RISK ANALYSIS OF THE AXOLOTL (*AMBYSTOMA MEXICANUM*) IN THE NETHERLANDS

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Commissioned by:
Invasive Alien Species Team
Netherlands Food and Consumer Product Safety Authority
Ministry of Economic Affairs

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Cover photos: Axolotls caught in Franeker (photo by st DA Dier in Nood), introduction location in Franeker (photo by S. van de Koppel)

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SUMMARY

This report presents the risk analysis of the axolotl (*Ambystoma mexicanum*) in the Netherlands. The study was initiated to provide insight into the potential effects of the occurrence of non-native populations of axolotl in the Netherlands, their present distribution, introduction pathways, and the probability of establishment and spreading. The risk analysis consists of both risk assessment and a general description of risk management, and is based on data analyses, (inter)national literature, interviews, and field expertise.

The axolotl is a salamander species of the family Ambystomatidae (mole salamanders). The species is endemic to the Valley of Mexico in central Mexico and it is commonly maintained in captivity. Optimal habitat consists of large, interconnected lakes with clear, relatively cold water, abundant aquatic vegetation, and absence of large, predatory fish. Free living specimens of the axolotl were discovered in March 2013 in the city of Franeker, Friesland province, the Netherlands. After removing two of at least three specimens, no sightings have been reported since.

Risk assessment

The probability of introduction of axolotl into the Netherlands is high. Since the supply of the species as pet is large, purchase prices are very low, and it is easy to breed, chances of introduction by deliberate release from captivity are estimated to be high. However, only one introduction of more than one axolotl has been recorded worldwide (i.e. in Franeker, March 2013). Probably this is caused by a limited chance of successful establishment.

Due to a wide habitat availability in the Netherlands and axolotl's climate tolerance, adaptability in habitat selection, and high reproduction success, establishment of populations of axolotl in the Netherlands seems to be likely. However, its vulnerability to predation and possible other factors (e.g., diseases) will strongly limit establishment chances. Overall, the probability of establishment of populations of axolotl in the Netherlands is considered to be low. No successful establishment has been recorded anywhere across the globe.

The probability of axolotls spreading in the Netherlands is low to moderate. Dispersal rate will be relatively low due to site fidelity, but reproduction is likely and the species is rather opportunistic regarding habitat selection. Hence, spreading through interconnected water systems is likely. Nevertheless, predation might strongly limit dispersal probability and dispersal following introduction in isolated waters is considered impossible.

In relation to axolotl's requirements concerning climatic conditions and preferred habitats, most parts of the Netherlands are suitable for the species. However, vulnerability of the introduction location to impact caused by axolotls largely depends on the level of isolation of the introduction location and its conservation value for endangered species, mainly amphibians.

Ecological damage might be caused by competition, predation, and transmission of diseases. As the species is aquatic, ecological damage will be limited to other aquatic species, mainly amphibians and fish. Economic and social damage are unlikely.

Risk management

Eradication: effective eradication measures for future introductions might be needed to prevent further establishment, spread, and possible impacts. Several methods of eradication are proposed. Which (combination) of methods should be used, highly depends on the site characteristics.

Control: due to the fact that population control management and additional monitoring is permanent, accompanied by high costs, control of axolotl populations is not considered an option. However, if introduction has taken place in an interconnected water system and total eradication is considered impossible, control of an established population can be used as last resort in order to prevent dispersal and impact of axolotls.

1 INTRODUCTION

Axolotl (*Ambystoma mexicanum*) is endemic to the Valley of Mexico in central Mexico (Contreras *et al.* 2009, Recuero *et al.* 2010). All over the world, the species is commonly maintained in captivity, either for research purposes or as pet (Schreckenberg & Jacobson 1974, Voss *et al.* 2009, Recuero *et al.* 2010). Within its natural range however, this salamander species is highly threatened by habitat loss, water pollution, overharvesting, and introduction of exotic species (Shaffer 1989, Contreras *et al.* 2009, Voss *et al.* 2009, Recuero *et al.* 2010).

Free living specimens of the axolotl were discovered in March 2013 in the city of Franeker, Friesland province, the Netherlands. The species was found in a ditch covered by ice in a residential area. Two specimens were captured and removed. At least one specimen was able to escape (pers. comm. Fam. Dorenbos). No sightings have been reported since.

It is possible that the species still occurs in the area. Little is known about survival probability, chances of establishment, and dispersal capacity of axolotls in Franeker, or any other introductions in the Netherlands. Presence of axolotls can display a negative impact on humans and native biodiversity, e.g., by competition, predation, or spread of diseases.

This study was initiated to provide insight into the potential effects of the occurrence of non-native populations of axolotls in the Netherlands, their present distribution, introduction pathways, and the probability of establishment and spreading. Relatively little is known about what measures should be taken to prevent a sustainable establishment of (populations of) the species. Subsequently, this study deals with both risk assessment and risk management of axolotls in the Netherlands.

Project definition
The Invasive Alien Species Team of the Netherlands Food and Consumer Product Safety Authority (Ministry of Economic Affairs) has commissioned Natuurbalans-Limes Divergens BV, as member of the Dutch Expertise Center for Exotic species (Nederlands Expertise Centrum Exoten, NEC-E) to perform a concise risk analysis of axolotls in the Netherlands. The risk analysis includes the following objectives:

- To develop a clear picture of the present distribution, probability of entry, establishment, and spreading of axolotls in the Netherlands.

- To provide insight into the potential effects of axolotls on native species and/or communities, economy (e.g., loss of profits or even economic opportunities), and social aspects (e.g., recreational values and public health).

- To provide insight into measures that can be taken from the perspective of risk management with regard to eradication and control (prevention of establishment is explicitly not included in this risk analysis).

Report structure
Chapter 2 deals with the methods of current risk analysis. Chapter 3 provides a concise general description of the species, which focuses on aspects that are of direct interest to the risk analysis. Chapter 4 presents the risk assessment, divided into probability of introduction, establishment, and spreading, vulnerable areas, and impact. Chapter 5 deals with the options for risk management (eradication and control). Finally, the most important conclusions and recommendations are presented in chapter 6.

