

Where do African clawed frogs come from? An analysis of trade in live *Xenopus laevis* imported into the USA.

JOHN MEASEY

Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University,
Stellenbosch, South Africa

Email: john@measey.com

Phone: 021 808 2385

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Abstract

The African clawed frog, *Xenopus laevis*, is one of the most widespread and high impact invasive amphibians on earth. The initial purpose of the trade for pregnancy testing saw some hundreds of thousands of animals exported to laboratories throughout the world. Together with the use of this species as the standard laboratory amphibian, it is probably the most widespread amphibian on the planet and has established invasive populations on four continents. Trade figures for imports of live animals into the United States of America suggest that trade for medical and scientific purposes is now minimal (a few hundred animals per year), while the pet trade imports 1.83 million live animals over the last 15 years. Surprisingly, 75% of these animals are imported from Hong Kong. Only 5 600 animals were imported from South Africa, and this trade ceased in 2003. Nearly 200 000 individuals were imported from Chile and the majority of these were reported as being wild caught, suggesting that the invasive population there is being exported for the US pet trade. The implication of large numbers of *X. laevis* is likely to be an increase in the number of invasive populations, as well as movement of individuals that may be carriers of disease, already shown to be present in shipments. Import data for *X. laevis* into the US suggest that very few African clawed frogs come from Africa, with the vast majority of Asian origin.

Keywords: captive breeding, import, invasive species, pet trade, Pipidae

Introduction

The global trade in wildlife is practically impossible to quantify (KARESH et al. 2005, ROSEN & SMITH 2010) but is probably worth billions of dollars (BROAD et al. 2003). The relationship between this trade and the establishment of invasive species is not direct, but the link between the number of propagules and the establishment of invasive species is well known (LOCKWOOD et al. 2009). Unlike trade in most mammals, the majority of reptiles and amphibians are traded as live specimens (ROSEN & SMITH 2010, but see WARKENTIN et al. 2009), increasing the chance that the trade will result in propagules for invasive populations. One of many impacts the amphibian trade may have is the potential spread of disease (FISHER & GARNER 2007, ROWLEY et al. 2007), and it has been argued that this is a reason to ban all trade (KRIGER & HERO 2009). Although this was not seen as a realistic option (GARNER et al. 2009), trade in salamanders to the USA has recently been banned following an outbreak of *Batrachochytrium salamandrivorans* attributed to this trade (YAP et al. 2015). An alternative to banning trade is to follow a best practice that minimises the importation of diseased

animals, and trading from captive bred stocks about which the health status is known is likely to minimise the risk of spreading disease.

Analysis of the proportion of captive bred to wild caught amphibians has previously been conducted for the USA (HERREL & VAN DER MEIJDEN 2014, SCHLAEPFER et al. 2005, SCHLOEGEL et al. 2009), but other countries appear not to capture such information on importation, and it is often impossible to assign the origin of imports once they have arrived (PEEL et al. 2012, TAPLEY et al. 2011). The trend in US data suggested an increase in the imports of captive bred amphibians to around two thirds of nearly 6 million animals in 2002 (SCHLAEPFER et al. 2005). Since then, the overall level of imports of amphibians and reptiles into the US appears to be in decline from over 8 million animals in 2002 to around 5.5 million in 2008, with a steady growth in trade of captive bred animals (HERREL & VAN DER MEIJDEN 2014). Although captive bred animals should be carefully monitored to ensure they are disease free, studies have shown that disease is currently prevalent (PEEL et al. 2012), and exported both on the amphibians themselves and in the water that contains them (KOLBY et al. 2014). Of particular note, is the prevalence of pipid frogs in this international trade (70.6% of trade from Hong Kong, KOLBY et al. 2014), including the African clawed frog, *Xenopus laevis*, a species which has a long history of trade.

