Physiological properties and tailored feeds to support aquaculture of marbled crayfish in closed systems

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Abstract

The marbled crayfish (*Procambarus virginalis*) is a new freshwater crayfish species, which reproduces by apomictic parthenogenesis, resulting in a monoclonal, all-female population. The animals have become a popular source for nutritional protein in Madagacar and are increasingly being considered for commercial aquaculture. However, their potential has remained unclear and there are also significant ecological concerns about their anthropogenic distribution. We show here that the size and weight of marbled crayfish is comparable to commonly farmed freshwater crayfish. Furthermore, purification of chitin from marbled crayfish shells revealed a high chitin content, which can be utilized for the synthesis of chitosan and other bioplastics. To allow the further evaluation of the animals in closed aquaculture systems, we used a factorial modeling approach and formulated tailored feeds that were matched to the marbled crayfish amino acid profile. These feeds showed superior performance in a feed trial, with a noticeable feed conversion rate of 1.4. In conclusion, our study provides important data for a balanced assessment of marbled crayfish as a new species for sustainable aquaculture and a feed that allows their culture in closed systems.

Introduction

The marbled crayfish (*P. virginalis*) is a novel freshwater crayfish species that emerged in the German aquarium trade about 25 years ago[1,2]. Notably, marbled crayfish represent the only known freshwater crayfish species that reproduce by obligate apomictic parthenogenesis, a mechanism that results in the formation of an all-female, globally monoclonal population[3-5]. Through anthropogenic releases, marbled crayfish have been introduced to various freshwater systems, where they have formed numerous stable populations[6].

While the introduction of marbled crayfish raises considerable ecological concerns, it also creates opportunities for human exploitation. This is exemplified by the spread of the animals on Madagascar, where their distribution area has increased 100-fold over the past 10 years[5,7,8]. This dramatic increase is largely fueled by anthropogenic distribution, as marbled crayfish have developed into valuable source of dietary protein[9]. The rapid spread of the animals is also supported by their high tolerance in various habitat parameters and their high population densities[9]. The latter point may be related to the monosex population structure of marbled crayfish, which is considered to allow higher stocking densities, due to less aggressive behaviour[10]. Taken together, these characteristics suggest that marbled crayfish are an interesting candidate for aquaculture production[11]. However, their potential benefits need to be carefully balanced against their potential negative ecological impacts. As such, more data, in combination with measures that prevent the uncontrolled spread of the animals, are urgently needed.

Freshwater crayfish are increasingly popular livestock for aquaculture with a global value that now exceeds 10 billion US dollars[12]. They are a rich source of nutritional protein and contribute to the increasing global demand for it. From the 550 known crayfish species[13], aquaculture production is mainly pursued with the red swamp crayfish, *Procambarus clarkii*, a species that is native to Mexico and USA[14]. In the last decade, the production of *P. clarkii* has rapidly increased[12], as the species has proven to be particularly robust and suited for mass production. China is currently the main producer and consumer of *P. clarkii*, with a production of more than 720,000 tons in 2015[15].

Crayfish shells are also rich in chitin[16]. Chitin and its derivatives, such as chitosan, are important raw materials for many industries and have found frequent use in wound bandages, filter materials and for the production of biodegradable plastics[17]. Shrimp and crab shell waste currently represents the main source for chitin production[18] but the increasing demand for chitin is counteracted by the increasing popularity of shrimps with low shell content.

Crayfish farming is usually done in open systems that are based on the omnivorous feeding patterns of the animals. Freshwater crayfish utilize nutrients from the bottom of the trophic food web, transfer energy to higher trophic levels and build a major proportion of the benthos biomass[19]. The animals also mediate nutrient and energy flow of ecosystems by being prey for predators and utilizing all sources of food from the ecosystem[20-22]. Similarly, it has been described that the primary food source of marbled crayfish is autochthonous and allochthonous detritus, while other sources like zoobenthos, algae and macrophytes are utilized to a lesser extent[23]. However, open culture of freshwater crayfish can create significant ecological problems[24].

