EAZA Reptile Taxon Advisory Group

EAZA Best Practice Guidelines

European Pond Turtle
Emys orbicularis

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EAZA Best Practice Guidelines disclaimer

Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.
Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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Cover photo: A captive European pond turtle. ©Parc Animalier de Sainte Croix

Introduction

The European pond turtle (*Emys orbicularis*) is a one of Europe's freshwater turtles, that can be seen as a good indicator of the quality of freshwater bodies. Unfortunately, the species is threatened or even extinct in most countries within its original distribution range. Its populations suffer from various threats, mainly of anthropogenic origin. To prevent, stop or limit the decline of this species different *in-situ* as well as *ex-situ* actions are being implemented.

The reinforcement of remaining populations, or the (re-)creation of new populations in areas where the species has become extinct, has proven to be a successful strategy. As in many cases the translocation of wild individuals is not possible without weakening remaining populations, ex-situ breeding is necessary to be able to provide sufficient individuals for releases in the wild. Therefore, zoos, private breeders and other holders can play an important role in the conservation of the species. A great amount of experience and knowledge on the captive husbandry of European Pond Turtles is already present among current holders. The aim of these Best Practice Guidelines was to collect the available information and to provide institutions the tools to optimize animal welfare and breeding results, with consequently a larger number of individuals available for reintroduction and reinforcement projects. To collect much information on the experiences of institutions and private breeders, a questionnaire was compiled (see annex 2) and sent to 52 institutions and private breeders. Despite several reminders, only 16 completed the questionnaire, but fortunately these included some of the most successful breeders of European pond and the information obtained provided a very good base for the compilation of these Best Practice Guidelines.

Institutions not able or willing to breed European pond turtles, but keeping the species for its important educational role, can also use these guidelines to optimize the husbandry of their animals.

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The EEP of *Emys orbicularis* is working under the responsibility of the EAZA Reptile Taxon Advisory Group.

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Section 1: Biology and field data

1.1 Taxonomy

Order: Testudines
Family: Emydidae
Genus: *Emys*
Species: *Emys orbicularis*
Subspecies: see below
Common name: European pond turtle

Emys orbicularis is the sole recent member of the Emydidae, all other members of this family can be found in the New World. *Emys* must have reached Eurasia from the east, over the Bering Bridge (Fritz, 1998). The species got extinct in the northern regions (incl. Scandinavia, United Kingdom and the Netherlands) during the Pleistocene, but expanded later northwards from a range of southern refugia, marking the different genetic clades (see below).

Until 1989, little was known about the taxonomy of *Emys orbicularis*. A few subspecies had been described, but the diversity of the species was greatly underestimated. That changed when the herpetologist Uwe Fritz started his impressive work on this species. His studies firstly focussed mainly on morphological characters, but when new techniques became available, he refined his work with the description of a large number of different haplotypes. Other researchers elaborated on the work of Fritz and helped to further understand the taxonomical status of the European pond turtle. What was thought to be a single species, turned out to be a complex of different species, subspecies and haplotypes. A portrait of the diversity within the European pond turtles is a fascinating picture of the climatological and geographical history of Europe and Asia Minor, and an excellent view of the dispersion and evolution of a species that could easily be overlooked by researchers and zoologists. In this chapter we will give an overview of the history of taxonomical studies and will summarize our current knowledge on the taxonomy of *Emys orbicularis*.

1.1.1 Morphologic studies

Extensive morphological studies have been hindered by a lack of materials in museum, the bad quality of many museum specimens and the difficulties in observing and capturing specimens in the wild. Many specimens in museums are of unknown localities and if a captive animal was conserved its real origin would often be unknown, especially considering that for hundreds of years pond turtles were traded all over Europe, with animals being transported for hundreds of kilometres from their capture localities. Even when the locality of capture is known, there is also the risk that it was an introduced, released or escaped individual.

There are many factors that make a good arrangement based on phenotypic variation of the European pond turtle very difficult. From the many studies that are mentioned below, it is clear that there is much natural variation of coloration within populations and therefore within proposed subspecies. Furthermore, the distribution of different subspecies/phenotypes is not clear-cut, and there is much integration between subspecies (Mascort, Bertolero, & Arribas, 1999). This situation is even more complicated due to the fact that humans have transported and released pond turtles all over Europe, therefore mixing populations that were formerly separated by mountain chains and large distances.

One final complication is that this turtle shows considerable changes in colouration throughout the different life stages (Najbar & Szuszkiewicz, 2006), and only adult animals can be compared to define colour differences between populations. All these factors maybe the reason why for so long only few people have attempted to unravel the systematics of the European pond turtle. It has also resulted in the description of several subspecies, which revealed later to be only sexual differences within an existing subspecies or a different coloration due to reddish clay in the habitat of the individual (Fritz, 1992).

As mentioned in the introduction, Uwe Fritz is the main researcher on *Emys orbicularis* taxonomy and distribution. Since 1989 a large number of publications shed light on the history of the species (and its allies).

It was Linnaeus who published in 1758 his “Systema Naturae”, which included “*Testudo orbicularis*”. In 1831 Eichwald mentioned two varieties, *iberica* (from Astrakhan, Russia) and *persica* (Mazanderan, Iran). One year later Valenciennes (1832) described *hellenica* from Peloponnesus, Greece (Fritz, 1992).

For a period of more than 150 years, no thorough research was conducted on the species, until 1989, when Fritz described *Emys orbicularis luteofusca* from the Konya Province in central Turkey. This subspecies, in which the males are at least 14cm and the females 17.5cm, differs from others in having a very small head and the carapace yellowish-brown. There is, however, considerable variation in the colouration of individuals (Fritz, 1989).

In 1992 Fritz redefines *Emys orbicularis orbicularis* as the taxon from Central Europe, and *Emys orbicularis hellenica* as the taxon from the eastern Mediterranean region. Several other described forms of turtles are discussed and either considered as synonyms of already named taxa or rejected (Fritz, 1992).

In 1993 Fritz describes two other new subspecies. *Emys orbicularis fritzjuergenobsti*, the “Eastern Spanish pond turtle”, is found on the eastern (Mediterranean) coast of Spain. This pond turtle is of average size (14cm for the males; 9 to 14cm for the females) but has a relatively large head. It differs from other subspecies in the long and narrow carapace with a light brown to yellowish brown primary colour. Young animals are usually of a darker colour. Males and females have a yellow iris with black cross; one male had a brown-yellowish-red iris. The cross is in males less visible than in females. The second new subspecies, *Emys orbicularis occidentalis* or the “Western pond turtle”, originates from Morocco, Algeria, Tunisia, southern and western Portugal. The males are 9 to 14 cm long, the females 10 to 15cm. It differs from related subspecies by very wide carapace, with very wide yellow spots. Males and females have a yellow to yellow-whitish iris, females with a very visible black cross (Fritz, 1993a).

In a second publication in the same year, Fritz divides the Anatolian pond turtles in six groups, with three new subspecies which he named a year later (Fritz, 1993b). *Emys orbicularis colchica*, from Colchis and east Turkey is a subspecies of median size with a dark colour. The second new subspecies is *Emys orbicularis orientalis*, from Turkmenia and northern Iran. It differs from other subspecies by its very short central intergular seam. The third new subspecies is *Emys orbicularis kurae*, from the Kura river drainage and the downslope of the Greater Caucasus. It differs by a very light colour and very light coloured forelegs with a wide yellow band (Fritz, 1994).

Fritz continues his work with the description of the Sardinian pond turtle, *E. o. capolongoi* from Sardinia and surrounding islands and of the very dark Lanza's pond turtle, *E. o. lanzai* from Corsica (Fritz, 1995) (Fritz, 1995). From south-western Spain he describes the Spanish pond turtle, *E. o. hispanica* (Fritz, Keller, & Budde, 1996) and finally from south-eastern Turkey the Eiselt's pond turtle, *E. o. eiselti* (Fritz, Baran, Budak, & Amthauer, 1998).

To finish (for the moment?) the long list of described subspecies, the Ingauna pond turtle, *E. o. ingauna* was described from the Ingauna mountain chain, western Liguria (Italy). This subspecies is reported to be recognizable by its small size and uniform chestnut-brown carapace (Jesu, Piombo, Lamagni, Ortale, & Genta, 2004).

Zuffi and Ballasina studied a large dataset of Italian turtles, and noticed that there are four, and possibly five, different "biometric ecotypes" in the country. However, temperature experiments on growing juveniles showed that these may influence colouration, patterns and markings. Therefore, genetic studies are necessary to define which and how many subspecies can be found in Italy (Zuffi & Ballisana, 1998).

As a rule of thumb, we can say that European pond turtles from the north are generally large and dark coloured, whereas subspecies from the south are smaller and distinctly lighter coloured. As always, there are exceptions, with some very dark, almost black animals described from Turkey.

No new subspecies have been proposed since then, but there are indications that other populations are different enough to be lumped into new subspecies (Fritz, 1998), and genetics even suggest that some subspecies could be raised to the species level. On the other hand, additional research (both morphological and genetic) suggest that some subspecies are just local variations of earlier described subspecies, and should be rejected. More research may give us a better picture of the diversity of *Emys orbicularis*, but the widespread introductions and local extinctions will probably make a full picture impossible.

1.1.2 Genetic studies

The first genetic studies on the European pond turtle are those of (Lenk, Joger, Fritz, Heidrich, & Wink, 1998). With their preliminary study (187 samples) of the mitochondrial cytochrome b gene they identified 13 different haplotypes, corresponding to six clades.

Clade I: Eastern Balkan
Clade II: Central Europe – Balkan
Clade III: Southern Italian
Clade IV: Adriatic
Clade V: Tyrrhenian
Clade VI: Ibero-Maghrebian

Haplotype Ia: Eastern Balkan region, Turkey, Bulgaria, Romania, Denmark and eastern Poland. Mixed population of Ia and Ib were found on the Bulgarian shore.
Haplotype Ib: Bulgarian shore (Black Sea), Turkey, Thásos island and Evvoia island (both belonging to Greece).
Haplotype IIa: this was the most abundant haplotype, encountered in the south on Thásos island (with Ib), and from Hungary in the east to Central France in the west (Brenne).
Haplotype IIb: was found in all relict populations in Germany (Brandenburg and Saxony).
Haplotype IIc: this haplotype was only found in Velenceitó, Hungary.

Haplotype IId: was found in one specimen from Silkeborg, Denmark.
Haplotype III: was obtained from two populations in southern Italy, including Sicily (where also an animal with Haplotype V was found).
Haplotype IVa: was found along the Adriatic coast and on the Greek island Evvoia.
Haplotype IVb: was found across Peloponnisos and Kefallinfa island (Greece).
Haplotype V: was found in the eastern Provence, Corsica, Sardinia and western Italy, and one specimen on Sicily.
Haplotype VIa: was found in eastern Spain.
Haplotype VIb: was found in southern Portugal.
Haplotype VIc: was found in Morocco.

There were not enough samples to compare the genetic results with those of the morphologic research (see above) and to link the proposed subspecies precisely to the haplotypes.

The authors stress that some of the results should be taken with care, as human releases can cause some unnatural distribution of haplotypes. This was especially clear from the results of the samples collected in Germany, which resulted in six different haplotypes, while based on samples of neighbouring countries only one group (II) was expected.

A second analysis of 423 samples completed further the picture of the phylogeography of *Emys orbicularis* (Lenk, Fritz, Joger, & Winks, 1999). A total of seven new haplotypes are being described, bringing the total to 20. These are divided into seven major lineages. The distribution of the lineages can be compared to the earlier study, but the additional samples give a more complete picture:

Lineage I: Eastern Europe and Asia Minor
Lineage II: central Europe and central Balkan
Lineage III: southern Italy
Lineage IV: around the Adriatic Sea
Lineage V: northwest coast of Mediterranean
Lineage VI: Iberia and northern Africa
Lineage VII: Caspian region

More sampling resulted in more lineages and more haplotypes. Nine lineages with 44 haplotypes are reported in a new study (Fritz, Guicking, Lenk, Joger, & Wink, 2004). One of the new lineages (VIII) was encountered in southern Turkey, another (IX) in a captive turtle of unknown origin. The authors focussed in the publication on the situation in Germany, where they encountered among 75 samples eleven different haplotypes belonging to six lineages. Haplotype IIb is endemic to eastern Germany and western Poland, and only in the north-eastern states of Brandenburg and Mecklenburg-Western Pomerania a very small native population still exists. It has been argued that a relict population with Haplotype IIa survived in Hesse (southern Germany), however Fritz and his colleagues conclude that these animals must have been introduced by humans and may originate from other regions where this haplotype can be found. All other identified haplotypes originate from almost all other parts of the distribution range of *Emys orbicularis*. It is interesting to note that most introduced haplotypes in former East Germany originate from countries in Eastern Europe (Bulgaria, Hungary and Romania), while the animals sampled in former West Germany have a much greater variety of origins. This may reflect the tourist destinations and pet trade routes.

Further work on genetics shows the complexity of the situation in France (Fritz, et al., 2005). The results of analysing 106 samples reveal the existence of three lineages (II, V and VI) with six haplotypes. Haplotype IIa was found in the south-east and central part of France; from that haplotype three others derived, IIg was found in the south-east and IIh and IIi in the south-west. Haplotype Va (from Italy) was found on Corsica and in the Var Department, and finally Haplotype VIa (from Spain) in the south-west. Interestingly, IIa and Va were found together in the Camargue, and it is unclear if this is a natural situation and if hybridisations occurs.

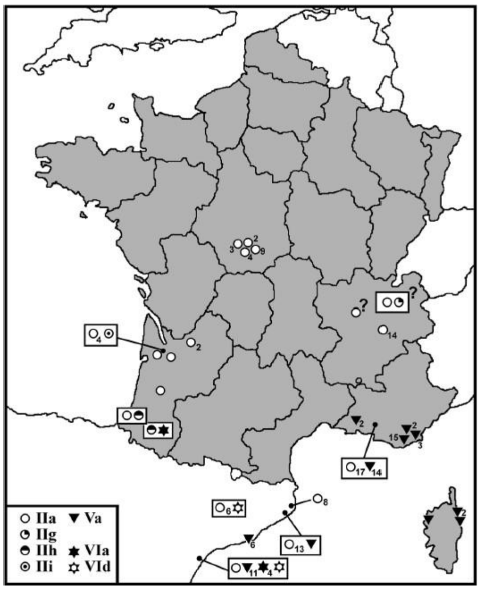


Figure 1 : Distribution of mtDNA haplotypes (cyt b) in France. Taken from: Fritz et al., 2005.

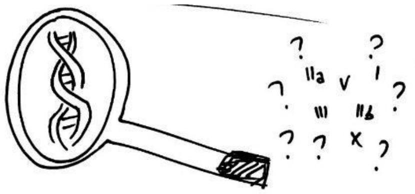
The authors continue to collect more samples, and in 2007 they publish the results of the analysis of more than 1100 samples (Fritz, et al., 2007). The sample area is much larger, giving a better picture of the diversity of the species. Eight lineages with 51 haplotypes occur, while the formerly know Lineage III (from Sicily) is now considered as a separate species, *Emys trinacris*, with 5 haplotypes.

One new lineage and 16 new haplotypes are subsequently described from Anatolia and the Ponto-Caspian Region, bringing the total to nine lineages and 67 haplotypes for *Emys orbicularis* (Fritz, et al., 2009). For this study, some 3000 individuals were examined and 184 were sampled. Data was compared with earlier results of samples from the region (Fritz, et al., 2007). The new lineage is Lineage X, originating from the southern coast of Turkey, with two haplotypes (Xa and Xb). Lineage I is the most diverse, with 23 haplotypes.

The results of an analysis of subfossil DNA revealed that European pond turtles living in Great Britain and Sweden belonged to Lineage II (Sommer, et al., 2009).

Three more haplotypes (Iy, IIn and IVl) from Poland can be added to the long list (Prusak, et al., 2011). Additional samples in Poland (Prusak, et al., 2013), making a total of 146 samples for Poland, showed that Haplotype Ia is restricted to eastern Poland and all the others (Iy, IIb, IIn and IVl) were found in western Poland. However, only IIb is considered as native for western Poland, the others are supposed to be the result of human translocations. Animals with the Haplotype IVl are probably hybrids, with ancestors coming from the Bay of Venice. Animals with Haplotype Ia in western Poland are probably also the result of translocations from the eastern regions.

Four additional haplotypes are discovered in wild caught animals (IIm – western Germany; Ix – southern Portugal) and captive animals (IIL – Zoo Frankfurt; VIg – Madrid rescue centre). The Haplotype Ix for Portugal is considered to be non-native (Velon-Anton, Wink, Schneeweiß, & Fritz, 2011).



A study on hybridisation in France (Raemy, Fritz, Cheylan, & Ursenbacher, 2017) using MtDNA, confirmed the presence of Lineage II in the central and western populations; in the south and east animals of Lineages II, IV and V were encountered, which confirms the already known contact zone (Fritz, et al., 2005). However, if nuclear DNA was analysed, the situation was considerable different. Most of the animals in Central France (Brenne) were pure Lineage II, only one out of 34 individuals showed introgression with Lineage V. In southern and eastern France, much hybridization between Lineage II and Lineage V was detected, partly recent and partly old. Introgression with Lineage IV was detected at two localities in the south, and is probably the result of human translocations.

(Vamberger, et al., 2015) analyse samples of 623 individuals, and report four new haplotypes of Lineage V (e – h) and nine new haplotypes of Lineage IV (l-t). At the same time they report that Haplotype IIIa is the same as IIIc, and therefore the first is rejected.

The current knowledge on the different lineages and their current distribution ranges is summarized in *Figure 2* (Pöschel, et al., 2018). Different taxa correspond to different colours. The morphologically different subspecies of *E*. *orbicularis* correspond to distinct mtDNA lineages as shown in the inset. Merging colours indicate hybrid zones. The wide-ranging subspecies *E*. *o*. *orbicularis*, distributed from the Atlantic coast of France to Central Asia, harbors two distinct mitochondrial lineages, which are less divergent than the other lineages

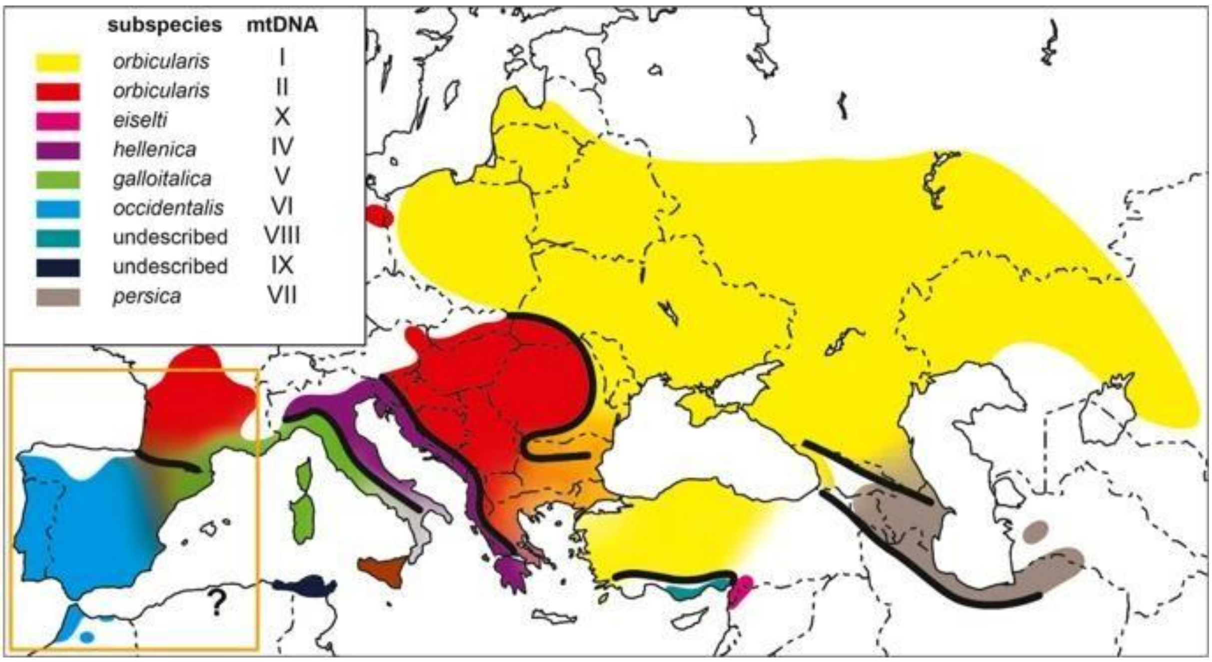


Figure 2: Distribution of Emys orbicularis *and its lineages. Taken from: Pöschel et al., 2018.*

In **summary** we now have, based on the above-mentioned studies, a total of nine lineages and 87 haplotypes, with an additional lineage and five haplotypes of *Emys trinacris*. Some of the haplotypes were from captive animals, and their origin is unknown. Some others were possibly captured on a locality where they, or their ancestors, were released by humans. However, due to the large number of samples we have a good picture of the distribution ranges of the different lineages and the closely related haplotypes.

Haplotype	Geographic Distribution
Ia	Central and eastern Poland; Lithuania; Ukraine (incl in Crimea near Sebastopol); Don, Volga, Ural and Turgay river basins in Russia and Kazakhstan; Black Sea regions of Romania, Bulgaria, Turkey and Georgia; western and central Georgia; Kladovo (Serbia)
Ib	Eastern Greece; Bulgaria; western Turkey
Ic	Central Anatolia; Turkish Black Sea region; southern Crimea (Ukraine); Dagestan and Kalmykia (Russia)
Id	Central Anatolia (Konya Province, Turkey)
Ie	Northern Crimea (Ukraine)
If	Izmir, Denizli and Manisa Provinces (south-eastern Turkey)
Ig	Central Anatolia; western and central Turkish Black Sea region; south-central Turkey
Ih	North-eastern Ukraine
Ii	South-eastern Crimea (Ukraine)
Ij	Unknown
Ik	Central Turkish Black Sea region
Il	South-eastern Ukraine
Im	South-central Turkey.
In	Edirne Province, western Turkey
Io	Western Turkey
Ip	Western Turkey
Iq	Western Turkey
Ir	Western Black Sea coast, Turkey
Is	Western Black Sea coast, Turkey
It	Eastern Crimea (Ukraine)
Iu	Central Turkey
Iv	South-western Turkey
Iw	South-western Turkey
Ix	Unknown (found in Portugal, but probably non-native)
Iy	Western Poland
IIa	Navarra, Huesca and northern Mediterranean coast of Spain; western, southern and central France; Danube basin; south-eastern Balkan Peninsula (Greece, Bulgaria, Serbia); Slovakia
IIb	North-eastern Germany; western Poland (mainly Oder basin)
IIc	Balaton and Velence Lakes (Hungary)
IId	Unknown
IIe	Unknown
IIf	Platamonas (Macedonia, Greece)
IIg	Département Aïn (France)?
IIh	Département Pyrénées-Atlantiques (France)
IIi	Départment Gironde (France)
IIj	Unknown
IIj	Unknown
IIk	Unknown
IIl	Unknown (captive in Germany)
IIm	Western Germany
IIn	Western Poland
IIIa	Rejected, probably the same as IIIc
IIIb	Unknown (*Emys trinacris*)
IIIc	Sicily (*Emys trinacris*)
IIId	Sicily (*Emys trinacris*)
IIIe	Sicily (*Emys trinacris*)

IVa	Apulia and Adriatic coast of Italy; west coast of Balkan Peninsula (not Cephalonia and Peleponnesus); Corfu and Evvoia (Greece)
IVb	Cephalonia (Greece)
IVc	Peleponnesus (Greece)
IVd	Southern Apulia (Italy); Tivat (Montenegro)
IVe	Unknown
IVf	Unknown
IVg	Peleponnesus (Greece)
IVh	Calabria; southern Apulia (Italy)
IVi	Southern Apulia (Italy)
IVj	Southern Apulia (Italy)
IVk	Unknown
IVl	Western Poland (native?); Peloponnese Region (western Greece)
IVm	Peloponnese Region (western Greece)
IVn	Peloponnese Region (western Greece)
Ivo	Peloponnese Region (western Greece)
IVp	Peloponnese Region (western Greece)
IVq	Fiesa, Slovenia
IVr	Lučina, Neretva delta, Croatia; Metkoviči – Opuzen, Neretva delta
IVs	Opuzen, Neretva delta
IVt	Piemonte: Vercelli: Fontanetto Po (Italy)
Va	Northern Mediterranean coast of Spain; southern France; west coast of Apennine Peninsula; Calabria; Basilicata; southern Apulia (Italy); Corsica; Sardinia
Vb	Calabria and southern Apulia (Italy)
Vc	Calabria and Basilicata (Italy)
Vd	Calabria (Italy)
Ve	Corsica: Étang de Biguglia (France)
Vf	Sardinia: Olbia-Tempio: Arzachena (Italy)
Vg	Corsica: Étang de Biguglia (France)
Vh	Corsica: Étang de Biguglia (France)
VIa	Alto Trás-os-Montes (Portugal); Galacia, Hueva, Madrid and Ciudad Real (Spain); Mediterranean coast of Spain; perhaps Département Pyrénées-Atlantique (France)
VIb	Algarve (Portugal)
VIc	Middle Atlas (Morocco)
VId	Alentejo (Portugal); León, Huesca and Ebro Delta (Spain)
VIe	Galicia (Spain)
VIf	Rif Mountains (Morocco)
VIg	Unknown (rescue centre Madrid)
VIIa	Araxes River (Armenia); Azerbaijan; Gilan, Mazandaran and Golestan (Iran); possibly between Volga and Ural Rivers (Kazakhstan)
VIIb	Unknown
VIIc	Azerbaijan; Mazandaran (Iran)
VIId	Mazandaran (Iran)
VIIe	Azerbaijan
VIIf	Azerbaijan; Golestan (Iran)
VIIg	Gilan (Iran)
VIIIa	Anamur (southern Turkey)
VIIIb	Silifke/Mersin Province (south-central Turkey)
IX	Unknown
Xa	South-central Turkey
Xb	South-central Turkey

Table 1: Summary of recognized haplotypes and their locality/distribution.

1.1.3 Summary

This chapter summarized the diversity of *Emys orbicularis* and the difficulties identifying animals. Although it may seem excessive to treat the subject this detailed in Best Practice Guidelines for captive husbandry, we think that it is important to stress the diversity of the species, and the importance to conduct genetic tests on the animals.

There are differences in phenotype between animals of different regions and subspecies, but it is impossible to identify with certainty an individual. Even the locality of capture will not give any certainty about its identification, as it has been shown that many European pond turtles have been and still are being moved throughout its distribution range and release in “wrong” regions. The differences between the different subspecies/haplotypes are not only genetic, but also morphologic and behavioural. There are some indices that hybrid or allochthonous have less chance to survival in the wild, underlining the importance to treat the genetic aspect of the captive European pond turtles seriously. Zoos should therefore test their animals, either to breed pure offspring or to offer their pure animals to institutions that want to breed pure animals for release projects.

Figure 3: Two adult European pond turtles. © Parc Animalier de Sainte Croix

1.2 Morphology

1.2.1 Size and weight

The European pond turtle is a small turtle. Different adult sizes and weights are reported, with an average of 15cm (carapace length) and 550g for adult females and 12 cm and 330g for males, depending on the population considered (Zuffi & Ballisana, 1998; Zuffi, Odetti, & Meozzi, 1999; Zuffi M. , 2004; Auer & Taskavak, 2004; Mazanaeva & Orlova, 2004). Usually turtles from the south-western populations are smaller than the ones from the north-eastern populations (Bonin, Devaux, & Dupré, 2006). For example, female turtles in western Poland were on average 17.1cm and 790 g and males 15.4cm and 554g (Najbar & Szuszkiewicz, 2006), while in the Central Polish population the mean straight carapace length was 18.18cm for females and 16.35cm for males with a body mass or respectively 970g and 670g (Mitrus & Zemanek, 2004). Females are rarely more than 21 cm (Bonin, Devaux, & Dupré, 2006), and reproductive females in Italy are only 12 cm (Zuffi M. , 2000)

Size and weight are important to consider as there is a correlation between body size and clutch size (Zuffi, Odetti, & Meozzi, 1999). It can also give a good indication of the health status of an individual (Benoit Quintard, pers. comm.).

