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Genetic additive components of the exoskeleton mineral profile and their genetic relationship with growth traits in *Penaeus vannamei*

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Introduction: The aquaculture industry faces challenges due to the limited availability and environmental impact of fishmeal. As alternative diets are adopted, mineral supplementation through feed, water, or substrate has become essential to sustain shrimp performance. A promising but unexplored approach is genetic selection to enhance mineral content, offering a sustainable complement to supplementation strategies. This work represents the first study of genetic parameters for mineral content in the most widely produced aquaculture species, the white leg shrimp.

Methods: To this aim, 388 shrimp from 79 full-sib and 18 half-sib families of the PMG-BIOGEMAR® breeding program selected focused on growth by harvest weight under low salinity industrial conditions in Ecuador were analyzed. Animals were sampled at harvest size and transferred to the University of Las Palmas de Gran Canaria to measure weight and length, and mineral concentrations of their exoskeletons by inductively coupled plasma mass spectrometry. Genetic parameters for these traits were obtained by Bayesian estimation method.

Results and Discussion: Genetic parameters for these traits were obtained by Bayesian estimation method. Interestingly, the concentration of Cu, Na, K, Mg, Ca, and P presented medium heritability (from 0.20 to 0.42), the highest being Cu. While Fe, Mn, and Zn showed low heritability (from 0.07 to 0.18), the lowest being Fe. Overall, the genetic correlations estimated among minerals suggest that their levels can be improved simultaneously through genetic selection, offering a viable strategy to face the future challenges of shrimp production in low-salinity environments. However, the lengthy time and high costs of the mineral analysis

could prompt the consideration of modulating mineral content through indirect selection based on a growth trait with high genetic correlations (positives or negatives) with mineral content. Thus, positive indirect selection can be applied to increase Ca and Mg levels, while negative indirect selection for Fe, Zn, and Cu may help maintain balanced concentrations and mitigate the effects of anthropogenic inputs.

KEYWORDS

calcium, genetic correlation, heritability, minerals, shrimp

1 Introduction

Ecuador is one of the main contributors to the high global production level of *P. vannamei* species (6.8 million tons), exporting more than 1.2 million tons in 2023 (CNA, 2024; FAO, 2024). This impressive statistic has been sustained over the last few years, driven by the incorporation of genetic breeding programs (Shin et al., 2020), the transfer of know-how (Martínez Soler et al., 2023), and the high level of knowledge about production techniques among local farmers, which will also enable expected future improvements.

Genetic selection for growth in the population used for the present study (PMG-BIOGEMAR[®]) has reduced the on-growing period in Ecuadorian industrial estuaries, from seeding to harvest (Shin et al., 2023; Martínez Soler et al., 2024). Guan et al. (2016) observed a reduction in the frequency of moulting in river prawns when growth was accelerated due to nutritional factors. This resulted in an extended interval between each moulting event. Previous studies have compiled the expression of a large group of genes associated with exoskeleton formation, degradation, regulation, synthesis, mineral absorption/reabsorption, and hardening in *P. vannamei* (Gao et al., 2017). This highlights the importance of establishing genetic relationships between growth and exoskeleton quality in terms of mineral composition.

The high prices of fishmeal and the limitations imposed by its shortage (Tacon and Metian, 2008) are causing a reliance on vegetable meals, which are deficient in the essential minerals and trace elements that fishmeal provides. Furthermore, the phytic acid present in plants binds with divalent cationic minerals making their assimilation more difficult (Lall, 2002; Huang et al., 2017). Mineral supplementation in diets offers a potential solution, but it represents a considerable expense for farmers (Jannathulla et al., 2020). This is driving the aquaculture industry to explore alternative strategies for modulating mineral content in aquatic species.

The structural integrity of the exoskeleton is of significant interest to shrimp farmers, with a focus on reducing breakage or softening, which can impact consumer and traders' acceptance and shrimp resilience (Truong et al., 2022). Exoskeletal firmness is determined by several factors, with mineral content being a significant one. Calcium (Ca) is the predominant mineral in the exoskeleton of *P. vannamei*, with an important function in hardening. Phosphorous (P) is also an important element for metabolic processes and exoskeleton formation along with Ca; the exoskeleton has the highest P content of the entire shrimp body (Jannathulla et al., 2020). Beyond their role in exoskeletal structure, minerals are also essential for other

physiological processes. For example, Copper (Cu) is the core element of hemocyanin, a key molecule in innate immunity, and influences the expression of genes related to antioxidant and immune responses (Li et al., 2018; Shi et al., 2021) and Magnesium (Mg) is essential for osmoregulation and protein synthesis in shrimp (Jannathulla et al., 2020).

