# Standardisation of alien invasive Australian redclaw crayfish *Cherax quadricarinatus* sampling gear in Africa

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Freshwater crayfish are damaging invaders across southern Africa; however, monitoring techniques and efforts are disparate across the region as different sampling methods have been used. To develop a standard method for assessing redclaw crayfish Cherax quadricarinatus abundance, a survey was conducted to assess for differences in detection and catch per unit effort (CPUE) in Lake Kariba. Two sampling approaches were compared: opera traps baited with cooked maize meal historically used in crayfish surveys in Zimbabwe, and Promar collapsible traps baited with dry dog food, which have been used for assessments in South Africa and Swaziland. Baits were compared in the Barotse Floodplain in Zambia using the Promar trap. Detection probability (P<sub>capture</sub>) and CPUE were significantly lower for opera traps baited with cooked maize meal  $(P_{capture} = 0.41; CPUE = 1.19 \pm 0.24 \text{ ind.} \text{trap}^{-1} \text{.} \text{night}^{-1})$  compared to the Promar traps baited with dry dog food ( $P_{capture} = 0.67$ ; CPUE = 4.53 ± 0.82 ind•trap<sup>-1</sup>•night<sup>-1</sup>). The  $P_{capture}$  and CPUE for Promar traps baited with dog food ( $P_{capture} = 0.89$ ; CPUE = 4.29 ± 0.83 ind-trap<sup>-1</sup>-night<sup>-1</sup>) was significantly higher than for maize meal baited traps ( $P_{capture} = 0.29$ ; CPUE =  $0.25 \pm 0.17$  ind-trap<sup>-1</sup>-night<sup>-1</sup>). Sex ratio and carapace length of crayfish sampled did not differ between sampling methods. Due to higher CPUE, the authors consider the Promar collapsible trap baited with dog food approach as the better method for determining crayfish population abundance and suggest that comparisons of abundance take this into consideration by applying conversion factors if different methods are applied.

# INTRODUCTION

Multiple alien and invasive freshwater crayfish have escaped from captivity and invaded African freshwater ecosystems (Madzivanzira et al., 2020). One species of concern is the Australian redclaw crayfish *Cherax quadricarinatus* (Von Martens, 1868), which has invaded the Zambezi River Basin and several other rivers in southern Africa, where it is rapidly spreading (Madzivanzira et al., 2020). In the Zambezi Basin, wild populations of *C. quadricarinatus* were reported in the Kafue River, Lake Kariba and the Barotse Floodplain after accidental and intentional introductions (Madzivanzira et al., 2020). In order to understand the invasion process, implement management measures or undertake impact assessments, comparable data on presence and abundance are required (Larson and Olden, 2016; Madzivanzira et al., 2020).

Common sampling methods for crayfish include active methods, such as collecting by hand, electrofishing and direct underwater or bankside observation, and passive methods such as the use of baited traps (Williams et al., 2014; Larson and Olden, 2016). Baited traps are the most commonly applied method (Larson and Olden, 2016). While a variety of traps have been used in crayfish surveys in Africa (see Madzivanzira et al., 2020), two designs – opera traps (Marufu et al., 2014, 2018) and Promar collapsible traps (Nunes et al., 2017) – are the most commonly applied methods for sampling *C. quadricarinatus* in southern Africa. Bait also differs between surveys, with choices including cooked maize meal (e.g. Marufu et al., 2014, 2018) and dry dog food pellets (e.g. Nunes et al., 2017). As both trap design and bait type have been shown to influence freshwater crayfish catch rates (Huner, 1988; Huner and Paret, 1995), different sampling methods can result in disparate results in surveys seeking to estimate abundance or even the distribution of crayfish. Research into developing standardised field sampling approaches is therefore required (Madzivanzira et al., 2020).

