AQUATIC CONSERVATION: MARINE AND FRESHWATER ECOSYSTEMS
Aquatic Conserv: Mar. Freshw. Ecosyst. **24**: 831–840 (2014)
Published online 2 April 2014 in Wiley Online Library
(wileyonlinelibrary.com). DOI: 10.1002/aqc.2454

Assessment of genetic structure, habitat suitability and effectiveness of reserves for future conservation planning of the Euphrates soft-shelled turtle Rafetus euphraticus *(Daudin, 1802)*

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ABSTRACT

1. The endangered Euphrates soft-shelled turtle, *Rafetus euphraticus*, is endemic to the Mesopotamian River Basin in the Middle East. Within recent decades, populations of this aquatic species have been heavily depleted and severely fragmented owing to habitat alteration and destruction by drainage and hydroelectricity dam constructions. Continuing habitat loss and fragmentation are considered the main drivers for the population decline of *R. euphraticus*.
2. Intraspecific genetic variability was investigated using two mitochondrial gene fragments for 31 specimens covering most of the distributional range of the species. Habitat suitability models were computed using a combination of bioclimatic and remote sensing variables as environmental predictors to assess habitat suitability, habitat fragmentation and coverage by designated protected areas across the range of *R. euphraticus*.
3. Beyond single substitutions in two sequences, no significant genetic variation could be detected in *R. euphraticus*. Models show habitat suitability to be high throughout the range of the species, although only a fraction is currently covered by reserves. Habitat suitability and coverage of reserves is highly variable among countries. South-western Iran appears to be of major importance for future conservation strategies. Suitability models are in concordance with the habitat selection patterns of *R. euphraticus*.
4. The existing reserve system is considered insufficient and has to be significantly improved in order to sustain viable populations of *R. euphraticus*. To counter continuing fragmentation and alteration by dam construction, future conservation measures should focus on highlighted priority areas.

Received 17 July 2013; Revised 9 January 2014; Accepted 5 February 2014

KEY WORDS: genetic variation; habitat suitability modelling; habitat fragmentation; protected areas; conservation planning; Middle East

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INTRODUCTION

The Euphrates soft-shelled turtle, *Rafetus euphraticus* (Daudin, 1802), is a highly aquatic trionychid turtle that is restricted to the Mesopotamian River Basin in Turkey, Syria, Iraq, and Iran (Taşkavak and Atatür, 1995, 1998; Ghaffari *et al*., 2008; Biricik and Turğa, 2011). The Mesopotamian River Basin encompasses 950,876 km^2 and is formed by the Euphrates and Tigris rivers and their tributaries, stretching from south-eastern Turkey to the Persian Gulf in south-western Iran. The northern part of the range of *R. euphraticus* is characterized by a semi-arid climate, while the southern portion comprises large floodplains and marshes as well as seasonal and permanent wetlands. While *R. euphraticus* historically inhabited lentic and lotic habitats all across the Mesopotamian River Basin, the species is now heavily affected by habitat destruction and fragmentation caused by conflicts and wars in the recent past, as well as by continuing drainage and a rapidly increasing number of dams constructed across its distributional range (Taşkavak and Atatür, 1995; Partow, 2001). As a result, populations are severely declining in Turkey and Iran, while data are lacking for Syria and Iraq (Gramentz, 1991; Taşkavak and Atatür, 1995; Ghaffari *et al*., 2008; Biricik and Turğa, 2011). Consequently, *R. euphraticus* was listed as 'Endangered' by the IUCN Red List of Threatened Species in 1996 (Biricik and Turğa, 2011; IUCN, 2013a).

Considering that levels of genetic diversity are generally low among turtles, many species of which are highly threatened, the assessment of genetic structure to reveal potential evolutionary significant units (ESUs) is important for planning conservation management strategies (Janzen *et al*., 1997; Asian Turtle Conservation Network (ATN), 2006; Alacs *et al*., 2007; IUCN, 2013a). Genetic analyses of the closely related Swinhoe's soft-shelled turtle, *Rafetus swinhoei* (Le *et al*., 2010; Duong *et al*., 2012) and the more distantly related Nile soft-shelled turtle, *Trionyx triunguis* (Güçlü *et al*., 2009; Gidis *et al*., 2011) found genetic diversity to be low. However, there is no information on the intraspecific genetic structure of *R. euphraticus*.

