near St. George, UT	23	15	23

[a] MCAGCC = Marine Corps Air Ground Combat Center.

[b] Population occurring in the Eastern Mojave Recovery Unit assigned to the Northeastern Mojave sample group for purposes of data analysis owing to geographic proximity.

Table 2. Observed microsatellite motifs in Mojave desert tortoises, *Gopherus agassizii*, compared with that of the originally described species or population.

Locus	Species originally described	Original repeat motif	Observed motif in Mojave population	Range of Mojave alleles	Range of Sonoran alleles
Edwards et al. 2003					
Goag3	*G. agassizii* (Sonoran)	$(CAA)_6$	$(CAA)_6$	6–7	6–9
Goag4	*G. agassizii* (Sonoran)	$(CAA)_{24}$	$CAA)_{24}$	12–32	7–30
Goag5	*G. agassizii* (Sonoran)	$(GAT)_8$	$GACGAA(GAT)_2GACGAA$	null	6–38
Goag6	*G. agassizii* (Sonoran)	$(TC)_8(AC)_{11}$	$(TC)_8(AC)_{11}$	17-–67	15–52
Goag7	*G. agassizii* (Sonoran)	$(AC)_3(GC)_5(AC)_{11}$	$(AC)_8(AT)_2GC(AC)_3(GC)_3(AC)_9$	13–28	12–28
Goag32	*G. agassizii* (Sonoran)	$(AC)_6$	$(AC)_6$	6	5–6
Schwartz et al. 2003					
GP26	*Gopherus polyphemus*	$(GT)_{12}$	$(GT)_7$	7	6–9
GP55	*G. polyphemus*	$(GT)_9$	$(GT)_7$	7–30	7–34
GP102	*G. polyphemus*	$(GT)_5(CT)_{13}(CA)_5$	$(TC)_2(TG)_2CG\ [(TG)_8(TC)_{14}]$[a]	19–42	19–36
GP15	*G. polyphemus*	$(GA)_{15}(GT)_8$	$(GA)_{14}(GT)_{20}$	13–52	13–56
GP19	*G. polyphemus*	$(GT)_9/(GT)_3(GA)_6$	Allele 1; $(GT)_3/(GT)_2GAAA(GA)_4$ Allele 2; $(GT)_7ATGTATGT/(GT)_2GAAA(GA)_5$	11 and 21	6, 11, and 21
GP30	*G. polyphemus*	$(GT)_{13}$	$(GT)_5(CT)(GT)_4$	10–17	5–29
GP81	*G. polyphemus*	$(GT)_{11}(GA)_{10}$	$(GT)_9GACA(GA)_8$	16–28	18–22
GP61	*G. polyphemus*	$(GT)_{12}$	$(GT)_4AT(GT)_6$ & $(GT)_{16}$	11–38	9–43
GP96	*G. polyphemus*	$(GA)_{11}$	$(GA)_7$	7	7
FitzSimmons et al. 1995					
Cm58	*Chelonia mydas*	$(CA)_{13}$	$(TA)_5(GA)_3GC(GT)_3$	12	12–13

[a] Complex repeat; unable to obtain entire sequence.

ern Recovery Unit, and sequenced their mtDNA for a total evidence analysis (Kluge 1989; Ernisse and Kluge 1993) of unique haplotypes only. Unweighted maximum parsimony analyses were performed on potentially informative characters by using PAUP* 4.0b10 (Swofford 2002). Most parsimonious trees were obtained by using the heuristic tree search algorithm with random addition of individuals, 10,000 replicates while retaining minimal trees only and holding 10 trees at each replicate, tree bisection-reconnection branch swapping with the steepest descent, and collapsed zero-length branches. All multistate characters were evaluated as nonadditive (unordered). Nodal consistency was assessed by using nonparametric bootstrap proportions (Felsenstein 1985) and decay analysis (Bremer 1994) performed in PAUP*. Relative nodal support was assessed by using bootstrapping with 10,000 random pseudoreplicates of the data, with each pseudoreplicate being replicated twice.

Bayesian inference was also used to hypothesize matriarchal history (Huelsenbeck and Ronquist 2001; Buckley et al. 2002; Nylander et al. 2004; Ronquist 2004). MrModeltest 2.2 (Nylander 2004) was used to select the best evolutionary model based on the Akaike Information Criterion (Akaike 1974, 1979). Hierarchical likelihood ratio tests (Goldman 1993) compared log-likelihood scores of 56 models. Bayesian inference, conducted by using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), started with random trees. Six Markov chains were used, and the data set was run for 3×10^6 generations. Trees were sampled every 100 generations. Two independent analyses with different starting trees were run and the fluctuating values of likelihood were graphically monitored (Huelsenbeck and Bollback 2001). Log-likelihood scores of sample points were plotted against generation time to establish stationarity (Huelsenbeck and Ronquist 2001). The analysis was a priori required to achieve a split frequency standard deviation of ≤ 0.005. After discarding 25% of the sampled trees as burn-in, the remaining trees were used to generate a 50% majority rule consensus tree.

Nuclear DNA. — We used several methods of analyses to assess gene flow and population differentiation. Each of the methods had different assumptions and relied on different properties of the data, as noted below.

Population Structure. — We used 1) traditional techniques that a priori defined sample groups and 2) an a posteriori genotypic clustering method to analyze population structure. Individuals for which more than 3 loci did not amplify were discarded. Allelic frequency distributions for unique (study site or region restricted) and private alleles ($> 5\%$ in a sample group or region) were examined. Loci that exhibited more than 7 alleles were examined by using the log-likelihood-based (G-based) exact test (Goudet et al. 1996) in GENEPOP 3.1 (Raymond and Rousset 1995). A triangular contingency table and a modified version of the Markov-chain random walk algorithm (Guo and Thompson 1992) were used in ARLEQUIN 2.0 (Schneider et al. 2000) to detect significant departures from the Hardy-Weinberg equilibrium (H-W). The multiple tests were not Bonferroni corrected because we looked for trends only and not a precise application of statistical tests. The trends would have remained with a Bonferroni correction but the levels of significance (p-values) would have been raised, possibly to the extent of no significance. Default parameters in GENEPOP and ARLEQUIN were used for all Markov-chain tests and permutations.

Linkage equilibrium is assumed by some statistical tests and, thus, was necessary to confirm. GENEPOP tested for linkage disequilibrium (nonrandom association between loci) among all pairs of loci in the entire sample and within each group by using the method of Garnier-Gere and Dillmann (1992).

Population genetic structure was assessed under nonequilibrium conditions (Pearse and Crandall 2004; Manel et al. 2005). We used STRUCTURE 2.1 (Pritchard et al. 2000) to a priori define cohesive genetic units. Because it does not provide a good measure of genetic structuring in populations that exhibit nonlinear patterns of isolation-by-distance (IBD; Kimura and Weiss 1964; Pritchard et al. 2000), as do Mojave desert tortoises, STRUCTURE was used as a guideline only. An extension to the program by Falush et al. (2003) accounts for correlations between linked loci that arise in admixed populations. We evaluated the 15 sample groups (K populations) with 4 simulations of 500,000 iterations for each K by using the default parameters for an admixture model with a prior mean Φ_{ST} (F_{ST} sensu Weir and Cockerham 1984) of 0.06 (0.05 SD), based on the mean generated from our data set. (We initially also tried the analysis with a lower number of runs by using prior mean Φ_{ST} of 0.01, without a noticeable difference in the outcome.) The best model had the smallest value of K and the largest likelihood values.

To reduce the strongest effects of multilinear IBD, we performed an analysis on the Western Mojave Recovery Unit but first removed the northern- and southernmost samples. The analysis included sample groups 1–10 and used 1,000,000 iterations with a prior mean of Φ_{ST} at 0.01.

Population differentiation was also assessed by using WHICHRUN 4.1 (Banks and Eichert 2000), which calculates the likelihood of a given individual originating from either of 2 or more candidate populations. If the groups identified by STRUCTURE and/or the 6 units hypothesized in the Recovery Plan were distinct and not interconnected by frequent gene flow, then WHICHRUN should assign an individual to its source population with a high likelihood score and assign it to other populations with low scores. Stringency for population allocation used a selection criterion of the log of the odds ratio (LOD) for the 2 most likely source populations. The chance of error is equal to the inverse of this ratio; assignments with a LOD of at least 2 had a ≤ 0.01 chance of error.

Traditional equilibrium-based F-statistics, using analysis of molecular variance (AMOVA) in GENEPOP, were also employed to infer population structure. Inbreeding coefficients (Φ_{IS}; F_{IS} sensu Weir and Cockerham 1984) were calculated for each locus in each sample group. Genetic distances based on pairwise Φ_{ST} were calculated among groups and individuals by using GENEPOP and were visually assessed by producing a multidimensional monotonic scaling plot (MDS) that used the program NTSYS (Exeter Software, NTSYS pc 2.1, Setauket, NY). Goodness of fit was measured by using the Stress test (Kruskal and Wish 1978). Mantel tests obtained from NTSYS assessed correlations between genetic and geographic distances among sample groups. The Φ_{ST} values estimated population structure and gene flow by assuming mutation-drift or migration-drift equilibrium with symmetric migration in both directions for all pairwise combinations of populations. The Φ_{ST} values also assumed an island model that may not be met in desert tortoises, especially because they have experienced recent demographic declines (see Whitlock and McCauley 1999).

Demographic History. — Two very different models assessed historical changes in population density. First, BOTTLENECK (Piry et al. 1999) was used to test for evidence of historical changes in effective population sizes and deviations from equilibrium conditions for each of the sample groups, regions, and the entire population. Populations with recent reductions in effective population size should show an excess of heterozygosity (Cornuet and Luikart 1996; Spencer et al. 2000). Significance of the observed deviations, assuming the infinite alleles model, was determined by the Wilcoxon test as well as the Sign test method of Piry et al. (1999). Second, the M-ratio test of Garza and Williamson (2001) was used to investigate changes in population density and to evaluate bottlenecking, where M is the ratio of the total number of alleles (k) to the overall range in allele size (r). When rare alleles are lost during a population bottleneck, the number of allele size classes is reduced to a greater extent than the range in allele size. Value M is reduced in populations known to have declined in size. In total, 20 populations had the required number of individuals for applying this test. Bottlenecking was assumed to have occurred if M was above the critical value M_C (Garza and Williamson 2001). Congruent findings from the 2 tests would suggest that the results were not biased for any single method or set of assumptions.

Human-Mediated Translocations. — We compiled published and unpublished data and interviewed biologists in state and federal wildlife and land management agencies, then mapped localities of releases or escapes of captive tortoises and translocations of wild tortoises. The results of WHICHRUN assessed the source of an individual tortoise and assignments or misassignments to specific populations. BOTTLENECK, G-based exact tests in GENEPOP, and estimates of inbreeding values (Φ_{IS}) provided information on population trends. Significant deviations from H-W, estimates of recent gene flow and distributions of haplotypes from previously described analyses also provided valuable information.

RESULTS

MtDNA Evaluation. — Estimations of maternal history and population structure were based on *G. agassizii* from the Mojave population and the outgroup taxa (Table 3). All sequences were deposited in GenBank (Accession no. DQ649394–DQ649409).

