


Historical perspectives on global exports and research of African clawed frogs (*Xenopus laevis*)

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Trade in live animals has been associated with populations of invasive species as well as the spread of disease. The African clawed frog, *Xenopus laevis*, was exported from its native region of southern Africa for use in pregnancy testing, and later for laboratory use as the model amphibian. We use historical export figures and publication records to detail the size and extent of the global trade. In addition, we explore the link between exports, scientific use, and invasive populations and chytrid outbreaks. Exports reached 400 000 animals in the first 30 years from 1940, but only 86 000 were sent outside Africa. Exports out of Africa peaked in the 1950s, while scientific publications using *Xenopus laevis* grew in the 1970s, coinciding with a rise in invasive populations and chytrid outbreaks. We show a lag between exports of *Xenopus laevis* and a rise in invasive populations of around 15 years. Our data demonstrate the global reach of the exports of *Xenopus laevis* from South Africa, and a later, much wider distribution via the scientific network which was supplied by secondary means outside of South Africa. We contend that our data demonstrate that by 1970, *Xenopus laevis* was the world's most widely distributed amphibian: institutions in 48 countries were supplied with live colonies on all continents except Antarctica. There is some evidence linking exports and scientific studies with invasive populations, but others appear to be linked to secondary distributors of this species.

Keywords: African clawed frog; Anura; historical biology; invasion biology; invasion debt; propagules; trade; *Xenopus*

INTRODUCTION

The global trade in wildlife is practically impossible to quantify, but is certainly worth billions of dollars (Karesh *et al.*, 2005). The legal, international side of this trade shows trends that are ever increasing, and as they do so, the risks associated with wildlife trade become manifest. An increased threat of disease and the introduction of invasive species have been identified as resulting from wildlife imports into the USA (Smith *et al.*, 2009). Disease can be transmitted to both humans (e.g. monkeypox), or to wildlife (e.g. paramyxovirus), with costs being associated with socioeconomics and health-care on the one hand, and to threat of extinction on the other (Karesh *et al.*, 2005). Invasive species are a global problem which impact severely on biodiversity, cost governments an estimated \$1.4 trillion annually (Ricciardi *et al.*, 2011), and impact on the lives of individuals and communities the world over. Despite the very real problems associated with the global wildlife trade, there have been surprisingly few studies that have attempted to assess the historical trends for any taxa, no doubt due to the difficulties in piecing together data from disparate sources.

Biodiversity does not always respond to the immediacy of changes in the magnitude of disturbance (Essl *et al.*, 2015a, b). Time lags between the cause and effect in ecosystems are very problematic with respect to policy decisions as they often fall outside of typical political cycles (Essl *et al.*, 2015a). They also pose problems to researchers in the field as they

rely on good historical evidence for events in previous generations that may have only recently had significance. However, when historical details are available, this can lead to meaningful policy changes which in turn can block invasion pathways (Wilson *et al.*, 2009). The trade in live animals represents a significant invasion pathway, and is increasingly implicated for movement of disease, but few studies have attempted to reconstruct the historical basis for the trade and associated invasions.

Global trade in live amphibians has grown steadily over time (Herrel & van der Meijden, 2014; Schlaepfer *et al.*, 2005), and the result has been the widespread introduction of invasive populations as well as disease (Van Wilgen *et al.*, 2008). One amphibian, the African clawed frog *Xenopus laevis*, became the centre of speculation (after Weldon *et al.*, 2004) about the origin and spread of the chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), widely thought to be associated with the catastrophic global decline of amphibians (Pounds *et al.*, 2006; Stuart *et al.*, 2004). Intensive research into *Bd* since its description in 1999 has shown that there are multiple strains of this pathogen, many of which appear to be endemic to areas outside of Africa (Rosenblum *et al.*, 2013). For example, the fungus was present in North and South America (and Korea) more than 100 years ago (Fong *et al.*, 2015; Rodriguez *et al.*, 2014; Talley *et al.*, 2015). This has led to two hypotheses, namely that catastrophic chytrid outbreaks have derived from indigenous strains, or that virulent strains have been

transported along with the trade in amphibians (Rachowicz *et al.*, 2005). It is therefore important to trace the introduction of novel, and possibly more virulent, strains into local systems via trade and/or invasive amphibian populations (Goka *et al.*, 2009; Rosenblum *et al.*, 2013). Invasive populations of African clawed frogs have now been recorded on four continents, and in some cases populations are thought to be beyond eradication (Measey *et al.*, 2012). This frog has a special place in the history of 20th century science as it became one of four vertebrate species universally recognised as standard biological models representing all vertebrates (Travis, 2006). The history of the global trade in this species, therefore, has relevance both to invasion biology and the spread of disease.

The history of how African clawed frogs rose to prominence in academia has recently been compiled by Gurdon and Hopwood (2000). These frogs appeared on the international scene in the 1930s. During the 1940s, trade was mostly associated with the use of live animals for human pregnancy testing (Hogben, 1930; Shapiro & Zwarenstein, 1946). These animals were sourced from private dealers in the South African Cape region (Cape Town and surrounds) until the late 1930s, when the trade was regulated and the Cape Provincial Administration (CPA) became the main suppliers (Hey 1949). The pregnancy test was in use until an immunological assay was developed in the 1960s. *Xenopus laevis* became the amphibian of choice for embryologists as a result of year round availability of embryos, and the establishment of normal tables (Nieuwkoop & Faber, 1956). Due to the hardy nature of laboratory colonies of *X. laevis* (see Measey *et al.*, 2012), other scientists were attracted from fields which included biochemistry, cellular studies and genetics. The number of scientific fields that use *X. laevis* continues to grow, and with them laboratory colonies. Despite assertions that *X. laevis* is the world's most widely distributed species (e.g. Measey *et al.*, 2012), the global distribution remains undocumented.

