# Length-weight relationship and condition factor of papain-fed *Cherax quadricarinatus* craylings

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**Abstract**. In aquaculture, the growth and health of high-value species like the Australian red claw, *Cherax quadricarinatus*, can be enhanced through optimized diets. Supplementing feed with papain, a proteolytic enzyme from *Papaya carica*, improves protein digestion and nutrient absorption, supporting growth and health condition. This study investigates the effect of varying dietary levels of papain on the length–weight relationship (LWR) and condition factor of *C. quadricarinatus* over a 45-day culture period. The experimental treatments consisted of varying inclusion levels of papain added to commercial diets: T1 (0.0% $kg^{-1}$), T2 (0.07% $kg^{-1}$), T3 (0.08% $kg^{-1}$), T4 (0.09% $kg^{-1}$), and T5 (0.10% $kg^{-1}$). A total of 225 craylings were randomly assigned to 15 culture units in a completely randomized design. After 45 days of culture, all treatments significantly enhanced growth performance: the T3 group recorded the highest, while the control (T1) group recorded the lowest growth. Based on the findings of the LWR analysis, papain supplemented in the diet of *C. quadricarinatus* at an inclusion level of 0.08% promote weight gain. The positive allometric growth observed in T3 (b=3.6090), suggests that papain improves nutrient absorption, resulting in better growth performance, in other treatments exhibited negative allometry. Since the condition factor remained stable across all treatments, the addition of papain does not compromise the health or physical condition of the crayfish.
**Key Words**: crayfish, growth, feed additives, *Papaya carica*.

**Introduction**. The Australian red claw crayfish (*Cherax quadricarinatus*, von Martens 1868) is recognized as one of the most rapidly cultivated freshwater crustaceans. Its widespread aquaculture potential is attributed to its high market demand, fast growth rate, adaptability to diverse environments, and resilience under varying culture conditions (Saoud et al 2013; Reynolds et al 2013). This species is a member of the Parastacidae family and was initially distributed in northern Australia and southern Papua New Guinea. This species has been introduced to the Philippines and numerous tropical and subtropical regions, and successfully cultured due to its omnivorous feeding habit, capacity to thrive in unregulated and extreme environments, and moderate tolerance to environmental factors such as temperature (Jones 1990; Ackefors 2000; Rigg et al 2020; Haubrock et al 2021). Sustainable cultivation of this species in the Philippines is being promoted through enhanced management practices, driven by its economic profitability and export potential (Cagauan 2007; Salayo et al 2022). Due to increased demand, significant efforts should be made to enhance aquaculture production (Pavasovic 2008; Saoud et al 2012), including less costly dietary enhancements through supplementation.

Nutrition plays a critical role in aquaculture productivity by influencing growth, feed efficiency, and stress tolerance (Carter & Mente 2014). Improving nutrition and health through feed additives, including enzymes, probiotics, and plant-based supplements, has been the focus of new studies (Hossain et al 2024). Feed inclusion of papain, a proteolytic enzyme derived from papaya (*Carica papaya*), improves feed digestibility, increases the availability of amino acids, and enhances feed conversion (Amri & Mamboya 2012; Feng et al 2022). The papain supplementation has shown beneficial effects in various aquatic species including fish. The diets containing 2%-5% papain improved growth performance, feed utilization, and digestive enzyme activity on *Trachinitus blochii* and *Cyprinus carpio* (Singh et al 2011; Rostika et al 2023). In Freshwater lobster, growth, feed efficiency, and stress tolerance were all improved by the addition of 0.24–0.31% papain (Rachmawati et al 2018).