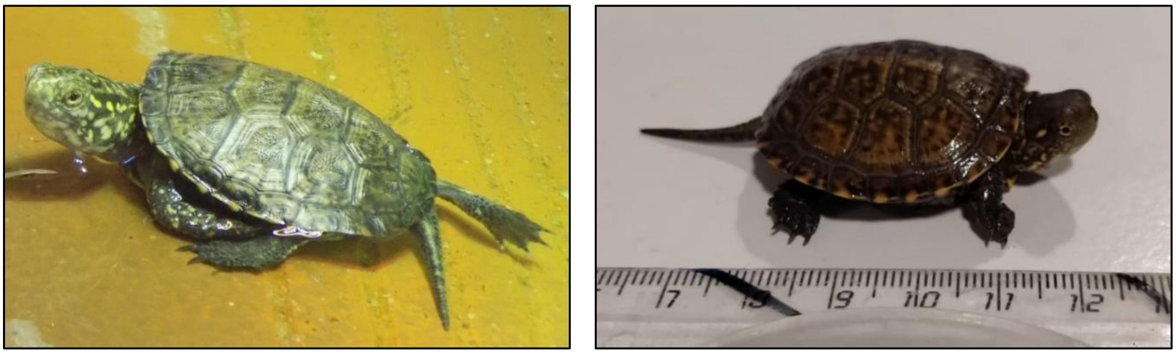
Figures 4 and 5: Adult Emys orbicularis. *© Parc Animalier de Sainte Croix*

The eggs are around 30mm long and 20mm width (Zuffi, Odetti, & Meozzi, 1999; Mazanaeva & Orlova, 2004; Avanzi & Millfanti, 2003; Bonin, Devaux, & Dupré, 2006) and in contrast with body size, egg size does not vary within or between populations (Zuffi M. , 2004).

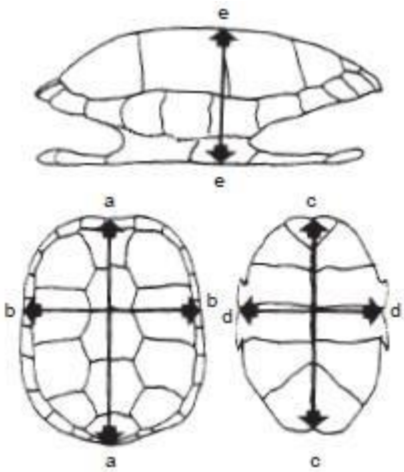
Hatchlings are around 26mm long and weight around five grams with natural incubation (Mazanaeva & Orlova, 2004; Bonin, Devaux, & Dupré, 2006; Rössler, 2000; Mitrus, 2000; Servan & Pieau, 1984) but only three grams in artificial incubation (Mazanaeva & Orlova, 2004) or 5 grams after artificial incubation of eggs collected in the wild (Mitrus, 2000).

Captive bred turtles may grow faster than wild turtles. In La Réserve de la Haute Touche, the one-year old captive-born turtles weighted approximatively 40g (Vallet, 2011), compared with the one-year old wild-born weights of 11.8g (Servan & Pieau, 1984). Mitrus reports that 9 month old captive born hatchlings weighted 15 to 21 grams (Mitrus, 2000). Skipping hibernation may result in a faster growth of the hatchlings. In captivity, turtles can reach 125g when they are only two years (Vallet, 2011). If no alterations in morphology, physiology and behaviour due to this quick growth can be observed, the quick growth in captivity can be positively used in reintroduction programmes, as predation being reduced in larger turtles (Vallet, 2011).

Zuffi and Ballasina showed that in captive conditions individuals of some populations grow faster when kept at higher temperatures, while individuals of other populations seem to suffer from these environmental conditions (Zuffi & Ballisana, 1998).



Figures 6 and 7: Hatchlings of Emys orbicularis. *© Zoodyssée*



Dorsal view (bottom left): a—a, carapace length; b—b, carapace width. Ventral view (bottom right): c—c, plastron length; d—d, plastron width. Lateral view (top): e—e, carapace height. Shape of carapace was redrawn from Lanza (1983) and Zuffi & Gariboldi (1995*b*), modified.

Figure 8: Carapace shape and measurement of Emys orbicularis *(Zuffi, Odetti, & Meozzi, 1999)*

1.2.2 Coloration

The European pond turtle is a polytypic taxon (Bonin, Devaux, & Dupré, 2006). Depending on age, subspecies and geographic distribution, the colour is olive-dark green to black with yellow spots more or less pronounced on the shell and skin (Avanzi & Millfanti, 2003). When the turtle becomes older, the spots can be less visible and the turtle appears to be uniform green, grey or black (Bonin, Devaux, & Dupré, 2006). The yellow spots and its long tail gave its name to this turtle. *Emys* meaning mouse for its long tail and *orbicularis* for the shape of the spots. The juvenile colour is brighter, and spots become visible only at around two or three years old. Indentations are noticeable on the marginal scutes (Bonin, Devaux, & Dupré, 2006).

Growth rings are visible on scales. They are sometimes considered as a useful tool for age determination in young turtles up to an age of approximately 18 years, but in older individuals, the shell shows a progressive loss of growth rings (Schneeweiß, 2004a). Furthermore, observations on wild animals in Spain showed that animals may not form each year a growth ring and that rings can fade away (Keller, Andreu, & Ramo, 1998). Therefore, this method is not completely reliable and should be handled with care.

Figure 9: Two adult European pond turtles with different colour pattern. © Parc animalier Sainte Croix

1.2.3 Sexual dimorphism

Females have a faster growth than males and once adult they are significantly bigger than males (Zuffi M. , 2004). Males have the posterior part of the plastron slightly convex, while this is flat in the females. The nails of males are longer and slightly hooked. The tail of the males is longer and wider than in the females, with a more distant cloacal opening (Avanzi & Millfanti, 2003).

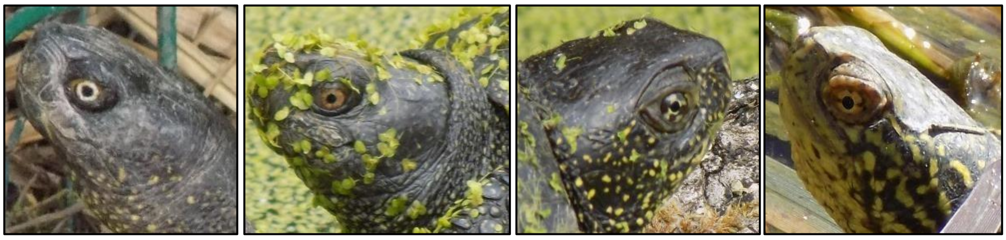


Figure 10: Different iris colours in Emys orbicularis*. © L. Berthomieu*

Iris colour can sometimes be used in sex identification, but there are also differences between subspecies. Juveniles have a light-yellow iris colour. Depending on the subspecies, mature females are thought to have a white or yellow iris, mature males a light brownish to red iris (Auer & Taskavak, 2004; Bonin, Devaux, & Dupré, 2006). It has also been suggested that the change of eye-colour in the male is related to sexual activity (Najbar & Szuszkiewicz, 2006).

1.3 Longevity

Three categories of ages can be defined: hatchling (0-1 years old), juveniles and adults. A turtle is considered as adult when it reaches its sexual maturity (see Chapter 1.6 for the age of the sexual maturity). In the wild ages of up to 120 years have been recorded (Jablonski & Jablonska, 1998). In higher latitudes, mortality of the turtles is highest during overwintering (Mitrus & Zemanek, 2004).

Aging turtles

Polish researchers Jablonski and Jablonska (1998) encountered during their studies females that had remains of dates engraved on their carapace. The engravings had been made by inhabitants of local villages, who captured animals on their way to or from nesting sites. They succeeded in finding some of these people, now very old, who used to mark turtles when they were young and pasturing cows. This taught us that turtles may use the same nesting sites for more than 70 years! The oldest females had the date 1892 on their back. Based on the size of the engravings, the animals must have been at least 20 years when being engraved, making them at least 120 years when recaptured!

Citizen science avant la lettre.

1.4 Distribution and conservation

1.4 1 Geographical distribution

The current distribution range is indicated in *Figure 2*. Archaeological findings show that the original distribution range was much larger, but postglacial climatic change caused extinction in many countries (the Netherlands, Belgium, the United Kingdom, Sweden and possibly others), as temperatures became too low for breeding (van Wijngaarden-Bakker, 1999) and the steppe-like vegetation in the early Holocene gradually changed into forest, making the habitat unsuitable for nesting of turtles (Fritz, 1998). But the changes in climate cannot be the only explanation for the extinction in many parts of its range (Fritz, 1998). Archaeological findings show that European pond turtles have been a human food source for thousands of years (Fritz, 1998), but increased human activities had also a large impact on the habitat of the European pond turtles.

E. orbicularis is found in semi-natural lentic (flowing) and lotic (stagnant) waters (Puky, Gemesi, & Schad, 2004). The species prefers calm waters with silty bottom such as ponds, rivers and swamps, but can also be found in canals and other artificial water bodies (Servan J. , 1991; Lebboroni & Chelazzi, 1991) (Puky, Gemesi, & Schad, 2004; Zuffi, M, & Foschi, 2004; Thienpont, 2005; Bonin, Devaux, & Dupré, 2006). Viable populations exist even in forest ponds at high elevations (Puky, Gemesi, & Schad, 2004) and in brackish water (Mazanaeva & Orlova, 2004). Location can change with season; in one study the turtles are observed during spring in freshwater swamps, while during summer they estivate or move to irrigation canals (Ottonello, Salvidio, & Rosecchi, 2005).
Spatial distribution seems to be related to aquatic vegetation, offering protection and food (Lebboroni & Chelazzi, 1991). It can be found in areas with abundant floating aquatic vegetation (*Potamogeton spec., Nymphea alba, Spirodela polyrrhiza, Typha latifolia* and *Acorus calamus*) (Meeske, 1999) and reed beds (Servan J. , 1991; Thienpont, 2005).
Species using upland environment only for nesting are generally regarded as strictly aquatic species. However, some authors consider *E. orbicularis* as a semi-aquatic species (Ficetola & De Bernardi, 2006) as they use the upland not only for nesting but also for hunting (terrestrial items in the turtle diet), aestivation, hibernation or travelling. Therefore, quality of upland habitat is as important as wetlands in conservation programmes.

1.4 2 Threats

Throughout the distribution area, the threats reported by authors (Kotenko, 2004; Puky, Gemesi, & Schad, 2004; Thienpont, Cadi, Quesada, & Cheylan, 2004; Cordero-Rivera & Ayres Fernandez, 2004; Broggi & Grillitsch, 2012; Cheylan & Poitevin, 1998) on European pond turtle populations are more or less similar:

- Loss, degradation and fragmentation of their habitats
    - draining of ponds
    - changes in the ecosystem of ponds due to water loss for irrigation
    - water and soil pollution
    - increased extinction risk due to small population size
    - conflicts with current pond management practices: for example, the willow *Salix cinerae*, often present in the most important overwintering microhabitat, is frequently cut over winter. Moreover, often the water level is lowered in winter. Both seriously threaten the survival of overwintering pond turtles
    - fire

- Introduction of exotic species (predators as the black-bass or competitors as the pond slider)
- Hybridisation due to translocation/introduction of *E. orbicularis* of other origins
- Accidental death
    - accidental capture and death in fish and rodent traps
    - traffic accidents
    - recreational fishing (Nemos, Cadi, & Thienpont, 2004)
- Harvesting turtles for commercial purposes (for food, to make ashtray of the shells, to sell turtles as pets). As recently as 2012, one batch of 1327 animals was confiscated in Serbia (Nikolic & Golubovic, 2017)!
- Direct disturbance (e.g: increased recreational use of water bodies and their banks, people and dogs trampling soil and swimming)

Solutions proposed rely on the creation of a network of protected wetlands, control of exotic turtles, implementation of environmental education programmes, research and study programmes and the use of ex-situ programmes to reinforce or recreate populations.

Competition with slider turtles (*Trachemys scripta*) and other exotic turtles has often been mentioned as a possible threat for the European pond turtle. Although the severity of the threat is not completely known, there are several indications that the exotic species could harm *E. orbicularis* through:
- Competition for basking sites (Cadi & Joly, 2000)
- Transmission of (exotic) diseases (Jablonski, Mrocek, Gruľa, & Christophoryová, 2017)
- Competition for food resources (Arvy & Servan, 1998)

1.4.3 Conservation status

The conservation status of *Emys orbicularis* on the international red list of IUCN (International Union for Conservation of the Nature) is Near Threatened for Europe (van Dijk & Sindaco, 2004) and Vulnerable for the EU (Cox, 2009) , meaning that the species depends on conservation efforts to prevent becoming threatened. Conservation status on national red lists depends on the country and ranges from “Least concern” in France, Spain or Croatia to “Critically endangered” in Germany, Austria or Ukraine (note: some countries do not have a Red List for reptiles, or information on national protection could not be found).
The European pond turtle is not a CITES species, but it is listed in the annex II of Bern Convention (1979) and in the annexes II and IV of the European Directive 92/43/CEE « Habitats-Fauna and Flora ». In addition, some countries protect this species and its habitat with national laws (see *Table 2*).

Country	National Red List Status	Protection by national laws
Spain	Vulnerable/Endangered	Yes
Portugal	Endangered	Yes
France	Least concern	Yes (Arrêté du 19 novembre 2007)
Switzerland	Critically endangered	Yes (Ordonnance Fédérale sur la Protection de la Nature et du Paysage)
Germany	Critically endangered	Yes (Federal nature conservation law (2002))
Austria	Critically endangered	Yes (In 6 federal states)
Czech Republic	Critically endangered	Yes (Law 114/1992)
Slovenia	Endangered	Yes (Decree on Protected Wild Animal Species 57/1993)
Macedonia	Vulnerable	Yes
Poland	Endangered	Yes
Hungary	Near threatened	Yes
Slovakia	Critically endangered	Yes (Law 543/2002, Ordinance of Department of Environment 24/2003
Albania	Near threatened	No
Serbia	?	Yes
Croatia	Near threatened	Yes, strictly protected species (Official gazette 99/09)
Greece	?	Yes (Presidential Degree 67/1981)
Romania	?	Yes (Law no. 49/2011)
Lithuania	?	Yes
Latvia	?	Yes
Bulgaria	Lower risk/Near threatened	Yes (Biodiversity Protection Act of Bulgaria, 2002)
Ukraine	Critically endangered	Yes
Belarus	Near Threaten	?
Turkey	Vulnerable	Yes (Land and Hunting Law but no protectives measures)
Russia	?	?

Table 2 : National conservation status and law protection in home range countries

Several countries have implemented conservation actions for the survival of the species. These actions may include research and reintroductions/reinforcements.

In France, conservation projects are managed through a National Action Plan and include research (distribution, ecology, dispersion, genetics, pathology, impact of invasive species), conservation activities (habitat protection, creation of connectivity, reintroduction, capacity building of governmental parties) and communication (education of general public, capacity building of all actors involved in the conservation of the European pond turtle, sharing of literature) (Anonymous, 2020). In Poland, eggs are collected and incubated in captivity, or hatchlings are collected to be reared during their first winter in aqua-terrariums to increase survival rates (Maciantowicz and Najbar, 2004; Mitrus and Zemanek, 1998). In Spain, Switzerland, Italy, Hungary, Portugal and Germany conservation actions include, depending on the country, the restoration of water bodies, the control of exotic species, protection of remaining habitat, protection of nests, education, reintroduction and population reinforcements with captive bred or free-ranging animals and communication (Lacomba Andueza et al., 2004; Mitrus and Zemanek, 1998; Quesada, 1999, Martinez Silvestre and Velarde, 2017).

1.5 Diet and feeding behaviour

For a long time *E. orbicularis* was regarded as a strictly carnivorous species, feeding on insects, crustaceans, molluscs, worms, salamanders, frogs, and fishes (Ficetola & De Bernardi, 2006; Ciçek & Ayaz, 2011). Vegetable matter found in faeces were considered as eaten by mistake. However, recent studies show that the European pond turtle is an opportunistic predator, its feeding regimes ranging from carnivorous to omnivorous depending on environmental conditions (Ottonello, Salvidio, & Rosecchi, 2005). A study on the Iberian population of European pond turtle demonstrates the use of water lily seeds (*Nymphea alba*) as an emergent food resource during summer. This water lily fruit consumption was observed in all age classes (Calvino-Cancela & Cordero-Rivera, 2010). The same authors have observed individuals eating flowers and petals of water-lilies and leaves of the common cattail (*Typha latifolia*). In this study nearly 100% of the diet of the turtles was vegetable. They explain that the European pond turtle is able to shift its diet to an almost complete vegetarian diet during summer months. Their hypothesis is that summer is a period with declining activity, so energy requirements are lower. Consequently, even though plants have a lower energy value as they are easy to obtain, they are favourite by turtles.

Studies in the French Camargue (Ottonello, Salvidio, & Rosecchi, 2005) and in Turkey (Ciçek & Ayaz, 2011) show that the European pond turtle is an opportunistic predator, with a diet ranging from mostly carnivorous to omnivorous. In the French Camargue, plants were present in 89% of adults' and 25% of juveniles' faeces (Ottonello, Salvidio, & Rosecchi, 2005). These authors observed a difference in the abundance of plants in turtle diets depending on the reproductive or the post-reproductive state. During spring, turtles are usually in fresh water marshes and their diet is mostly carnivorous, but during summer - when marshes dry out and the animals move to irrigation canals - the diet turns to be more omnivorous with an increase of plant matter and terrestrial invertebrate consumption. It is possible that the change in diet reflects seasonal availability of food items. This seasonally is also observed in Turkey (Ciçek & Ayaz, 2011). Based on an analysis of stomach contents, *E. orbicularis* should be regarded as carnivorous during breeding season and omnivorous during post-breeding, as they observed between breeding and post-breeding season an increase from 4.4% to 22.2% in plant consumption (especially seeds and roots). Insects and other invertebrates were the major part of the diet during breeding season, while vertebrate (fish and amphibians) and plant material are the main food item during post-breeding season. In Italy, plants were also present in a majority (63%) of analysed faeces, with a dominance of watermilfoil (*Myriophyllum*). The authors even conclude that this plant is really important as shelter and food resource and explains the distribution of the turtles in this area (Lebboroni & Chelazzi, 1991).

The plant material observed in the gastric content in Turkey were *Typha angustifolia, Phragmites australis, Juncus* sp., *Carex* sp. and *Potamogeton* sp. Roots and seeds of aquatic forms are the major plant material eaten (Ciçek & Ayaz, 2011). Additionally to this plant material, the diet was also composed of gastropods, earthworms, insects, fish and amphibians.

The intestinal tracts of 14 adult *E. orbicularis* captured in the Ukraine contained a large variety of vertebrate and invertebrate parts (Kotenko, 2000). Although plant rests were found, most plant material was supposed to have been swallowed incidentally, with the exception of shoots of reed, leaves, stems and roots of other vascular plants. Turtles were also observed to nibble on dead (shot) birds.

Invertebrates are an important part of *E. orbicularis'* diet. In the Camargue, aquatic invertebrates such as Coleoptera (beetles), Decapoda (crustaceans), Odonata (dragonflies), Gastropoda (snails) and Heteroptera (bugs) were the main preys (Ottonello, Salvidio, & Rosecchi, 2005). This was also true for the turtles in Italy (see table 3) (Lebboroni & Chelazzi, 1991). In juveniles, the dragonfly-nymph is the major item found in faeces. Terrestrial and flying invertebrates such as adult Libellulidae (dragonflies), Coenagrionidae (damselflies), Araneae (spiders) and Formicidae (ants) and termites are an important part of the diet, as can be amphibian larvae and crustaceans (e.g. American crayfish (Procambarus clarkii)) was observed (Lebboroni & Chelazzi, 1991; Kotenko, 2000; Ottonello, Salvidio, & Rosecchi, 2005; Calvino-Cancela & Cordero-Rivera, 2010).

Invertebrate type	Presence in faeces
Diptera larva	36%
Odonata – nymph	59%
Odonate – other juvenile life stages	14%
Aquatic insects	27%
Terrestrial insects	72%
Unidentified insects	40%
Araneae	27%

Table 3 : Presence of invertebrates in faeces (Lebboroni & Chelazzi, 1991).

As well as *E. orbicularis* was considered strictly carnivorous, it was also considered to be strictly aquatic. A species is regarded as strictly aquatic if it uses upland environment only for nesting. But we should rather considered *E. orbicularis* as a semi-aquatic species, as it hunts as well in water as on land (Matz & Vanderhaege, 2004; Ciçek & Ayaz, 2011). Terrestrial prey items such as adult Odonata, Anisolabididae, Cicadidae, adult Hymenoptera, Formicidae, Cerambycidae, Staphylinidae, Muscidae and Culicidae are also found in stomach contents (in 8.7% of samples), land is used for basking and European pond turtles are known to travel considerable distances overland between water bodies.

1.6 Reproduction

1.6.1 Sexual maturity

Sexual maturity is usually linked to individual size. Males are sexually mature when they are approximately 12cm and females when they reach 15cm (Matz & Vanderhaege, 2004). In the Ukraine sexual mature females were slightly smaller (Kotenko, 2000). Fritz compared growth and age of sexual maturity in different parts of the distribution range and showed that individuals from northern populations, where weather is cooler, reach a certain size and thus sexual maturity later than animals of the southern populations (Fritz, 1998).

Country	Age in years	Source
Russia	6-9	(Mazanaeva & Orlova, 2004)
Northern Europe	10-12	(Rogner, 2009)
Ukraine	Females: 5-6	(Kotenko, 2000)
Poland	Males: 11 / Females: 15	(Mitrus & Zemanek, 2004)
France	Males: 8-9 / Females: 11-12	(Baron & Duguy, 2000)
Spain	Males: 4-5 / Females: 6-7	(Keller, Andreu, & Ramo, 1998)
Italy	Males: 7-8 / Females: 11-12	(Zuffi, M, & Foschi, 2004)

Table 4: Examples of estimated ages of sexual maturity depending on the distribution area.

1.6.2 Sex ratio

The sex ratio varies considerably between different *E. orbicularis* populations, and depending on the location the sex ratio is male or female biased (Cordero-Rivera & Ayres Fernandez, 2004). At one locality in Switzerland adult sex ratio was reported to be 1:1,47 (Mosimann & Cadi, 2004), while it was 1:4 in Turkey (Auer & Taskavak, 2004) and 1:2 in France (Servan, Zaborski, Dorizzi, & Pieau, 1989). These figures are based on captured animals, and it is very well possible than in certain periods of the year one of the sexes is more active than the other and therefore easier to capture.

In turtles, sex determination is temperature dependant. Artificial incubation protocols show that in *Emys orbicularis* the pivotal temperature to obtain a 50/50 sex ratio is 28.5°C (Pieau, 1982) with a very small tolerability around this temperature : at 28.3°C males are more numerous and at 28.7°C females are (Presteau, 2008a). However, in wild populations in north-eastern Germany, females predominate despite temperatures that rarely exceed 28.5°C in natural nests, and a genotypic sex determination under natural incubation conditions may be more important than soil temperature (Schneeweiß, 2004b). This had also been suggested in a study in France (Servan, Zaborski, Dorizzi, & Pieau, 1989). Jablonski and Jablonska (1998) argue that there is a majority of females (1:4), caused by higher temperatures at the upper part of the nest. Due to these higher temperatures the upper embryos not only develop into females, but also hatch earlier. When leaving the nest, the lower eggs (not yet ready to hatch) are left uncovered and exposed to predators (Jablonski & Jablonska, 1998).

1.6.3 Reproduction characteristics

Freshwater turtles such as *E. orbicularis* have a reproductive strategy with few offspring (small number of eggs in each clutch) and a long life span (Servan J. , 1986).

Mating is mostly performed in spring and early summer, with a very low occurrence in autumn (Lebboroni & Chelazzi, 1991). The mating period peak is April-May (Baron & Duguy, 2000; Novotny, Danko, & Havas, 2004). The laying period lasts from May to July (Matz & Vanderhaege, 2004) (Bonin, Devaux, & Dupré, 2006). Young turtles hatch after 2.5-3months later from July to September (Mazanaeva & Orlova, 2004). For late disposed clutches or clutches produced in a relatively cold fall, the hatchlings may hibernate in the nest chamber and emerge next spring (Servan, Zaborski, Dorizzi, & Pieau, 1989; Andreas & Paul, 1998; Novotny, Danko, & Havas, 2004; Rössler, 2000). However, during very cold winters the nest may not provide enough protection and many hibernating offspring may die (Schneeweiß, 2004b).

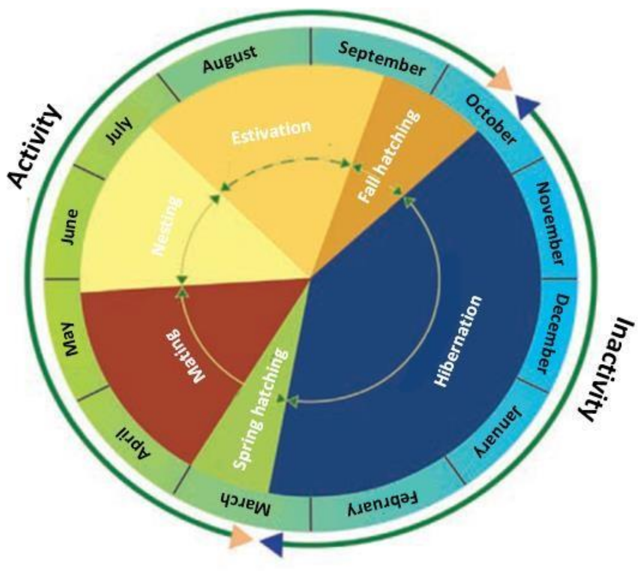


Figure 11: Annual life cycle in Emys orbicularis *(adapted from Cistude Nature).*

1.6.4 Nesting area characteristics

Substrate

The nesting area is usually located on well drained sites (Schneeweiß, 2004b), with substrates that are easily capable of being dug (Rogner, 2009). Different reported substrates are sandy soil (Lebboroni & Chelazzi, 1991; Schneeweiß, 2004b; Zinenko, 2004), sandy soil with silt (Mazanaeva & Orlova, 2004), silty-clay (Lebboroni & Chelazzi, 1991) or fine-grained gravel (Mazanaeva & Orlova, 2004; Thienpont, 2005). The substrate should allow a good oxygenation of the eggs.

The main feature of a nesting site may be that it has a high degree of surface heating. Therefore the vegetation on the nesting area is usually sparse, like steppe-meadow vegetation with some bushes (Jablonski & Jablonska, 1998; Schneeweiß & Steinhaurer, 1998; Lebboroni & Chelazzi, 1991; Lyet & Cheylan, 2001; Zinenko, 2004).

Location

Nesting areas are usually situated close to basking sites, but can be hundred meters from the pond or the riverbanks (Zuffi M. , 2004; Mazanaeva & Orlova, 2004) in areas which will not be flooded (Rogner, 2009). When an adequate site is not available, females can travel several kilometres to find a suitable nesting area (Jablonski & Jablonska, 1998).

Exposure

Nesting sites are more often situated on south-facing slopes (Schneeweiß, 2004b; Zinenko, 2004; Zuffi M. , 2004). Some are on horizontal grounds or on east facing slopes. The south and east exposition improves the insolation rate and thus the incubation conditions for the clutches (Novotny, Danko, & Havas, 2004). Sun exposure should be not less than 5 to 6 hours (Mazanaeva & Orlova, 2004) and a minimum of 600 sunny hours is necessary for embryo development (Anonymous, 2004).

The quest for a nest

The nesting behaviour of *Emys orbicularis* is one of the many amazing features that makes this species so special and interesting. Good nesting sites are often rare and used for many generations (centuries?) by a population of pond turtles. The distance between the breeding grounds and the water ranges from several dozens of meters to more than four kilometres, and the journey can take several days. Females first move along water courses as close as possible to the nesting place. They start their overland trip at dust. The females seem to use the shortest way, making use of forest roads. If disturbed, they may hide under leaves or branches or even dig a hole before continuing their way. When temperature drops under 8°C, all females stop their journey and hide under leaves, until temperature rises again next day.

Once arrived on the nesting site, the female tries to find the best place for deposing her eggs. She starts digging her nest with her hind legs, the front ones being planted on the ground. If necessary, she wets the soil with cloacal bladder water. Using hind legs alternately, she scoops out a broad, pear-shaped cavity of appr. 8cm wide and 11cm deep, with a narrow vertical opening. The eggs are laid and the female fills up the nest with earth, beats the earth and masks it with plant material that she collects nearby.

And then the journey back to the water starts…

1.6.5 Egg laying characteristics

Turtles lay an average of ten eggs, the number ranging from 3 to 16; clutch size differs per region (see table 5) and may also depend on the age and physical condition of the female (Rogner, 2009). In Austria, clutch size was positively corelated to the size of the female (Rössler, 2000).

When females lay multiple clutches, time between two egg depositions was around 34 days in a study in Italy (Zuffi & Odetti, 1998). The authors suggest that within a population there is a relationship between body size and frequency of reproduction and between populations there is an inverse relationship between latitude and clutch frequency. Usually the northern population lay a single large clutch a year (Zuffi M. , 2004). One hypothesis is that smaller females (which is generally the case in southern populations) lay smaller clutches and to compensate the deficit they lay multiple clutches yearly instead of one large clutch, resulting eventually in a similar reproductive outcome. However, female turtles in Austria were relatively large with and average size of 17.6cm, but multiple clutches were common (Rössler, 2000). In Austria the time between two egg depositions was 23 to 27 days (Rössler, 2000).

The size of the female may also have consequences on hatchling weight: the larger female, the heavier the hatchlings (Molmy, 2013).

