EAZA Amphibian Taxon Advisory Group

Best Practice Guidelines for
The Lake Pátzcuaro salamander (*Ambystoma dumerilii*)

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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.

Cover image: *Ambystoma dumerilii* © Chester Zoo.

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Introduction

The Lake Pátzcuaro Salamander, or Achoque, is a Critically Endangered species of aquatic salamander endemic to a single lake located in Michoacán state of Mexico. The species is directly threatened by extinction due to a multitude of factors. Therefore, the maintenance of this species in captive colonies may be of vital importance to enhance future conservation efforts.

This best practice guideline is the result of collaboration between multiple individuals and institutions within Mexico and within EAZA that are dedicated to the conservation of this unique and enigmatic species.

Key husbandry points

1. Maintaining stable water temperatures and parameters and providing seasonal temperature changes as required.

2. Providing larvae with large and consistent amounts of live aquatic foods to ensure normal growth rate.

3. Stocking density of larvae during rearing to prevent cannibalism.

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

Contents

Section 1. Biology and field data

1.1 Taxonomy

The genus *Ambystoma* currently contains 33 extant species (Frost, 2021), ranging from southeastern Alaska, south through Canada, occurring throughout most of the USA and the Sierra Madre Occidental and central Mexico; they are absent from the Florida peninsula, Nevada, southern California, southwestern Arizona, Baja California and lowland tropical Mexico (Frost, 2021). Most species metamorphose into a terrestrial adult form that return to water for reproduction. The genus also contains species that exhibit facultative neoteny, and four species (including *Ambystoma dumerilii*) live in a permanent natural state of neoteny and therefore retain larval characteristics whilst reaching a reproductive state of adulthood.

ORDER: *Urodela* Duméril, 1805

FAMILY: Ambystomatidae Gray, 1850

GENUS: *Ambystoma* Tschudi, 1838

SPECIES: *Ambystoma dumerilii* (Dugès, 1870)

COMMON NAMES:

Dumeril's salamander
Lake Pátzcuaro Salamander
Achoque

1.2 Morphology:

1.2.1 Weight:

Adult specimens of *A. dumerilii* are known to weigh up to at least 226g, the average weight of N = 51 adult individuals weighed 120.91g ± 44.27g (Bland, 2018 unpublished data) and 127.76 ± 181.1g (Escalera-Vázquez *et al*, unpublished data). Female individuals on average typically weigh more than males, thus although lengths may be comparable female individuals are heavier bodied than males.

1.2.2 Length:

Sexually mature individuals of *A. dumerilii* of both sexes measure greater than 122mm snout-to-vent length (SVL), with a total length of at least 282mm and no sexual difference in body length (Brandon, 1970a). *Ambystoma dumerilii* may represent one of the largest species in the genus *Ambystoma*.

1.2.3 Colouration:

Ambystoma dumerilii is tan brown to greenish on the dorsum with a paler belly tending to grey. Some individuals have darker brown-grey spots across the dorsum, but these are not in particular contrast to the background colour. The fore and hind feet may have darker tips and webbing, this darker pigmentation may extend to the underside of the tail, the rostrum of the head and the underside of the cloaca. In extreme situations, some dark pigmentation may appear on the dorsum, particularly in males. The fimbriae of the external gills are usually red in colouration.

1.2.4 Description:

This species is a neotenous ambystomatid salamander with large hyperfilamentous external gills, a wide mouth and a large caudal fin running up to and close to the head. *Ambystoma dumerilii* shares the same general appearance as *A. andersoni*, *A. mexicanum* and *A. taylori* but all four species possess noticeable differences in specific identifiable characters. *Ambystoma dumerilii* in particular possesses unique characters that make it an unmistakable and easily identifiable species of *Ambystoma* (Brandon, 1992).

Ambystoma dumerilii (Fig. 1) has a notably large spatulate flattened head and rostrum, and gills bearing particularly numerous fimbriae, which attach around most of the rachis and the rakers of the third gill arch are particularly few in number compared with other closely related species. The body, which is widest in the thoracic region and tapers towards the hind legs, is short and stocky, the hind feet have diminutive, webbed toes. The skin is generally smooth, but there are small, domed protuberances on the head. The caudal fin is fleshy. The eyes are small and situated far forward on the head close to the snout.

Fig 1. Individuals of *A. dumerilii* collected from Lake Pátzcuaro, note flattened head and highly developed gill fimbriae © Escalera-Vázquez & A.Bland.

1.3 Physiology:

Ambystoma dumerilii, being a neotenic species respire via large highly vascularised and hyperfilamentous external gills, and although this species does possess lungs and may be observed to occasionally take a gulp of air from the surface, the primary method of respiration is through the gaseous exchange via the gills in the aquatic environment. Being a neotenous species *A.dumerilii* possess typical larval characteristics such as external gills and lack of skin proteins found in metamorphosed adult salamanders.

As observed in the closely related species *A. mexicanum*, this species is also capable of the of tissue and limb regeneration (A. Bland, pers. Obsv.; Lee and Gardiner 2012; Cano-Martinez *et al*., 2007 Bryant *et al*., 2002 and Wallace, 1981).

1.4 Longevity:

Longevity in this species has not been widely recorded and is unknown in nature, and many captive colonies have only been established relatively recently. There is a report of a female individual living for 16 years in a long maintained colony in Pátzcuaro (Sor Orfelia Morales, pers.comm).

1.5 Zoogeography, ecology and conservation

1.5.1 Distribution:

This species is endemic to Lake Pátzcuaro in Michoacán state (19.6333° N, 101.6333° W) situated 1920m asl (Shaffer, 1989) (Fig. 2). Pátzcuaro lake is one of the last of twenty five relict lakes that used to occur in the central region of Mexico. It is one of the most important natural lakes in Mexico due to its ecological, historical, social, cultural, economical and fishing value to the local human community. It is part of the Trans-Mexican volcanic belt, where geologically recent drying of the climate has isolated several species of *Ambystoma* within several freshwater systems (Shaffer, 1989). This species is considered to be solely distributed within this lake and has not been recorded in any areas of the surrounding wetlands.

Around the lake there are twenty six different human settlements (Cities, villages and rural communities) and six islands within the lake, four of which are inhabitat by people. One of these islands in particular (Janitzio), is densily populated.

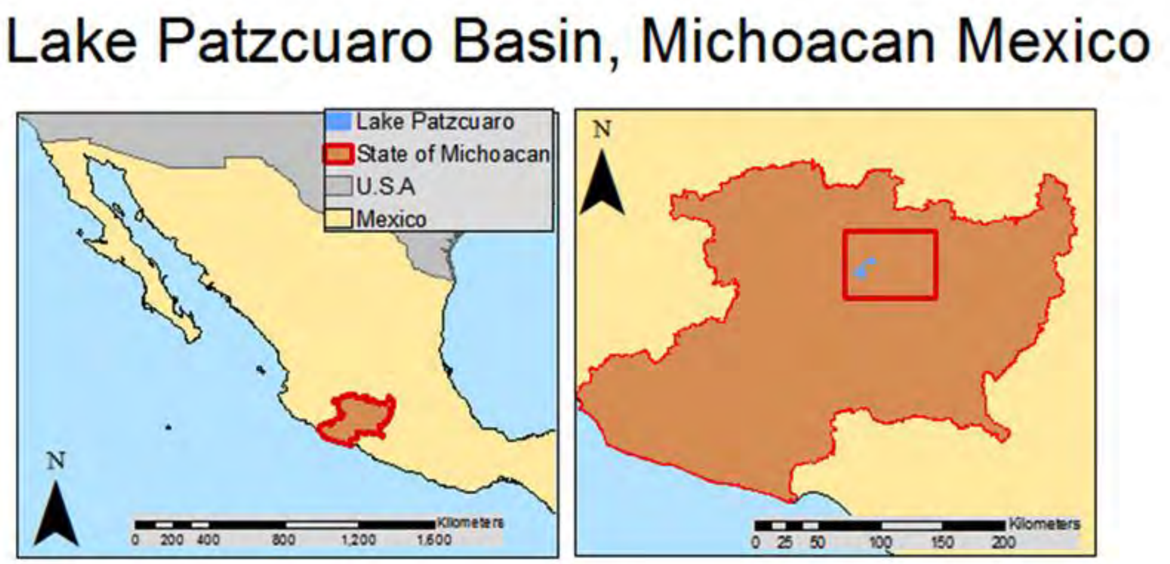


Fig 2. Location of Lake Pátzcuaro situated in Michoacán state of Central Mexico and a satellite image of the lake itself. https://maps.google.com/

1.5.2 Habitat:

Lake Pátzcuaro is a freshwater lake. Due to the geology of the region this has created unique water parameters, particularly with regards to dissolved minerals and hard alkaline water. The annual yearly temperature cycle of Pátzcuaro can be seen in Fig. 3. Chemical parameters for the lake collected in 2019 by Escalera-Vázquez *et. al* are presented in Fig. 4 and show that Lake Pátzcuaro is characterised as having a high pH of between 9.20 – 9.66. Shaffer (1989) recorded Lake Pátzcuaro as having an average water temperature of 14°C - 25°C at various depths, a pH of 8.1 – 8.8 and a conductivity of 275 – 760µS/cm. The lake has a maximum depth of 11m and an average depth of 5m – 11m, due to anthropomorphic changes such as pollution and filling of the lake, it is possible that historic measurements are subject to change as the environment of the lake deteriorates (Torres, 1993).

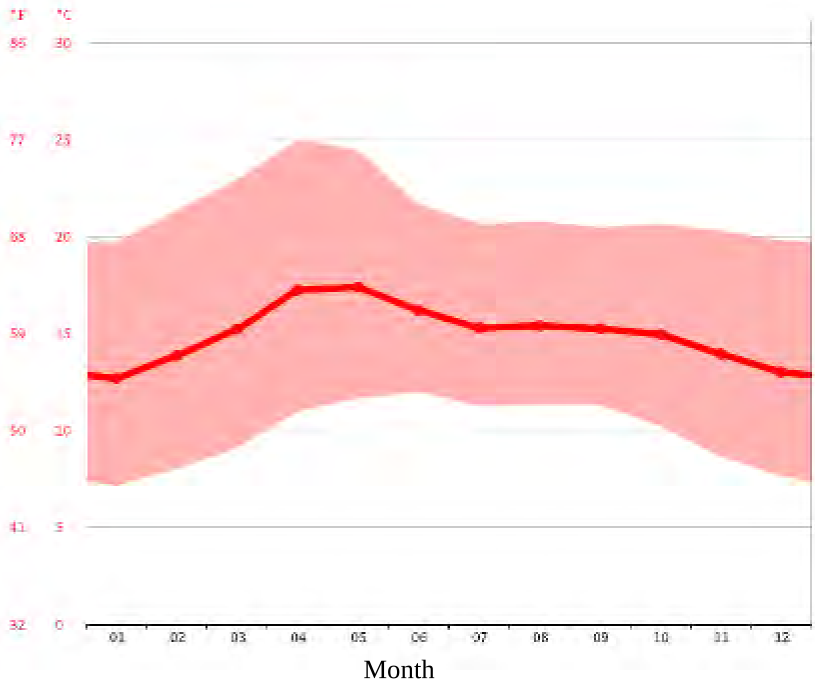


Fig 3. Yearly Temperature cycle of Pátzcuaro. (www.climate-data.org)

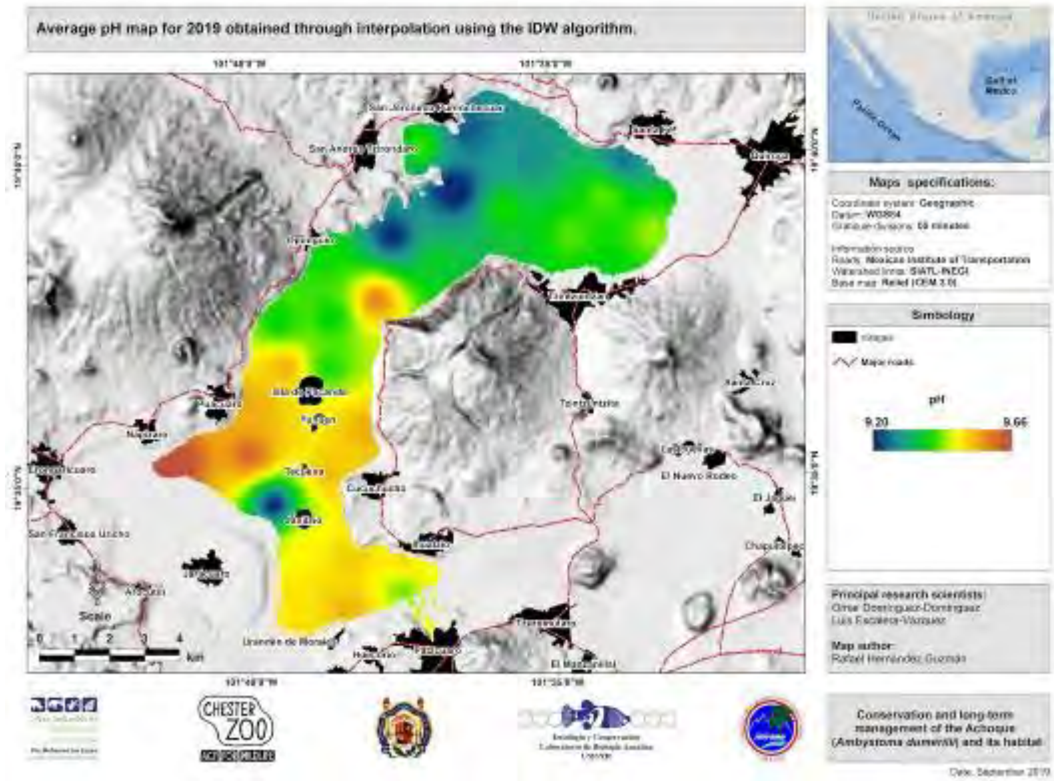


Fig 4. pH data of Lake Pátzcuaro collected Escalera-Vázquez in 2019. Map created by Hernández 2019.