Here, this study presents field experiments to compare the sampling approaches employed by Marufu et al. (2014, 2018) and Nunes et al. (2017) in Lake Kariba, Zimbabwe, and to assess the influence of bait choice in the Barotse Floodplain, Zambia. The central hypothesis was that there would be no difference between methods with regard to detection probability (the proportion of traps containing at least one crayfish), catch rate (number of individuals per trap per night), crayfish size and sex ratio. This study further re-calculates catch data collected using the sampling method with the lowest CPUE, in order to have cohesive population abundance estimates throughout the region.

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## DATES

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## MATERIALS AND METHODS

#### Study site

The gear comparison study was carried out at 10 locations in the Sanyati Basin in the Zimbabwean section of Lake Kariba, between 27 November and 5 December 2018 (Fig. 1). Lake Kariba is the world's largest man-made lake (by volume) and is located along the Zambezi River and shared between Zambia and Zimbabwe, forming part of the Middle Zambezi Biosphere Reserve (Magadza et al., 2020). During the survey in Lake Kariba, the average  $\pm$  SE water temperature was 28.39  $\pm$  0.40°C, pH 7.66  $\pm$  0.10, dissolved oxygen 7.43  $\pm$  0.83 mg·L<sup>-1</sup>, conductivity 229.50  $\pm$  4.90 µS·cm<sup>-1</sup>, TDS 147.65  $\pm$  2.76 mg·L<sup>-1</sup>, salinity 0.07  $\pm$  0.01 and tur-bidity 16.92  $\pm$  2.76 NTU.

The bait comparison experiments were carried out at 3 locations on the Barotse Floodplain in Zambia between 16 and 18 October 2019 (Fig. 1). During the survey in the Barotse Floodplain, the average  $\pm$  SE water temperature was 26.15  $\pm$  0.08°C, pH 7.78  $\pm$  0.24, dissolved oxygen 9.21  $\pm$  0.97 mg·L<sup>-1</sup>, conductivity 177.17  $\pm$  14.89  $\mu$ S·cm<sup>-1</sup>, TDS 116.50  $\pm$  9.70 mg·L<sup>-1</sup>, salinity 0.06  $\pm$  0.01 and turbidity 17.47  $\pm$  3.14 NTU.

## Comparison of sampling approaches

The opera crayfish trap (dimensions:  $100 \times 50 \times 30$  cm; mesh size: 10 mm) and the Promar collapsible crayfish trap (dimensions:  $61 \times 46 \times 20$  cm; mesh size: 10 mm) were used in this survey (Fig. 2). The opera trap entrance has a fixed circular area of 78.5 cm<sup>2</sup> whilst that of

the Promar collapsible trap is flexible and can widen to a rectangular area of 460 cm<sup>2</sup>. The opera traps were baited with approximately 100 g of cooked maize meal (hereafter referred to as opera<sub>mm</sub>) and Promar collapsible traps were baited with 100 g of Bobtail dry dog food pellets, steak flavour (hereafter referred to as Promar<sub>df</sub>). Both baits were placed in nylon stockings which were tied inside the trap and suspended  $\approx$  5 cm from the bottom of the trap (Fig. 2b).

At each sampling locality the physio-chemical variables (temperature, pH, dissolved oxygen saturation, turbidity, electrical conductivity and salinity) were measured using an AP-700 Aquaread multimeter and traps were deployed in pairs, comprising of an opera<sub>mm</sub> sampling gear and one Promar<sub>df</sub> gear deployed at least 10 m apart to minimise potential interaction between the methods. A total of 98 and 102 opera<sub>mm</sub> and Promar<sub>df</sub> gears, respectively, were deployed over night at depths of  $\leq 5$  m. On retrieval, the number of crayfish caught in each trap was recorded and each crayfish was measured for carapace length (CL), weighed (to the nearest gram) and sexed.