2 METHODS

2.1 COMPONENTS OF THE RISK ANALYSIS

Research on the following components was conducted in order to cover the complete risk analysis: risk assessment and risk management.

2.1.1 Risk assessment

Probability of introduction
The probability of introduction was determined by the possible introduction pathways of the species into the Netherlands, both present and future.

Probability of establishment
The probability of establishment was determined by the current dispersal range of the species and presence of potential habitat in the Netherlands related to (a)biotic requirements of the species.

Probability of spreading
The probability of spreading was determined with regard to the dispersal capacity of the species by natural means and by human action.

Vulnerable areas
Areas or habitats in the Netherlands that are at risk of invasion were selected and possible effects of the presence of axolotls within these areas were discussed.

Impact
Based on the results of the three probabilities mentioned above, the impact of the species was determined and the ecological effects and risks were scientifically characterised, using the Invasive Species Environmental Impact Assessement (ISEIA) protocol. Subsequently, the (possible) ecological, economic, and social impacts as a result of the presence of the species were discussed.

2.1.2 Risk management

Based on the results of the risk assessment, measures were identified and proposed to counteract the introduction, establishment, and spreading of and damage caused by the species. The risk management dealt with measures for:

- *Eradication*
- *Control*

The (dis)advantages of each measure were investigated in terms of effectiveness, feasibility, and costs.

2.2 INVESTIGATION OF SOURCES

The risk analysis was based on existing data and interviews. Several databases and other sources of information were used. These are discussed in further detail below.

2.2.1 Literature

National (e.g., reports and species distribution atlases) and international literature (e.g., scientific articles via ISI web of knowledge, Google Scholar, Wiley Interscience, and Zoological Records) was investigated in order to get a clear view of existing knowledge regarding the subject. Additionally, several websites, e.g., IUCN Red List, Animal Diversity Web, and Encyclopedia of Life, were used.

2.2.2 Distribution data

Data on the present distribution of axolotls in the Netherlands was obtained using several databases. The extensive database of RAVON Foundation was consulted, together with several general websites, e.g., www.waarneming.nl and www.telmee.nl. Also several organisations, e.g., reptile zoo SERPO and local herpetological societies and nature groups, were consulted.

2.2.3 Interviews

Information on trade, breeding, and releases in nature were gathered, among others, through interviews with several organisations and breeders of axolotls. Interviewed organisations are DoeZoo in Leens, Reptile Zoo and asylum SERPO in Delft, Reptile Zoo and asylum Iguana in Vlissingen, pet trade organisation Dibevo, municipality of Franeker, waterboard Wetterskip Fryslân, the salamander society, and the animal ambulance.

2.3 FIELDWORK

The introduction location in Franeker was visited on 27 August 2013 by two ecologists of Natuurbalans - Limes Divergens BV. Using electrofishing, the introduction location and direct vicinity was examined for the presence of axolotls. Additionally, the finders of the axolotls were interviewed.

3 GENERAL SPECIES DESCRIPTION

This chapter presents a short species description of the axolotl. Aspects that are of importance for the risk analysis are considered, mainly distribution, habitat, reproduction, and diet of the species.

Scientific name
Ambystoma mexicanum.

Common names
Axolotl, Mexican axolotl.

Family
Ambystomatidae (mole salamanders).

Conservation status
IUCN red list: critically endangered.
CITES: appendix II.

Species description
Axolotl exhibits paedomorphosis or neoteny, retaining larval characteristics in the adult, reproductive stage, like external gills and tail fins. The species is considered strictly aquatic as metamorphosis is relatively very rare (Swingle 1922, Shaffer 1989, Voss *et al.* 2009). Axolotls grow to an average length of 20-25 cm, but can reach lengths over 30 cm. It naturally has a darkly greyish to brownish coloured body (Majchrzak 2004). For pet trade and biological research several colour varieties are bred, like melanoid, white, albino, and axanthic (Voss *et al.* 2009).

Natural distribution range
Axolotl (*Ambystoma mexicanum*) is endemic to the Valley of Mexico in central Mexico (Contreras *et al.* 2009, Recuero *et al.* 2010). Within its natural range, it is critically endangered due to habitat loss caused by urban growth, water pollution, overharvesting, and introduction of large, predatory fish, resulting in three isolated and highly threatened populations (Shaffer 1989, Contreras *et al.* 2009, Voss *et al.* 2009, Recuero *et al.* 2010). Two wild populations persist in southern Mexico City: one in an urban-rural water system in the Xochimilco region, and a second in the remnants of Lago de Chalco (Contreras *et al.* 2009, Recuero *et al.* 2010). A third population was recently found in Lago Viejo, an artificial lake in the public recreational area of Chapultepec Park, in the heart of Mexico City (Recuero *et al.* 2010). Figure 1 presents the current distribution of the three axolotl populations.

Distribution outside natural distribution range
All over the world, the species is commonly maintained in captivity, either for research purposes or as pet (Schreckenberg & Jacobson 1974, Voss *et al.* 2009, Recuero *et al.* 2010). Several breeding colonies exist across the globe (Recuero *et al.* 2010); more axolotls live in captivity than are free living in remaining parts of its natural distribution range (Voss *et al.* 2009). However, only one record of more than one free living axolotl outside its natural distribution range has been recorded worldwide.

At least three free living specimens of the axolotl were discovered in March 2013 in the city of Franeker, Friesland province, the Netherlands. The species was found in a ditch covered by ice in a residential area. Two specimens were captured and removed. At least one specimen was able to escape (pers. comm. Fam. Dorenbos). No sightings have been reported since. Moreover, no sightings of the species have been registered by RAVON Foundation.