Aquatic vegetation is present within the lake, which likely provides oviposition sites for this species; shallow areas usually contain emergent vegetation, a general view of the lake displaying these features can be seen in Fig. 5. Currently lake Pátzcuaro is heavily polluted, as the lake acts as a basin for surrounding villages and the four inhabited islands within the lake, which produce varius waste materials including nitrogenous waste that runs off into the water system; the water of the lake is turbid, with a clay mud substrate and visibility is poor (Torres, 1993).

The exploitation of the lake dates back to the pre-colombian periods when endemic species of fish historically occurred such as Silverside Pike (Pez blanco, *Chirostoma estor estor*), Pátzcuaro chub (acúmara, *Algansea lacustris*), goodeido (Chehua, *Alloophorus robustus*), Mexican silverside (Charales, *Chirostoma sp*.), Pátzcuaro Allotoca (Chorumo, *Allotoca diazi*) and Blackfin goodea (*Tiro*, *Goodea luitpolldii*). Five introduced species of fishes are currently found within the lake in higher abundance and in direct competition with the endemic/native species (Carp, *Cyprinus carpio*; Herbivorous carp, *Ctenopharyngodon idella*.; Tilapia, *Oreochromis* sp.*;* and Largemouth bass, *Micropterus salmoides*) (Velarde, 2012).

Pollution and poor water quality of freshwater systems are known to affect macro invertebrate abundance and diversity (Xu *et al.*, 2014), which in turn may affect survivability of salamander larvae that depend on an abundance of these food sources. These environmental changes are also a direct threat to the endemic fish species.

During the last decade there has been a severe negative impact of deforestation, erosion and siltation which has reduced the depth of the lake by 2.6m (Vargas and Guzmán-Ramírez, 2009). The reduction of the lake surface has also been affected during the last 50 years, with a reduction of 40 km^2 (Vargas and Guzmán-Ramírez, 2009).

Fig 5. Lake Pátzcuaro. Note the turbid water and emergent vegetation. © A.Bland.

1.5.3 Population:

The wild population of *A. dumerilii* is considered to have suffered a significant decline and reviewing harvesting information of this species via local fisherman highlights this. The species is considered to be close to extinction (IUCN, 2020; Huacuz, 2002), although the presence of this species within Lake Pátzcuaro is still confirmed, only very small numbers of animals are found occasionally, and further investigation is underway to determine an accurate population density using mark recapture methods. It is reported by local fishermen that they often find the spawn of this species attached to fishing nets when retrieved from the water (Fig.6), thus a reproducing population is known to exist, although success and survival rates of spawn and larvae are unknown.

The decline in this species population has been attributed to deterioration in water quality due to pollution, filling of lake Pátzcuaro, historic over exploitation and introduced fish species (Shaffer, 1989). The introduced external parasite, *Lernea cyprinacea,* is often found on wild collected individuals and although it is known to be an introduced species it is unknown what effect this parasite is having on the wild population (Poly, 2003). Non-native fish were introduced to lake Pátzcuaro as a food source for people as early as the 1940's (primarily the largemouth bass *Micropterus salmoides*) (Shaffer, 1989; IUCN, 2020) and it is noteworthy that *A. dumerilii* are one of the few ambytsomatid species capable of persisting alongside these highly competitive and predatory threats, albeit in smaller numbers, and this warrants further investigation with regard to the limits of competition between these species.

Fig 6. Spawn of *A. dumerilii* attached to a fishing net retrieved from lake Pátzcuaro. © Universidad Michoacana.

1.5.4 Conservation status:

This species is assessed as considered Critically Endangered by the IUCN (IUCN, 2020), and faces extinction in the wild. The Extent of Occurrence is less than 100km^2 and the distribution restricted to a single lake that is not considered a protected area. The total number of mature wild individuals is unknown, but the population has been observed to have severely decreased over the past 30 years. Conservation of the remaining habitat including reducing pollution and control of invasive species are considered to be urgent actions (IUCN, 2020).

Although not inhabiting a protected area, *A.dumerilii* is a protected species under category Pr by the Government of Mexico (Subject to special protection NOM-059-SERMARNAT-2010) and is listed on CITES Appendix II. This protection primarily aims to regulate harvest and trade of this species, most importantly at the local level as this species is used as a local food source and for medicinal purposes (IUCN, 2020).

Although the species is listed withinin the Mexican Red List, no real protective measures have been implemented for the species and the habitat. The habitat protection for Lake Pátzcuaro and the Achoque is possible in the long term. This could be achieved if efficient policies of resource conservation in a drainage perspective is implemented.

The Achoque has represented an important fishery resource for the establishment and development of human settlements in the Lake Pátzcuaro area. Historically, they have had considerable value for the *purhrpecha* culture, because of its importance to survival as a food source, but also as a medicinal remedy for the treatment of respiratory diseases and anemia, as well as believed to supply energy for children, the elderly and women during lactation (Mendoza, 2012; Calderón and Rodríguez, 1986).

The species is maintained within four local *in-situ* captive breeding colonies and also within several European collections, which are members of EAZA, and American institutions within AZA membership. These populations, particularly *in-situ*, have conservation relevance and also provide opportunity to study the species behaviour and biology in order to inform conservation planning. Individuals maintained in captivity may be considered for future reintroductions if necessary.

Of the four *in-situ* management units that have been established, one has been working with the species for sixteen years (Convent of the Dominical order), where a large reproductive colony has been established, although the principal objective of this colony is the commercial production of the achoque. Of the three remaining established colonies, two are primarily aimed for conservation purposes and one for educational activities. The results of these colonies are preliminary and no conservation impacts can thus far be delineated. The management units that are already established and part of this species action plan are:

UMA Jimbani Erandi

The nuns of the Monastery of the Dominical Order *"Predicadores María Inmaculada de la Salud"*, founded in Patzcuaro since 1747, have been engaged for more than 150 years in the development of a syrup for the treatment of respiratory diseases, using the Achoque as raw material. The decrease in Achoque populations in the natural environment during the last decades encouraged them to begin a captive breeding program for this amphibian. The nuns have designated a whole area of the convent for the breeding of this species, with several systems with controlled conditions of aeration and light, as well as filtration systems and water oxygenation, quarantine areas, production and maintenance of live food, among others. In addition to the production of syrup, the other aims of this center are the reproductive conservation by captive management, and the building of an environmental awareness initiative by guided visits to the installations.

UMA CRIP-Pátzcuaro

The "Centro Regional de Investigaciones Pesqueras Pátzcuaro" (CRIP), is a federal research center for fisheries which is currently promoting the development of technological programs in the aquaculture of several native species from the Lake. Within its priority species, the Achoque is to be considered for development of a large scale production culture, since the medicinal and food importance around the Pátzcuaro Lake region is in high demand. As part of its vision and mission, the CRIP has contemplated the transference of technical and practical knowledge to the local people interested in the Achoque's production. This center has the keepers and the facilities to realise these goals.

PIMVS Jimbani Xarhantani

This is a local initiative that is managed by Gerardo León Murillo. This initiative began in 2017, from individuals donated by the UMA Jimbani Erandi. Currently this breeding center has ten breeding adults. The aim of this PIMVs is the reproductive conservation by captive management, the production to meet the local demand of this resource, and promote an environmental awareness in the people by exhibition of the Achoque and providing appropriate educational information. The consolidation of this highly productive center depends on the possible economic support and timely technical support to ensure continuos production of the Achoque.

Universidad Michoacana:

This center is part of the Laboratory of Aquatic Biology pertaining to the Biology Faculty of the Universidad Michoacana de San Nicolás de Hidalgo, localised in Morelia city, Mexico. The aim of this facilities include reproductive conservation, the development of several research lines as part of captive management, reproductive and genetic control and environmental education. As a university center, it has the mission of contributing towards the training and preparation of future professionals in Conservation Biology. Currently this breeding center has ten breeding adults, and operates the appropriate facilities to maintain the reproductive stock, and carry out research, outreach and awareness activities.

In order to facilitate the effective conservation of this species, over the last year (2020 – 2021) these *in-situ* project teams have established a collaborative network with 27 local artists to create eleven murals in eight locations around the lake (Fig. 7), this aims to bring the conservation of the species into the day to day life of the local communities. To date (2021), six of these have been completed. These are intended to create a visual talking point about the conservation of *A. dumerilii* and the lake's habitat, which will serve as a launchpad for future activities with the local communities. Further to these, twelve radio messages have been recorded in Spanish and Purepecha for transmission via the local radio station, which broadcasts to all of the communities in the Lake Patzcuaro area, and also has listeners in Morelia, Mexico City, a number of other Mexican state capitals, as well as in the US, in Portland, Salem, San Jose, Nashville, Miami, Washington, Chicago, Kansas City and Raleigh. In combination with this, a touring exhibition visited local schools displaying live *A. dumerilii*, which also resulted in teachers being trained in their husbandry to enable the species to maintained within these schools for educational purposes. School visits to the Monastery of the Dominical Order have also been facilitated as part of the local education initiative for local schoolchildren.

Within EAZA collections, this species is held as an *ex-situ* population in European zoos which facilitates educational initiatives through public exhibitry, aiming to raise awareness regarding the conservation of this species, and the plight of amphibians as a taxanomic group. This population also enables the continuation of species specific research which may benefit conservation efforts in the field, such as validating techniques such as PIT tagging, and captive husbandry focussed research which may enhance the captive breeding program both *in* and *ex – situ*.

Fig 7. A mural created by local artists aiming to create visual talking points within the local community.

1.6 Diet and feeding behaviour

1.6.1 Food preference:

Neotenic ambystomatid salamanders are generalist carnivores and will readily accept a broad range of food items. The wild diet of *A. dumerilii* may consist of a variety of aquatic invertebrates and small fish, as well as conspecifics, eggs or parts of conspecifics, as observed in the similar species *A. mexicanum* (Zambrano *et al.*, 2010). The guts of wild ambystomatid salamanders often contain plant and algal matter, but these are incidentally swallowed and are not considered nutritionally important (Zambrano *et al.*, 2010).

In a previous study on the feeding selection of *A. dumerilii* the diet was anecdotally restricted to a list of the probable food items consumed, based on the available taxa in the lake (crustaceans, insects, and molluscs) (Brandon 1970). To verify that the entire diet is represented by gut sampling, the number of new food items discovered should gradually decrease to zero as the number of stomachs analysed increases (Holomuzki and Collins, 1987).

In a later study investigating *A. dumerilii* stomach contents by Huacuz (2002) samples consisted of twelve adults obtained from commercial catches with gill nets, and fifteen preserved specimens collected from the market between 1995 and 1998 by the Herpetology Laboratory of the Faculty of Biology of Morelia (UMSNH). The results were expected to be similar to other *Ambystoma* species (Licht 1975; Rudolph 1978; Petranka and Petranka 1980, Stenhouse *et al*., 1983; Hutcherson *et al*.,1989) however, adult

Ambystoma dumerilii only contained remains of the endemic crustacean *Cambarellus patzcuarensis* (Fig. 8)

Adult *Ambystoma andersoni*, which are found in Lake Zacapu, were found to consume a range of taxa (*C. montezumae, Physa, Plumatella, H. azteca, Acarí, Erpobdella, Asellus, Anisoptera*), all of which are also available in Lake Patzcuaro (although *C. patzcuarensis* is found in place of *C. montezumae*). As *A. andersoni* stomach analysis required 60 samples to adequately encapsulate all food items, and *A. dumerilii* saw only *C. patzcuarensis* in 27 samples, it is clear that *A. dumerilii* shows trophic specialisation in both its method of predation and in its prey selectivity. Further evidence for this is found when observing feeding behaviours in wild caught adult specimens in captivity, as it can take days or even weeks for these specimens to become conditioned to accept food items other than *C. patzcuarensis* (Huacuz, 2002).

Fig 8. Native dwarf crayfish *Cambarellus patzcuarensis* likely form a large part of the wild diet of adult *A. dumerilii*. © Universidad Michoacana.

The size and quantity of food items consumed varies according to the size and life stage of the animal from hatchling larvae through to mature adults. Larvae predominantly feed on large amounts of small aquatic invertebrates.

1.6.2 Feeding:

Ambystoma dumerilii possess wide mouths and powerful buccal apparatus adapted to capturing prey by suction. Water is pumped from the buccal cavity through gill slits, filtered by gill rakers, to efficiently suck prey items into the mouth; this species feeds largely benthically. Gape size is relatively small compared with head size, and this proportion is smaller than for other closely related species.

The sense of smell is very important in locating food for juvenile, sub adult and adult individuals, while small larvae largely identify prey items based on movement and will not typically accept dead food until they have grown somewhat. Based on observations in other species of neotenic *Ambystoma* (*A. mexicanum*), during the first weeks of development the larvae prefer prey with slow swimming speed as is the case of rotifers and cladocerans; in fact, like in *A. tigrinum* and *A. mexicanum* (Chaparro *et al*. 2011), the small larvae of *A. dumerilii* have a higher preference for cladocerans, whose size is better matched to the gape size of larvae. Animals detecting food scents will search by pressing the snout to the substrate and making sweeping motions; they will typically also snap at anything that moves close to their snout. *Ambystoma dumerilii* will frequently stand on its snout and/or front limbs with the body arched back downwards toward the substrate when searching for food (C. Michaels, pers. obs.).

