

# EAZA Amphibian Taxon Advisory Group Best Practice Guidelines for the Sardinian brook salamander *Euproctus platycephalus*

Version 1



FONDAZIONE  
BIOPARCO  
di ROMA



FRENCH  
URODELA  
GROUP

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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 “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country.

Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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## **Introduction**

The information in this Best Practice Guideline has come from a variety of sources including an extensive literature review, the experience of the authors and others in the captive husbandry of *Euproctus platycephalus* as well as direct observations of the species in the field.

Amphibian husbandry is a rapidly evolving field and there are many aspects that require further research. UV-B provision for captive newts and salamanders is an area which requires further research. A full suite of water parameters is not currently available from the field. It has only been possible to recommend water parameters based on captive husbandry experience and a more evidence based approach utilising parameters from the field should be developed in order to make evidence based husbandry recommendations.

Captive diets for both larval and post metamorphic amphibians are likely to differ from diets consumed by larval and post metamorphic amphibians in the field. Replicating the wild diet in captivity will likely be precluded by the limited number of invertebrate species that can be reared on scale required for them to form viable live food colonies.

## **Key husbandry points**

1. This species is sensitive to mildew and pathogenic fungus that attack the eggs and larva. Good water quality and well oxygenated aquariums are essential to help prevent this.
2. The provision of appropriate seasonal temperature regimes.
3. Monitoring and management of water quality.

These guidelines have been reviewed and approved by the Amphibian TAG members.

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## Section 1. Biology and field data

### 1.1 Taxonomy

ORDER: Caudata (Fischer von Waldheim, 1813)

FAMILY: Salamandridae (Goldfuss, 1820)

GENUS: *Euproctus* (Gené, 1838)

SPECIES: *Euproctus platycephalus* (Gravenhorst, 1829)

COMMON NAMES: Sardinian brook salamander, Sardinian mountain newt / salamander

### 1.2 Morphology

**1.2.1 Weight:** Adults weigh between 3.0 – 8.5 g (Zirichiltaggi pers. comm.).

**1.2.2 Length:** Adults reach a maximum total length of 140 mm but are usually smaller at 100 - 120mm. Length of adults from snout to vent is approximately 75 mm (Bovero *et al*, 2003). Northern populations of the species tend to be larger than specimens found in the south of their range (Angelini *et al*, 2014).

**1.2.3 Colouration:** Varies between individuals and at different ages. The dorsal colouring in adults and juveniles is a dark olive or brown with yellow, black or green spots. Colouration tends to darken with age, especially in males. Some juveniles and young adults have an orange/yellow vertebral stripe that fades with age and eventually disappears. The ventral surface is a cream colour on the sides becoming yellow in the middle. Numerous black spots are present on the ventral parts; distribution of spots is greatly reduced in females. With age, the black spots tend to enlarge with some of the oldest individuals having completely dark ventral areas. At 2 weeks old, larvae are greyish in colour with black eyes and distinguishable pink gills (French Urodela Group, 2005).

**1.2.4 Description:** *E. platycephalus* is sexually dimorphic (Fig. 1 and 2). The head of this species is very depressed with a wide snout. Males have proportionally larger and wider heads when compared to females (Bovero *et al*, 2003). The upper jaw is larger than the lower jaw. Ventral and dorsal skin is relatively smooth, fine tubercles are present on the dorsal surface. The tail is oval in cross- section and is broad at the base and compressed laterally with small upper and lower crests. The males are longer and heavier than the females (Bovero *et al*, 2003). The females are more stream-lined with a finer and longer tail than the males. Males have longer and wider hind legs than females and especially visible protruding spurs present on the rear of the hind leg just above the foot (French Urodela Group, 2005). These spurs appear at 12 to 14 months of age and are present throughout life.

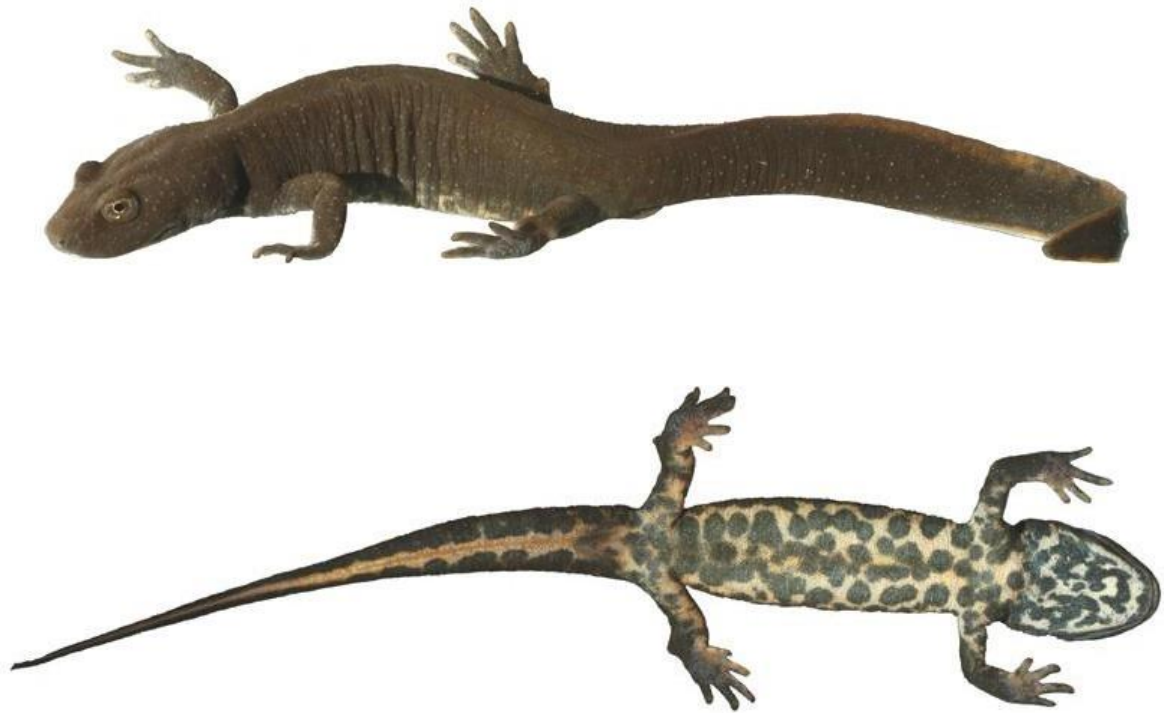


Figure 1: Male *E. platycephalus* (French Urodela Group, 2005)

