A head-starting trial for the reintroduction of the pool frog *Pelophylax lessonae* to England

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ABSTRACT - Head-starting tadpoles was trialled to assist the programme for the reintroduction of the northern clade of the pool frog *Pelophylax lessonae* to England. Ten spawn clumps (estimated 1228 eggs) were removed from the reintroduction site and hatched under captive conditions. Survival rate of the eggs varied greatly between clumps, from 11% to 100%, but overall survival to the free-swimming tadpole stage was approximately 50%. Survival rates thereafter were high; 97% for tadpoles reared indoors and 81% for tadpoles grown for part of the larval period outdoors in artificial ponds. Releasing head-started tadpoles substantially increased metamorph productivity at the reintroduction site. The head-starting methodology described is labour intensive but provides an approach that could be used in secondary reintroductions of this species. It therefore merits further development for its potential contribution to the reintroduction of the northern pool frog to England.

INTRODUCTION

After extinction in England the northern clade of the pool frog *Pelophylax lessonae* (the northern pool frog) has been subject to a carefully planned reintroduction (Buckley & Foster, 2005). Over a four-year period (2005 to 2008) frogs were translocated from Sweden to a specially prepared site in Norfolk. Individual frogs have fared well and a population has become established (Foster et al., 2018). Nevertheless, the population has remained small (approximately 50 adults) which puts it at risk of extinction from stochastic events. The specific habitat requirements of the northern pool frog mean that it is unlikely to spread to new sites naturally. Hence the highly desirable establishment of further populations is reliant on translocation (Buckley & Foster, 2005), but while the first population remains small there are insufficient post-metamorphic animals to provide the necessary donor stock. Further importation of frogs from Sweden would be legally complicated and the pool frog is a rare species in that country anyway.

Head-starting is a management technique that rears early life stages (eggs, larvae, juveniles) in captivity before releasing them into native habitats (Smith & Sutherland, 2014). It boosts population productivity by protecting these life stages from the high rates of mortality normally experienced in the wild. Head-starting tadpoles has been recommended as a cost-effective method of establishing new populations of crawfish frogs *Lithobates areolatus* with minimal cost to the donor population(s) (Stiles et al., 2016) and within the British Isles it has been used as a successful technique in the recovery of the agile frog *Rana dalmatina* on Jersey (Ward & Griffiths, 2015).

In 2012 a limited head-starting trial was undertaken for the northern pool frog reintroduction programme. One hundred and thirty-eight eggs and hatchlings were taken into captivity from the established population and 113 of these were released back into the donor population as well-grown tadpoles. The current trial further investigated the potential of taking spawn from the wild, rearing tadpoles in captivity and subsequently releasing them to supplement the numbers of metamorphs produced naturally. Both trials aimed to release tadpoles immediately prior to metamorphosis to capitalise on the rapid larval growth stage but to avoid the relative difficulties of rearing large numbers of juvenile frogs. Both head-starting trials were carried out under licence from Natural England.

METHODS AND RESULTS

Collection of spawn

The behaviour of adult frogs at the reintroduction site was monitored to anticipate spawning. Behaviour indicative of imminent spawning included amplexus or the movement of female frogs towards male choruses. Spawn clumps were deposited on top of mats of vegetation, floating at the pond surface, or adhering to stems of broad-leaved pondweed *Potamogeton natans*, just below the surface. All of the clumps were found some distance from the pond shoreline and were collected by wading into the pond or from an inflatable dinghy, taking care not to disturb remaining spawn in the process. Each female produces two to five small spawn clumps in a single spawning (Sjögren, 1991). Effort was made to find spawn in different locations within a pond, or from different ponds, to maximise the number of donor females. Ten clumps of spawn were collected from two ponds (six clumps on 8 May, two on 29 May and two on 2 June).

The number of eggs in nine of the clutches was counted from photographs. One of these spawn clumps contained 246 eggs, more than twice the mean number of eggs in

the others (mean=109, n=8), suggesting that in the former case two clumps were deposited simultaneously. Using the mean value of 109 to substitute for the number of eggs in the clutch where eggs were not counted, the estimated total number of eggs taken was 1228. These were equivalent to the reproductive output of one or two females, based on clutch sizes of six females which ranged from 587 to approximately 2,000, dependent on body size (Sjögren, 1991).

Head-starting facility

The spawn was hatched and the subsequent tadpoles reared in a private home approximately 50 km from the reintroduction site. No amphibians, or other animals, were kept at this residence, greatly reducing biosecurity risks. All equipment used in maintaining spawn and tadpoles was dedicated to the rearing protocols (i.e. not used for any other purpose) as a biosecurity measure.

