EAZA Amphibian Taxon Advisory Group

Best Practice Guidelines for the natterjack toad (*Epidalea calamita*)

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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 “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. All forms/templates are available to download on the EAZA Member Area. 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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Introduction

This Best Practice Guideline is based on information from the published literature on natterjack toads, and from first-hand experiences working with the species both *in*- and *ex-situ* in three institutions in the northern region of the species' distribution area.

The natterjack toad is an endemic European amphibian that hibernates in the cold months. During hibernation they burrow themselves deep into the ground, and they are not seen again before the spring arrives. This might be one of the reasons why it is not a common species held in zoos. Currently the species is only registered in eight institutions in Europe. However, it is a symbol of precipitous global amphibian declines, and it is therefore a part of several conservation projects in multiple EAZA institutions, including Copenhagen Zoo in Denmark, Riga Zoo in Latvia, and Fota Wildlife Park in Ireland. The natterjack toad is currently (2025) listed as Least Concern on the IUCN Red List (Ogrodowczyk et al. 2024). In several regional Red Lists have been updated, and the natterjack toad is currently categorized as threatened in most of its northern distribution area primarily due to loss of habitat. As a part of the conservation projects for natterjack toads, new ponds are established, and old ones are restored in their natural habitat. Moreover, natterjack toads from different locations are captive bred in the zoos and are then released in the wild at the same places as their genetic origin, in accordance with the IUCN Reintroduction Guidelines (IUCN/SSC, 2013) and the EAZA Guidelines for Releasing Animal into the Wild. Further assistance can be provided by the EAZA Conservation Translocation Working Group when designing such projects.

The natterjack toad is therefore a good example of a species where captive breeding can be used directly to increase the wild populations if their natural habitats are conserved.

This Best Practice Guideline is meant as a guide for keeping and breeding natterjack toads, but it may also serve as an inspiration for starting conservation projects, where captive bred natterjack toads can be released to the wild.

Section 1: Biology and field data

Biology

1.1 TAXONOMY

- Class: Amphibia
- Order: Anura (Fischer von Waldheim, 1813)
- Family: Bufonidae (Gray, 1825)
- Genus: *Epidalea* (Cope, 1864)
- Species: *Epidalea calamita* (Laurenti, 1768)
- Common name: Natterjack toad

The species has previously been classified as *Bufo calamita.*

1.2 MORPHOLOGY

1.2.1 Weight

Natterjack toads exhibit snout-vent length polymorphism related to latitudinal and altitudinal gradients (see section 1.2.3). Studies have found that males in Nordrhein-Westfalen (Germany) weigh 6.4 g to 29.9 g, whereas males in Andalusia (Spain) weigh 5.3 to 35 g. Female natterjacks weigh 8.3 g to 54.8 g in a study of a German population, where in a Spanish study they weigh 4 g to 66 g. Generally, prior to mating the egg mass in females makes them weigh more than males since there is no sexual dimorphism in their size (Sinsch, 2009).

At the time the tadpoles start metamorphosis they weigh 79.2 mg $\pm$ 5.78 to 88.2 mg $\pm$ 19.1 (Griffiths et al., 1991).

1.2.2 Length

The snout-vent length in adults varies from 39 mm to 95 mm (Beebee et al., 2012); the average size is 50 mm to 80 mm (Leskovar et al., 2006). As in many anuran species, natterjack toads' size-related life-history traits vary along latitudinal and altitudinal gradients. The latitudinal variation of size follows roughly a converse Bergmann cline, where body size decreases with higher latitudes.

However, not all populations fit this pattern. A population exists in Spain with a snout-vent length smaller than expected. This might be an evolutionary by-product of optimized lifetime fecundity (Sinsch et al., 2010).

The size of an egg ranges from 1.0 to 1.8 mm (Sinsch, 2009; Tejedo, 1992a); egg clutches are laid in strings that can be 1-2 m in length, depending on the number of eggs. The egg string is relatively thin with a width of 2-6 mm (Fog et al., 2001; Sinsch, 2009). The newly hatched tadpoles measure 4-6 mm (Rannap et al.; 2012; Sinsch, 2009) and reach a maximum length of 22-30 mm before they go through metamorphosis (Sinsch, 2009). The newly transformed toadlets are 4-11 mm long (Fog et al., 2001; Sinsch, 2009).

1.2.3 Colouration

The most characteristic colouration trait for natterjack toads is the yellow vertebral (fig. 3). The stripe can sometimes have a whiter colour tone. In other cases, the stripe can be reduced or missing (Sinsch, 2009).

Dorsally, the fundamental colours are brownish grey to greenish olive with distinct marbling. The marbling colours can be greenish, brownish, reddish, or yellowish, and they can be fairly extensive. Large warts and the parotoid glands can have an orange to reddish brown colour. Ventrally, natterjack toads are whitish or greyish with some darker spots irregularly distributed. Their irises are greenish yellow with a black horizontal pupil (Sinsch, 2009; Zamora-Camacho & Comas, 2019).

In the breeding season the vocal sacs of the males have a blueish, purplish or reddish colour (Zamora-Camacho & Comas, 2019) (fig. 6). Moreover, the three inner digits of the males' forelimbs get an ochreous or brown-coloured rough keratinization (Fog et al., 2001; Sinsch, 2009).

Eggs from natterjack toads are black with a roughly marked grey spot (fig. 8). The tadpoles are also black with a dark grey abdomen (Fog et al., 2001; Sinsch, 2009) (fig. 7), however the yellow stripe along the back can develop before completing metamorphosis (fig. 2).

Newly metamorphosed toadlets can be quite dark in colour, but they quickly develop the yellow vertebral stripe if not already present.

Figure 1: Young natterjack toad (Mikkel Jézéquel).

Figure 2: Individuals with the yellow stripe along the back before complete metamorphosis (Fota).

Figure 3: The yellow stripe on the back of a young natterjack toad (Evelina Puzo, Riga Zoo)

Figure 4: Adult natterjack toad (Copenhagen Zoo).

Figure 5: Abdomens of male (left) and female (right) natterjack toads. Notice the darker color of the male's vocal sac (Copenhagen Zoo).

Figure 6: Croaking male (Lisa Pochinda).

Figure 7: Natterjack toad tadpoles (Mikkel Jézéquel).

Figure 8: An egg string from a natterjack toad. The black eggs are fertilized, while the grey ones are not (Copenhagen Zoo).

Figure 9: Newly metamorphosed natterjack toad. Notice the dark color of the toadlet (Elvīra Hrščenoviča, Riga Zoo).

Figure 10: Natterjack toad toadlets. The yellow stripe on their back often becomes visible soon after metamorphosis (Copenhagen Zoo).

1.2.4 Description

The natterjack toad is a small European toad species. It has a compact body with relatively short hindlimbs. The size of the hindlimbs makes them walk rather than run.

Males tend to have longer and stronger forelimbs than females, which probably evolved due to their role during amplexus (Sinsch, 2009; Zamora-Camacho, 2018). If males have bred, it is often possible to see that they have large upper arms, and this is a characteristic that can be used to separate males from females. Males also have large vocal sacs that turn blueish, purplish, or reddish in the breeding season, where they also croak. Due to the croaking, the skin around their throats gets “wrinkly,” and the colour pattern around the throat almost “disappears” because it has been distended (Fig. 6). In general, the colour around their necks keeps being darker. Despite these differences, it can still be challenging to distinguish the sexes from each other. A method for sexing the individuals is to hold the animal with one hand grabbing the animal by their back thereby pretending to be a male in amplexus. If it is a male, it will often make a small squeaking sound as a way to communicate to the other male that it cannot be mated.

Natterjack toads belong to the family *Bufonidae*, of which members have specialized poison glands in their skin, and they have parotoid glands visible as parallel bulges at the toads’ temples. These poison glands can secrete strong acting skin poisons that are rich in cardiotoxic steroids. A natterjack toad carries a pair of prominent parotoids and a plethora of poison gland openings all over its body, indicating that the species is capable of secreting high amounts of skin poison (Stawikowski & Lüddecke, 2019).

Natterjack toads have four digits on their forelimbs and five digits on their hindlimbs. They have very little interdigital toe webbing. On the longest digit on their hindlimb they have two tubercles underneath the toe.

1.3 PHYSIOLOGY

1.3.1 Body temperature

Natterjack toads are thermal conformers, which means they regulate their body temperature through their behaviour. Two studies have monitored the behavioural regulation of body temperature in 38 free-ranging adults in two populations in Spain and Germany respectively. The body temperature of natterjack toads in Spain varied +0.3℃ during winter to a maximum of 32.2℃ during summer. In Germany it varied +0.5℃ during winter and 37.4℃ during summer. The highest measured body temperature in the studies was 38.8℃ (Beebee et al., 2012; Sanuy & Sinsch, 2010; Sinsch & Leskovar, 2011).

The upper thermal body temperature of tadpoles is 39.8℃ . This high thermal tolerance appears to be an adaptation to life in the shallow sunny ponds where natterjacks breed (Beebee et al., 2012; Duarte et al., 2012).

1.4 LONGEVITY

The longevity of natterjack toads in the wild is up to 9 years (Leskovar et al., 2006). When natterjack toads are held in captive settings, their potential longevity exceeds that in the field by almost threefold. Males can live more than 24 years, while females can live more than 26 years (Sinsch & Lehmann, 2006).

In the wild the survival rate from egg to toadlet can vary considerably from year to year and from location to location. A comprehensive study of 1,480 egg strings from Western Germany measured the number of egg strings that gave rise to toadlets in four breeding areas from 1986 to 1991. In the least-successful area only 2% of the egg strings made it to the toadlet stage, whereas in the most successful area 49% of the egg strings made it to the toadlet stage (Sinsch, 1992).

Field data

1.5 CONSERVATION STATUS/ZOOGEOGRAPHY/ECOLOGY

1.5.1 Distribution

Natterjack toads are endemic to Europe. Here they are widely distributed, ranging from the Iberian Peninsula in the west and as far east as the Baltic coast with several isolated populations in Ireland and Great Britain (Reyne et al., 2019). The countries in which natterjacks can be found include Austria, Belarus, Belgium, Czechia, Denmark, Estonia, France, Germany, Ireland, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Spain, Sweden, Switzerland, Ukraine, and the United Kingdom (Ogrodowczyk et al. 2024) (fig.11). Since the previous red list assessment in 2009, the species is now considered extinct in the Russian Federation (Kaliningrad).