Country	**Nesting period**	**Average clutch size (min – max)**	**Number of clutches per year**	**Incubation time (days)**	**Source**
Slovakia	Mid-May to the end of June	12.2 (6-16)	2	74-89	(Novotny, Danko, & Havas, 2004)
Ukraine	End of May to middle of July	9-11 (6-22)	?	?	(Zinenko, 2004)
Ukraine	June to first half of July	10.1 (6-16)	1 or 2	47-150	(Kotenko, 2004)
Ukraine	June to first half of July	6-9 (5-16)	1	55-65	(Szczerbak, 1998)
France	End of May to end of June		1-3		(Servan & Roy, 2004)
Russia	Mid-May to end of July	5.5 (2-11)	3	90-110	(Mazanaeva & Orlova, 2004)
Poland	Mid-May to June	14.4 (7-23)	1	85-113	(Mitrus & Zemanek, 2000)
Germany	June	14.25 (10-18)	1	?	(Andreas & Paul, 1998)
Germany	Late May to mid-June	12.7 (8-18)	1	?	(Schneeweiß, Andreas, & Jendretzke, 1998)
Spain	May to July	7	2	83	(Diaz-Paniagua, et al., 2014)
Austria	End of May to first days of July	12.4 (8 – 17)	2	90-117	(Rössler, 2000)
Italy	May to July	4.9	1-2	80-90	(Zuffi M. , 2000)
Hungary	June, firsts days of July	6 (4-9)	1	?	(Farkas, 2000)
Hungary	June; second clutch end of July or early in August	12	2	?	(Farkas, 2000)
Dagestan (south)	Half May to the end of July	2-10	3	?	(Mazanaeva & Orlova, 2004)
Dagestan (central)	Half May to the end of July	3-13	2	90-110	(Mazanaeva & Orlova, 2004)

Table 5 : Egg laying characteristics.

1.6.6 Incubation time

Incubation time may depend on the average temperature in the nest (Rössler, 2000).

Year	Mean temperature (°C)	Maximum temperature (°C)	Incubation time (days)	First hatching
1997	22.6	32.2	98-117	13th of September
1998	23.5	34.1	90-108	1st of September
1999	22.6	32.7	91-98	30st of August

Table 6: Temperature of nest and incubation time (Rössler, 2000).

1.6.7 Nesting behaviour

Egg laying usually occurs in the late afternoon or early evening (Mazanaeva & Orlova, 2004; Cadi, Nemoz, Thienpont, & Joly, 2004; Novotny, Danko, & Havas, 2004) and lasts about 1.5-2 hours (Mazanaeva & Orlova, 2004). The female digs a roughly jug-shaped nest of an overall depth of 10-12cm with their hind limbs. She carries water in her bladder that she can use to loosen the substrate. The opening is elliptically shaped and has a width of approximatively six centimetres, then widens into a chamber (Novotny, Danko, & Havas, 2004). If the female is disturbed, the laying can be incomplete (Anonymous, 2004). When the laying is ended, the female closes the entrance, the substrate being moistened to create a plug.

1.6.8 Hatching success

In Austria, a hatching success of 77.3% was reported for 21 nests (Rössler, 2000).

1.6.9 Nest predation

The loss of eggs and hatchlings through predation can reach 85% (Zuffi M. , 2000) and approximately only 1% of the animals survive and become breeders (Anonymous, 2004). Usually, predation occurs within 48 hours after laying and the main predators are foxes, martens and badgers, but other animals as dogs, wild boars, crows, nightjars, other birds and even rodents may prey on eggs and hatchlings. Nests can be damaged because of mechanical compression of herbivorous (Zuffi M. , 2004). Fishes such as Northern Pikes (*Esox lucius*) can be predators once hatchlings reach water.

1.7 Behaviour

1.7.1 Basking

Most of the year basking is the major activity of European pond turtles, except from May to June when feeding becomes predominant (Lebboroni & Chelazzi, 1991). Basking is essential for the European pond turtle as it increases body temperature and thus activates metabolism.

Two kinds of basking can be observed (Meeske, 1999):

- Atmospheric basking: the turtle is lying above the water surface, at the shore or on an emerged trunk.
- Aquatic basking: the turtle is floating at the surface or lying in shallow water with part of the carapace above the water surface. This kind of basking mainly occurs at the end of June and in July, when daily temperatures are high (Meeske, 1999).

The basking area should have different characteristics: be suitable for thermoregulation, have enough inter-individual space to avoid conflicts between males and be protected from external disturbances

(Lebboroni & Chelazzi, 1991). The European pond turtle is shy and when disturbed it will hide under water, leaving its basking place (Daugey, 2005). Reproduction is influenced by stress hormone so a decrease in reproductive success can be observed in individuals with high levels of stress. It is therefore important that the turtles can find places with little disturbances. Selection of a basking place results from a trade-off between heat gain and risk avoidance (Cadi & Joly, 2003). In Brenne (France), European pond turtles bask in reed areas where large helophytes (*Phragmites australis*) shelter them from the wind and therewith create a warmer area. The turtles have also been reported to bask on emerged branches, abandoned bird nests, broadleaf cattail (*Typha latifolia*) and muskrat nest (Servan J. , 1991). European pond turtles show fidelity to the basking area, and juveniles smaller than 8 cm basked separately from adults (Lebboroni & Chelazzi, 1991; Meeske, 1999).
Basking occurs when air temperature is more than 14°C and cloud coverage under 50%. Differences in behaviour between subadults and adults were observed, basking bouts being shorter for the former (an average of 30 minutes compared with one to four bouts for adults. The different basking patterns could be explained by an age-depending thermoregulation strategy (Bresson, 2012).

1.7.2 Hibernation

The annual cycle of *Emys orbicularis* can be divided into two phases: an activity period from April to October and a lethargic to hibernation period from November to March. The onset and duration of these periods are influenced by climatic conditions (Cadi et al., 2004), but usually activity decreases quickly from late September onwards and the turtles became dormant during the first December week (Cadi, Nemoz, Thienpont, & Joly, 2004). Mazanaeva and Orlova (2004) described that most turtles disappear when air temperature drops to 6-7°C and water temperature to 3-4°C. As long as the water surface is not frozen, turtles are still surfacing for air. The turtles become dormant when the water surface gets ice-covered (Novotny, Danko, & Havas, 2004). Hatchlings may decide to hibernate in the nesting chamber when temperatures are too low for emergence. However, when ground temperature fall below -7°C during the winter, none of the hatchlings may survive (Schneeweiß & Jablonsky, 2000).

Characteristics of the hibernation area
European pond turtles hibernate mostly under water, but terrestrial hibernation is reported. The turtles concentrate in groups of 20-30 individuals in a small water area for hibernation (Thienpont, Cadi, Quesada, & Cheylan, 2004; Mazanaeva & Orlova, 2004). To reach good hibernating ponds, animals may travel hundreds of meters.
Several factors seem to be crucial for the choice of the hibernation area:

- Shallow water (Schneeweiß & Steinhaurer, 1998; Parde, Hurstel, & Lefevre, 2000).
- Dense vegetation cover offering protection against freezing. Overwintering sites were typically associated with three plant communities (S*alicion cinereae*, *Phragmition sp.* and *Magnocaririon sp.*) (Schneeweiß & Steinhaurer, 1998; Parde, Hurstel, & Lefevre, 2000; Thienpont, Cadi, Quesada, & Cheylan, 2004).
- Mud depth allowing the turtles to bury themselves when temperatures decrease. Turtles dig overwintering pits in the sand and silt among the rhizomes of reeds. The silt layer should be at least 30 centimetres to avoid the turtles to freeze (Mazanaeva & Orlova, 2004).
- Many decomposing dead leaves, providing stable thermal conditions.

Emergence

Timing of emergence from wintering depends on the locality and the climate. While the animals become already active at the second half of February in France (Duguy, 2000), in other regions it begins in the second half of March to early April (Lebboroni & Chelazzi, 1991; Mazanaeva & Orlova, 2004). The turtles spend a long time basking and become more and more active, with an activity peak in May. In the Mazanaeva and Orlova (2004) study, turtles become active when water temperature rises to 6-9°C and when the average daily temperature increases from 15°C to 20°C.

1.7.3 Locomotion

Two different aspects of locomotion should be considered: home range movements and long-distance movements for egg-laying or changing ponds.

Home range

Home ranges reported are 7.74+/- 3.63 ha for males and 12.51+/- 8.38 ha for females (Cadi, Nemoz, Thienpont, & Joly, 2004).

Long distance movements

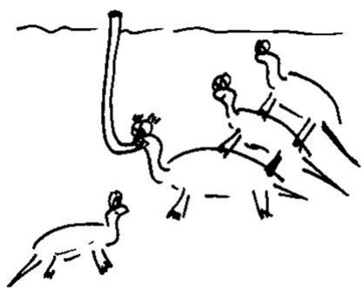
Males can travel far without returning their resident territory, allowing gene flow between populations, while females are more sedentary (Baron & Duguy, 2000). However, females can travel distances of several hundreds of meters to more than four kilometres to find a suitable nesting area (Kotenko, 2004; Novotny, Danko, & Havas, 2004; Thienpont, 2005). In some area, seasonal migrations are common. In the Ukraine, animals move in autumn from small and warm water bodies to lakes with springs that remain cold in the summer but where the water on the bottom in not freezing during winter (Szczerbak, 1998). In spring, the turtles migrate back to the ponds.

1.7.4 Social behaviour

The European pond turtle is a gregarious species, living in groups of 10 to 20 individuals (Bonin, Devaux, & Dupré, 2006). They can be observed basking in groups of up to five or six specimens (Meeske, 1999).

1.7.5 Sexual behaviour

In the European Pond Turtle mating is always aquatic. Males turn around female and bite them in the legs and neck. They climb on the female and grasp the female's shell with their hooked claws. The male can remain on the female for a long period (several hours). Sometimes several males climb on a female, which can lead to the drowning of the female (Lebboroni and Chelazzi, 1991 ; Bonin et al., 2006). During mating season, fights between males are frequent (Lebboroni & Chelazzi, 1991; Wolff, 2004; Bonin, Devaux, & Dupré, 2006).



Section 2: Management in Zoos and Aquariums

Enclosure

European pond turtles can be kept either indoors or outdoors. Considering that this is a European species, adapted to the climate in Europe, we recommend to keep the species in natural, outdoor ponds. However, in these Best Practice Guidelines we will also discuss the requirements for indoor enclosures, as some institutions may have important reasons to keep their animals indoors.

2.1 Enclosure for adult turtles

2.1.1 Small indoor enclosure (aqua-terrarium)

The use of an aqua-terrarium is not recommended for adult turtles, except for quarantine and animals that need special care. The aqua-terrarium can also be used to house hatchlings and juveniles younger than 3 years, at least during fall and winter (Avanzi & Millfanti, 2003; Rogner, 2009). Concerning specific recommendations for such animals, please refer to chapter 4.

Some institutions keep European pond turtles an indoor pond in a greenhouse. Besides the constant temperature, the major difference with an outdoor enclosure is the presence of windows or skylights between the sun and the animals. The use of UV-b transmissible windows and skylights such as acrylite PO-4 Acrylic Sheet or Polycast Solacryl SUVT (Boyer & Boyer, 2005) is recommended, otherwise artificial UV lights need to be added.

2.1.1.1 Boundary

For an aquaterrarium either glass or plastic can be used. The choice of material is important as it should be resistant to UV. The recommended dimensions are discussed below. For a large indoor enclosure, boundary recommendations are the same as for outdoor enclosure (see below).

2.1.1.2 Water

Tap water is generally a poor substitute for the naturally occurring acidic bacteria-laden water from which many freshwater reptiles are derived. Tap water contaminated with faeces forms a suitable medium for opportunistic pathogens, such as bacteria and fungi that can cause lesions. In a natural situation, the warm acidic-bacteria laden water forms a bioactive substrate which could interfere with the growth of pathogens. Therefore, it could be interesting recreating this bioactive medium by acidifying the water and providing organic material that maintain acid-living bacteria. Different solutions exist: diluted mixtures of tea, addition of peat to filters or the use of swamp mud and live plants. An alternative that seems to help fresh water reptiles avoiding skin lesions is the addition of some salt to the water (1 cup of table salt per 80L) (Rossi, 2005). Water quality is further discussed in the chapter on environment.

Substrate at the bottom of the aqua-terrarium is not necessary and makes cleaning more difficult (Rogner, 2009).

2.1.1.3 Furnishing

A land area is essential in an aquaterrarium, as turtles need to bask and dry and adult females need an emerged area compound of loose substrate so they can dig to lay eggs (Rogner, 2009). Even if males are not present, nesting areas should be provided for adult females to avoid egg binding. The minimum nesting area should approximatively be four to five times larger than the length of carapace (Boyer & Boyer, 2005). The substrate should be 15-25cm thick and 3cm above the water surface. Different substrates are suggested for aquatic turtles: sand, peat, soil (Matz & Vanderhaege, 2004; Wilke, 2009). Some herpetologists use bioactive substrate systems (BSS) for their reptiles (Rossi, 2005). The idea is to provide an environment where beneficial bacteria compete with pathogenic bacteria and fungi, but its use in aquatic turtle aquaterrariums has not yet been described in literature. Rocks can be placed near the water to avoid that the turtles drag substrate into the water (Matz & Vanderhaege, 2004).

This nesting area can also be used as a basking area. Different materials can be used to create basking areas: flat rocks resting on submerged bricks or a piece of wood, a floating piece of cork, a wooden or plastic floating platform, etc. Well-fixed branches or heavy trunks (e.g. *Sambucus nigra*) can be used by the animals for basking (Rogner, 2009).

In order to increase the volume of water usable by the turtles, the emerged area can be hung above the water surface, provided that the turtles cannot be trapped under it. The use of floating plants such as bamboo leaves, dry reed or aquatic plants are important to provide hiding places (Rogner, 2009; Wilke, 2009).

2.1.1.4 Maintenance: cleaning & disinfection

Because of faeces and food leftovers the aqua-terrarium can get dirty quickly. To keep the water clean, two solutions are possible:

Full water change

Depending on the number of animals, the recommended frequency of emptying is three times a week for an aqua-terrarium with 45 litters of water and once a week for an aqua-terrarium containing 230 litters. Abrupt change in water temperature can be harmful to the turtles, therefore the temperature after cleaning should be the same as before (Boyer & Boyer, 2005).

A solution to decrease the cleaning frequency is to feed the animal in a container, but this solution increases animal handling and can be stressful.

Filtration

An alternative to full water change is mechanical, biological and/or chemical filtration. The choice of the appropriate filter is important and should be adapted to the volume of the aquaterrarium. Insufficient filtration can be the cause of bacterial overgrowing and urea excess. One of the most common consequences of dirty water is shell infection (Boyer & Boyer, 2005).

An appropriate filter should filter one to three times an hour the volume of the aquarium (Wilke, 2009; Prestreau, 2008b). General small tropical fish filtration systems are useless because turtles produce much more waste than fish (Boyer & Boyer, 2005). Therefore, we recommend the use of aquatic turtle filters or filters designed for large fish (Koi) or high stocking densities. The best filters for turtles are large combined biological and mechanical filters, such as those designed for Koi fish, rapid sand filters, propeller driven fluidized bead filters and external canister filters. In addition, ozone filtration and UV sterilization can be used. For large setups, pumps designed to clean swimming pools can be used (Boyer & Boyer, 2005).

In order to avoid drowning, especially of youngsters, it is important to use a filtration system that does not create an important water flow (Rogner, 2009) or to protect the entrance of the pump with an inlet filter or a grid placed some centimetres before the inlet so turtles cannot be sucked against the entrance (Wilke, 2009).

An aquarium with aquatic turtles should not smell strongly; if otherwise, the cleaning protocol should be reassessed.

As disinfectants are not active on organic matter, a good cleaning of the aqua-terrarium and furnishing with hot water and detergent is necessary before disinfection. When cleaning the furnishings, avoid placing items from different aqua-terraria in the same sink, to avoid the spread of pathogens from group to group (some disinfectants do not work against some pathogens) (Rossi, 2005).
Disinfectants used should preferably have a broad spectrum of activity, be easy to use, be fast acting and without risk for the animals. It is important to follow the manufacturer's instructions, as too much dilution may render the product ineffective and it can be toxic when too concentrated. Chemicals should not be mixed, as some are not compatible and a mixture may be either ineffective or toxic. Contact time is the key to effectiveness and depends on the product. Disinfected surfaces and objects should be rinsed well after the action period. Disinfection of porous items such as wood is difficult; if they are suspected to be contaminated with pathogens they should be thrown away. Some materials such as plastic can catch compounds and release it later in the water, with toxics effects on the animals. Ammoniac and bleach are excellent, inexpensive and readily available products, but should not be mixed. Ammoniac can be rinsed with water. When using bleach, add a dechlorinator to the water or place the furnishing during a few hours into the sunlight before using it again for the turtles (Slomka-McFarland, 2006).
During maintenance procedures, turtles must be kept in an aquarium containing water with the same temperature as in their usual aqua-terrarium, with a basking area and some plants to hide.

2.1.1.5 Water quality
European pond turtles can be maintained in water with a neutral pH (Boyer and Boyer, 2005). Some holders keep their turtles in slightly to even strongly acidic water with pH down to around 5. This reduces the bacterial load and is safe to the turtles (anonymous reviewer).

2.1.1.6 The theory of lighting
Lighting can positively or negatively influence a reptiles' immune-neuro-endocrine network (Brames & Baines, 2007). When keeping European pond turtles outdoors, environmental lighting conditions are comparable to those in the wild (in most European countries). But when animals are kept indoors, lighting is an important management aspect to consider in order to keep the animals healthy. Sunlight spectrum is composed of visible, ultraviolet and infra-red radiation. Artificial lighting should provide a spectrum similar to sunlight.

Visible light
Many lamps are now available to illuminate reptile aqua-terraria, with a broad range of light spectrum. The assumption is often made that the lamp that emits light most similar to natural light is the most suitable. Actually, natural light is variable during seasons and days. It is recommended to use a light with a colour temperature of about 5000°K (Gehrmann, 2005). The majority of the lamps emit in the visible spectrum. Some can only be used for this purpose, while others have a broader spectrum in the UV range and can be used as UV source (see below).

There are many types (LED, HID, …) and vendors of these lamps (X-Reptile, Aquarium Systems, Arcadia, Solar Raptor, Exoterra, ..).

Ultra-violet

There are three bands of ultraviolet radiation within the UV spectrum : UVA (315-400nm), UVB (290-315nm) and UVC (100-290nm) (Adkins et al., 2003).

UVA radiation is important for reptile welfare. It is essential for some species in social behaviours, because reptiles use it to recognize conspecifics or food items. UVC is dangerous for all living beings and is cancerogenic (UVA and UVB also, but to a lesser extent). UVB radiation is essential for reptile health because it contributes to metabolism, reproductive cycles and appetite. UVB is a key part in vitamin D3 synthesis (necessary to convert 7-dehydrocholesterol into vitamin D) and consequently on calcium metabolism (vitamin D is metabolised in calcitriol). A vitamin D3 deficiency is the cause of metabolic bone disease (MBD). Because they are growing, juveniles are more sensitive to vitamin D3 deficiency, but problems can be observed in adults with long lasting deficiency. Louisville zoo succeeded to breed yellow-spotted Amazon river turtles under weak UVB irradiance without evidence of nutritional secondary hyperparathyroidism (Adkins, et al., 2003). However, long-term suboptimal lighting contributed to other problems such as ulcerative dermatitis. UV has other roles beyond vitamin D3 production : it modules the cutaneous immune system, increases pigment formation, induces production of endorphins and destroys some bacteria, fungi and viruses on the surface of the skin (Baines, et al., 2016).

When talking about reproductive success, UV is an important factor. Studies have shown that a deficiency in either vitamin D3 or calcium may result in non-viable egg production (Ferguson, et al., 2010) and hatching failure (Packard & Clark, 1996). Because of increased need in calcium for egg production, reproductive females need sufficient UVB and calcium intake to avoid metabolic bone disease. However, UVB radiation can also have detrimental effects and can be deleterious if the turtle is overexposed, leading to skin and eyes damage, reproductive failure and even death (Adkins, et al., 2003; Baines, et al., 2016).

In summary, UV is essential for the captive maintenance and breeding of many chelonians.

It should be noted that the best and most balanced source of ultraviolet light is the sun, when possible access to natural sunlight is preferred (BIAZA-RAWG, 2015). Access to natural sunlight can be an important factor to maintain health and increase successful reproduction. Basking temperature and ambient temperature must be correct to ensure basking behaviour and so UV exposition (Baines et al., 2016). For *Emys orbicularis* individuals kept indoor we recommend the use of artificial UV lighting, which is not necessary if the European pond turtles are housed at least 6 months outside (Rogner, 2009).

In nature, UV irradiance and exposure changes continuously, depending on numerous factors such as sun position and animal behaviour. In addition, it is important to know the range of irradiance appropriate for the species and the gradients created by the artificial lighting product to produce the desired effect (Baines, et al., 2016). Ferguson et al. (2010) allocated species in different groups depending on their basking behaviour: Ferguson zones or UVB zones. *Emys orbicularis* is considered as a type 3 species, meaning an open or partial sun basker and thermo-regulator. It is important to provide a UV gradient to the captive animal that enables the animal to self-regulate its exposure from zero (full shade) to the maximum indicated for that zone. As most (or all) artificial sources of light lose some value over time, they must be checked regularly and changed if necessary. Whatever the source chosen, we recommend checking regularly how much UV the reptiles receive by using a UV meter. Different meters exist, we recommend to use the Solarmeter 6.5 UV index Meter (UVI) or the ZooMed Digital UV Index Radiometer (UVI).

2.1.1.7 Lighting in practice

Providing suitable lighting for reptiles is not easy and the knowledge on the best methods and materials is evolving constantly. Considering the fast development of this knowledge, it seems to be pointless to discuss in these guidelines old references that were based on outdated studies. Thence we advise zoos to monitor constantly the developments that are published in scientific journals and on professional websites.

The publication of Baines et all. (2016) can serve as a baseline for developing a good lighting management of an enclosure for European pond turtles. Other useful resources, that are constantly being updated and provide many references for further reading, are:

http://www.uvguide.co.uk/

https://www.facebook.com/groups/AdvancingHerpHusbandry/

https://www.facebook.com/groups/ReptileLighting/

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

Fortunately, most zoos keep European pond turtles in natural ponds outside, and no artificial lighting is necessary. The main exceptions are the rare zoos that keep their animals inside, and the zoos that rear hatchlings and juveniles inside.

Lamp choice

Be careful, the term “full spectrum” lamp is use wildly and some of these lamps do not emit UV radiation. Consequently, do not rely on this mention alone to choose your lamp (Gehrmann, 2005). The suitability of any light source is governed by two main features: its quality (the spectrum) and quantity (the irradiance received by the animal). Depending on the type of lamps, the UVB gradient will be very different (shape of the beam, output of the lamp) (Baines, et al., 2016). There are two major groups of lamps emitting UV: tubes and spots.

Fluorescent tubes

Fluorescent tubes emit low intensity visible light, little heat and uniform UVB gradients. Fluorescent tubes are ideal for providing UVB to all species in a small vivarium (Baines, et al., 2016). However, there are different types of fluorescent tubes. Some designed to provide UVA and UVB and others to simulate “daylight”, emitting UVA but only a very low level of UVB.
The power of a tube is proportional to its length. Its length should range from two-thirds to three-quarters of the length of the aqua-terrarium.

Tubes are not directly connected to 220V plug, a ballast is necessary. The power of the ballast should match tube power (1-2W difference is no problem, but not 10W) (Presteau, 2008a). Specific ballasts designed for aqua-terraria should be splash proof (rubber joints). Two types of ballasts exist, magnetic and electronic. The advantage of electronic ballasts compared with magnetic ones is that they increase energy efficiency and reduce flicker. Some ballasts are

designed to create an artificial "dawn" and "sunset". There is a large number of suppliers for both types of ballasts.
To increase UVB and visible light output, one can combine tubes or use a reflector. Thanks to a reflector it is possible to double the output of the tube. When combining a reflector with old tubes it is possible to use them longer.

Bulbs

- **Metal halide lamps** are particularly good sources of lighting, their extremely high output of visible light and suitable levels of UVB output makes them excellent simulators of sunlight. These lamps are currently recommended for reptiles.

Other types of lamps can be used to provide UVB to reptiles are :

- **Compact fluorescent lamps** which produce a more focused UVB output - than tubes, with a low intensity visible light and little heat
- **Mercury vapour flood lamps**: the UVB output is more intense and concentrated in a smaller area than tube. These lamps also produce heat and more intense visible light, albeit in a quite non-natural spectrum. They are not recommended.
- **Incandescent lamps** and **Halogen lamps** (either bulbs or tubes) can be used for heating, they may radiate a small amount of UVA and no UVB (Gehrmann, 2005), but only at very low levels.
- **LED lamps** alone are not suitable as the only light source, as they do not produce UVA and the reptiles do not see them as (natural) white light, but probably as strong coloured (pink?) light (Wunderlich, 2021).

Position/Distance

It's important to provide a suitable UV gradient in the captive animal's environment, in order allow them to self-regulate their exposure. Therefore, a full range of UVB should be provided from zero to the maximum UV Index for the species. The European pond turtle is considered to be a "type 3 species", for which this maximum ranges from 2.9 to 7.4 (Ferguson, et al., 2010). For aquatic turtles this gradient is usually present, the higher level is restricted to the basking area with a gradient to zero when the animal are under water.
Depending on the chosen lamp, the zone of adequate irradiance is different. The effective UV coverage needs to be at least as wide as the whole body of the animal. The lamps should always be positioned above the animal to have the eyes protected by the shade of the eyelids and the head (Baines, et al., 2016).

Although some manufacturers and scientific publications (Adkins, et al., 2003; Avanzi & Millfanti, 2003; Baines, et al., 2016; Presteau, 2008a) give indications of the ideal setup for the lamps, the only reliable method to establish the safe positioning and distance is by using an UV-meter.

Materials

In order to prevent thermal and UV burn, all bulbs must me inaccessible and/or protected with wire mesh. An important point to keep in mind is that every material located between the turtle and the UV source filter a part of the UVB. Wire-mesh between the lamp and the animal can decrease UV transmission by 40-50%, a plastic or glass top filters most or all the UV (Adkins, et al., 2003; Ferguson, et al., 2010). So the chosen mesh must maximise light and UV transmission. Air permeable materials are more desirable than solid ones to maximize UVB transmission. The UV transmission by different materials has been studied (Gehrmann, 2005; Prestreau, 2008b). When choosing glass to build your aqua-terrarium, do not forget that on top of UVB filtering some materials create a greenhouse effect. Therefore it should never be installed behind windows.

Control

Whatever the material chosen, we recommend checking how much UV the reptiles receive by using a UV meter. It is important to be able to measure the quantity of UV that permit vitamin D3 synthesis (295-315nm), for each type of material the filtration of the UV wave bands is different. For example, most UV-transmitting acrylics disproportionally reduce the shorter wavelength within the UVB band more than longer wavelength.

Different meters exist, we recommend to use the Solarmeter 6.5 UV index Meter (UVI) or the ZooMed Digital UV Index Radiometer (UVI).

UVB irradiance tolerances and requirements vary with species but also with age, reproduction and health. In addition, thermal preference can influence UVB exposure (Ferguson, et al., 2010). An animal can avoid an UVB source, or expose itself too much, if the UVB is not of an appropriate level, if the temperature at that location is too hot or too cold, or if the visible light is not suitable (Ferguson et al., 2010). Therefore it is important to monitor the animals' response to the used lighting, and to assure that temperature regulation in the enclosure is not connected to UVB exposure. UVB levels must be suitable, while the ambient temperature is also important as UVB radiation does not convert any vitamin D3 if the heat on the skin is too low.

All manufacturers recommend changing UV tubes after 10 months, when using it 12 hours a day. When the tube is new, the UV index is higher than the nominal value and it usually needs 4 to 6 weeks of use to reach this value. The tube keeps this nominal value approximatively 6 months, after which UV irradiance decreases. If the tube is changed too often, it can create an overexposure, while changing it too late can lead to UV deficiency. The industry standard "burning-in" period is 100h, and some users recommend a burning-in period before using new tube. Prestreau (2008a) explains that if buying the tube during spring, the decrease in UV output begin in September-October, which corresponds to natural conditions. Therefore a new tube should ideally be installed in March, when turtles wake up from hibernation, and can be used for one year. If this schedule is not used, the date of installation should be noted to remember the date it needs to be changed.

The use of an UV meter is the best solution to determine if changing the UV source is necessary.

To conclude this chapter, we need to emphasise that there is still much to learn about the requirements for UV, optimal use of artificial lamps and the way we need to provide UV to our animals. Requirements depend on age, sex and reproductive status (Adkins, et al., 2003), and probably even subspecies/haplotype. Even when following the previous recommendations, it is important to observe the animals, use a Solarmeter to monitor the UV levels and adapt parameters when necessary. Additional research, in the wild and captivity, is necessary to further understand the UV requirements of European pond turtles.

2.1.1.8 Photoperiod

Photoperiod is important for reptiles, as it has been shown to influence a variety of physiologic rhythms including thermoregulation and reproduction (Gehrmann, 2005). A poor management can result in reproductive failure or disease. The hypothesis is that inappropriate photoperiod and temperature fluctuations result in repeated reproductive failure as a result of abnormal vitellogenesis, with chronic resorption of yolk and ultimately ovarian granulomas or tumours. An abnormal photoperiod can also lead to obesity (Rossi, 2005).