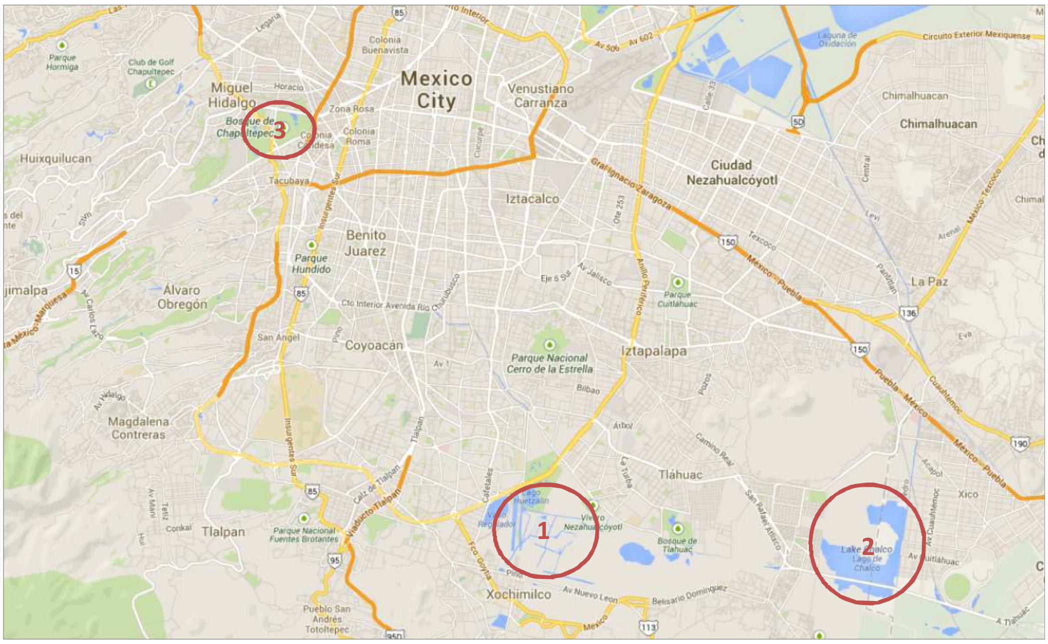

Figure 1. Current distribution of axolotl within its natural distribution range. Three populations are distinguished: (1) Xochimilco region, (2) Lago de Chalco, (3) Chapultepec Park / Lago Viejo (Google Maps layer and distribution data in Contreras *et al.* 2009 and Recuero *et al.* 2010).

Habitat

Axolotl's habitat consists of relatively large, permanent freshwater lakes, situated in dry areas at mid to high altitude above 1600 m above sea level (Shaffer 1989). Its original habitat in the entire Valley of Mexico consisted of a lacustrine system of large, interconnected lakes. Its current habitat is formed by the remaining small lakes of this lacustrine system, combined with artificial channels and temporary wetlands (Contreras *et al.* 2009, Recuero *et al.* 2010), and the artificial Lago Viejo (elevation 2240 m) with a surface of 6 ha, maximum depth of 1.8 m, and a high degree of eutrophication (Recuero *et al.* 2010).

Optimal habitat consists of clear, cold water with low ammonium and nitrate concentrations, and abundant aquatic vegetation. As the species is vulnerable to predation, absence of large, predatory fish or enough shelter is a necessity. Axolotls are generally found at a pH of 7.0 to 9.2 and water temperatures of 16 to 23 °C (Shaffer 1989, Contreras *et al.* 2009). The species is adapted to intermediate temperature ranges; the middle of its adaptive temperature range is 18.2 °C (Bachmann 1969). Embryos can even tolerate water temperatures ranging from 5 to 25 °C (Voss *et al.* 2009).

Reproduction

Reproductive age:	generally around 1 year (Brandon 1972, Voss *et al.* 2009).
Reproduction method:	oviparous; internal fertilization (Voss *et al.* 2009).
Clutch size:	100 - 300 eggs, which are attached to rocks or vegetation (Majchrzak 2004). In captivity clutches up to 1000 eggs are known (Bruins 1999, Clare 2012).
Embryonic period:	10 - 20 days (Bruins 1999, Majchrzak 2004); approximately 20 days at 20 °C (Voss *et al.* 2009).

Under laboratory conditions breeding is possible multiple times in a year; males can even mate with an interval of several weeks. Females are able to produce two to five clutches per year (Voss *et al.* 2009). Breeding seems to be very easy, also for amateurs at home. One amateur breeder reported that reproduction occurred spontaneously in her aquarium at room temperature (pers. comm. Kamphorst).

Other breeders indicate that the chances of reproduction are high under Dutch climatic circumstances, based on their own observations in garden ponds (e.g., pers. comm. Kenter, Achterhof, Lippe).

Life span

Axolotls can live past 10 years (Voss *et al.* 2009). Expected life span in laboratory is 5 to 6 years (Majchrzak 2004). If metamorphosis is induced, axolotls generally die shortly after metamorphosis (Majchrzak 2004).

Diet

Before introduction of large, predatory fish, axolotls were considered the top predator within their natural habitat. The species is an opportunistic feeder; it eats everything that moves and is smaller than itself. Their diet is comparable to that of predatory fish, mainly consisting of small fish, snails, crayfish, and several invertebrate species. Axolotls also eat conspecifics and other ambystomatids (Shaffer 1989, pers. comm. Achterhof).

Predators

The main predators of axolotls are (large) fish and conspecifics. Moreover, humans harvest the species for medicinal purposes and even for food (Smith 1969, Shaffer 1989, Majchrzak 2004, Recuero *et al.* 2010).

4 RISK ASSESSMENT

The risk assessment examines the situation in which no measures for prevention, eradication, or control are taken. It focuses on the potential risks of the presence and invasiveness of axolotl in the Netherlands. Therefore, the probability of introduction, establishment, and spreading are discussed, resulting in an overview of vulnerable areas and an evaluation of the potential impact of the species.

4.1 Probability of introduction

Axolotls are commonly maintained in captivity (Schreckenberg & Jacobson 1974, Voss *et al.* 2009, Recuero *et al.* 2010). The supply of the species as pet is large and purchase prices are very low. On advertising sites, like marktplaats.nl, axolotls are sold for less than € 5,- each. Hence, axolotl is very popular as pet; additionally it is easy to breed and rear in captivity (Voss *et al.* 2009), even by hobbyists (Clare 2012).