Another issue involving mineral concentration in shrimp is that increasing levels of heavy metals in waters from different origins (McRae et al., 2016) which can be absorbed by aquatic animals (Viana et al., 2021). In the case of Zinc (Zn), an excess can alter immune, antioxidant, and detoxifying responses (Liang et al., 2022). An excess of Cu can damage the hepatopancreas structure, inhibit haemolymph immunity, and increase intestinal pathogens (Qian et al., 2020).

Iron (Fe), Cu, Zn, sodium (Na), and manganese (Mn) levels in the *P. vannamei* exoskeleton are not strongly influenced by their dietary concentrations (Huang et al., 2017). Similarly, potassium (K) content in the exoskeleton does not vary significantly with changes in its concentration in the water, suggesting the presence of an ion regulatory mechanism that allows this species to maintain stable levels (Cheng et al., 2006; Jannathulla et al., 2020). The ability of the shrimp exoskeleton to remain mineral content stable despite variations in diet and environmental conditions (water) may indicate the existence of a genetic component underlying phenotypic variation.

While nutrition and management can contribute to regulating mineral content in shrimp, genetic selection produces continuous, cumulative and permanent gains (Toro and López-Fanjul, 2007). However, the capacity of using genetic selection to regulate the mineral content in aquatic animals, either directly or indirectly through growth, remains unexplored. Hence, the main objective of this study was to estimate the heritability in exoskeletal mineral composition and the genetic correlations with growth-related traits in a genetically selected *P. vannamei* population (PMG-BIOGEMAR[®]), cultured in low-salinity water under extensive industrial conditions.

2 Materials and methods

2.1 Genetic line

Shrimp analyzed in the present work belonged to the fourth generation (G₄) of the PMG-BIOGEMAR[®] project, developed

between the University of Las Palmas de Gran Canaria (ULPGC, Gran Canaria, Spain) and the Almar Group (Guayaquil, Ecuador), to implement a genetic breeding program at BIOGEMAR S.A. company (San Pablo, Ecuador) (Shin et al., 2020). The elite breeders from all generations of the PMG-BIOGEMAR[®] were evaluated by BLUP (Best Linear Unbiased Prediction) and selected by the Optimal Contribution Selection (OCS) method (Meuwissen, 1997; Grundy et al., 1998; Woolliams et al., 2015) for weight at harvest size.

To obtain the G₄, mating was carried out at BIOGEMAR S.A. using artificial insemination method, crossing each selected male with one or two selected females, during six consecutive days. This involved 120 contributing females and 88 males, to produce 138 full-sib and 50 half-sib families. Fertilized females were individually placed in different spawning tanks. At the nauplii V stage, 15,000 individuals per family were transferred to the postlarvae facilities, where all families obtained per day of crossing were mixed in six different tanks (one per mating day), until postlarvae stage-12 (PL-12). Hence, age and larvae tank effect are the same and only one of them was considered in the model (larvae tank). Then shrimp from the six tanks were mixed and a representation of the entire progeny was kept in the genetic selection nucleus at BIOGEMAR S.A. for potential use as elite breeders and another batch was sent to the industrial estuaries in PRODUMAR S.A. (Durán, Ecuador). Environmental conditions differed greatly at the genetic nucleus (BIOGEMAR S.A.) and at industrial ponds (PRODUMAR S.A.), in salinity ≥ 35 and 2–10 psu, and density ~ 300 and ~ 30 animals/m³, respectively. In both environments, temperature was maintained at average of 25.59 ± 0.04 °C, and pH 8 ± 0.02 . A random sample of 388 adults (G₄), from the same industrial estuary of PRODUMAR S.A. were harvested at commercial size (119–124 days of culture) to carry out the present study. All shrimp were harvested on the same day; they differ in age according to the day of spawning. After harvesting, they were physically tagged with numerical eye rings, a pleopod was taken for DNA extraction and the shrimp were sent to the ULPGC laboratories for analysis (Martínez Soler et al., 2024).

2.2 Analyzed traits

Weight in fresh was recorded at the time of harvest in Ecuador. Total length (TL) in frozen was measured at ULPGC facilities, using a vernier calliper (0.0 cm precision), according to previous standardized methodology (Shin et al., 2023; Martínez Soler et al., 2024). Then, exoskeletons were carefully removed from each shrimp, washed with ultra-pure water to remove the rest of the body flesh, and sent to the Servicio de Toxicología Clínica y Analítica of the ULPGC (SERTOXX; <https://www.ulpgc.es/servicios-cientificos/sertox>).