Morphometric indices are valuable for analyzing the growth, condition, and health status of fishery and farmed aquaculture species, as they describe condition factors (K) and length–weight relationships (LWR), which are among the most essential indices (Simon & Stewart 2014; Lopeztegui-Castillo 2021; Paramo et al 2024) in aquaculture species. LWR provides estimates of biomass, growth predictions, and assessments of the impact of diet and environmental conditions (Zhang et al 2024). Higher K indicates better physiological health and denotes improvements in nutritional, health, and cultural conditions for the aquaculture species (De Carvalho-Souza 2023). In aquaculture crustaceans, these two indices form the basis for assessments of growth and nutritional condition and are therefore important (Lopeztegui-Castillo 2021). LWR and K value in *C. quadricarinatus* are influenced by diet, especially during the early stages, which are periods of sensitive growth and critical nutrient utilization (Jones 1990; Pavasovic 2008). However, the impacts of papain on freshwater crayfish, particularly on morphometric indices such as LWR and K, are not well studied. To address this gap, the current study investigated the length–weight relationship and condition factor of papain-fed of *C. quadricarinatus* craylings to promote greater efficiency and sustainability in production.

## Material and Method

***Study site and duration***. The experiments were carried out at the Multi-Species Hatchery of the College of Oceanography, Fisheries, Environmental Science and Technology (formerly known as the College of Fisheries), Mindanao State University–Tawi-Tawi College of Technology and Oceanography (COFEST, MSU-TCTO), located in the Barangay of Sanga-Sanga, Bongao, Tawi-Tawi, Philippines (5°02.233'N 119°44.580'E, in DMM format), for a 45-day culture period from August to October 2025 (Figure 1).

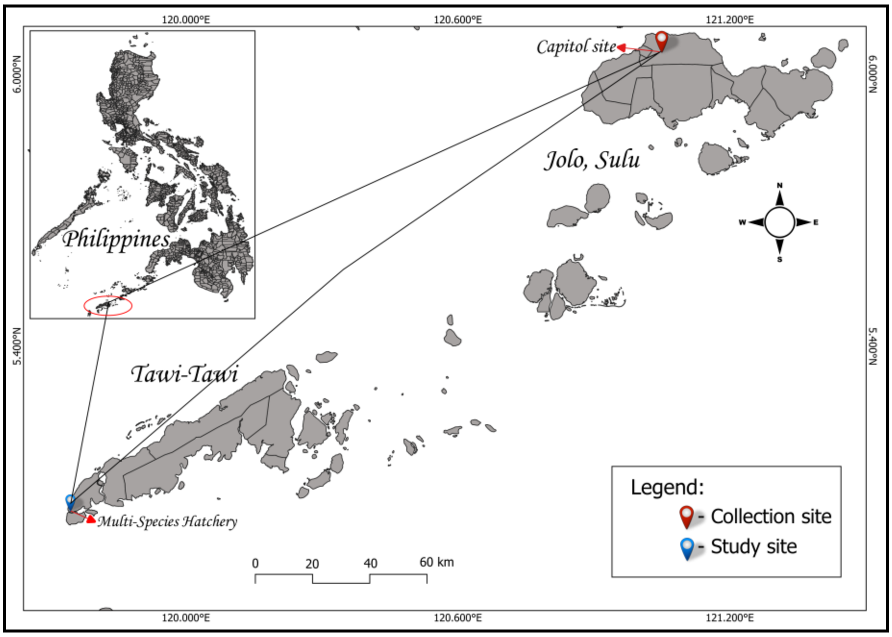


Figure 1. Map of the study site and the source of the experimental organism (generated using QGIS version 3.30.3).

***Source of Cherax quadricarinatus***. In this study, a total of 225 *C. quadricarinatus* craylings with an average length and weight of 1.65±0.16 cm and 0.4±0.03 g, respectively, were obtained from Jolo, Sulu, Philippines, and were transported via boat to the culture site. Craylings were placed in an ice chest with dimensions of 15.24 x 10.16 x 7.62 $cm^3$, with holes for air, acclimated for 30 minutes at ambient temperature, followed by a one-week conditioning period to adapt to the new environment, before the start of the experiment.

***Layout and design***. The experimental layout followed a Completely Randomized Design (CRD), with five treatment groups and three replicates (Table 1). A total of fifteen experimental units were used in this study, each containing 15 craylings, for a total of 225 craylings distributed among culture units. Treatments were randomly allocated to experimental units using a CRD. Randomization of treatments was conducted using the random numbers presented by Walpole (1968). Random numbers were selected by closing the eyes and pointing to values in the table, which were then ranked from highest to lowest to assign treatments, to minimize experimental bias, and ensure a random distribution of treatments among the replicates.

