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Reproduction Frequency, Sex Ratio, Growth Rates, Growth Performance indices, Condition Factors and Natural Mortality of Red Claw Crayfish (*Cherax quadricarinatus*, Von Martens, 1868) in Lake Kariba, Zambia.

Mauris Chinyama Makeche[1*], Gordon Hangoma Mudenda[2] and Geofrey Makwelele[3]

[1]Institute of Basic and Biomedical Sciences, Levy Mwanawasa Medical University, Lusaka, Zambia.
[2]Department of Biological Sciences, School of Natural Sciences, University of Zambia, Lusaka, Zambia.
[3]Department of Biological Sciences, School of Mathematics and Science Education, Chalimbana University, Chongwe, Zambia.
E-mail: mcmakeche@gmail.com
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ABSTRACT

The research was conducted from Lake Kariba because Lake Kariba is the largest man-made Lake in Zambia that has diverse ecological habitats that are rich in fish biodiversity. Crayfish were sampled from Kabbyobbyo and Mundulundulu in Siavonga. A total of 639 *C. quadricarinatus* samples were collected from Lake Kariba between November and December, 2022. Traps, locally called Kamono, were used to collect Crayfish. Length-weight relationships were determined by linear regression in Microsoft Excel to determine growth rates, r^2 and allometric growth forms. Reproduction frequencies of the sampled Crayfish were determined using length-frequency histograms. The growth performance indices, Condition factors and natural mortality parameters were determined from the linear regression of the length-weight relationship. The results showed that Crayfish is a multiple spawner and it has a sex ratio of 1:1. The growth performance indices ranged between 2.6 to 4.6. Natural mortality coefficients were less than 1.0 and the Condition factors were all above 1, suggesting good adaptability to the habitat at Lake Kariba.

Key words: *Cherax quadricarinatus*, Lake Kariba, reproduction, Condition factor, natural mortality.

INTRODUCTION

Cherax quadricarinatus, Red claw crayfish, is a freshwater crustacean belonging to the family Parastacidae (Austin *et al*., 2010; FAO, 2011). Feral populations have been established in most tropical and subtropical freshwater systems across the globe from its native range of the tropical river catchments of Northern Australia and South-Eastern Papua New Guinea (Belle *et al*., 2011). Aquaculture and ornamental aquarium trade are notably the main pathways behind the rapid spread of Crayfish globally. *C. quadricarinatus* is a hardy crustacean; it can be found in coastal streams and freshwater habitats, with a preference for the slower moving waters of rivers, lakes and lagoons (Austin *et al*., 2010). *C. quadricarinatus* reproduction frequency is dependent on water temperature and day length, and normally spawns between September and April in freshwater bodies (Nunes *et al*., 2016). *C. quadricarinatus* is a multiple spawner which is capable of spawning several times (3 to 5 times) each year as long as water temperatures remain above 18°C (Karplus *at el*., 1997; Jaklic and Vrezec, 2011).

Length-weight studies are important in estimating various parameters on fish species such as reproduction frequency, growth forms, condition factors and natural mortalities (Osho and Usman, 2019). Length-weight relationships are also important in assessing the wellbeing of a fish populations (Makeche *et al*., 2023). If the relationship between the length and weight of fish is isometric, it implies that the increase in fish weight is proportional to the cube of its length. Any other result depicts an allometric relationship, which can either be positive ($b > 3$) or negative allometric ($b < 3$)(Froese, 2006).

A Condition factor, which is obtained from length-weight measurements, can be used as an indicator of the health and sustainability of a fish stock. Various factors such as sex, seasons, environmental conditions and food availability all affect Condition factors of fish (Amponsah *et al*., 2020). Condition factors are important in determining the ideal harvesting size, fish maturity and adaptability (Saha *et al*., 2021). Fish species with Condition factor values greater than or equal to one (≥ 1) are in good condition and healthy while fish species with Condition factors less than one (< 1) are in a bad condition and unhealthy (Saha *et al*., 2021).

Growth performance indices and growth rates are used in determining the type of growth a living organism exhibits. Growth of *C. quadricarinatus* is moderately fast ($r^2 > 0.5$) with longevity of approximately 4 years (Mukuka, 2019). This species has a growth performance index of between 2 (Makwelele, 2017) and 4 (Marufu *et al*., 2018; Mukuka, 2019).

In Zambia, Crayfish are usually caught as a by catch (Makwelele, 2017). *C. quadricarinatus* death due to natural causes are low and usually result from diseases, competitors, spawning stress and other severe environmental factors (Nunes *et al*., 2016). Makwelele (2017) reported natural mortality values ranging from 0.06 to 0.08 from the Kafue Floodplains while Marufu *et al.* (2018) reported a natural mortality of 0.99 in Lake Kariba. Mukuka (2019) recorded an above-average natural mortality of 1.45 from the Upper Zambezi. Estimates of natural mortality parameters are important in improving the conservation of fish species (Hossain *et al*.,2019; Saha *et al*., 2021). Natural mortality is an important component which is used to estimate exploitation ratios of a fish stock in an aquatic habitat. An exploitation ratio which is less than 0.5 denotes under-exploitation while an exploitation ratio value above 0.5 depicts over-exploitation. A natural aquatic habitat is optimally exploited if its exploitation ratio is 0.5 (Makeche *et al*., 2020).

