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Head Starting European Pond Turtle (*Emys orbicularis*) for Reintroduction: Patterns of Growth Rates

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Abstract.—**Few data are available on the head-starting of the European Pond Turtle (*Emys orbicularis*) and some seem to indicate unique features during first year of development. A slow growth rate might have important consequences on the usefulness of head-starting in this species. We head-started 12 *E. orbicularis galloitalica* hatchlings for eight months. We individually marked hatchlings and kept them in an aquarium equipped with UV-b light tubes. Water temperature was kept at 24°C and we provided them a basking site at 30° C. We first fed hatchlings small fresh items, then shifted to larger prey. We measured and weighed hatchlings weekly and released them as a part of a reintroduction project. The survival rate of released individuals was 83% after one year. Released turtles started breeding when they were six years old, and attained an adult body size similar to turtles from natural populations very quickly. Our results suggest that *E. orbicularis* head-starting can be successful.**

Key Words.—captive rearing; conservation; feeding ecology; growth rates; hatchlings; turtle captive management

Introduction

The lack of information about the captive requirements of many species is a major concern for head-starting projects because microhabitats and feeding ecology of reptile hatchlings could make the difference between success and failure of the project (Mitrus and Zemanek 1998). In turtle head-starting programs, growth rate of hatchlings is a further crucial issue. The time needed for the hatchlings to reach the minimum safe size for release can influence the entire project; Chelonians are generally slow-growing, and if newborns must be maintained in captivity for several years, costs rise noticeably (Ewert 1989).

The European Pond Turtle, *Emys orbicularis*, is an Emydid turtle that is widely distributed throughout Europe, North Africa, and Asia Minor (Fritz and Andreas 2000). Populations of European Pond Turtle are considered endangered in several parts of the range (Ficetola et al. 2013; Fritz and Chiari 2013). Although Germany, Italy, Spain, and Poland have undertaken conservation plans, including head-starting, to preserve and increase populations (Fritz and Andreas 2000; Mitrus 2005; Ficetola et al. 2013; Fritz and Chiari 2013), the success of these initiatives is seldom assessed. We studied growth rates of hatchling *E. obicularis* in captivity, because Ewert (1989) and Mitrus and Zemanek (1998) suggested that hatchlings grow slowly during their first six months and are difficult to feed. If true, this could complicate raising hatchlings to body sizes appropriate for release. We also present data on post-release survivorship and breeding activity of turtles we released.

Materials and Methods

In September 2003, we acquired the first group of eight hatchling *E. orbicularis galloitalica* from a private breeder. The animals were less than one month old and had a mean straight carapace length (SCL; ± SD) of 27.9 ± 1.6 mm and mean body mass (BM) of 6.1 ± 0.9 g. In September 2005, we obtained four more hatchlings with mean SCL of 27.0 ± 1.1 mm and mean BM of 6.6 ± 0.5 g. Both groups were composed of siblings. We marked each hatchling on the carapace with a tag made of a small square of gauze with a written number, which we glued with a drop of epoxy resin.

We reared hatchlings in a 10 × 30 × 30 cm terrarium. We divided the terrarium into two parts: a shallow basin and a terrestrial area (Mitrus and Zemanek 1998). The aquatic part consisted of a 50 × 30 × 15 cm plastic tub. We made the terrestrial area a deep layer of peat moss, which allowed the hatchlings to bury themselves. The basin was connected to the terrestrial area by means of a small wood ramp. We equipped the tank with a basking spot lamp and two reptile fluorescent tubes (Sylvania 18w GroLux, by Sylvania S.p.A., Milano, Italy and ReptaSun Full Spectrum Reptile light 18W by Fluker Farms, Port Allen, Louisiana, USA). We set a 12 h day/12 h night photoperiod. We kept the temperature at the basking site at 30°C, and we maintained a diurnal thermal gradient of 24–30 °C in the enclosure. At night, the water temperature dropped to 20 °C and in daytime, the water temperature was around 24 °C. We maintained this temperature year-round to keep turtles from entering torpor thereby accelerating growth.