Habitat alterations resulting from dam construction are known to have severely diminished populations of *R. euphraticus* (Gramentz, 1993; Taşkavak and Atatür, 1995), but no comprehensive assessment spanning its range has been conducted so far. Although the range of *R. euphraticus* encompasses many designated reserves, an assessment of the effectiveness of these reserves to sustain viable populations is currently lacking. Species distribution models using species occurrence records and bioclimatic variables have been used successfully to assess habitat suitability and the effectiveness of existing reserves for conservation planning (Araújo *et al*., 2004, 2007; Hannah *et al*., 2007; Rödder and Schulte, 2010; Rödder *et al*., 2010). This study investigated whether genetically distinct units exist in *R. euphraticus*, and determined habitat suitability and fragmentation, as well as coverage by designated reserves across its distribution range, in order to give recommendations for future conservation management.

METHODS

The level of genetic variation in the mitochondrial DNA of *R. euphraticus* was assessed to identify potential ESUs that need to be considered in conservation planning. Thirty-one tissue samples were collected from 12 localities covering much of the distribution range of the species (Table 1, Figure 1). Tissue samples were obtained either by clipping carapace edges of living turtles or by taking muscle or liver tissue from preserved specimens. DNA was extracted using the Qiagen DNA tissue extraction kit (Qiagen Benelux B.V., Venlo, Netherlands). Two mitochondrial gene fragments, cytochrome b (cytb) and NADH dehydrogenase subunit 4 (ND4), were amplified by polymerase chain reaction (PCR) using the primers GluDG/Peil (Engstrom *et al*., 2007, modified from Palumbi *et al*., 1991) and ND4/LEU (Arevalo *et al*., 1994), respectively. All amplified fragments were sequenced in both directions by a commercial company (Macrogen, Korea). Sequences were checked with the original chromatograph data using the program CodonCode (CodonCode Corporation, Dedham, MA, USA) and subsequently aligned with the MUSCLE algorithm (Edgar, 2004). The final dataset comprises 778 and 711 base pairs for cytb and ND4, respectively. Sequences are available in GenBank (Table 1). The dataset was supplemented

Table 1. List summarizing geographical locations as displayed in Figure 1, scientific collection numbers, and GenBank accession numbers of tissue samples used to assess the genetic structure of *Rafetus euphraticus* (for geographic location of collection sites see Figure 1)

Code	Locality	Locality ID	GenBank accession numbers (cytb, ND4)	
RE01	Iraq, Dookan Lake	1	KJ401000	KJ400974
RE02	Iraq, Dookan Lake	1	KJ401001	KJ400975
RE03	Iraq, Dookan Lake	1	KJ401002	KJ400976
RE04	Iraq, Mosul	2	KJ401003	KJ400977
RE05	Iraq, Habbaniya Lake	3	KJ401004	KJ400978
RE06	Iran, Loore River	4	KJ401005	KJ400979
RE07	Iran, Loore River	4	KJ401006	KJ400980
RE08	Iran, Loore River	4	KJ401007	KJ400981
RE09	Iran, Loore River	4	KJ401008	
RE10	Iran, Rofaiye, Hawizeh Marshes	5	KJ401009	KJ400982
RE11	Iran, Rofaiye, Hawizeh Marshes	5	KJ401010	KJ400983
RE12	Iran, Zavie Mash Ali	6	KJ401011	KJ400984
RE13	Iran, Zavie Mash Ali	6	KJ401012	
RE14	Iran, Karkheh Dam Lake	7	KJ401013	KJ400985
RE15	Iran, Karkheh Dam Lake	7	KJ401014	KJ400986
RE16	Iran, Karkheh Dam Lake	7	KJ401015	KJ400987
RE17	Iran, Karkheh Dam Lake	7	KJ401016	KJ400988
RE18	Iran, Karkheh Dam Lake	7	KJ401017	KJ400989
RE19	Iran, Karkheh Dam Lake	7	KJ401018	KJ400990
RE20	Iran, Karkheh Dam Lake	7	KJ401019	
RE21	Iran, Karkheh Dam Lake	7	KJ401020	
RE22	Iran, Karkheh Dam Lake	7	KJ401021	KJ400991
RE23	Iran, Karkheh Dam Lake	7	KJ401022	KJ400992
RE24	Iran, Karkheh Dam Lake	7	KJ401023	
RE25	Turkey, Atatürk Dam	8	KJ401024	KJ400993
RE26	Turkey, Atatürk Dam	8	KJ401025	
RE27	Turkey, Batman River	9	KJ401026	KJ400994
RE28	Turkey, Diyarbakir, Euphrates River	10	KJ401027	KJ400995
RE29	Turkey, Halfeti, Euphrates River	11		KJ400996
RE30	Turkey, Halfeti, Euphrates River	11	KJ401028	KJ400997
RE31	Turkey, Halfeti, Euphrates River	11	KJ401029	KJ400998
RE32	Turkey, Birecek	12		KJ400999
	Turkey, Birecek	12	AY259554	AY259604