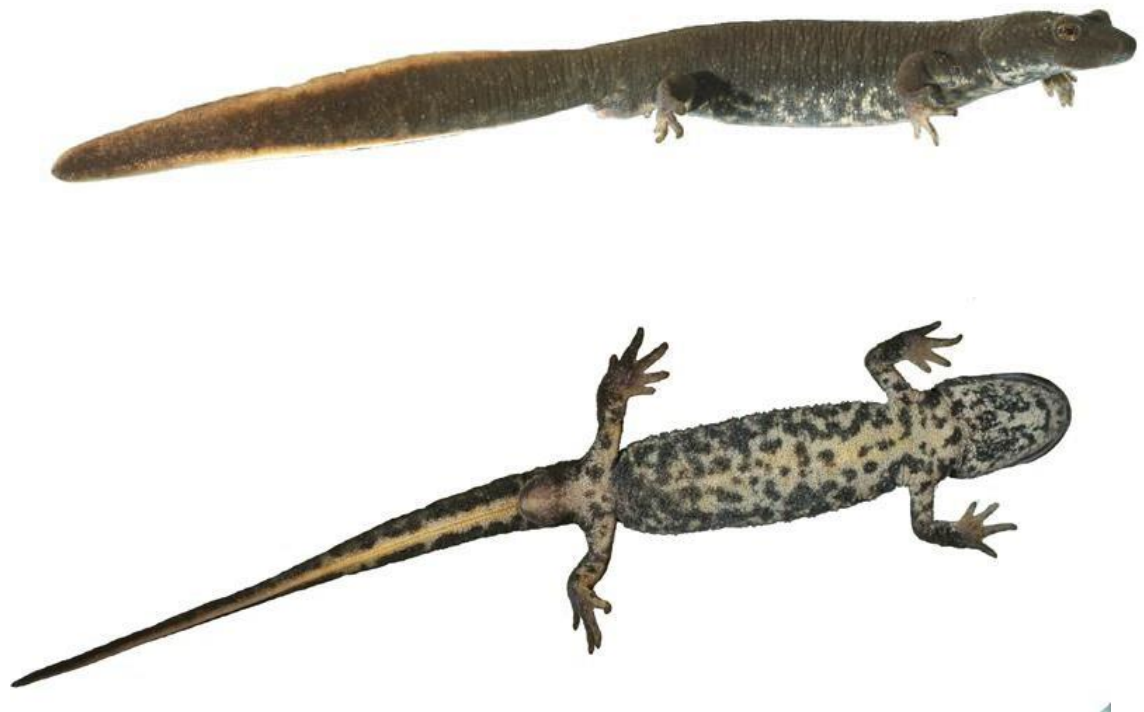


Figure 2: female *E. platycephalus* (French Urodela Group, 2005)

The cloaca of the female opens distally and is cone shaped (Fig. 3). The hook shaped cloaca of the male (Fig. 4) is directed posteriorly and opens dorsally. A large pseudopenis is evaginated during mating (Bovero *et al*, 2003).



Figure 3: Male cloaca in form of a hook (Left). Figure 4: Conical cloaca of the female (Right) (French Urodela Group, 2005)

At 3 to 4 months of age the head is visibly flattened and the top jaw is more prominent. In males, the spurs become evident and the tail thickens at the base. Gills are present throughout the larval stage. The gills regress just before metamorphosis and eventually disappear; in very few wild localities, neoteny has been observed the retention of larval characteristics at the adult stage (Voesenek *et al*, 1987; Macale & Vignoli pers. obs.). In captivity, many adult animals retain very small gill remnants behind the paratoid gland, which due to their vestigial size are probably not physiologically important (pers. Obs. C Michaels).

**1.3 Physiology:** *E. platycephalus* absorbs dissolved oxygen in the water through its gular sac as well as cutaneously. Oxygen content of the water is a determining factor in the distribution of the species (Maillet & Schultschick, 2013).

**1.4 Longevity:** The average age in the wild ranges between 6.4 -8.5 years (Bovero *et al*, 2003; Angelini *et al*, 2008) however a life span of 17 years has been observed in the wild for males and 9 years for females (Bovero *et al*, 2003). The maximum recorded life span for this species in captivity is 9 years (French Urodela Group, 2005), however they usually live for about 5 - 7 years in captivity.

## Field data

### 1.5 Zoogeography, ecology and conservation

**1.5.1 Distribution:** *E. platycephalus* is endemic to the island of Sardinia, Italy (Fig. 5), and survives in three isolated regions of the island; the Limbara and Albo Mountains in the north east, the mountains of Gerrei and Sarrabus in the region of Sette Fratelli to the south east and the Gennargentu Mountains in the centre of Sardinia. All three regions range in altitude from 50 m to 1,800 m a.s.l. however the species is most abundant



between 500 to 600 m a.s.l. (French Urodela Group, 2005). There are no reliable records of the species on the west side of the island (IUCN *et al*, 2008).

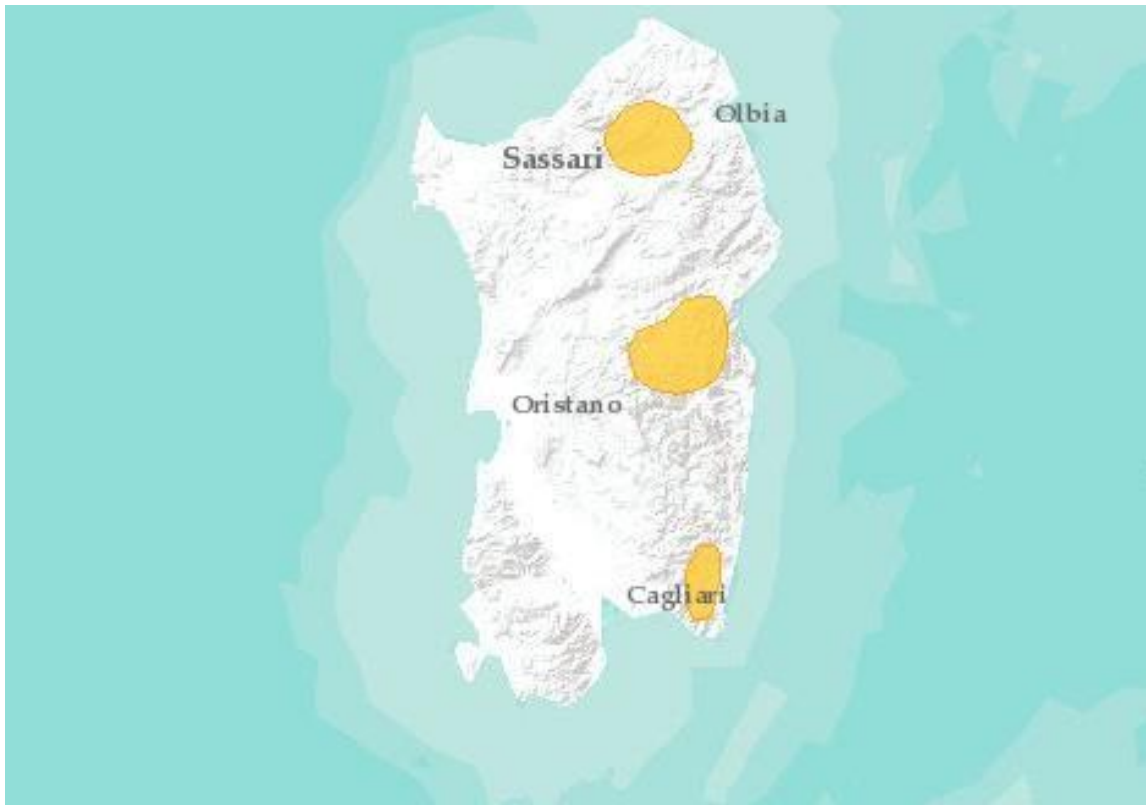


Figure 5: The distribution of *E. platycephalus*. Orange polygons represent where *E. platycephalus* are present (IUCN *et al*, 2008).



Figure 6 (Left) Deep pool habitat of *E. platycephalus* (Trenton Garner ©). Figure 7 (Right) Stream habitat of *E. platycephalus* (Trenton Garner ©).

**1.5.2 Habitat:** *E. platycephalus* is the most strictly aquatic species of the genus (Maillet & Schultschik, 2013). Whilst aquatic, *E. platycephalus* inhabits streams, pools (Fig. 6) and small lakes on the main mountain systems of Sardinia (Arnold & Ovenden, 2002). The species tends to occur in streams with little water (Fig. 7), that lack dense aquatic vegetation and where the substrate is interspersed with rubble and boulders (Maillet &



Schultschick, 2013). The species has also been found in muddy bottomed lakes without any rocky refugia (Voesebeck et al., 1987).