## **Bait comparison**

The bait study was carried out in the Barotse Floodplain to compare bait efficiency in the Promar collapsible traps. Twenty-eight pairs of Promar collapsible traps baited with either cooked maize meal or dog food were deployed overnight, at least 10 m apart, at depths of  $\leq$ 5 m. On retrieval, the number of crayfish caught in each trap was recorded and each crayfish was measured for CL, weighed (to the nearest gram) and sexed.

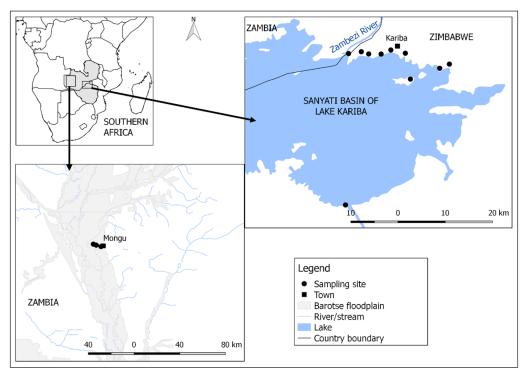


Figure 1. Sampling sites in: (a) the Sanyati Basin of Lake Kariba where opera traps baited with cooked maize meal and Promar collapsible traps baited with dog food were compared; and (b) sampling sites on the Barotse Floodplain where baits were compared



Figure 2. (a) The opera trap used for crayfish surveys in Zimbabwe and (b) the Promar collapsible trap used for crayfish surveys in South Africa and Swaziland

#### **Data analysis**

The STATISTICA software package (version 7.1; Statsoft, Inc.) was used to conduct the analysis. Differences in the habitat as well as physio-chemical variables in this survey were not considered to be influencing factors, as the gears with different baits were deployed simultaneously in the same environment. Detection probability  $(P_{capture})$  was expressed as the number of traps containing at least one crayfish as a proportion of all traps set. Catch rate was expressed as catch per unit effort (CPUE) as the mean number of individuals caught per trap per night set. The Shapiro-Wilk test of normality failed to reject the null hypothesis that CPUE data were normally distributed for the Promar<sub>df</sub> sampling method (w = 0.86, p = 0.08) and rejected the null hypothesis that CPUE data were normally distributed for the  $\mathsf{opera}_{\mathsf{mm}}$  sampling method (w = 0.82, p = 0.03), and hence the data were log-transformed. A t-test was used to test for CPUE differences between the sampling methods. The  $P_{\text{capture}}$  were analysed with contingency tables, and the differences in  $P_{\rm capture}$  between the two gears were tested with a Chi-square statistical test. The relationship between CPUE from the sampling methods was explored using linear regression to estimate a conversion factor for data standardisation. A Kolmogorov-Smirnov two-sample test was used to test whether CL distributions from the sampling methods differed significantly. Finally, sex ratios between the sampling methods were compared using contingency tables and tested using Chi-square analysis.

