The Management and Release of Ex-Captive Moorish Tortoises (*Testudo graeca graeca*) into wild populations in Southeastern Spain

Risk Assessment: Some Practical Problems & Recommendations

A Report by The Tortoise Trust

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Introduction

This report was prepared as an overview and general summary of the risks and difficulties associated with gathering, holding and releasing ex-pet tortoises into wild populations for conservation purposes. On the surface, such strategies often appear to offer immediate benefits, and may be attractive to the general public and the media. However, virtually without exception, all major international conservation organisations and many individual expert researchers have expressed serious concerns about this approach on scientific and technical grounds.

This brief report reviews those concerns and examines specifically how they apply to the tortoises of this region. These tortoises are extremely important as they represent the only mainland European populations of this particular species, and they are under severe pressure from habitat loss and habitat modification due to the intensification of agriculture, building developments, and the construction of roads and railways, etc.

There are three primary areas of concern:

1) The risk of introducing disease to wild populations.
2) The risk of 'genetic contamination' where animals of a different genetic make-up are introduced into an existing population.
3) The general difficulties of management for large groups of tortoises.

Each of these areas of concern are discussed in detail.

The primary objective of any conservation program must be to balance conservation gains with available resources to maximum effect, also to achieve a good outcome in terms of animal welfare and to maximise public support for continued conservation efforts.

Resources are always limited, so it is vital that they are used intelligently and to maximum real effect. It is also the case that conservationists must ensure that they act in a way that gains the confidence and support of the general public. **It is essential that any action that cannot be fully justified in both scientific and ethical terms must be avoided.** This is even more true today than it was many years ago, when social media will very quickly generate extremely negative publicity on a world-wide scale when things go wrong. At the same time, this power of open communication can be employed to educate and inform the public, and to change damaging behaviours. It is critical everyone involved in conservation is aware of this. The objective must be to achieve genuinely positive results with the most economical use of resources, while generating and maintaining public awareness and support. Any program that fails to do this has no hope of success.

Rescue and Recuperation Centres: Disease Risks

Centres where injured tortoises can receive expert veterinary care and that can function in an educational capacity can have an important role in any conservation strategy. However, it must also be realised that unless they are established and operated according to carefully considered protocols they can have very undesirable consequences. The most critical of these problems are the potential for centres to act as “epidemic amplifiers”, where cross-contamination results in uncontrolled spread of diseases not only within the centre, but also to wild populations, and also in the matter of “genetic contamination” where genetically different species and even genetically different individuals from separate population clusters within the same species are mixed or re-located randomly.

It is now accepted by most specialists that large collections of tortoises from different geographical origins gathered in one location represent an extremely high risk in terms of disease transmission.

Typically, conditions at rescue centres often present ideal conditions for mass cross-contagion and infection:

1) Overcrowding is a major risk factor. Multiple tortoises feeding and mating in the same enclosure is extremely dangerous, as if just one infected animal is introduced it is almost certain that the pathogen will spread to the entire group very rapidly.

2) Contaminated substrate (soil and plants) may contain viable virus particles for up to 24 weeks depending upon weather conditions and season. As tortoises are in close contact with the ground, often burying themselves for extended periods, this is an important potential mode of transmission.

3) Not all rescue centres have adequate disease prevention protocols in place. Specialists in reptile and amphibian viral infections recommend the use of disposable gloves, disinfectant foot baths, the use of disposable shoe coverings when moving from different enclosures or touching different tortoises, and an approved method of hand-washing with disinfectants that have high efficiency against these pathogens (Green, 2009, Highfield, 2011, Highfield, 2014)). All feeding utensils and other items used for maintenance must be thoroughly cleaned and disinfected to adequate standards. Movements of humans or other animals between enclosures should be minimised. It should be noted that birds, small rodents and even insects present a theoretical risk of transmission.

4) All enclosures should use double-barrier separation and sufficient distance from each other and care must be taken to transfer nothing, including soil particles, from one enclosure to another. Handling, enclosure cleaning and feeding should always be carried out in the order of Low Risk > High Risk. For example, animals that have been proved disease free should be handled or fed first, with medium risk animals next, leaving high risk and infected animals to the very last. Of course, full cleaning and disinfection protocols must also apply.

Achieving truly safe levels of operation at such centres is unfortunately extremely challenging in a technical sense and are also financially very costly, while their benefits to conservation are very limited. These limitations and hazards need to be recognised and acknowledged. While often viewed by the public in an overenthusiastic way, the reality is that the problems of achieving safe

operation are enormous, and the consequences if something goes wrong are very grave indeed and may be impossible to reverse. So, while it is acknowledged that they can play a useful supporting role, it is important this is kept in perspective, and is not exaggerated. Alternative strategies should be investigated, and 'rescue centres' should never be the primary strategy in conserving threatened species. Alternatives, such as conserving key habitats, habitat management and threat reduction (fencing roads and predator control, for example) are not only infinitely lower in risk, but are also considerably more effective at lower overall cost.

