

Basic Information and Husbandry Guidelines for *Tylototriton vietnamensis*, Vietnamese Crocodile Newt





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# 1. Characterisation

Scientific name: Tylototriton vietnamensis (Böhme, Schöttler, Nguyen & Köhler, 2005) Vernacular name: Vietnamese crocodile newt Snout-vent length: 5-20 cm total length, slightly more than half is accounted for by the tail CC#Amphibians category: || IUCN Red List: Endangered: endangered (EN) Protection status CITES (Washington Convention on International Trade in Endangered Species): Appendix II Protection status according to EU Species Protection Regulation: Appendix B Protection status in Germany: "specially protected" Housing: Rainforest terrarium with water part Required equipment: For 3-5 animals terrariums with a base area of approx. 50-80 cm length and 40-50 cm width. Water part of about 1/4 to 1/3 of the area, good exit possibilities are important. Substrate of gravel, earth or similar natural substrates. Furnishing with moss cushions, bark pieces, stones, planting.Simple lighting. Temperatures in summer approx. 23-25 °C, in winter approx. 18-20 °C. Simulation of dry season in the winter half-year and rainy season in the summer half-year. Incubation of the clutches at maximum humidity. Ideally, larvae should be reared in small groups in in small boxes filled with stagnant, slightly acidic water with daily feeding and daily water change or alternatively in a large aquarium. Diet: Common feeder animals such as crickets, earthworms, isopods, etc. Feeding of adults 1-2 times a week, juveniles daily. Larvae are fed with small live food such as water fleas, Artemia,

Tubifex etc., feed interval for larvae in

groups should be ideally several times a day.



# 2. Why is *Tylototriton vietnamensis* a Citizen Conservation species?

The Vietnamese crocodile newt was only recently discovered; from the beginning it was clear that it inhabits a very small range. As knowledge has increased and the species status of related crocodile newts in the region has been clarified, it has become apparent that *T. vietnamensis* actually occurs at only a few localities. Modeling indicates that no significant, previously undiscovered range expansions are to be expected. Based on this small-scale distribution, the species must be considered potentially endangered.

The Vietnamese crocodile newt is an obligate forest dweller that can cope with some habitat disturbance and is not necessarily dependent on primary forests, but large-scale logging and habitat conversion for agriculture have rendered large areas of its range uninhabitable. Only small, fragmented remnant forest stands remain. The species has therefore now been classified as "critically endangered" both in its country of origin, Vietnam, and on the IUCN Red List.



*Tylototriton vietnamensis* in action for environmental education - here the display in the aquarium of the Cologne Zoo Foto: Thomas Ziegler



In addition to the main threat from habitat destruction, as with all crocodile newts, collection of the animals for traditional medicine and for the international pet trade represents an additional endangerment factor. The genus *Tylototriton* has therefore been listed in Appendix II of the Washington Convention on International Trade in Endangered Species.

The Vietnamese crocodile newt is a prototype for the global threat to amphibians and the endangerment of biodiversity through deforestation and can therefore serve well as an ambassador for these issues in captivity. At the same time, the species is part of a comprehensive in-situ / ex-situ biodiversity conservation project in Vietnam, which is being carried out by the Cologne Zoo, among others, and thus demonstrates the possibilities of such a holistic species conservation approach in line with the "One Plan Approach" to biodiversity conservation.

In addition, a large part of the knowledge about the Vietnamese crocodile newt could be gained by keeping it in terrariums, so that extensive biological knowledge important for species conservation could be gained from this species already relatively short time after its discovery. *Tylototriton vietnamensis* also exemplifies this important aspect of wildlife conservation.

And finally, it is an attractive, charismatic and easy to keep species, which allows interesting observation possibilities for show facilities as well as private keepers and is easy to maintain in captivity.



The Vietnamese crocodile newt is endangered | Foto: Benny Trapp / Frogs & Friends



Tylototriton vietnamensis occurs only in small, fragmented remnant forest areas in the north of Vietnam | Foto: Thomas Ziegler



# 3. Biology and Conservation

# 3.1 Biology

# 3.1.1 Systematics

The Vietnamese crocodile newt belongs, within the caudate amphibians, to the salamander family (Salamandridae). The genus *Tylototriton* has become the subject of intensive systematic research in recent years, which has led to an enormous gain in knowledge.

It has become apparent that some of the species previously assumed to be widespread actually conceal many independent species, which have then been redescribed. As a result, the genus *Tylototriton* is now the most diverse of the Salamandridae, with more than 30 species, and this revealed that some crocodile newts have a very small distribution and are therefore highly endangered.