To facilitate aquaculture in closed systems, feeds are designed to meet specific needs of the cultured animals, targeting specific production parameters (e.g., growth, feed efficiency, economics). The amino acid profile determines the feed quality as it directly affects the protein gain and, thus, the growth of the cultured animal. For example, methionine is often the first limiting amino acid for fish and crustaceans and its deficiency can directly affect animal growth[25]. Fish meal is often used as a preferred source of dietary protein, especially for new species, as it is known to be highly digestible and has a well-balanced amino acid composition. However, using fish meal in aquaculture is ecologically unfavorable. Natural alternatives, such as soybean and rapeseed meal are common alternative ingredients, however, they lack methionine and lysine. Supplementation with crystalline amino acids and the development of defined feeds is therefore required to meet the amino acid demands for crustaceans[26].

Here we determine key characteristics of marbled crayfish in the context of commercial aquaculture and describe the development of feeds that are tailored to the amino acid composition of this species. We evaluated growth performance and feed utilization of adolescent marbled crayfish to increasing levels of dietary methionine. Our results provide novel resources for the development of the animals into a sustainable aquaculture livestock.

Results

Size and weight analysis

P. clarkii from commercial aquaculture is usually harvested at a weight of 20-25 g[15]. Among wild-caught marbled crayfish from Germany and Madagascar, we observed several animals that exceeded this weight, including one animal with a length of 12.6 cm (Fig. 1A) and a weight >45 g (Fig. 1B). We also analyzed available morphometric data of marbled crayfish populations from Madagascar[9] and additional collections from Germany. The results showed that a target weight of 20 g correlated with a total length of 94 mm (Fig. 1C), which is often exceeded in existing wild populations. As these populations are usually located in a challenging environment (cold climate, presence of predators, nutrient-poor habitat), their growth can likely be augmented and accelerated by the development of tailored feeds.

Analysis of chitin content

To further assess the commercial potential of marbled crayfish, we also analyzed the chitin content of their shells and compared it to shrimp shells. Parallel extraction of chitin from shrimps and marbled crayfish showed that the mean chitin content of marbled crayfish shells was significantly higher than for shrimp (*L. vannamei*) shells (31.6 % vs. 25.1 %, $p<0.05$, Fig. 1D). We also observed that *L. vannamei* has relatively thin shells (3.4 % relative exoskeleton weight compared to total weight), while marbled crayfish had a relative exoskeleton weight of 16 %, which is 4.7 times more than shrimps.

Amino acid profile of marbled crayfish

High-performance feeds represent a key component for successful aquaculture. In order to design the basic composition of tailored feeds for marbled crayfish, we analyzed amino acid profiles (see Methods for details). A comparison of the whole-body amino acid content of the marbled crayfish and *L. vannamei* revealed an amino acid profile with similarities and some notable differences (Tab. S1): relative to Lys (100%), the ratios of several essential amino acids were higher for the marbled crayfish. These included Cys (by 7%), Thr (by 12%), Trp (by 3%), Arg (by 14%), Ile (by 8%), Leu (by 10%), Val (by 7%), His (by 11%) and Phe (by 9%).

Feed formulation

In addition, the amino acid content of a common aquarium pet feed (NovoPleco), which was used as a control feed in this study, was analyzed. The comparison of this feed and the marbled crayfish on the amino acid profile revealed that the ratio of Arg relative to Lys is lower in the control feed compared to the crayfish whole-body profile (125% versus 129%, Fig. 2). This suggests that control feed sold for crayfish does not contain the ideal amino acid profile and illustrates the need of tailored feed development.

