A comprehensive evaluation of the nutritional value of semiterrestrial isopods, *Ligia exotica*: a potential new aquatic feed?

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DOI:
10.21203/rs.3.rs-20446/v1

SUBJECT AREAS
Animal Science

KEYWORDS

Ligia exotica, nutritional analysis, distribution, aquaculture

Abstract

Background

The semiterrestrial isopod, *Ligia exotica* represents one of the oldest documented species introductions of marine organisms and is known as an intermediate form between marine and strictly terrestrial isopods. They are considered to make a significant contribution to nutrient cycling and ecosystem services in the near shore environment and in helping to maintain the biodiversity of the coastal zone. The special biological role and its environmental plasticity contribute to its potential value for academic research. In order to explore the practical value for food & feed of *Ligia*, this study focused on growth rate under laboratory rearing conditions and detailed analysis of the overall nutrient content of the species in comparison to two other aquatic food media (krill and fish meal).

Results

Evaluation of the growth rate of juveniles demonstrated a weight gain rate of 13,026.76% to70 days after hatching (DAH) and a total specific growth rate of 6.97%, which suggests it is a relatively fast-growing species of the *Ligiidae* family. Compared with Antarctic krill meal and commercial white fishmeal, *Ligia* meal is closer to krill in amino acid content and contained 43.45% (wet weight) crude protein. Its essential amino acids content was the lowest (35.21% in total amino acids) but the proportion of flavor amino acids was 49.37%, higher than that of krill meal (38.98%) and fish meal (39.80%). In particular, the content of taurine (9.45 g/100 g) was much higher than that of the other two meals. Amino acid score (AAS) and Chemical score (CS) show that the most restricted amino acids of isopod meal are methionine and cysteine, which are less than half of those of krill meal and fish meal. The extremely unbalanced amino acid composition may affect the absorption and utilization by consumers. In terms of fatty acids, isopod meal contains 8.99% (wet weight) crude fat and more saturated fatty acids (33.66%). The total polyunsaturated fatty acids (PUFA) are only 23.61%, lower than 40.50% of krill meal and 47.27% of fish meal. The Σ n-3 PUFA content is even lower with only 12.12%. A total of 12 vitamins were examined. The VK_{1}, VE, VB_2, VB_3, VB_5 content of isopod meal were significantly higher than those of krill meal and fish meal. Similarly, most of the 11 mineral elements are the highest in isopod meal including the ubiquitous elements of calcium and

potassium, and the trace elements ferrum, chromium, and selenium.

Conclusions

Ligia offers potential as an alternative natural food source especially in aquaculture given the growth rate under culture and the overall nutrient content (as demonstrated by the preliminary trials on cuttlefish) but *Ligia* collected in most of the field would be deemed unfit for human consumption because of the relatively low nutritional value and heavy metal content exceeding the provided standard. At the same time, *Ligia* isopods offer some potential to become a crustacean model animal for commercial aquaculture crustaceans for research on physiological and other aspects because of their position in ecological food chain and unique semi-terrestrial characteristics that allow them to be cultured out of seawater. Further study is warranted to elucidate its biological characteristics.

Introduction

Ligia is a genus of isopods (Isopoda; Crustacea), which is commonly known as rock lice or sea slaters based on its appearance. Most *Ligia* species live on tidal zone cliffs and rocky beaches, as well as dams, ports and docks and tolerate a wide range of temperatures and salinity. They are distributed across almost the entire coastline of China and have naturally high biomass. Coastal *Ligia* exhibits a mixture of terrestrial and marine characteristics, drying out easily, needing moist air and proximity to water. Although they have gills and can exchange gas under water, they only do so when escaping terrestrial predators or being dislodged by wave action. They do not move swiftly in the water and are open to marine predation. They are well adapted to rocky surfaces but avoid exposed sand, which opens them to terrestrial predation and desiccation[1]. The fertilized eggs of *L. exotica* develop into juveniles in the brood pouch (oöstegites) of females until they can live independently. It takes about 5wk from egg deposition to release[2]. Further details of their biology, including types, habitats, reproduction, food, growth, physiology *et al* can be found in an academic website A Snail's Odyssey[3], and Taiti *et al* (2003)[4], Renate Eberl (2012)[5].

Ligia isopods are omnivorous detrivores and feed by chewing on organic debris on the shore. *Ligia* isopods themselves are often used as bait by fishermen and they are the primary prey for mangrove crabs, fish, birds, and lizards[6] and even small mammals[7]. *Ligia* are considered to play an active role in nutrient recycling and energy flow in the near shore environment and in supporting the biodiversity of the coastal zone[8].

Our preliminarily study has confirmed that *Ligia exotica* can provide a high-quality natural diet for cultured cuttlefish *Sepia pharaonis*[9]. *Ligia* isopods are also utilized in traditional Chinese medicine for the treatment of muscle injury, swelling and pain, or to overcome malnutrition in children[10]. Extracts from *Ligia exotica* were proven to have obvious proliferation inhibitory effects on a range of biochemical and cellular functions such as cervical cancer cells HeLa, stomach cancer cell SGC-7901 and NCI-60 human tumor cell in vitro and have an inhibitory effect on mouse sarcoma S180-induced transplantable cancers by intraperitoneal injection in tumors over 7d[11]. A novel nucleoside, elucidated as 3’-*O*- (α-D-glucosyl) inosine, had been isolated from *Ligia exotica* but no bioactivity identified[12].

As far as we are aware, there is no published report on the nutritional analysis of *Ligia* isopods although there is evidence of potential nutritional as well as medicinal value. To explore this potential value further we established a 70-day culture experiment on juveniles of *Ligia* to evaluate their growth performance and compared the nutritional content of *Ligia exotica* meal with two other regular used marine feed sources, white fish meal and Antarctic krill (*Euphausia superba*) meal. The objectives included whether *Ligia* can be artificially cultured on a large scale and to document their growth rate and how well the nutritional value compared with the reference feed sources. It is hoped that the approach adopted here can provide information on this potential as a feed source within animal culture as well as contributing to increasing our understanding of basic crustacean physiology.

Results

Growth performance of juvenile Ligia exotica

(n = 3 groups, each contain 10 individuals from the same female. ±Bar means standard error)

As shown in Fig. 1, the average body weight of the new-hatched juveniles of *Ligia exotica* is 0.24 ± 0.005 mg. The increase of body weight (BW) in the early stages is not significant until 15 days after hatching (DAH), after that BW increases from 0.85 ± 0.02 mg to 6.37 ± 0.04 mg at 45 DAH. From then on, the weight gain of individuals begins to accelerate significantly, reaching 12.69 ± 0.01 mg at 55 DAH and 16.37 ± 0.41 mg at 60 DAH. The exponential growth equation of juveniles *Ligia* is $y = 0.3485e^{0.3119x}$, and the correlation index R^2 is 0.93, which demonstrate a good positive correlation of body weight gain over time. The final BW value of *Ligia* at 70 DAH is 31.06 ± 1.06 mg. Weight gain rate of juvenile *Ligia* is (final BW- initial BW) / initial BW × 100% is 13026.76%. Specific growth rate per day is (ln final BW - ln initial BW) / DAH × 100% is 6.97%.

General nutritional components of Ligia

Table 1. General nutritional components of isopod meal, antarctic krill meal and fishmeal (%, wet weight ± SD)

	Crude protein (%)	Crude fat (%)	Crude ash (%)	Moisture (%)	Cholesterol (mg/100 g)
isopod meal	43.45^a ± 0.68	8.99 ^{a}±0.15	27.14 ^{c}±0.67	10.89 ± 0.07	146.67 ^{a}±4.16
krill meal	61.5^b ± 0.84	13.97 ^{c}±0.34	15.12 ^{a}±0.14	9.66 ± 0.10	370.33^c ± 1.53
fishmeal	60.17^b ± 1.69	11.12 ^{b}±0.33	18.95 ^{b}±0.99	10.24 ± 0.04	215^b ± 7.93
Numerical values marked with the same letter a, b or c are not statistically significantly different.					

As shown in Table 1, the crude protein of isopod meal is less than both the krill meal and fishmeal ($F = 225.18$, $df = 2$). The crude fat ($F = 224.02$, $df = 2$) and cholesterol content ($F = 1430.29$, $df = 2$) of the isopod meal is lower whereas, the crude ash content ($F = 237.64$, $df = 2$) of isopod meal is higher than krill and fishmeal.

Amino Acids Composition

Table 2 shows the composition and content of 18 amino acids and taurine in isopod meal, antarctic krill meal and fish meal.

Table.2 Amino acids composition of isopod meal, antarctic krill meal and fish meal (%, dry weight)

