

## Habitat use and dispersion of translocated European pond turtle (*Emys orbicularis*) in Lake Bourget and meta-population project over the Haut-Rhône

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**Abstract:** We conducted an experimental release of European pond turtles (*Emys orbicularis*) in an area of natural habitat from which the species had apparently disappeared around the early 20th century. Three groups of turtles were released in April 2000 (nine females and four males), May 2001 (five females and eight males), and April 2002 (six females and three males), and were monitored until September 2002 using telemetry (over 10 000 locations). Movements based on telemetry are summarised for each individual. The release is considered a success. All 35 turtles survived in the wild. They established their home ranges near the release point, with no tendency to move in a particular direction. Maximum distance travelled was 5120 m from the release point, but the majority did not disperse. Clutches were observed on the artificial nesting site and hatchlings emerged in 2002. This three years program shows that turtles were able to survive in their new environment and to settle, forage and reproduce at the release site. Translocation can be considered a viable conservation strategy for this locally endangered species. However, as a sustainable supply must be used for the next releasing steps, a farming project has to be implemented to supply several new cores along the Rhône river.

**Key words:** conservation strategy, dispersal, *Emys orbicularis*, home range, telemetry, translocation.

**Resumen:** Uso del hábitat y dispersión de galápagos europeos (*Emys orbicularis*) translocados en el Lago Bourget y proyecto de meta-población en el Haut-Rhône. – Se ha llevado a cabo una reintroducción experimental de galápagos europeos (*Emys orbicularis*) en un área de hábitat natural de la que la especie aparentemente había desaparecido desde principios del siglo xx. Se liberaron tres grupos de tortugas en abril de 2000 (nueve hembras y cuatro machos), en mayo de 2001 (cinco hembras y ocho machos) y en abril de 2002 (seis hembras y tres machos) a los que se realizó un seguimiento individual mediante telemetría (más de 10 000 datos). La reintroducción puede considerarse un éxito, ya que los 35 individuos sobrevivieron en estado salvaje. Su dispersión fue muy limitada, sin tendencia a ninguna dirección en particular y con desplazamientos cortos (la distancia máxima recorrida fue de 5120 m desde el punto de liberación), observándose puestas y neonatos en el año 2002. Los tres años de programa muestran que los galápagos pudieron sobrevivir, establecerse, alimentarse y reproducirse en el área elegida por lo que la translocación puede considerarse una estrategia de conservación viable para esta especie localmente en peligro. Sin embargo, cuando son necesarias liberaciones periódicas, es necesario contar con un proyecto de cría en cautividad para establecer nuevos núcleos de población a lo largo del río Rhône.

**Palabras clave:** dispersión, *Emys orbicularis*, espacio vital, estrategias de conservación, telemetría, translocación.

## INTRODUCTION

In the context of the decrease of biodiversity (SOULÉ, 1986), the reintroduction of organisms into the wild is often considered by wildlife professionals as a solution for increasing population numbers (GRIFFITH *et al.*, 1989; KLEIMAN *et al.*, 1994). Diverse terminology (translocation, relocation, reintroduction or restocking) has been used to describe this intentional movement of animals (DODD & SIEGEL, 1991). Translocation is broadly defined as the intentional release of captive and/or wild animals into the wild for the purpose of reestablishing an extirpated population, or augmenting a critically small population (GRIFFITH *et al.*, 1989). Translocations of threatened species are becoming more numerous, but detailed studies of those programs are rarely available (GRIFFITH *et al.*, 1989). Due to the lack of information on the consequences of various release strategies, new programs often use an arbitrary approach to select individuals and methods for population restoration. Data collected from well-monitored projects, combined with demographic modeling and experimental design monitoring, can be helpful in making these choices (SALTZ, 1998). Post-release monitoring of translocated animals is not simply determining the number of survivors, but also interpreting the ensuing species-habitat relationships. Underpinning such studies is the understanding of how the target species behaves in the new habitat and how characteristics of the recipient habitat correlate with the distribution of individual animals in the translocated populations (DICKINSON *et al.*, 2001). Hence, how the translocated species distribution can be predicted from habitat variables should be central to monitoring (HEATWOLE, 1977). Because they directly induce management tools on the release site,

monitoring of dispersal behavior and habitat use after release through the annual cycle is very important.