Weldon *et al.* (2007) attempted to quantify the trade in *X. laevis*, but their quantification of the export trade prior to 1998 was derived from the published annual reports of the Cape Provincial Administration Department of Nature Conservation whose Jonkershoek and Pirie fish hatcheries were the official suppliers of *Xenopus laevis* to both national and international markets. This series is problematic for several reasons. Firstly, data on *X. laevis* sales were not published for 11 of the 34 years (1941–1974) in which the CPA traded in *X. laevis*. Secondly, the published data indicated exports in only 11 out of 23 years, and destination is recorded by continent only. Weldon *et al.* (2007) thus only provided a total sales figure of 340 000 *X. laevis* for two thirds of the period in which the CPA participated in the trade. As they were unable to detail exports over the period 1941–1997 they failed to demonstrate their central contention of global pathogen dissemination pathway via trade in *X. laevis*.

We accessed the unpublished archives of the Jonkershoek fish hatchery housed in the Cape Town Records Centre (TBK) of the National Archives and Records Services (NARS) which provide a much more complete and higher resolution reconstruction of the CPA trade in *X. laevis*. The archive contains data on every individual sale made by the hatchery for 28 of 34 years (the orders for 1956 and 1970–1974 could not be traced, although the latter are thought to have been retained at Jonkershoek), including the number and sex of frogs and the name and street address of the purchaser. This allowed Jonkershoek's annual *X. laevis* sales to be quantified

by sex and destination and the latter to be plotted at global, continental and national scales.

The source of animals for invasive populations, and thus putatively introducing chytrid fungus into the environment, is not well understood, but is thought to originate from pregnancy clinics, scientific laboratories and the pet trade (Kraus, 2008; Measey *et al.*, 2012). In this study, we attempt to reconstruct a complete trade record of exports of *X. laevis* from South Africa, and trace this through the first 50 years of its use and establishment in science as the global model amphibian. To approximate the existence of colonies of *X. laevis* in scientific laboratories throughout the world, we used publications in the scientific literature which featured '*Xenopus laevis*' in their title as a proxy. In addition, we show the relative timing of the establishment of known invasive populations and chytrid fungal infections, and discuss how the trade in animals from South Africa or laboratory colonies may have impacted this.

MATERIALS AND METHODS

The Jonkershoek *X. laevis* sales archive is housed in the Cape Town Record Centre (TBK) in the PAF (C-SJ Group) Cape Provincial Administration: Department of Nature Conservation: Jonkershoek Fisheries (1912–1970). The archive comprises 11 box files (B1, B13–22 and B84) containing 28 individual order files and related correspondence in roughly chronological order including a single file (B84) of orders filled by the Pirie hatchery, King Williams Town, in the summer rainfall region in the period 1949–1957 (see Appendix 1). The files were deposited in 1976 in accordance with the requirement of the then Archives Act (No. 6 of 1962). The archives have been housed in the TBK since receipt and have not been weeded. They can be searched on the National Automated Archival Information Retrieval System (NAAIRS) accessible at <http://www.national.archives.gov.za/>. They were located on the NAAIRS by performing keyword searches of all NARS repositories (RSA) with the terms 'frog(s)', '*X. laevis*' and 'padda(s)' (the Afrikaans word for frog/s). The files were called up at the main Cape Town Archives Repository in Roeland Street, Cape Town, read in series sequence, and the individual order data captured on a spreadsheet using the fields; invoice no., receipt no., SA, export, year, month, day, purchaser, address, city, female XI no., male XI no., total XI no., cost per frog (cents), cost of shipment (cents), transport (cents), packing (cents), total cost of shipment (cents). The orders were sorted into chronological order, cleaned to remove any duplicates and analysed by year, quantity, sex and destination (Tables 1 & 2 Supplementary Information). The *X. laevis* sales data contained in the published reports of the CPA, Department of Nature Conservation annual reports used by Weldon *et al.* (2007) were also extracted to check and to fill in gaps (1970–1974) in the archival series. The ancillary correspondence in the archival files and published annual reports was read for the qualitative information they contained about the trade.

A search in Web of Knowledge (WoK: Thomson Reuters, previously known as the Science Citation Index) was made for all publications with '*Xenopus laevis*' in their title from 1930 to 1980. We recognise that this excludes many iterations that may have been relevant (e.g. *X. laevis*, African clawed frog, clawed frogs, platannas), but consider this a representative sample. We then recorded the town/city/state and country of the corresponding author and determined latitude and

longitude for these from Google Maps (maps.google.com). Between 1930 and 1972, WoK did not record the address of the corresponding author in their database. For these first 655 publications we ascertained the address from journal websites and by sourcing the publication for 505 of them, the remainder could not be determined. While we acknowledge that the corresponding author of a paper with '*Xenopus laevis*' in the title may not signify that a laboratory colony of *X. laevis* were present in that town, we suggest that this is likely in the majority of cases. In addition, we searched Google Scholar using the South African commercial suppliers listed in Nace *et al.* (1971) together with the term '*Xenopus*', and listed the countries from the resulting publications.

The Global Mapping project Bd-Maps (<http://www.Bd-maps.net/>) database records all instances of the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd* – the fungus that gives rise to chytridiomycosis), together with dates when specimens were collected, their latitude and longitude, and species in which *Bd* was diagnosed. We downloaded all data for wild caught individuals between 1930 (the earliest recorded specimen being 1933) and 1980. We then used the co-ordinates to plot the recorded instances of this disease around the globe. Lastly, we plotted the positions of invasive populations of *X. laevis* using Measey *et al.* (2012), Kraus (2008), Tinsley and McCoid (1996) and associated literature searches. We plotted populations as present within a decade if they are reported as established at any time within that decade, and continued to plot them until they were reported as absent.