We fed the 2003 hatchlings daily. We reduced the frequency of feedings for 2005 hatchlings to avoid overgrowth problems, as an excessive amount of food can cause a very fast growth, resulting in abnormalities of the carapace, deformities, and depigmentation (Mader 1996). We thus fed these turtles every other day. We offered small food items for the first two months because the hatchlings had very small mouths. We started hatchlings with sludge worms (*Tubifex*), and Chironomus larvae, then shifted to mealworm (*Tenebrio molitor*) larvae, small crickets (*Acheta domestica*), and small fish (*Atherina boyeri*). Once a week we offered raw chicken. We introduced finely cut romaine and dandelion leaves, and pieces of zucchini in the second month. We provided some pieces of cuttlefish bone in the water as a calcium supplement.

Data analyses.—We weighed and measured each hatchling weekly on Monday mornings before they were fed. We weighed (to 0.1 g) individuals with a 30 g spring scale and a caliper to measure (to 1 mm) straight carapace length (SCL). We used one way ANOVA to assess differences in weight at release between the 2003 and 2005 individuals. We used the analysis of covariance (ANCOVA) to assess the relationship between biometric parameters of hatchlings (BM, SCL) and those measured at the end of the head-starting period, while controlling for differences between years. We defined BM gain as the difference in body mass and SCL gain as the difference in carapace length of individuals between hatch and release. We also used ANCOVA to assess relationships between hatchling parameters and BM / SCL gain. We performed this analysis both using the gains values and using the proportional gain (i.e., BM gain / starting BM, and SCL gain / starting SCL) as dependent variables. For all tests, $\alpha = 0.05$

Reintroduction.–The study focused on a restored wetland network in northern Italy where the reintroduction of *E. orbicularis* was ongoing. The area chosen for the reintroduction was in the boundaries of Parco Agricolo Sud Milano, a regional park extending in the suburbs of the city of Milan (Ficetola et al. 2013). We selected a suitable wetland area on the basis of ecological requirements of *Emys orbicularis*: (1) presence of a network of wetlands and forested areas; (2) microhabitats for nesting and hibernation activities; (3) presence of riparian vegetation; (4) absence or eradication of competing non-native species of turtles (Cadi and Joly 2003; Ficetola et al. 2004; Ficetola and De Bernardi 2006; Sperone et al. 2010; Perez-Santigosa et al. 2011).

The selected area was a groundwater pond network embedded into 1 ha deciduous woodland in a sand quarry. The wetland network consisted of three ponds connected by small channels. The three ponds measure 520 m^2 (Pond 1), 190 m^2 (Pond 2) and 935 m^2 (Pond 3). The maximum depth of the three ponds was 4–5 m (ponds 1 and 2), and 0.6 m for Pond 3. The entire area was already surrounded by a 2 m tall fence. To minimize loss of animals, we first restricted releases to ponds 1 and 3. We built an inner fence around both ponds, created basking sites (Ficetola et al. 2004), and built a sand bench to be used for nesting. We constructed the sand bench with 50% sand and 50% dirt, with an average height of 2.5 m and a length of 15 m with a southwestern exposure. Largemouth Bass (*Micropterus salmoides*) are predator fish that poses a major threat to pond turtle hatchlings (Britson 1998). Adult Largemouth Bass up to 60 cm long were present in Pond 1. We removed as many Largemouth Bass as we could using line and pole with artificial bait to lower their abundance. We created two nursery areas of shallow water to provide safe microhabitats for hatchlings and to prevent predation by bass. These nursery areas were protected with Hazelnut (*Corylus avellana*) and Sedge (*Carex elata*) branches tied together in bundles to create a wooden weave and sedge matte as a shelter and to keep the large basses out of the nursery area.