From the 1930s to 1960s, African clawed frogs were in use in around 31 countries of the world in tests for human pregnancy (SHAPIRO & ZWARENSTEIN 1934, VAN SITTERT & MEASEY 2016). The early trade came direct from and was controlled by the Cape Provincial Authority (CPA) Jonkershoek, near Cape Town. Subsequent invasions that followed this usage and the scientific trade (MEASEY et al. 2012) have been investigated with all showing origins from the South African Cape (LILLO et al. 2013, LOBOS et al. 2014, but see DE BUSSCHERE et al. 2016). The origin is of significance as *Xenopus laevis* has long been viewed as a species complex (e.g. MEASEY & CHANNING 2003), and a taxonomically robust view confining the species to southern Africa has only recently been proposed (FURMAN et al. 2015). Invasive populations of African clawed frogs have considerable environmental as well as economic impact compared to most other invasive amphibians (e.g. impacts on aquaculture), including direct predation on other amphibians (MEASEY et al. 2015). Their known impacts from invasive populations have led to controls and/or bans of trade in this species from 12 US states as well as some countries (e.g. Australia; SOMMA 2015).

At the height of the South African exports in 1959, nearly 26 000 *X. laevis* were being shipped to 14 countries (although 69% was supplied to African universities for dissection; VAN SITTERT & MEASEY 2016). The lag between this trade and establishment or discovery of invasive populations is thought to be around 15 years (VAN SITTERT & MEASEY 2016). In addition to invasive populations, exports of *X. laevis* from South Africa have been found to include fungal pathogens and parasites (WELDON et al. 2004), which can persist in invasive populations (KUPERMAN et al. 2004). After the 1980s, most

trade of African clawed frogs came from captive bred animals due to trade bans associated with the economic boycott of the apartheid regime in South Africa (VAN SITTERT & MEASEY 2016). Trade from South Africa resumed in the late 1990s, but appears to be in decline (WELDON et al. 2007). Most trade from South Africa appears to be associated with scientific use. However, it has been reported that African clawed frogs can be purchased as pets in the USA as well as other countries, and the pet trade appears to be increasing as a pathway for invasive populations (MEASEY et al. 2012). The purpose for which animals are traded is important in determining whether trade represents significant pathways to invasive populations, as well as the movement of their pathogens into the environment.

In this study I first quantify the size and trend of the trade in African clawed frogs imported to and exported from the USA over the past 15 years. Secondly, I aim to ascribe the purpose of the trade (science, pets or medical), to determine the proportion of wild caught trade versus captive bred, and to detail the countries involved in trade of live *X. laevis*.

Methods

Import data for all records relating to African clawed frogs (*Xenopus laevis*) were requested from the U.S. Fish and Wildlife Service under the Freedom of Information Act. Records supplied detailed the number of individuals in every shipment entering the USA from 9 November 1999 to 2 June 2015. Only trade in live animals was considered further. Preserved specimens accounted for only 118/1 856 of records and 5 069/1 842 274 individuals, and other records included only two for eggs.

The taxonomic designation of *Xenopus laevis* has long been in flux with a large number of taxa that were traditionally considered to be sub-species of *X. laevis* (see KOBEL et al. 1996). However, recent phylogenetic investigations have proved most of these sub-species are full species in their own right (FURMAN et al. 2015). For this analysis, I consider only animals which belong to southern African *X. laevis* (formerly *X. l. laevis*) from South Africa, Lesotho, Swaziland, Zimbabwe and Malawi (and parts of Namibia, Botswana and Mozambique: see FURMAN et al. 2015, MEASEY et al. 2012).

Animals that were listed as wild caught and shipped from African countries where *X. laevis* does not occur were treated separately. Most of these are considered to have traded animals that were formally considered to be subspecies of *X. laevis* (see KOBEL et al. 1996), but which now are recognised to be full species (FURMAN et al. 2015).

Results

Data was received of 1 856 shipments which ranged from single animals to 11.5 thousand individuals (mean 992.6; SE 21.55). More than 1.83 million live African clawed frogs are documented as having been imported into the USA between 9 November 1999 and 2 June 2015. The majority of these frogs were imported from Asia (82%), a trade which appears steady over the entire period (Figure 1). Over 10% of *X. laevis* (190 000) were imported from Chile, although imports appear to be declining since 2004. Imports of African origin represent only 5.6% and have crashed since 2007 (Figure 1): it is not clear that frogs of African origin were all *X. laevis* and only 0.3% come from countries to which *X. laevis* is indigenous (in this dataset, only South Africa). Frogs imported from European or North American origin represent <1% of the total and shipments from these areas appear to have dwindled over this period (see Figure 2). During the entire period, African clawed frog imports (88.6% of records) from 19 countries on five continents, while exports (11.4% of records) were made to 54 countries on the same five continents (Table 1).