One additional difficulty is that many of the diseases that affect tortoises are not obvious visually and can be difficult, or even impossible, to detect, certainly without extensive (and very costly) laboratory screening techniques. Some of these diseases are highly contagious with extremely high mortality rates. It is important to note that there are many cases in the veterinary literature of large-scale outbreaks of epidemic disease that are associated with rescue centres and with similar large scale collections of tortoises (MacArthur, 1997). These diseases can have devastating consequences. For example, one group of 370 tortoises illegally imported into Italy from Libya and subsequently seized by the authorities, began to display symptoms of mouth infections (stomatitis). Within six months all but 40 were dead (330 mortalities). Laboratory analysis using polymerase chain reaction (PCR) and virus isolation revealed the presence of Chelonian Herpes-Virus (CHV) in affected animals and tissue. This particular disease (CHV) has been implicated in multiple similar outbreaks world-wide and represents an extremely grave threat to both captive and wild tortoises. **Chelonian herpes-virus is very different from herpes-virus as typically seen in humans; in tortoises it has a near 100% mortality rate within a few weeks of first symptoms.** This virus has also been reported from captive *Testudo graeca* at a conservation centre in Spain (Muro, J, 1998). An adult female tortoise with zero clinical signs of disease and appearing entirely healthy was relocated to the Catalonian Reptile and Amphibian Rehabilitation Centre (CRARC) and six months later developed symptoms of rhinitis (nasal discharge) and stomatitis (mouth infection). The tortoise died from the infection shortly afterwards. Laboratory testing confirmed the presence of a herpes-virus infection. As a consequence of this incident, leading researchers and specialists made a series of recommendations concerning the management of tortoises that are intended for release into the wild, or are kept in close proximity to tortoises (of any species) that are intended for release. The most important recommendations were as follows:

1) Recognition that 'silent carriers' or animals with no visible symptoms present a very serious risk and that visual inspection alone is entirely inadequate to determine if a tortoise is carrying a potentially lethal and highly contagious disease that could spread in an uncontrolled manner among already endangered wild populations.

2) Testing tortoises for herpes-virus is critical and essential before releasing any into the wild. **Tortoises should not be released into nature unless a minimum of three separate diagnostic tests have proved negative.**

There is no single 100% effective test, and the time after infection can also affect the reliability of results. However, PCR, ELISA (enzyme-linked immunosorbent assay) and serum neutralization (SNT) tests have a confidence factor of at least 97% and repeat tests at intervals of 10-12 weeks apart can increase the overall reliability to an acceptably high degree of confidence.

It is also extremely important that every single tortoise that dies at a rescue centre is comprehensively autopsied by specialists and tissue samples are subjected to full laboratory analysis, including electron microscope histology (a very useful method of detecting viral inclusion bodies), with virus isolation and identification of any results, combined with a general report that

identifies any other diseases present and provides a reliable over-view of body condition and nutritional status, etc. Such a protocol can provide an effective 'early warning' of problems that may be present, but have not yet been observed in living animals.

It should be noted that tests for parasites alone or checks relying upon detecting visual symptoms of disease are completely inadequate and are entirely unacceptable where animals are to be released . The consequences of releasing tortoises that are 'silent carriers' of highly contagious diseases into wild populations are so grave that absolutely no chances should be taken. Some of these diseases (Chelonian herpes-virus is only one of them), are capable of wiping out entire populations. Therefore, it is absolutely vital that all possible precautions should be taken.

While Chelonian herpes-virus represents one of the best-known risks, as stated above, it is certainly not the only contagious disease that occurs in these species that must be considered (Jacobson, 2007). There are a number of equally serious threats. For example:

Ranavirus

This group of iridoviruses has been repeatedly associated with mass mortalities of fish, amphibians and reptiles. The virus has been positively identified in several different tortoise and turtle species. It is highly contagious and there is evidence that it can “cross the species barrier”, with infected tortoises or turtles infecting amphibian populations and vice-versa. This virus has been summarised as follows: “*Infection is highly fatal in turtles, and the potential impact on endangered populations could be devastating”* (Allender, 2011). Wildlife rescue and rehabilitation centres are again identified as potential major vectors of cross-infection and transmission. Mass mortalities in tortoises and turtles have been recorded. Tortoises that may have been exposed to other exotic animals at pet dealers or during international transport, or housed in zoological collections with inadequate hygiene should be regarded as high risk.

A PCR test does exist but should be conducted separately from the PCR test for herpes-virus.

Adenovirus

Adenoviruses are mostly found in lizards and snakes, but have also been detected in turtles and tortoises. Infections have been associated with anorexia, oral lesions, and diarrhoea. Adenoviruses can be detected in cloacal swabs in live animals and in liver and intestine of dead animals via PCR.

