



3.4 Carapace length and exoskeleton weight help predict chitin yield of giant freshwater prawn (*Macrobrachium rosenbergii*) cultured in perennial reservoirs across different agroecological zones in Sri Lanka

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Abstract

Chitin, the second most abundant polysaccharide, is found in the exoskeletons of crustaceans, including the GFP (*M. rosenbergii*), which is an important commodity in CBF in Sri Lanka. We examine the chitin yield of GFP inhabiting perennial reservoirs (n=25) scattered across different agroecological zones (n=6) in Sri Lanka, as well as the relationships between chitin biomass from GFP exuviae and morphometric characteristics (GFP length) and yield characteristics (exoskeleton weight) of GFP collected from various agroecological zones. The discarded inedible exoskeletons of GFP are used to extract chitin through a process that includes preconditioning, demineralisation and deproteinisation. The chitin yields significantly differ ($p < 0.05$) among GFP-inhabiting reservoirs across agroecological zones. The highest biometric characteristics of GFP are observed in reservoirs with annual rainfall of 900 mm or less, which may enhance the production of GFP through CBF in Sri Lanka. The highest chitin yield ($18.30 \pm 5.24\%$) was obtained from GFP exoskeletons inhabiting reservoirs with 900 mm of annual rainfall. Strong correlations were observed between chitin yields and carapace length, as well as the shell weight of GFP inhabiting reservoirs across the 3 derived agroecological zones. The results support the hypothesis that the agroecological status of reservoirs influences the quantity of chitin extracted from the exoskeletons of GFP. Thus, the study concludes that the chitin yield of GFP can be predicted using either carapace length or exoskeleton weight, which could assist in establishing a small-scale chitin production industry in Sri Lanka.

Keywords: culture-based fisheries, agroecological zones, relationship, predicting, chitin

Introduction

Sri Lanka is blessed with large, perennial and small, seasonal reservoirs (Chandrasoma et al. 2015). As of 2019, a total of 1,398 perennial reservoirs, including 43 major perennial reservoirs, 92 medium perennial reservoirs and 1,263 minor perennial reservoirs, were scattered across 25 administrative districts of Sri Lanka (Anon 2022). Additionally, numerous small tanks are dispersed throughout the dry zone, and local scientists have found that developing CBF in these seasonal, small reservoirs is more effective for boosting inland fish supply (Amarasinghe and Wijenayake 2015; Silva et al. 2006). Although the perennial reservoirs are mainly impounded for agricultural use, hydroelectricity generation and drinking water supply, they are rarely utilised for fishery development (De Silva and Amarasinghe 1996; Nissanka and Amarasinghe 2000). The reservoirs in Sri Lanka are scattered across various agroecological zones. Agroecological zoning (AEZ), as outlined in studies by the Food and Agriculture Organisation of the United Nations (FAO 1996), defines different zones based on combinations of soil, landform and climatic characteristics. The specific parameters used in this definition emphasise climatic and edaphic requirements of crops and the management systems under which they are cultivated. Each zone presents a similar combination of constraints and opportunities for land use, offering a foundation for targeted recommendations to enhance the existing land use situation, either by increasing production or mitigating land degradation (FAO 1996). Annual rainfall is the primary factor influencing the division of agroecological zones in Sri Lanka (Premarathne and Pushpakumara 2025).

From the earliest recorded history, inland fisheries have played a key role in supporting the subsistence economy of Sri Lanka (Jones et al. 2021). The large-scale development of inland fisheries began only in the early 1950s with the introduction of tilapia, as well as Indian and Chinese carp, into the dry zone reservoirs of Sri Lanka. CBF in Sri Lanka began in the 1960s by stocking Mozambique tilapia in some reservoirs. CBF, which involves direct stocking and recapturing strategies, significantly increases yields in both perennial and non-perennial reservoirs. Introducing CBF in village reservoirs is the most important strategy for enhancing inland fisheries (Amarasinghe and Wijenayake 2015). Exotic tilapia, Chinese and Indian major carps, along with indigenous GFP (*M. rosenbergii*), are the primary commodities utilised for CBF in both perennial and seasonal reservoirs across Sri Lanka (Deepananda et al. 2014). In 2019, the annual fish production in Sri Lanka's aquaculture sector was 10,710 mt, with 1,195 mt attributed to GFP catch (Ministry of Fisheries and Aquatic Resources Development 2022). The GFP culture is gaining popularity among crustacean farming due to several key factors, including its profitability, which is a source of income for rural families. It enables inland farming on both small and large scales (not limited to coastal areas) and supports polyculture with carp, tilapia and barbs. Additionally, GFP culture has lower feed costs, as these organisms are omnivorous and have a wide availability of food resources in the reservoirs, while reducing input costs. It also reduces expenses for pesticides and herbicides, as GFP feeds on fish parasites and weed seeds in paddy fields while enhancing soil fertility (Chowdhury et al. 1993).