1.7 Reproduction

Since the 1970's, various studies have been conducted on the reproduction of the species under natural conditions (Brandon 1970; Gómez 1989; Uribe *et al*. 1994). The knowledge generated by these authors is related to the establishment of the age of maturity and the period that includes the reproductive cycle and the gametic cycles. However, this is a very limited knowledge for the understanding of their reproductive strategies (Huacuz, 2002). *Ambystoma dumerilii* reproduce seasonally and recent preliminary data suggest that reproduction predominantly takes place during Winter (November-January). Following internal fertilisation eggs are deposited by the female that undergo aquatic development and produce free-swimming larvae.

1.7.1 Developmental stages to sexual maturity:

Ambystoma dumerilii is a neotenic species and therefore individuals retain basic larval characteristics throughout their lives (Fig. 9), although distinctive larval appearance and developmental stages are present during early life. Brandon (1972) described newly hatched *A. dumerilii* as measuring 11.5mm – 12.0mm in total length, lacking balancers (paired stalk like organ usually found positioned behind the eye in salamander larvae), lacking gill fimbriae on dorsal surfaces of rami, possessing small front limb swellings and are light brown in colour. When larvae reach 35mm in snout vent length pigmentation is progressively developed, all limbs are developed and toes are formed with the species characteristic and diagnostic toe webbing and from this stage gill rami become more hyperfilamentous as in adults. This form is now maintained into adulthood with the only changing feature being colouration; juvenile and sub adult individuals display a paler yellowish colouration that darkens during maturity. In young larvae, the gill filaments are attached only on the posterior surfaces of the rachis, more like *A. mexicanum*, but spread further around the rachis as the animal matures to create the characteristic appearance of adult gills.

Fig 9. *Ambystoma dumerilii* during early larval stage, this general body form with external gills is retained into adulthood. © A.Bland.

A small number of individuals in captivity have been observed to spontaneously metamorphose once adult; however metamorphosed specimens of *A. dumerilii*, have never been observed to survive or reproduce in the terrestrial form. Occurrences of spontaneous metamorphosis are most likely to occur during maturation or reproductive cycles when hormone changes are taking place (Brandon, 1976), it may also be linked to high stocking density and poor water quality, high temperatures or inappropriate diet including oily fish (C. Michaels, pers. Obs.). Likewise, in other neotenic species such as *A. andersoni* and *A. taylori* metamorphosis has only been recorded in captivity, transformed indivuduals have not been found in nature (Krebs and Brandon, 1984). In observations in the laboratory of Universidad Michoacana the transformed individulas of *A. dumerilii* refuse to eat at all, and without assisted feeding, they eventually die.

When males attain sexual maturity they develop a swelling of the cloaca in the breeding season. See section **2.5.1** (Fig. 27).

1.7.2 Age of sexual maturity:

Based on observations of captive specimens, sexual maturity may be reached at approximately 18-24 months of age. Although in other cases as observed at the Jimbani Erandi colony from the Dominical covent in Pátzcuaro, it has been observed that sexual dimorphism can begin to appear between 8 and 12 months (Pérez-Saldaña *et al*., 2006). In *Ambystoma dumerilii*, biometric data show that there is a correlation between snout-vent length and sexual maturity, animals between 108mm and 122mm SVL are mature or are maturing, all individuals with sizes greater than 122mm SVL are considered sexually mature in individuals raised by Brandon (1970a). As with many species male individuals may be capable of reproducing earlier than females. Although females may be capable of reproduction at this early age fewer eggs will be produced, and of the eggs that are laid, many will not be fertilised or viable.

1.7.3 Seasonality of cycling:

The spermatogenic cycle of *A. dumerilii* begins in September when the testes reach their maximum size and contain more cysts with primary spermatocytes and spermatids than empty cysts or with spermatozoa, on the other hand, in December, the testes show fewer cysts with primary spermatocytes and are more empty of sperm (Uribe *et al.* 1994; Gómez, 1989). The oogenesis of *A. dumerilii* occurs in six maturation stages, in spring the females present previtelogenic oocytes, in summer there are follicles of all maturation stages except mature oocytes, in females collected in autumn all development phases are observed follicular in the ovary; and in the oviducts oocytes were found, which indicates the beginning of ovulation, which continues during the winter months. The females analyzed in the study by Brandon (1970a) presented eggs in the oviduct in the months of December, January and February (Huacuz, 2002). See Fig. 3 for general yealy temperature cycle of Pátzcuaro.

With this profile it is further supported that this species reproduces seasonally, observation of captive reproductions confirms that breeding is usually triggered by periods of water temperature fluctuations, both decreases or increases (Saldaña *et al*. 2006). Changes in photoperiod are also likely to play an important role in reproductive cycling and provide a trigger for reproductive behaviour (Fig. 10), with decreasing of day length during the initial winter spawning period, and an increased day length in spring.

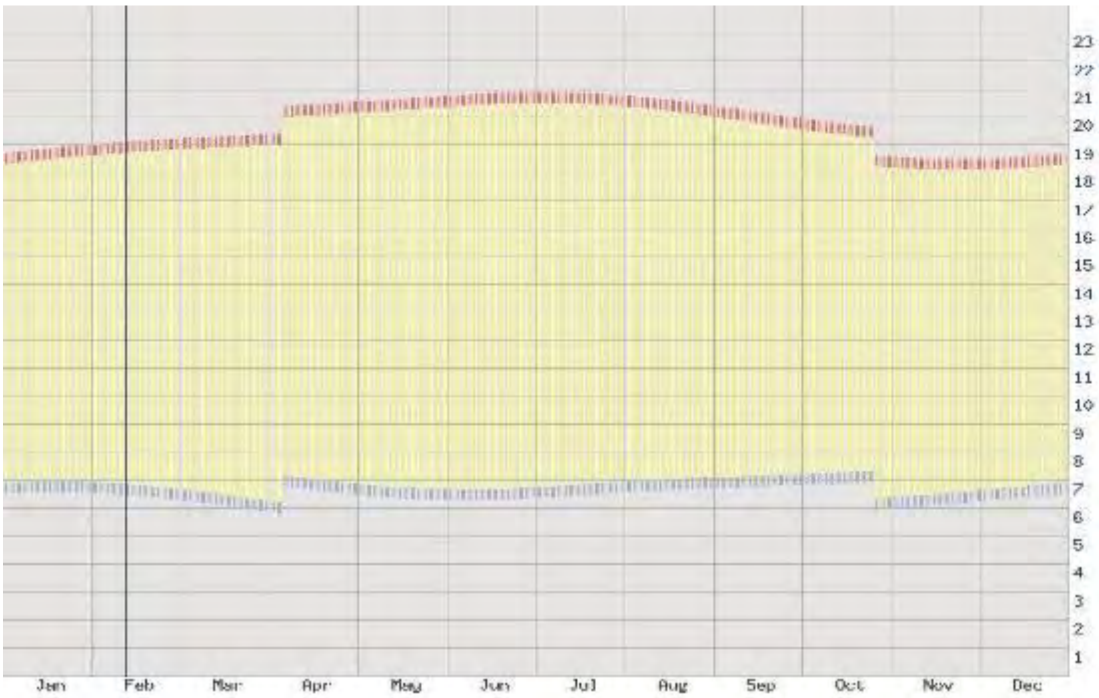


Fig 10. Yearly photoperiod cycle of Pátzcuaro, Mexico showing seasonal change of day length. (https://www.citipedia.info/)

1.7.4 Clutch size:

Mature female individuals are known to lay in excess of 1000 individual eggs (Brandon, 1970b), usually attached loosely to aquatic vegetation (Huacuz 2002) or objects suspended in the water column. It was observed by Brandon (1970a) that one female contained hundreds of eggs which all measured approximately 1.8mm in diameter.

1.8 Behaviour

Due to the difficulty to observe and rarity of this species in nature all behavioural observations in this species have been described in captive specimens in laboratory conditions or from zoological collections. It may be inferred that many behaviours such as activity patterns, courtship, oviposition and feeding behaviour are representative of wild behaviour.

Activity:

Activity patterns in the wild are not known, although from captive observations increased nocturnal activity is observed. Opportunistic activity during the daytime has also been observed (A. Bland, pers. obs.). It can be assumed that there would be an increased level of general activity during the breeding seasons of winter and spring. Movements of animals throughout the lake during both breeding season and regular seasons are currently unknown.

1.8.2 Locomotion:

Locomotion is achieved largely by walking along the substrate. The large tail fin may be used to accelerate walking or to swim in the water column. *Ambystoma dumerilii* is the only neotenic ambystomatid to possess fully webbed digits on all limbs, which may be used to aid locomotion. The amount of air in the lungs is regulated to allow them to float in the water column while foraging. When startled this species is observed to hold all limbs close to the body and use undulating body motions with the head positioned downwards to rapidly swim through the water; this rapid response in movement would aid in predator avoidance. Animals may climb into aquatic vegetation or swim into the water column with a short burst of tail movement aimed towards the substrate to pursue prey.

1.8.3 Predation:

Historically, in the absence of introduced predatory fish species, adult *A. dumerilii* would have been apex aquatic predators in Lake Pátzcuaro. *Ambystoma dumerilii* could be predated upon by larger conspecifics and water birds such as herons, and although not reported, it is likely that they may also be predated on by the snake *Thamnophis eques patzcuarensis*, a species known to feed upon fish and amphibians and is common in the Lake Pátzcuaro area. *Ambystoma dumerilii* is the only species of neotenic ambystomatid salamander that has thus far been known to persist alongside introduced predatory carp (Brandon, 1970a). Although it is likely that the introduction of these species and others has had a negative impact on the population if not on the adults themselves.

Of the introduced fish species, those mentioned in **1.5.2** "Habitat", it could be considered that they are not predators of adult specimens of *A. dumerilii* following studies by Toledo *et al.* (1992). With this consideration, a first effort was made to know the impact of carp, *Cyprinus carpio* on *A. dumerilii*. Huacuz (2002) analysed stomach contents of carp from local fishermen and in the market during the months of February and March, months in which there should be eggs of *A. dumerilii* and larvae within the lake, 40% (n = 10) of their contents from the full stomachs presented complete or parts of *C. patzcuarensis* and in 4 (16%; n = 4) remains of egg yolk of *A. dumerilii*. The catching localities of the carp where egg remains were found are Tarerio, Ucazanastacua and Ihuatzio. There is no record of when the carp was introduced into the lake, so it is difficult to establish how much this species has impacted on the recruitment of young *A. dumerilii* to the adult population since that time, but this information is important, because the predation by the carp on this species appears to be upon the eggs.

In relation to trophic competition, carp can be considered omnivorous, bentophagous and detritophagous, this species consumes mainly copepods, cladocerans and zooplankton. When they become larger (>10cm long) it begins to feed on bottom fauna, insect larvae, worms, molluscs and crustaceans (García-Berthou, 2001). The carp analysed from the lake basically consumed algae, macrophytes and, to a greater extent, crustaceans (*C. patzcuarensis*). It can be observed that the carp consume in general the same trophic resources that *A. dumerilii* should be consuming. The hypothesis could be considered that carp competes with and displaces *A. dumerilli* for trophic resources. Also, carp are carriers of parasites that were not naturally found in the ecosystem such as *Bothricephalus acheilognathi, Agulus* and *Lernaea*.

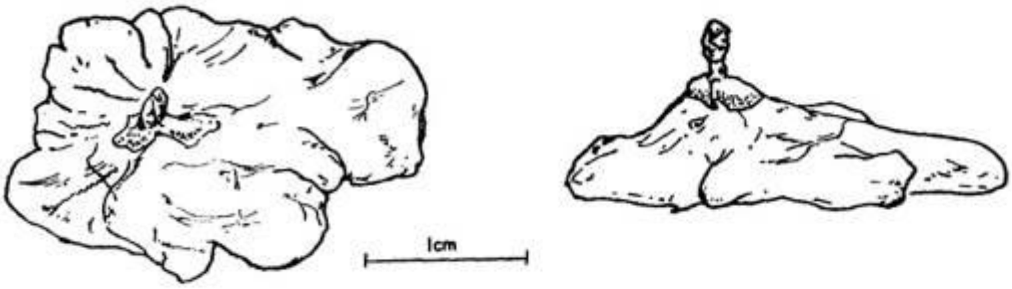
When handled and otherwise agitated this species produces excessive mucous through the skin which may bubble or foam, this likely acts as a predator deterrent and may be irritable or distasteful as in known to occur in other species of *Ambystoma* (Mason *et.al.,* 1982).

1.8.4 Vocalisation:

These salamanders do not vocalise, although a quiet croaking or squeaking may be emitted by passive expulsion of air if seized and lifted out of the water.

1.8.5 Sexual behavior:

Sexual behavior in this species has been observed via captive observations in laboratory conditions (Brandon, 1970b) or zoological collections. The male initiates courtship behaviour by arching the base of the tail and swimming around the female, occasionally lifting the tail and showing the swollen cloaca. Receptive females generally tolerate the male and do not swim away; this will lead to the female then following the male with the snout close to his cloaca. Spermatophores will then be deposited by the male on the substrate, on smooth stones or objects slightly raised from the ground (Fig. 11). Up to 16 spermatophores may be deposited in a single courtship sequence (Brandon, 1970b).