Mycoplasma

This class of organism has been directly associated with a steep decline of Desert tortoises in the South-Western USA over the past 30 years. It typically results in a chronic rhinitis (nasal discharge) and other upper-respiratory tract symptoms. Additionally, there are many similarities between the conservation and disease management situations of the Desert tortoise in the United States and the situation facing *Testudo graeca graeca* in Spain. One important lesson learned from experience in the United States is that disease spread by the release or escape of captive desert tortoises is considered a major threat to wild populations (AIDTT 1996). In addition, concern has been raised that the addition of more distantly related individuals could cause genetic contamination of local populations. We consider both concerns are valid. It should be noted that in Desert Tortoise areas there are active public education campaigns aimed at discouraging accidental or deliberate releases of pet tortoises for these reasons. We believe that a similar approach in Spain would be helpful and would greatly reduce the danger of such contamination. Mycoplasma based diseases have recently

been recorded at tortoise rescue centres in Europe (Lecis, 2011). The authors of that paper specifically warn that *"These results highlight the potential role played by wildlife recovery centres in the spread and transmission of pathogens among wild chelonians and to humans"*.

Picornavirus

Picornaviruses (also known as virus "X") are regularly detected in tortoises. They are often found with other infectious agents, particularly with herpesviruses and mycoplasma. In juvenile animals, they are associated with softening of the carapace. In adult animals, they have been detected in conjunction with rhinitis, stomatitis, ascites, and sudden death. Picornaviruses are best detected in oral swabs in live animals (www.laborkin.de – laboratory for clinical diagnostics). This particular family of viruses should be regarded as extremely high risk. Over one hundred imported Sulawesi tortoises (*Indotestudo forsteni)* in the United States showed signs of severe disease, including anorexia, lethargy, mucosal ulcerations, erosions of the oral cavity, nasal and ocular discharge, and diarrhoea that were traced to infection with this virus. Mortality is extremely high and the disease appears to be very easily transmitted. The present authors are currently investigating a possible case of this virus affecting a group of juvenile tortoises.

There is one very important fact that should be considered, and that is that "new" viruses are being discovered all the time. For example, just 25 years ago we had almost no knowledge of viruses affecting tortoises at all. In fact this author (A. C. Highfield, 1990) was one of the first to report on viral epidemic diseases in tortoises. Since then, a range of different diseases have been revealed. In the list above, the Picornaviruses have only been reported in the last 5 years and their effects are only just becoming known. Therefore, we can never absolutely guarantee that animals are 100% "safe" as we cannot test for diseases that we have yet to discover. Even so, such undiscovered diseases may represent a very serious threat.

The risks of "new" diseases being introduced to natural populations is amplified massively where different species from different geographical locations are kept in close proximity. This applies even more so where animals may have had contact with tortoises that may have been kept as pets, or where there has been secondary contact with pet-trade animals.

The pet trade has featured in many high profile serious disease outbreaks. This author (A. C. Highfield) has been personally involved in investigating several cases. One such case involved a reptile dealer in the UK who imported over 150 Russian tortoises (*Testudo horsfieldii*) that he sold to numerous customers. Those customers then experienced disease outbreaks in their own collections involving multiple fatalities in each case. 100% of the affected animals died. In total, it is estimated that several hundred tortoises were affected and died. The dealer involved was prosecuted and convicted of several crimes.

Reptile keepers who keep large groups of mixed species from different origins are also at high risk (Highfield, 1996). The more animals who enter the group, or who have contact in any way, the greater the risk becomes. Although a suitable quarantine period (this author recommends a minimum of 18 months) is useful in reducing the risk and should be mandatory, it should not be considered as 100% protection. Many of these diseases may be carried without symptoms for very long periods – we are aware of one case where a Leopard tortoise (*Geochelone pardalis*) appears to have carried a herpes-infection for 10 years before displaying any symptoms. The only reliable way

to reduce risk is to use the testing protocols discussed above.

Even keepers who have a small number of animals are a high risk source of animals if release into the wild is the objective. Many of these keepers do not maintain any form of quarantine, keep no proper records, have no effective hygiene measures, and may allow contact with other pet species, including other tortoises of unknown origin and history.

It must be stressed that one single brief contact is sufficient to transmit some of the diseases listed above.

To give a practical example, we are aware of some people in Almeria who kept a few local tortoises in their gardens, but also kept other tortoises purchased from pet dealers, including Russian tortoises (*Testudo horsfieldii*) and Turkish Spur-thighed tortoises (*Testudo ibera*) in the same garden. Both of these species have frequently been implicated in the spread of epidemic diseases, including Chelonian Herpes-virus and Picornavirus. In fact, one study suggested that up to 20% of some wild tortoise populations in Turkey were “passive carriers” of Virus-X or picornavirus (Marschang, 2007).

If just one *Testudo graeca* from one of these keepers was taken to a rescue centre and allowed contact with other tortoises that were ten released it would technically be capable of infecting the entire local wild population with tragic and irreversible consequences. This risk must not be underestimated. It is very real. In the author's opinion it is better to be over-careful than not careful enough. Once such a disease has “escaped” it is too late.

Different species, and even different populations of the same species, may have different immune responses and different levels of resistance to “new” diseases. This is of course also the case with humans and there are many historical cases of travellers acting as vectors for “new” diseases (that may be common and relatively harmless among Europeans) that cause devastation to remote tribes, for example. This is certainly also the case with tortoises. The principle is exactly the same.