Seven haplotypes were observed among the 125 *G. agassizii* from the Mojave population (Table 3). Five localities had a single haplotype, and 1 region, the Northeastern Mojave, had 3 sympatric haplotypes, likely a result of the greater extent of sampling at this locality. One haplotype, MOJ-A01, occurred in all but the Northeastern Recovery Unit. Similarly, haplotype MOJ-B01 was common in the Northeastern and Upper Virgin River recovery units but also occurred in low frequency in the Western Mojave and Eastern Colorado recovery units (Table 3). Haplotype MOJ-A02 occurred in 2 nearby localities in the Southern Mojave. MOJ-A03 was found in the nearby Western Mojave and Southern Mojave recovery units. In contrast, haplotypes MOJ-A04 and -B02 occurred at single locations only. Haplotypes within the Mojave population differed at most by 4–5 bp, or only 0.6%, and haplotypes MOJ-B01–03 differed from one another by 1–2 bp only, as did MOJ-A01–04.

Maternal History. — The phylogenetic evaluation was based upon 60 potentially cladistically informative nucleotide positions. In total, 842 nucleotide positions did not vary between the outgroup and ingroup taxa. Autapomorphies occurred at 22 nucleotide sites. The cladistic analysis of the sequences yielded 2 most parsimonious solutions (length $= 77$ steps, $CI = 0.81$, $RI = 0.95$, $RC = 0.76$). By using *G. flavomarginatus* as the primary outgroup, *G. berlandieri* was resolved as the sister group to all maternal lineages of *G. agassizii*. The consensus trees (Fig. 2) had 2 strongly supported lineages at the base of the tree, one containing Sonoran samples and the other containing samples from the Mojave population. Within the Mojave population, 2 major sublineages were resolved: Haplogroup A, "broadly distributed," and Haplogroup B, Northeastern Mojave. Both lineages contained 1 haplotype that was relatively broadly distributed (Table 3), along with alternative haplotypes. The 2 most basal nodes for *G. agassizii* were strongly supported having bootstrap proportions of 100% and decay indices of 9–10 steps for the Sonoran and Mojave lineages, respectively (Fig. 2). Within the Mojave, Haploclades A and B were only weakly supported; bootstrap proportions = 53%–65% and decay values were 1–2 steps.

When using MrModeltest, the general time reversal plus invariant sites (GTR + G) model was selected for use in the Bayesian inference analysis ($-\ln L = 2111.7654$; $K = 9$; $AIC = 4241.5308$). Bayesian inference resulted in

Table 3. The distribution of mitochondrial deoxyribonucleic acid haplotypes from the Mojave desert tortoise, *Gopherus agassizii*.

Desert region/ recovery unit[a]	Group	Haplogroup A				Haplogroup B			Total
		MOJ-A01	MOJ-A02	MOJ-A03	MOJ-A04	MOJ-B01	MOJ-B02	MOJ-B03	
Western Mojave	1	2				1			3
	2	10		1					11
Central Mojave	3	6							6
	5	2							2
Southern Mojave	6	6	2						8
	7	7		1					8
	8	3							3
	9	5	1						6
	10	6							6
Northeastern Mojave	11					40	1	6	47
Eastern Mojave	12	8							8
Northern Colorado	13	3			1				4
Eastern Colorado	14	6				1			7
Upper Virgin River	15	1				4		1	6
Total		65	3	2	1	46	1	7	125

[a] Within the Mojave Desert, 2 major sublineages were resolved: Haplogroup A "broadly distributed", and Haplogroup B, Northeastern Mojave (Fig. 2). The greater relative sampling in the Northeastern Mojave (group 11) reflected an attempt to locate a haplotype from Haplogroup A.

a tree that was identical to the maximum parsimony consensus trees. The Bayesian posterior probabilities were higher than the bootstrap proportions (Fig. 2).

Microsatellite Evaluation. — Of the 16 loci surveyed in 628 desert tortoises (Table 1), 11 were highly variable and informative: Goag03, Goag04, Goag06, Goag07, GP15, GP19, GP30, GP55, GP61, GP81, and GP102. Five loci showed insufficient variation and were excluded from our analyses: GP26, GP96, Cm58, Goag05, and Goag32. For locus Goag03, only 2 study sites exhibited variation: groups 11 and 15 (Northeastern Mojave and the Upper Virgin River recovery units, respectively). For all microsatellite loci used in this study, individual genotypes were summarized by regional groups and are available from the Internet home page of RWM (www.zoo.utoronto.ca/drbob/publications).

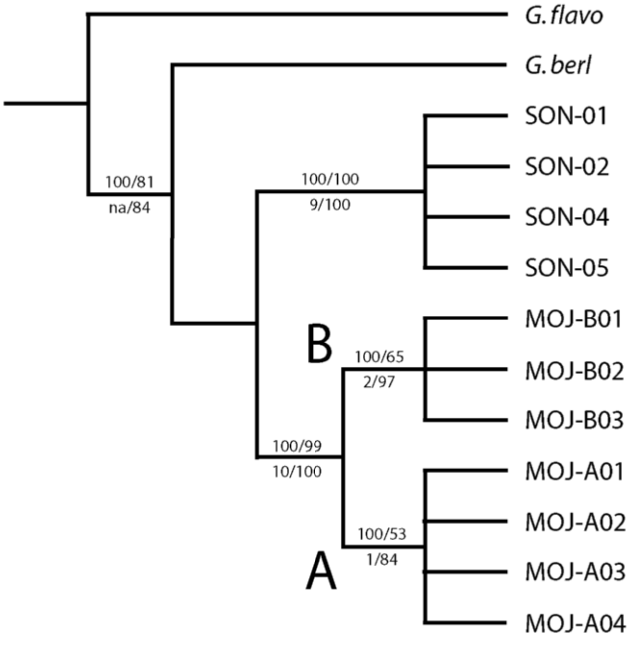

Figure 2. A 50% majority rule consensus tree based on maximum parsimony and Bayesian inference evaluations of the mitochondrial deoxyribonucleic acid sequence data from tortoises, genus *Gopherus*. SON = Sonoran and MOJ = Mojave populations of the desert tortoise (*Gopherus agassizii*) and outgroups G. berl (*G. berlandieri*) and G. flav (*G. flavomarginatus*). Numbers above the branches are given as frequency of resolution in the maximum parsimony evaluation/bootstrap proportions, and below as Bremer support/Bayesian posterior probabilities. Na = not applicable, and letters at nodes denote haplogroup lineages of Mojave populations discussed in text.

Major differences occurred between repeat motifs at some microsatellite loci in *G. agassizii* when compared with species or the population for which the locus was originally isolated, including GP19, GP30, GP61, GP81, and GP102 (Table 2). We were not able to precisely determine the motif for GP102 in *G. agassizii*. Homozygous amplicons were vague in the middle of the sequences, suggesting that 2 alleles were present. Fragment analysis did not allow determination of a heterozygous state (difference in repeat motifs) when amplicon lengths were equal. We did not clone these products to determine the competing sequences but rather made an arbitrary assignment of repeat numbers. Consequently, data for GP102 were not necessarily reflective of all possible heterozygous states.

Locus GP61 exhibited 2 different motif states; alleles having more than 16 repeats had a simple dinucleotide motif, $(GT)_{16+}$. However, alleles scoring in the range of 10–12 repeats had a compound motif, $(GT)_4AT(GT)_6$. As in the Sonoran population (Edwards et al. 2004), heterozygous individuals had both motifs. The simple motif had a greater range of allelic states than the compound motif.

Schwartz et al. (2003) originally described the compound motif for GP19 in *Gopherus polyphemus* as $(GT)_9/(GT)_3(GA)_6$. We found a dramatically derived state

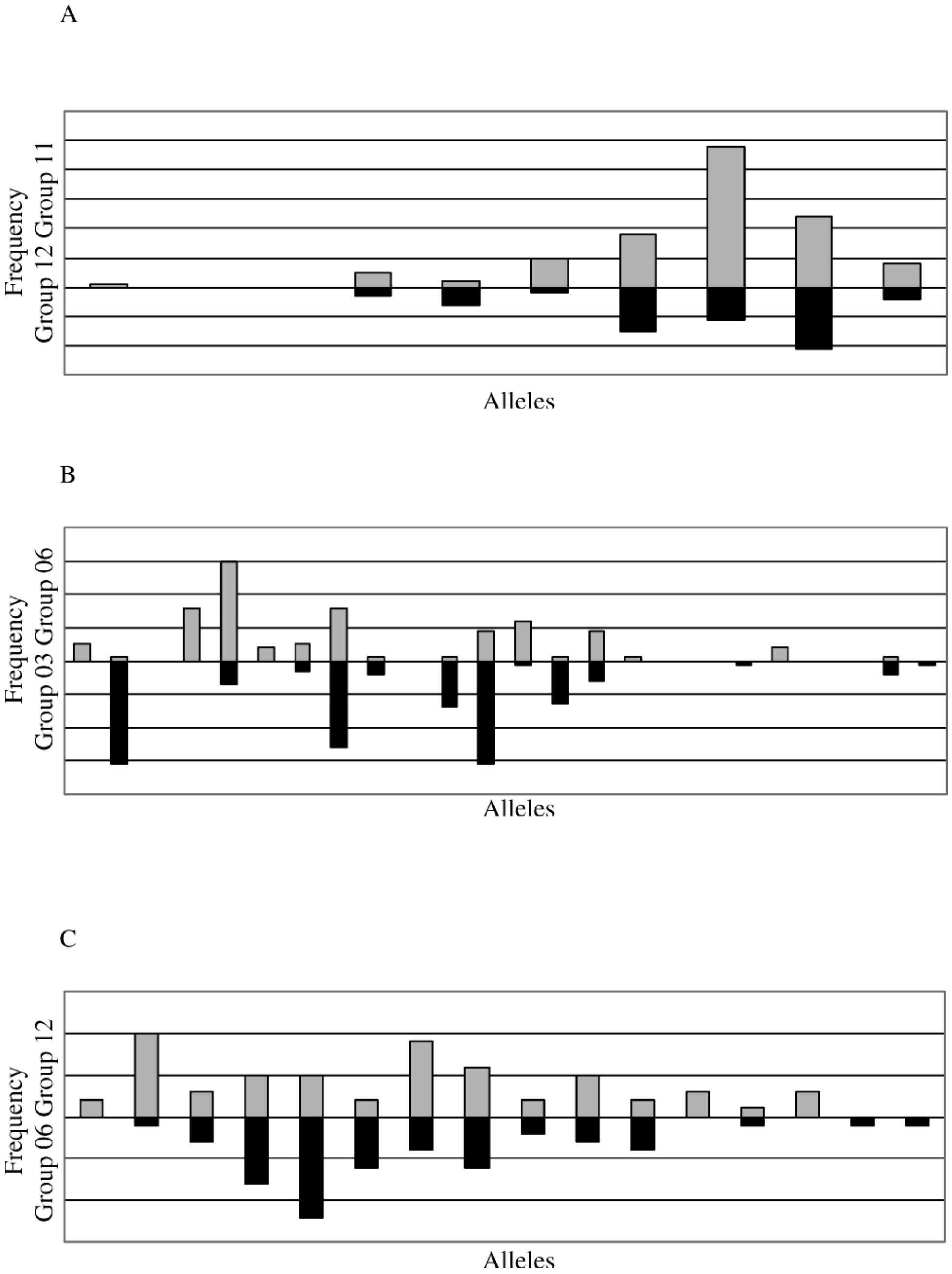

Figure 3. Comparison of allelic frequencies between sample groups of desert tortoises, *Gopherus agassizii*, from the Mojave population by using the G-based exact test for genotypic differentiation. Sample groups refer to Table 1. A: Locus GP81, $p = 0.024$, SE = 0.002; B: Locus GP102, $p < 0.001$, SE < 0.001; C: Locus Goag04, $p = 0.031$, SE = 0.003.

in our Mojave samples of *G. agassizii*, such that allele 11 sequenced as $(GT)_3/(GT)_2GAAA(GA)_4$ and allele 21 sequenced as $(GT)_7ATGTATGT/(GT)_2GAAA(GA)_5$. Consequently, we could not use analyses that required a stepwise mutation model, such as R_{ST} (Slatkin 1995).