Five macrominerals (Ca, Na, P, Mg, and K) and four microminerals (Cu, Fe, Mn, and Zn) were encompassed in this study. Elemental standards were obtained as certified reference material from CPA Chem (Stara Zagora, Bulgaria), either as multi-elemental or individual acid solutions. Before the measurement of the mineralogical profile of each exoskeleton, samples were individually homogenized with the Precellys

Evolution homogenizer (Bertin Technologies, Fontaine, France) to obtain 300 mg of final weight for digestion. Each sample was fully digested by adding 3 mL of 65% nitric acid distilled in a sub-boiling system with a PTFE-TFM purification unit (SubCLEAN, Milestone SRL, Sorisole, Italy), and 0.5 mL of 30% ultrapure hydrochloric acid using a High-Performance Microwave Digestion System (ETHOS UP, Milestone SRL, Sorisole, Italy), with controlled temperature, power, pressure, and time conditions.

After digestion, exoskeletal mineral concentrations were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) with the Agilent 7900 (Agilent Technologies, Tokyo, Japan), equipped with standard nickel cones, a MicroMist glass concentric nebulizer, and an Ultra High Matrix Introduction (UHMI) system. Quantification was carried out using MassHunter v.4.2 ICP-MS Data Analysis software by Agilent Technologies. The internal standard (ISTD) solution was introduced in each sequence to evaluate the reproducibility of counts per second of the ions (CPS), associated relative standard deviation (RSD) values, and the recovery rates of the four isotopes (⁴⁵Sc, ⁷²Ge, ¹⁰³Rh, and ¹⁹³Ir). Before sample determination, the entire procedure underwent in-house validation using two certified reference materials (CRM): fish muscle (ERM-BB422, Joint Research Centre-Institute for Reference Materials and Measurements, Belgium) and non-defatted lobster hepatopancreas (LUTS-1, National Research Council, Canada). In addition, recovery studies using 4% (v/v) nitric acid solutions fortified with the analytes at three concentration levels were conducted. Thus, the recovery percentages were appropriate and ranged from 83 to 119% for the studied minerals. Two calibration curves (macroelemental and microelemental) were specifically designed for optimal quantification and suitable regression coefficients ($r > 0.995$) were obtained. RSDs were generally below 5%, however, they occasionally increased to 8% for Cu and Zn, which were discarded as up to 12 individual measurements per sample were considered. Digested reagent blanks, CRM, and fortified matrices were included in all analyses for quality control (results were accepted when the concentration of the element was within 20% of the deviation of the theoretical value). Three aliquots were analyzed per individual. For each aliquot, the instrument performed four measurements, and the mean value was calculated. The final reported value corresponds to the median of the three aliquots, which was used to minimize the influence of out-of-range values.

2.3 DNA extraction and family assignment

DNA was extracted from the shrimp pleopods at the laboratories of the Servicio de Acuicultura y Biotecnología de Alta Especialización (SABE, ULPGC; <https://www.ulpgc.es/servicios-cientificos/sabe>), using the DNA Blood Kit (QIAGEN[™]) and BioSprint 96[®] equipment according to the protocol established by the manufacturer. The NanoDrop-800[™] v.3.7 spectrophotometer (Thermo Fisher Scientific) was used for quantification before normalization to 30 ng/μl by the TECAN robot (Tecan Schweiz AG, Switzerland) and Freedom Evoware[®] Standard v2.5 software.

Normalized DNA samples were sent to the Center for Aquaculture Technologies (California, USA) to perform genotyping and parentage assignment. Parentage assignment was carried out using 166 SNPs of the AQUARRAY LD™ (Ion Torrent platform, Thermo Fisher Scientific™) by marker exclusion (99% match SNPs) and likelihood methods (minimum 35; maximum 104). Marker exclusion and likelihood methods were implemented using R software.

2.4 Data analysis

Descriptive data analysis for mineral concentrations was carried out using SPSS (v25) software to calculate the mean \pm standard error (SE), standard deviation (SD), minimum, maximum, and coefficient of variation (CV) (Table 1).