A B

Fig 11. Illustrated spermataphore of *A.dumerilii* shown arterially (A) and laterally (B) showing gelatinous base and stalk contain sperm at cap. Taken from Brandon (1970b).

The tip of the spermatophores contain active sperm. The female collects the spermatophore into the cloaca where fertilisation takes place. Oviposition typically takes place 24 – 48 hours after the female has collected spermatophores and individual eggs are subsequently deposited over a period of between 24 hours or in some instances 72 hours. The female attaches eggs to aquatic vegetation or objects such as rocks. Fig. 12 shows the eggs of *A. dumerilii* as illustrated by Brandon (1970b) and in life.

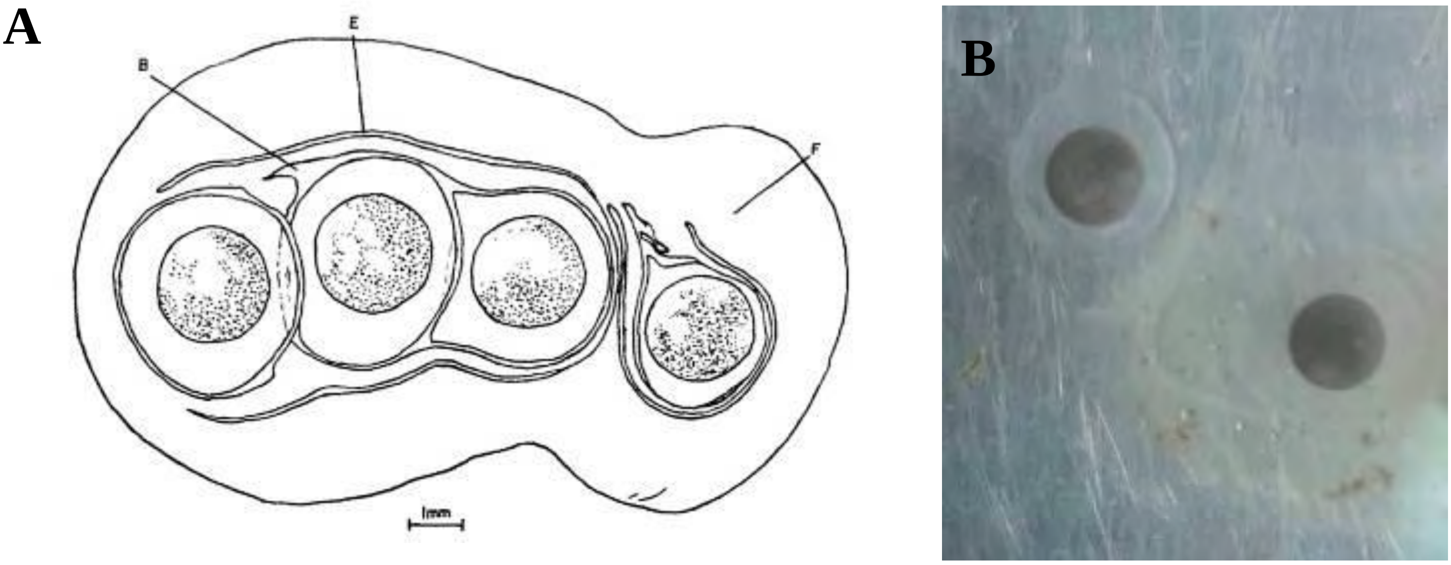


Fig 12. (A) Eggs of *A. dumerilii* after membranes are swollen with absorbed water showing (B) Inner envelope, (E) Outer Envelope & (F) Jelly. Taken from Brandon (1970b). (B) *A. dumerilii* eggs in life. © A.Bland.

SECTION 2. Captive management

2.1 Enclosure:

This species should be maintained in large aquaria. As they are neotenic and therefore fully aquatic a land area is not required. Metamorphosed individuals are generally not known to survive without specific husbandry conditions, although In the event of an individual metamorphosing see section **2.6**. There are three styles of housing used with success with this species detailed within this section.

Fig 13. A densely planted aquarium measuring 110 x 60 x 40cm housing up to four *A.dumerilii.* © A. Bland/Chester Zoo.

The most commonly utilised housing for this species in zoos is a large indoor aquarium (Fig. 13), this may be in the form of a glass aquarium, or a large non-transparent or darkened plastic container (Fig.14). The benefits of the plastic container method include cost, when simply housing specimens in an off show facility, ensuring the animals feel secure therefore reducing potential stress due to the darkened walls and when space is a limiting factor.

Plastic containers have been used with success with maintaining numbers of this species at the captive population in the *Colección Científica Nicolaita* (DGVS-CC-322-MICH/20) from *Facultad de Biología, UMSNH*. Varius sized plastic containers can used to accommodate all life stages of this species.

The glass aquariums will be commonly used when exhibiting specimens in public zoos or aquariums, and may also be used in an off show capacity. Due to the transparancy of all sides it is important that the aquarium is densely planted and includes refuge sites to aid in providing security.

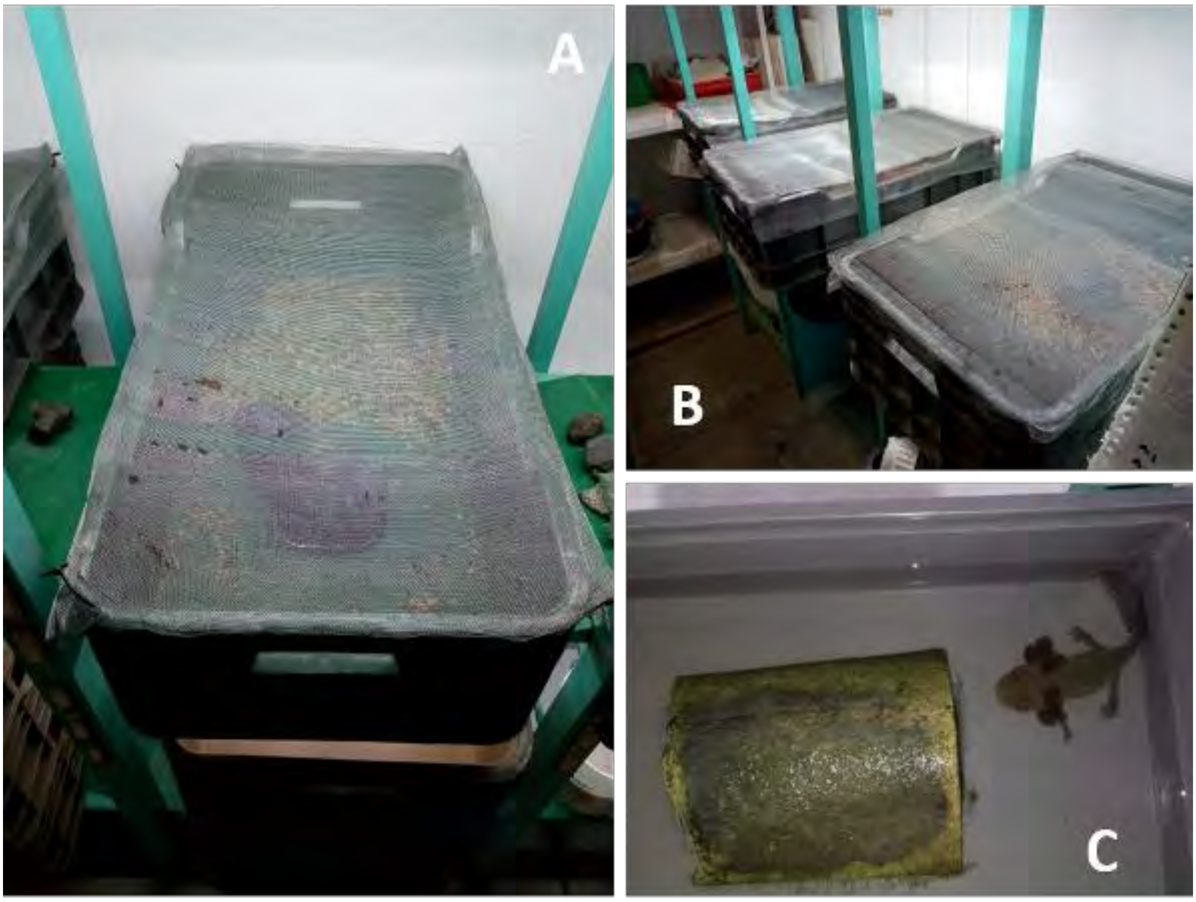


Fig 14. Plastic containers measuring 60 x 40 x 20cm. A) single plastic container; B) Module of containers; C) Adult female *A.dumerilii* within container. ©.Jose G. Delgado-Hernández/ *Colección Científica Nicolaita, UMSNH*.

A third housing methodology for this species is the use of artificial ponds under semi-controlled conditions (Fig. 15 A & B), due to biosecurity implications of this housing type, it is recommended that these enclosure are not outdoors when outside of the native locality of the species, and even in this instance secured from cross contamination of any native amphibian species. This kind of housing is utilized at several in country collections. If not outdoors, artificial ponds may be maintained within secured non-insulated buildings or green houses to ensure natural temperatures whilst ensuring biosecurity. Benefits of artificial ponds include lower maintanaince schedule due to the larger volume of water and a more natural “bio-active” environment and seasonality, another advantage of this bio activity is the development of Phytoplankton and Periphyton which promote the presence of zooplankton such as Rotifers, Cladocerans and Ostracods which provide a constant food source for larvae, juvenile and adult animals. As *A. dumerilii* spends most of its time at the bottom of the container, considerable height is not necessary (eg. ≤30cm); in fact, in order to facilitate monitoring and handling, it is recommended that the containers have a lower height.

Fig 15. Artifitial ponds. A) rectangular pond lined with plastic measuring 300 x 100 x 30cm, corresponding to the juvenile growth module of the Colección Científica Nicolaita, UMSNH; B) Circular ponds lined with plastic measuring 120 cm diameter X 40 cm heigh, corresponding to larvae and juvenile growth modules of the Jimbani Erandi colony. © Universidad Michoacana.

2.1.2 Substrate:

Ambystoma dumerilii can be maintained without substrate when maintained in glass or plastic aquaria. Although, if substrate is to be provided with this species fine sand can be used, and this must be rinsed thoroughly before introduction to aquaria. The wide mouths and voracious appetites of these salamanders makes them prone to ingesting stones, which can cause impaction of the gastrointestinal tract, therefore fine gravel substrates small enough to be injested must be avoided. Any other decorative stones used in aquaria must be of a size large enough not to be accidentally consumed by the salamanders. Bare bottom aquaria facilitate removal of faeces, uneaten food and general ease of maintenance; a layer of sand may enable a more natural look and form a more “bioactive” naturalistic aquarium, allowing aquatic plants to root and provide a substrate for aquatic invertebrates. These may provide food for the salamanders and also aid in the breakdown of waste.

If using artifictial ponds, the development of periphyton produces a layer of sediment which is used by individuals as refuge and favors the establishment of macroinvertebrates within the environmrnt. Although up to now the importance of this layer of sediment has not been assessed, the reduced occurrence of diseases (eg. absence of *Saprolegnia* and bacterial diseases) and the good health condition present in individuals, supposes that this layer must contain a good load of microbiota that can have a positive effect on the digestive system and on the skin.

2.1.3 Furnishings and maintenance:

Aquaria should be furnished with refugia such as stones, pipes and terra cotta, unglazed tiles arranged to form caves in which animals can shelter (Fig. 16). Any refugia should be immovable to avoid collapse. Enclosures can also be planted with aquatic plants; rooted forms may be frequently disturbed so floating species are most practical. A broad range of species can be used successfully, *Elodea sp*. are very useful for maintaining this species as they provide dense cover, are tolerant of low temperature and are also readily accepted as spawning sites by reproductive females.

Fig 16. Ceramic shelters as pictured make ideal areas or refugia within aquaria for *A. dumerilii.* © A. Bland

Sedimentary calcareous rocks such as limestone, tufa and chalk can be used to buffer water hardness, as well as providing refugia. Any furnishings should be free of sharp edges to avoid injuries to the animals; enough hides should also be present for all animals to seek refuge simultaneously.

2.1.4 Environment:

As this species occurs within a highland lake where the water temperature is generally cool, temperatures should be kept below 18°C and should not exceed 20°C for any length of time. In winter, temperatures should be reduced according to the natural temperature cycle. *Ambystoma dumerilii* continues to feed at temperatures as low as 10°C when maintained in a collection in the United Kingdom (C. Michaels, pers. obs.). In the *ex-situ* management units located at Patzcuaro city, winter water temperature ranges between 16°C to 18°C.

Temperatures above 22°C have been observed to present health problems in specimens of all life stages caused by infectious and nonifectious diseases, such as fungal infections and nitrogenous toxicity (See section **2.6** Health). In all management facilities, during spring and summer, invariably there is an observed increase in the prevalence of Saprolegniasis. Particularly in the Jimbani Erandi unit, another fungal disease also appears, and based on the pathology is associated with *Mucor* spp., another common fungal agent also recorded in *A. mexicanum* (Servin-Zamora, 2011). Low water temperatures can be maintained by either cooling the room in which aquaria are held or by the use of inline aquarium chillers. Be aware that electrical equipment including pumps and lights can raise aquarium temperatures significantly above room temperature.

2.1.5 Water:

As with all amphibians *A. dumerilii* presents a high permeability of the skin, which is vital for water absorption and respiration (Katz, 1986) therefore it is very susceptible to water toxicity, and also a variety of chemicals within the aquatic environmet may be problematic in both, *in-situ* and *ex-situ* conditions.

