RESEARCH ARTICLE



First population assessment of the Asian Water Dragon (Physignathus cocincinus Cuvier, 1829) in Thua Thien Hue Province, Vietnam

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Academic editor: K. Henle | Received 23 October 2017 | Accepted 20 February 2018 | Published 15 March 2018 http://zoobank.org/AE66A752-6A50-4955-9D6F-2074EC2A77FD

Citation: Nguyen TQ, Ngo HN, Pham CT, Van HN, Ngo CD, van Schingen M, Ziegler T (2018) First population assessment of the Asian Water Dragon (*Physignathus cocincinus* Cuvier, 1829) in Thua Thien Hue Province, Vietnam. Nature Conservation 26: 1–14. https://doi.org/10.3897/natureconservation.26.21818

Abstract

The Asian Water Dragon, *Physignathus cocincinus* Cuvier, 1829, was originally described from southern Vietnam. Wild populations of this species are strongly affected by harvesting, particularly in central Vietnam, while potential negative impacts are not yet foreseeable. This study provides the first population size estimation using a capture-recapture approach and an evaluation of threats to and trade levels in *P. cocincinus* in Thua Thien Hue Province, central Vietnam. Field surveys were conducted at 14 sites of known occurrences of the species in Thua Thien Hue during April and June of 2016 and 2017. The estimated population size of the species amongst these sites ranged from 232 to 250 individuals. The mean abundance varied from 1.98–2.64 individuals/100 m along inhabited streams. This study revealed harvest activities for local food consumption and to supply the pet trade. Accordingly, imports of several thousand living individuals per year from Vietnam into the EU were documented. It was further demonstrated that the level of negative impacts on different sub-populations is in accordance with respective abundances and the incidence of adults. This finding indicates that rising harvest levels in concert with decreasing habitat

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quality, might seriously affect the wild population of *P. cocincinus* in the future. Thus, the authors herein emphasise the necessity for increased conservation measures and for monitoring of the species as well as its natural habitat in order to warrant a sustainable use of the species in the future.

Keywords

Population size, capture-recapture method, trade, conservation, risk assessment

Introduction

The Asian Water Dragon, Physignathus cocincinus, was originally described by Cuvier, 1829 from southern Vietnam, subsequently recorded from southern China, Myanmar, Thailand, Laos and Cambodia and reported as introduced in Taiwan (Smith 1935, Taylor 1963, Cox et al. 1998, Nabhitabhata et al. 2000, Stuart and Emmett 2006, Stuart et al. 2006, Grismer et al. 2008a, b, Suzuki et al. 2015, Uetz et al. 2017). Typical habitats of the species consist of undisturbed rocky streams within evergreen lowland rainforest, but sometimes the species can also be found in densely vegetated streams nearby villages and in agricultural areas (Ziegler 2002, Nguyen et al. 2009). In Vietnam, the Asian Water Dragon is considered as a species with a wide geographic range. However, habitat degradation represents an emerging threat and there is rising evidence for strong local use of the species. In addition, its attractive appearance, as well as its semi-aquatic lifestyle, made the species a highly desirable target species in the international pet trade (UNEP-WCMC 2017). As a consequence, the species was recently listed in the Vietnam Red Data Book (2007) as Vulnerable. Furthermore, the species was listed in Appendix D on the "Council Regulation (EC) No. 338/97 on the protection of species of wild fauna and flora by regulating trade therein" in order to identify potential unsustainable levels of trade in the species in good time. However, basic knowledge on the current population status of *P. cocincinus* and anthropogenic impacts on the species is still lacking. Population size estimations provide essential baseline information for the assessment of the conservation status of species and populations and are crucial for wildlife management strategies (Reed et al. 2003, Traill et al. 2007, Ngo et al. 2016). The present study provides the first assessment of the population size of *P. cocincinus* in Vietnam, based on surveys conducted in Thua Thien Hue Province, as well as an evaluation of threats in order to assess its conservation status and as a basis for timely development of adequate conservation management to warrant both the persistence and the sustainable use of the species.

Methods

Study sites

Survey sites were selected based on previous reports on known occurrences of *P. cocinci*nus in Phong Dien Nature Reserve (NR) in Phong Dien District, Sao La NR in A



Figure 1. Map of Thua Thien Hue Province in central Vietnam showing the survey sites: **1** Phong Dien NR in Phong Dien District; (2) Sao La NR in A Luoi District; and **3** Bach Ma NP in Nam Dong District.

Luoi District and Bach Ma National Park (NP) in Nam Dong District, Thua Thien Hue Province, Vietnam (Nguyen et al. 2009) (Figure 1). The vegetation throughout the study area is dominated by evergreen broadleaf forest, intermixed with bamboo forest. The region is characterised by a tropical climate and seasonal monsoons, with an annual average temperature of 24.9 ± 0.51 °C, an annual average precipitation of 3243.2 ± 780.6 mm and an annual average humidity of $86.8 \pm 1.05\%$ (General Statistics Office of Vietnam 2013, Nguyen et al. 2013).

Field surveys

Field surveys were conducted in April and June 2016 and 2017, respectively, during the active season of the Asian Water Dragon. During previous surveys, *P. cocincinus* was found to be a riparian species, being exclusively recorded in the immediate proximity to or above the water at the stream banks. Thus, the riverine vegetation of selected rocky streams was sampled from downstream to upstream by walking through the water and intensively investigating both sides of the streams equally. The surveyed streams were characterised by a width of about 1–12 m, depths of up to 200 cm and dense vegetation along and above the stream. Since *P. cocincinus* is a diurnal species, night excursions were conducted between 1900h and 2400h, when animals were expected to rest on branches above the water (Fig. 2A).



Figure 2.A Habitat of *Physignathus cocincinus* in Nam Dong District, Thua Thien Hue Province **B** Resting perch in front of waterfall **C** Adult male **D** Adult female. Photos: Hai Ngo.

In total, 14 sections of 14 different streams with lengths varying from 320 to 1400 m (see Table 1) were surveyed. Thereof, two streams were in Phong Dien NR (Phong Dien District), at elevations between 43 and 90 m a.s.l, eight streams were within Sao La NR (A Luoi District) at elevations between 176 and 820 m a.s.l and four streams were within Bach Ma NP (Nam Dong District) at elevations between 105 and 269 m a.s.l. Each stream was repeatedly surveyed in intervals of several days. At each point of investigation, the team consisted of 2–4 persons. Coordinates and elevations for each captured individual were recorded with a GPS Garmin 64. These streams were selected based on information about the presence of *P. cocincinus* obtained during previous surveys and interviews with local people. Lengths of stream sections were dependent on habitat suitability and accessibility.

All encountered individuals were captured, individually marked with a permanent pen (Edding Eraser) and released at the same spot after taking measurements (Fig. 2B, C). This marking technique has the advantage of being non-invasive, not affecting the animals, inexpensive and enables the short-term identification of animals, while marking lasts until the next shedding of the animals (for details see Ngo et al. 2016). Each spot was marked by a label made of waterproof paper to identify exact positions of captured and recaptured animals. It was observed that animals occupied previous perches after marking, indicating that capturing does not affect the behaviour and perch choice of animals. Generally, individuals of all ages and sexes were found in a likewise manner. To estimate

Stream	District	Physignathus cocincinus	Elevation (m. asl)	Length (m)	Habitat type
1	Phong Dien	Present	44–90	1,160	Primary forest
2	Phong Dien	Present	43–75	1,300	Primary forest
3	A Luoi	Present	706–780	320	Secondary forest
4	A Luoi	Present	720-820	500	Secondary forest
5	A Luoi	Present	623-770	420	Secondary forest
6	A Luoi	Present	176 – 250	950	Secondary forest
7	A Luoi	Present	179-214	700	Secondary forest
8	Nam Dong	Present	129-179	1,400	Primary forest
9	Nam Dong	Present	173-269	1,300	Primary forest
10	Nam Dong	Present	111-145	1,000	Secondary forest
11	Nam Dong	Present	105-129	460	Secondary forest
12	A Luoi	Absent	550-740	1,000	Secondary forest
13	A Luoi	Absent	650-875	450	Secondary forest
14	A Luoi	Absent	690	_	Secondary forest

Table 1. Survey sites in Thua Thien Hue Province, central Vietnam.

population sizes, a non-invasive "Capture-recapture Method" was applied using both the "Lincoln-Peterson Index" in case of one-time mark and recapture event and the "Schnabel Index" in case of several mark and recapture events (e.g. Caughley 1980; Schlüpmann and Kupfer 2009; Smith and Smith 2009). The Petersen-Lincoln estimation requires several assumptions, namely a closed population, the same capture probability for all animals and that markings neither affect animals nor will be lost between sampling. Surveys were repeated along each site within the timeframe of only three days, as migration events were not expected to occur due to the sedentary behaviour of *P. cocincinus*. Based on the reasons mentioned above, it was also expected that the assumptions on markings and capture probability would be fulfilled. Estimated population sizes only refer to survey sites and might not be representative for the entire population of the species in Central Vietnam.

To assess the population structure of *P. cocincinus*, lizards were categorised into three age classes based on the snout-vent length (adult with SVL > 140 mm, sub-adult with 100 mm < SVL ≤ 140 mm and juvenile with SVL ≤ 100 mm). As sexual dimorphism is only expressed in adult *P. cocincinus* (e.g. presence of well-developed nuchal and dorsal crests in adult males; see Fig. 2C, D), accurate sex determination of living animals thus could only be performed in mature individuals. To test for differences in population structure and frequency of snout-vent length between April and June, 2016 and 2017, a c² test with α = 0.05 was applied. Statistical analysis was carried out with the SPSS software, version 16.0 (SPSS Inc., Chicago).

Threat evaluation

In order to assess levels of harvesting and use of the Asian Water Dragon in Thua Thien Hue Province, 21 hunting groups were interviewed between February and October 2016, including six groups from Phong Dien District, seven groups from A Luoi District and eight groups from Nam Dong District. Each group contained two or three lizard hunters. Some rapid surveys were also conducted in local markets and restaurants in district towns of Phong Dien, A Luoi, Nam Dong and in Hue City to obtain evidence of local trade and consumption of lizards. Single impacts on local sub-populations were ranked according to severity as "low" defined as being observed only once or less, "medium" defined as being observed several times or "high" defined as being recorded frequently. Furthermore, several internet platforms were screened to obtain an overview of the availability, demand, prices and evidence of trade in the species. Since the species is listed on Annex D of the Council Regulation (EC) No 338/97, which requires an import notification at EU border customs, retrospective data were obtained for the recorded imports of *P. cocincinus* from Vietnam into the EU from 2010 to 2016 from the UNEP-WCMC CITES trade database (UNEP-WCMC 2017). Taking into account that CITES trade database records might be incomplete (D'Cruze and Macdonald 2017) and considering this dataset only represents imports into the EU and does not encompass imports into other states, this data however provides a rough impression on current trade levels.

Results

Population status

During the present study, P. cocincinus was found along 11 of 14 investigated sites within three districts of Thua Thien Hue Province. In 2016, the authors only surveyed A Luoi District and recorded a similar number of individuals of *P. cocincinus* in April and June along all five sites, counting 97 and 95 individuals, respectively. In 2017, a total of 167 animals (thereof 31 adults) were captured in April in Phong Dien and Nam Dong districts and 172 individuals were observed in June in all three districts, of which 28 were adults. Higher fluctuations in population sizes were observed between the two consecutive surveys in April and June 2017 in Phong Dien and Nam Dong than those in the year before in A Luoi. The estimated total population size at the survey area was about 250 animals in April 2017. Even though two additional sites in A Luoi District (viz. five sites in total) were included in the estimation, the estimated population size decreased to 233 individuals in June 2017 (Table 2). With respect to the sub-population in Phong Dien District, site 2, the estimated population size was distinctly higher in April than in June 2017 (124 vs. 87 individuals, respectively). In Nam Dong District, transect 9, the number of observed individuals also significantly decreased from 25 individuals in April to 2 individuals in June and only 3 individuals were recorded along transect 11 in June 2016. The authors failed to observe any adult specimens of P. cocincinus in the transect 8 in Nam Dong District in June, whereas the species had been earlier recorded in April 2017 (Table 2).

In total, most individuals were encountered in Phong Dien District, where the highest incidence of adult individuals was also recorded compared to the other two

District	Phong	g Dien			A Luoi			Nam Dong				Total
Stream	1	2	3	4	5	6	7	8	9	10	11	
April-2016												
Total (observed)	*	*	4	4	6	25	21	*	*	*	*	60
Mature (obs.)	*	*	1	4	0	7	2	*	*	*	*	14
D[ind/100 m of stream]	*	*	1.25	0.8	1.43	2.63	3.0	*	*	*	*	1.98
Ń	*	*	6	6	12	35	38	*	*	*	*	97
June-2016												
Total (observed)	*	*	4	5	5	22	22	*	*	*	*	58
Mature (obs.)	*	*	3	2	1	5	0	*	*	*	*	11
D[ind/100 m of stream]	*	*	1.25	1.0	1.19	2.32	3.14	*	*	*	*	1.98
Ń	*	*	5	9	6	42	33	*	*	*	*	95
April-2017												
Total (observed)	13	86	*	*	*	*	*	26	25	17	*	167
Mature (obs.)	0	18	*	*	*	*	*	5	6	0	*	29
D[ind/100 m of stream]	1.12	6.62	*	*	*	*	*	1.86	1.92	1.7	*	2.64
Ń	*	124	*	*	*	*	*	74	52	*	*	250
June-2017												
Total (observed)	33	67	*	*	*	25	22	*	2	20	3	172
Mature (obs.)	8	17	*	*	*	2	1	*	0	0	0	28
D[ind/100 m of stream]	3.84	5.15	*	*	*	2.63	3.14	*	0.15	2.0	0.65	2.51
Ń	56	87	*	*	*	35	24	*	*	30	*	232

Table 2. Summary of observed individuals as well as densities and population size estimates (\hat{N}) of *Physignathus cocincinus* in Thua Thien Hue Province, central Vietnam in April and June, 2016 and 2017. D: Density; ind: individuals; Asterisks indicate missing data.

sub-populations. The mean density of *P. cocincinus* along suitable stream transects was estimated to be around 25–26 individuals per km of transect. By comparing different habitat sites, the highest abundance of *P. cocincinus* was found in April 2017 at site 2 in Phong Dien District (about 6.6 individuals per 100 m stream length). In A Luoi District, being situated at elevations of 623–820 m, densities ranged from 0.8 to 1.4 individuals per 100 m stream length along sites 3, 4 and 5. The authors failed to observe any lizards along the other three sites, namely 12, 13 and 14, in April and June 2017. In comparison, densities along transects of lower elevations (from 49–269 m), even though in a slightly disturbed habitat, were about or higher than two individuals per 100 m stream length during at least two surveys (sites 6 and 7).

Comparing the data achieved in the different months, the investigated population of *P. cocincinus* in Thua Thien Hue Province consisted mainly of juveniles (139 individuals) in April, followed by sub-adults, adult males and adult females (60 %, 19 %, 11 %, 10%, respectively; Fig. 3A). In June, the population structure significantly differed ($c^2 = 50.401$; df = 3; a < 0.001), with sub-adults (113 individuals) constituting the major proportion, followed by juveniles, adult females and adult males (50 %, 33 %, 10 %, 7 %, respectively; see Fig. 3A). Frequency histograms of SVL (Min–Max = 42.4–260 mm) showed a tendency for a monthly variation in the presence of age classes of *P. cocincinus* from Thua Thien Hue Province ($c^2 = 77.87$; df = 6; a < 0.001). Indi-



Figure 3. Population structure of *Physignathus cocincinus* from Thua Thien Hue Province, central Vietnam (April vs. June, respectively); B. Frequency histogram of snout-vent length of *P. cocincinus* for the months April and June, 2016 and 2017.

viduals with the shortest SVL (40–60 mm) were mostly found in June, while juveniles with higher SVL (60–100 mm) and adults accounted for a larger number in April. Sub-adults with SVL larger than 100 mm were observed more frequently in June (Fig. 3B).

Threat evaluation

According to interviews with local hunters and the authors' own observations, wild P. cocincinus populations are affected by negative anthropogenic impacts in all investigated districts (Table 3). In two of three districts, namely A Luoi and Nam Dong, the total impact is even considered to be high. Harvesting of the Asian Water Dragon has been frequently recorded by the team in all surveyed districts of Thua Thien Hue Province. Interviews with 21 local hunter groups in the remote villages within the species distribution range revealed that adult lizards are heavily collected by hand and/or with traps (Fig. 4A) from February to October, while the number of captured animals is usually highest in May and June. With respect to quantities, a total of about 1,000 kilograms of animals collected by hunters was recorded from the three study sites in Thua Thien Hue Province in 2016, which corresponds to more than 2,000 individuals considering a mean mass of 450 g. Eggs of P. cocincinus were also collected and soaked in rice wine for traditional medicine (Fig. 4D). It was regularly recorded that captured animals were sold in local markets and then finally were transferred to local restaurants as food (Figs. 4B and C). The price of living animals was approximately 250,000 Vietnam dong (about \$12) per kilogram in remote districts and fetching

District	Trapping/ hand collection	Use for traditional medicine	Consumption in restaurants	Incidence on local markets	Habitat degradation	Total impact
Phong Dien	High	Low	Medium	High	Low	Medium
A Luoi	High	_	Medium	High	High	High
Nam Dong	High	Low	High	High	High	High

Table 3. Impacts on *Physignathus cocincinus* at study sites based on interview data and own observations.



Figure 4. Threats to *Physignathus cocincinus* in Thua Thien Hue Province: **A** A trapped individual in Nam Dong District **B** Specimens collected for food in Hue City **C** The Asian Water Dragon served as food in a restaurant in Hue City **D** Eggs of *P. cocincinus* preserved in alcohol in Nam Dong District.

up to 450,000 Vietnam dong (about \$20) per kilogram in restaurants in Hue City. In addition, the Asian Water Dragon became very popular in local pet markets due to its striking appearance. *P. cocincinus* was frequently observed being offered for sale for prices ranging from 150,000 (about \$7) to 450,000 Vietnam dong (about \$20) / individual, depending on size and/or colouration in local pet shops and on several Vietnamese internet platforms, such as Facebook from Hanoi and Ho Chi Minh City.