While reintroduction success is never guaranteed (GRIFFITH *et al.*, 1989), recourse to experimentation gives the possibility to compare the behaviour of released individuals with that of wild ones, but also to explore the possible alternatives within the framework of the reintroduction in progress. We can deduce the solution maximising chances of success. This step requires a rigorous protocol, that makes it possible to distinguish the impact of: (1) the origin, age and size of the individuals, (2) the reintroduction methods, and (3) the release site characteristics and management. The choice of the "source" population and individuals constitutes one of the first questions. According to different study, the genetic, demographic or behavioural characteristics can differ and determine variable aptitudes for releasing (MAYOT *et al.*, 1998; WAUTERS *et al.*, 1997). The release methods (site, season, meteorological conditions, densities...) can also influence individual behaviour and survival (BRIGHT & MORRIS, 1994; LOVEGROVE, 1996; LETTY, 1998). Lastly, the release site characteristics can affect the success of the project according to its intrinsic capacities (food and cover availability, predation risks...) and the importance of the ecological differences with the site of origin (KRAUSS *et al.*, 1987; BRIGHT & MORRIS, 1994; SORCI *et al.*, 1996). The experimental step is also the occasion to identify the best techniques for monitoring and to prepare the implementation of a long-term study.

Most translocation programs concern flagship or keystone species (WILSON & STANLEY-PRICE, 1994). In this context, tortoises and turtles are a good model because of their charismatic position. Because so many people are concerned about those

animals and their welfare, it is relatively easy to obtain support for conservation actions that, superficially at least, seem to benefit them. One set of these strategies is translocation, though of course it can be limited in regard of animal numbers.

Quoted in the Habitats Directive of the European Commission (Annexes II and IV) and in the Bern Convention (Annexe II), the European pond turtle (*Emys orbicularis*) is now considered an endangered species. In this context, many conservationists start reintroduction programs (FERRI, 2000; LACOSTE *et al.*, 2000; SCHNEIDER, 2000).

The European pond turtle disappeared from Savoy Department around the early 20th century; the last regional wild population endures some 30 km away, beyond a mountain range. Besides, the Rhone "corridor" is so fragmented that a spontaneous comeback of the species is not possible—however good the local habitat quality—. Thus, in 1994, the Savoy Nature Conservancy (SNC) planned an experimental reintroduction in collaboration with the Regional Natural Environment Conservatory and the University Claude Bernard Lyon 1. The SNC is managing most marshes around Lake Bourget (45 km<sup>2</sup>), directly connected to the Rhône river. To compensate for marsh and pond decline, 13 ha of reed and bushes were cleared of bushes, embanked and re-flooded.

In order to receive the agreement of the French Environment Ministry, a four-step program was based on IUCN (1998) recommendations: (1) feasibility study to evaluate the chances of success of the project, particularly with the identification and elimination of the extinction factors. (2) Preparatory phase to lay out the site and constitute the stock of individuals, including all protection measures to insure the maintenance of the reintroduced population

and information of the public. (3) The releasing phase that takes into account the biological parameters necessary to limit the risks for the individuals. (4) The monitoring phase to retrieve a maximum of information from this experience for the next project. On the basis of a technical report (MIQUET, 1994), the French National Council for Nature Protection approved the project. The program was also integrated in the Life Nature program "Lake Bourget" and funded by the European Council from 2000 to 2002; it is conceived as a first step in a meta-population perspective (CADI & FAVEROT, 2004).