Also *T. vietnamensis* belongs to these only relatively recently (2005) described species and was previously considered to belong to *T. asperrimus*. Therefore, animals from Vietnam described as *T. asperrimus* in older literature may also represent *T. vietnamensis* or other crocodilie newts described later. For the differentiation of *T. vietnamensis* from other crocodile newts, see RAUHAUS & ZIEGLER (in press).



The Vietnamese crocodile newt was not scientifically described until 2005 | Foto: Thomas Ziegler



#### 3.1.2 Description

*Tylototriton vietnamensis* is a small representative of the crocodile newts. Typical for the genus are the many warts on the skin of the upper side and on the sides, which are rather small in *T. vietnamensis*. Also typical are the large parotoid glands (ear glands) on the back sides of the head. Characteristic of the genus are three warty, longitudinal, well-developed dorsal ridges reminiscent of crocodiles.

Females reach a snout-vent length of up to 8.4 cm and are clearly more massive than the males, which grow to a maximum length of 7.5 cm. The laterally flattened, tapering tail is slightly longer than the head and body and has a fringe above and below. The head is broader than the trunk and somewhat flattened. Webbed toes are absent except for the very base of the toes.

Vietnamese crocodile newts are uniformly black, brown to gray in color. While on land, they are noticeably darker than in water. In sharp contrast to this rather dusky base coloration, finger and toe tips and the underside of the tail are bright yellow to red-orange in color. Some animals show similarly colored rib knots, occasionally the uppermost part of the tail is also yellow to red-orange.

## 3.1.3 Sex differences

Females grow larger and have a much stockier build. The sexes can also be identified by carefully spreading the cloacal lips by hand: Females then show a more punctate cloacal opening; in males it is slit-shaped.



Particularly noticeable are the large parotoid glands on the posterior skull and the well-developed dorsal ridges | Foto: Thomas Ziegler



Above the larger and stockier female, below the male I Foto: Anna Rauhaus



#### 3.1.4 Distribution and Habitat

The Vietnamese crocodile newt is known from only a few localities in northern Vietnam. The type locality is the Tay Yen Tu Nature Reserve in Bac Giang Province. Other occurrences are known from Mau Son mountain in Lang Son province and Yen Tu and Dong Son - Ky Thuong nature reserves in Quang Ninh province.

These localities are relatively small remnants of lowland rainforest with granite rocks, dominated by hardwood, bamboo and shrubs. *T. vietnamensis* inhabits altitudes range from about 180 to 980 m above sea level. Humidity in the habitat varies between 68 and 100 %, temperature between about 24 and 34 °C. The region is characterized by the summer monsoon.



Exploring the habitat in the nature reserve Yen Tu I Foto: Thomas Ziegler



#### 3.1.5 Reproduction in the Wild

Breeding occurs during the rainy season between April and July. At the beginning of this period, small waterholes - shallow pools, large puddles, temporary shallow lakes - are formed, which are then immediately visited by the males. They clearly prefer the water at this time of the year and only very few males can still be found on land – provided sufficient rainfall ensures such temporary water bodies are available.

These mating waters, located in the forest, are small, shaded, and muddy; often they are only a few inches deep. Newts prefer areas that are at least half shaded by trees. Waters on cleared areas are therefore not used.

Unlike males, females probably spend short periods in the water only for courtship. Eggs are not laid there, but up to 2 m from the bank under foliage on the soil of the forest floor.

Clutch size varies greatly; clutches of 5-85 eggs have been recorded so far.

The eggs have a diameter of about 6-14 mm. After oviposition, they are transparent and then turn increasingly brownish, but remain translucent.

The larvae measure about 15.5-18 mm after hatching. According to the limited data available so far, metamorphosis in the wild occurs at a length of about 4.5 cm and a weight of 0.6 g in October.



Such puddles and temporary pools serve as larval habitat for Vietnamese crocodile newts I Foto: Thomas Ziegler



These two larvae of *Tylototriton vietnamensis* were found in the temporary pool | Foto: Thomas Ziegler



# 3.2 Threat Situation and Protection

The Vietnamese crocodile newt has only a very small distribution area. The species is dependent on forests, which are increasingly being cleared in its native country to make room for agricultural land. Mining is also a threat, as the range is located in a region with large coal deposits and the impacts of the mines (infrastructure, pollution) are present, even close to the borders of the protected areas.

Niche modeling by Bernardes et al. (2013) has shown that potentially undetected occurrences of this newt would only be expected in highly fragmented habitats threatened by deforestation. Accordingly, no significant expansion of the distribution range can be assumed, and the discovery of many more occurrences is virtually impossible. Overall, only a few small and highly fragmented habitats remain available to this newt.