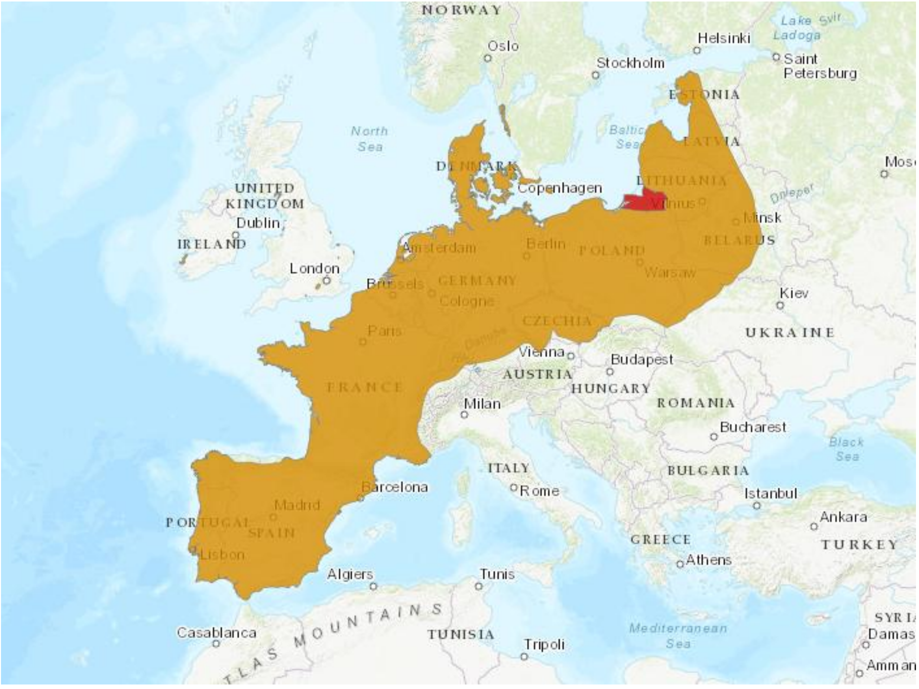

Figure 11: Distribution of natterjack toads. Illustration from Ogrodowczyk et al. 2024.

1.5.2 Habitat

Natterjack toads in Spain and Portugal occupy a wide range of habitat, and they can be found in altitudes as high as 2500 meters in southern Spain. Elsewhere, the natterjack toad is mainly a lowland species. They occupy open, unforested habitats, often in nutrient poor environments with light sandy soils, and they prefer areas with temporary shallow ponds. These habitats include, e.g., river valleys, gravel pits, coastal marshes, sand dunes, and lowland heaths (Beebee et al., 2012; Fog et al., 2001; Reyne et al., 2019)

Some natterjack toads breed in brackish water. They are known to have a relatively high salt tolerance compared to many other amphibian species. Lethal limits for spawn are between 4.1% to 5.5% (Beebee et al., 1993). Tadpoles can tolerate higher concentrations of salt, which may be an adaptation to water evaporation in the temporary breeding ponds. Sinsch et al. (1992) showed that tadpoles were able to tolerate 7 to 8% salt, while adults can tolerate around 16%. The mean population performance under different salt concentrations differs markedly among populations in embryonic and larval stages (Gomez-Mestre & Tejedo, 2005). Moreover, natterjack toads can breed in permanent, large, nutrient poor lakes with scattered, thin forests of reeds (Fog et al., 2001). The banks in all types of breeding ponds should have areas where they are gradually sloping, so the toadlets easily can emerge onto land.

The choice of breeding places relates to tadpoles being vulnerable to predators and interspecific competition (Bardsley & Beebee, 1998; Denton & Beebee, 1997; Griffiths et al., 1991). They prefer their spawn having water almost to themselves. Species, such as the great crested newt (*Triturus cristatus)* and common backswimmer (*Notonecta glauca)*, are known to eat natterjack toad tadpoles (Sinsch, 2009) (see section 1.8.3.). If the tadpoles share the water with other amphibian species, their development is known to be affected negatively. This can lead to inhibited growth, prolonged development, higher mortality, and emerging as smaller toadlets (Bardsley & Beebee, 1998; Griffiths et al., 1991).

To maintain genetically healthy and robust populations of natterjack toads, it is essential that there is connectivity among different breeding ponds. The distance that natterjack toads are capable of travelling varies in its distribution area. Estimates obtained for populations in Central Europe and the United Kingdom indicate a core area of 600 m around the breeding site, and connectivity is maintained with a maximum distance of 2250 m between the breeding ponds. In contrast, estimates for Spanish populations indicate a core area of 5 km around the breeding site, and connectivity is maintained with a maximum distance of 12 km between breeding ponds (Sinsch et al., 2012). Natterjack toads show a preference for bare environments and low vegetation when they move (Stevens et al., 2006, personal comment from radiotracking project at Copenhagen Zoo, 2021).

During the day, natterjack toads hide in self-dug galleries, in mammal lairs, in clefts, or under stones (Hemmer & Jakobs, 1974; Sinsch, 2009). They are only known to dig by themselves if the soil has a grain size of 3 mm or less (Hemmer & Jakobs, 1974). Hiding spots and appropriate grain sizes are therefore also important in their habitats.

Natterjack toads also inhabit agricultural areas (García-Muñoz et al., 2009, 2011; Sanuy & Sinsch, 2010). If the breeding ponds are placed in an agricultural area, attention should be paid to pollutants. Natterjack toad larvae survival and growth are negatively affected by ammonium nitrate and copper sulphate (García-Muñoz et al., 2009, 2011a, 2011b). Moreover, amphibians are also generally negatively affected by pesticides, and they can potentially die in the fields during ploughing (Brühl et al., 2013; Ockleford et al., 2018; Personal observation, Caroline Pedersen).

1.5.3 Population

The population of natterjack toads are declining. This is particularly true in the northern parts of its range. Natterjacks are currently under substantial or serious threat in Austria, Belgium, Czechia, Denmark, Estonia, Germany, Ireland, Netherlands, Switzerland, and the United Kingdom (see table

1). The main threat to natterjack toads is loss of specialized habitats such as coastal meadows, heaths, and dunes. These habitats are changed by natural encroachment of scrub and woodland, afforestation, acidification of breeding ponds, agricultural development, infilling of breeding sites (e.g., temporary pools, sand, and gravel quarries), increased mechanization of sand and gravel extraction, and infrastructure development for tourism (Ogrodowczyk et al. 2024). For instance, Baltic coastal meadows are a primary habitat for natterjack toads, but these are among the most threatened habitats in Europe. According to aerial photos, 60 to 83% of the coastal meadow habitat was lost in Estonia from 1950 to 2000. Moreover, from the 1930s to 2000s, 91% of the natterjack toad populations living in coastal meadows in Estonia disappeared (Rannap et al., 2007). In Britain, habitat change of coastal dunes, upper saltmarshes, and inland heaths is the primary reason for natterjack toads vanishing from 70 to 80% of its British range from the late 1800s to 1970 (Beebee et al., 2012). In Switzerland, more than 60% of the known Natterjack toad occurrences have disappeared during the last 30 years (Schmidt & Zumbach, 2005).

1.5.4 Conservation status

The latest IUCN assessment for the natterjack toad is from 2023. The species is categorized as “Least Concern (LC)” with decreasing population trends (Ogrodowczyk et al. 2024). In several countries natterjack toads are nationally categorized as a threatened species (see Table 1).

To protect the natterjack toad, it is listed on Appendix II of the Bern Convention and on Annex IV of the EU Habitats Directive, and it is protected by national and sub-national legislation throughout much of its range. Moreover, the natterjack toad is listed in many regional, national, and sub-national Red Data Books and Lists and is present in many protected areas (Ogrodowczyk et al. 2024).

*Table 1: National (or regional) conservation status for the natterjack toad (FV: favourable; XX: unknown; U1: unfavourable-inadequate; U2: unfavourable-bad) (*Information on habitat and conservation status from https://nature-art17.eionet.europa.eu/article17/species/summary/.)*

Country	*Habitat	*Current period Conservation status	*Previous period Conservation status	Other classification
Austria	Alpine	U2	U2	CR (Gollmann, 2007)
	Continental	U2	U2	
Belarus	ND	ND	ND	
Belgium	Continental	U2	U2	VU (Jooris et al., 2012)
	Atlantic	U2	U2	
Czechia	Continental	U2	U2	CR (Jeřábková & Zavadill, 2020)
Denmark	Continental	U2	U2	

	Atlantic	U2	U2	EN (Moeslund, Nygaard et al., 2019)
Estonia	Boreal	U2	U2	EN (Kübarsepp, 2020)
France	Continental	U2	U2	LC (IUCN France et al., 2015)
	Atlantic	U2	U2	
	Alpine	U1	U1	
	Mediterranean	U1	U1	
Germany	Continental	U2	U1	CR (Meyer et al., 2020)
	Atlantic	U2	U1	
Ireland	Atlantic	U2	U1	EN (King et al., 2011)
Latvia	Boreal	U2	U1	EN (Čeirāns, 2022)
Lithuania	Boreal	XX	U1	VU (Rimšaitė, 2021)
Luxembourg	Continental	U2	U2	
Netherlands	Atlantic	U1	U2	VU (Delft et al., 2007)
Poland	Continental	FV	FV	
Portugal	Mediterranean	XX	XX	LC (Cabral et al., 2005)
	Atlantic	XX	XX	
Russia	ND	ND	ND	
Spain	Alpine	U2	U1	U2 (Calmaestra, 2020)
	Atlantic	U2	FV	
	Mediterranean	U2	FV	
Sweden	Boreal	U1	U1	NT (SLU Artdatabanken, 2020)
	Continental	U2	U2	
Switzerland				EN (Schmidt & Zumbach, 2005)
Ukraine	ND	ND	ND	
United Kingdom	Atlantic	U2	U2	EN (Foster et al., 2021)

1.6 DIET AND FEEDING BEHAVIOUR

1.6.1 Food preferences

Natterjack toads feed opportunistically based on what they can find and handle in their habitat (Sinsch, 2009). However, insects make up most of their food intake. A study of a coastal population in the South of England indicates the most important food items are smaller beetles, leatherjackets, caterpillars, and centipedes (Mathias, 1971). Another study in a salt marsh area of Doñana National Park in south of Spain found it to be leatherjackets, ants, smaller beetles, and ticks, and a study from an island population of the Swedish west coast found it to be ants and smaller beetles, primarily weevils (Andrén & Nilson, 1985; Valverde, 1967) There are a few insect species that natterjacks are known to actively avoid, including European wasps (*Vespula germanica*), cabbage butterfly caterpillars (*Pieris brassicae*), and Colorado beetles (*Leptinotarsa decemlineata*) (Sinsch, 2009).