To estimate heritabilities and genetic correlations between traits, the following bi-trait model expressed in matrix annotation was implemented:

$$y = Xb + Za + e$$

where, y is a vector of all studied traits for growth and mineral concentrations of the exoskeleton (TL, Weight, Ca, P, Mg, K, Na, Mn, Fe, Zn, Cu) as continuous traits (studied in pairs); b is a vector of fixed effects (sex, spawning tank, and larvae tank); a is a vector of random additive genetic effects of the individual shrimp distributed following a normal distribution $\sim N(0, A\sigma_a^2)$, where A is a relationship matrix calculated through pedigree information; e is a vector of random residual effects, and following a normal distribution $\sim N(0, I\sigma_e^2)$, where I is an identity matrix. X and Z are incidence matrices with the corresponding effects in vectors b and a , respectively. All studied traits were analyzed pairwise. Estimates were obtained by Bayesian estimation method through the marginal posterior means. GIBBS2F90 software from BLUPF90 (Miszta et al., 2022) was used implementing a Gibbs sampling chain of 100,000 iterations, with a burn-in period of 20,000 and a thinning interval of 80 cycles. The convergence of Gibbs sampling chains was checked with Geweke's diagnostics using R software. Three fixed factors were included in the model: sex, larvae tank, and spawning tank, that are the ones that most affect shrimp growth. The sex effect was excluded from the mineral content analyses, as no significant differences were detected between males and females.

3 Results

3.1 Pedigree assignment

The total percentage of shrimp subjected to analysis in the present study was successfully assigned, resulting in a total of 79 full-sib and 18 half-sib families. The minimum number of offspring per family was 1 and the maximum 15. The average was 5 organisms per family. Only 57% of all families obtained during effective crosses for G_4 were represented in the present study.

3.2 Phenotyping

Mean \pm SE for weight and TL were 18.64 ± 0.14 g and 13.28 ± 0.04 cm, respectively. In the studied population, weight ranged from 10.00 g to 26.00 g, and TL ranged from 10.70 cm to 15.40 cm. The SD and CV was 0.71 and 5.36% for TL, and 2.77 and 14.88% for weight, respectively. For exoskeleton mineral content, results are shown in Table 1, as mean \pm SE, SD, minimum, maximum, and CV. The main elements present in the exoskeleton were Ca and P, with mean concentrations of 51.64 ± 0.80 g/kg and 9.22 ± 0.13 g/kg, respectively. Cu and Fe exhibited the highest CV, whereas Ca, P, and Mg showed the lowest.

3.3 Heritabilities and genetic correlations

According to Geweke's diagnostics, convergence of the Gibbs sampling chain was attained for all variables studied. Heritability estimates and genetic correlations for growth traits and exoskeleton mineral composition in *P. vannamei* are presented in Table 2. TL showed high heritability (0.62), whereas weight exhibited moderate heritability (0.41), with a very high positive genetic correlation between the two traits (0.98). Most of the macro- and microminerals analyzed presented moderate heritabilities, ranging from 0.20 to 0.42 (Ca, P, Na, K, Mg, and Cu), with Cu showing the highest value (0.42). In contrast, Fe, Mn, and Zn showed low heritabilities, ranging from 0.07 to 0.18, with Fe being the lowest (0.07). Genetic correlations between exoskeleton mineral content and growth traits were generally positive and high for Ca and Mg (0.64–0.75), positive but low for Na (0.24 with TL and 0.11 with weight), negative and moderate-to-high for Fe, Cu, and Zn (–0.34 to –0.56), and not significantly different from zero for K and Mn

TABLE 1 Descriptive statistics (mean \pm standard error, standard deviation, minimum, maximum, and coefficient of variation) for the exoskeletal mineral composition of *Penaeus vannamei* at harvest size ($n=388$) and produced under industrial conditions in Ecuador (PMG-BIOGEMAR®).

Statistical	Ca (g/kg)	P (g/kg)	Mg (g/kg)	K (g/kg)	Na (g/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)
Mean \pm se	51.64 ± 0.80	9.22 ± 0.13	1.89 ± 0.03	1.17 ± 0.02	0.88 ± 0.01	3.84 ± 0.07	17.70 ± 1.21	8.77 ± 0.17	6.91 ± 0.19
SD	15.58	2.43	0.55	0.39	0.27	1.33	23.47	3.20	3.62
Min	7.47	2.20	0.45	0.25	0.25	0.61	1.44	1.96	1.60
Max	95.65	15.74	3.75	2.65	1.94	8.27	190.14	26.73	27.24
CV	30.18	26.36	29.07	33.33	31.00	34.61	132.59	36.52	52.45

SE, Standard error; SD, Standard deviation; Min, Minimum; Max, Maximum; CV, Coefficient of variation; Ca, Calcium; P, Phosphorous; Mg, Magnesium; K, Potassium; Na, Sodium; Mn, Manganese; Fe, Iron; Zn, Zinc; Cu, Copper.