Only 5 600 animals were imported from South Africa during this 15 year period, and these imports appeared to have ceased in 2003. Hong Kong is the origin of 75% of the *X. laevis* traded, although other areas appear to have increased exports to the US, including China with between 10 and 35 000 animals a year since 2007 (Figure 2).

Trade in African clawed frogs for scientific and medical purposes represented less than 0.5% of the trade into the US, around 2 800 individuals in 15 years (Figure 1A). Trends in this trade appear to be sporadic over the period, with shipments being just a few hundred animals each time. Most animals imported for medical purposes originated in Europe, while most animals for scientific work came from Africa (but see above). The pet trade made up 99.5% of the trade into the US, a trend unchanged over the period.

Reporting on whether animals were wild caught or captive bred must be interpreted with caution. Over the entire period 52% of all animals imported were recorded as being wild caught, and the majority of these from Asia. Since 2010, reporting may have improved as 97.5% of individuals were recorded as captive bred, and Chile is reported as supplying the majority of the remaining wild caught African clawed frogs. Excluding reports for wild caught animals from areas where they do not occur in the wild, the only potential imports of wild caught animals are from South Africa (which appears to have ceased in 2003) and Chile. In fifteen years of imports from Chile totalling some 191 000 individual *X. laevis*, 59% were reported to have been wild caught (and the rest captive bred).

Discussion

The trade in African clawed frogs has changed dramatically in terms of the purpose of the trade, the origin of the frogs and the numbers of animals traded. The first 20 years of the trade (from 1940) is characterised by small numbers of animals being sent from Jonkershoek, South Africa to public health laboratories around the world for use in pregnancy testing (but mostly within the British Empire; VAN SITTERT & MEASEY 2016). By the 1960s, a chemical pregnancy test saw the decline of sales from South Africa, with most African clawed frogs being exported for scientific investigations. By the 1980s, trade to the US from South Africa was stopped and sales became focussed from dealers outside of South Africa for scientific studies as well as the rise of the pet trade (VAN SITTERT & MEASEY 2016). Actual numbers of *X. laevis* being imported for medicine and science were only a few hundred individuals per year, compared to many thousands per year in the 1940s (VAN SITTERT & MEASEY 2016). In the last 15 years, the size of the trade for medicine and science is only 0.1% of imports with the pet trade commanding 99.6%.

The movement of wild caught animals brings with it possibilities of movement of disease and parasites. Data shows that the quantity of wild caught *X. laevis* from their native range in southern Africa has dwindled with nothing being imported to the USA for over 10 years. This agrees with laboratory suppliers of *X. laevis* who concur that live animals have not been imported from the native range for many years (XENOPUS EXPRESS, pers. comm. www.xenopus.com). However, there are significant quantities of wild-caught animals being exported from areas outside the range of *X. laevis*. Most of these animals are being exported from Nigeria. The previous difficulties with taxonomy of the species have meant that Nigeria and Cameroon both contained a disjunct population of what has long been known to be distinct taxon from the southern African *X. laevis* and more closely related to *X. poweri* from Botswana and Zambia (EVANS et al. 2004). This has since been confirmed in a more recent and comprehensive phylogeny (FURMAN et al. 2015). Therefore, there is now an urgent need for authorities dealing in trade to update their taxonomy (HJARDING et al. 2014). Whether or not *X. poweri* from Nigeria pose a potential invasive threat in the USA is currently unknown, but pathogens of concern are known to be present in the region, including pipids which have tested positive for *Bd* (PENNER et al. 2013, REEDER et al. 2011). The consignment in 1999 of 1 500 wild-caught *X. laevis* originating in Madagascar is presumably an error as no pipids of any description occur there. These and other presumed anomalies should be treated with suspicion (including data prior to 2010), including wild caught *X. laevis* being reported from Canada, China, Czech Republic, Hong Kong, Indonesia, Singapore and Switzerland, as no native or invasive populations occur there. However, the biggest consignments of wild caught *X. laevis* come from Chile, presumably from their invasive populations. This trade is of significance as it signals a potential conflict of interest between stakeholders interested in invasive populations of *X. laevis* in Chile.