Some dinucleotide loci exhibited imprecise pherograms (e.g., stutter peaks) when the number of repeats exceeded 25. A score of "35" could not be differentiated from "34" or "36". Consequently, pherograms were scored by using a standardized rule set for consistency with error on the conservative side. Loci GP15, GP61, GP102, and Goag06 may have reached the upper limits of our ability to detect repeat numbers, because larger amplicons had very low intensity pherograms. Generally, alleles with more than 55 repeats were not scored, and, thus, we likely missed some alternative alleles.

The distributions of allele size classes for most loci were not normally distributed. Some were highly skewed, and others exhibited multiple peaks (Fig. 3). Unique and private alleles were detected in several sample groups at some of the more variable loci. In some cases, private alleles comprised a high proportion of the alleles observed within a population. For example, sample group 14 had 4 alleles at GP30; the private allele composed 25% of all alleles (Table 4) but it occurred at a frequency of < 5%.

Table 4. Distribution of unique and private alleles in 15 sample groups (summarized in Table 1) of desert tortoises from the Mojave population.[a]

Sample	GP61			GP19			GP102			GP30			GP55			GP15			GP81			Goag4			Goag06			Goag7			Goag3		
group	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%	T	U	%
1	14			2			7			9	1	11	9			17			7	2	29	16			26			5			1		
2	15			2			14			8	1	13	8	1	13	24	1(1)	4.2	5			17	1	5.9	22			4			1		
3	9			2			9			5			8	1	13	15			5			13			16			4			1		
4	11			2			11			7			11			14			6			12			19			4			1		
5	13			2			11			7	1	14	12	1	8.3	16			5			15			21			5			1		
6	10			2			10			4			6			9			6			13			18			4			1		
7	16			2			13	1	7.7	8	2	25	7			21			7			12			21			4			1		
8	14			2			15			7			11			20	1	5	7	1	14	11			33			5			1		
9	11			2			12			6	1	17	7	1(1)	14	10			7			12			22			5			1		
10	13			2			7	1(1)	14.3	4			7			14	1	7.1	7			9			14			4			1		
11	15			2			16	1	6.3	8			6			19			8	1	13	16			19			6			2		
12	18			2			12			9			7			15			7			14			15	1	6.7	4			1		
13	13	1	7.7	2			12			10	2(1)	20	5			14			7			11	1	9.1	14			5			1		
14	15	1(1)	6.7	2			12	1(1)	8.3	4	1	25	9			13			6			11	1(1)	9.1	21			5			1		
15	11			1			12	1(1)	8.3	7			3			15			5			10			15			3			2		
Total	27			2			24			21			17			37			11			22			49			5			2		

[a] T = total number of allelic states observed in a sample group; U = number of alleles unique to the sample group; parenthetic values are the number of unique alleles that occur at a frequency > 5% (private alleles) in a sample group relative to the total number of alleles; % = the percentage of alleles that are unique in a sample group [(U/T) × 100]. No private alleles in a population occurred at a frequency > 8%.

The frequency of occurrence for the relatively rare, private allele was always ≤ 8%.

Most sample group pairwise comparisons between distributions of allelic frequencies (Fig. 3) were found to be significantly different by the G-based Exact test (Goudet et al. 1996). Three sample groups deviated from H-W in exhibiting a greater number of heterozygotes than expected (Table 5). By using a 5% cutoff, about 1 deviation is expected for each locus, except for Goag3. Three loci showed excessive deviations from expectations in the form of heterozygote deficiencies: GP30, G81, and Goag06. In total, 24.5% of the data points showed deviations from H-W, with 8.6% owing to Goag06 alone (Table 5).

Garnier-Gere's and Dillmann's (1992) test rejected the null hypothesis for linkage disequilibrium (equilibrium for locus pairs) for 45 (of 165) locus pairs within 15 sample groups. Nine sample groups had a percentage of total pairwise comparisons with p-values > 0.05 (range 0.0%–26.7%). However, locus pairs did not consistently exhibit disequilibrium among groups.

Bayesian likelihood values for all runs by using STRUCTURE typically stabilized after 50,000–100,000 iterations after burn-in. The analyses obtained the lowest average Ln for 6 subpopulations (Table 6). These subpopulations were concordant with the recommendations in the *Recovery Plan*. Because substantial differentiation was observed in the Western Mojave Recovery Unit, as revealed by Φ_{ST} values, we removed populations 11–15 and performed a new analysis to reduce the affects of IBD. This analysis suggested that the current Western Mojave Recovery Unit supported 4 subpopulations (Table 6): sample groups 1–2, 3–5, 8, and 6–7 plus 9–10 (Fig. 4).

A 2-dimensional, monotonic MDS plot displayed population differentiation among sample groups (Fig. 5). It had a stress of 1.39, a fair to good fit by Kruskal's and Wish's (1978) index. The 15 sample groups clustered complementary to their geographic proximities, as anticipated when assuming gene flow. Geographically distant sample groups 11 and 15 were noticeably separated from the other groups.

Population assignment tests correctly placed the majority of individuals back to their sample groups with high stringency (Table 7). Individuals not assigned to a sample group were frequently assigned to a geographically nearby group or to one within the same region. Geographically proximate groups 12 and 13 occurred near the boundary of 2 desert regions, the eastern Mojave Desert and northern Colorado Desert (Fig. 1). The population assignment evaluations had difficulty distinguishing individuals between these 2 recovery units. Whereas, 80% of the samples from group 11 were correctly assigned, only 48% of 31 samples from group 12 were correctly assigned. However, 87% of tortoises from group 12 were correctly assigned to groups 12 and 13 combined, indicating that, in this case, geographic proximity was a better predictor of genetic structuring

Table 5. Summary of deviation from Hardy-Weinberg expectations for 11 variable microsatellite loci and 15 sample groups of the desert tortoise, *Gopherus agassizii.* Sample groups refer to Table 1.

Locus	No. comparisons	No. heterozygote excess	No. heterozygote deficiency	Range in no. of repeats
GP61	15	0	2	11–38
GP19	14	0	0	11–21
GP102	15	1	1	19–42
GP30	15	0	7	10–17
GP55	15	0	3	7–30
GP15	15	0	2	13–52
GP81	15	0	6	16–28
Goag4	15	1	0	12–32
Goag06	15	0	13	17–67
Goag7	15	1	0	13–28
Goag3	2	0	0	6–7

than recovery unit. A similar trend was discovered for tortoises in group 13.

When sample groups were combined to reflect current recovery units, and when sample groups 12 and 13 were combined, assignment scores of $\geq$ 80% were obtained (Table 7). For the Western Mojave Recovery Unit, we deleted geographically distant sample groups (1, 2, 11–15) and re(-)ran the assignment test. We combined samples 3–5 and samples 6–10, because they had higher proportions of misassigned individuals than all other units (Table 7). Although not given in Table 7, the percentage of individuals correctly assigned to the proposed Central Mojave (samples 3–5) and Southern Mojave (samples 6–10) recovery units combined was 52% each, with 24% being assigned to the combined unit as the second most likely assignment and 13% assigned to the adjacent Western Mojave Recovery Unit.

Finally, we combined the sample groups to reflect geographic regions, which reflected the current recovery units (Table 7). This treatment recognized variation within the Western Mojave Recovery Unit. In total, 8 regions were identified. Assignment scores ranged from 59.6% to 95.7%. The more fine-grained analyses, those that included a greater number of subdivisions, yielded lower assignment scores.

Geographic substructuring was further assessed by breaking and recombining specific units. The assignment tests produced 96%–98% accuracy when the distribution of tortoises was divided into 2 groups: Northeast (11, 15) and Central (1–10, 12–14), respectively. When geographically proximate groups were split and recombined, the assignment tests invariably decreased, some to less than 50% (sample groups 2, 6, and 8).

The hierarchical analysis of molecular variance indicated the absence of panmixia; significant genetic structuring was discovered. The AMOVA revealed that 93.9% ($p < 0.001$) of the observed variation was partitioned among individuals within sample groups ($\Phi_{IT} = 0.939$), whereas only 6.1% of the variation was among the sample groups ($\Phi_{ST} = 0.061$, $p < 0.001$). The positive significant correlations between genetic distance (pairwise Φ_{ST}) and geographic distance accounted for approximately 65% of the observed variation (Mantel test; $r^2 = 0.646$, $p = 0.002$).

By using BOTTLENECK, we detected a significant excess in heterozygosity in 2 sample groups, 11 and 15, the Northeastern Mojave and Upper Virgin River recovery units. The Wilcoxon Test with the (infinite alleles model [IAM]) detected an excess in both groups but the Sign Test (IAM) method of Piry et al. (1999) identified group 15 only. No deficit or excess in heterozygosity was detected when the data for all groups were combined. All sample sets fit the expected beta distribution (Cornuet and Luikart 1996), thus providing no evidence for bottlenecking. By using the method of Garza and Williamson (2001) to detect potential reduction in population size, all values of M fell above the critical value M_C. However, the results may not be reliable, because this test assumed stepwise mutation.

Human-Mediated Translocations. — Native Americans undoubtedly moved desert tortoises from one place to another (as implied in Schneider and Everson 1989). The distances were probably limited, except for annual gatherings for mourning ceremonies (i.e., Las Vegas Band, Southern Paiute: Kelly, no date) and the result may have been death for the tortoises.

Throughout the 20th century, tortoises were captured for domestic pets and were translocated for various purposes. Captive tortoises currently or formerly kept by residents of desert communities often escape or are deliberately released into adjacent desert lands. The sources of the captives may or may not be local relative to the point of escape or release. Escaped captives are so common that a publication gives actions to take when a former captive is found (Berry and Duck, 2006). Captives have been observed wandering within city limits or nearby in Ridgecrest, Barstow, Ft. Irwin, Victorville, and Twentynine Palms in the Western Mojave Recovery Unit; Needles in the Eastern Mojave Recovery Unit; Las Vegas in the Northeastern Mojave Recovery Unit; and St. George in the Upper Virgin River Recovery Unit. Tortoises are often taken to or released at protected areas such as parks and Natural Areas (Howland 1989; Ginn 1990; Jennings 1991; Connor and Kaur 2004).