To promote a soft release of head-started juveniles, we built an acclimation cage (1 × 2 m) that was left in place in water near the shore of the main pond for two months before the release of turtles. We allowed aquatic vegetation to grow into the cage. We made the cage of 1 cm wire mesh, covered on the top to prevent predation by herons and crows (*Nycticorax nycticorax*, *Ardea cinerea*, *Egretta garzetta* and *Corvus corone*). We secured the cage in a shallow shore of the pond, partially submerged in water, and provided two cork-bark basking sites (Alberts 2007). We put two groups of juveniles, each individual about eight month old, into the acclimation cage; one group in May 2004 and May 2006, which we left in the cage for one month. During the acclimation period, we did not feed turtles. In June of each year, we released them into Pond 1 during sunny mornings.

We measured and weighed juveniles just before release, but no relevant SCL and BM change occurred during the last month in both groups. During the summer after the release, we monitored turtles twice a week using visual point counts at basking sites. After the release, we recaptured the turtles once a year, at the end of hibernation period (March) using an open-top basking trap (Drobenkov 2000). We assessed differences in survival between the 2003 and the 2005 turtles using a chi-square with continuity correction. We also performed monthly surveys of the pond network by means of visual inspection to assess events of mortality and predation. We assessed reproductive status of females by palpation of the inguinal region. This approach allows to record the presence of oviductal eggs (Zuffi et al. 1999). To confirm the reproductive status assessed through palpation, one female thought to have eggs was radiographed (Zuffi et al. 1999).

Results

Both BM and SCL of turtles increased over time while in captivity, with higher rates of increase for both measures in the 2003 cohort than in the 2005 cohort (Fig. 1). These differences between years were significant (BM: $F_{1,10} = 19.6$, $P = 0.001$; SCL: $F_{1,10} = 5.7$, $P = 0.039$). Also, differences for body mass between groups were larger than those for SCL. For mass, between years variation accounted for 66% of variability, while for SCL between years variation accounted for 36% of variability. We did not observe any growth slowdown in winter months (Fig. 1) as ob-