The chemical parameters of water considered more important for the health of captive amphibians (Odum and Zippel, 2011) are:

1) General Hardness. This is the measure of the amount of minerals dissolved in the water and depending on the basic requirements of the species, minerals can be incorporated, or on the contrary, water can be diluted (see below for the case of *Ambystoma*).

2) Dissolved Oxygen as O_2. The concentration of dissolved oxygen is relevant in particular for larval amphibians and aquatic adults, as the case with *A. dumerilii* and other neotenic species. It is also important to faciliatate the bacteria that metabolize the organic matter, and therefore maintaining a suitable environment.

3) Gas Supersaturation. This will mainly be by nitrogen and carbon dioxide, but sometimes even oxygen. This may occur during winter when the water is cold and capable of holding higher levels of disolved gases, and in *Ambystoma sp.* this can be the cause of the gas bubble disease (See section **2.6** Health).

4) Nitrogen as Ammonia/Ammonium NH_3/NH_4^+. Metabolic waste products derived from respiration, faeces and from bacterial decomposition of organic matter. This toxicity can induce metamorphosis in the case of neotenic species, or in the most extreme case, death of an individual.

5) Nirogen as Nitrite NO_2^-. This is formed in the aquatic environment by the oxidation of ammonia/ammonium by nitrifying bacteria, and presents a toxicity similar to that of free molecular ammonia and presents a toxicity compariable to that of ammonia.

6) Nitrogen as Nitrates NO_3^-. This is formed by the action of nitrifying bacteria on nitrites, and although this is substantially less toxic than ammonia and nitrites, it can produce sublethal effects on amphibians.

7) pH. This is the measures of the level of acidity and alkalinity of an aqueous solution, and although *Ambystoma* may tolerate a wide range (6.5 to 8.5 sensu Servin-Zamora, 2011), incorrect pH can have sublethal effects as a stressor.

8) Chlorine as Cl_2 and Chlorines. Chlorine is generally used as an antibacterial agent in municipal water supplies and may be present in concentrations of over 9 mg/l, which can be greater than the minimum lethal concentrations for many gill breathing animals.

9) Phosphates PO_4^{-3}. An excess of this chemical can have sublethal effects associated with neurological and osteological problems.

10) Copper. This is a very common component of potable water plumbing systems, that in synergy with other substances, such as ammonia, forms other chemical complexes that can be very toxic to many aquatic organisms.

Water chemistry should replicate conditions in Lake Pátzcuaro as accurately as possible, with relatively high pH and hardness. In areas where municipal tap water is sufficiently hard, this can be used provided it is treated first with water conditioner and/or carbon filtration to remove heavy metal contaminants and chlorine/chloramine. Although standing water will allow chlorine to gas off, heavy metals, chloramine and other toxic compounds will not be removed by this method. Ideally, and especially if well water (which is pressurised) is used, water should be allowed to stand for 12-24 hours before being added to aquaria to allow the pH to stabilise as dissolved gases equilibrate with the atmosphere.

Where tap water is not sufficiently hard, minerals can be added to tap or Reverse Osmosis water to increase hardness to desired levels. The simplest method is to dissolve Sodium Hydrogen Carbonate (also known as Sodium bicarbonate; $NaHCO_3$), but more complex solutions can be used (Table. 1).

Laboratories working with the ecologically similar and the more commonly kept species *A. mexicanum* typically use Holtfreter's or Steinberg's solutions (see Table. 1), diluted to 50% concentration to maintain their specimens (Kim *et.al.,* 1996). Some institutions modify these mixes; for example Indiana University Axolotl Colony omits $NaHCO^3$ from Holtfreter's solution and instead uses the same weight of Magnesium Sulphate ($MgSO^4$) (J. Claire pers. comm.). Until a specific solution to create water parameters in line with that of the lake are developed the use of solutions such as this are recommended. Chester Zoo's Pátzcuaro solution, current as of June 2021 (Table. 1), has been used with success maintaining Goodeid fish from Pátzcuaro (Goodwin, pers. Comm.) and is suggested as a possibility for better replication of water parameters closer to those found in Lake Pátzcuaro. Further trials maintaining *A. dumerilii* within these parameters are required to strengthen this suggestion.

Table 1. Recipe for 100% concentration of Holtfreter's and Steinberg's Solution commonly used to maintain colonies of *A.mexicanum,* and a Pátzcuaro solution developed and used by Chester Zoo for maintaining Goodeid fish.

Medium	Components to make 100% concentration (per litre of water); this is then diluted to 50% with RO water	Weight in grams
Holtrefeter's Solution	NaCl	3.46
	KCl	0.05
	$CaCl_2$	0.1
	$NaHCO_3$	0.2
Steinberg's Solution	NaCl	3.4
	KCl	0.05
	$Ca(NO_3)_2.4H_2O$	0.08
	$MgSO_4.7H_2O$	0.205
	Tris (a sort of buffer)	0.56
	Add HCl to pH 7.4	
Chester Zoo Pátzcuaro Solution	Na_2CO3	0.287
	$MgSO_4.7H_2O$	0.0014
	$KHCO_3$	0.150
	$CaCl_2$	0.059
	$MgCl_2$	0.149
	$NaHCO_3$	0.456

As mentioned above, high water quality should be maintained to avoid the presence of ammonia (the principal waste product), nitrite and minimise concentrations of nitrate in aquaria. This can be achieved through the use of biological filtration (see section 2.1.6) and appropriate water changes. Frequency and magnitude of water changes will depend on circumstances, but ideally no more than 10-20% should be exchanged at a given time and it is preferable to do frequent, small water change rather than infrequent large changes. Water used to top tanks up should be similar in chemical parameters and temperature to the water already in aquaria.

2.1.6 Filtration:

Ambystoma dumerilii are messy feeders and produce large amounts of waste; hence appropriate biological and mechanical filtration is required for their proper maintenance.

Suitable filters include external canister filters, internal box and airstream sponge filters for small and medium tanks. Very large systems or ponds might be filtered using bubble-bead, sand or other similar large filters. Filters should be cleaned in aquarium water at intervals according to the manufacturer's advice. It is very important when setting up filtration for this species to ensure that water flow rate is not too high, as this species occurs in still water and high flow rate causes stress and will therefore have a negative impact on the health of the animals. In this respect, the previously mentioned airstream sponge filters (Fig. 17) work remarkably well in different sizes for all life stages of this species.

Fig 17. Air line sponge filters as pictured above are ideal for producing biological filtration without causing a high flow rate within the aquarium.

Biological filtration is critical in maintaining good water quality. Nitrifying bacteria are cultured in filter media and convert highly toxic ammonia to less toxic nitrite and finally even less toxic nitrate. These bacteria will also establish themselves on substrate, décor and even aquarium glass but filters provide the necessary high surface area to deal with the ammonia load produced by *A. dumerilii*. Large bacterial cultures should be present before animals are added to tanks and these can be established, or matured, in several ways. Mature media from existing aquaria can be transferred to new systems; this can be particularly useful if splitting existing groups of animals or setting up systems for offspring. If this is not an option, either because there are no existing systems or because new systems need to be biosecure from existing aquaria, biological filters can be grown from scratch (see Odum and Zippel, 2008 for methods). The latter method is much slower and more difficult to achieve.

Recently hatched larvae may also be maintained during the early periods with no filtration, in small containers of standing water (Fig. 18), although it is necessary when using this method to replace at least 50% of the water every 24 hours to prevent toxic waste accumulating in the environment. this is an exception to the general recommendation for water changes detailed in **2.1.5** "water" which only applies in this circumastance.

Fig 18. Intensive larvae production using small plastic containers of standing water. A) Larvae growth module in Colección Cientifica Nicolaita, UMSNH; B) Three week old larvae. © Jose G. Delgado-Hernández/ *Colección Científica Nicolaita, UMSNH*.

Live plants also use nitrogenous waste as a nutrient source and will remove ammonia, nitrite and nitrate (in that order of preference) from the water column. Vigorously growing live plants can significantly boost the ability of a system to process waste products and should be included in aquaria for axolotls where possible.

Zeolite may also be used within filters to absorb ammonia in place of a biological filter; regular water tests should be used to detect when the medium has become saturated and needs to be changed. To ensure water quality and parameters are maintained in stable conditions it is important that routine water testing is undertaken weekly, this can be achieved by using standard aquarium chemical drop tests, dip tests or by the use of digital readers. Each method offers a varying degree of accuracy.

2.1.7 Dimensions:

Aquarium dimensions will vary according to the size of animals housed within and the capacity of filtration systems. However, as these animals are largely benthic, aquaria should maximise floor area providing that water depth is deeper than the total length of the largest individual. See Section **2.1.**

2.1.8 Lighting:

This species should be dimly lit, as they are reasonably photophobic, using lamps that emit little heat, such as T5 fluorescent tubes or LEDs. If brighter lighting is to be used, sufficient plant cover must be present to allow individuals to regulate their own exposure to light. The exposure level of UVB irradiance for this species is unknown in nature, due to aquatic plants and turbidity, but in any case, these animals do not appear to require this lighting to remain healthy over many generations in captivity. Providing adequate lighting is important in creating the photoperiods necessary to signal seasonality to the salamanders, these seasonal changes are considered an important factor in successful reproduction. Although it is not known how important UVB rays can be in *Ambystoma* (as in the case of reptiles), it has been observed that in artificial ponds, individulas of *A. dumerilii*, at some time of the day settle in a space where it reaches direct sunlight. For this reason it may be beneficial to utilise a UVB emitting spot lamp in one focal point above aquaria when maintained indoors, to allow specimens some self regulatory exposure to UVB. It is recommened that UVB provision be measured using equipment such as a Solarmeter 6.5 ® to ensure animals are not exposed to harmful levels UVB exposure. Although natural exposure levels of UVB in nature are unknown, it is generally not recommended to expose indivuals to a UVB index higher than 2.0 in one area until additional data may further inform this environmental parameter.

2.2 Feeding

These salamanders readily feed on a variety of live or frozen thawed food items in captivity, loss of appetite is normally attributed to ill health or potentially reproductive activity. Live food items are preferable as they have little effect on water quality and movement of the live prey attracts the salamanders.

2.2.1 Basic diet:

Larvae of this species should be offered suitably sized live invertebrate prey, including, but not limited to, *Daphnia*, *Cyclops*, assorted infusoria, *Artemia* (washed to remove salt), grindal (*Enchytraeus bucholzi*) and white (*Enchytraeus albidus*) worms, micro and banana-worms (*Pangrellus* spp.) and *Tubifex*. Larger animals will continue to feed on invertebrates of increasing size, but will also begin to accept prey based on smell as well as movement and can be offered strips of dead fish, specially formulated axolotl pellets (many fish pellets contain iodine, which may induce metamorphosis) and dead shrimp, crayfish and prawns. In addition to live invertebrate prey including earthworms, glassworms (*Chaoborus*), waxworms, crickets, locusts, and snails (whole or crushed). If possible food analogous to the wild diet of *Cambarrellus* sp. should be offered, or *Cambarrellus* sp. themselves if possible, if not possible due to regional difficulties species of river shrimp (*Palaemon varians*) are readily accepted and offer a food option similar to that of the wild, sourcing of these should be considered as they are not often reproduced regulary in aquaria.

Important: animals offered freshwater fish (catfish, *Tilapia,* trout) and rodent (mouse) as a substantial portion of the diet are known to develop oedema/ascites as a result of kidney failure, which is linked to excessive protein content in the diet. Therefore, vertebrates should only form a small amount of the diet.

Although bloodworms (Chironomidae) are readily eaten, they are usually passed undigested and should not generally be used as a frequent food source. Larger salamanders will also accept meat products such as pinky mice and beef heart strips. However these are high in fat and protein and should not be used as a staple food source.

Animals of all sizes will regard conspecifics of smaller size as potential prey, especially when hungry; therefore it is vitally important when rearing juveniles of this species that close attention is paid to the sizes and density of the specimens being maintained together. Initial growth rates are fast and cannibalism is a common behaviour even in the presence of invertebrate food items. If individuals are not eaten completely by larger siblings, it is commonplace for smaller salamanders to present with missing limbs or damaged fins in particular the tail tip. Although not ideal, if separated missing limbs are usually regenerated without issue, although energy used to regenerate limbs when young may slow general growth rates.

Although stomach contents of animals in nature include algae and silt, these do not represent part of the diet and are only consumed along with food items by mistake.

2.2.3 Method of feeding:

Small larvae should be presented with live foods added to the aquaria in large enough amounts that there are always prey items available. This will limit cannibalism and maximise growth rates.

Larger animals may be scatter fed, especially when housed individually, but this may cause indiscriminate biting among groups of animals and lead to inadvertent injury. Therefore, larger animals are best tong fed where possible, or smaller food items provided in several different spots to minimise contact between animals during feeding. One to two food items per individual should be offered for adult specimens at a frequency of every other day. During winter food intake may decrease, but many ambystomatid salamanders seem to readily eat throughout all seasons. Although care must be taken not to overfeed in one sitting, as this species will voluntarily continue to feed and too many items in one sitting may be regurgitated.

2.3 Social structure:

A.dumerilii have no particular social structure.

2.3.1 Basic social structure:

As above.

2.3.2 Changing group structure:

As *A. dumerilii* has no particular social structure animals may be moved between groups without social implications. Increased interest may be initially shown in new individuals, and new individuals will show increased activity whilst they adapt to their new environment.

