

MANUAL

FOR THE DIFFERENTIATION OF CAPTIVE-PRODUCED AND WILD-CAUGHT TURTLES AND TORTOISES (TESTUDINES)



Manual for the differentiation of captive-produced and wild-caught turtles and tortoises (Testudines)

This document was prepared by Species360 under contract for the CITES Secretariat.

Principal Investigators:

Prof. Dalia A. Conde, Ph.D. and Johanna Staerk, Ph.D., Species360 Conservation Science Alliance, <u>https://www.species360.org</u>

Authors:

Johanna Staerk^{1,2}, A. Rita da Silva^{1,2}, Lionel Jouvet^{1,2}, Peter Paul van Dijk^{3,4,5}, Beate Pfau⁵, Ioanna Alexiadou^{1,2} and Dalia A. Conde^{1,2}

Affiliations:

¹ Species360 Conservation Science Alliance, <u>www.species360.org</u>,² Center on Population Dynamics (CPop), Department of Biology, University of Southern Denmark, Denmark, ³ The Turtle Conservancy, <u>www.turtleconservancy.org</u>, ⁴ Global Wildlife Conservation, <u>globalwildlife.org</u>, ⁵ IUCN SSC Tortoise & Freshwater Turtle Specialist Group, <u>www.iucn-tftsg.org</u>. ⁶ Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT)

Images (title page):

First row, left:Mixed species shipment (image taken by Peter Paul van Dijk)First row, right:Wild Testudo marginata from Greece with damage of the plastron (image taken by Henrik Bringsøe)Second row, left:Wild Testudo marginata from Greece with minor damage of the carapace (image taken by Henrik Bringsøe)Second row, middle:Ticks on tortoise shell (Amblyomma sp. in Geochelone pardalis) (image taken by Andrei Daniel Mihalca)Second row, right:Testudo graeca with dog bite marks (image taken by Beate Pfau)

Acknowledgements:

The development of this manual would not have been possible without the help, support and guidance of many people. We thank all the members of Species360 for supporting the development of the Zoological Information Management System (ZIMS), and for their dedication on the record keeping of all the animals under their care. We further thank the Species360 Conservation Science Alliance for their support. We are especially grateful for the support of Markus Monzel and Ronny Bakowskie (Deutsche Gesellschaft für Herpetologie und Terrarienkunde, DGHT), Chris R. Shepherd (Monitor Conservation Research Society), Kanitha Krishnasamy (TRAFFIC Southeast Asia) and John Werth (Pan-African Association of Zoos and Aquaria; Nelson Mandela University; The South African Institute of Electrical Engineers). The information on parasites is strongly based on information provided by Prof. David Modrý (Faculty of Veterinary Medicine, Dept. of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences Brno) and Prof. Andrei D. Mihalca (Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca) to whom we are truly grateful for sharing their knowledge. We further thank our colleagues: Gerald Benyr (Austrian CITES Scientific Support Agency for Animals), Henrik Bringsøe (Køge, Denmark), Victor Loehr, Joe Flanagan (Houston Zoo), Thais Morcatty (Oxford Wildlife Trade Research Group), Mike Pingleton, Mona van Schingen (Federal Agency for Nature Conservation, CITES Scientific Authority), Daniel Natusch, and Peter Praschag (Turtle Island, Conservation Center for Endangered Turtle and Tortoises) for providing their expert knowledge. We further thank the CITES Secretariat for their comments and support.

Table of contents

ntroduction	.1
Glossary	.2
How to use this manual	. 3
Part 1: Inspection key	.4
Part 2: Criteria to determine wild-caugth vs. captive-produced origin	. 5
Part 3: Illustrated guide	.6
Part 4: Advanced methods to determine wild-caugth vs. captive-produced origin \acute	10
References	13

Tables

Table 1 – Criteria to determine wild-caught vs. captive-produced origin	5
Table 2 – Recommended resources for a review of the species most relevant facts	
	1
Table 3 – Summary of advanced methods to determine wild-caught vs. captive-	
produced origin1	2

Appendices

Appendix A: Supplemental information on advanced methods for the differentiation of captive vs. wild origin

Appendix B: The Turtles and tortoises demographic traits database for CITESlisted species

Introduction

Annually, about 865,000 specimens of live tortoises and freshwater turtles (Testudines) listed in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) are legally traded. The vast majority of those originate from captive-breeding and ranching facilities (CITES, CoP17 Doc. 73). CITES requires documentation of the source of a specimen and applies strict conditions that have to be met when a specimen is declared as produced in captivity. The high number of transactions of specimens claimed to be produced in captivity, however, has raised concerns about the potential misuse of source codes. Claims of commercial captive-breeding (source code C) is especially questionable for many turtles and tortoises as it requires considerable amounts of money and expertise and many turtles and tortoises grow slowly and mature late, making commercial captive-breeding unprofitable (Nijman & Shepherd, 2009; TRAFFIC, 2013). Custom officers and inspection officials are challenged to identify misuse of source codes, given the high numbers of exports and imports. The aim of this manual is therefore to provide guidance for inspection officials and customs officers and CITES Management and Scientific Authorities to aid in the identification of potential misuse of source codes.

This manual aims to implement **CITES Decision 17.291 a ii**:

Developing guidance concerning the differentiation of specimens of tortoises and freshwater turtles (Testudines) originating from the wild, from those from captive and ranching production systems.

I. CITES Source Codes

CITES-listed species in the international trade require permits issued by the CITES Management authority. CITES permits must contain a source code that indicates the source of the specimen being traded. Source codes have strict definitions that must be met.

Source code C: Captive-bred

Applies to specimens bred in captivity of Appendix I, II or III listed species, whether or not they were bred for commercial purposes;

- Refers only to specimens born or otherwise produced in a controlled environment
- Parents mated in a controlled environment or were in a controlled environment when the development of the offspring began

Government authorities of the exporting countries must be satisfied that the breeding stock

- was established in accordance with the provisions of CITES and relevant national laws and in a manner not detrimental to the survival of the species in the wild
- is maintained without the introduction of specimens from the wild, except for the occasional addition of animals, eggs or gametes, in accordance with the provisions of CITES and relevant national laws and in a manner not detrimental to the survival of the species in the wild as advised by the Scientific Authority
- has produced offspring of second generation (F2) or subsequent generation (F3, F4, etc.) in a controlled environment; or is managed in a manner that has been demonstrated to be capable of reliably producing second-generation offspring in a controlled environment; and

Further, regarding the trade in specimens of Appendix-I species bred in captivity CITES recommends that the trade in a specimen bred in captivity be permitted only if it is marked in accordance with the provisions on marking in the Resolutions adopted by the Conference of the Parties and if the type and number of the mark are indicated on the document authorizing the trade. **Full text: Resolution** Conf. 10.16 (Rev.) <u>https://cites.org/eng/res/10/10-16C15.php</u>

Source code F: Farmed

Animals born in captivity (F1 or subsequent generations) that do not fulfill the definition of "bred in captivity" in Resolution Conf. 10.16 (Rev.), as well as parts and derivatives thereof).

Adapted from Conf. 12.3 (Rev. CoP17) Permits and certificates

Source code R, ranched:

Rearing in a controlled environment of animals taken as eggs or juveniles from the wild, where they would otherwise have had a very low probability of surviving to adulthood.

Adapted from Conf. 12.3 (Rev. CoP17) Permits and certificates

An additional CITES source code D is defined below, which is only applicable to Appendix I species.

Source code D, captive-bred Appendix I species:

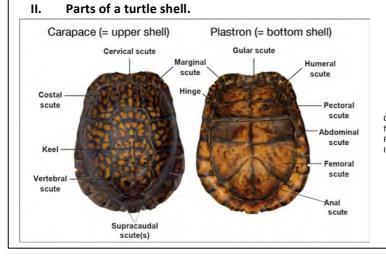
Appendix I animals bred in captivity for commercial purposes and Appendix I plants artificially propagated for commercial purposes as well as parts and derivatives thereof, exported under the provisions of Article VII, paragraph 4 of the Convention.

Adapted from Conf. 12.3 (Rev. CoP17) Permits and certificates

Please note: At present, only one species of tortoise or freshwater turtle can be traded under source code 'D': *Astrochelys radiata*, for which two facilities in Mauritius are registered (<u>https://www.cites.org/eng/common/reg/cb/species.html</u>)

For a guide on the correct application of source code, see also:

Guide to the application of CITES source codes (2017)- also available in Chinese, Indonesian, Khmer, Lao, Malay, Thai and Vietnamese



Credit: Peter Paul van Dijk and Ernie Cooper, original figure from CITES document: <u>SC70 Doc. 61 Annex 6</u> (Guide to Photographing Live Tortoises and Freshwater Turtles for Identification)

How to use this manual:

Four main approaches are used in this manual to identify the true source of a specimen:

- Part 1: An inspection key
- Part 2: Criteria to determine wild-caught versus captive-produced origin
- Part 3: An illustrated guide
- Part 4: Advanced methods to determine wild-caught versus captive-produced origin

The inspection key can be used by inspection officials and custom officers for an initial assessment on whether the traded specimens should be further investigated with regards to the plausibility of the reported source code.