Table 1

Concentration of papain (% $kg^{-1}$) of feed

| *Treatments* | *Inclusion level of papain* |
|---|---|
| T1 (Control) | 0.0% |
| T2 | 0.07% |
| T3 | 0.08% |
| T4 | 0.09% |
| T5 | 0.10% |

***Preparation of feed inclusion***. The experimental diets and papain were procured from an online store. The proximate analysis of papain is presented in Table 2 (Macalood et al 2013), while the proximate composition of the crayfish feed is shown in Table 3.

Table 2

Proximate analysis of papain

| *Nutrients* | *Contents* |
|---|---|
| Crude protein | 57.24% |
| Moisture | 17.76% |
| Ash | 7.00% |
| Crude fat | 5.21% |
| Crude fiber | 0.67% |

Table 3

Proximate analysis of fry master crayfish feed provided by Aquacray farms

| *Nutrients* | *Contents* |
|---|---|
| Crude protein | 38% |
| Crude fat | 8% |
| Crude fiber | 16% |
| Coarse ash | 16% |
| Moisture | 12% |

The diets were prepared by spreading them into aluminum trays and weighing the required amount of papain according to the specified inclusion levels. Papain was dissolved in 10 mL of distilled water and stirred thoroughly until totally dissolved. The solution was distributed into labelled spray bottles for each treatment to avoid any contamination. The papain solution was uniformly sprayed onto the designated feed treatments and mixed thoroughly to ensure even distribution. Following mixing, the feeds were spread into aluminum trays lined with aluminum foil and covered with filter cloth to prevent contamination by dust and insects, and were dried at room temperature. This method was adapted from Jumah et al (2020). Once completely dried, the feeds were packed in properly labelled Ziplock plastic bags and stored in a cabinet at a room temperature.

***Feeding****.* The craylings were fed twice daily at 6 AM and 6 PM at 5% of their body weight (Jones & Ruscoe 2000). Total feed consumption was computed as the difference between the feed offered and the feed remaining, amounting to 7.81 g over the entire experimental period.

***Growth monitoring and water parameters***. Sampling was performed after 45 days of culture. The craylings were not fed for 24 hours prior to sampling, and data collection began at 6 AM. Each craylings was weighed using an analytical weighing scale (0.01 g) after lightly blotting it with a towel and an absorbent cloth to remove excess water. The total length was taken using a digital Vernier caliper (mm). During the experiment, water parameters were monitored. The pH was 7.36, the temperature 29$^{o}$C, and the salinity 2.5 ppt. A multifunctional parameter was used to measure pH and temperature, while salinity was measured using a refractometer (ATC).

***Data analysis***. The measurement of the length-weight relationship of the crayfish involved the use of the following formula (Pauly 1983):

$$W = aL^{b}$$

Where:
W - body weight (g);
L - total length (cm);
a - the intercept;
b - the growth coefficient.

Linear regression analysis of the log-transformed W and L data enabled the calculation of a and b from the regression equation log W=a+b log L. Evaluation of the regression strength and precision was performed using the coefficient of determination ($r^2$) (Golmard et al 2007). As described by Riedel et al (2007), when b=3, growth is considered isometric; when b<3, growth is described as allometric negative; and when b>3, the growth is considered allometric positive.

The condition factor was calculated according to Pauly (1984) using the following formula:

$$K = 100 \times W/L^3$$

Where:
K - condition factor;
W - body weight;
L - total length.

The significance of the association between the variables was assessed using the $r^2$ value, and all data analyses were conducted in Microsoft Excel 2021 (Sahami et al 2013). The data on K value were analyzed using one-way Analysis of Variance (ANOVA) in JASP v.0.95.4, to determine significant differences among treatments at a p-value of<0.05.