For example, it became clear very early in the study of tortoise infectious and contagious diseases that susceptibility to many diseases is very different even between the different Mediterranean tortoise species (*Testudo graeca, Testudo ibera, Testudo marginata, Testudo hermanni* and Testudo *kleinmanni)*. Very high rates of mortality are noted when Turkish *Testudo ibera* are mixed with Spanish or North African *Testudo graeca graeca*, for example. This danger is made worse by the fact that many keepers (including some professionals) often make incorrect species identifications, resulting in frequent accidental mixing. These differences even appear to extend to differences in nematode (intestinal parasite) species and tolerances between captive vs. wild tortoises resulting from exposure to 'new' species of parasites originating in pet trade animals (Chavarri, 2012). That study suggests “important differences in the nematode fauna of captive and free-living tortoises and questions one more time if the reintroduction of captive animals presents a risk for the wild population”.

Treatment of affected tortoises

It must be emphasised that not all tortoises that present with symptoms of a 'runny nose' or a mouth infection are suffering from a fatal viral infection. These conditions can be an entirely temporary situation caused by common bacterial organisms, or by poor environment, or other simple factors. With the correct treatment the vast majority will go on to make a full recovery. We are aware of

many tortoises that have had these symptoms, have recovered and that have not only survived for decades, but have subsequently bred 100% disease-free and healthy offspring. Both sets of symptoms are common, and only a small minority will prove to be caused by dangerous, contagious viral pathogens.

It is completely inappropriate to routinely employ euthanasia for all animals that display such symptoms.

Diagnostic tests now exist that can reliably identify the causes, and any following action should be based only upon such tests, and not on guesswork. With expert veterinary care, most non-viral and non-mycoplasma cases will recover to perfect health.

It is vitally important that all tortoise conservation programs can command the support of the general public. This is not possible if unjustifiable euthanasia is routinely employed. Where there is no option, such as in the case of a terminally sick tortoise suffering from a confirmed viral infection the public will sympathise and will understand the necessity of this. If, however, easily curable animals are euthanised the public will not sympathise and will not understand. The conservation of a species must have the widest possible support from land owners, the general public, from animal welfare supporters and from veterinarians and conservation biologists. It can only hope to do this if it is managed using effective and ethically acceptable protocols and procedures.

Sick or suspect tortoises must be housed separately from any intended for release and full quarantine measures must be in place and followed carefully. Once recovered they may continue to be of value in education or in some cases, in captive breeding. Euthanasia should be avoided unless it is fully justifiable on the basis of clinical results and a thorough ethical assessment has been carried out for every individual case.

Suggested guidelines for disease risk reduction:

No tortoise should be released into an existing wild population until several criteria have been met. These include

a) An extended period of quarantine of at least 18 months.

b) Three separate negative PCR/ELISA tests for each of all known viral diseases for which a test exists over a period of at least 6 months.

c) An individual risk assessment. One solitary animal from a known location close to its natural habitat that had never been exposed to other exotic species would be relatively low risk. An animal from a large group of mixed species, of unknown origin, or where pet trade animals have been kept would be classed as extremely high risk. In this author's opinion high risk animals should not be released into the wild. It is generally accepted within the worldwide conservation biology community that the release of ex-pets into the wild invariably involves an exceptionally high degree of risk, and that it is essential that adequate disease prevention protocols are in place at all stages of the project. The degree of risk may outweigh any possible benefit and alternative strategies that present a lower level of risk should always be considered first.

d) Identification of parasites to species level present in the intended release population and

comparison with parasites present in any animals intended for release.

e) Accurate and reliable taxonomic identification of all animals intended for release or those that might be in contact with animals intended for release. If in doubt – do not release.

f) Final check to determine a satisfactory weight and good general condition of health. Tagging and microchip identification is essential to produce long term data on survival.

g) Careful monitoring of all release sites for at least 15 years, with random PCR testing and general population density and health status checks.

h) All release sites must be suitable of supporting the target population and must be within the optimal bio-climatic zone. Suitable vegetation must exist and human induced hazards to survival such as roads and introduced predators must be minimised. Paved roads will require tortoise-proof fencing and tunnels to allow for population movement. Animals should only be released after a full site survey and only at suitable times of the year. There is strong evidence in multiple studies that tortoises released into unsuitable habitats have very poor survival rates, and that stress in tortoises (from an unsuitable release site or poor vegetation) can result in a depressed immune response and consequently in high rates of disease.