RESULTS AND DISCUSSION

The archival reconstruction of Jonkershoek *X. laevis* sales significantly revises Weldon *et al.*'s (2007) estimates of the total number of animals sold from 340 000 to over 400 000 animals. It also yields a near complete list of all destinations to which *X. laevis*, collected from the winter rainfall population of the southwestern Cape, were exported by the CPA over the period 1941–1969 (excluding 1956). This provides baseline first confirmed arrival dates for *X. laevis* from the winter rainfall population in 97 destinations, many of which are novel in the literature (Table 1, supplemental data). Destinations supplied by the CPA occur in 32 countries in Asia, Australia, Africa, Europe and North America. An additional six countries were supplied by private suppliers (Table 3, supplemental data), expanding the continents supplied to include South America, and covering all continents except Antarctica. During the same period, we show that publications on *X. laevis* emerge from all six continents with 10 additional countries having publications with the species name in the

title. Thus by 1970, live colonies of the African clawed frog, *Xenopus laevis*, had been distributed to 48 countries on all continents except Antarctica, making it the world's most widely distributed species of amphibian. In comparison, the Cane Toad, *Rhinella marina*, was introduced to 28 countries in North and South America, Asia and Australia, while the North American Bullfrog, *Lithobates catesbeiana*, has been introduced to 36 countries in Asia, Europe, North and South America (Kraus, 2008). However, the comparison is not entirely equal as these latter records refer to introductions of alien populations, while those described for *X. laevis* refer to laboratory colonies.

To best illustrate the change in historical use over time, we have broken the period into 10-year time slices (Figure 1). Here we record results from our historical searches and discuss their consequences.

1930s

Research on *X. laevis* was predominantly South African and Cape Town based, and covered a broad variety of topics including endocrinology, developmental biology, and reproduction. Other research was in London (L. Hogben, S.S. Alexander & C.W. Bellerby), Edinburgh (F.A.E. Crew), Aberdeen (F.W. Landgrebe) and Baltimore (H. Jensen) and was associated with individual scientists who used this species in anatomical studies or the diagnosis of pregnancy (see Gurdon & Hopwood, 2000). Prior to the commencement of trade from the CPA, animals were purchased from private dealers by both local and overseas users.

Although the distribution of the pathogen *Bd* is shown as being restricted to continental Africa, it should be remembered that other instances which predate this period are not shown (Fong *et al.* 2015; Huss *et al.* 2013; Talley *et al.* 2015).

1940s

In response to the development of frog pregnancy testing in the 1930s, the CPA, concerned about the sustainability of private dealers' wild harvesting of *X. laevis*, sought to supplement this supply with frogs cultivated in its Jonkershoek hatchery. The hatchery, however, failed in its efforts to breed *X. laevis* and so from 1941 supplemented cultivation with wild caught *X. laevis* from private farm dams in the southwestern Cape (Hey, 1949). The hatchery relied on farmers to alert them to well-stocked dams and despatched catching teams within a 150 km radius of the hatchery during spring to trap in dams, paying farmers 1–3d per frog (equivalent to USD 0.65 today). The wild caught *X. laevis* were held at Jonkershoek and distributed according to orders received by post. Frogs sold nationally were transported by rail in milk tins, and internationally by sea in custom-built metal cases each holding 100 frogs. The first air shipment of *X. laevis* was made in 1949, but this only became routine in the late 1960s and then only for ex-Africa sales.

During the 1940s, a total of 74 020 African clawed frogs were shipped from CPA Jonkershoek, with 43.5% of these being shipped to destinations outside of Africa (Figure 1; Table 1, supplemental data). After African shipments (most of which were inside South Africa), North America took 32% of animals, including two shipments to Ottawa, Canada, of a total of 1125 individuals and another consignment to Orlando, Florida, of 6650 *X. laevis* (see below).

The first report of an alien population of *Xenopus laevis* came from Ascension Island dating back to at least 1944 when a

Table 1. Jonkershoek *Xenopus laevis* sales by destination and decade 1941–1969.

Continent	Number of destinations	1940–1949	1950–1959	1960–1969
Africa	35	41 833	118 176	152 163
Asia	6	450	1 036	500
Australasia	8	1 445	2 325	344
Europe	39	6 019	15 263	16 219
North America	16	24 273	14 175	4 368
Total	104	74 020	150 975	173 594