Tadpoles feed on phytoplankton, periphyton (a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus attached to submerged surfaces in aquatic environments), plant parts, microbes, detritus, carcasses, and spawn. It should be noted that coprophagy plays an important role in the feeding objects that are difficult to digest (Sinsch, 2009).

1.6.2 Feeding

Adult natterjack toads are nocturnal and forage during the night (Reyne et al., 2019). The toadlets forage during day and night (Buckley & Beebee, 2004; Sinsch, 2009).

When natterjack toads are foraging, they use both vision and smell. Hemmer & Schopp (1975) found that natterjacks are more likely to react to prey when they receive visual stimuli. However, the study also found that natterjacks standing in “smelling distance” of their prey in the dark, they will stand still and wait, indicating they also use their sense of smell when they forage.

When natterjack toads catch a small prey item, they use their tongue to throw it directly into the throat. If prey is larger, they hold on to it with their jaws and use rubbing movements with their hands in combination with withdrawal of the tongue. When they are going to swallow, they use their eyes. While closing their eyes, they squeeze their eyes hard together. The eyeballs’ strong retractor muscles then push the eyeballs into the oral cavity which squeezes the food into the gullet (Sinsch, 2009).

1.7 REPRODUCTION

1.7.1 Developmental stages to sexual maturity

Natterjack eggs usually hatch within 10 days if the environment has favourable conditions (Reyne et al., 2019). The exact time for the embryogenesis depends on the water temperature. In the high summer the eggs can hatch after two days, whereas they will hatch after more than 14 days in the early spring in Northern Europe (Sinsch, 2009).

Metamorphosis occurs approximately six weeks after hatching, but the exact speed of development depends on water temperature, nutrient availability, and intra- and interspecific competition (Griffiths et al., 1991; Reyne et al., 2019; Sinsch, 2009). Griffiths et al. (1991) found for instance that tadpoles of common frogs (*Rana temporaria*) inhibit the growth, prolong the development, and reduce survival rate of natterjack toad tadpoles. Moreover, they found that the faeces of common frog tadpoles contain a unicellular organism known to cause inhibition in laboratory cultures. Tadpole densities in the breeding ponds affect the mass at metamorphosis as it decreases with high tadpole density (Reques & Tejedo, 1997). Genetic differences may also play a role in mass at metamorphosis.

The growth rate after metamorphosis is almost 30 times greater than the growth rate during the tadpole phase. In their first year the toads attain a snout-vent length of 14-27 mm, whereas they only grow to a snout-vent length of a minimum 3.9 mm in their second year (Beebee et al., 2012; Denton & Beebee, 1993; Sinsch, 2009).

1.7.2 Age of sexual maturity

The minimum age at which both sexes of natterjack toads can attain sexual maturity is at the age of about two, after their second hibernation. However, most individuals reproduce for the first time when they are three years old. (Leskovar et al., 2006).

1.7.3 Seasonality of cycling

The seasonality of cycling varies with thermal conditions at different latitudes. In northern, cold, wet habitats, hibernation happens during winter, and reproduction takes place in spring or early summer (April to July). In the southern, warmer, drier habitats, toads skip hibernation and reproduce in the late winter or early spring (mid-January to April) and avoid summer aridity by aestivating (Reyne et al., 2019, Sinsch, 2009, Tejedo, 1992a, Zamora-Camacho & Comas, 2019) (fig. 12). Several climatic factors initiate reproductive activities: a combination of rainfall over a short period, though not necessarily on the day of spawning, and preferably following a time period of dry weather; minimum temperatures on the day before spawning of at least 10℃; and a series of preceding minimum temperatures of at least 5°C (on average) over two to four nights (Banks & Beebee, 1986).

Natterjack toads have a prolonged breeding period compared to other anurans. However, even though the annual breeding period lasts for about four months, the individual reproductive activity is considerably shorter (Sinsch, 1992). The breeding period can be separated into early, main, and late breeding periods. This means there are different temporal populations at the same breeding site (Sinsch, 1992). However, there is usually a large spawning effort early in the season (Banks & Beebee, 1986). The temporal structure of natterjack metapopulations seems to represent a successful reproduction strategy to reproduce in biotopes, where the formations of suitable breeding sites can be unpredictable. Moreover, the temporal spacing of reproductive activity reduces the male-male competition for females as well as the intraspecific competition of tadpoles in the usually small ponds (Sinsch, 1992).

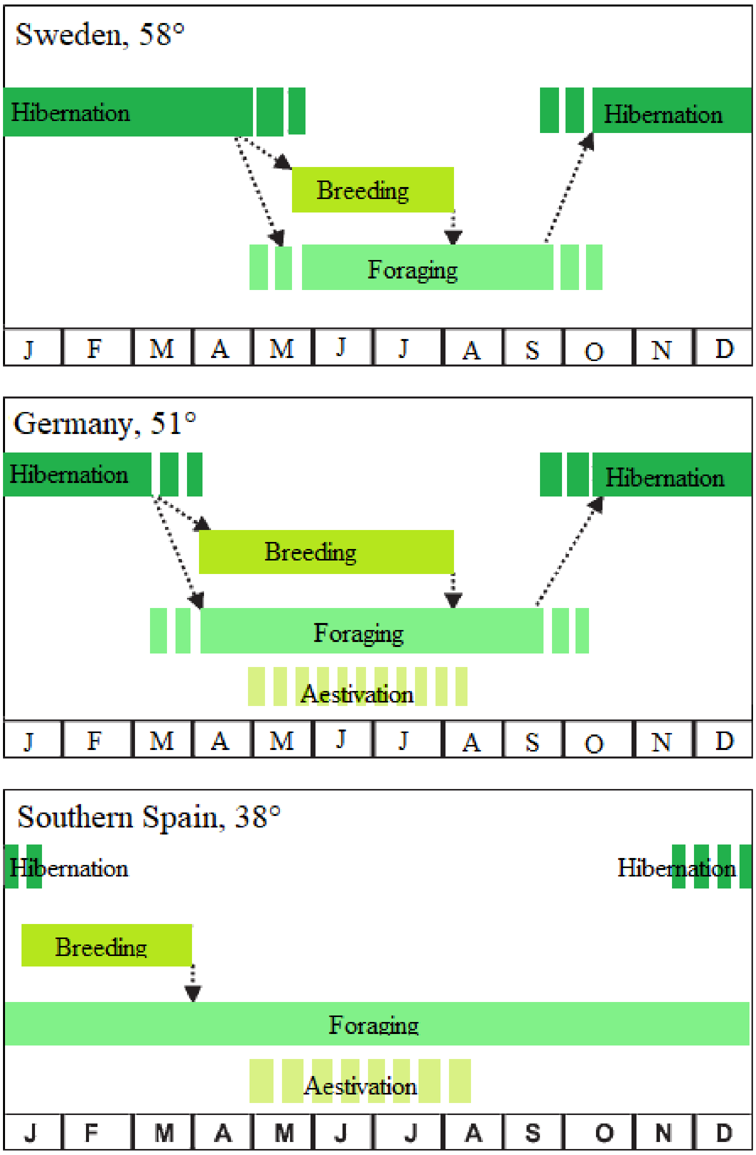

Figure 12: Seasonality of cycling at different latitudes for adult natterjack toads. The striped lines show the period for an activity is climate dependent, and in some years the activity can be absent. The dotted arrows indicate that the activity change is accompanied by a residence shift. The figure is edited and translated from Sinsch (2009).

1.7.4 Gestation period/incubation

Natterjack toad eggs usually hatch within 10 days if the environment has favourable conditions (Reyne et al., 2019). However, the exact time for the embryogenesis depends on the water temperature. In the middle of the summer, when the water temperature is high, the eggs can hatch after two days, whereas in the early spring in Northern Europe the eggs will hatch after more than 14 days (Sinsch, 2009). Laboratory studies have shown that embryogenesis takes 30 days if the water temperature is 9.5℃, whereas it only takes one day if the temperature is 35℃ (Beebee, 1983; Sinsch, 2009).

1.7.5 Clutch/litter/brood/offspring size/number

Each female in the breeding season lays normally 2,000 to 4,000 eggs; a single egg string can contain 700 to more than 7,000 eggs (Banks & Beebee, 1986; Reyne et al., 2019; Sinsch, 2009). The fertility varies among populations, but the body size of the female is also an important factor (Reyne et al., 2019; Sinsch, 2009). Egg strings that contain more than 7,000 eggs have only been found in Spanish populations (Sinsch, 2009).

In Sweden and England up to 20% of the earliest breeding females are observed to breed twice during a breeding season (Andren & Silverin, 1992; Beebee & Denton, 1996).

1.7.6 Hatching details and season

The seasonal timing of spawning varies with thermal conditions at different latitudes (see section 7.3.1). Spawning almost always occurs during the night, but if the male and female are in amplexus for many hours, spawning can also occur during the day. While the spawning occurs, the amplectant pair stay in shallow water at the shore with their nasal passageway in the air. During the spawning there are rest periods that last 10 to 15 minutes, followed by ejection of a part of the egg string simultaneously with emission of seminal fluid (Sinsch, 2009). The egg string is up to two meters in length and relatively thin with a width of 2 to 6 mm. There are two rows of eggs in the string; if the egg string is stretched out, the eggs are arranged in a single row (Fog et al., 2001). After spawning, the female leaves the breeding pond and there is no parental care (Sinsch, 2009). The time for the eggs to hatch varies with water temperature (see section 1.7.4).

1.8 BEHAVIOUR

1.8.1 Activity

Adult natterjack toads are nocturnal and forage during the night. During the breeding season, males gather around edges of breeding ponds in the first few hours after dusk to attract females (Arak, 1988a). However, the seasonality of sexual behaviour varies with climatic conditions at different latitudes (see section 1.7.3). During the day, they hide in 15-40 cm self-dug galleries, in mammal lairs, in clefts, or under stones (Hemmer & Jakobs, 1974; Sinsch, 2009) (fig. 13). They are only known to dig by themselves if the soil has a grain size up to 3 mm. The toads have been recorded to dig at a speed of 3-4 cm/min (Hemmer & Jakobs, 1974). When they dig, they do not create open galleries. It can better be described as soil redistribution, where they get buried while digging. When they hibernate or aestivate, they burrow themselves deeper into the ground. The toads have been recorded to burrow themselves down almost 2 m during hibernation, but the depth depends on the moisture of the soil and the frost penetration (Sinsch, 2009). North of the Pyrenees (Spain) the natterjacks mostly burrow themselves down to a depth of 20-120 cm (Adrados, 2015; Lindenthal et al., 1991).