On the basis of the amino acid profile of the marbled crayfish and our experience, tailored feeds were formulated with the ideal amino acid profile determined for marbled crayfish using a factorial modeling approach (Tab. 1). Additionally, methionine levels were varied from 0.45% to 0.7% (Tab. 2) and the control feed was used as a reference diet (D1). Diet 2 (D2) was formulated with a methionine concentration of 0.45%, which is methionine deficient when compared to the amino acid profile of the marbled crayfish and the analyzed pet shop feed. The methionine concentration of D3 was matched to the pet shop feed, with a concentration of 0.52%. D4 was matched to the amino acid profile of the crayfish with a methionine concentration of 0.6%. D5 was designed to have a methionine surplus compared to the amino acid profile and the competitor feed, with a methionine concentration of 0.7%. Our feeds were thus designed to cover specific nutritional requirements of the marbled crayfish and to determine the effects of different methionine levels on the growth of the animals.

Feed trial

In order to test the tailored feeds and validate the methionine requirement determined in the factorial modeling approach, we designed a feeding trial, where multiple independent groups of adolescent marbled crayfish were fed for three months. No other food source was provided to the animals. The animals were counted, measured and weighed once per week.

Out of 100 animals at the beginning of the trial, 30 animals survived. However, this includes a significant number of 40 animals that were removed for molecular analyzes. Additional deaths were mostly due to cannibalism, which is prevalent among freshwater crayfish and the corrected survival probability was 52% among all groups (Fig. 3). A statistical comparison between the groups showed no significant differences (Tab. S2), which allows the direct comparison of all groups. Interestingly, the tailored feeds showed a noticeable effect on the growth of the animals. When compared to the control feed (D1), tailored feed D3 (methionine matched) resulted in an increased weight gain. At the end of the trial, a mean weight gain of 2.81 g was observed for D3, while only 1.43 g was observed for D1 (Fig. 4). Consistently, the total mass gain of D3 was with 9.61 g more than the double weight compared to D1 with a total mass gain of 4.36 g (Tab. 3). Also, the feed conversion ratio of D3 was significantly ($p<0.05$. ANOVA) better than for the control feed (1.38 to 2.41, Tab. 3). These results illustrate the superior performance of tailored feeds.

A comparison of all tailored feeds showed that animals fed with D5 (surplus methionine) and D3 (methionine matched to control) showed a faster weight gain compared to D2 (methionine deficient) and D4 (methionine matched to requirement). During the early stages of the trial (until week 8), D5 showed the best performance, while D3 showed the best performance during the late stages of the trial (Fig. 4). Also, the feed conversion rates of D5 and D3 were significantly better than for D2 (Tab. 3). These findings raise the possibility that D5 is more suited for juveniles and adolescents, while D3 is better suited for adults. In agreement with this notion, D5 showed the highest total mass gain at the end of the trial with 10.11 g (Tab. 3)

Effects of astaxanthin additives

As pigmentation can strongly affect consumer acceptance, we also analyzed how feed additives can affect the pigmentation of marbled crayfish. A carotenoid-free synthetic feed caused almost complete loss of pigmentation in adult animals (Fig. 5, left). The addition of astaxanthin (0.2% Carophyll Pink) to the D2-D5 feeds, resulted in a brown pigmentation of the carapace with blue to green walking legs and chelipeds (Fig. 5). In comparison, animals that were fed with common pet food or were captured from wild populations usually appeared in darker shades (Fig. 5). These findings further emphasize the importance of specific feed additives for obtaining the desired animal phenotypes.

Discussion

The increasing demand for nutritional protein and bioplastics requires the development of sustainable and ecologically friendly production strategies. Due to its unique biological makeup and efficient feed conversion, the marbled crayfish represents an excellent in this context, if it can be produced in closed and controlled systems. Our study provides first data about the amino acid requirements of marbled crayfish. This information enabled us to formulate diets and feeds with superior performance. When diets are imbalanced and do not meet the animal's requirements for all amino acids, part of the amino acids are catabolized for energy rather than for protein synthesis[27,28]. This leads to inadequate protein and feed utilization and high ammonia production in the water[27,28]. Our findings suggest that tailored feeds, optimized to the amino acid profile of the marbled crayfish, and with adjusted methionine concentrations, improve the growth performance of cultured marbled crayfish.