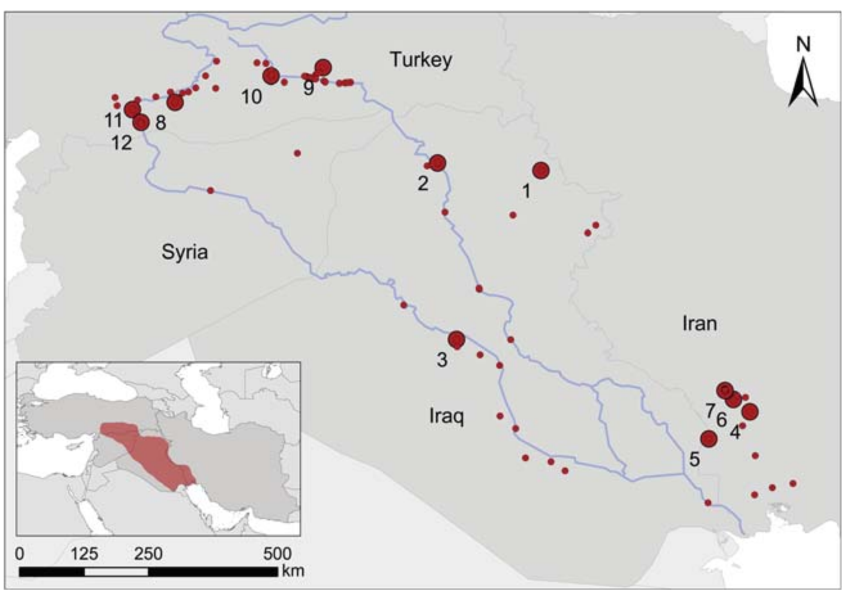

Figure 1. Distributional range of *Rafetus euphraticus* shown in translucent red, species occurrence records used for HSMs displayed as small red dots and genetic sampling sites as listed in Table 2 displayed as large red dots. Labels refer to sampling site IDs listed in Table 1.

by one cytb (AY259554) and one ND4 (AY259604) sequence of a single individual accessible on GenBank.

Habitat suitability models (HSMs) were used to determine habitat suitability throughout the distributional range of *R. euphraticus*. Ninety-one georeferenced species occurrence records, covering the entire range of the species, were compiled from fieldwork, scientific collections (Naturhistorisches Museum Wien, Vienna, Austria and the Natural History Museum, London, UK), online databases (Global Information Facility GBIF: http://www.gbif.org and HerpNet: http://www.herpnet.org), and scientific publications (Kinzelbach, 1986; Stadtlander, 1992; Taşkavak and Atatür, 1998; Taşkavak, 1999). When necessary, records were georeferenced using the Global Gazetteer, version 2.1 (global gazetteer: http://www.fallingrain.com/world). A combination of modified remote sensing variables obtained from the EDENext project (EDENext: http://www.edenext.eu) was used as environmental predictors comprising pre-processed remote sensing variables derived from Moderate Resolution Imaging Spectroradiometer (MODIS) sensors of two NASA satellites with a spatial resolution of 30 arc seconds and a temporal resolution of 8-day averages (MOD11A2) and 16-day averages (MCD43B4) (Mu *et al*., 2007; Scharlemann *et al*., 2008). The raw set of remote sensing variables comprised monthly averages of average day and night time land surface temperatures, normalized vegetation index (NDVI), enhanced vegetation index (EVI) and middle infra-red coding for water content in the vegetation collected between 2001 and 2005. Based on bioclimatic variables and the remote sensing dataset, a new set of environmental predictors, describing annual seasonal variation, was computed using the *dismo* and *raster* packages (Hijmans and van Etten, 2012; Hijmans *et al*., 2012) for *Cran R* (R Development Core Team, 2012). The final set of environmental predictors comprised 12 variables (Table 2), clipped to the distribution range of *R. euphraticus*.