Care of spawn

Spawn clumps were held separately in small plastic containers (used margarine tubs [Fig. 1] and food storage boxes) and maintained at room temperature. Immediately after hatching the tadpoles moved little and did not require feeding. As they became mobile they began to feed on algae growing on the remaining spawn and on fragments of adhering vegetation. At this point the tadpoles were transferred to larger containers by pipette. Some eggs failed to develop and survival rate varied between clumps from 11% to 100%. Some hatchlings were malformed, mostly oedematous. None of these survived long after hatching. Six hundred and nine healthy tadpoles were produced from an estimated 1228 eggs, giving a survival rate from egg to free-swimming tadpole of approximately 50%.

Figure 1. A clump of pool frog spawn collected from the field and hatched out in a used margarine container.

Care of tadpoles

Tadpoles were housed in plastic food storage containers, increasing in size from two to six litres, and then transferred to ten-litre plastic containers (domestic washing-up bowls) as they grew. The containers were partially filled with a mixture of water taken from ponds at the reintroduction site and tap water. Tadpole stocking densities ranged from approximately 30 per litre initially, reducing as they grew to approximately three per litre. Water hornwort *Ceratophyllum demersum*, also taken from the reintroduction site, was added to each container to provide refuge and surfaces for periphyton growth, upon which tadpoles could feed. Boiled spinach, as used by Orizaola et al. (2010), was provided, initially daily, then increasing to three or four times a day, to ensure ad libitum feeding (Fig. 2). Tadpoles did not consume a variety of pelleted food that was offered (fish and rabbit pellets) nor algae wafers, although during the early stages a little goldfish flake food (Aquarian) was consumed.

Figure 2. Boiled spinach was the only easily available food identified which northern pool frog tadpoles fed upon readily

For most of the time the rearing containers were kept indoors, moving them daily to benefit from sunlight from south-, east- and west-facing windows. On most days the rearing containers were also temporarily moved outside on to the flat roof of a two-storey building (Fig. 3). This exposed them to direct sunlight in a location relatively safe from potential predators, with no resident amphibians and, hence, minimal risk of pathogen transfer. Nevertheless, rearing containers left in full sun had to be monitored closely to ensure that overheating did not occur.

During the early stages containers were cleaned by transferring tadpoles, using a pipette or small hand net (a

Figure 3. Rearing containers were moved onto a flat roof to benefit from exposure to sunlight on most days. Mesh lids protected against predation by birds.

plastic tea strainer), to a container of fresh water (prepared as above) at intervals of three to five days. As the tadpoles grew larger and were transferred to ten-litre containers, cleaning was carried out by siphoning detritus from the bottom. This was carried out once or twice a day when the tadpoles were large and growing fast. The survival of tadpoles maintained under these conditions was 97%. At least three of the mortalities were related to trauma sustained during cleaning.

Completion of tadpole development outdoors

One hundred tadpoles were transferred (50 on 22 June and 50 on 24 June) to two artificial ponds constructed outdoors, 15 km from the reintroduction site. These ponds were 76-litre plasterers' baths sunk into the ground and filled with approximately 55 litres of tap water inoculated with pond water from the reintroduction site and with tadpole densities of approximately one per litre. The ponds were protected from birds by a fruit cage, but additionally, they had closely fitting mesh lids to exclude other amphibians that may have been present in the area, grass snakes and large, predatory aquatic invertebrates. Tadpoles released into these ponds had a high rate of survival (81%). Nevertheless, growth and development were slower than that of tadpoles grown indoors. On 1 August the first individuals from the artificial ponds were released at the reintroduction site and by this date all but 11 of the tadpoles reared indoors had reached a similar developmental stage.

Release of tadpoles

Development rate of individuals varied and tadpoles reached the pre-metamorphic stages asynchronously. Eighteen releases were carried out between 20 June and 27 August 2013. The first two releases (20 and 24 June) took place earlier than was ideal, due to lack of rearing space, and involved relatively small, less developed tadpoles. Tadpoles released after this, in July and August, had all developed to at least the point of having small hind limbs. Tadpoles were transported back to the reintroduction site in ten-litre plastic food canisters with a large hole cut from the centre of the lid to allow ventilation. These transport containers were partially filled with a mixture of tap water and water from the rearing containers and packed with water hornwort. Tadpoles were released shortly after arrival at the reintroduction site rather than allowing further time for acclimation in the transport containers. Five hundred and seventy-five well-grown tadpoles or pre-metamorphic froglets were released at four ponds within the reintroduction site.