Figure 5. Threats to *Physignathus cocincinus* in Thua Thien Hue Province: **A.** Illegal timber logging in Nam Dong District; **B.** Building a highway from Nam Dong to A Luoi District.

With respect to the international trade in the species, a total of 43,100 specimens have been officially exported by Vietnam into the EU between 2010 and 2016 according to the UNEP-WCMC trade database. Thereof, most specimens had an unknown origin and some were stated to be "wild caught", while the purpose was "for commercial use" in any case. Germany was the country with by far the most imports. It can be assumed that the majority of exported specimens with unknown origin are actually wild individuals, since there is no knowledge about breeding facilities. According to interviews with locals, there have been some people keeping and rearing *P. cocincinus* to supply local restaurants for a short period. However, these facilities were recently closed, because it is allegedly more convenient to collect wild animals than to spend time and efforts in breeding them, as long as animals can be found in the wild.

It was found that habitat loss and habitat alterations, such as road building and illegal timber logging (Figs. 5A and B), are further critical threats to *P. cocincinus* in Thua Thien Hue Province. For the building of highways from Nam Dong to A Luoi District, huge parts of the forest have been cleared, which resulted in the fragmentation of forest streams, thus affecting stream-adapted species like *P. cocincinus*. The construction of roads furthermore has facilitated illegal logging, because accessibility of forested regions has significantly increased and now almost every area within Bach Ma NP and Saola NR can be easily reached. Phong Dien District is the only one of the three investigated sites, which is hardly affected by habitat degradation.

Discussion

In order to manage the wild populations of the Asian Water Dragon properly and to implement conservation measures accordingly, the assessment of its population status, abundance and distribution is important. In total, the estimated population size probably does not encompass all occurrences of *P. cocincinus* in Thua Thien Hue Province, however due to the strong association of the species with small freshwater streams,

which only represent small areas of the Province, it can be assumed that the total population is not disproportionately higher. A detailed further assessment on the availability of suitable habitats would be helpful.

Generally, *P. cocincinus* was found in monthly densities of 2.6 and 2.5 individuals per 100 m in April and June, 2017, respectively. However, three stream transects (3, 4 and 5) in A Luoi District, located at higher elevations (623–820 m), revealed a constantly lower density of *P. cocincinus* than found in other streams, although the habitat was not disturbed. It seems that not only hunting activities, but also elevation and habitat quality of each stream, might influence the abundance of the species. Recently, van Schingen et al. (2015) explained the abundance of the Crocodile Lizard (*Shinisaurus crocodilurus*), another semi-aquatic lizard occurring in northern Vietnam, to mainly depend on the stream properties and water quality that varied along elevation gradients. Thus, these hypotheses explaining abundances of *P. cocincinus* need to be tested in further studies.

Regarding the sex ratio of the species, investigations in 2016 and 2017 showed that the number of adult males was slightly higher than that of adult females in April. However, adult females were observed more frequently in June, which might be an indication for targeted harvest. Most of the local hunters, who have been interviewed, preferred collecting adult males of *P. cocincinus*, because of their bigger size compared to adult females, an important aspect in terms of food consumption. There was a significant difference in age structure in *P. cocincinus* between April and June, with juveniles accounting for the dominant percentage in April and sub-adults being predominant in June. This trend can be explained by the fact that juveniles of P. cocincinus reach the sub-adult stage relatively fast (after only two months), indicating that the survival rate is relatively high in the species. Overall estimates of the population size of *P. cocincinus* in the survey areas within Thua Thien Hue Province, central Vietnam, revealed that the population remained relatively stable between April and June with 250 to 232 individuals respectively in 2017. This trend was constantly observable at each survey site, except for the sub-population in Nam Dong District with a distinct decline in encountered individuals from April to June. This might be explained by the fact that the population in Nam Dong District is heavily affected by hunting activities. Reed et al. (2003) suggested that a population size of at least 3,000–7,000 mature individuals is required to maintain stable populations over a long time period. Thus, an ongoing decline in the number of mature individuals could negatively impact the genetic diversity and reproductive capacity of P. cocincinus in Thua Thien Hue Province. The compilation of impacts on P. cocincinus at different sites indicates that the total impact is "high" in A Luoi and in Nam Dong and only "medium" in Phong Dien. Accordingly, sizes of sub-populations were smaller and lower numbers of adult individuals were recorded in A Luoi and Nam Dong than those in Phong Dien. These findings indicate that anthropogenic impacts negatively affect population sizes. Furthermore, Phong Dien was the only site with hardly any habitat degradation, which indicates the potential and value of intact habitats to buffer harvesting activities.

Conclusions

In total, several critical threats to the population and habitat of *P. cocincinus* were recorded in Thua Thien Hue Province, namely harvesting for food consumptions and to supply trade, as well as habitat fragmentation through road construction and timber logging. It is assumed that similar impacts are certainly to be observed in other areas in Vietnam. Besides local use of the species, the recorded imports of more than 43,000 animals from Vietnam into the EU for commercial use during recent years indicate an increasing international interest in the species. Most of those specimens had an "unknown" origin. According to interviews with local people involved in the trade in P. *cocincinus*, there are no attempts at breeding of the species in captivity, since harvesting from the wild is currently the most economic way. Thus, it is expected that most of the reported imports will represent wild individuals. In addition, this data represents only the tip of the iceberg of actual international trade levels in the species, since only imports into the EU are recorded. In addition to the international trade, the species is also harvested in large amounts for national use. According to these high levels of exploitation, it is recommended that continuous monitoring of wild populations be undertaken in order to warrant the sustainable use of the species. It is also recommended to extend monitoring activities on further populations in the North and South of Vietnam. Moreover, captive breeding could be an alternative, as currently undertaken at the Me Linh Station for Biodiversity in northern Vietnam and in Phong Dien District as well as in Hue City in Central Vietnam. Such breeding programmes could later be transferred to local communities to reduce the pressure on wild populations and supply the high demand for the species. The listing of the species in CITES Appendix III and the establishment of a sustainable export quota could support a shift from the use of wild harvested specimens to captive bred ones. Furthermore, it is herewith recommended to improve conservation measures in the natural habitats in collaboration with the local authorities to enable the long-term persistence and sustainable use of the species.

Acknowledgements

We are grateful to the directorates and staff of the Bach Ma National Park, Phong Dien and Sao La Nature Reserves (Thua Thien Hue) for support of our field work and issuing relevant permits. We thank S. V. Nguyen (IEBR), T. Pagel and C. Landsberg (Cologne Zoo) for support of our research. We thank T. V. Nguyen, T. Q. Le, T. V. Nguyen (Hanoi), B. V. Ngo and L. C. Nguyen (Thua Thien Hue) for their assistance in the field. We thank G. Benyr (Vienna) and U. Schepp (Bonn), as well as the subject editor K. Henle (Leipzig) for their constructive comments to improve an earlier version of the manuscript. This research was funded by the Vietnam Academy of Science and Technology and the People's Committee of Thua Thien Hue Province (grant number: VAST.NĐP.01/17-18) as well as Cologne Zoo.

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RESEARCH ARTICLE



Movement ecology of brown bears (Ursus arctos) in the Romanian Eastern Carpathians

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Academic editor: N. Constant Received 14 December 2017 Accepted 19 March 2018	Published 17 April 2018

Citation: Pop IM, Bereczky L, Chiriac S, Iosif R, Nita A, Popescu VD, Rozylowicz L (2018) Movement ecology of brown bears (*Ursus arctos*) in the Romanian Eastern Carpathians. Nature Conservation 26: 15–31. https://doi.org/10.3897/ natureconservation.26.22955

Abstract

Brown bear movement patterns are driven by their opportunistic feeding behaviour, with their complex life history and seasonality playing an important role in habitat selection. Within a large unfragmented forest habitats persisting over decades in the Romanian Carpathians and a prohibitive hunting management during 40 years of communist centralised game management, information about brown bear movements and spatial ecology is lacking. Using data obtained from 13 brown bears fitted with GPS telemetry collars, we estimated home ranges and core activity areas and we investigated the daily, seasonal and altitudinal movements of brown bears in the Eastern Romanian Carpathians and surrounding high hills. The median MCP95% home ranges of brown bears was 629.92 km² and the median size of core activity areas (estimated as 50% kernel density) was 36.37 km², with no significant differences between males and females. The mean daily distance travelled, measured as daily displacement length, was 1818 m and an analysis of seasonal movements indicated significant differences between seasons (greatest movements during the Hyperphagia season). The GPS-collared brown bears travelled between a minimum altitude measured at ~234 m and a maximum at ~1634 m. Analysing the spatial overlap between the estimated home range and the game management units (GMU) limits, we obtained a median number of 8 GMUs overlapping totally or partially with estimated home range polygons. Our study, using GPS telemetry, highlights the

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complex spatial ecology of the brown bear in the Romanian Carpathians, with larger home range size than those estimated in other European brown bear populations and with daily movements that vary by season and within a large altitude range. Our study supports the implementation of brown bear monitoring at a regional scale, rather than focusing on county level GMUs as the monitoring unit.

Keywords

Daily movements, home range, Romanian Carpathians, Ursus arctos

Introduction

Understanding animal movement patterns is crucial for wildlife management and conservation (Kernohan et al. 2001). Movements of individuals in their forays for food, switching between habitats, defending cubs or food resources, are of high interest for understanding the ecology of brown bears in heterogeneous landscapes (Martin et al. 2013). The use of telemetry to obtain movement data has been adopted in brown bear research studies for decades, e.g., movement patterns in Kodiak Island or denning ecology in Yellowstone (Berns and Hensel 1972, Craighead and Craighead 1972). Telemetry was also used to provide information about brown bear habitat suitability in the Alps (Guthlin et al. 2011) or predicting human-bear conflicts in the Dinaric Mountains (Jerina et al. 2012). Analysis of animal movement data leads to a deeper understanding of key environmental resources required for population persistence (Margules and Pressey 2010), which aids resource managers in developing wildlife management strategies. Worldwide, brown bear population management (e.g., for North American, Dinaric, Scandinavian, Alpine brown bear populations) has been heavily influenced by the results of telemetry studies over the past four decades. In Croatia, telemetry studies have been critical for quantifying home range sizes, the overlap between individual home ranges, as well as the gender differences in home range sizes (Huber and Roth 1993). These data have been used in developing the brown bear management plan for the Republic of Croatia.

Brown bear movement patterns and spatial ecology are driven by their complex life history and seasonality plays an important role in their selection of habitats (Pop et al. 2018). Brown bears are opportunistic omnivores (Bojarska and Selva 2012, Kavcic et al. 2015) and their movements are heavily influenced by food resource availability (Nielsen et al. 2006, Stofik et al. 2013, Ciucci et al. 2014, Kavcic et al. 2015). In addition to food availability, other factors such as reproductive stage, den site availability or avoidance of conspecifics or predators, can affect the movement of individuals, which further poses challenges to understanding space use patterns (Nathan et al. 2008). Furthermore, environmental characteristics such as the reproduction period, air temperature or daylight length are also important in understanding the seasonality of brown bear movements (Nielsen et al. 2006, Martin 2009, Ware et al. 2013).

Large unfragmented forest habitats, persisting over decades in the Romanian Carpathians and surrounding high hills (Rozylowicz et al. 2011, Patru-Stupariu et al. 2013), and a prohibitive hunting management during 40 years of communist centralised game administration, had contributed to the maintenance of the largest and most stable European brown bear population (Chapron et al. 2014, Popescu et al. 2016). Romania's brown bear population inhabits the Carpathian mountains and surrounding high hills, which are characterised by low human density, but with intensive use of natural resources (e.g., timber and non-timber forest products) and livestock grazing. The highest brown bear densities, of up to 11 individuals/100 km² occur in Eastern Carpathians (Cazacu et al. 2014, Popescu et al. 2017). However, no studies to date have documented the movement ecology of Romanian brown bears at individual and population levels, leading to suboptimal management strategies (Popescu et al. 2016).

In Romania, brown bear ecology data are collected via opportunistic observations at feeding stations or using methodologies for counting track signs (Popescu et al. 2016) which lack statistical rigor and ignore the complex bear space use patterns (Lindstedt et al. 1986, Dahle and Swenson 2003). These monitoring deficiencies lead to a poor understanding of brown bear movements and habitat selection. Published data on space requirements or home range sizes of Romanian brown bears have been based on expert opinion and varied from 15–20 km² for an individual (Comsia 1961) to 200 km² for males and 50 km² for females (Maanen et al. 2002). Management decisions, made at the level of game management unit (GMU), may be suboptimal, given that GMUs are on average ~120 km² in size, thus potentially failing to capture the full extent of movement and space use of a single individual.

The aim of the study is to investigate brown bear movement ecology and space use in the Eastern Carpathians and surrounding high hills (Subcarpathians), Romania. We take advantage of a large dataset of Romanian brown bear GPS telemetry gathered between 2011 and 2015 within the framework of several large carnivore conservation projects to quantify annual and seasonal home ranges. We specifically evaluate whether differences in home range sizes exist between males and females, as well as between adults and sub-adults (Dahle and Swenson 2003). To test the validity of using GMUs for brown bear management, we analyse the overlap between individual home ranges, core activity centers and the game management units. Lastly, we investigate the daily, seasonal and altitudinal movements of brown bears across four different seasons with varying food availability (Ciucci et al. 2014, Roellig et al. 2014).

Methods

Study area

The Romanian Eastern Carpathians and Subcarpathians (elevation = 200–2303 m) (Figure 1) vary along an altitude gradient from urban and agricultural landscape on the low hills and main valleys, to deciduous, mixed and coniferous forests to subalpine shrubs and grasslands. Below 1200 m, habitats are dominated by deciduous forests with *Fagus sylvatica*, *Quercus* spp., *Carpinus* spp., *Acer* spp. and *Fraxinus* spp., in a heterogeneous landscape with secondary pastures, orchards and agricultural land. Mixed forests (*Picea abies, Abies alba, Fagus sylvatica*) are present mainly between 1000 m and



Figure 1. Study area (dashed area) in the Eastern Carpathians and Subcarpathians covering mountain and hill area; brown bear GPS fixes used in the study (black dots).

1400 m. Between 1400 m and 1700 m, forests are dominated by coniferous species (*Picea abies* and *Abies alba*) and *Vaccinium* spp. and *Rubus* spp. in the understory. The subalpine and alpine region (above 1700 m) are dominated by *Pinus mugo*, *Juniperus* spp. while the herbaceous layer is by *Festuca* spp., *Agrostius* spp. and *Poa* spp. (Rey et al. 2007). The large mammal community also includes prey species such as wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*). Top predators such as wolf (*Canis lupus*) and the Eurasian lynx (*Lynx lynx*), have also a permanent presence in the study area.

Telemetry data

To evaluate the spatial ecology of brown bears, we captured 13 individuals using a bespoke 7.5 m³ cuboid cage with two independent gliding doors. Bears were captured between 2011–2014 (April–September) in 13 locations (Table 1). All field procedures and the immobilisation of the bears were made under veterinary supervision and carried out in accordance with verified protocols (www.carnivoremari.ro). Bears were tranquilised using medetomidine–zolazepam–tiletamine or medetomidine-ketamine drug combinations (Fahlman et al. 2011). The drugs were delivered with a gas

Bear code	Sex	Age class	Monitoring period	Monitoring days	Hours between fixes	No. of successful fixes
Bear2	male	subadult	2013	233	1	4836
Bear4	female	subadult	2013-2014	368	1	8413
Bear5	male	adult	2011-2012	300	1	3969
Bear6	male	adult	2011-2012	405	1	8392
Bear8	female	adult	2012-2014	738	1	12313
Bear10	male	adult	2012-2013	324	1	5646
Bear12	male	adult	2013-2014	420	1	6212
Bear13	female	subadult	2014-2015	386	2	1231
Bear14	male	subadult	2014-2015	309	2	2021
Bear15	male	adult	2014-2015	387	2	1781
Bear16	female	adult	2014-2015	360	2	1725
Bear17	male	adult	2014-2015	270	2	1024
Bear18	male	subadult	2012-2013	308	2	2444

Table 1. GPS collared brown bears in Eastern Romanian Carpathians and Subcarpathians.

driven rifle or with a blow pipe. The eyes were lubricated to avoid corneal abrasions or ulceration and a blindfold was placed to protect the eyes and decrease visual stimuli (West et al. 2007).

Bears were fitted with 8 GPS ProLight collars with GSM transmission (Vectronic Aerospace Gmbh) and 5 G2110 collars with Iridium transmission (Advance Telemetry System, USA). Collars were equipped with mortality sensors and with a 12 months planned drop-off activation system. The GPS collars were programmed to collect fixes between 1 to 2 hours. Data were received on a compatible GSM station or through Iridium Data Service available with the collars and pre-processed in order to remove locations with coordinates errors.

The monitoring interval for each individual varied between 233 days (Bear2, subadult male) and 738 days (Bear8, adult female) (median = 360 days, interquartile range - IQR = 387–308- days; Table 1). During the study, we collected 59997 successful fixes (median = 3969, IQR = 6107–1781). The lowest number of locations was 1024 fixes in 270 days (Bear18, male) and the largest 12313 fixes in 738 days (Bear8, female) (Table 1).

Data analysis

To analyse the seasonal variation of home ranges and daily distances covered, we divided annual data into 4 seasons (Szabo et al. 2013): Winter sleep season (season 1, 15 November – 31 March); Den exit and reproduction season (season 2, 1 April – 15 June); Forest fruits season (season 3, 16 June-31 August); and Hyperphagia season (season 4, 1 September – 14 November). The classification captures the denning period and the variation in food availability with possible influences in bear behaviour. By analysing the seasonal movements (only for bears monitored all four seasons), we

expected to observe small home ranges and movements during the Winter sleep season and larger home ranges and movements during Hyperphagia.

We estimated home ranges size for each individual using the 95% minimum convex polygon (Dahle et al. 2006), hereafter MCP95% and core areas within the home range by using kernel density estimator (KDE) for 50% isopleths with reference bandwidth estimated by adehabitatHR R package (Worton 1989, Cale.g., 2006), hereafter KDE50. After partitioning data by seasons, gender and age, we tested seasonal and gender/age-specific differences in home range size using non-parametric Mann-Whitney or Kruskal-Wallis tests (Kernohan et al. 2001). We also analysed the spatial overlap between individual home ranges and the game management units limits in order to assess the suitability of the game management units size for the brown bear monitoring and management. For the home ranges-GMU spatial overlap analysis, we considered only GMUs with >5% of area overlapped with each individual home range or home range core activity areas.