Chitin is a vital compound in insect cuticles and the cell walls of fungi, which account for about 500–5,000 kg/ha of chitin in the soil (Pillai et al. 2009). Consequently, large amounts of chitin are synthesised naturally (Einbu 2007). Chitin, which is composed of N-acetyl-glucosamine (GlcNAc), is the second most ubiquitous naturally abundant mucopolysaccharide after cellulose on Earth (Dutta et al. 2002). The chitin is hard and inelastic (Subasinghe 1999), and the colour of the chitin flakes or powder is either white or yellowish. Crustaceans lack a backbone; they typically possess a hard exoskeleton that protects their soft internal bodies from predators and various diseases. The cuticle of crustaceans is composed of 4 distinct layers. From outermost to innermost, these layers include the epicuticle, exocuticle, endocuticle and a membranous layer (Simkiss and Wilbur 1989). The outermost epicuticle is a barrier against the external environment due to its waterproof properties, with a thickness ranging from 1 to 5 µm. The exocuticle accounts for one-fifth of the cuticle and contains unevenly distributed calcium carbonate within canals of pores and chitin-protein fibrils. Before molting, these 2 layers are formed, and mineralisation occurs afterwards. In the endocuticle, chitin aligns with protein fibres in a specific arrangement of lamellae (Lowenstam and Weiner 1989). Consequently, 3 significant components can be identified in the cuticle: chitin, protein and calcium carbonate. The relative proportions of these components can vary even within a single individual (Cribb et al. 2009). Chitin in crustaceans appears as a protein-polysaccharide complex, and several different proteins can bind to it to form a chitin/protein complex (Cribb et al. 2009). Following ecdysis, most crustaceans consume exuviae to meet their chitin requirements (Luo 2019). The value addition

of GFP at processing factories usually removes the exoskeleton. Also, scientists have identified the importance of chitin for biomedical purposes. Considering the importance of extracting chitin from GFP, which is a significant biomass of the GFP exoskeleton, the present study examines the influence of the agroecological status on the quantity of chitin biomass in the GFP exuviae obtained from agroecologically different reservoirs in Sri Lanka.

Materials and methods

The present study involved extracting chitin biopolymers from the exoskeletons of male and female GFP, collected from a total of 25 reservoirs across 4 administrative districts in the North Central, North Western, Southern and Uva provinces of Sri Lanka: Anuradhapura, Puttalam, Hambantota and Monaragala. The study reservoirs were assigned to different agroecological zones based on the criteria used by FAO (1996), which helps categorise Sri Lanka into 46 agroecological zones (Table 3.4.1). Consequently, 5 reservoirs in the Hambantota district belong to the lowest annual rainfall zone, DL5, while the other 2, H3 (Muruthawela) and H6 (Udukiriwala), are in the IL1b zone. The Anuradhapura and Puttalam district reservoirs, along with 4 others in Monaragala district, M2 (Kiriibbanwewa), M3 (Urusita), M7 (Habaralu) and M8 (Mahawwewa), belong to the DL1b zone. The remaining 3 reservoirs (M4 – Handapanagala, M5 – Hambegamuwa, M6 – Buduruwagala) are in DL1a. M9 (Sugaladevi) belongs to the DL2a zone, and M1 (Muthukandiya) is in the IL2 zone, representing the zone with the highest annual rainfall among the 6 agroecological zones in the present study. The distribution of study reservoirs across different agroecological zones is shown in Figure 3.4.1. In the present analysis,