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Taxonomic Problems and Risks

There are four widely accepted species of terrestrial tortoise to be found in the Mediterranean area. Members of the *Testudo graeca* complex (Spur-thighed tortoises), *Testudo hermanni* (Hermann's tortoise), *Testudo marginata* (Marginated tortoise) and *Testudo kleinmanni* (Egyptian tortoise). The taxonomy of the *Testudo graeca* complex is particularly confused with numerous subspecies and species proposed (Bonin, Devaux and Dupre, 2006). There is rarely much agreement on validity. It is clear that there are a large number of different geographical forms, each with unique morphological and sometimes behavioural features. Their taxonomy, however, is much in dispute. Indeed, it is difficult to find two experts who even agree as to how many species there really are. This is one reason why we refer to the '**Testudo graeca complex**' – an admission that this is a group of tortoises that we simply do not understand well enough yet and which is very much in doubt There are two primary geographical groups within the *Testudo graeca* complex: those occurring in North Africa and Southern Spain, and those occurring in Turkey, the Caucasus and the Middle East. The North African group contains the prototypical *Testudo graeca* L. 1758. The Caucasian group is dominated by *Testudo (graeca) ibera*. Many commentators consider this to be a separate species entirely. A detailed review of this confused and controversial taxonomic situation is beyond the scope of the present text. However, it should be noted that these different forms do have significantly differing patterns of behaviour, different structural features, different responses to common pathogens and can prove to be mutually incompatible. Their taxonomic status and an ability to differentiate them in practice therefore also has important welfare and conservation implications. In general terms their environmental and dietary requirements are similar. *Testudo graeca* complex tortoises, *Testudo hermanni* and *Testudo marginata* are all relatively common in captivity. The Egyptian tortoise, *Testudo kleinmanni*, however, is a CITES Appendix I species and trade is strictly controlled.

Two widely accepted species occur in Spain.

Testudo hermanni in the North East of the country, and *Testudo graeca* in the South.

The recognised subspecies involved are *Testudo hermanni hermanni* and *Testudo graeca graeca*.

The taxonomic classification of *Testudo graeca* is particularly difficult and is certainly not fully understood. It is commonly believed, for example, that the form of *Testudo graeca graeca* found in Spain is identical to to those found throughout North Africa, Tunisia, Libya, Algeria and Morocco. This is categorically not the case, as revealed by recent research. Several different subspecies (or possibly species) have already been described from these countries and this number is almost certain to grow as further studies are carried out (Highfield, 1989, 1990a, 1990b). Currently these include:

- *T. g. soussensis* (South Morocco)
- *T. g. marokkensis* (North Morocco)
- *T. g. nabeulensis* (Tunisia)
- *T. g. cyrenaica* (Libya)
- *T. g. whitei* (Algeria)

There are also a large number of visibly quite different tortoises with unique morphological characters that are as yet formally described, but that may be formally described and named separately in future. Tortoises from these different populations and geographical areas may differ in size, in colouration, in body shape, in marking patterns, in scale structure and in several other details.

Individual tortoise populations, or 'clusters' may have been separated by mountains, rivers, valleys or other inhospitable habitat zones for extremely long periods, and as a result, undergone unique and different responses to those specific conditions prevailing in their own habitat. Populations or subpopulations that have been separated for long periods of time can differentially adapt and diverge. Taxonomists also describe tortoise populations as exhibiting powerful trends in morphological plasticity (Fritz, 2007). In some cases there are also distinct genetic differences between them, however there is also often an apparent conflict between the degree of morphological variation and the genetic results. The significance of this is not yet fully understood, and demonstrates yet again that our present level of knowledge remains inadequate.

For example, tortoises fromTunisia and adjacent Algeria reveal seven distinct genetic haplotypes and a very considerable range of morphological variation. Due to political and similar logistical difficulties, many of these areas have never been comprehensively studied, so we have a very incomplete understanding of the true taxonomic and population status of many of these tortoises. This makes reintroductions either from these areas or to them very unwise.

Unfortunately, several such actions have taken place. One group based in Italy “reintroduced” ex-pet tortoises to Morocco. Unfortunately, the precise origin of these tortoises was unknown, their full history was unknown, and totally inadequate genetic and disease screening protocols were employed. This has several important implications. Firstly, the risk of introduction of 'alien' pathogens, particularly viral diseases as discussed previously, and also 'genetic contamination' of already 'at risk' native populations. This view is shared by the IUCN (2004) **“The release of**

captive tortoises from different populations into *T. graeca* habitat represents dangers of genetic pollution as well as introduction of pathogens". In addition, there are reports that tortoises that were seized from a Ferry from North Africa were released in South Eastern Spain some years ago. This was plainly done with good intentions, but the fact remains that we cannot confirm that these tortoises were identical genetically to native Spanish tortoises, and we do not even know if they were the same species – if from Morocco they could have included *T. g. soussensis* or *T. g. marokkensis* or even some other variety, species or subspecies that has not yet been formally described. We simply have no idea what they were. Neither can we be sure that they were free of all diseases that might present a grave threat to native Spanish populations. It was simply *assumed* they were identical because the common reference books (many of which are years out of date) said they were the same thing. **This is a very dangerous and false assumption and is not a satisfactory basis for a serious and successful conservation effort.**

The key aim of conservation must be to ***preserve and protect genetic diversity and uniqueness.***

If we introduce 'alien' species, particularly those capable of hybridising with the native populations, we totally destroy that diversity and uniqueness. It can never be undone. The damage is permanent.