2.3.3 Sharing enclosure with other species:

Mixed species enclosures including *A. dumerilii* are not recommended. *Ambystoma dumerilii* are highly predatory and will consume or attempt to consume any other animals smaller than them; in the case of some spined fish (e.g. *Corydoras*) or toxic species, this can lead to the deaths of both animals. Many fish species will attack the gills of axolotls and can cause severe injuries. Many parasites and pathogens of fish are also communicable to aquatic amphibians and this presents an additional risk.

2.3.4 Population management:

Ambystoma dumerilii is listed as an EAZA EEP species and therefore specimens maintained in European Zoos and Aquariums are part of a managed breeding program, as the European population originates from relatively few founding animals it is important to make efforts to reduce levels of inbreeding. In Mexico there are also several in-country captive populations in the state of Michoacán, which also form part of a managed breeding population to ensure the survival of the species. These institutions are outlined within section **1.5.4**, examples of these facilities can be seen in Fig. 19 a & b. These facilities are based locally to Lake Pátzcuaro. The largest captive population of this species currently held is at the Monastery de Dominicas de Orden Predicadores (Fig. 19b), where a group of nuns are dedicated to the management and protection of this species.

Populations may be managed by housing adult animals in same-sex groups or individually to prevent unplanned breeding. Unwanted spawn may be destroyed by freezing if removed immediately or by immersion in MS222 if it has already developed. Larvae left to hatch in adult enclosures will almost certainly be eaten. Although this species will spawn when housed in 1.1 pairs, sperm storage is also possible and so parentage may not be guaranteed as observed in other species.

Hybridisation between *A. dumerilii* and *A. mexicanum* is possible (Brandon, 1972; 1977) and is probably possible between *A. dumerilii* and *A. andersoni,* as well as with other *Ambystoma* species within the *tigrinum* species complex. Although many hybrids metamorphose and die, some do not and are capable of reproducing. Given the situation of wild populations and the small captive populations of genetically pure animals it is not advisable to produce hybrids in captivity unless for specific research purposes. In these cases, hybrids should not be allowed to enter the general captive population.

Fig 19. a) Breeding center Centro Regional de Investigaciones Pesqueras, Pátzcuaro, and b) Monastery of the Dominican Order in Pátzcuaro. © A.Bland.

2.4 Breeding

Reproducing patterns of temperature, photoperiod and freshening of water bodies to simulate rains is key to breeding this species in captivity. Once appropriate seasonality has been provided breeding attempts may take place, these usually are most effective during the start of winter in November and at the beginning of spring as temperatures and photoperiod again begin to change. In a captive setting with accurate climate control equipment such as aquarium chillers and timed artificial lighting, breeding attempts may be attempted throughout the breeding season by manipulating the environment, primarily temperature and light. The separation of males from females outside of the breeding season is important in stimulating breeding once sexes are reunited; animals may be introduced to one another's aquaria, or introduced simultaneously to a specific breeding tank where the male is removed after courtship, and subsequently the female following oviposition.

2.4.1 Mating:

Salamanders do not mate in a traditional sense; there is no form of copulation between the sexes. Instead, following a series of courtship behaviours as previously described, the male deposits a spermatophore, which is then collected by the female via her cloaca. Eggs are then internally fertilized prior to oviposition. Conspecific males may become intolerant of one another during periods of courtship and reproduction. This may result in bites, which are capable of creating wounds, these usually do not present much risk to the overall health of the specimen due to the regenerative capabilities of the species, nevertheless if injuries occur it is important to closely monitor the salamander and maintain good quality water parameters to prevent infection.

2.4.2 Egg laying:

Several hundred to a thousand or more eggs are laid individually or in loose groups on aquatic plants and other aquatic structures, where they are weakly stuck. In rare cases some females will produce small strings of eggs; these usually develop normally. In normal circumstances, a female will deposit all her eggs over a 24 or 48-hour period where she is best left undisturbed. Small numbers of eggs deposited randomly are usually non-viable.

2.4.3 Hormone induced reproduction:

There is currently no requirement for hormone-induced reproduction in this species, as providing the seasonality requirements are provided reproduction usually takes place willingly.

2.4.4 Hatching:

Eggs are either best removed from aquaria and incubated in shallow trays of fresh water of the same parameters of the spawning aquarium, which is refreshed at regular intervals of at least every 24-48 hours, allowed to develop in a mature aquarium separate from adults, or left to develop in the spawning tanks with the adults removed to prevent damage or predation. The disadvantage of the latter method is that upon hatching larvae will require moving to small aquaria and this risks causing them damage. Depending on temperature, following a developmental period of two to three weeks' larvae will begin to hatch by vigorous movements within the egg that cause the breakdown of the egg capsule. Once free of the egg the larvae are then free swimming but a large yolk reserve still remains.

2.4.5 Development and care of young:

Eggs are abandoned immediately after oviposition and there is no parental care. It is recommended to remove adults from aquaria containing eggs as they will willingly feed upon them and are likely to cause general displacement or damage to developing spawn.

During the initial days following hatching larvae spend much of their time sitting motionless on the floor of aquaria where they absorb the yolk reserve and continue development, this may take up to seven days and during this time they do not feed and therefore should not be offered food as this may cause unnecessary stress. During this stage it is also important that the larvae are not moved or disturbed unless absolutely necessary, as they are incredibly delicate and unnecessary manipulation can cause damage or even death of individuals. The first weeks of life in larvae are critical for their survival and development and this period is considered to be the more complex stage for management mainly due to 1) They have to be fed with large amounts of small sized live foods; 2) High stocking densities and heterogeneous development promote cannibalism, and 3) Wounded larvae caused by bites of other larvae favour the attack of pathogens leading to disease. In the Colección Científica Nicolaita experiments have been undertaken in order to determine the optimal larvae density during the first weeks of life, and the results showed that during the first eight weeks, 5 and 10 larvae in a container of 25 x 18 x 6cm with a volume of one liter of water were the optimal density, these individuals showed normal development and increased survival rate (Fig. 20)

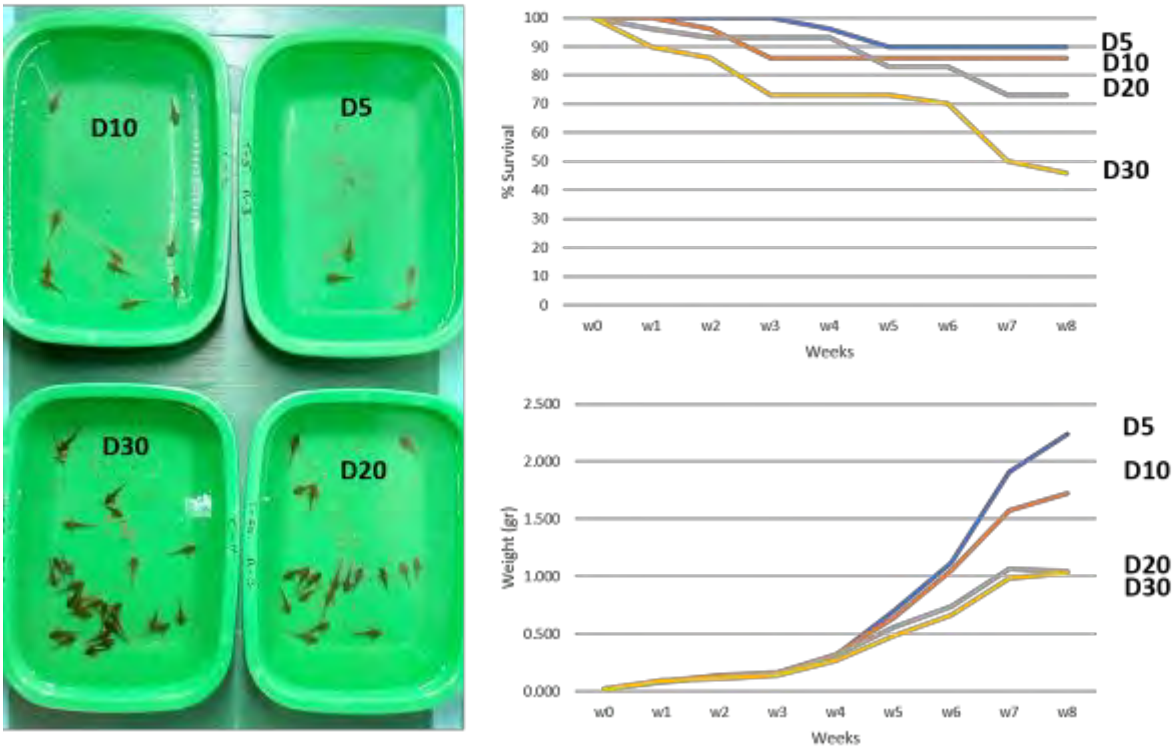


Fig 20. Experiment for optimal larvae density. D5= five larvae per container; D10 10 larvae per container; D20= 20 larvae per container; D30= 30 larvae per container. © Universidad Michoacana.

Specimens should be housed in small containers filled with water from mature aquaria and of appropriate parameters (Fig. 21), if biological filtration is to be used it should be in the form of an air line sponge filter which aerates water without causing water movement; excess water movement will be detrimental to small larvae. If no filtration is to be used water in containers must be replaced every 24 – 48 hours and waste removed, this can be achieved using a small pipette or siphon. Once yolk is absorbed and feeding begins, live foods must be continuously maintained in containers providing constant feeding opportunities; live food items outlined in section **2.2.1** should be used and size of invertebrate prey can be increased in accordance to growth of larvae.

Fig 21. Newly hatched larvae of *A. dumerilii* that have just begun to feed. © A. Bland.

Container size should be increased according to growth until young may be housed in more standard aquaria at which point husbandry generally follows that of adults albeit with an increased feeding regime. Initial growth is fast, and individuals can reach sub adult sizes within 8 – 12 months. A group of larvae (N = 310) marked with visual implant elastomer (VIE) had growth rates monitored by weight, starting with an average weight of 4.08g, this increased to an average weight of 39.64g over a five month period (Fig. 22).

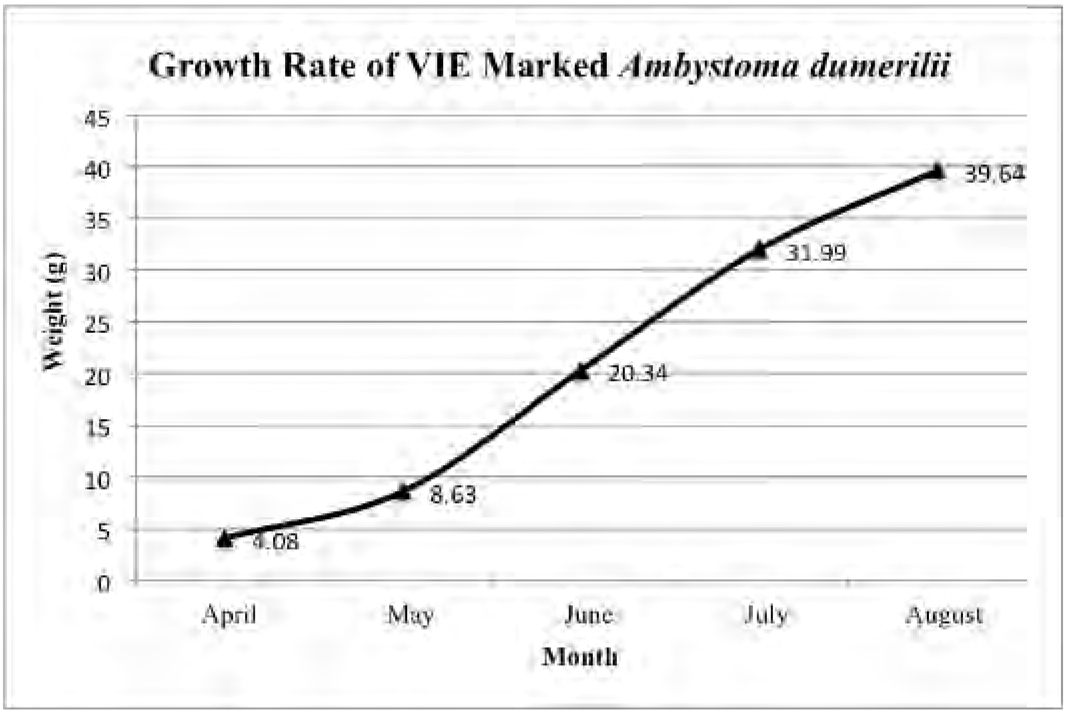


Fig 22. Growth rate in weight (g) of N= 310 larval *A. dumerilii* over a five month period. © A. Bland unpublished data.

When rearing larvae of *Ambystoma* sp. the most commonly encountered issue is likely to be cannibalism. Unless larvae are housed individually, which in many instances may not be practical, a certain level of cannibalism may be unavoidable, but this may be reduced by closely monitoring stocking densities and growth rates; only house specimens of similar size together, as these salamanders will consume any conspecific that will fit into the mouth. Cannibalism may result in total loss of individuals, or lead to specimens with missing limbs or nipped tails (Fig. 23). Once the skin is broken this leaves salamanders vulnerable to fungal skin infections that again may result in loss of animals. Minor injuries are usually of little consequence as damaged skin and limbs will be regenerated, but this may have a negative effect on the growth rate of the larvae during this stage. Groups may go through phases of cannibalism where the behaviour is particularly persistent; it may also be observed that when specimens attack one another it may cause a short period of frenzy type behaviour where injury may occur to multiple animals. During periods such as this keepers must be vigilant with observation and remove and regroup specimens accordingly.