TABLE 2 Heritabilities (on the diagonal) and genetic correlations (above de diagonal) (95% highest posterior density intervals) for growth traits and exoskeletal mineral content of *Penaeus vannamei* at harvest size from the PMG-BIOGEMAR[®] breeding program attained by Bayesian approach using Gibbs sampling.

Trait	TL	Weight	Ca	P	Na	K	Mg	Fe	Cu	Mn	Zn
TL	0.62 (0.358, 0.967)	0.98 (0.930, 1.000)	0.69 (0.067, 0.999)	- 0.01 (-0.990, 0.910)	0.24 (-0.613, 0.999)	- 0.01 (-0.990, 0.910)	0.75 (0.182, 0.999)	- 0.35 (-0.999, 0.962)	- 0.37 (-0.991, 0.240)	0.08 (-0.975, 0.999)	- 0.53 (-0.999, 0.186)
Weight		0.41 (0.256, 0.865)	0.64 (-0.120, 0.999)	0.34 (-0.595, 0.995)	0.11 (-0.765, 0.925)	- 0.15 (-0.978, 0.666)	0.71 (0.098, 0.999)	- 0.34 (-0.995, 0.905)	- 0.56 (-0.985, 0.070)	0.05 (-0.868, 0.999)	- 0.49 (-0.990, 0.323)
Ca			0.26 (0.014, 0.567)	0.72 (0.117, 0.998)	0.87 (0.521, 0.999)	0.72 (-0.025, 0.999)	0.97 (0.875, 1.000)	0.18 (-0.960, 0.998)	0.52 (-0.388, 0.999)	0.85 (0.355, 1.000)	0.31 (-0.837, 0.997)
P				0.20 (0.034, 0.45)	0.81 (0.306, 0.999)	0.78 (0.216, 0.999)	0.68 (0.036, 0.995)	- 0.03 (-0.998, 0.971)	0.53 (-0.344, 0.999)	0.67 (-0.101, 0.999)	0.75 (0.136, 0.999)
Na					0.34 (0.179, 0.749)	0.97 (0.886, 0.999)	0.96 (0.8384, 1.000)	0.21 (-0.894, 0.998)	0.85 (0.449, 0.999)	0.79 (0.210, 0.999)	0.67 (-0.444, 0.999)
K						0.28 (0.076, 0.520)	0.86 (0.417, 1.000)	0.18 (-0.926, 0.999)	0.91 (0.670, 1.000)	0.70 (-0.172, 0.999)	0.75 (0.144, 0.999)
Mg							0.28 (0.095, 0.686)	- 0.06 (-0.991, 0.959)	0.69 (-0.030, 0.999)	0.73 (-0.000, 0.999)	0.39 (-0.529, 0.998)
Fe								0.07 (0.01, 0.210)	0.10 (-0.982, 0.992)	0.55 (-0.571, 0.999)	0.27 (-0.943, 0.999)
Cu									0.42 (0.174, 0.8107)	0.59 (-0.390, 0.999)	0.71 (0.060, 0.999)
Mn										0.10 (0.0142, 0.287)	0.62 (-0.202, 0.999)
Zn											0.18 (0.049, 0.517)

TL, Total length; Ca, Calcium; P, Phosphorous; Mg, Magnesium; K, Potassium; Na, Sodium; Mn, Manganese; Fe, Iron; Zn, Zinc; Cu, Copper. The values for heritabilities and genetic correlations are shown in bold.

(-0.15 to 0.08). For P, the genetic correlation was lower for TL than for weight (-0.01 and 0.34, respectively). Most genetic correlations among macro- and microminerals were positive and high, except for Fe, which showed relatively low values (-0.06 to 0.21). Particularly strong positive correlations were observed between Ca and P (0.72), Na and K (0.97), K and Mg (0.86), Na and Mg (0.96), Mg and Ca (0.97), K and Cu (0.91), and Zn and Cu (0.71) (Table 2).

4 Discussion

4.1 Phenotyping

The mineral composition of the exoskeleton of *P. vannamei* presented by other authors showed higher concentrations of all elements analyzed than those observed in our population at similar sizes (Cheng et al., 2006; Huang et al., 2017; Jannathulla et al., 2020).

The observed differences may be attributed to the analytical methodology employed, as these authors opted for optical emission spectroscopy (ICP-OES) instead of mass spectrometry (ICP-MS) with different equipment. According to Agilent's protocols, ICP-MS is the best option when element concentrations in tissues or products are below 10 ppb. Phan-Thien et al. (2012) demonstrated that ICP-MS afforded more accurate and precise quantification of mineral concentrations in peanuts than ICP-OES, with a 10% variation in these concentrations. Therefore, the ICP-MS technique offers the greatest versatility and sensitivity for a wide range of elements.