Ensemble HSMs were performed using the *biomod2* package vers. 2.1.15 (Thuiller *et al*., 2013) for *Cran R* including the following algorithms: Surface Rectangular Envelopes (SRE), Maxent, Generalized Boosting Models (GBM), Artificial Neural Networks (ANN), and Multivariate Adaptive Regression Splines (MARS). Models were trained using a randomly selected subset of species occurrence

Table 2. List of derived environmental predictors and contributing remote sensing and bioclimatic variables used for SDMs. Column two lists abbreviations of derived predictors used; column three and four list contributing remote sensing and bioclimatic variables

		Contributing variable denotation	
ID	Derived variable	Remote sensing variable	Bioclimatic variable
X01	Mean of Middle Infra-Red in the Quarter with Highest Scores	MODIS V4 Band 03 Synoptic Months: Middle Infra-Red	Bio10: Mean Temp. of Warmest Quarter
X02	Mean of Middle Infra-Red in the Quarter with Lowest Scores	MODIS V4 Band 03 Synoptic Months: Middle Infra-Red	Bio11: Mean Temp. of Coldest Quarter
X03	Seasonality of Middle Infra-Red	MODIS V4 Band 03 Synoptic Months: Middle Infra-Red	Bio 4: Temp. Seasonality (standard deviation *100)
X04	Annual Mean Temp.	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temp.	Bio1: Annual Mean Temp.
X05	Mean Temp. of Warmest Quarter	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temp.	Bio10: Mean Temp. of Warmest Quarter
X06	Mean Diurnal Range of Temp.	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temp.	Bio 2: Mean Diurnal Range (Mean of monthly (max temp - min temp))
X07	Isothermality (Bio2/Bio7) (* 100)	MODIS V4 Band 07 + 08 Synoptic Months: Day- + Night-time Land Surface Temp.	Bio 3: Isothermality (Bio2/Bio7) (* 100)
X08	Min NDVI of Monthly Scores	MODIS V4 Band 14 Synoptic Months: Normalised Difference Vegetation Index (NDVI)	Bio 6: Min Temp. of Coldest Month
X09	Mean EVI in the Quarter with Highest Scores	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Bio10: Mean Temp. of Warmest Quarter
X10	Mean EVI in the Quarter with Highest Scores	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Bio11: Mean Temp. of Coldest Quarter
X11	Annual Range of EVI	MODIS V4 Band 15 Synoptic Months: Enhanced Vegetation Index (EVI)	Bio7: Temp. Annual Range (Bio5-Bio6)
X12	Slope	Slope	

records (80%), while the remaining 20% were used to assess model performance in a total of five iterations per algorithm, applying the receiver operating characteristic curve (ROC) (Swets, 1988), Cohen's Kappa and the True Skill Statistic (TSS) (Allouche *et al*., 2006). For model building, 1000 pseudo-absences were randomly created within a circular buffer of 100 km surrounding the species records and a weighted ensemble was computed based on all models with ROC>0.7. The minimum training presence was selected as presence/ absence threshold. Results were used to build a consensus map based on an ensemble using the partial weighting mean option displaying current distribution and habitat suitability.

Habitat suitability was compared with the known distribution of *R. euphraticus* and the impact of dam constructions and drainages which, according to Partow (2001), are major drivers of habitat fragmentation. Data on dam locations and construction times were obtained from the Food and Agriculture Organization of the United Nations (FAO, 2013). In addition, coverage of the current occurrence of *R. euphraticus* and suitable habitat with terrestrial protected areas according to IUCN standards (categories I–VI) (IUCN, 2013b) was analysed to highlight potential future conservation areas. Categorizing reserves according to the management objectives of the IUCN protected area management categories system is recognized as a standard for defining protected areas by international bodies and numerous national governments (IUCN, 2013b). Protected areas assigned to the following categories were selected: (Ia) Strict nature reserve; (1b) Wilderness area, (II) National park, (III) Natural monument or feature, (IV) Habitat/species management area; (V) Protected landscape, (VI) Protected area with sustainable use of natural resources (IUCN, 2013b). ESRI shapefiles on protected areas were obtained from the World Database of Protected Areas (IUCN, UNEP-WCMC, 2013. http://www.protectedplanet.net).

RESULTS

Except for single substitutions in three cytb sequences (RE 06, cytb of RE 14, AY259604) the specimens examined shared the same haplotype for the mitochondrial cytb and ND4 genes, i.e. no relevant intraspecific genetic variation could be found in *R. euphraticus*.