Methionine is often the limiting amino acid to promote growth in aquatic animals if feeds contain mainly plant proteins[25]. To cover these needs, different feeds with various methionine levels were tested. Indeed, the lowest growth rate was detected for the feed with the lowest methionine level. This shows that methionine is a growth-limiting nutrient for marbled crayfish, similar to other crayfish, shrimps and fish[26,29,30]. Interestingly, the feed with the highest methionine level mainly promoted growth at earlier developmental stages, while later stages performed best with a feed that had an intermediate methionine level. These observations indicate different needs of methionine in different stages of crayfish development. Studies in other aquatic animals show that growth rates reach a plateau[31,32], or even decrease when the methionine requirements of the animals are overfed[33,34]. Our results suggest that methionine levels of 0.7% optimally support the growth of juvenile and early adolescent marbled crayfish with up to 2 grams of body weight.

As pigmentation of crayfish can strongly influence consumer choices, we also analyzed the effect of pigmentation-relevant feed additives. It has long been known that crayfish pigmentation is dependent on nutritionally supplied astaxanthin[35] and our findings confirm this dependency for marbled crayfish. Indeed, the use of astaxanthin-free feeds led to a complete loss of pigmentation. While this translucent phenotype appears undesirable for aquaculture farming, it could be favorable for scientific experiments, as it permits the analysis of organs and embryos in live animals.

Taken together, our study establishes the marbled crayfish as a promising new candidate species for sustainable aquaculture. A size and weight comparison of wild-caught marbled crayfish revealed comparable features to commercially harvested *P. clarkii*, which is the dominant species in crayfish aquaculture[15]. This is also consistent with the close genetic relationship of the two species[36]. Additional potential for commercialization was provided by the observation that marbled crayfish shells contain almost 6 times more chitin per animal compared to *L. vannamei* shells, which currently represent the main source of chitin. As important questions about the ecological impact of marbled crayfish remain to be resolved, aquaculture should be limited to closed systems. Our feeds are ideally suited to promote the growth of juveniles and adolescents in a closed hatchery and nursery environment, where a balanced feed composition is also important to maintain high water quality. Finally, our results establish marbled crayfish as highly efficient feed converters, which further illustrates their potential for sustainable aquaculture production. Sensitive detection methods based on environmental DNA are available[37] and can be used for monitoring and safeguarding marbled crayfish aquaculture.

Methods

Size and weight measurements. Morphometric data from marbled crayfish in Madagascar were taken from published datasets[9]. Animals from Murner See (Germany) were caught manually while diving. Total length was measured using a manual caliper and weight was recorded using a portable scale with 0.1 g precision. Animals from Reilinger See (Germany) were caught with traps and total length was measured with a manual caliper.

Growth prediction. To predict marbled crayfish growth rates, morphometric data of different animals (Madagascar and Germany) was analyzed. Precisely, a local polynomial regression using the locally estimated scatterplot smoothing (LOESS) method in R (version 3.6.1) was performed on the total length and weight of the animals (n=1,537). The prediction was performed by using the predict function on the total length of the animals (n=347) from a German lake.

Isolation of chitin. The following procedure is based on slightly modified literature protocols[38,39] to remove $CaCO_3$ and protein from shellfish shells. Molts from marbled crayfish and peeled shells from pre-cooked shrimps were collected and air dried. Shells were subsequently ground to powder with a Thermomix® (level 8, 30 s). From each species, 7 g of powdered shells were weighed out and added portion wise to a magnetically stirred solution of 1 M HCl (50 ml) in a wide necked 250 ml Erlenmeyer flask at 23 °C. Note: care must be taken to add the shells slowly as vigorous foaming occurs, which can cause overflowing. After the addition was complete, an additional 10 ml of 1M HCl was used to rinse the sides of the flask to ensure all shells were submerged. After 75 min, the resulting slurry was vacuum filtered through a porosity #3 sintered glass Büchner funnel using a membrane pump as a source of vacuum. The solid was repeatedly rinsed with deionized H_2O (6 x 50 ml) until the filtrate was neutral to pH indicator paper. After air drying with vacuum on the filter for 15 min, the powder was transferred to a 250 mL Erlenmeyer flask containing 1 M NaOH (75 mL), where it was magnetically stirred at 23 °C. After 24 h, the resulting slurry was vacuum filtered through a porosity #3 sintered glass filter Büchner funnel as before. The solid was repeatedly rinsed with deionized H_2O (6 x 50 mL) until the filtrate was neutral to pH indicator paper. The resulting solid was air dried with vacuum for ~20 min, transferred to a 100 mL round bottom flask, dried under high vacuum (~5 x 10^{-2} mbar) for 24 h, and weighed on an analytical balance. This protocol was performed in triplicate for each species.