The criteria in Part 2 and the illustrated guide in Part 3 can aid Inspection officials, custom officers and CITES Management and Scientific Authorities in differentiating wild-caught from captive-produced specimens.

In Part 4 we provide additional supporting information that can aid CITES Management and Scientific Authorities to differentiate between wild-caught and captive-produced specimens:

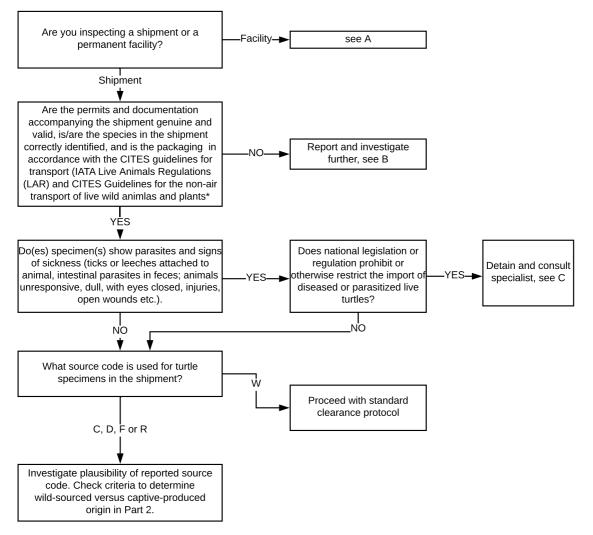
- A table summarizing the species most relevant facts that should be reviewed, including relevant references (Table 2)
- A table reviewing advanced scientific methods that can be used to differentiate wild-caught and captive-produced origin (Table 3), further explained in Appendix A.
- A link to the "<u>Turtles and tortoises demographic traits database for CITES-listed species</u>", which provides a database of reproductive traits extracted from the scientific literature and from the Species360/ZIMS database, further explained in Appendix B.

An important note:

The information presented in this guidance will assist relevant personnel to identify cases where testudines claimed to be captive-bred are in fact wild-caught. However, all of the methodologies presented in this guidance are indicators only, and cannot be used to unequivocally determine the source of specimens. Instead, relevant personnel should use these indicators to identify instances of suspicion, for which in-depth inspection and follow-up is required.

Part 1: Inspection key

This key can help to identify situations where there is concern about the true source of specimens entering trade.



- A. For the inspection of permanent facilities please refer to the general information provided in CITES document AC30 Inf. 25: <u>Guidance for Inspection of captive-breeding and ranching facilities</u> (Lyons, Jenkins & Natusch, 2017) – also available in <u>Chinese</u>, <u>Indonesian</u>, <u>Khmer</u>, <u>Lao</u>, <u>Malay</u>, <u>Thai</u> and <u>Vietnamese</u> and the <u>Inspection Manual for use</u> in <u>Commercial Reptile Breeding Facilities in Southeast Asia</u> (TRAFFIC, 2013).
- B. Report and investigate further. Please note, even if only part of the shipment does not match documentation, still the entire shipment has to be stopped, reported and investigated. If species identification cannot be determined please use proper photo documentation (see SC70 Doc. 61 Annex 6 (Guide to photographing Live Tortoises and freshwater Turtles for Identification, see https://cites.org/sites/default/files/eng/com/sc/70/E-SC70-61.pdf) and refer to the rapid species identification task force (SC70 Doc. 61, p. 6, item 24 available at: https://cites.org/sites/default/files/eng/com/sc/70/E-SC70-61.pdf) and refer to the rapid species identification task force (SC70 Doc. 61, p. 6, item 24 available at: https://cites.org/sites/default/files/eng/com/sc/70/E-SC70-61.pdf) and refer to specimens, default/files/eng/com/sc/70/E-SC70-61.pdf). Concerning export of live specimens, poor packaging for shipping may be indicative of wild origin. Because captive breeding turtles and tortoises requires significant investment of time and resources, exporters should be more likely to invest in adequate packaging to ensure survival during shipping. Because production of wild specimens does not require such investment, the impetus for ensuring adequate package is reduced.
- C. Report to health and quarantine. Parasites and signs of disease can transmit diseases and may be indicative of wild origin; based on national legislation or regulation, such cases should be referred to the health and quarantine specialist.

NOTE: * CITES Guidelines for the non-air transport of live wild animals and plants: (https://cites.org/eng/resources/transport/index.php)

Part 2: Criteria to determine wild-caught versus captiveproduced origin

Check if one or more criteria apply. If in doubt, expert consultation is offered by the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group (TFTSG) and its members (<u>www.iucn-tftsg.org</u>).

CRITERIA	WILD-CAUGHT ORIGIN	CAPTIVE-PRODUCED ORIGIN
Uniformity and age / size	Specimens of different size, age, shape and coloration. Old adults with worn shell, or groups of adults of different sizes/ages.	Usually, specimens similar in size, shape, age and coloration indicate captive-produced origin. Howeve very young animals of similar size may indicate wild- caught origin in some species (see point below on "Only juveniles")
Damages/ injuries	Minor and major scratches, chipped or scarred shells, severe burn marks, scar-related, open wounds, stubbed tails, missing toes, holes drilled into shell	Animal looks clean and flawless. Little or no signs of scratches, damages, injuries, abrasion or wear.
Behavior and Health	Animal unresponsive, dull, eyes closed, weak, dehydrated, underweight, fresh injuries, retraction into shell. Fresh injuries, or infections of older injuries, are sometimes found in shipments of wild-caught turtles and tortoises. Signs of disease like noisy breathing, nasal discharge or plaques in the mouth should be assessed by a specialist. Loose stool often occurs in frightened chelonians and is not necessarily a sign of disease.	Animal alert, responsive, bright eyed, heavy-weight. However, some species are known for their shyness, will close up and be completely unresponsive even after years of captivity. In healthy chelonians it need quite some strength to (carefully) pull a leg out - to avoid biting try with a hind leg.
Parasites	Freshly caught wild chelonians often have external parasites either attached to soft parts or crawling on shell. Check in folds of neck and tail for ticks in tortoises or leeches in freshwater turtles. If found, inform health and quarantine.	Parasites in specimens that are captive-bred indoors are uncommon, but parasites may be present in outdoor enclosures of when wild and captive-bred animals are kept together. Farmed freshwater turtles may show leeches. If parasites are found, contact health and quarantine or consult expert.
Only juveniles	Usually, shipments of mostly juveniles are likely of captive- produced origin. However, some species are prone to egg- poaching, where poachers dig up nests and the resulting wild sourced hatchlings may be shipped as "captive bred" or "ranched". Check for Pig-nosed turtles, and side-necked turtles (e.g. <i>Carettochelys insculpta</i> and <i>Podocnemis unifilis</i> (<i>P. unifilis</i> usually shipped as ranched)) from countries where egg collection is popular (Indonesia, Papua New Guinea etc.) (Burgess & Lilley, 2014).	Young turtles and tortoises from captivity are easily shipped (all small size or similar size), and difficult to find in large quantities in the wild for most species. Therefore shipments of young turtles and tortoises will most probably be captive-produced. Exceptions are a few species that nest <i>en masse</i> in predictable locations; these are prone to egg poaching. However watch out for signs of egg collection to sell hatchlings.
Shell growth	Signs of slow growth such as narrow growth rings or smooth looking shells can be indicators of wild origin in some species (see pictured guide)	In some species of tortoises, animals raised quickly to adult size will show signs of excessive growth (unusually wide, sometimes pale, growth rings) and/or pyramidal shell growth (see illustrated guide) However, pyramiding may occur naturally for example in Star tortoises, and rarely occurs in freshwater turtles.
Color		Color morphs or unusual color strains or patterns of captive-bred specimens are popular among breeders Albino turtles (which lack all pigments), amelanistic (without black pigment) and hypo-melanistic (reduced black pigmentation) and leucistic (excessive yellow pigment) specimens are known for several tortoise and freshwater turtle species.
High numbers/ Breeding success	High numbers of individuals in a single shipment that are known to be difficult to breed in captivity may be wild- caught. An indicator on whether a species is difficult to breed is the species reproductive biology (e.g. late sexual maturity, few eggs laid per year a). Further research into the species most relevant facts and breeding biology may be necessary (see Part 4).	Only few species of turtles and tortoises are easy to breed in high numbers, such as for example the Chinese Softshell turtle (<i>Pelodiscus sinensis</i>). See further information. Further research into the specie most relevant facts and breeding biology may be necessary (see Part 4).

Part 3: Illustrated guide

Uniformity and age/size

Shipments of turtles or tortoises of mixed species and/or turtles and tortoises that are different in size, age, shape, and/or coloration are highly indicative of wild-caught origin.



Shipment of wild *Malayemys sp.* Animals of various sizes, coloration and shape. Damages of the shell indicate wild origin.

Shipment of wild Keeled box turtles (*Cuora mouhotii*). Animals of various sizes, coloration and shape. The yellow spot in the animal at the bottom of the picture is a burn mark typical for wild individuals.