Site	Habitat type	Altitude (m a.s.l.)	Mean annual temperature (°C)
Rio Pisciaroni (Limbara mountains)	Stream	960–1,000	11.0
Riu Fundu di Monti tributary (Limbara mountains)	Stream	970–990	11.0
Gorroppu (Supramonte mountains)	Residual pond	625	13,7
Perdasdefogu Gennargentu mountains	Cave Brook	486	13.8
Rio Picocca tributary (Sette fratelli mountains)	Stream	150–200	15.9
Rio Picocca tributary (Sette fratelli mountains)	Stream	200–300	14.8
Rio Picocca tributary (Sette fratelli mountains)	Stream	300–400	15.5
Rio Maidopis (Sette fratelli mountains)	Stream	450–550	13.9
Rio Suergiu Mannu tributary (Sette fratelli mountains)	Stream	550–600	14.2

Table 1. Habitat type, altitude and temperature of *E. platycephalus* study sites after Angelini et al, (2014).

Specimens have been encountered during day time surveys by lifting rocks in streams, snorkeling and sifting through leaf litter (Bielby *et al*, 2013). During hot, dry years larvae are forced to endure warm stagnant waters (Maillet & Schultschick, 2013). During hot, dry periods adults may leave the water where they then aestivate (Maillet & Schultschick, 2013). On land, they are found among roots and under stones and logs (Arnold & Ovenden, 2002) that are sometimes close to its aquatic habitats (Arnold & Ovenden, 2002; Zirichiltaggi pers. comm.). Studies have shown that *E. platycephalus* are more likely to be found in relatively calm sections of rivers and streams with cool water temperatures (Lecis & Norris, 2004) ranging from 12°C – 16°C (Zirichiltaggi pers. comm.),

a marginal absence of fish, less riparian vegetation and in well oxygenated water (Arnold & Ovenden, 2002; Lecis & Norris, 2004; Urodela Group, 2005). Mean annual water temperatures for *E. platycephalus* habitats are presented in table 1.

**1.5.3 Population:** This generally rare species can be locally common in suitable habitat. Historically, one of the largest populations of the species occurred in the Gola di Gorroppu in the Gennargentu region. The Gola di Gorroppu population has recently become threatened through damage to its habitat caused by tourists and illegal fishing activities. During surveys in 2012 and 2015 *E. platycephalus* was not encountered at all along the river. However, the species is still present in the small tributaries of the main river Rio Flumineddu (Macale & Vignoli unpublished data). In one well-studied population, the sex ratio is male dominated. Between 1999 and 2001 it was known from 14 sites, whereas in 1991 it was present in 30 sites (and even in 1991 it was absent from nine other sites where it had been previously observed) (IUCN *et al*, 2008). However, recent ongoing studies on species distribution report the species is actually present in more than 50 sites across its range, and may be present in more (Vignoli *et al*, unpublished data).

**1.5.4 Conservation status:** The species is listed as Endangered by the IUCN because its area of occupancy is less than 500 km<sup>2</sup>. Its distribution is severely fragmented, and there is a continuing decline in the extent and quality of its habitat, (IUCN *et al*, 2008). The numbers of *E. platycephalus* have reduced drastically in recent years due to a number of reasons. In the past, Sardinia was covered in forests providing a good hydraulic system to the area which resulted in frequent precipitation. Recently, anthropogenic influences such as forest fires, removal of trees to support development of railways and tourist sites and increasing agricultural development has resulted in desertification. Gradually, as the ground has become less able to support vegetation, temperatures have increased, precipitation has reduced and streams and lakes have dried up resulting in localised disappearances of *E. platycephalus*. The three main evolutionary units are most likely isolated with no gene flow between the populations.

In the region of Gennargentu, tourism and farming have played an important role in the drying up of mountain streams. Domestic livestock such as goats are now found in the area resulting in the pollution of the remaining streams from faeces and erosion. In low elevation areas pollution from agricultural processes such as fertilizers and pesticides are an ever increasing problem (French Urodela Group, 2005). The treatment of water bodies with DichloroDiphenylTrichloroethane (DDT) in the 1950's in the battle against malaria may also have contributed to the decline of the species (Böhme *et al*, 1999).

The three areas now inhabited by the species are considered as biogenetic reserves (IUCN *et al*, 2008) as they are especially valuable for nature conservation in Europe. This species is listed on Appendix II of the Berne Convention and on Annex IV of the EU Natural Habitats Directive; it is also protected by regional legislation (Regional Law n. 23/1998 (art. 5, c. 3). The Gola di Gorroppu has been designated as a Site of Community Importance under the Habitats Directive (IUCN *et al*, 2008). Monte Limbara and Sette Fratelli are now regional parks with Gennargentu under the process of being declared a national park. Programmes to remove trout from the species' habitat would assist in the

recovery of populations (IUCN *et al*, 2008), however it is unknown whether there are any plans to do so.

Disease is also a threat to *E. platycephalus*. Specimens in the Sette Fratelli mountains have been found to be infected with *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of chytridiomycosis (Bovero *et al*, 2008). Specimens in nature exhibited clinical symptoms of chytridiomycosis including digit loss and patchy skin discoloration (Bovero *et al*, 2008). More recently, clusters of *Bd* infection were recorded in the North of Sardinia and *E. platycephalus* within this area had a relatively high prevalence of infection (Bielby *et al*, 2013). The optimal temperature range for *Bd* is 17-25°C. *E. platycephalus* inhabits areas which overlap with the optimal environmental suitability for the chytrid fungus for much of the year (Bovero *et al*, 2008).

Recently, the emergence of the chytrid fungus *Batrachochytrium salamandrivorans* (*B. sal*) in Europe resulted in rapid population declines in *Salamandra salamandra*. It is likely that *B. sal* originated in Asia and it is highly pathogenic for urodeles (Martel *et al*, 2014). In the laboratory, *E. platycephalus* exposed to *B. sal* developed lethal chytridiomycosis (Martel *et al*, 2014). *B. sal* would likely pose a significant threat to Sardinia's diverse salamander assemblage, should it reach the island.

There is a captive breeding program to ensure this species persistence in captivity. Returning the species back into the wild at present may not be a viable option in the short term as the threats to the habitat and the species are still present. Since 2010, an ex situ conservation program has been undertaken at the Fondazione Bioparco of Rome in collaboration with the Department of Science, University Roma Tre. *E. platycephalus* founder individuals (n=23) from the three main evolutionary units have been successfully maintained and bred. The F1 and F2 amount to nearly 200 individuals. In accordance with Sardinian authorities (Assessorato della Difesa dell'Ambiente and Ente Foreste), a restocking/reintroduction plan involving individuals born at the Bioparco is under consideration. The conservation program consists also of an in situ activity funded by EAZA aimed at monitoring natural *E. platycephalus* populations and discovering new sites where the species is present. The species is currently under the Regional Collection Plans (RCP) for speciose *taxa* category 2: research (b) (Furrer & Gibson, 2008) under which the species is undergoing general research; the species has been recommended for a clearly defined pure or applied research that includes knowledge of natural history, population biology, taxonomy, husbandry or disease and health management (Koldwey *et al*, 2008).