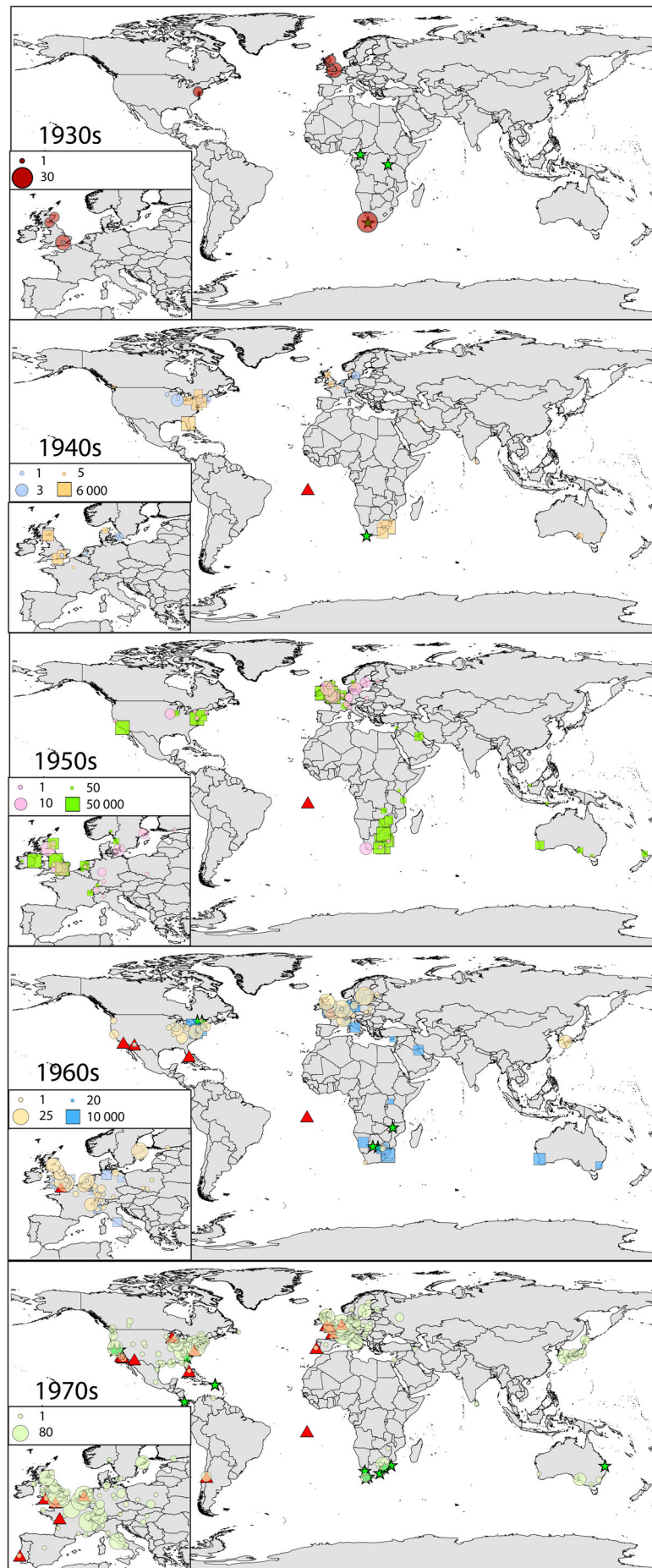


Figure 1. Decadal distribution of the African clawed frog (*Xenopus laevis*) through publications in journals (circles), shipments from the Cape Provincial Authority (squares) and invasive populations (triangles). Instances of chytrid outbreaks (from www.bdnnet.org) are plotted as stars. The sizes of circles and squares are proportional to the number of publications and export of individuals, respectively (note the key in each panel indicates relative sizes).

specimen was collected (Loveridge, 1959). Loveridge noted making a collection of animals near the summit of Green Mountain in the early 1950s. Tinsley and McCoid (1996) presumed these to have been released from exports during World War II, but we note that no exports were destined for Ascension Island, so we assume that these animals were intercepted (or escaped) from consignments destined for the USA when a boat docked there.

Interestingly, exports and research during the 1940s appear disassociated. Research was restricted to the USA, and a few locations in Europe, and notably absent in South Africa. This may be primarily due to the impact of World War II on research. Prominent work at this time included work on steroid hormones and information about breeding and care (F. Parker in Chicago, IL, USA and J.V. Thorborg in Copenhagen, Denmark). Meanwhile, exports were biased towards the USA, Canada, UK, Australia, Sri Lanka, Yemen, and some other European countries. Most exports made during this period appear to be linked to pregnancy testing.

1950s

Jonkershoek abandoned efforts at cultivation after the war and all animals during the 1950s were from wild caught populations. The export demand levelled off due to the establishment of commercial breeding populations in the USA and the UK and the substitution of *Xenopus laevis* with local species for pregnancy testing (see Frazer & Wohlzogen, 1950; Mainini, 1947; Mello, 1949; Robbins *et al.*, 1947; Wiltberger & Miller, 1948). Jonkershoek also prioritised supplying the national public health and research demand at cost before exporting. It is possible that these commercial breeding populations also engaged in exporting to third countries, for example Japan. The Director of Nature Conservation in the CPA, Douglas Hey, was also opposed to the commercialisation of the export trade on principle and prioritised supplying the national public health and research demand at cost before exporting any surplus stock. The bulk of its export trade was likewise directed into the public health and research network of the wider British Empire.

The vast majority of animals (78%) exported from Jonkershoek were supplied for dissection to tertiary education institutions in southern and eastern Africa. It is notable that most of these were inside the known distribution of *X. laevis* (see Furman *et al.*, 2015), and those that were not have local species that could have been adequate. Exports expanded to an increasing number of global destinations, and peaked in terms of numbers exported out of Africa. California (Los Angeles) received its first shipment of approximately 10 000 African clawed frogs during the 1950s (see below).

Many areas producing research were supplied by Jonkershoek, although outside of the main areas of research (South Africa, Europe and USA) many exports occurred to areas without any publications in the 1950s (e.g. Australia, New Zealand, Indonesia, Yemen), suggesting that the main demand was for pregnancy testing in state public health systems. In Europe, a sizeable area in the East received no animals from Jonkershoek, although it produced many publications, suggesting the continued presence of private dealers in the market (both in South Africa and elsewhere) despite, or perhaps because of, Jonkershoek flooding it with cost price animals. Alternatively, these laboratories may have been seeded with stocks from collaborating scientists (see below).

The only known invasive population during the 1950s was still on Ascension Island.

1960s

The steady, rising demand for female *X. laevis* for pregnancy testing from both state and private pathology laboratories in Britain and its empire, including South Africa, comprised 31.5% of sales. This demand disappeared by the mid-1960s when the frog test was replaced by chemical pregnancy tests. The slack was taken up by surges in demand from the tertiary education sector in South Africa and developmental biologists abroad, who had adopted *X. laevis* as a model amphibian for developmental work (see Gurdon & Hopwood, 2000). This was reflected in an increase in the volume of CPA sales to private dealers (11.1% of all sales).

Significant scientific advances were being made using *X. laevis* as a model organism. In 1962, J. Gurdon discovered the reversibility of cell specialisation through replacement of DNA in *Xenopus* eggs (Gurdon, 1962). This work eventually led to his receiving a joint Nobel Prize in Physiology in 2012. The total number of *X. laevis*-related publications rose to 352 (Figure 2).