Furthermore, it is widely used in biological research with several breeding colonies across the globe. Since long time axolotl is used as model organism for evolutionary and developmental biology (Schreckenberg & Jacobson 1974, Voss *et al.* 2009, Recuero *et al.* 2010). The species is used in research on, for example, tissue regeneration, metamorphosis, germ cell induction, gastrulation, neural crest and lateral line formation, and electrophysiology (Roy & Gatien 2008, Voss *et al.* 2009).

As axolotls are commonly kept and cultured, there is a serious risk that the species is deliberately released from its captive environment, for example if the owner is no longer interested in its pet or as a result of a large surplus of breeding. Expression of similar behaviour of pet owners are the frequent introductions of frogs, salamanders, and fish (e.g., sunfish). Hence, release from captivity is considered the most important introduction pathway of axolotl into the wild, and probability of introduction by this pathway is high. However, only one introduction of more than one axolotl has been recorded worldwide (i.e. in Franeker, March 2013). Probably this is caused by a limited probability on successful establishment (see §4.2), mainly due to its vulnerability to predation.

4.2 Probability of establishment

Not all introductions result in the establishment of populations. The most important underlying factors are propagule pressure, availability of suitable habitat, climatic factors, and reproduction success.

No literature is available on the relationship between propagule pressure, i.e., the quality, quantity, and frequency of introduced organisms, of axolotl, and probability establishment. Nevertheless, it is obvious that more than one specimen or at least a gravid female is needed to establish a population of the species. Hence, if more specimens or a gravid female are let loose, a population might be able to establish, provided that the other requirements are met.

Another important predictor of probability of establishment is habitat availability. The Netherlands is located in a delta region; wetlands, lakes, pools, ponds, rivers, streams, and ditches are abundant all over the country. Large, interconnected water systems are available in high quantities. Thereby, the Netherlands offers a vast amount and diversity of aquatic habitats. Hence, it is expected that habitat availability is not limiting establishment chances of axolotl. Moreover, axolotl has proven to be able to survive in suboptimal habitats like artificial channels and recreational lakes with a high degree of eutrophication (Contreras *et al.* 2009, Recuero *et al.* 2010). Hence, axolotl might adapt easily to new habitats. Nevertheless, presence of predators, mainly large, predatory fish, but also bird species like herons, may strongly limit habitat suitability (Shaffer 1989) and subsequently establishment chances, especially as most bred colour varieties are very striking and do not provide any camouflage.

A third main factor is the suitability of the climate. Within its natural distribution range, axolotl lives at mid to high altitudes (Shaffer 1989) and is adapted to intermediate temperature ranges (Bachmann 1969). Axolotls are generally found at water temperatures of 16 to 23 °C (Shaffer 1989, Contreras *et al.* 2009), but embryos can tolerate a wide range of 5 to 25 °C (Voss *et al.* 2009). For keeping axolotls in aquariums a water temperature of 10 to 20 °C is advised by Clare (2012). Bruins (1999) advices 15 to 20 °C in summer and 5 to 10 °C in winter. Temperatures above 24 °C are too stressful and often lethal (Clare 2012). Clare (2012) also states that maintaining axolotls in outdoor ponds in temperate regions is possible. The animals can endure periods with ice cover on the pond, as long as winters are not particularly harsh or long (Clare 2012). Observations on free living axolotls in Franeker confirm this statement, as the caught specimens were healthy and lively after spending several days underneath a layer of ice (pers. comm. Fam. Dorenbos). Hence, regarding climatic conditions in its natural distribution range and observations on axolotls in Dutch climate, it is expected that the species has a relatively high chance of survival in the Netherlands (also pers. comm. Kenter, Achterhof, van der Made).

Finally, climatic conditions are also considered the most important possible inhibitor of reproduction success. As axolotls naturally occur at mid to high altitudes (Shaffer 1989) and embryos can tolerate a wide range of water temperatures (Voss *et al.* 2009), it is expected that the temperate climate does not form a limitation on reproduction success of the species in the Netherlands (pers. comm. Kenter, Achterhof). Moreover, with its low reproductive age and large clutch size (up to 1000 eggs in captivity; Bruins 1999, Clare 2012), rapid reproduction is likely, provided that predators are absent or occur only in low densities.

In summary, based on the ecology of the species, its habitat requirements, climate tolerance, and reproduction success, establishment of populations of axolotl in the Netherlands seems to be likely. However, its vulnerability to predation will strongly limit establishment chances. This might explain the fact that successful establishment of the species has not been recorded anywhere across the globe. Besides predation, other factors (e.g., diseases) might prevent successful establishment, but these factors could not be derived from current knowledge. Hence, uncertainty remains about the establishment chances. Considering the high introduction risk but the absence of successful establishments anywhere across the globe, the probability of establishment is considered to be low.

4.3 Probability of spreading

If an alien species becomes established, the probability of spreading, is the third key aspect regarding the risk of the new species.

Axolotl is considered as a sedentary species with reduced mobility due to high fidelity to home ranges (Contreras *et al.* 2009). Nevertheless, its strong reproductive capacity (Bruins 1999, Clare 2012) might lead to very high densities (rough estimate of one individual per square foot of lake bottom) (Shaffer 1989), which could in turn lead to density-dependent dispersal, i.e. more rapid dispersion over larger distances. Furthermore, colonisation rate of axolotl is not expected to be inhibited by specific habitat requirements. An aquatic species can readily find appropriate corridors and habitat in the Netherlands and dispersal is hardly limited by infrastructural barriers.

Although Smith (1969) states that “axolotl frequently transforms in nature” and “numerous records exist of spontaneous transformation”, most authors agree that spontaneous transformation or metamorphosis is (very) rare (Swingle 1922, Shaffer 1989, Voss *et al.* 2009) and axolotl can be considered as “strictly aquatic species” (Recuero *et al.* 2010). Hence, if axolotls are introduced into an isolated water (pond, lake, fen, etc.), spreading into the surrounding area is impossible. Within interconnected water systems of ditches, streams, canals, and rivers, however, spreading is likely, but only if predators are absent or occur only in low densities.