A further threat, as with all crocodile newts, is the collection of the animals for traditional medicine as well as for the pet trade; under a false name, *Tylototriton vietnamensis* has also already been detected in the international pet trade.

The species is therefore listed as "endangered" (EN) in both the IUCN Red List and the Vietnamese National Red List. On the IUCN list, the upgrade from "potentially endangered" (near threatened, NT) to EN occurred in 2016 after new knowledge of its only small range and habitat threats were incorporated into the assessment.

The most important conservation measure is the preservation of the nature reserves in the distribution area. The Cologne Zoo, together with the Institute of Ecology and Biological Resources

(IEBR) and the Vietnamese Melinh Station for Biodiversity, are caring for the species both through basic research and support of conservation efforts in the field and through the establishment of an ex situ population in human care, to be achieved through a network of husbandries in local, German and international facilities. Citizen Conservation also belongs to this husbandry network.



The small remnant forest stands where *T. vietnamensis* occurs are threatened by coal mining, among other things | Foto: Thomas Ziegler



# 4. Keeping and Breeding

The following Information on captive husbandry is based on experience at the Cologne Zoo (ZIEGLER & RAUHAUS 2019a; RAUHAUS & ZIEGLER in press; pers. comm.), supplemented by information from Lisa Breitenbach, who was the first Citizen Conservation participant to successfully breed Vietnamese crocodile newts repeatedly.



Anna Rauhaus working with Vietnamese crocodile newts at Cologne Zoo | Foto: Benny Trapp / Frogs & Friends



# 4.1 Restrictions and Documentation Requirements

Until CC is established as an independent organization, the CC animals are the property of the association Frogs & Friends e. V., which manages them for the Citizen Conservation #Amphibians project. This also applies to all offspring. Owners are not allowed to sell or give away offspring on their own. The animals must not be mixed with other *Tylototriton vietnamensis* that do not belong to the CC program.

According to the Citizen Conservation contract and guidelines, deaths should be reported directly to the CC office by email (amphibien@citizen-conservation.org), so that a decision can be made whether a necropsy is advisable, if the cause of death is not obvious. The animals should be stored in a cool place until this decision is made. A separate information sheet on the shipment of dead animals and precautionary preservation is available. The bi-annual reporting of the stock is contractually stipulated. This is currently done on the reporting dates of 1.3. and 1.9. using the appropriate reporting forms, which are to be replaced in the near future by an online procedure.

Reporting of offspring to the CC-#Amphibians office can be done at about six months of age, when the number of juveniles expected to reach adulthood becomes manageable. Also, special observations, encountered problems, etc. should be reported to the CC office at the bi-annual reporting or in between to increase knowledge of the husbandry and reproduction of this species.

If keepers are no longer able or willing to keep the animals or their offspring - whereby CC assumes that the keeping of the animals has been carefully considered beforehand and that the long-term nature of such an undertaking has been planned for and taken into account - the CC office must be informed as early as possible that a transfer is imminent, so that a successor for the keeping of the animals can be organized. CC guarantees the acceptance of the animals.

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## 4.2 Transport

The transfer of CC animals including their offspring may only take place in consultation with the CC office.

Before each change of location within CC, a swab sample of the animals must be taken in accordance with the CC instructions and submitted for examination for the chytrid fungus *Bd* and *Bsal*. In addition, a fecal sample should be sent for parasitological examination (use CC's veterinary examination form, available from the CC office; CC bears the costs). After negative test results are available or confirmation that there is no parasite load requiring treatment, the transfer can take place.

The delivery will be made either personally or by an agency certified for animal transport. An information sheet from the CC office provides information on appropriate contact persons. For transport, the crocodile newts are placed in small, sturdy boxes with air holes lined with damp cellulose paper or moss. The air holes should not have an inward rim to prevent skin injury to the newts. Larvae can be transported in tightly sealed plastic bags or boxes filled about 1/3 with stagnant water.

The boxes or bags are placed in a thermostable, larger container (Styrofoam box, insulated bag) for protection against external weather conditions and secured against slipping, flinging around and falling over. In winter or summer, it may be necessary to place a coolpack or a hot water bottle or respectively a heatpack in the container. Caution, such items must be safely separated from the animal containers to prevent overheating or hypothermia.



Employees of the Cologne Zoo packing Vietnamese crocodile newts into a transport box | Foto: Thomas Ziegler



Protected in a styrofoam box, even a longer transports can take place like here during a repatriation action of the Cologne Zoo to Vietnam | Foto: Kölner Zoo



#### 4.3. Socialization

Vietnamese crocodile newts can be kept in small groups, depending on the size of the terrarium. Intraspecific aggression is usually not an obstacle - but see the notes on raising larvae together.