Europe and USA were responsible for most published papers, but a number came out of Japan. There are no specific records of sales from Jonkershoek, pointing to the presence of private dealers, and/or the inter-institutional exchange of animals enabled by cheap and rapid air transport. It is notable that most publications do not match exports from Jonkershoek. There are occasional examples of publication lag, and indications that some laboratories may have taken animals left after pregnancy clinics ceased using frogs (e.g. Dublin, Ireland).

Chytrid infection outside the African continent was found from specimens of *Rana clamitans* from Saint-Pierre-de-Wakefield, Quebec in 1961 (Ouellet *et al.*, 2005). This site is only 30 km from the city of Ottawa (see above and Table 1, supplemental data) where consignments were delivered in the 1940s, with aquatic corridors (Riviere Gatineau) and small lakes permeating the system.

This period coincided with the first invasive population established in Europe, on the Isle of Wight, by deliberate introduction around 1962, and associated with a pregnancy testing clinic (Tinsley & McCoid, 1996). At the same time, multiple introductions were made in California, many of which are still present as invasive populations today (see Measey *et al.*, 2012). Of note is the simultaneous shipment of animals, publication of papers, and the development of an invasive population in Tuscon, Arizona. This invasive population is believed to have resulted from a deliberate introduction (Tinsley & McCoid, 1996). Another population in Florida coincided with large shipments made to Orlando in the 1940s. Many of these introductions in the 1960s have been recorded as deliberate (Kraus, 2008; Measey *et al.*, 2012; Tinsley & McCoid, 1996).

1970s

Exports declined from more than 40% of sales in the mid-1940s to less than 10% by the mid-1970s. This reflected the CPA policy of giving precedence to the national market and refusing to supply foreign wholesale dealers, not a decline in international demand which was increasingly supplied by private dealers in South Africa and abroad (see Nace *et al.*, 1971). Nace *et al.* (1971) listed seven ex-South African

commercial suppliers; three in the USA (one in Cockysville, MD and two in Oshkosh, WI), two in the UK (Littlehampton and Oxford) and one each in the Netherlands (Ermelo) and (then) Czechoslovakia (Svermova). Three were private companies, three researchers and one private individual. More than half (55.6%) of Jonkershoek *X. laevis* were sold to the tertiary education sector for teaching and research almost entirely in South Africa (93.8% of tertiary education sales) (see Figure 2).

Research output had quadrupled, with the USA and most countries in Europe having produced many papers. Research was spreading east into Russia, and Japan rose to prominence. Australia was also starting to produce research on *X. laevis*, including many of the cities that had received exports from Jonkershoek in previous decades.

Infections of *Bd* were found on the East and West coasts of North America including Quebec, Canada (Ouellet *et al.* 2005). Australia also recorded the first presence of *Bd*, which also appeared in the Caribbean and Central America, areas that have no known historic connection with *X. laevis*. During the 1970s invasive colonies of this species were widespread in the USA, Europe and Japan (although Japan's invasive population was not recorded until the 1990s; Measey *et al.*, 2012). Additional invasive populations appeared in Santiago (Chile) and Lisbon (Portugal) within the same decade that the first publications appeared in those countries. The Lisbon invasion is thought to be directly related to escape from a research facility (see below), and while the origin of the Chilean invasion is unknown, it seems likely that it too originates from research.

1980s onwards

In the penultimate decade of the 20th century South African exports of *Xenopus laevis* were constrained by anti-apartheid sanctions abroad. The passing of the Comprehensive Anti-Apartheid Act in the United States in 1986 closed the lucrative American market for five years and importers everywhere were encouraged to source *X. laevis* from suppliers other than those in South Africa. The export trade rebounded strongly after the abolition of apartheid in 1994 despite new conservation controls on the wild harvesting of *X. laevis* in the Western Cape. More than 71 500 *X. laevis* were exported from the province under the new permit system in six years (1998–2004) alone (Weldon *et al.*, 2007), roughly three quarters of the total exported by Jonkershoek over 35 years (1941–1974).

Relationships between exports, publications, introduced populations and disease

Our data show a surprisingly poor link between exports of *X. laevis* from the CPA and the far greater numbers of institutions where research was being undertaken on *X. laevis*. Although large numbers of animals were shipped from Jonkershoek to many major research areas, especially in the UK and the USA, publications arose from areas which had apparently received no animals from the CPA. We attribute this discrepancy both to the (unrecorded) trade by private dealers in South Africa since 1970, but also to secondary trade from laboratories undertaking research and passing on viable colonies for use. We acknowledge that our use of '*Xenopus laevis*' in the title of a research publication does not guarantee the presence of a laboratory colony at the corresponding author's address. Especially as some of the publications would not have relied on live animals, and others may have resulted from the work being carried out at other institutions. In addition, we

did not include many other publications on this species that, for example, did not have the full Latin name in the title. However, we feel that this does give a conservative estimate of the geographic areas in which researchers kept colonies of African clawed frogs. In addition, our search accurately located laboratories which kept live animals when these were not reflected in exports from the South African CPA, for example in Lisbon, Portugal.

Examples of secondary movement of laboratory stocks can be seen in our literature records. In 1968, a consignment of 200 *X. laevis* (120 females and 80 males) were sent to Dr M. Balls at the University of East Anglia in Norwich, who had been publishing work on the African clawed frog since the early 1960s (Balls, 1962). The same department has hosted researchers working with *X. laevis* and this has continued to the present day. The physiologist, Dr G. Shelton, also worked on *X. laevis* and trained various students including Dr M. Emilio (e.g. Emilio & Shelton, 1974). Emilio continued working on African clawed frogs when she returned to Lisbon, Portugal (e.g. Costa *et al.*, 1989). The invasive population of *X. laevis* in Lisbon is believed to have been established in the late 1970s when the basement animal facility, which housed Dr Emilio's frogs, flooded (see Measey *et al.*, 2012). Whether or not this sequence resulted in an invasive population descended from the same animals (or even including the same animals given their longevity, see Measey, 2001) that were shipped in 1968 is unknown, but this example does serve to show the potential for connectivity between shipments from the CPA, researchers and the secondary movement of colonies of *X. laevis*.