Temperate-zone reptiles should be exposed to light during 15 hours during summer, 12 hours during spring and fall and nine hours during winter (Rossi, 2005) or more specifically for *Emys orbicularis* 14 hours during summer and 10 hours during winter (Baines, et al., 2016). But it is easier to consider the natural light cycle for the (sub-)species in its natural habitat (Adkins, et al., 2003; Prestreau, 2008b). Lighting should be increased gradually during the morning and decrease gradually in the evening. During the night, a weak intensity incandescent light is used to imitate moon light helping turtles to orientate (Rogner, 2009).

2.1.1.9 UV and dietary supplementation

One controversial issue concerning UV radiation in reptiles is that much literature states that UVB is unnecessary for species in which vitamin D is obtained through the diet, meaning carnivorous and insectivorous species. Therefore some herpeto-culturists do not use UV lighting and prefer to use a dietary source of vitamin D3 alone. However, much skill and experience is needed to ascertain a correct level of supplementation, as failure to provide adequate amounts of vitamin D3 results in metabolic bone disorder or hypervitaminosis. Furthermore, there are no quantitative studies determining optimal doses of dietary vitamin D3 for a reptile maintained without access to UVB. Ferguson et al. (2010) recommend that lizard and snake keepers rely primarily on UVB exposure for provisioning of vitamin D, and we follow this advice for European pond turtles.

2.1.1.10 Temperature

Physiologic processes of reptiles that are known to be thermally sensitive include metabolic rate, digestion, growth, cardiovascular function, acid-base regulation, water balance, reproduction, immune function and activities such as locomotion and prey acquisition (DeNardo, 2002). European pond turtles are, just like other reptiles, ectotherm animals, whose regulation of body temperature depends on external sources, such as sunlight or heated surfaces. Experience shows that maintaining most reptiles for prolonged periods at temperature ranging from 15-21°C is potentially harmful because it is too cold to allow normal digestion and immune system response but too warm to allow hibernation (Rossi, 2005).

Land area

For reptiles kept in inside enclosures it is important to create the possibility to choose the ideal temperature during each part of the day. For species of temperate zones, at the coldest spot the temperature should be 20°C, while there should be another spot of 24-25°C (Prestreau, 2008c), while other authors prefer higher temperatures (see Table 9). To provide the animals an optimal choice of temperatures, the heating lamp with visible and UV light should be located at one end of the terrarium and never in the centre, except in large indoor enclosure of more than 4m²

(Prestreau, 2008c). During the day the temperature at the basking area should ideally be 5-6°C higher than the water temperature (Prestreau, 2008b).

Basking area surface temperature	**Ambient temperature - day**		**Ambient temperature - night**	
	Summer	**Winter**	**Summer**	**winter**
35°C	25-30°C	3-7 °C	18°C	20-23°C

Table 7: Temperature recommendations for Emys orbicularis *(Baines et al., 2016).*

Heating lamp choice

Different lamps are available to create heat at the basking point:

- Mercury vapour and metal halide lamps
- Halogen flood lamps
- Incandescent light bulbs (50W to 100W)
- Infrared bulbs (with the exception of red lights)
- Quartz-halogen lamps, such as the type of orang-glow patio heaters
- Ceramic heating elements can be used to heat a room, but not for basking, as they produce the wrong wavelengths (Muryn, 2020)

Distance and position

The size of the optimum upper temperature zone needs to fit animal size (whole animal body must be included) (Baines et al., 2016) and the number of animals that need to bask. Minimal distances should be respected to avoid severe burnings and the use of a thermometer is highly recommended for checking the temperature at the hot spot. UVB and visible light gradient should be coordinated, therefore the UV and visible light sources should be placed close together or come from the same bulb simulating sunlight (Adkins, et al., 2003; Baines, et al., 2016). If the room is oriented to the South or East, be careful not to place the aqua-terrarium near a window because during summer the temperature inside would be too high (Prestreau, 2008c).

	Heating lamp		**UV tube**		**Visible light**
	Time slot	Duration of lighting	Time slot	Duration of lighting	Local lighting conditions
January	12h-14h	2h	10h-16h	6h	
February	11h-15h	4h	9h30-16h30	7h	
March	10h-16h	6h	9h-17h	8h	
April	9h-17h	8h	8h30-17h30	9h	
May	9h-19h	10h	9h-19h	10h	
June	8h-20h	12h	8h30-19h30	11h	
July	8h-20h	12h	8h-20h	12h	
August	8h-20h	12h	8h30-19h30	11h	
September	9h-19h	10h	9h-19h	10h	
October	10h-18h	8h	9h30-18h30	9h	
November	10h-16h	6h	9h-17h	8h	
December	11h-15h	4h	9h30-16h30	7h	

Table 8: Recommendations for the timing of light and UV provision (Prestreau, 2008b).

Water temperature should be the same as the ambient temperature, so around 24-28°C (Boyer & Boyer, 2005). In order to increase water temperature, different tools can be used, such as heating mats or heating cables under the aqua-terrarium or submersible aquarium heaters. In larger setups, an in-line heater can be plumbed to the filtration system. When using submersible aquarium heaters, you should provide barriers around it to prevent contact burns on the turtles (Boyer & Boyer, 2005; Wilke, 2009). Ambient and water temperature should be regularly monitored with a thermometer, ideally near the water heater, at the opposite side of the aqua-terrarium and at the basking area.

2.1.1.11 Dimensions

The recommended size of an enclosure differs very much between the personal perceptions of a holder and is rarely based on scientific research. If is also important to follow national legislation, if available. Some authors published size recommendations for individually kept turtles (which is not recommended for a social species) or groups of turtles. We recommended for a fully grown pair of European pond turtles an enclosure with a length of at least 150cm, a width of 75cm and a water depth of at least 60cm. For each additional individual, 20% should be added. In addition, to avoid escape it is recommended that the high of the walls around emerged area is at least 1.5 times the length of the animal (Adkins, et al., 2003) and smooth, as these turtles are very good climbers.
Considering that captive reproduction is more likely to occur when larger enclosures are used (Rossi, 2005), we recommend choosing the largest aqua-terrarium possible. The most common shape, rectangle, is structurally sound, available and minimizes angles into which a reptile might collide. Unusual shapes are associated with more injuries (Rossi, 2005). The aqua-terrarium should not be placed near a door or a window, preferred is a place near a wall or in the middle of a room (Prestreau, 2008b).

2.1.2 Large inside enclosure

For large inside enclosures, recommendations are comparable to outdoor enclosures (see below).
An unheated greenhouse for breeding European pond turtles for releases was constructed in Latvia (Pupins, Pupina, & Pupina, 2016) . The glasshouse is 104 m², with a pound of 35m² for 20 adults and a smaller pond of 8m² for 8 subadults, both with an average depth of 70cm and a maximum of 120cm. The pools are concrete made and are filled with water from a natural pond water near the greenhouse and equipped with a filter. At the bottom of the pond plastic boxes with aquatic plants (*Acorus calmus*) were installed, and some planks are added on water surface for basking. In order to allow females to lay their eggs, 2 plastics boxes (2m² and 20cm height) with humid fine sand were installed in enclosure of the adults.
This study showed that successful reproduction can be observed in countries situated in high latitude with the use of an unheated greenhouse (Pupins, Pupina, & Pupina, 2016).

Figures 12 and 13: Indoor enclosure at Hluboka Zoo. © Hluboka Zoo

2.1.3 Outdoor enclosure

2.1.3.1 Boundary

As it is important to keep the turtles in their enclosure, we need to erect a fence. European pond turtles have great abilities to escape and we should consider the risk of predation from a variety of species. Different materials have been proposed, such as wood, wire-mesh or fibre cement (Wilke, 2009) (Hartmut, 2007). Zoodyssée (France) used a certain type of PVC (Komacel®) to build their fences, but as it was not only expensive but it also deformed due to UV-exposure; they recommend now the use of steel sheets (CORTEN or painted metal sheets). Réserve de la Haute-Touche (France) uses a UV-resistant PVC at the bottom (see Figure 15-16) or highest part of the fence. *Emys orbicularis* is a good climber so if a wire mesh fence is being used, it should be 50cm high with the upper part angled inside the enclosure (Rogner, 2009). Glass panes are used in Hluboka Zoo (Czech Republic). To create underwater view, the use of glass is possible.

Turtles (and their predators!) are able to dig, so the fences should be buried at least 50 cm deep (Wilke, 2009). To avoid rats that may predate on eggs or hatchlings entering the enclosure the use of one meter high galvanized 19x19 mm wire mesh fences, buried 50-60 cm in the ground, and the addition of electric wires on the outside of the enclosure, some centimetres above the floor, is recommended.

If natural incubation or housing hatchlings is foreseen, a net should be place over the enclosure to avoid predation by storks, herons, crows and other potential predators.

Figure 14: Boundary in Hluboka Zoo. © Hluboka Zoo

Figure 15: Zoodyssée enclosure boundary. © L. Berthomieu

Figures 16 and 17: Fences at Réserve de la Haute-Touche (left) and Sainte-Croix (right). © L. Berthomieu

2.1.3.2 Terrestrial area

In the enclosure of semi-aquatic turtles, two areas should be considered: terrestrial area and aquatic area.

As European pond turtles sometimes travel on land for basking, foraging for plants or insects and for egg laying, the terrestrial area is an important part of their enclosure. Special attention should be paid to the nesting area. Different substrates for nesting areas are mentioned in field data (see before), and ideally we should copy the natural situation. Some breeders recommend the use of clay soil so that the nest plug is more easily observed (Georges, 2015). Other authors recommend the use of sand to facilitate digging by turtles and limit the growth of a layer of herbs or grass. The use of coloured substrate to facilitate nest location has also been suggested (Georges, 2015). The creation of a small nesting area (1m²) prevents long lasting nest search, however competition for nesting area should be take into consideration and therefore nesting area size depends on the number of breeding females in the enclosure.

Structure	Nesting area size (m²)	Number of adult females	Substrate	Exposure	Shelter on the nesting area
Village des Tortues	4		Soil	South	No
Uwe Stulle	2	4	Humus	North	Yes
Papiliorama	1.05	11	soil, sand	North-east	Yes
Editha Kruger	2	5	Soil, sand	?	Yes
Bern Zoo	6	8	Sand	South	No
Mulhouse Zoo	1.5	?	River sand	South	No
Réserve de la Haute-Touche	5	30	Soil, sand	?	No
Zoodyssée	1	8	Soil	South	No
Poznan	1.5	4	Sand	?	Yes
Lodz	1	8	Sand	East	No
Goldau	5	2	Sand	Southwest	No
CEPEC	15	?	soil	South	Yes
Chomutov	4	8	Sand, soil	East	Yes
PCA	20	16	Sandy clay	South	No
Hluboka	4	4	Fine white sand mixed with peat	South	Yes

Table 9: Nesting area characteristics of different private breeders and institutions.

Figures 18 and 19: Nesting areas at la Réserve de la Haute Touche (left) and Mulhouse(right). © L. Berthomieu

The nesting area should be at a place where it can never be flooded and ideally exposed south as this reflects the wild situation. Just like in the wild, we recommend keeping vegetation short between the pond and the nesting area, to facilitate the accessibility of the turtles. As explained before, females can travel long distances to find nesting area, so the creation of a snail-shaped path to access nesting area to increase and simulate travel length has been suggested (Scibek, 2014).

Figure 20: Nesting area at Zoodyssée © L. Berthomieu

2.1.3.3 Aquatic area

There are many ways to create a pond. One can choose to simply dig in a fiberglass or PVC tank or pre-formed pond, as these are easy to install and cheap. However, the borders are often very steep, and animals may have difficulties with climbing on the borders, especially in spring when the animals are not yet very active. A more natural looking pond can be made with PVC or EDPM pond liner, which allows also more freedom with the shape of the pond. In some regions it is possible to make an impermeable pond with naturally occurring clay. Making a concrete pond is also possible, but it is more work and more expensive.

Wolff (2004) describes his pond of 12 meters long and four meters wide, created with PVC liner. The deeper part is 1.5 meters deep which rises gradually to the banks. Three square meters of the pond is swampier and heavily vegetated. The basking place is located on the shallow water area. No substrate is added except in the deepest area where gravel is added to allow plants to attach, and slit/mud where turtles can hibernate. Plants are growing in buckets on the bottom of the pond (Wolff, 2004).

The creation of several ponds with different sizes to allow males to retreat when chased by competing males is sometimes recommended (Rogner, 2009).

The PVC or EPDM liner is installed on a 5-10 cm tick sand layer (Avanzi & Millfanti, 2003) to protect it against stones in the soil. Once installed, rocks can maintain the edges of the liner and a layer of 5-6cm of sand and stones covers the borders of the liner (Avanzi & Millfanti, 2003), but this may not be ideal for the turtles when they want to leave the water and a more natural border with soil or clay may be better. The deeper part of the pond should be at least 100 cm to avoid during summer the water becoming too warm, causing overgrowth of algae and to avoid freezing during winter. In very cold areas, a deeper pond may be necessary to protect the turtles during the winter against the low temperatures.

Border with gradual slopes are important, as in early spring the animals are not yet active enough to swim well and they need to walk on the bottom of the pond to come to the surface (Richter, 2015).

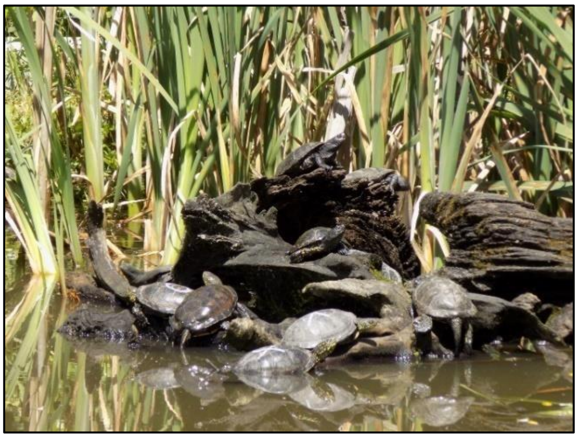
A six meter diameter pond with two meters wide flat edges and an area of one meter deep and two meters wide in the middle. The banks have a slight slope of 7°, becoming more abrupt (45-50°) towards the middle (Wilke, 2009).

2.1.3.4 Furnishing and maintenance

Vegetation

Different plants can be planted on the banks (e.g.: *Yucca* spp, *Typha latifolia, Cortedaria spp, Arundi donax, watercress, Carex* spp*, Phragmites* spp, *Hippuris vulgaris*, *Calta palustris*, *Sagittaria sagittifolia, Juncus trifidus*) and inside the pond (e.g. : *Potamogeton* spp*, Juncus ensifolius, Caltha palustris, Lytrhum salicaria, Elodea canadensis*, *Myriophyllum spicatum*, *Nymphaea* spp*, Eichhornia crassipes, Ceratophyllum* spp*, Nuphar lutea*, *Nymphoides peltata*) (Avanzi & Millfanti, 2003; Richter, 2015).

It may be important is to be sure that none of the plants is toxic, such *as Nerium oleander, Arum* spp*, rodhodendron* spp*, Iris* spp and *Clyclamen* spp (Avanzi & Millfanti, 2003), although the animals may avoid eating such plants on a natural way.



Figures 21 and 22: Basking areas at Zoodyssée and la Réserve de la Haute-Touche. © L. Berthomieu

Basking furniture

To allow turtles to bask, rocks and branches are installed inside the pond or on the banks. To increase basking surface is also possible to build rafts with branches, the turtles can also use them for hiding.

Other furniture

The use of shelters in the nesting area can incite turtles to nest under the shelter (Georges, 2015), which may decrease the risk of egg predation by birds.

Filtration

The need of artificial filtering depends on many factors, such as the number of turtles, the size of the pond and the way the pond has been created. In many cases it is possible to rely on a natural balance, provided that a natural biotope with plants, insects, worms, crustaceans, bacteria, algae and protozoa has been constituted. The addition of small fishes, such as roaches, may help in the creation of this new ecosystem (Avanzi & Millfanti, 2003), but in a natural pond where waterfowl has access these fishes may arrive at a natural way. Zoodyssée relies on natural filtration with a lagoon system (see figure 25), Sainte Croix has a slow circulation of water between the pond and a large natural lake.

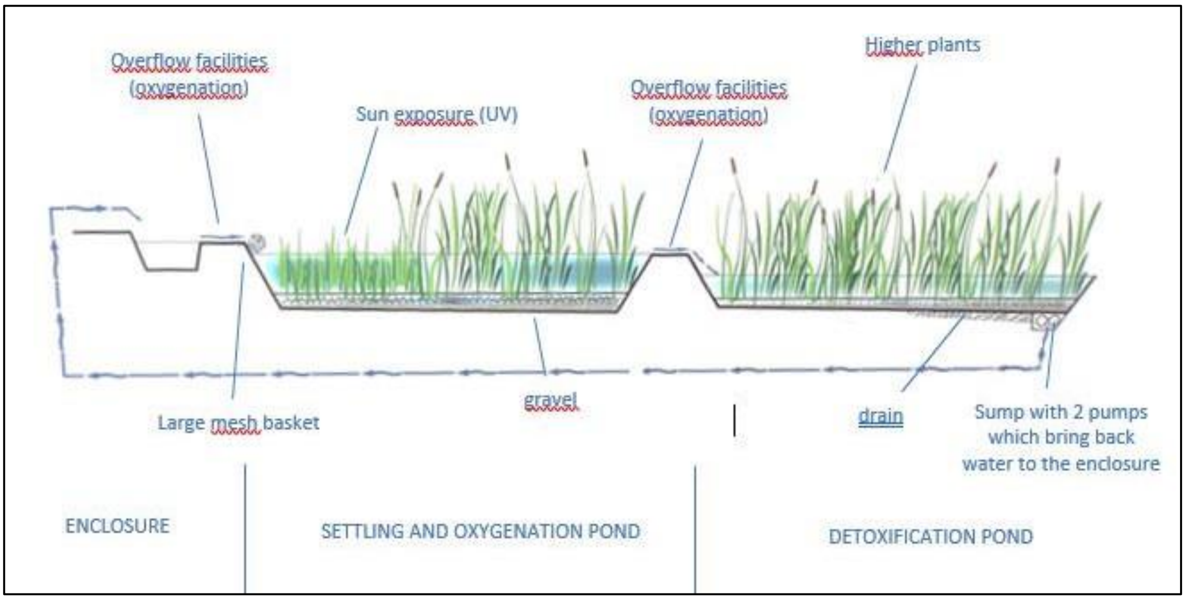


Figure 23: Lagoon system at Zoodyssée (scheme adapted from Zoodyssée signage).

Pumps or filters which create much water flow should not be used because they will mix the water of different temperatures and the mean temperature might become too low for the turtles (Rogner, 2009). It is important to maintain a natural gradient in temperature between the shallow and deeper parts of the pond, even if a filter is used (Kruger, pers. comm., 2018). Therefore, if a filter is being used, it should not take water from the deepest point of the pond and return it via a turbulent outlet, but the inlet for the filter should lie in the upper half of the pond and the water should be returned to the pond via a gentle steady flow. Distributing the outflow of the filter over a large stone or rock is a solution to obtain additional oxygenation.

2.1.3.5 Dimensions

There is much variation in enclosure size between the different responding institutions (*Table*). We recommend a natural pond with a considerable amount of vegetation and a thick layer of silt for hibernation. One m² per turtle should be sufficient, but with more space the animals have more opportunities to express their natural behaviour.

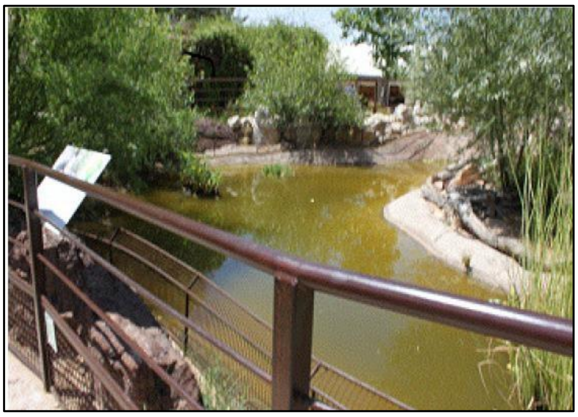
Institution	Enclosure size (m²)	Pond size (m²)	# of turtles	Density /m² + m²/turtle	Depth (max.)	Thickness of silt
Zurich	2500	668	7	0.0028 / 357	50	15-25
Sainte Croix	150	100	14	0.14 / 7.1	1.5	50
Village des tortues	70	15	?	?	40	10
Uwe Stulle	11	9	9	0.8 / 1.2	100	?
Papiliorama	3.4	2.6	3	0.9 / 1.1	95	10
Editha Kruger	50	35	10	0.2 / 5	100	10
Bern Zoo	200	80	9	0.05 / 22	80	10
Mulhouse Zoo	10	2	8	0.8 / 1.25	50	30
Réserve de la Haute-Touche	400	240	53	0.13 / 7.5	100	30
Zoodyssée	150	130	47	0.3 / 3.2	90	20
Poznan	73.9	8.5	7	0.09 / 10.6	40	2
Goldau	70	48	10	0.14 / 7	100	40
CEPEC	20	2	50	2.5 / 0.4	70	15
Chomutov	6	2	18	0.3 / 3	40	0
PCA	1200	250	22	0.02 / 54.5	200	20
Hluboka	30	15	7	0.2 / 4.3	140	?

Table 10: Outdoor enclosure dimensions in different institutions.


Figures 24 and 25: Outdoor enclosures at Sainte-Croix (left) and la Réserve de la Haute Touche (right). © L. Berthomieu


Figures 26 and 27: Outdoor enclosure for breeders (left) and exhibition enclosure (right) at Mulhouse Zoo. © L. Berthomieu

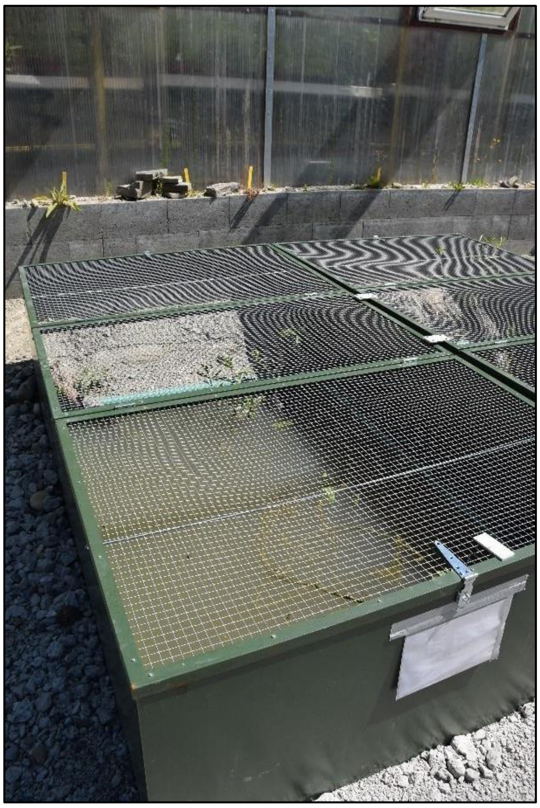
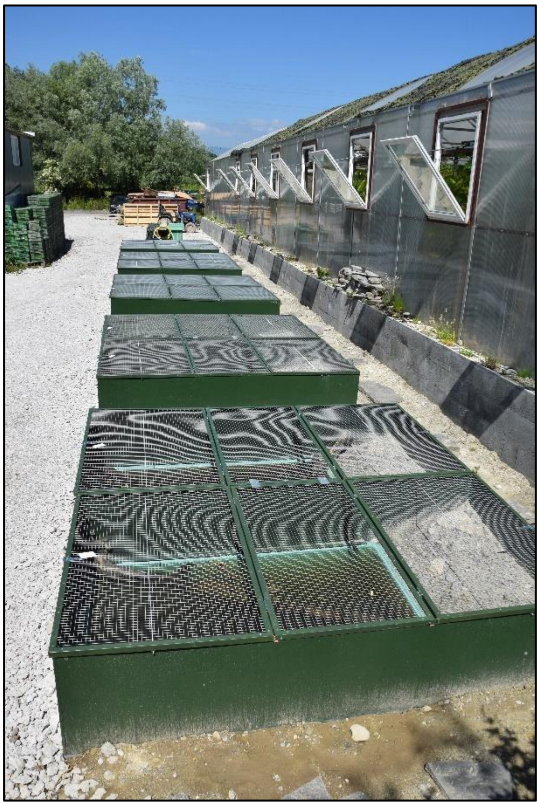

Figures 28 and 29: Outdoor enclosure at Zoodyssée. © L. Berthomieu



Figures 30 and 31: Outdoor enclosure Village des Tortues (© S. Gagno) and at la Ferme des Crocodiles. © P. Scibek

Figures 32 and 33: Outdoor enclosures at Bern Zoo (left) (© Zoo Bern) and Hluboka (right). © Zoo Hluboka

Figures 34 and 35: Outdoor enclosure at Hluboka. © Zoo Hluboka

Figures 36 and 37 : Outside enclosures for breeders at Papillorama. © Papillorama.

2.1.4 Enclosure for offspring

As all institutions that responded to the survey use incubators for breeding (because of predation risk and/or unsuitable climate), enclosures to house the offspring are necessary. Most institutions have indoor nurseries where the youngsters remain during at least the first year of their life.

2.1.4.1 Indoor nursery

Zoos and private breeders use a variety of systems to rear their offspring (*Table 1*). Generally the animals are reared in plastic containers or aquariums of various sizes.

Water

Rogner (2009) recommends being careful with water height in order to avoid drowning. He recommends housing the hatchlings in 50x40x20 cm plastic containers which are kept inclined so that the water is two centimeters depth at the bottom and there is no water on the upper part.

However, results of the survey (see Table 10) show that many other breeders keep hatchlings in deeper water without problems of drowning.

Institution	Length (cm)	Width (cm)	Height (cm)	Water depth (cm)	Hatchlings per container	Volume/ individual (L)	Material
Uwe Stulle	80	40	40	15	10	4.8	Plastic
Papiliorama	60	40	30	6-10	8-10	1.9	Plastic
Editha Kruger	80	35	?	10	5-7	4	Glass
Bern	100	50	20	4	?	?	PVC
Mulhouse	60	40	30	6-7	10	1.4	PVC
Haute Touche	40	30	20	15	9-10	1.8	Ceramic sink
Zoodyssée	60	40	30	5	8-12	?	Plastic
Lodz	100	60	60	50	1	30	Glass
Chomutov	76	50	30	2	10	0.8	Glass
Hlboka	60	50	40	5-7	?	?	glass

Table 10: Characteristics of the indoor nursery of private breeders and breeding institutions.

Substrate

Hatchlings and juveniles do not need loose substrate such as sand, but it is essential that they can bask. Different solutions can be used to allow the hatchling to bask, such as the use of a tile, branches or rocks. In the survey executed by the ESB, 25% (n=16) use tiles, 31% (n=16) branches and 19% (n=16) rocks.

Furnishing

Additionally to the basking structure some plant material can be added to allow hatchling climbing on it or hiding. The plants used by breeders are *Elodea canadensis* (Rogner, 2009), plastic plants, *Ceratophyllum demersum, Chlorophytum comosum, Lysimachia nummularia Cyperus alternifolius*, swamp species or bamboo leaves. Plants cover of the nursery tank varies between 25% to 75 %.

Environment

Currently, we do not have studies on difference of needs between young and adults (Adkins et al., 2003), so we refer to the chapter about adult inside enclosures for recommendations.

Temperature

Georges (2015) explains that it is essential to avoid overheating of the water and to allow the turtles to cool; a circadian cycle with a cooling during night should be used.

Structure	Water temp.	Hot spot temp.	Heating system	Brand	Distance from basking area
Papiliorama	21-24	27	Metal Halide lamp	Bright Sun UV Jungle, LUCKY REPTILE	20cm
Editha Kruger	25	35	UV Metal Halide Lamp	Bright Sun UV, LUCKY REPTILE	30cm
Bern Zoo	?	35	UV Metal Halide Lamp	REPTILES EXPERT	35cm
Mulhouse Zoo	?	/	UV Mercury Vapor Lamp	Solar-Glo, EXO-TERRA	40cm
Réserve de la Haute Touche	?	/	UV Mercury Vapor Lamp	SOLAR RAPTOR	40cm
Zoodyssée	Ambient	28-30	UV Mercury Vapor Lamp	Ultra Sun, REPTILES-PLANET	25cm
Lodz	?	30	UV Mercury Vapor Lamp	EXO-TERRA	50cm
Chomutov	?	32	Incandescent light bulb	REPTISPA	20cm
Hluboka	21-25	32-38		SunLux UV	?

Table 11 : Examples of temperature characteristics in the indoor nursery.

Lighting

The young turtles need both visible and UV light. Zoos use different systems. Requirements have been discussed elsewhere in the BPG.