Amino acids(g/100g)	isopod meal	krill meal	fish meal
Aspartate (Asp) [f]	4.16 ± 0.03^{a}	4.97 ± 0.04^{b}	6.68 ± 0.02^{c}
Threonine (Thr)[e]	1.86 ± 0.02^{a}	1.92 ± 0.03^{b}	2.98 ± 0.03^{c}
Serine (Ser)	1.38 ± 0.05^{a}	2.02 ± 0.02^{b}	3.18 ± 0.02^{c}
Glutamate (Glu) [f]	4.66 ± 0.02^{a}	7.06 ± 0.02^{b}	9.62 ± 0.00^{c}
Glycine (Gly)[f]	6.96 ± 0.02^{c}	2.04 ± 0.02^{a}	4.43 ± 0.03^{b}
Alanine (Ala)[f]	2.11 ± 0.03^{a}	2.56 ± 0.05^{b}	4.35 ± 0.03^{c}
Valine (Val) [e]	3.90 ± 0.04^{c}	2.38 ± 0.03^{a}	3.44 ± 0.02^{b}
Methionine (Met) [e]	0.54 ± 0.01^{a}	1.46 ± 0.02^{b}	1.72 ± 0.04^{c}
Isoleucine (Ile) [e]	1.19 ± 0.02^{a}	2.41 ± 0.02^{b}	2.91 ± 0.04^{c}
Leucine (Leu)[e]	4.26 ± 0.02^{b}	3.90 ± 0.01^{a}	5.50 ± 0.03^{c}
Tyrosine (Tyr) [e]	1.76 ± 0.01^{b}	1.68 ± 0.00^{a}	2.40 ± 0.01^{c}
Phenylalanine (Phe) [e]	1.84 ± 0.02^{a}	2.39 ± 0.04^{b}	2.84 ± 0.01^{c}
Lysine (Lys) [e]	3.36 ± 0.02^{a}	3.72 ± 0.01^{b}	5.39 ± 0.04^{c}
Histidine (His)	0.99 ± 0.04^{a}	2.01 ± 0.01^{c}	1.62 ± 0.02^{b}
Arginine (Arg)	4.24 ± 0.00^{b}	2.94 ± 0.02^{a}	4.50 ± 0.03^{c}
Proline (Pro)	2.37 ± 0.03^{b}	1.68 ± 0.04^{a}	3.15 ± 0.00^{c}
Cysteine (Cys) [e]	0.09 ± 0.03^{a}	1.34 ± 0.02^{b}	1.37 ± 0.02^{b}
Tryptophan (Trp) [e]	0.61 ± 0.01^{a}	0.88 ± 0.04^{b}	0.62 ± 0.01^{a}
taurine[f]	9.45 ± 0.08^{c}	2.93 ± 0.04^{b}	2.04 ± 0.05^{a}
ΣAA	55.61 ± 0.77^{b}	50.11 ± 0.37^{a}	68.55 ± 0.30^{c}
ΣEAA	19.57 ± 0.58^{a}	20.73 ± 0.68^{b}	27.74 ± 0.42^{c}
ΣNEAA	36.73 ± 0.57^{b}	29.41 ± 0.18^{a}	40.63 ± 1.07^{c}
ΣFAA	27.45 ± 0.47^{b}	19.54 ± 0.9^{a}	27.28 ± 0.11^{b}
ΣEAA / ΣAA(%)	35.21 ± 0.46^{a}	41.38 ± 1.01^{b}	40.47 ± 0.50^{b}
ΣEAA / ΣNEAA(%)	53.29 ± 1.12^{a}	70.52 ± 2.07^{b}	68.34 ± 0.81^{b}
ΣFAA / ΣAA(%)	49.37 ± 0.48^{a}	38.98 ± 1.76^{b}	39.80 ± 0.18^{b}
Note: Values are means of triple determination ± standard error			
ΣAA is total amino acids,ΣEAA is total essential amino acids，Σ NEAA is total nonessential amino acids，ΣFAA is total flavor amino acids.			

Amino acids marked [e] means essential amino acids, while [f] means flavor amino acid.

Numerical values marked with the same letter [a, b] or [c] are not statistically significantly different.

The total amino acid content (ΣAA) of isopod meal is higher than that of krill meal but significantly lower than the total amino acids of fish meal ($F = 989.81$, $df = 2$). The contents of the nine essential amino acids (ΣEAA) differ with the lowest values in *Ligia* ($F = 181.36$, $df = 2$). While the ratio of essential amino acids to the total amount of amino acids (ΣEAA / ΣAA) of isopod meal is the lowest in those three substrates ($F = 17.92$, $df = 2$). Surprisingly however, the content of taurine, a beneficial non-protein amino acid, is much greater in *Ligia* than that of krill meal and fish meal ($F = 784.36$, $df = 2$). In terms of the content of five flavored amino acids, the amino acid content of isopod meal is higher than that of krill powder ($F = 91.28$, $df = 2$), and its proportion to the total amino acid (ΣFAA / ΣAA) is also higher than both of krill meal and fish meal ($F = 51.10$, $df = 2$).

Nutritional Evaluation Of Amino Acids

The amino acid score (AAS), chemical score (CS, the limiting amino acid index) and essential amino acid index (EAAI) were calculated by converting the data in Table 2 into milligrams of amino acid per gram of nitrogen (× 62.5). The results were compared with the amino acid scoring standard pattern suggested by FAO/WHO and the amino acid pattern of whole egg protein as described later.

Table 3

Comparative analysis of Amino Acid Score (AAS), Chemical Score (CS) and Essential Amino Acid Index (EAAI) of antarctic krill meal and isopod meal

Amino acids		FAO evaluation standard(mg/gN)	Egg protein standard(mg/gN)	Score of isopod meal	Score of krill meal	Score of fish meal
AAS	Ile	250		0.30 ± 0.01 [a]	0.60 ± 0.01 [b]	0.73 ± 0.03 [c]
	Leu	440		0.61 ± 0.11 [b]	0.55 ± 0.06 [a]	0.78 ± 0.05 [c]
	Lys	340		0.62 ± 0.04 [a]	0.68 ± 0.02 [b]	0.99 ± 0.07 [c]
	Thr	250		0.47 ± 0.05 [a]	0.48 ± 0.05 [b]	0.75 ± 0.08 [c]
	Val	310		0.79 ± 0.06 [c]	0.48 ± 0.06 [a]	0.69 ± 0.04 [b]
	Trp	60		0.64 ± 0.1 [a]	0.92 ± 0.04 [b]	0.65 ± 0.1 [a]
	Met + Cys	220		0.18 ± 0.01 [a]	0.80 ± 0.01 [b]	0.88 ± 0.02 [c]
	Phe + Tyr	380		0.59 ± 0.05 [a]	0.67 ± 0.01 [b]	0.86 ± 0.00 [c]
CS	Ile		331	0.22 ± 0.03 [a]	0.46 ± 0.03 [b]	0.55 ± 0.07 [c]
	Leu		534	0.50 ± 0.02 [b]	0.46 ± 0.01 [a]	0.64 ± 0.03 [c]
	Lys		441	0.48 ± 0.02 [a]	0.53 ± 0.01 [b]	0.76 ± 0.05 [c]
	Thr		292	0.40 ± 0.04 [a]	0.41 ± 0.04 [b]	0.64 ± 0.06 [c]
	Val		410	0.59 ± 0.05 [c]	0.36 ± 0.04 [a]	0.53 ± 0.03 [b]
	Trp		106	0.36 ± 0.06 [a]	0.52 ± 0.02 [b]	0.37 ± 0.05 [a]
	Met + Cys		386	0.10 ± 0.06 [a]	0.45 ± 0.06 [b]	0.50 ± 0.01 [c]
	Phe + Tyr		565	0.40 ± 0.03 [a]	0.45 ± 0.08 [b]	0.58 ± 0.02 [c]
EAAI				34.13 ± 0.48[a]	45.16 ± 0.64 [b]	55.93 ± 0.57 [c]
Numerical values marked with the letter a, b or c are statistically significantly different.						

Amino Acid Score (AAS) and Chemical Score (CS) reflect the relationship of protein composition and utilization ratio from different perspectives. As can be seen from Table 3, the superscript letters indicate clearly that in most of the case, the lowest scores of amino acids are in isopod category, while Ile and Met + Cys may be the most distinct that less than half of those of krill meal and fishmeal ($F = 3024.7$, $F = 2392.19$ respectively for AAS, and $F = 2935.5$, $F = 2392.19$ for CS. All $df = 2$). Methionine and cystine are therefore the main limiting amino acids of isopod as suggested by their content. EAAI index reflects how close the essential amino acid content of material is to the standard protein (egg protein). Comparing the values of EAAI of isopod meal, krill meal and fishmeal, indicates that the protein quality of the isopod is worse than that of krill meal and fishmeal ($F = 1294.4$, $df = 2$).

Most of the highest AAS and CS scores of fishmeal demonstrate that fishmeal is rich in essential amino acids and it is well-balanced in composition, therefore easy to be digested and absorbed by human.

Nutritional Composition Of Fatty Acid

Table 4

Fatty acids content of isopod meal, Antarctic krill meal and fishmeal

Fatty acids	isopod meal	krill meal	fishmeal
C14:0 / C16:0	1.95 ± 0.04 [a]/ 27.33 ± 0.01[a]	8.90 ± 0.02 [c] / 22.89 ± 0.01[b]	5.90 ± 0.04 [b] / 17.32 ± 0.02[c]
C17:0 / C18:0	None/ 4.39 ± 0.02 [c]	0.26 ± 0.03 / 1.20 ± 0.02 [a]	0.58 ± 0.02/ 2.78 ± 0.01 [b]
Σ SFA	33.66 ± 2.01[b]	33.13 ± 1.21 [b]	26.88 ± 1.27[a]
C16:1 / C17:1	11.22 ± 0.03 [c] / None	6.93 ± 0.02 [a] / None	8.31 ± 0.03 [b] / 0.32 ± 0.02
C18:1 n9 C / C20:1 n9	7.48 ± 0.01 [a] / None	18.0 ± 0.02 [c] / 1.64 ± 0.02 [b]	15.29 ± 0.01 [b] / 0.85 ± 0.03 [a]
C22:1 n9 / C24:1 n9	None / 0.57 ± 0.02	None / None	1.19 ± 0.02 / 0.81 ± 0.05
Σ MUFA	19.28 ± 1.22[a]	26.57 ± 0.46 [b]	26.61 ± 0.75 [b]
C18:2 n6 c / C18:3 n6	10.11 ± 0.04 [c] / 0.33 ± 0.01 [c]	3.10 ± 0.05 [b]/ 0.40 ± 0.01[b]	1.79 ± 0.02 [a] / 0.04 ± 0.00 [a]
C18:3 n3	3.41 ± 0.02 [c]	0.94 ± 0.02[b]	1.05 ± 0.01 [a]
C20:2 n6/ C20:3 n6	0.5 ± 0.02 [a]/ 0.29 ± 0.02[a]	4.21 ± 0.04 [c] / 0.32 ± 0.03[a]	2.06 ± 0.03 [b] / 12.94 ± 0.04[b]
C20:3 n3 / C20:4 n6	0.31 ± 0.02 / None	None / 0.51 ± 0.06	0.31 ± 0.01 / 0.70 ± 0.02
EPA C20:5 n3	6.54 ± 0.01[a]	18.30 ± 0.02 [c]	14.70 ± 0.02[b]
DPA C22:5 n3	0.62 ± 0.02 [b]	0.44 ± 0.03 [a]	1.13 ± 0.02[c]
DHA C22:6 n3	1.27 ± 0.03[a]	12.30 ± 0.02[b]	12.95 ± 0.06[c]
EPA + DPA + DHA	8.40 ± 0.27[a]	31.01 ± 1.56[c]	28.70 ± 1.09[b]
Σ n-3 PUFA	12.12 ± 0.3[a]	31.96 ± 1.57[b]	30.06 ± 1.09[b]
Σ n-6 PUFA	11.50 ± 0.70[b]	8.55 ± 0.19[a]	17.18 ± 1.60[c]
Σ PUFA	23.61 ± 0.99[a]	40.50 ± 1.76[b]	47.27 ± 2.69[c]
Σ n-3 PUFA /Σ n-6 PUFA	1.06 ± 0.04[a]	3.74 ± 0.1[b]	1.76 ± 0.1[c]
Note: Values are means of triple determination ± standard error			

Numerical values marked with the same letter [a, b] or [c] are not statistically significantly different.

SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids;

ND: Not Detected

Table 4 shows the fatty acid composition of isopod meal, krill meal and fish meal. There are 12 fatty acids including 3 saturated fatty acids (SFA), 3 monounsaturated fatty acids (MUFA) and 6 polyunsaturated fatty acids (PUFA) in isopod meal. 14 fatty acids were detected in krill meal, including 4 SFA,4 MUFA,6 PUFA, while all 4 SFA,6 MUFA and 7 PUFA were found in fish meal. The content of saturated fatty acid (SFA) in isopod meal is like that of krill but higher than that in fish meal ($F = 18.00$, $df = 2$), which may show that its fatty acid characteristics are closer to those of terrestrial animals. The content of monounsaturated fatty acids (SFA, F = 71.11, df = 2), and EPA and DHA are the lowest in the isopod (F = 367.63 and F = 311.70 respectively, while df = 2). Although the content of n-6 PUFA is slightly higher in the isopod than that of krill meal (F = 55.69, df = 2), the total content of PUFA is far lower than either krill meal or fishmeal (F = 117.81, df = 2).

Comparison Of Vitamin Composition

Table 5
Vitamin composition of isopod meal, Antarctic krill meal and fishmeal

Vitamin (mg/100 g)	Isopod meal	Krill meal	Fish meal
VA (retinol)	□0.05	□0.05	0.19 ± 0.05
VD_3 (cholecalciferol,μg/100 g)	□2	□2	□2
VK_1 (phylloquinone,μg/100 g)	64.0 ± 3	□1	□1
VE (tocopherol)	9.32 ± 0.14^{c}	2.53 ± 0.03^{b}	0.82 ± 0.01^{a}
VB_1 (thiamine)	ND	0.04 ± 0.00	ND
VB_2 (riboflavin)	1.68 ± 0.00^{b}	0.12 ± 0.00^{a}	0.12 ± 0.00^{a}
VB_3 (niacin)	2.83 ± 0.28^{b}	1.41 ± 0.41^{a}	ND
VB_5 (pantothenic acid)	2.43 ± 0.52	ND	ND
VB_6 (pyridoxine, mg/kg)	ND	ND	ND
VB_{12} (cobalamin, mg/kg)	ND	0.849 ± 0.18	ND
Folic acid (mg/kg)	ND	ND	ND
VC (ascorbic acid)	□1	□1	□1
Note: ND, Not Detected.			

Values are means of triple determination ± standard error.

Values with the same letter $^{a, b}$ or ^{c} indicates that the differences are not statistically significant between mean values at the $p < 0.05$ level.

As shown in Table 5 the vitamin composition of isopod meal is relatively comprehensive. Among the four fat-soluble vitamins, the content of VA is lower in the isopod than in fish meal, while the contents of VK1 and VE ($F = 1.81 \times 10^5$, df = 2) are much higher than those in krill and fish meal. In addition, the contents of water-soluble vitamin VB_2 ($F = 1.30 \times 10^5$, df = 2),VB_3 (t = 19.13, df = 2)and VB_5 are the highest in isopod meal among the three materials.

Comparison Of Mineral Composition

Table 6

Minerals composition of isopod meal, Antarctic krill meal and fishmeal

Minerals(mg/kg)	Isopod meal	Krill meal	Fish meal
calcium	90283 ± 618.41 [c]	21536.04 ± 1484.63[b]	18575.06 ± 1058.80 [a]
potassium	5403.4 ± 59.88 [c]	2378.68.15 ± 136.90 [a]	3352.11 ± 118.47 [b]
sodium	8117.13 ± 126.96 [b]	10592.23 ± 615.4[c]	4033.63 ± 418.27 [a]
magnesium	4862.67 ± 40.55 [c]	4517.53 ± 204.63 [b]	1256.98 ± 102.84 [a]
copper	31.00 ± 2 [b]	70.76 ± 7.62 [c]	4.74 ± 7.62 [a]
ferrum	882.67 ± 7.37 [c]	84.27 ± 10.08 [b]	22.10 ± 1.13 [a]
zinc	62.95 ± 2.76 [b]	52.04 ± 2.85 [a]	74.40 ± 4.22 [c]
chromium	2 ± 0.14	None	None
selenium	2.69 ± 0.12 [c]	1.54 ± 0.13 [b]	0.4 ± 0.00 [a]
manganese	60.67 ± 2.52 [a]	2.6 ± 0.2 [c]	25 ± 2 [b]
total phosphorus (%)	0.43 ± 0.02 [c]	1.42 ± 0.04 [a]	1.15 ± 0.08 [b]

Note: Values are means of triple determination ± standard error.

Table 7

Nutritional items and their determination methods

Nutrient components	Code and name of Chinese national determination standard		Brief description of methods and equipment
Moisture	GB 5009.3–2016	Determination of moisture in feedstuffs	direct drying method
Crude protein	GB 5009.5–2016	Determination of crude protein in feeds	Kjeldahl method
Crude ash	GB 5009.4–2016	Animal feeding stuff-Determination of Crude ash	550℃in muffle furnace
Crude fat	GB 5009.6–2016	Determination of Crude fat in feeds	Soxhlet extraction
Taurine	GB 5009.169.2016	Determination of taurine in food	neighbor grows responds with o-Phthalaldehyde
Amino acids	GB 5009.124–2016	Determination of amino acids in foods	amino acid analyzer
Cystine	GB/T 18246 − 2000	Determination of amino acids in feeds	oxidation hydrolysis
Tryptophan	GB/T 18246 − 2000	Determination of amino acids in feeds	Alkali hydrolysis, RP-HPLC
Vitamin A & Vitamin E & Vitamin D_3	GB 5009.82–2016	Determination of vitamin A, D, E in food	RP-HPLC
Vitamin B_1	GB 5009.84–2016	Determination of vitamin B_1 in food	HPLC
Vitamin B_2	GB 5009.85–2016	Determination of vitamin B_1 in food	HPLC
Vitamin B_3	GB 5009.89–2016	Determination of niacin in food	HPLC
Vitamin B_5	GB 5009.210–2016	Determination of pantothenic acid in food	HPLC
Vitamin B_6	GB/T 14702 − 2018	Determination of vitamin B_6 in premix	HPLC
Vitamin B_{12}	GB/T 17819 − 2017	Determination of vitamin B_{12} in additive premix	HPLC
Vitamin C	GB 5009.86–2016	Determination of ascorbic acid in food	HPLC
Vitamin K1	GB 5009.158–2016	Determination of vitamin K1 in food	HPLC plus fluorescence detection
Folic acid	GB/T 17813 − 2018	Determination of folic acid in premix	HPLC
Nicotinic acid	GB 5009.89–2016	Determination of niacin and nicotine in food	HPLC
Pantothenic acid	GB 5009.210–2016	Determination of pantothenic acid in food	HPLC
Potassium & Sodium	GB 5009.91–2017	Determination of potassium and sodium in	FAAS

		potassium and sodium in food	
Magnesium	GB 5009.241–2017	Determination of Magnesium and sodium in food	FAAS
Calcium	GB 5009.92–2016	Determination of calcium in food	FAAS
Copper	GB 5009.13–2017	Determination of copper in food	graphite furnace atomic absorption
Chromium	GB 5009.268–2016	Determination of multiple elements in Food	ICP-MS
Ferrum	GB 5009.90–2016	Determination of multiple elements in Food	Flame atomic absorption spectrometry (FAAS)
Zinc	GB 5009.14–2017	Determination of zinc in food	FAAS
Manganese	GB/T 13885 − 2017	Determination of the content of manganese in feed	Atomic absorption spectrometry
Total phosphorus	GB/T 6437 − 2018	Determination of phosphorus in feeds	spectrometry
Cholesterol	GB 5009.128–2016	Determination of cholesterol in food	HPLC

2.2 Evaluation of nutritional quality of amino acids

Based on the amino acid scoring standard model recommended by FAO & WHO39 and the amino acid model using egg protein as an ideal protein reference40, the Amino Acid Score (AAS), Chemical Score (CS) and Essential Amino Acid Index (EAAI) from eight essential amino acids for humans were calculated from the following formulae (Pellett & Yong, 1980). The higher the scores and indices that the substrates received, the more similarity they are with the ideal protein model, and the better the protein quality for human consumption.

AAS=

CS=

EAAI=

where *aa* is the amino acid content of the sample (%); $AA_{FAO\&WHO}$ is the content of the same amino acid recommended by FAO & WHO (%) (shown in Table 3); AA_{egg} is the content of the same amino acid in whole egg protein (%); n is the number of essential amino acids compared (n = 9). A, B, C, ⋯; I is the content of essential amino acid of sample protein (mg/g N), AE, BE, CE, ⋯; IE is the content of essential amino acid of whole egg protein (mg/g N).

3 Statistic Analysis

Following the Chinese national determination standard method to analyze the nutritional components of substrates, the analysis of each samples was repeated three times by the same tester to obtain data for statistical analysis. When conducting the fatty acid and vitamin content analysis, the concentration of some parameters that were too low to be detected (ND) and were considered zero with no statistical analysis undertaken. Before analyzing all the data statistically, their normality was tested by Shapiro-Wilk test method in SPSS 24.0 software. It indicated that all the original data follow the normal distribution.