Lake Kariba is an important fishery in Zambia which ranks third in terms of fish output, after Lake Tanyanyika and Lake Bangweulu (Mudenda *et al*., 2012; Lake Kariba Fisheries Research Unit, 2015). Lake Kariba has many aquaculture farmers who create employment besides fish production for export (ZAEDP, 2019).

The study had four main objectives: 1. to determine the reproduction frequency and sex ratio of *C. quadricarinatus* at Lake Kariba, 2. To determine the growth forms of *C. quadricarinatus* at Lake Kariba, 3. to determine the Condition factor of *C. quadricarinatus* at Lake Kariba, and 4. to determine the natural mortality coefficients of *C. quadricarinatus* at Lake Kariba.

MATRIALS AND METHODS

Study region and sampling

The research was conducted from Lake Kariba because Lake Kariba is the largest man-made Lake in Zambia that has diverse ecological habitats that are rich in fish biodiversity (Mudenda *et al*., 2012; Lake Kariba Research Unit, 2015).

Crayfish were sampled from Kabbyobbyo and Mundulundulu in Siavonga (Fig.1). Kabbyobbyo lies along latitude -16°53'S and longitude 28°57'E. Mundulundulu study site was located along latitude -16°54'S and longitude 28°71'E. A total of 639 *C. quadricarinatus* samples were collected from Lake Kariba between November and December, 2022.

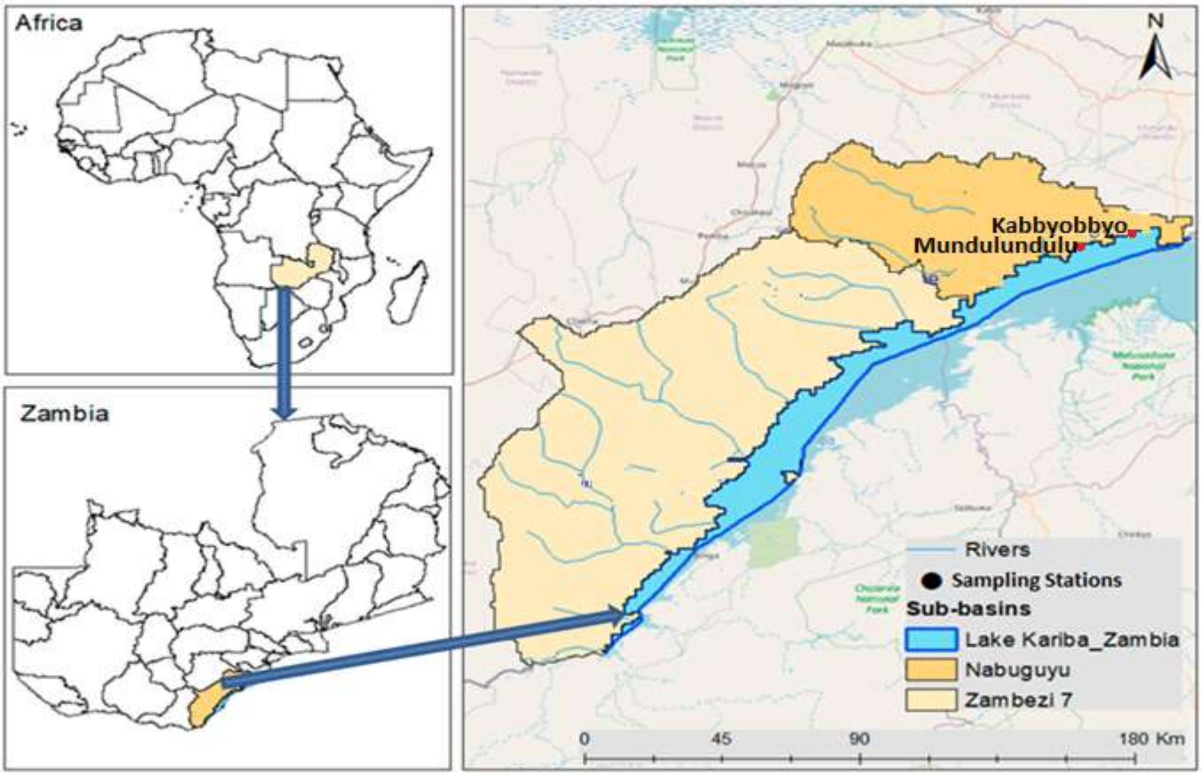


Fig-1: showing Lake Kariba with sampling stations

(**Source:** modified from http://www.bing.com/images)

The study followed the guidelines of the Declaration of Helsinki. Traps locally known as Kamono were used to trap sample Crayfish. The caught specimens were handled ethically by immediately immersing them in 10% formalin. The sampled Crayfish were weighed, measured and sexed. This sampling procedure was repeated for three nights at each study site. total length (TL), carapace length and Body weight (BW) were measured using 30cm fish measuring board and electronic scales.