## **1.6 Diet and feeding behaviour**

**1.6.1 Food preference:** The only study on the diet of *E. platycephalus* performed in the wild (Sotgiu *et al*, 2008) reports that it is a carnivorous species that feeds on small invertebrates. Males prey on more varied taxa than females (Zirichiltaggi pers. comm.) due to the sexual dimorphic morphology of the species; there is a tendency for the males to take larger prey in addition to the smaller prey that females may be limited to (Wells, 2007). The diet of *E. platycephalus* varies seasonally according to activity levels and nutritional needs. *E. platycephalus* larvae feed on mosquito larvae, blood worms

(Chironomids and *Glycera sp.*), sludge worms (*Tubifex tubifex*), very small aquatic crustaceans and amphibian larvae (*Hyla sarda* and *Discoglossus sardus*). Oophagy can also occur in some cases (Sotgiu *et al*, 2008) as well as cannibalism on small larvae (Macale & Vignoli unpublished data).

**1.6.2 Feeding:** Sight is the dominant sense in prey detection. Movement of the prey is the stimulus that triggers the feeding response. Olfaction is also used to find prey but is secondary to sight and is used in dim or dark conditions (Stebbins *et al*, 1995). Water vibration sensed by the lateral line is also used to detect prey. Once prey is located the mouth is opened very rapidly and the prey is sucked into the mouth.

## 1.7 Reproduction

**1.7.1 Developmental stages to sexual maturity:** Once hatched, the young are between approximately 5 mm in length, at 6 months they are 20 - 30 mm, 60 - 70 mm at 1 year and 80 - 90 mm at 2 years. Time to metamorphosis is temperature dependent and in captivity can occur at 7 months (French Urodela Group, 2005). However, in some cases, this can occur between 14 - 15 months of age (Alcher, 1980; Zirichiltaggi pers. comm.) and some paedomorphic individuals may retain gills into reproductive maturity.

**1.7.2 Age of sexual maturity:** *E. platycephalus* reaches sub-adulthood at about 17 to 18 months. At 18 months, both males and females are sexually mature. In captivity, first mating can occur from the age of 13 months onwards (Macale & Vignoli pers. obs.). Southern populations of *E. platycephalus* reach sexual maturity earlier than northern populations with sexual maturity being positively correlated with temperature (Angelini *et al.*, 2014).

**1.7.3 Seasonality of cycling:** Mating occurs all year round (Pasmans *et al*, 2014) and observations have shown that reproduction peaks in the summer months in the centre of Sardinia.

**1.7.4 Clutch size:** The number of eggs laid varies from between 50 - 230 (Böhme *et al*, 1999). The fecundity is positively correlated to female body size. Eggs are 3 mm in diameter. With the gelatinous envelope they are 4 to 5 mm in diameter (Böhme *et al*, 1999).

**1.7.5 Birth details and seasons:** Eggs are laid over a long period from 3 - 6 months. Oviposition sites are usually swept by light oxygenating currents that are thought to prevent fungal infection. Females usually select crevices and cracks or the underside of flat rocks to lay groups of eggs where they are relatively safe from predation by conspecifics and other animals; the cone-shaped cloaca allows them to access these sites.

## 1.8 Behaviour

**1.8.1 Activity:** *E. platycephalus* are diurnal (Andreone & Luiselli, 2000) and are possibly the most aquatic member of the genus. They can be found in water throughout the year in nature. There are, however, reports of *E. platycephalus* hibernating and aestivating on land in the vicinity of water during the autumn months of September through to November (Böhme *et al*, 1999). In captivity, young animals especially may try to leave the water when water temperatures rise above around 20.0 °C (pers. obs. C. Michaels).

**1.8.2 Locomotion:** In water, *E. platycephalus* either walks along the bottom or swims in the water. Being a riverine species, it is largely benthic and usually only leaves the substrate to breathe from the water's surface.

**1.8.3 Predation:** *Natrix maura* has been observed preying on *E. platycephalus* (Zirichiltaggi pers. comm.). Adults can prey on their own larvae. Cannibalism is also common in larvae and sub-adults and may occur even when individuals do not present marked size difference (Macale & Vignoli pers. obs.).

**1.8.4 Sexual behavior:** Mating takes place in the water and usually occurs in April and May once water temperatures begin to rise after winter, or after aestivation in the autumn (Fig. 8). Males actively search for females and upon approach the male will ripple the tail slightly. When a female is encountered the male grips her in the trunk with his jaws, this supports mating in running water. Females will struggle and bite until they are freed if they are not ready or willing to mate (Salthe, 1967). When a male encounters a receptive female, he will find a suitable place for mating, carrying the passive female in his jaws. This searching behaviour can last for several hours. The male then curves his body so that his tail lies over the tail base of the female, and his hook-like extended cloaca lies under her tail in the cloacal region. The male then strokes the female's cloaca with his hind limbs (Salthe, 1967) for a period of time before transferring his spermatophore. Spermatophore transfer can take place directly or with the aid of the spurs on the hind legs of the male (Böhme *et al*, 1999). The conical shape of the female cloaca supports fertilisation in flowing water during the rainy season as it assures penetration of the males' spermatophore. The female shows no observable responses during courtship, being held captive throughout (Salthe, 1967). There is a tendency for females to select larger males for mating. In a species with such a forced mating technique, selection for larger males is stronger (Bovero *et al*, 2002). Reproductively active males will often pursue females and attempt to mate even when the female is not receptive, although usually the female is eventually released (C. Michaels pers. obs). Females impeded to mate for one year (by keeping them separated from males) produced only unfertilised eggs. This would suggest that sperm storage in this species cannot allow females to reproduce over two seasons.



Fig. 8. Shows a breeding pair in amplexus. The pair often bites each other during mating. Bite marks can usually be seen on females due to the well developed jaws of the males (French Urodela Group).



## SECTION 2. Management in zoos and aquaria.

### 2.1 Enclosure



Figure 9. Enclosures for *E. platycephalus* at ZSL London Zoo.

*E. platycephalus* may be maintained in aquaria throughout their life (Fig. 9 and 10). The enclosure must have a tight fitting lid to prevent animals escaping.

**2.1.2 Substrate:** Sand of a fine grade is a suitable substrate, as it is fine enough to pass through the alimentary canal and out of the cloaca. However, sand should be thoroughly rinsed before use and the sand bed must not be so deep as to create anoxic zones that may produce toxic hydrogen sulphide gas. At ZSL London Zoo *E. platycephalus* a blockage of the cloaca occurred when a single specimen ingested a fine gravel substrate. A substrate free aquarium is also suitable as it facilitates the removal of waste solids and prevents prey items from escaping into the substrate where they eventually die and decompose. A terrestrial area is not essential as *E. platycephalus* can remain aquatic throughout the year in captivity; however see below (section 2.4.4) for a discussion of terrestrial enclosures, particularly for juveniles.



Figure 10. Left Amphibian quarantine facility at Fondazione Bioparco di Roma. Centre and Right, Amphibian Ark Laboratory at Fondazione Bioparco di Roma, the facility houses and breeds *E. platycephalus* destined for potential release.