The hibernation in populations from Central Europe does not seem to be a direct reaction to low temperatures but partly controlled by endogenous changes. In a laboratory study, natterjack toads were kept at summer conditions during their normal wintertime. The findings revealed a varying activity in loci that controls the production of enzymes used in the intermediary metabolism during the season (Sinsch, 1990).

The tadpoles are active during both day and night. Their motion intensity relates to the water temperature. They move more during warm days compared to colder nights. On sunny days they prefer to be in the warmer surface water at a depth of 1 cm (Sinsch, 2009).

Toadlets forage during both day and night their first days on land. Some studies indicate that they quickly switch to being only nocturnal, whereas others have observed toadlets basking a week after they emerged on land (Buckley & Beebee, 2004; Sinsch, 2009).

Figure 13: Burrowing natterjack toads (Elza Birbele, Riga Zoo)

Figure 14: Natterjack toad skeleton (Elina Gulbe, Riga Zoo)

1.8.2 Locomotion

Natterjack toads have relatively short hindlimbs (fig. 14). Their shinbones are especially shorter than those of other toads. The morphology of their legs makes them move exclusively by means of intermittent runs, instead of leaping as in many anurans. The sprint speed is directly related to hindlimb length.

Males are faster than females. In one study, the average maximum sprint speed of males was 67 cm/s, whereas it was 47 cm/s for females. This is partially explained by males having longer limbs. However, the longer forelimbs in males are not related to higher speed. These have probably evolved so males can get a better grab of females during amplexus. Males are also more conspicuous to predators, mainly while searching for and defending their territories, and while calling to attract females, which could be other reasons why the longer hindlimbs have evolved (Zamora-Camacho, 2018). The authors of this study also found that habitat type affected the locomotion pattern. Natterjack toads living in agricultural systems had more numbers of runs per meter compared to natterjack toads living in pine groves. This suggests an increased vigilance against predators in agrocultural systems and is probably related to the more open habitat (Zamora-Camacho, 2018).

Another study tested the flight strategy of natterjack toads and how it is affected by refuge proximity. Natterjack toads started their flight at a faster speed to reach the refuges faster when refuges were placed nearby. Moreover, they had fewer numbers of runs per meter (i.e., they made fewer stops when running), suggesting they want a straighter and faster flight towards the refuge. When the refuges were placed further away, the number of runs per meter was greater. This could suggest that when a refuge is further away, natterjack toads respond firstly with slow intermittent movements that could confound predators or rely on the unpalatability of their deterrent skin secretions, and only shift to less intermittent, faster sprints if the threats persist (Zamora-Camacho, 2020).

1.8.3 Predation

Adult natterjacks toads generally do not have many predators due to their poisonous glands (Reyne et al., 2019). However, some of their recorded predators include reptiles (snakes): grass snake (*Natrix natrix*), viperine snake (*Natrix maura*); mammals: brown rat (*Rattus norvegicus*), European hedgehog (*Erinaceus europaeus*), American mink (*Mustela vison*), stoat (*Mustela erminea*), Eurasian otter (*Lutra lutra*), red fox (*Vulpes vulpes*), and badger (*Meles meles*); and birds: Black-headed gull (*Larus ridibundus*), Eurasian wigeon (*Anas penelope*), grey heron (*Ardea cinerea*), Eurasian magpie (*Pica pica*), white wagtail (*Motacilla alba*), house sparrow (*Passer domesticus*), carrion crow (*Corvus corone*), and tawny owl (*Strix aluco*) (Adrados, 2015; Sinsch, 2009). When crows eat natterjacks, they flip them over and peck a hole in the abdomen (Adrados, 2015).

A defensive behaviour of natterjacks is to drastically inflate their body, leading to an artificial increase of body size. Additionally, the animal straightens its legs to lift its body far from the ground. These mechanisms make the natterjack appear a lot larger and more defensive than it is. After inflation and leg extension the natterjack lowers its head and presents its pair of parotoid glands towards the source of agitation. If a predator attacks the natterjack frontally in this posture, it would be confronted with a high dosage of the toad’s chemical defence, since the glands store a large proportion of its toxin resources (Stawikowski & Lüddecke, 2019).

Another defensive behaviour is the secretion of skin poison. The poison appears as white droplets on the skin surface and is often released in small amounts on the natterjack toad’s back, legs, or parotoids. They are therefore capable of releasing their toxic secretions quite extensively at once across their whole body. The two types of defensive behaviour can be combined or happen independently from each other (Stawikowski & Lüddecke, 2019).

It was once thought that eggs, embryos, and tadpoles had deterrent gustatory stimulants or toxins to protect them from predators; however, it is now known that they do not have those abilities for protection (Sinsch, 2009). To avoid predators, tadpoles can only change their behaviour. It is not clear exactly what kind of behaviour they use since contrary observations have been made. In some observations, tadpoles aggregate when predators are around, but in other cases they do not. Other tadpoles have been observed to reduce their movement frequencies, while others have been seen swimming towards the bottom and staying there without moving. When tadpoles from *Bufo bufo* and *Rana tempoaria* live in the same pool, no change in the swimming activity of natterjack tadpoles has been observed (Sinsch, 2009).

The known predators of eggs and embryos include: crayfish: red swamp crayfish (*Procambarus clarkii*); amphibians (tadpoles): European common frog (*R. temporaria*), common tree frog (*Hyla arborea*), yellow-bellied toad (*Bombina variegata*), parsley frog (*Pelodytes punctatus*), common spadefoot toad (*Pelobates fuscus*), Iberian spadefoot toad (*Pelobates cultripes*), and common toad (*B.bufo*); reptiles: viperine snake (*N. maura*); and birds: mallard (*Anas platyrhynchos*) (Sinsch, 2009).

The known predators of tadpoles include insects (larvae): Southern hawker (*Aeshna cyanea*), migrant hawker (*Aeshna. mixta*), green emerald damselfly (*Chalcolestes viridis*), vagrant darter (*Sympetrum vulgatum),* broad-bodied chaser *(Libellula depressa),* downy emerald *(Cordulia aenea)*, great diving beetle (*Dytiscus marginalis*), *Dytiscus pisanus*, *Copelatus haemorrhoidalis*, *Agabus sturmii, Agabus melanocornis, Agabus bipustulatus, Agabus affinis, Rhantus pulverosus, Rhantus bistriatus, Hydrochara carabidoides,* and water beetle (*Acilius sulcatus*); insects (larvae and imagoes): *Colymbetes fuscus*, *Ilybius fuliginosus;* insects (imagoes): common backswimmer (*Notonecta glauca*) and *Nepa cinerea*; annelids: *Nephelis vulgaris*; bony fishes: three-spined stickleback (*Gasterosteus aculeatus*), green sunfish (*Lepomis cyanellus),* pumpkinseed (*Lepomis gibbosus)*, common rudd (*Scardinius erythrophthalmus)*; amphibians (larvae and adults): great crested newt (*Triturus cristatus)*; and birds: mallard (*Anas platyrhynchos),* black-headed gull (*Larus ridibundus),* species from the family *Laridae,* and rook (*Corvus frugilegus*) (Sinsch, 2009).

1.8.4 Social behaviour

After newly metamorphosed toadlets have left the water ponds, they stay nearby in the following days. They are observed to stay in numbers of hundreds under the same refugia (Sinsch, 2009). However, when leaving their birth location in the wild, they walk alone and show no sign of social behaviour.

The only time during the year natterjack toads gather is during the breeding season. Here the males croak loudly to attract females to the breeding ponds. When many males croak simultaneously the chorus can get so loud that it can be heard more than a kilometre away (Adrados, 2015; Sinsch, 2009).

1.8.5 Sexual behaviour

When the breeding season begins, male natterjacks gather around the edges of breeding ponds and call loudly to attract females for the first few hours after dusk (Arak, 1988a). The nightly sex-ratios are always male-biased, resulting in an intense vocal competition between males for females. While all males attending the pond are sexually mature and capable of calling, not all of them use calling as a strategy when attempting to mate (Arak, 1988a).

Three different mating tactics have been observed among males. One is *calling males* that call actively most of the time. Second is *satellite males* that silently adopt a quiet position close to a calling male. Small males, with low intensity calls, most frequently adopt the satellite tactic and prefer to parasitize the loudest callers. The third is *searcher males* moving most of the time. Some use a tactic where they swim quietly around, whereas others utter calls at any moment during their swim (Arak, 1988a; Tejedo, 1992b). Males can switch between the different tactics during the night. It has been observed that they do not switch tactics randomly, but their decisions seems to depend on their immediate circumstances such as the acoustic stimulus produced by their neighbours (Arak, 1988a). When a female comes near a male, a study has observed two different tactics for males to mate with her. One tactic is to wait for the female to crawl out onto the bank and initiate contact (usually by nudging the male's vocal sac) before it clasps her. The other tactic is to dash towards the approaching female and attempt amplexus in water. Females vigorously try to escape from dashing males by diving underwater or running fast alongside the pond. Waiting is the most frequent tactic used by males that are alone at their calling site. It also results in a higher probability of successful amplexus than dashing. When a satellite male is present, callers usually begin dashing when they detect an attempt of interception by a satellite male. A study found that dashing in general can be interpreted as a form of sexual defence (Arak, 1988a).

If a mating attempt is successful it results in axillary amplexus (fig. 15 and 16). In amplexus the male sits on the back of the female and clings to her by having his arms around her armpits. During this process both individuals' cloacae are juxtaposed to ensure successful external fertilization. The duration of amplexus depends on the water temperature. If the water temperature is above 10℃ degrees, the amplexus lasts for about two to five hours. If the temperature is lower the amplexus typically lasts 10 to 12 hours with a maximum of 24 hours before spawning (Arak, 1988b; T. J. C. Beebee, 1979; Sinsch, 2009).

Figure 15: Natterjack toads in amplexus with the male on top of the female (Steen Drodz Lund)

Figure 16: Spawning female while in amplexus (Fota)

The number of females that spawn varies from year to year. A five-year long study in England found that no more than 64% of the known females in a breeding area deposited spawn each year. In a year where it was particularly dry, less than 50% of the females bred, even though some water was present in the ponds well into May. Particularly striking was that females not spawning still visited the breeding pond in spring, and some even went in amplexus with males for short periods before they dislodged and returned to their foraging areas. This is a marked contrast to males, since all males in the study made some effort to breed every year (Denton & Beebee, 1993). These findings also correspond with another study in Germany that found large variations in the total number of egg strings laid each year. In the least successful year 87 egg strings were found in the study areas, whereas in the most successful year there were 539 egg strings (Sinsch, 1992).