Structure	Brand	Type	Ballast	Power	Distance from water (cm)	Distance from basking area (cm)
Papiliorama	Bright Sun UV Jungle, LUCKY REPTILE	UVB Metal Halid Lamp	Yes	50W	25	20
Editha kruger	Bright Sun UV, LUCKY REPTILE)	UVB Metal Halid Lamp	Yes	70W	30	30
Bern Zoo	REPTILES EXPERT	UVB Metal Halid Lamp	Yes	70W	35	35
Mulhouse Zoo	Solar-Glo, EXO-TERRA)	UV Mercury Vapor Lamp	Self-ballasted		40	40
Réserve de la Haute Touche	SOLAR RAPTOR	UV Mercury Vapor Lamp	Self-ballasted	100W	40	40
Zoodyssée	Ultra Sun, REPTILES-PLANET	UV Mercury Vapor Lamp	Self-ballasted	100W	25	25
Lodz	EXO-TERRA	UV Mercury Vapor Lamp	Self-ballasted		20	
Chomutov	REPTISPA	UV Mercury Vapor Lamp	Self-ballasted	100W	25	20
Hluboka	SunLux UV			35W	15	10

Table 12 : Examples of lighting systems. All institutions use spots.

Some holders adapt the lighting hours to the seasons of the year.

Structure	Spring	Summer	Fall	Winter
Papiliorama	6	/	7	5
Editha Kruger	8	/	8	/
Bern	12	/	/	/
Haute Touche	12h30	/	/	/
Zoodyssée	12	14	10	8
Lodz	/	12	/	/
Chomutov	10	12	10	8
Hluboka	10	12	10	10

Table 13: Examples of photoperiod protocols (hours/day.)

Filtering

Hygiene is important for young turtles, and a good filtering system is necessary to keep the water clean.

Structure	Brand	Flow (L/h)	Power (W)	Individual/collective
Uwe Stulle	EHEIM	30	3	individual
Papiliorama	Trixie M380	380	7	individual
Editha Kruger	EHEIM	500	8	individual
Haute-Touche	EHEIM prof.2275	1250	16	collective
Zoodyssée	Aqua-Flow 100	50-200	3.5	individual

Table 14: Examples of filtering systems.

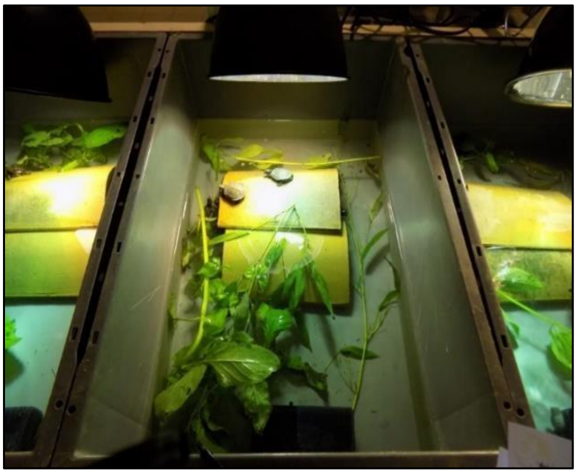
La Réserve de la Haute Touche uses a collective filtering system, all nursery tanks are connected on the same closed water system. At Zoodyssée each tank has its own filter.

Avanzi and Millfanti (2006) recommend using a filter whose flow per hour is at least twice the total amount of water and to clean the filter once a month.

Some breeders do not use a filtering system but change the water once a day to once a week (survey results). When changing water in indoor aquarium, always use ambient temperature water as a sudden change in temperature can be harmful for the turtles.

Figures 38 and 39: Indoor nursery at Mulhouse Zoo. © L. Berthomieu

Figures 40 and 41: Indoor nursery at la Réserve de la Haute-Touche. © L. Berthomieu



Figures 42 and 43: Indoor nurseries at Zoodyssée (left) (© L. Berthomieu) and Editha Kruger (private breeder) (right). (© E. Kruger)


Figures 44 and 45: Editha Kruger's indoor nursery. © E. Kruger


Figures 46 and 47: Indoor nurseries at Bern Zoo (left) (© Zoo Bern) and Latvia (right)

2.1.4.2 Outdoor nursery

In some structures hatchlings and juveniles are transferred from the indoor nursery to an outdoor nursery, before being reintroduced or transferred into the adult enclosure. Depending on the breeder, the young turtles are either transferred immediately after hatching to an outdoor nursery, or only when they reach a certain size or age. Ages reported range from one month to one year old. One breeder keeps his turtles inside until they reach seven centimetres.

Young turtles of less than 2 years old may be only 2-3 centimetres wide, and the wire-mesh of the enclosure should have a gauge of only less than 15mm x 15mm to prevent escaping.
Rogner (2009) recommends to house juvenile outsides from May to the beginning of September and to keep them inside the rest of the year, until they are 3-4 years old. His outside enclosure is 3*2 meters with a pond in the middle and 20-30 cm wide gravel banks. The turtles are protected from predation with a wire mesh (netting to protect fruit trees can also be used). When keeping hatchlings and juveniles outdoor, it is very important is to protect them against predators. In the ESB survey 60% (6/10) of the breeders have a protection for the outdoor nursery, different examples can be found in the following chapter.
The outdoor nursery could be completely separated from the adults' enclosures, but as in Zoodyssée it can also be installed within the adult pond, but in a well-protected area.

Figures 48 and 49 : Outdoor nursery at Réserve de la Haute-Touche. © L. Berthomieu

Figures 50 and 51: Outdoor nursery at PCA (left) and Zoodyssée (right). © L. Berthomieu

Figure 52: Outdoor nursery at Bern Zoo. © Zoo Bern

2.2 Feeding

As already summarized in before, wild European pond turtles have an omnivorous diet that may vary between populations, habitats and life stages. Besides large numbers of animal prey (mostly arthropods but also a variety of other species), plant material is an important part of the natural diet of this turtle. When composing a diet for captive specimens, it is important to take into account the natural needs of the species, including seasonality.

European pond turtles are kept in a wide variety of enclosures, which affects the diet that should be provided to the animals. Turtles living in large, natural ponds can be almost or completely self-sufficient, which seems to be the ideal situation. In this case a major part of the diet is composed of the food items that turtles find by themselves in their enclosure; they feed on aquatic and terrestrial gastropods, insects and crustaceans which are naturally present (Rogner, 2009). Turtles living in smaller enclosures need to be supplied a well-balanced diet to assure their health and reproductive success.

2.2.1 Suitable food items

Fish

Freshwater are used to feed turtles (Rogner, 2009; Scibek, 2014). The fish can be given whole or in pieces. Examples of suitable species are roach, eel, lance, guppy, pollock, salmon and trout (Schilliger, 2004).

Two points are important to remember:

- Avoid the use of fishes which contain thiaminase (ex: gold fish, carp, catfish, smelt, herring, …)
- Feeding frozen fish would better be avoided. Freezing destroys the thiamine (vitamin B1) and increases the activity of thiaminase (Schilliger, 2004). If fed, it should be in moderation and as part of a balanced diet (Boyer & Boyer, 2005). An advantage of freezing is to decrease the parasite contamination, but freezing fish may not eliminate all parasites (Boyer & Boyer, 2005).
- Saltwater fish and mussels are not recommended as a food source, because of reported iodin issues (Martinez Silvestre & Velarde, 2017).

Richter (2015) reports to have small fish (*Leucaspius delineates*) in the pond with the turtles. Most of these fish are too fast for the turtles to catch, but older (they only live two years) and weak fish are eaten and provide a good source of food.

Crustaceans/seafood/molluscs/worms

Feeding only *Gammarus* is not recommended, as it is not clear if they contain all necessary nutrients and the Calcium/Phosphorus ratio may be unsuitable for turtles (Rineau, 2017). Furthermore, *Gammarus* may be a vector and reservoir of the bacteria *Beneckea chitinivora* responsible of ulcerative disease of the shell (Guillon, 2010). Other seafood items such as shrimp, cuttlefish and squid can also be a part of the diet of aquatic turtles (Firmin, 2004) . Mussels are rich in thiaminase so should better be avoided.

Bloodworms, earthworms, tubifex worms, slugs, snails (with shell) can be the major part of the diet. Earth worm are nutritious. Snail and slug can be fed but are potential vector for trematodes (Boyer & Boyer, 2005) .

Meat

Fresh meat can be used to feed European pond turtles (Scibek, 2014). However, plain meat is deficient in calcium, vitamins and minerals. The use of whole animals such as mice or baby mice (more than 3 days) soaked in fish mucus provides a better balanced nutrition than just pieces of meat. A minority of the diet can be made with lean raw beef/veal/chicken liver/gizzard/heart, if supplemented with calcium power (Boyer & Boyer, 2005; Schilliger, 2000). Raw chicken should be avoided because of the risk of salmonellosis (Rogner, 2009). To avoid obesity and lipidosis the use of fat meat such as ham should be avoided, except in the month before hibernation (Firmin, 2004).

Insects

A variety of insects (crickets, waxworms, mealworms, flies, moths, grasshoppers) can be given, but in moderation because insects are calcium deficient (Boyer & Boyer, 2005).

Vegetables and fruit

Adult turtles become more omnivorous, so gradually the diet should contain increasing levels of dark leafy greens, dandelion leaves and/or watercress (Boyer & Boyer, 2005). Aquatics plants such as water hyacinth, duckweeds, water lettuce and watercress are also appreciated. To avoid intoxication, do not use plants treated with chemicals. In addition, some flowers such as azaleas, daffodils, tulips and hemp are toxic to reptiles and it is better to ban these from the enclosure (Rossi, 2005).
Mature fruits are appreciated (Firmin, 2004) but too mature fruits can contain bacteria and should be avoided (Rineau, 2017). Suitable fruits are pears, apples, melons, grapes and figs. Carrots, sweet potatoes and squash can be used to provide beta-carotene.

Industrial food

While dry cat or dog food have often reported to be fed to aquatic turtles (Firmin, 2004; Rineau, 2017), the current availability of more balanced commercial turtle food make the use of this unsuitable food redundant. The same is true for feeding commercial freshwater fish food, that has often been used for aquatic turtles (Boyer & Boyer, 2005; Rogner, 2009).

Rineau (2017) analysed the contents of Vita Terra® Omnivore Pellets, Zoo Med® Aquatic Turtle Food (hatchling/growth/maintenance formulas) Exo-Terra® Aquatic Turtle (hatchling/juvenile/adult), and concluded that these products are suitable and considered to be “complete foods”, no other food is necessary.

Other brands that produce suitable food are Arcadia Earth Pro Turtle Gold and Earth Pro Omni Gold and various Repashy products (Goetz, pers. comm.), but there are certainly other brands that could be used, and this market is in full expansion with new products being available each year.

As there are so many brands and types of commercial food available, and their availability may differ between countries, it might be difficult to choose the most suitable food and it is impossible to make a recommendation. All professional products will give detailed information on their contents, which can then be compared to the (known) nutritional requirements indicated in this paper, to decide if the product is suitable.
The amounts fed to the turtles are usually indicated on the products; animals living in a natural pond will eat plants and invertebrates, and less commercial food is necessary.

2.2.2 In-house preparations

Although there is complete food available on the market, some holders prefer to use in-house preparations for their animals. Such preparations are often based on own or other's experiences. Special preparations are described to feed juveniles or ill *Emys orbicularis.*

Some examples of recipes of special mixture for aquatic turtles are listed below.

<table>
<tr><td>Ingredients:
- 60 bags of powdered gelatine
- 1.5kg of fresh trout
- 0.72kg of fresh shrimp
- 0.54kg of fresh mussel
- 1.5kg of beef escalope
- 2.5L of water</td><td>
- 6 eggs
- 1.5L of milk
- 3 jars of carrots, 3 jars of garden vegetables, 3 jars of spinach
- 0.3kg of rabbit or poultry liver
- 60 g Korvimin multivitamin powder for reptiles
- 2 bags of red food colouring</td></tr>
<tr><td colspan="2">Recipe:
- Add the 60 bags of gelatine in a 12L container > Add slowly 2.2L of water with constant stirring > Allow jelly to set during 20 minutes > Gelatine should be liquid at 40°C
- Rinse shrimp and mussel > Chop trout, beef, liver, shrimp and muzzle and mix in another container > Add the 9 jars with vegetables > Add milk and eggs with slow stirring > Add multivitamin powder > Dilute red food colouring in 0.3L of water and add it to the mixture > Stir the mixture so that it become homogeneous > Heat up the mixture to 40°C so it is the same temperature as gelatine
- Refresh the gelatine with some centilitres of fresh water and rapidly add the mixture and stir
- Divide the preparation in smaller containers > Let it cool down and put it for some hours in a fridge
- When it hardens put in in freezer</td></tr>
<tr><td colspan="2">Note: The ratio Ca:P of this recipient has inadequate ratio of 0.76 (Pfau & Wiechert, 2003). Therefore we recommend to complement with calcium.</td></tr>
</table>

Table 15 : Recipe to prepare 12 litres of Artner's pudding (Artner, pers.comm. 2018).

<table>
<tr><td colspan="4">Pudding at the zoo of Mulhouse (Technical manual of Mulhouse zoo)</td></tr>
<tr><td colspan="4">Volume: 1.5-5L</td></tr>
<tr><td>Ingredients:
- 90g of broccoli heads
- 125g of red shrimps
- 230g of trout</td><td>
- 190g of courgette
- 100g of mussels
- 2 eggs</td><td>
- 400g of carrot
- 75g chicken liver
- 200ml of milk</td><td>
- 200g of red beet
- 75g of chicken heart
- 100g of jelly with Madeira</td></tr>
<tr><td colspan="4">Recipe:
- Cook the vegetable and chop them > Blend shrimps, mussels, liver, heart and trout and add it to vegetables and eggs > Add milk and water until obtain a liquid preparation > Add jelly > Boil
- Divide the preparation in smaller containers > Let it cool down then put it in freezer</td></tr>
</table>

Table 16: Recipe to prepare 1,5 – 5 litres of pudding at the zoo of Mulhouse (Technical Manual of Mulhouse zoo).

Ingredient:	
- 500g of gelatine powder - 200g of dried chopped shrimps - 300g of beef (tenderloin or roast and heart) - 6 chicken eggs (or better quail eggs) - 1kg of vegetables (carrots, chard, broccoli, dandelion) - 2.5L of water	- 2000g of fresh water fish - 900g of fresh or frozen shrimps (with shell) - 300g of rabbit, poultry or beef liver - 1,5L of skimmed milk - 3 tablespoons of vitamin/mineral (Korvimin ZVT®) - 70g of calcium for reptiles
Recipe: - Add the 500g of gelatine in a 12L container > Add slowly 2L of water with constant stirring > Allow jelly to set during 20 minutes - rinse the shrimps and rehydrate in milk - boil vegetables - chop fish, meat, liver and vegetables, add the eggs and the shrimps with the milk > stir together to obtain homogeneous preparation - Heat up at 80-90°C and add vitamins and calcium - Mix gelatine and preparation - Divide the preparation in smaller containers and put immediately in freezer (quick cool down is essential)	

Table 17 : Recipe to prepare pudding at the Petite Camargue Alsacienne (Molmy, 2013).

The pudding should be defrosted at 4°C before feeding the turtles. Pudding is cubed (1cm²x2 mm thick per juveniles) and should be eaten within 10 to 15 minutes (Rust 2007, pers. comm. with Parc Animalier de Sainte-Croix). Private breeder Rust feeds its European pond turtle with Artner's pudding from hatchling to 10 years old.

2.2.3 Nutrition of young turtles

At Mulhouse zoo, hatchlings receive their first meal one week after the hatching. As for adults, the quantity of food given is gradually decreased from mid-August to mid-November when they stop feeding the juveniles (the quantity is divided by two every months).

Scibek (2014) recommends to start feeding juveniles with insects, switching later to homemade pudding. Rogner (2009) recommends the use of small earthworms, mosquito larvae, micro-crustaceans such as copepods, water fleas. In Mulhouse zoo, juveniles are offered some blood worms (*Chironomus* spp), micro-crickets and fruit flies. In Zoodyssée the juveniles are fed every two or three days with frozen blood worms (equivalent to 4 sugar cubes for 42 juveniles). In Zoodyssée cricket larvae, earthworms and duckweed are given once a week. When the hatchlings are 2-3months old, they give them some 2-3cm long guppies to train them to hunt.

Young turtles can also be fed with industrial food (see above).

2.2.4 Vitamin and mineral supplementation

Some information about the vitamin requirements in chelonians is reported:

Vitamin	Requirement
A	1500-10000 UI/kg dry matter
B1	20-30 mg/kg dry matter
D3	2000-50000 UI/kg dry matter
E	400 UI/kg dry matter 20-80 UI/kg dry matter

Table 18 : Vitamin requirements of chelonians (Rineau, 2017).

Usually, natural food found in an outdoor enclosure is thought to be well balanced in vitamins and minerals (Rogner, 2009). The recommended ratio calcium on phosphorus is 1.5:1 to 2:1 (Rogner, 2009; Rineau, 2017). The complicated subject of calcium/vitamin D3 supplementation has been discussed earlier.

Pfau and Wiechert (2003) explain that freshwater turtles kept in captivity have often an unbalanced diet concerning calcium:phosphorus ration, with a deficiency in calcium. The turtles can compensate a part of the deficiency extracting calcium from water through their cloacal and mouth mucosae. As the turtles may look actively for calcium sources in their enclosure, keepers should provide access to egg shells or cuttlebones (Pfau & Wiechert, 2003). To avoid mineral and vitamin deficiencies, examples of products which can be used are Ornivita®, Vita Tortue® and Korvimin ZVT®.

2.2.5 Feeding practices

Turtles are diurnal species and should be fed during the day. As *Emys orbicularis* prefers to feed in the water the use of floating items to feed the turtles is preferred. In aqua-terrariums this will make the removal of uneaten food easier. Turtles kept in aqua-terrariums can be fed in a separate aquarium, to limit water pollution and to check individual food intake (Rogner, 2009).

In order to be able to check individual health and facilitate catching, it can be interesting to handfeed the turtles or to habituate the turtles to come on the edge to feed. Rogner (2009) advices to prefer live prey to incite turtles to hunt.

2.2.6 Quantity

The quantity of food given depends on age, gender and activity of the reptile but also on the ambient temperature. Indeed, food intake is higher in optimal temperatures (Firmin, 2004). As there has not been much research on the nutritional needs of European pond turtles, it is difficult to calculate the necessary daily required amount of food. There are two models to calculate the energy need:

Donoghue (2006) proposes the following formula:
BE = k*SMR = k *32*P^0.86)
SMR = Standard Metabolic Rate (kcal/day), P in kilogrammes
This formula is appropriated to turtles kept in aqua-terraria, which do not hunt, but probably over estimated turtles needs.

Activity	k
Stress, growing	2.5
Growing	2
Ovogenesis	1.5
Active turtle	1.25
Rest	1

The second model for reptiles is the more general Field Metabolic Rate:
FMR (kJ/day) = 0.796 * P^0.889 (P in grammes)

Neither of these models have never been checked on aquatic turtles living in a natural enclosure (Rineau, 2017).

In a more practical way, one can assume that in adult turtles the quantity to food is approximatively 5% of their weight (Firmin, 2004). If it is observed that food is left from the precedent feeding, the quantity given should be reduced.

2.2.7 Seasonality

The digestive metabolism of reptiles varies with temperature and therefore with the season. In addition, *Emys orbicularis* is a hibernating turtle so it is important that the feeding protocol takes into consideration the transition to and from this period.

At Mulhouse zoo, feeding their adult turtles starts 15 days after observing the first turtles coming out of hibernation. Food quantities are gradually increased to reach the full daily diet in May. To prepare for hibernation, the quantity of food given is decreased gradually from mid-July until hibernation. Other holders (Rust, 2007, pers. comm. with Parc animalier de Sainte-Croix) stop feeding the adults at the end of august.

Turtles should not be fed if the temperature is less than 16°C, because the digestive metabolism is to slow.

2.2.8 Major pathologies induced by inappropriate feeding

It is important to remember that an inappropriate feeding is a cause of health problems:

- Underweight, when the diet is deficient in energy or quantity of food given is too low. Juveniles are the animals of most concern.
- Obesity, when food items are too fat (such as insect larvae, adult insects contain more proteins and less fat) or when ad libitum feeding. The risk of obesity increases if the turtle does not hibernate and will not use its fat reserves. A turtle is considered as obese if its weight is higher than 110% of its theoretical weight. This weight can be assess using Donoghue's equation ($W = 0.191 \times L^3$, W= theoretical weight and L : plastron length) or referring to the weight of wild animals
- Gout: excess of proteins
- Overgrowth with deformities: excess of proteins
- Metabolic Bone Disease: calcium or vitamin D3 deficiency
- Vitamin A deficiency
- Vitamin B1 deficiency: feeding food with thiaminase
- Vitamin E deficiency and steatite : food with too much fat

Fish		
Sardine	rich in purines thiaminase	Articular and visceral gout Hypovitaminosis B
Anchovy	rich in purines thiaminase	Articular and visceral gout Hypovitaminosis B
Herring	thiaminase	Hypovitaminosis B
Carp	thiaminase	Hypovitaminosis B
Smelt	thiaminase	Hypovitaminosis B
Mackerel	thiaminase	Hypovitaminosis B
Silure	thiaminase	Hypovitaminosis B
Sculpin	thiaminase	Hypovitaminosis B
Shad	thiaminase	Hypovitaminosis B
Gold fish	Thiaminase mycobacteria	Hypovitaminosis B
Crustaceans and molluscs		
Mussels	thiaminase	Hypovitaminosis B
Gammarus	Bacteria Too much phosphorus	Septicemic Cutaneous Ulcerative Disease
Snail	Trematodes	
Slug	trematodes	
Insects		

All insects	Calcium deficiency Low level in Vitamin A	
	Meat	
Liver	rich in purines	Articular and visceral gout
Brains	rich in purines	Articular and visceral gout
Kidney	rich in purines	Articular and visceral gout
	Vegetables and fruits	
Broccoli	rich in thiocyanate	Goitre: possible intensification problems in case of already existing deficit in iodine (Rineau, 2017)
Kale	rich in thiocyanate	
Brussel sprouts	rich in thiocyanate	
Green cabbage	rich in thiocyanate	
Turnip	rich in thiocyanate	
Spinach	oxalates	
Rhubarb	oxalates	
Potato	oxalates	
Cabbage	oxalates	
Red beet leaves	oxalates	
	Vitamin and mineral supplementation	
Overdose		Hypervitaminosis A or D3

Table 19 : Food to be avoided or used in limited quantity.

2.2.9 Feeding protocols for turtles

Feeding protocols differ much between zoos, which is partly caused by different housing conditions (availability of naturally occurring food in the enclosure or not).

Adults should be fed one to three times a week (Boyer & Boyer, 2005; Firmin, 2004; Rogner, 2009; Schilliger, 2000), but this should be adapted to temperature and climate. For example, with good weather they can be fed every 2-3 days and only once a week when the weather is bad.
Juveniles have higher nutritional needs, so they should be fed more often than adults, ideally daily.

Institution	Feed themself	Red meat	Poultry	Fish*	Special mixture	Pond fish food	Cat food	freshwater turtle food	Insects**	Microcrustacean***	Other****	Feeding frequency (/week)
Zurich Zoo (CH)	■											0
Village des Tortues (F)	■						■					1-2
Uwe Stulle (private)					■		■	■				3
Papiliorama (CH)				■				■		■	■	6
Editha Kruger (private)	■		■	■		■	■				■	3-7
Bern Zoo (CH)	■			■					■	■	■	3
Mulhouse Zoo (F)	■			■								2
Réserve la Haute-Touche (F)	■			■							■	3
Zoodyssée (F)	■			■							■	4
Poznan (Pl)		■		■					■			2-3
Lodz (Pl)				■								2
Goldeau (CH)	■				■							4
CEPEC (F)						■		■			■	5
Chomutov (CZ)				■					■		■	2
PCA (F)	■			■								2
Hluboka (CZ)	■	■		■							■	3

Table 20 : Food items provided by different institutions to adult turtles.

** Fish reported to be used are smelt, pangasius, trout, sprat, herring, stickleback, roach,* Rutilus sp. *and carp.*

*** Insects reported to be used are cricket and locust.*
**** Micro-crustaceans reported to be used are* Gammarus *sp. and sand shrimps.*
***** food items reported in "other" are squid, snails, slugs, mice, frogs, tadpoles, dog food, earthworms, mealworms (*Tenebrio*),* Zophobas morio*, rodents and mouse pinkies.*

Reported feeding frequencies range from zero (turtles only feed by themselves) to daily. Some breeders indicate that feeding frequency depends on the weather. 25% (4/16) give avec vitamin and mineral mixture to their breeders.

Institution	Red meat	Poultry	Fish*	Special mixture	Pond fish food	Cat food	Aquatic turtle food	Insects**	Microcrustaceans***	Other****	Feeding frequency (/week)
Uwe Stulle (private)	X			X			X		X		7
Papiliorama (CH)			X					X			6
Editha Kruger (private)		X	X					X		X	7
Bern Zoo (CH)			X							X	7
Mulhouse Zoo (F)				X			X				3
Réserve de la Haute-Touche (F)			X				X	X			7
Zoodyssée (F)			X				X	X			4
Lodz (Pl)			X					X	X		3
CEPEC (F)				X			X				5
Chomutov (Cz)			X					X			7
PCA (F)							X				
Hluboka (Cz)	X		X				X	X	X	X	3-5

Table 21 : Food items provided by different institutions to hatchlings.

** Fish reported to be used are smelt, trout, gambusia, stickleback,* Rutilus sp. *and carps.*
*** Insects reported to be used are mosquitoes, bloodworms,* Chironomus sp. *and mealworms (*Tenebrio sp.*).*
**** Micro-crustaceans reported to be used are* Gammarus sp. *and Daphnia sp.*
***** food items reported in "other"" are earthworms, tubifex and snails.*

According to the EEP survey, zoos vary in their management of the hatchlings, offering food is started at the first day or only after one week of age. Several breeders use yolk absorption as criterium. 45% (5/11) of the breeders that breed turtles reported to give a mineral and vitamin supplementation.

2.3 Social structure

2.3.1 Basic social structure

In the wild, European pond turtles live in populations of males and females. In captivity, in relatively small enclosures, aggression between males may be a problem. It is therefore recommended to have a second enclosure to separate, if necessary, some of the males or to give dominated the possibility to seek shelter from male aggression (Wolff, 2004).

2.3.2 Sharing enclosure with other species

Reptiles and amphibians from different geographic areas should not be maintained together because of the risk of disease transmission. This is especially important for animals that are bred for releases in the wild. Chelonians appear to naturally carry a number of commensal protozoans that may be pathogenic for snakes and lizards so should not be housed together. Avoid mixing species which occupy the same ecological niche (eat same food, same microhabitat). For example two species of turtles that eat the same food at the same time of the day may compete directly or indirectly. Mixing turtles with small fish may be a nice enrichment, as the turtles can feed on their eggs, hatchlings and dead fish. Finally avoid the use of species which could destroy the nest and/or predate eggs or hatchlings.

2.4 Breeding

2.4.1 Mating

Mating takes place in the water. Currently, one of grey areas in the European pond turtle breeding management is the affiliation of the offspring. When keeping together several females and males, it is unknown which males really contribute to reproduction. If there are only few males in the colony that mate, the genetic diversity of the produced offspring is limited compared to the potential. Reintroducing animals with a low genetic variability is not recommended when the goal is to create sustainable wild populations. Researchers currently work on genetic tests to identify paternal affiliation, which are already successfully used in Mulhouse Zoo and the Petite Camargue Alsacienne centre.

One possible solution can be to keep turtles in couples or small groups. Rogner (2009) proposes to install after hibernation in pairs in a 60*30*30 cm aqua-terrarium with 10 cm of water, and to separate them after mating. Afterwards the female is kept in a 100*40*40 cm aqua-terrarium, to which in May a 40x25x25 cm box filled with moist soil is added as nesting area, allowing the female to lay her eggs. After laying, the eggs are collected to be artificially incubated and the female is released outdoor.

At Papiliorama the turtles are kept in small outdoor enclosures in small groups of one male with two females. Maternal filiation is easier to determinate as egg-laying can be monitored (see below) and females will probably lay on different days and in different nests. At least the contribution of a larger number of males to the population is assured.

Figure 53 : Enclosures for small breeding groups at Papiliorama. © Papiliorama.

Although these solutions are very useful to control the affiliation of the offspring, they are generally not an option if the turtles are in a public exhibition. Zoos that want the turtles in an education exhibit and have a controlled breeding programme, may follow the example of Mulhouse zoo with an exhibition enclosure visible by visitors and a breeding enclosure behind the scenes. The educational enclosure could be a good solution to keep turtles unsuitable for the breeding programme (hybrids, unknown origin, wrong haplotype, …).

2.4.2 Egg laying and incubation

2.4.2.1 Collection and handling of the eggs

The majority of European pond turtle breeders collect the eggs to incubate them artificially. Artificial incubation is useful to maximize the number of turtles produced, while decreasing the loss of eggs due to predation. It allows successful hatching in areas where environmental conditions do not enable natural incubation with a high hatching rate. To collect the eggs, one first needs to locate the nest. Different solutions can be implemented to facilitate finding the location.