**Results**. The value of the condition factor and the length-weight regression analysis for *C. quadricarinatus* throughout the 45-day culture period under different dietary treatments, including the total length, body weight, regression parameters, standard error, coefficient of determination, and growth type, are summarized in Table 4.

The results presented in Table 4 show that the highest b value (3.6090) was observed in T3 (0.10% $kg^{-1}$ papain), while the lowest b value (2.3125) was observed in the control group (T1; 0% $kg^{-1}$ papain) throughout the culture period. Most of the treatments were observed to exhibit negative allometric growth because the values for the regression coefficient (b) indicated this (b<3), meaning length was gained faster than weight. Day 45, T3 (0.08% $kg^{-1}$ papain) was the only treatment that consistently exhibited positive allometric growth (b>3), showing that the crayfish from this treatment were gaining weight faster than length. The relationship between length and weight across all treatments was consistent, with $r^2$ values ranging from 0.8231 to 0.9992. Lower standard errors (0.030–0.147) suggest that the regression parameters were consistent. Regarding the K, all crayfish across all treatments maintained good physical condition throughout the culture period. The crayfish were well fed and physiologically stable as reflected by their K throughout the experiment. The T2 and T3 treatments showed K values higher than those in the control treatment, indicating better somatic condition and growth, but no significant differences among treatments (p>0.05). The overall growth trend indicated that diets supplemented with papain improved LWR and K throughout the culture period, promoting even growth and effective nutrient utilization.

Table 4

Length–weight regression analysis and condition factor of *Cherax quadricarinatus* throughout the 45-day culture period under different dietary inclusion levels

| *Cultures duration* | *Treatment* | *Log a* | *Log b* | *SE* | *$r^2$* | *Growth type* | *K value* |
|---|---|---|---|---|---|---|---|
| Day 0 | T1 (0.0% $kg^{-1}$) | 1.4363 | 2.8908 | 0.127950 | 0.9969 | Allometric (-) | $0.95 \pm 0.10^a$ |
| | T2 (0.07% $kg^{-1}$) | 1.3575 | 2.4273 | 0.137770 | 0.9992 | Allometric (-) | $1.02 \pm 0.10^a$ |
| | T3 (0.08% $kg^{-1}$) | 1.4479 | 2.6195 | 0.146720 | 0.9670 | Allometric (-) | $0.98 \pm 0.13^a$ |
| | T4 (0.09% $kg^{-1}$) | 1.5557 | 2.8335 | 0.137410 | 0.9376 | Allometric (-) | $1.08 \pm 0.16^a$ |
| | T5 (0.10% $kg^{-1}$) | 0.8300 | 1.4185 | 0.049770 | 0.9535 | Allometric (-) | $0.83 \pm 0.02^a$ |
| Day 45 | T1 (0.0% $kg^{-1}$) | 1.1837 | 2.3125 | 0.030438 | 0.8231 | Allometric (-) | $0.56 \pm 0.02^a$ |
| | T2 (0.07% $kg^{-1}$) | 1.5063 | 2.8118 | 0.070796 | 0.8980 | Allometric (-) | $0.61 \pm 0.05^a$ |
| | T3 (0.08% $kg^{-1}$) | 1.9959 | 3.6090 | 0.078452 | 0.9964 | Allometric (+) | $0.59 \pm 0.05^a$ |
| | T4 (0.09% $kg^{-1}$) | 1.2119 | 2.3492 | 0.056869 | 0.9974 | Allometric (-) | $0.58 \pm 0.02^a$ |
| | T5 (0.10% $kg^{-1}$) | 1.5327 | 2.8619 | 0.049063 | 0.9228 | Allometric (-) | $0.55 \pm 0.03^a$ |

LWR-length–weight relationship; *a* and *b*-the estimated coefficients of the regression model; SE-the standard error associated with parameter *b*; $r^2$-the degree of correlation between length and weight. Growth type: +A=positive allometric, -A=negative allometric. Values are means of triplicate. T1 (0% $kg^{-1}$ papain); T2 (0.07% $kg^{-1}$ papain); T3 (0.08% $kg^{-1}$ papain); T4 (0.09% $kg^{-1}$ papain); T5 (0.10% $kg^{-1}$ papain); n=15.