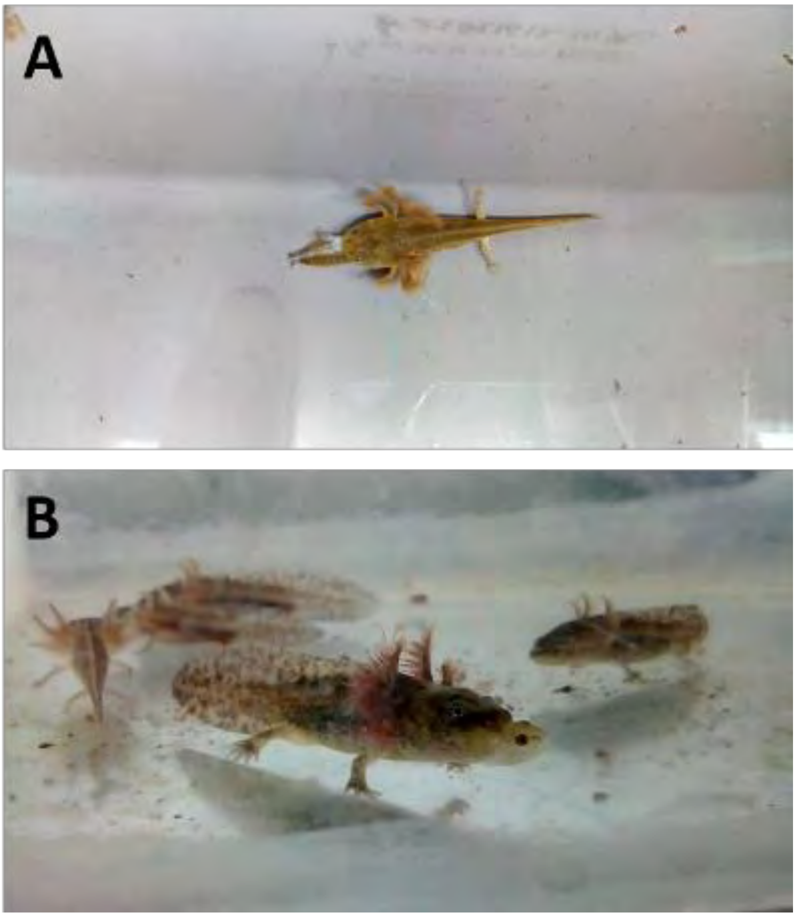


Fig 23. Cannibalism in larvae of *Ambystoma*. A) cannibalism in larvae of *A. dumerilii*; B) cannibalism in larvae of *A. andersoni*.

2.5 Handling

2.5.1 Individual identification and sexing:

If identification of specific individuals is necessary they are best individually identified using artificial marking techniques, as skin patterns are subtle and subject to change both through ontogeny and metachrosis. The method of marking individuals will depend on life stage of the animal; typically with young animals being marked using visual implant elastomer (VIE) and adults using subcutaneous passive integrated transponder (PIT tag). Both methods have been used with success to individually identify salamanders in varius studies (Marold, 2001; Bailey, 2004; Whitman *et.al*, 2016).

Fig. 24 Larva of *A. dumerilii* is gently restrained within soaked paper towel and VIE injected sub-cutaneously. © Chester Zoo.

In order to implant VIE salamanders should be gently restrained out of water, this is best undertaken by resting the animal within soaked paper towel using aquarium water, the VIE is then injected sub cutaneously at the chosen site (Fig. 24). Fig. 25 shows larvae marked with Visual Implant Elastomer (VIE), which involves the injection of a small amount of fluorescent plastic elastomer under the skin, which illuminates under UV light. The position and colours are varied to identify multiple individuals. VIE should be injected in areas of lighter skin where pigments are less likely to obscure the tags. Suitable areas on small individual are within the transparent areas of the dorsal fin and tail, although this is a less reliable method of marking in this species in the long term, as the mark will be obscured through the dorsum as further pigmentation develops during growth. This method of marking also has a lower retention rate in this species, therefore VIE is more suitable for short term monitoring of small individuals until PIT tagging is a suitable option. For managing populations VIE marking may be used for larval individuals if necessary, for example, if it is required to identify individuals or groups of specific parentage and genetic origin, but this may not be necessary if groups of individuals of the differing genetic origin can be housed separately.

Fig 25. A Juvenile *A. dumerilii* marked dorsally with VIE illuminated with a UV light. © A.Bland.

Sub-adult and adult individuals may be marked via subcutaneous PIT tag, this method is slightly more invasive than VIE tagging, though done properly no negative health effects have been observed following tagging thus far in this species, it therefore is important that this is carried out only by experienced keepers or under veterinary supervision. Due to the more invasive nature of PIT tagging, involving the breaking of the skin, it should be noted that although not yet observed in this species, that is had been recorded that the breaking of the skin in amphibians during marking can disrupt skin micro biome temporarily (Antwis *et al.,* 2014a; 2014b), and therefore this must only be performed on healthy animals by experienced keepers. The tag used with success with this species is a Trovan ID-162B/1.4 Animal Implant Mini Transponder, which is 8mm in total length and 1.4mm in diameter. Although this tag has been used with success in this species, smaller alternatives such as Biolog nano PIT tags measuring 7mm in total length and 1.6mm in diameter or similar may offer a slightly less invasive alternative.

The PIT tag is implanted subcutaneously entering dorsally and positioned laterally directed downward placing the tag within a costal fold (Fig. 26), this position reduces migration of the tag and also prevents the tag loss whilst the entry site heals, as the tag is held in place by gravity. This method requires two handlers, one to restrain the animal whilst the second implants the tag. This has shown to be a reliable method of individually identifying adult individuals (97.5% retaining tag N=80 >175 days, Bland, unpublished) and thus considered beneficial to their management in captivity and additionally to facilitate field surveying via mark recapture.

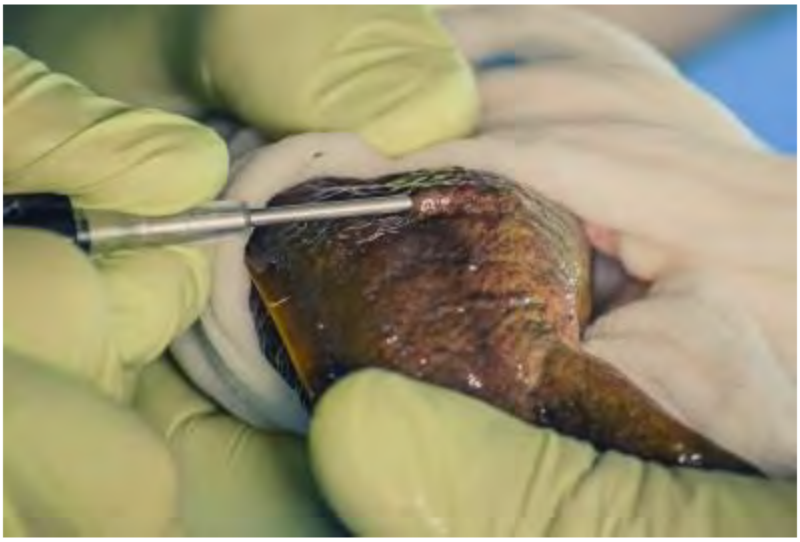


Fig 26. PIT tagging of adult *A. dumerilii*, tag is placed laterally within a costal fold. © Chester Zoo.

Once adult, this species is relatively easy to sex. Mature females are generally heavier bodied than males, although the key feature in distinguishing sexes is the shape of the cloaca as illustrated in Fig. 27. . In non adult specimens, there are no notable characterisitcs to indicate sex.

Fig 27. Swollen cloaca of male (Left) is clearly noticeably in comparison to the cloaca of female (Right). This may be more pronounced in males during the breeding season. © A. Bland.

In the male, the cloaca is generally swollen and rounded in appearance, which may be more developed and evident during the reproductive season, whereas in contrast the female's cloaca has no notable swelling, although a small amount of swelling may be observed during egg laying, in contrast to the male this is very minor. If unsure of sex of individuals, it is best to directly compare specimens if possible, if mature the differences between the sexes cloacas should be evident as in Fig. 27.

2.5.2 General handling:

Animals should not be handled unless entirely necessary; in these instances follow the guidelines on catching and restraint, below.

2.5.3: Catching / Restraining:

This species should be manually caught and restrained as little as possible. It is preferable to capture and move animals in containers of water to avoid damage to skin and gills. Larvae can be captured and transported using turkey basters, siphons or large pipettes. Larger animals can be captured by hand, locking forefinger and thumb around the neck of the animal to prevent escape while the rest of the hand provides support (Fig. 28). Nets should be avoided if possible as the fabric can abrade skin and gill tissue. Animals should be out of the water for as little time as possible to prevent desiccation of skin and gill tissue. Providing hands have been cleaned, when restraining clean wet hands will suffice for brief restraint, for extended purposes and in the instances of medical examination or PIT tagging where the skin is to be broken, powder free Nitrile gloves can be worn to maintain a level of sterility.

Fig 28. Manual restraint of *A. dumerilii*. © A. Bland

Should the animal need to be restrained on a surface, it should be placed on wet paper towel in an environment without strong airflow in order to minimize evaporative and capillary water loss. Animals can be restrained by pressing the head down gently but firmly with a flat hand and using the other hand to restraint the pelvic girdle and tail.

Any extended procedures should be carried out under sedation. Animals can be sedated for surgery by constant immergence in MS222 solution in 50mmg/l of water as described by Burns. *et.al* (2019) for surgery in *A. mexicanum*. Sedation for non-surgical anesthesia, for example for use in blood sampling, an aqua sed solution of 2ml/1l of water has proven effective (J.lopez, Pers.comm). Procedures should occur in a tray of shallow, oxygenated water of similar chemical parameters to the home aquarium. Alternatively, the animal may be placed on wet paper towel and periodically dipped, sluiced or poured over in water to prevent desiccation of tissues (Fig. 29).

Fig 29. Anaesthatised specimens are placed in a tray containing wet paper towel and are periodically re moistened with water to prevent dessication. © A. Bland

2.5.4 Transportation:

Post-hatching *A. dumerilii* are best transported in water, similar to fish. Traditional bags should be filled partly with air and partly with water (Fig. 30a), while modern breather bags should be used preferentially and should be entirely filled with water as the membrane allows gases to pass through. Any bags or other transport containers should be packed securely in insulated boxes (Fig. 30b). Chill packs or air conditioning should be used if the boxes will be exposed to high temperatures; heat packs are rarely necessary, but animals should not be allowed to become much colder than 10°C. Transportation should not occur during periods of very warm or very cold weather unless absolutely necessary, or unless climate control can be guaranteed throughout the journey.

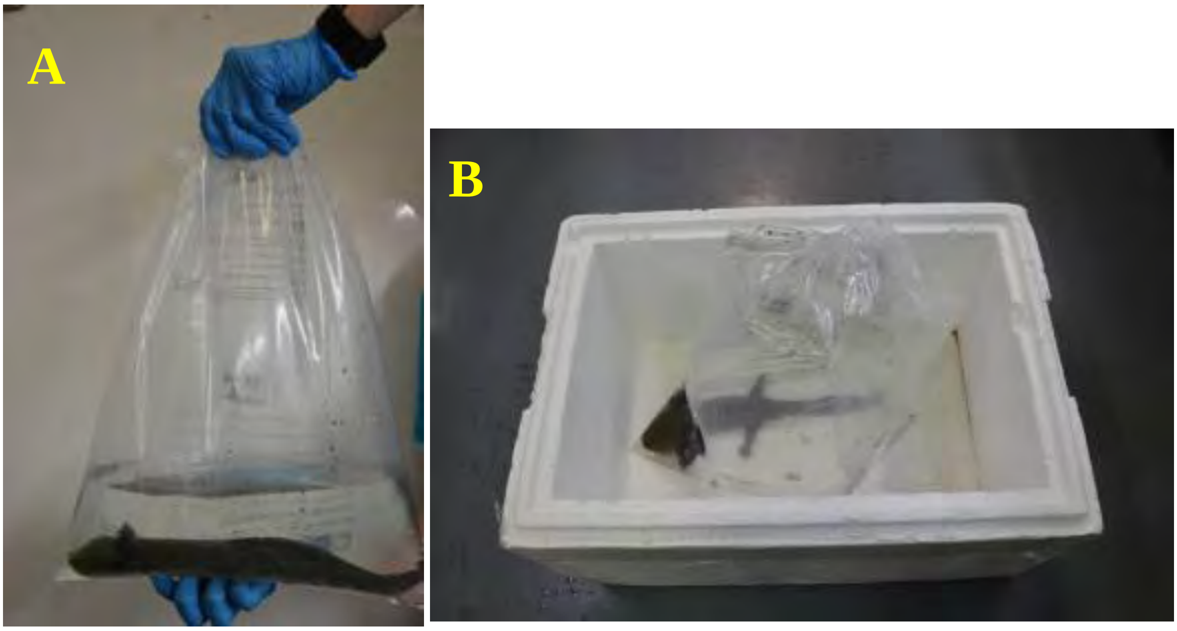


Fig. 30. (A) Bagged individual for transportation, these are then placed within secured insulated boxes (B). © A. Bland

Animals should not be fed for at least 24 hours before transportation to reduce the risk of contamination of transport containers with waste products; ideally for adults, no food should be provided for 5-7 days prior. Animals should be transported individually to avoid cannibalism or injury during transit.