Damages, injuries

Damages to the shell, such as scars, minor and major scratches, chipping of the shell, stubbed tails, missing toes or injuries are highly indicative of wild-caught origin.



Marginated tortoise, *Testudo marginata* (wild, Greece). Minor scratches on the shell of a wild tortoise. Seldom seen in captive-bred tortoises. Also note the clearly visible growth rings.

Physical damage to the shell is a frequent occurrence in wild turtles and tortoises and includes minor and major scratches, chipping, or badly scarred shells. Wild-caught animals can show various abnormalities, open wounds, stubbed tails, or missing toes (Brenner *et al.*, 2009; TRAFFIC, 2013; Benyr, 2014). Scratches on the shell are common especially in land tortoises that live in scrub habitats (especially those originating from drier environments, e.g. the Mediterranean or desert). Some tortoises may show more severe injuries that occur from traffic accidents or agricultural machinery, such as ploughs. Significant damage may also occur from tortoises or freshwater turtles falling off walls (Meek *et al.*, 1981; Sos, 2012), from domestic animals, such as sheep or cows accidentally stepping on tortoises, from birds of prey, foxes, dogs and other carnivores that may attack, gnaw on a turtle's shell, or bite off limbs (especially of land tortoises) (pers. comm. H. Bringsøe; Meek *et al.*, 1981). Some species may occasionally present holes drilled through the edge of the shell, which is done by collectors to temporarily secure animals captured in the wild (TRAFFIC, 2013).



Sicilian pond turtle, *Emys trinacris* (wild). Plastron scars as usual in freshwater turtles.

Marginated tortoise, *Testudo marginata* (wild, Greece). A goat or sheep has stepped on the shell.

Burn marks caused by forest fires are highly indicative of wild-caught origin.

Forest fires or the common practice of agricultural burning kills many tortoises; surviving individuals often possess burn marks on their shell.



Terrapene carolina (wild) The yellow areas are severe burn scars, probably from bush fires.

Testudo hermanni (wild, Greece) Severe damage by fire on the upper hind shell. The white parts are the exposed boney elements underlying the horn scutes. Around the white parts the horn scutes are still partly intact but shape and coloration are irregular.

Behavior and health

Animals that are unresponsive, dull, with eyes closed, weak, dehydrated, underweight, sick or with fresh injuries are indicative of wild-caught origin.



Shipment of mixed species, wild and captive: *Mauremys reevesii* (likely captive, round with black lines); *Trachemys sp.* (unknown origin, ornamented carapace); *Manouria impressa* (likely wild, single individual with spikes); *Indotestudo sp.* (likely wild, far right, rounded). Interesting is the difference in behavior between captive and wild specimens. Captive ones are alert with head sticking out; wild ones are unresponsive. Captive-bred individuals can be habituated to the presence of humans, small spaces, the constant availability of food and water, and the absence of predators (El Balaa & Blouin-Demers, 2011). Signs of captive origin therefore include that the animals are well-fed, are responsive or occasionally even aggressive towards humans (Benyr, 2014). This, however, may not apply to captive animals that are sick or were kept under poor husbandry or after longtime transportation, which is stressful for most animals. When animals are taken from the wild, the animals are often kept under poor conditions prior to transport and may show signs of disease, and be desiccated or under-fed and therefore light in weight. Wild animals are unaccustomed to being handled or to take food from humans and are more likely to show signs of stress and disease, withdraw into their shell, or are unresponsive, lethargic, dull, and/or refuse to eat.

Parasites

The presence of ticks or leeches is indicative of wild-caught origin.



Amblyomma sp. in *Geochelone pardalis*. Typical for African Geochelone species.

Hyalomma aegyptium in *Testudo graeca*. Tick that typically occurs in the Mediterannean, Middle East and Central Asia (Brianti *et al.*, 2010).



Placobdella sp. in *Emys orbicularis*. Leeches usually do not occur in turtles in captivity. Only sometimes in farmed animals kept in outdoor ponds or if wild and captive individuals are kept together.

Tick hidden in the cavity of a leg in a wild *Testudo marginata*.

External parasites such as ticks or leeches can be highly indicative of wild-caught origin. Some species of ticks and leeches require several host species and have well defined geographic boundaries that can help track the origin of the specimen that they are attached to. For example, the tick *Hyalomma aegyptium* is typical for Testudo species and occurs in the Mediterranean, Middle East and Central Asia (Brianti *et al.*, 2010); *Amblyomma sparsum* typical for African *Geochelone* species (Mihalca, 2015). Leeches can usually not survive long in captivity and are typical vectors (carriers) of other blood parasites (hemogregarines); the presence of both can be highly indicative of wild-caught origin. Whereas, parasites such as ticks or leeches are generally not present in captive-bred turtle individuals that are bred indoors, but leeches may be present in farmed turtles, or if wild-caught and captive-produced individuals are raised together or in outdoor enclosure in range countries. Often require several host species, and with well defined geographical boundaries. The correct sampling and the determination of the type of parasite is therefore of crucial importance and needs to be assessed by an expert (for further details, see Appendix A).

Shell growth

Narrow growth rings and a smooth, continuous profile of the shell can be indicative of wild-caught origin in some species.



Red-footed tortoise, wild (*Chelonoidis carbonarius*). A freshly imported wild-caught female; her carapace (left) has a smooth, flowing appearance, but note the dings, dents, and scratches evident along her sides; this is typical for a tortoise that lives in dense undergrowth, and not typical of captive animals. Her plastron (right) is worn smooth like a river pebble, from a lifetime of abrasion from natural substrate. Plastra of captive-bred animals are typically much rougher in appearance and feel.

All species of turtles (except soft-shell turtles: *Trionychidae, Carettochelys insculpta,* and *Dermochelys coriacea*) grow their shell by forming new epidermal keratin layers beneath and beyond the scute of the previous growth cycle, lifting the old scute off the underlying bone and connective tissue (Zug, 1991). This type of growth leads to the formation of concentric growth rings around the edges of the older scutes. Growth rates of captive testudines are often accelerated as compared to animals living in the wild. The width of the growth rings can be used to determine whether the turtle has shown periods of irregular or excessive growth, indicating a period where the animal has been kept in captivity. Recently added wider rings in an animal with otherwise narrow rings can be an indication of a wild animal that has recently been taken into captivity. Shells of wild chelonians typically have a smooth, continuous profile due to slow growth and narrow growth rings (especially in old animals) and/or due to abrasion or wear, or can be slightly raised.

Pyramidal growth and wide growth rings due to periods of excessive growth can be indicative of captive-production in some tortoise species.



Red-footed tortoise, captive (*Chelonoidis carbonarius*). A captive individual with pyramid growth and wide growth rings often seen in captive-raised turtles and tortoises.

Red-footed tortoise (*Chelonoidis carbonarius*). The picture is of a wild-caught adult that has spent some time in captivity, and shell development has altered as a result.

Several dietary and environmental differences in captivity, commonly lead to the development of pyramidal shaped horny plates, referred to as pyramidal growth syndrome (Gerlach, 2004). Pyramidal growth is more prevalent in tortoises, but occasionally occurs in freshwater turtles. In the wild, this condition is rare to non-existent (Wiesner & Iben, 2003; Heinrich & Heinrich, 2016) but a few species, have naturally domed scutes (e.g. *Geochelone elegans, Psammobates spp.*) (Wiesner & Iben, 2003) or may have ridges on the carapace scutes (*Stigmochelys pardalis*) (pers. comm. Pingleton, M.). Experienced breeders that offer good husbandry are able

to minimize or avoid pyramid growth in their animals. Therefore, the presence of this condition is a fairly reliable indicator that the specimen was raised in captivity.

Transportation

Transportation not according to CITES transportation guidelines – (animals crammed, overly taped etc.).



Seizure of black spotted turtle, *Clemmys guttata*. Individuals are crammed and overly taped and were illegally smuggled in suitcases.



Seizure of radiated tortoise (*Astrochelys radiata*) and ploughshare tortoise (*Astrochelys yniphora*).

Illegally traded shipments, possibly concerning wild-collected turtles, may be packaged in inappropriate (non-IATA-compliant) packaging, or shipped 'disguised' as another item. Shipments of legally traded tortoises and freshwater turtles, whether wild or captive-produced, are likely to adhere to required standards of packaging and shipping. Responsible breeders invest considerable time and money into the captive breeding of their animals and usually have an interest in ensuring secure, hygienic transportation, documentation, and high standards of animal welfare, including shipping to prevailing standards.

Part 4: Advanced methods to determine wild-caught versus captiveproduced origin

Whether a particular trade should raise concerns on the potential misuse of source codes will depend on many factors and on the specific situation, species, and country from which the species is exported. Research on the species most relevant facts can be of crucial importance in the evaluation of a fraudulent claim of captive origin, such as the species breeding biology, its threat status, national legislations, and countries of concern. Resources for such a review are provided in Table 2. Moreover, in some cases advanced methods and expert assessment may be necessary. An overview of advanced scientific methods that may be useful in the differentiation between wild-caught and captive-produced individuals is provided in Table 3.