In summary, the probability of the species spreading from an area where it is introduced and has been able to establish is considered to be low to moderate. Reproduction is likely to take place and the species is rather opportunistic regarding habitat selection. Although dispersal rate might be relatively low due to reduced mobility, total dispersal distance can be fairly large after some years of presence, as dispersal is hardly limited by infrastructural barriers and the species might show density-dependent dispersal. Nevertheless, predation might strongly limit dispersal probability and dispersal following introduction in isolated waters is considered impossible.

4.4 Vulnerable areas

In relation to axolotl's requirements concerning climatic conditions and preferred habitats, a large part of the Netherlands is estimated to be suitable as habitat for axolotl. The species is adaptive regarding habitat and food resources (Shaffer 1989).

The most important pathway of introduction, i.e., deliberate release from captivity, is not bound to specific areas. Animals can be dumped within built-up areas, but introduction into natural areas is also possible, as is illustrated by repeated introduction of sunfish in marshes. As distances between natural areas and urban areas in the Netherlands are seldom large, colonisation of natural areas is possible, provided that axolotls are let loose in interconnected water systems. If no large predators are present, colonisation, reproduction, and dispersal are likely, but impact will be limited, as these systems (e.g., polder ditches) often do not house vulnerable amphibian species. Impact in isolated pools, ponds, and fens could be more severe, especially at locations of high conservation value for endangered amphibian species.

4.5 Impact

As concluded in the previous paragraphs, the probability of introduction of axolotl in the Netherlands is high and the probability of establishment and spreading is low to moderate. The possible ecological, economic, and social impacts are discussed below. However, up to this moment no successful establishment of axolotls outside their natural range has been recorded. Therefore, the impact assessment is mainly based on expert judgement and the level of uncertainty is relatively high. The ecological risks were also assessed using the Invasive Species Environmental Impact Assessement (ISEIA) protocol (appendix, Branquart 2009).

4.5.1 Ecological damage

Competition
Axolotls may compete for food and space with other amphibians and fish of comparable size, as their diets are comparable (Shaffer 1989). Moreover, axolotls are able to reach very high densities (Shaffer 1989), making them potentially strong competitors.

Predation
Besides competition, ecological damage might be caused by predation, as axolotls are top predator in absence of larger, predatory fish (Shaffer 1989). The effect of predation on populations of common species is not expected to cause significant ecological damage. However, if introduced in isolated waters with high conservation value, axolotls may predate on several rare, endangered species (mainly amphibians),

resulting in a more pronounced negative effect on prey populations. Adversely, several native species might become important predators of axolotls, for example herons and larger fishes, thereby limiting population growth and decreasing possible ecologically damaging effects of predation by axolotls.

Hybridization

Hybridization is known as important ecological damaging factor with regard to other invasive species (Mack *et al.* 2000, Grosholz 2002). Axolotls hybridize with Lake Patzcuaro salamander (*Ambystoma dumerilii*), marbled salamander (*Ambystoma opacum*), and eastern tiger salamander (*Ambystoma tigrinum*) and produce fertile progeny (Smith 1969, Brandon 1972, Brandon 1977). Also crosses have been made with Jefferson salamander (*Ambystoma jeffersonianum*), long-toed salamander (*Ambystoma macrodactylum*), spotted salamander (*Ambystoma maculatum*), mole salamander (*Ambystoma talpoideum*), and small-mouthed salamander (*Ambystoma texanum*), but fertility was either not tested or progeny was infertile (Brandon 1977). All those species are ambystomatids, so they are relatively closely related to axolotl, and do not live in Europe. No occurrences of hybridization with other (native) species are known. Moreover, native Dutch amphibians are phylogenetically distant to axolotl. Thus, it is very unlikely that axolotl causes ecological damage to native species due to hybridization.

Diseases

As release from captivity is the most important introduction pathway, diseases in captive axolotls are most important possible causes of ecological damage:

- *Batrachochytrium dendrobatidis*: highly transmissible parasitic chytrid fungus which can be abundant in captive colonies of axolotl. This fungus causes chytridiomycosis, but captive individuals seem not to be sensitive to this disease (Recuero *et al.* 2010). Chytridiomycosis threatens amphibians worldwide, causing serious population declines and extinction of entire populations and even species (amongst others: Skerratt *et al.* 2007, Fisher *et al.* 2009, Voyles *et al.* 2009).

- Ranavirus: associated with significant declines and local extinction of amphibian populations (Rijks *et al.* 2011). In an experiment three axolotls were inoculated with the *Ranavirus*. None of the individuals showed any clinical signs and after nine weeks the *Ranavirus* could not be detected in the axolotls (Pasmans *et al.* 2008). According to the researchers, this could indicate that axolotls are not suitable hosts of the virus, but it is plausible that individuals become sensitive to *Ranavirus* only under severe stress, especially in combination with heavy infection (Pasmans *et al.* 2008).

- Clare (2012) sums up a great variety of bacteria, fungi, and parasites found in captive axolotls:
  - Bacteria: *Acinetobacter*, *Aeromonas hydrophila*, *Alcaligenes*, *Chondrococcus columnaris*, *Mima*, *Proteus*, *Psuedomonas*, *Salmonella*.
  - Fungi: *Saprolegnia*.
  - Parasites: ciliates, *Dactylogyrus*, *Gyrodactylus*, *Hexamita*, *Opalina*, Platyhelminthes, Protozoan parasites (like *Costia, Trichodina*, *Vorticella*).

Moreover, Recuero *et al.* (2010) found three parasites in adult *Ambystoma* in Chapultepec:

- *Eustrongylides* sp. (larval stadium): fish are the most important intermediate host of this parasitic nematode; also amphibians can be infected. *Eustrongylides* is associated with significant population declines in fish-eating birds, its final host. No declines in amphibian populations caused by this nematode are known (Recuero *et al.* 2010).

- *Lernaea*: ectoparasitic copepod which generally infects fish. It can cause limb abnormalities in amphibians, but this is probably not the case in Chapultepec (Recuero *et al.* 2010).