## MATERIALS AND METHODS

### Individual selection

To determine the genetic characteristics of the Rhône-Alpes populations, blood samples were obtained in populations from Brenne, Allier and North Isère (last population in Rhone-Alpes and analyzed for mitochondrial DNA variation (U. Joger, Heidelberg University, Germany); they all indicated the subspecies *Emys orbicularis orbicularis* (LENK *et al.* 1999). With the agreement of the French authorities, the Brenne was chosen as a supply of wild adult turtles. In collaboration with J. Servan (National Natural History Museum, France), we captured 35 individuals in three groups (1999, 2000 and 2001) from different ponds to maximize genetic diversity.

All chosen turtles had reached maturity with carapace length of  $150.4 \pm 13$  mm and body mass of  $584.8 \pm 137$  g. Sex was determined by secondary sex characteristics (ERNST *et al.*, 1994). Before they were committed to this trial, a rigorous health screening and behavior examination was undertaken to ensure that the turtles were healthy, to reduce the risk of spreading disease and to minimize the probability of

release failure (VIGGERS *et al.*, 1993). All turtles received an individual and permanent marking with notches on the marginal scutes (CADI, 2003).

### Acclimation pond

In summer 1999, the first ten individuals from Brenne and six others from a rescue operation in Isère department were "acclimatized" all together on site. They were held in a 400-m<sup>2</sup> pre-release enclosed pond situated on the border of the release site since August 1999 to May 2000 in order: (1) to allow them to become familiar with the release site, (2) to ensure they would not be totally disorientated once released (BERRY, 1986; LOHOEFNER & LOHMEIER, 1986; DIEMER, 1989), and (3) to monitor the health of the turtles following the potential stress of transport (JACOBSON, 1993). The same acclimatization phase was done with the other groups (respectively in August 2000 and August 2001).

### Release site

The reintroduction site was the southern part of the Lake Bourget (WGS84 coordinates: 5° 50' 25" E 45° 39' 50" N) (Fig. 1); it is considered suitable for the species, large enough to allow dispersal of individuals, and connected to other suitable habitats (around the lake Bourget). The ultimate release site is 13 ha of reed and bushes, that were cleared of bushes, embanked and re-flooded, within a 110 ha of strictly protected nature reserve managed by the Conservatoire du Patrimoine Naturel de Savoie. A survey of the wild habitats was made to determine the most important habitat resources, taking into account the above requirements (CADI *et al.*, 2003). The "Aigrettes pond" is connected to several habitats: channels, ponds, lake littoral and also unfloodable south facing meadows. Three nesting "dunes" were specially laid out

as artificial nesting site inside the protected perimeter (Fig. 1), from soil excavated during pond construction, and inclined 25° in south exposition. The vegetation was grazed between April to May then from July to August to allow egg laying (SERVAN, 1988; ROVERO & CHELAZZI, 1996; SCHNEEWEISS *et al.*, 1998). The totality of the site is forbidden to navigation, fishing and hunting. The public is guided to an observatory with a concealed access, where visitors can enjoy a good view of the environment. Captures of introduced Slider turtles (*Trachemys scripta elegans*) are realised every year in order to limit their presence on the site (successful breeding was observed since 2002) and the competition risk (ARVY & SERVAN, 1998; CADI & JOLY, 2000) and parked in several ponds of "La Ferme aux Crocodiles" zoological facility (Pierrelatte, France).

### Release procedure and monitoring

The three turtles groups were released during spring, in April 2000 (nine females and four males), May 2001 (five females and eight males), and April 2002 (six females and three males), when food, water and vegetation cover are most available, and were all monitored until September 2002. Global sex ratio was slightly biased in favor of females (1:1.33), as it is known in wild populations of Brenne (SERVAN *et al.*, 1989; KELLER, 1997; PIEH & SÄTTELE, 1998). After release, human contact was reduced to a minimum and no further intervention was made to help the turtles in any way. The individuals were released in the morning in one group at the center of the release site, in order to allow them to find a suitable shelter before nightfall and to give them the maximum distance available for dispersal in any direction. The release point was marked and used as a reference point from which future movements could be measured.