Many species of turtles and tortoises are very difficult to breed in captivity, for examples because they are stress-sensitive, are difficult to keep in groups (e.g. solitary forest turtles), show intraspecific aggression (aggression towards other individuals of the same species) or reproduce and grow slowly. The slow reproduction of some species coupled with high costs of maintenance of the species in captivity (including costs for food, space, electricity, veterinary care etc.) makes breeding of many testudines unprofitable on a large commercial scale (Sigouin *et al.* 2017). Knowledge on the reproductive traits of a species can therefore

give a crude estimation on the biological limits, i.e. on the number of offspring that can maximally be produced. If the demand exceeds the reproductive potential, illegal laundering will become more profitable and is more likely to occur. To assess the reproductive potential of a species some research and understanding into the species life history is necessary. Some traits that are important to consider include: Age at first reproduction, clutch size, number of clutches per year, inter-birth-intervals, survival rates of eggs and juveniles and the species reproductive lifespan.

To aid in the assessment of biological breeding limits we developed a <u>database of demographic life history</u> <u>traits</u> that can help to inform the CITES Management and Scientific Authorities (APPENDIX B). The data provided from Zoos and Aquariums should be taken with caution because zoos and aquariums are not breeding species for commercial purposes, but may give a crude idea on whether breeding is possible. We aim to continue adding information to this database as more data becomes available from field biologist, zoos and aquariums, and registered commercial breeders.

Type of Information:	Suggested references:
Taxonomy	Reference list of identification guides: pp. 71-81 in CoP17 Doc. 73
	https://cites.org/sites/default/files/eng/cop/17/WorkingDocs/E-CoP17-73.pdf
	CITES taxonomic names & Appendices: <u>https://speciesplus.net/species</u>
	Global species list: Catalogue of Life: <u>http://www.catalogueoflife.org</u>
Legal	FAO, IUCN and UNEP. ECOLEX – the gateway to environmental law www.ecolex.org
	UNEP – The Species+ website: <u>www.speciesplus.net</u>
	CITES Checklist: <u>http://checklist.cites.org</u>
	Legal Atlas: <u>https://www.legal-atlas.net</u>
	IUCN Red List: <u>http://www.iucnredlist.org</u>
Threat status	IUCN Red List (global and regional): http://www.iucnredlist.org
	National Red List: http://www.nationalredlist.org
	IUCN SSC Tortoise and Freshwater Turtle Specialist Group: The Top 25+ most endangered
	species of tortoises and freshwater turtles in the world: http://www.iucn-
	tftsg.org/trouble/
Species geographical range	CITES Checklist: <u>http://checklist.cites.org</u>
	IUCN Red List: <u>http://www.iucnredlist.org</u>
Trade volumes	UNEP-WCMC CITES Trade Database: <u>https://trade.cites.org</u>
	Guide to using the CITES Trade database:
	https://trade.cites.org/cites_trade_guidelines/en-CITES_Trade_Database_Guide.pdf
	See Appendix A for total numbers of live individuals exported for commercial trade from
	2010-2016 for different sources.
Species/Countries of concern	This information can be obtained from various online sources, such as news reports or
	from organizations such as the wildlife trade monitoring network TRAFFIC -
	https://www.traffic.org
Captive breeding operations	CITES – Register of captive breeding operations
	https://www.cites.org/eng/common/reg/e_cb.html
	The EU Wildlife Trade Regulation Captive Breeding Databasewww.captivebreeding.unep-
	wcmc.org
	Species360 Zoological Information Management System (ZIMS)
Described and the data	https://www.species360.org - see Appendix A and B
Reproductive data	Turtles and tortoises demographic traits database for CITES-listed species This information
	can be obtained from various sources online, see also database Appendix B.

Table 2: Recommended	resources for a	review of the	species mos	t relevant facts:
----------------------	-----------------	---------------	-------------	-------------------

Table 3. Summary of advanced methods to determine wild-caught versus captive-produced origin

Parasites	The identification of parasite species that require one or several intermediate host species to complete		
	their life cycle (so called heteroxenous species) are of high importance to determine if an individual is		
	wild caught. These types of parasites are very unlikely to occur in captivity, because the respective host		
	species are usually not present in captive environments (see Appendix A).		
Stable	It is possible to use the isotope signatures in a specimen's tissue sample to reconstruct past		
isotope	environmental and climatic conditions, its diet, or trophic level. Isotopic analysis can potentially be		
analyses	used as a regulatory tool for the turtle/tortoise trade such as in determining 1) if batches of captive		
	specimens also include wild-caught individuals and 2) if wild-caught specimens have been fraudulently		
	claimed as captive-bred (see Appendix A)		
Blood	Blood parameter differences found between captive bred and wild caught individuals are mostly		
parameters	attributed to differences in diet, environment, health and age. Parameters include counts of blood		
-	cells, and concentrations of: lipids, natrium, kalium, urea, haematocrit and haemoglobin, among		
	others, which in many cases have been found to be significantly different between wild and captive-		
	bred individuals (see Appendix A)		
Genetic	Forensic genetics can be used for species and source population identification and can assist with the		
samples	identification on whether an animal was captive-produced or wild-caught for example by determining		
	the likelihood that a traded specimen is in fact the offspring from a claimed breeding stock. It is also		
	possible to assign genetic samples to a particular geographic origin (see Appendix A).		
Marking	Individual marking techniques can be of high value in assisting enforcement authorities to trace the		
	origin of a specimen. The unique details of the mark, such as the number code need to match the		
	number on the accompanying permit to ensure that specimens are the same as those referred to in		
	the documentation (see Appendix A)		
Passport	The Pan-African Association of Zoos and Aquaria (PAAZA) is developing the PAAZA Animal Passport		
	that links animal identification with a national database. Therefore, authorities will have ready access		
	to a secure digital database to trace the origin of an individual. In the process, it would also assist		
	Enforcement with animal identification through transponder/microchip and DNA data, which is linked		
	through the various Studbook databases (see Appendix A)		

References:

- Benyr, G. (2014) Die Unterscheidung von Wildfängen und Nachzuchten bei Reptilien: Ihre Bedeutung für den Artenschutz. Projektendbericht im Auftrag des BMLFUW (Ministerium für ein lebenswertes Österreich) Abt. I/8, Nationalparks, Natur- und Artenschutz
- Brenner, D., Lewbart, G., Stebbins, M.,& Herman, D., W. (2009). Health survey of wild and captive bog turtles (Clemmys muhlenbergii) in North Carolina and Virginia. dx.doi.org, *33*, 311–316.
- Brianti, E., Dantas-Torres, F., Giannetto, S., Risitano, A., Brucato, G., Gaglio, G., & Otranto, D. (2010).
 Risk for the introduction of exotic ticks and pathogens into Italy through the illegal importation of tortoises, Testudo graeca. *Medical and veterinary entomology*, 24(3), 336-339.
- Burgess, E. A. and Lilley, R. (2014). Assessing the Trade in Pig-nosed Turtles *Carettochelys insculpta* in Papua, Indonesia. TRAFFIC. Petaling Jaya, Selangor, Malaysia.
- CITES Seventeenth meeting of the Conference of Parties, CoP17 Doc. 73, Tortoises and freshwater turtles (Testudines spp.) Available at:

https://cites.org/sites/default/files/eng/cop/17/WorkingDocs/E-CoP17-73.pdf

- El Balaa, R., & Blouin Demers, G. (2011). Anti predatory behaviour of wild caught vs captive bred freshwater angelfish, Pterophyllum scalare. *Journal of Applied Ichthyology*, 27(4), 1052-1056.
- Gerlach, J. (2004). Effects of diet on the systematic utility of the tortoise carapace. African Journal of Herpetology, *53*(1), 77-85.
- Heinrich, M. L., & Heinrich, K. K. (2016). Effect of supplemental heat in captive African leopard tortoises (Stigmochelys pardalis) and spurred tortoises (Centrochelys sulcata) on growth rate and carapacial scute pyramiding. *Journal of Exotic Pet Medicine*, 25(1), 18-25.
- Lyons, Jenkins & Natusch (2017). Guidance for Inspection of Captive Breeding and Ranching Facilities. https://cites.org/sites/default/files/eng/com/ac/30/Inf/E-AC30-Inf-25.pdf
- Meek, R., Inskeep, R. (1981). Aspects of the field biology of a population of Hermann's tortoise (Testudo hermanni) in Southern Yugoslavia. *British Journal of Herpetology*, *6*, 159-164.
- Mihalca, A. D. (2015). Ticks imported to Europe with exotic reptiles. *Veterinary parasitology*, *213*(1-2), 67-71.
- Nijman, V., and Shepherd, C. (2009). Wildlife trade from ASEAN to the EU: Issues with the trade in captive-bred reptiles from Indonesia. TRAFFIC Europe Report for the European Commission, Brussels, Belgium. Available at: <u>https://www.traffic.org/site/assets/files/9837/issues-with-the-trade-in-captive-bred-reptiles-from-indonesia.pdf</u>
- Sigouin, A., Pinedo-Vasquez, M., Nasi, R., Poole, C., Horne, B., & Lee, T. M. (2017). Priorities for the trade of less charismatic freshwater turtle and tortoise species. *Journal of Applied Ecology*, *54*(2), 345-350.
- Sos, T. (2012). Two extreme cases of regeneration in Testudo graeca ibera Pallas, 1814. *Bihearean Biologist*, 6(2), 128-131
- TRAFFIC (2013). Inspection Manual for use in Commercial Reptile Breeding Facilities in Southeast Asia. Report prepared by TRAFFIC. Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Geneva, Switzerland. p.81. Available at: <u>https://cites.unia.es/cites/file.php/1/files/cb-captive-breeding-manual-en.pdf</u>
- Wiesner, C. S., & Iben, C. (2003). Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (Geochelone sulcata). Journal of animal physiology and animal nutrition, 87(1 - 2), 66-74.
- Zug, G., R. (1991). Age Determination in Turtles. Society for the Study of Amphibians and Reptiles, Herpetological Circular 20. Lawrence, Kansas, U.S.