- *Saprolegnia*: pathogenic fungus which can infect all stages of amphibians. In combination with stress factors or other diseases, it can cause high mortality in amphibian populations (Recuero *et al.* 2010).

In summary, all above mentioned bacteria, fungi, parasites, and viruses, and possibly other species not yet known in axolotls, might cause ecological damage to native fauna, especially amphibians. As a variety of these diseases is also associated with captive axolotls (Clare 2012), introduction from captivity can lead to introduction and/or spread of these (new) diseases. Probably the most important are the chytrid fungus and Ranavirus, as these are linked to serious amphibian population declines and extinctions (Skerratt *et al.* 2007, Fisher *et al.* 2009, Voyles *et al.* 2009, Rijks *et al.* 2011).

4.5.2 Social damage

No negative effects of axolotls on humans are known (Majchrzak 2004). Axolotl is a harmless species which does not pose a threat to humans. The above mentioned diseases could be of concern to native fauna, not to humans, as transmission to humans is unlikely and most diseases are harmless to humans. Hence, axolotls are not considered of public health importance. Other forms of social damage (e.g., fear factor caused by larger, dangerous animals) are hardly applicable to axolotls.

4.5.3 Economic damage

No economic damage, besides possible costs for risk management, is expected to be caused by the presence of axolotls in the Netherlands.

4.6 Case study: axolotls in Franeker

In this section, the three probabilities and possible impact are assessed relating to the axolotls in Franeker, after a short general background of this introduction is given.

Background

In March 2013 at least three axolotls were discovered in a ditch covered by ice in a residential area of Franeker. After some days spent under the ice cover, two specimens were captured and removed. At least one specimen was able to escape (pers. comm. Fam. Dorenbos). No sightings have been reported since.

Probability of introduction

The axolotls in Franeker were probably introduced in March 2013, a short time before discovery since the individuals were seen close to each other. It is expected that the animals were dumped by a local resident, as the location is situated in a residential area. However, the exact source of this introduction remains unknown. According to Fam. Dorenbos at least three specimens were present. The introduction location is part of an extensive water system of ditches through the whole residential area, connected to a larger watercourse bordering Franeker in the east. The exact introduction location is a ditch (width: 3 m; depth: 25 cm in summer; thickness mud layer: 50 cm), with hardly any aquatic vegetation and bordered by reed.

Probability of establishment and spreading

Shortly after discovery, Fam. Dorenbos captured and removed two specimens. At least one specimen was able to escape. As management has taken place, no firm conclusions can be drawn about the probability of establishment and spreading of the species, based on the case in Franeker. During ecological research using electrofishing on 27 Augustus 2013, no axolotls or other amphibians were discovered. Several species of (predatory) fish were discovered: common bream (*Abramis brama*), common rudd (*Scardnius erythropthalmus*), European eel (*Anguilla anguilla*), European perch (*Perca fluviatilis*), gudgeon (*Gobio gobio*), northern pike (*Esox lucius*), and roach (*Rutilus rutilus*), ranging from juveniles up to adults of 50 cm (mainly European eel and northern pike).

The remaining specimen(s) might have escaped to other parts of the water system. However, it is more likely no axolotl could survive within the area. The species is vulnerable to predation by large, predatory fish (Shaffer 1989, Majchrzak 2004, Recuero *et al.* 2010), especially because the introduced axolotls lacked camouflage due to their white skin. It might also be an easy catch for large birds like herons. Hence, survival in the particular system is unlikely. This seems to be confirmed by the fact that the species has not been seen in the area since the two specimens were captured. The finders of the axolotl visited the location on a very regular basis; several times a day during approximately eight weeks. As the species was also not discovered during electrofishing, it is likely that axolotls did not establish nor spread into the surrounding area.

Impact
No occurrence of ecological, social, or economic damage caused by the axolotls in Franeker has been recorded. As most specimens were captured in an early stage and no sightings have been reported since, the potential impact of the species cannot be inferred fully from the case in Franeker.

Conclusion
The risks of the presence and the invasiveness of axolotl in Franeker approach nil, partly because two specimens were captured in an early stage. As it is expected that the remaining axolotl(s) did not survive, it can be concluded that the species has not been able to establish and disperse, probably due to a combination of population management (i.e., capturing and removing two specimens) and presence of predators.

5 RISK MANAGEMENT

In this chapter measures are identified and proposed to counteract and control introduction, establishment, and spreading of and damage caused by axolotls in the Netherlands. The presented measures are based on the results of the risk assessment.

5.1 ERADICATION

Effective eradication measures for future introductions of axolotls in the Netherlands might be needed to prevent further establishment, spread, and possible impacts. These measures are designed to completely remove (a population of) the species.

Appropriate eradication measures should be undertaken as soon as possible following a future introduction to increase the chance of success of such measures. When an invasive species has become widespread, total eradication is often not feasible anymore. In order to successfully eradicate a species, rapid devotion of ample resources during a sufficient long time span is essential (Mack *et al.* 2000, Genovesi 2001). If introduced species are detected in an early stage and measures are taken accordingly, establishment is not likely (Genovesi 2001). However, if the species remains undetected for a longer period of time, the probability of establishment increases (Mack *et al.* 2000).

Capturing and removing axolotls is the most obvious eradication measure. If appropriate eradication measures are undertaken as soon as possible, the chance of completely removing the species is high. Costs are involved, but the economic damage is relatively low. Eradication is even considered the most cost effective and ethical solution once an alien species is introduced (Genovesi 2001). The best method for capturing and removing axolotls depends on the characteristics of the introduction site. We distinguish isolated waters (e.g., pools, ponds, and fens) and interconnected water systems (e.g., ditches, streams, canals, and rivers).

As axolotl is strictly aquatic, dispersal from isolated waters is impossible. Hence, eradication measures can be totally focused on the introduction location itself. Especially in small waters, eradication is relatively easy. With increasing size of the water, efforts for total eradication increase accordingly. Introduction into interconnected water systems confronts us with more difficulties, as dispersal throughout the system is possible. Hence, before total eradication can be guaranteed, the total distribution range should be known, followed by the right (combination of) eradication measure(s). Isolating the population within interconnected waters would at least prevent further spreading and limits the area to be treated.