**2.1.3 Furnishings and maintenance:** Plants such as java moss (*Taxiphyllum barbieri*), Brazilian water-oest (*Elodea densa*) and similar cold-tolerant plants can be placed in the tank to improve oxygenation of the water, aid in the removal of toxic nitrogenous waste (ammonia, nitrite and nitrate), provide shelter and can also be used as a support for eggs. Plant density should never be high (see descriptions of natural habitat) as this will reduce water flow and accumulate organic matter. Boulders can be stacked to form caves and refugia. Ideal refuges are relatively narrow horizontal cracks, which can be formed by stacking flat rocks or tiles. These should be slightly higher than the animals so that they can fit tightly inside. Ensure that any rocks are stable as newts may dislodge them and become trapped. If a substrate is employed, the rocks should be placed on the bottom of the aquarium before substrate is added to avoid instability caused by rocks eventually sinking into the substrate. These rocks can also act as oviposition sites. Spot cleaning should be carried out daily (especially the day after feeding) using a turkey baster, siphon or net to remove detritus. After egg laying the adults can be removed and the eggs can be reared in situ, or eggs can be transferred to tanks similar to those used for adults for rearing (see below for details).

**2.1.4 Environment:** Aquaria should not be exposed to direct sunlight as they may over heat; additionally, newts do not like very bright light and may become stressed if unable to access darker refugia. reproduction occurs normally with only artificial lighting. Natural photoperiods should be used to expose the species to its natural daylight hours; 9 - 10 hours of light mid winter and 14 - 15 hours of light mid summer.

Water temperature should be kept between 8.0 °C - 15.0 °C during the winter months of December through to February, 15.0 °C - 20.0 °C in the spring months of April through to June and peak at a maximum of 22.0 °C during the summer months of July through to September. Cooling during the winter months at low temperatures is the key to the successful breeding of this species. Avoid keeping individuals that originate from the region of Sette Frattelli, in temperatures below 10.0 °C.

The eggs of this species should be housed in a tank, in a shady area to prevent the formation of algae (French Urodela group, 2005).

**2.1.5 Water:** Regular 10-20% water changes should occur to ensure the maintenance of good water quality. These should remove accumulated debris and uneaten food and not just water; a siphon or aquatic vacuum cleaner is best used for water changes, rather than installing a tap in the side or base of tanks. Larger water changes may dramatically

alter water parameters and this may be highly stressful. 10-20% water changes should be carried out at regular intervals, larger water changes can stimulate mating and egg-laying which corresponds with the beginning of the spring rains in its natural habitat. At ZSL London Zoo the species breeds without such drastic water changes; low winter temperatures are the main stimulus.

Field data do not currently exist regarding the mineral composition of water in the field. However, this species does not appear to be overly sensitive to water hardness or pH provided that pH is maintained between 6.5 and 8 and some carbonate hardness is present to allow robust biological filtration.

*E. platycephalus* may be kept in aged tap water treated with water conditioners or passed through a carbon filter, if parameters are suitable. If tap water is to be used the water provider should be contacted to get detailed information on water parameters. Water parameters from tap water can vary greatly on a day to day basis and even more between geographic areas and so 'tap water' is not a standard composition. Alternatively rain water can be collected and used, but care must be taken to avoid airborne contaminants, particularly in cities and industrial areas. Alternatively, remineralised reverse osmosis (RO) water may be used. Pure RO should never be used with amphibians as its osmotic potential may interfere with osmoregulation in the salamanders. If tap water is used, water conditioners or carbon filters should be used

Parameter	Winter November - February	Spring March - May	Summer June - August	Autumn September - October
Temperature range (°C)	10-12	14-16	18-22	14-16
pH	6.9-7.1	6.9-7.1	6.9-7.1	6.9-7.1
Alkalinity ppm	30	30	30	30
Ammonia ppm	0	0	0	0
Nitrite ppm	0	0	0	0
Nitrate ppm	<25	<25	<25	<25

Table 2. Water parameters for *E. platycephalus* maintained at ZSL London Zoo.

following the manufacturers guidelines. An air diffuser can be used in the water storage container to keep the water well oxygenated. It is important to note that while allowing

tap water to stand for 24hrs removes chlorine by evaporation, it does not remove chloramine, heavy metals and a number of other contaminants (including very low levels of cyanide) from water. If tap water is used, a suitable chelator (available commercially as water conditioner for aquaria) or activated carbon should be used before adding to the aquarium.

It is not possible to manage what you do not measure. Water should be tested on a regular basis, this will allow the performance of filtration systems to be assessed, can identify one off problems or recurring issues and assess corrective measures.

Fluctuating water parameters can be very stressful for aquatic organisms. It is better to have slightly suboptimal parameters than wildly fluctuating parameters. At ZSL London Zoo RO water is mixed with tap water (in London, very hard and alkaline) at a 9:1 ratio. Therefore, replacement water should be as similar in composition, temperature, pH and other parameters as possible. Water should also be added to aquaria gently such as to disturb the animals as little as possible. See table 2 for any overview of water parameters at ZSL London Zoo.

At Fondazione Bioparco di Roma *E. platycephalus* are housed in 75 L tanks connected to a 150 L sump and water quality is maintained with frequent 25% water changes. The tanks were filled with reverse osmosis processed/purified water reconstituted by adding 50% tap water and a bio conditioner (Sera GmbH Aquatan). Water parameters as follows; general hardness (GH), 3 degrees; carbonate hardness (KH), 2 degrees; pH, 7.6; nitrates, nitrites, ammonium and phosphates, absent. Water temperature ranges from 8-10°C in January to 20-22°C in July. The water level and water temperature is adjusted according to seasons in order to simulate the natural oscillations. Water temperature is recorded with a data logger recording 24 measurements per day.

*E. platycephalus* is a stream-dwelling species and as such is more sensitive to accumulation of nitrates and organic matter than many pond-dwelling newts. Stocking density, feeding, water change regimens and filtration capacity (see below) should be designed to maintain negligible or low levels of nitrates as well as to handle ammonia and nitrites.

**2.1.6 Filtration:** For larger aquaria or aquaria with many animals, large external filters are vital. A sump may also be used to increase water volume and filtration capacity. For small aquaria with a pair or trio of animals internal box filters are sufficient. Filter media should be cleaned on a regular basis (depending on the size of the filter and the stocking density). Media should be cleaned in tank water rather than tap water as the sudden change in water parameters, as well as chlorine/chloramine in tap water, can be detrimental to the nitrifying bacteria which are vital for biological filtration. It is important that filters are cleaned before they become heavily clogged with detritus, rather than once they are very dirty, as otherwise water flow through the media is hampered and biological filtration capacity will be reduced. At ZSL, filters are cleaned every other week to maintain optimal water flow through media.

Juvenile and sub-adult animals can easily find their way into the internal mechanisms of filters. For this reason it is vital that any way into the filter is blocked up with plastic

mesh or sealed with silicone. For small juveniles, the use of internal sponge filters or external filters with finely meshed in- and out-flows may be preferable to internal power filters. The filter should not create anything more than a moderate current (Maillet & Schultschick, 2013) and areas protected from any current, which can be created using rocks, tiles etc., are important.

**2.1.7 Dimensions:** 30 x 60 x 30 cm (50 L ) aquariums can be used as a minimum to house three adult individuals. For larvae, tanks of 40 x 25 x 30 cm (20 L) can be used to house a maximum of 15 individuals of the same size.

**2.1.8 Lighting:** The UV-B requirements of caudate amphibians are not known and there is no published evidence, at present, to suggest that UV-B radiation is required. The UV-B requirements of *E. platycephalus* in the wild and in captivity are currently unknown. At Durrell Wildlife Conservation Trust sub adult *E. platycephalus*, reared without UV-B provision were radiographed and bone formation and mineralisation appeared good and there was no change in bone mineralisation when larvae were radiographed after 5 months of being kept beneath a UV-B emitting lamp array.

