Australian Government

Department of the Environment and Energy

Department Risk Analysis

Application to add *Nothobranchius furzeri* (Turquoise Killifish) to the Environment Protection and Biodiversity Conservation Act 1999 *List of Specimens taken to be Suitable for Live Import*

February 2019

INTRODUCTION

Purpose of the proposed import

Monash University seeks to import Turquoise Killifish (*Nothobranchius furzeri*) into Australia for medical research purposes.

Importation of these animals would contribute to medical research in Australia as this species makes an ideal model for studies examining the effects of aging and associated medical disorders due to its short lifespan and rapid generation time.

Fish imported for this purpose will be used to establish breeding colonies in appropriate laboratory facilities for ongoing research use.

Background

Under s303EC of the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act), the responsible Minister may amend the *List of Specimens taken to be suitable for live import* (Live Import List) by including a specimen on the list. There are two parts to the List:

- Part 1 comprises specimens that can be imported without a permit under the EPBC Act and
- Part 2 comprises specimens that require a permit under the EPBC Act to be imported. Import restrictions generally apply to the species listed on Part 2, such as 'Eligible non-commercial purpose only, excluding household pets'. Additional conditions may also be applied when the permit for import is issued.

Before amending the Live Import List, the Minister must consult with appropriate Ministers and other persons, and consider a report assessing the potential environmental impacts of the proposed amendment. When submitting an application to the Department to amend the Live Import List, all applicants are required to provide an accompanying report that addresses specific terms of reference.

The Department undertakes a risk assessment using the information in the applicant's report and any other sources of relevant information. The Department also considers comments and information received through the public consultation process (including states and territories). The application and accompanying report for the proposed import of Turquoise Killifish was released for public comment in July/August 2018.

Biology and Ecology of Turquoise Killifish (*Nothobranchius furzeri*)

Introduction

Turquoise Killifish (*Nothobranchius furzeri*) is a small, annual, extremophile fish species native to transient freshwater systems in south-eastern Africa. Turquoise Killifish belongs to one of four distinct species groups of annual fishes in the order *Cyprinodontiformes*. This species is distributed in scattered populations corresponding to their preferred ephemeral water sources throughout Northern South Africa, eastern Zimbabwe, and Mozambique (see Fig 1) (Cellerino *et al.,* 2016).)

Description

The average length of Turquoise Killifish is reported at 3.2cm with a maximum length of 6.5cm (Fishbase, 2019). The average body weight reported for males is 3.8 ± 1.1 grams with a range of 0.5 – 6.7 grams and 2.2 ± 0.6 grams with a range of 0.2 - 3.6 grams for females (Applicant's report). Growth rates of *Nothobranchius* in the wild have been reported to be similar to those in captivity. Strong sexual dimorphism can be seen in this species with males brightly coloured and females dull. There is also a tendency towards male colour polymorphism with both a "red" and "yellow" (referring to the caudal fins' primary colour) colour morph occurring in varying ratios in different parts of the species' distribution (Cellerino *et al.,* 2016). This appears to be linked to sexual selection with males competing strongly to mate with females, with the larger, more vigorous males usually having more success (Cellerino *et al.,* 2016; Reichard, 2015).

Turquoise Killifish is an arid environment specialist and had adapted its rapid lifecycle and annual life habit to be able to survive in fragmented, turbid and often highly disturbed pools or other temporary watercourses. While the total area of this species' distribution is significant, the suitable habitat within this area is far more limited. This is due to this species strict requirement for specific soil types to support the survival of their eggs in diapause (hibernation) over the dry season. These 'quaternary vertisol soils' correspond strongly with the known distribution of this species. Despite these specialist traits, Turquoise Killifish may also be found in more permanent water sources and co-exist with other fish species (Cellerino *et al.,* 2016).

During courtship a female will select a male and whilst being clasped by the male will oviposit an egg into the sediment at the bottom of the pond. This action will be repeated throughout the day, sometimes with the same male, sometimes with multiple males until the female has no further eggs to lay (Cellerino *et al.,* 2016). Females can lay anywhere from 20 to 120 eggs per day dependant on female body size and resource availability (Reichard and Polacik, 2019).

Generally the environments the Turquoise Killifish inhabits are only inundated with water for an average of 75 days each season (Cellerino *et al.,* 2016) and this usually requires that eggs laid during this time will need to enter diapause to await the next rains. This period is generally 10 months throughout this species' range. However in extreme circumstances, embryos have been recorded as surviving in diapause for a period in excess of three years and successfully hatching after this period. If water remains available eggs may hatch immediately ("skipping" diapause) and rapidly develop into a second generation (Cellerino *et al.,* 2016).