Reproduction frequencies

Reproduction frequency of the Red claw Crayfish at Lake Kariba was determined using length frequency histograms constructed using Microsoft Excel, 2019 (Microsoft Corporation, 2019). The number of peaks on length-frequency Histograms were used to determine the reproduction frequency of sampled Crayfish.

Sex ratio

The sex ratio of Crayfish at Lake Kariba was determined by dividing the number of Males by the number of Females as earlier determined by Theocharis *et al*. (2023).

Length-weight Relationships

The growth form coefficient (b) was determined by linear regression of the length-weight relationship (LWR) in Microsoft Excel, 2019 (Microsoft Corporation, 2019) using the equation: $W = aL^b$; where W = weight in grams, L = total length in centimeters, a is a scaling constant and b is the allometric growth form. This equation was logarithmically-transformed and expressed as: log Weight = a + b log Total length (Froese, 2006). The growth exponent coefficient, r^2, was used to denote the growth rate of the sampled specimens (Makeche *et al*., 2023).

Growth performance indices

The Von Bertalanffy growth parameters k and L_∞ were estimated using the Ford-Walford, 1946 (Sparre and Venema, 1998) method and Pauly's equation of 1979 (Sparre and Venema, 1998), that is, $k = -\frac{1}{\Delta t}$ x ln b (Sparre and Venema, 1998); where b is a constant obtained by regression analysis of L(t) values of the sample size and Δt is change in time and $L_\infty = \frac{L\,max}{0.95}$ (Sparre and Venema, 1998); where L_{max} is the maximum total length measurement recorded. Growth performance indices (Φ′) were then estimated using the equation by Pauly and Munro (1984) expressed as: $\Phi' = \log_{10}(k) + 2\log_{10}(L\infty)$, where;

k = Von Bertalanffy growth coefficient and L_∞ = Von Bertalanffy asymptotic length.

Condition factors

Based on the expression of Fulton (1904): $K = 100\times (W/L^3)$, where W is body weight (g), and L is Total Length (cm), Fulton's condition factor (K) was estimated. To obtain a Condition factor value close to unit, a scaling factor of 100 was used (Froese, 2006).

Natural mortality

The natural mortality (M_w) of *C. quadricarinatus* was determined from the equation: M_w =1.92 year-1 $*(W)^{(-0.25)}$) (Peterson and Wroblewski (1984); where, M_w = natural mortality at mass W; and $W = a*L^b$, a and b are the regression variables of length and weight (total length against body weight).

Statistical Analysis

The collected results were analyzed using linear regression and T-test using Microsoft Excel, 2019 (Microsoft Corporation, 2019). Linear regression was conducted using Microsoft Excel, 2019 (Microsoft Corporation, 2019) to determine the growth form coefficient 'b'. T-test analysis was conducted using Microsoft Excel, 2019 to determine significant differences (P = 0.05), if any, between the determined variates. One-way ANOVA was computed using Microsoft Excel, 2019 (Microsoft Corporation, 2019) to determine significant differences (P = 0.05), if any, among the regression variates.

RESULTS

Reproduction frequency results

The display of the length-frequency histograms in Fig. 2 shows defined peaks, an indication that the *Cherax quadricarinatus* has well-defined spawning seasons at Lake Kariba.

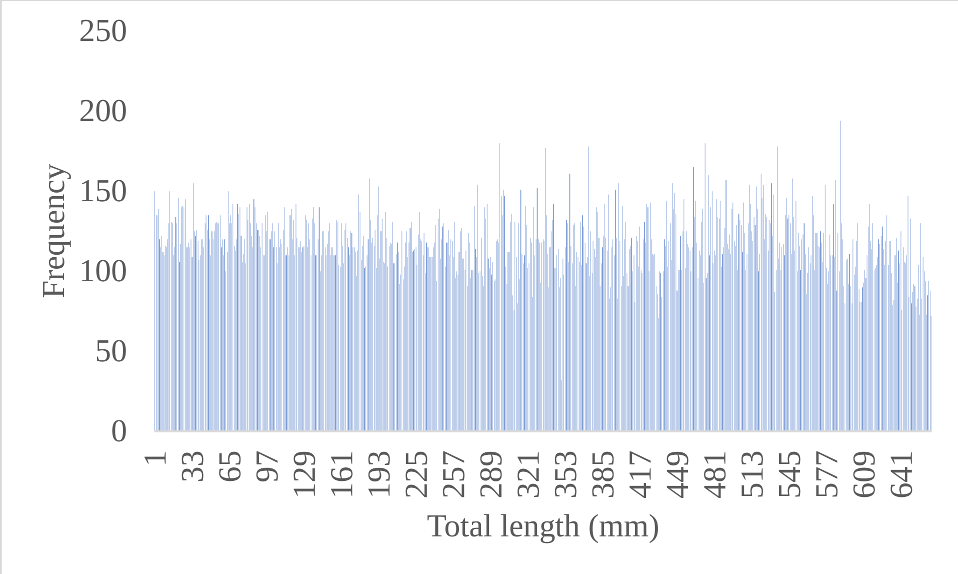


Fig-2. Length-frequency histogram of *C. quadricarinatus* at Lake Kariba.