Radiotransmitters (Biotracks, U), we attached to the upper central anterior margin of the carapace of each turtle with aluminum machine screws, and plumber's epoxy was molded into the seams. Transmitter packages were less than 5% of each turtle's body mass (15–16 g). All individuals transmitter frequencies were searched each day from the end of March to the beginning of October, then once a week during the rest of the year (receiver Mariner 57 148–149 Mhz and Yagi antenna). We were able to detect signals very precisely up to 500 m from the turtles. Because females usually dig their egg chambers in the afternoon or evening (KOTENKO, 2000; MITRUS & ZEMANEK, 2000), all individuals transmitter frequencies were searched each day during the afternoon then two times during the beginning and the middle of the night. Occasional long-range (> 1 km) movements by some individuals required searches be made by foot, car or boat with mounted antennae. All turtles were

located in aquatic or in terrestrial habitats. Their positions were mapped onto a rectified scanned image of a 2000 true-color aerial photograph (scale 1:7000) of the study area using Map Info 6.5 geographic information system (GIS) software. Comparisons of the under laid image with field observations indicated that telemetry location displacement was generally less than 5 m.

Wetland habitats within the study area were surveyed during 1999–2000 to determine habitat types. Six habitat types were identified: open water, *Salicion cinerea*, Phragmition, Magnocaricion, Nymphaeion, Potamion, and *Alnion glutinosae*. The perimeter of each landscape element belonging to one of the six habitat types was digitised into the GIS using the rectified image of the study-area photograph as a template. The boundaries of the study area used for habitat proportions were defined with the shoreline of the ponds (other are "terrestrial habitat"). Each telemetry location

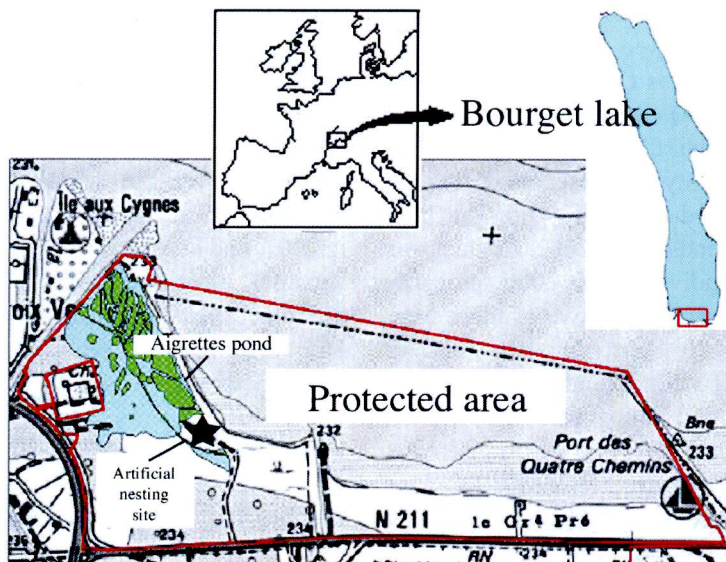


FIGURE 1. Location of the study site in Lake Bourget, south eastern France.

FIGURA 1. Localización geográfica del enclave en el lago Bourget, Francia sudoriental.

was assigned to a habitat type using the Map Info feature-join function. Comparisons of the overlaid locations on the digitised habitat types corresponded with field observations. Distances between each location and the release point were calculated, as well as distance of each telemetry location from the previous daily point (distance between a daily location and the last one) and distance of each individual location from each other individual (daily distance between each individual and the other). Home range size was computed using kernel area method (WORTON, 1989). Data were analysed with the Ranges VI computer program (Anatrack, UK), based on 95% contours (KENWARD & HODDER, 1996).

## RESULTS

We successfully located turtles with transmitters 9,422 times out of 10,553 (i.e. in 10.7% of searches, transmitters either were not heard or were impossible to locate precisely). Of 35 individuals, 28 (six from the first group, 12 from the second and nine from the third) were followed until the end of the study (transmitter was changed once for first group individuals). Mean of monitoring duration is 663 days after release for both first groups. No mortality was observed during the monitoring period. We only present here results until the end of July 2002. Contact was lost for seven individuals in 2000, probably because of battery failure (two of them were rediscovered in 2001, carrying a transmitter out of order); five individuals were lost in 2001, to a total of 11 altogether.