Thousands of tortoises were released in the southwestern deserts by humane societies, California Department of Fish and Game, Nevada Department of Wildlife Resources, Utah Division of Wildlife Resources, State and National Park personnel, academicians and others (Fig. 6). Data are limited before the 1960s, but releases were documented for California and Utah (Hardy 1945; Woodbury and Hardy 1948; Jaeger 1950, 1955). Woodbury and Hardy (1948) surveyed Beaver Dam Slope, Utah (Northeastern Mojave Recovery Unit) for tortoises between 1936 and 1946. At least 6.1% of 281 tortoises found showed signs of previous captivity. Releases also occurred in the

Table 6. Inferred population structure obtained from the software program STRUCTURE 2.1 for all samples, and for a subset of samples from the current Western Mojave Recovery Unit (sample groups 1–10).[a]

All samples ($n = 628$)

	Ln (variance below)				
K	Run 1	Run 2	Run 3	Run 4	Average Ln
1	–25,140.5	–25,144.0	–25,143.6	–25,143.3	–25,142.9
	99.7	106.1	106	105.8	
2	–24,362.2	–24,360.6	–24,360.8	–24,361.2	–24,361.2
	463.9	460.7	462.6	463.3	
3	–23,644.7	–23,646.2	–23,647.9	–23,648.6	–23,646.9
	568.4	570.5	572.8	574.9	
4	–23,283.3	–23,275.4	–23,269.5	–23,272.6	–23,275.2
	827.5	810.6	800.5	804.8	
5	–23,134.7	–23,038.1	–23,030.7	–23,042.5	–23,061.5
	1049.5	1056.0	1041.2	1062.6	
6	–22,881.4	–22,886.7	–22,883.4	–22,893.2	–22,886.2
	1249.2	1260.3	1251.2	1275.1	
7	–23,042.2	–22,840.3	–24,213.8	–24,745.5	–23,710.5
	1921.8	1521.7	4220.5	5220.9	
8	–22,901.4	–23,454.5	–23,144.8	–22,964.3	–23,116.3
	1712.3	3043.6	2204.3	1858.5	
9	–23,538.9	–24,007.6	–22,951.0	–23,041.1	–23,384.7
	3494.4	4412.3	2335.7	2230.9	
10	–22,857.7	–24,696.7	–22,900.7	–22,900.7	–23,339.0
	2208.1	5872.7	2262.5	2280.9	
11	–23,305.8	–24,272.3	–24,176.7	–24,377.2	–24,033.0
	3318.1	5406.3	5027.1	5490.7	
12	–23,236.8	–24,848.4	–23,590.5	–34,317.7	–26,498.4
	3426.8	6666.9	4129.0	25,502.9	
13	–24,346.5	–23,339.1	–34,657.2	–28,975.2	–27,829.5
	5879.4	3820.1	26,339.3	15,064.1	
14	–31,546.3	–560,553.8	–31,303.2	–24,971.2	–162,093.6
	20,362.5	1,077,674.6	19,809.4	7242.0	
15	–133,340.8	–28,256.8	–27,197.9	–41,616.9	–57,603.1
	223,973.3	13,936.0	11,869.1	40,664.7	

Western Mojave samples ($n = 459$)

K	Run 1	Run 2	Run 3	Run 4	Average Ln
1	–17,343.6	–17,342.7	–17,338.4	–17,339.0	–17,340.9
	99.8	97.2	90.7	90.8	
2	–16,870.6	–16,871.0	–16,870.0	–16,873.2	–16,871.2
	405.0	406.7	405.5	411.5	
3	–16,968.7	–16,715.6	–16,722.3	–16,626.4	–16,758.3
	1218.3	693.6	847.8	657.2	
4	–16,438.7	–16,434.3	–16,432.9	–16,438.4	–16,436.1
	874.5	863.0	860.4	871.3	
5	–16,380.9	–16,404.5	–16,419.0	–18,206.9	–16,852.8
	1068.9	1114.4	1143.6	4629.7	
6	–16,742.5	–16,392.3	–16,418.5	–17,106.1	–16,664.9
	1876.6	1163.9	1217.5	2750.5	
7	–16,778.8	–17,811.3	–16,450.6	–18,021.6	–17,265.6
	2430.1	4440.4	1540.5	4871.7	
8	–16,343.7	–18,314.1	–18,520.9	–16,417.4	–17,399.0
	1837.0	5698.8	5924.8	1746.6	
9	–20,559.6	–17,456.7	–16,346.8	–19,067.6	–18,357.7
	10,289.0	4207.3	1842.1	7354.0	
10	–18,184.4	–406,665.0	–19,777.8	–21,971.6	–116,649.7
	5770.3	780,420.0	8955.7	13,321.4	

[a] K = the number of populations set as the a priori for the simulation; Ln = the log likelihood of the data averaged over all iterations after burn-in (with variance reported below); and the average Ln for all 4 runs for a given simulation. (For all simulations: 250,000 iterations per run with a burn-in of 5000).

vicinity of St. George and the Upper Virgin River Recovery Unit (Hardy 1945).

From the late 1960s to the mid 1970s, the California Department of Fish and Game sponsored numerous captive releases and kept records for > 800 individuals (Fig. 6). Their last official release was the rehabilitation experiment at the Quarterway and Halfway Houses in the Living Desert Reserve and Ft. Soda, respectively, in the late 1970s. Among 200 tortoises initially in the program, 30 survived, only to be moved to private lands in the Antelope Valley (Cook et al. 1978; Weber et al. 1979; Cook 1983).

In Nevada, the first documented releases of captive tortoises occurred on the Desert Game Range in 1973 (B.L. Burge, *pers. comm.*, December 2005; Fig. 6). In the late 1970s and early 1980s, employees of the Nevada

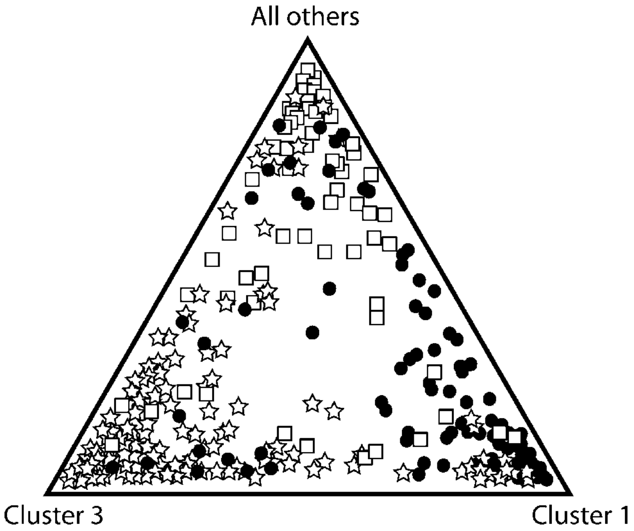

Figure 4. Triangle plot of the estimated membership coefficients for each individual in the Western Mojave Recovery Unit. Symbols correspond to sampling groups (given in Table 1) when the number of populations (K) is K = 3: circles = sample groups 1 and 2, squares = sample groups 3–5, stars = sample groups 6–10. Note the general clustering in the corners of each group and the overall pattern of admixture (gene flow). The cluster of stars in the circle samples depicts individuals mostly from Group 8, which is geographically the most proximate to the circle sample group.

Department of Wildlife Resources released hundreds of captive tortoises onto desert lands (R.J. Turner, *pers. comm.*, December 2005).

State and federal agencies approved the release of numerous captive and wild tortoises in 1997 at a long-term release site in southern Nevada (Field 1999). Additional translocation projects occurred throughout Nevada between 1990 and 2005 (Corn 1991; Nussear 2004; Charles Le Bar, *pers. comm.*, December 2005).

Between 1973 and 1983, the Utah Division of Wildlife Resources released at least 195 captive tortoises on Beaver Dam Slope (Coffeen, *pers. comm.*, December 2005; Coffeen 1984, 1985). In 1980, a general survey conducted throughout 324 km^2 of the area revealed that 21.9% of 105 located tortoises were marked captives (Minden 1980). Tortoises were also released on the historical Woodbury and Hardy (1948) site; when the study site was surveyed in 1981, 23.3% of the 73 tortoises observed were marked captives (Minden and Keller 1981). In the mid to late 1980s, captive tortoises were released in the Upper Virgin River Recovery Unit at Grapevine Pass and Red Cliffs Recreation Area (Coffeen 1986); 71 captive tortoises were also released at Hurricane Cinder Knolls (McLuckie, unpubl. data, 2006).

Evidence exists of a substantial transfer of tortoises from the western Mojave Desert in California to Utah. In April of 1970, 2 wardens arrested a commercial collector who claimed to have taken thousands of tortoises from the Western Mojave Recovery Unit of California between the 1960s and April 1970 and sold them commercially in Salt Lake City, Utah (Berry 1984). Some of these tortoises may have been released on the Beaver Dam Slope and north of St. George in the 1970s and early 1980s in what are now the Northeastern Mojave and Upper Virgin River recovery units.

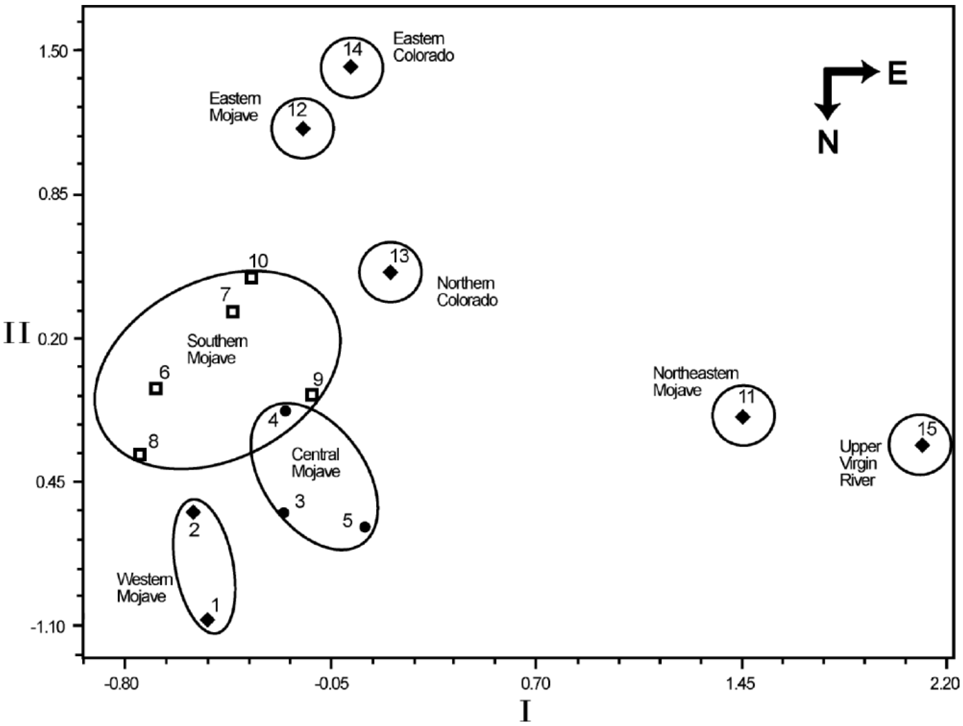

Figure 5. A 2-dimensional scaling plot of genetic distances (Φ_{ST}) for 15 sample groups of desert tortoises, *Gopherus agassizii*, from the Mojave population. Open squares and solid circles indicate samples from the southern and central Mojave Deserts, respectively.