APPENDIX A

Supplemental information on advanced methods for the differentiation of captive vs. wild origin

- 1. Parasites
- 2. Stable isotope analysis
- 3. Blood parameters
- 4. Genetic samples
- 5. Individual Marking and Passport

1. Parasites:

Parasites can serve as a reliable indicator of wild origin of traded specimens because many parasites have highly complex life-cycles and restricted geographic ranges. Of particular importance are species that require one or several intermediate host species to complete their life cycle (so called heteroxenous species). These are very unlikely to occur in captivity, because the respective host species are usually not present in captive environments. Not all parasites will be indicative of wild origin, the correct sampling and the determination of the type of parasite is therefore of crucial importance. Groups of parasites with high forensic value in chelonians include the protozoan blood parasites Haemogregarines and Haemosporidia, which can be detected in blood smears; internal parasites such as Trematodes (flukes), Cestodes (tapeworms), Acanthocephalans (thorny-headed worms) and Nematodes, particular Spirurida & Filaria (roundworms). Their stages (ususally eggs) can be detected in fecal samples, and sometimes in organs or under the skin, larvae of filaria, so called microfilariae, are detected in blood smears. External parasites include ticks and leeches.

Blood parasites:

Haemogregarines (Protozoa) stages detectable in blood and tissue of turtles and tortoises often transmitted via blood-sucking invertebrates (leeches, ticks, mosquitoes etc.) (Kreier, 1977), normally not present in captivity. Infections can be detected in blood smears and infection persists in wild-caught individuals even after years of rearing in captivity (Široký *et al.*, 2004). *Haemolivia* spp. and *Hepatozoon* spp. are especially important in determining the geographic origin of land tortoises (e.g. *Haemolivia mauritanica* does not occur in Central Europe; *Hepatozoon fitzsimonsi* is only present in African terrestrial tortoises such as in some species of the genus *Kinixys* (Cook *et al.*, 2014)). The genus *Haemogregarina (Placobdella* in Europe) can be an important indicator found mostly in aquatic turtles (DvořÁkovÁ *et al.*, 2015). For example, *Haemogregarina sp.* was detected in 100% of the wild individuals, but not in captive individuals in *Dermatemys mawii* (Rangel-Mendoza *et al.*, 2009).

Haemogregarina stepanowi can be found in the distribution range of European turtle species of the genus *Emys* and *Mauremys* (Özvegy *et al.*, 2015; Javanbakht & Sharifi, 2014).

Haemosporidia (Protozoa) - detectable in blood smears; can be proof of wild origin; further morphological and genetic examination can determine geographic region of origin for example in *Testudo graeca* and *T. horsfieldi* (Javanbaght *et al.*, 2015).

Internal parasites:

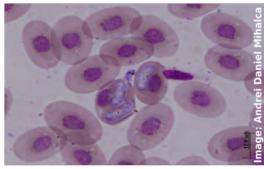
- **Trematodes** (flukes) Eggs detectable in feces. Require intermediate hosts, (often molluscs) that cannot complete life cycle in captivity.
- **Cestodes** (tapeworms) Eggs and proglottids detectable in feces, sometime in organs or abdominal cavities (in dead specimens only), and under skin; highly suggestive for wild origin, transmission in captivity highly unlikely
- Acanthocephalans (thorny-headed worms) live in gut, eggs and larva with obvious horns detectable in feces; presence is highly suggestive of wild origin, transmission in captivity extremely

unlikely;

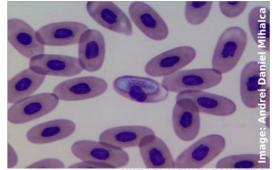
Nematodes, particular Spirurida & Filaria (roundworms) – eggs of Spiruida in feces and presence of microscopic larvae in blood smears in Filaria suggest wild origin. Transmitted by ingested insects as intermediate hosts or by blood-sucking insects.

External parasites:

- Ticks present externally on the specimen, either attached or unattached. Often require several host species, and with well defined geographical boundaries. *Hyalomma aegyptium* is typical for Testudo species and occurs in the Mediterranean, Middle East and Central Asia (Brianti *et al.*, 2010); *Amblyomma sparsum* typical for African *Geochelone* species (Mihalca, 2015).
- Leeches: externally attached to aquatic turtles. Strong indicator as they cannot survive long in captivity. However, may change host in facilities shared between wild and captive animals. Typical vector of blood parasites (hemogregarines). If leeches are present blood should also be examined. Presence of both is highly indicative.



Haemogregarina stepanowi in Emys orbicularis



Hemolivia mauritanica in Testudo graeca



Spirhapalum polesianum in Emys orbicularis

How to:

Imported animals should be physically inspected, preferably by a veterinarian or trained staff. Visible parasites should be documented with protocol and photo-documentation. Parasites are then collected from the body and, if possible from the oral cavity. Ticks and leeches are commonly found on soft parts of turtles and tortoises hidden in skin folds, but also crawling on the shell looking for a place to attach. If subcutaneous parasites are present, swellings or nodules should be photographed and can be surgically removed by a veterinarian for further examination. Fecal samples can be taken non-invasively, and ideally fresh in quantities of 5-10g (depending on the individuals body size) or, if individual sampling is not possible, can be taken from the transportation box. In groups of individuals samples should be taken of 10%-30% of individuals depending on group size. All samples should be labeled (animal ID, data and place of collection), stored cool in a closable container (e.g. plastic tube with lid), and dispatched in a cooled box as soon as possible to a laboratory. If storage exceeds three days samples can be preserved in formalin or ethanol. For blood analysis a veterinarian is required who can take a blood smear and a sample of full blood in pure absolute ethanol. Carcasses of dead animals can be submitted for necropsy. Gloves and disinfectants should be used when taking samples, as some parasites can transmit diseases to humans.

2. <u>Stable isotope analyses:</u>

Isotopes are variants elements, such as hydrogen, carbon, nitrogen or oxygen, that slightly differ by their masses. Because the different isotopes vary their ratios and compositions across geographic regions or trophic levels, it is possible to use the isotope signatures in a specimen's tissue sample to reconstruct past environmental and climatic conditions, its diet, or trophic level. Isotopic analysis can potentially be used as a regulatory tool for the turtle/tortoise trade such as in determining 1) if batches of captive specimens also include wild-caught individuals, and 2) if wild-caught specimens have been fraudulently claimed as captive-bred (Wood, 2012). Isotope analysis can be of strong forensic value, highly accurate and difficult to falsify (Van Schingen et al., 2016) but is highly dependent on the reference data to compare with. At best the isotope signature of all wild populations should be compared with the sample of unknown origin. To assess the variation in the samples would be a first rough estimate. The isotopic variation among samples is expected to be much lower in captive specimens, because they always have the same food source than in wild animals that have diverse prey. However, it is important to note that one cannot conclude that individuals with similar isotope signature come from the same region, only that physiologically similar individuals with different isotope signature are probably not from the same habitat (Wood, 2012; Benyr, 2014). Further limitations of the method include a lack of established protocols due to the limited amount of studies conducted so far, high costs, and time expenses. The method may further not be applicable to species with vast distribution ranges and species that are food and habitat generalists (Van Schingen et al., 2016). Moreover, a number of factors can influence isotope signature in an individual and lead to error, such as heat stress, age, sex, nutritional stress etc.; therefore the method is only applicable when dealing with samples that are similar to samples of unknown origin (Wood, 2012). In previous studies, isotope analysis has been used to accurately differentiate wild and captive crocodile lizards, Shinisaurus crocodilurus (Van Schingen et al., 2016), to reconstruct resource use over decades for the loggerhead sea turtle, Caretta caretta (Vander Zanden et al., 2010), to determine dietary switches in the desert tortoise, Gopherus agassizii (Murray & Wolf, 2012), and has recently been discussed as a tool to regulate trade in three species of the genus Testudo (Wood, 2012).