Most males show a rather strict lifelong fidelity to the site of first breeding. In a study 90% of the included males showed a strict site fidelity of "This place has worked once it will surely work again". In contrast, females do not show side fidelity. This also means that interactions between local breeding ponds are based on females and first-time breeder males from other areas/ponds (Sinsch, 1992).

Section 2: Management in Zoos and Aquariums

2.1 ENCLOSURE

Up to five different types of enclosures are used for natterjack toads. These different types will from now on be described as *tadpole enclosure* (fig. 17-22), *walk-on-land enclosure* (fig. 23-25), *breeding enclosure* (fig. 26-30), *storage enclosure,* and *hibernation enclosure* (fig. 31-32). Natterjack toad eggs are placed in *tadpole enclosures* after being laid. Here they hatch and stay as tadpoles until they begin to develop hind limb buds. They are then taken into a *walk-on-land enclosure*, where they fulfil their development into toadlets. The *walk-on-land enclosures* are slightly tilted and filled with a bit of water to create a water area and a land area. The adults selected for breeding in each season are placed in *breeding enclosures* with both a land and water area, whereas the rest are placed in *storage enclosures* with no water . During hibernation the natterjack toads are placed in *hibernation enclosures* with plenty digging opportunities .

<u>Tadpole enclosure:</u>

Figure 17: A tadpole enclosure seen from above. The enclosure contains a few water plants, where the egg strings can be entangled (Copenhagen Zoo).

Figure 18: The breeding facilities in Copenhagen Zoo. Here several tadpole enclosures are placed next to each other. From the pipe in the ceiling an oxygen pump is led to each enclosure. Moreover, notice the plastic boxes next to the enclosures. Extension cords are placed in these, and thereby avoid being wet (Copenhagen Zoo).

Figure 19: Tadpole rearing units in Fota Wildlife Park.

Figure 20: Tadpole rearing unit in Fota Wildlife Park.

Figure 21: An egg string from a natterjack toad. The black eggs are fertilized, while the grey ones are not (Copenhagen Zoo).

Figure 22: An egg string entangled in water plants in a tadpole enclosure (Copenhagen Zoo).

Walk-on-land enclosure:

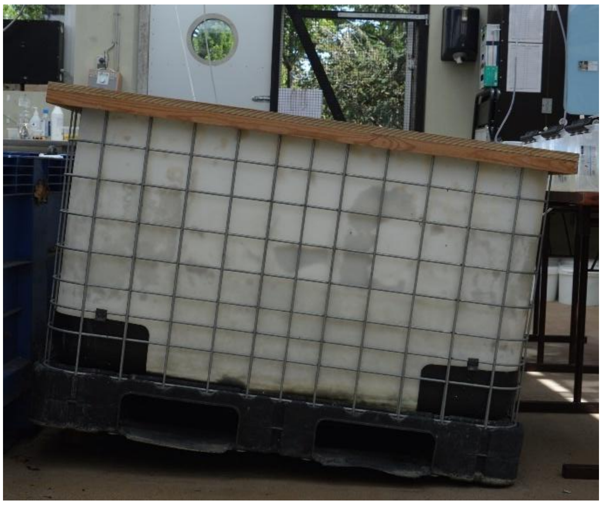

Figure 23: A walk-on-land enclosure seen from the side. The enclosure gets slightly tilted to create both a water area and a land area, so the tadpoles can emerge on land (Copenhagen Zoo).

Figure 24: A walk-on-land enclosure seen from above (Copenhagen Zoo).

Figure 25: Walk-on-land enclosure in Fota Wildlife Park.

Breeding enclosure:

Figure 26: The foundation for a breeding enclosure in Copenhagen Zoo. This is what the enclosure looks like before it is prepared for containing natterjack toads in the breeding season (Copenhagen Zoo).

Figure 27: A breeding enclosure with natterjack toads (Copenhagen Zoo).

Figure 28: A closer picture of the water area in a breeding enclosure (Copenhagen Zoo).

Figure 29: A closer picture of the land area in a breeding enclosure. Approximately one half of the area has sand as a substrate, whereas the other half has sphagnum (Copenhagen Zoo).

Figure 30: Breeding enclosure in Fota Wildlife Park.

<u>Storage - and hibernation enclosure:</u>

Figure 31: A cold store where storage - and hibernation enclosures are kept (Copenhagen Zoo).

Figure 32: A storage enclosure (Copenhagen Zoo).

2.1.1 Boundary

Natterjack toads run rather than leap, so the boundaries in their enclosures do not have to be very high. However, they can stretch their body quite a lot, so if the barriers are not higher than approximately 30 cm, it is recommended that a lid is placed on top of the enclosure if it contains adult natterjack toads. Moreover, if some of the furnishing in the enclosure creates elevations near the boundaries, a lid should also be considered.

2.1.2 Substrate

The recommended substrate is different for each type of enclosure. Substrate suggestions for each enclosure type are presented in table 2.

Table 2: Recommended substrate for each type of enclosure.

Type of enclosure	Substrate
Tadpole enclosure (fig. 17-22)	Do not need to be provided with any substrate.
Walk-on-land enclosure (fig. 23-25)	The walk-on-land enclosures are slightly tilted to create a land part and a water part. The substrate on the land should consist of a flat layer of sand. This substrate makes it easier for the toadlets to find food, since the live food items will not be able to hide from them. Sand is also a good substrate for digging. The substrate should be kept moist. Bark, leaves, sphagnum, and moss can be used additionally as substrate. There does not need to be substrate in the water area.
Breeding enclosure (fig. 26-30)	One half of the land area should be provided with a thin layer of sand, while the other half should be provided with a thin layer of sphagnum. Leaves and moss can be dispersed around as well. There does not need to be substrate in the water area.
Storage enclosure (fig. 31-32)	The substrate in the storage enclosure is basically the same as in the land area in the breeding enclosure. However, there should be more options for borrowing and hiding.
Hibernation enclosure (fig. 31-32)	During hibernation natterjack toads need to have a deeper layer of substrate to burrow themselves deeper down as they often do during hibernation in the wild (Adrados, 2015; Lindenthal et al., 1991; Sinsch, 2009).The substrate can consist of materials such as sphagnum, sand, leaves mixture (of e.g., red oak), and moss. These

	materials are also good for creating porosity since they do not get sluggish during winter.

2.1.3 Furnishings and maintenance

The recommended furnishing for each type of enclosure is presented in table 3.

Table 3: Recommended substrate for each type of enclosure

Type of enclosure	Furnishings and maintenance
Tadpole enclosure (fig. 17-22)	Natterjack toads lay their eggs around water plants. Soon after an egg string is laid in a breeding enclosure it is transferred to a tadpole enclosure (see section 2.4.2). A tadpole enclosure contains a few water plants that the egg strings can be wrapped around. Good plant species for this include the grasses creeping bentgrass (*Agrostis stolonifera*) and floating sweet-grass (*Glyceria fluitans*), the water plant *Ceratophyllum* sp., and *Valisneria* sp.
Walk-on-land enclosure (fig. 23-25)	Plants, such as the grasses creeping bentgrass (*Agrostis stolonifera*) and floating sweet-grass (*Glyceria fluitans*), the water plant *Ceratophyllum* sp. and *Valisneria* sp. should be placed together with bark pieces and small sticks for crossing from water to land. Then the newly metamorphosed toadlets have good hiding possibilities. Those objects should also be placed on the land with dry leaves.
Breeding enclosure (fig. 26-30)	There need to be several plants in the water area since natterjack toads lay their eggs strings around plants. Suitable plants include creeping bentgrass (*Agrostis stolonifera*) and floating sweet-grass (*Glyceria fluitans*), the water plant *Ceratophyllum* sp. and *Valisneria* sp. Moreover, small branches can be put in the water as well. Large stones in the water create small islands, where the toads can sit. On land, branches in different sizes, bark pieces, and stones are used to create opportunities for hiding.
Storage enclosure (fig. 31-32)	Branches in different sizes, bark pieces and stones are used to make opportunities for hiding.

Hibernation enclosure (fig. 31-32)	Branches in different sizes, bark pieces and stones are used to make opportunities for hiding. In the *hibernation enclosures* there need to be extra opportunities for hiding compared to the other enclosure types.

2.1.4 Environment

Natterjack toads should be kept at temperature and light conditions which correspond to their natural outdoor conditions. To initiate the onset of hibernation, the temperature is lowered during the colder months from October to mid-April. The temperature can gradually be decreased before October to imitate the seasonal temperature fluctuations more naturally. The natterjack toads can eventually be placed outdoors in their active season if the environment corresponds with what it would be like in their natural habitat. However, they need to be taken in before below-zero temperatures occur at night. In Denmark that is typically before 1st of November. To stimulate termination of hibernation, the temperature can gradually be increased before April. Table 4 presents yearly fluctuations in the minimum and maximum temperatures in the breeding facilities from two institutions.

Table 4: Yearly fluctuations in temperature in the breeding facilities in Riga Zoo and Fota Wildlife Park.

Riga Zoo				Fota Wildlife Park	
Month	Temp.			Temp.	
	Min	Max	Note	Min	Max
Jan.	1	10		4	10
Feb.	4	8		6	14
March	4	9		7	20
April	9	11	22-25 in amplexarium	10	22
May	18.5	24	22-25 in amplexarium	14	24
June	20	25	up to 32 under heat lamp	15	25
July	20	25	up to 32 under heat lamp	17	27
August	20	25	up to 32 under heat lamp	15	25
September	20	25	up to 32 under heat lamp	12	21
October	10	12		8	17
November	6	11		8	14
December	6	6		5	10

The photoperiod during hibernation in the cold store is kept at 5h:19h (L:D) or with no lights at all except when during periodical controls every 15 days. It is important that there is no harsh light pointing directly into the *storage enclosure*s. In Riga Zoo, no alterations have been observed in the more than 20 years of holding the species that could be related to UVB deficiency when in hibernation. To stimulate termination of hibernation in the cold store the temperature can gradually be increased before April, and the photoperiod is set to 12h:12h.