The overall habitat suitability model performance was high with 'excellent' ROC values being obtained for 19 models (mean $ROC_{test} = 0.93 \pm 0.01$; mean $Kappa = 0.63 \pm 0.02$; mean $TSS = 0.75 \pm 0.02$), which indicates that the model possesses a strong ability to discriminate between suitable and unsuitable habitats. Variables with the highest contribution to the model are X07 (temperature isothermality) (19.2%), followed by X04 (annual mean temperature) (13.4%), X02 (mean of middle infra-red in the quarter with lowest scores) (11.2%), X01 (mean of monthly middle infra-red in the quarter with highest scores) (11.1%), X06 (mean diurnal temperature range (mean of monthly (max temp–min temp))) (8.7%), X12 (slope) (8.7%), X05 (mean temperature of warmest quarter) (8.5%) and X03 (seasonality of middle infra-red) (5.9%), while the remaining variables on average added less than 5% to the final habitat suitability model (Table 2).

The assessment of the spatial extent of suitable habitat for *R. euphraticus* shows the species to be restricted to floodplains, streams, rivers, and marshes of the Mesopotamian Plains, which is bordered by the Taurus Mountain Range in the north and the Zagros Mountain Range in the east. While 22% of the species distribution range is considered to be covered with suitable habitat only a fraction (0.5%) currently falls within the boundaries of designated protected areas (Figure 2). Listing suitability as proportion of country surface indicates Syria (40%) to be most suitable, followed by Iraq (34%), Turkey (26%) and Iran (14%). Ranking countries by suitable habitat that is currently protected according to IUCN criteria as a proportion of suitable habitat suggests Iran (5.7%) to be of major importance, while coverage of protected areas with suitable habitat is low in Turkey (0.6 %) and absent in Syria and Iraq.

DISCUSSION

Although previous analyses of the genetic structure of wide-ranging trionychid turtles detected sequence

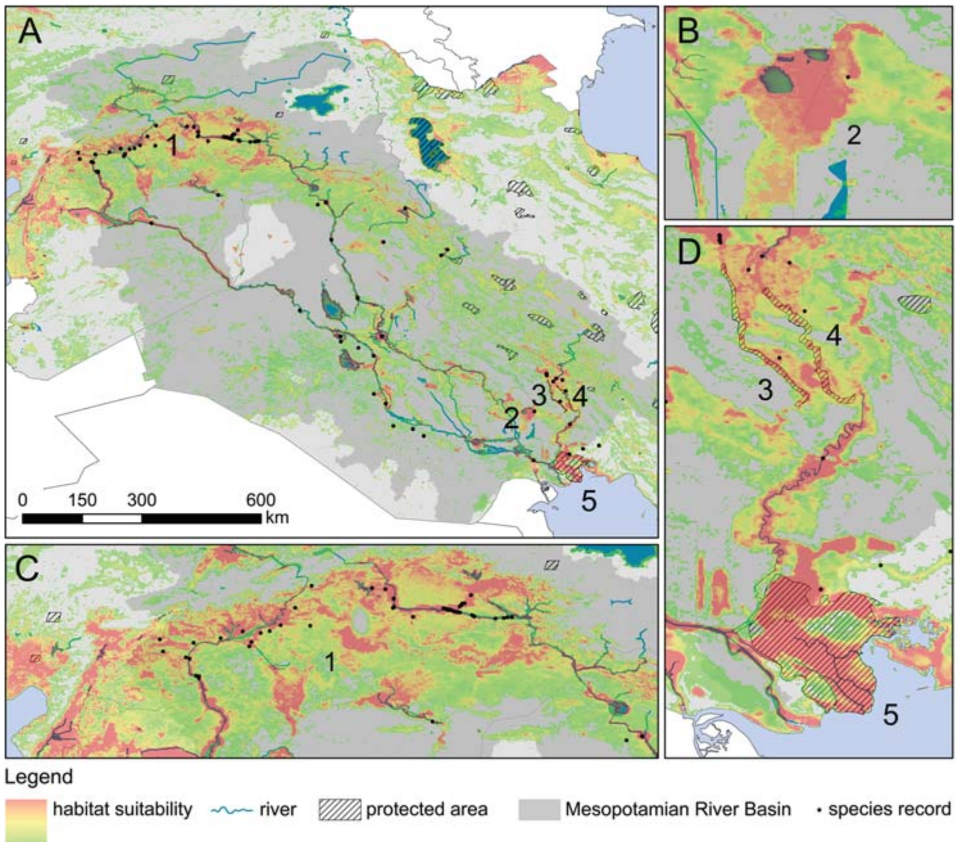

Figure 2. (A) Potential habitat suitability for *Rafetus euphraticus* across its range, with suitability ranging from high (red) to low (green) and particularly important future conservation priority areas: (1) Plains in south-eastern Turkey; (2) Havizeh Marshes, (3) Karkheh Protected Areas; (4) Dez Protected Areas; (5) Shadegan Wildlife Refuge. (B) Potential habitat suitability at the Havizeh Marshes. (C) Potential habitat suitability at the Plains in south-eastern Turkey. (D) Potential habitat suitability at (3) the Karkheh and (4) Dez Protected Areas and (5) the Shadegan Wildlife Refuge. Protected areas refer to IUCN standards (I–VI) (IUCN, 2013b).