Levene's test was adopted to deal with nutritional parameters with specific values for Analysis of Variance (ANOVA, two tailed) in three raw materials (isopod meal, antarctic krill meal and fishmeal). If the data followed the assumption of homogeneity of variances, Duncan's multiple range test (multiple F test) was used to identify the difference in means. Meanwhile Fisher's least significant difference (LSD) would be employed as references to confirm the statistical differences. If the data violated the assumption of homogeneity of variances, Welch's Anova was used and post-hoc methods of Dunnett's T3 test employed to identify the significance or otherwise of the differences. Statistical significance is $p < 0.05$. Mean ± standard deviation was used to describe the statistical data. By employing the analytic hierarchy process (AHP) technique, the following structural analysis model was established for evaluating the nutritional value of fish meal, isopod meal and krill powder: amino acids, fatty acids, vitamins and minerals (Fig. 6).

Numerical values marked with the same letter $^{a, b}$ or ^{c} are not statistically significant different at the $p < 0.05$ level.

The mineral composition of *Ligia exotica* is shown in Table 6. Ubiquitous mineral elements such as calcium (F = 3995.94, df = 2), potassium (F = 590.06, df = 2) and magnesium (F = 658.60, df = 2) are most abundant in the isopod. Trace mineral element ferrum (F = 13185.74, df = 2) is also the richest in the isopod, whilst copper content (F = 160.04, df = 2) in both isopod and krill meal is higher than that in fishmeal. Chromium (F = 33.59, df = 2) and selenium (F = 406.02, df = 2) are also the highest in the isopod.

Comprehensive comparison of amino acids, fatty acids, vitamins and minerals

Based on the group decision hierarchy process software YAAHP (Yet Another Analytic Hierarchy Process Software, V10.0) to summaries of expert judgment, the weighted index nutritional value was calculated, as shown in Fig. 2.

For human or animal consumption, the importance of amino acids, fatty acids, vitamins and minerals are different (blue numbers in the middle of Fig. 2 show the weight of the elements, the larger the number, the greater the importance of the index, and the following blue numbers in the bottom of Figure mean the same). On this basis, fishmeal is the most preferable substrate, and isopod meal is the worst, due largely to its imbalance in nutritional elements.

In order to better visualize the differences of nutrient composition of the three food materials, a radar chart (Fig. 3) was constructed including essential amino acids, flavor amino acids, essential amino acid index, Σ PUFA, vitamins (8 parameters) and minerals (9 parameters). The rank order of the three food materials for each of the main nutrient category is based on the top rank having the highest value in the number of parameters, followed by the second and third respectively, considering that numerous parameters are involved. For example, in the mineral category, isopod substrate has six the highest parameters, followed by krill with two and fishmeal one (Table 8), so they are ranked first, second and third respectively. To facilitate comparison with other factors in the radar chart, numbers "50", "40", "30" were assigned to the first, second and third ranked materials respectively, while factors of ΣFAA / ΣAA(%),ΣEAA / ΣAA(%), EAAI andΣPUFA were scored based on the actual values from Table 5 and Table 6.

Table 8

Table of relative scores

Value of *j & k**	Interpretation
1	*j* and *k* are equally important
3	*j* is slightly more important than *k*
5	*j* is more important than *k*
7	*j* is strongly more important than *k*
9	*j* is absolutely more important than *k*
2, 4, 6, 8	intermediate value in two adjacent judgments
* *j&k* Do not only mean the comparison between different nutritional indicators, but also the comparison between same indicators in different substrates.	
The judgment matrices of each were imported into the group decision system and tested for consistency by YAAHP(Yet Another AHP)V.10.0 software. Upon testing, all the matrices form from the scores of three experts met the consistency requirement (consistency ratio = 0.0981, 0.0000, 0.0398, respectively). The total sequencing weight value was obtained through arithmetical average, which calculated from the matrices given by experts.	

As can be seen from Fig. 3, there are clear differences between the assessed nutritional value of isopod, krill and fish meal. Isopod substrate scores better in minerals and vitamin content, and has a certain flavor stimulating effect (view from ΣFAA / ΣAA(%)). However, fatty acid content, especially ΣPUFA, is far lower than that of krill meal and fish meal. Fish meal scores best in EAAI andΣPUFA. Unsaturated fatty acids are known to have beneficial physiological functions such as improving blood microcirculation and increasing the activity of brain cells. The closer the protein composition is to the egg protein, the easier it absorbed and utilized by humans (view from EAAI). To sum up, the nutritional value of isopod is inferior to that of krill and fish meal.

Discussion

Ligia species are distributed all over the world and *Ligia exotica* is probably the most widely distributed among about 30 species of the genus. Based on the data presented in Hourado *et al*[13] , the sites sampled in this present study and after reviewing the literature on *Ligia exotica*, we have compiled a comprehensive list of locations where *Ligia* are known or generally available (Appendix 1) and constructed a global distribution map (Figure 4) generated by ArcGIS software.

Appendix 1. Information on the distribution of *Ligia exotica* worldwide

(mainly adapted from Hurtado *et al*[13])

Locality Names	ID	Sources	Lat	Long
Goodland, FL, USA	1	Hurtado *et al*. 2018	25°55'57''N	81°39'21''W
Sunshine Skyway Bridge North Rest	2	Hurtado *et al*. 2018	27°39'14''N	82°40'41''W

Bridge North Rest Area, St. Petersburg, FL, USA				
Cedar Key, FL, USA	3	Hurtado *et al*. 2018	29°8'8''N	83°2'11''W
Eastpoint, FL, USA	4	Hurtado *et al*. 2018	29°44'21''N	84°52'25''W
Pensacola, FL, USA	5	Hurtado *et al*. 2018	30°25'11''N	87°11'36''W
Biloxi Small Craft Harbor, Biloxi, MS, USA	6	Hurtado *et al*. 2018	30°23'31''N	88°53'8''W
Long Beach Harbor, Biloxi, MS, USA	7	Hurtado *et al*. 2018	30°20'41''N	89°8'42''W
Avery Island, LA, USA	8	Hurtado *et al*. 2018	29°54'57''N	91°54'14''W
Galveston, TX, USA	9	Hurtado *et al*. 2018	29°17'43''N	94°48'28''W
Palacios, TX, USA	10	Hurtado *et al*. 2018	28°44'18''N	96°24'6''W
Municipal Harbor, Port Aransas, TX, USA	11	Hurtado *et al*. 2018	27°50'24''N	97°3'50''W
South Padre Island, TX, USA	12	Hurtado *et al*. 2018	26°4'44''N	97°10'9''W
San Juan de Ulúa Fort, Veracruz, Mexico	13	Santamaria *et al*. 2013[14] and Hurtado *et al*. 2018	19°12'34''N	96°7'51''W
Jetty by Adolfo Ruiz Cortines statue, Veracruz, Mexico	14	Hurtado *et al*. 2018	19°11'40''N	96°7'24''W
Cumberland Island, GA, USA	15	Wetzer 2001[15]; Hurtado *et al*. 2018	30°51'N	81°27'W
Chaguaramas Bay, Trinidad, Trinidad and Tobago	16	Hurtado *et al*. 2018	10°40'57''N	61°37'21''W
Praia de Calhetas, Cabo de Santo Agostinho, Brazil	17	Hurtado *et al*. 2018	8°20'38''S	34°56'43''W
Praia do Paraíso, Pernambuco, Brazil	18	Hurtado *et al*. 2018	8°21'S	34°57'W
Rio de Janeiro, Brazil	19	Hurtado *et al*. 2018	23°2'50''S	43°31'10''W
Lagoa Azul, Ilha Grande, Costa Verde, Brazil	20	Hurtado *et al*. 2018	23°11'S	44°18'W
Hilo Harbor, Hawai'i, HI, USA	21	Hurtado *et al*. 2018	19°43'57''N	155°3'26''W
Pearl Harbor, O'ahu, HI, USA	22	Hurtado *et al*. 2018	21°21'50''N	157°57'37''W
Honolulu Harbor, O'ahu, HI, USA	23	Taiti *et al*. 2003[16]; Hurtado *et al*. 2018	21°18'9''N	157°51'53''W
Vilankulos, Mozambique	24	Hurtado *et al*. 2018	21°59'52''S	35°19'30''E
Beira, Mozambique	25	Hurtado *et al*. 2018	19°50'53''S	34°53'35''E
Durban Harbor, KwaZulu-Natal, South Africa	26	Hurtado *et al*. 2018	29°52'19''S	31°1'30''E
Blue Lagoon, Umgeni River Mouth, KwaZulu-Natal, South Africa	27	Hurtado *et al*. 2018	29°48'36''S	31°2'8''E
Niigata, Japan	28	Hurtado *et al*. 2018	37°54'58''N	139°2'11''E
Kanagawa, Japan	29	Hurtado *et al*. 2018	35°9'25''N	139°36'43''E
Fukuoka, Japan	30	Hurtado *et al*. 2018	33°35'N	130°24'E
Kitadaito son, Okinawa, Japan	31	Hurtado *et al*. 2018	25°56'45''N	131°17'56''E
Okinawa, Japan	32	Hurtado *et al*. 2018	26°28'46''N	127°55'40''E
Ulleungdo Island, South Korea	33	Hurtado *et al*. 2018	37°30'6''N	130°51'11''E
Boryeong, South Korea	34	Hurtado *et al*. 2018	38°4'53''N	127°38'16''E
Lutao, Taitung, Taiwan, China	35	Hurtado *et al*. 2018	22°45'6''N	121°9'42''E
Pingtung County, Taiwan, China	36	Hurtado *et al*. 2018	22°29'44''N	120°36'52''E
Rushan, Shandong, China	37	Yin *et al*. 2013[17]	36°50'59''N	121°36'50''E