Table 3.4.1: Agroecological zones of Sri Lanka and their annual rainfall

	Climate zone	Agroecological zone		Annual rainfall (mm)
1	WET ZONE	UP COUNTRY	WU1	3,100
2			WU2a	2,400
3			WU2b	2,200
4			WU3	1,800
5		MID COUNTRY	WM1a	3,300
6			WM1b	2,900
7			WM2a	2,200
8			WM2b	1,800
9			WM3a	1,600
10			WM3b	1,400
11		LOW COUNTRY	WL1a	3,200
12			WL1b	2,800
13			WL2a	2,400
14			WL2b	2,200
15			WL3	1,700
16	INTERMEDIATE ZONE	UP COUNTRY	IU1	2,400
17			IU2	2,100
18			IU3a	1,900
19			IU3b	1,700
20			IU3c	1,600
21			IU3d	1,300
22			IU3e	1,400
23		MID COUNTRY	IM1a	2,000
24			IM1b	2,000
25			IM1c	1,300
26			IM2a	1,800
27			IM2b	1,600
28			IM3a	1,400
29			IM3b	1,200
30			IM3c	1,100
31		LOW COUNTRY	IL1a	1,400
32			IL1b	1,100
33			IL1c	1,300
34			IL2	1,600
35			IL3	1,100
36	DRY ZONE	LOW COUNTRY	DL1a	1,100
37			DL1b	900
38			DL1c	900
39			DL1d	900
40			DL1e	900
41			DL1f	800
42			DL2a	1,300
43			DL2b	1,100
44			DL3	800
45			DL4	750
46			DL5	650

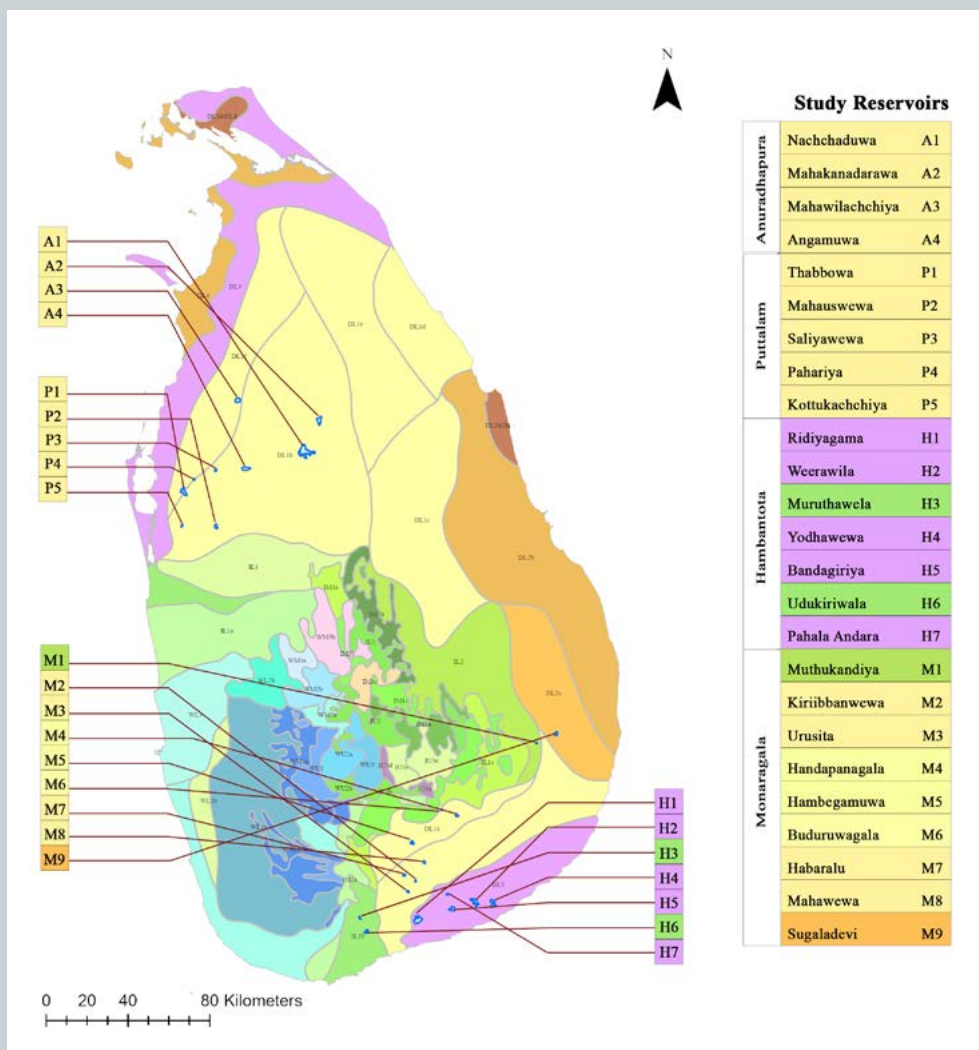


Figure 3.4.1: The map of Sri Lanka, showing the locations of study reservoirs across different agroecological zones in Sri Lanka