The following methods of eradication can be used:

- Seine (Dutch: zegen);
- Dip-net (Dutch: schepnet);
- Fish pots (Dutch: fuiken);
- Electrofishing;
- Pumping the location until it is dry;
- Biological control, e.g., introduction of predatory fish;
- Chemical control, e.g., use of specific poison.

Which (combination) of the proposed methods should be used, highly depends on the site characteristics. It is necessary to make a sound ecological judgement of each future introduction to develop a customized eradication program. Additionally, since the public reaction to eradication measures is hard to predict, it is advisable to design a communication plan in order to raise public support and prevent sabotage of the measures. The public reaction will depend on the final destination of the captured individuals, the ecological value of the specific area, and the likeability of the species.

The most important advantage of total eradication is that the project is finite. Within limited time, it is possible to eliminate the species from the introduction site, thereby preventing further spreading. Once totally eradicated, no extra measures and accompanying costs are involved. A disadvantage of total eradication is that, depending on the situation, it may be a very intensive, possibly costly method during a short time span. Moreover, permission of property owners is necessary and temporary damage to (protected) native flora or fauna could occur.

5.2 Control

Another way of risk management is formed by measures to control an established population of the species. This means that further dispersal and impact of the species is minimised. If introduction has taken place in an isolated water, further dispersal is inhibited by the fact that axolotl is strictly aquatic; additional control measures are not required. If introduction has taken place in an interconnected water system and total eradication is considered impossible, control of an established population can be used as last resort in order to prevent dispersal and impact of axolotls.

Control measures suitable to axolotls in interconnected water systems could focus on making the location unsuitable for axolotl. Due to its vulnerability to predation, a possible control measure is introduction of large, predatory fish (biological control). In many water systems, such fish species are already present, thereby providing a natural control mechanism of axolotl. Intensive monitoring of the population is necessary to investigate the effectiveness of such control measures since there is no practical experiences with these measures yet.

Due to the fact that population control management and additional monitoring is permanent, accompanied by high costs, this possibility of risk management is not considered an option if eradication is estimated to be possible. Moreover, control measures aiming at making a location unsuitable for axolotl, will consequently also negatively impact (protected) native fauna and/or cause adverse impacts on the whole ecosystem for a long period of time.

6 CONCLUSIONS AND RECOMMENDATIONS

Free living specimens of the axolotl were discovered in March 2013 in the city of Franeker, Friesland province, the Netherlands. Two specimens were captured and removed. At least one specimen was able to escape. The current study was initiated to provide insight into the potential effects of the occurrence of non-native populations of axolotl in the Netherlands, their present distribution, introduction pathways, and the probability of establishment and spreading. Besides risk assessment, the risk analysis deals with possible measures regarding risk management of the species.

6.1 RISK ASSESSMENT

The risk assessment defines probabilities of introduction, establishment, and spreading, and vulnerable areas, and describes potential impacts by ecological, social, and economic damage.

The probability of introduction of axolotl into the Netherlands is high. The supply of the species as pet is large, purchase prices are very low, and it is easy to breed (Voss *et al.* 2009, Clare 2012). Hence, release from captivity is considered the most important introduction pathway of axolotl into the wild. However, discussion remains why only one introduction of the species has been recorded worldwide, despite of the fact that it is commonly kept and cultured. Probably this is caused by a limited chance of successful establishment which can be related to its high vulnerability to predation, but the risk analysis could not resolve the exact underlying mechanism.

Based on the ecology of the species, its habitat requirements, climate tolerance, and reproduction success, establishment of axolotl in the Netherlands seems to be likely. Axolotl naturally lives at mid to high altitudes (Shaffer 1989), is adapted to intermediate temperature ranges (Bachmann 1969), and embryos can tolerate a wide temperature range (Voss *et al.* 2009). Moreover, the Netherlands offers a vast amount and diversity of suitable habitats, and the species may be able to adapt easily to new habitats. However, its vulnerability to predation will severely limit establishment chances. Besides predation, other factors (e.g., diseases) might prevent successful establishment, but these factors could not be derived from current knowledge. Considering the high introduction risk but the absence of successful establishments anywhere across the globe, the probability of establishment is considered to be low.

The third key aspect regarding the risk of the presence and the invasiveness of the species, i.e., probability of spreading, is estimated to be low to moderate. Axolotl is considered sedentary with reduced mobility due to high fidelity to home ranges (Contreras *et al.* 2009). Moreover, dispersal is inhibited by the fact that the species is considered as “strictly aquatic species” (Recuero *et al.* 2010); spreading from isolated waters is considered impossible. Spreading is only likely following introduction and establishment in interconnected water systems, but only if predators are absent or occur in low densities. In such scarce systems, dispersal is hardly limited by infrastructural barriers and density-dependent dispersal might enable the species to disperse more rapidly over large distances, reinforced by its strong reproductive capacity (Bruins 1999, Clare 2012).

If axolotl would successfully establish a population in the Netherlands, ecological damage may occur. Ecological damage could be caused by competition, predation, and transmission of diseases, posing a serious threat to native (aquatic) species and ecosystems. However, as no risks for native biodiversity caused by axolotls have been recorded, impact by ecological damage is highly speculative. This is illustrated by Verbrugge *et al.* (2010) in general for recently established species: ”If a species has only recently established the full extent of their impacts may not yet be known“ (Verbrugge *et al.* 2010). Therefore, risk classification by the ISEIA protocol (Branquart 2009) was mainly based on expert judgement, as is required if data is scarce (Verbrugge *et al.* 2012). According to the ISEIA classification, the environmental risk level of axolotls in the Netherlands is low. Nevertheless, ecological damage could occur, especially in vulnerable ecosystems inhabited by endangered amphibian species. Economic and social damage are unlikely; no negative effects of axolotls on humans are known (Majchrzak 2004).