First, it is possible to work on nesting area size. The smaller the nesting area is, the easier it can be inspected. The female turtle will generally close here nest with a plug, to protect the eggs. When the substrate of the nesting area is composed of a majority of soil, the entrance of the nest can be detected by looking for the plug composed of compact moist soil (*Figure*). When the nesting area substrate is compound of a majority of sand, the plug is less characteristic and to facilitate localisation institutions used different methods:

- the use of substrate of different colours: the use of a layer of dark soil under the sand, when the turtles dig the dark substrate will be visible on the surface
- raking of the nesting area at the end of afternoon so a disturbance of the substrate will be more visible
- the use of camera traps. If the females have external identification, this method can even be used to monitor the maternal filiation of clutches

- someone can observe the turtles in the evening without disturbing them and collect the clutch immediately after the laying or remember the location and collect the eggs the next morning.

Figure 54: Nest plug on Zoodyssée nesting area. © L. Berthomieu

At Mulhouse Zoo and at the Petite Camargue Alsacienne (PCA) a system created at the Institut Pluridisciplinaire Hubert Curien in Strasbourg is being used. This system is adapted from a system used in studies on penguins. The system is made of two-meter long antenna, positioned on both sides of a data acquisition system. When a turtle equipped with a special transponder crosses an antenna, a text-message is sent with the date, hour, identification of the turtle and the number of the crossed antenna. The cost of such installation is 4000 to 5000€. Some of the components of the device installed at Mulhouse Zoo and the PCA are no more available, so employees at IPHC work on an alternative system with some adaptations. The adaptation proposed is to increase the length of the antenna and to use sun power for power supply.

For more information on this device, please contact Jean-Yves Georges, Université de Strasbourg CNRS, IPHC UMR7178 (jean-yves.georges@iphc.cnrs.fr).

Figures 55 and 56: Nesting area with dug-in antennas in PCA during and after building. © Zoo Mulhouse.

At the Réserve de la Haute Touche, females that are observed on the nesting area in the early evening are collected, injected with 10UI/kg of oxytocin and installed on a grid in a plastic box with water at the bottom (*Figure*). The females will lay the eggs, that fall through the mesh (3x3cm) without the female being able to crush them, and that stay in the water all night long to be collected for incubation the next day.

The advantages of this protocol are that the maternal affiliation is known and that the risk of predation on the eggs is decreased to zero. However, the impact on animal welfare should be evaluated, as with this induced laying the natural behaviour is entirely modified: the female cannot build its nest and the oxytocin causes contractions which could be painful. Another research point is to evaluate the impact of this protocol on the incubation and hatching success to be sure that the water does not, for example, increase the number of rotten or moulded eggs. In the wild, turtles lay on areas which cannot be flooded, so the eggs are never under water. Chelonian eggs typically do not survive submersion for extended periods (Innis, 2008), but La Haute Touche is very successful in breeding European pond turtles and this methods seems to work well.

Figure 57: Laying containers at Réserve de la Haute-Touche. © L. Berthomieu

Once a nest is located, the second step is to collect the eggs and the sooner the better. Unlike bird eggs, there is no chalaza (bands that hold the yolk) in the eggs of turtles, so they should be kept in the exact position as they were in the nest, to avoid the embryo to be choked under vitellus (yolk). Consequently, the eggs should not be tilted or rolled and the top of the eggs should be marked (Rogner, 2009). However, some herpetologist state that there is no special precautions when handling the eggs during the first 24 hours (Innis, 2008).

The necessary tools to collect the eggs are a plastic box with a layer of sand, a small shovel or spoon, a wax pencil and a paint brush.

One should dig carefully around the nest with the shovel, then finish with the hands and eventually to use the paintbrush to get off the remaining sand/soil from the eggs. Eggs should never be washed or disinfected, as the shell is covered with an active substance with some antiseptic properties that should not be removed (Presteau, 2008a). Each egg should be weighted and measured. With a wax pen the upper part of the egg is marked to avoid changing the position of the egg, especially during transport and hatching, when a hatchling could push aside other eggs (Rogner, 2009; Wolff, 2004). The eggs are transported in the box with a space of 2cm between each egg and they are buried a little in the sand to prevent movement during transport. With a wax pencil (2B or 4B) the eggs can be numberer to allow individual follow-up. Other pencils, markers or nail polish could damage the shell or chemicals could enter the eggs. The shell is still soft and delicate so handling of the eggs should be reduced to a minimum.

The maximal limit to collect the eggs proposed is 48 hours (Anonymous, 2004) but it should preferably be done in the first 24h after laying.

2.4.2.2 Incubation

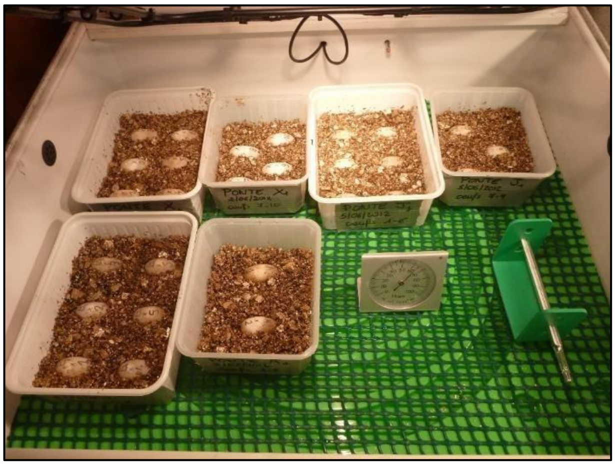
Substrate

For incubation different substrates are used. Some breeders do not use substrate, but the eggs are laid on a grid. The grid is positioned in a transparent plastic box with 2cm of water at the bottom. The box is closed with a plastic lid and is kept closed during the whole incubation time.

In the wild, the European pond turtle builds a nest which looks like a pear-shaped cavity where eggs are laid (see chapter Reproduction), allowing to have always some air around them. This is important to keep it in mind when choosing the type of substrate. If the substrate is too fine (e.g. fine sand) the aerial exchange would be insufficient. The usual substrates used for reptile eggs incubation are Vermiculite or Perlite or a 2-4mm gravel (Rogner, 2009). The use of a mix with 2/3 of peat and 1/3 of sand is also reported for the incubation of chelonian eggs (Presteau, 2008a). The addition of peat acidifies the substrate, which may facilitate the opening of the shell during hatching. In the natural environment, the acidity of the substrate and carbon dioxide emitted by the embryo decreases egg shell hardness. If in artificial incubation the substrate is not sufficient acid, hatchlings can have difficulties to break the egg shell (Rogner, 2009).

The substrate should be moist, it is advised to soak the Vermiculite one hour in water and let drain off. In general, a ratio of 1:1 water: vermiculite by weight is successful (Innis, 2008). A plastic box can be filled with a layer of 2-5 cm substrate (Presteau, 2008a; Rogner, 2009), which should be compressed to keep air between substrate grains. Some keepers periodically spray the substrate with water. If desired, the entire egg box can be precisely weighed and water added as needed to maintain a constant weight (Innis, 2008). Boxes can be labelled with clutch number, date and number of eggs.

Eggs are placed at least 2-4cm from each other. They should be half buried in the substrate, but the upper surface should be visible in order to follow egg evolution.

Figures 58 and 59: Vermiculite preparation and egg disposition. © Mulhouse zoo

Depending on the breeder, the boxes are closed or not. Rogner (2009) advices to put a slightly moistened linen cloth on the boxes to prevent condensation dripping on the eggs and keep the inside humidity stable.

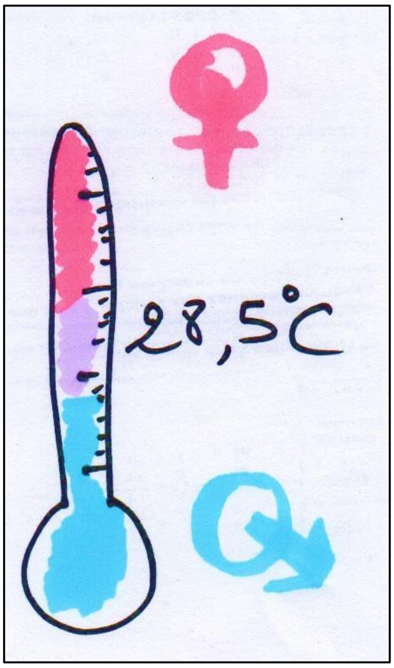
Incubator type

Different incubators designed for reptiles are available on sale and can be used to incubate European pond turtle eggs. Some examples are the JAEGER Kunstglucke FB 80; the JAEGER FB 50; the HEKS Snake 2018 and the GRUMBACH Compact SR. At the Réserve de la Haute Touche a GRUMBACH incubator designed for bird eggs is modified with a thermostat adjusted for reptile egg incubation and used successfully.

To function properly, it is important that the incubator will not be installed in a room with extreme conditions, the room temperature should be monitored and maintained around 20-22°C. The incubator should not be installed near a window, to avoid overheating from the sun. In addition, the room should be enough ventilated to prevent that the air humidity is too high.

When an incubator is not available, it is also possible to build your own incubator. The method described by Prestreau (2008a) and Innis (2008) to incubate eggs of aquatic or semi-aquatic turtles is au bain-marie. Put two or three bricks in the middle of a 60-100cm long aquarium and add water to the height of the bricks. Install a plastic container with the boxes containing your substrate and eggs to incubate on the bricks. Cover the boxes with lids in which five to six holes are drilled. Cover the aquarium with an angled lid (1-2cm lower on one side). This is important to allow condensation sliding along the lid without falling on the eggs and cause them to rot. The water is warmed with a submersible aquarium heater. Install a thermometer and a hygrometer in a box with substrate but no egg, use this thermometer to check temperature (see chapter XX for the chosen temperature) in the boxes (Prestreau, 2008a).

Temperature and hygrometry



As described before, in the European pond turtle the gender of the embryo is partly determined by the incubation temperature of the egg (temperature-dependent sex determination, TSD). *Emys orbicularis* is one of the first turtle in which temperature-dependent sexual differentiation of the genital system was shown. Under laboratory conditions with constant incubation temperatures, *E. orbicularis* embryos develop male characteristics at temperatures below 28.0°C and female characteristics above 29.5°C. In between, males and females will hatch. According to Pieau (1998), there may be differences between clutches, populations or species. When studying animals from the Brenne population (France) in laboratory conditions, she found that all eggs incubated below 28°C yielded males, while all eggs incubated above 29.5°C yielded only females. In the transitional range between 28 and 29.5°C there is much variation between clutches. For clutches, the pivotal temperature to obtain a 50/50 sex ratio is between 28 and 29°C, in case of the Brenne population it is 28.7°C (Pieau, 1998). The thermosensitive period is 11-12 days long (Pieau, 1982). Temperature begins to influence sexual differentiation at embryonic stage 16, a stage in which gonads are still histologically undifferentiated. In order to obtain 100% males, exposure at 25°C is required from stage 16 to 21 while to obtain 100% females an exposure at 30°C from stage 16 to 22 is required. With shorter exposure, sex ratio obtained is different and intersex individuals are observed. The incubation temperature applied before stage 16 does not influence the sexual differentiation of gonads. The sensitive period begin at this stage and after this thermosensitive period the temperature does not affect sex differentiation anymore (Pieau, 1982).

It might seem more interesting to reintroduce females than males, as the more females are present the more offspring can be produced. However, Girondot et al. (1998) showed that on the long term this may not have an effect on the population size, as the released females may react on a low number of males by producing more male offspring. As the unequal sex-ratio is disadvantageous for the long-term genetic variability in the population (only few males will share their genes with many females), it is not advised to manipulate the sex of the offspring born in captivity.

We recommend not to use temperatures exceeding 33°C, because embryonic development does not profit or is even disrupted at these high temperatures (Schneeweiss, 2004b).
Two solutions are possible to obtain males and females. Either the incubation temperature is close to 28.6°C (between 28 and 29.5°C) or to obtain a defined sex ratio, eggs from the same clutch can be separated and incubated in different incubators, one with “female” temperature and the other with “male” temperature. Prestreau (2008a), relying on personal data, advices to use temperature 2°C under or above the critical temperature because the closer to the critical temperature the incubation temperature is the higher embryonic mortality is.

The temperature sensitive stage in sex determination last approximatively 12 days (Rogner, 2009). It is important to respect these temperatures between the 30th and 42nd day. Haderer (2009) advices to gradually decrease temperature to 25°C after the 42nd day) (Haderer, 2009).

In natural conditions, there is a change in temperatures between day and night. Prestreau (2008c) recommends to copy this daily temperature cycle with artificial incubation. He observed that if incubation temperature is kept constant, the hatchlings plastron is sometimes deformed and that the offspring is often less resistant. He also noted that the upper part of the shell can become deformed during the first years, comparable to UVB or calcium deficiency. Wolff (2004) lowers at night the temperature with 2-4°C for approximately 4 to 5 hours. Mulhouse Zoo follows Haderer's advice by decreasing incubation temperature by 6°C during night (between 22h-6h). Other zoos, such as Zoodyssée, switch off the incubator during the night (the temperature in the incubator stays at approximatively 22°C).
The recommended hygrometry for incubation is 80-90% (Rogner, 2009; Wolff, 2004). High hygrometry is important at the beginning and the end of incubation; Mulhouse Zoo incubates at 90% hygrometry and advices in their technical sheet to place if necessary, a glass of water in the incubator to keep a high hygrometry.

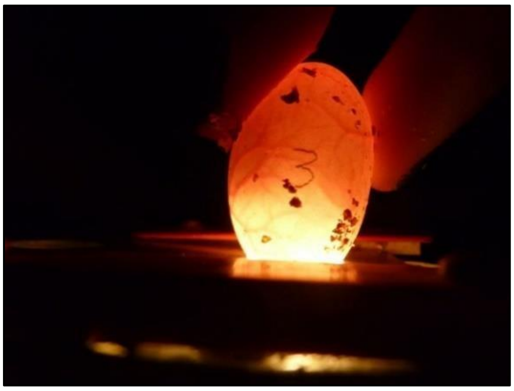
We advise to check daily the temperature and hygrometry in the incubator; at the same time one should check the eggs (e.g. remove rotten eggs). The best is to install the thermometer and the hygrometer in the same conditions as the eggs (e.g. in an incubation box). Drops on the inside of the lid of the incubator should be removed, and once a day the incubator should be aired by removing shortly the lid (Haderer, 2009).

The chosen parameters can influence the incubation time. The reported incubation times range from 60 to 90 days (see table 28).

Breeder	**Incubator**	**Incubation temp. during day (°C)**	**Incubation temp. during night (°C)**	**Hygrometry (%)**	**Incubation time (days)**
Uwe Stulle	JAEGER	31	31	80	60
Papiliorama	HEKS Snake 2018	31-32	31-32	80-90	80
Editha Kruger	JAEGER, FB 50	31	Ambient temp.		65
Bern	GRUMBACH, Compact SR	30-31	20	80	60
Mulhouse		28 (males incubator) 30 (females incubator)	T° day minus 6°C	90	?
Réserve de la Haute Touche	GRUMBACH	26 (males incubator) 30 (females incubator)	26 (males incubator) 30 (females incubator)	?	80-90
Zoodyssée	GRUMBACH, Compact SR	29	Ambient temp. (22°C)	?	65-70
Lodz	Self-made	27-30	26-28	90	60
Chomutov	JAEGER, FB80-R	28-30	28-30	90	60
Wolff	?	28-29°C (males) 30-32°C (females)	T° day minus 4-6°C	80	64-70 (males) 61-65 (females)

Table 22: Incubation conditions used in different breeding centres

Eggs
Many chelonian eggs have a slightly yellow to orange hue when freshly laid (Innis, 2008). When fertilised, a small white patch appears on the dorsal surface of the egg during the first or second day and its size gradually increases during incubation period (Innis, 2008; Haderer, 2009).



Figures 60 and 61: Fertilized eggs and egg candling. © Zoo Mulhouse

It is also possible to check if the eggs are fertilised by candling. Within several weeks, most eggs will show blood vessel development (Innis, 2008). The use of a light source such as an ophthalmoscope allows to observe these vessels while transilluminating the egg. However, it should be kept in mind that this method is not mild and be the cause of embryonic development disruption. Because of the lack of yolk strings (chalazas) in turtle eggs, they should not be rotated after the first few days of incubation (Innis, 2008; Presteau, 2008a). Innis (2008) reports successful chelonian egg monitoring using an avian egg cardiac monitor.
As egg candling requires handling the eggs, it increases risks of embryonic mortality. Therefore we do not recommend the use of this method; if the incubator is regularly checked to detect rotten eggs, there is no risk to keep unfertilized eggs in the incubator.

Abnormal eggs can be a sign of inadequate incubation parameters. If withered, deflated eggs are observed, hygrometry should be increased (Presteau, 2008a). Cracked eggs can be the sign of excessive humidity. Cracked eggs can be repaired with chalk, ideally chalk sticks made of calcium carbonate ($CaCO_3$). Calcium carbonate powder can also be purchased in drugstores. Some mineral water is added to the powder and the preparation is then applied on the crack (Presteau, 2008a). Hygrometry in the incubator should be decreased to avoid new cracks. Some zoos also try to use surgical glue for repairing cracked shells.
A swollen egg is the sign of rotting and should be removed from the incubator.

Maintenance
To avoid pathogens transmission between clutches we recommend cleaning and disinfecting the incubator at the end of the incubation period. Examples of disinfectant usable are Ovaclean ® and Ark-klens ®.

2.4.3 Contraception

Breeding of pure *Emys orbicularis* of certain haplotypes is necessary to reinforce wild populations or for reintroductions. However, breeding of hybrids, animals of unknown origin and animals of "unwanted" haplotypes is not recommended. Sexes can be separated or clutches can be destroyed after laying. The advantage if the latter method is that the animals can express natural behaviours, but it can be time-consuming to detect nest and nests can be missed. Unwanted offspring can be euthanised, depending on national legislation.

2.4.4 Breeding results

When incubation and hatching is well monitored, problems can be detected and it might be possible to determinate which parts of the breeding should be improved to increase the production of viable individuals. In the annexes an Excel document can be found, that could be used by different breeders to enter their data and to compare breeding results of different breeders.

A breeding centre can be considered as well managed when with 15 adults females 50 to 100 hatchling are produced yearly (Anonymous, 2004).

2.4.5 Hatching

The hatchling may take one to three days to emerge from the egg (Presteau, 2008a; Innis, 2008; Haderer, 2009). Cracks can be observed some days before hatching. During this time, the yolk sac remnant is generally resorbed. As disturbing the hatchling at this time may traumatize the yolk sac, we recommend not to help turtles during hatching. Rogner (2009) recommends to put hatchlings with an unabsorbed yolk sac in an egg-shaped cup in the incubator, with the head out of the water while the yolk sac is under water. Prestreau (2008a) advices to disinfect a cup with chlorhexidine and fill it with gauze moistened with a sterile saline solution (cotton should not be used). The turtle is put on the moist gauze and the cup is installed in the incubator (60% hygrometry maximum) until yolk sac is absorbed. Once the yolk sac is absorbed, hatchling can be transferred to the nursery. Wolff (2004) makes a shallow ditch in the substrate, puts the hatchling in it and covers the hatchling with a small piece of foam. The animal usually stays in the cover and slowly absorbs the yolk.

Prestreau (2008a) reports that seven to ten days may pass between the hatching of the first egg and the last egg of the same clutch. When observed the first hatching at Mulhouse zoo, the boxes are closed with a perforated lid to be able keeping track of the hatchlings from the same clutches. Prestreau (2008a) recommends not to remove the shell after hatching, to allow the hatchling eating it and getting some minerals.

Once emerged from the egg, the hatchling should be moved to a nursery enclosure appropriate for the species. Aquatic species are generally able to swim as soon as emerged from the egg (Innis, 2008), but need to be able to emerge easily from the water. Haderer (2009) advices to keep hatchlings during one week in a box with 1cm of water and aquatic plants, and transfer them afterwards to the indoor nursery. Weighting and measuring turtles can be interesting to monitor their development. The weight of a hatchling is around 5g.

2.4.6 Development and care of young

Housing conditions and feeding are discussed in earlier chapters. After hatching either hatchling are kept in indoor nursery or, to copy natural conditions, in a natural outside enclosure (Anonymous, 2004). Sexual maturity is reached at various ages, depending on the haplotype and rearing conditions.

2.4.7 Population management

According to the Reptile RCP (Goetz, Aparici Plaza, Van Lint, Fienieg, & Hausen, 2019), *Emys orbicularis* has direct conservation roles (insurance, source, *in-situ* research, conservation education and *in-situ* support) and an indirect role (rescue). Therefore it was decided to create an EEP for the species.

Direct Roles	Programme characteristics required	Benefit	Feasibility	Risk	Role recommended by TAG?	Will EAZA contribute to deliver this role?	Notes
Insurance	For pure threatened subspecies	**HIGH**	**MEDIUM**	**LOW**	**YES**	**YES**	Genetic analysis required
Source	Healthy population of animals that are individually identified, sexed and genotyped	**HIGH**	**HIGH**	**MEDIUM**	**YES**	**YES**	(re)-introduction projects in several countries are in need for more animals for release. Ideally, these animals should not come from only a few institutions with the same founders, but the genetic variability of the released animals should be higher than the current situation.
Research (*in-situ*)		**LOW/ MEDIUM**	**HIGH**	**LOW**	**YES**	**NO**	Research on certain aspects of the biology can help reintroduction and conservation projects. Ground work is done. Should be part of package when reviewing a project.
Conservation education		**HIGH**	**HIGH**	**LOW**	**YES**	**YES**	Zoos can educate about this species, but also about the risk of releasing exotic turtles in nature (e.g. the problem of red-eared sliders
***In situ* support**		**HIGH**	**HIGH**	**LOW**	**YES**	**YES**	Zoos can support with (funding) research and conservation projects.
Indirect Roles	**Programme characteristics required**	**Benefit**	**Feasibility**	**Risk**	**Role recommended by TAG?**	**Will EAZA contribute to deliver this role?**	**Notes**
Rescue	See above + husbandry guidelines	**HIGH**	**MEDIUM**	**LOW**	**YES**	**YES**	Zoos can play a role in the rescue of confiscated/abandoned animals. This has not only a welfare and educational role, but selected animals can contribute to the gene pool of the breeding population

Table 23: Roles of the Emys orbicularis *EEP (Goetz, Aparici Plaza, Van Lint, Fienieg, & Hausen, 2019)*

European zoos keep a large number of *Emys orbicularis*. Unfortunately, many zoos have difficulties catching their animals (especially those that live in large natural ponds) and most animals have not been genotyped. Many animals are confiscated or abandoned specimens, and from experience we

know that these have a variety of haplotypes (with many from the Mediterranean region) and a large percentage is hybrid.
Although education is an important role for the European pond turtles in European zoos, the zoos should work towards a situation that the necessary number of pure *Emys orbicularis* for (re)-introduction programmes can be produced. To achieve this goal, several actions by individual zoos are necessary.

1) Each zoo should identify, sex and genotype all individuals kept in the zoo

Once this is done, and depending on the outcome, zoos can:

2a) Keep only the pure and recommended haplotypes, and make all necessary preparations to breed and rear, if requested by the EEP (in concertation with *in-situ* conservation projects), pure European pond turtles

<u>or</u>

2b) Keep all hybrid and unrecommended haplotypes without breeding, and transfer pure animals of recommended haplotypes to institutions that want to breed with these haplotypes. The zoos can use the animals for the education role.

Zoos that decide to keep a breeding group should not only prepare installations for the breeding groups, but should also have facilities to keep the offspring until they are ready for release, unless other zoos are willing to take the animals during this period. There are different opinions about the best age to release captive born animals in the wild. In some projects the turtles are transferred to an acclimation enclosure near the reintroduction area when they are 4-5 years old and larger than 10 centimetres to limit predation (Anonymous, 2004), to be released some time afterwards. Other projects release animals at the age of 3 years, or even during their first year of life. Releasing very young animals will probably result in a greater loss due to predation, but has an advantage that no enclosures for rearing are necessary. Further research in the best methods will be necessary.

The EEP will, with the help of the zoos in range countries, try to stay in contact with different (re-) introduction programmes to determine what number of which haplotypes are necessary for the project, to establish the breeding recommendations for zoos.

As indicated above, one of the major aims of the EEP is to determine the haplotype of each individual, in order to be able to breed pure turtles and stop the hatching of hybrids. The EEP works together with research institutes to conduct the genetic testing of *Emys orbicularis*. With the cytB marker (mtDNA) the haplotype group can be defined. However, to define de purity of the specimen (potentially hybrid of 2 haplotypes) nuclear DNA analyses with microsatellite markers is required. Genetic analysis (mtDNA and Nuclear DNA) can be done with different types of samples on living specimens.

2.5 Hibernation management

2.5.1 Adult individuals

Adult turtles can either hibernate indoors or outdoors. Typically outdoor chelonian hibernation lasts five to six months, but indoor hibernation can be shorter (Boyer & Boyer, 2005). Whatever the method chosen, hibernation is only recommended for healthy specimens with good body weight, because weight loss during hibernation can be considerable. A physical examination several weeks before hibernation is advisable (Boyer & Boyer, 2005). Before hibernation, food quantity should be gradually be decreased to allow turtles to empty their guts before hibernation, so that no fermentation will be possible.

2.5.1.1 Outdoor hibernation

Outdoor hibernation is potentially more dangerous because there is less control over environmental conditions. Feasibility depends on the constitution of the outdoor pond, as the layer of silt should be thick enough (minimum 20 centimetres), with enough vegetation and water depth to avoid that the turtles freeze. However, the thickness of silt should not be too excessive because it the cause of suboxic or even anoxic conditions, thus preventing the extra-pulmonary oxygen uptake via the gut which usually occurs under the ice. If ice appears at the surface of the pond, it should not be broken to not disturb the hibernating animals (Rogner, 2009).

2.5.1.2 Indoor hibernation

To prepare the turtles for hibernation, feeding is stopped approximately one week in advance (Matz & Vanderhaege, 2004; Rogner, 2009). Boyer and Boyer (2005) advise that when turtles decrease their food intake during fall, to withhold food for one to two weeks but keep them at 21-27°C, to give the turtle time to clear their gastrointestinal tract. Only afterwards the external heat source can be removed. When kept indoor, lighting and temperature are easy to manage. From November lighting should be reduced to ten hours and temperature decreased to 20°C. One week before hibernation light is switched off, water temperature is decreased to 12°C and the aquarium is covered with black cardboard (Rogner, 2009).

Boyer and Boyer (2005) recommend selecting a room that can be kept between 2 and 10°C. The ideal target temperature is 5°C. Temperatures above 10°C are too high and below 2°C too cold. With temperatures of above 10°C a torpor is created, but not a true hibernation, and the turtle will use its fat reserve with its ongoing metabolism. A turtle should not lose more than 1% of its body weight per month of hibernation (Morris, 2002). During torpor a turtle may lose too much fat, and in case of excessive weight loss should be taken out of hibernation.

When choosing for indoor hibernation, the “dry” or the “wet” method can be used. When using the “dry method”, the turtles can be kept individually in a fridge or cool room in boxes with holes on the sides and with a layer of flax or wet peat so the turtle can burry itself (Matz & Vanderhaege, 2004). Wild turtles can hibernate in a slightly humid soil to decrease evaporative water loss through the skin and lungs. Captive turtles hibernating indoors risk dehydration due to low humidity, and hydration is critical to a successful hibernation. If the hibernating turtle empties its water stores, it should be brought out for rehydration in shallow water (Morris, 2002).

Other breeders let their turtles hibernate in water. The Ferme aux Crocodiles uses for hibernation containers of 116x154x80cm, with 20 centimetre water and different floating and emergent items inside (the number of turtles in each container is not specified (Scibek, 2014), while Hlubloka Zoo keeps the turtles individually in buckets in a cool room (figure 65). Turtles are also reported to be kept in individual aquaterraria of 30x20x20cm in a fridge (Rogner, 2009). A fridge, a wine cooler, basement or a cool room (6-10°C) are locations to make turtles hibernate. In the container, the water should be one to two centimetres above shell. If necessary, water can be renewed using water with same temperature as the previous one. When kept in a fridge, it should be opened daily and the door should be moved several times for air turnover (Matz & Vanderhaege, 2004).

Figure 62: Wet hibernation system in a cool room in Hluboka Zoo. © Hluboka zoo

2.5.1 Young individuals

Hibernation of juveniles is disputed. Some experts believe that turtles less than 3-4 years old should not hibernate (Boyer & Boyer, 2005). One of the reasons is to increase the turtles' growth. Some breeders let them hibernate from their second year but only during a short period of one month (Rogner, 2009). Others transfer juveniles to the outdoor nursery and let them hibernate outdoor from their first year. In Hluboka Zoo, the outdoor nursery is partially covered with polystyrene plate and the temperature is monitored by a thermostat and aquarium heater at about 5°C. At the Centre d'Etudes de Protection d'Elevage des Chéloniens (CEPEC), the turtles hibernate in their outdoor nursery when they are three months old. Some breeders consider the absence of hibernation as a risk factor for mortality in hatchlings (Perconte-Duplain, 2017). Considering the fact that in nature young *E. orbicularis* hibernate from their first winter and that in his study there is a higher (though not statistically different) mortality in a breeding structure where their youngsters do not hibernate, he recommends to hibernate juveniles their first winter (Perconte-Duplain, 2017).