Invasive populations of *X. laevis* in Chile are believed to have been released into the wild near Santiago in 1973 (LOBOS & MEASEY 2002, MEASEY et al. 2012), but now cover 21 200 km²,

including areas where animals have undergone secondary introductions (LOBOS et al. 2013). SOLÍS et al. (2010) found chytrid fungus in invasive populations of *X. laevis* in Chile, moreover all specimens were asymptomatic. Meanwhile, *Bd* is thought to be driving population declines in local amphibians (Soto-Azat et al 2013). The current study shows that tens of thousands of *X. laevis* are being imported into the US from populations of invasive animals in Chile. Given that they are indeed wild caught, there is a chance that animals exported from Chile could be infected with the chytrid fungus. Whether this strain of the fungus originates from Chile or southern Africa is currently unknown (SOLÍS et al. 2010). The trade from Chile to the USA appears to be regular, but has a downward trend. Data from this study show that there is some trade from other countries with known invasive populations (UK and France), but all animals were small quantities (<100) of captive bred animals usually for medical or scientific purposes and are thus presumably not from invasive populations.

The majority of animals imported live to the USA are declared to be captive bred. Captive bred and laboratory *X. laevis* are not necessarily free of chytrid or Ranavirus (KOLBY et al. 2014, PEEL et al. 2012, SCHLOEGEL et al. 2009), but they are less likely to be carrying other pathogens and parasites. Most of these animals originated in Hong Kong, a locality well known for amphibians in the pet trade as well as for the trade of frogs for human consumption. ROWLEY et al. (2007) detailed the enormity of trade in amphibians entering Hong Kong in 2006, approximately 4.26 million individuals, although only 33 000 of these were part of the pet trade and scientific use, the rest being for human consumption. A breakdown of these 33 000 shows that only 240 individuals were *X. laevis* within this period from the USA (220) and France (20) suggesting that these were supplied by commercial suppliers for scientific use. During the same period (1 December 2005 to 30 November 2006) Hong Kong exported 110 914 *X. laevis* to the USA (data from this study), a large disparity with the previously reported figures. ROWLEY et al. (2007) also tested *X. laevis* being sold in the Mong Kok pet market for *Bd*, but found that all 7 were *Bd* negative (Figure 1C). Interestingly, samples of *X. laevis* shipped from Hong Kong to the USA were sampled by KOLBY et al. (2014) in 2012 who found that frogs were positive for *Bd* with low-level infections (but not Ranavirus – see also SCHLOEGEL et al. 2009), while the water they were shipped in had exceptionally high densities of *Bd* zoospores. Individuals sampled were all juveniles (ca. 5cm SVL) and were from an albino stock (J. KOLBY pers. comm.). Similarly, all *X. laevis* seen in Hong Kong were also albino juveniles (J. ROWLEY pers. comm., see Figure 1C). Therefore, it seems likely that the trade from Hong Kong is all of similarly sized and captive bred individuals. Trade data between 2006 and 2012 suggests that *X. laevis* was the second most important species imported into the USA from Hong Kong (673 859 individuals), with imports of another pipid (*Hymenochirus curtipes*) being more than double (1 468 130 individuals). While Hong Kong remains the stipulated origin of all of these animals, it is possible that many originate from mainland China (or elsewhere), and shipped to Hong Kong prior to export (J. ROWLEY pers. comm.).