Once hatched, offspring develop rapidly and are sexually mature at an average of 19 days after emergence. There is no significant parental care of either the eggs or the offspring recorded in the literature and there is no record that this species is able to store sperm (Cellerino *et al.,* 2016; Reichard, 2015; Reichard and Polacik, 2019; Valdesalici and Cellerino, 2003). Considering the rapid lifecycle of this species this is an unlikely adaptation for Turquoise Killifish to have developed.

Breeding seasons are dictated by the availability of water and will continue throughout the lifespan of this species. This strategy is an adaptation to the arid and unpredictable

environments that this species lives in and allows them persist in areas with the correct soil substrate despite low, or at times, non-existent rainfall (Cellerino *et al.,* 2016).

Habitat/ Special adaptations

N. furzeri is a freshwater species that resides in temporary pools. The water characteristics include pH ranges from 6.5 - 7.0; degree of hardness ranges from 4 - 15dH; and temperature ranges from 23°C - 30°C (Fishbase, 2019). It has also been noted that populations of this species are confined to an altitude range between 16 and 422 meters above sea level (Cellerino *et al.,* 2016). In addition to these requirements, this species has strict requirement for specific soil types (vertisol type) to support the survival of their eggs in diapause during dry periods. While some of the habitats that Turquoise Killifish live in are vegetated with grasses and *Nymphaea* species many are not and this does not appear to be a requirement for this species (Cellerino *et al.,* 2016).

Diet

Killifish, including Turquoise Killifish, mostly feed on macroinvertebrates in the wild as this is the most available prey item in the temporary waterways they inhabit. This species does appear to have a preference for not consuming hard bodied terrestrial insects although this has been recorded in food constrained environments. Studies on the diet of four wild populations of Turquoise Killifish indicated that they preferred to feed on small crustaceans such as members of *Cladocera* and *Copepoda* species. Cannibalism has not been recorded in the wild for this species and there is no indication that this species predates on other fish species or their offspring (Cellerino *et al.,* 2016).

Home range and social structure

Turquoise Killifish are confined to the small temporary pools and waterways in which they hatch. In the wild, on attaining sexual maturity at approximately 19 days old (Reichard, 2015) this species will breed continuously in the same pool until either the pool dries up or the fish dies (Cellerino *et al.,* 2016) at a maximum of 12 weeks of age (Reichard, 2015).

Distribution and endemism (as regards conservation status)

Range Description:

Turquoise Killifish has a scattered distribution determined by the presence of appropriate soil types within this range. Its distribution spans the area between the Save river in Zimbabwe, the Lebombo ridge in South Africa and Limpopo River in Mozambique. **(See Fig 1 below)**

Fig.1:
*Distribution map from **IUCN website** (accessed Jan 2019) with the orange shaded areas representing the current distribution.*

Reason for import (captive breeding program etc.)

The importation of Turquoise Killifish is for research on ageing biology. Ageing is an inevitable, multi-systemic disorder characterized by a progressive decline in the ability of organs to perform their physiological functions. *N. furzeri* has the shortest known maximum lifespan of a vertebrate species that can be bred in captivity making it an ideal model for the study of ageing biology, and an optimal platform to develop therapies for ageing and age dependent diseases such as cancer, obesity and diabetes. *N. furzeri* is a very important research tool for research on ageing and has the potential to reveal the many unresolved questions pertaining to this inevitable condition (Applicant's report).

Conservation status

- Turquoise Killifish (*Nothobranchius furzeri*) is not listed on the *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) appendices CITES, 2019).
- The International Union for Conservation of Nature (IUCN) Red List has assessed *Nothobranchius furzeri* as being of 'Least Concern' (IUCN, 2018).

Risk assessment

The Department used the Australian Freshwater Fish Risk Assessment Model developed by Mary Bomford (2008) to assess the risks posed by the importation of Turquoise Killifish (*Nothobranchius furzeri*)((**Appendix A**). The results indicate that the species has:

- a **moderate** risk of establishing a wild population in the Australian environment if released. However it would be worth pointing out that this assessment model does not take in to account the specific habitat requirements of this species for oviposition.
- a **moderate** risk of becoming a pest if it were to establish. Again it is worth noting that this score is based upon the introduction of another species in the same Genus, *Nothobranchius guentheri*, to only two point location in coastal California and Honolulu, Hawaii which ultimately failed to establish (USGS, 2019).
- a theoretical Invasive Plants and Animals Committee (formerly Vertebrate Pest Committee) threat category of **Moderate** (using Table 2.3 in Bomford, 2008).