the study reservoirs scattered across 6 agroecological zones were grouped into 3 categories based on the annual rainfall (Table 3.4.2). Here, Category 1 represented the 5 reservoirs scattered in areas with <650 mm annual rainfall, and Category 2 included 13 reservoirs scattered in areas with annual rainfall of ≤900 mm. Category 3 consisted of 7 reservoirs with an annual rainfall of >900 mm.

A total of 12 intact (undamaged and no missing appendages) GFP – 6 males and 6 females – were purchased at the fish landing sites of each study reservoir from local fishers (n=25). Collected GFP were arranged in polystyrene boxes layered with ice to prevent damage and losses. Sex differentiation was achieved by identifying the ‘lump’, a spine-like structure located on the ventral side of the first abdominal somite, which is found only in male GFP. The GFP samples were transported to the Fish Post-Harvest Laboratory in the Department of Fisheries and Aquaculture, University of Ruhuna, to complete the experimentation. Total length (TL), taken as the length from the tip of the antennule’s plate to the end of the telson, and carapace length (CL), taken from the base of the eyestalk to the posterior end of the carapace for both sexes, were measured separately using a measuring board and a vernier caliper, respectively. The total body weight of the

respective GFP individuals was measured separately using a top-loading balance (Type 3600, Mettler, Switzerland). Then, the chitin extraction was made separately for male and female GFP at the laboratory, following the procedure outlined in Figure 3.4.2.

The normality of all collected data was assessed using the Shapiro-Wilk test ($p>0.05$). Mean separations were done using the one-way ANOVA and Tukey’s (HSD) studentised range tests at $\alpha=0.05$. Pearson’s product-moment correlation was performed to determine the relationship between the extracted chitin biomass and the carapace length and exoskeleton weight. Regression and correlation analyses were made with the combined sexes of the GFP. Minitab version 20 was used for all calculations and statistical comparisons.

Results

Morphometric and yield characteristics

Morphometric and yield characteristics of male GFP showed higher values than those of females. The highest values for TL, CL, body weight (BW), and exoskeletal/shell weight (SW) were recorded in GFP that inhabited reservoirs in DL1b agroecological zone receiving 900 mm of annual rainfall. The mean, minimum and maximum value ranges of morphometrics for blue-claw

Table 3.4.2: Distribution of study reservoirs in 6 different agroecological zones and categories of the reservoirs used for the present analysis

Reservoir category	Agroecological zone	Annual rainfall (mm)	Reservoir code (see Figure 3.4.1 for the reservoir name)
Category 1	DL5	<650	H1, H2, H4, H5, H7
Category 2	DL1b	≤900	A1, A2, A3, A4, P1, P2, P3, P4, P5, M2, M3, M7, M8
Category 3	DL1a	>1,100	M4, M5, M6
	DL2a	>1,300	M9
	IL1b	>1,100	H3, H6
	IL2	>1,600	M1