Additionally, we analysed the daily displacement length (m/day) by calculating the Euclidian distance between consecutive fixes at a time interval of 24 hours. Using non-parametric Mann-Whitney test, we tested for seasonal and gender/age-specific differences in daily displacement length and for altitudinal variation between individuals during different seasons in order to test the influence of changes in vegetation distribution and phenology on individuals' movements.

For statistical analyses and graphics, we used packages adehabitatHR (Cale.g., 2006), dunn.test (Dinno 2017), maptools (Bivand 2017) and ggpubr (Kassambara 2017) for R 3.4.3 (R Core Team 2017) and ArcMET movement ecology toolbox for ArcGIS Desktop 10.3.1 (Wall 2014).

The data, underpinning the analysis reported in this paper, are deposited in the Dryad Data Repository at http://dx.doi.org/10.5061/dryad.jk127ng.

Results

Brown bear home ranges

The median of MCP95% home ranges of brown bears in Eastern Carpathians and Subcarpathians was 629.92 km² (IQR = 925.66–131.12). Both the largest and smallest home ranges were recorded for adult males (max = 3142.94 km², min = 73.35 km²) (Figure 2). We did not find statistically significant differences between the MCP95% of males and females (Mann-Whitney W = 17, p = 0.94), but ranges varied widely for males; we did not find differences between adults and subadults (Mann-Whitney W = 14, p = 0.44), but the subadult bear movement showed higher individual variation.

The number of core activity areas, estimated using KDE50, varied between min 1 and max 4 areas (median = 2). The median size of core areas per bear was 36.37 km^2 (IQR = 84.71-11.91), with minimum and maximum recorded for adult males (min = 1.04 km^2 , max = 363.81 km^2) (Figure 3).



Figure 2. Brown bear home range (MCP95%) size by age class (**A**) and sex (**B**) in Eastern Romanian Carpathians and Subcarpathians (box = interquartile range, horizontal line = median, whiskers = $1.5 \times$ interquartile range, points = outliers).



Figure 3. Core area size by age class (**A**) and sex (**B**) in Eastern Romanian Carpathians and Subcarpathians (box = interquartile range, horizontal line = median, whiskers = 1.5 × interquartile range, points = outliers).

Seasonally, the smallest MCP95% home range size (2.98 km^2) was estimated during the Winter sleep season for a subadult male and the largest home range (2036.11 km^2) during Hyperphagia season for an adult male (Table 2). Moreover, we did not record statistically significant differences between seasonal home ranges estimated using MCP95% (Kruskal-Wallis = 3.86, df = 3, p = 0.28).

	Sex	Age class	MCP95% (km ²)						
Bear			Winter sleep	Den exit and reproduction	Forest fruit season	Hyperphagia			
Bear2	М	subadult	29.88	22.98	24.04	54.50			
Bear4	F	subadult	2.98	6.54	425.66	69.52			
Bear5	М	adult	56.95	49.79	16.71	23.89			
Bear6	М	adult	344.92	54.95	109.88	854.02			
Bear8	F	adult	77.30	94.59	57.87	85.71			
Bear10	М	adult	27.25	115.11	188.43	113.39			
Bear12	М	adult	252.72	211.75	1195.78	2036.11			
Bear14	М	subadult	46.82	34.24	210.78	1222.29			
Bear15	М	adult	177.14	14.36	201.84	79.06			
Bear18	М	subadult	7.76	1561.42	3.03	1047.06			
Median			51.88	52.37	149.155	99.55			
IQR			152.18-27.90	109.98-25.79	208.54-32.49	998.8-71.90			

Table 2. Brown bears MCP95% seasonal home range sizes in Eastern Romanian Carpathians and Subcarpathians (M = males, F = females).

Overlap between brown bears home ranges and Game Management Units

The mean size of the 272 GMUs within our study area was 126.97 km² (stdev = 32.56 km^2 , min = 70.56 km², max = 294.56 km²). The number of GMUs overlapping with MCP95% individual home ranges varied between 2 and 32 GMUs (median = 8, IQR = 17-4) with males home range size overlapping more GMUs compared to females and subadult individuals home range size overlapping more GMUs than adults (Figure 4).

The number of GMUs overlapping with KDE50 home range core areas individual home ranges varied between 1 and 10 GMUs (median = 3, IQR = 7-2), with males overlapping more GMUs than females (Figure 5).

Brown bear daily displacement length

The mean daily displacement length calculated for brown bears with data in all seasons was 1818.78 m (stdev = 2338.29 m/day, max = 20833.7 m/day). Seasonal movement analysis indicates statistically significant differences between seasons (Kruskal-Wallis = 82.87, df = 3, p < 0.001), i.e., between Winter sleep season and all other seasons (Table 3). The most active season in terms of daily displacement length was the Hyperphagia (Figure 6) with a mean value of 2097 m (stdev = 2855.72). The Den exit and reproduction season is also an active season for bears, with an average daily displacement length of 1884 m (stdev = 2257.21). The mean daily distances estimated during Forest fruits season (1842 m, stdev = 1917.60) and Winter sleep (1274 m, stdev = 1848.58) are lowest. The GPS-collared brown bears travelled between a minimum altitude of ~234 m and a maximum of ~1634 m. The average altitude of locations was ~886 m (stdev



Figure 4. Number of Game Management Units (GMUs) overlaping subadults/adults bears (**A**) and females/males (**B**) MCP95% annual home ranges in Eastern Romanian Carpathians and Subcarpathians (box = interquartile range, horizontal line = median, whiskers = 1.5 × interquartile range).



Figure 5. Number of Game Management Units (GMUs) overlaping subadult/adult brown bears (**A**) and females/males (**B**) core areas (KDE50) in Eastern Romanian Carpathians and Subcarpathians (box = interquartile range, horizontal line = median, whiskers = 1.5 × interquartile range).



Figure 6. Seasonal variation of the mean daily displacement length of brown bear in Eastern Romanian Carpathians and Subcarpathians : I – Winter sleep ; II – Den exit and reproduction ; III – Forest fruits; IV – Hyperphagia. (Horizontal line = median value, box = interquartile range, the vertical width = density of the data within a season, points = outliers).



Figure 7. Seasonal variation of the altitude of brown bear locations in Eastern Romanian Carpathians and Subcarpathians: I – Winter sleep ; II – Den exit and reproduction ; III – Forest fruits ; IV – Hyperphagia. (Horizontal line = median value, box = interquartile range, the vertical width = density of the data within a season, points = outliers.

= 242.56), while the mean altitudes of locations by seasons were: ~933 m for Winter sleep (stdev = 196.35); ~932 m for Den exit and reproduction (stdev = 204.86); ~968 m for Forest fruit (stdev = 239.12); and ~761 m for Hyperphagia (stdev = 246.27). The season with the lowest recorded altitude (234 m) was Hyperphagia and the season with the highest recorded altitude was the season of Winter sleep (1634 m) (Figure 7). The Kruskal-Wallis test indicates statistically significant differences between the altitude occupied by brown bears in the four seasons (Kruskal-Wallis = 252.03, df = 3, p < 0.001), i.e., between the Hyperphagia season and all other seasons (Table 4).

Season	Winter sleep	Den exit and	Forest fruits
	··	reproduction	
Den exit and reproduction	W = -6.51, p < 0.001	-	-
Forest fruits	W = -8.35, p < 0.001	W = -1.61, p = 0.32	-
Hyperphagia	W = -7.64, p < 0.001	W = -0.53, p = 1.00	W = 1.28, p = 0.66

Table 3. Comparison of seasonal daily displacement length of brown bears in Eastern Romanian Carpathians and Subcarpathians (Dunn test with Bonferroni adjustment, $\alpha = 0.05$).

Table 4. Comparison of seasonal altitude of brown bears GPS fixes in Eastern Romanian Carpathians and Subcarpathians (Dunn test with Bonferroni adjustment, $\alpha = 0.05$).

Season	Winter sleep	Den exit and reproduction	Forest fruits
Den exit and reproduction	W = 0.51, p = 1.00	-	-
Forest fruits	W = -1.61, p = 0.32	W = -2.17, p = 0.09	_
Hyperphagia	W = 11.40, p < 0.001	W = 11.39, p < 0.001	W = 13.99, p = < 0.001

Discussion

Our analysis of brown bear movement and space use from GPS telemetry data showed that previous predictions on bear movement ecology and home range sizes from the Romanian Carpathians (e.g., Comsia 1961, Maanen et al. 2002) were not realistic. The estimated median MCP95% home range (~630 km²) and median home range core areas (~36 km²) were more than three times larger than published information. Furthermore, our estimated home range size was higher than brown bear home ranges in the Northern or Southern Europe (Dahle and Swenson 2003, Dahle et al. 2006, Gavrilov et al. 2015). Individual seasonal home ranges varied from a few square kilometres to over 2000 km² (see Table 3) and, for the Hyperphagia season, home ranges had larger values (see Figure 3), although we did not detect significant differences between seasons. Our results also show that there are no differences between age groups or between males and females, which contradicts findings from other European studies (Dahle and Swenson 2003). However, we did detect higher variation in home range size for males and subadults.

The number of core areas identified for each bear varied between 1 to 4, while the median area of activity centres (KDE50) was estimated at under 40 km², demonstrating that bears may intensively use several areas during a year and such areas can be larger than a GMU. The median number of 8 GMUs overlapping with individual home ranges (MCP95% home ranges) and 3 GMUs overlapping with home range core areas (KDE50 core areas), suggest that the size of GMUs in our study area (mean = 126.97 km²) might not be suitable for planning bear management and especially as a population census base unit, as done presently. Thus, GMUs as sampling units might be a biased approach that can lead to poor management decisions at the population level, such as overharvesting (Popescu et al. 2016) or unnecessary supplementary feeding (Selva et al. 2017). Planning further census techniques should consider that male home ranges overlap more GMUs than those of the females (see Figures 4 and 5), mostly as a result of the brown bear mating system (Dahle and Swenson 2003).

This study presents the first extensive assessment of space use and movements for brown bears in the Romanian Carpathians. Our results show larger home range size in Romania compared to bears in Bulgaria (MCP100% and Kernel 99% isopleth for home ranges and Kernel 50% isopleth for core areas, Gavrilov et al. 2015) and Sweden (MCP95%, Dahle et al. 2006) and comparable to those reported in Italy (MCP100% and Kernel 95% isopleth for home ranges and Kernel 50% isopleth for home ranges and Kernel 50% isopleth for home ranges and Kernel 50% isopleths for core areas, Preatoni et al. 2005). The larger home range sizes from our study could be due to external factors such as human disturbances due to logging activities, heterogeneous landscapes or food availability. In particular, the home range sizes may have been influenced by the presence of supplementary feeding locations (3–5/100 km² in study area, personal estimation). Bear daily displacement length comparisons revealed minor differences between seasonal movements: the Winter season displacement distances were smaller compared to the Hyperphagia season (Figure 6). The daily mean distance, estimated in our case, corroborates those obtained in other studies – median daily distance of 1500 m in Croatia (Huber and Roth 1993) and averaged 2450 m in Greece (Mertzanis et al. 2005).

Seasonally, Hyperphagia resulted in increased home ranges and daily movements in all individuals. During the Hyperphagia season, we recorded the lowest altitude for an adult female and a highest variability of altitude between individuals, showing a tendency to seek out food resources in different types of habitats varying along an altitudinal gradient (Pop et al. 2018). Considering both the daily movement and altitude results, we observed a variability of the daily, seasonal and altitudinal movements of brown bears on relation to the seasonal and altitudinal natural food resource availability. The greater anthropogenic food availability at lower altitudes, such as orchards or crops, might also influence the brown bear distribution (Pop et al. 2012), especially during the Hyperphagia season. Seasonal movements of brown bear in the Carpathians should be analysed in relation to seasonal changes in bear diet, approach often addressed in other studies (Stofik et al. 2013, Ciucci et al. 2014) to explain changes in brown bear movements.

As the main limitation of the study was related to the small numbers of bear females (4) and subadults (5), some of our results should therefore be interpreted with caution (Preatoni et al. 2005). Using a larger dataset and considering the seasonal and altitudinal gradient of the food availability (Roellig et al. 2014), may result in differences between seasonal movements and/or between genders. Future brown bear spatial ecology studies in the Romanian Carpathians should consider both external (e.g., food availability, predation risks, presence of mating partners) and internal factors (e.g., health, age, reproductive stage) (Nathan et al. 2008, Martin et al. 2013) recommended using a landscape approach (Di Minin et al. 2016).

In summary, our study, using GPS telemetry, highlights the complex spatial ecology of the brown bear in the Carpathians, with home range sizes larger than those estimated in other European brown bear populations and daily movements that vary by season and within a broad altitude range. Human disturbance caused by traditional activities such as logging, hunting, agriculture, could not be considered when describing the movements, but it represents a key factor influencing brown bear movement and habitat selection (Martin et al. 2010), and should be considered in further studies. Notably, our study supports the implementation of brown bear monitoring at a regional scale, rather than focusing on GMUs as the monitoring unit (Popescu et al. 2016). As brown bears may use multiple GMUs annually or during a single season, a regional approach to monitoring based on remote cameras and track counts (Popescu et al. 2017) or non-invasive DNA techniques (Proctor et al. 2010) is likely to yield better results and will require coordination between multiple adjacent GMUs. Additionally, conservation initiatives should cover large areas, which seems to be difficult in Romania given the tendency of conservationists to work at a local scale (Nita et al. 2016). This study provides important information for future brown bear research and conservation, such as habitat selection studies, habitat connectivity and population estimates for Romania's brown bear population.

Acknowledgments

The research was supported by a grant of the Romanian National Authority for Scientific Research (www.uefiscdi.ro), PN-III-P2-2.1-PED-2016-0568. We thank our colleagues from ACDB, Vrancea, Covasna and Harghita EPAs, WWF Romania, Buzau County Council and to game wardens for the efforts made during the trapping and monitoring activities. The bears were fitted with GPS collars within the projects: LIFE08 NAT/RO/000500 LIFEURSUS, Protecting and promoting the biodiversity in the ROSCI0190 Penteleu and ROSCI0229 Siriu and Open Borders for Bears between Romanian and Ukrainian Carpathians. We thank Diana Zlatanova, Gabriel Chisamera and an anonymous reviewer for their valuable comments and suggestions on the manuscript.

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RESEARCH ARTICLE



Conservation of the endangered freshwater mussel Solenaia carinata (Bivalvia, Unionidae) in China

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Academic editor: <i>M. Halwart</i> F	Received 28 March 2018	Accepted 4 April 2018	Published 23 April 2018
http://zoo	bank.org/B180DCB3-8D06-	4D0A-B0EA-148A013DD000	5

Citation: Cao Y-L, Liu X-J, Wu R-W, Xue T-t, Li L, Zhou C-H, Ouyang S, Wu X-P (2018) Conservation of the endangered freshwater mussel *Solenaia carinata* (Bivalvia, Unionidae) in China. Nature Conservation 26: 33–53. https://doi.org/10.3897/natureconservation.26.25334

Abstract

Despite the diversity and economic and ecological value of freshwater mussels, relatively little is known about their biology (especially for species outside of Europe and North America). *Solenaia carinata* is an endangered freshwater mussel, is endemic to China and is now only distributed in Poyang Lake basin. However, its conservation status is not clear. Thus, for this study, surveys were conducted at 41 sites along the lower reaches of the Ganjiang River to study the conservation status of *S. carinatus*. The results showed that *S. carinata* had a restricted distribution and extremely low density. In addition, the habitat sediments where *S. carinata* was located were mainly composed of silt (particle size <0.0625 mm). RDA analysis showed that the density of *S. carinata* was correlated to dissolved oxygen, temperature, turbidity and chlorophyll-a. Microsatellite analysis showed that *S. carinata* had a low genetic diversity (mean H_o: 0.419; mean H_E: 0.484; mean PIC: 0.430). At the same time, we firstly report the glochidia of *S. carinata* and describe its morphological characteristic. Surprisingly, its reproduction period and morphological characteristics were different from that of others freshwater mussels. Therefore, this study clarified the resource conditions, endangered status and threat factors for *S. carinata* and it provided a theoretical basis for the conservation and management of its resources.

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Keywords

Solenaia carinata, endangered, conservation, glochidia, microsatellite

Introduction

Freshwater mussels are large benthic animals and important components of the freshwater biotic community, they can be important filter feeders and play an important role in aquatic ecosystems (Vaughn and Hakenkamp 2001, Bogan 2008, Geist 2010, Lopes-Lima et al. 2017b). However, over the past few years, the overall situation of freshwater mussels is really alarming (Williams et al. 1993, Lydeard et al. 2004, Strayer et al. 2004, Geist 2010). It is reported that, in a total of 297 freshwater mussel species in North America, there were 19 presumed extinct in 1993; by 2008, the number of extinct freshwater mussels reached 37 (Bogan 1993, 2008, Christian and Harris 2008). The IUCN Red List of Endangered Species listed 247 species of freshwater mussels above the near the threatened level (IUCN 2017). To date, conservation efforts for freshwater mussels are almost non-existent in East and Southeast Asia. The IUCN conservation status assessment for unionoid species could not be completed due to a lack of information on their status, threats and other conservation-related issues (IUCN 2017). National Red Lists for Unionida are available for only four of the 17 countries (i.e. Vietnam, South Korea, Japan and Russia) where they are found. None of the 228 unionoid species in the region is protected by international legislation (Zieritz et al. 2018). Therefore, freshwater mussels are considered one of the most endangered groups of animals in the world, which has caused great concern as they provide the following services (inter alia): providing food resources to higher trophic levels, cleaning the water, controlling of the amount and composition of suspended particles, cycling nutrients and providing of habitat for other organisms (Strayer et al. 2004, Aldridge et al. 2007, Bogan 2008, Vaughn 2012).