China				
Weihai, Shandong, China	38	Yin *et al*. 2013	37°26'14''N	122°9'42''E
Qingdao-Zhanqiao, Shandong, China	39	Yin *et al*. 2013	36°3'41''N	120°19'10''E
Qingdao-Hongdao, Shandong, China	40	Yin *et al*. 2013	36°10'58''N	120°16'57''E
Qingdao, Shandong, China	41	Hurtado *et al*. 2018	36°3'58''N	120°22'10''E
Zhujiajian Island, Zhejiang, China	42	Hurtado *et al*. 2018	29°54''N	122°53'E
Lianyungang, Jiangsu, China	43	Yin *et al*. 2013	34°46'32''N	119°26'34''E
Nantong, Jiangsu, China	44	Yin *et al*. 2013	32°5'7''N	121°35'51''E
Zhujiazian, Zhoushan Islands, China	45	GenBank	29°52′12″N	122°23′55″E
Parangipetta, India	46	Hurtado *et al*. 2018	11°29'24''N	79°45'36''E
Punta Carretas, Montevideo, Uruguay	47	Hurtado *et al*. 2018	34°56'06'' S	56°09'40'' W
Orchid Island, Taiwan	48	Hurtado *et al*. 2018	22°04'51''N	121°30'44''E
Karachi, Pakistan *	49	Kazmi. 1993[18]	24°45'6''N	66°9'42''E
Jordan coastline, Gulf of Aqaba, Red Sea *	50	Ismail. 1990[19]	28°43'8''N	34°41'28''E
Bandra, India *	51	Joshi & Bal. 1959[20]	19°3'39''N	72°49'35''E
Mergui Archipelago, Myanmar *	52	Barnard. 1936[21]	11°21'5''N	98°0'48''E
Aldabra, Seychelles Archipelago *	53	Ferrara & Taiti, 1985[22]	4°40'11''S	55°28'18''E
Pacific seashores Kamogawa, Japan	54	Horiguchi *et al*. 2006	35°1′ N	140°1′ E
Pacific seashores Shimoda, Japan	55	Horiguchi *et al*. 2006	34°4′ N	138°6′ E
Adyar Beach, Madras, Tamil Nadu, India *	56	Ravindranath. 1974[23]	13°0'56''N	80°16'18''E
Patos Lagoon, Rio Grande do Sul, Brazil *	57	Lopes *et al*. 2006[24]; Hurtado *et al*. 2018	32°2'44''S	52°4'39''W
Sunday Island, Victoria, Australia *	58	Green. 1962[25]; Hurtado *et al*. 2018	38°43'22''S	146°37'36''E
Tiaoshun island, Zhanjiang, China	59	This study	21°16'59''N	110°24'8''E
Naozhou island, Zhanjiang, China	60	This study	20°54'28"N	110°33'38"E
Old Port, Marseille, France *	61	Roux P. 1828[26] and Hurtado *et al*. 2018	43°17'38.75''N	5°21'47.61''E
Manado Post, Manado, Indonesia	62	Undap *et al*. 2013[28]	1°28'22.84''N	124°49'50.23''E
Qinglan port, Hainan, China	63	This study	19°34'1.73''N	110°49'27.35''E
Note: * means Presumed longitude and latitude				

L. exotica represents one of the oldest documented introductions of marine organisms[13], discovered at the docks in Marseille, France, originally described by Roux[27]. This is the northernmost location, while the southernmost location is Sunday Island, Australia[26]. They are widely distributed in tropical and temperate regions, including the Seychelles archipelago and Hawaiian Islands suggesting significant colonization ability, but the species has not been found in the Antarctic or Arctic. The southern coast of the United States and the coast of East Asia are two major hot spots. East Asia is traditionally considered to be the origin of *L. exotica*. In China, *L. exotica* is mainly distributed on the rocky coast south of Tanggu district (36°N), Tianjin city[29] and in Taiwan. Areas in eastern China account for 70% of Chinese human population, whose activities not only bring a large amount of nutrients to the coastal waters by way of waste discharge and disposal, but also provide habitats to *L. exotica* in form of wharves and dams. *Ligia sp*. plays an important role as a scavenger/detritivore, feeding on a large range of organic matter plant debris and animal corpses brought by tides.

The only published study on *Ligia* growth we are aware of to date is that of Carefoot[30] on the field population and growth of *Ligia pallasii* Brandt. Further work examined the nutritional requirements of *Ligia pallasiii* using artificial feed and demonstrated that this species is able to grow from 56.5mg to 111.6mg over 40 weeks of culture[31]. The resulting specific growth rate (SGR) is only 0.24%. In contrast, for the Pacific white shrimp *Litopenaeus vannamei*, the SGR of genetically selected high growth lines could reach 29.25%[32]. In present study, juvenile *Ligia exotica* have an SGR of 6.97% after 70 days of culture.

The primary aim of this present study was to examine the nutritional value of *Ligia* as a potential new natural food source in aquaculture based on our previous study that confirmed that *L. exotica* provide a good diet for juvenile cuttlefish[9]. However, it is still unknown how long this feed could support the growth of juvenile cuttlefish if be used as a sole diet. In comparison to both krill and fish meal, the nutritional value of protein and amino acids of *Ligia* isopod is lower in almost all evaluation indexes, such as crude protein content, ΣEAA / ΣAA (%), ΣEAA / ΣNEAA (%), and EAAI. In particular the two amino acids with the lowest values for *L. exotica*, methionine and cystine, are present at less than half of that of krill meal and fishmeal. The imbalance of these amino acids may affect the digestion and absorption of predators from isopod food. However, isopods have a relative high value of ΣFAA / ΣAA (%) and the contents of taurine are 4 to 5 times those of fish meal and krill. As a sulfonic acid, taurine is a found in high concentrations in animal tissues and has been attributed a wide diversity of roles in some mammals, e.g., as an essential dietary requirement for cats, and a critical supplement for marine fish feed[33].

Ligia contains more saturated fatty acid (SFA) than fishmeal and krill, but carries few polyunsaturated fatty acid (PUFA), which is important to humans and other animals. This also reduces its potential nutritional value. Interestingly, however, the isopod has superior vitamin content as concentrations of VK_1, VE, VB_2, VB_3 and VB_5 are all far higher than in krill and fishmeal. It should be noted that the vitamin content in substrate is highly variable, influenced by several factors, such as origin and composition of the animal, meal processing method, and product freshness[34]. Under the processing methods of this study, the three substrates went through a process of heating and drying at high temperature, so for unstable vitamins such as VC, VB_1 and folic acid problems with detection may have occurred. In addition, part of fat-soluble vitamins in fish meal were lost during oil extraction. The mineral composition analyses show that calcium accounts for a very large proportion of body content in the isopod.

Isopods have a high tolerance to heavy metals contaminants in intertidal environment and their body concentration of metal elements are highly affected by the environment. In areas with severe anthropogenic contamination, heavy metal elements are transmitted through food chains and can accumulate in isopods[35]. For example, high concentrations of copper in *Ligia* from the Santa Rosalía area are consistent with mining activities at this location. Industrial and municipal sewage discharges appear to influence the high concentrations of zinc (326 µg/g) and lead (144 µg/g) in *Ligia* observed at Guaymas[7]. The area we sampled Jinsha Bay, Zhanjiang City is a hot spot for human activity and waste discharge, therefore it is likely that *Ligia* would accumulate any heavy metal pollution. The body tissues of Ligia are also very high in iron content. According to Chinese national food safety standards (GB 2762-2017) and the standard for the use of food fortifiers (GB14880-2012), the mineral content of *L. exotica* is outside of the standards as food for human consumption. Since the intertidal zones are exposed to pollution from both marine and terrestrial sources, isopods could potentially be used as biomonitors of pollution in these habitats in a similar way to terrestrial isopods in soil ecotoxicology[36].

In China, crustacean farming for food (represented by species such as Pacific white shrimp and American crayfish) is a significant industry. The annual output of white shrimp is more than 1.5 million tons, and its gross value is more than 8.7 billion dollars. *Litopenaeus vannamei* also has a mineralised cuticle that sheds regularly to allow for growth. *Ligia extocia*, given its abundance and large geographic range therefore has the potential to become a model animal for crustacean studies related to aquaculture, to better understand some of the physiological properties of crustacea such as the shrimp that are economically important. For example, the calcium translocations and transepithelial movement during the moulting cycle of *L.vannamei*, and dietary calcium requirement in low salinity environments[35]. This undoubtedly has great theoretical and applied value.

In conclusion, *Ligia extocia* has potential to serve as an alternative natural food source in aquaculture or animal farming given the growth rate under culture, acclimatization ability and the fact that it can be cultivated either in or out of water. It is especially suitable for cuttlefish which prefer live crustacean as diet. However, the unbalanced amino acid composition and lower content of PUFA may limit its practical value. *Ligia* collected in the field are deemed unfit for human consumption because of the heavy metal content exceeding the provided standard. Considering its unique semi-terrestrial ability and its role in the material cycle of the coastal zone, further study is warranted to elucidate its biological characteristics.

Materials And Methods

1 Growth rate determination of juveniles *Ligia exotica*

Ligia exotica[38] (Fig.5) were collected at the embankment of Tiaoshun Island in Zhanjiang City, Guangdong Province, China (N21 °28, E110 °39'), and were cultured in a 40cm ×20cm ×30cm aquarium, with oyster shells stacked on the right side and a plastic baffle with small holes through which seawater can pass was fixed to the left 10cm of the aquarium. Sea water reached half the height of the oyster stack and a filter pump was installed. Daily feeding of *Ligia exotica* was with tilapia fish pellet feed placed on dry oyster shells.

1.1 Isolated culture of gravid females

When gravid females were observed, especially where fertilized eggs in the marsupium were found to change color from orange to black, they were immediately isolated into a plastic box with a layer of cotton covered with a layer of gauze on the bottom. A piece of paper was placed on the gauze and thoroughly wetted with clean seawater. Tilapia pellet feed was spread on the paper as a food source. The cotton, gauze and seawater were changed every two days.

1.2 Culture of juveniles

When developed to a certain extent, the 50-60 juveniles would crawl out from the brooding female. The time of birth was recorded, and the mothers removed from the plastic box to avoid cannibalism. Juveniles were divided into groups of 10 and cultured in a constant temperature incubator at 28 ℃. The culture conditions are described above.

1.3 Sampling

Each group of juveniles were sampled every 5 days and the culture experiment lasted for a total of 70 days, providing 15 temporal samples. After being frozen at -20 ℃, they were placed at room temperature for 20 minutes to volatilize the water on the body surface and were weighed with a high-precision electronic balance. The replicate groups of juveniles were weighed on the same days after hatching from different mothers.

2 Analysis of nutritional components of *Ligia exotica* and comparative substrates

2.1 Analysis method

The frozen *Ligia exotica* contained individuals caught in the field, were subsequently dried at 75℃ for one day in an oven, ground into powder and stored at -20 ℃ until analysis.