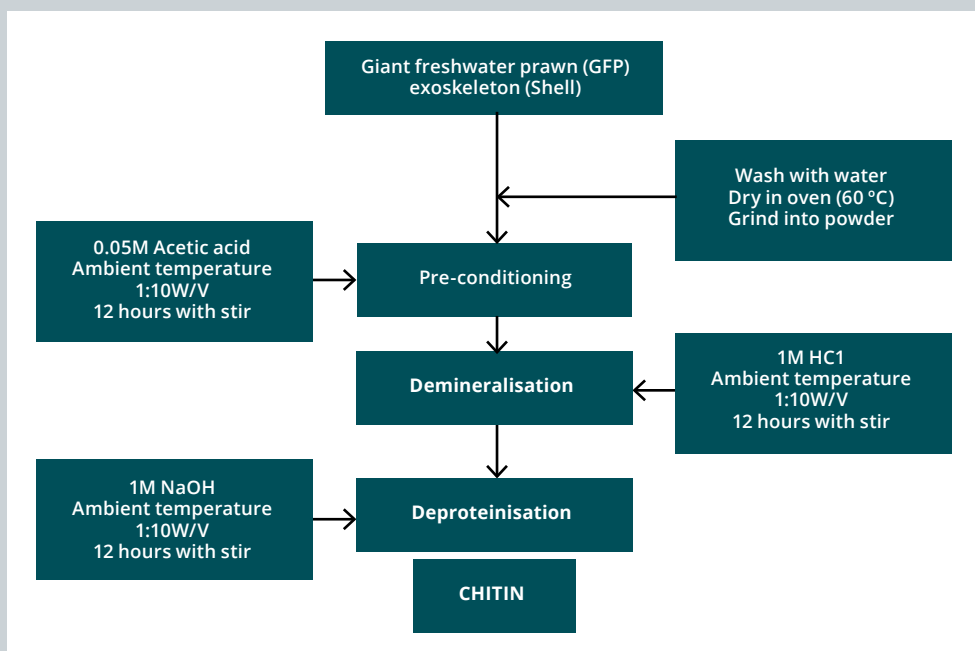


Figure 3.4.2: The flow chart, illustrating the procedure used in isolating chitin from the GFP shells

males (n=150) and females (n=150), and a total of 300 combined sexes GFP, are summarised in Table 3.4.3.

Variations in Chitin across agroecologically different reservoirs

The highest chitin weight and percentage of chitin per SW for male GFP were 9.90 ± 5.49 and 18.45 ± 5.16 , respectively, recorded from the reservoirs in the DL1b agroecological zone, which has an annual rainfall of 900 mm. Conversely, the lowest chitin weight and percentage of chitin per SW for female GFP were 2.31 ± 1.05 and 18.15 ± 5.34 , respectively, also recorded from the reservoirs in DL1b. Descriptive statistics of chitin weight (g) and chitin percentage per SW recorded from blue-claw males, females, and combined sexes of GFP are summarised in Table 3.4.4.

Chitin weight versus morphometric and yield characteristics

The simple linear regression analysis performed on males, females and combined sexes of GFP showed a significant correlation ($p < 0.05$) between the chitin yield and the morphometric (CL) and yield characters (SW). The strongest relationship between CL and chitin weight was reported from the Category 1 reservoirs, followed by Category 3 ($r = 0.80$) and Category 2 ($r = 0.78$) reservoirs, respectively. Also, the strongest to lowest relationship between shell and chitin weights was shown from Category 1 to Category 3 reservoirs, respectively. The correlation and simple linear regression results for combined sexes of the GFP are summarised in Table 3.4.5 and depicted in Figure 3.4.3.

Table 3.4.3: Reservoir category and TL, CL, BW and SW of male, female and combined sexes of GFP collected from agroecologically different reservoirs in Sri Lanka