The Histogram shows that *C. quadricarinatus* has a breeding frequency of 5 within one breeding season at Lake Kariba.

Sex ratios

The sampled *C. quadricarinatus* species at Lake Kariba were divided into 375 Males AND265 Females. This gave a sex ratio of almost one Male to one Female.

Male length-weight relationship and condition factor at Lake Kariba

Male weight measurements ranged from 8g to 178g with a mean of 40 g at Lake Kariba. Length measurements ranged from a low of 32mm to a high of 194mm with a mean of 117mm. The Fulton's condition factor for Male *C. qaudricarinatus* at Lake Kariba was 2.5 at Lake Kariba. There was significant correlation between Crayfish length and Crayfish weight ($r_{374, 0.01} = 0.8002$). The adjusted r^2 was 0.800. ANOVA results showed a significant regression of length on weight (P = 0.000) (Table 1). The Linear regression equation of Crayfish length and Crayfish weight at Lake Kariba was described by the mathematical model: log Weight = -3.68 + 2.54 log Total length. The growth form coefficient 'b' of Male *C. quadricarinatus* at Lake Kariba was 2.54. The calculated 'b' value implies negative allometry because it is less than 3. The graphical representation of the length-weight relation is given in Fig. 3.

Table 1: Length-weight regression ANOVA results of Male *C. quadricarinatus* at Lake Kariba

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	16.75108	16.75108	1489.809	3.7805E-132
Residual	372	4.182686	0.011244		
Total	373	20.93377			

df means degrees of freedom, SS means sum of squares, MS means mean sum of squares and F means the computer-generated comparison value. ANOVA means analysis of variance.

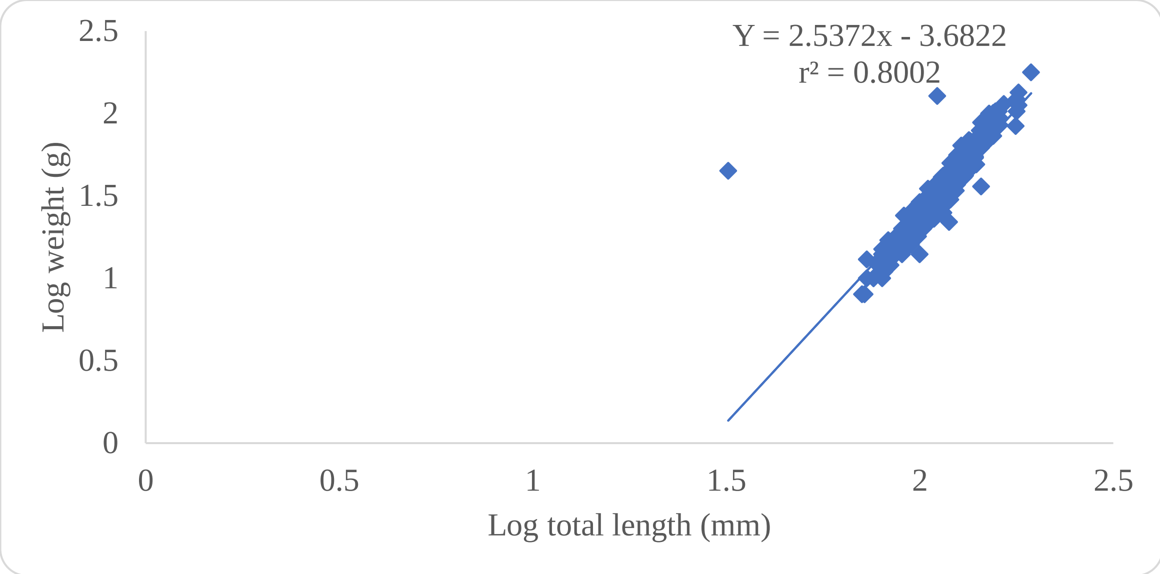


Fig-3: Graph of length-weight relationship of Male *C. quadricarinatus* at Lake Kariba.

Graph depicting the logarithmic relationship between Crayfish length and Crayfish weight at Lake Kariba. There was a significant correlation between fish growth and fish weight ($r^2 = 0.8002$). The graph shows a positive correlation between Crayfish length and Crayfish weight.