In addition to the disjunction between CPA supplies and research, there appears to be no direct link between CPA supplies or research and invasive populations. While some invasive populations did have clear links with research (e.g. France, Portugal, see Measey *et al.*, 2012), others result from these animals being kept inside the workplaces or homes of people who were curious or had a misplaced sense of ethics. The secondary trade in *X. laevis* as pets appears to be responsible for several invasive populations (e.g. Humberside, UK and Florida, USA, see Measey *et al.* 2012), as well as an unknown number of individuals released into the environment. Our findings illustrate that *X. laevis* were not shipped from South Africa for the pet trade. Clearly, animals originally intended for pregnancy clinics or scientific laboratories have moved into the pet trade, probably through secondary distributors, and from there into the environment. This secondary spread, from areas supplied by South Africa to other laboratories has not been investigated, yet the historical scale of these secondary movements is likely to be of relevance.

We did find some support for the suggestion that CPA exports were chronologically concordant with certain chytrid outbreaks. An early chytrid outbreak in North America occurred north of Ottawa, Canada in 1961, 10–14 years after 1125 individuals were sent there. Extensive studies of chytrid in this region suggest that in subsequent years, the fungus spread to several other species throughout the St Lawrence River Valley of Québec including the American bullfrog, *Lithobates catesbeiana* (Ouellet *et al.*, 2005). Although chytrid is known to have been present in North America prior to this record (Huss *et al.*, 2013; Talley *et al.*, 2015), it is thought that novel strains of the pathogen from other areas may have higher impacts on native species, and that these are facilitated

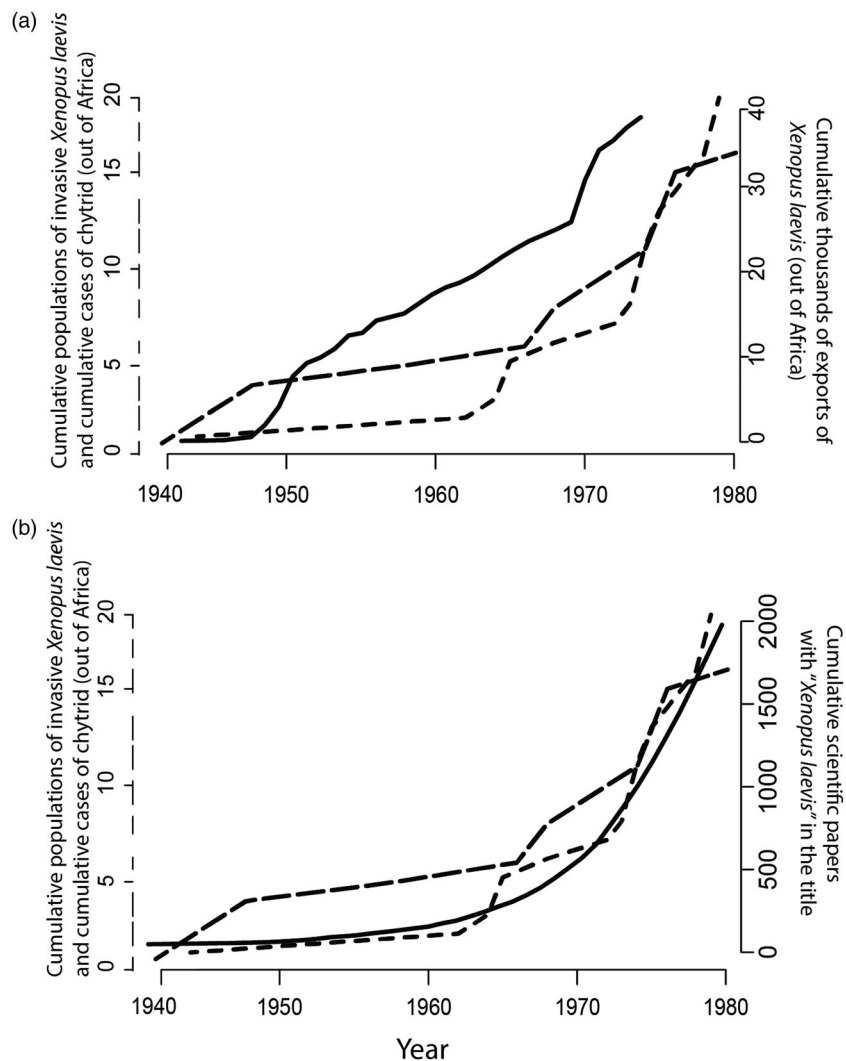


Figure 2. Cumulative numbers of invasive populations of African clawed frogs (*Xenopus laevis*) and outbreaks of chytrid fungus are shown on the left abscissa. (a) shows the cumulative exports of *X. laevis* from the Cape Provincial Authority while (b) shows the cumulative number of scientific papers with '*Xenopus laevis*' in the title.

by invasive anurans including *L. catesbeiana* (see Goka *et al.*, 2009; Rosenblum *et al.*, 2013). This is notable as trade in this species is also thought to be responsible for disseminating chytrid globally (e.g. Goka *et al.*, 2009; Schloegel *et al.*, 2009).