All individuals selected for breeding are moved to the *breeding enclosures* in mid-April. The *breeding enclosures*, together with *tadpole and walk-on-land enclosures*, are placed in areas that follow the natural outdoor temperatures and photoperiods. UVB radiation can be provided by using either or both fluorescent and mercury vapour bulbs. A Solarmeter®[1] Model 6.5 can be used to keep track of the UVA and UVB radiation. In Fota, the general enclosure is kept as Ferguson zone 1 (UVI 0-0.7) while it is kept as Ferguson zone 2 (UVI up to 1.4) under the basking light.

The environment in the enclosures should be kept humid (between 70-100%) by spraying with water. This also stimulates breeding activity. It is also recommended to create variation in the humidity. For example, after a longer period with drought, it can be good to spray the land part greatly with water to make it muddier. While the toads hibernate, it is also important to check the humidity frequently. The humidity can be very dry, so sometimes you need to spray the substrate with water.

The water used in the enclosures is tap water which has been left to stand to release any dissolved chlorine (off-gassing) for min. 24 hours. Alternatively, it can be run through a carbon filter. Reverse-Osmosis water and/or Seachem Prime[2] water treatment is also used successfully to counter any effects of chlorine treatment. Table 5 gives an overview of different water parameters in the tap water in Copenhagen, Riga and Fota.

Table 5: Water parameters for tap water in the three institutions.

Parameter	Range
pH at 25 °C	7.1 – 8.1
Alkalinity (mmol/L)	5.36 – 6.8

[1] Solar Light Company, LLC, 100 East Glenside Avenue, Glenside, PA 19038, USA
[2] Seachem Laboratories, 1000 Seachem Drive, Madison, GA 30650, USA

Ammonium + ammonia (mg/L)	< 0.005 – 0.026
Nitrite (mg/L)	< 0.001 – 0.013
Nitrate (mg/L)	0.93 – 22
Chlorine (mg/L)	0 – 110
Calcium (mg/L)	46 – 150 (only data from Copenhagen)
Phosphor, total (mg/L)	0.010 – 0.012 (only data from Copenhagen)

Water plants help creating a good aquatic environment. They provide shelter and structures for the females to wrap the egg string and absorbe nitrogenous waste. Moreover, daphnia are placed in the *tadpole enclosures* to eat the unicellular algae that can make the water murky. In the *tadpole enclosures* it is recommended to regularly siphon out feed remnants and excrements. This can be done by making a “whirlwind” in the water with an arm and then immediately after using a siphon system to remove the water with dirt into another bucket. It is important to remove the water into a bucket since the water will probably contain some tadpoles. The caught tadpoles can be moved back into the *tadpole enclosure* with a net. This technique can be used when the tadpoles have reached at least a length of approximately 1.5 cm and can withstand the process.

Every day, 10-50% of the water in the *tadpole enclosures* is changed once or twice and gets filled up with new water. Tap water can be used after aging it for 24 hours to ensure that it is off-gassed, or the tap can be equipped with an inline carbon filter. If the tap water contains added chlorine, it is important to pay close attention to chlorine levels as chlorine in public water supplies likely exceeds lethal tolerance limits for aquatic amphibians (National Research Council (US) Subcommittee on Amphibian Standards, 1974). In Denmark, additional chlorine is not added to the tap water, so it should be noted that the following advice is not based on personal experience but on articles found online. Free chlorine (Cl_2) can be removed by letting the water gas off. This process accelerates if the water is well aerated and warmed. In some locations the water contains chloramines (NH_2Cl, $NHCl_2$ or NCl_3), which are stable compounds that do not readily dissipate from water. Chloramines and chlorine can be removed with hypo sodium thiosulfate ($Na_2S_2O_3$). First, a saturated solution of sodium thiosulfate in water should be made by adding it to the water until no more chemical will dissolve. Then, to dechlorinate tap water, add one drop of the saturated thiosulfate solution for each gallon of water. Sodium thiosulfate can be toxic, so care must be made not to use too much of it. Moreover, it should be noted that when thiosulfate acts with chloramines, toxic ammonia is released in small quantities, which may present a separate problem (García-Muñoz et al., 2011; Odum &

Zippel, 2011). Seachem Prime can also be added to the water to remove chlorine, chloramine and to detoxify ammonia.

Every type of enclosure that contains water needs an oxygen pump and an air stone. The air stone serves as a backup if an oxygen pump suddenly breaks. The oxygen pump is held at the bottom with the use of a hag stone. The oxygen pump should pump oxygen into the water at a minimum effective level.

The *tadpole enclosure* also contains an external aquarium filter. Copenhagen Zoo uses a performance canister filter from the brand Fluval, model 307. It works well and it is easy to get replacement items for it. The water is led through a filter pad and bio balls. The filter media should be checked and cleaned once a week. The filter pad will turn black when used for some time and will need to be replaced with a new one. The bio balls sometimes need to be rinsed with water (not directly from the tap, see explanation on pp. 34). This cleaning should take place in the enclosure to avoid a sudden change in water parameters. There is a potential for tadpoles to find their way into the internal mechanisms of the filters. Therefore, it is vital that any pathways into the filters are blocked with fine mesh.

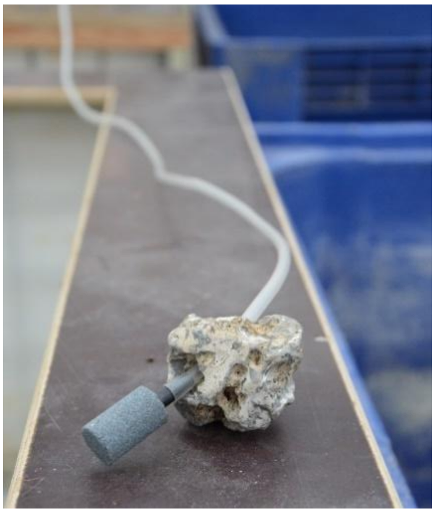

Figure 33: An oxygen pump secured in a hag stone. In this way the pump is kept at the bottom of the enclosure (Copenhagen Zoo).

It should be noted that the oxygen pump and the external aquarium filter in the tadpole enclosure should create very little current since tadpoles are filter feeders and swim constantly. Tadpoles could quickly become exhausted if they must swim against a current.

2.1.5 Dimensions

The *tadpole enclosures* in Copenhagen Zoo usually measure 110 x 90 x 50 cm and contain a little less than 500 L water. The enclosures are used storage boxes from a catering centre, and they all contain a drain. It is estimated that each enclosure contains up to 2,000 tadpoles. In Riga Zoo, plastic boxes measure 34 x 27 x 11 cm and house approximately 200 tadpoles, Fota Wildlife Park uses 40L plastic storage containers with a density of 50 tadpoles per box. Studies suggest that amphibians will metamorphose to smaller sizes when kept at higher densities, and they will then be very small when they walk on land (Dash & Hota, 1980; Newman, 1994). Therefore it is important to avoid over-crowding by using the densities as mentioned above.

The boxes used as *tadpole enclosures* can be the same as those used for *walk-on-land enclosures*. The *walk-on-land-enclosures* are just tilted slightly to create a water area and a land area. Tadpoles can also be moved to Exo Terra fauna terrariums measuring 36 x 21 x 16 cm, which are tilted.

The *breeding enclosure* at Fota Wildlife Park is a round 150 cm cattle trough sitting on an angle. Riga Zoo uses glass aquariums measuring 57 x 26 x 25 x cm with a water level of 7-10 cm. In Copenhagen Zoo the enclosures for breeding measures 227 x 310 cm with the land area making up 75% of the area, while the water area makes up the remaining 25%. It is better to have a large water area so breeding pairs can move away from the others and not be disturbed while they are in amplexus. The water area has a depth of 20 cm, and there is a slight incline between the water and land area. At the beginning of the breeding season there are often 8 individuals per breeding enclosure, but this can be increased to 20 individuals. Though they are a bit different, the systems of the three zoos show great breeding success.

The boxes used for *storage* and *hibernation enclosures* measure 57 x 76 x 30 cm in Copenhagen, and 30 x 20 x 17 cm in Riga.

2.2 FEEDING

2.2.1 Basic diet

Natterjack toads will eat everything that moves and has the correct size while their larvae are filter feeders. Examples of basic diets are given in table 6.

Table 6: Examples of food items for tadpoles, juveniles, and adult toads.

Life stage	Copenhagen Zoo	Riga Zoo	Fota Wildlife Park
Tadpoles	Spirulina in powder form[3] (right after hatching to start the feeding process slowly) Crushed fish flake[4] Spirulina-egg white[5] smeared on stones and kept	Fish flakes (JBL[6] NovoBel), 0.005 g per aquarium, once a day Pollen, some pieces per aquarium, 3 x per week	Fish flakes (JBL Novo Vert and Tetra Pro Algae), once a day Green leaf lettuce (previously frozen), ad libitum

[3] Avian, Aves New Products B.V., Drostenkamp 3, 8101 BX Raalte, The Netherlands
[4] TROPICAL Tadeusz Ogrodnik, Opolska 25, 41-507 Chorzów, Poland
[5] DANÆG Products, Maglegårdsvej 13, 4000 Roskilde, Denmark
[6] JBL GmbH & Co. KG, Dieselstraße 3, 67141 Neuhofen, Germany

	in the refrigerator until they are used for feedings (fig. 34) Blanched Chinese cabbage, potatoes, and carrots (fig. 35-36). Cuttlefish bone (to ensure they get enough calcium).	Branches with algae and lichens, in the water all the time Dried dandelion leaves, 0.005 g per aquarium, once a day Snails (aquarium ramshorn snails, smashed), about 5 snails, 3 x per week	
Juveniles	Springtails, fruit flies, weevils for newly metamorphosed toadlets; micro worms, firebrats, woodlice when a bit bigger. Food is shaken in a calcium or vitamin preparation (Dendrocare[7]).	Crickets, cockroaches, and wax worms, 2-4 insects per animal, 2-3 x per week	Springtails and/or fruit flies once a day. Food is shaken in either Dendrocare or Nekton[8] Rep three times a week
Adults	Meal worms, grasshoppers, crickets, flies, maggots. Food is shaken in Dendrocare. 1-2 insects per animal once a day but no feeding during hibernation.	Crickets, cockroaches, and wax worms, 2-4 insects per animal, 2-3 x per week. In winter, the adults not in brumation are feed 2-3 x a week with 2-4 pill bugs per toad.	Crickets, locusts and cockroaches, dusted with Dendrocare or Nekton Rep. In winter the adults not in brumation are offered one or two crickets once a week, in early spring and late summer/autumn they are fed 2-3 x a week, and daily in breeding season.