Female length-weight relationship and condition factor at Lake Kariba

Female weight measurements ranged from 11g to 197g with a mean of 46g at Lake Kariba. Length measurements ranged from a low of 43mm to a high of 205mm with a mean of 126mm. The Fulton's condition factor for Female *C. qaudricarinatus* at Lake Kariba was 2.3 at Lake Kariba. There was significant correlation between Female Crayfish length and Female Crayfish weight ($r_{265, 0.01} = 0.817$). The adjusted r^2 was 0.816. ANOVA results showed a significant regression of length on weight (P = 0.000) (Table 2). The Linear regression equation of Female Crayfish length and Female Crayfish weight at Lake Kariba was described by the mathematical model: log Weight = -3.7+ 2.54 log Total length. The allometric growth form 'b' of Female *C. quadricarinatus* at Lake Kariba was 2.54. The calculated 'b' value implies negative allometry because it is less than 3. The graphical representation of the length-weight relation is given in Fig. 4.

Table 2: Length-weight regression ANOVA results of Female *C. quadricarinatus* at Lake Kariba

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	11.00246	11.00246	1163.365	1.5076E-98
Residual	263	2.487309	0.009457		
Total	264	13.48977			

df means degrees of freedom, SS means sum of squares, MS means mean sum of squares and F means the computer-generated comparison value. ANOVA means analysis of variance.

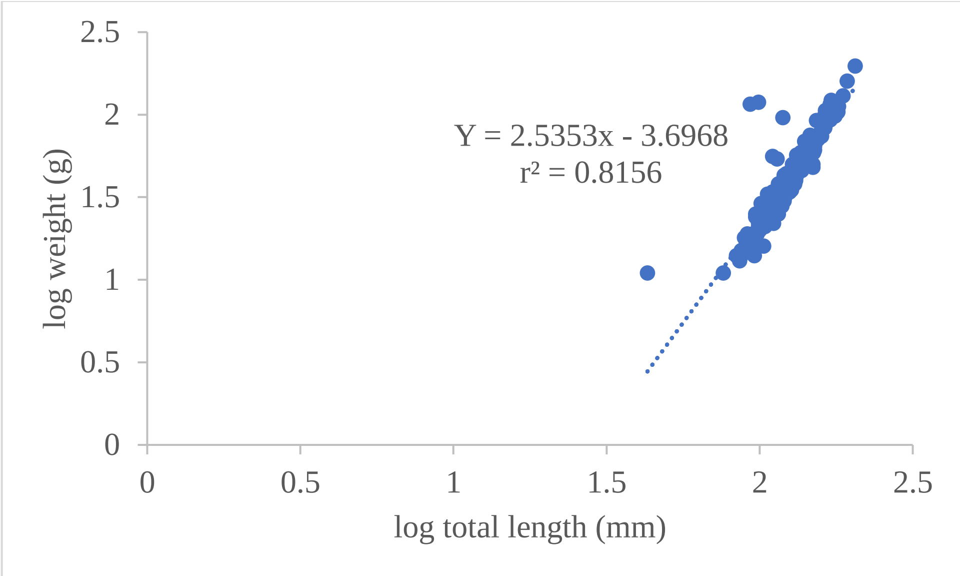


Fig-4: Graph of length-weight relationship of Female *C. quadricarinatus* at Lake Kariba

Graph depicting the logarithmic relationship between Crayfish length and Crayfish weight at Lake Kariba. There was a significant correlation between fish growth and fish weight (r^2 = 0.816). The graph shows a positive correlation between Crayfish length and Crayfish weight.

Overall length-weight relationship and condition factor at Lake Kariba

Weight measurements ranged from 8g to 178g with a mean of 37g at Lake Kariba. Length measurements ranged from a low of 32mm to a high of 194mm with a mean of 118mm. The Fulton's condition factor for combined data of *C. qaudricarinatus* at Lake Kariba was 2.4 at Lake Kariba. There was significant correlation between Crayfish length and Crayfish weight ($r_{265, 0.01}$ = 0.75). The adjusted r^2 was 0.75. ANOVA results showed a significant regression of length on weight (P = 0.000) (Table 3). The Linear regression equation of Crayfish length and Crayfish weight at Lake Kariba was described by the mathematical model: log Weight = -3.6+ 2.5 log Total length. The allometric growth form, 'b', of *C. quadricarinatus* at Lake Kariba was 2.5. The calculated 'b' value implies negative allometry since it is less than 3. The graphical representation of the length-weight relation is given in Fig. 5.

Table 3: Length-weight regression ANOVA results *C. quadricarinatus* at Lake Kariba

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	20.57443	20.57443	1995.241	2.2624E-202
Residual	665	6.857313	0.010312		
Total	666	27.43174			

df means degrees of freedom, SS means sum of squares, MS means mean sum of squares and F means the computer-generated comparison value. ANOVA means analysis of variance.