The climate match, comparing the native range of the species to Australian climates, indicates that Turquoise Killifish has a **moderate** climate match to Australia **(Appendix B**). This species has a highest Climatch class of nine and these matches correspond to coastal regions in central and northern Queensland. However, as noted above, despite the displayed distribution of Turquoise Killifish in Figure 1 this species is confined within this range to primarily small, temporary water courses and pools with the appropriate soil type to support their eggs during their diapause. Therefore, although climatically this species would appear to be suitable to the corresponding Climatch areas in Queensland, this match would likely also need to correspond to the correct soil type and rainfall/ water supply regime in order to allow this species to establish successfully.

This species has been used as a laboratory model species in a number of countries without incident. Another species within this Genus, *Nothobranchius guentheri*, has been intentionally introduced to the United States of America as a biological control agent of mosquito larvae, however this represents only two single locality reports in California and Honolulu, Hawaii and all following reports have indicated that this species was unsuccessful in establishing itself (FAO, 2019; USGS, 2019).

Risk mitigation

The risk assessment indicates that the species has a moderate potential for establishing in Australia if it were released (with the caveats mentioned regarding habitat requirements above). However as this species is to be used as a model species for medical research it will be contained in appropriate laboratory facilities for the containment of live animals. This containment will assist in preventing the escape of this species into suitable habitat.

The Department considers that any risks posed by this species would be adequately mitigated by listing the species under Part 2 of the Live Import List with standard conditions relating to the import of live animals for research purposes.

Concerns raised and responses

The Department undertook consultation with relevant ministers (or their delegates), government agencies and the public in July – August 2018. The Department received responses from the ACT and SA:

- The ACT was supportive of the application to allow import for research purposes.
- The SA Department of Environment and Water was supportive of the application to import Turquoise Killifish for research purposes provided that this species was appropriately contained in a PC2 laboratory or the equivalent to prevent release into the wild. The SA government noted that the soils this species requires are abundant in SA and that it would

likely compete with a number of native species were it to establish. The possibility of this species posing a disease transmission risk was also raised. This however will need to be addressed by the Department of Agriculture and Water Resources as this falls within their area of responsibility as the primary quarantine agency for Australia.

Response from second round of consultation with Commonwealth, state and territory Departments.

The Govdex comment round was undertaken during February of 2019. Two comments were received from the same two agencies that provided comments in the first round of consultation, the South Australian and ACT governments, with the same reservations that were already raised in the first round of consultation and are addressed in the risk mitigation section of this assessment.

Conclusion

Having undertaken an analysis and reviewed the available information, the Department recommends listing Turquoise Killifish (*Nothobranchius furzeri*) on Part 2 of the Live Import List with conditions: **Research only. High security facilities only.**

Permits would be required for each import, the security of the facilities would be assessed and further conditions can be placed on individual imports as required.

APPENDIX A: AUSTRALIAN FRESHWATER FISH RISK ASSESSMENT MODEL

Australian Government
Department of the Environment, Water, Heritage and the Arts

Exotic Freshwater Fish Risk Assessment Model

Instructions: Complete the risk assessment by typing values in the pale green cells. The numerical result of the assessment is then calculated automatically. Context-sensitive help will appear when cells with a red triangle are selected.