## 2.2 Feeding

**2.2.1 Basic diet:** Food variety is fundamental for successful keeping and breeding of amphibians. Diet differs between young larvae and adult individuals. Larvae can be fed *Daphnia* that are clean and free of *Cyclops* (some *Cyclops* species can consume salamander larva) or small worms (e.g. Micro, Walter and banana nematode worms (*Panagrellus spp.*), vinegar eels (*Turbatrix aceti*), *Tubifex tubifex*, white and grindal worms (*Enchytraeus albidus* and *E. bucholzi*), earthworms or bloodworms (Chironomidae) cut into pieces. It is important that *Tubifex* and bloodworms are well washed for several days before being added to the aquarium as potentially pathogenic organisms, as well as heavy metals and other pollutants which are often abundant in the environments where these worms are collected. The appropriate prey species can be chosen from these and other organisms based on the size of larvae. When maintained in mature tanks, larvae will often find enough microfood living in the aquarium to grow to the point of accepting white- and grindal worms and larger *Daphnia*. At ZSL London Zoo, for example, larvae longer than 15mm long are often removed from parental tanks where they have hatched and developed unnoticed in rock crevices or within internal filters. Adult specimens can be fed a variety of food such as bloodworms and small earth worms of up to 6 cm in length. *Trichopterid* and *Plecopterid* larvae along with *Gammarus* and other stream-dwelling aquatic invertebrates are favourably accepted by the adults (French Urodela Group, 2005). At Fondazione Bioparco di Roma *E. platycephalus* are fed ad libitum on frozen mixed invertebrate food (*Daphnia*, *Chironomus*, *Tubifex*, and other aquatic invertebrates) and reared *Artemia salina* naupli. Many aquatic invertebrates are not readily cultured in captivity and harvest from the wild may not be practical. It should be noted that the vast majority of aquatic live-foods commercially available are collected from wild or semi-wild settings and so are unlikely to be less of a disease risk than invertebrates collected by institutional staff themselves. Live captive bred tadpoles have been offered by some private breeders (Pasmans *et al*,



2014), but this poses a risk with regard to pathogen transfer and may not be an ethically sound option in the zoo setting.

*E. platycephalus* is prone to obesity and body condition should be monitored and feeding regimens altered accordingly. Larger newts may also outcompete smaller individuals for food and food intake of individuals should be monitored to ensure that all animals are feeding properly.

**2.2.3: Method of feeding:** Feeding should occur during the day. Food should be dropped into the aquarium close to the individuals. Uneaten dead prey items should be removed within 24 hours to avoid water pollution.

## **2.3 Social structure**

**2.3.1 Basic social structure:** In captivity a 1:2 sex ratio should be maintained as repetitive mating with the females can cause stress and eventually lead to death of the female. Larvae should be allocated according to size with each tank and provided that there is ample food and refugia they can be reared in groups. Individuals that have originated from the same sites should be housed together (French Urodela Group, 2005) as individuals from different sites may have different disease histories. Mixing populations may have negative ramifications for possible future reintroductions as well as the potential for outbreeding depression between genetically differentiated populations. Moreover, animals from different sites / regions may have slightly different environmental optima.

**2.3.2 Changing group structure:** Group structure for larvae should be changed according to their size. Only individuals of the same size should be housed together to avoid cannibalism.

**2.3.3 Sharing enclosure with other species:** This should be avoided as *E. platycephalus* is a predatory species and males may also attempt to amplex individuals of other species, risking injury or drowning. There is also a risk of spreading pathogens between the species which may have a detrimental impact on both species. Fish that are not consumed by the newts will damage gills, feed on eggs and larvae and stress adults, which do not live in streams containing many fish in the wild.

## **2.4 Breeding (Fig. 11).**

**2.4.1 Mating:** First successful matings can be anticipated when specimens reach 13 months of age (Macale & Vignoli pers. obs.), but younger animals may begin to amplex without successful reproduction. Sexual maturity is linked more to size than age and animals kept at the warmer end of their range and provided with abundant food are likely to grow faster and so reach maturity sooner; the implications of unnaturally rapid growth for health and fitness are unknown. In captivity, mating events occur most frequently in spring and autumn but may be witnessed throughout the year (Pasmans *et al*, 2014). Mating occurs in water and can last up to several hours. Multiple shelters



need to be available for adult females to provide an escape from repetitive mating with the males. After mating the female may have several bite marks on her back (Maillet & Schultschik, 2013), these should heal rapidly but should be monitored, particularly for fungal infections.

**2.4.2 Egg laying:** Females lay their eggs over a period of 3 to 5 ½ months (Böhme *et al*, 1999). They deposit and attach their eggs to the underside of stones and at the base of plants, lodged between the twigs, roots and pebbles (Arnold & Ovenden, 2002, Alcher,