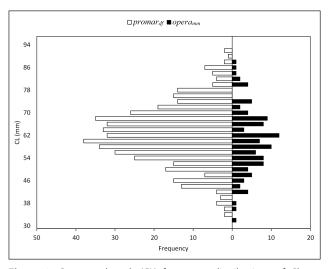
## RESULTS

#### **Comparison of sampling approaches**

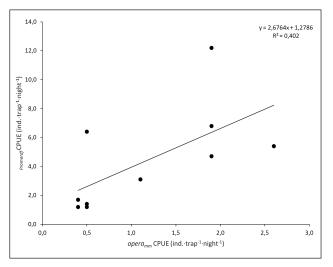
In Lake Kariba, 572 crayfish were caught during this survey. Of these, 116 were caught by the opera<sub>mm</sub> method and 456 were caught using the Promar<sub>df</sub> method. Overall  $P_{\text{capture}}$  for the opera<sub>mm</sub> method (0.41) was significantly lower ( $\chi 2 = 13.90$ , df = 1, P = 0.0002) than that for  $Promar_{df}(0.67)$  method. Mean (± SE) CPUE for the opera<sub>mm</sub> method (1.19  $\pm$  0.24 ind trap<sup>-1</sup> night<sup>-1</sup>) was significantly lower (t (18) = 2.84, P = 0.01) than that for the  $Promar_{df}$  method (4.53)  $\pm$  0.82 ind trap<sup>-1</sup> · night<sup>-1</sup>). The CL distribution of C. quadricarinatus (see Fig. 3 for CL distributions) did not differ significantly (K-S, D = 0.05; p = 0.98). The overall female to male ratio for opera<sub>mm</sub> (1:1.06) was not significantly different ( $\chi 2 = 0.54$ , df = 1, P = 0.46) from that of the Promar<sub>df</sub> method (1:1.22). Regression analysis indicated a weak correlation between CPUE of the two sampling approaches ( $R^2 = 0.40$ ) which was best explained by the relationship: CPUEPromar<sub>df</sub> = 2.68·CPUEopera<sub>mm</sub> + 1.28 (Fig. 4). Data collected using operamm can therefore be up-calculated using the equation in Fig. 4 (see Table 1 for final CPUEs).

Table 1. Standardised CPUEs from Marufu et al. (2014, 2020) using regression parameters from Fig. 4

Site	CPUE (ind <sup>-1</sup> ·trap <sup>-1</sup> ·night <sup>-1</sup> .)	Slope	Constant	Predicted standardised CPUE (ind <sup>-1</sup> ·trap <sup>-1</sup> ·night <sup>-1</sup> )
1	2.00	2.68	1.28	6.64
2	0.00	2.68	1.28	1.28
3	4.00	2.68	1.28	12.00
4	0.20	2.68	1.28	1.82
5	2.17	2.68	1.28	7.09
6	1.33	2.68	1.28	4.85
7	1.67	2.68	1.28	5.75
8	0.50	2.68	1.28	2.62
9	0.20	2.68	1.28	1.82
10	0.00	2.68	1.28	1.28
11	0.67	2.68	1.28	3.07
12	0.50	2.68	1.28	2.62



**Figure 3.** Carapace length (CL) frequency distributions of *Cherax quadricarinatus* in the opera<sub>mm</sub> and Promar<sub>df</sub> methods in the Sanyati Basin, Lake Kariba, November–December 2018



**Figure 4.** CPUE of the opera<sub>mm</sub> sampling method versus the Promar<sub>af</sub> sampling method from 10 sampling localities in the Sanyati Basin, Lake Kariba, November–December 2018

## **Bait study**

In the bait experiment on the Barotse Floodplain, 121 of the 128 *C. quadricarinatus* were caught in traps baited with dog food and 7 in traps baited with cooked maize meal. Overall  $P_{\text{capture}}$  for traps with dog food (0.89) was significantly higher ( $\chi 2 = 23.63$ , df = 1, P = 0.000001) than that for cooked maize meal (0.29). Mean ( $\pm$  SE) CPUE for dog food bait (4.29  $\pm$  0.83 ind·trap<sup>-1</sup>.night<sup>-1</sup>) was significantly higher (t (2) = 3.43, P = 0.03) than cooked maize meal bait (0.25  $\pm$  0.17 ind·trap<sup>-1</sup>.night<sup>-1</sup>). Insufficient crayfish specimens were captured with maize meal bait for meaningful comparison on CL and sex ratio.

# DISCUSSION

The objective of this study was to determine the more effective of the two sampling approaches used for *C. quadricarinatus* in southern Africa, an important step towards standardising crayfish sampling methods in Africa (Madzivanzira et al., 2020). On Lake Kariba, detection probability and CPUE were significantly higher for Promar<sub>df</sub> than for opera<sub>mm</sub>. Sex ratio and size structure did not, however, differ between sampling approaches. In addition, the bait type experiment demonstrated that the use of dog food pellets as bait resulted in higher CPUE and superior detection probability over cooked maize meal. The results demonstrate that, CPUE data from the opera<sub>mm</sub> method require up-calculation by a factor of 2.68 to enable comparisons with data obtained using the Promar<sub>df</sub> method for *C. quadricarinatus*.

