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SHORT COMMUNICATION

Combating adult invasive American bullfrog *Lithobates catesbeianus*

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Abstract American bullfrog *Lithobates catesbeianus* is considered as one of the world's worst alien invasive species. Currently, the species is present over almost all continents and is suspected to cause substantial ecological damage. Knowledge about its population density, as well as catchability of the species with commonly used sampling gear, is very useful when considering the development of sound population control programs. Using the multiple mark-recapture method, we investigated the density, sex ratio, and mobility of an adult bullfrog population inhabiting several neighbouring small shallow ponds at the peak of reproduction (mid June–mid July). We estimated the density at 4.3 adults/100 m shore length and the sex ratio (males/females) to 1.64. On average, males and females moved 25 and 83 m, respectively. Catchability of adults, using one double fyke net for 24 h, was rather constant and equalled 0.7 % of the population size. At first sight, this capture technique seems not to generate large-scale adult removal. However, as a companion study revealed that double fyke nets are highly effective for capturing tadpoles, control or even eradication of isolated populations may be enhanced when this proportion of the adult segment is equally removed.

Keywords Alien invasive species · Population control · Mark-recapture · *Rana catesbeiana* · Shallow water bodies

American bullfrog *Lithobates catesbeianus* is an alien invasive species suspected to cause substantial ecological damage around large parts of the globe (Lowe et al. 2000; Adams and Pearl 2007). Negative impacts on native biota entail competition, predation, and transmission of pathogens (D'Amore 2012). As for native amphibian populations of northwest Europe, potential negative effects (competition and predation) are expected on edible frog *Pelophylax kl. esculentus* and common toad *Bufo bufo*, because they share the same type of habitat (permanent eutrophic water bodies) (Louette and Bauwens 2013). Other amphibian species are less likely to come into direct contact with bullfrogs as they mostly occupy a different kind of breeding habitat (i.e. temporal fishless water bodies). However, they can still be impacted via transmission of viruses and funguses (Sharifian-Fard et al. 2011; Martel et al. 2013). Furthermore, a very broad range of prey species can be eaten by the voracious bullfrog adults (Adams and Pearl 2007).

Controlling or eradicating bullfrogs remains an ongoing challenge. Several strategies and types of capture method have been proposed and applied (Doubledee et al. 2003; Govindarajulu et al. 2005; D'Amore 2012). However, the effectiveness of only a few of these strategies and techniques has really been quantified (Louette 2012; Louette et al. 2013). In this study, we assessed the population density, sex ratio, mobility, and catchability of the species with a commonly used sampling gear (double fyke nets) for an adult bullfrog population inhabiting several neighbouring small shallow ponds. Information on these crucial variables is highly important when developing sound population control programs.

An adult population of American bullfrog was investigated at the peak of reproduction (mid June–mid July) during 2 years (2012–2013) on a former outdoor fish farm at Balen (northeast Belgium; 51° 09′N, 05° 08′E). The species has appeared in the neighbourhood in the 1990s, and its population has grown since then (Jooris 2005). The study site has a total area of 5 ha of which 2.8 ha are small shallow water bodies. Twelve rectangular rearing ponds (average surface area 2,200 m^2, average perimeter 200 m, and maximum depth of 1 m) are present and are all separated by 10-m wide and 0.5-m high

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dikes. Additionally, eight small breeding ponds (average surface area 200 m^2, average perimeter 75 m, and maximum depth of 0.5 m) are located in the centre of the site, also separated by similar dikes. The site is surrounded in the east and the west by willow-alder forests, in the south by grasslands, and in the north by a 10-m wide lowland river bordered by grasslands. Dikes between the ponds are grazed by horses leading to rather short vegetation. Along the shoreline of the ponds, taller vegetation is present, consisting of grasses and herbs, while common reed *Phragmites australis* and soft rush *Juncus effusus* grow in the shallow of the water. Adult bullfrogs reside in this zone along the shore during the mating season (in northwest Europe peaking from June until August, Jooris 2005), from which they both court and feed along the shore and in the water (Clarkson and deVos 1986). Some ponds have transparent water harbouring submerged and floating macrophytes; other ponds are turbid and lack aquatic vegetation.