[7] DendroCare, Het Ronsel 17, 5527 GR Hapert, The Netherlands
[8] NEKTON GmbH, Hoheneichstraße 19, 75210 Keltern – IKG Dammfeld, Germany

The best diet for optimal growth from tadpole to toadlet in natterjack toads is currently unknown. Therefore, keepers offer tadpoles many different food items to hedge their bets for optimal growth.

A small study investigated the effect of dietary protein on early life history and morphological traits in natterjack toad tadpoles by feeding them with three different commercial fish diets with varying protein (32%, 38.3%, and 46.2%). High-protein diets revealed significantly longer bodies and wider heads and hindlimbs. Feeding with 46.2% protein promoted better growth, development, and survival compared to the two other fish diets (Martins et al., 2013).

Figure 34: Tadpoles fed with spirulina-egg white smeared on a stone (Copenhagen Zoo).

Figure 35: Blanched potatoes used to feed tadpoles (Copenhagen Zoo).

Figure 36: Blanched cabbage used to feed tadpoles (Copenhagen Zoo).

2.2.2 Method of feeding

Natterjack toads are fed every 1-2 days during daylight hours. During the breeding season they eat a lot and should be offered food every day. Outside the breeding season they eat less, and during hibernation they eat nothing at all. If there are many leftovers from the day before, or if they have been fed with large food items, a day can be skipped. The food is given on the land area, often on a place where there is a flat area with few hiding spots. This makes it more difficult for the food items to hide from the natterjack toads. All food items are given alive to the natterjack toads, otherwise they will not eat their food. Before each feeding the animal keepers from Copenhagen Zoo knock on the enclosures, so the natterjack toads learn to associate the sound with food. Moreover, it is recommended that they are fed with a different food item at their next feeding to give variation in their food intake. Uneaten food should be removed the day after it is offered, however small amounts of carrot and leafy greens may be left for the uneaten crickets to consume.

Tadpoles are fed at least once a day. It is better to feed a little at a time, so there are not many leftovers, keeping the water clearer and reducing the amount of nitrogenous waste.

2.2.3 Water

It is important to consider water quality in keeping any amphibians since all rely on some form of moisture. Monitoring water quality is vital to successfully rearing healthy animals. The optimal water conditions are unknown for most amphibian species, but Odum & Zippel (2011) developed some general guidelines that can be followed. It is important to keep the water parameters as steady as possible because fluctuating water parameters create stress for the individuals. It is better to maintain constant conditions, even if they are slightly sub-optimal (Wells et al., 2015). Further information about water parameters and treatments used for natterjack toads in Copenhagen Zoo can be found in the last part of section 2.1.4.

2.3 SOCIAL STRUCTURE

2.3.1 Basic social structure

Tadpoles are kept in *tadpole enclosures* until they start to develop hind limb buds. Tadpoles that are close to reaching full metamorphosis are kept together with toadlets from the same year. Afterwards, individuals from the same year are kept together until they reach sexual maturity. When the natterjack toads reach sexual maturity, all individuals can be kept together. In all the different life stages males and females are kept together. In the breeding season some males can be removed to remove the pressure on females if males show a strong desire to mate which may be stressful to the females.

2.3.2 Changing group structure

The group structure is only changed during the breeding season in the *breeding enclosures*. If breeding activity is absent for a while, it can it be beneficial to add new natterjack toads or switch some of the individuals between *storage enclosures*. Introducing new individuals can elicit increased social activity incl. increased calling which often leads to a new bout of breeding activity. Moreover, individuals in the *storage enclosures* have been able to hear the croaking males without being able to participate in the mating attempts by themselves and are therefore sometimes more eager to breed.

If there are too many males in a *breeding enclosure*, they can potentially disturb pairs in amplexus, discouraging them to lay eggs. For confirmation, observations should be made of where amplexus are seen but no egg strings are laid afterwards. Some males should then be removed from the *breeding enclosure.*

2.3.3 Sharing enclosure with other species

In principle it is possible for natterjack toads to share enclosures with other amphibian species, however it is not recommended especially if the animals are being bred or maintained for translocation purposes where biosecurity levels must be kept high (see section 2.7). Natterjack toads are capable of hybridizing with both the European green toad *(Bufotes viridis*) and common toad (*Bufo bufo*) (Fog et al., 2001; Strömberg et al., 1991). Moreover, natterjack toad eggs and tadpoles are eaten by other amphibian species (see section 1.8.3). Furthermore, multiple studies have shown that natterjack toad tadpoles sharing a pond with other amphibian species such as the common toad or the common frog (*Rana temporaria*), larvae will grow more slowly, suffer from higher pre-metamorphic mortality and emerge as smaller toadlets compared to keping them by themselves (Bardsley & Beebee, 1998; Griffiths, et al., 1991).

2.4 BREEDING

2.4.1 Mating

To start mating it is important that the males croak. In the wild they normally start croaking when they gather around the breeding ponds. The same behaviour is seen when they are placed in the *breeding enclosure* because there is an aquatic area. The croaking attracts the females. If mating is successful it will result in the pair being in amplexus, and the male will fertilize the egg string laid by the female.

If mating does not occur, it can be stimulated by making “rain”. Moreover, it is recommended that there is variation in the humidity in the enclosure. “Rain” has the potential to stimulate breeding even more if it has been absent for a while.

If breeding activity is absent for a while, it can it be beneficial to add new natterjack toads/switch with some of the individuals in the *storage enclosures*. Introducing new individuals can elicit increased social activity incl. increased calling which often leads to a new bout of breeding activity. Moreover, individuals in the *storage enclosures* have been able to hear the croaking males without being able to participate in the mating attempts by themselves and are therefore sometimes more eager to breed.

If there are too many males in a *breeding enclosure*, they can potentially disturb pairs in amplexus, discouraging them to lay eggs. To confirm this, observations should be made of where amplexus are seen but no egg strings are laid afterwards. Some males should then be removed from the *breeding enclosure.*

2.4.2 Egg laying and development

Egg strings are often laid during the night or early in the morning. Each female lays 3000 to 4000 eggs. Most females lay a single egg string per season, but it is observed that some lay twice (Andren & Silverin, 1992; Beebee & Denton, 1996).

Natterjack toads entangle the egg strings in plants and branches in the water. When an egg string is observed it should be cut free from the plants and removed from the *breeding enclosure* to a *tadpole enclosure*. The way to remove an egg string is by taking a bucket and pushing it gently under the water, so the egg string can be moved to the bucket by the vacuum, thereby avoiding being out of the water.

2.4.3 Hatching

After a couple of days, the eggs will hatch in the *tadpole enclosures*. The exact time depends on temperature (see section 1.7.1). Afterwards the feeding of tadpoles will begin (see section 2.2.2).

2.4.4 Development and care of young

Keepers must pay attention to the feeding of natterjack toad tadpoles since it can be challenging to feed them properly (see sections 2.2.1 and 2.2.2). During the tadpoles' development it is important to keep an eye on them every day. When they begin to develop hind limb buds, they need to be transferred to a *walk-on-land enclosure* so they can emerge on land when they develop lungs. Once emerged, the newly metamorphosed toadlets should be offered small food items such as fruit flies (see section 2.2.1). The newly metamorphosed natterjack toads should be offered food more frequently than juveniles and adults.

2.4.5 Population management

The current (2025) captive population, registered in the software ZIMS in spring 2025, is limited to nine collections (nine institutions in Europe).

In the three sending institutions, natterjack toads are primarily bred with the aim of releasing individuals to the wild in adherence to the IUCN Guidelines for Reintroductions and Other Conservation Translocations (IUCN/SSC, 2013), EAZA Field Conservation Standards and the Chapter 4.3: Releasing animals to the wild from the EAZA population management manual. The multiple populations originate from different geographical areas, and it is important that these are kept separately due to potential differences in their genetics. Geographic isolated populations may have adaptations specific to the microhabitat they originated from and thus interbreeding of populations may dilute these adaptations.

If there is a need to control the population, males and females should be allowed to breed but the still underdeveloped eggs should be discarded or euthanized (depending on the legality of the respective country). Breeding limited by keeping males and females separate is not advised as females may develop follicular stasis or become infertile when not able to mate for a prolonged time.

2.5 BEHAVIOURAL ENRICHMENT

Providing a naturalistic captive environment with opportunities for hiding and digging provides natterjack toads with opportunities to express a range of natural behaviours.

2.6 HANDLING

2.6.1 Individual identification and sexing

Individuals can be identified by the patterns on the abdomen. Each toad has a unique colour pattern. The patterns are visible and stable when the toads are one year old and do not seem to change thereafter. For individual identification, a picture of the pattern can therefore be taken after this age. The pictures can then be laminated, and information about the specific toad can be written on the back of the picture (Fig.37). The information may include age, gender, disease records, place of origin etc. A good way to keep an overview of which individuals are in which enclosure is by having a plastic sheet with all the laminated pictures hanging on the enclosure. The pictures can be removed/switched if changes are made with the group structure in the enclosure (Fig. 38).

Another way of identifying adult individuals is by PIT-tagging (Passive Integrated Transponders) (Camper & Dixon, 1988). This procedure is not very complicated and can be performed by a veterinarian. However, the cost of the PIT-tags must be considered.

It can be difficult to determine the sex of a natterjack toad. Males typically have larger upper arms than females if they have bred. Moreover, they have a large vocal sac that turns blueish, purplish, or reddish in the breeding season. where they also are recognized by their croaking. Due to croaking, the skin around their throat gets "wrinkly" and the colour pattern around the throat almost "disappears" because it has been distended. In general, the colour around their neck gets darker. A method for sexing the individuals is to hold the animal with one hand grabbing the animal by their back thereby pretending to be a male in amplexus. If it is a male, it will often make a small squeaking sound as a way to communicate to the other male that it cannot be mated.

Figure 37: A natterjack toad being handled with the aim of determine the individual based on the abdomen pattern. Four laminated pictures of abdomen patterns from different individuals are placed on the ground (Mikkel Jézéquel).

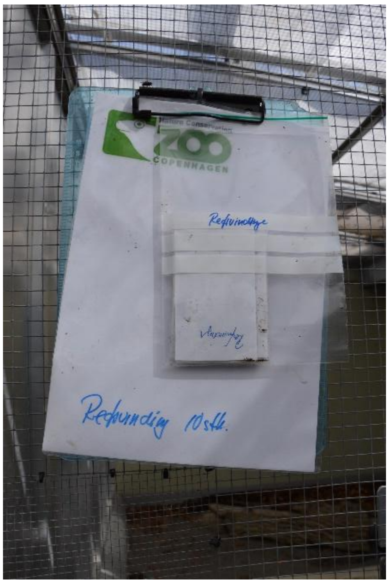

Figure 38: Plastic sheet with laminated abdomen pictures on an enclosure with individuals from the city Refsvindinge (Copenhagen Zoo).