The middle and lower reaches of the Yangtze River in China are diverse and abundant in freshwater mussels and they are also hotspots for biodiversity in East Asia. However, more than 80% of freshwater mussels in the region are considered near threatened or threatened (Wu 1998, Prozorova et al. 2005, Shu et al. 2009, Xiong et al. 2012; Zieritz et al. 2018). Poyang Lake is surrounded on three sides by mountains and is fed by five large rivers (Ganjiang, Fuhe, Xiuhe, Xinjiang and Raohe), forming a complex and highly interconnected river–lake–wetland system in a monsoondominated sub-tropical climate (Zhang et al. 2013). Poyang Lake basin has the typical natural ecosystem characteristics of the Yangtze River basin, its natural environment is healthy and freshwater mussel species are extremely rich. However, approximately 75% of freshwater mussels are endemic to China and the population of freshwater mussels continues to decline (Wu et al. 2000b, Xiong et al. 2012). Threats to freshwater mussels include extreme climate changes, sand excavation, pollution, dam construction and overharvesting, but human disturbance is the major factor affecting freshwater mussels (Wu 1998, Zhang et al. 2013, Lopes-Lima et al. 2017b, Zieritz et al. 2018). These factors have resulted in a decrease in freshwater mussel diversity and a change in their community structure (Wu 1998, Zhang et al. 2013, Zieritz et al. 2018).

S. carinata is an endemic unionoid species in China and currently, it is only distributed in Poyang Lake basin (Wu 1998, Lopes-Lima et al. 2017a). In 1877, Heude found fossil fragments in the lower reaches of the Yangtze River and thought they were a new species; however, since that time, there were no more relevant reports on this species until 1991, when Liu and Wu found live specimens of *S. carinata* in Wucheng Town, Poyang Lake region and gave an additional description (Liu and Wu 1991). In addition, the fragmentary fossil specimens, found in the quaternary of the conglomerate layer of the Yellow River bank, indicated that *S. carinata* once lived in northern China (Savazzi and Yao 2010). *S. carinata* preferred to inhabit silt or hard clay, with its narrow front end and strong axe helping it dig down into the sediment to resist erosion from flood waters (Liu and Wu 1991, Wu 1998, Savazzi and Yao 2010). Due to their delicious taste and high nutritional value, like other *Solenaia* species, *S. carinata* was also targeted by local people for food (Liu and Wu 1991). However, over the past few years, due to environmental degradation and overfishing, the numbers of *S. carinata* have fallen sharply.

There are few studies on *S. carinatus* and specifically, there is little basic information on the species. The purpose of this study is to survey the distribution, density, age structure and habitat characteristics of *S. carinata* in the lower reaches of the Ganjiang River and to clarify its status, endangered condition and threat factors to provide the basis for its protection. In addition, we also hope that the government departments of China can strengthen the protection for freshwater mussel diversity using the information from this study.

Materials and methods

Study area

The Ganjiang River (116°01'–116°22'E, 25°57'–29°11'N) is the largest river running north to south in Jiangxi Province, China, flowing into Poyang Lake and it is one of the important tributaries (7th largest) of the Yangtze River. The Ganjiang River covers a total catchment area of 82809 km² and its main channel is 823 km. In addition, it has a complex river system and is in the mid-subtropical humid monsoon climate zone. Precipitation is abundant with an average of 1580.8 mm/year.

Sampling method

A total of 41 sampling sites were established in three major tributaries of the lower reaches of the Ganjiang River (the main distribution area of *S. carinata*) from September 2016 to March 2017 (Figure 1). A triangular mussel harrow (550 mm wide,



Figure 1. Sampling map of S. carinata.

20 mm mesh, rake tooth spacing 15 mm) was used for quantitative sampling. The harrow was dragged slowly by hand in the shallows and the sandy beach of the river (0.5-2.0 m of depth) that had a total area of 2.2 m². The distance between each site was approximately 2 km and each sampling site had 5 quadrats (distance between each quadrat was 100 m). Shell length, shell width and shell height (mm) of live mussels
was measured with a dial caliper (\pm 0.1 mm). Live mussels and attached dreissenids were weighed separately (\pm 0.1 g). The specimens were counted and then returned to their original habitat. In addition, when we found glochidia, the whole animal was preserved in 75% alcohol and was taken back to the laboratory.

Habitat characteristic analysis

Temperature (T), turbidity (TURB), salinity (Sal), pH and dissolved oxygen (DO) were measured by a water quality meter (AQUAREAD, AP-800) and Chlorophyll-a (Chl-a) was measured by a chlorophyll meter (HL-168C06, made in China).

The habitat sediment samples of *S. carinata* were collected by a special pipe, emptied into sealable plastic bags and taken back to the laboratory for sediment particle size analysis. Samples were oven-dried at 105 °C for 24 hours (Gordon et al. 2004) and then sieved through 3 sieves with mesh sizes of 4 mm, 2 mm and 0.0625 mm with hand shaking for 30 minutes. Percentages of pebbles (>4 mm), granules (2–4 mm), sand (0.0625–2 mm) and silt (<0.0625 mm) in the sediment samples were determined (Wentworth 1922).

Analysis of age structure

Analysis of age structure used mussel shells that were obtained by hand-collection from the shallows and the sandy beach of the river. The method of thin section was used for the analysis of age structure (Hua et al. 2001). First, we cut from the top of the shell to the ventral margin along the maximum vector direction of the growth line and we made the inner section at right angles to the growth line. At the same time, we made a second cut on half of the shell and then chose the thin layer of shells which were bonded to a glass slide by a colourless transparent epoxy glue (Hua et al. 2001). Finally, the prepared inner sections were burnished until they were smooth and polished (Hua et al. 2001). Moreover, they were observed with a TCA-10 model stereomicroscope (3664×2748 pixel resolution) and then we recorded the number of growth lines (Figure 2).

Morphological observation of glochidia

Mature glochidia were collected from the demibranches of *S. carinata* gravid females and fixed in 75% ethanol for investigation under light microscopy. Measurements of shell length, shell height and hinge length of 30 glochidia were taken under a light microscope equipped with a calibrated ocular micrometer.

For SEM, specimens were washed with 0.65% saline water, then fixed for 12 h in glutaraldehyde (4 °C), macerated with 0.1 mol/l phosphate buffer (pH = 7.2) three



Figure 2. The internal section of S. carinata.

times, dehydrated in a graded ethanol series (30 min each at 30%, 50%, 70%, 90%, 95% and three times in 100%) and transferred to isoamyl acetate for 30 min. They were critical-point dried with liquid CO_2 and coated with gold. Observations were made with a scanning electron microscope (S-570) and photographs were taken.

Redundancy analysis

Redundancy analysis (RDA), a multivariate direct gradient analysis technique, was used to evaluate the variations in density in relation to environmental variables. Detrended correspondence analyses indicated that the *S. carinata* dataset had a short gradient length, indicating that the linear model of RDA was more appropriate than canonical correspondence analysis (CCA; ter Braak and Verdonschot 1995). Similar to our regression analysis, we performed RDA to assess the correlations between *S. carinata* densities; we then performed RDA to determine how the physicochemical parameters correlated to *S. carinata* density. All variables were entered in the analysis after a forward selection procedure to show their importance in explaining the total variability in *S. carinata* density. The significance (P<0.05) of the RDA gradient was assessed by Monte Carlo permutation tests and their importance was measured by the eigen values of the first 2 axes (ter Braak and Verdonschot 1995). All *S. carinata* density and environment data were $\log_{10(X+1)}$ transformed to meet the assumptions of multivariate normality and to moderate the influence of extreme data. All the ordinations were performed using CANOCO 4.5 (ter Braak and Verdonschot 1995).

Microsatellite analysis of genetic diversity

A total of 27 specimens of *S. carinata* were collected in the lower part of the Ganjiang River. The specimens were taken to the laboratory where tissues were dissected and preserved at -80 °C. DNA was extracted from the foot tissue for genetic analysis using the TIANamp Marine Animals DNA Kit (TianGen). We used 19 primer sets (scastt1, scastt2, scastt3, scastt4, scastt5, scastt6, scastt7, scastt8, scastt9, scastt10, scastt11, scastt12, scastt21, scastt21, scastt22, scastt23, scastt24, scastt27 and scastt33) devel-

oped by Sun et al. (2016) for PCR amplification of microsatellite loci. Amplification conditions are described in Sun et al. (2016). Amplification products were analysed on an ABI 3730 automated sequencer and scored using GENEMAPPER v. 3.7 (Applied Biosystems) with a ROX-labelled size standard.

The number of alleles (N_A), observed heterozygosity (H_O) and expected heterozygosity (H_E) and tests for deviation from Hardy-Weinberg Equilibrium (HWE) were calculated by POPGENE v. 1.32 (Yeh et al. 2000). CERVUS v. 3.03 (Kalinowski et al. 2007) was used to calculate the polymorphism information content (PIC).

Results

The distribution of S. carinata

The distribution of *S. carinata* in the Ganjiang River is shown in Figure 3. *S. carinata* was presented in five of the 41 surveyed sites (12.2%, sites 11, 12, 14, 27 and 29). Historical information on the distribution of *S. carinata* described this species as colonising the lower stream of the Ganjiang River, Xiuhe River, Fuhe River, Xinjiang River and Poyang Lake (Figure 3).

The mean density of S. carinata

There were significant differences in the mean densities of *S. carinata* amongst the discovery sites (p<0.05) (Figure 4). The mean density of *S. carinata* was the highest at site 27 (1.54 ± 1.35 ind./m²) and the mean densities of the other four sites were 0.55 ± 0.81 ind./m² (site 14), 0.18 ± 0.41 ind./m² (site 29), 0.09 ± 0.20 ind./m² (site 11) and 0.09 ± 0.20 ind./m² (site 12), respectively.

Age structure of S. carinata

The age range of *S. carinata* was 2–8 years and the number of 5-year-old individuals was the greatest, accounting for 29.6% of the total number of individuals. The number of 2-year-old individuals was the next highest, accounting for 22.2% of the total individuals and the number of 8-year-old individuals was the lowest, accounting for 3.7% of the total number of individuals (Table 1).

Habitat characteristic of S. carinata

The habitat sediments of *S. carinata* were mainly composed of silt and the silt content at site 11 was 90.83%, followed by the silt content at site 27 being 84.59% (Table 2).The



Figure 3. The distribution of *S. carinata* (Red triangle: In the study; Black triangle: Historical research; Green line: In the survey area; Historical data sources: Liu and Wu. 1991; Wu et al. 1994; Wu et al. 2000b; Liu et al. 2008; Xiong et al. 2011; Xiao et al. 2012; Zhang et al. 2013; Huang et al. 2013).



Figure 4. The mean density of S. carinata along the 5 sites with mussels in the Ganjiang River.

temperature range of all sites was 11.20–29.10 °C; the pH range of all sites was 6.68–7.10; the dissolved oxygen range of all sites was 3.46–11.10 mg/l; the salinity range of all sites was 0.02–0.10 mg/l; the turbidity range of all sites was 7.20–107.00 mg/l; the

Number of samples	Shell length(mm)	Shell height(mm)	Shell width(mm)	Body- weight(g)	Age
1	126.4	25.6	16.8	75.6	2
2	137.2	27.6	20.1	80.1	2
3	145.3	30.4	22.4	81.7	2
4	145.5	30.6	22.6	82.1	2
5	157.6	32.4	24.3	101.1	2
6	164.3	34.4	25.5	109.5	2
7	171.1	26.9	35.4	113.5	3
8	178.0	37.4	28.3	122.1	3
9	195.3	39.4	30.2	144.2	4
10	198.4	40.4	30.9	151.3	4
11	205.6	40.8	30.9	174.5	4
12	210.4	41.2	32.1	180.7	5
13	210.7	40.2	31.4	181.6	5
14	217.6	40.9	27.1	193.5	5
15	218.7	42.1	31.7	200.5	5
16	220.4	42.2	31.9	205.4	5
17	221.3	42.2	28.9	208.5	5
18	222.2	41.4	32.6	209.5	5
19	222.6	42.2	31.9	211.4	5
20	225.8	41.8	32.9	217.9	6
21	230.0	46.2	33.5	234.8	6
22	230.5	46.4	34.1	236.3	6
23	238.8	48.2	35.1	289.8	7
24	240.0	48.4	35.3	307.1	7
25	244.3	48.8	35.8	317.9	7
26	245.0	48.9	36.1	320.1	7
27	253.1	50.1	40.1	340.9	8

Table 1. Shell shape data and age structure of S. carinata.

Table 2. Characteristics of sediment particle size of S. carinata

Particle size	Site 11	Site 12	Site 14	Site 27	Site 29
Silt (%)	90.83	18.52	75.44	84.59	23.08
Sand (%)	9.17	61.23	23.01	15.41	76.48
Granules (%)	0	8.21	1.55	0	0.44
Pebbles (%)	0	12.04	0	0	0

Note: Grading standards for particle sizes are from Wentworth (1922).

chlorophyll-a range of all sites was 5.75–13.54 mg/l (Table 3). The results of RDA analysis showed that the mean density of *S. carinata* was positively correlated with dissolved oxygen, temperature, turbidity and chlorophyll-a (Figure 5).

Factors	Site 11	Site 12	Site 14	Site 27	Site 29	All sites	
						Mean ±SE	Range
Т	20.3	20.4	20.5	19.3	19.2	20.3 ± 7.1	11.2-29.1
рН	6.74	6.72	6.72	7.03	7.04	6.94 ± 0.16	6.68–7.10
DO	7.18	7.13	7.22	8.01	8.19	7.60 ± 1.20	6.46-11.10
Sal	0.05	0.05	0.05	0.04	0.04	0.05 ± 0.01	0.02-0.10
TURB	47.7	26.4	36.8	57.2	57.8	49.4 ± 22.9	7.2–107.0
Chl-a	6.14	6.91	6.44	12.15	13.54	8.40 ± 2.00	5.75-13.54

Table 3. Environmental factors of *S. carinata* (T: temperature; DO: dissolved oxygen; Sal: salinity; TURB: turbidity; Chl-a: Chlorophyll-a).



Figure 5. Analysis of correlation between environmental factors and mean density of *S. carinata* (T: temperature; DO: dissolved oxygen; Sal: salinity; TURB: turbidity; Chl-a: Chlorophyll-a).

The morphology of glochidia of S. carinata

Mature glochidia of *S. carinata* were from December to February of the following year. *S. carinata* had marsupium in all 4 demibranches, the hinge length of glochidia was $31.8 \pm 2.9 \mu$ m and the length and height of the shell of $58.9 \pm 1.8 \mu$ m and $51.6 \pm 1.8 \mu$

 $2.2 \mu m$, respectively. Moreover, its glochidia were classified as small according to Davis et al. (1981) (Gln = shell length × shell height).

The results of the morphology of glochidia showed that they were ovate subtriangular, nearly symmetric and moderately inflated (Figures 6A, B). The hook styliform was bluntly rounded at the distal end, the microstylets were sparsely and evenly distributed on the hook and the micropoints were distributed densely on the ventral margin of valves, but did not extend to the hinges (Figure 6C). The exterior surface of the shells were densely but shallowly pitted (Figure 6D). The sensory hairs had tufts in one pair, located near the ventral margin of the valve (Figure 6E). Long larvae threads (a diameter of 0.5μ m) wrapped around each other in the shell and protruded out of the shell (Figure 6F).

Genetic diversity of S. carinata

A total of 27 individuals of *S. carinata* were successfully genotyped for all 19 microsatellite loci. The results showed that 63 alleles were detected amongst the three sampling locations. The number of alleles (N_A) at each sampling location ranged from 2 to 6 and the mean number of alleles (N_A) was 3.32; the observed heterozygocity (Ho) ranged from 0.143 to 0.796, the mean Ho was 0.419; the expected heterozygosity (H_E) ranged from 0.155 to 0.767, the mean H_E was 0.484; the PIC ranged from 0.124 to 0.708 and the mean PIC was 0.430. After the Bonferroni correction for multiple comparisons, no loci were deviations from HWE (Table 4).

Discussion

Solenaia are mainly distributed in East Asia, Thailand and India's Assam (Liu and Wu 1991, Wu 1998, Deein et al. 2003, Lopes-Lima et al. 2017a). Heude (1874–1885) reported that the genus Solenaia had 11 species and live specimens were mostly distributed in the south of the Huaihe River, specifically in the middle and lower reaches of the Yangtze River. Moreover, fossil specimens were widely distributed in the Quasi Algor basin of Xinjiang Province, Jilin Province, Hebei Province and Shanxi Province (Heude 1874–1885, Savazzi and Yao 2010, Xu et al. 2003). The fossil specimens of S. *carinata* were once reported as occurring in the quaternary of the conglomerate layer in the Yellow River bank, but now live specimens are only distributed in Poyang Lake basin (Liu and Wu 1991, Savazzi and Yao 2010). S. carinata do not move throughout their lives because they always insert themselves into the silt. Therefore, they were highly selective in their habitat choice, especially in the sediment. Moreover, they preferred to inhabit the silt and stable mussel beds. The decrease in the distribution range of S. carinata was likely caused by human disturbance, long-term environmental changes and habitat destruction (Wu 1998, Xiong et al. 2012, Zhang et al. 2013, Zieritz et al. 2018). In this study, *S. carinata* only occurred in 5 of the 41 surveyed sites in the lower



Figure 6. The morphology of glochidia of *S. carinata* (**A** lateral **B** ventral valve **C** hook with large spines **D** the exterior valve sculpture **E** sensory hair tufts **F** the larvae thread).

Locus	N,	Ho	H	PIC	F.
scastt1*	4	0.551	0.657	0.614	0.165
scastt2	2	0.434	0.463	0.357	0.041
scastt3*	2	0.153	0.155	0.134	-0.077
scastt4	3	0.341	0.526	0.449	0.401
scastt5*	5	0.659	0.718	0.652	0.048
scastt6	3	0.143	0.321	0.264	0.529
scastt7	2	0.240	0.265	0.213	0.073
scastt8	2	0.210	0.265	0.213	0.073
scastt9	3	0.189	0.290	0.236	0.350
scastt10*	4	0.551	0.767	0.697	0.231
scastt11	4	0.403	0.533	0.449	-0.093
scastt12	3	0.551	0.532	0.432	-0.093
scastt19	4	0.510	0.377	0.592	0.209
scastt21	5	0.617	0.569	0.527	-0.106
scastt22	3	0.474	0.517	0.458	0.102
scastt23*	2	0.353	0.467	0.348	0.123
scastt24	3	0.331	0.521	0.447	0.360
scastt27*	3	0.454	0.489	0.382	0.039
scastt33	6	0.796	0.761	0.708	-0.066

Table 4. Population genetic parameters in three populations of *Solenaia carinata*. N_A : the number of alleles, H_O : observed heterozygosity; H_E : expected heterozygosity.