### Post-release dispersal

Nearly the three group stayed all year long on the reintroduction site. Some females nevertheless left to explore the lake. All these "migrants" (with the exception of one with

loss of contact) returned to hibernate within the release pond. This shows the close connection between the pond and the lake, turtles exploration capacity, and their homing capacity.

The three groups did not present the same displacement behaviours during the first month after release (Fig. 2). Most females of the first group left the site three weeks after the release. The second and third groups remained on the site. Over the whole monitoring, individuals remained within a few hundred meters around the release point (Fig. 3), and some stayed in the pond (eight out of 35 individuals).

### Home range formation

Home range was computed for each sex and groups over two activity cycles (from one hibernation event to the next, including all terrestrial and aquatic locations). Male (mean  $11.82 \pm 9.46$  ha) and female (mean  $19.05 \pm 21.91$  ha) home range sizes are not different (Mann-Whitney  $U$  test:  $U = 81$ ,  $p = 0.627$ ). Even if group 1 home range size (mean  $21.31 \pm 22.21$  ha) looks larger than that of group 2 (mean  $9.38 \pm 4.98$  ha) and group 3 (mean  $8.41 \pm 3.09$  ha), this difference is not significant (Kruskal Wallis  $H$  test:  $H = 3.26$ ,  $p = 0.198$ ,  $df = 2$ ). Individual home ranges computed over the whole year overlay at  $58.87\% \pm 25.52$  in 2000, at  $68.75\% \pm 14.26$  in 2001, and at  $67.96\% \pm 15.21$  in 2002. If we consider all three groups, there was no difference between sexes (males  $64.98\% \pm 16.36$ ; females  $59.67\% \pm 24.85$ ; Mann-Whitney  $U$  test:  $H = 87$ ,  $p = 0.846$ ).

### Distances

Figure 4 shows the annual and cyclical changes in distances moved daily. Movements start in May and end in November; they strongly increase from May, and decrease in August. This cycle is repeated in the

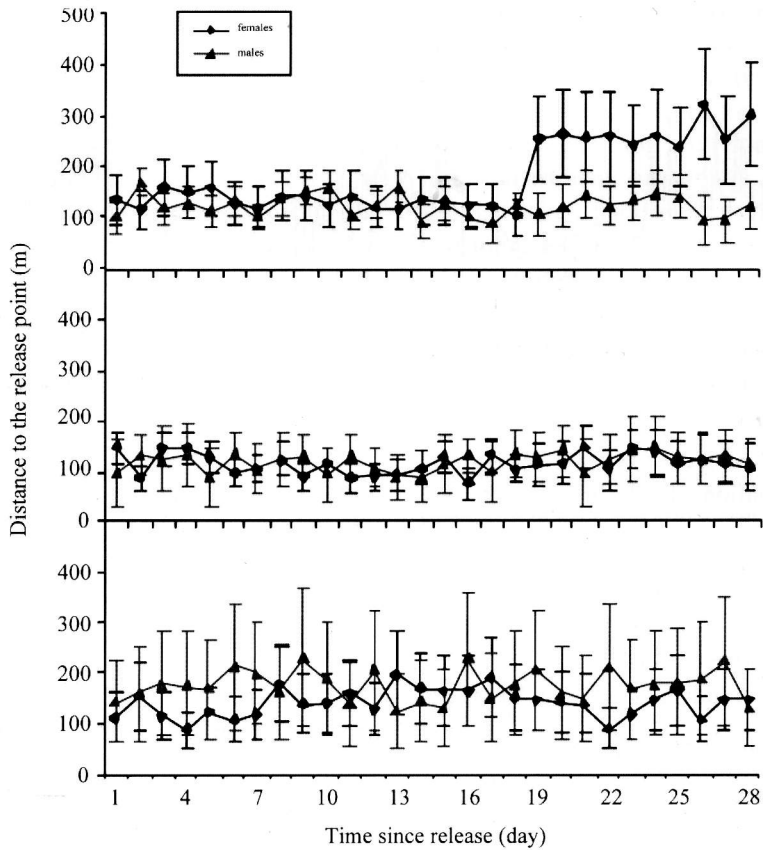


FIGURE 2. Mean distance to the release point during the first month after release for both males and females from each group.