Citizen Conservation generally recommends keeping them in species tanks, i.e. without other amphibian species. Keeping invertebrates (e.g. water snails in the water part; isopods and earthworms in the land part) in the same terrarium is possible and recommended. In principle, it is not allowed to keep CC animals together with conspecifics that do not belong to the CC program!

When keeping several CC groups of *Tylototriton vietnamensis*, please take care not to mix the animals and report them separately by group in the bi-annual population reports. The CC program is based on coordinated breeding, so special attention must be paid to the genetic background of the animals to avoid unnecessary inbreeding and narrowing of the gene pool within the CC population.

Therefore, animals may only be bred after consultation with the CC office or on instruction of the studbook keeper.



Water snails live together with newt larvae in the rearing tanks of the Cologne Zoo | Foto: Thomas Ziegler



#### 4.4 The Terrarium

Vietnamese crocodile newts are maintained in terrariums with a water section. The terrarium can be made of glass or plastic and must have adequate ventilation in the lid and/or sides.

As a rule of thumb for determining the terrarium size, the AG Urodela of the DGHT recommends the following for two salamanders: To determine the area in square meters, take the total length of the newts in centimeters and multiply by 0,01. For each additional animal, this area should be multiplied by 1.25. For two adult *Tylototriton vietnamensis*, about 15 cm long, this would result in an area of 15 x 0.01 = 0.15 m2 = 1500 cm2.

This would correspond to terrarium dimensions of 50 x 30 cm (length x width). For three animals 1500 cm2 x 1,25 = 1875 cm2 would have to be estimated, which would correspond to 50 x 40 cm and so on.



View into an amphibian breeding room of the Cologne Zoo | Foto: Christian Niggemann



In the Cologne Zoo, the animals are kept in pairs or in groups of up to five in terrariums measuring 55 x 50 x 35 cm and 120 x 60 x 60 cm (length x depth x height). Lisa Breitenbach maintains a group of three adult animals in a terrarium measuring 70 x 30 x 26 cm.

All terrariums have a water part. It should be about 1/5 to 1/4 the size of the of the total area. This can be created by gluing a glass strip of max. 10 cm height on the bottom, which separates the water part from the land part. Alternatively, the entire terrarium can have a water level, and the substrate then rises significantly toward one side of the terrarium so that an land area is available. It is important to have a largely "barrier-free" access and especially exit for the crocodile newts. This is made possible by stones or a shallow shore zone of clay or gravel.

The water should have a low degree of hardness and a slightly acidic pH (6.4-6.8) - reflecting natural conditions. The maximum water depth is about 5 cm. The water part is usually visited by the newts only for mating; the males sometimes spend several weeks in the water.

Moss cushions or other hiding places near the shore are suitable for egg laying.

The substrate in the terrarium should be filled 8-15 cm high. For this purpose gravel or e.g. a clay mixture over a gravel-earth layer can be used. It is important to have a mixture of drier and wetter areas. Lisa Breitenbach recommends a layered construction of the substrate: first a 2 cm high foamed clay ball layer as drainage, over which a garden fleece is placed so that the actual substrate does not mix with the foamed clay. On top of this fleece, deciduous forest soil is then spread, which has previously been disinfected in an oven at 70 °C for 20 minutes. A hilly structure provides different moisture levels.



View into a terrarium for Tylototriton vietnamensis | Foto: René Zelger / Citizen Conservation



Hiding places must be available in both the wetter and drier parts of the terrarium. Moss helps to retain moisture and is therefore well suited for Crocodile newt terrariums. Suitable hiding places and structural elements include pieces of cork bark, clay bowls, caves in a designed back or side wall, bamboo cut in half, etc. A layer of leaf litter (oak, beech or bamboo leaves) not only looks attractive, but provides further hiding places and a natural structure.

The terrarium can be planted with live plants. Not only visually, but also geographically suitable are, for example, Vietnamese cannon flower (*Pilea cadierei*), emerald tree (*Radermachera sinica*), bonsai bamboo (*Pogonatherum paniceum*) and various ferns (*Asplenium*).

Additionally, small invertebrates can be introduced into the terrarium, such as springtails, earthworms, and the isopod *Porcellio laevis*. These feed on organic waste and thus ensure a cleaner terrarium, at the same time serving as a permanent staple food supply for the crocodile newts. However, as the isopods can also fall into the water part and drown, regular cleaning must be ensured.