2.6.2 General handling

Natterjack toads must be handled carefully and only when necessary. It is best to handle them with clean humid hands or powder free vinyl, latex, or nitrile gloves. Powder free vinyl, latex or nitrile gloves protect the toad's skin from abrasion, contamination between the toad and the handler, and from the spread of pathogens. The gloves should be changed between species or individuals as appropriate.

Tadpoles are caught with a fine mesh net and afterwards immediately moved into a bucket of water.

2.6.3 Catching/restraining

When natterjack toads are caught, for instance to check their abdomen pattern for individual identification, they are best held by a grip right above their hindlimbs. In this way you have full control over the natterjack toad (fig. 37).

2.6.4 Transportation

During transportation natterjack toads should be placed in a moist plastic box so they do not get stressed and dehydrated. Soaked moss is a good material for keeping the environment humid in the box. There should not be any water in the transportation box since this can cause drowning. Breathing holes in the box lid can be made with a soldering iron. They must be made from the underside of the lid so there will not be any sharp plastic edges where the toads can cut themselves (fig. 41). Regarding feeding during transportation, natterjack toads can easily do without food for some time. The temperature while natterjack toads are being transported should not be higher than 20°C. If they are transported in warmer weather, freezing elements can be placed on top of the lid.

Tadpoles are transported in a bucket with water where an air stone has been placed (fig. 39). The air stone should preferably have been put in the water a day before the transportation and is then removed before the lid is closed. It is recommended that the bucket is completely filled with water, so the water does not slosh around and shake the tadpoles which can be harmful to them (fig. 39). If the transportation time is longer than three hours and it is possible to break along the way where the bucket stands still, the lid should be taken off to let the tadpoles have some oxygen. Tadpoles should not be transported too early in their development, i.e., when they are less than 2 cm long. At the same time, they should not be transported too late in their development when they are close to walking on land. They can then potentially drown during transportation and contaminate the water.

Egg strings are transported in plastic bags (fig. 42). The air in the plastic bag is sucked out so the water cannot slosh around which might break up the strings. In the water, some grass, e.g., creeping bentgrass (*Agrostis stolonifera*), should be placed where the egg strings can be tangled around (Fig.

43). When the egg strings are removed from the plastic bag they should not come out of the water (see section 2.4.2).

When the eggs strings are placed in the plastic bag, they should be kept under temperature conditions at around 5℃. This prolongs the time before the eggs hatch.

Figure 39: Preparation for a transportation of natterjack toad tadpoles. An oxygen pump is placed in each bucket before transportation (Copenhagen Zoo).

Figure 40: Tadpoles being transported. The buckets are completely filled with water, so the tadpoles do not slosh around (Copenhagen Zoo).

Figure 41: Toadlets in a moist plastic box. Before transportation a lid with breathing holes is placed on the box (Copenhagen Zoo).

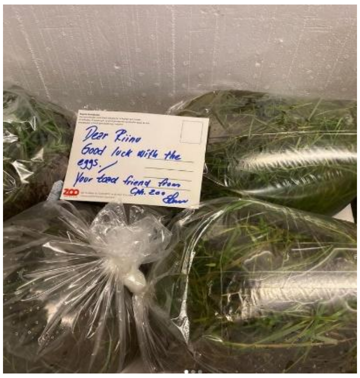

Figure 42: Plastic bags with egg strings ready for transportation (Copenhagen Zoo).

Figure 43: A close-up picture of an egg string in a plastic bag (Copenhagen Zoo).

2.6.5 Safety

As a defence mechanism, natterjack toads are capable of secreting strong acting skin poison from their parotoid glands that are rich in cardiotoxic steroids. It is known that this skin poison from the family Bufonidae can cause severe intoxication in vertebrates due to the high amounts of cardiotoxic steroids (Stawikowski & Lüddecke, 2019). The skin poison from natterjack toads is not known to be harmful to human skin, but powder free gloves are recommended to be worn when handling to protect the toad's skin from abrasion, contamination between the toad and the handler, and from the spread of pathogens. The gloves should be changed between species or individuals as appropriate. It is furthermore important to be aware that the poison can be very harmful to the eyes. If a toad is to be handled without gloves, hands should be washed both before and after handling. Moreover, it should be noted that natterjack toads normally only secrete skin poison when they are very stressed. It is not something that normally happens if the toads are used to being handled.

2.7 VETERINARY: CONSIDERATIONS FOR HEALTH AND WELFARE

Newly arrived individuals from the wild should be quarantined and kept in separate facilities from the current captive populations for at least 6-8 weeks, and their health and physical condition checked. Routine skin screening for chytridiomycosis (*Batrachochytrium dentrobatidis*-Bd and *Batrachochytrium salamandrivorans*-Bsal) and Ranavirus should be performed.

If the population is kept as part of a breeding and reintroduction project, the IUCN reintroductions and translocations guidelines (IUCN/SSC, 2013) need to be followed and the IUCN manual for control of infectious diseases (Pessier & Mendelson, 2017) should be consulted. A pre-release health screening must always be performed. This includes examinations on bacteriology, virology, fungal diseases and faecal parasitological examination. If the hygiene and quarantine rules are respected (i.e. cleaning of materials, boots, aquariums etc.) a health screening is necessary after each new acquisition of animals; and in any case, every time a toad release is scheduled, a pre-release health screening should be done. If an animal is showing signs of disease or is not in optimal physical condition, it should be excluded from the reintroduction project.

Biosecurity levels must always be high. Biosecurity is maintained by having the head-starting operation set up in its own facility (e.g. a converted shipping container) and managed by staff that have no interactions with the other amphibians, reptiles, or fish in the collection. If staff that work with reptiles, amphibians, or fish must also work with the natterjacks for reintroduction then the natterjacks should be serviced before the other animals on a given day. For reintroduction programmes, a high level of biosecurity must be always maintained.

Especially if the animals will be part of an *ex situ* breeding and re-introduction program, it would be highly beneficial to carry out genetic tests to check the genetic origin, the level of inbreeding, etc. of the founder animals.

Spindly-leg-syndrome can occur in natterjack toad tadpoles. It is a musculoskeletal abnormality that is associated with captive-rearing of aquatic amphibian larvae. It results in underdeveloped limbs that cannot support the body of newly metamorphosed toadlets (Lassiter et al., 2020). Water composition and overfeeding are two components linked to the problem (see section 2.2.1).

Liquid accumulation (i.e. oedema) in the abdomen of natterjack toads is sometimes observed. It is currently unknown what causes the condition. However, the liquid can be sucked out with a syringe by a veterinarian. The liquid can sometimes have a bluish/greenish colour. It has once been observed that a natterjack toad was bloated, without any liquid accumulation in the abdomen. It turned out it was caused by an infection in the abdomen. The toad was treated daily with 0.017 mL Baytril Vet mixture 2.5 mg/ml for five consecutive days and recovered.

During a pilot study about radio tracking of natterjack toads in Denmark conducted by Copenhagen Zoo, some of the toads developed sores around the front legs. The sores were then smeared once a day with eye drops for dogs (Isathal 10 mg/g) to support the healing process (Carsten Grøndahl, pers. comm.). If an infection (a swollen limb) was observed, it was treated with antibiotics. We used a Baytril Vet mixture 2.5 mg/ml that was given orally once a day until the swelling had disappeared. The amount of Baytril was found by using the following formula:

$$amount\ of\ Baytril\ (ml) = \frac{\left(\frac{weight\ of\ the\ toad\ (g)}{5}\right)}{100\,\frac{g}{ml}}$$

2.8 SPECIFIC PROBLEMS

The captive natterjack toad populations from different locations are often based on a few individuals. Currently no signs of negative effects due to inbreeding have been observed in the three institutions. However, this should be kept in mind as a potential future problem. In a replicated pond experiment it has for instance been observed that inbred larvae grow more slowly than outbred larvae (Rowe & Beebee, 2005). If the actual relationships and genetic makeup between breeding animals is unknown, a general guiding principle to lower the potential of inbreeding is to reproduce generations separately, i.e. to make sure only animals in the parental generation breed with animals from their, i.e. parental, generation, only F1 animals breed with F1 animals etc.

A study has found that water composition likely plays a role in developing spindly leg syndrome. Harlequin frog (*Atelopus* spp.) tadpoles swimming in water treated by reverse osmosis and then reconstituted, were significantly less likely to develop spindly leg syndrome compared to tadpoles reared in tap water. The reverse osmosis treatment might have removed a factor causing spindly leg syndrome, or the reconstitution might have added a mineral lacking in the tap water. Moreover, the study also found that overfeeding of tadpoles in tap water increased the incidence of spindly leg syndrome (Ciani et al., 2018). Two important components regarding developing spindly leg syndrome are found to be calcium and phosphate in Harlequin frogs. Calcium supplementation significantly reduces the incidences of toadlets developing spindly leg syndrome. Moreover, phosphate supplementation significantly reduces incidences of spindly leg syndrome in low calcium treatment. This clearly links spindly leg syndrome to the calcium: phosphate homeostatic system (Lassiter et al., 2020).

2.9 RECOMMENDED RESEARCH

It is currently unknown how far natterjack toads travel in the most northern part of its distribution area, such as Denmark and Sweden. Earlier studies have indicated that the migration distances vary with latitudes, and it is therefore relevant to know the migration distances at specific places to optimize conservation efforts for the species. This knowledge can for instance be used to ensure the possibility for connectivity among breeding ponds. A pilot study about radio tracking of natterjack toads has been carried out in Denmark in 2021 by Copenhagen Zoo. However, the radio tracked individuals were captive bred. The next step in this project will be to radio track wild natterjack toads. Radio tracking of wild individuals can also increase our knowledge about hibernation places, habitat preferences, activity level during the day etc.

Another important area for research is further investigations of what is causing spindly leg syndrome in natterjack toads. It has been found that the two water components calcium and phosphate play the main role for incidences of spindly leg syndrome in Harlequin frogs. It will be interesting to investigate this on natterjack toads as well. In connection to tadpole development the optimal diet for tadpoles still needs further research.

Other potential areas for research include natterjack toads' use of UV-light, such as for instance: Does UV radiation affect tadpole development? And why do natterjack toad adults sometimes sunbathe? Finally, it is also currently unknown what is causing frequent oedema in the abdomen of some captive natterjack toads.

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