Another explanation for differences with previous studies could be the environmental conditions prevailing in the country of production, including factors such as diet or ocean chemical profile. Jannathulla et al. (2020) obtained their animals from a local Indian farm, and Huang et al. (2017) from a commercial hatchery in China. The order from highest to lowest exoskeletal concentration of the corresponding minerals was similar to that shown by Huang et al. (2017), namely Ca, P, K, Mg, Na, Fe, Cu, Zn,

and Mn. The exception was Mg, which was higher than Na, and Fe, which was higher than Zn in the animals in this study. To the best of our knowledge, no studies on the exoskeletal mineral composition of *P. vannamei* from Ecuadorian population culture under industrial conditions have been published to date, therefore, the data cannot be compared.

Moulting can significantly affect the mineral concentrations of exoskeletons (Truong et al., 2022) with different ratios of each mineral at different times in the process. It is important to note that each industrial estuary in the PRODUMAR company in Ecuador has a production area of 5,000 hectares, which makes it challenging to synchronize the moulting time for all animals. However, there is general synchronization in the estuaries regarding this phenomenon in shrimp and it is standard practice to harvest during the inter-moult period when the mineral concentration remains more stable (Truong et al., 2022). Unfortunately, it was not possible to ascertain the precise day of moulting for each animal in the present study. Therefore, in future work, it would be beneficial to be able to measure the exact time of moulting, perhaps using transcriptomic methods (Gao et al., 2017), for controlling and measuring this source of variation.

4.2 Heritabilities for growth and exoskeletal mineral traits in shrimp

The results of the present study, obtained using Bayesian statistical method indicate a slightly higher heritability for weight and length than that reported for the same population by Martínez Soler et al. (2024), using the Restricted Maximum Likelihood (REML) statistic method. As highlighted by Waldmann and Ericsson (2006), it is important to be aware that estimates obtained through Bayesian framework may be subject to overestimation depending on the level of heritability. The estimates produced by the two methodologies tend to be more similar when the heritability is higher, and the number of individuals is greater. The medium-high heritability obtained for growth-related traits in this population provides further evidence to support the hypothesis that the genetic contribution to increase these traits in *P. vannamei* is significant (Tan et al., 2017), making them the most interesting traits for producers, in addition to survival and disease resistance (Zhang et al., 2017). The medium heritability attained for Ca, P, Na, K, Mg, and Cu content, along with the high genetic correlations between them, indicate that the concentration of these elements in the exoskeleton of *P. vannamei* is subject to genetic control. These results have the potential to enhance the commercial value of shrimp, as farmers could modulate mineral content in this tissue, given that the exoskeleton plays an important role in the survival, growth and productive performance of the species (Tong et al., 2024). Furthermore, the exoskeleton is considered a valuable and reusable product in the context of the circular economy (Barikani et al., 2014; Moreno-Sader et al., 2021). Genetic variation in mineral concentrations in other species of plants or terrestrial animals has been demonstrated (Phan-Thien et al., 2012; Singh et al., 2024). Despite the crucial role of minerals in aquatic animals, particularly in the aquaculture industry, this area has yet to be explored in

genetic terms. The reasons may be the high cost and lengthy process involved in the analysis besides animal loss due to invasive methods. Thodesen et al. (2001) examined differences between parental half-sib families in apparent absorption coefficients of elements in Atlantic salmon, identifying a significant impact of the sire component on K, Ca, Mg, Zn, and Fe. In the present study, the highest additive genetic variances were obtained for Cu, Na, K, Mg, Ca, and P. The highest heritability of Cu indicates a significant influence on its content in the exoskeleton. The important role of Cu has been reported in the hardening and repair of crustacean exoskeletons (Terwilliger, 1999; Rao and Anjaneyulu, 2008a), innate immunity (Li et al., 2018; Shi et al., 2021) and moulting process (Rao and Anjaneyulu, 2008a, b). This mineral is imported and exported from the hepatopancreas to the haemolymph during the moulting process, specifically from the post-moult to pre-moult stage (Arumugam, 1989; Rao et al., 2008b). It is understood that this fact is linked to the synthesis of hemocyanin, a Cu transporter and structural component of the new exoskeleton, which occurs in the hepatopancreas (Gellissen et al., 1991; Spindler et al., 1992; Rao et al., 2008b). During the moulting process, hemocyanin in *P. vannamei* is transported from the hepatopancreas to the hypodermis by the haemolymph. The addition of Cu to the diet of *P. vannamei* has a significant impact on its concentration in the hepatopancreas (Shi et al., 2021), from where it is transferred to the other tissues, including the exoskeleton, through the haemolymph in the form of hemocyanin (a Cu-binding protein) (Rao et al., 2008b). This indicates that Cu in the exoskeleton is less dependent on environmental factors such as diet and more dependent on genetic aspects of the species.