Invasion debt

Despite more than 200 000 individual *X. laevis* being shipped from South Africa in the 1940s and 1950s (Table 1), it was not until 15 years after global dissemination of this species in the 1960s and 1970s that invasive populations started to grow. In many cases, it appears that this lag is owing to a move from the use of the species for pregnancy testing, to laboratory animals, and then into the pet-trade (Measey *et al.*, 2012). Figure 2 shows the numbers of invasions to have undergone a sharp increase in the 1960s. Invasions are ongoing, with more being uncovered in the 21st century (Measey *et al.*, 2012; Peralta-García *et al.*, 2014). In addition, the cryptic nature of this principally aquatic species may make detection difficult and it is possible that more invasive populations exist, but are yet to be discovered.

The lag between the export of African clawed frogs by the CPA and a rise in invasive populations of around 15 years (Figure 2a) could represent original animals shipped, or 3 to 7 generations depending on the location (see Measey, 2001). We show that the rise in invasive populations occurs simultaneously with a rise in chytrid outbreak, although we do not suggest causation. The late 1960s and 1970s show an exponential increase in the use of *X. laevis* in laboratories worldwide, and this coincides with increases in both invasive populations and chytrid outbreaks (Figure 2b).

Genetic implications of trade

Around 60% of African clawed frogs were sold to buyers in 30 locations within the range of *Xenopus laevis* and other *Xenopus* species in Africa (see Figure 1). It seems possible that some of these animals may have made their way out of laboratories and into their local environment. Jonkershoek falls into a clade of *X. laevis* which is known to occur in the southwestern Cape: 'Cape clade'. Although the full extent of this clade is not well understood, it occurs from around Vredendal in the north, to Knysna in the east (Furman *et al.*,

2015; Measey & Channing, 2003). The Cape Fold mountains appear to harbour at least three other clades that extend north and east, and while these are not thought to represent separate species, their genetic divisions are thought to amount to 4–5 My of evolution (Furman *et al.*, 2015). Should exports from Jonkershoek be released at their destinations, they would be in the presence of a different genetic clade. This would make such releases genetically identifiable, and potentially cause changes in the genetic make-up of *X. laevis* in some of its native range.

Within Africa, but outside of the range of *X. laevis*, there are no introductions known, although it is not possible to say that none have occurred. As *X. laevis* is able to hybridise with many of its congeners (Graf & Kobel, 1991), any such populations may pose a threat to local species, although no threatened *Xenopus* species are known from the areas to where *X. laevis* were shipped. Outside of Africa, we would expect that populations of invasive *X. laevis* would belong to the ‘Cape clade’, as has already been found in Italy, Chile and Portugal (De Busschere *et al.*, 2016; Lillo *et al.*, 2013; Lobos *et al.*, 2014), however the French invasion appears to have a far more complex history with clades from throughout the native range being represented (De Busschere *et al.*, 2016). By the time the CPA withdrew from the market the export demand for *X. laevis* was for developmental biology research (Gurdon & Hopwood, 2000). In the early 1970s, Nace *et al.* (1971) compiled a comprehensive list of 11 global suppliers of *X. laevis*; 5 in South Africa (including Jonkershoek), 3 in the USA, 2 in the UK and 1 in the Netherlands. One each of the American (J. Cook: Cockeysville, MD), British (Gerrard and Haig: Littlehampton) and South African (D. Muller: Johannesburg) private dealers sourced their *X. laevis* from Jonkershoek. Two of the other three South African private *X. laevis* dealers were located in Cape Town (D. Wood: Snake Farm, Fish Hoek) and Stellenbosch (P. van den Elzen) all in the winter rainfall region, and ‘Cape clade’. All supplied only wild caught *X. laevis*, from within the ‘Cape clade’ (van Elzen and Jonkershoek, also dealing in *X. gilli*: Nace *et al.*, 1971).

However, we are also aware that there is the possibility that exports came from elsewhere in South Africa, within the regions of genetic clades other than the ‘Cape clade’. The last South African supplier mentioned by Nace *et al.* (1971) was from Port Elizabeth (D.M. Pinker: Walmer) in the summer rainfall region. A private shipment of 10 000 *X. laevis* was reported from Port Elizabeth to an unknown foreign destination (South African Shipping News and Fishing Industry Review). Currently, there have been no studies that would suggest which clade would be found in the area, and this adds to a growing need to define the endemic intraspecific biodiversity of this species. In addition, the CPA was known to fill orders with animals from the Pirie hatchery near King Williams Town, Eastern Cape. However, close inspection of these records show that they were all sent to South African institutions on the east coast (e.g. Durban, East London). It is not yet known whether the native genetic lineages in these areas are the same as those from the Pirie source.

Private dealers

While it may be true that there are no quantitative data on the private frog trade for the period 1974–1997 (Weldon *et al.*, 2007), its scope can be delineated through the use of a bibliographic proxy. The only private South African dealer to register on the Google Scholar keyword search was the Snake Farm in

Fish Hoek which yielded 215 discrete institutional reports in scientific journals from 14 different countries over a period of 37 years 1968–2005. The most striking contrast with CPA sales was the complete absence of South African, African or Australasian institutions. While some of the pathology laboratories in these regions likely stopped buying *X. laevis* after decolonisation and/or the discontinuation of frog pregnancy tests in the 1960s, the continued demand of the tertiary education sector was primarily for male *X. laevis* for teaching and thus invisible to this source.

Four countries (28.6%) accounted for 79.1% of all published papers utilising Snake Farm supplied *X. laevis*; the USA (86 papers), Germany (34 papers), Switzerland (26 papers) and the UK (24 papers). Three other countries, Israel (15 papers), Belgium (11 papers) and France (8 papers), accounted for a further 15.8% with the remainder made up by Chile (3 papers), Canada (2 papers), Italy (2 papers), China (1 paper), Greece (1 paper), Japan (1 paper) and Spain (1 paper) (see Figure 1).