Table 7. Population assignment tests for desert tortoises from the Mojave population and 8 desert regions or recovery units. The initial evaluation treated all 15 sample groups separately. The second treatment combined tortoises into units reflecting the recovery units recommended in the 1994 *Recovery Plan* except for combining sample groups 12 and 13. The third treatment considered populations on the basis of existing and proposed recovery units.

Sample group	No. samples	No. correctly assigned	% Correctly assigned	% With LOD > 2[a]	No. assigned to same region or neighboring group	% Assigned to same region
1	62	42	67.7	58.1	8	80.6
2	83	26	31.3	19.3	16	50.6
3	19	10	52.6	47.4	3	68.4
4	31	11	35.5	22.6	11	71.0
5	47	25	53.2	51.1	12	78.7
6	26	12	46.2	42.3	11	88.5
7	71	20	28.2	19.7	37	80.3
8	74	34	45.9	35.1	13	63.5
9	27	8	29.6	14.8	14	81.5
10	19	10	52.6	52.6	5	78.9
11	60	48	80.0	78.3	0	80.0
12	31	15	48.4	38.7	12 (to group 13)	87.1
13	18	10	55.6	27.8	3 (to group 12)	72.2
14	37	28	75.7	59.5	0	75.7
15	23	22	95.7	91.3	0	95.7
Combined groups						
15	23	23	100			
11	60	51	83.3	10		
12, 13	49	41	81.6	8.2		
14	37	35	91.9	5.4		
1–10	459	377	80	8.5		
Region						
Western Mojave	164	139	84.8			
Central Mojave	97	66	68.0			
Southern Mojave	198	118	59.6			
Northeastern Mojave	60	49	81.7			
Eastern Mojave	31	17	54.8			
Northern Colorado	18	13	72.2			
Eastern Colorado	37	33	89.2			
Upper Virgin River	23	22	95.7			

[a] LOD = log of the odds ratio.

DISCUSSION

Maternal History. — Two distinctive maternal lineages exist, one associated with the Sonoran population in Arizona and the other with the Mojave population. By using *G. flavomarginatus* as the outgroup, the sister group to *G. agassizii* was *G. berlandieri* (Fig. 2). This resolution differed from that of Lamb et al. (1989). Rooting with the same outgroup, they found that the Sonoran *G. agassizii* was the sister group of *G. berlandieri* and exclusive of the Mojave population. The difference could have resulted from several factors. Lamb et al. (1989) evaluated restriction fragment length polymorphisms, and we used more precise sequences. They also had greater taxonomic and geographic sampling. Although we might have reached a similar conclusion if we had used the same coverage, this was unlikely. The difference likely resulted from their use of presence/absence coding of nonhomologous fragment lengths.

Within Mojave population samples, little differentiation occurred among the 7 haplotypes (Fig. 2). Two primary maternal sublineages occur in the Mojave population, but the minor level of differentiation was not indicative of taxonomic differentiation. In contrast, the substantial sequence differentiation between Mojave and Sonoran (Arizona) populations is consistent with the hypothesis that *G. agassizii* consists of more than one species (Berry et al. 2002).

Descriptive Statistics of Microsatellite nuclear DNA (nDNA). — The motif differences in interspecies amplification of microsatellite loci indicated that evaluation of data required species-specific and even population-specific sequence information. Loci amplified between species (and within species too; Estoup et al. 2002.) did not necessarily follow assumptions of the stepwise mutation model.

Deviations from H-W could have several sources. Excess of homozygotes at some loci (e.g., Goag06) could have resulted from nonamplifying alleles, as a consequence of motif anomalies. Translocations of tortoises throughout the Mojave population also might have contributed to the excess of heterozygosity. For cases of heterozygotic deficit, ambiguities associated with high numbers of repeats might have artificially inflated the number of observed homozygotes or elevated Φ_{IS} values if translocated tortoises had very different allele frequencies

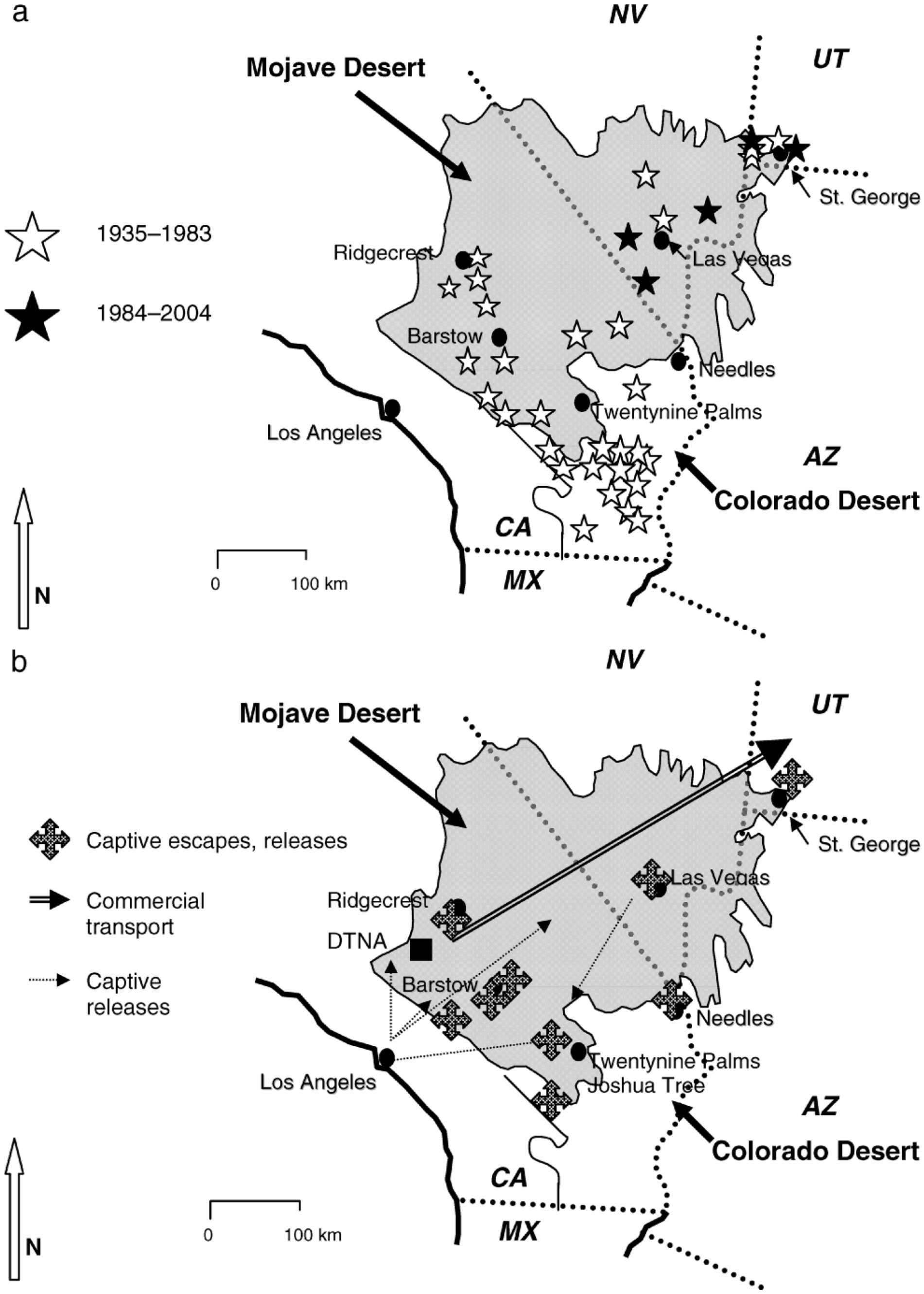

Figure 6. (a) Locations of captive desert tortoises, *Gopherus agassizii*, released by the California Department of Fish and Game, Nevada Department of Wildlife, Utah Division of Wildlife Resources or by others, as described in government reports and university theses and dissertations. The shaded area indicates the limit of the Mojave Desert. (b) Locations of areas where captives escaped or were released outside of desert towns. Tortoises were taken from the Los Angeles basin and released at places such as the Desert Tortoise Research Natural Area (DTNA) or Joshua Tree National Park. There were also large-scale commercial transfers of tortoises.

(a Wahlund effect, lower than expected heterozygosity owing to population substructuring). Technical difficulties of accurately scoring heterozygotes with high numbers of repeats surely contributed to the estimates of heterozygosity deficiencies at Goag06 and possibly at other loci (Table 5). Unfortunately, the proportions of misscored loci cannot be accurately partitioned from the data set to examine for a Wahlund effect (e.g., Chapuis and Estoup 2007).

In total, 24.5% of the data points showed deviations from H-W in the form of heterozygote deficiencies (Table 5). Such deviations may not significantly affect our conclusions. Dankin and Avise (2004) showed that 20% of the data points can deviate from H-W, without affecting the accurate determination of parentage. Empirically, the great correspondence between the results of the microsatellite analyses and ecological boundaries supports our assumption of the utility of the data irrespective of their deviations from H-W expectations.

For tortoises, IBD (isolation-by-distance) affected the probability of individuals mating with one another and violated the assumption of panmixia for statistical tests. Significant pairwise associations of some loci (Table 5) may have reflected an absence of panmixia (i.e., a Wahlund effect), mating systems or problems in resolving alleles. However, because significant linkage disequilibrium was not observed in all groupings, this explanation was unlikely. The greater than expected deviations from H-W were strongly paralleled by Φ_{IS} values. Some deviations from H-W owed to technical constraints (e.g., Goag06), but this was unlikely for other loci (e.g., GP30, GP81). Some positive inbreeding coefficients and departures from H-W may have been because of population structure. However, inbreeding was unlikely to have occurred because most loci did not have significant Φ_{IS} values within a sample group.

Gene Flow. — Genetic structuring was strongly associated with geography (Slatkin and Maddison 1990), IBD, and the limited dispersion of individual tortoises (Mantel test; $r^2 = 0.646$, $p = 0.002$). The results of the AMOVA indicated the absence of panmixia. IBD was also reported by Britten et al. (1997) for allozyme and mtDNA data, and by Edwards et al. (2004) for Sonoran tortoises. Microsatellite variability was greater within than among sample groups, suggesting that the Mojave metapopulation was relatively homogeneous, i.e., the common alleles were broadly distributed. Gene flow likely occurred throughout populations in California, at least until the recent proliferation of anthropogenic barriers. The distribution of low-frequency, unique microsatellite alleles supported the hypothesis that the genetic structure resulted from gene flow and not common ancestry. Indeed, Edwards et al. (2004) noted that desert tortoises were ideal organisms for applying the IBD model, because they are distributed across the landscape in patches, and the difficulty of dispersion is a function of geography.