Two readily available aquatic food substrates were used for comparative purposes. Antarctic krill (*Euphausia superba*) meal was purchased from China National Fisheries Corporation. It was rapidly cooked at 80~95℃ for 20~25 min, dehydrated and dried on board when caught at sea and transported to the laboratory to be stored at -20 ℃.

Fishmeal was white fish meal (degreased) imported from Russia, which was mainly composed of the pacific cod *Gadus macrocephalus*. When delivered to the laboratory, the samples were divided into several bags, stored at -20 ℃ and sampled at random during the experiment.

A range of nutrient components were analyzed from *Ligia* and the comparative substrates as described in Table 7.

2.2 Evaluation of nutritional quality of amino acids

Based on the amino acid scoring standard model recommended by FAO & WHO[39] and the amino acid model using egg protein as an ideal protein reference[40], the Amino Acid Score (AAS), Chemical Score (CS) and Essential Amino Acid Index (EAAI) from eight essential amino acids for humans were calculated from the following formulae (Pellett & Yong, 1980). The higher the scores and indices that the substrates received, the more similarity they are with the ideal protein model, and the better the protein quality for human consumption. (see Equations in the Supplementary Files)

where *aa* is the amino acid content of the sample (□); $AA_{\mathrm{FAO\&WHO}}$ is the content of the same amino acid recommended by FAO & WHO (□) (shown in table 3); AA_{egg} is the content of the same amino acid in whole egg protein (□); n is the number of essential amino acids compared (n=9). A, B, C, ⋯; I is the content of essential amino acid of sample protein (mg/g N), AE, BE, CE, ⋯; IE is the content of essential amino acid of whole egg protein (mg/g N).

3 Statistic Analysis

Following the Chinese national determination standard method to analyze the nutritional components of substrates, the analysis of each samples was repeated three times by the same tester to obtain data for statistical analysis. When conducting the fatty acid and vitamin content analysis, the concentration of some parameters that were too low to be detected (ND) and were considered zero with no statistical analysis undertaken. Before analyzing all the data statistically, their normality was tested by Shapiro-Wilk test method in SPSS 24.0 software. It indicated that all the original data follow the normal distribution.

Levene’s test was adopted to deal with nutritional parameters with specific values for Analysis of Variance (ANOVA, two tailed) in three raw materials (isopod meal, antarctic krill meal and fishmeal). If the data followed the assumption of homogeneity of variances, Duncan’s multiple range test (multiple F test) was used to identify the difference in means. Meanwhile Fisher’s least significant difference (LSD) would be employed as references to confirm the statistical differences. If the data violated the assumption of homogeneity of variances, Welch's Anova was used and post-hoc methods of Dunnett's T3 test employed to identify the significance or otherwise of the differences. Statistical significance is $p < 0.05$. Mean ± standard deviation was used to describe the statistical data.

By employing the analytic hierarchy process (AHP) technique, the following structural analysis model was established for evaluating the nutritional value of fish meal, isopod meal and krill powder: amino acids, fatty acids, vitamins and minerals (Figure 6).

Nutritional value was assessed based on the considered views of 3 nutritional experts in a small advisory committee, including expertise in human nutrition education (Lingnan Normal University. China), in swine nutrition (Jiangsu AnYou Biotechnology Group Co., Ltd. China), and one researcher with expertise in aquaculture nutrition (Ocean University of China, China). According to the scoring criteria in Table 8, each of them rated the nutritional components of the substrates and assessed the accuracy of the nutritional value judged by the four nutritional indicators.

The judgment matrices of each were imported into the group decision system and tested for consistency by YAAHP（Yet Another AHP）V.10.0 software. Upon testing, all the matrices form from the scores of three experts met the consistency requirement (consistency ratio=0.0981, 0.0000, 0.0398, respectively). The total sequencing weight value was obtained through arithmetical average, which calculated from the matrices given by experts.

List Of Abbreviations

Amino Acids include

Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Thr: Threonine; Val: Valine; Trp: Tryptophan; Met: Methionine; Cys: Cysteine; Phe: Phenylalanine; Tyr: Tyrosine

AAS: Amino acid score

BW: Body weight

CS: Chemical score

DAH: Days after hatching

EAAI: Essential amino acid index

FAAC: Flame atomic absorption spectrometry

GB: Chinese national determination standard

HPLC: High performance liquid chromatography

ICP-MS: Inductively coupled plasma- mass spectrometry

MUFA: Monounsaturated fatty acids

ND: Not Detected

PUFA: Polyunsaturated fatty acids

RP-HPLC: Reversed-phase chromatography

SFA: Saturated fatty acids

SGR: Specific growth rate

ΣAA: Total amino acids

ΣEAA: Total essential amino acids

ΣNEAA: Total nonessential amino acids

Declarations

Ethics approval and consent to participate

The study received approval from the institutional review board of Lingnan Normal University.

Consent for publication

I give my permission for the following manuscript to appear in the print, online, and licensed versions of Journal of Animal Science and Biotechnology and for the journal to grant permission to third parties to reproduce this manuscript.

Availability of data and materials

The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing financial interests.

Funding

1. The natural science research fund of Lingnan Normal University (1170918267).
2. The science and technology research project of Zhanjiang, China (2018B01103 and

2018B01051 respectively).

3. The public service platform of biomedical resources research and development of south China sea (2017C8B2).

Authors' contributions

Lele Xu, Liyun Wang designed the research study, analyzed the experimental parameters. Xiang Jiang and Yulin Sun collect the experimental material in the field. Yongqin Li wrote the paper and Haiyong Zhao polish the article in English. Haifeng Mi provide the essential laboratory and equipment to carry out the analysis. Yao Liu gave a substantial contribute to the data statistics, and Daohai Chen supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Acknowledgements

The authors would like to thank Miss Huiqi Zhou, Yirong Huang for the isopod nutritional analyses, Miss Yingzi Lei for the juveniles culture of *ligia exotica*, while Mr. Jianqiang Liu for the parent isopod collection in the field. The authors acknowledge all projects of different level to fund this research and thank to the experts who participated in the nutritional rating questionnaire. Finally, we sincerely thank Professor Paul Giller MRIA from University College Cork (UCC) for comments and advise on earlier versions of the manuscript.

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Figures

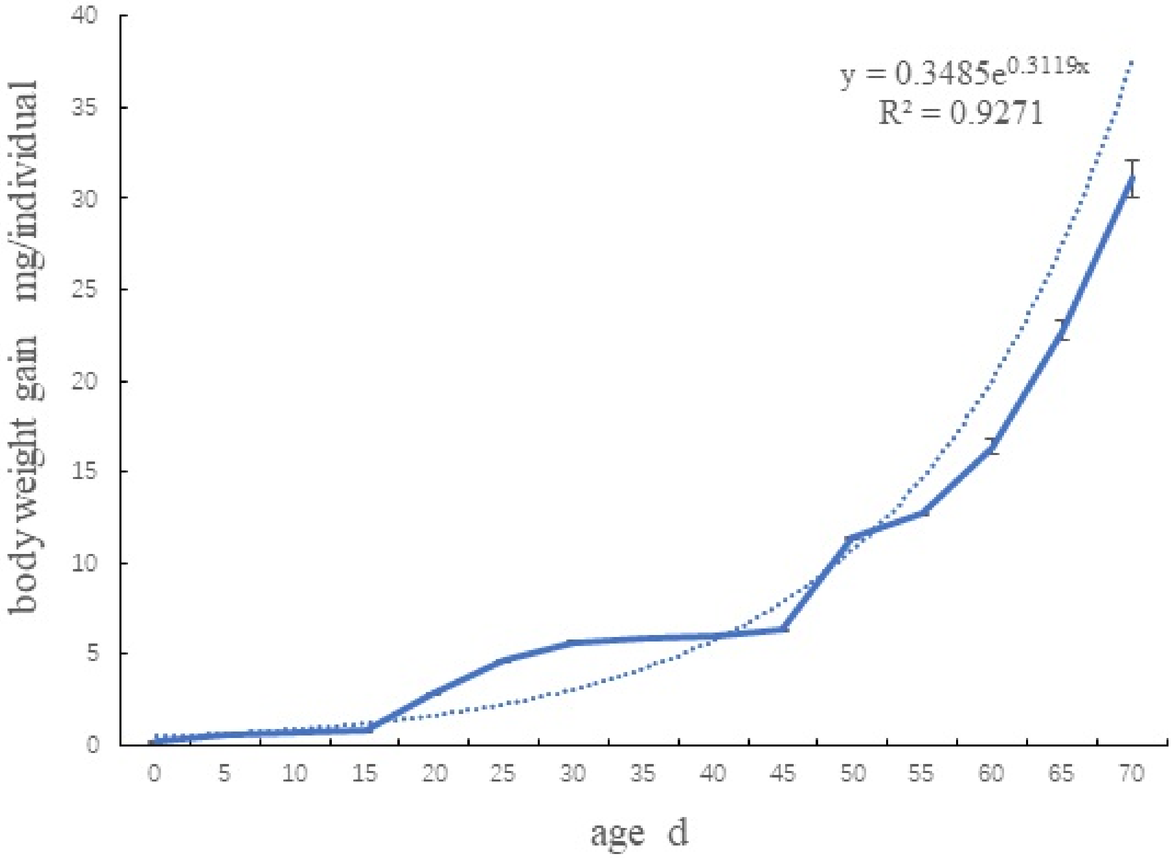
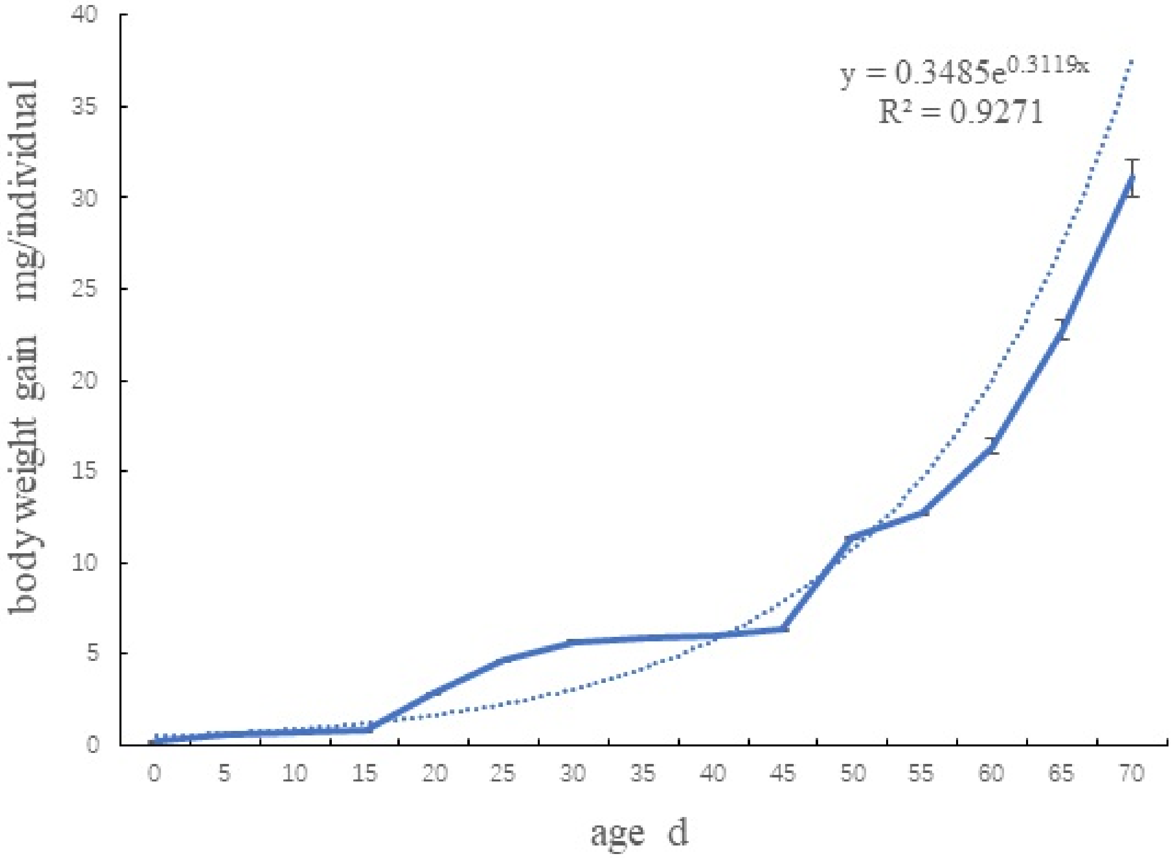