Despite the many uncertainties in the U.S. Fish and Wildlife Service trade data (e.g. taxonomy, captive vs. wild bred and exact origin), it has been used extensively in analyses because it exists and is freely available. It is not known what proportion of world trade in the African clawed frog the U.S. trade represents as the data is not available. As trade in amphibians reaches unprecedented levels (HERREL & VAN DER MEIJDEN 2014, SCHLAEPFER et al. 2005, SCHLOEGEL et al. 2009), it becomes increasingly important for other trading regions to capture this data and make it freely available (PEEL et al. 2012, TAPLEY et al. 2011). While it is possible for the quality of the U.S. Fish and Wildlife Service trade data to improve, its very existence is laudable. The data not only makes it possible to determine where in the world African clawed frogs are imported from, but also which U.S. states they are imported into.

Half of all individual *X. laevis* arrived in New York, while Florida, Georgia, Illinois, New Jersey, Michigan and California make up the states that have received over 10 000 animals in the period. Interestingly, it is illegal to own, transport or sell *X. laevis* in 11 US states including New Jersey and California (SOMMA 2015; DODD 2013). This restriction makes sense in terms of potential invasions in that there are large areas with suitable climate for *X. laevis* in California (MEASEY et al. 2012). California received over 36 000 animals (2% of all imports) over the period investigated. It is presumed that the current invasive population in California became established as a result of the less than 10 000 sent to California in the 1950s (VAN SITTERT & MEASEY 2016).

Most of the current invasive populations of African clawed frogs saw their origins in the early export of animals from South Africa. In a recent review of the trade, VAN SITTERT & MEASEY (2016) document the export of only 86 000 individuals out of Africa, less than 5% of the animals that have been imported into the US in half the time. VAN SITTERT & MEASEY (2016) also suggest that there is a lag between the transit of animals and the establishment of invasive populations of around 15 years. There is some evidence that suggests that animals from the pet trade are the source of recent invasive populations. Lily Pond in Golden Gate Park, San Francisco was the subject of an eradication program of *X. laevis* believed to have arrived as unwanted pets (MEASEY et al. 2012) with many thousands of individuals removed the eradication was still ongoing in 2013.

Conclusion

From 1930s to 1970s, African clawed frogs used in pregnancy diagnosis and laboratory experiments were likely to have come from South Africa, and specifically the south-western Cape, with steadily increasing numbers of animals being moved between laboratories (VAN SITTERT & MEASEY 2016). The pet trade during this time was probably small, but is likely to have been made up from captive bred animals from laboratory stocks and suppliers in the UK and US. With the close of trade from

South Africa in the 1980s, the majority of African clawed frogs traded were captive bred. Over the last 15 years, the trend has reached an extreme such that an African clawed frog in the US is very unlikely to have come from Africa, and is most likely to have originated in a captive breeding facility in Hong Kong or on the Chinese mainland. However, these animals are unlikely to be disease free (KOLBY et al. 2014). Ironically, wild caught African clawed frogs in the US trade are most likely to be from invasive populations in Chile, or if from Africa they are unlikely to be *X. laevis*. The enormity of trade in *X. laevis* suggests that propagule pressure is higher in the past 10 years (1.07 million live animals imported into the US) than in any previous decade throughout the 85 year trade history. It has been claimed that by 1970, *X. laevis* was the world's most widely distributed amphibian (VAN SITTERT & MEASEY 2016), but today this distribution network has increased. The trade in African clawed frogs will most likely result in many more invasive populations of this high impact amphibian.

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Salamandra (in press)

Table 1: Live individual African clawed frogs exported from and imported into the USA
(from 9 November 1999 to 2 June 2015)

Exports	education	medical	scientific	pet trade	other	total
Africa	0	20	0	789	0	809
Europe	0	0	0	3290	0	3290
Australasia	0	0	7	33342	0	33349
North America	12	608	435	197435	61	198551
South America	0	0	0	92	0	92
Imports	education	medical	scientific	pet trade	other	total
Africa	0	0	1003	103904	0	104907
Europe	472	0	500	14943	176	16091
Australasia	0	0	4	1523851	10	1528935
North America	0	0	5	1122	23	1150
South America	0	0	800	190435	500	191735

Countries listed as the origins of imports of *Xenopus laevis*: Africa (Benin, Cote d'Ivoire, Madagascar, Malawi, Nigeria, Tanzania, South Africa); Europe (Switzerland, Czech Republic, Germany, France, Great Britain, Italy, Ukraine); Australasia (Australia, China, Hong Kong, Indonesia, Japan, Malaysia, Philippines, Singapore); North America (Canada, United States); South America (Chile, Nicaragua, Peru). See text for possible errors.