Pair in terrarium; moss, foliage and stones are suitable materials for decorating the terrarium | Foto: Anna Rauhaus



## 4.5 Lighting, Temperatures, Humidity

The lighting of the terrarium does not play a special role for the newts, they do not like it particularly bright. The lighting of the terrarium can therefore be based on the requirements of the plants. Common systems such as LED light bars or fluorescent lamps are possible. Indirect lighting via the light in the room is also sufficient for the newts. Artificial lighting can be turned on for 12 hours a day in summer, and somewhat shorter in winter (approx. (approx. 10-11 hours).

Between March and October the terrarium temperature is 23-25 °C. In summer, peaks above 30 °C are tolerated for short periods. Cooler retreats in the terrarium are important. In winter, the values drop to 18-20 °C; short periods of lower temperatures also do not cause any problems for the newts.

## 4.6 Feeding and Care

Crocodile newts eat the usual range of "salamander foods". Well suited for adults are earthworms, crickets and waxworms. Adults are fed about twice a week. In winter, they eat only irregularly and much less. It is important that the feeders have a high quality diet. Crickets are kept in plastic boxes and fed a varied diet of oatmeal, fruit and vegetables (grated carrots, peppers, cucumber, etc.). The feeder animals are dusted with vitamin-mineral supplements.

Daily sprinkling by hand (flower sprayer) or via a sprinkler system provides the necessary moisture. Frequency and amount depend on terrarium size and ventilation area.

In winter the animals are kept drier. In the Cologne Zoo, the water levels in the terrariums are lowered from October onwards. The water section is gradually drained to a large extent except for small remnants for basic moisture supply, thus simulating the dry season. During this time the terrarium is sprayed very little and only in some places (moss cushions). From the beginning of March, sprinkling is heavily increased and the water levels should gradually rise.

If gravel is used as substrate, it should be flushed regularly. Soil or soil-loam mixtures rarely need to be replaced. Recognizable contaminants (feces, food remains) are regularly collected from the terrarium.



## 4.7 Breeding

Already towards the end of the summer half-year, the females clearly increase in girth. Crucial for triggering the mating season is the temperature drop in winter described above in conjunction with drier housing and fewer feedings, as well as the subsequnetly simulated rainy season. Crocodile newts are sensitive to fluctuations in atmospheric pressure. Therefore, it is recommended to start the rainy season in the terrarium when a low pressure area is coming up in spring from March on. For this purpose, the terrarium is intensively sprayed from now on (several times a day), and the water level in the water part of the terrarium is significantly increased. At the same time the temperatures should also rise. The males now move into the water within 1-2 days after the beginning of the "rainy season", where they wait for the females. During the weeks they spend in the water, the males show a lighter coloration and a clearly changed skin structure - a typical "aquatic appearance" for many newts.

Animals of both sexes develop much more appetite in the spring. Males also consume food in the water.

The females also come into the water for one or more days to mate with the males. Air pressure probably plays a role in this as well. At the Cologne Zoo, mating was observed when low pressure areas or thunderstorms were occurring outside. Water temperatures during observed matings were 18-19 °C in the morning and 21-22 °C during the day.



Most of the year the pairs of Tylototriton vietnamensis live on land | Foto: Anna Rauhaus



Typical for crocodile newts, the pairs perform a circle dance during mating. The male repeatedly bumps the female with his head and undulates his tail in the direction of the female. Finally, it deposits several spermatophores (sperm capsules) and directs the female over them by means of the gyratory movements with the cloaca.

The whole procedure can take several hours. Occasionally, a ventral amplexus could be observed at Cologne Zoo - the male hooks a foreleg under a foreleg of the female for a few seconds. Lisa Breitenbach observed a complete amplexus before mating.

An attempt at Cologne Zoo to artificially motivate a female to reproduce by placing her in water resulted in mating, but no egg laying. Clutches were only laid if the female had previously joined the male in the water of her own free will.



During the mating period the males go into the water part of the terrarium | Foto: Anna Rauhaus



When the female joins the male in the water, the "circle dance" begins | Foto: Anna Rauhaus



At Cologne Zoo, egg-laying occurred from late January to August, with the majority in March and April. Some females produce two clutches in one year. The first breeding pair at Cologne Zoo, which originated from a clutch imported from Vietnam, layed eggs exclusively in March or April.

The oviposition usually takes place within 24 hours, but also up to one week after the observed mating. For this purpose the female prefers a place covered with moss on the ground near the water. The clutch is often hidden under leaves or pieces of bark. Egg-laying is usually completed during the first night, but can sometimes extend through the next day. The female usually remains with the freshly laid clutch until nightfall the following day.

In the Cologne Zoo the clutches comprised between 95 and 178 eggs, with a diameter of 5.8-10.2 mm directly after laying. In the first hours they swell strongly, the diameter is then 7.9-12.5 mm.