The present study reveals that the exoskeletal Ca content is also influenced by a genetic component. This element can be absorbed by shrimp from three sources: the external environment (gills), diet, or recycled from the old exoskeleton. Then, it is stored in the gut or hepatopancreas (epithelial cells) and transferred to the new exoskeleton through the haemolymph. The Ca concentration in the exoskeleton varies according to the moulting cycle, rather than when shrimp are cultured in different salinities (Li and Cheng, 2012). This evidence supports our findings and demonstrates the intrinsic ability of shrimp to regulate the Ca concentration in their cuticle.

The structural integrity of the exoskeleton is of significant concern to shrimp farmers, particularly regarding reducing breakage or softening, which can influence buyers' decisions in competitive markets. This is especially critical when the primary export markets require the shrimp to be sold whole. Exoskeletal firmness is influenced by multiple factors, with mineral composition being the most significant. Ca and P are the predominant minerals in the exoskeleton of *P. vannamei* (Table 1), playing a crucial role in its formation and hardening (Jannathulla et al., 2020). Selecting *P. vannamei* breeders for their exoskeletal Ca content will enable the production of higher-quality shrimp with a more robust exoskeleton.

Due to the high cost of analyzing mineral content, and because this was the first time that its genetic basis was explored, a total of 388 shrimp from 79 full-sib and 18 half-sib families, and an average of five descendants per family was used to balance cost against the

accuracy of the estimates. To consolidate the present results, it could be interesting to increase the number of families and offspring in future studies.

4.3 Genetic correlations between exoskeletal minerals and growth traits in shrimp

The medium heritabilities obtained for some minerals indicate that they can be included in breeding programs for the species to improve the quality of cultured shrimp. However, it may be challenging to consider this as a selection criteria in the industrial sector as it is not a commercial trait of interest that directly affects the price of the final product. The mineral content of the exoskeleton affects the hardness and firmness of the product (Jannathulla et al., 2020), which is of significant concern to shrimp farmers, particularly regarding breakage or softening. This is a consequence of the mineral content, not a direct trait, like color or morphology. Furthermore, the determination processes are neither straightforward nor cost-effective. There is an opportunity to leverage genetic correlations with growth and morphological traits, which are easier and faster to measure, by indirect selection. This approach was previously proposed by Martínez Soler et al. (2024) for meat quality traits in the same population.

Exoskeletal Ca and Mg content present the highest positive genetic correlation with growth traits, in line with the observations made in Atlantic salmon (Thodesen et al., 2001). It is worth noting that exoskeletons in the present study had a higher concentration of Mg than Na, which differs from the results presented by Jannathulla et al. (2020) and Huang et al. (2017). The high and positive genetic correlation between Ca and Mg and growth traits means that genetic selection for growth in our Ecuadorian population has the potential to increase the content of these minerals in the exoskeleton, enhancing the quality of the shrimp in terms of external hardness, appearance, and firmness in a cost-effective way. Furthermore, these medium and positive genetic correlations could ensure that the quality of the exoskeleton is not compromised by accelerated growth when shrimp are genetically selected.

Several authors have reported that low concentrations of Mg (approximately <30 mg/L) in low salinity waters lead to significantly reduce levels of Mg in the shrimp carapace, haemolymph and whole body, which in turn is associated with decreased growth and survival (Roy and Davis, 2010; Galkanda-Arachchige et al., 2020), thereby a common practice among commercial producers to enhance species performance is adding Mg and K in specific ratio to the medium (Davis et al., 2005; Roy et al., 2009; Saoud et al., 2003; Galkanda-Arachchige et al., 2020). However, in large-scale pond systems, such as those used in Ecuador, this practice represents a significant additional production cost. In this context, the high genetic correlations with growth traits—particularly TL—together with the high heritability estimated for TL and the moderate heritability obtained for Mg content, indicate that indirect selection to increase Mg content in the shrimp exoskeleton through selection on TL represents a viable and complementary alternative for producers, by enhancing the shrimp's capacity to incorporate Mg and meet its physiological requirements.