The problem of ex-pets

The first problem with using tortoises that have been kept as pets for conservation purposes is that we normally have no idea at all of their original origin. This means it is totally impossible to return them to the site where they were taken from. There may be some few exceptions where this is known with a high degree of confidence, but in the vast majority of cases it cannot be known. This is not a minor issue – it is a critical issue.

The second problem is that we cannot reliably know what other tortoises they may have been in contact with. The problem of diseases has already been discussed, and even though other tortoise contacts might appear to have been disease-free, we now know that a significant percentage could very well be passive or "silent" carriers of lethal chelonian herpes-virus strains (Martel, 2009).

Disease transmission is certainly not the only problem, however. Some of these different species, subspecies and un-described "varieties" of *Testudo* are capable of hybridising. So, for example where we have a pet keeper who has mixed the Turkish-origin *Testudo ibera* with North African or Spanish origin tortoises, viable offspring can result. Although serious herpetologists and experienced keepers will be aware of these dangers (disease and hybridisation), most ordinary members of the public who may have kept tortoises are not aware. Such mixing of species and hybridisation is therefore quite common.

If we therefore encourage pet keepers to surrender such animals for release into the wild, and particularly if we frighten keepers into making uncontrolled random releases, we are, in effect, encouraging genetic vandalism (in addition to massively increasing the risk of a devastating disease being introduced to already threatened wild populations). We are already aware of tortoises being released at random, or even "dumped" in boxes in the streets as keepers react to the new regulations banning the keeping of *Testudo graeca graeca*. Such incidents are already being reported in the

press. The danger presented by such actions is incalculable. **It would take only one single serious incident to introduce a devastating disease, or to completely destroy the ancient and unique genetic integrity of an isolated population.**

It needs to be recognised that tortoises that have been kept as pets are not safe to be released into the wild, except in a very few special cases where their origin and disease status can be fully verified. Anything less carries such a high risk that the dangers far outweigh any possible benefit to conservation.

The taxonomic status of *Testudo graeca graeca* in Spain

If we acknowledge (as we must) that the tortoises previously "lumped together" as *Testudo graeca* from various North African countries do, in fact, constitute a whole 'new' group of different species and subspecies complexes, that differ from one another on morphological and genetic grounds, then we must also keep an open mind with regard to the status of *Testudo graeca* populations within Spain.

The simple question we must ask is "*are we sure they are a homogenous group and are we sure they are all the same tortoise"?*

For many years, the answer to that would have been "yes".

There are clear morphological differences from one population to another, however. For example, some populations have a larger average body size than others, and colouration also varies substantially, with some displaying uniformly darker patterns, and others a bright yellow. The full significance of this is presently unknown and insufficient studies have been carried out.

Today, we must hesitate before reaching any certain conclusion. The most remarkable example of how wrong such basic assumptions can be is to be found in the case of the Desert Tortoise (*Gopherus*) in the South-Western United States. It would be right to say that this is almost certainly the most intensively studied, most documented tortoise species on Earth. Literally thousands of scientific papers have described it in every aspect of its biology and behaviour. Entire books have been written about it. Hundreds of genetic studies have been conducted. We thought we knew what it was.

Then, in 2011, completely new research (Murphy, 2011) demonstrated that what had been believed to be one species by experts everywhere, was in fact, two distinct species.

"A new study shows that the desert tortoise, thought to be one species for the past 150 years, now includes two separate and distinct species, based on DNA evidence and biological and geographical distinctions.

This genetic evidence confirms previous suspicions, based on life history analysis, that tortoises west and east of the Colorado River are two separate species.

The newly recognized species has been named Morafka's desert tortoise (Gopherus morafkai) and represents populations naturally found east and south of the Colorado River, from Arizona extending into Mexico.

The originally recognized species, the Agassiz's desert tortoise (Gopherus agassizii) is listed as threatened under the federal Endangered Species Act. It represents populations naturally found west and north of the Colorado River in Utah, Nevada, northern Arizona and California"

(Source: Press Release from US Geographical Survey)

It must be strongly emphasised that this tortoise (Desert tortoise) is without doubt the most intensively studied and documented on Earth. It has featured in literally thousands of scientific papers, had had entire books written about it, and hundreds of different taxonomic and genetic studies have been carried out over a period of decades. Yet, despite this, new evidence only recently emerged that changed our entire understanding of it.

By contrast, *Testudo graeca* in Spain has had infinitely fewer studies conducted and some populations have barely been studied at all.

However, some recent work is highly significant, and confirms previous suspicions that the genetics and taxonomic status of *Testudo graeca* in Spain are very far from being fully understood.

The most important recent work by far is that of Graciá, et. Al (2011) in their paper "Genetic patterns of a range expansion: The spur-thighed tortoise *Testudo graeca graeca* in southeastern Spain".