On arrival, animals should be allowed to slowly acclimatize to ambient temperatures and water parameters through the use of a drip syphon. As a rough guide, pH should change by no more than 0.1 and temperature no more than 1°C per hour.

2.5.5 Safety:

2.6 Specific problems: considerations for health and welfare

Ambystoma dumeriliii may occasionally spontaneously metamorphose. This is more common in male animals and typically occurs at sexual maturity, or due to stressors including high temperatures, inappropriate diet or illness. This species has lost the ability to produce adult amphibian skin proteins (Brandon, 1976) and therefore cannot osmoregulate effectively if it becomes terrestrial; it also continuously sheds fine flakes of skin as a metamorphosed animal (Michaels, pers. obs.). The limited scientific literature suggests that metamorphosed individuals typically die within a few months (Brandon, 1976); however in these instances animals were maintained in terrestrial conditions. Animals maintained in a shallow aquatic enclosure with water just deeper than the height of the animal lived without issue for nearly two years until death occurred from a husbandry error. It is unclear if different metamorphosis triggers may result in varying degrees of viability. However, the reason for spontaneous metamorphosis is not always clear, but high temperatures and poor water quality can be significant contributors, although the phenomenon does also occur under apparently good environmental conditions. It was observed by Brandon (1970a; 1976) that an increase instance of spontaneous metamorphosis occurs during reproductive seasons where hormonal changes or activity are taking place.

Neoteny is a result of thyroid inactivity and metamorphosis can be artificially induced in part by administration of iodine (Swingle, 1923) or thyroxine. Food high in either chemical, including many brands of fish pellets, should therefore be avoided. Purposeful induction of metamorphosis is not considered ethical as it results in compromised animals, which do not occur in nature.

Most diseases observed in amphibians maintained in captivity are related directly or indirectly to the husbandry and management of the species; these can be noninfectious (eg. Tumors, nutritional deficiencies or overloads, chemical toxicity and inadequate husbandry or environmental management) or infectious caused by several agents (eg. bacterial, mycotic and mycotic-like, protozoan and metazoan parasites) (Densmore and Green, 2007). In Mexico, captive colonies of *Ambystoma* have reported the following diseases as the most common (Servin-Zamora, 2011).

Noninfectiuos Diseases: Skin irritation from poor water quality; injuries caused by objects or congeneres; ingestion of foreign bodies; gas bubble by gas supersaturation, and tumors or malformations.

Infectious Diseases: Mycotic diseases such as saprolegniasis, and Chytridiomycosis; bacterial diseases as the red leg by *Aeromona hydrophila*, *Flavobacteriun* sp., *Pseudomonas* sp., *Citrobacter* sp. and *Enterobacter* sp., and Chlamydia by *Aeromona* sp.; viral diseases such as ranavirus that cause similar signs to bacterial diseases, and parasitic diseases such as the copepod *Lerneae* spp., the protozoos Opalina spp., *Costia necatrix*, *Protoopalina* spp., *Proteromona* spp., *Chilomastix* spp. and *Balantidium* spp.

Individuals showing signs of infectious disease must be immediatley isolated from cohabitants for treatment and the remainder of the group monitored for signs of infection. Some diseases such as red leg syndrome may even present as sudden death, with few or no overt signs of infection (Densmore and Green, 2007).

In *A. dumerilii* the disease observed most commonly in captivity is the infection of the skin and gill fimbriae with *Saprolegnia* water mould (previously considered a fungous) (A. Bland, pers. obs.), illustrated in Fig. 31, and can quickly overcome a weakened individual leading to death or euthanasia on ethical grounds. The root cause of such infections are not entirely understood but it is observed that outbreaks occur during times of stress, for example during reproductive seasons, stress caused by high stocking density, or if maintained within the incorrect environment such as high temperatures (>22°C), low pH value (<7) and poor water quality. Outbreaks of *Saprolegnia* do not appear to be infectious amongst individuals but instead reflect an underlying health issue with an individual. Therefore treatments of such infections have mixed success if the underlying issue is not identified and rectified if possible. Mild infections may include a small amount of “cotton wool” like growth attached to a gill, this may gently be removed and in some cases require no treatment and infection may not reoccur. Severe infections are considered to be when large clumps of *Saprolegnia* are appearing on the gills, removal of which removes fimbriae from rachis and causes visible damage or disfigurement of gill and when removed reappear within a short time period. Infections may appear upon the dorsal surface, this appears to be displayed usually in severely immunocompromised individuals.

Fig. 31 A compromised male individual of *A.dumerilii* presenting with *Saprolegnia* infection on the dorsal surface of head and gill fimbriae. This specimen also displayed oedema of the thoracic cavity highlighting an underlying health condition. © A. Bland/Chester Zoo.

Effective treatments remain very much experimental and have mixed success. Minor infections may be corrected without medical treatment of the individual if environmental trigger/stressor can be identified and corrected. Anecdotally, many minor infections appear to be most likely linked to the incorrect environment (high temperature and incorrect pH) that can easily be corrected, severe infections are usually a sign of a serious underlying health condition.

A second commonly encountered health issue is oedema and ascites as mentioned in section **2.2.1** (Diet), which presents typically with an abdomen bloated and distended with fluid which causes buoyancy issues, usually due to kidney failure. Once this condition is presented there is as yet no succesfull treatment and euthanasia is the best option. This issue is highly likely linked to diet, therefore the best prevention is through proper dietary management of the species.

Ambystoma dumerilii are known to be capable of carrying *Batrachochytrium dendrobatidis* (Bd) without displaying signs of clinical disease (Michaels *et al.*, 2018), therefore it is of utmost importance that adequate testing is undertaken when acquiring new individuals into an established collection. Bd loads in the salamanders can be very low which can affect detection rates. It is the recommendet that any new specimens are swabbed for Bd and Bsal infection three times, one month apart, in order for optimum detection (A. Weissenbacher, pers. comm.). When swabbing this species for Bd, particular attention should be paid to the keratinised mouthparts, as these act as focal areas for Bd and Bsal and therefore aid in an increased detection rate (see Appendix **3.2**). If specimens of *A. dumerilii* test positive for Bd, chemical antifungal treatments may be used to clear infection following methodology detailed by Michaels *et al.* (2018).

In wild specimens observed during surveying the wild population in Lake Pátzcuaro introduced ectoparasites are commonly observed attached to the gills. These can be removed by gently restraining the animal in hand and using fine tweezers to remove the parasites (Fig. 32). These parasites are believed to have been introduced to the lake via non native introduced fish species.

Fig 32. Manual removal of introduced external parasites of wild collected *A. dumerilii.* © Universidad Michoacana.

2.7 Recommended research

Compared to the commonly kept Axolotl, *A. mexicanum,* the specific reproductive triggers of *A. dumerilii* are less well understood, and the development of specific detailed protocols to reliably induce reproduction would be useful. This species also appears to produce a higher number of infertile eggs when compared to similar species, whether this is caused by environmental of genetic factors may require further investigation.

Neotenic ambystomatids such as *A. dumerilii*, have been reported by some individuals to be highly sensitive to the mineral and salt content of aquarium water, and the development of species-specific optimal recipes for artificial solutions in which to house animals would be welcomed. Investigation and development of optimal water parameters links also to investigating the relationship of specific water parameters such as pH and temperature and how they relate to outbreaks of *Saprolegnia* of the gills and dorsal surface. This would benefit the husbandry and welfare of this species in captivity, as this is usually the most commonly encountered health issue. Additionally, reliable, safe and effective treatments for such infections are in need of development in order to remove the uncertainty and experimental element of attempting to treat *Saprolegnia* infections in salamanders.

Preliminary bioassays using saline solutions as permanent media for rearing larvae to combat *Saprolegnia* infection based on common salt NaCl (At several concentrations: 2.5%, 5% and 7.5%), showed that permanent exposure results in eventually lethal effects, even with the lowest concentration, less than 50% survival was obtained (Fig.33).

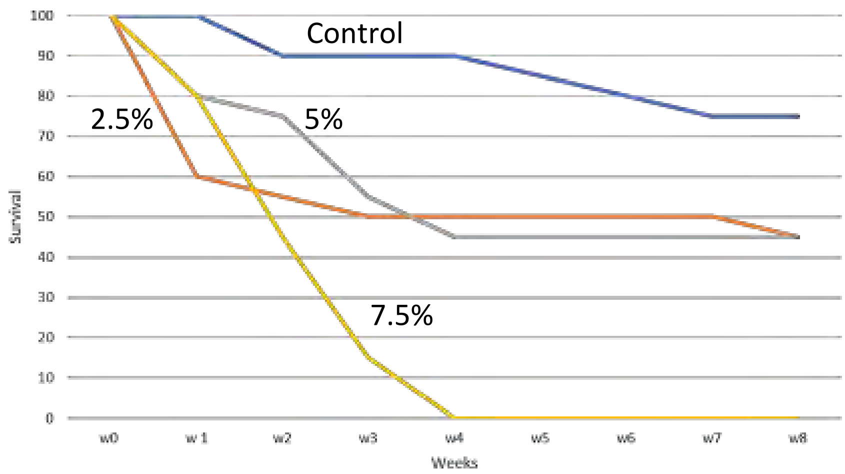


Fig 33. Survival in larvae of *A. dumerilii* during the first weeks using several concentration of saline solution based on common salt (NaCl). © Universidad Michoacana.

These results suggest that the poor ion content of the common salt (NaCl), rather than helping the osmotic balance, affected its performance. On the contrary, the successful use of ion-rich saline solutions such as the holtsfreter solution as a permanent medium in *A. mexicanum* larvae (Blancas-Arroyo *et al*., 2014) indicates that the same result can probably be achieved with *A. dumerilii*. However, the main drawback of the use of this solution is that its preparation requires different mineral salts that are not easy to obtain in every region housing the species. Based on the above, it would be important to continue testing other solutions that are easy to prepare to counteract human error during preparation and to improve accessibility to all institutions working with this species, eg., the enriched salt mixes for marine aquariums. When altering water chemistry for maintaining this species the key factor is to maintain consistency in the required parameters following aquarium maintainenance such as water changes, as fluctuating parameters may also cause stress which could lead to health issues. See table 1. Section **2.1.5** for current options of aqueus solutions for maintaining *A. dumerilii*.

From an ecological perspective much knowledge is still to be gained regarding the wild habits of this species with regard to activity patterns and movements within the lake, natural sites of spawning, ecological niches of larvae and a thorough estimate of the population and demographics of the wild population. Data on UVB exposure and how this may inform further development of husbandry practices is also lacking and therefore in need of further research. All of this information is empirical to the successful conservation of this species in the wild. Much of these research questions are currently being studied by researchers at the Universidad Michoacána de San Nicolás de Hidalgo.

Section 3 – Acknowledgments, References and Appendix.

3.1 Acknowledgements:

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Juan Antonio Tello Ballinas
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Gerardo León Murillo
Arturo Vergara Iglesias
Giselle Ibáñez Rubio
Laura L. Rodríguez Cedillo
Rafael Hernández Guzmán
Rubén Hernández Morales
Yurixhi Maldonado López
Yvonne Herrerías Diego
Federico Hernández Valencia
Javier Lopez

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3.3 Appendix

Appendix 1 – Equipment for PIT Tagging and VIE marking.

Visual Implant Elastomer (VIE) is available through North West Marine Technology, Inc. and is available in 10 colours enabling a large number of individually identifiable combinations.

https://www.nmt.us/visible-implant-elastomer/

Passive Intergrated Transponders (PIT Tag) successfully used with this species are Trovan ID-162B/1.4 Animal Implant Mini Transponders and applicator (Fig. 34).

https://www.trovan.com/en/products/trovan-fdx-b/preprogrammed-transponders

Fig 34. Suitable PIT tag used with adult *A. dumerilii*.

Appendix 2 – Procedure for swabbing for Bd/Bsal.

Testing for Bd and Bsal is done via swabbing followed by a qPCR test, usually a combined PCR from a singular swab sample is used to test for both pathogens, although ensure to check with testing laboratory processing samples if they require a singular swab sample or one for each pathogen.

This is usually best undertaken with two people. Both participants should wear Nitrile powder free examination gloves, one individual gently restrains the salamander out of water whilst the second uses a sterile cotton swab to take the sample (Fig. 35). The swab is gently rolled across the salamander, ensuring that the rolling of the swab takes place whilst in motion across the area, to improve in the collection of skin samples containing Bd or Bsal. The samples may be taken from the ventral surface and the inside of the limbs, but most importantly the keratonised mouth parts of the salamander as these will be focal area for chytrid. Ensure a standardised approach, it is recommended that each area is swabbed three times during a single swabbing for approximately the same amount of time. Ensure that the swab is correctly labelled and once completed return swab to sealed container and store in a cool dry place until required for testing. Change gloves between handling and swabbing if dealing with multiple individuals.

Fig 35. The salamander is gently restrained whilst swabbed, ensuring the mouth parts are a focal area for the sample collection. © A. Bland

Appendix 3 – Legislation in effect controlling movement of Salamanders and specifically *A. dumerilii.*

Ambystoma dumerilii is listed within Appendix II of CITES, therefore it is the responsibility of the holding institution to ensure correct documention is obtained and protocols are followed concerning the movement of this species between countries and collections.

Within Europe, all tailed amphibians are subject to strict regulation and health screening regarding importation and movement between collections. This is to protect against Bsal within collections and also in the wild within Europe, where this disease is currently spreading. It is the responsibility of the holding collections to ensure that all movements of this species conform to the regulation of the European Convention on this subject. More information can be found here:

https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0320&from=CS