The genetic correlations between Cu, Zn, and Fe and growth traits were negative, which could be advantageous for the species. An excess of Cu and Zn can have a negative impact on *P. vannamei* (Liang et al., 2022; Qian et al., 2020). An excess of Fe in the animal can lead to lipolysis, impaired intestinal immune function, and microbiota disruption (Jiao et al., 2022). Moreover, replication of white spot syndrome virus (WSSV) in *P. vannamei* requires Fe, as this element acts as a cofactor for the viral ribonucleotide reductase (Lin et al., 2002). In this context, our results showed that the negative genetic correlations between the contents of these three minerals and growth traits indicate that strong selection pressure on harvest weight and length is associated with lower levels of Cu, Zn, and Fe, which may be beneficial for the species.

Special attention should be paid to the negative genetic correlations with Cu since is the core of hemocyanin and an essential mineral in crustaceans compared with other animal groups (Truong et al., 2022). It is crucial to ascertain whether the genetic component also influences hepatopancreas content and how genetic correlations behave with growth traits in this organ. Mn does not exhibit significant heritability and is not genetically related to growth traits in the species in line with that observed for Na, K, and P.

4.4 Genetic correlations between exoskeletal minerals

The results of the present study demonstrated a general tendency of high and positive genetic correlations among the exoskeletal minerals studied. This suggests that selection for one of them increases the concentration of the others in this tissue, except in the case of Fe. This was also observed in the milk of dairy cattle (Singh et al., 2024). In nutritional terms, in *P. vannamei* species, an increase in Ca demanded an increase in P, otherwise an excess of Ca has negative effects on growth and tissue mineralization (Cheng et al., 2006). Moreover, Huang et al. (2017) demonstrated that the exoskeletal Ca content is reduced when diets contain low levels of P, suggesting that the reduced tissue calcification in shrimp is the result of a lack of P. These previous observations reinforced our results, as a very high and positive genetic correlation was obtained between both elements. The genetic correlation between Cu and Zn was also high, which may be related to the activity of Cu/Zn superoxide dismutase (SOD) (Shi et al., 2021). In this study, under low salinity and density, Na, Mg and K exoskeleton content reported a high and positive genetic correlation, and medium heritability, showing the important role is playing of genetic regulation. It is in concordance with the relatively stable concentration of Na and K elements in the exoskeleton of animals cultured under different salinity concentrations (Jannathulla et al., 2020), which can be related with the Na-K-ATPase enzyme activity, of which Mg is an essential cofactor (Huang et al., 2017). This could be indicative of a scarce genotype by environment interaction, which could be investigated in future studies in Ecuador, where shrimp are cultivated across a broad range of salinities (low, medium and high).

The results are highly relevant for improving shrimp quality, as they reveal moderate to high heritability for several of the analyzed

mineral traits. These findings suggest a promising genetic basis for the enhancement of mineral composition through selective breeding. In future studies, the implementation of genomic selection could improve the accuracy of estimated breeding values by capturing within-family variation. Genomic selection is particularly interesting for traits that require invasive sampling, such as mineral composition, as it reduces the number of individuals needed for phenotyping, thereby minimizing costs without compromising selection efficiency (Blay et al., 2021).

5 Conclusion

This study demonstrates, for the first time in *P. vannamei*, that mineral content in the exoskeleton exhibits additive genetic variation, ranging from low (Fe and Mn) to moderate (Ca, P, Na, K, Mg, Cu, and Zn). Genetic correlations among mineral contents were generally strong and positive, except for Fe. Growth-related traits showed positive genetic correlations with Ca, Na, and Mg, whereas negative genetic correlations were observed with Fe, Cu, and Zn.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving animals in accordance with the local legislation and institutional requirements because crustacean are excluded. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MM: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft. HSS: Methodology, Supervision, Writing – review & editing, Project administration. ÁL-F: Writing – review & editing, Data curation, Software. MJZ: Writing – review & editing, Supervision. RG: Supervision, Writing – review & editing, Methodology. LP: Writing – review & editing, Methodology, Project administration. OP: Writing – review & editing, Data curation, Formal analysis. MZP: Writing – review & editing, Data curation, Formal analysis. NR: Writing – review & editing, Data curation, Formal analysis. ÁR: Writing – review & editing, Data curation, Formal analysis. JF: Supervision, Writing – review & editing. RT: Writing – review & editing, Conceptualization, Resources. JAL: Writing – review & editing, Funding acquisition. ER: Writing – review

& editing, Project administration, Resources. JML: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

Authors LP Mesa was employed by company Biotechnology and Marine Genetic S.A. BIOGEMAR. Authors RT, JAL, and ER Abad was employed by company PRODUMAR S.A.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The authors JF and RT declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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