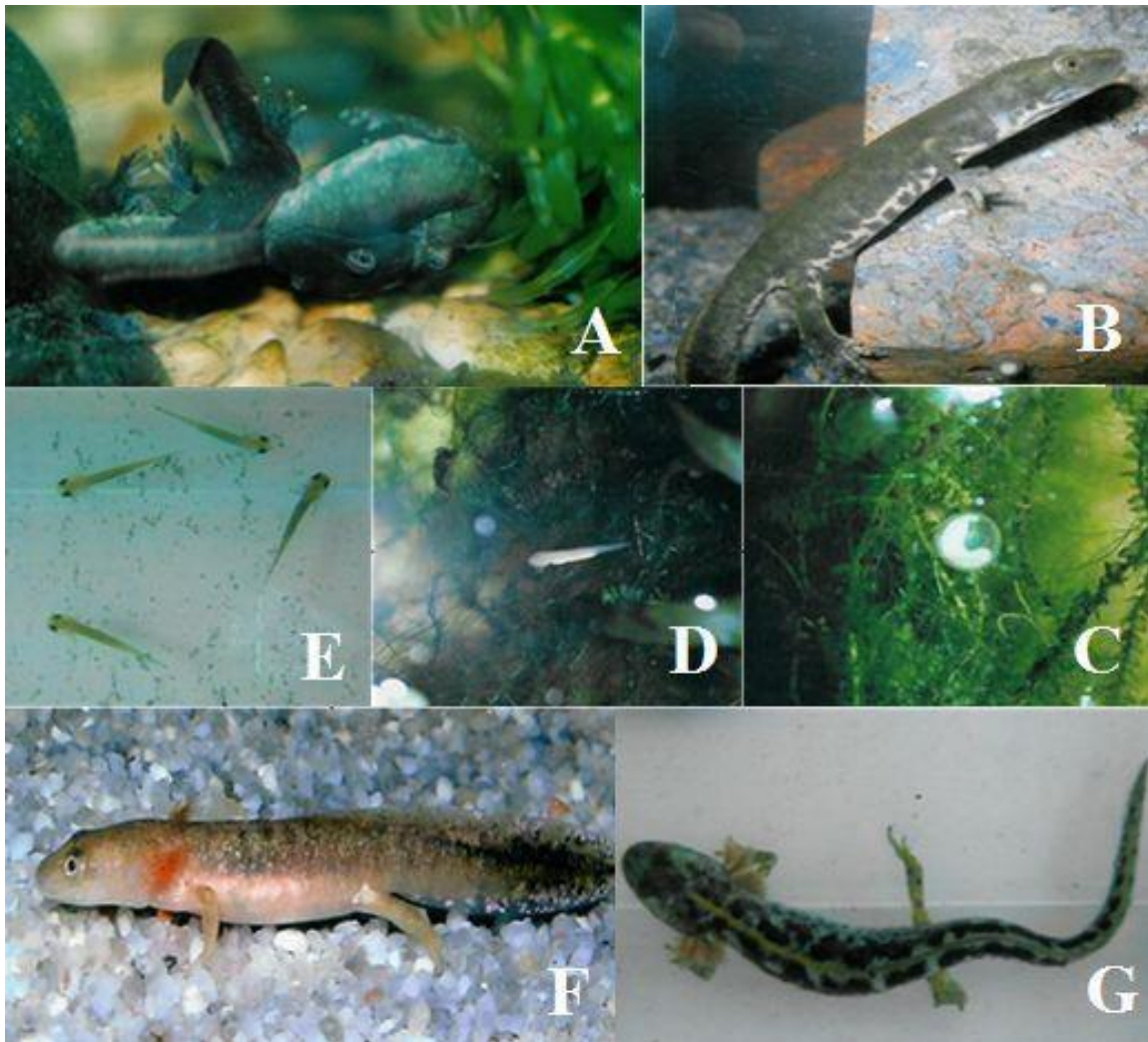


Figure 11: Breeding, reproductive and larval stages of *E. platycephalus*. A. Mating. B. Female with eggs. C. Egg in the final stage of development. D. Newly hatched larva. E. Larvae at 2 weeks post hatching. F. Larva at 2 months old. G. Larva at 12 months old (French Urodela Group).

1975, 1980, 1981) by extending their conical cloaca into a tube. Females will also lay eggs on artificial woolen spawn mops (B. Tapley pers. obs.). The eggs firmly adhere to the substratum. When the substratum consists mainly of sand, the females oviposit almost the totality of their eggs in the sand within the superficial stratum (0.5-2 cm) (Alcher, 1981; Macale & Vignoli pers. observ.). In this condition, the eggs are easily collected by dragging the substratum with a net of appropriate size (the sand grains must be drained out and the eggs kept). The eggs are translucent and the whitish embryo is visible inside. Moldy or infertile eggs should be removed to prevent the spread of mould to the developing eggs. Fungicide treatments such as methylene blue

may help keep fungus in check, but optimal water quality is the first line of defense. UV filters may also reduce fungal infection by limiting the number of pathogen propagules in the water column (Maillet & Schultschick, 2013; Macale & Vignoli pers. observ.). Increasing the tannin content of the water, by adding dried oak leaves or similar, has also anecdotally reduced *Saprolegnia* infection of the eggs (B. Tapley pers. obs.). Maintaining eggs in clean water with a mature biological filter reduces the chances of fungal outbreaks.

**2.4.3 Hatching:** The larvae hatch after about 4-5 weeks depending on the temperature. Embryonic development takes approximately 37.5 days at 15 °C and 12.5 at 24.5 °C (Böhme *et al*, 1999). 17.0 °C – 20.0 °C is the optimum temperature for embryonic development. The hatched embryo is between 4 to 5 mm and can be discerned by their black eyes and lack of balancers on the body (French Urodela Group, 2005).

**2.4.4 Development and care of young:** Larval development is temperature dependent. At 15.0 °C development takes from 376 - 453 days, at 20.0 °C development takes 184 to 260 days (Böhme *et al*, 1999). The larvae are very sensitive to pollution at this stage. It is not necessary to feed the larva during the first 10 days after hatching as they sustain themselves on their yolk reserves. On average, larvae start feeding 10-15 days after hatching (Maillet & Schultschick, 2013). The yolk sac is visible as a yellow line in the abdomen of hatchlings and this can be used as guide to when feeding should commence. Larvae will also develop noticeably during this period. Water must be well oxygenated and partial water changes performed frequently as larva are very sensitive to mildew. In addition, density plays a role in regulating newt growth. In experimental tanks, larvae tend to grow slower and show aggressive behavior at high densities. Also survival rates is negatively affected by high larval density.

As with many other salamandrid newts, the juvenile phase or eft is naturally terrestrial and juveniles may seek to leave the water by climbing the aquarium glass after metamorphosis. This is particularly noticeable when temperatures are towards the higher end of the optimal range. Juveniles may develop hydrophobic skin (when animals submerge they will appear silvery with air trapped on the skin). In the majority of cases, these animals will continue to feed in the water and will quickly become completely aquatic, particularly when maintained in cool conditions and when water quality remains good. If juveniles are kept under terrestrial conditions, terraria should include a compacted soil-based substrate (do not use coconut fibre/coir only, or paper towels), ideally a mixture of peat compost, coco fibre, sand and loamy topsoil should be used. This should be compacted and covered with leaf litter, moss sheets (not *Sphagnum* due to its acidity) and flakes of stone, cork bark or tile to form piles with crevices at varying heights and humidity, from damp at the base to dry at the top. Terrestrial animals should be maintained at similar temperatures to aquatic animals, although they may tolerate slightly higher temperatures, and can be offered various terrestrial prey including crickets, earthworms and woodlice. A water dish should be present with easy ingress/egress points and the terrarium should be sprayed regularly so that it is kept damp but not wet. Animals can be reintroduced to water by placing them in a shallow aquarium with stones positioned so the animals can rest with their heads out of the water but not get completely dry. They should be fed in the water to encourage aquatic behaviour. Animals that refuse to become at least semi-aquatic after a week should be

returned to terraria, particularly if they are not feeding in the transitional enclosure. This process maybe easiest in the autumn or under cooler conditions, when animals would naturally start to return to the water in the wild.

**2.4.5 Population management** Populations of *E. platycephalus* should be kept according to their geographical origin / collection site. This is due to the potential for differences in the genetic makeup of populations who are prevented from dispersing and breeding in the wild state due to physical barriers (e.g. unsuitable habitat, natural and man-made obstacles). Studies on the genetic population structure show significant geographical population differentiation (Lecis and Norris, 2004). These populations may have adaptations specific to the microhabitat they originated from, and interbreeding of populations may dilute these adaptations, or produce other deleterious effects associated with hybridisation. This is of particular importance when maintaining populations where the ultimate goal is reintroduction. Twenty-two wild caught adult salamanders (12 males and 10 females) from three distinct populations have been maintained at Fondazione Bioparco di Roma since 2010. Promiscuity among individuals from different populations is excluded as some of the animals may be destined for reintroduction.

## **2.5 Handling**

**2.5.1 Individual identification and sexing:** Photographic identification can be used as an individual identification method (Ferner, 2007). *E. platycephalus* has its own naturally occurring variation in markings. Photographs of the ventral areas of each individual will provide individual recognition techniques from the variation of black dot patterns that are present on the males and females. Photographs should be sharp and shadow free, with good colour rendition (Heyer *et al*, 1994) and can be taken most easily by placing the newt on a glass plate and photographing from beneath (newts have a strong righting reflex and it is difficult to restrain them upside down without causing significant stress). In some cases, significant changes in extension (increase) of black dot patterns, are observed, especially in females (Macale & Vignoli, pers. observ.). This changes can be an issue for individual recognition and further research is required for individuals marking techniques (for suitable marking methods refer to Ferner, 2007).

At Fondazione Bioparco di Roma each specimen is identified with an alpha-numerical code associated to a corresponding plastic tag as well as a picture of its gular chromatic pattern, the sex, and any distinguishing features of the specimen (eg., neoteny, polydactyly, etc.). The identification tag follows the specimens for all the time it is hosted the facilities of the Bioparco Foundation.