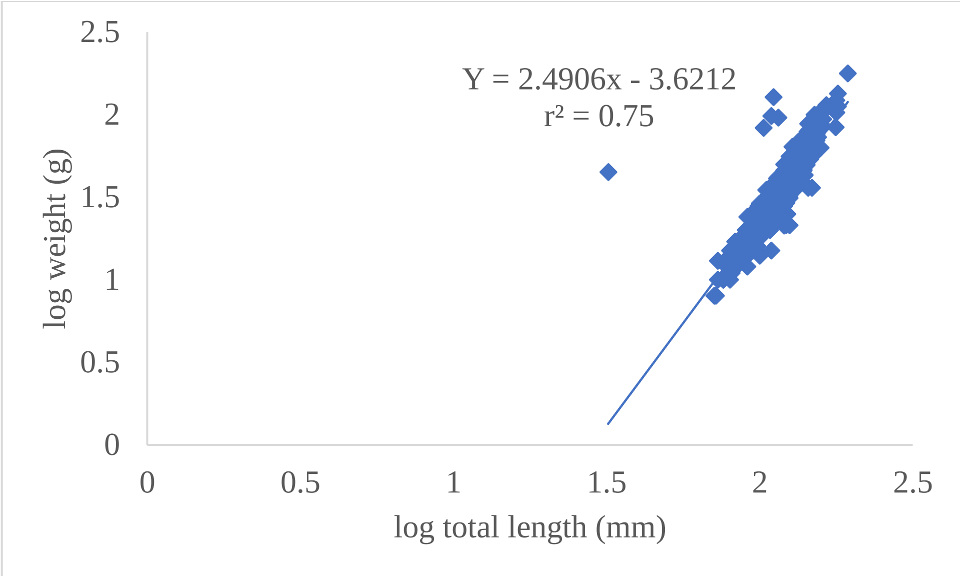
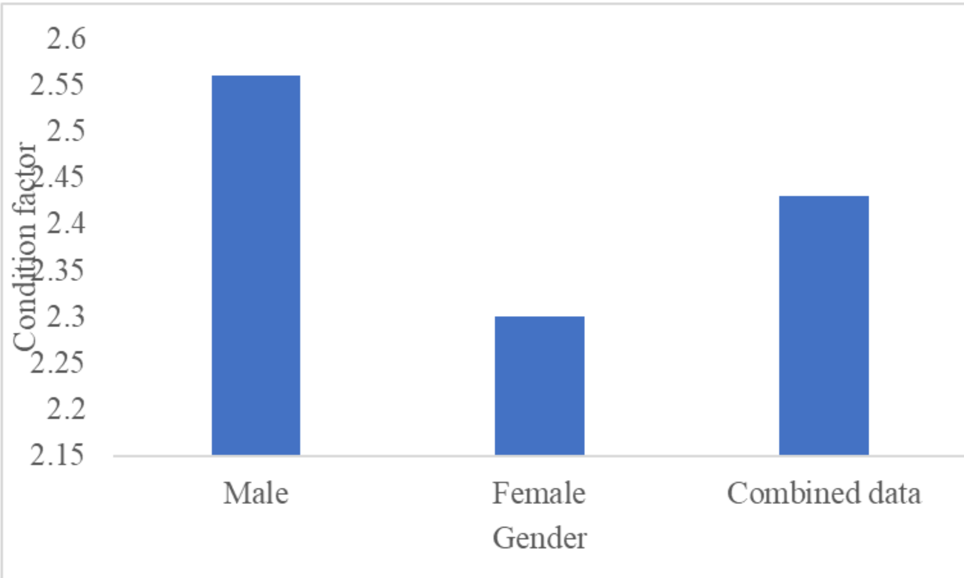


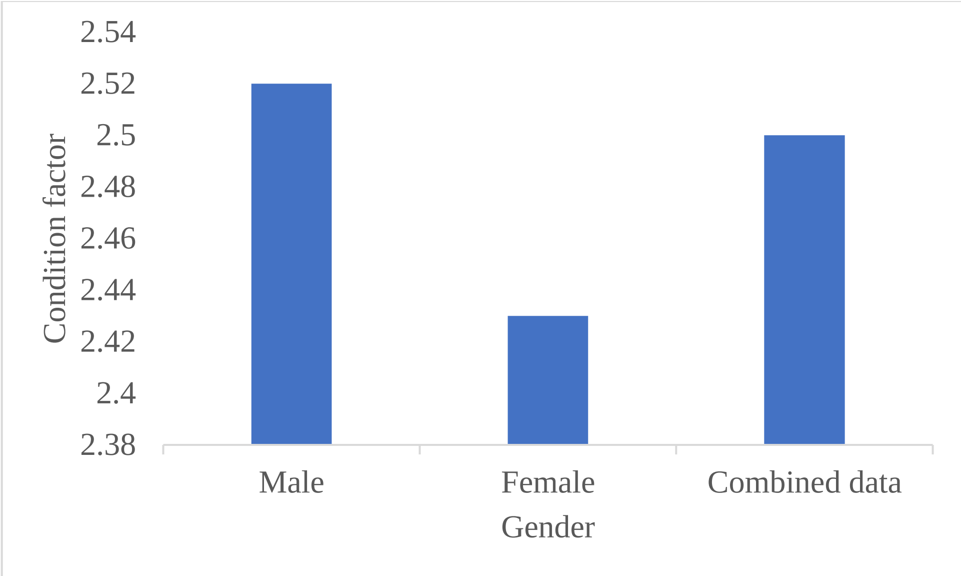
Fig-5: Graph of length-weight relationship of *C. quadricarinatus* at Lake Kariba.