Figure 1

Growth curve (body weight gain over time) of juvenile Ligia exotica (n=3 groups, each contain 10 individuals from the same female. ±Bar means standard error)



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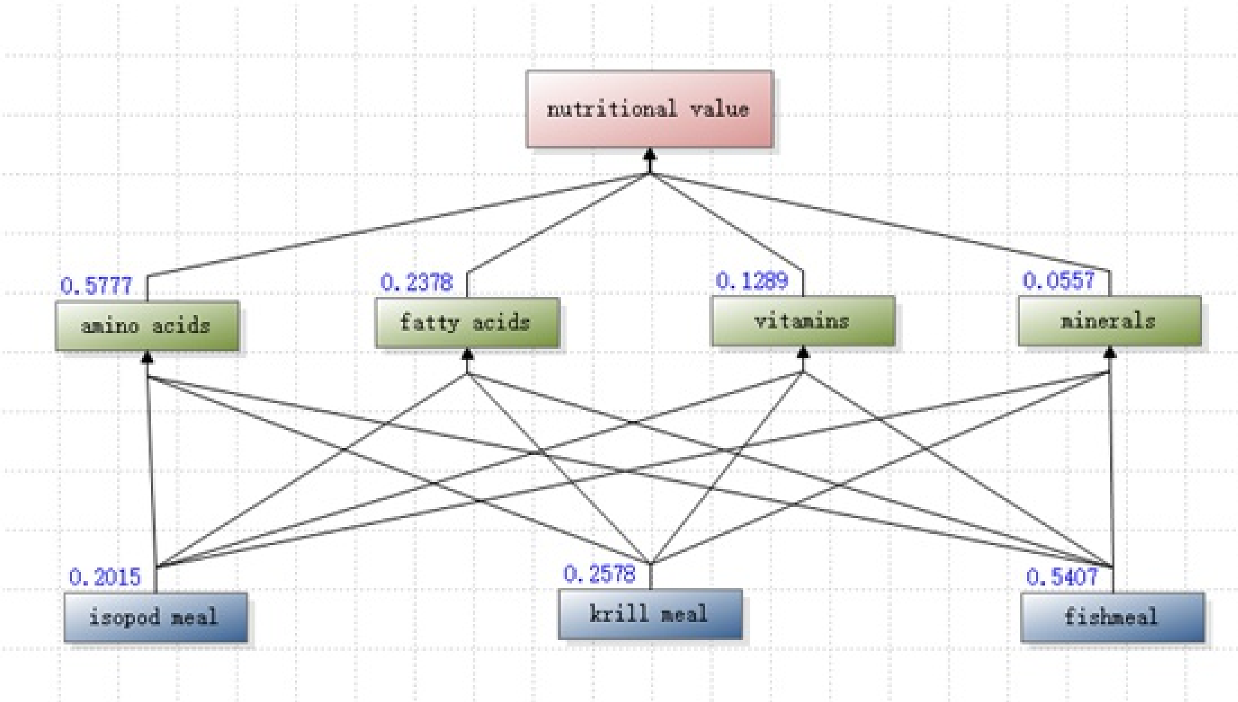
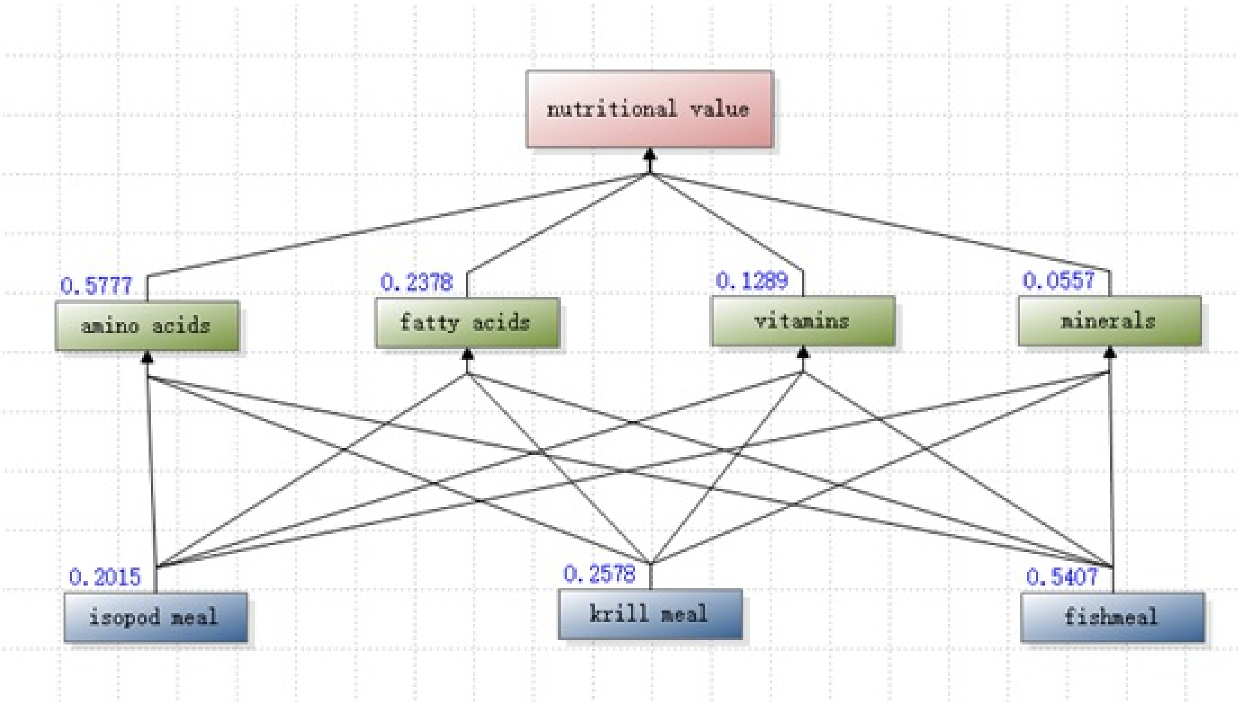


Figure 2

Nutritional value of three substrates based on group decision analytic hierarchy process of expert judgement



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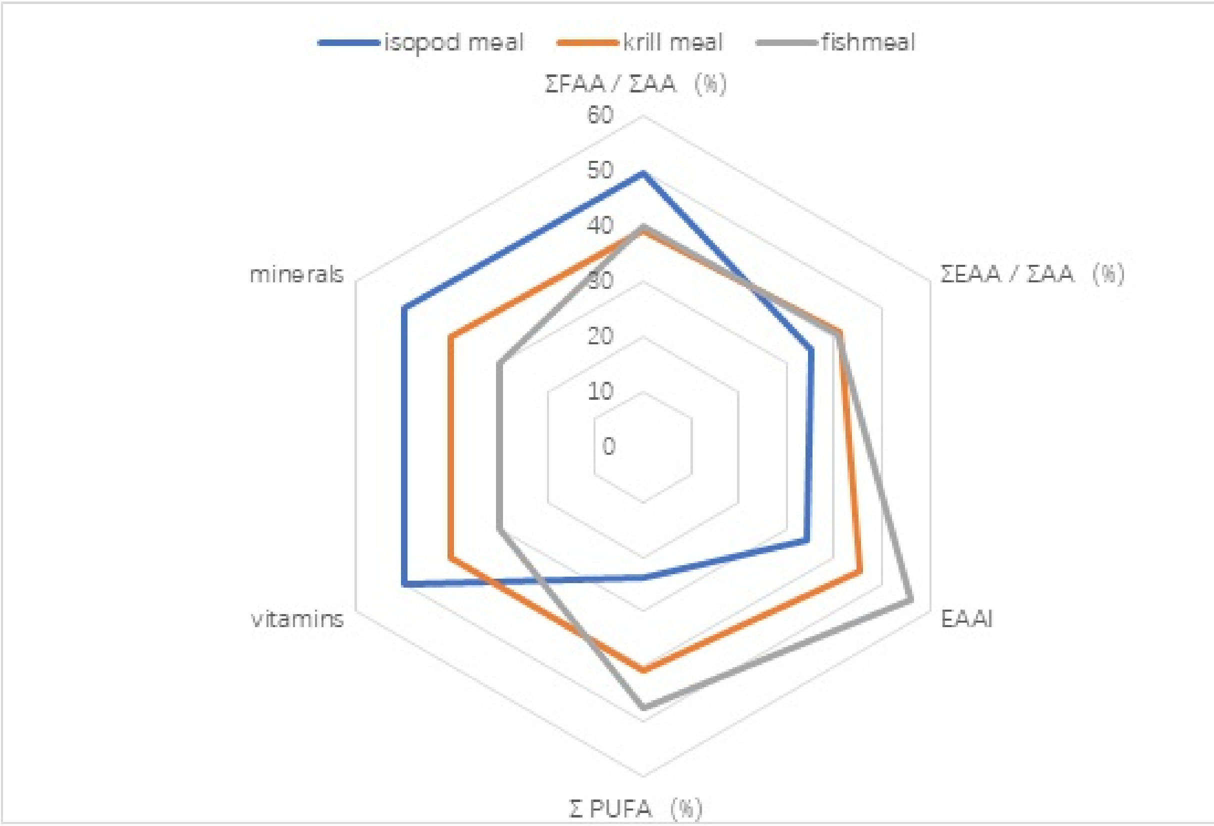