Bottlenecking. — The excess of heterozygosity in samples from the Northeastern Mojave and Upper Virgin River recovery units could have resulted from recent bottlenecking. However, this possibility was not supported by the ratio of the total number of alleles to the overall range in allele size. Population declines in the Northeastern Mojave and Upper Virgin River recovery units have been well documented in recent years (USFWS 1980; Minden and Keller 1981; Fridell and Coffeen 1993; McLuckie et al. 2004). Although other regions also experienced population declines (Berry and Medica 1995; Brown et al. 1999; Christopher et al. 2003), they did not show genetic evidence of bottlenecks. This inconsistency may have been because of at least 4 factors. First, our samples were collected over 10 years and this could have precluded the effects of recent declines. Second, the time frame for sampling may have been too short for observing a shift in heterozygosity for a long-lived species with a long generation time. Garrigan and Hedrick (2003) reported that 5–10 generations were required to genetically detect bottlenecks. Moreover, Dinerstein and McCracken (1990) did not see bottleneck effects in the greater one-horned rhinoceros by using microsatellite DNA, despite well-documented evidence. Consequently, conclusions on the genetic structure of populations should not be based on molecular evidence alone but should accompany field observations. Third, polyandry, if common, and especially when combined with sperm storage, could have increased the effective population size (Sugg and Chesser 1994). Sperm storage for up to 3 years has been documented in the desert tortoise (Palmer et al. 1998) and anecdotal evidence suggests that it may occur for much longer. (One isolated captive female tortoise produced viable clutches for 15 years after her last known association with a male tortoise; P. Gould Glasco, *pers. comm.*, May 2006.) A controlled investigation of polyandry in the western Mojave Desert found that all females produced polyandrous clutches over a period of 2 years (Murphy, Edwards, Bratton, and Hagen, in prep.). And fourth, the observed increase in heterozygosity in the Northeastern Mojave and Upper Virgin River recovery units may also be a reflection of translocated tortoises. The translocation of gravid females or those that were storing sperm would serve to compound the possible explanations for excess heterozygosity.

Human-Mediated Translocations. — Translocations and releases of animals, especially if uninformed, can have negative genetic consequences (Allendorf and Luikart 2007). The historical releases and translocations of tortoises could have affected our results in the form of deviations from the H-W, increased heterozygosity and estimates of recent gene flow, anomalous distributions of some haplotypes, and increased Φ_{IS} values (through a Wahlund effect). The geographically disjunct occurrence of some haplotypes (MOJ-A01 with -B01 and -B03 in the Upper Virgin River; Table 3) could be caused by translocations. Because the widespread MOJ-A01 haplotype was absent in our initial survey of 7 tortoises in the Northeastern Recovery Unit only, we sequenced 40 additional samples: in total, 40 were MOJ-B01, 6 were MOJ-B03, and 1 was MOJ-B02. Because MOJ-A01 was absent from the Northeastern Recovery Unit, its presence in Upper Virgin River Recovery Unit was likely because of relocated tortoises. Our samples from the Northeastern Mojave were taken from relatively remote areas where the releases of captives were less likely.

Several other incidences of geographic mixing are evident: MOJ-B01 is geographically and genealogically associated with other members of Haplogroup B, but it also occurs in sympatry with Haplotypes MOJ-A01 (Haplogroup A) in the Western Mojave Recovery Unit (Table 3), specifically at the Interpretive Center at the Desert Tortoise Research Natural Area. This finding is concordant with documentation of multiple captive tortoise releases at the Natural Area (Howland 1989; Ginn

1990; Jennings 1991; Connor and Kaur 2004). Haplotype MOJ-B01 also occurs with MOJ-A01 in the Eastern Colorado Recovery Unit. Very long distance dispersion is the alternative explanation for the widespread occurrence of some haplotypes. Given the extent of documented translocations, the dispersion hypothesis is unlikely, particularly because our data lack other evidence of population expansion or recent ancestry.

Translocated tortoises could compromise the genetic integrity of a population by disruption to coadapted gene complexes in local environments or loss of fitness through outbreeding depression. In particular, Beaver Dam Slope, Utah, has a high frequency of released captive tortoises (Woodbury and Hardy 1948, Minden 1980, Minden and Keller 1981). Although we do not have genetic samples from this area, the excess of heterozygotes in the adjacent Upper Virgin River and Northeastern Mojave recovery units, in the absence of a decrease in the ratio of the total number of alleles to the overall range in allele size, could reflect first- or second-generation offspring from translocated tortoises. A similar problem may exist at the Desert Tortoise Research Natural Area and Joshua Tree National Park in the Western Mojave Recovery Unit. Outbreeding depression can lead to reduced fitness via disease in hybrid populations (Goldberg et al. 2005, Allendorf and Luikart 2007). The high levels of assignments of tortoises to the correct region (Table 7) indicate that, in some cases, survival rates of released tortoises may be low, e.g., the early California reintroduction experiments (Cook et al. 1978; Cook 1983; Weber et al. 1979).

Regional Differentiation. — The STRUCTURE analysis identified from 5 to 8 genetically structured units. These findings support the hypothesis of population structure in the *Recovery Plan* and the Desert Wildlife Management Units described in the Western Mojave Recovery Unit. When considering the close geographic proximity of some of our sample groups (e.g., groups 12 and 13), this result was consistent with our assumption that the Mojave population is genetically structured and that these genetic data were informative for designating recovery units. Sample group 8 may have the most admixture between the "Central" and "Southern" areas of the Western Mojave Recovery Unit. This subanalysis suggested that the Western Mojave Recovery Unit could be subdivided into at least 3 geographic groups. Although STRUCTURE is not a good measure of structure in populations that exhibit nonlinear patterns of IBD (Pritchard et al. 2000), the findings were congruent with the *Recovery Plan* and natural barriers to gene flow. Thus, we used these results as evidence for the assessment of recovery units.

The null hypothesis of a single, homogeneous, panmictic Mojave population was rejected. Although most alleles were broadly distributed, most sample groups significantly differed from one another in allelic frequencies (Table 7). Because the G-based exact test is sensitive to different sample sizes, as in our data, the imbalance in samples might have accounted for the high number of significant differences. However, this does not appear to be true. Most individuals ($> 80\%$) were reassigned (Table 7) back to their sample group. The accuracy of the assignments implies genetic divergence.

The population assignment was viewed as a conservative result. Our data set was limited to 11 variable microsatellite loci only. Additional loci would have likely increased the accuracy of the assignments and the distinctiveness of each recovery unit.

Congruent patterns of genetic differentiation from different regions or taxa lend credence to conclusions. Comparatively, desert tortoises from Mojave and Sonoran populations had almost identical genetic structuring at local and regional levels. The AMOVA of microsatellites from the Sonoran population revealed that 96.3% ($p < 0.001$) of the diversity occurred in individuals within study sites ($\Phi_{IT} = 0.963$), whereas only 3.7% ($p < 0.001$) of the variation was among sites ($\Phi_{ST} = 0.037$) (Edwards et al. 2004). The same result occurred in a geographically equivalent sized subset of our data; $\Phi_{ST} = 0.037$ ($p < 0.001$). In both studies, a significant positive correlation occurred between genetic distance (pairwise Φ_{ST}) and geographic distance.

Recovery Units Revisited

The authors of the *Recovery Plan* proposed 6 recovery units to capture the known genetic, morphological, ecological, and behavioral diversity in desert tortoises as of 1993 (USFWS 1994). Their original objectives agree with the views of Crandall et al. (2000), specifically to preserve the options for adaptive diversity and evolutionary processes, maintain a network of populations, reduce the likelihood of further contraction of the geographic range, and minimize homogenization of the gene pool or pools by anthropogenic activities. The recovery units in the *Recovery Plan*, with some exceptions described below, appear to reflect natural, biological differences in populations and to fall within the DPSs described in government policy (US Department of the Interior and US Department of Commerce 1996).

We emphasize, however, that the genetic evidence presented here is not necessarily concordant with or related to morphological, ecological, and behavioral differences observed in the tortoise populations. Genetic evidence is only one factor among many that should be considered in managing desert tortoises (Crandall et al. 2000; DeSalle and Amato 2004; Green 2005). No direct evidence suggests that the mtDNA and microsatellite markers reflect the observed phenotypic differences and local adaptations, although the assumption is that identified genetic markers may serve as surrogates for these and other character traits (Pearman 2001). Behavioral differences between populations can be genetically linked, as in the case of garter snake food habits (Arnold 1981) and morphological variability in turtles can be heritable (Myers et al. 2006).

In the absence of data linking genotypic markers with specific phenotypic characters or adaptations in desert tortoises, we are confined to delineating recovery units based on available information, such as the differences in mtDNA and microsatellite markers described here, as well as differences in vegetative communities, physical attributes of the habitat, climate (e.g., mean number of freezing days annually, mean annual precipitation, amounts of precipitation occurring in summer), choice and availability of forage plants, cover sites (burrows, dens), and denning behavior.

The direct translation of molecular data into management units is subjective. On one extreme, it is possible to define 2 recovery units, based on the arbitrary subdivision of assignment values. However, the STRUCTURE analysis indicated the presence of at least 6 genetically cohesive units. Although this evaluation was compromised by multidimensional IBD, when we reduced the effects of IBD, 4 additional genetic units were identified in the Western Mojave Recovery Unit: sample groups 1–2, 3–5, 8, and 6–7 plus 9–10 (Fig. 4). Ultimately, the designation of recovery units must synthesize all relevant factors to achieve effective management.

Our analyses indicate that the Western Mojave Recovery Unit should be divided into 3 regions (western, southern, and central) and 3 corresponding recovery units: Western Mojave, Central Mojave, and Southern Mojave (Table 8, Fig. 7). Although the analysis by using STRUCTURE discovered 4 genetic units within the Western Mojave, the segregation of 1 site (8) would not facilitate effective management. Our proposed recovery units are similar to the 3 Desert Wildlife Management Areas described in the *Recovery Plan* and are concordant with the western, southern, and central regions of the Mojave Desert described by botanists and climatologists (Rowlands et al. 1982; Rowlands 1995a, 1995b). The western, central, and southern Mojave regions differ primarily in the amounts of summer rainfall, number of freezing days, and mean January minima and mean July maxima temperatures, as well as in species richness (vegetation) and types and composition of plant species with different metabolic pathways, e.g., C_3, C_4, and Crassulacean acid metabolism (CAM). The redefined Western Mojave Recovery Unit (Fig. 7) receives precipitation primarily in winter and $< 10\%$ of rainfall occurs in summer (Rowlands 1995a; Table 8). The summer flora is very limited, and tortoises rely heavily on the succulent green forbs and herbaceous perennial plants available in late winter and spring (Jennings 1993, 2002; Oftedal 2002; Oftedal et al. 2002). The proposed Central Mojave Recovery Unit is the hottest and driest of the 3 regions and is low in botanical diversity (Rowlands, 1995a). Of the 3 regions, the proposed Southern Mojave Recovery Unit has more summer precipitation and a higher richness of C_4 and CAM plant species (Rowlands 1995a). Until ca. 100 years ago, the Southern Mojave Recovery Unit was physically separated from the proposed Central Mojave and Western Mojave recovery units by the Mojave River; human activities have since reduced or eliminated the flow along much of the river.