Note: *: significant deviations from Hard-Weinberg equilibrium (p<0.05).

reaches of the Ganjiang River and this result indicated that its distribution range was limited in the region. Compared with *S. oleivora*, although both have similar morphology and life styles, the population size, density and distribution of *S. carinata* were much smaller than that of *S. oleivora* and the ratio of *S. carinata* and *S. oleivora* from the catch of local fishermen was 1:37.5.

Environmental factors, such as sediment, dissolved oxygen and water turbidity, significantly affect the distribution of freshwater mussels (Zhao et al. 2016, Negi and Mamgain 2013). The ability of freshwater mussels to spread was weak and they did not move; thus, they are less tolerant of the environment. In general, muddy areas had more abundant organic matter and freshwater mussels prefer to live in this habitat (Akiyama and Maruyama 2010). *S. carinata* is a relatively large-bodied mussel, remaining almost completely sessile as an adult (more so than most unionids) and it burrows in soft mud and clay sediments near riverbanks (Liu and Wu 1991, Yao and Wu 2001, Liu et al. 2017). We found that the habitat sediments of *S. carinata* were mainly composed of silt and the moderate ratio of mud or sand can form a hard substrate structure, which kept mussel beds stable and protected from the impacts of water flow.

Some studies have shown that environmental factors such as water temperature, DO and turbidity influence benthic community structure. Dissolved oxygen was one of the important factors which affects the distribution and abundance of macrozoobenthos. For example, a positive correlation was found between the macrozoobenthos diversity and dissolved oxygen at all of the sites during the present investigation which is in accordance with the findings of Joshi et al. (2007) who reported maximum benthic diversity during the winter season when the amount of dissolved oxygen is higher and the temperature is low (Negi and Mamgain 2013). Chlorophyll-a was another factor that affected the distribution and abundance of macrozoobenthos. Within a certain range, phytoplankton can provide rich food for macrozoobenthos and promote their reproduction and growth. However, when water had a high concentration of chlorophyll-a, algae density was also higher, which would restrain the biomass and distribution of macrozoobenthos (Stout and William 1985). Turbidity was also an important environment factor by its influence on the distribution of macrozoobenthos (Martin et al. 2008). In this study, we also found that the distribution of S. carinata was correlated to dissolved oxygen, water temperature, turbidity and chlorophyll-a. In addition, strikingly, the number of S. carinata was greater in water with high turbidity, which indicated that high turbidity of water could provide rich food sources for the reproduction of S. carinata.

Freshwater mussels are selective and adaptive to habitats (Vaughn 2012). Some studies called a region or niche of mussels gathered as "mussel bed" (Brainwood et al. 2008, Vaughn 2012). A "mussel bed" was the result of long-term adaptation of mussels to the hydrological situation and characteristics of the river (Vaughn 2012). Diversity and richness varied greatly amongst different microhabitats (Hornbach et al. 2010). Different mussels had different diffusion abilities and changing water level was one of the important factors that affected the survival and dispersion of mussels (Spooner et al. 2012; Jones and Neves 2011; Vaughn 2012). The average water level in the lower reaches of the Ganjiang River was 13.51 m (Wusong elevation), the lowest water level was 9.09 m (January) and the highest water level was 17.82 m (July). There were lots of "mussel beds" in the lower reaches of the Ganjiang River, especially in the low water level zone. When the water level of Nanchang reaches dropped to approximately 8.0 m (Wusong elevation), some "mussel beds" are exposed. The movement ability of mussels varied amongst the different water levels. The life style of S. carinata is almost completely sessile as an adult (more so than most unionids), remaining burrowed in soft mud and clay sediments near river banks (Figures 7A, B). Although S. carinata usually inhabited the low water level zone (9-10 m), when the water level was below 9m or much more than 10 m, the survival of S. carinata would be affected. Therefore, the coupling between the survival of S. carinata and the distribution of "mussel beds" and changes in water levels is the results of a long-term adaptation of the mussels to the water level in the river.

The reproduction characteristics of freshwater mussels and the morphology of glochidia were important to its classification and phylogeny (Heard and Gluckert 1970; Wu et al. 1999, Graf and Foighil 2000, Lopes-Lima et al. 2017a). *S. carinata* had a marsupium in all 4 demibranches. The reproduction type of *S. carinata* was similar to that of *Lamprotula*, such as *L. caveata*, *L. cornuum-lunae*, *L. scripta* and *L. leai* and, for this reason, *Lamprotula* and *Solenaia* were previously thought to have a close relation-



Figure 7. The life style of *S. carinata* (**A** *S. carinata* used its narrow front end and strong axe to keep digging down to resist erosion from flood waters **B** *S. carinata* preferred to inhabit silt or hard clay).

ship (Wei et al. 1994, Wu et al. 2000a). However, the glochidia of *S. carinata* were hooked, while those of *Lamprotula* were hookless. It is unfortunate that no information is available on potential host fishes.

Glochidia of *S. carinata* were small-sized and numerous, which could promote population viability based on increasing the number of juveniles and shrinking the morphology size of glochidia. This study indicated that the reproduction period of *S. carinata* was from December to February the following year. In this period, Poyang Lake basin was in its dry season, which resulted in many freshwater mussels and fish being easily harvested. However, the reproduction period of *S. oleivora* was in May and, in this period, the middle and lower reaches of the Yangtze River were in their wet season and fish activity was high, which was beneficial to the parasitism of glochidia. Droughts not only affected the survival of freshwater mussels, but also led to a reduction in host fishes. These factors were likely to be reasons for a sharp decline in the *S. carinata* population.

Higher levels of genetic diversity amongst populations of aquatic organisms could improve evolutionary potential for dealing with habitat change, effects of pathogen infection and other selective forces (Freeland et al. 2011, Liu and Yao 2013, Wu et al. 2013, MacDonald et al. 2017). However, the genetic diversity of *S. carinata* (mean H_0 : 0.419; mean H_E : 0.484; mean PIC: 0.430) was lower than *Sinohyriopsis cumingii* (mean heterozygosity ranged from 0.617 to 0.750), *S. oleivora* (mean H_0 ranged from 0.501 to 0.620; mean H_E ranged from 0.598 to 0.701) and *L. caveata* (mean H_0 : 0.455; mean H_E : 0.835; mean PIC: 0.795) (Luo 2006, Min et al. 2015, Xu 2014), which indicated that *S. carinata* was less adaptive to the environment and populations were prone to decay and extirpate. Narrow-range endemics like *S. carinata*, as well as rare and imperilled species, often have lower genetic diversity and may have experienced genetic bottlenecks (Hamrick and Godt 1989). Genetic bottlenecks in a narrowrange endemic could be the result of the initial founder event that led to speciation in *S. carinata* (Freeland et al. 2011).

Human activities such as dam construction, sand mining, water pollution and overfishing could seriously affect the survival of freshwater mussels (Burlakova et al. 2011, Geist 2011, Lopes-Lima et al. 2017b). Large-scale and unregulated sand mining operations not only damaged the habitats of freshwater mussels, but also resulted in

refloated heavy metals and toxic substances in the water, which could be dangerous for freshwater mussels. Similarly, construction of dams would change the river hydrological characteristics, which affected the colonisation of juveniles and reduced the survival rate of juveniles (Mueller et al. 2011). In addition, dam construction also affected the survival of host fishes. For S. carinata, we should not only protect and manage their own river habitats, but also prevent the discharge of pollutants in upper reaches of the river and sustain appropriate flow rates, especially in the dry season (Saunders et al. 2002). Recently, global climate change had resulted in an earlier and prolonged dry season. The water levels in the Ganjiang River have also continued to decline and some reaches of the river are below the drought alarm level. During this period, S. carinata have been exposed to drought conditions for a long time, which has led to death and, moreover, it has also been easy for fishermen to capture lots of S. carinata by using their tools. Freshwater mussels are highly dependent on host fishes. However, fishermen overfished host fishes due to commercial interests, which could affect the survival and communication of freshwater mussels amongst lakes. Therefore, it is necessary to prevent overfishing to protect freshwater mussel diversity.

Despite the diversity and value of freshwater mussels, relatively little is known about the biology of many of these species (especially species outside of Europe and North America) (Zieritz et al. 2018). Currently, a special conservation area for freshwater mussels has not been established in China. In addition, studies on freshwater mussels are mainly concentrated on resource surveys in some regions (e. g. Yangtze River; Wu 1998, Xiong et al. 2012; Zhang et al. 2013), but there are still many gaps in the information on the conservation status of these species and research efforts are almost non-existent (Lopes-Lima et al. 2014). To a large extent, the lack of protection for freshwater mussel species in China is due to the lack of knowledge on their status, threats and other conservation-related issues. Scientific studies should specifically investigate their threats and known host fish identities in China (Zieritz et al. 2018). The lack of Chinese freshwater mussel species in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is particularly surprising in that the commercial use and trade of these animals is most likely more intense in China than anywhere else in the world (Fiske and Shepherd 2007, Ali and Cartier 2013, Ng et al. 2016). Therefore, conservation biology research on freshwater mussels in China is very important. Overall, urgent management measures devoted to the conservation of freshwater mussels are required to: 1) raise ecological protection awareness; 2) prevent commercial capture; 3) strengthen protection efforts for host fishes and habitats of freshwater mussels and 4) prolong the closed fishing season appropriately, which can provide a good ecological environment for the reproduction of freshwater mussels.

Acknowledgements

Funding for this research project came from the National Nature Science Foundation of China (No. 31772412 and 31260515), and the Leading Talent Training Plan of

the GanPo Outstanding Talents 555 Project (No. 18000041). The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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REVIEW ARTICLE



The challenge and opportunity of behaviour change methods and frameworks to reduce demand for illegal wildlife

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Academic editor: M. Grodzinska-Jurcak	Received 3 December 2017	Accepted 9 April 2018	Published 25 April 2018
http://zooba	nk.org/43717C98-86B8-4AEE-	90B0-45C7131346EB	

Citation: Wallen KE, Daut EF (2018) The challenge and opportunity of behaviour change methods and frameworks to reduce demand for illegal wildlife. Nature Conservation 26: 55–75. https://doi.org/10.3897/natureconservation.26.22725

Abstract

Biodiversity conservation is contingent upon managing human behaviour and, at times, changing behaviour. This is particularly relevant to the illegal trade in wildlife and wildlife products, both flora and fauna. Driven by dynamics of consumer demand and illicit supply, mitigation of illegal trade requires a fuller appreciation of human behaviour and methods to change it. In various sectors, social influence, behavioural insights, social marketing and human-centred approaches trend towards mainstream practice and policy application. However, in the context of conservation and wildlife trafficking, these approaches and their usefulness are not well-articulated nor application widespread. Here, we provide a practical overview of relevant behaviour change methods and frameworks. We discuss their usefulness and potential application to mitigating the illegal wildlife trade, in general and consumer demand, in particular.

Keywords

Consumers, human-centred design, nudges, social marketing, social norms, theory of change, wildlife trade

Introduction

Conservation is contingent upon human behaviour and, at times, changing behaviour (Reddy et al. 2016, Schultz 2011, St. John et al. 2010). This is particularly relevant to and salient within the context of the illegal wildlife trade (IWT) (Biggs et al. 2017, Burgess 2016). Driven by various dynamics of consumer demand and illicit supply, the harvest and sale of flora and fauna overexploits and threatens various species (Oldfield 2003, Rosen and Smith 2010). As such, mitigating IWT necessitates that conservation professionals and policy-makers are aware of and acknowledge the cognitive, social and institutional elements that influence human behaviour and the methods and frameworks of behaviour change.

In various sectors, behaviour change methods and frameworks have become central to practice and policy, e.g. health, finance, transportation and public utilities (Organisation for Economic Co-operation and Development [OECD] 2017, World Bank 2015). In the context of conservation and IWT, their use is not well-documented nor their application mainstream; though some organisations have begun to focus on behaviour and emphasise strategic application of behaviour change methods and frameworks. For instance, TRAFFIC, a wildlife trade monitoring network of researchers and practitioners hosted by the World Wide Fund for Nature (WWF) and the International Union for the Conservation of Nature (IUCN), has developed and made publicly available a Wildlife Consumer Behaviour Change Toolkit (available at http:// www.changewildlifeconsumers.org). Oxford University, in late 2016, started the Oxford Martin Programme on the Illegal Wildlife Trade, a collaborative initiative focusing on consumer demand and behaviour change aspects of IWT. In late 2017, WWF's Fuller Symposium focused exclusively on the science of behaviour change and effective methods that influence behaviour (available at https://www.worldwildlife.org/pages/ the-nature-of-change). These and similar initiatives aim to provide essential resources, knowledge exchange and network-building that enable a community of practice to meet a common goal of reducing demand for illegal wildlife via social and behaviour change (Lertzman and Baragona 2016).

Our discussion of behaviour change and IWT mitigation, like others', focuses on the role of consumer demand, which is recognised by many, including governments and non-governmental organisations (NGOs), as a significant and inherent driver of IWT (Daut et al. 2015, Executive Order 2013, McHale and Hayes 2014, Traffic 2008, WWF 2012). In addition, researchers and practitioners suggest a consumer demand/ behaviour focus for several reasons: (1) enforcement and regulatory strategies often fail to adequately account for underlying social drivers of illegal trade (Challender and MacMillan 2014), (2) demand for illegal wildlife is more appropriately viewed as being wealth driven, not poverty driven (Duffy 2010), (3) mitigation strategies often neglect consumer preferences and behaviour (Nijman 2010, Veríssimo et al. 2012) and (4) regulations often focus on enforcement but neglect demand reduction and local community engagement (Roe 2015, Wellsmith 2011). Here, we complement these initiatives and published works by providing a practical overview of behaviour change methods and frameworks. We also provide examples specific to IWT, highlighting successes and failures. Our overview of select methods and frameworks serves to highlight the challenges and considerations that should be taken into account when conservation professionals and policy-makers chose to apply and evaluate behaviour change. Furthermore, to lend additional practical and policy relevance, we introduce the concept of Theory of Change (ToC) as a process for conservation professionals and policy-makers to examine the position and suitability of behaviour change strategies alongside other efforts.

Basic considerations of context and scale for behaviour change

Human behaviour is complex, relative and context-dependent. Reasonably specific and small-scale behaviour change involves navigating, coordinating and influencing multiple cognitive and social processes, stakeholder groups and environmental or institutional circumstances. Implementing household energy-saving measures, for example, can involve coordination amongst residents, utility providers, policy regulators and commercial companies. In the context of the wildlife trade, an illustrative example of coordinating multiple stakeholders to change behaviour is the plume feather trade of the early 1900s. At the time, overexploitation of migratory birds for decorative feathers caused dramatic population declines, particularly of egrets (Souder 2013). Mitigating impacts required coordinating the behaviour of wealthy consumers, retail merchants and policy-makers. The coordination of these stakeholders via social influence, stigmatisation, education, outreach and lobbying resulted in the development of the Weeks-McLean Law in 1913, which later transformed into the Migratory Bird Treaty Act of 1918 (Souder 2013).

As implied by these examples, the behaviour of any one stakeholder interacts with those of others. That is, individual behaviour is influenced by both internal and external factors. Whereas cognitive factors such as beliefs, values, attitudes and emotions directly or interactively influence behaviour, they also interact with institutional factors like informal social norms or cultural taboos and formal regulations like treaties or directives. As such, central to changing behaviour is to understand the context in which behaviour operates and manifests. Each setting poses different contextual factors that constrain or afford certain behaviour change strategies. These may include cultural practices, economic conditions, governance structures, geography or associations with other illicit trade activities like human trafficking and organised crime (Brashares et al. 2014, Gore 2011). For example, Parry et al. (2014) highlight the failure of comprehensive bans and punishments designed to mitigate demand for wildlife in Amazonia because enforcement is scarce and illegal behaviour deterrents few. They suggest a more context-specific approach in which locals are allowed to trade harvest-tolerant species, with increased protection and enforcement focused on more vulnerable species.

Alongside this example, a body of literature is developing that evinces the need for context-specific behaviour change approaches (Kahler et al. 2013, Steinmetz et al. 2014). While perhaps the idea of context-dependence is a truism, consideration of context is crucial to implementing effective on-the-ground behaviour change and consumer demand reduction (Brashares et al. 2014, Gifford and Nilsson 2014).

Scale is another element that behaviour change strategies must consider. Behavioural influences are multi-layered and can manifest and operate at various scales depending on the lens through which they are viewed, i.e. the individual, group or society (World Bank 2015). For example, behaviour can be viewed through the lens of a person making decisions via automatic judgements, without deliberation or deep thinking (based on habit, instinct or imitation). From another perspective, a person behaves more deliberately via active consideration of what others around them do or think, perhaps based on prevailing social norms and cultural values (Reddy et al. 2016). The illegal trade of wildlife is a system that operates at multiple spatial and temporal scales, from local–global and individual–societal, as species and products often traverse several scales from harvest to consumption (Gao and Clark 2014, Traffic 2008). Delineating the multiscale nature of IWT and associated behaviour is necessary to identify relevant behaviour, define context and choose appropriate behaviour change methods and frameworks.

Behaviour change methods: strategies and tools

The relevance and suitability of the behaviour change approaches which are outlined vary in relation to the context and scale of the behaviour targeted for change. Behaviour change methods will also vary in terms of how passive (indirect) or active (direct) the mode of influence or persuasion is. For instance, the messages and graphics in campaign materials like signs, brochures or websites are passive modes of influence, which are often static and the likelihood of an individual interacting with them is uncertain. In contrast, a block leader approach is an direct, active mode of influence that uses face-to-face and interpersonal communications to increase the likelihood of an individual interacting with the mode of influence (Abrahamse and Steg 2013). Here, we summarise four behaviour change methods and provide a general overview of their application and, when applicable, application specific to IWT (Fig. 1a). For more thorough descriptions and discussions of these conceptual approaches to behaviour change, we recommend further reading of referenced materials.