Neither the proportion of females to males as well as the CLs differed between the two sampling approaches. However, it should be noted that although the two sampling approaches captured a wide range of crayfish sizes, with total and carapace length ranges of 65 to 197 mm and 32 to 91 mm, respectively, juveniles with CL less than 32 mm were poorly represented overall. As crayfish trapping is a passive sampling method that is not only dependent on overall crayfish abundance, but also behaviour, this will unavoidably interact with trapping results in potentially complex ways (Dorn et al., 2005). Large and aggressive crayfish, which normally favour baited traps (Brown and Brewis, 1978; Capelli and Magnuson, 1983), often tend to defend traps as habitat and exclude smaller individuals (Ogle and Kret, 2008), which may account for the observed low percentage of juveniles.

Effectiveness of the dog food bait in attracting crayfish may be related to its high crude protein content (180 g·kg<sup>-1</sup>) compared to that of cooked maize meal (50.84 g·kg-1). Baits with high-protein content are considered effective for crayfish (Huner and Barr, 1980). Other high-protein baits have been found to produce similar catch results as that of the dog food (Larson and Olden, 2016). Somers and Stechey (1986) did not find any significant CPUE differences for Cambarus bartonii (Fabricius, 1798) and Faxonius virilis (Hagen, 1870) between liver, fish, chicken, canned cat food and dog food baits. Mhlanga et al. (2020) showed that the CPUE of traps baited with liver, cooked maize meal and fish heads were not significantly different. These baits can be suitable for crayfish exploitation purposes but not for standardised surveys (Mhlanga et al., 2020). Dog food is suitable as a standard bait as it is easy to handle, store and requires less time to prepare for fieldwork and can be standardised. Other baits - for example, fish - would need to be standardised according to species, size and condition, which would make the standardisation process across the region cumbersome. An example is the sampling of Procambarus clarkii (Girard, 1858) in Mimosa Dam, Free State Province in South Africa, using fish heads (of common carp Cyprinus carpio, Orange River mudfish Labeo capensis and moggel, Labeo umbratus) (Barkhuizen et al., n.d.).

Although baited traps are biased towards large male crayfish, they have been the preferred method of sampling crayfish in many

population and distribution studies, as traps can be successfully deployed in most habitats (Gherardi et al., 2011; Stebbing, 2016; De Palma-Dow et al., 2020). Recently, a novel 'triple drawdown' (TDD) method was developed to sample invasive populations of P. leniusculus in northern England (Chadwick et al., 2020). This method revealed large numbers of juveniles which, however, were not detectable using traditional methods. However, this method is only possible in smaller streams and with the correct technical infrastructure. Other crayfish sampling methods that can be used include snorkel surveys and environmental DNA (eDNA) (Chadwick et al., 2020), but these seem to be less commonly used owing to the required technical expertise (Stebbing, 2016). Moreover, some aquatic environments in Southern Africa are characterised by poor light conditions and low water clarity may limit the application of underwater surveys, leaving baited traps to be the most suitable method.

Standardisation and affirmation of crayfish trapping gear capacities in the southern African region is essential information for further work on the subject (Madzivanzira et al., 2020). Due to higher  $P_{\rm capture}$  and CPUE across multiple sampling sites, the Promar collapsible crayfish trap baited with dog food could be considered as the most appropriate gear for crayfish abundance studies in Africa. The findings that the two gears used in this study differed in their efficiency suggests that application of a correction factor is necessary when comparing population abundance estimates in the region.

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# **AUTHOR CONTRIBUTIONS**

All authors conceived the ideas and designed the methodology; OLFW provided the funding; TCM and JS conducted the fieldwork; TCM and JS analysed the data; TCM led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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