**Discussion**. This study evaluated the effects of different papain concentrations on the length-weight relationship and condition factor over a 45-day culture period. It was evident that most growth patterns of *C. quadricarinatus* were generally negative allometric (b<3) across most treatments, suggesting that length increased more than weight. However, treatment T3 (0.08% $kg^{-1}$ papain) was the only treatment to achieve a positive allometric growth pattern (b=3.6090), suggesting that this level of papain aided in weight gain enhancement during the growth period, resulting from efficient feed use and nutrient uptake. The lowest growth rate (b=1.2781) was also observed for the control (T1), with regression coefficients for the other treatments ranging from 1.2781 to

3.6090. The greater the $r^2$ coefficient (0.8231–0.9992) in weight-length relationship, the more mutually dependent. The precision of the LWR estimations was very strong, with length also supporting the high K. Thus, dietary supplemental papain, may improve this species growth performance. Variations in b values across studies may be attributed to differences in feed formulation, experimental duration, and developmental stage. Hobbs et al (1989), Mazlum et al (2007), and Aydın et al (2015) stated that negative allometric growth is frequent during the early developmental stages, when the crayfish primarily expends energy on increasing length rather than weight.

Previous studies have shown that K values can vary across treatments due to differences in feed digestibility, nutrient composition, and nutrient abundance (Baltaci et al 2020). The consistency of K values throughout the study demonstrates that all diets were nutritionally adequate to maintain *C. quadricarinatus* in good physiological condition. Similar observations have been reported by Le Cren (1951) and Zhang et al (2024), where increased K values reflected improved health and nutrient assimilation. The dominance of negative allometry and the stable K value in this study imply that the crayfish may not have had sufficient time to attain isometric or positive allometric growth by the end of the allocated 45-day culture period. Crayfish in shorter culture periods that are reported to be, as reported by Mazlum et al (2007) and Aydın et al (2015), tend to invest energy in exoskeletal elongation rather than somatic growth. Lindqvist & Lahti (1983) and Aiken & Waddy (1987) noted that crayfish growth is significantly affected by the intervals between molts, stocking density, and the nutritional value of the feed provided. This suggests that longer culture periods, allowing *C. quadricarinatus* to shift energy from exoskeletal growth to somatic mass gain, would facilitate the attainment of isometric or positive allometric growth. Overall, the present study indicates that the addition of papain, specifically at T3 (0.08% $kg^{-1}$ papain), improves growth and condition factor of *C. quadricarinatus*. This is a consequence of papain's proteolytic activity, resulting in enhanced protein digestion and absorption. This is likely to increase the species' growth efficiency and improve physiological condition. Further extensions of culture periods and adjustments to dietary enzyme inclusion may further enhance the growth performance and physiological condition of the species.

**Conclusions**. Dietary papain has potential as a natural feed additive to promote growth in *C. quadricarinatus* as demonstrated in this 45-day study. Most growth response treatments in the study were classified as negative allometric, indicating a greater increase in length than in weight of the crayfish. The results suggested that the optimal level of papain in the diet was 0.08% $kg^{-1}$, resulting in a positive allometric relationship (b=3.6090), indicating that greater weight gain resulted from improved feed utilization and nutrient absorption. The condition factor was similar across all treatments, indicating that all diets were sufficiently balanced to support healthy physiological condition and extend the culture period. Despite the short culture period may have limited full growth, the strong length–weight correlations ($r^2$=0.8231–0.9992) observed in the papain-treated groups indicate a positive growth response to papain supplementation. Growth responses promote the findings that intermediate levels of dietary papain support effective growth performance. Papain supplementation in the diets of *C. quadricarinatus* would thus be an effective way to increase the productivity and nutritional efficiency. To validate these results and assess the broader applicability of papain supplementation, further studies should be conducted to evaluate its long-term effects on craylings until they reach marketable size, as well as its influence on survival, feed conversion efficiency, and overall production outcomes.

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**Conflict of interest**. The authors declare that there is no conflict of interest.

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