Successful reproduction in *Paramesotriton chinensis* after more than a decade of reproductive inactivity, with observation of parental care

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ABSTRACT - Amphibians may be held in captivity without successful reproduction, due to the absence of environmental cues, for many years. However, there are few data concerning the potential for amphibians to recover reproductive capacity after such hiatuses or their impact on fecundity and viability. In one male and two female *Paramesotrition chinensis* newts, I report successful reproduction including previously unreported oviposition site guarding behaviour, but very low rates of viability, over two breeding seasons after more than a decade of aseasonality and reproductive inactivity, following the introduction of appropriate seasonal environmental variation. These findings suggest that reproduction can be achieved in animals that have not reproduced for many years, but that possibly age-related fertility issues may compromise the degree of reproductive recovery.

INTRODUCTION

Many amphibians breed annually in response to regular circannual environmental cues, which may be important in driving hormonal cycles needed to produce gametes and secondary sexual characters over long periods or in triggering hormonal cascades leading to spawning in the short term (Duellman and Trueb, 1986). In order to breed amphibians in captivity, most species require annual environmental cycling relevant to their geographic and microhabitat origin as well as exposure to suitable mating and spawning conditions at the appropriate time in the year. In many species, so-called 'reproductive triggers' may be difficult to identify or to replicate and, in these cases, individuals that are otherwise healthy may never reproduce. For this reason, among others, a number of ex situ conservation breeding programmes have failed to breed target species despite holding founder animals for many years (Gagliardo et al., 2008; Michaels et al., 2014; Tapley et al., 2015). There are few data, however, on the impact of long reproductive hiatuses on the reproductive capacity of captive amphibians. Although individuals of many amphibian taxa may live for years or even decades (e.g. Bowler, 1975; Wagner et al., 2011; also see Max-Planck-Gesellschaft, 2002), it is possible that populations of non-breeding adults may be doomed to extinction well before animals themselves senesce due to compromised reproductive ability. This paper describes success, albeit limited, in recovering reproductive capacity in Chinese warty newts (*Paramesotriton chinensis*) after more than a decade of near aseaonality and no reproduction.

METHODS

One male and two female *P. chinensis* were acquired from a private collection in August 2014. *P. chinensis* is a large, robust newt species (see Fig. 1) with a wide distribution in China, where it inhabits stream pools and surrounding broadleaf forest at medium altitude (Gu et al., 2004). Although it is listed as Least Concern by the IUCN (Gu et al., 2004), it is heavily exploited for the pet trade (Rowley et al., 2016) and this combined with habitat loss and degradation, as well as its very slow rate of maturation, means that its population is rapidly declining. In many respects, this species is similar in terms of husbandry and biology to other more range-restricted *Paramesotriton* (Pasmans et al., 2014).

Figure 1. Dorsal aspect of and juvenile *P. chinensis*, the result of the 2014-2015 breeding period, and portrait of an adult female (inset top) and of eggs laid in clusters between leaves of *M. pteropus* (inset, bottom).

The newts had originally been purchased in 2003 from the pet trade, originating in the wild, probably in the vicinity of the animal trading hub of Shanghai, China. However, over the subsequent 11 years the newts were maintained under quasi-aseasonal conditions (i.e. conditions were not completely stable, but the changes throughout the year were very minor – see below) and did not reproduce. During this period the animals were maintained in soft tap water (alkalinity <20 mg/L, pH c. 6.5) at between approximately 14 and 22 °C and with an invariant 12:12 photoperiod.