Rogner (2009) recommends to decrease water temperatures from the end of October and to keep the juveniles from half November to half February in a dark cool room of between 5 and 10°C. In a personal communication to Parc Animalier de Sainte Croix, captive breeder Rust mentioned that from the end of September he decreases the photoperiod, to switch off the light completely at half November. At the end of November, the hatchlings are housed in a fridge between 4-6°C, in a box (four turtles per box), with one centimetre of water and much aquatic vegetation (ex: *Ceratophyllum*). The turtles are checked every once or twice a week.

Georges (2015) reports that some breeders add salt and black tea to the water in the hibernation boxes, because of their purifying properties.

Figures 63 and 64: Hibernation system in Mulhouse Zoo. © Zoo Mulhouse

2.6 Handling

2.6.1 Individual identification

Individual identification is essential to implement a consistent management of the captive population of *Emys orbicularis*. Genetic management is possible only if breeders are individually identified, and if the (potential) parents of each individual are registered. In addition, in some countries such as France individual and permanent identification of protected animals is compulsory.

Different methods are described to identify individuals in field studies (Servan J. , 1986), but some are not consistent with captive population identification (ex: balloons, radio-tracking, GPS) and they will not be described in these guidelines.

Transponder

As transponders are widely used in zoos and have been standardized between countries, this is the best solution. There is a small risk that the transponder is lost or stops functioning, but the system is generally very reliable. One disadvantage is that catching is necessary to read the transponder to identify the individual. Therefore, we advise to add a second (external) method, as described below.

Because of the small size of hatchling and juveniles, inserting a transponder may be difficult for very young animals. However, small transponders of 1.14mm x 8mm (e.g. Biolog-FD Tiny®) are available and can be used in turtles with a 5cm plastron size. Currently, the smallest transponders available are the Biolog-Nano® and the AgnThos Pico-ID®, the microchips being only 1.2mm x 7mm and the needle for application has a diameter of only 1.6mm. Mulhouse Zoo identifies *Emys orbicularis* hatchlings with these microchips, which are implanted subcutaneously in the nuchal area.

For small turtles, the microchip is usually implanted subcutaneously in the left thigh. If the skin is too thin, it can be implanted intramuscularly in the quatriceps femoris muscle. If this is not possible, it can be implanted in coelomic cavity. For medium-sized turtles, the microchip is implanted subcutaneous or intramuscularly at quadriceps femoris level or in the lateral left side of the tail.

To avoid the transponder dropping out of the insertion wound, a small amount of chirurgical glue can be applied on the wound. The transponder should not be injected just before the hibernation period, as the skin will not heal very well during this period.

Nail polish, painting or permanent marker signs
Individuals can be identified with a code (letter, sign, number, …) on the shell, made with painting, nail polish or a permanent ink marker. This method is easy to implement, painless for the animal and allows recognition from a distance. It can be useful to recognise which female is laying. However field data shows that after two to eight weeks the marking is no longer readable because of silt, scale loss or deterioration of the markings (Servan J. , 1986). Consequently, regular catching is necessary to keep the marking efficient. This method is also quite unsightly when turtles are presented to the public.
The code depends on the institution; to avoid confusion, this method should be combined with a transponder.

Figures 65 and 66: Painted identification Zoo Mulhouse. © Zoo Mulhouse

Notches or holes on marginal scutes
A common identification method is to make notches or holes in the marginal scutes. Depending on the size of the individual, different tools can be used. Smooth files are the best for marking young juveniles, second-cut files for older juveniles or adults (Nagle, Kinney, Whitfield-Gibbons, & Congdon, 2017). Drilling is also an option and is faster than notching with files, but can be difficult in the European pond turtle because the marginal scutes do not protrude well beyond the plastron. A four to five millimetre diameter drill bit can be used but marginal scute width should be at least three times larger than the drill bit diameter. To avoid injuries, be careful to keep the tail and limbs out of the path of the rotating drill bit, which can be difficult when the turtle struggles from handling. On very small turtles notches can be applied using fingernail clippers or scissors (Nagle, Kinney, Whitfield-Gibbons, & Congdon, 2017).

Even though this method of marking seems to be unharmful for turtles, it may be considered as mutilation and therefore sometimes not permitted either by country law or through an institutional ethics code.

Figures 67 and 68: European pond turtles marked with a drilled hole in a scute. © L. Berthomieu

Once the notches/holes made, the reading of the identification depends on the numbering of marginal scutes and the code chosen. Different numberings are possible. A well-known coding system is the CAGLE code (Cagle, 1939). Marginal scales of each side are number from 1 to 12, the first one being the one next to nuchal scale. One or several scales can be notched, so there are more than 50 000 combinations.

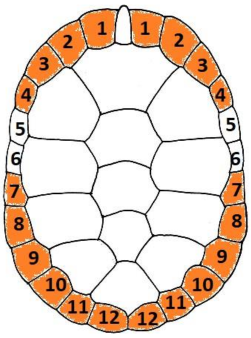


Figure 69: CAGLE numbering

Others numbering systems have been proposed and are used by different breeders and institution.

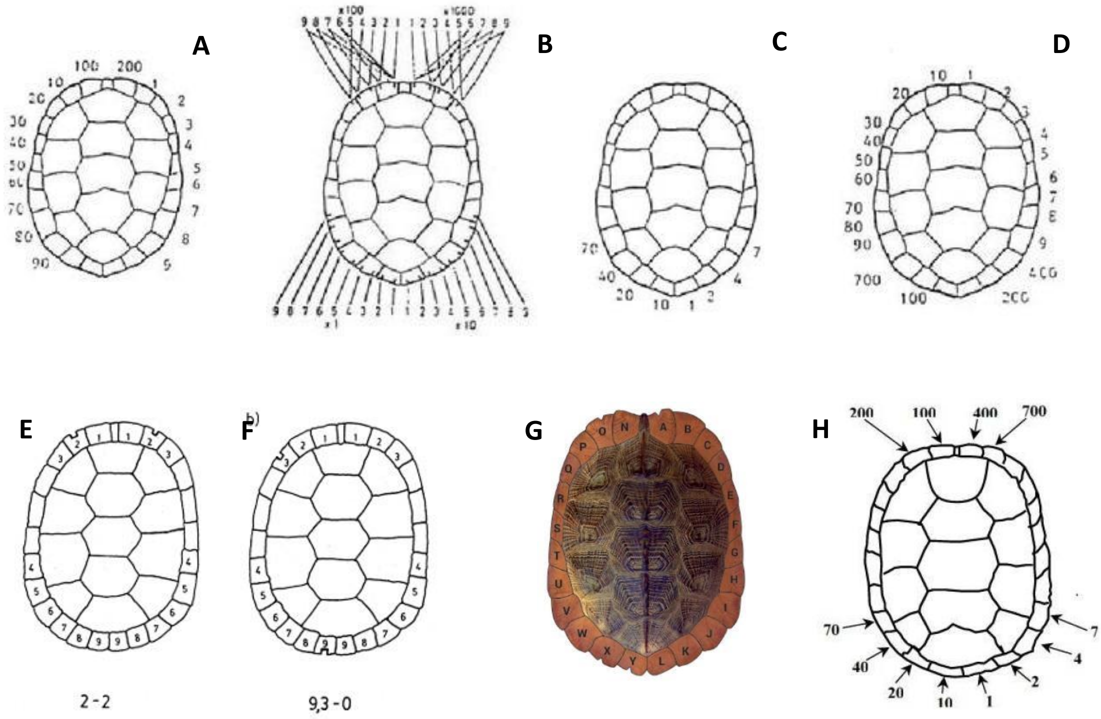


Figure 70: Different numbering systems - A: SHEALEY numbering; B: PEREZ, COLLADO and RAMO numbering; C: MAJOR numbering; D: STUBBS et al. numbering (Servan, et al., 1986); E and F: URSENBACHER numbering and coding (Ursenbacher, unpubl.); G: North American Coding (Nagle, Kinney, Whitfield-Gibbons, & Congdon, 2017); H: CEYTE numbering (Ceyte, 2013).

With the described methods, there are thousands possible combinations to identify individuals. Because of the diversity in numbering/coding marginal scutes, this can lead to some confusion if the turtle changes institutions. Therefore, the animals should also be equipped with a transponder.

Unlike the painted code, this method is permanent. Data shows that the notches/holes are still present 8 to 15 years later (Servan, et al., 1986). This method is harmless if no notches or holes are made on the marginal scutes along the bridge, as this could interfere with growth or cause a fracture the shell, creating a breach to the internal body cavity (Servan, et al., 1986; Nagle, Kinney, Whitfield-Gibbons, & Congdon, 2017). So notching can only be done from M1 to M4 and from M7 to M12 (according to CAGLE numbering).

Shell burning
Burning of scutes (marginal or plastron) using a red-hot iron is a method sometimes used to identify individuals (Servan, et al., 1986).

Marks/rings/colour strips
Either a hole is made on a scale and a ring/mark (plastic, metal) is inserted through this hole or the mark is fixed on a limb (Servan, et al., 1986). The major problem is that the animal can lose the ring, or even worse that it can drown, while getting caught in branches, plants, etc. Therefore this method is not recommended.

Pearls

Mulhouse Zoo has also experimented with the use of colour pearls stitched on the shell, to recognize hatchlings. It may be possible to use several pearls on an individual (one colour for the clutch and the other one for the individual). This method can be a good solution while waiting for the turtle to be large enough for transponder identification. However, in Mulhouse Zoo, shell deformation was observed to be caused by the pearl.

Photographic identification

In France, pictures of the upper shell and plastron are compulsory for small individuals not identified with microchips. Pictures of the head can be added. The pictures should be scaled with a graduated ruler or grid pattern.

Phalanx amputation

An old identification method is phalanx amputation. Today this method is not acceptable anymore for animal welfare and ethical reasons.

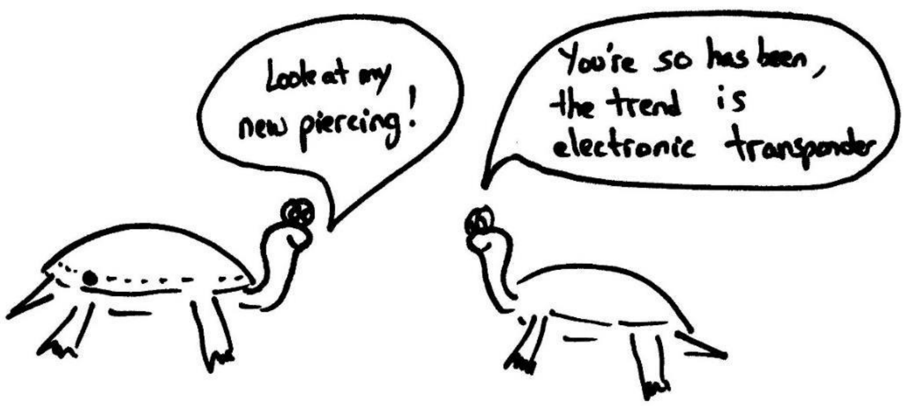
Conclusion

We recommend the use of transponder identification, as this is the most reliable method and an important prerequisite for animal transfer. As this method requires to catch the animal for identification, it can be combined with a second method which can be used to recognize individual at distance in daily management. We recommend that all zoos use the CAGLE numbering to create a standard that will avoid confusion when an animal is transferred between zoos. Combining methods is useful because an individual is still identifiable if one of the marks is not usable.

Example of national legislation :

According to French legislation, because *Emys orbicularis* is a protected species, all captive animals should be identified individually using a permanent method (transponder) in the month after hatching. If identification by transponder is not possible because of biological or morphological characteristics, animals can be identified later, but if the animal needs to be transferred the breeder should be able to guarantee the animal's traceability with dated pictures (plastron and upper shell) with a scale (ruler or grid pattern).

European pond turtles imported into France and originating from another country must be identified within eight days after arrival, except if they are already identified with a transponder or with another approved method. Inserting the transponder must be realised by a vet. After implantation, the vet and owner must complete an identification document. The location



2.6.2 Sexing

In order to manage the captive turtle population, it is important to sex the individuals. The usual method relies on sexual dimorphism (see earlier chapter). However, when the turtles are young, they are difficult to sex. In one study *E. orbicularis* with a carapace length of 110mm and exhibiting seven scute annuli (male) could be sexed (Auer & Taskavak, 2004), but that means that they were already several years old. Mulhouse Zoo has been testing other methods; cystoscopy works well, but sexing through hormonal analyses (oestradiol, progesterone, testosterone, FSH and LH, were still without significant results (Quintard, pers. comm).

Figure 71: General view of the endoscope insertion in the cloaca. © B. Quintard, Mulhouse Zoo

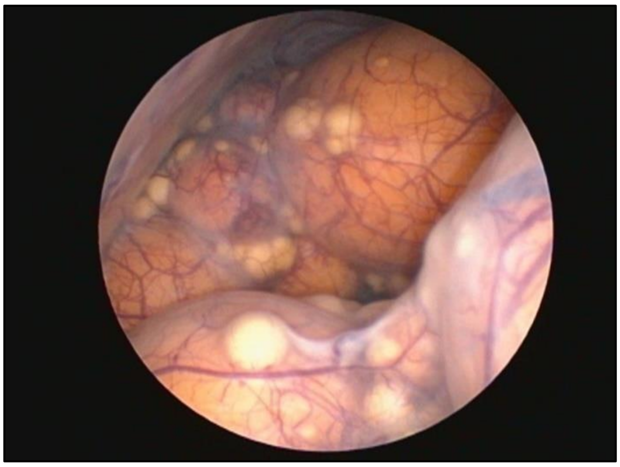


Figure 72: Endoscopic view of the ovaries through the vesical wall during cystoscopy. © B. Quintard, Mulhouse Zoo

2.6.3 Handling

Because of zoonosis risks (e.g. salmonellosis and *Mycobacterium* ubiquitous in aquatic systems) we advise to wear gloves when handling turtles and to efficiently clean yours hands when finished. With their claws aquatic turtles can easily tear the gloves if not resistant enough, personal experiences show that nitrile rubber gloves are more resistant than latex gloves. A turtle bite is painful so always be careful how to handle the animal.

2.6.4 Catching

From the transfer of animals to a health check-up, different reasons can lead to the necessity of catching individuals. For young housed in an indoor nursery, it can be quite easy to catch them by hand. But animals living outdoor and/or in an enclosure with a pond, this method is usually not practicable. In this chapter, we present different methods to catch turtles. Two kinds of methods exist: passive and active.

Active methods

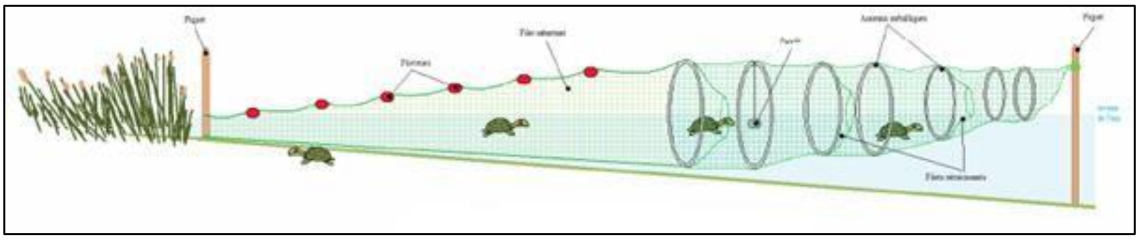
Active methods are hand catching and the use of a landing net. These methods cannot be used if the pond is too large, except when using a boat. However, when disturbed the turtle will quickly hide into the water or silt (Servan, 1986). So we recommend this method only for structures with small ponds. As explained previously, turtles can easily be captured by hand when they are indoor (nursery or aqua-terrarium) and also if the catching occurs during pond emptying.

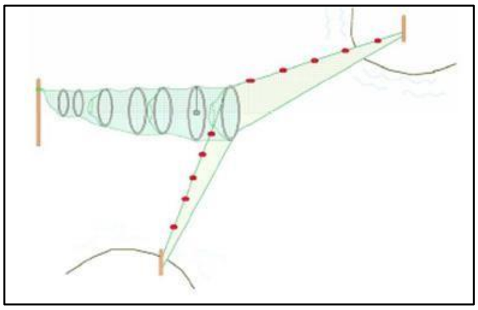
Passive methods

A variety of passive methods has been proposed by researchers that need to catch turtles in the wild.

<u>Hoop net (Verveux net)</u>

This is one of the favourite methods used during sampling protocols in the field (Tomas, 2009; Ceyte, 2013; Tanguy & Gourdain, 2011). This net is composed of successive chambers which become smaller towards the end. In front of the first hoop a net guides the turtle in the trap.
The net is positioned along the bank. When using a hoop net, the end of the net should be out of the water to allow turtle to breath (Tomas, 2009). Bait such as sardine can be used to encourage turtles to enter (Ceyte, 2013).





Figures 73 and 74:Installation of a Verveux net. Taken from: Tomas, 2009.

Because of its large size, this net is not very practical in small ponds and quite expensive (100-200€).

<u>Fish trap</u>
A bait such as sardine can be used to encourage turtles to enter. A study on invasive turtles (*Trachemys* spp*, Pseudemys* spp*, Graptemys* spp*., Chrysemys* spp) catching shows that compared with the Verveux net and the box trap, better results are obtained using this trap (Gendre & Cases, 2011). This trap is not very expensive, approximatively 10€.

Figure 75: Rigid fish trap. © J. Vermeer

Fish traps and Verveux nets are the traps recommended for catching wild *Emys orbicularis* in the French National Action Plan.

<u>Box trap</u>
The major point of this trap is the one-way entry for the turtles. With some efforts, it is possible to build such a trap and adapt it to the size of the pond:

1) Make a cage of wire mesh (ex: chicken mesh), or adapt an existing cage (like a commercial fox trap)
2) Create two openings: one on the top (to fix lure and handle animal) and one on the smaller side at 3 cm from the bottom.
3) Circle several times a stick (e.g: pencil) with wire and keep a long part), thread them on a wire stake
4) Add a mesh trap on the opening at the top and install the system described previous at the inside of the box in the opening so that the animal can enter but not go out (Tomas, 2009).

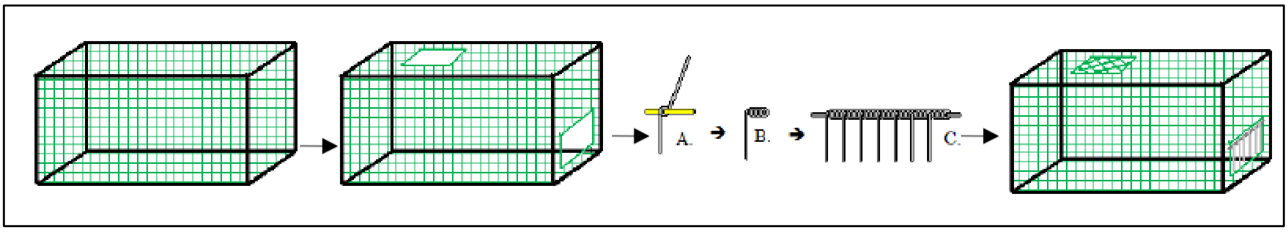
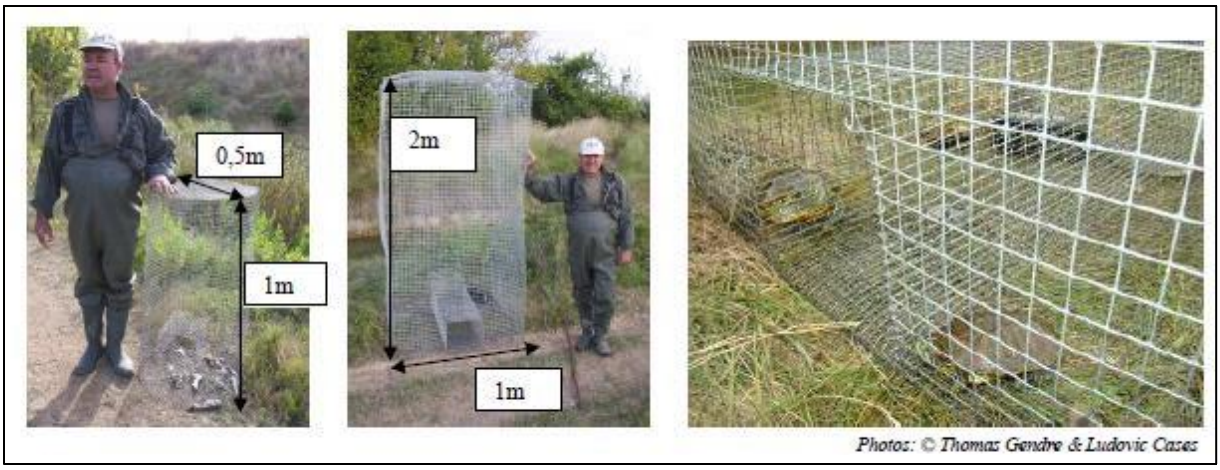


Figure 76: How to build a box trap. Taken from Tomas, 2009.

This trap can be use in the water or on the border of the pond. If such trap is used in the water, the box should be equipped with floating structures or should be height enough that a part on the trap is above the water level, allowing the animals to breathe at water surface. Bait such as sardine can be used to encourage turtles to enter.

Fresquet-cage

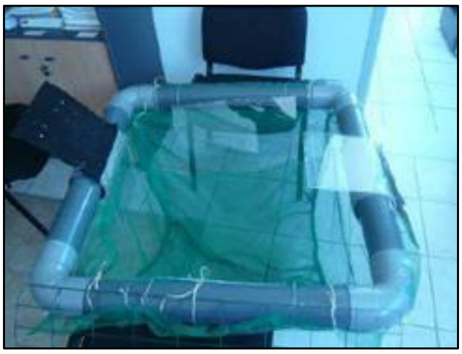
The use of this type of trap showed excellent results to catch invasive turtles (Fillon & Gendre, 2014). This is a huge rectangular or cylindric cage, with an entrance larger than in a fish trap so that turtles enter easier. The trap is simple posed on the bottom of the pond, and the turtles seems to enter easily when they are walking under water, but due to the tunnel they find it difficult to find a way out. This cage can be built cheaply and easily, using mesh and some wire. The major inconvenience is that this trap is bulky. The height of the trap is chosen depending on the pond depth so that turtle can emerge to breath. A bait such as sardine can be used to encourage turtles to enter.



Figures 77-79: Images of the Fresquet cage. Taken from Fillon & Gendre, 2014.

Basking trap

This trap is made of a floating frame (wood or PVC) with a net inside. The principle of this trap is that the turtle climbs on the frame to bask, falls on the net and cannot climb back thanks to frame shape. A rocking system can be added to help the fall. This type has also been used to capture invasive sliders (Gianaroli, Lanzi, & Fontana, 2001).



Figures 80 - 82: Basking trap. Taken from Tomas, 2009; Gendre & Cases, 2011 and © Julie Peinado.

Build your own basking trap:
Insert empty plastic bottles (with lid) in a 75cm long PVC tube, assemble 4 tubes and corner pieces with glue or duct tape. Fix a net to this frame. Use a piece of mesh to create a ramp to help turtle climbing on the trap.

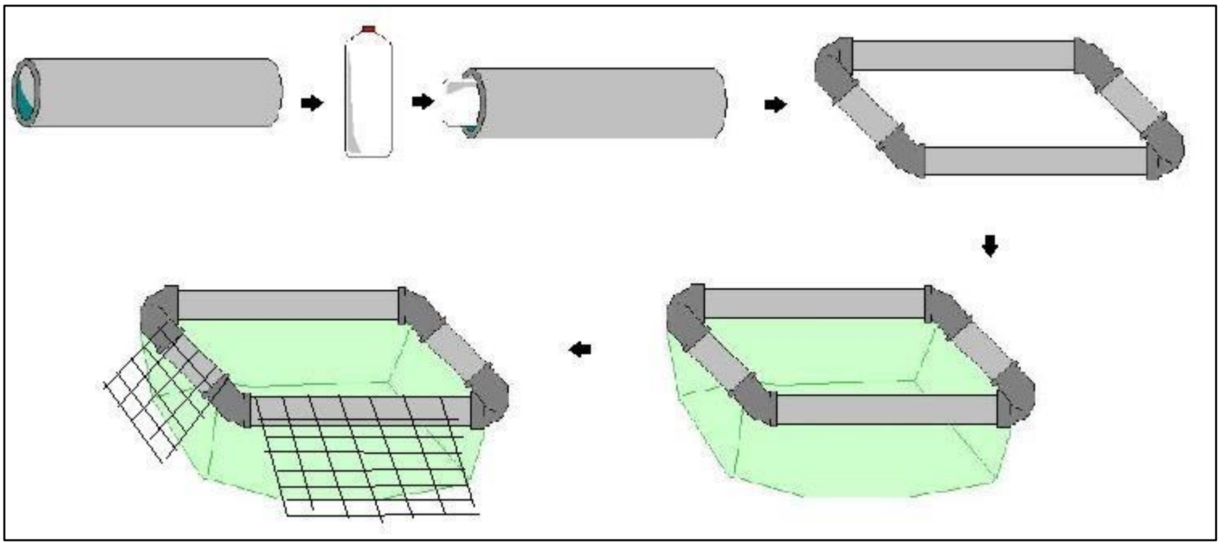
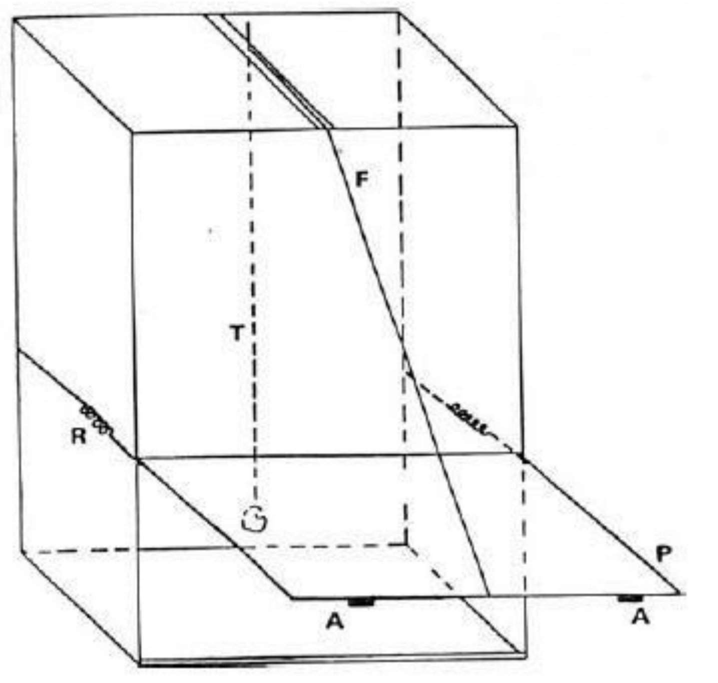


Figure 83: Build your own basking trap.

<u>Servan trap</u>
Servan proposed a catching method especially adapted to *Emys orbicularis* (Servan J. , 1986) . This is a rectangular trap of 45cm long and 25cm wide, the height depending on depth of the pond.
The materials used are aluminium framework, plastic mesh (mesh: 5 or 10mm), two springs, a magnet and wire. Bait is hung to a shaft which is connected with the trap door thanks to the wire. When the turtle catches the bait, the shaft disconnects from the wire and thanks to springs the door close. The magnet keeps the door closed



T = shaft, F= wire, A=magnet, R= springs

Figure 84: Scheme of the Servan trap. Taken from Servan, 1986.

Table summarized advantages and disadvantages of the different traps.

Trap type	Advantages	Disadvantages
Hoop net (Verveux)		- *Expensive (> 100€)* - *Difficult to install* - *Only for large ponds*
Fish trap	- *Inexpensive (10€)*	- *Without float risk of drowning*
Basking trap (with or without rocking system)	- *Easily made* - *Inexpensive* - *No drowning risk*	- *Heavy and bulky* - *Sunny days necessary* - *Adaptation time*
Servan trap	- *Hand made* - *Inexpensive* - *No drowning risks* - *Specially designed for Emys orbicularis*	- *Turtles caught only one by one* - *Heavy and bulky* - *Difficult to construct*
Box trap	- *Hand made* - *Cheap*	- *bulky*
Fresquet trap	- *Hand made* - *Inexpensive* - *No drowning risk*	- *bulky*
Landing net		- *turtles can hide rapidly in the silt* - *Time consuming*
Hand catching	- *no cost*	- *turtle can hide rapidly in the silt* - *Time consuming*

Table 23: Advantages and disadvantages of the different traps.

2.6.5 Transportation

Before transporting animals between institutions it is important to check national legislation. *E. orbicularis* is not listed by CITES, but at national level the species may be protected and transport permits, identification with a transponder, prove of origin and other requirements may need to be fulfilled to transfer the animals legally.