FIGURA 2. Distancia media recorrida desde el punto de liberación durante el primer mes para machos y hembras de cada grupo.

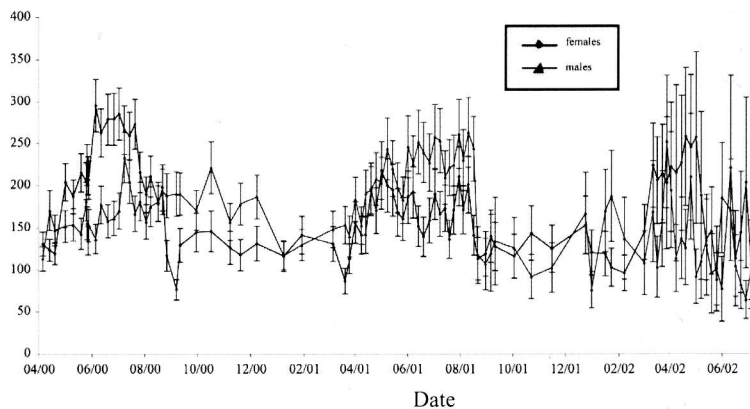


FIGURE 3. Mean distance to the release point since the first group release and over the three activity seasons monitoring.

FIGURA 3. Distancia media recorrida desde el punto de liberación del primer grupo tras los tres periodos de actividad monitorizados.

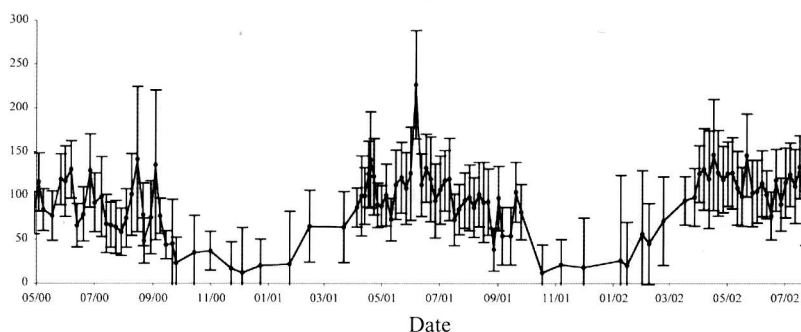


FIGURE 4. Variation of the daily distance (from May 2000 to July 2002).

FIGURA 4. Variación de la distancia diaria recorrida (desde mayo de 2000 hasta julio de 2002).

three activity periods that we monitored. During hibernation, turtle movements are very low.

In 2001 during the egg-laying period (June), we noted a peak of daily displacements of females. This phenomenon was not observed in 2000 and 2002: females stayed in the Aigrettes pond around the artificial nesting site.

### Habitat selection

Over the whole annual cycle, habitat use showed a strong selection for aquatic vegetation. Despite the proximity of the Bourget lake, open waters were not used (only 2.1% total locations). Turtles always stayed in habitats with high plant density. Rooted floating vegetation habitat (*Nymphaeion* and *Potamion*) and reedbed (*Phragmition*) were strongly selected, with respectively 52.7% and 35.2% of locations. Only 1.4% of the locations were made on dry land. The other 8.6% were dispatched between semi-aquatic *Salicion cinerea*, and dry land.

During overwintering, 91% of locations were found in a small clump of *Salicion cinerea* in the reed. Other winter locations were found in *Phragmition*.