Determination of amino acid requirements using factorial modeling. One randomly selected crayfish (14.77 g), which had been fed standard crayfish pet food was euthanized and freeze-dried (final mass: 4.35 g). The whole-body amino acid profile of this sample was analyzed using ion-exchange chromatography (AMINOLab®, Evonik Nutrition & Care, Germany), except for tryptophan, which was estimated using HPLC. In addition, the amino acid content of a common pet shop feed (NovoPleco), which was used as a control feed, was analyzed. Using the amino acid profile of the crayfish, amino acid composition or gain were calculated for a body weight gain of 1.5 g. The utilization of different amino acids absorbed across the gut was assumed to be 50-60%, and the maintenance requirements for different amino acids were considered to be 15-25% of the amino acids absorbed (i.e., on digestible basis). Amino acid requirements calculated with a factorial modeling approach are presented in Tab. 1 on digestible basis and total basis (assuming 85% digestibility for amino acids), following established procedures. Absolute requirement values derived as mg were converted into % feed assuming 1.5 g as the feed requirement for 1.5 g body weight gain.

Experimental diets. The detailed ingredient and nutrient composition of the diets used in this study are provided in Tab. S3. A basal diet was formulated using soybean meal, soy protein concentrate, fish meal and krill meal as the main protein sources. A basal diet (D2) was formulated to contain 29% crude protein and 18.31 MJ/kg gross energy. The amino acid profile of the diet except for methionine was balanced considering the requirements predicted using factorial modeling approach (Table 1). The basal diet (D2) was formulated to be low in Met (0.45%) and Met + Cys (0.86%) and was supplemented with increasing levels of methionine dipeptide (AQUAVI® Met-Met): 0.07% (D3), 0.15% (D4), and 0.25% (D5). Feed contents were validated by amino acid analysis (Tab. S3). The D2-D5 feeds were produced in pellets of 2 mm size.

Feed trial. Each feed was tested for three months. In total, 100 adolescent animals (size: 1.75 cm. SD: 0.25 cm. weight: 0.11g. SD: 0.05 g) were used in the study. Per feed, 20 animals were kept in 4 tanks (25.6 x 18.1 x 13.6 cm), with five animals per tank, at 20 °C under natural daylight. The animals were fed daily at 17:00 with 0.08 g of feed. Higher amounts of feed were offered occasionally but refused by the animals. Because of the specific feeding behavior of crayfish (prolonged feeding time; preference to stay hidden), it was not possible to determine accurate feed intake. A fixed amount of feed was provided in each tank, and no uneaten feed was recovered. Daily mean feed intake per tank was calculated by dividing the amount of feed fed to a tank on a given day by the number of animals survived on that day. Calculated daily mean feed intake value was summed over the whole experimental period to calculate the total mean feed intake in each tank. All animals were measured and weighed once per week. Water parameters were checked daily for temperature and once per week for NH_4, NO_3, NO_2 and O_2 (JBL Test sets). The results confirmed good water quality for the entire duration of the trial (Tab. S4).

Feed trial data analaysis. Survival probabilities for all animals and the groups D1 to D5 were calculated in R using the CRAN packages survival (version 2.44-1.1) and survminer (version 0.4.6). Kaplan-Meier plots were generated, and survival probabilities were calculated for each feed group and for all animals. To investigate differences in survival between feeds pairwise p-values were calculated using a log-rank test. Finally, p-values were adjusted using the Benjamini-Hochberg procedure. To assess differences in the means of feed conversion rates among the five feed groups a one-way analysis of variances (ANOVA) was performed. As the total variances between groups was statistically significant (p-value = 0.00239) a more detailed pairwise comparison between all groups was performed using Tukeys multiple comparison of means.