divergence in mitochondrial DNA within species of up to 8% (Weisrock and Janzen, 2000; Engstrom *et al*., 2004; McGaugh *et al*., 2008), except for single substitutions in three sequences, no differences were found in the genetic structure of *R. euphraticus*. Given the wide distributional range of the species, stretching from eastern Turkey to south-western Iran, the observed genetic uniformity is surprising. However, results for the closely related species *R. swinhoei* from Vietnam and China, also using cytb, ND4, and one nuclear gene R35, showed no significant genetic diversity as well (Duong *et al*., 2012). The observed uniformity in two mitochondrial genes (each >700 bp) in *R. euphraticus* indicates the absence of distinct ESUs in the study area. It suggests also that *R. euphraticus* expanded recently to its current extent or that, until recently, there were high levels of gene flow among the different populations. However, these results are based on a rather limited number of tissue samples (31) from only 12 localities. Further analyses, including a larger sample size, additional genes and higher resolution markers, are required to conduct adequate MUs (management units) and conservation management strategies that retain a maximum of genetic diversity.

While the model showed that a major portion of the interconnected system of lotic and lentic water bodies in the Mesopotamian River Basin represents potentially suitable habitat, the known distribution of *R. euphraticus* seems to be restricted to patches in south-eastern Turkey, the Euphrates River Basin in Iraq, and the Mesopotamian Marshes in south-western Iran (Figure 1). During the late 1990s, large portions of the Mesopotamian River Basin were drained to reclaim land for agricultural purposes, reducing wetlands to a fraction of their former extent (Partow, 2001). Since 1960, the number of dams completed, mainly for hydroelectric power and flood control, has increased dramatically (1960 = 18, 1970 = 42, 1980 = 84, 1990 = 163, 2000 = 246, 2012 = 126). Most of these dams are either situated within the Mesopotamian River Basin or upstream (Figure 3), affecting the area by water level alterations. A particularly large number of dams are situated in southern Turkey.

Dams reduce flood pulses that sustain downstream wetlands, while saline return drainage from irrigation and dam retention of sediments reduce marshland fertility and related ecosystem processes (Partow,