Figure 3

A radar chart illustrating the comprehensive nutritional evaluation of isopod meal, krill meal and fishmeal

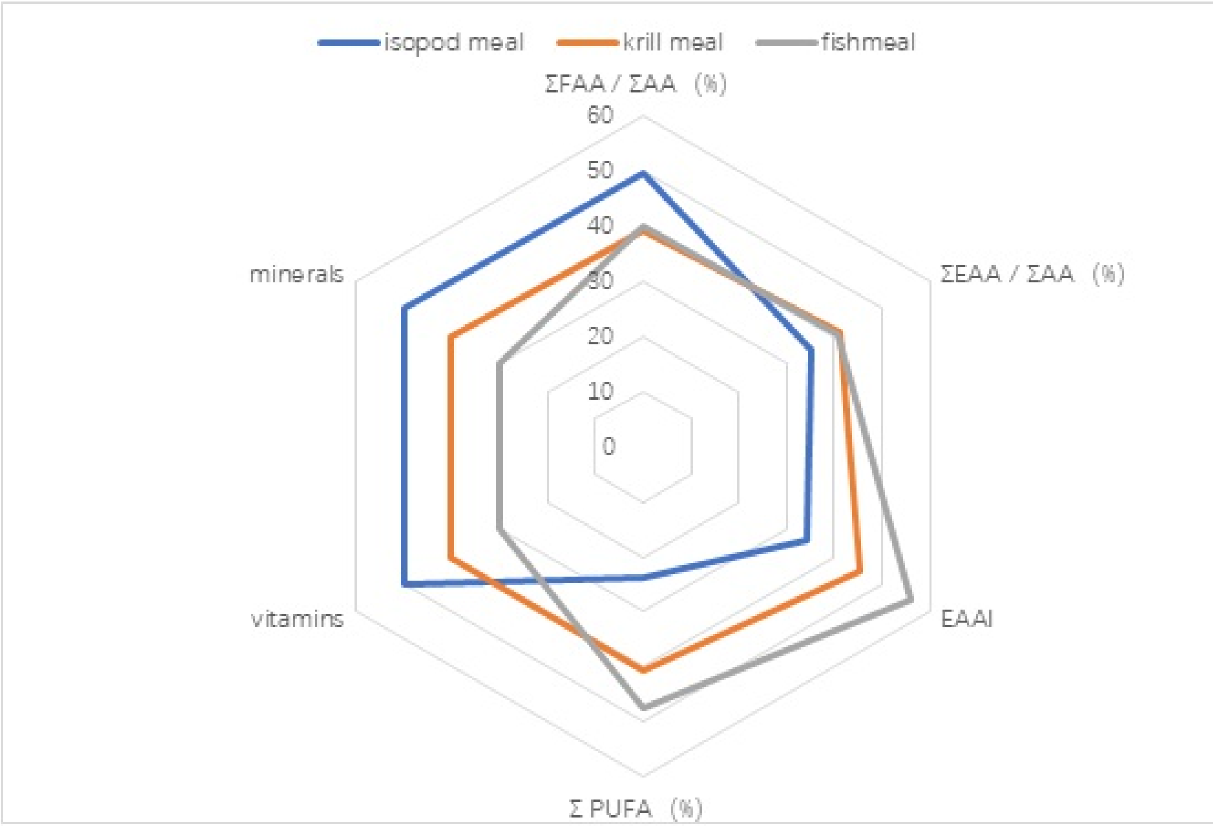

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Figure 4

Currently documented global distribution of L. exotica (red dots). Mainly adapted from Hurtado et al13. Map source: National Geographic World Map (ESRI). Downloaded from ArcGIS online, 2019. Sample locations were generated with ArcGIS. Version 10.5, Esri, USA from excel based on Appendix 1. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 5

External morphology of Ligia exotica (scale bar=0.6 cm)

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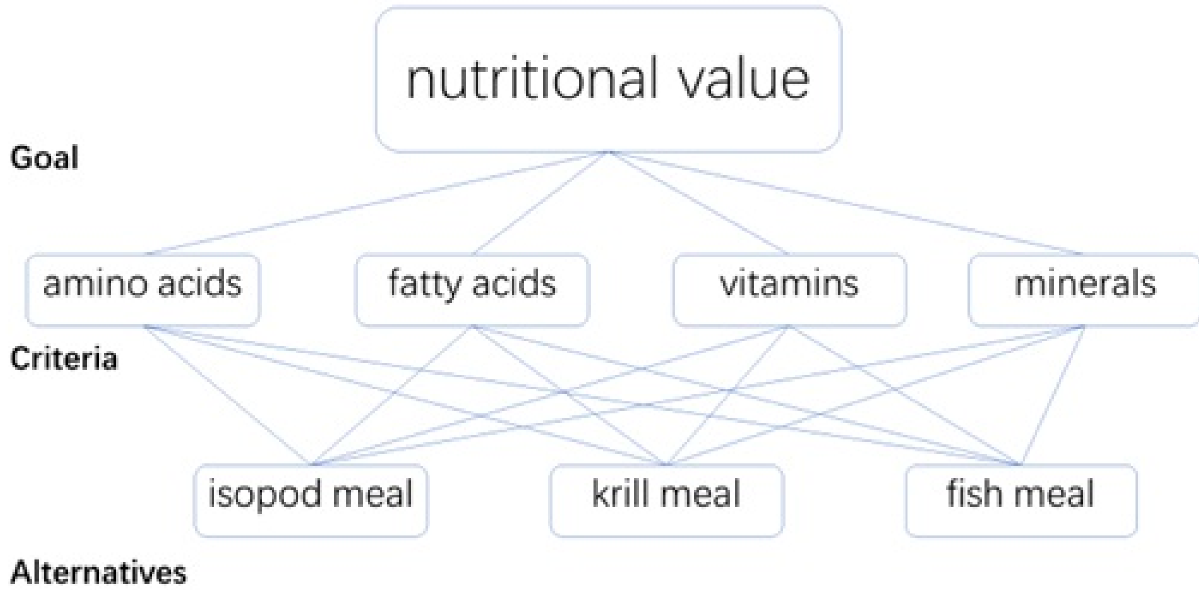

Figure 6

Analytical hierarchy process model of nutritional value for L.exotica

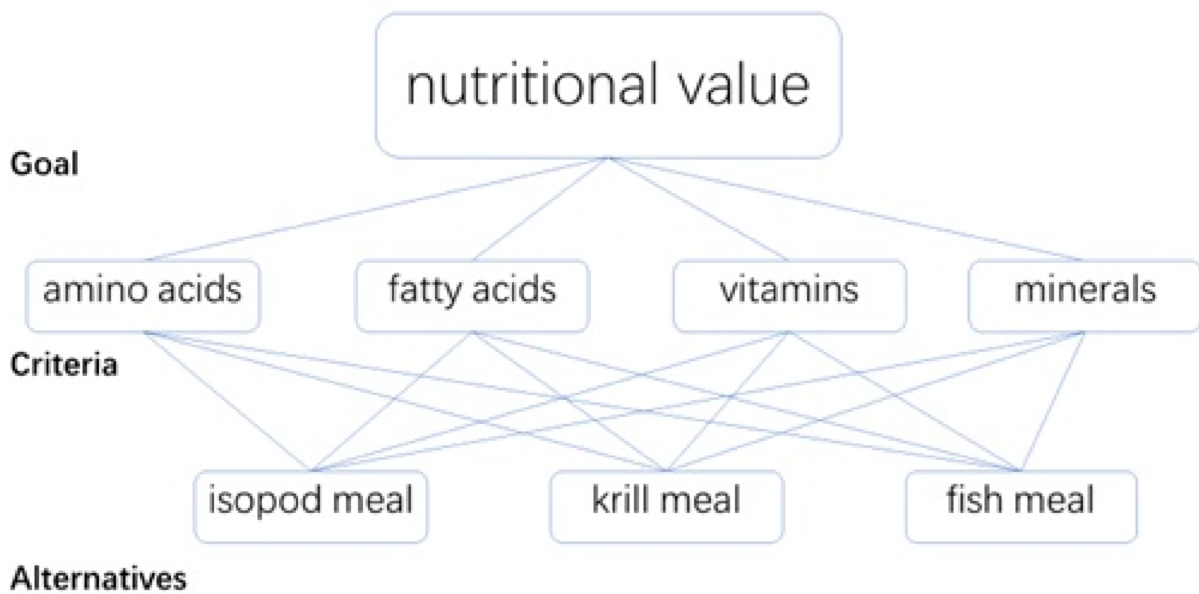

Figure 6

Analytical hierarchy process model of nutritional value for L.exotica

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