Fig 1: Numbers of African clawed frogs (*Xenopus laevis*) imported into the USA over the last 15 years. (A) Erratic and low numbers are imported for scientific (squares) and medical (circles) purposes. (B) The pet trade demonstrates a steady Asian (triangles) led market trading an order of magnitude more live animals than other regions. Numbers of live individuals from South America (squares) have reduced, while numbers imported from Africa (diamonds) have crashed. (C) *X. laevis* on sale in a Hong Kong market (photo Jodi Rowley).

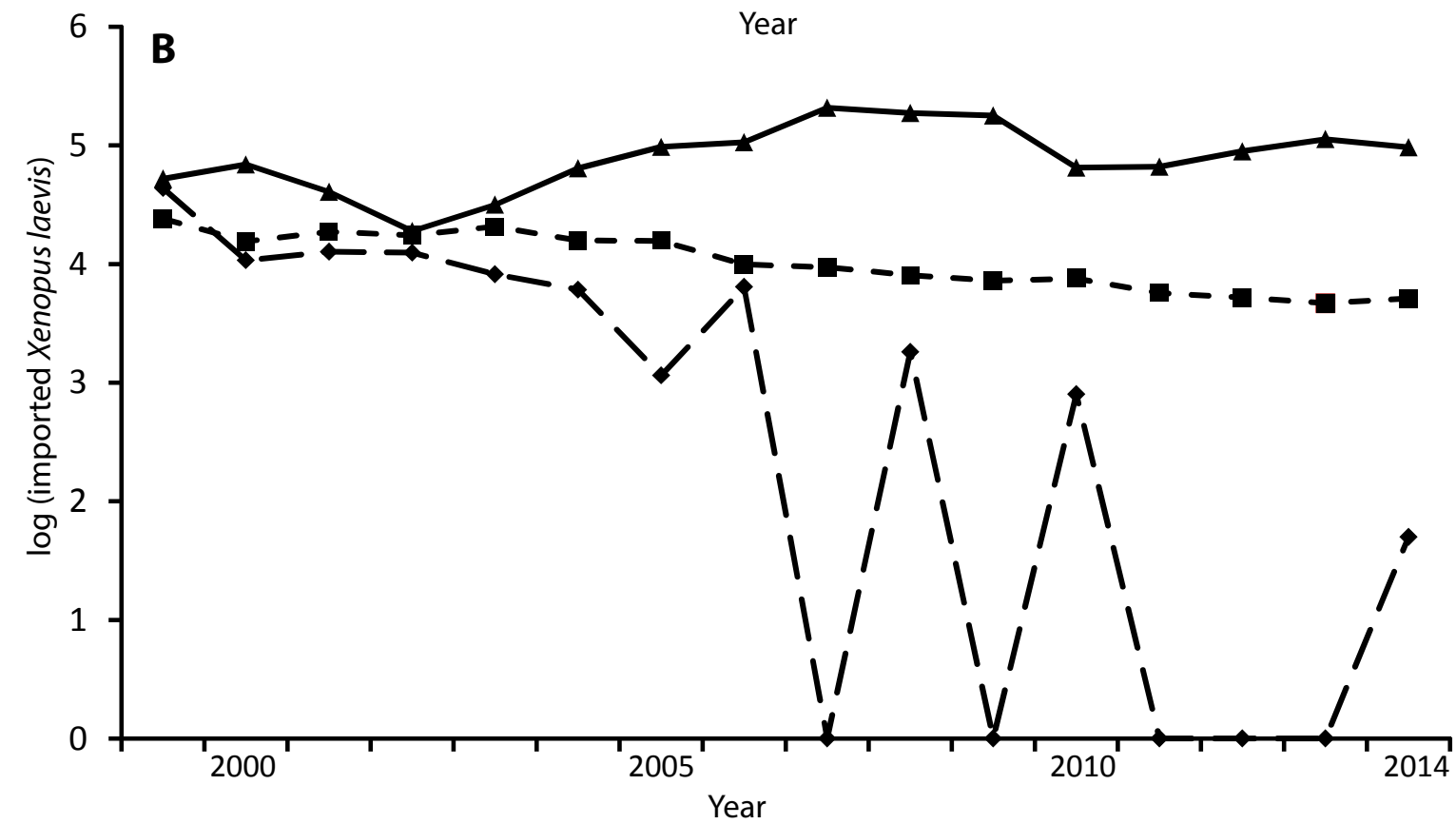
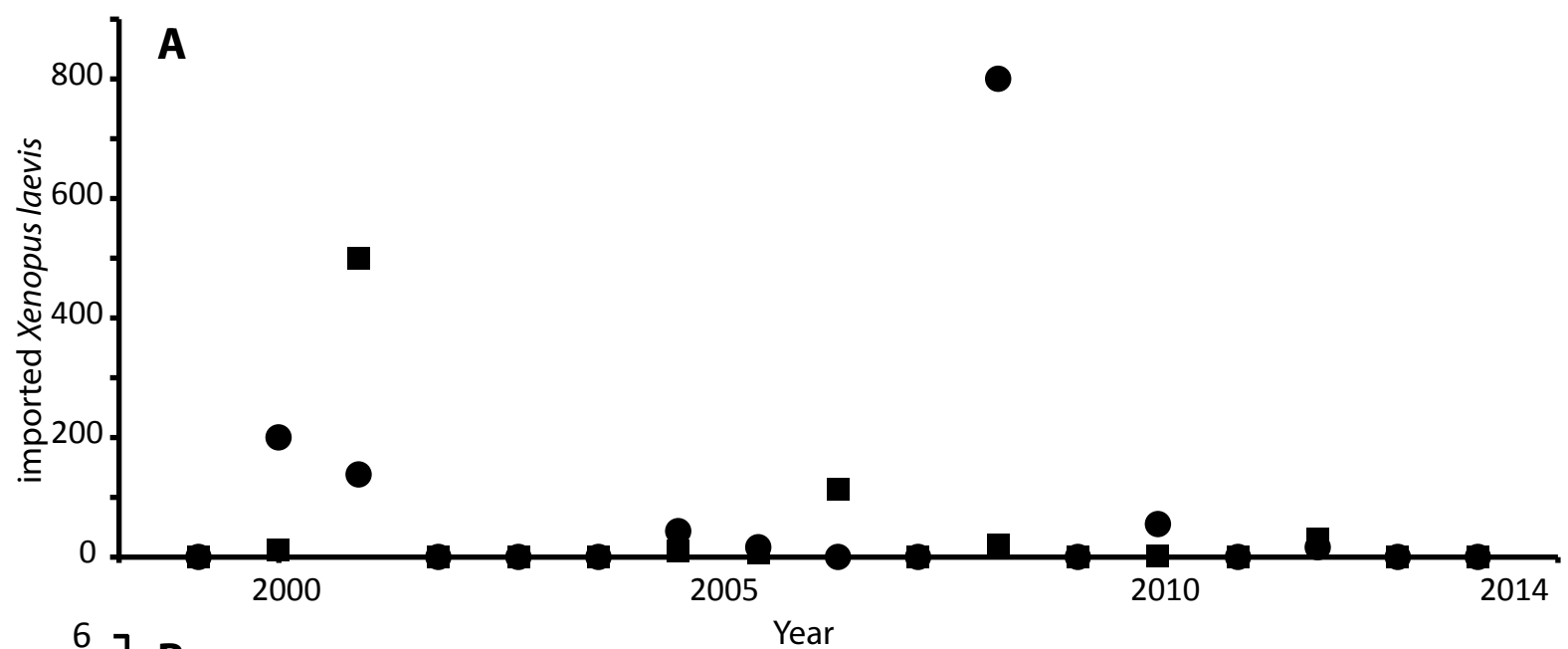


Fig 2: Total live trade of African clawed frogs, *Xenopus laevis*, imported into the USA over the last 15 years. The thickness of the lines is proportional to the size of the trade (see legend).

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