Graph depicting the logarithmic relationship between Crayfish length and Crayfish weight at Lake Kariba. There was a significant correlation between fish growth and fish weight ($r^2 = 0.75$). The graph shows a positive correlation between Crayfish length and Crayfish weight.

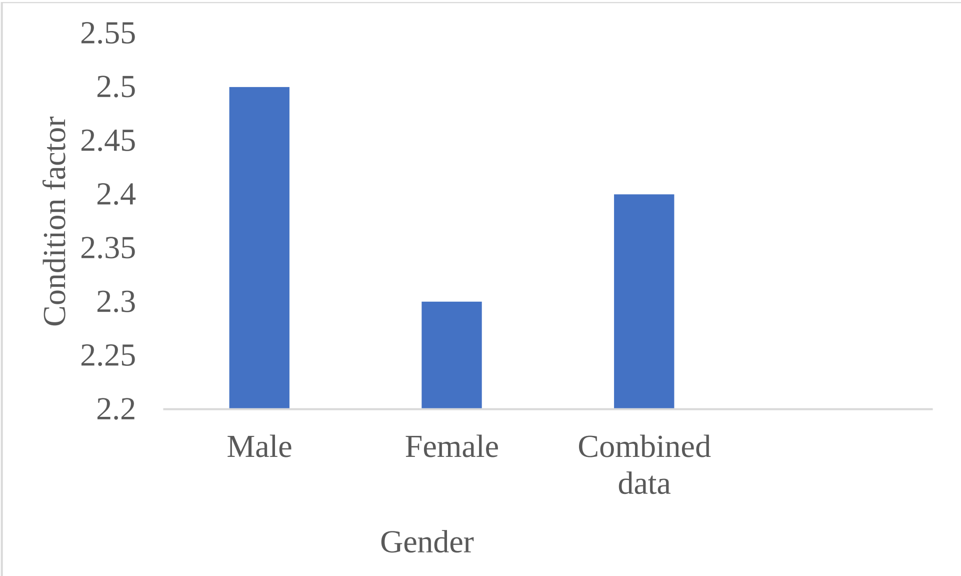
The Histograms which were constructed to show the Condition factors at the study sites within Lake Kariba are shown in Fig. 6a, b and c.



(a)



(b)



(c)

Fig-6: Histogram of Condition factors at Lake Kariba study sites (a) Kabbyobbyo (b) Mundulundulu and (c) Overall data. The y-axis shows the Condition factor at each study site while the x-axis shows the gender of the sampled *C. quadricarinatus*. Male *C. qaudricarinatus* showed larger Condition factors than Females. Condition factors were all above-average.

The larger growth exponent coefficients of Females (r^2 = 0.816) relative to Males (r^2 = 0.80) were validated by T-test results (Table 4) which showed no significant difference between Male and Female lengths (p = 0.0102). The T-test results showed that the growth rates of *C. quadricarinatus* were independent of gender. The T-test results showed that the habitat at Lake Kariba did not favour any particular gender of Crayfish.

Table 4: T-test results of Kariba Male against Kariba Female.

t-Test: Two-Sample Assuming Unequal Variances

	Kariba Male	*Kariba Female*
Mean	60.03475936	62.44150943
Variance	128.4197001	140.2172098
Observations	374	265
Hypothesized Mean Difference	0	

df	553
t Stat	-2.576622724
P(T<=t) one-tail	0.005117615
t Critical one-tail	1.647613736
P(T<=t) two-tail	0.010235229
t Critical two-tail	1.964263051

Df means degree of freedom, t Stat means the statistical t test value, P means critical p-value.

Growth performance indices and natural mortality results

Growth performance indices ranged from a low of 2.6 among Female *C. quadricarinatus* at Mundulundulu to a high 4.6 of at all other study sites (Table 5). All the growth performance indices were supported by above-average growth rates (r^2 values above 0.5) at all study sites and across both gender. Natural mortality values of *C. quadricarinatus* ranged from a low of 0.0386 among Females at Mundulundulu to a high of 0.0869 among Females at Kabbyobbyo. Combined data gave a natural mortality value of 0.0472 at Mundulundulu and 0.0829 at Kabbyobbyo. The difference between natural mortality values imply that the habitats were different.

Table 5: Growth parameters, condition factors, growth performance indices and natural mortality parameters of *C. quadricarinatus* at Lake Kariba.

	Kab									Mun								
	N	TL	W	a	b	r^2	K	M_w	Φ′	N	TL	W	a	b	r^2	K	M_w	Φ′
Male	289	114	38	-3.487	2.44	0.768	2.56	0.0783	4.6	85	124	48	-4.49	2.93	0.939	2.52	0.0386	4.6
Female	139	125	45	-3.26	2.32	0.747	2.30	0.0869	4.6	126	128	51	-4.052	2.71	0.851	2.43	0.0263	2.6
Total	428	118	40	-3.38	2.38	0.766	2.43	0.0829	4.6	211	126	50	-4.16	2.77	0.876	2.5	0.0472	4.6

N = number of *C. quadricarinatus* sampled, TL = mean total length in millimeters, W = mean weight in grammes, a = intercept, b = regression slope, r^2 = coefficient of determination, K = Fulton's condition factor, M_W = natural mortality and Φ′ = growth performance index. Kab means Kabbyobbyo study site while Mun means Mundulundulu study site.