Adult bullfrogs were sampled during 16 separate capture occasions (four times a week for four consecutive weeks, mid June–mid July) in 2012, and eight capture occasions (four times a week for two consecutive weeks, begin July–mid July) in 2013. We used as sampling gear double fyke nets (height and width of the first hoop 80 and 90 cm respectively, three narrowing funnels in each fyke, leader net 7 m, and mesh size 8 mm). To prevent drowning of adults, a closed empty plastic bottle was placed in the last compartment of each fyke, ensuring that frogs were able to surface and take air. At each sampling occasion in 2012, we randomly sampled ten ponds, whereas six ponds were randomly sampled at each capture occasion in 2013. For every subsequent sampling occasion in each year, a new randomization of sampled ponds was made. We placed each time one double fyke net 2 m out and parallel to the shore of the longest side of the pond for 24 h. Fyke nets were every 2 days alternated between the opposite banks of the ponds under investigation. Captured adult frogs (>10 cm snout-vent length) were sexed (as males when having a yellow throat, all other individuals as females). They were all individually recognizable marked by a subcutaneous high-pressure injection of biological pigment in a unique pattern combination on the web of the hind legs (Jet Injector, Schuco International, London, UK). Individuals were released in the centre of their respective pond, as well as bycatch of bullfrog tadpoles and fish. As all captured adults were individually recognizable marked, the travelled distance between two catch locations could be determined for recaptured individuals.

We estimated the population density of adult bullfrogs (males and females separately) in the site with a multiple mark-recapture analysis for closed populations. In this method, the total number of individuals present at the site can be estimated from repeated sampling occasions. It is possible to estimate the probability of capture on a given occasion, based on the number of individuals marked at a given occasion and recaptured on a later capture occasion. Using the Schumacher and Eschmeyer adjustments of the Schnabel method, calculations were made and a population estimate obtained (see formula in Krebs 1989). To meet the assumption of population closure (no immigration and emigration, no recruitment or mortality) during the sampling period, we considered two periods (mid June–end June and begin July–mid July) to estimate the population size in 2012. In 2013, the same exercise was performed from begin July–mid July.

The population size in the study site varied slightly over periods and years (Table 1). Taking the overall average over the three periods, the population size was estimated at 129 adult individuals (80 males and 49 females) of which 39 % was actually captured during the sampling campaign. Converting to densities, 4.3 individuals/100 m shore length (3,000 m in the study site) were present, or when taking water surface area (2.8 ha in the study site) as estimate, 46 individuals/ha water surface. These densities fit within the data reported in earlier studies. Densities of bullfrog seem to range widely, from only 4 individuals to more than 1,000 individuals/ha of water surface, densities which in some cases probably also cover juvenile individuals (Currie and Bellis 1969; Schwalbe and Rosen 1988; Shirose et al. 1993; Govindarajulu et al. 2006).

We observed a skewed sex ratio (males/females) towards males (average 1.64 (SE 0.01) over the three periods) (see also Shirose et al. 1993). Because of their more active display (territorial behaviour), one would expect males to be more vulnerable to predation (with an important contribution of cannibalism Adams and Pearl 2007) than females. However, contrary to expectations, females seemed to be present in lower numbers than males. It is known that females need a longer time span to mature (Shirose et al. 1993). Because this almost grown segment of the female population does not take part in the reproduction, they only sporadically join the mating sites (Emlen 1968). Other studies report sex ratio's in balance but were based on data gathered during longer sampling periods (Durham and Bennett 1963; Schroeder and Baskett 1968; Clarkson and deVos 1986).

Adult mobility during the sampling campaign differed substantially between males and females (Fig. 1). The median distance travelled between the first and second capture location for individuals that were at least once recaptured in the 1-month sampling period of 2012, amounted to 25 m for males (based on 16 individuals) and 83 m for females (based on 14 individuals). This more than threefold difference may be explained by the territorial behaviour males exerted at the time of sampling (attracting females by calling and displaying), while females travel around looking for partners (Emlen 1968). Earlier studies report travelling distances during the year to be relatively short, ranging between a few meter to more than 100 m (Willis et al. 1956; Currie and Bellis 1969; Stinner et al. 1994; Berroneau et al. 2007).