Morphometric and yield characteristics	Reservoir category	Agro-ecological zone	Male			Female			Combined sexes		
			N	mean±SD	Min-max	N	mean±SD	min-max	N	mean±SD	min-max
Total length (cm)	C1	DL5	30	28.35±3.75 ^a	19.90–33.60	30	24.41±3.12 ^a	18.40–31.00	60	26.40±3.96 ^a	18.40–33.60
	C2	DL1b	78	28.38±3.15 ^a	20.20–35.40	78	24.80±2.95 ^a	17.60–33.10	156	26.58±3.53 ^a	17.60–35.40
	C3	DL1a, DL2a, IL1b, IL1b2	42	26.48±2.31 ^b	18.60–31.00	42	22.57±2.37 ^b	18.40–28.50	84	24.52±3.05 ^b	18.40–31.00
Carapace length (cm)	C1	DL5	30	7.74±1.29 ^{ab}	4.95–9.40	30	6.01±0.88 ^{ab}	4.17–7.58	60	6.88±1.40 ^{ab}	4.17–9.40
	C2	DL1b	78	8.18±1.10 ^a	4.70–10.50	78	6.22±1.03 ^a	4.20–10.50	156	7.20±1.45 ^a	4.20–10.50
	C3	DL1a, DL2a, IL1b, IL2	42	7.21±0.84 ^b	4.26–8.50	42	5.72±0.77 ^b	4.17–8.00	84	6.46±1.09 ^b	4.17–8.50
Body weight (g)	C1	DL5	30	285.30±129.50 ^a	70.00–499.90	30	138.51±52.73 ^{ab}	45.91–263.50	60	211.90±122.80 ^a	45.90–499.90
	C2	DL1b	78	308.00±95.70 ^a	67.40–553.70	78	152.77±67.87 ^a	55.80–519.00	156	230.37±113.57 ^a	55.80–553.68
	C3	DL1a, DL2a, IL1b, IL2	42	222.60±68.60 ^b	55.70–369.00	42	121.88±46.39 ^b	45.91–256.00	84	172.25±77.18 ^b	45.91–369.00
Exoskeleton weight (g)	C1	DL5	30	46.34±24.73 ^{ab}	9.03–98.35	30	18.94±7.99 ^{ab}	6.39–39.16	60	32.64±22.87 ^{ab}	6.39–98.35
	C2	DL1b	78	47.26±26.43 ^a	7.84–120.02	78	26.17±24.19 ^a	6.10–176.78	156	36.72±27.38 ^a	6.10–176.78
	C3	DL1a, DL2a, IL1b, IL2	42	36.07±13.54 ^b	5.49–61.08	42	14.95±4.84 ^b	6.39–24.46	84	25.51±14.66 ^b	5.49–61.08

Note: Superscript letters indicate statistically significant differences between groups. Values that share the same superscript do not differ significantly from one another, whereas values with different superscripts differ significantly based on post-hoc comparisons ($p<0.05$).

Table 3.4.4: Chitin weight and chitin percentage per SW for male, female, and combined sexes of GFP inhabiting agroecologically different reservoirs

Chitin yield	Agroecological zone	Male			Female			Combined sexes		
		N	mean±SD	min-max	N	mean±SD	min-max	N	mean±SD	min-max
Chitin (g)	DL5	30	7.47±4.03 ^{ab}	1.04-16.09	30	2.57±1.33 ^{ab}	0.74-6.40	60	5.02±3.87 ^{ab}	0.74-16.09
	DL1b	78	9.90±5.49 ^a	0.88-25.41	78	3.35±2.46 ^a	0.85-17.83	156	6.62±5.37 ^a	0.85-25.41
	DL1a, DL2a, IL1b, IL2	41	5.63±2.58 ^b	0.78-14.35	42	2.31±1.0 ^b	0.74-6.70	84	4.01±2.61 ^b	0.74-14.35
Chitin (%)	DL5	30	16.19±2.86 ^{ab}	11.53-28.43	30	13.59±3.38 ^b	4.81-20.18	60	14.89±3.37 ^b	4.81-28.43
	DL1b	78	18.45±5.16 ^a	3.78-34.04	78	18.15±5.34 ^a	6.91-38.79	156	18.30±5.24 ^a	3.78-38.79
	DL1a, DL2a, IL1b, IL2	41	15.79±3.46 ^b	7.80-28.43	42	15.02±3.13 ^b	11.06-29.70	84	15.43±3.29 ^b	7.80-29.70

Note: Superscript letters indicate statistically significant differences between groups. Values that share the same superscript do not differ significantly from one another, whereas values with different superscripts differ significantly based on post-hoc comparisons (p<0.05).

Table 3.4.5: Correlations between extracted chitin weight and morphometric characters, as well as yield characteristics of GFP inhabiting agroecologically different reservoirs in Sri Lanka

Variable 1	Variable 2	Category 1			Category 2			Category 3		
		r	p value	r	p value	r	p value	r	p value	
In carapace length (CL)	In chitin weight	0.89	<0.001	0.78	<0.001	0.80	<0.001			
In shell weight (SW)		0.95	<0.001	0.82	<0.001	0.94	<0.001			