Education and awareness

Providing educational and awareness-raising materials and resources are intuitive and often default behaviour change strategies (Daut et al. 2015). These modes of behaviour change tend to focus on information-provisioning, often based on a "bank model",



Figure 1a. Infographic overview of selected behavior change methods relevant to reducing consumer demand for illegal wildlife and wildlife products.

wherein it is assumed that individuals lack the necessary information and provisioning information will change behaviour, accordingly (Freire 1970). This type of behaviour model is sometimes referred to as an information- or knowledge-deficit model (Owens 2000). In the form of information-provisioning, the efficacy of education and raising awareness as a behaviour change method are greatest when individuals are motivated by a pre-existing self-interest to (voluntarily) engage in the desired behaviour (Rothschild 1999). That is, behaviour change is most likely to occur when individuals are provided with information that aligns with a pre-existing personal interest to engage in the target behaviour. For instance, Frantz and Mayer (2014) and Carmi et al. (2015) suggest pre-existing emotions and "connection to nature" are important mediators of the relationship between knowledge and behaviour in the context of environmental education. With the absence of emotions or other pre-existing factors or motives, the prospect of changing behaviour diminishes (Lo et al. 2012).

In the context of IWT, when a consumers' interests and/or motivation align with those of the education and awareness-raising efforts, these can be effective behaviour change tools that provide the information for purchasing decisions. For example, consumers who purchase exotic pets, meats or souvenirs may be unaware they are purchasing illegal wildlife or wildlife products and merely require this type of information to refrain from purchasing (e.g. Moorhouse et al. 2017). This consumer segment is not actively attempting to purchase illegal wildlife and their consumption may be entirely unintentional or inadvertent. Other examples of targeted education campaigns by NGOs have

led to decreased demand for parrots in Saint Lucia (Jenks et al. 2010), rhino horn in Yemen (Vigne and Martin 2013) and shahtoosh in India (Misra 2003). Education can also be used proactively to subvert the creation of future consumers by educating younger generations (Daut et al. 2015). While not at the scale of current consumers, an inter-generational approach to behaviour change via education can be part of a larger behaviour change framework that strategically incorporates other methods outlined below.

Outreach, relationship building and trust

Outreach activities, typically, consist of initiatives designed to provide or facilitate services or goods that improve wellbeing and/or initiate behaviour that are viewed as more sustainable or beneficial. For example, outreach to reduce consumer demand for illegal wildlife may take the form of direct and indirect public education or public advertisements (Daut et al. 2015). While the term outreach encompasses various types of initiatives or interventions, including education and raising awareness, we distinguish outreach as a behaviour change approach because it often emphasises relationship building and garnering trust amongst stakeholders to enact collaborative behaviour change (Stern 2017). For instance, trusting the mode (e.g. workshop, training programme) or medium (e.g. governmental management agency, NGO), by which information and/or resources are provided, is a significant predictor of behaviour (Stern 2008). Likewise, efforts to influence culturally-entrenched behaviour can be difficult to accomplish without building a two-way relationship and dialogue with the target stakeholder groups (Stern and Coleman 2015). For many local or regional-scale IWT or conservation initiatives, the efficacy of education programmes, training workshops or community events/meetings hinges on the relationship and trust between stakeholders and those seeking to influence their behaviour.

Outreach activities coupled with robust stakeholder relations can be an effective tool to influence behaviour and reduce consumption of endangered or illegally-traded wildlife (Cvetkovich and Winter 2003, Perez 2008, Steinmetz et al. 2014). For example, outreach with traditional medicine practitioners has resulted in reduced use and, consequently, demand for illegal wildlife used in medicinal products (Traffic 2015). In Thailand, Steinmetz et al. (2014) combined long-term (4–6 years) outreach activities with social-psychological principles, such as social norms and motivations, to successfully reduce illegal poaching. These examples, while relying on synergies between two or more behaviour change approaches, highlight that a strong, trusting relationship between stakeholders and organisations is often a prerequisite for effective behaviour change.

Social influences

Social influence refers to the change in behaviour which one individual causes in another, intentionally or otherwise (Turner 1991). That is, the behaviour and expectations of others (un)consciously influences behaviour. Particularly influential are those within an individual's social groups, such as family or friends, neighbours or community members and members of a meaningful social group (Cialdini and Trost 1998). Concepts like social norms, social comparison and social learning are representative of this phenomenon. For instance, interactions with others from a social group foster cognisance and adherence to informal rules that guide individual behaviour (social norms), allows for comparison of behaviour to that of others (social comparison) and provides an opportunity to learn behaviour from others (social learning). Social influence techniques and methods are common across many fields of study and practice, as well as being central to many behaviour change strategies (Wallen and Daut 2017). Below, we summarise three common methods.

Normative social information and feedback is a method that relies on perceptions of and responses to information about behaviour that is common and/or approved, i.e. normative behaviour. This method of behaviour change uses specific normative social information to convey what behaviour other group members do and/or approve or expect. Following that information, a person is provided information about their personal behaviour in relation to others' behaviour and/or approval, which acts as a feedback mechanism (Schultz et al. 2018). This facilitates reflection and assessment of one's behaviour in direct relation to the prevailing social norm(s) of the focal group. With such information and feedback, an individual is assumed to align their personal behaviour with that of the groups in order to avoid perceived or actual social punishments (ostracism, shame) or to perceive or receive actual social rewards (praise, respect).

Public commitments, written or verbal, are used to bind an individual or entity to a specific opinion, goal or behaviour (Kiesler 1971). Commitment-making techniques can be effective at influencing short- and long-term behaviour change when they use monitoring and enforcement mechanisms to reinforce the agreed upon behaviour (Lokhorst et al. 2013). Public commitments, in the form of a written or spoken statements, are an example of this. They oblige an individual to align their behaviour with their commitment and, subsequently, be consistent in their behaviour with the knowledge that others are able to monitor their behaviour in relation to their public commitment (Cialdini 2001).

Block leaders are influential members of a social network that are used as agents for social influence and change. By capitalising on existing social networks and key members within the network, this method increases the likelihood that information about (un)desired behaviour reaches target individuals or groups. At the local scale, a block leader method is an effective influence approach given the inherent face-to-face interaction (Abrahamse and Steg 2013). For additional discussion and review of these and other methods, see Abrahamse and Steg (2013), Abrahamse et al. (2005), and Osbaldiston and Schott (2012).

With respect to IWT, emphasis on local-scale community engagement and behaviour change represents an opportunity to more widely incorporate social influence methods (Biggs et al. 2017, Cooney et al. 2017, Roe 2015). In general, past IWT projects that used these social influence methods have been positive to mixed. For example, public commitments by celebrities, regional leaders and local community members are used extensively by organisations, such as WildAid (http://www.wildaid.org/) and Education for Nature Vietnam (http://www.envietnam.org/). However, the efficacy of their social influence approaches on consumer behaviour is not well-understood (Duthie et al. 2017). Public commitments by governments and businesses provide more tangible examples of behaviour change, albeit by institutions that facilitate consumer behaviour rather than individuals (Hall 2013; International Fund for Animal Welfare 2014). With regards to block leader approaches, Rare's longstanding Pride campaign is an example of a programme that uses influential members of local social networks to promote and persuade others to engage in desired behaviour or refrain from undesired behaviour (Jenks et al. 2010). Given the limited application of social influence approaches to reduce consumer demand for illegal wildlife, we provide hypothetical examples of their use in Table 1 to demonstrate potential on-the-ground applications.

Behavioural insights and nudges

Behavioural insights and the term nudge originate from various cognitive science disciplines that have identified specific cognitive biases, boundaries, heuristics and habits that influence behaviour and increase the predictability of behaviour (Kamenica 2012, Thaler and Sunstein 2008). This behaviour change method builds on the assumption that the behavioural choices available to an individual within any setting are limited. That limitation of choice is referred to as the decision context (or choice architecture). A nudge is an alteration to the decision context that changes behaviour in a predictable way without significantly prohibiting alternative options or altering incentives (Thaler and Sunstein 2008). For example, in many settings, a default choice (or default option) steers individuals towards a reasonably predictable behaviour. Common examples of defaults are not being automatically: enrolled in a retirement fund (e.g., 401K), registered to vote or listed as an organ donor. Vice versa, these include automatically: enrolling to receive email marketing, contributing a low level to your retirement fund or donating a portion of a tax return to charity. Nudges, generally (but not exclusively), are interventions that attempt to change behaviour by replacing or making the default choice more or less easy, intuitive, attractive, social and/or timely. That is, changing the default choice changes behaviour, e.g. an individual is automatically enrolled in a retirement fund, registered to vote or contributes the maximum level to a retirement fund each pay period. Other types of nudges incorporate aspects of priming, cognitive biases, perceptual cues and social influence and can be combined with other behaviour change approaches (OECD 2017). For instance, as a perceptual cue, reducing the size of a plate or bowl can reduce the amount of food consumed as the perception of portion size is relative to the dish on which food is served.

The term behavioural insights is commonly used to denote the application and integration of nudges into public policy design and evaluation. Relative to other sectors, nudges are uncommon in conservation and wildlife trade policy. Though not explicitly

Illegal wildlife product	Desired behavior change	Target audience	Social influence approach
Bushmeat of protected-species (i.e., that may be served at restaurants in urban areas)	Consumers select bushmeat legally harvested with commercial quotas	Restaurant customers, e.g., tourists, businesspersons, or military personnel ^b	Normative social information: place visible signs and other messages at relevant locations to expose consumers to information that (1) bushmeat consumption is socially disapproved and (2) a majority does not consume bushmeat. <i>Public commitment:</i> encourage senior and/or influential military and business personnel to pledge they will not consume illegal bushmeat and denounce illegal consumption.
Protected songbird species Illegally-harvested as pets or for singing competitions in Indonesia or Amazonia	Select legally-harvested or captive- bred songbirds	Songbird club members and hobbyists	Block leader: Recruit club founders/ leaders to use and promote the use of only captive-bred or legally-capture species for singing competitions; block leaders talk to other clubs to persuade them. <i>Normative social feedback</i> : Create clubs or singing competitions with prestigious participants and prizes that only allow certified captive-bred birds; do not allow clubs and individuals using illegally-harvested birds to participate.
Asian and African pangolin ^c scales as traditional medicine in Viet Nam ^{d,c}	Select alternative modern medicine or other traditional medicine from legally-traded species	Affluent, urban consumers	<i>Public commitment:</i> obtain public pledges from traditional medicine practitioners, physicians, or celebrities stating they will not purchase or consume pangolin products.

Table 1. Three hypothetical IWT scenarios^a and suggested social influence methods to reduce consumer demand for illegal wildlife and wildlife products (adapted from Wallen and Daut 2017).

Each scenario should start with a baseline evaluation to better understand the context and scale of consumption to determine the most appropriate method. ^bMilitary personnel, businesspersons, and tourists are significant consumers of novelty bushmeat.

^cTrade and consumption of Asian pangolins is illegal in Viet Nam.

^dA first step would be identifying areas where products are sold, the merchants, and the consumers.

"Over the past 15 years the demand for pangolin has increased as the traditional medicinal market has reemerged

designed as a nudge, Indonesia's fatwa policy against all hunting of and trade in endangered species incorporates aspects of social norms and taboos to create a new default that nudges individuals towards more socially desirable and religiously approved behaviour (Meissner 2015). Nudges can be used to redirect behaviour towards a familiar or existing alternative without having to create a new behaviour. For example, as humans tend to be loss averse, a behavioural nudge could frame an existing IWT behaviour as having short- and long-term economic, social, or health costs and risks. Loss-aversion nudges can also be designed to eliminate a default choice and direct individuals to consider losses incumbent in a non-preferred alternative (Keller et al. 2011).

Nudges relevant to IWT and consumer demand may involve the development of text message programmes or smart-phone applications that provide information about known IWT retailers and alert consumers to alternative choices. The principles of behavioural insights can also be used to nudge institutions and governments. For instance, the combination of local behaviour change working synergistically over time (e.g. education, outreach, public commitments) can nudge governments by changing their default option. That is, prior to local-level efforts towards consumer demand reduction, a government's default may be lax IWT policies, negligible monitoring and enforcement and limited motivation to improve policy. With a constituency of stakeholders shifting their behaviour and support for demand reduction policies, a government's default option can be nudged from the bottom-up. Hong Kong's domestic ban on shark fin soup and legislation introduced to ban the ivory trade after widespread advocacy from NGOs, influential celebrity endorsements and social marketing campaigns are examples of this type of indirect, bottom-up nudge (Environment Bureau 2013).

Behaviour change frameworks: implementation and evaluation

Understanding the context and scale of behaviour to identify an appropriate behaviour change method(s) is followed by implementation and evaluation, which requires the use of adaptive, flexible and iterative multi-stage frameworks (World Bank 2015). Many behaviour change frameworks parallel adaptive or learning-based management, which are known as effective and robust management frameworks (Williams and Brown 2012). Importantly, these types of frameworks allow for systematic pilot testing, implementation and evaluation that is coupled with iteration, reflection and learning (e.g. McKenzie-Mohr 2011, McKenzie-Mohr and Schultz 2014, Schwartz et al. 2017). Recent work outlining the diversity of products (flora, fauna), actors (motivations, values), networks (small/large-scale, distribution), scales (local, national, international) and contexts (culture, geography) that define an IWT system serve as a pivot to look more closely at such frameworks (e.g. Phelps et al. 2016).

Evaluation is an essential element of any behaviour change framework or strategy (Ferraro and Pattanayak 2006, Olmedo et al. 2017). For example, marketers use a pretest, concept test or copy test process to evaluate if elements of a campaign elicit the



Figure 1b. Infographic overview of selected behavior change frameworks relevant to reducing consumer demand for illegal wildlife and wildlife products.

desired response(s) (Kotler and Armstrong 2017). In the context of IWT, recent studies highlight the benefits of and insights gained when evaluation is made as an explicit and crucial step for the behaviour change process (Duthie et al. 2017, Olmedo et al. 2017). Below, we outline frameworks that enable the development, implementation and evaluation of behaviour change (Fig. 1b).

Social marketing

Social marketing is a systematic, staged-planning framework that applies marketing principles to *sell* social good and solve a social problem by changing and maintaining beneficial behaviour (Andreasen 1995). Social marketers plan, design, implement, evaluate and adapt programmes by viewing an issue from the perspective of a specific and targeted audience (i.e. a "know your audience" principle). A social marketing campaign, generally, has three overarching objectives: (1) provision of information or create awareness to ensure a target audience understands basic concepts and ideas and their relevance to them, (2) make a desired behaviour easier by reducing or removing barriers and (3) develop and manage relationships with a target audience (Wright et al. 2015). These objectives are achieved using a stepwise, iterative process. Typically, this process consists of six steps or stages: (1) plan, (2) scope, (3) develop, (4), implement, (5) evaluate and (6) follow-up (Burgess 2016, Hopwood and Merritt 2011, Jenks et al. 2010). See Table 2 for more details of each step.

The flexibility and scope of a social marketing framework has led to contextspecific variants. We outline two relevant to IWT: community-based social marketing (CBSM) and conservation marketing (ConsMark). CBSM represents a social marketing approach that stems from the pro-environmental and sustainability fields

Plan	An initial planning stage can help determine if social marketing is the appropriate framework for the scale and context of the target behavior change. Questions to consider are: what issue or challenge is being addressed, what resources are available to draw on, what associated policies (formal and informal) exist, what are potential risks or consequences of changing the behavior, what is the timescale partners have to work with, and can an evaluation strategy be created.
Scope	During the scoping stage, the behavior change method(s) are selected. Questions to consider during this stage are: who is the target audience and behavior, what are their motivations and barrier to desired behavior(s), what has been done previously, and what factors will affect the issue and behaviors based on the selected method(s). This stage can also be used to facilitate collaboration with stakeholders to co-identify important variables and co-develop methodology.
Develop	After planning and scoping to identify the behavior, audience, and method, the intervention requires operationalization and proof-of-concept via pretesting (pilot testing) with a subset of audience. During this step, set goals and identify specific, measurable, achievable, realistic, and time-bound objectives, and evaluate those based on pretest results. The pretest and goal setting can help maintain engagement and relations with the audience and external partners. Pretest results will determine needed adjustments.
Implement	The behavior change strategy is implemented at full scale. Though each campaign will differ, important tasks during this stage are monitoring, gathering feedback, being prepared to respond to problems, and publicizing benefits and ease of behaviors to garner support and engagement.
Evaluate	This stage is a formal review of the interventions objectives. Evaluation may take the form of qualitative and/or quantitative assessment. A desired outcome of this stage is a report with recommendations for improvement. Importantly, as the effects of a behavior change intervention may develop and emerge over time, either immediate and gradual, evaluation should planned for multiple time periods post-intervention (e.g., 1-month, 3-month, 6-month, 1-year, 2-year, etc.).
Follow-up	Evaluation results are presented to stakeholders and a collective plan is created to move forward. At this stage, it is important to recognize and acknowledge positives and negatives as both assist in determining if modification to the intervention is needed or if the chosen methods were appropriate based on feedback from stakeholders. This stage represents the main recourse for feedback, iteration, and adaptation.

Table 2. General outline and description of social marketing stages (step) and process.

(McKenzie-Mohr 2011). The CBSM framework shares several similarities with social marketing. This includes the stepwise process to implement behaviour change: (1) identify a specific behaviour or set of behavioural actions related to the desired outcome, (2) uncover barriers to and benefits of types of behaviour, (3) develop strategies and/or design a programme to overcome those barriers and promote benefits, (4) pilot the selected strategy or programme and (5) broadly implement and evaluate the strategy or programme. As with its parent framework, CBSM emphasises preliminary identification of behaviour to target, removal of behavioural barriers and an iterative process of implementation, monitoring and evaluation. With its explicit focus on community- and local-scale issues, the principles of CBSM facilitate a scalable and context-specific behaviour change framework, which is a growing priority for IWT interventions to reduce consumer demand (Roe 2015).