### Reproduction

Four females were observed on the nesting

dunes in 2000 but no clutch was observed. In 2001, three females were monitored on the artificial nesting site; two of them were observed digging. Unfortunately, one of the two clutches was predated by a small mammal within an hour after laying, giving us no time to fence it. The other was empty. In 2002, seven nests were detected and protected (Table 1). Two of them gave five hatchlings in September 2002 and further seven hatchlings in April 2003. No emergence was observed in the remaining four clutches.

TABLE 1. Reproduction of the European pond turtle on release site dunes in 2000, 2001 and 2002 (because the possibility of overwintering in the hole, number of hatchlings in 2002 could increase in spring 2003).

TABLA 1. Reproducción del galápago europeo en las dunas de hábitat translocado 2000, 2001 y 2002 (dada la posibilidad de hibernación en el nido, el número de neonatos de 2002 podría aumentar en la primavera de 2003).

	2000	2001	2002
Number of females released	9	14	20
Number of trials	4	3	10
Number of known clutches	0	2	7
Predation percentage	—	50%	0%
Number of hatchlings	0	0	14 (3 clutches)

## Discussion

The dispersal behavior expressed in this experimental release was restricted, indicating an ability to adapt to new environmental conditions. The majority of translocated individuals did not move far away from the release site, only exploiting the vicinity. No homing phenomenon occurred to the acclimation pond, contrary to what was reported in other chelonian species (CHELAZZI & FRANCISCI, 1980; LEBBORONI & CHELAZZI, 2000). We do not know whether a homing phenomenon would occur in individuals translocated directly in the wild. Moreover, it is impossible to be sure that without the acclimation phase, individuals would not immediately disperse from the release site.

Turtle locations show that the annual cycle can be divided into two parts: an activity period from April to October and a lethargic, hibernation period from November to March. Similar observations have been made in other parts of pond turtle range (Italy, ROVERO & CHELAZZI, 1996; Hungary, FARKAS, 2000; Ukraine, KOTENKO, 2000). Hibernation duration can be influenced by weather conditions.

In temperate zones, all turtles hibernate (ROLLINAT, 1934; PARDE *et al.*, 2000) mostly under water (CADI, 2003). Movements are restricted during hibernation; they increase as soon as weather conditions allow (increase in temperature and photoperiod). The long immobile period of the 2000/2001 winter is explained by the presence of a thick layer of ice that impeded movement for more than a month. The progressive scattering of locations over the whole pond in the course of the activity period corresponds to the colonization of all aquatic biotopes. As no territorial behavior has ever been observed in freshwater turtles (BURY, 1978), this phenomenon could be due to avoidance of competition for food and basking sites. Even

if turtles could easily cross the bank to go to the Bourget lake, this suggests that home range size is limited by physical barriers (dykes or canals, see BURY, 1978). As we found in neighboring wild populations (from Isere department, around 30-km away from Bourget lake), habitat selection seems to be tied more to abiotic than biotic (vegetation) criteria: minimal water levels, access to sunlight, and vegetation density are common to all the habitats used (CADI, 2003; CADI & MIQUET, 2004,).

High survivorship of the released individuals was partly attributable to their age: their carapace size protects them from native predators. The observation of clutches and hatchlings on the artificial nesting site show the efficiency of this part of the management plan. While hatchlings will insure population durability, reproduction is a very important step for a reintroduction program.

This first attempt to release European pond turtle in part of their former range was encouraging, and suggested that reintroduction in the wild could be an effective conservation tool for this species. Translocated individuals can survive in the new natural habitat for at least three years. Most tortoise and turtle behavior have a basic social structure and no parental care (BRATTSTROM, 1974). Their generalist diet (ROLLINAT, 1934; LEBBORONI & CHELAZZI, 1991; KOTENKO, 2000) could explain why the transition to the wild was successful. It was not necessary to undergo a training period prior to release and post-release management was not required. Furthermore, for the majority of chelonian species, stress induced by capture, transport, and release procedures appear minimal in comparison with that experienced by birds and mammals (MCARTHUR *et al.*, 1986, LYLES & MAY, 1987; WINGFIELD *et al.*, 1997). For these



reasons, translocation may be considered a viable conservation strategy for this long-lived species with delayed maturity, low mortality and innate behavior. But because of the decline of wild population (it could become difficult to find source populations), captive breeding programs seem necessary for long term management.