r: correlation coefficient

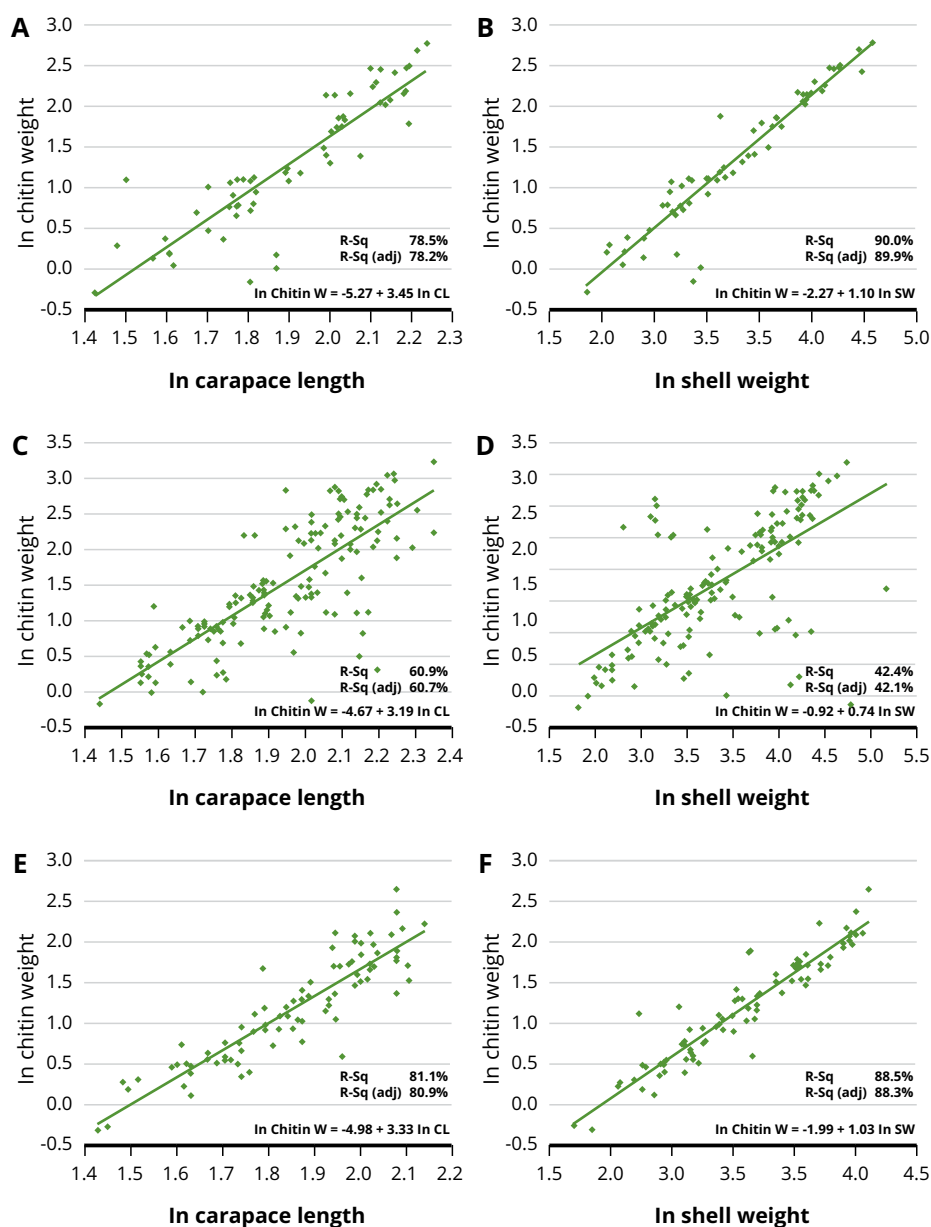


Figure 3.4.3: Simple linear regression between chitin weight and CL and SW of GFP captured from agroecologically different reservoirs in Sri Lanka

Note: a & b for Category 1 reservoirs; c & d for Category 2 reservoirs; and e & f for Category 3 reservoirs.



Discussion

The present findings reveal that the exoskeletons of GFP are an important source of chitin, which is considered a precursor to chitosan, regarded as the wonder drug of the 21st century. Aligning with morphometric and yield characteristics, the highest chitin yield was obtained from male GFP due to their higher SW compared to females. Concerning the biometric characteristics, the higher SW has been reported from males, dwelling in reservoirs in the DL1b agroecological zone, which receives an annual rainfall of 900 mm. The overall outcome of this study indicates that reservoirs located in DL1b agroecological zone with an annual rainfall of 900 mm provide suitable habitats for GFP, an important species in CBF, not only as a food commodity but also as a source for extracting high-quality biopolymer chitin from their exoskeletons. Strong correlations have been observed between chitin weight and CL, as well as with SW in GFP, inhabiting the reservoirs across the 3 categories of agroecological zones. Thus, the present study demonstrates that the chitin yield of GFP can be predicted using either CL or SW.