Conservation marketing is a related framework that has developed in the conservation field (Wright et al. 2015). The ConsMark framework is defined as the ethical

application of marketing strategies with the objective of advancing conservation goals (Veríssimo and McKinley 2016). Apart from social marketing principles, the Cons-Mark framework also draws on aspects of branding, social media and conservationspecific concepts to guide and promote behaviour (Smith et al. 2010). Such marketing campaigns have been successful at encouraging sustainable seafood consumption and orca conservation (Wright et al. 2015). Social marketing principles have also been applied to conserve golden snub-nosed monkeys (DeWan et al. 2013), tigers (Saypanya et al. 2013) and to reduce wild meat consumption (Chaves et al. 2017). Importantly, ConsMark researchers and practitioners are beginning to emphasise and promote the crucial step of evaluation (Jenks et al. 2010, Veríssimo et al. 2017). For example, Duthie et al. (2017) evaluated the efficacy of celebrity endorsements designed to raise awareness and mitigate illegal poaching of wildlife. Results of this study were mixed, which suggest celebrity endorsements should be used with caution and in conjunction with standard marketing pre-test and post-test procedures.

Human-centred design

The general principles of human-centred design (HCD) are derived from participatory action research (Reason and Bradbury 2008). The framework endeavours to develop, refine and implement a context-specific and locally-suited behaviour change strategy by working directly and collaboratively with the individuals and communities affected, i.e. those who will be using the strategy to solve the issue (IDEO 2015). An HCD framework, generally, uses a stepwise methodology consisting of (1) defining, (2) diagnosing, (3) designing, (4) testing and (5) scaling. See Table 3 for more details. Within the social sector, organisations like ideas42 (http://www.ideas42.org) and IDEO (htt-ps://www.ideo.org/) use a HCD framework to develop and implement user-orientated solutions to social issues such as water conservation, poverty alleviation, smallholder farming and urban sustainability (Tantia 2017).

While HCD is not widespread within conservation, it has similarities to community-based conservation but differs with its explicit focus on behaviour, viewing issues through a behavioural lens. In the conservation sector, it has been used, for example, to design frameworks that increase participation in conservation incentive programmes, engender local stewardship of resources and sustain long-term efficiency of programmes (e.g. Sorice and Donlan 2015). In the environmental and conservation fields, HCD has the potential to be an effective tool that can address iniquitous problems, which are epitomised by issues in illegal wildlife trade.

Empathy is a central tenet of HCD. This shifts researchers' and practitioners' focus from what to conserve and how to conserve it, to what fundamental stakeholder needs are met by a behaviour and how those needs can be met in alternative ways. That is, HCD recognises that the objectives of conservation may not align with the needs, motivations and values of stakeholders. For example, mitigating the illegal trade of songbirds in Brazil and Indonesia may require designing behaviour change intervention that do **Table 3.** General outline and description of a human-centered design program to develop and implement strategies in collaboration with individuals and communities.

Define	Defining an issue within the HCD framework ensures desired outcomes are made explicit (i.e., changing behavior) but also ensures that no a priori assumptions for causes or solutions are implied. That is, the problem is defined only in terms of the behavior(s) targeted for change.
Diagnose	The diagnosis stage generates hypotheses for how and why a behavior(s) occurs. That is, using a behavioral lens that draws on insights from the social and behavioral science literature, collaborators identify potential behavioral constraints and affordances. This stage focuses on gathering information about context and scale. After gathering information and generating hypotheses, qualitative research that focuses on how behaviors occur (rather than why) is conducted via participant observations, semi-structured or unstructured interviews, and focus groups.
Design	A successful diagnosis stage will generate and prioritize possible behavioral constraints and affordances, enabling the design of potential solutions to change and maintain the desired behavior(s).
Test	At this stage, the proposed behavioral intervention(s) is implemented, tested, and evaluated. However, if a proposed intervention is complex, pretesting or prototyping is used (similar to social marketing pilot testing).
Scale	Scaling involves applying the designed and tested intervention to different spatial and/or temporal scale, i.e., taking from the local to regional or national level. For some interventions, scaling may involve process optimization and incorporating technology. In this sense, scaling could, for example, first involve lowering the cost of intervention development and delivery without compromising quality and then identifying more efficient communication tools, e.g., switching from an passive mode of communication to active mode. However, as behavioral solutions are dependent on the mode and medium of delivery, scaling must be done with behavioral principles in mind. For example, some interventions rely on building relationships and trust with local communities such that scaling up can be difficult. In these cases, it may be more effective to re-implement the HCD process at other local scales.

not necessary align with conservation goals. That is, stopping the trade of songbird species may be incongruent with local needs and require that consumption is shifted away from illegal to legally harvested species. Empathising with the needs and values of stakeholders facilitates the development of interventions that directly respond to them at the scale and context in which they make choices. This perspective also allows for stakeholders to learn and become active leaders in the continued implementation and redesign of approaches, without continuous external support (Gelcich and Donlan 2015).

Theory of change: tailoring and targeting approaches and frameworks

The implementation frameworks presented above can incorporate one or several of the behaviour change methods in the form of cognitive, structural, environmental or technological interventions (Heberlein 2012). An obvious caveat to these behaviour change frameworks and methods is that they may be only part of a larger IWT consumer demand reduction campaign. The frameworks, which we present, are a means of identifying and implementing one or more of the methods. IWT and demand reduction are, however, complicated issues, which likely require mixing approaches, coupling those with regulations and understanding the resulting interactions (Challender et al. 2015). Therefore, it is important that professionals and policy-makers ask how behaviour change fits with other conservation efforts and which are appropriate by developing a theory of change (ToC) (Biggs et al. 2017, Oxford Martin School 2016).

A ToC is a decision support tool that outlines expectation of what and how actions (activities) will occur to reach a desired outcome, including causal linkages and the sequence of actions. Importantly, it also makes explicit the assumptions that underlie each step of that sequence. Education approaches, for example, assume individuals are self-interested and motivated to voluntarily engage in the desired behaviour. A theory of change would explicitly outline this assumption and develop steps accordingly: the first being to measure individuals' motivations, then implement the education activity and, finally, evaluate the effect of education on the desired behaviour. A ToC may also identify gaps, such as the need for more qualitative approaches to better understand a specific context or provide in-depth baseline information on the behavioural process or scale of behavioural influences.

A major benefit of developing a ToC is that it provides a conceptual map of what each action or step does, its impact and how it leads to behaviour change; providing a clear description of how change occurs or why it did not occur. A ToC further enables stakeholders to develop explicit and transparent principles and best practices, moving them towards theory- and practice-based approaches that navigate the nuances and challenges of IWT mitigation and consumer demand reduction (Phelps et al. 2016). Biggs et al. (2017), provide one of the more recent and detailed examples of a ToC specific to IWT. Similarly, but not exclusive to IWT, Jenks et al. (2010) outline a ToC for Rare's Pride programme, which facilitates behaviour change in various conservation and natural resource contexts.

Importantly, the development of a ToC serves as a convergence for professionals, policy-makers and community members involved in IWT mitigation and demand reduction to more fully and explicitly elaborate on the tools and methods needed to attain desired reduction and change. That is, a ToC is not exclusively a social science tool to outline and map social scientific elements (though it does endeavour to bring those to the forefront); it enables various and diverse stakeholders to mutually understand how and why a system functions and change develops. A ToC can also serves as a communication tools for the various sectors, groups and individuals embedded within an IWT system to collaborate and develop a holistic understanding of how change happens, can be implemented and is sustained given a diversity of regulatory, economic, political, socio-cultural, technological and environmental factors in operation. In this sense, a ToC is an important tool to connect various methods and frameworks of behaviour change (Fig. 1b).

Conclusion

Human behaviour, while responsible for illegal wildlife demand and consumption, also represents the means by which strategies to address IWT will more readily succeed. Changing behaviour often requires removing barriers to more desirable or equitable behaviour. Barriers may exist at the scale of the individual, e.g. ignorance that a purchase is illegal or that there is a legal alternative, which may merely require education or better information. More complex barriers are often embedded within sociocultural or political contexts, requiring more dedicated relationship building or social influences that complement regulatory policies. Reducing consumer demand for illegal wildlife requires identifying and specifying behaviour that needs changing, identify those who engage in this behaviour and why and what is(are) the most appropriate means to remove barriers and influence more desirable or equitable behaviour. This necessitates that professional and policy-makers are aware of various approaches to and understand the practicality of behaviour change methods and frameworks applicable to IWT issues. It also requires that there is a social and behaviour change community of trained researchers and practitioners who are aware of useful methods and have access to tested knowledge. As an overview of behaviour change methods and frameworks, this article endeavours to benefit IWT professionals and policy-makers, as well as the broader social and behaviour change community.

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RESEARCH ARTICLE



Abundance and survival rates of three leaf-litter frog species in fragments and continuous forest of the Mata Atlântica, Brazil

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Academic editor: C. Knogge Received 28 March	2018 Accepted 9 April 2018 Published 30 April 2018				

Citation: Steinicke H, Pe'er G, Henle K (2018) Abundance and survival rates of three leaf-litter frog species in fragments and continuous forest of the Mata Atlântica, Brazil. Nature Conservation 26: 77–96. https://doi.org/10.3897/ natureconservation.26.25339

Abstract

Habitat destruction and fragmentation alter the quality of habitats and put populations under the risk of extinction. Changes in population parameters can provide early warning signs of negative impacts. In tropical forests, where habitat loss and fragmentation are vast, such indicators are of high relevance for directing conservation efforts before effects are irreversible. Most of our knowledge from tropical ecosystems originates from community level surveys, whereas our understanding of the influence of habitat conversion on vital rates of species is limited. This study focused on the influence of anthropogenic habitat fragmentation on the survival probability and abundance of three leaf-litter frog species (*Rhinella ornata, Ischnocnema guentheri* and *I. parva*) in forest patches of the Atlantic rainforest of South-east Brazil compared to a continuous forest. The species differ in their matrix tolerance: high for *R. ornata* and low for *I. guentheri* and *I. parva* and, thus, we examined whether their survival and abundance correspond to this classification. *Ischnocnema guentheri* showed highest abundances in all study sites and low mortality in the forest patches compared to the continuous forest; *I. parva* was encountered only in isolated fragments, with very low mortality in one isolated fragment; and the matrix tolerant species had generally low abundance and showed no clear pattern in terms of mortality in the different sites. Our counter-intuitive results show that even matrix sensitive amphibian species may show high abundance and low mortality in

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small forest patches. Therefore, these patches can be of high value for amphibian conservation regardless of their degree of matrix aversion. Landscape level conservation planning should not abandon small habitat patches, especially in highly fragmented tropical environments.

Keywords

Amphibia, Brazilian Atlantic Forest, habitat fragmentation, abundance, mark-recapture, survival

Introduction

Habitat destruction and fragmentation are amongst the major causes for the loss of terrestrial biodiversity (Saunders et al. 1991, Pimm and Raven 2000, Laurance and Cochrane 2001, Henle et al. 2004a). Besides loss of suitable area and isolation of remaining habitats, the fragmentation process may result in changes of habitat geometry and the physical environment, thus influencing habitat quality parameters both directly (Lovejoy et al. 1986, Saunders et al. 1991, Laurance 2000, Fahrig 2003) and indirectly, e.g. through edge effects (Murcia 1995, Ewers and Didham 2006). This in turn can influence individual traits (Steinicke et al. 2015) as well as the extinction risk of populations and the composition of communities within patches (Saunders et al. 1991, Davies et al. 2001, Wiegand et al. 2001, Hokit and Branch 2003, Ewers et al. 2007, Zurita et al. 2012). Together, loss and change of the quality of habitats and isolation of remaining patches affect the persistence of species in fragmented landscapes (Settele et al. 1996, Halverson et al. 2006, Watling and Donnelly 2007).

Whether a species in a fragmented habitat is vulnerable to extinction or not, depends not only on the spatial configuration of the landscape (Gunton et al. 2017), but also on demographic parameters and on the ecological traits of species (With and Crist 1995, Henle et al. 2004a). Species with narrow habitat requirements, i.e. habitat specialists, are likely to be more affected than habitat generalists, as the chance that their niche is represented in remnants of a fragmented landscape is smaller (Henle et al. 2004a). Habitat specialists also often exhibit lower tolerance to the matrix surrounding the remnants (Gascon et al. 1999, Bentley et al. 2000, Henle et al. 2004a, Hoehn et al. 2007) and therefore they are considered to be more prone to extinction than habitat generalists (Sarre et al. 1996, Henle et al. 2004a).

Tropical forest species are often assumed to be more sensitive to fragmentation than temperate ones (Henle et al. 2004a), partly due to their high specialisation levels. Habitat loss and fragmentation are particularly severe in tropical forests. The Brazilian Atlantic Forest (Mata Atlântica) is one of the global biodiversity hotspots severely impacted by habitat loss and fragmentation. Only 11-16% of its original area remains wooded with native forests and these remnants are highly fragmented, isolated and disturbed to an extent where many of them only retain intermediate secondary forest (Oliveira and Fontes 2000, Ribeiro et al. 2009). Despite its severe fragmentation, the Mata Atlântica still maintains high species richness, including a large number of en-

demic species (Tabarelli et al. 2005), with particularly high endemism of amphibians (Myers et al. 2000, Haddad et al. 2013).

Compared to the temperate zone, much less is known about the effects of fragmentation on amphibians from tropical forest ecosystems. As for the majority of fragmentation studies in tropical forests, the available studies for amphibians focus primarily on the community level or genetic variability (e.g. Pineda and Halffter 2004, Bell and Donnelly 2006, Dixo et al. 2009, Lion et al. 2014, Riemann et al. 2015, Almeida-Gomes et al. 2016). Existing studies have revealed differences amongst amphibian taxa in response to habitat fragmentation (Almeida-Gomes et al. 2016), finding that ground-living species (Pineda and Halffter 2004), naturally rare species and species with a high habitat specialisation are more affected than others (Gascon et al. 1999, Watling and Donnelly 2007, Almeida-Gomes et al. 2016). Sensitivity to fragmentation may further depend on their reproductive mode and the proximity of water bodies to the forest patches but results obtained so far are not consistent (Cannatella 2008, Bickford et al. 2010, Lion et al. 2014, Almeida-Gomes et al. 2016).

Effects of fragmentation on the survival of species are governed by changes in demographic processes. However, knowledge about demography in relation to habitat fragmentation is scarce (but see e.g. Funk and Mills 2003, Holland and Bennett 2010), especially in tropical regions (e.g. Freitas et al. 2003). For amphibians, in general, few studies have assessed the population dynamics of tropical species (but see Funk and Mills 2003, Grafe et al. 2004, Ryan et al. 2008, McCaffery and Lips 2013). Thus, for a better understanding of the persistence of species in fragmented landscapes, information on population dynamics should receive top priority in tropical ecological research (Bierregaard et al. 1997).

This study aims to address this still existing gap in ecological knowledge. We studied the abundance and mortality of three leaf-litter amphibian species, differing in their level of matrix tolerance, in forest fragments and in a comparable site of continuous forest in the Brazilian Atlantic Forest in south-eastern Brazil.

Our focus on abundance and mortality stems from the assumption that population size and fluctuation are two key factors determining whether species are able to maintain stable populations or are prone to stochastic extinction (Henle et al. 2004a, Henle et al. 2004b). Therefore, these population parameters can serve as important early warning signs, potentially indicating a pending extinction debt (McCarthy et al. 1997, Kuussaari et al. 2009), whereas abundance data alone are insufficient to detect such trends. For instance, van Strien et al. (2011) showed that metapopulation dynamics may change some tens of years before an actual change in occupancy is visible. Additionally, mortality rates may aid in identifying whether species observed in a fragmented landscape perform better in non-fragmented than in fragmented habitats. As habitat specialists tend to be more fragmentation-sensitive than generalists, we expected that the relative performance would reflect the degree of matrix tolerance of the three leaf-litter frog species studied by us. We discuss the role of small fragments for the conservation of Neotropical leaf-litter frog species.

Material and methods

Study area

We carried out our study on the Atlantic Plateau of São Paulo in the Mata Atlântica of Brazil at an altitude of 860–1075 m above sea level (Metzger et al. 2006). The study region comprised a continuous forest area at the Morro Grande Reserve, approx. 10,000 ha in size and a neighbouring fragmented landscape (Figure 1) surrounding Caucaia do Alto (23°40'S; 47°01'W) in the municipalities of Cotia and Ibiúna, 40 km southwest of São Paulo. Oliveira and Fontes (2000) classified the original forest as lower montane rainforest that forms a transition between the coastal Atlantic rain forest and the Atlantic semi-deciduous forest. The fragmented landscape comprises 31% secondary forest cover, 39% open areas (mainly agricultural and pasture), 17% tree plantations (mainly *Eucalyptus* and pine plantations) and 15% human settlements (Uezu et al. 2005, Metzger et al. 2006, Dixo et al. 2009). In both the continuous landscape and the fragmented one, primary forest has almost completely disappeared due to historical clear-cuts followed by regeneration. Mature forest ages are estimated between 60 and 80 years (Metzger et al. 2009).

We selected four study sites: one control site within the continuous forest of the Morro Grande Reserve ("control"), one small fragment (Alcides), 5 ha in size, which is connected by a corridor to a larger forest area ("connected") and two small isolated patches, 5 ha each. One of the latter (Carmo Messias) is surrounded by a mixture of habitats including riparian habitat, ("isolated 1"), the other (Dito) is close to a settlement and its only neighbouring forest patches are small and degraded ("isolated 2"; Table 1). All sites are characterised by secondary forest that has not experienced major logging or disturbance for at least 60 years. All studied forest patches and the site in the continuous forest were devoid of permanent/large water bodies within the forest area or within a radius of 200 m from the forest patches or within that distance to any of the survey sites.

Selected species

Based on capture numbers from Dixo (2005), we chose three leaf-litter dwelling, forest species that were expected to be sufficiently abundant for a capture-mark-recapture study in all sites: *Rhinella ornata* (Spix, 1824; Bufonidae), *Ischnocnema guentheri* (Steindachner, 1864; Brachycephalidae) and *Ischnocnema parva* (Girard, 1853; Brachycephalidae). All three species are listed as of "Least Concern" (IUCN 2013). We classified the three species according to their tendency to tolerate or avoid the surrounding agricultural matrix as elaborated in the following.