Species identification and sources

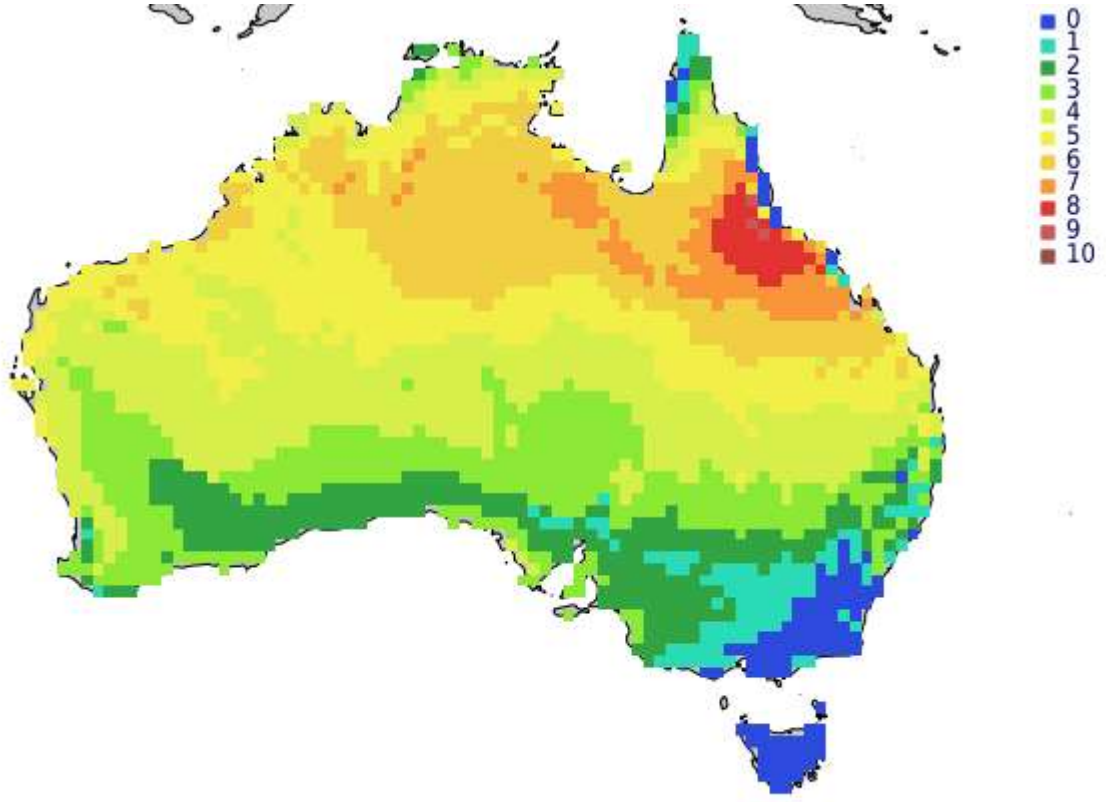
Common name	Turquoise Killifish
Scientific name	*Nothobranchius furzeri*
Date assessed	7-Feb-19
Literature Search Type And Date:	Google, IUCN, Google Scholar, Science Direct

Scores	Value	Explanation
A. Climate Match Score (1–8)	5	**Moderate**
B. Overseas Range Score (0–4)	1	5–10 grid squares
C. Establishment Score (0–3)	1	Never introduced
D. Introduction Success Score (0–4)	2	Success rate of >0.25 ≤ 0.5 OR Never introduced
E. Taxa Risk Score (0–5)	0	**Very low**

Summary	*Score*	*Rank*
Establishment Risk	9	**Moderate**

Note: The taxonomic family risk score is based on raw frequencies. It does not account for other variables, or interactions with them, that are likely to affect to the risk of establishment. **Hence this risk assessment should be interpreted in the light of any other information you may have.**

APPENDIX B: CLIMATCH PREDICTED RANGE.



REFERENCES

Bomford, M. (2008). *Risk assessment models for establishment of exotic vertebrates in Australia and New Zealand*. Invasive Animals Cooperative Research Centre, Canberra

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (2019). Appendices I, II and III. https://www.cites.org/eng/app/appendices.php

Cellerino, A, Valenzano, R, Reichard, M (2016). *From the bush to the bench: the annual Nothobranchius fishes as a new model system in biology.* Biological Reviews 91, pp. 511-533. doi: 10.1111/brv.12183

Fishbase (2019) Accessed February 2019. http://www.fishbase.org/summary/9855

Food and Agricultural Organisation (FAO) (2019) Database on the Introductions of Aquatic Species. Accessed 05/02/2019. http://www.fao.org/fishery/factsheets/search/fs/introsp/species/Nothobranchius+guentheri/en#

International Union for Conservation of Nature (IUCN) (2018). The IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/63305/12651421 .

Reichard, M (2015) *Annual Fishes: Life History Strategy, Diversity and Evolution*. Eds. Berois, N, Garcia, G and De Sa, RO. Chapter 9, The Evolutionary Ecology of African Annual Fishes pp.133-158. CRC Press, Taylor and Francis group.

Reichard, M and Polacik, M (2019) *Nothobranchius furzeri, an 'instant' fish from an ephemeral habitat*. doi:10.7554/eLife.41548

United States Geological Survey (2019) Nonindigenous Aquatic Species Database. Accessed 05/02/2019. https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=315#

Valdesalici, S and Alessandro, C (2003) *Extremely short lifespan in the annual fish Nothobranchius furzeri.* Proceeding of the Royal. Society London. B Biological Science. 270, S189–S191.