The authors of this work report on several significant conclusions, and also make some important practical recommendations. In brief, they find:

a) "we were able to detect a structured regional pattern of genetic diversity with individuals that were well differentiated from the Algerian outgroup"

Commentary: The Spanish regional population clusters are genetically different from an Algerian tortoise control group of *Testudo graeca*. Previous assumptions that tortoises from North Africa are genetically identical to those of southeastern Spain is incorrect.

b) *"In past decades, public administration and NGOs have developed management schemes for these captive animals (i.e. captive breeding, introductions or translocations) with the aim of reinforcing wild populations . However, the execution of these actions, without regard to whether or not the species presents a structured regional pattern of genetic diversity,* ***threatens the evolutive potential of the species*** *in this context through the loss of adaptive complexes "*

Commentary: Releases of seized animals originating in North Africa is a serious threat to the genetic and morphological diversity and integrity of native Spanish populations.

c) *"these manipulative management tools threaten the incipient evolutive potential of the species due to the loss of possible local adaptations. Under a principle of caution (Cooney, 2004), we recommend a better characterization of the genetic structure of T. g. graeca and the delimitation of genetic units at a regional scale, before carrying out these actions"*

Commentary: Introductions of animals from one location to another threaten these populations as they potentially 'degrade' and undermine the very specialised local adaptations to microclimates, habitats and behavioural traits that those populations may have evolved over millennia of isolation.

Further research and much more detailed knowledge of the specific genetic structure of these various populations is required before any further releases or supposed 'reintroductions' take place. Further, because tortoise population have 'adapted' to specific habitats and microclimates over millennia, releasing tortoises into those habitats that do not have these adaptations is highly likely to compromise the survival prospects of released, incompatible, individuals. An obvious example would be releasing light coloured, smaller body-size tortoises into a population of darker coloured, larger average body-size tortoises. The effects this might have on their ability to thermoregulate, to evade predators, or to blend into the vegetation specific to that area is difficult to predict.

Conclusion and recommendations

1) The routine release of ex-pets is unjustified on conservation grounds and doing so presents a very serious threat to the genetic integrity of wild tortoise populations.

2) We presently have an inadequate understanding of the genetic variability present in wild population and sub-populations of *Testudo graeca graeca*. Until we have more knowledge, supposed “reintroductions” should cease.

3) Under no circumstances whatsoever should tortoises of unknown origin ever be released.

4) Tortoises that have mixed with (or inter-bred with) other Mediterranean tortoise species must never be released on the dual grounds of conservation genetics and disease prevention.

5) All possible steps must be taken to avoid releasing hybrid animals.

6) The public should be educated not to take tortoises from the wild. Healthy tortoises that are found should be left alone. If in immediate danger (on a road, for example) they should be moved the shortest possible distance to safety. Injured or sick tortoises may need specialist attention and the public should have a contact point (for example, a website) where this can be obtained, and where further expert advice is available.

7) Tortoises already in captivity should not be released without full disease prevention precautions as described previously, and without being able to establish with 100% certainty their point of origin. A full individual risk assessment is required in all cases. Where this is not possible, the safest course of action is to leave the situation as it is, ensuring that any keeper is made fully aware of the dangers presented by accidental or deliberate releases.

8) There is zero conservation benefit to confiscating ex-pet tortoises. The management problems of maintaining large groups of tortoises are considerable, the financial cost is prohibitive, and once mixed in a large group, such animals can never be released on both disease prevention and genetic grounds.

9) Any reintroductions **MUST** be conducted on a geographical “population by population”

basis, not merely on the basis that "*all Testudo graeca graeca*" are identical. Any such actions must be fully supported by recent genetic evidence and must have the support of relevant specialists and experts who should be consulted at all stages of the program.

10) The most effective conservation actions are often the simple ones. Conserve key habitats. Do not put roads or railway lines through key habitats. If roads are present, they must be fenced and 'underpass' tunnels allowing tortoises to cross safely must be installed. If the habitat is conserved and correctly managed, very few other interventions will be required. Particularly important habitats of threatened species should be fully protected by law, with severe penalties for infringement.

11) It is often preferable (and usually much safer) to do nothing at all than to take actions which have a high risk of causing the very opposite effects to those intended. All conservation actions should be based on solid science and "must do no harm" as one of their most important priorities. Activities which have an unacceptably high risk factor and which could have unpredictable consequences have no place in any serious conservation strategy.

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The Captive Management of Testudo Graeca and the Problems Associated With High Density Groups

Testudo graeca graeca occur in semi arid scrub to semi desert habitats where there is sufficient edible vegetation during periods of activity. The terrain is sloped with a well drained and often rocky substrate. Scrubby vegetation provides partial to total shelter from the sun, enabling the tortoises to selectively position themselves for optimum thermoregulation.

Vegetation is most abundant in late winter and early spring when tortoises emerge from hibernation and are most active. Mating occurs in Spring and gravid females excavate nests to lay their eggs. As the dry season approaches in June, edible vegetation becomes more scarce and to conserve body fluids and energy, tortoises bury down into the substrate and enter into a period of aestivation. Tortoises will remain inactive until mid to late August and when the first heavy rainfall occurs, tortoises emerge from aestivation to drink. After rehydrating they will bury down again until early autumn. Tortoises emerge from aestivation by mid-September, when the lower temperatures and increased rainfall result in the recovery of edible vegetation in the habitat. The eggs laid the previous Spring begin to hatch in the same time period. By late autumn the lower temperatures reduce the activity level of the tortoises. As the temperature continues to drop, they will reduce and then cease feeding and begin to bury into the substrate to hibernate.