On the basis of these results which emphasize the sedentary nature of reintroduced individuals and the rapid adaptive response of the species (no mortality and successful reproduction in the third year), we discuss the necessity to continue the release program and how. Whether restocking or reintroducing populations, models show that from a demographic point of view, a small founder population of adults or subadults shows low extinction probabilities: around 10 adult or 12 subadult females are sufficient to reach the 5% threshold (CADI, 2003). In this context, the released population may be large enough. Note that the numbers obtained through these models are far below those advised by population geneticists. The reconstitution of a matrix of well-connected subpopulations is one way to alleviate genetic concerns. For those reasons, the program is continuing with wetlands management and protection in other parts of the Bourget lake (particularly in East and the North part) and along the Rhône river, ultimately connecting with the last wild population.

The management policies must provide an optimal use of the resources available to realize the conservation objectives as rapidly as possible (LINDBURG, 1992). It is necessary to determine an optimal size and age ratio for cost-effective reintroduction to the wild. The release of one year old juveniles, much more economical (no captive breeding, no delays due to growth), however necessitates many more individuals (hundreds of each sex) because of their low survival rate (CADI &

MIQUET, 2004). The difference between juveniles and adults stems from first reproduction delays: a significant proportion of juveniles will not survive to reproduce. Released individuals need to be large enough to withstand predation (LEBBORONI & CHELAZZI, 1998; JABLONSKI & JABLONSKA, 1998; ZUFFI, 2000). In addition, they should have achieved a sufficiently advanced stage of development to optimize their resistance to environmental factors (such as climate and food availability) and risks (predation) without being too old to compromise their potential to settle at a given site. In the case of adult release, the delay necessary to get sufficient number of mature individuals (e.g. from available young from captive breeding adults) may be a strong constraint. Maturation of adults slows down the build up of stock available for a reintroduction program and increases the costs per released turtle, which might in turn reduce the allocation of resources to other conservation programs.

Therefore, we propose to use immatures. Alongside this first experimental release, two pools were set up in "Mottets enclosure" (near the release site) and in The "Ferme aux Crocodiles" zoological facility in order to monitor the growth of hatchlings coming from Brenne, which were incubated in the Jacques Monod Institute (Paris VI) in collaboration with Pr C. Pieau. The breeding success is around 95%, far higher than the one observed in natural conditions (5-10% due to predation, climatic hazards...); sex is determined by incubation temperature conditions (PIEAU, 1982). Twenty percent of the young obtained are returned back to the wild population and the others are kept in two natural enclosures. Conversely the slow growing rate of turtles renders it necessary to maintain them in captivity for a long time before they reach the size at which they can avoid most predators (around 70-80 mm and



5 years: in captivity the growth rate is higher than in the wild, as a result of unlimited availability of high quality food and water). This approach has been tested successfully by different authors and described as a "head-starting" procedure, that has been repeatedly proposed for turtles, including *Emys orbicularis* (HEPPELL & CROWDER, 1996; MOLL & MOLL, 2000; HERLANDS *et al.*, 2004; MITRUS, 2005). In any future reintroduction program we propose to release subadults at each release site, to allow sexual partners to meet once they reach maturity. To avoid inbreeding, it will be important to use unrelated individuals. For this reason, such a program requires sex determination of captive-raised juveniles and genetic analyses, in order to have a balanced sex ratio and sufficient DNA variability. This sustainable solution should allow an efficient stocking in the long term (from a demographical and a genetical point of view), with an insignificant impact on source populations.

On this basis, several groups should be released along the Rhône river, in proper and protected habitat, as a major environmental action for this Natura 2000 site.

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