Monitoring released animals

Tadpoles were released into ponds where pool frogs from the parental population had not spawned. This allowed comparison between head-started and free-ranging productivity. Metamorphosing northern pool frogs bask on floating vegetation in their natal ponds and remain in and around these ponds for several days prior to dispersing. Metamorph emergence was monitored using a standardised count procedure developed during the course of the reintroduction. A count was the number of metamorphs seen on a circuit of each pond. Monitoring was carried out on

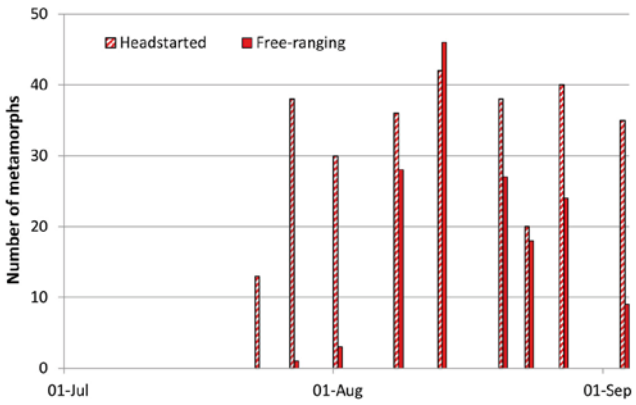

Figure 4. Counts of free-ranging and head-started metamorphs in 2013

nine occasions from 23 July to 3 September. Metamorphs were identified as individuals judged to have recently transformed. These included froglets during the stage of tail absorption and those assumed to have just completed tail absorption. The ponds surveyed for metamorphs included two where spawning occurred earlier in the year, four ponds at which head-started tadpoles had been released and four nearby neighbouring ponds to which metamorphs dispersed. The total number of metamorphs observed during each survey visit was recorded as a site count.

Metamorph emergence

Head-started individuals began to metamorphose earlier than free-ranging tadpoles and were found at all four of the release ponds. For eight of the nine monitoring visits the release ponds yielded higher metamorph counts than were obtained from the two free-ranging (spawning) ponds (Fig. 4). Metamorphs at the release ponds contributed from 44% to 100% of the site counts, which ranged from 13 to 95.

DISCUSSION

Husbandry

Collection of spawn and rearing tadpoles mostly indoors at high densities was relatively successful. Although survival of spawn to the free-swimming tadpole stage was only approximately 50%, this seemed to reflect the viability of the spawn itself rather than any effects of capture and captive husbandry.

Once they reached the free-swimming stage subsequent survival rates of tadpoles were high and they fared well under the captive rearing conditions described. Factors that may have contributed to the success of rearing tadpoles were warmth, exposure to direct and indirect sunlight, plentiful food, frequent maintenance (feeding, cleaning and observation) and adequately conditioned water and rearing containers.

The majority of the tadpoles were reared (mostly) indoors, at high densities, in preference to the use of artificial ponds outdoors. The artificial ponds appeared to work fairly well in that they provided a lower maintenance, low-stocking-density option. The slower growth and development rates of tadpoles in the artificial ponds were probably due to

logistical difficulties in providing frequent (at least daily) feeding and monitoring. Hence, the artificial ponds did not achieve their full potential as a rearing environment.

Effectiveness of head-starting

Head-starting tadpoles made a significant contribution to the numbers of metamorphic pool frogs in 2013. For eight of the nine monitoring visits metamorph counts from the release ponds were higher than those from the spawning ponds. Furthermore, releasing head-started metamorphs at four ponds, other than the two where spawning occurred naturally, spread transforming froglets over more of the reintroduction site than would otherwise have been the case. The quality and fitness of head-started tadpoles has been questioned (Mendelson & Altig, 2016) and the subsequent survival of head-started metamorphs compared with those left on site is unknown. Nevertheless, the positive early indications have encouraged ongoing development of this approach to provide stock for a secondary reintroduction, which will be subject to long-term monitoring needed to determine the ultimate value of head-starting northern pool frogs.

Logistics and effort

Evaluation of the cost-effectiveness of head-starting tadpoles must consider logistical issues and resources available. Although it has been recommended as a cost-effective means of establishing new populations in one case (Stiles et al., 2016) high costs in terms of labour, finance and other resources have been highlighted in another (Ward & Griffiths, 2015). The head-starting methodology described here for rearing tadpoles indoors was certainly highly labour intensive, but inexpensive in terms of other resources.

Effective biosecurity is a requirement throughout conservation translocations (IUCN/SSC, 2013). Rearing tadpoles in a private home, with no other captive animals present, is an example of how 'modified spaces' can provide effective amphibian rearing facilities (Barber, 2012). This is in contrast to working within an existing facility, such as a zoo, that may house a cosmopolitan amphibian collection. Within such a facility, isolation of reintroduction stock to avoid disease transmission between species (Pessier & Mendelson, 2017) and, ultimately, into the wild with reintroduced amphibians (Walker et al., 2008) may require construction of new buildings or the use of modified shipping containers (Barber, 2012).

In spite of its positive aspects, the current trial yielded only 575 well developed tadpoles/metamorphs. The release of large numbers of animals (>1000) is a significant factor in the success of amphibian translocations (Germano & Bishop, 2009) and to produce more well-grown pool frog tadpoles will require greater investment of financial resources and time.

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