References

1. Scholtz G*, et al.* Ecology: Parthenogenesis in an outsider crayfish. *Nature* **421**, 806 (2003).

2. Lyko F. The marbled crayfish (Decapoda: Cambaridae) represents an independent new species. *Zootaxa* **4363**, 544-552 (2017).

3. Martin P, Kohlmann K, Scholtz G. The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring. *Naturwissenschaften* **94**, 843-846 (2007).

4. Vogt G, Huber M, Thiemann M, van den Boogaart G, Schmitz OJ, Schubart CD. Production of different phenotypes from the same genotype in the same environment by developmental variation. *J. Exp. Biol.* **211**, 510-523 (2008).

5. Gutekunst J*, et al.* Clonal genome evolution and rapid invasive spread of the marbled crayfish. *Nat. Ecol. Evol.* **2**, 567-573 (2018).

6. Chucholl C, Morawetz K, Groß H. The clones are coming – strong increase in Marmorkrebs *Procambarus fallax* (Hagen, 1870) *f. virginalis* records from Europe. *Aquat Invasions* **7**, 511-519 (2012).

7. Jones JPG*, et al.* The perfect invader: a parthenogenic crayfish poses a new threat to Madagascar's freshwater biodiversity. *Biol. Invasions* **11**, 1475-1482 (2009).

8. Kawai T, Scholtz G, Morioka S, Ramanamandimby F, Lukhaup C, Hanamura Y. Parthenogenetic alien crayfish (Decapoda: Cambaridae) spreading in Madagascar. *J. Crust. Biol.* **29**, 562-567 (2009).

9. Andriantsoa R*, et al.* Ecological plasticity and commercial impact of invasive marbled crayfish populations in Madagascar. *BMC Ecol* **19**, 8 (2019).

10. Malecha S. The case for all-female freshwater prawn, Macrobrachium rosenbergii (De Man), culture. *Aquacult. Res.* **43**, 1038-1048 (2012).

11. Jurmalietis R, Grickus A, Elstina A. Marbled crayfish (Procambarus virginalis) as a promising object for aquaculture industry. *Environment. Technology. Resources. Proceedings of the International Scientific and Practical Conference* **1**, 92-95 (2019).

12. FAO. Fisheries statistical database, Global aquaculture production. (ed^(eds) (2016).

13. Crandall KA, De Grave S. An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. *J. Crust. Biol.* **37**, 1-39 (2017).

14. Loureiro TG, Anastácio PM, Araujo PB, Souty-Grosset C, Almerão MP. Red swamp crayfish: biology, ecology and invasion - an overview. *Nauplius* **23**, 1-19 (2015).

15. Wang Q, Ding H, Tao Z, Ma D. Crayfish (Procambarus clarkii) cultivation in China: a decade of unprecedented development. In: *Aquaculture in China: success stories and modern trends* (ed^(eds Gui JF, Tang Q, Li Z, Liu J, De Silva SS). John Wiley & Sons (2018).

16. Kaur S, Dhillon GS. Recent trends in biological extraction of chitin from marine shell wastes: a review. *Crit. Rev. Biotechnol.* **35**, 44-61 (2015).

17. Park BK, Kim MM. Applications of chitin and its derivatives in biological medicine. *Int J Mol Sci* **11**, 5152-5164 (2010).

18. Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs* **13**, 1133-1174 (2015).

19. Holdich DM. *Biology of Freshwater Crayfish*. Wiley-Blackwell (2002).

20. Correia AM, Anastácio PM. Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecol. Res.* **23**, 729-734 (2008).

21. Grey J, Jackson MC. 'Leaves and eats shoots': direct terrestrial feeding can supplement invasive red swamp crayfish in times of need. *PLoS ONE* **7**, e42575 (2012).