Sexual dimorphism in the growth of male GFP compared to females has been documented in several studies (Nagamine and Knight 1980; Thanh et al. 2009; Lalrinsanga et al. 2012; Indarjo et al. 2021). The findings align with those studies, showing that the length and weight are significantly higher in male GFP than in females. This reveals that perennial reservoirs of Sri Lanka are ideal for GFP CBF, which yield higher harvest biomass through cultivating larger prawns that attract the export market. Literature confirms that most studies based on agroecological zoning in Sri Lanka have

aimed to address resource management, agricultural production and land-use planning, making it an essential tool for sustainable development (Premarathne and Pushpakumara 2025). Although the present study deviates from conventional techniques, it assists in building a novel approach between agroecological zones and CBF in Sri Lanka. Chitin extraction is a common practice that utilises a large quantity of exoskeletons from crustaceans, internal skeletons of cephalopods (cuttle bones and squid pens/gladius), molluscan shells and fish scales. However, the extraction of this polymer from individual GFP exoskeletons has received less attention in scientific studies. Therefore, for the first time in Sri Lanka, this study has assessed the effects of the agroecological status of GFP residing in dry zone reservoirs and the sexual dimorphism of GFP on the chitin biomass extracted from their exoskeletons. Our findings reveal that male GFP exoskeletons can be used to extract a higher percentage of this polymer than female GFP. The percentage of chitin yield recorded in this study ($18.30 \pm 5.24\%$) aligns with the findings on chitin from shrimp shell waste by Samar et al. (2013), chitin from *P. monodon* shell by Islam et al. (2016), and chitin extracted from deep-sea mud shrimp by Rasweefali et al. (2021). Lobster shells have been reported to yield low biomass, although crabs yield higher biomass. The relatively low chitin yield may be attributed to de-polymerisation and loss of chitin particles during washing (Sewvandi and Adikari 2002). This suggests that innovative methodologies are needed to extract high chitin biomass.

Additionally, the significant findings of this study indicate that the yield of chitin is higher in the GFP inhabiting reservoirs within agroecological zones with annual rainfall of 900 mm (Category 2) compared

to those with less or more than 900 mm (Categories 1 and 3, respectively). The most plausible explanation for this finding is the greater growth of GFP in reservoirs located in agroecological zones with 900 mm of annual rainfall (Category 2), which helps increase SW. According to prior research by Sanuja et al. (2024), most reservoirs in the agroecological zone with 900 mm of annual rainfall (Category 2) can be classified as eutrophic reservoirs, while others are categorised as mesotrophic reservoirs. Although mesotrophic reservoirs are considered healthy aquatic systems with moderate transparency, primary production, alkalinity, well-oxygenated water, and sediments containing moderate amounts of organic matter (Kim and Kim 2021), better results have been observed in eutrophic reservoirs, which are regarded as slightly unbalanced aquatic systems primarily due to anthropogenic nutrient inputs. The CL of GFP is considered the most convenient and accurate morphometric character that can be measured in the field. Therefore, establishing strong correlations and simple linear relationships helps predict the chitin yield of GFP. The present findings can address the lack of scientific research on CBF in agroecological zones in Sri Lanka.

Conclusion

The findings indicate that the amount of chitin extracted from GFP exuviae depends on the agroecological state of the reservoir they inhabit. The highest chitin yield is observed in reservoirs housing GFP, particularly in the agroecological zone DL1b, which experiences an annual rainfall of 900 mm. These findings support the proposed hypothesis that the agroecological status of the perennial reservoirs influences the quantity of chitin extracted from the exoskeletons of

M. rosenbergii and that the chitin yield of GFP can be predicted using either CL or SW. Furthermore, the empirical results recommend collecting GFP from other dry zone perennial reservoirs and expanding the study to include seasonal reservoirs (non-perennial) in Sri Lanka to compare the quantity of chitin extracted from GFP inhabiting these 3 types of reservoirs as well.

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