The clutches are usually deposited on land close to the water's edge | Foto: Thomas Ziegler



Freshly laid eggs between moss | Foto: Thomas Ziegler



#### 4.8 Incubation

Eggs can also develop successfully in the terrarium, but we recommend separate incubation, to provide more controlled conditions and because of the danger of eggs and larvae being eaten by the parents. In the Cologne Zoo a male has already been observed eating eggs, and a larva hatched in the terrarium has disappeared without a trace from the water part there. Various methods have proven to be successful.

In the Cologne zoo the eggs are laid individually or in small clusters next to each other on slightly damp cellulose paper (paper towel), which is then placed on a grid plate as often used in aquariums. This grid plate is then placed in a closed plastic box filled with some water, so that the eggs are well above the water level, but exposed to very high humidity. Alternatively, a plastic grid floating on the water can be used, with cellulose paper placed on top. Once or twice a day, the container is opened for inspection and ventilation. Dead eggs are removed.

The cellulose paper must be replaced regularily; this is necessary once or twice during the incubation period.

If egg cases of still living larvae are damaged, they are placed in water and inside an incubator at the Cologne Zoo, where they usually hatch (see item 4.12, "Husbandry problems"). Of course, other incubation methods are also possible, but this procedure has proven itself well at the Cologne Zoo with a very high hatching rate:

thus, 136 larvae hatched there from the 140 eggs of the first F2 clutch.

Alternatively, other incubation methods were tested (for details see ZIEGLER & RAUHAUS 2019a, RAUHAUS & ZIEGLER in press): in a Petri dish with a small amount of water, on sea almond leaves, bamboo leaves or a moist clay dish. However, the results at the Cologne Zoo were significantly worse with these methods, and the workload due to mold growth on the substrate was higher in some cases.



The eggs should be incubated outside of the terrarium I Foto: Lisa Breitenbach / Citizen Conservation



## 4.9 Larval Development

The duration of larval development in the egg is highly dependent on temperature, ranging from as little as nine days to nearly four months from egg deposition to hatching. The higher the incubation temperature, the longer the development seems to take until hatching. Already at values above 26 °C a significant delay occurs. Remarkable, however, is a pronounced heat tolerance for crocodile newts: even at values of up to 38 °C larvae still developed. However, the fitness of larvae remaining in the egg for longer periods decreases, they are thinner and more often show problems with feeding.

At temperatures of 22-24 °C during the day and 20-22 °C at night, development proceeds as follows according to experience at Cologne Zoo:

After one day: formation of the neural crests. This can be seen with the naked eye in fertilized eggs.

After 5-6 days: first movements of the larvae in response to shocks, head,

tail and gill rudiments are visible.

After approx. 12 days: eyes dark, pigmentation well advanced, gills pronounced, larvae wriggling in the egg.

After approx. 28 days: hatching

Superficial fungal growth during incubation does not seem to affect larval development. Right after hatching, larvae are up to 22 mm long. They can jump into the water on their

own - jumps of up to 12 cm have been documented at Cologne Zoo!

With the hatching of the first larvae the water level in the incubation container should be increased, which also induces the other larvae to hatch. Presumably, this behavior corresponds to the reaction to precipitation in the natural habitat and ensures that the temporary water bodies actually carry water.



Larvae at an early stage of development | Foto: Anna Rauhaus



Older larva | Foto: Thomas Ziegler



Large larva of *Tylototriton vietnamensis* before metamorphosis | Foto: Anna Rauhaus



#### 4.10 Rearing of the larvae

In practice, different rearing methods for the larvae of the Vietnamese crocodile newt have now been tested, mainly through the work of the Cologne Zoo (RAUHAUS & ZIEGLER in press).

Rearing is possible in small groups and rearing boxes with a high maintenance effort. Best results are obtained when group sizes are kept small and larvae are sorted by size during development. Then the survival rate is greatest, and the young are larger and stronger when they come ashore compared to larvae of different growth rates reared together.

Alternatively, much more time-efficient rearing in large groups in spacious aquaria is possible. The survival rate of the larvae is lower, but one get strong, large young animals. At the Cologne Zoo, larvae were initially reared in groups of no more than five animals, later in groups of 5-10 animals. Plastic boxes with 10 liters of water each were used. The smaller the group size, the easier it is to control the rearing.