Table 1 Summary of the number of unique adult American bullfrog *Lithobates catesbeianus* individuals that were captured, the population size estimate (*N*) with the 95 % confidence interval (between brackets), the catch per unit of effort (CPUE, number of individuals in one double fyke net for 24 h with the SE between brackets), and the catchability (CPUE/*N* in %, with the 95 % confidence interval between brackets) for males, females, and total (males+females) in the three different multiple mark-recapture sampling campaigns

	Males			Females			Total		
# Unique individuals captured	35	36	16	25	28	11	60	64	27
N	80 (61–114)	99 (57–369)	62 (33–576)	48 (34–83)	61 (42–113)	38 (20–233)	128 (95–197)	160 (99–482)	100 (53–809)
Catch per unit of effort	0.7 (0.1)	0.6 (0.1)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.3 (0.1)	1.3 (0.1)	1.1 (0.1)	0.7 (0.1)
Catchability (q)	0.8 (0.6–1.1)	0.6 (0.2–1.1)	0.6 (0.1–1.1)	1.0 (0.6–1.4)	0.8 (0.4–1.1)	0.7 (0.1–1.3)	0.9 (0.6–1.2)	0.7 (0.2–1.1)	0.6 (0.1–1.2)

The catchability of the species in each of the different capture occasions was fairly constant for both males and females and averaged 0.7 and 0.8 %, respectively (Table 1). Taking the overall population into account, we observed a catchability of adult bullfrogs to equal 0.7 %. This percentage seems to be rather low, especially compared to the catchability of tadpoles (6 %), using the same catching gear (Louette et al. 2013). A different behaviour probably lies at the basis of this observation. As double fyke nets are highly effective for actively swimming species (Louette and Declerck 2006), purely aquatic tadpoles are more likely to be captured than adults having a rather amphibian and sedentary lifestyle. Adults post most of the time along the shoreline of water bodies and only occasionally jump into the water to feed or mate. Hence, encounter with double fyke nets placed in the water is more uncertain.

Insights in crucial variables, such as population size and catchability of the species with selected sampling gear, are essential when a population control program is under consideration. Earlier, a companion study reported the control of small and isolated bullfrog populations to be feasible when intensively applying double fyke nets as catching gear for the removal of bullfrog tadpoles (Louette et al. 2013). In that study, the simultaneous use of eight double fyke nets in a pond over 12 separate capture occasions is put forward, leading to the drop of tadpole numbers below a critical threshold. Using the adult density and catchability data of this study in the above specified control program, a bycatch of 46 % of the adult population would equally be generated (being four out of nine present adults on the 200-m shoreline of a given pond; see also Louette et al. 2013). This proportion of the adult segment will not take part in reproduction anymore. When depletion together with hindering of any further reproduction (e.g. removing egg masses, fencing reproduction ponds) is

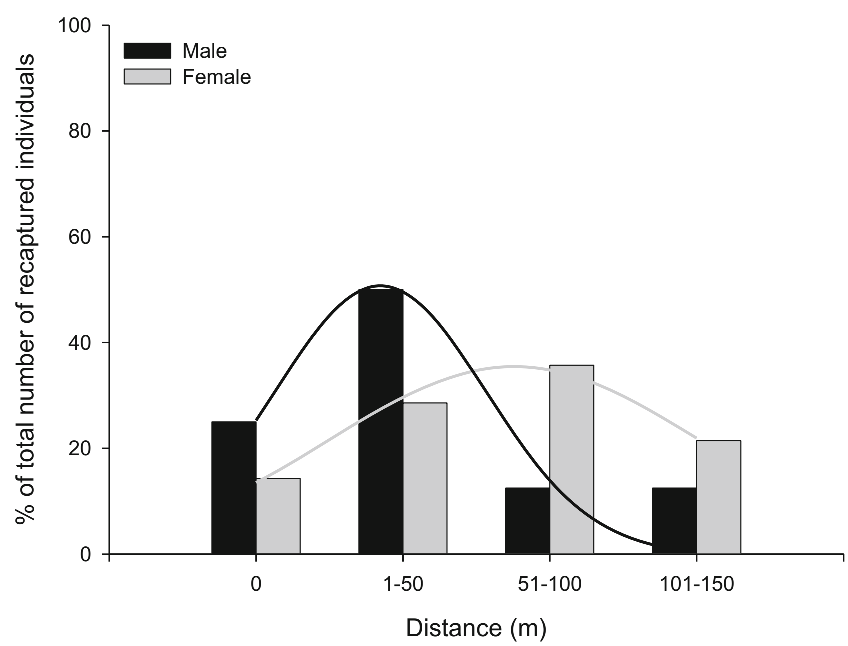

Fig. 1 Mobility of adult American bullfrog *Lithobates catesbeianus* in the study site over a 1-month timeframe (mid June–mid July 2012) of the multiple mark-recapture sampling campaign. Within the segment of recaptured individuals, the percentage of individuals (males and females separately) for each travelled distance class (distance between the first and second capture locations) is presented. A Gaussian curve shows the distribution of the distance travelled for both sex classes

maintained over years, this may lead to the control or even eradication of isolated populations.

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