How to: Tissues have different turnover rates and the tissue to be sampled depends on what best reflects the period of interest. Tissues that may be useful include claw, carapacial and plastral keratin, blood, and in dead specimens: bones, liver, and muscle (Wood, 2012). Of particular importance seems to be metabolically inert tissue, such as scute material, which can be sampled non-invasively and to which material is continuously added over the lifetime in non-shedding species, because isotope signatures can be preserved indefinitely (Wood, 2012). Sampling should be conducted by a veterinarian and the samples of specimens with unknown origin should be compared with reference samples from wild specimens that should ideally cover the species entire distribution range.

3. Blood parameters:

Health assessments measuring haematocrit and haemoglobin concentration in blood samples and body size have previously been used in crime convictions by comparing wild and confiscated animals (Henen *et al.*, 2013). Similar procedures might apply to captive and wild animals. For example, Swimmer (2000) found significantly higher levels of corticosterone, lactate, triglyceride, glucose, and calcium in Hawaiian wild as compared to captive green turtles (*Chelonia mydas*). In loggerhead sea turtles (*Caretta caretta*), count of red blood cells and haemoglobin levels were higher in captive as compared to wild turtles (Basilie *et al.*, 2012). Rangel-Mendoza *et al.* (2009) found differences between wild and captive individuals in several hematological parameters in the critically endangered Central American river turtle (*Dermatemys mawii*) and Brenner *et al.* (2002) in bog turtles (*Clemmys muhlenbergii*). Captive bog turtles had higher white blood cell counts, a lower percentage of heterophils, and lower levels of Natrium, Kalium, and urea nitrogen.

Studies in other reptiles have found similar results, such as an increase in white and red blood cells in wild tuatara, which has been related to the higher tick burden (Cartland et al., 1994). Obese captive

tuatara showed an increased plasma lipid concentration, such as cholesterol as compared to their wild counterparts (Cartland *et al.*, 1998). Differences in blood parameters have been mostly attributed to differences in diet between captive and wild individuals, such as better nutritional status and the richer diet of captive versus wild animals and lower protein intake in wild animals (Basilie *et al.*, 2012). Moreover, higher densities in captivity may result in elevated stress levels, such as corticosterone or testosterone in some populations (Benyr, 2014). Blood parameter samples of specimens with unknown origin should be compared with reference samples from wild specimens that should ideally cover the species entire distribution range. Moreover, blood values may be affected by many factors such as the age, health status, the method of blood withdrawal and the method of analysis.

How to: A veterinarian can collect Blood samples. Experts for example from veterinary or wildlife forensics institutions may be able to make further conclusion, if reference values for the different populations are available or can be collected.

4. Genetics:

Forensic genetics is an advanced technique that can be used for species identification and source population identification and may therefore also be used to identify whether an animal was captivebred or wild sourced. One advantage is that it can be applied to live organisms but also to derivatives and parts. DNA-analysis can be used to determine if a specimen belongs to a CITES listed species, or to assess how related is an individual to another, in this sense genetic analyses can be used to determine the likelihood of traded specimen to in fact be the offspring from a claimed breeding stock. It is also possible to assign genetic samples to a particular geographic origin. Likewise, it was possible to assign wild and captive European pond turtles to their region of origin using the genetic marker cytochrome b (Velo-Anton et al., 2011). The method of identification of genetic origin requires that genetic population data exists from the various regions and that the populations have genetic signatures that are sufficiently distinct from one another (Ogden *et al.*, 2009). This may not be the case, for example if populations regularly exchange genetic material or if populations have only been recently geographically separated. Genetic data is probably still lacking for many testudines species, but due to advances in genetic technologies they are increasingly stored in genetic databases such as GenBank (Benson, 2008). Captive populations often show reduced genetic diversity as compared to wild populations, for example due to inbreeding. This may be used to assist the identification of whether an individual stem from a captive population.

How to: Genetic samples can be collected by a veterinarian for example as blood or tissue samples from live animals, but as well as from parts and derivatives for example with the help of a standard DNA sampling kit. Specific Wildlife forensic DNA kits including an instruction manual have for example been published by the Forensic Working Group (FWG) part of the Partnership for Action against Wildlife Crime (PAW). However, the right molecular marker should be used depending on the questions. Further guidance on wildlife forensics methods can be obtained from TRACE (Tools and Resources for Applied Conservation and Enforcement) – the wildlife forensics network https://www.tracenetwork.org/wp-

content/uploads/2012/08/Wildlife_DNA_Sampling_Guide_web.pdf

5. Individual Marking and Passport

5.1 Individual marking

Individual marking techniques can be of high value in assisting enforcement authorities to trace the origin of a specimen. The unique details of the mark, such as the number code need to match the number on the accompanying permit to ensure that specimens are the same as those referred to in the documentation. European regulations (The EC Wildlife Trade Regulation and some national legislations) require that all live vertebrates of species listed on Annex A being used for commercial purposes (Council Regulation (EC) No 338/97), are permanently and uniquely marked. Annex A listed testudines should be marked with an unalterable microchip transponder, or where this is not

possible, a ring, band, tag, tattoo or another appropriate method. Some Member States have additional guidelines or national legislations on which marking technique are to be used for certain specimens/species. Germany for example requires photo-documentation or other specific marking methods for several testudines species, including *Geochelone radiata*, *Testudo hermanni*, and *Testudo marginata*, (for more information visit <u>www.dght.de</u>) (European Commission, Practical information on Marking and Labelling). The International Zoo Veterinary Group (IZVG) reviewed and identified methods to permanently mark juvenile tortoises in trade, that are too small to be marked by microchips (Walter *et al.*, 2009).

How to: Check whether the unique details of the mark, such as the number code need to match the number on the accompanying permit to ensure that specimens are the same as those referred to in the documentation.

5.2 Passport

An interesting approach that has been developed by the Pan-African Association of Zoos and Aquaria (PAAZA) is the PAAZA Animal Passport that links animal identification with a national database. The PAAZA Animal Passport was introduced as a secure, on-line electronic entry of animal identification to create a National database (South African Department of Environmental Affairs) of initially CITES and TOPS (Threatened or Protected Species) captive Wildlife. This would Assist CITES and regional National TOPS Permitting by having ready access to a secure digital database. In the process, it would also assist Enforcement with animal identification through transponder/microchip and DNA data, which is linked through the various Studbook databases. Because of the linked data recorded in the Studbook, the Animal Passport would strive to ensure that captive born is captive born and also be a good resource in creating internationally viable breeding populations, particularly for endangered species. Most tagging methods can be accommodated into the database since it is an alphanumeric protocol. With reference to Turtles, both RFID and Satellite tags can be used and recorded. RFID can be recorded by handheld readers (researchers) or automatically through embedded ground readers at specific breeding locations (as with many other species) (pers. comm. John Werth, Executive Director, PAAZA).

References

- Basile, F., Di Santi, A., Ferretti, L., Bentivegna, F., & Pica, A. (2012). Hematology of the Mediterranean population of sea turtle (Caretta caretta): comparison of blood values in wild and captive, juvenile and adult animals. *Comparative Clinical Pathology*, 21(6), 1401-1406.
- Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2008). GenBank. *Nucleic acids research*, *36* (Database issue), D25.
- Benyr, G. (2014) Die Unterscheidung von Wildfängen und Nachzuchten bei Reptilien: Ihre Bedeutung für den Artenschutz. Projektendbericht im Auftrag des BMLFUW (Ministerium für ein lebenswertes Österreich) Abt. I/8, Nationalparks, Natur- und Artenschutz
- Brenner, D., Lewbart, G., Stebbins, M.,& Herman, D., W. (2009). Health survey of wild and captive bog turtles (Clemmys muhlenbergii) in North Carolina and Virginia. dx.doi.org, *33*, 311–316.
- Brianti, E., Dantas-Torres, F., Giannetto, S., Risitano, A., Brucato, G., Gaglio, G., & Otranto, D. (2010).
 Risk for the introduction of exotic ticks and pathogens into Italy through the illegal importation of tortoises, Testudo graeca. *Medical and veterinary entomology*, 24(3), 336-339.
- Cartland, L. K., Cree, A., Sutherland, W. H. F., Grimmond, N. M., & Skeaff, C. M. (1994). Plasma concentrations of total cholesterol and triacylglycerol in wild and captive juvenile tuatara (Sphenodon punctatus). *New Zealand journal of zoology*, *21*(4), 399-406.
- Cartland-Shaw, L. K., Cree, A., Skeaff, C. M., & Grimmond, N. M. (1998). Differences in dietary and plasma fatty acids between wild and captive populations of a rare reptile, the tuatara (Sphenodon punctatus). *Journal of Comparative Physiology B*, *168*(8), 569-580.