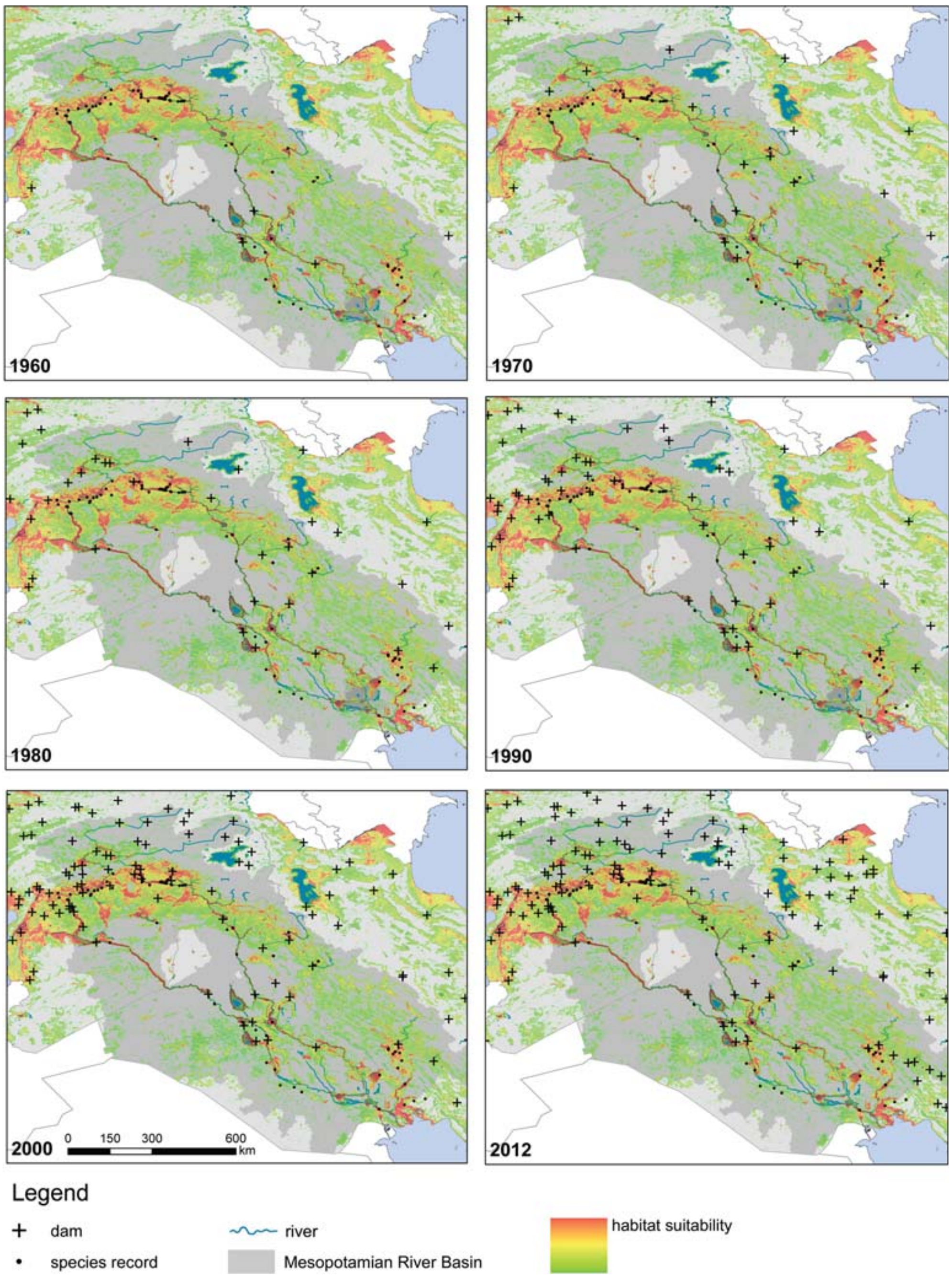

Figure 3. Current habitat suitability ranging from high (red) to low (green) and habitat fragmentation owing to increasing dam construction and drainage of the Mesopotamian Marshes between 1960 and 2012.

2001). Dam construction and channelization have been reported to fragment habitat and cause severe changes of microhabitat conditions causing multiple problems for freshwater turtles, such as *R. euphraticus* (Dodd, 1990; Gramentz, 1993; Taşkavak and Atatür, 1995, 1998). The construction of the Keban and Atatürk Dams on the Euphrates River were reported to strongly decrease water level fluctuations and temperature (Gramentz, 1993; Taşkavak and Atatür, 1995, 1998). As a consequence, growth of aquatic vegetation eliminated basking spots while the rising water level inundated potential nesting sites (Gramentz, 1993; Taşkavak and Atatür, 1995, 1998). Hence, habitat conditions for resident turtle populations changed dramatically, causing severe population declines (Gramentz, 1993; Taşkavak and Atatür, 1995). Unfortunately, the species is currently affected by the construction of 19 additional proposed dams and projects already under construction, which will cause further fragmentation of the remaining habitat and may increase the probability of local extinction (Gramentz, 1991) (Figure 4).

As no comprehensive dataset on water quality, current or substrate characteristics is available for the area, these parameters could not be incorporated into the HSM although they certainly affect turtle distribution. In addition, barriers to the movement of turtles, such as dams, as well as general accessibility cannot be captured by HSMs. Both drawbacks lead to overestimations of suitable range sizes to an unknown extent.

As the area designated as 'suitable' is already heavily disconnected, the portion that is suitable as well as accessible for turtles may be even smaller. The HSM in comparison with real occurrence records suggests that dam construction along with drainage and channelization have already caused severe fragmentation and hampered population connectivity for decades. Given the home range sizes, territoriality and habitat selection of *R. euphraticus* (Ghaffari *et al.*, in press), only large interconnected wetlands are considered suitable future conservation areas. Regarding habitat suitability, size, accessibility and coverage with reserves as well as recent and future dam constructions, the interconnected system of wetlands located in south-western Iran, including the Shadegan Wildlife Refuge (IUCN category IV), and the Karkheh and Dez Protected Areas (IUCN category V) which are connected by the Karun River, is considered a particularly important stronghold for *R. euphraticus*, although the recent construction of the Dez, Karkheh, and Gotvand Dams and the current