6.2 Risk management

Risk management deals with measures to counteract and control introduction, establishment, and spreading of and damage caused by axolotls in the Netherlands. Recommendations for these measures are based on the risk assessment. Risk management is subdivided into measures for prevention, eradication, and control of the species. For the purpose of this risk analysis, measures for prevention were explicitly not included.

Effective eradication measures for future introductions might be needed to prevent further establishment, spread, and possible impacts. These measures are designed to completely remove (a population of) the species. In order to successfully eradicate the species, early detection and rapid devotion of ample resources during a sufficient long time span are essential (Mack *et al.* 2000, Genovesi 2001). Capturing and removing axolotls is the most obvious eradication measure. If appropriate eradication measures are undertaken as soon as possible, the chance of completely removing the species is expected to be high. Costs are involved, but the economic damage is relatively low. Several methods of eradication can be used. Which (combination) of methods should be used, highly depends on the site characteristics. Additionally, since the public reaction to eradication measures is hard to predict, it is advisable to design a communication plan in order to raise public support and prevent sabotage of the measures.

Finally, control management of populations can be used to minimise dispersal and impact of an established population of axolotls. Due to the fact that control management is permanent, accompanied by high costs, this kind of management is not considered an option. Only if introduction has taken place in an interconnected water system and total eradication is considered impossible, control of an established population can be used as last resort. Control measures can focus on axolotl's vulnerability to predation, e.g., by introduction of large, predatory fish. Negative side effects can arise, as control measures aiming at making a location unsuitable for axolotl, will consequently also negatively impact (protected) native fauna and/or cause adverse impacts on the whole ecosystem for a long period of time.

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APPENDIX. ISEIA PROTOCOL

The Invasive Species Environmental Impact Assessement (ISEIA, Branquart 2009) protocol is used to scientifically assess the environmental risks (not impacts on human interests, e.g., public health or economic damage) of non-native organisms and to identify whether preventive and mitigation actions are of concern. In this appendix the results of the ISEIA for axolotls in the Netherlands are presented.

Risk categories

Using the ISEIA protocol, species are placed in one of the following risk categories.

- Category A (black list, score 11-12): species with a high environmental risk.
- Category B (watch list, score 9-10): species with a moderate environmental risk on the basis of current knowledge.
- Category C (low environmental risk, score 4-8): species that are not considered as a threat for native biodiversity and ecosystems.

Scoring system

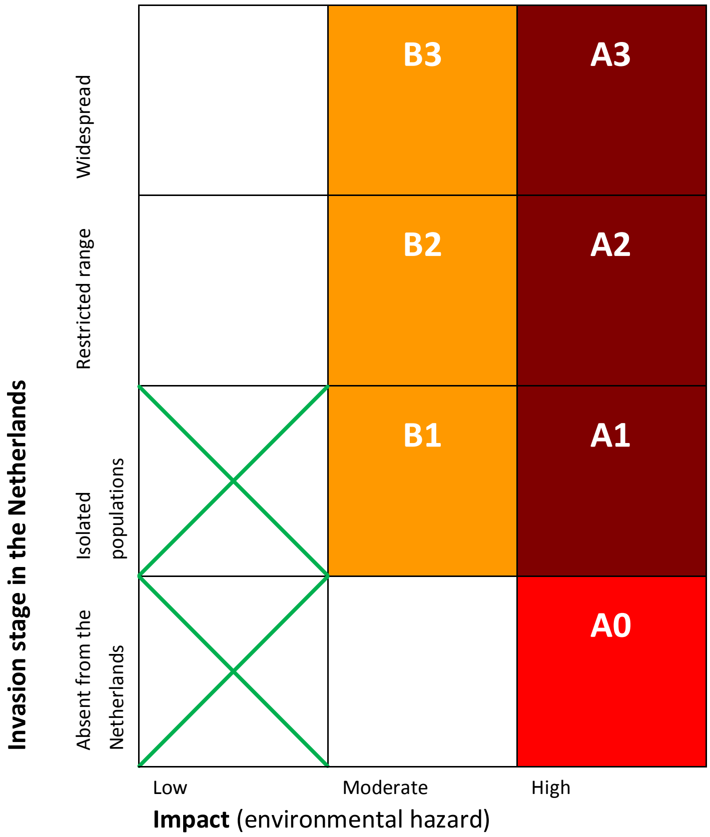
The scoring system used in the ISEIA depends on the availability of information.

- Low level of uncertainty (information documented in literature):
    - Score 1 = low
    - Score 2 = medium
    - Score 3 = high
- High level of uncertainty (information poorly documented):
    - Score 1 = unlikely
    - Score 2 = likely
- No information available:
    - No score = deficient data

Assessment for axolotl in the Netherlands

Section	Category	Score	Reasoning
Dispersion potential or invasiveness	Medium risk	2	Dispersal rate is fairly low due to high side fidelity, but reproduction is likely and total dispersal distance can be large after a few years of presence.
Colonisation of high conservation value habitats	Medium risk	2	Diversity of aquatic habitats is suitable, but as it is strictly aquatic, it cannot reach isolated waters (with highest conservation value) by natural dispersal.
Adverse impacts on native species	Likely	2	No data from invasion histories available. Expert judgement: competition and predation are likely; disease transmission is possible; hybridization is impossible.
Alteration of ecosystem functions	Likely	2	No data from invasion histories available. Expert judgement: axolotl can be aquatic top predator in absence of fish; disruption of food webs by predation is possible; other alterations are unlikely.
Global ISEIA score	**C**	**8**	

<u>Conclusion:</u> the global ISEIA score of axolotls in the Netherlands sums up to 8. This means that the environmental risk level is low, i.e., category C. Axolotl is not considered as a threat to native biodiversity and ecosystems; only possibly to vulnerable ecosystems inhabited by endangered amphibian species. Considering the invasion stage in the Netherlands, axolotl can be categorized as either “absent” or “isolated populations”, depending on the current occurrence of the species. The outcome of the total list system of the ISEIA protocol is presented in the figure underneath, indicated by the green crosses.



Black list: A1 – A3
Alert list: A0
Watch list: B1 – B3