Later, rearing in large common groups of up to 150 larvae in a 75 liter aquarium was also tested at the Cologne Zoo. In contrast to the rearing conditions described in more detail below, water changes were omitted in this husbandry and feeding was sporadic. The survival rate of the larvae was significantly lower in this aquarium rearing, but the successful juveniles were significantly larger when they came ashore, in some cases exceeding 7 cm. Cannibalism among the larvae may also have occurred with this rearing method, which was never observed with small group and constant feeding, even with strong differences in size between the larvae kept together.



Larvae rearing facility at Cologne Zoo | Foto: Anna Rauhaus



Among larvae kept together the faster-growing individuals soon become dominant. Smaller, suppressed larvae are bitten, and there is occasional loss of tail tips, caudal fin hems, or even limbs (which can be regenerated; in the case of toes and fingers, this is possible even after larvae have left the water). If the rearing boxes provide enough hiding places, the smaller larvae will retreat. When rearing in small groups, it is advisable to sort the larvae by size, because the smaller larvae grow better among conspecifics their own size. In addition the suppressed animals tend to be more susceptible to diseases, which may also affect the rest of the larvae.

Group size and feeding of the larvae has a great influence on the speed of larval development and on the size of the young land-living juvenile newts. According to experience at the Cologne Zoo, the duration from hatching to metamorphosis varies between two and six months, depending on husbandry conditions. A rule of thumb is: The smaller the group size during rearing, the larger the larvae will grow, the longer they will stay in the water and the larger the juvenile newts will eventually be. Therefore, especially when gaining first experiences with this species, we recommend rearing in small group sizes of maximum 5 larvae. The water used should be well stagnant. Lisa Breitenbach successfully used stagnant rainwater with leaf brew.

The substrate is sand from an aquarium that has already been running and developed bacterial communities, boiled oak leaves and hiding places in the form of PVC tubes are used as furnishings. The tank is planted with aquatic plants such as Java fern (*Microsorum pteropus*), waterweed species (*Hygrophila difformis, H. siamensis, H. polysperma*) and Java moss (*Taxiphyllum barbieri*). Some post horn snails help to remove food remains. It is best to let the rearing boxes "run in" a few days before the larvae move in.

The following information on growth and development time is based on the experience of the Cologne Zoo with the husbandry system "5-10 larvae in 10 liter containers".



View into a rearing tank for larvae | Foto: Lisa Breitenbach / Citizen Conservation



Like a creature from another world: portrait of a larva | Foto: Anna Rauhaus



The larvae hatch with a length of 18-22 mm. The front and hind legs are already developed, but the fingers and toes are only partially formed.

The body coloration of the larvae is yellowish, the legs are whitish, the gills are slightly orange. The body is black spotted.

The larvae might hatch with a small yolk sac remnant and begin feeding immediately after hatching. They practically have to swim in the food - even one day without food can negatively affect development.

Artemia, very small water fleas and copepods serve as starter food. Initially they are fed two to three times a day, when the larvae grow up to six to eight times, depending how much the larvae eat. All feeders are well rinsed and watered before feeding.

When the larvae have reached a length of 25 mm, *Tubifex* pieces are added to the diet, and from approx. 35 mm onwards, earth worm pieces, whole Tubifex and Enchytraeidae are added. Even larger larvae also eat red mosquito larvae and sometimes young shrimps.



Tank for large larvae just before going on land | Foto: Thomas Ziegler



Larvae become quite trusting. Larger, more dominant animals quickly learn that the keeper's appearance is related to food. They then swim up and look up expectantly. This gives them an advantage over their smaller, shyer and hidden siblings in the same rearing tank. Basically, such dominant larvae grow larger, but also stay longer in the water, while smaller, suppressed animals metamorphose earlier and with smaller body size and go ashore. Partial water changes are performed two to three times daily, depending on feeding. At the Cologne Zoo, feces and food remains as well as a maximum of 1.5 liters of water are siphoned off during this process. Stale water is used for refilling. This should be done very carefully, as the larvae have little resistance to the water flow and are washed away.

After one month the larvae measure 27-40 mm, their stature becomes visibly stronger, and they increasingly show a yellowish brown ground coloration with a grayish marbling or dot pattern. Now the limbs are also pigmented. The ventral side is transparent, and the gills are orange. The tail fringes are partly white spotted, the orange tail stripe is sometimes already visible. In some animals the later tubercles on the sides are already visible as lighter spots. The animals now also eat the snails that may live with them in the rearing containers.

After about 6-7 weeks the larvae are about 50 mm long, they become darker and the tubercle rows become more visible. Now also the orange coloration of fingers and toes as well as the tail stripe gradually emerges. However, some of the larvae do not darken until shortly before meta-morphosis and retain the lighter "juvenile" coloration until then.