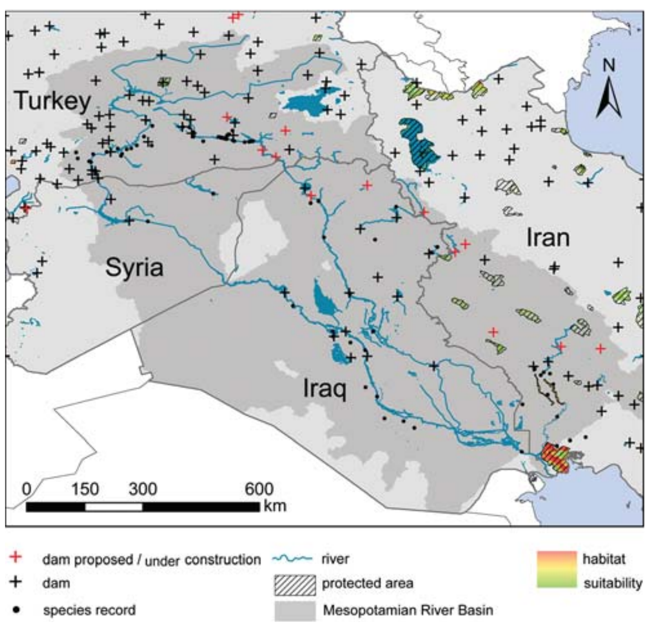

Figure 4. Present and future dam constructions in Turkey, Iraq, Syria and Iran, including projects currently under construction as well as proposed dams. Habitat suitability within protected areas according to IUCN standards (I–VI) (IUCN, 2013b) ranging from high (red) to low (green).

upstream construction of the Bakhtiari and the Seimare Dams may cause some habitat alterations. In addition, the plains in south-eastern Turkey, at present harbouring a significant portion of the species, are considered potential high priority conservation areas. Unfortunately, coverage of protected areas supporting *R. euphraticus* in south-eastern Turkey is less than 1% while the area is heavily fragmented by several dams. These issues should be addressed to increase the effectiveness of any conservation initiatives undertaken in the area. In addition, the restored Havizeh Marshes located on the border between Iraq and Iran represent a potential stronghold for *R. euphraticus*. In Iraq, there are potentially suitable habitat stretches along the Euphrates and Tigris rivers, including adjacent floodplains and lakes. However, as suitable habitat along the Tigris and Euphrates rivers is already heavily disconnected by dams, we suggest conservation approaches to focus on the wetlands and rivers south of Baghdad.

Throughout its range *R. euphraticus* is affected by water pollution through pesticides, fertilizers, oil, garbage, and industrial chemicals. The species is prone to entanglement and drowning in fishing gear and is considered by fishermen to be a competitor for fish (Ghaffari *et al*., 2008). As a result, specimens are intentionally killed when caught (Ghaffari *et al*., 2008); thus, to sustain viable populations, hunting, fishing, and pollution need to be reduced to a minimum and patrols put in place. As suggested by Lowry *et al*. (2005), fishing gear should be modified to prevent accidental capture of turtles. Capacity building and environmental education among local residents should be conducted to raise awareness for turtle conservation and to stop the dispatch of accidently caught specimens. A project to protect the remaining *R. euphraticus* populations in south-western Iran, carried out by the Pars Herpetologists Institute between 2009 and 2012 has proved successful and resulted in a significant behavioural change amongst the local population, providing confidence for future projects. To restore population connectivity and gene flow among populations, we suggest equipping dams with suitable passes to facilitate migration of turtles and other aquatic species. To our knowledge, such passes exist at present only to assist migratory fish.

ACKNOWLEDGEMENTS

We thank James Hanken and José Rosado of Harvard University for providing tissue samples from Habbaniye Lake and Mosul in Iraq. We are indebted to Barbod Safei Mahroo, Anna Sophia Bachmann, Hana Raza and Nabil Musa for their assistance during the field surveys in Iran and Iraq. We are grateful to Morris Flecks for his valuable comments on the genetic part of this manuscript, Ursula Bott and Jeffrey E. Dawson for proof reading the manuscript.

This project was supported by the Turtle Conservation Fund (TCF), the German Academic Exchange Service (DAAD) and the Mohamed bin Zayed Species Conservation Fund (MBZ) for which we are very grateful.

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