Of the three species, *R. ornata* is the most tolerant to the agricultural matrix. It can be found in undisturbed continuous forest as well as in disturbed forest fragments (Heyer et al. 1990, Dixo 2005). It migrates to temporary and permanent ponds within



Figure 1. Map of the study area. Circles around the patches represent buffers of 100 m to assess the level of isolation in terms of forest patches within a reachable distance (see Table 1). Patches are from north to south "connected", "isolated 1" and "isolated 2".

forests and open areas for breeding (Izecksohn and de Carvalho-e-Silva 2001, Haddad and Prado 2005). Reproduction starts at the end of the dry season in late July or the beginning of August (Dixo 2005). It is distributed throughout the Atlantic Forest from Espírito Santo in the north to Paraná in the south (Frost 2017).

Ischnocnema parva is the least matrix tolerant of the three species, not only avoiding the external matrix but also forest edges (Heyer et al. 1990, IUCN et al. 2006). Females deposit egg clutches terrestrially and development is directly within egg capsules

Table 1. Characterisation of the study sites. Habitats around each study site are ordered according to ranking of border-length. Forest cover within 100 m represents the area of forest within a buffer of that distance, whilst the total area of forest patches represents the total area of all patches that partly or fully occur within this buffer.

	Patch size	Habitats neighbouring the site	Distance to nearest patch	Forest cover within a 100 m buffer / Total area of forest patches
control			Continuous forest	
connected	5.11 ha	Open, plantation, riparian, corridor, settlements	Connected by corridor	67.4 / 203.25 ha
isolated 1	5.41 ha	Settlement, open, plantation	20 m	0.37 / 2.65 ha
isolated 2	4.88 ha	Open, riparian, plantations	43 m	1.44 / 4.35 ha

(Heyer et al. 1990, Izecksohn and de Carvalho-e-Silva 2001, Haddad and Prado 2005). Reproduction occurs between October and December. It is distributed in the southeast of the Mata Atlântica; the populations in the state of São Paulo may be an undescribed cryptic species (Frost 2017).

In terms of matrix tolerance, *I. guentheri* is positioned between the other two species. It occurs primarily within forest habitats but has been found, albeit very rarely, in cleared and degraded areas (Heyer et al. 1990) and it also occurs at the forest edge (HS and KH pers. observ. in the study area). Females deposit egg clutches terrestrially, hidden below tree trunks and stones (Kwet and di-Bernardo 1999, Izecksohn and de Carvalho-e-Silva 2001). Frogs develop directly within the egg capsule (Heyer et al. 1990, Izecksohn and de Carvalho-e-Silva 2001). Reproduction occurs between August and December (Heyer et al. 1990). The *I. guentheri* species group is distributed over large parts of the Mata Atlântica; the populations in the state of São Paulo may be an undescribed species (Frost 2017).

Both *I. guentheri* and *I. parva* are assumed to be territorial species that do not migrate over long distances, but data on individual movement behaviour are scarce.

Sampling

We sampled frogs by hand along three parallel transects, 100 m each in each study site (meaning that, in the fragments, most of the patch excluding the edge was covered). We performed sampling during two rainy seasons, from October 2003 until March 2004 and November 2004 until March 2005. We implemented a robust design of mark recapture studies (Pollock 1982), surveying each site for five consecutive nights (21:00-01:00 h local time) (hereafter, 'secondary periods') before sampling the next site. Once all sites had been sampled, the next round of sampling started again at the first site. In total, we conducted five sampling rounds. These five sampling rounds serve as 'primary periods', each containing the five consecutive nights per site as 'second-ary periods'. Thus, the total search effort was 25 nights per site (15 nights per site in 2003/2004 and 10 nights per site during 2004/2005).

We photographed all body sides of all captured animals using a digital camera with a macro lens and a flash bulb and used pattern for individual identification (Donnelly et al. 1994, Henle et al. 1997). Once photographed, we released each animal at the point of capture.

Data analysis

Due to low recapture rates within primary periods, we used the number of individuals captured within a primary period as a measure of relative abundance. For the survival analysis, we combined all captures from the secondary sampling periods of a primary sampling period to analyse survival probabilities between primary periods. We calculated local survival probabilities (φ) and capture probabilities (p) using programme MARK (White and Burnham 1999). Local survival is defined as the probability of an individual to survive and remain on the study site from one primary capture period to the next. As periods between primary sessions differed, we standardised the parameters in terms of weekly survival rates.

We fitted several candidate Cormack-Jolly-Seber models to the recapture data to determine the most parsimonious model (Table 2). We used the notation of Lebreton et al. (1992) for model specification. Following the recommendation of Burnham and Anderson (2001), we chose the set of candidate models *a priori*. The most general model we used assumed time specific survival and capture probabilities $[\varphi(t); p(t)]$; the most restrictive one assumed constant survival and constant capture probabilities $[\varphi(.); p(.)]$. The model selection in MARK is based on the corrected Akaike Information Criterion (*AICc*) (Hurvich and Tsai 1989), an adjustment for the *AIC* for small sample sizes and overdispersion (Buckland et al. 1997). Models with a $\Delta AICc \leq 2.0$ are usually regarded as equally parsimonious. If $\Delta AICc$ was less or equal to this value, then we selected the more restrictive model representing time-independent estimates.

To determine whether estimated survival probabilities differed significantly between study sites, we calculated log-based 85% confidence intervals and checked for overlap. Non-overlap of 85% confidence intervals is equivalent to a 5% significance level in a two-sided test ($\alpha \le 0.05$) (Buckland et al. 1993).

Results

Abundances

In total, we captured 632 individuals of the three focal species at least once, representing 116 individuals of *R. ornata*, 383 individuals of *I. guentheri* and 133 individuals of *I. parva. Rhinella ornata*, the most matrix tolerant species, was more abundant in the isolated patches and less abundant in the connected patch and in the control site (Figure 2). *Ischnocnema guentheri* was most abundant in the isolated patches, less abundant in the

	Model tested	AIC _c	ΔAIC _c	AIC _c weight		
		R. 0	rnata	· · · ·		
	$\varphi(t) p(t)$	5.439	0.000	0.851		
	φ(t) p(.)	9.173	3.733	0.132		
	φ(.) p(.)	13.234	7.795	0.017		
	φ(.) p(t)	43.479	38.040	0.000		
control		I. guentheri				
	$\varphi(t) p(t)$	18.569	0.000	0.560		
	φ(.) p(.)	19.829	1.261	0.298		
	φ(t) p(.)	21.363	2.794	0.138		
	φ(.) p(t)	28.657	10.089	0.004		
		I. guentheri				
	φ(.) p(t)	35.455	0.000	0.846		
connected	φ(.) p(.)	40.068	4.614	0.084		
	φ(t) p(t)	41.250	5.795	0.047		
	φ(t) p(.)	42.695	7.240	0.023		
		R. ornata				
	φ(.) p(.)	45.443	0.000	0.890		
	φ(.) p(t)	50.137	4.694	0.085		
	φ(t) p(.)	53.303	7.860	0.020		
isolated 1	$\varphi(t) p(t)$	55.103	9.660	0.007		
Isolated 1		I. gu	entheri	1		
	φ(t) p(t)	145.685	0.000	0.607		
	φ(t) p(.)	146.589	0.904	0.386		
	φ(.) p(.)	154.716	9.031	0.007		
	φ(.) p(t)	161.432	15.747	0.000		
	R. ornata					
isolated 2	φ(.) p(.)	10.624	0.000	0.528		
	φ(t) p(.)	12.345	1.721	0.223		
	φ(t) p(t)	12.345	1.721	0.223		
	φ(.) p(t)	16.649	6.024	0.026		
	I. guentheri					
	φ(.) p(t)	70.224	0.000	0.699		
	φ(.) p(.)	73.019	2.795	0.173		
	φ(t) p(t)	73.927	3.702	0.110		
	φ(t) p(.)	77.454	7.230	0.019		
	I. parva					
	φ(t) p(.)	97.256	0.000	0.466		
	φ(t) p(t)	98.058	0.802	0.312		
	φ(.) p(t)	98.823	1.567	0.213		
	φ(.) p(.)	105.087	7.830	0.009		

Table 2. Overview of models considered and results of model selection based on the corrected Akaike Information Criterion (AIC_{c}) for estimating survival probability (φ).

connected patch and had the lowest abundance at the control site (Figure 2). The least matrix tolerant species, *I. parva*, was only captured in the isolated patches, mainly in the most isolated study site "isolated 2", but was neither captured in the "connected" fragment nor in the "control" site.



Figure 2. Comparison of the number of captures of *Rhinella ornata* and *Ischnocnema guentheri* in the study sites. The median, first and third quartile, minimum-maximum range and outliers of capture rates are indicated.

The number of captures per primary period of *R. ornata* tended to be larger in all fragments compared to the control site and of *I. guentheri* in the isolated sites compared to the control site. However, the difference between the control site and any of the three fragments was not significant for both species, likely due to low power associated with the small number (5) of primary periods (Wilcoxon test with Bonferroni correction: $\alpha > 0.05$ for both species).

Survival

The number of animals captured allowed the calculation of survival probabilities for *R. ornata* for all sites except for the "connected" fragment, for *I. guentheri* at all study sites and for *I. parva* only for the fragment "isolated 2". For all but two cases (*R. ornata* at the "control" site and *I. guentheri* at the "isolated1" patch), models with time-independent survival yielded the lowest *AICc* or rendered a $\Delta AICc$ within the threshold and were therefore used for parameter estimation. In the two exceptions, the *AICc* values suggested time-dependent models of survival but they had uninformative large confidence intervals due to seasonally poor recapture rates. Therefore, we also provide results from time-independent survival models for these two cases.

The estimated local survival probabilities of *R. ornata* were high for "isolated 1" (φ = 0.99) and the "control" site (φ = 0.94), but relatively low for "isolated 2" (φ = 0.68),

Species	Selected model	Survival estimate	Standard error	95 % (85 %) Confidence interval				
				lower	upper			
Control								
R. ornata	φ(.) p(.)	0.938	0.117	0.228 (0.455)	0.998 (0.996)			
I. guentheri	φ(.) p(.)	0.726	0.132	0.418 (0.504)	0.907 (0.874)			
Connected								
I. guentheri	φ(.) p(t)	0.933	0.015	0.898 (0.909)	0.957 (0.951)			
Isolated 1								
R. ornata	φ(.) p(.)	0.992	0.023	0.289 (0.649)	0.999 (0.999)			
I. guentheri	φ(.) p(.)	0.979	0.010	0.947 (0.959)	0.992 (0.990)			
Isolated 2								
R. ornata	φ(.) p(.)	0.676	0.121	0.415 (0.485)	0.860 (0.822)			
I. guentheri	φ(.) p(t)	0.927	0.012	0.901 (0.908)	0.947 (0.943)			
I. parva	φ(.) p(t)	0.974	0.013	0.931 (0.946)	0.991 (0.988)			

Table 3. Summary of the results of survival estimates in the different study sites.



Figure 3. Comparison of weekly survival probability estimates φ of *R. ornata* and *I. guentheri* at the study sites. Data of *R. ornata* in the "connected" site were not sufficient for estimation. The survival estimate, upper and lower 85% confidence intervals (boxes) and the upper and lower 95% confidence intervals are indicated (minimum and maximum lines).

but, due to the large confidence intervals, the estimates were not significantly different (Table 3, Figure 3). Survival probabilities of *I. guentheri* were high in all three patches compared to the control site with a significantly higher value in "isolated 1" (Table 3, Figure 3). The most specialised species *I. parva* proved to have a very high survival probability in the patch "isolated 2". In fact, in this fragment, *I. parva* was the species with the highest survival probability (Table 3).

Discussion

General discussion

Forest fragmentation reduces habitat availability, increases edge effects and leads to the isolation of subpopulations from each other (Saunders et al. 1991, Laurance 2000, Fahrig 2003). The combined effect of reduced patch size and quality is known to negatively affect the abundance and survival rates of populations (Marsh and Pearman 1997, Hokit and Branch 2003, Henle et al. 2004a) and to alter trophic chains and species communities in forest fragments (Henle et al. 2004a). The reduction of habitat generally leads to a stronger reduction of possible niches for specialists compared to generalist species (Sarre et al. 1996, Henle et al. 2004a, Kimmel et al. 2010). Furthermore, increased edge effects are known to cause changes in microhabitat quality inside forests (Saunders et al. 1991), which affects forest specialist species particularly strongly (Laurance 2000). The isolation of remnants also leads to the isolation of populations, which may negatively affect survival rates (Halverson et al. 2006, Watling and Donnelly 2007). Highly specialised species therefore do often vanish with habitat fragmentation (Saunders et al. 1991, Laurance 2000). Less specialised species may persist in fragmented landscapes but with lower abundance, may exhibit source-sink dynamics or show negative trends over longer terms, eventually leading to their extinction (i.e. extinction debt). Alternatively, they may be pre-adapted to the changed ecological conditions in fragmentation or may hold the flexibility to adapt alternative strategies or habitat usage that may enable them to sustain populations in the long run.

As our studied species are all forest specialists, we anticipated all of them to exhibit negative effects of fragmentation, especially in the small and isolated forest patches. We further expected a ranking of the strength of response amongst the three species, where the most matrix intolerant species, *I. parva*, should exhibit the strongest response and thus lower abundance and/or local survival rate in the fragments compared to the control area.

Our results therefore seem counter-intuitive at first sight. *I. parva*, the species, which could be expected to be most sensitive to fragmentation and patch isolation as it avoided the forest edge, was found neither in the continuous forest site nor in the connected fragment, but only in isolated patches, in which it showed a very high survival probability. The abundance of the most matrix tolerant species, *R. ornata*, was near-significantly higher in the "isolated 1" fragment than at the control site, with no consistent pattern with respect to its survival in the different sites. The intermediate species in terms of matrix tolerance, *I. guentheri*, that tolerates forest edges and has been found, albeit very rarely, just within the matrix, did not show significant changes in abundance between the different fragments, had its lowest abundance in the continuous forest site and exhibited significantly higher survival probabilities in the forest fragments. Likewise, in an experimental fragmentation study in central Amazonia, survival in *Anomaloglossus stepheni* (as *Colostethus stepheni*) was not related to fragmentation (Funk and Mills 2003).

These results clearly demonstrate that the ranking order of species according to their matrix tolerance does not explain the trends from a continuous forest to the most isolated patch, as the small forest fragments did provide sufficient suitable habitat to host stable populations for all three species, even the most matrix intolerant one. Bickford et al. (2010) obtained similar results for anurans in rainforest fragments in Singapore, in which abundance was not related to fragment size or isolation.

For species that are less tolerant to the matrix, matrix-avoidance and low permeability of the matrix leads to a decrease in the exchange of individuals (Hanski 1991, Saunders et al. 1991, Fahrig and Merriam 1994, Gascon et al. 1999, Rosenberg et al. 1997, Hoehn et al. 2007). This consequently enhances inbreeding (Madsen et al. 1996) and potentially reduces survival due to inbreeding depression (Brook et al. 2002, Halverson et al. 2006), though increased intra-patch mobility may (partially) compensate for these effects (Lange et al. 2013). Likewise, an increase in available resources may lead to an increased abundance of leaf litter amphibians, as the abundance of leaf-litter invertebrates often increases in tropical forest fragments and as they are important prey for leaf-litter amphibians (e.g. Whitfield and Donnelly 2006).

In addition, the disappearance of predators that are sensitive to fragmentation can lead to a higher abundance and survival probability of species less sensitive to fragmentation (Terborgh 1974, Ogle 1987, Henle et al. 2004a) as was the case for *I. guentheri* in our study. Ants, spiders, snakes, mammals and especially understorey birds (such as obligatory ant-followers) are amongst the main predators of tropical leaf-litter anurans (McCormick et al. 1982, Poulin et al. 2001, Toledo et al. 2007). These groups are known to be sensitive to the fragmentation of tropical forests (Stouffer and Bierregaard 1995, Harrison and Bruna 1999, Vasconcelos et al. 2006). Their disappearance in fragmented landscapes and especially from small isolated patches is well-documented in the Mata Atlantica (Zurita et al. 2006, Zurita and Bellocq 2010), including the studied landscape (Uezu et al. 2005, Martensen et al 2008, Banks-Leite et al. 2010).

We must add one note of caution. Low mortality in small patches does not necessarily mean more stable populations compared to a continuous forest, since the lower local survival in the latter could also be due to higher emigration rates away from the sampled area, especially for the edge and matrix intolerant species, *I. parva* and *I. guentheri*. While in the small and isolated patches one can assume relatively closed populations, this assumption may not hold in a continuous environment and we cannot separate the two without tracking the movement of individuals.

Implications for conservation

Our findings support recent studies, indicating that small habitat patches can have an important conservation value (Bickford et al. 2010, Lion et al. 2016). This is especially true for taxonomic groups with limited spatial requirements that may also benefit from the absence of predators. In the case of temperate wetlands, there is evidence that even small isolated habitat patches can have a high value for sustaining amphibian diversity (Semlitsch and Bodie 1998, Riemann et al. 2015). Tscharntke et al. (2002) reached similar conclusions for herbivorous insects and Ogle (1987) for endemic carnivorous snails in New Zealand forests. Our results and those of Bickford et al. (2010) concur with these findings for small fragments in a tropical forest. Our study further concurs with recent studies that point to mosaic landscapes as important for protection of species under fragmentation. Such heterogeneous landscapes maintain complementary elements and, combined with a benign management (which is often typical in rural landscapes), could support high species richness (Haslem and Bennett 2008, Norris 2008, Ranganathan et al. 2008) provided they retain all essential resources, such as suitable water bodies or terrestrial breeding sites for tropical amphibians. Though certainly less valuable than large continuous tropical forests, the heterogeneity of such landscapes should be maintained and protected from further intensification and degradation in order to safeguard biodiversity in face of habitat loss and fragmentation.

Acknowledgements

We are grateful to C. Dormann and B. Gruber for help with statistical analyses. M. Dixo and J.-P. Metzger for technical support and scientific advice, C. Guimaráes-Steinicke and A. Bispo on behalf of numerous persons for their help in the field, R. Nali (SABESP) for accommodation, private landowners for letting us work on their properties, two anonymous reviewers and C. Knogge for their helpful comments and to C. Knogge for administrative and logistic support. This project was funded within the Brazilian-German bilateral research programme "Science and Technology for the Mata Atlântica" by the German Federal Ministry of Education and Research (BMBF FKZ 01LB020A1).

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