At about eight weeks of age, or about three months after egg laying, metamorphosis begins. The gill branches and caudal fin seams regress, and the skin becomes increasingly rough. It is now of

great importance that the animals can leave the water at any time without problems. For this purpose, the rearing boxes are redesigned, or the animals are placed in a "terrestrial tank". Easily accessible shoreline transition zones are crucial, which can be created by a rising gravel layer, but also by cork pieces, roots, rocks, etc.



On land! | Foto: Thomas Ziegler



#### 4.11 Rearing of the young Newts

After leaving the water on going on land, juveniles measure approximately 53-75 mm and weigh 1.1-2.8 g according to data from the Cologne Zoo (RAUHAUS & ZIEGLER in press). Freshly metamorphosed animals on land still show remnants of the gill branches, which disappear completely after a few days.

Around the time of going ashore, the animals molt; if they have already gone ashore before, they return to the water once more for this purpose. Only after this first molt - if the skin is still very smooth, it has not yet occurred - the young animals should leave the transition tank and be placed in the rearing terrarium.

After metamorphosis, the small crocodile newts are uniformly black or dark brown in color, except for the now bright orange finger and toe tips and the equally bright tail stripe.



Rearing box for young Vietnamese crocodile newts | Foto: René Zelger / Citizen Conservation



After the first molting after metamorphosis, the terrestrial animals at the Cologne Zoo are kept in boxes with 40 x 30 cm floor space. The rearing takes place in groups of 5-12 animals. As with the larvae, the smaller the group size, the better the control possibilities. Temperatures and lighting correspond to the conditions of the adult animals.

Gravel placed at an angle is used as substrate. Water is poured in high enough to create a small "pond" in the lowest gravel area, while the rest of the area serves as a land area. Mosses, stones, oak leaves and pieces of cork bark provide structure, hiding places and areas of varying moisture. The juveniles have a large appetite after the transformation. They will eat micro crustaceans, small earthworms, firebrat, *Drosophila* and *Tubifex* offered in a dish or on a small plate. It is important that the feeders are well powdered with vitamin-mineral supplements. Feeding of the young land-living newts is done daily and then gradually reduced as they grow.

Boxes are inspected daily and droppings, moldy leaves, etc. are removed. The complete cleaning is done weekly at the beginning, later every two weeks. The gravel is rinsed and the leaves are replaced. After about a quarter of a year the juveniles measure 70-95 mm, after about half a year about 80-110 mm. At the age of one year the sexes can already be distinguished reasonably well, some males then already go into the water and show the typical water coloration. At the age of 1.5 years, after the second winter, the young animals in the Cologne Zoo were about 10 cm long.

The males weighed between 6,5 and 8,8 g, the females 13,5-18 g. Now they have reached sexual maturity. First offspring are now already possible.

While the rearing of juveniles taken from the wild has proven to be problematic (RAUHAUS et al. 2018, SPARREBOOM 2014), it is quite unproblematic for terrarium offspring.



Offspring in terrarium | Foto: Anna Rauhaus



Cologne Zoo offspring from 2018 and 2019, photographed in July 2019 | Foto: Thomas Ziegler



#### 4.12 Husbandry Problems

Occasionally, egg are damaged before the larvae are ready to hatch. In the Cologne Zoo, such eggs were then placed into water and inside the incubator, where the eggs often developed well. However, they then usually hatch at an earlier stage of development and cannot yet move independently. Such "preemies" can be transferred to a Petri dish filled with water, where they grow for a few days, even without feeding, by resorbing their yolk sac. They remain in the Petri dish until they can no longer lie on their sides but can "stand" upright and swim.

During larval rearing, some attention must be paid to proper water conditions. If too much fresh water is used, the larvae will quickly develop gas accumulation in their abdomen, so only a partial water change of no more than about 15% should ever be performed. If the water has too high of a degree of hardness, degeneration of the gill branches may occur. This can be remedied by replacing the water with water of lower hardness as soon as possible. In Cologne Zoo, problems have frequently occurred because introduced freshwater polyps of the genus *Hydra* have injured the small crocodile newt larvae with their stinging cells, which then no longer liked to stay on the ground. To solve this problem, great pond snails (*Lymnaea stagnalis*) were introduced into the rearing tanks.

When the larvae start to eat snails at the age of more than one month, snail remains in the shells can lead to a problematic deterioration of the water quality. This must therefore be kept an eye on, especially if there is an accumulation of empty snail shells in the rearing tanks.



Fungal infection in a larva of *Tylototriton vietnamensis* | Foto: Anna Rauhaus



View into the amphibian quarantine behind the scenes of the Cologne Zoo | Foto: Thomas Ziegler



# 5. Further Literature

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