In the author's collection, newts were housed in a 120x30x30 cm (LxWxH) aquarium lit by T8 fluorescent lamps ('Freshwater lamp', Arcadia), filled to a depth of 28 cm with a 8:2 mix (pH c. 7.5, alkalinity c. 35 mg/L) of reverse osmosis and London tap-water treated with chelating water conditioner (sodium thiosulphate; API) and furnished with stacks of terracotta roof tiles to provide shelter. Potted *Vallisneria* sp. and loose Java fern (*Microsorum pteropus*) were also provided. Life support systems included an external canister filter (EF-2, All Pond Solutions) and an aquarium chiller (DC 750, Deltec). A gentle current replicating the stream-pool habitat of this species was created using an internal pump (Eheim Compact Pump 1000) to drive water through the life support system, but refugia from water flow were available. *P. chinensis* requires several months of temperatures consistently below 10 °C followed by a slow increase in temperature during spring time (reflecting annual variation in temperature in their natural range; World Weather Online, 2016) in order to successfully breed, and this may be facilitated by varying photoperiod according to natural patterns (Pasmans et al., 2014; H. Jansens pers. comm.). Therefore, water temperature and photoperiod were

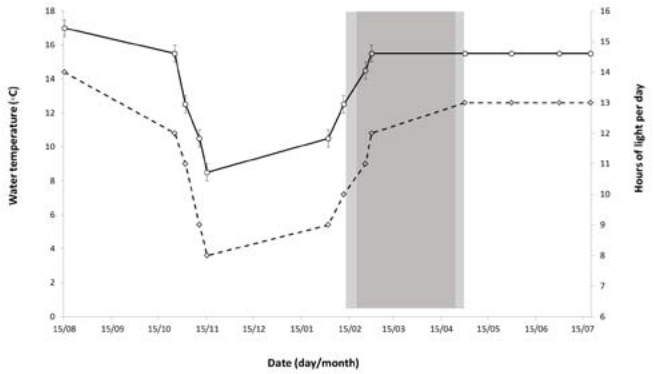

Figure 2. Annual variation in water temperature (continuous line) and photoperiod (broken line) used to house Paramesotriton chinensis; parameters remained constant between 15/04 and 15/08. Error bars represent max-min variation in temperature around the setpoint of the chiller. Oviposition period shown in light (2014-15 season) and dark (2015-16 season) grey blocks.

varied to mimic natural seasonality (Fig. 2). Partial water changes of between 10 and 20% were performed weekly during summer, autumn and spring, and every 2-3 weeks during winter. Food, comprising aquatic invertebrates (Chironimidae, *Gammarus pulex*, *Asellus aquaticus*, *Lumbricus terrestris*) and pieces of defrosted freshwater fish (*Oncorhyncus mykis*s; *Rutilus rutilus*) and prawn (*Paenaeus monodon*), was offered weekly, but food intake and activity decreased during the coolest months with animals barely moving and accepting a meal once every 2-3 weeks.

Eggs produced in the 2014-15 breeding period were removed from the aquarium in batches soon after laying and maintained under identical water parameters at 16-18 °C in separate aquaria to avoid predation by the adults. In 2015-16, when no juveniles were to be reared, eggs were removed from the aquarium on discovery and destroyed by freezing.

RESULTS

In late October in both 2014 and 2015, females began to display pseudo-oviposition behaviour. The cloacae of the females became more triangular and elongated in profile. At the same time, the iridescent blue stripe on the tail of the male intensified and his cloaca became more noticeably swollen, with papillae visible inside the lips. Active courtship (similar to that described in *P. caudopunctatus* by Sparreboom, 1983; Sparreboom, 1986) occurred between early November and early February (2014-15 reproductive period) and between late October and February (2015-16 reproductive period). One of the two females successfully reproduced and laid eggs in 2014-15 and both females reproduced in 2015-16. In 2014-15, the oviposition period lasted from 11/02/2015 to 30/4/2015 (79 days) and in 2015-16 from 19/02/2016 to 25/04/2016 (66 days); see Fig. 2. Between 0 and 7 eggs were produced per day and laying rate was highest at 15-16 °C. Oviposition started at a temperature of 10-11 °C (2014-2015) and 12-13 °C (2015-2016). Almost all eggs were laid in rows between *M. pteropus* leaves (see Fig. 1) in areas of moderate water flow. *M. pteropus* was selected over all other plants in the enclosure, which had different leaf forms. Breeding females became slightly defensive of favoured nesting sites with a 'boundary' of around 5-10 cm radius of the site, attacking the other female if approached and driving her away from the eggs. Defensive behaviour was less vigorous against the male, but he was also kept from approaching oviposition sites.