Turtles, even aquatic species, should never be transported in water (not even one centimetre!), to avoid drowning. They should ideally be transported in individual compartments, although this may be difficult when transferring large numbers of hatchlings. Substrate used is a wet cloth (towels), finely woven to avoid that the turtle can get its claws trapped. The container height should be less than the shell width, so the turtle cannot turn upside down. By adding ice packs during the transport overheating can be prevented and the animal will slow down its metabolism. Transport boxed should be attached during transport, to avoid abrupt movements. The animals must be transferred in a cooled, calm and dark vehicle.

When a large number of individuals should be transported, a compartment container is easily made. As larger turtles can easily damage the cardboard, it is better to use wood or plastic.

La Réserve de la Haute Touche uses a expanded polystyrene (EPS) containers with wet towel paper for transports. In three large containers they transport around 120 hatchlings, while adults are transported in individual wooden boxes.

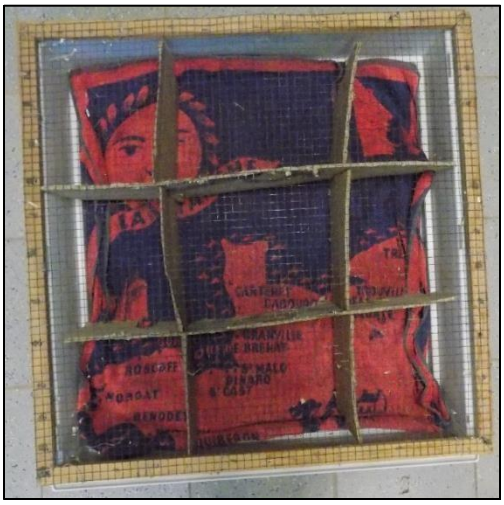


Figure 85: Home-made transport container (for sliders) at Parc Animalier de Sainte Croix. © L. Berthomieu

Figures 86 and 87: Box for the transport of young turtles. A wet cloth may be more suitable than saw-dust. Careful, the young turtles can climb out and a lid is needed. © J. Vermeer

2.6.6 Safety

European pond turtles are relatively harmless animals, but some precautions are necessary. As mentioned before, there is the risk of zoonosis; *E. orbicularis* can be vector of salmonellosis, so turtles should be handled with gloves and people should carefully wash their hands after being in contact with the animals or their surroundings. The turtles can also bite or scratch a handler, so the animals need to be handled with care.

2.7 Veterinary considerations for health and welfare

2.7.1 Weight

Weight is an important parameter to measure when a health-check-up is proceeded. To determine the ideal weight of a turtle, Donoghue's equation is commonly used (Schilliger, 2004):

$$\text{Weight (g)} = 0{,}191 \times \text{Length}^3 \text{ (cm)}$$

Ideally each turtle should be weighted at least once a year, preferably just before hibernation.

2.7.2 Clinical biochemistry

	(Metin, et al., 2006) *n=10 captive animals*		(Yilmaz & Tosunoglu, 2010) *n=42 wild animals*		(Schönbächler, 2020) *n=41 w+c animals*	
Parameter	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Total protein (g/L)	25.00	14.70-33.60	R: 32.85 PR : 35.52	25.00-40.00 10.00-55.00	33.20	15.20-72.20
Albumin (g/L)	7.20	4.70-11.00	-	-	10.00	3.00-21.00
Globulin (g/L)	17.80	5.20-26.40	-	-	25.60	-
Glucose (mmol/L)	2.91	1.55-4.38	R: 3.97 PR: 3.62	2.51-6.61 2.16-7.61	4.74	1.90-9.81
Calcium (mmol/L)	2.32	1.87-3.39	-	-	2.73	2.09-4.23
Phosphorus (mmol/L)	1.55	1.13-2.03	-	-	0.95	0.53-2.19
Creatinine (µmol/L)	46.85	20.33-86.63	-	-	-	-
Urea (mmol/L)	10.93	7.41-15.96	R: 11.13 PR: 19.09	2.99-23.81 7.33-28.93	-	-
Uric acid (µmol/L)	-	-	-	-	42.5	-
Triglycerides (mmol/L)	0.44	0.16-0.79	R: 1.06 PR: 1.13	0.08-3.05 0.11-4.85	-	-
Cholesterol (mmol/L)	1.48	0.98-1.79	-	-	-	-
Sodium (mmol/L)	125.76	118.00-136.0	-	-	135.8	115.40-145.00
Potassium (mmol/L)	3.98	3.10-5.60	-	-	5.42	2.90-7.88
Chloride (mmol/L)	93.94	89.00-101.00	-	-		
Iron (µmol/L)	13.34	8.96-18.27	-	-		
ASAT (µkat/L)	2.14	1.40-3.40	-	-	1.7	0.64-112
ALAT (µkat/L)	Male: 0.21 Female: 0.10	0.10-0.35 0.05-0.15	-	-	-	-
GGT (U/L)	2.15	1.00-4.00	-	-	-	-
Amylase (µkat/L)	8.09	4.40-14.59	-	-	-	--
Lactate dehydrogenase (µkat/L)	19.93	9.77-31-97	-	-	-	

Table 24: Blood chemistry of Emys orbicularis *(R: Reproduction period; PR : Post-reproduction period).*

2.7.3 Haematology

Blood can easily be collected either from the caudal or the jugular vein with a 21 gauges needle and a 5mL syringe. The maximal amount of blood collected from an individual should not exceed 0.4% of body weight.

	Value	[min-max]
Red Blood Cells (/mm^3)	454523	[360000-620000]
White Blood Cells (/mm^3)	5965	[3300-8000]
Hematocrit (%)	22.24	[16-19.5]
Haemoglobin (g/dL)	6.76	(4.8-8.4]
MCV (fl)	494.63	[370.25-613.64]
MCH (pg)	150.83	[108.89-191.80]
MCHC (%)	30.58	[24.39-38.34]

Table 25: Blood count (n=42 wild animals) (Yilmaz & Tosunoglu, 2010).

	Size (µm)	[min-max]
Erythrocyte	21.7 (length) *13.2 (width)	[17.5-25.0] [10.0-20.0]
Eosinophils	15.8	[11.3-20.0]
Basophils	13.3	[11.0-16.3]
Lymphocytes	8.7	[6.3-12.5]
Monocytes	13.7	[10.0-18.8]
Thrombocytes	7.5	[5.0-11.3]
Heterophils	19.6	[17.5-25]

Table 26: Blood cells characteristics in Emys orbicularis *(n=10 captive animals) (Metin, et al., 2006).*

Parameter	*n*	Range	Mean	Median
Hematocrit %	115	3.6-24.6	14.3	14
Corr. Leucocyte count (*10E3/ul)	82	0.57-8.47	3.37	3.3
Heterophils relative (%)	76	8.96-47.69	25.57	25
Eosinophils relative (%)	76	0.91-40.25	13.84	12
Basophiles relative (%)	76	4.19-33.8	18.99	19.25
Monocytes relative (%)	76	0.5-9.08	4.16	4
Lymphocytes relative (%)	76	12.89-68.41	37.19	36
Heterophils (*10E3/µl)	76	0.13-2.19	0.86	0.78
Eosinophils (*10E3/µl)	76	0.03-1.55	0.44	0.35
Basophiles (*10E3/µl)	76	0.08-1.55	0.62	0.58
Monocytes (*10E3/µl)	76	0.01-0.44	0.15	0.12
Lymphocytes (*10E3/µl)	76	0.11-4.04	1.33	1.06

Table 27: Hematology of clinically healthy Emys orbicularis *from Switzerland (Schönbächler, 2020).*

In this species all blood cells are nucleated, which makes analyses impossible with an automatic analyser. Note that in addition of the usual erythrocytes, some micronucleate erythrocytes are presented at 0.0016 (for males) and 0.003 (for females) for 1000 erythrocytes. Micronucleus are an indicator of genetic damage and an elevated number of micronucleate cells indicate poor health (Metin, et al., 2006).

2.7.4 Hospitalisation

The most important physical requirement of sick reptiles is heat, as temperature is essential for their metabolism (Rossi, 2005). Furthermore, medicine pharmacokinetics can be influenced by body temperature (DeNardo, 2002). In a clinic, reptiles should be housed in conditions that provide a thermogradient covering temperatures well above and below preferred temperature of the species as the preferred temperature for a sick animal may be higher than that one of the healthy animal. At the same time the enclosure must allow the animal to escape these high temperatures. Additionally, the animal should be offered a place to hide when in hospitalisation to reduce stress (Rossi, 2005).

2.7.5 Quarantine

For reptiles a recommended period of three months of quarantine is advisable, with series of faecal examinations (parasitology and bacteriology, including at least *Salmonella*, *Campylobacte*r, *Shigella* and *Yersinia*) at the beginning and the end of the period. Testing new animals for herpesvirus, ranavirus and *Mycoplasma* is also recommended.

2.7.6 Disinfection

To avoid pathogen transmission between ill animals, a cleaning and disinfection protocol is important. The first step is a thorough cleaning, as for a disinfectant to be efficient it is imperative to remove organic debris.

Bleach (sodium hypochlorite), phenolics and ammonia are good disinfectants, the latter is also effective against coccidia and cryptosporidium.

2.7.7 Pathologies

Specific literature on *E. orbicularis* pathologies is not abundant, but it is supposed that pathologies described for other freshwater turtles also can affect the European pond turtle. Therefore we describe a non-exhaustive list of pathologies, but for more details about treatment we refer to specific veterinary literature. It should be kept in mind that in reptiles the most common health problems are related to inadequate husbandry conditions, therefore the focus will be on these aspects. And we refer to the different chapters of this guidelines dealing with housing and feeding.

As captive born European pond turtles are often reintroduced to the wild, it is important to learn more about the possible diseases that could unintentionally be transmitted to wild turtles. In a recent study in Switzerland, 40% of the examined turtles were tested positive for *Mycoplasma* spp. (Schönbächler, 2020). The captive population was only slightly more affected than the free-ranging population, and with the exception of only one free-ranging population, all captive and free-ranging populations were affected. In only one positive individual, dyspnoea was observed.

Shell and skin

In his veterinary thesis, (Boit, 2017) sums up the different pathologies and causes that can affect freshwater turtles tegument (skin and shell):

- Mechanical causes
    - traumatic fracture of the shell
    - plastron abrasion caused mainly by a rough substrate.
- Nutritional causes:
    - Hypovitaminosis A (see chapter Feeding)
    - Hypervitaminosis A
    - Metabolic bone disease
    - Hypertrophic osteodystrophy
- Parasites:
    - Flatworms such as *Temnocephala brevicornis*
    - Leeches
    - Mycosis (*Mucor*, *Rhizopus*, *Fusarium*, *Geotrichum*)
    - Algae
    - Spirochetes

- Bacterial causes
    - Superficial dermatitis caused by *Pseudomonas*, *Aeromonas*, *Citrobacter*, *Morganella*, *Proteus, Serratia, Klebsiella* and sometimes *Streptococcus*
    - Abscesses and pyogranuloma
    - Dermatophilosis
    - Ulcerative Shell Disease (USD)
- Systemic infection with integumental repercussion
    - *Aeromonas* infections
    - Pseudomonasis
    - Salmonellosis
    - SCUD (Septicaemic Cutaneous Ulcerative Disease)
    - Mycobacteriosis
- Viral causes
    - Papilliomatosis
    - Grey patch disease
- Neoplasia

Some specific studies on wild populations of European pond turtles described integument pathologies. In Hungary, 49.33% of the examined animals had macroscopic tegument abnormalities. The most common macroscopic lesions found on the skin were abscesses, dermatitits, SCUD, keratin layer abruption, blepharoconjunctivitis and pododermatitis. 67.33% of the animal showed lesions on the plastron carapace or skeletal system. Bacterial infection was diagnosed in 76.66% and mycosis in 33.33% of the animals (Aleksic-Kovacevic, et al., 2014). Shell dermatitis and soft skin lesions were also the most common clinical finding in both captive and free-ranging turtles (Schönbächler, 2020).
In France, the algae genus *Vaucheria* was found on the whole body of *E. orbicularis* specimens. They explain this phenomenon as a dystrophic disorder in the aquatic habitats. As the alga cover the whole body of the turtle, it can cause problem during hibernation when this species uses cutaneous breathing (Olivier, Koenig, Suet, Ficheux, & Fayolle-Sanna, 2014).

Respiratory tract
The major problems are rhinitis and pneumonia. The symptoms which can be observed are discharge from the nose, irregular to open mouth breathing, apathy and disinterest of food. A turtle with pneumonia is unable to swim and dive in a normal way.
Different pathogens like viruses, mycosis, parasites or bacteria can be the cause, and the treatment depends on the identified cause.

Digestive tract
Several endoparasites are described in wild populations of *E. orbicularis*. A total of 14 species were reported from the Steppe Zone of Ukraine (Kotenko, 2004). Three species of *Eimeria* were discovered on *E. orbicularis*, of which two new to science (*Eimeria gallaeciaensis* and *Eimeria emydis*) and *Eimeria mitraria* (Segade, Crespo, & Ayres, 2006). In Iran two nematodes, *Serpinema microcephalis* and *Falcaustra araxiana*, and one trematode, *Telorchis assula*, were identified in wild European pond turtles (Hossein, Rajabloo, & Gholamhosseini, 2016) . The pharynx parasite *Polystomoides ocellatum* was found in some populations and exotic parasite species in nearly the totality of populations in (Héritier, Palacios, & Verneau, 2014), suggesting the transfer of parasites from exotic turtle species such as Florida sliders.
Parasites can also be found in lungs, kidneys, the urinary bladder and blood. Helminths found in Turkey are *Ptagium lazarewi, Serpinima microcephalus, Spironoura armenica and Spiroxys contortus* (Yildiriham & Sahin, 2005)

In a large number of samples from captive and free-ranging turtles in Switzerland, nematodes (resembling *Falcaustra*), protozoa (*Blastocystis* spp.), trichomonads, hexamitids and eggs of monogenean and digenean trematodes were detected, but at very low numbers. Pseudoparasites (*Malamoeba*, *Monocystis* and *Ascaridia* were also reported (Schönbächler, 2020). In three free-ranging and one captive individual (2.8% of samples) *Polystomoides* spp. was encountered in the oral cavity.

In the choice of an anti-parasite treatment, vets should remember to never use ivermectins because of the toxicity for the species (and chelonians in general).

Metabolic pathologies

Hypovitaminosis A
In aquatic turtles, a bilateral palpebral oedema is commonly a symptom of vitamin A deficiency (Schilliger, 2004). Turtles can be complemented with vitamins but it is essential to have a well-balanced feeding protocol (see the chapter on nutrition).

Metabolic bone disease
Metabolic bone disease is the consequence of an insufficient Ca:P ratio in the diet and/or Vitamin D deficiency, often created through a lack of UV radiation provided. The symptoms of metabolic bone disease are unspecific. Turtles are lethargic, general swellings and shell softening can be observed. X-rays show a decrease bones density, deformed long bones, scoliosis, pathologic fractures and soft tissue mineralisation.

Reproductive pathologies
The absence of adequate oviposition sites and continuing disturbances during egg-laying are risks factors for egg binding. If placement in a proper and calm environment with suitable parameters do not lead to egg-laying, medical treatment should be considered. After a dorsoventral X-ray (45kV, 20mAs) to estimate the number of eggs, 10 UI/kg oxytocin are injected IM. The animal is then placed in a dark, plastic container with a 10 cm layer of sand. Injections can be repeated every 90 minutes until all eggs are laid (Quintard & Georges, in press).

Other pathologies

Mutilation
Mutilation of the tail or legs due to bites can be observed. To limit this phenomenon crowding should be avoided.

Drowning
Drowning can be observed mainly in hatchlings, but also occurs in adults. Deep water or a too powerful pump can be a cause of drowning in hatchlings. Hluboka zoo reports several adult death caused by drowning when transferring them in an outdoor enclosure. The solution was the creation of a smaller enclosure in the outdoor enclosure, with a small shallow pond and transparent fences, next to bigger tank. Turtles are kept in this part of the enclosure until they have adapted to the new environment.
In nature, during mating season, females can drown because too many males climb on her to mate and she is enable to surface. We therefore recommend female biased sex ratios.

2.8 Recommended research

It is essential to be sure not to produce hybrids, and all zoos should genotype their animals. Research on easier and cheaper methods should be developed and supported.

It is essential to determine which captive males really contribute to the production of offspring for introductions, and the development of useful paternity tests should be supported. In addition, the results of this research could be used to manage breeders and avoid inbreeding.

If the different institutes use the “breeding results sheet” proposed in these guidelines, it may help us define breeding parameters which affect reproductive results and consequently be used to improve recommendations given in the guidelines.

There are still some aspects of the husbandry requirements for this species that are insufficiently understood. One of these is the UV requirements. Animals from different haplotypes and origins may have different needs, and we recommend studies during all parts of the year and in different localities to obtain information that can be used for improving the husbandry in captivity.

Section 3: References

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Annex 1 : *In situ* conservation links

There are many conservation projects for *Emys orbicularis*, many of them have been summarized in the Herpetology Notes, volume 6, published in 2013.
European zoos are involved in a large number of *in situ* conservation projects, some useful links on current and former projects can be found here:

Austria:
https://www.sumpfschildkroete.at/

France:
The national action plan: https://www.ecologie.gouv.fr/sites/default/files/PNA_Cistude_2020_2029.pdf

Germany:
http://www.xn--sumpfschildkrte-ltb.de/

https://rlp.nabu.de/tiere-und-pflanzen/amphibien-und-reptilien/sumpfschildkroete/

https://niedersachsen.nabu.de/tiere-und-pflanzen/aktionen-und-projekte/sumpfschildkroete/index.html

https://www.lubw.baden-wuerttemberg.de/-/europaeische-sumpfschildkroete-emys-orbicularis-linnaeus-1758

https://www.stalu-mv.de/ms/Themen/Naturschutz-und-Landschaftspflege/Artenhilfsprogramm-Sumpfschildkr%C3%B6te/

Italy:
http://www.lifeemys.eu/en/

Latvia:
https://webgate.ec.europa.eu/life/publicWebsite/index.cfm?fuseaction=search.dspPage&n_proj_id=4530

Spain:
http://www.lifepotamofauna.org/www.lifepotamofauna.org/ca/que-es/especies/especies-autoctones.html#estany-box

Switzerland:
http://www.karch.ch/karch/de/home/reptilien/reptilienarten-der-schweiz/europaische-sumpfschildkrote.html

Various countries:
http://www.glis.lt/life/?pid=59&lang=en

Annex 2 : Questionnaire distributed to holders and breeders

Survey on breeding procedures for the European Studbook for European Pond Turtle (*Emys orbicularis*)

Laurie BERTHOMIEU and Jan VERMEER for the European Pond Turtle ESB

Breeding programmes are necessary to be able to breed individuals which could be used for reintroductions or to reinforce weak populations.
Breeding success relies on factors which may influence:

- Mating and laying/nesting
- Hatching
- Survival of hatchlings

The results of this survey will be used to compile the first EAZA Best Practice Guidelines for management of the European Pond Turtle in captivity and will be used to determinate which factors in turtles management can influence the breeding success.

If possible, this questionnaire must be filled in by the reptile manager in partnership with animal keepers and all staff taking care of the turtles.

Instructions:
- For the questions with a small square ☐ , please tick the chosen answer.
- For open-ended questions (example: *others:*) write down your comments.
- Any pictures of your facilities (breeders' enclosure, eggs storage, incubator, juveniles' nursery, ...) would be a plus and highly appreciated (add the name of the photographer (copyright))

Many thanks for your help and your contribution to this project.

Characteristics of the colony

Age and gender:
Females: Hatchlings ([0-3] years old) Juveniles (]3-9]) Adults (>9 (breeders))
Males: Hatchlings ([0-3] years old) Juveniles (]3-7]) Adults (>7 (breeders))
Undetermined: Hatchlings ([0-3] years old) Juveniles (]3-9]) Adults (>9 (breeders))

Are males and females kept in the same enclosure during the entire year? ☐Yes ☐No
or only during breeding season? ☐Yes ☐No

Is the haplotype of each breeder determined? ☐Yes ☐No
Do you breed European pond turtle for (re-)introduction programmes? ☐Yes ☐No

Breeders

Are your breeders housed ☐ inside or ☐ outside during mating and nesting season?

How many times a day the turtles may be disrupted by humans during breeding and nesting period?
☐ once ☐ 2-5 times ☐ 6-10 times ☐ >10 times

Which reproductive pathologies did you observe over the last 5 years?
☐ egg binding ☐ prolapses of reproductive organs ☐ penile paralysis ☐ other:

How do you feed the adult turtles?
☐ feed themselves ☐ red meat (beef, ...) ☐ poultry ☐ fish (which one?), ☐ special mixture
☐ pond fish pellets ☐ cat food ☐ freshwater turtle pellets ☐ insects (which one?)
☐ microcrustacean (which one?) ☐ other:

How many times a week do you feed them?
Describe the feeding protocol during the year (beginning of feeding after hibernation, limit of temperature, ...):
Are the breeders given mineral and vitamin supplementation? ☐ Yes ☐ No

Outdoor enclosure

Total size of the enclosure: m^2
Is the pond ☐ natural or ☐ artificial?
Size of the pond: m^2 Maximal depth: cm
Thickness of silt: cm
Emptying and cleaning frequency:
Are basking area presents? ☐ Yes ☐ No
Total number of turtles in this enclosure?

Mortality rate (subadults and breeders):
2017: 2016: 2015: 2014: 2013:

Nesting/Eggs laying

Which size is the nesting area? m^2
Which substrate is located at the nesting area?
Can the nesting area be flooded? ☐ Yes ☐ No
In the enclosure, is the vegetation kept short between the pond and the nesting area? ☐ Yes ☐ No
What is the exposure direction of the nesting area?
Is there some shelter on the nesting area? ☐ Yes ☐ No
Is there other furnishing on the nesting are? ☐ Yes ☐ No
What furnishing?
Do you protect eggs from predators? ☐ Yes ☐ No
What protection?
How do you monitor eggs laying?
☐ different colour of substrate ☐ raking of the nesting area ☐ observer ☐ other:

How long (maximum) from the laying do you collect the eggs from the nest?
Is the collect ☐ total or ☐ partial?

Number of clutches collected?
2017: 2016: 2015: 2014: 2013:

Average size of clutch?
2017: 2016: 2015: 2014: 2013:
Total number of eggs collected?
2017: 2016: 2015: 2014: 2013:

Incubation

Do you have natural successful incubation? ☐Yes ☐No
Do you incubate the eggs artificially? ☐Yes ☐No

Collection and handling of the eggs

How do you collect the eggs (describe)?
Which precautions when collecting the eggs?
☐ no tilt ☐ no roll over ☐top of the eggs marked ☐ none ☐ cleaning (How?) ☐ other:
What do you do with lightly cracked eggs? ☐ remove ☐ incubate ☐ chalk on the crack + incubate

Incubation conditions

How are the eggs placed into the incubator?
☐ on a grid ☐ laid on substrate ☐ half buried on substrate ☐ totally buried in substrate
Which substrate is used?
☐ fine sand ☐ coarse sand ☐ soil ☐ vermiculite ☐ perlite ☐ no substrate ☐ other:

Do you separate eggs from different clutches? ☐Yes ☐No
Describe eggs storage (ex: use of boxes? number of eggs per box? ...)
Which incubating method do you use?
☐ Incubator Which brand? Which type?
☐ Other (describe):
Which temperature protocol do you use to incubate eggs?
During the day:
During the night:
What is the sex ratio obtained?
Hygrometry:
How often do you check incubation conditions (temperature and hygrometry)?
☐ less than once a day ☐ once a day ☐ twice a day ☐ more than twice a day
Average time of incubation?
Do you use egg-candling method to check fertilisation? ☐Yes ☐No
When during incubation period?
What do you do with infertile eggs? ☐ remove ☐ keep in incubation ☐ other:
Average proportion of abnormal eggs (on the total number of incubated eggs)?
rotten eggs (swollen eggs/ greenish egg) withered eggs cracked eggs
other:

Maintenance

How often do you disinfect the incubator?
Which disinfection protocol do you use (disinfectant chosen, ...)?

Hatching management

Do you help juveniles during hatchling (ex: break the shell, ...)? ☐Yes ☐No
If you do, when or why do you decide to help?
Average proportion of abnormal juveniles obtained (on the total number of hatched eggs)?

III

still-born twins deformed tail monster (ex: conjoined twins, ...)
unabsorbed yolk sac other:

How do you manage young with unabsorbed yolk sac?

Do you realize some care on young just after hatchling? ☐Yes ☐No
If so, what?

Do you keep hatchlings in the incubator after hatchling? ☐Yes ☐No
If so, for how long?

Reproduction results

If you keep documents with your reproduction results for the last 5 years can you send them attached with this survey?

Collected eggs: 2017: 2016: 2015: 2014: 2013:

Incubated eggs: 2017: 2016: 2015: 2014: 2013:

Cracked eggs: 2017: 2016: 2015: 2014: 2013:

Fertilised eggs: 2017: 2016: 2015: 2014: 2013:

Rotten eggs: 2017: 2016: 2015: 2014: 2013:

Viable hatchlings: 2017: 2016: 2015: 2014: 2013:
Abnormal hatchlings: 2017: 2016: 2015: 2014: 2013:
Average size of hatchlings: 2017: 2016: 2015: 2014: 2013:

Average weight of hatchlings: 2017: 2016: 2015: 2014: 2013:

Some pictures of your incubation system would be appreciated.

Offspring husbandry

When are the hatchlings moved outdoors?
☐ immediately after hatching ☐ at certain age (months?) ☐ at certain size (cm?)

Indoor nursery

* *Aqua-terraria*

Dimensions: length: cm; width: cm, height: cm
Material: Height of water: cm
How many hatchlings per aqua-terraria?

* *Water*

Do you check?
pH: ☐Yes ☐No water hardness: ☐Yes ☐No micro-organisms contamination: ☐Yes ☐No

* *Lighting*

Are your aqua-terraria furnished with UV (A and B) lamps? ☐Yes ☐No
Brand/type:
☐ tube or ☐ spot Tube length: Index: Power: W
Type of transformer:
Distance from water: cm Distance from basking area: cm
How often do you replace UV lamps?

How many hours the UV lamps are switched on during a day? (lightening protocol)
spring: summer: fall: winter:

* *Temperature*
Do you use heating system? ☐Yes ☐No
Which one?
☐ Water heater Characteristics: Brand/type: Power: W
What is temperature of water? °C
☐ Heater lamp
Which types? ☐ ceramic heat lamp ☐ infrared heater lamp
☐ halogen lamp ☐ other:
Brand/type: Power: W
How high above the basking area? cm
How many hot spots are presents in each terrarium?
What is the temperature at the hot spot? °C
Do you check temperature?
At the hot spot: ☐Yes ☐No In water: ☐Yes ☐No
* *Hygrometry*
What is the hygrometry? % Do you check it? ☐Yes ☐ No
* *Filtering system*
Do you use filtration pumps?
Flow: L/h Power: W Brand:
How many aqua-terraria for each pump?
Is there some grid to protect pump entry? ☐Yes ☐No
* *Maintenance*
How often water is changed?
Cleaning protocol (describe):
* *Aqua-terraria furnishings*
Is there an emerged area? ☐Yes ☐No Size: cm²
What it is composed of?
☐ substrate (which one?) ☐ branch ☐ tile ☐ rock ☐other:
Are there plants in aqua-terraria? ☐Yes ☐No
Which one?
Proportion of the aqua-terraria occupied by plants? %
Some pictures of your indoor nursery would be appreciated.

Outdoor nursery
Is there an emerged area? ☐Yes ☐No Size: cm²
How it is composed of?
☐ substrate (which one?) ☐ branch ☐ tile ☐ rock ☐other:
Are there plants? ☐Yes ☐No
Which one?
Can you describe outdoor compound (pictures would be appreciated)?
Do you protect offspring from predators? ☐Yes ☐No
What protection?

Feeding
What do you feed offspring?
☐ red meat (beef, ...) ☐ poultry ☐ fish (which one?), ☐ special pudding
☐ pond fish pellets ☐ cat food ☐ freshwater turtle pellets ☐ insects larvae (which one?)
☐microcrustacean (which one?) ☐ other:
At what age do you start to feed the offspring?
How many times a week are the offspring fed?

V

Is the offspring given mineral and vitamin supplementation? ☐Yes ☐No

Management

What is the size or age during the first hibernation?

Describe your hibernation protocol:

Raising results

Mortality rate before 1 week: 2017: 2016: 2015: 2014: 2013:

Mortality rate before 1 year: 2017: 2016: 2015: 2014: 2013:

Mortality rate between the 1st and 2nd year:
2017: 2016: 2015: 2014: 2013:

Average size (cm) when offspring is 1 year old:
2017: 2016: 2015: 2014: 2013:

Average size (cm) when offspring is 2 years old:
2017: 2016: 2015: 2014: 2013:

Average weight (g) when offspring is 1 year old:
2017: 2016: 2015: 2014: 2013:

Average weight (g) when offspring is 2 years old:
2017: 2016: 2015: 2014: 2013:

Thank you very much for answering this questionnaire. Should you have comments to add, please use the box below.

VI