- Cook, C. A., Lawton, S. P., Davies, A. J., & Smit, N. J. (2014). Reassignment of the land tortoise haemogregarine Haemogregarina fitzsimonsi Dias 1953 (Adeleorina: Haemogregarinidae) to the genus Hepatozoon Miller 1908 (Adeleorina: Hepatozoidae) based on parasite morphology, life cycle and phylogenetic analysis of 18S rDNA sequence fragments. *Parasitology*, 141(12), 1611-1620.
- DvořÁkovÁ, N., KvičerovÁ, J., HostovskÝ, M., & ŠirokÝ, P. (2015). Haemogregarines of freshwater turtles from Southeast Asia with a description of Haemogregarina sacaliae sp. n. and a redescription of Haemogregarina pellegrini Laveran and Pettit, 1910. *Parasitology*, 142(6), 816-826.
- European Commission, "Marking and Labelling", 2016. Available at http://ec.europa.eu/environment/cites/info_marking_en.htm. Accessed: 21st of October, 2018.
- Henen, B. T., Hofmeyr, M. D., & Baard, E. H. (2013). Body of evidence: forensic use of baseline health assessments to convict wildlife poachers. *Wildlife Research*, 40(4), 261-268.
- Highfield and the Tortoise Trust (2015). The Effect of Basking Lamps on the Health of Captive Tortoises and other Reptiles. Online article, available at:

http://www.tortoisetrust.org/articles/baskinghealth.html

- Javanbakht, H., & Sharifi, M. (2014). Prevalence and intensity of Haemogregarina stepanowi (Apicomplexa: Haemogregarinidae) in two species of freshwater turtles (Mauremys caspica and Emys orbicularis) in Iran. *Journal of Entomology and Zoology Studies*, 2(4), 155-158.
- Javanbakht, H., Kvičerová, J., Dvořáková, N., Mikulíček, P., Sharifi, M., Kautman, M., & Široký, P.
 (2015). Phylogeny, diversity, distribution, and host specificity of Haemoproteus
 spp.(Apicomplexa: Haemosporida: Haemoproteidae) of Palaearctic Tortoises. Journal of Eukaryotic Microbiology, 62(5), 670-678.
- Kreir, J. (Ed.). (1977). Gregarines, Haemogregarines, Coccidia, Plasmodia, and Haemoproteids (Vol. 3). Elsevier.
- Mihalca, A. D. (2015). Ticks imported to Europe with exotic reptiles. *Veterinary parasitology*, *213*(1-2), 67-71.
- Ogden, R., Dawnay, N., & McEwing, R. (2009). Wildlife DNA forensics—bridging the gap between conservation genetics and law enforcement. Endangered Species Research, *9*(3), 179-195.
- Özvegy, J., Marinković, D., Vučićević, M., Gajić, B., Stevanović, J., Krnjaić, D., & Aleksić-Kovačević, S. (2015). Cytological and molecular identification of Haemogregarina stepanowi in blood samples of the European pond turtle (Emys orbicularis) from quarantine at Belgrade zoo. *Acta Veterinaria*, 65(4), 443-453.
- Rangel-Mendoza, J., Weber, M., Zenteno-Ruiz, C. E., López-Luna, M. A., & Barba-Macías, E. (2009). Hematology and serum biochemistry comparison in wild and captive Central American river turtles (Dermatemys mawii) in Tabasco, Mexico. *Research in veterinary science*, 87(2), 313-318.
- Široký, P., Kamler, M., & Modrý, D. (2004). Long-term occurrence of Hemolivia cf. mauritanica (Apicomplexa: Adeleina: Haemogregarinidae) in captive Testudo marginata (Reptilia: Testudinidae): evidence for cyclic merogony?. *Journal of Parasitology*, *90*(6), 1391-1393.
- Swimmer, J. Y. (2000). Biochemical responses to fibropapilloma and captivity in the green turtle. *Journal of Wildlife Diseases*, *36*(1), 102-110.
- van Schingen, M., Ziegler, T., Boner, M., Streit, B., Nguyen, T. Q., Crook, V., & Ziegler, S. (2016). Can isotope markers differentiate between wild and captive reptile populations? A case study based on crocodile lizards (Shinisaurus crocodilurus) from Vietnam. *Global ecology and conservation*, *6*, 232-241.
- Velo-Antón, G., Wink, M., Schneeweiß, N., & Fritz, U. (2011). Native or not? Tracing the origin of wildcaught and captive freshwater turtles in a threatened and widely distributed species (Emys orbicularis). *Conservation genetics*, *12*(2), 583-588.

 Walter, O., Quest, R., Bradfield, T & Thornton, S.M. 2009.
 A study to identify the suitability of NonatecTM mini-microchips and Alpha•DotsTM as methods of uniquely marking juvenile Testudinids (tortoises). JNCC report, No. 459 Wood, E., M. (2012). A Potential Enforcement Tool for Regulating Trade in Tortoises: Stable Isotope Analysis.

APPENDIX B

The Turtles and tortoises demographic traits database for CITES Listed species

Many species of turtles and tortoises have a low reproductive potential; that is they grow slowly, mature late, and lay few eggs per year. The slow reproduction coupled with high costs of maintenance of the species in captivity (including costs for food, space, electricity, veterinary care etc.) makes breeding of many turtles and tortoises unprofitable on a large commercial scale. Knowledge on the reproductive traits of a species can give a crude estimation on the biological limits, i.e. on the number of offspring that can maximally be produced. If the demand exceeds the reproductive potential, illegal laundering will become more profitable and is more likely to occur. To assess the reproductive potential of a species some research and understanding into the species life history is necessary. Some traits that are important to consider include age at first reproduction, clutch size, life span, and inter-birth intervals.

Age at first reproduction (also: Age at sexual maturity, Age at maturity, Age at first offspring): The age at which an individual starts reproducing).

Many turtle and tortoise species mature late. Desert tortoises for example reach maturity only after 10-15 years. In captivity, some species may attain maturity earlier than in the wild. The highly traded Indian star tortoise (*Geochelone elegans*) for example may attain maturity around 6-7 years in the wild, but by 3 years in captivity (D'Cruze et al. 2016). It is therefore unlikely that a facility that has recently been established sells adult females that mature late produce a large number of offspring.

Clutch size: Average number of eggs per clutch.

Testudines can have clutch sizes as little as one egg per clutch up to ~200 eggs per clutch (e.g. 75-123 eggs/clutch for the Arrau turtle (*Podocnemis expansa*; Vanzolini, 2003); 65-193 eggs/clutch for the narrow-headed softshell turtle, (*Chitra indica*; Das & Singh, 2009). The average number of eggs laid per clutch multiplied with the number of clutches a species can lay per year indicates the yearly reproductive output of a female. This can give a rough estimate of upper limit of the number of offspring that a female can produce, but does not consider egg or hatchling mortality. The **recruitment** describes the actual number of hatchlings that survive, being a more realistic measure of the number of offspring a female can produce.

Maximum longevity: Maximum number of years a species can live

Many testudines species are long lived and some can live over a hundred years. Subtracting age at first reproduction from the maximum longevity can give an upper limit on the number of years a female can reproduce (called the **reproductive lifespan** of the species). However, it is important to consider that not all individuals survive to the maximum age. In fact, these constitute only a small proportion of the total population. This is because, of the initial number of individuals being born at a given time (i.e. cohort), the number of individuals still alive at different ages diminishes as time passes, which in turn reduces the number of females capable of reproducing.

Inter-birth intervals: Time between consecutive birth events

In many species, individual females do not reproduce every year, particularly among long-lived species. Thus, the reproductive potential of females of a species that reproduce every two years will be half of that of females of a species with same clutch sizes and reproductive lifespan but that reproduce every year. For example, in a wild population in the wet-dry tropics of northern Australia, Doody et al., 2006, recorded that females of the Pig-nosed turtles (*Carettochelys insculpta*) lay only one egg every second year.

Unfortunately, data on inter-birth intervals is not widely common, therefore it is not yet included in the database. However, we are working on obtaining this information from experts including registered captive breeders and field biologist.

IMPORTANT NOTE: The reproductive output in the wild can only give a crude measure on the reproductive output expected in captivity as these may vastly differ. Some species will not be able to reproduce in captivity at all, or the techniques may not yet have been developed, whereas some species that can easily be farmed may reproduce in much higher numbers than in the wild. For example, individuals experience higher growth rates, when resources are constantly available under captive breeding conditions.

The database includes all CITES listed species of turtles and tortoises (181): 31 in Appendix I, 130 in Appendix II, 29 in Appendix III, and one in Appendix I/III. We matched the CITES listed species with the IUCN Red List, of the 191 CITED listed species one is assessed as extinct in the wild (EW), 33 as critically endangered (CR), 35 as endangered (EN), 33 as Vulnerable (VU), 15 as near threatened (NT), 18 as least concern (LC), 4 as data deficient (DD) and 43 have not been evaluated (NE). However not for all listed species there is information. Demographic data is not available for all CITES-listed species. For example, data on age at first reproduction was only available for 31% of all species (55/181), clutch size for 74% of species (133/181) and number of clutches per year for 38% (71/181) of species. We included data on other demographic variables and the number of exports reported by the exporter in the WCMC CITES Trade Database (see Table B.1)

How to: Determine whether the species can likely be bred in captivity, given its reproductive potential, information on reproductive traits from the literature and derived from Species360 zoos and aquariums member institutions is provided in the Turtles and tortoises demographic traits database for CITES Listed Species (https://www.species360.org/serving-conservation/turtles-tortoises-cites/).