Climatic differences between all recovery units profoundly affect timing and availability of forage, as well as seasonal activities and very possibly depth of burrows and, thus, protection from freezing temperatures and the hot, dry summers. The existing eastern recovery units in the Mojave population have higher percentages of precipitation in the summer, thus supporting a more diverse and complex summer flora (Table 8; Rowlands 1995a, 1995b; Oftedal 2002). A winter flora is also available. Differences in the mean number of freezing days per annum contribute to seasonal activity periods and the types of winter hibernacula protecting the tortoises from freezing. The Northern and Eastern Colorado Desert recovery units are the warmest, with 1–16 freezing days/y compared with 29–127 freezing days/y in the Mojave. Northeastern recovery units are by far the coldest, possibly contributing to the well-developed dens and lengthy tunnels on Beaver Dam Slope (Woodbury and Hardy1948) that are rarely observed outside the Northeastern Mojave and Upper Virgin River recovery units.

Genetic assignments do not support a separation between the Eastern Mojave and Northern Colorado recovery units, possibly because we only had 4 sample groups from these regions. The close geographic proximities of the sample groups (Fig. 7) are unlikely to reflect the potential diversity occurring along a 250 km north-south axis. Until more data are gathered along the north-south axis, we do not recommend treating the 2 recovery units as one, because of major differences in climate, forage availability, and seasonal activities. These distinctions may be exactly the kind of ecological/adaptive differences worthy of conservation management, independent of the units delimited by neutral molecular variation (Crandall et al. 2000; Allendorf and Luikart 2007). Significantly, unlike the genetically restricted and legally inapplicable ESU, the legal application of DPS allows for and promotes such protection (US Department of the Interior and US Department of Commerce 1996).

The Northeastern Recovery Unit (group 11) and the Upper Virgin River Recovery Unit (group 15) showed the strongest differentiation (MDS plot, assignment test, and unique matriarchal lineage). They may be more genetically isolated than other areas. Both potentially show evidence of recent population reductions. Additional sampling of these regions is encouraged for evaluation of current management strategies. Unfortunately, under current legislation these and perhaps other demes cannot be protected solely on the basis of the degree of threat alone, as recently advocated by Green (2005).

Recovery Actions. — Populations that have become disjunct or mixed as a result of recent anthropogenic activities may be suitable for restorative actions (Crandall et al. 2000; Allendorf and Luikart 2007). One restorative action would be to remove deliberately or inadvertently

Table 8. Physical and biological attributes of proposed recovery units for the Mojave population.

Recovery unit	Mean annual precipitation (mm)	% Rainfall June–Sept	Mean no. freezing days annually	Mean July maximum temperature (°C)	Topography	Vegetation types
Western Mojave	90–150	3.1–9.9	33–84	35.4–37.4	Flats, valleys, alluvial fans, rolling hills, mountainous slopes	(1) Creosote Bush Scrub, (2) Mojave Saltbush-Allscale Scrub (endemic), (3) Indian Rice Grass Scrub-Steppe, (4) Hopsage scrub, (5) Cheesebush scrub (west Mojave type)
Central Mojave	109	18.3–20.7	57 +	39.1–42.9	Flats, valleys, alluvial fans, rolling hills, mountainous slopes, rock outcrops, badlands, sand dunes, lava flows	(1) Creosote Bush Scrub, (2) Big Galleta Scrub Steppe, (3) Hopsage Scrub, (4) Cheesebush scrub, (4) Desert Psammophytes
Southern Mojave	108	18.1–36.1	29–104	37.2–39.1	Flats, valleys, alluvial fans, rolling hills, mountainous slopes, rock outcrops, lava flows	(1) Creosote Bush Scrub, (2) Big Galleta Scrub Steppe, (3) Hopsage Scrub, (4) Cheesebush scrub, (4) Desert Psammophytes, (5) Blackbush Scrub
Eastern Mojave	112–208	27.5–37.7	34 +	34.8–36.1	Flats, valleys, alluvial fans, bajadas, rocky slopes	(1) Big Galleta-Scrub Steppe, (2) Succulent Scrub (*Yucca*, *Opuntia*), (3) Creosote Bush Scrub, (4) Cheesebush Scrub (eastern Mojave type), (5) Indian Rice Grass Scrub-Steppe
Northeastern Mojave	100–210	27.1–41.0	46–127	38.2–40.1	Flats, valleys, alluvial fans, rocky slopes, deeply cut washes	(1) Creosote Bush Scrub, (2) Big Galleta Scrub-Steppe, (3) Desert Needlegrass Scrub-Steppe, (4) Blackbush Scrub
Upper Virgin River	210	28.7	96 +	38.4	Rocks, caves, sandstone crevices, sand dunes	Transitional Vegetation: (1) Sagebrush Scrub, (2) Psammophytes, Great Basin (sand sage), (3) Blackbush Scrub
Northern Colorado	112–129	32.6–34.1	2–12	42.2–42.3	Flats, valleys, bajadas, rocky slopes, small washes	(1) Succulent Scrub (*Fouquieria, Opuntia*, *Yucca*), (2) Blue Palo Verde-Smoke Tree Woodland, (3) Creosote Bush Scrub (lava flows)
Eastern Colorado	96–100	32.3–34.4	1–16	40.5–42.2	Flats, valleys, alluvial fans, small washes, deeply dissected washes, rocky slopes	(1) Succulent Scrub (*Fouqueria, Opuntia, Yucca*), (2) Blue Palo Verde-Ironwood-Smoke Tree Woodland, (3) Creosote Bush Scrub (rocky slopes)

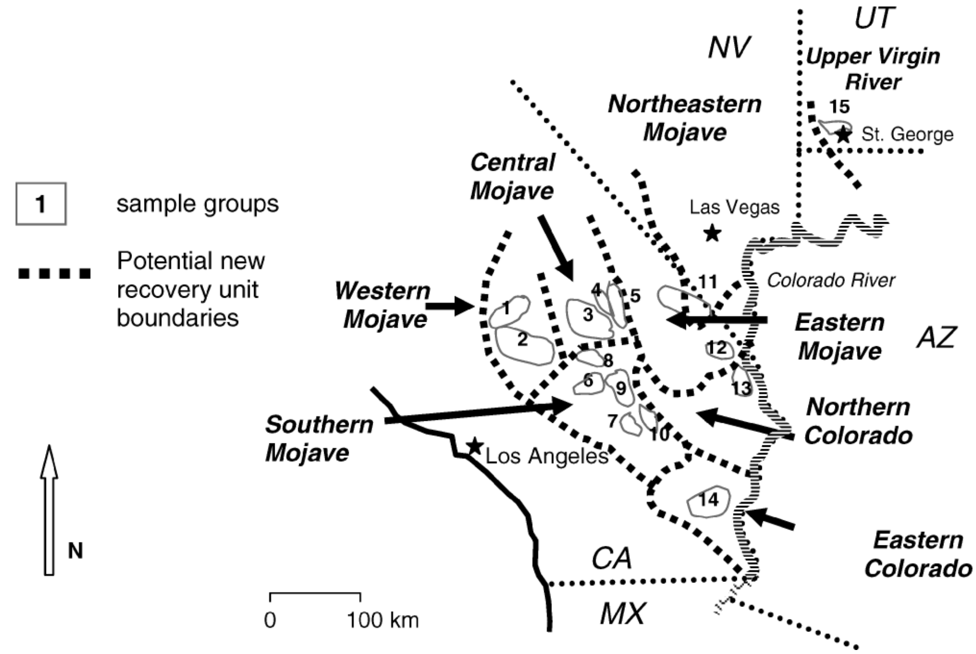

Figure 7. Sample groups of desert tortoises, *Gopherus agassizii*, shown with a new, preliminary alignment of recovery unit boundaries developed by using the mitochondrial deoxyribonucleic acid and microsatellite data presented in this study.

translocated tortoises from critical habitat. This strategy would be unreasonably difficult where populations are dense but may be a viable option where the area of interest and densities are limited, populations are declining, and most tortoises could be located and sampled. This strategy might be appropriate in the Upper Virgin River Recovery Unit and the Beaver Dam Slope Desert Wildlife Management Area (within the Northeastern Mojave Recovery Unit). Another restorative action would be to genetically test tortoises in the vicinity of frequently used recreation sites within national parks, research natural areas, and other protected areas: sites where visitors often release tortoises illegally, e.g., the Desert Tortoise Research Natural Area. The released tortoises from other populations could be identified and removed to a more appropriate place. In populations that have dropped below viable levels (e.g., Fremont-Kramer Desert Wildlife Management Area, Western Mojave Recovery Unit), informed and carefully planned augmentations or translocations could promote recovery, as has been done for a few other species (Allendorf and Luikart 2007). However, genetic planning is an essential part of such recovery efforts. Using tortoises within a well-defined recovery unit or local geographic area for headstarting or augmentation is far more desirable than translocating tortoises between recovery units. If local adaptations exist, then uninformed translocations of desert tortoises may do much more harm than good by introducing maladaptive genes into a locally adapted population.

Empirical studies need to be designed and tested to determine whether marker loci reflect specific adaptations with potential conservation value. For the Mojave population of the desert tortoise, the initial recovery units were defined on the basis of morphological, ecological, and behavioral differentiation, and the patterns of genetic variation parallel the earlier assessment in the *Recovery Plan*. Taken together, these 2 independent approaches strongly suggest the occurrence of local adaptation and evolutionary potential. Not only is it essential that this potential be conserved but also that underlying hypotheses be tested in the near future.

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Long-Term Sperm Storage in the Desert Tortoise (*Gopherus agassizii*)

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Many reptilian species exhibit the ability to store sperm in the female reproductive tract for extended periods of time (Gist and Jones, 1987; Birkhead and Moller, 1993). Sperm storage may be used to separate reproductive events such as copulation, fertilization, and hatching to optimize timing of these events (Birkhead and Moller, 1993). The redsided garter snake (*Thamnophis sirtalis*) ovulates and produces offspring in the spring but mate in the fall (Whittier and Crews, 1986). In this species, sperm from fall matings are thought to overwinter in the oviduct and fertilize ovum the following spring (Crews, 1984; Whittier and Crews, 1986). Reports of delayed egg laying in other species support the hypothesis that stored sperm are viable and used to fertilize subsequent clutches. Sperm storage has been inferred from oviductal flushings and/or observations of sperm in histological preparations of the oviduct (Gist and Jones, 1989; Gist et al., 1990). However, only controlled-mating experiments can demonstrate whether stored sperm are viable.

Sperm storage and multiple insemination may play a significant role in turtle reproduction (Gist and Jones, 1989). The ability to store sperm from previous matings and produce viable offspring using these sperm would be necessary for species whose male and female reproductive cycles do not coincide. Discordant cycles have been observed in temperate-zone turtles where time of mating and gamete maturation do not always occur simultaneously (Moll, 1979; Licht et al., 1985). In males of *Gopherus agassizii*, spermatogenesis begins in early summer and terminates with the onset of fall mating activity; females alternatively complete ovarian growth and lay eggs upon emergence from hibernation the following spring when male testes are fully regressed (Rostal et al., 1994).