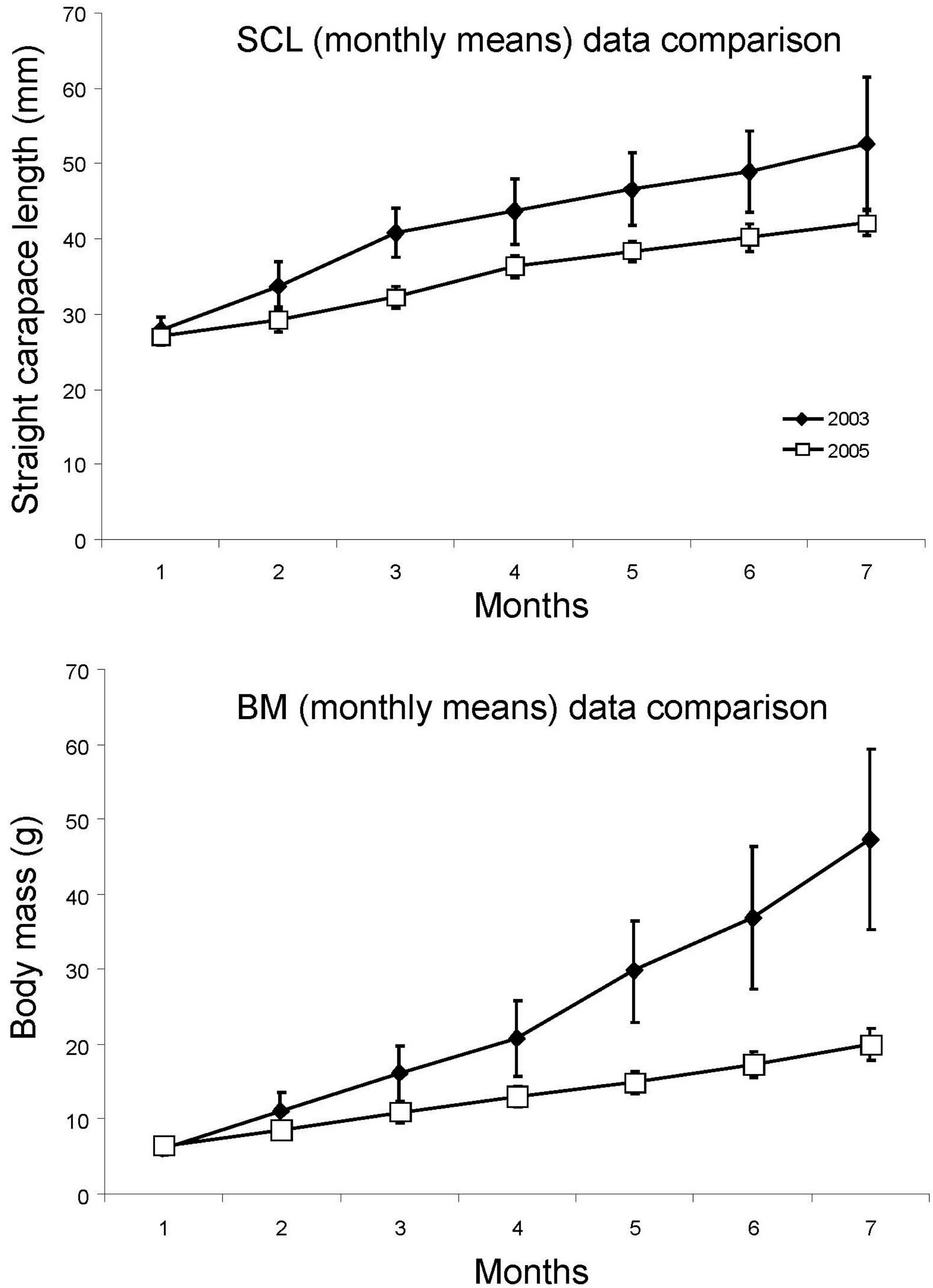

FIGURE 1. Comparisons of body mass and straight carapace length of hatchling European Pond Turtle (*Emys orbicularis*) while in captivity for 2003 (filled diamonds) and 2005 (open squares).

served in other head starting experiences with this species, even if hatchlings were not allowed to go into torpor (Mitrus 2005). The 2005 hatchlings were smaller than those from 2003 at release, but had firmer and correctly pigmented carapaces.

There was a positive and significant relationship between mass at hatching and at the end of head-starting (when we put turtles in the acclimation cage). Individuals that were the heaviest at hatching remained the heaviest during the whole captive period (F1,9 = 8.2, P = 0.019; effect of year: $F_{1,9} = 40.3$, $P < 0.001$; Fig. 2a). We noticed that the heaviest hatchlings gained significantly more mass ($F_{1,9} = 6.3$, $P = 0.033$; effect of year: $F_{1,9} = 40.3$, $P < 0.001$; Fig. 2b), possibly because they are more vital and ready to accept food after the hatch. The relationship between starting mass and the proportional gain of mass was not significant ($F_{1,9} = 0.1$, $P = 0.817$; effect of year: $F_{1,9} = 35.8$, $P < 0.001$). The relationships between SCL at hatching and SCL at release, between SCL at hatch and SCL gain, or between SCL at hatch and SCL proportional gain were positive but not significant (dependent, SCL at release: $F_{1,9} = 3.5$, $P = 0.095$; dependent, SCL gain: $F_{1,9} = 1.3$, $P = 0.289$; dependent, SCL proportional gain: $F_{1,9} = 0.3$, $P = 0.599$; Fig. 2 c-d).