In 2014-15, the breeding female produced a total of 111 eggs. However, viability to hatching was 11.7% with only thirteen eggs developing. Of these, four larvae were deformed and died shortly after hatching; the remaining 9 larvae (i.e. 8.1%) developed normally under similar environmental conditions as the adults at 16-18°C on a diet of *Panagrellus*, *Enchytraeus*, *Chironomus* and other aquatic invertebrates. In these animals, the larval phase lasted approximately 120 days. In 2015-16, a total of 114 eggs were produced by both females, but the proportion contribution of each animal could not be determined. Again, the vast majority (c. 102/114 or 89.5%) of eggs were infertile; however, no larvae were raised in 2016 due to housing capacity limitations.

DISCUSSION

Amphibians in captivity, including those held in some conservation breeding facilities, may undergo long periods of reproductive inactivity due to the absence of appropriate environmental stimuli. Amphibians may be long lived, especially in captivity, but the impact of reproductive hiatuses on the reproductive capacity of animals, and therefore the viability of such populations beyond the lifespan of founder adults, is largely unknown. The longitudinal studies required to investigate such phenomena may reduce the availability of relevant data as individual animals must be followed for long periods of time. The observations reported here show that reproduction can be triggered in at least this species of newt after a very long reproductive hiatus. Michaels et al. (2015) showed that reproductive activity could be triggered in *Xenopus longipes* after seven years of reproductive inactivity in the absence of appropriate environmental cues, but the present findings are the first data concerning a non-reproductive period of more than a decade and the first concerning a tailed amphibian.

Although reproduction was immediately triggered in one female after appropriate environmental cycling, the other female required two years of environmental cycling to regain reproductive function, presumably due to hormonal cascades requiring substantial environmental input to re-initiate. Moreover, the viability of the eggs that were deposited in both years was low, suggesting that recovery of full reproductive capacity may be problematic. The age of the animals in question is unknown, but the newts were adult with mature colouration (dorsally a putty green-grey and ventrally a faded yellow-orange, rather than the black, orange and yellow dorsal and vibrant orange ventral colouration seen in young adults) when purchased in 2003 and take at least 10 years to reach full adult size and coloration (H. Jansens, pers. comm.). Therefore these animals were likely at least 20 years old, if not older, approaching the maximum lifespan of around 30 years (H. Jansens, pers. comm.). Given that eggs produced by both females had low hatch rates and even lower rates of viability beyond hatching when offspring were reared, the most parsimonious explanation is that the fertility of the male animal was waning. There are, however, no data available to provide insight into either age or aseasonality on fertility or gametogenesis in the long term in amphibians. The clutch of eggs produced in the 2014-2015 breeding period was very large for this species (H. Jansens, pers. comm.; also see comparison with total number of eggs from two animals in the 2015-2016 reproductive period), which may be the result of accumulated fat reserves after having not invested in ova for a long period. Although these individuals were alive and in good body condition following the long non-reproductive period, these data should not be used to suggest that withholding seasonality from newts when reproduction is not required is appropriate.

Without seasonally cycled animals for comparison, and thorough internal investigation it is unclear whether the reproductive hiatus has had any negative impact on the health or longevity potential of these long-lived animals.

It is also significant that moderate oviposition site guarding, possibly a form of parental care, was elicited in females and that this behaviour survived reproductive hiatus. Such behaviour has been reported from *P. caudopunctatus* (Sparreboom, 1983; Rafaelli, 1989), but has not been observed in this species or any other congener. Reproductive function may therefore be recovered in amphibians after long non-reproductive periods in captivity, but the viability of populations comprising aging animals may be compromised by declining fertility. In the context of maintaining viable breeding populations of amphibians in captivity, for conservation, research or other purposes, this highlights the importance of developing appropriate breeding protocols for amphibians as early as possible after a population is established in captivity. Reference to climatic variation to which a species is exposed in nature may, as in this case, facilitate the provision of stimuli necessary to induce reproduction (Michaels et al., 2014).

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