In 1991, a large study was initiated at the Desert Tortoise Conservation Center to fill existing gaps in both basic and applied biology of the desert tortoise, *G. agassizii* (Spotila et al., 1994). A part of this large study was designed to delineate the reproductive cycle of male and female desert tortoises (Rostal et al., 1994). Reproductive groups consisting of three females and two males were maintained and monitored in seminatural enclosures from 1991–1993. Allozyme data were used to infer paternity and identify cases where stored sperm was used to fertilize eggs. Hatching success was recorded, and comparisons were made between clutches fertilized with sperm maintained in female reproductive tracts in excess of two years and clutches fertilized with sperm resulting from matings with males currently maintained in the enclosures.

MATERIALS AND METHODS

Fifty adult desert tortoises were placed in 10 seminatural enclosures at the Desert Tortoise Conservation Center (DTCC) in Las Vegas, Nevada. Tortoises at the DTCC were relocated from construction sites on the edges of Las Vegas in 1990 and 1991, as part of a research and conservation effort resulting from the recent listing of *G. agassizii* as a threatened species under the Endangered Species Act. Individuals were collected during late spring or early summer and weighed, measured, sexed, and tagged. Each reproductive group was comprised of three females and two males in individual 15 × 30 m field enclosures. Each enclosure contained five artificial burrows, natural vegetation, and two watering stations. Each enclosure was supplemented with alfalfa hay (Rostal et al., 1994). Animals were allowed to continue "normal" behavior (i.e., male-male combat and mating) and were kept in the enclosures for two complete breeding seasons (August 1991 to July 1993). Heparinized blood samples (3–5 mL) were collected via jugular venipuncture (Jacobson et al., 1992) and stored frozen for future analysis.

Females were observed closely, and ovaries and oviducts were scanned every two weeks during the nesting seasons by using an Aloka 500 V ultrasound scanner (Corometrics Medical Systems, Inc., Wallingford, CT 06492) to confirm

TABLE 1. POLYALLELIC ENZYME SYSTEMS DETECTED AND ELECTROPHORETIC CONDITIONS USED FOR THE DESERT TORTOISE, *Gopherus agassizii.*

Enzyme	Locus	Electrophoretic conditions[a]
Lactate dehydrogenase	Ldh-B	B
α-Naphthyl propionate	α-NP-Est	A
Malate dehydrogenase	mMdh-A	B
Glucose-6-phosphate isomerase	Gpi-A	B
Phosphogluconate dehydrogenase	Pgdh-A	B

[a] A: Lithium hydroxide pH 8.1 (Selander et al., 1971); B: Triscitrate pH 7.1 (Ayala et al., 1972).

when ovulation and subsequent egg deposition occurred (Rostal et al., 1994). Twelve clutches were collected during the second breeding season and incubated in individually sealed 850 mL tupperware containers. This allowed hatchling identification in specific clutches. Sixty-one hatchling tortoises were produced from these clutches, marked for identification, and transported to Georgia Southern University in 1993. Blood samples (0.5–1.0 mL) were taken from the identified offspring via jugular venipuncture in 1995 (tortoises were two years old).

Blood samples were thawed and proteins extracted from 0.5–1.0 mL whole blood by using 50 μl of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NaDP, pH = 6.8). Horizontal, starch-gel electrophoresis, using 12% starch gels composed of hydrolysed potato starch (Starchart Corp. lot W571-2), was conducted at 5 C. Electrophoretic conditions employed in this study are listed in Table 1. Allelic designations reflect relative differences in anodal electrophoretic mobility with the reference allele (= 100) being the most common allele found at a given locus (Rainboth et al., 1989). Other alleles were named relative to this standard and the origin (= 0).

To identify sperm storage, a comparison was made between genotypes within a clutch and genotypes of the mother and two males that occupied the same enclosure. Cases in which genotypes of offspring were not compatible with possible outcomes of matings between the female and two males were considered examples of sperm storage.

RESULTS AND DISCUSSION

Allele products of five polyallelic loci were scored; three of these were used in detection of long-term sperm storage. The latter were alpha-naphthyl propionate esterase (α-Np-Est), lactate dehydrogenase (Ldh-B), and glucose-6-phosphate isomerase (Gpi-A; Fig. 1). Products of the remaining loci were monoallelic or did not resolve well: adenosine deaminase (Ada-2), hemoglobin (Hb), lactate dehydrogenase (Ldh-A), malate dehydrogenase (sMdh-A), NADP-dependent malate dehydrogenase (sMdhp-A), purine nucleoside phosphorylase (Pnp-1), peptidase (Pep-A, Pep-B, Pep-D, Pep-S), phosphoglucomutase (Pgm-A, Pgm-B), pyruvate kinase (Pk-A), and superoxide dismutase (sSod-A). Offspring from five of 12 clutches produced in 1993 appeared to be the result of fertilization with sperm stored from matings prior to the study in 1991 (Table 2). Tentative evidence for multiple paternity was found in one additional clutch in which genotypes at multiple loci were

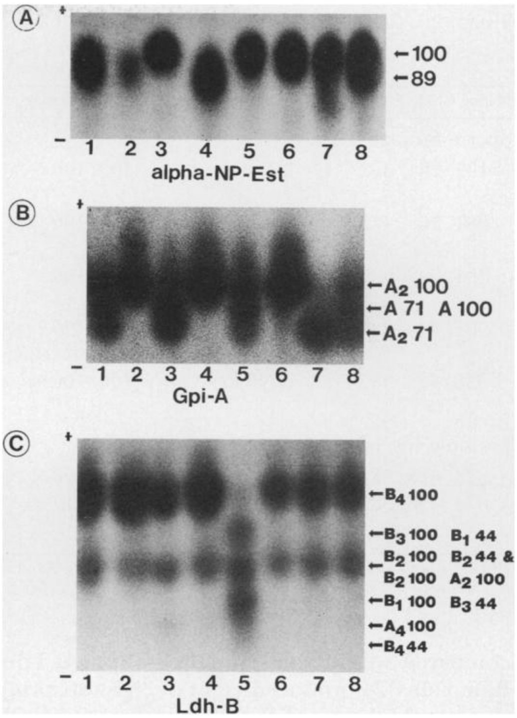

FIG. 1. Zymogram showing electrophoretic patterns of expression of gene products of the three enzyme systems used in this study. (A) Alpha-Napthyl propianate esterase; the individual in lane 4 is homozygous 89/89; specimens 3, 5, 6, and 7 are homozygous 100/100; and specimens 1, 2, and 8 are heterozygous 100/89. (B) Glucose-6-phosphate isomerase; lane 2, 4, and 6 individuals are homozygous 100/100; specimens 1, 3, 5, and 8 are heterozygous 100/71; and specimen 7 is homozygous 71/71. (C) Lactate dehydrogenase-A (designated with letter A before mobility number) and B (designated with letter B); specimen in lane 5 is heterozygous 100/44 at Ldh-B. All other specimens are homozygous for both the Ldh-A and Ldh-B loci.

TABLE 2. GENOTYPES OF EMBRYOS THAT SHOW LONG-TERM SPERM STORAGE AND/OR MULTIPLE INSEMINATION (MULTIPLE LOCI ANALYSIS)

Mating Group F, M1, M2	Locus	Genotype of female	Genotype of male 1	Genotype of male 2	Genotypes of offspring
Sperm Storage					
195, 264, 432	Ldh-B	100/100	100/100	100/100	100/100 (4) 44/100 (2)
081, 264, 432	Ldh-b	100/100	100/100	100/100	100/100 (4) 44/100 (1)
304, 292, 995	Ldh-B	100/100	100/100	44/44	100/100 (3) 44/100 (2)
901, 200, 282	α-NP-Est	100/100	100/100	100/100	100/89 (5)
336, 498, 382	α-NP-Est	100/100	100/100	100/100	100/100 (1) 100/89 (4)
Multiple Insemination*					
874, 498, 382	Ldh-B	100/100	100/100	44/100	100/100 (8)
874, 498, 382	Gpi-A	71/71	100/100	100/71	100/71 (5) 71/71 (3)

*Taking both loci into account a mating between female 874 and male 498 is most likely at the Ldh-B locus, whereas a mating between female 874 and male 382 is most likely for Gpi-A locus.

compared within single-family groups. At Ldh-B, female 874 produced eight of eight offspring that would be predicted from a mating with male 498. However, at Gpi-A, three of eight offspring produced were consistent with a mating between female 874 and male 382, whereas five of eight offspring produced were consistent with a mating with male 498. Looking singly at either locus, offspring genotypes are concordant with matings of one or the other male. If both loci are taken into account, it is impossible to assign paternity solely to either male. In this case, the possibility of sperm storage cannot be excluded, because a definitive third allele is not present in the offspring.

No reduction in fertility was observed in clutches resulting from sperm stored in the females reproductive tract prior to introduction into the reproductive study enclosures. Mean hatching success (± SE) for all clutches was 95.8% (± 2.34, n = 12), whereas hatching success for clutches fertilized by sperm stored greater than two years was 97.1% (± 3.19, n = 5). These data are consistent with observations of viable hatchlings produced by females isolated from males for two years in other enclosures at the DTCC (Rostal, unpubl. data). Studies of other species, however, have reported a decrease in fertility as time increased from the previous mating (Goin et al., 1978; Davenport, 1995). In one case involving a caiman (Davenport, 1995), the animal was brought from Suriname and kept in a zoological setting where a single clutch was collected 488 days after the last contact with a male. Environmental and physiological stress factors may have played a significant role in the reduction of fertility observed. In the diamondback terrapin, *Malaclemys terrapin*, a sharp decline in fertility (87.9% to 30% hatching) was noted after the second year following isolation of females from males (Goin et al., 1978). This reduction in fertility was thought to result from sperm depletion rather than degradation of sperm in the oviduct (Gist and Jones, 1987). Further research is needed on sperm viability in other species.

Data from this study demonstrate sperm storage in *G. agassizii* and are consistent with reports that turtle species can store sperm for extended periods of time (Gist and Jones, 1987, 1989). Sperm-storage tubules located within the albumen-secreting gland region of the oviduct have been observed in several turtle species and are thought to provide a suitable environment for the storage of viable sperm (Gist and Fischer, 1993). Sperm storage thus appears to be a normal reproductive process that explains observations of delayed fertilization and a means for successful matings that are asynchronous with ovulation.

As with other temperate species of turtle, reproductive cycles of male and female *G. agassizii* are not synchronized (Rostal et al., 1994). Gamete maturation in females concludes in early spring when ovarian follicles reach ovulatory size. During this time, male testes are fully regressed, with mature sperm produced prior to hibernation three to four months earlier. Fall mating behavior suggests that sperm are inseminated into the female and then stored in sperm-storage tubules until emergence from hibernation in the spring. Gist et al. (1990) reported finding sperm in oviducts of *Chrysemys picta* and *Trachemys scripta* during the fall when copulatory behavior was observed. In addition, sperm could only be recovered from males during the fall when testes were fully mature (Gist et al., 1990), providing additional evidence supporting the sperm-storage hypothesis.

Gist et al. (1990) suggested that male/female interactions during spring mating may be essential for proper growth and maturation of reproductive organs and gametes in females. In *G. agassizi*, mating activity is observed in both the fall and spring. Presence of shelled eggs in oviducts following spring emergence and before mating activities, however, supports the notion of a functional, fall-mating period (Rostal et al., 1994). Seasonal changes in environmental conditions may precipitate asynchronous mating systems and the evolution of sperm storage in temperate turtle species.

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