After the release of head-started turtles, recapture rate was high. The first cohort (hatched in September 2003 and released in June 2004; recaptured yearly between 2005 and 2007) showed a 100% recapture rate each year. We found two individuals of the second cohort (hatched in September 2005 and released in June 2006) dead nearby the release site; the two causalities were probably caused by some terrestrial predator, because we found pieces of carapaces with signs suggesting Brown Rat (*Rattus norvegicus*) gnawing. We recaptured the remaining individuals in spring 2007. Differences in survival one year after release were not significant between the 2003 and the 2005 batches ($\chi^2 = 1.88$, df = 1, P = 0.171). The average survival one year after release was 83%. We did not detect mortality events during the subsequent years. In 2008, part of the fence was removed and some turtles dispersed into nearby wetlands.

The first successful reproduction of released individuals was observed in 2009, when turtles were six years old. Radiography confirmed the reliability of palpation; the clutch size of the radiographed female was six eggs. At age seven, average SCL of released turtles was 12.4 ± 1.6 cm for of females, and 13.5 ± 1.3 for males.

Discussion

During two years of *Emys orbicularis* captive raising, we observed a rapid and steady growth of the hatchlings. Rapid growth was evident even during the first two months after the hatch, which is typically considered the most delicate period for the neonates of this species. Mitrus (2005) suggested that the tiny hatchlings of *E. orbicularis* hardly accept food in captivity, thereby limiting the effectiveness of this approach to head-starting. Mitrus and Zemanek (1999) also found very slow growth of *E. orbicularis* hatchlings in the first months after the hatch and an apparent incapacity to feed. In our study, food was readily accepted by all individuals of both groups by one week after hatching. Moreover, *Emys* hatchlings accepted plant foods readily, unlike previous observations on this species and on *Trachemys* hatchlings, that are often used as an experimental model. *Emys orbicularis* hatchlings are considered mostly carnivorous in their first year (Cadi and Joly 2003; Mitrus 2005).

One difference between these studies was that we kept turtles active continuously, even during winter months. Differences in growth rate might also be due to geographical differences among populations. Our study focused on a Mediterranean subspecies of *E. orbicularis* that typically emerges from the nest in September-October, and hatchlings might be able to find food immediately. Conversely, northern populations of this species seem more apt to overwinter in the nest (Drobenkov 2000; Fritz 2001). The differ-