The overall growth coefficient (r^2) at Lake Kariba was 0.75. The overall Condition factor and natural mortality coefficients for *C. quadricarinatus* at Lake Kariba were 2.4 and 0.07 respectively. The calculated value of the Condition factor shows that Crayfish is in good condition at Lake Kariba. Natural mortality values were below-average at both study sites ($M_w < 0.5$). The value of the allometric growth form for combined data at Lake Kariba was 2.49, depicting negative allometry. Negative allometry implies that the increase in body weight did not match increase in total length.

DISCUSSION

The results of the current study on breeding frequency of *Cherax quadricarinatus* agree with the findings of Levi *et al*. (1997) who found that Red claw Crayfish breeding occurs naturally during the summer months, when temperatures are more than 20 ºC. *C. quadricarinatus* mainly spawns during spring and summer while molting occurs mainly after the breeding season, but also between spawns (Karplus *et al*., 1997; Levi *et al*., 1997). Nunes *et al*. (2016) who reported that Crayfish normally breeds between September and April within their natural range in northern Australia freshwater bodies. The previous study by Makwelele (2017) in the Kafue Floodplain established that *C. quadricarinatus* has well-defined spawning seasons.

The sex ratio results are similar to the results obtained in the Upper Zambezi by Mukuka (2019), who also found a sex ratio of almost one to one. Both studies established that there are more Males than Females in most aquatic water bodies. The sex ratio results are also in agreement with those found in Mexico by Bortolini *et al*. (2007) and Belle *et al*. (2011) in Singapore. However, they are different from those found in Swaziland, South Africa, and Zimbabwe (Nunes *et al*., 2017b; Marufu *et al*., 2018) where Females were outnumbering Males.

The growth exponent coefficient (r^2) of *C. quadricarinatus* was above-average (more 0.5) at both study sites, implying maximum growth rate. These results are characteristic of invasive species as earlier found in *Oreochromis niloticus* by Chikopela *et al*. (2011), Makeche *et al*. (2023) and Nyirenda *et al*. (2024) in Zambia. The large growth exponents were supported by large growth performance indices at both study sites. These results are in agreement with earlier results by Marufu *et al*. (2018) ($\Phi' = 3.96$) from Lake Kariba, Mukuka (2019) ($\Phi' = 4.0$) from the Upper Zambezi and Nyirenda *et al*. (2024) who found similar values among *Oreochromis niloticus*, an invasive fish at Lake Kariba ($\Phi' = 4.58$). Makwelele (2017) found lower results ($\Phi' = 2.8$) from another part of Zambia. The difference between the current results and those obtained by Makwelele (2017) can be attributed to both the habitat and the sampling period. The current study was conducted during the rainy season when food materials are abundant while Makwelele (2017) conducted his study during the dry season when most natural habitats are nutrient-poor. The sampling season may also explain why Condition factor values of the present study were larger than those obtained by Makwelele (2017) (K = 1.8 to 2.3). These above-average values show that *C. quadricarinatus* is well acclimatized to its habitat (Makeche *et al*. (2023). The value of the Condition factor is a good indicator of the productivity of the habitat, ecological conditions and biological factors such as fatness, gonad maturity and habitat adaptability (Saha *et al*., 2021).

The Natural mortality rate estimates obtained in this study are similar to those obtained by Makwelele (2017) (M = 0.06 to 0.08) from the Kafue Floodplains and Makeche *et al*. (2020) on another invasive organism. The results of the current study are however different and lower than those those obtained by Marufu *et al.* (2018) (M = 0.99) from Lake Kariba and those found by Mukuka (2019). This may be due to several reasons. Firstly, it is always difficult to capture a representative length-frequency sample (real size distribution of the population) even with a large sample size due to gear selectivity. This has a compounding effect on the Von Bertalanfy growth equation parameter estimations which Mukuka (2019) used in estimating the growth coefficient, natural mortality, total mortality and asymptotic length. Also, the length-converted catch curve method which Mukuka (2019) used tends to over-estimate the total mortality when seasonal growth is considered (Gayanilo and Pauly, 1997).

CONCLUSION

The success of *C. quadricarinatus* in invading and establishing itself on Lake Kariba may not absolutely reflect on the species' inherently strong life-history strategies, instead, a number of external human-driven influences may have aided its current and future spread Lake Kariba. *C. quadricarinatus* is well-adapted to its habitat at Lake Kariba, as the growth exponents and condition factor results show. Although fish farmers may consider Crayfish to be a nuisance, it can be cultivated on a large scale as a reliable source of proteins while improving the economic returns of aquaculture farmers.

ACKNOWLEGEMENTS

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