**2.5.2 General handling:** This species should be handled very carefully and only when necessary. Nitrile or vinyl (rather than latex) gloves that are powder free (powder may irritate the skin of the individual) should be used during handling as they protect the skin from abrasion, contamination between the species and the handler and the spread of pathogens (CCAC, 2003). Gloves also reduce the transmission of heat from the handler to the specimen. Contact with the tail should be particularly avoided if possible as it will easily break off; this may influence future growth and reproduction as it will

deprive the individual of fat stores (Derickson, 1976; Bellairs & Bryant, 1985) and prevent males from employing it during reproductive attempts. When handled out of water 5 to 10 ml of aquarium water should be poured over the specimen to prevent it from drying out (Heyer *et al*, 1994). When moving eggs from their initial tanks to separate them, their primary support should always be used if possible, although if stuck to large or permanent tank feature (rocks, tank sides, filters etc.), they may be detached carefully using a sharp razor blade or scalpel. Eggs removed from their substrate must be kept particularly clean and it is best not to leave them loose on the bottom of the hatching aquarium and rather allow them to hatch in smaller trays of aquarium water before transferring the larvae to aquaria once they have hatched. When handling larvae or neotenic adults it is important to avoid contact with the gills as they are easily damaged. If any surgical procedures need to be carried out all equipment must be thoroughly sterilised before and after. Handling the species for measurement should be done carefully. It is important not to pull on the animal if measuring length as the vertebral column is quite flexible and doing so will stretch the animal and causes it harm (Heyer *et al*, 1994). The individual may become thermally stressed due to heat transfer from the hand of the handler so it is important to handle the individual quickly and efficiently (CCAC, 2003). Measurements are more easily and accurately taken by photographing the animal from directly above on a flat surface with a scale and using an image manipulation program (such as the free software ImageJ) to measure the animal based on the photograph; such photographs can be used for both measurements and individual identification.

**2.5.3: Catching / restraining:** The need to restrain individuals in captivity is quite rare. If restraint is necessary an open flat hand should be used to apply even pressure over the individual's entire body (CCAC, 2003). Anesthesia can be used during certain techniques involving surgery. Some anesthetics such as MS 222 are quite acidic, these require buffering to pH 7.0 to prevent damage to the skin especially with MS 222 (Heyer *et al*, 1994). Once the specimen is completely flaccid or does not move when nudged with a blunt probe it is completely anesthetised. Anesthetised specimens should be kept moist during any procedure, normally by placing the animal on damp paper towel or similar and periodically wetting the skin.

**2.5.4 Transportation:** Transportation of post metamorphic *E. platycephalus* should occur in a plastic container packed with wet paper towels or moss in a darkened condition. Sphagnum moss should be avoided as it is very low in pH and may harm animals during transport. Avoid transporting larvae as they are sensitive; if absolutely necessary they should be moved in fish bags with rounded or taped-up corners in aquarium water. Eggs can be transported on paper towel sealed inside a plastic bag or petri dish. The transportation container should be packed within a polystyrene cooler to prevent sudden changes in temperature and to provide a buffer against temperature extremes (CCAC, 2003). If water temperature needs to be increased for any reason a hot water bottle with cool water can be used. To cool the polystyrene container one can use freezer icepacks. In either case ensure that the cooling/heating pack is not directly in contact with transport containers; instead, wrap the cool/heat pack in a towel or cloth before putting it in the polystyrene box. Take temperature readings before the trip to determine how much of the cooling or heating element to use and for how long it retains its effectiveness (Indiviglio, 1997). The transportation container should be kept

out of direct sunlight. The packing containers should be placed in a rigid outer shipping container. To prevent jarring during transport, crushed newspaper or foam packing chips can be used to support the packing containers within the outer shipping container. It is advisable to avoid shipping if weather forecasts predict very hot or very cold temperatures (CCAC, 2003).

**2.5.5 Safety:** As with all amphibians, disposable powder free nitrile or vinyl gloves should always be worn to prevent toxins coming in contact with the skin and to avoid the spread of harmful microorganisms such as *Salmonella*, as well as irritant toxins from the skin of the newt itself, being transmitted to the handler.

**2.6 Specific problems: considerations for health and welfare:** This species is very sensitive to mildew and pathogenic fungus that attack the eggs, larvae and sometimes adults. Good water quality and well oxygenated aquariums are essential to help prevent this. Adult specimens are very sensitive to *Saprolegnia*, a fungus infecting the extremity of the tail and sometimes the limbs. A 3% methylene blue solution can be used to treat this (French Urodela Group, 2005); soak the individual in the solution for one hour. Alternatively, bathing animals in salt water (approximately 1 teaspoon of NaCl for each 8L of water) until the fungus dies, or in stronger solution (1 teaspoon per litre water) for 20 minutes per day are frequently used to treat aquatic caudates for fungal infections. Regular cleaning and good filtration of the aquarium help prevent this. The jaws of adult individuals sometimes succumb to an infection which is characterised by the removal of the skin on the infected area. The individual is not able to eat and eventually dies; further research is required to determine the etiology and how to treat this. Oedema has also been reported by the French Urodela Group (2005). The diagnosis of specific diseases in individuals is not easy as sick animals usually show similar symptoms with a variety of causal agents. Daily observation and visual examination is usually the best way to determine variations from the norm (CCAC, 2003) and good husbandry practice resulting in stable, optimal environmental conditions is preventative (and often a major contributor to recover) for the majority of reported health issues.



Figure 12: male *E. platycephalus* with distended cloaca due to gravel blockage.

Fine gravel (2-3 mm in diameter) caused a blockage in the gastrointestinal tract of an adult male *E. platycephalus* at ZSL London Zoo (fig 12.). The specimen recovered after the gravel was manually removed from the cloaca and the specimen was given a course

of anti-inflammatory drugs; however, the cloaca remained slightly deformed and the individual is prone to gas accumulation in the gut after feeding.

Amplectant struggles can, over several seasons, result in permanent damage to the digits and tail fin of animals, particularly females, but also males maintained together. The tail fin may appear toothed and animals may lose all digits, particularly on the front feet. Digits and tail fin may regenerate if animals are separated, but this has not been investigated. Anecdotal observation suggests that particular males may be more prone to causing this sort of damage than others.

At Fondazione Bioparco di Roma the ex situ phase of the conservation project is characterized by the adoption of the highest standards of hygiene for the housing of broodstock. For this purpose, a laboratory was created as quarantine area named "Amphibian Quarantine Facility", where the animals collected in nature are housed in order to subject them to the due veterinary analyses, which exclude any positivity to *Chytridium* spp. or other diseases. Such quarantine zone provides isolation of animals from other zoological and dall'Amphibian Ark Laboratory, the place where newts are reared and bred. The room is equipped with a quarantine 12-rack aquarium system where individuals of *Euproctus platycephalus* are individually hosted for a minimum period of 90 days according to the hygienic standards required by EAZA health protocols. During the quarantine, the incoming animals are screened for fungi and bacteria by means of swab samples of the skin.

At Fondazione Bioparco di Roma, *E. platycephalus* are maintained and bred as part of a potential reintroduction programme which was established in 2010. The chosen location for the Amphibian Ark Laboratory (AAL) consists of a building sufficiently distant and spatially isolated from other zoological facilities. The building is divided into three rooms, a first room used as a computer room where data on the water quality and temperature and all the behavioral observation are recorded. A second room is used as a dressing room, so to access to the third where the *E. platycephalus* are housed.

## 2.7 Recommended research

UV-B provision is an area that requires further research. Specific water parameters from the field including hardness, alkalinity, dissolved oxygen and pH are lacking. Obtaining these data would be beneficial to the captive management of the species. Given the risk that chytrids pose to this species the development of species-tested chytrid treatment protocols would also be beneficial.

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