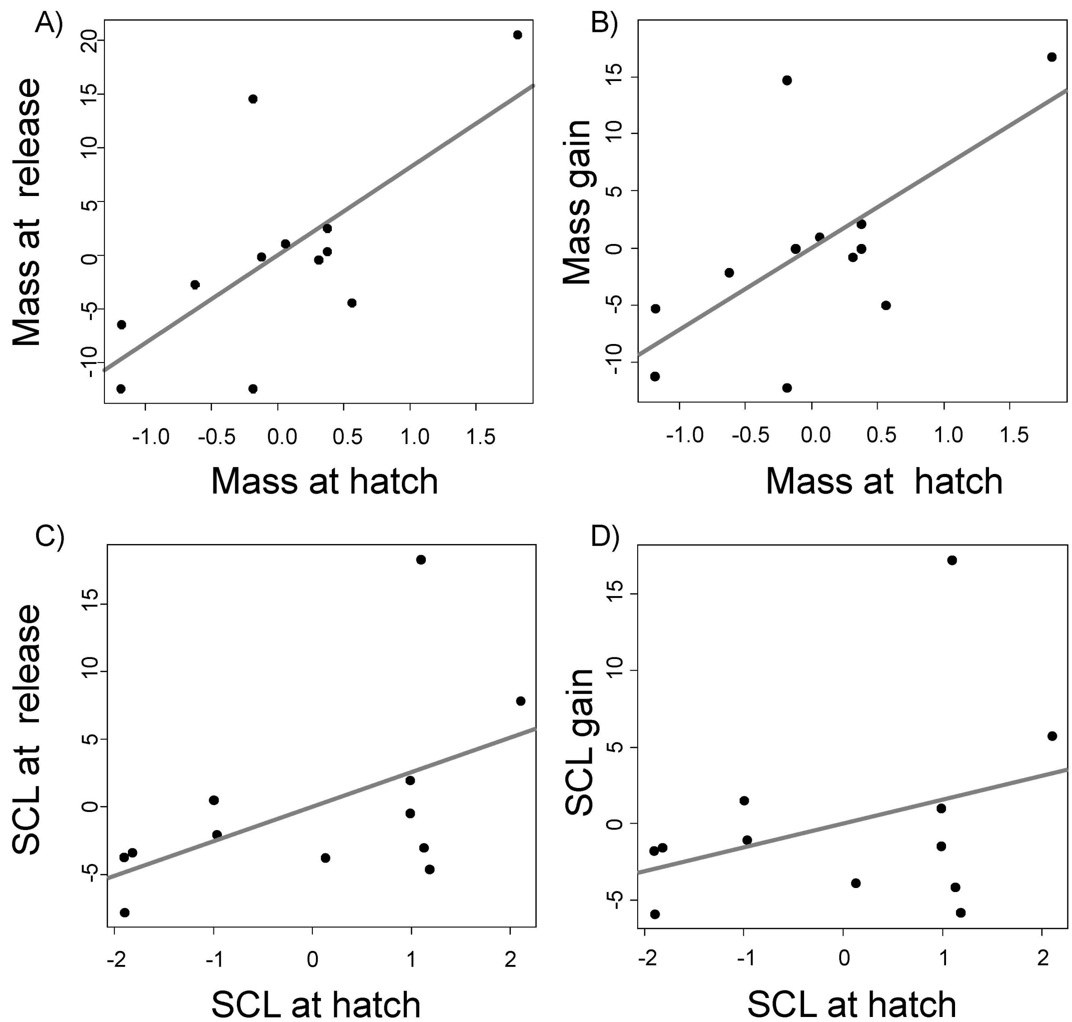

Figure 2. Partial regression plots showing the relationships between A) mass at hatch and mass at release; B) mass at hatch and mass gain; C) straight carapace length (SCL) at hatch and SCL at release; and D) SCL at hatch and SCL gain of hatchling European Pond Turtle (*Emys orbicularis*).

ences between our results for early growth and those from Mitrus and Zemanek (1999) could be caused by regional adaptation of the different populations that produced the hatchling studied.

We observed that the turtles of the 2005 group, which we fed every other day, showed a slower growth in comparison with the hatchlings born in 2003, which showed depigmented areas in each scute of the carapace. These might be symptoms of a too fast growth in aquatic turtles (Mader 1996). We observed that the 2005 juveniles were smaller than those from 2003 at release time, but had firmer and correctly pigmented carapaces. This issue could be of interest in head-starting projects: although a fast growth of the individuals is often desired, a high growth rate may cause problems to liver and kidney (Mader 1996).

In natural populations of E. orbicularis, sexual maturity is usually attained at 7–8 y in males, and >10 y in females (Girondot and Garcia 1999; Zuffi et al 2011). Head-started females released in nature reached sexual maturity at a much younger age; furthermore, the clutch size of the one individual that we radiographed was comparable to mature females of natural populations. Actually, the median clutch size of Italian populations of *E. orbicularis* is five eggs (Zuffi et al. 2007). In addition, head-started individuals attained a body size comparable or even larger than older, adult individuals. The average carapace length of adult *E. orbicularis* from Italian populations is about 12 cm in males and about 13 cm in females (Zuffi et al. 2007; 2011). In summary, our data show that head starting can allow fast growth rate, high survival, and early sexual maturity in *Emys orbicularis galloitalica*; therefore, this practice can be useful for population management and conservation programs.

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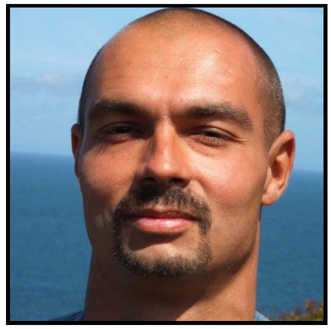

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