Methods:

To create the Turtles and tortoise's demographic traits database for CITES Listed species we compiled and standardized data from: i) existing peer-review databases and ii) from zoo and aquariums members of Species360 that hold turtles and tortoises. Furthermore, we retrieved the current IUCN Red List Status and the number of exports of live animals from the UNEP WCMC Trade Database.

Demographic data from existing databases:

We used data from our recently developed database to generate the Species Knowledge Index on Demography (Conde *et al. in prep*). For this database we compiled and standardized data from two databases and the literature. The available data covers 186 species of turtles and tortoises (complete database available in Appendix C) of the 344 extant species described in the Catalogue Of Life (Roskov et al., 2018). The data sources include the Amniote Life History Database (Myhrvold et al. 2015), AnAge (De Magalhaes & Costa 2009), which comprise an extensive literature search on demographic and other life history traits. Furthermore, we included data from Grimm *et al.* (2014) and Shine & Iverson (1995). The raw data and the references to it are provided in the database with its corresponding metadata.

Demographic data derived from Species360/ZIMS:

We obtained data on reproduction and maximum lifespan from the Species360 Zoological Information Management System (ZIMS) (Species360 ZIMS, 2018). Species360 is a Non-Profit Non-Governmental, international member driven institution that develops and manages the Zoological Information Management Software (ZIMS). Species360 has 1,100 member zoos and aquariums institutions across 96 countries, that share standardized records through ZIMS for 10 million historically and living animals across 21,000 species. For each species with records for more than 20 individuals, we extracted ages at first reproduction, maximum lifespan, and mean yearly recruitment (recruitment is the number of new born surviving birth/hatching). We excluded records for individuals that were either contracepted, managed as groups, that were older than one year when entering ZIMS, and that never gave birth in their lifespan. From the age at first reproduction and the maximum lifespan, we calculated the reproductive period of each species: Reproductive Period = (Maximum lifespan) -(age at first reproduction). We used the mean of the observed yearly recruitments of a species to determine the reproductive output. We highlight that most ZIMS members do not attempt to breed turtles and tortoises in large numbers. We further expect an underestimation of the recruitment potential because we do not have records of birth control (physical separation of sexes). Underestimation of the recruitment can also be induced by unavailable data on interbreeding periods. On the other hand, only reproducing animals were considered for the calculation, which may overestimate reproductive output. Still this information should be taken with caution since Zoos and Aquariums are not breeding the species for commercial purposes. Therefore, we are working with experts to further expand demographic information from farms and registered commercial breeders.

CITES UNEP-WCMC summary trade data:

We extracted data on exports, reported by the exporter, of live animals since 2005 per country and its source code from the UNEP-WCMC CITES Trade database (UNEP-WCMC, 2018) in case this information is needed to know if the species is commonly exported.

Table B.1. Names and description of variables contained in the Turtles and Tortoises demographic traits database for CITES Listed Species

database for CITES Listed	Species
Variable name	Variable description
Species.name.CITES	Species names as in the CITES Trade Database
Species.name.CoL	Species names according to taxonomy in the Catalogue of Life (http://www.catalogueoflife.org)
Common.name	Common name as in the CITES Trade Database
Family	Taxonomic family as in the CITES Trade Database
	CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) listing
CITES.Appendix	appendix in 2018 (https://www.cites.org/eng)
	Threatened category according to the IUCN Red List assessment (http://www.iucnredlist.org/)
IUCN.Status	version 2017-1
	Minimum age at first reproduction, in years from peer-reviewed data repositories (see
AFR.Minimum	metadata for the source of each record)
	Average age at first reproduction, in years from peer-reviewed data repositories (see metadata
AFR.Average	for the source of each record)
	Minimum number of clutches per year from peer-reviewed data repositories (see metadata for
CLY.Minimum	the source of each record)
	Average clutches per year, from peer-reviewed data repositories (see metadata for the source
CLY.Average	of each record)
021111001080	Maximum clutches per year from peer-reviewed data repositories (see metadata for the source
CLY.Maximum	of each record)
CET.Waxinum	
CC Mining and	Minimum clutch size per reproductive event, from peer-reviewed data repositories (see
CS.Minimum	metadata for the source of each record)
	Average clutch size per reproductive event, from peer-reviewed data repositories (see
CS.Average	metadata for the source of each record)
	Maximum clutch size per reproductive event, from peer-reviewed data repositories (see
CS.Maximum	metadata for the source of each record)
	Maximum lifespan in days from peer-reviewed data repositories (see metadata for the source of
ML.Maximum	each record)
	Number of individuals used to calculate demographic data from Species360 Zoological
Nr.Individuals	Information Management System (ZIMS) (2017), . <u>Species360.org</u> .
Nr.Institutions	Number of zoological institution from who data was used for the analysis
	Level of confidence of average age at first reproduction based on the number of individuals
Confidence	(<20: Very Low, 21-100: Medium, >101:High) in ZIMS. <u>Species360.org</u> .
	Average age at first reproduction, in years from the Species360 Zoological Information
AFR.ZIMS.Average	Management System (ZIMS) (2017), . <u>Species360.org</u> .
	Standard deviation of Average age at first reproduction, in years from the Species360 Zoological
AFR.ZIMS.Standard.deviation	Information Management System (ZIMS) (2017), ZIMS. <u>Species360.org</u> .
	Maximum Lifespan in years (maximum recorded lifespan from ZIMS data and from standardized
ML.ZIMS.Maximum	peer-review data repositories from Conde et al, in prep)
	Number of offspring per year, from the Species360 Zoological Information Management System
R.Per.Year	(ZIMS) (2017), <u>Species360.org</u> .
	Number of offspring per year (Standard deviation), from the Species360 Zoological Information
R.Per.Year.Standard.deviation	Management System (ZIMS) (2017), Species360.org.
C,R,F,D	Number of individuals commercially exported between 2010 and 2015 from a captive source
	(sources: C, R, F, D). from UNEP-WCMC CITES Trade Database
С	Number of individuals commercially exported between 2010 and 2015 from a captive-bred
	source (source: C), from UNEP-WCMC CITES Trade Database
R	Number of individuals commercially exported between 2010 and 2015 from a ranched source
	(i.e. animals reared in a controlled environment, taken as eggs or juveniles from the wild)
	(source: R), as reported from UNEP-WCMC CITES Trade Database
F	Number of individuals commercially exported between 2010 and 2015 that were born in
	captivity (F1 or subsequent generations that do not fulfill the definition of "captive-bred,
	source: F) from UNEP-WCMC CITES Trade Database
D	Number of individuals commercially exported between 2010 and 2015 listed in Appendix I as
-	(source: D from UNEP-WCMC CITES Trade Database
w	Number of individuals commercially exported between 2010 and 2015 from a wild source
	(source: W), from UNEP-WCMC CITES Trade Database

References

CITES trade statistics derived from the CITES Trade Database, UNEP World Conservation Monitoring Centre, Cambridge, UK. Available at: <u>https://trade.cites.org</u>. (2018).

Das, I., & Singh, S. (2009). Chitra indica (Gray 1830)–narrow-headed softshell turtle. *Conservation* biology of freshwater turtles and tortoises: a compilation project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs, (5), 027-1.

De Magalhaes J, Costa J (2009) A database of vertebrate longevity records and their relation to other life-history traits. *Journal of evolutionary biology* 22(8):1770-1774.

- D'Cruze, N., Choudhury, B. C., & Mookerjee, A. (2016). Geochelone elegans. The IUCN Red List of Threatened Species 2016: e. T39430A2926441.
- Doody J.S, Georges A, Young J.E. (2006). Twice every second year: reproduction in the pig-nosed turtle, Carettochelys insculpta, in the wet–dry tropics of Australia. J. Zool., 259(2), 179–188.
- Grimm A, Prieto Ramírez AM, Moulherat S, Reynaud J, Henle K (2014) Data from: Life-history trait database of European reptile species. *Dryad Data Repository*.
- Roskov Y., et al. (2018) Species 2000 & ITIS Catalogue of Life, 2018 Annual Checklist. Digital resource at http://www.catalogueoflife.org/annual-checklist/2018. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X.
- Shine R, Iverson JB (1995) Patterns of Survival, Growth and Maturation in Turtles. *Oikos* 72(3):343-348.
- Species360 Zoological Information Management System (ZIMS). Available at: <u>zims.Species360.org</u>, (2018)
- Myhrvold, N. P., Baldridge, E., Chan, B., Sivam, D., Freeman, D. L., & Ernest, S. M. (2015). An amniote life-history database to perform comparative analyses with birds, mammals, and reptiles. *Ecology*, *96*(11), 3109-3109.
- Vanzolini, P. E. (2003). On clutch size and hatching success of the South American turtles Podocnemis expansa (Schweigger, 1812) and P. unifilis Troschel, 1848 (Testudines, Podocnemididae). *Anais da Academia Brasileira de Ciências*, 75(4), 415-430.