When *Testudo graeca* are maintained in captivity it is essential that the environmental conditions of the wild habitat are replicated as closely as possible. In order to achieve this, rescue centres need to be in the same climatic zone of the tortoises being maintained and where the environmental features of their wild habitat are present.

Testudo graeca graze over a very large area and the wild habitat provides them with a wide variety of plant species. When maintained in captivity, these tortoises require large enclosures that will support the growth of the vegetation they feed upon. Providing enough natural graze becomes more difficult when enclosures are too small for the number of tortoises within that area and is virtually impossible in high density groups. High density groups (and too small enclosures) will result in the area being overgrazed and quickly stripped of all edible vegetation. When this occurs, the only option for rescue centres is to purchase commercially grown salad vegetables to feed the tortoises.

Unlike the high fibre, low protein and calcium-rich vegetation tortoises consume in the wild, the commercial produce often fed to them in captive conditions has a much lower fibre and calcium content. Commercial produce is also a poor source of other important trace minerals that would be present in the vegetation that wild tortoises consume and has different affects upon the digestive system. The use of such artificial diets is associated with numerous health problems and especially with growth-related bone disorders and consequent shell deformity.

Providing a correct diet is necessary to maintain the health of adult tortoises, and is absolutely critical for the proper growth and development of juvenile tortoises and for the egg development of gravid females. The diet of this species is quite specific and if the nutritional requirements are not met, juveniles will suffer from nutritional deficiencies and developmental deformities. On an incorrect diet, the eggs of females may not develop properly, resulting in related lower fertility, juvenile health problems and an increase in hatchling mortality.

Enclosure Design Requirements

The captive environment must replicate the wild habitat as closely as possible, and provide the features necessary for natural grazing, thermoregulation, estivation and hibernation, nesting, and the options to respond to seasonal changes within the habitat area.

Security: The perimeters of enclosures need to be secure to prevent tortoises from either climbing over or digging under the outer perimeter. Tortoises are excellent climbers, so the outer perimeters of all pens need to be of adequate height; at least twice as high as the largest tortoise is long. Any squared corners on lower fences/walls can give tortoises a foothold to climb over and should be covered to prevent escapes. Perimeters should also be set below the ground or placed on a solid foundation that is set into the ground.

Predators pose a serious threat to captive tortoises and animals that can attack and kill tortoises in southern Spain include: rats, dogs, foxes, boar, hedgehogs and even large birds. Small juveniles are especially vulnerable to predators, and their pens should have secure framed tops made of galvanised steel mesh.

Microclimates : Microclimates are an important feature in the natural environment of tortoises, as they provide protection from overheating and loss of body fluids (through respiration and evaporation) in extreme summer heat and prevent frost damage during the cold months in winter. When maintaining tortoises in a rescue centre, adequate shade and shelter and conditions in which tortoises can bury into the substrate must be provided. Failure to provide these stable microclimates will result in high mortality rates from dehydration, overheating and freezing.

Even during the spring and autumn when tortoises are most active, these animals still require microclimates to thermoregulate and respond to changes in temperature and humidity. Tortoises use scrubby vegetation to provide them with both full shade (to prevent overheating) and partial shade (to allow for thermoregulation). If these are not provided, tortoises will not be able to properly regulate their body temperature and if adequate shade is not available, tortoises (especially small juvenile tortoises) can quickly overheat and die.

Substrate: Substrate should be very well drained, and the enclosure should contain a variety of slopes, rocks, open basking areas, and a good provision of edible vegetation.
It should be suitable for tortoises to dig scrapes and bury down into and should provide suitable nesting areas for gravid females.

Tortoises will bury into the soil not just to protect themselves from temperature extremes, but to conserve body fluids in an arid environment. Tortoises bury down into the substrate to retain body fluids, and will suffer from chronic low level dehydration if they are kept in to conditions where the cannot dig down into the soil. Chronic low level dehydration causes renal problems and tortoises will often develop bladder stones that must be surgically removed. Due to their small size and lower body mass, juveniles are particularly vulnerable to chronic low level dehydration, and the health conditions described are a common cause of death in young tortoises.

Summary and recommendations:

The captive management of tortoises is far more complex and demanding than it first appears to be. They have extremely narrow and specific environmental and dietary needs that can be very difficult to meet adequately in captivity. The greater the number of tortoises involved, the more difficult this becomes as providing a suitable diet (in particular) can prove impossible. **The provision of commercial salad items is absolutely not an acceptable alternative to a healthy, natural diet.**

Wherever possible it is best to avoid keeping large numbers of tortoises in one location, in a limited area. There are many, many problems as discussed previously in this report. Additional problems occur with the difficulty of providing a suitable diet, and in meeting the very precise environmental requirements of this species. It is preferable not to concentrate large numbers of tortoises in one place, but to manage them as small, dispersed and isolated groups. This reduces disease risks and makes provision of an adequate diet and suitable habitat much easier.

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