Temporally, the proxy Snake Farm sales data clearly reflect the withdrawal of the CPA from the market in 1974, the combined deleterious effects of anti-apartheid sanctions abroad, and the increasing restriction of wild capture on the Snake Farm’s market after the mid-1980s. Spatially, the proxy Snake Farm sales data yield 63 locations outside Africa, 52 of which were not supplied by the CPA, together with approximate dates of first arrival. These locations are both in the CPA’s traditional European and North American export markets and in nascent markets in South America (Chile), Israel and East Asia (China and Japan).

CONCLUSION

More than 2100 orders comprising in excess of 400 000 *X. laevis* were filled by the CPA Jonkershoek in 28 years (1941–1969) to 104 specified global destinations. Pirie’s output was small, intermittent and only supplied the Eastern Cape and Kwa-Zulu Natal demand. A surprisingly high number of frogs were supplied for dissection within southern Africa, where dissection was already well established in tertiary education institutions (and this practice continues today). We provide details of 69 destinations and 86 000 animals that were sent outside Africa. We demonstrate that during this period, the number of colonies in laboratories around the world was much higher, with 254 locations producing publications with ‘*Xenopus laevis*’ in the title. The secondary trade that supplied these laboratories may be implicated in establishing invasive populations. We demonstrate an invasion lag for African clawed frogs between the movement of animals from Jonkershoek, and a rise in invasive populations which occurred some 15 years later. We contend that our data demonstrate the African clawed frog as the world’s most widely distributed amphibian, reaching all continents (except Antarctica) by the mid-1970s. Lastly, we provide new evidence on shipments of *X. laevis* which may be of interest to those studying the spread of novel strains of the chytrid fungus pathogen.

While this study represents a significant advance on previous attempts to reconstruct the CPA trade from its published reports alone, it is important to recognise that the CPA was not the only dealer in the national market and that private dealers effectively remain ‘ghosts’ glimpsed before 1997 only through proxies such as the scholarly output of their clients. African and ex-African private dealer sales records which are

comparable in quality and span to those of the CPA are urgently required. Only then will it be possible to map the full extent of the scale and scope of *X. laevis* trade over the second half of the 20th century. This will enable us to adequately address the many and far-reaching legacies and consequences which exist today.

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SUPPLEMENTAL DATA

Supplemental data for this article can be accessed here.

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APPENDIX 1. HISTORICAL RECORDS OF *XENOPUS LAEVIS* EXPORTS

Cape Town Archives Repository (KAB)

PAN30, A120/B/338, Supply of frogs from Jonkershoek trout hatchery. 1941–45.

PAS 3/192, P5, Inland fisheries. Culture of frogs. 1941–50.

Cape Town Records Centre (TBK)

PAF B1, C/A-Z (1), Jonkershoek fisheries. General. Includes applications for supply of fish and trout and frogs. 1944–45.

PAF B1, C/A-Z (2), Jonkershoek fisheries. General. Includes applications for supply of fish and trout and frogs. 1946.

PAF B1, C/A-Z (3), Jonkershoek fisheries. General. Includes applications for supply of fish and trout and frogs. 1947.

PAF B1, C/A-Z (4), Jonkershoek fisheries. General. Includes applications for supply of fish and trout and frogs. 1966–69.

PAF B13, C/FO(1), Jonkershoek fisheries. Frog orders. Miscellaneous. 1942–43.

PAF B13, C/FO (2), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1943.

PAF B13, C/FO (3), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1943–65.

PAF B13, C/FO (4), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1949.

PAF B14, C/FO (5), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1951.

PAF B14, C/FO (6), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1951–52.

PAF B14, C/FO (7), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1952.

PAF B15, C/FO (8), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1952–53.

PAF B15, C/FO (9), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1953.

PAF B16, C/FO (10), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1953–54.

PAF B16, C/FO (11), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1954.

PAF B16, C/FO (12), Jonkershoek fisheries. Frog consignments. Miscellaneous. 1954–55.

PAF B17, C/FO (13), Jonkershoek fisheries. Frog orders. Miscellaneous. 1956–57.

PAF B18, C/FO (14), Jonkershoek fisheries. Frog orders. Miscellaneous. 1957.

PAF B18, C/FO (15), Jonkershoek fisheries. Frog orders. Miscellaneous. 1958.

PAF B19, C/FO (16), Jonkershoek fisheries. Classified frog orders. Miscellaneous. 1958–64. PAF B20, C/FO (17), Jonkershoek fisheries. Frog orders. Miscellaneous. 1959–60.

PAF B20, C/FO (18), Jonkershoek fisheries. Frog orders. Miscellaneous. 1959–64.

PAF B21, C/FO (19), Jonkershoek fisheries. Classified frog orders. Miscellaneous. 1959–69.

PAF B21, C/FO (20), Jonkershoek fisheries. Frog orders. Miscellaneous. 1961–62.

PAF B22, C/FO (21), Jonkershoek fisheries. Frog orders. Miscellaneous. 1963–64.

PAF B22, C/FO (22), Jonkershoek fisheries. Frog orders. Miscellaneous. 1966.

PAF B22, C/FO (23), Jonkershoek fisheries. Frog orders. Miscellaneous. 1967–68.

PAF B22, C/FO (24), Jonkershoek fisheries. Frog orders. Miscellaneous. 1969–70.

PAF B84, PH16B (1), Pirie trout hatchery. *X. laevis* orders. 1949–57.

PAF B131, PS8/3 (1), Jonkerhoek fisheries. Transportation of frogs. 1946–66.

National Archives Repository, Pretoria (SAB)

HEN 1370, 161/1/7, Horse and cattle breeding industry. Toads and frogs. 1945–60.

HLD 74, 78/33 (2), Public health. Snake bite serum and supply of *Xenopus laevis* toads. Also export of live frogs. 1963–65.

LDB 3354, R4302, Frogs. General correspondence. 1941–59.