22. Ruokonen TJ, Kiljunen M, Karjalainen J, Hämäläinen H. Invasive crayfish increase habitat connectivity: a case study in a large boreal lake. *Knowl. Managt. Aquatic Ecosyst.* **407**, 08 (2012).

23. Lipták B*, et al.* Trophic role of marbled crayfish in a lentic freshwater ecosystem. *Aquat Invasions* **14**, 299-309 (2019).

24. Gherardi F. Crayfish invading Europe: the case study of Procambarus clarkii. *Aquatic Ecosystems* **416**, 1–15 (2006).

25. Bulbul M, Kader MA, Ambak MA, Hossain MS, Ishikawa M, Koshio S. Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, Marsupenaeus japonicus. *Aquaculture* **438**, 98-104 (2015).

26. Tan Q, Song D, Chen X, Xie S, Shu X. Replacing fish meal with vegetable protein sources in feed for juvenile red swamp crayfish, Procambarus clarkii: Effects of amino acids supplementation on growth and feed utilization. *Aquacult. Nutr.* **24**, 858-864 (2018).

27. Cowey CB, Walton MJ. Intermediary metabolism. In: *Fish Nutrition* (ed^(eds Halver JE). Second Edition edn. Academic Press (1989).

28. Council NR. *Nutrient Requirements of Fish and Shrimp*. The National Academies Press (2011).

29. Yan Q, Xie S, Zhu X, Lei W, Yang Y. Dietary methionine requirement for juvenile rockfish, Sebastes schlegeli. *Aquacult. Nutr.* **13**, 163-169 (2007).

30. Gu M, Zhang WB, Bai N, Mai KS, Xu W. Effects of dietary crystalline methionine or oligo-methionine on growth performance and feed utilization of white shrimp (Litopenaeus vannamei) fed plant protein-enriched diets. *Aquacult. Nutr.* **19**, 39-46 (2013).

31. Harding DE, Allen OW, Jr., Wilson RP. Sulfur amino acid requirement of channel catfish: L-methionine and L-cystine. *J Nutr* **107**, 2031-2035 (1977).

32. Alam MS, Teshima S, Ishikawa M, Koshio S, Yaniharto D. Methionine requirement of juvenile Japanese flounder Paralichthys olivaceus estimated by the oxidation of radioactive methionine. *Aquacult. Nutr.* **7**, 201-209 (2001).

33. Millamena OM, Bautista-Teruel MN, Kanazawa A. Methionine requirement of juvenile tiger shrimp Penaeus monodon Fabricius. *Aquaculture* **143**, 403-410 (1996).

34. Sveier H, Nordås H, Berge GE, Lied E. Dietary inclusion of crystalline D- and L-methionine: effects on growth, feed and protein utilization, and digestibility in small and large Atlantic salmon (Salmon salar L.). *Aquacult. Nutr.* **7**, 169-181 (2001).

35. Harpaz S, Rise M, Arad S, Gur N. The effect of three carotenoid sources on growth and pigmentation of juvenile freshwater crayfish Cherax quadricarinatus. *Aquacult. Nutr.* **4**, 201-208 (1998).

36. Owen CL, Bracken-Grissom H, Stern D, Crandall KA. A synthetic phylogeny of freshwater crayfish: insights for conservation. *Philosophical Transactions of the Royal Society London B: Biological Sciences* **370**, 20140009 (2015).

37. Mauvisseau Q, Tönges S, Andriantsoa R, Lyko F, Sweet M. Early detection of an emerging invasive species: eDNA monitoring of a parthenogenetic crayfish in freshwater systems. *Manag. Biol. Invasions* **10**, 461-472 (2019).

38. Percot A, Viton C, Domard A. Characterization of Shrimp Shell Deproteinization. *Biomacromolecules* **4**, 1380-1385 (2003).

39. Percot A, Viton C, Domard A. Optimization of Chitin Extraction from Shrimp Shells. *Biomacromolecules* **4**, 12-18 (2003).

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Author contributions

S.T. performed the experiments and analyzed the data. K.M. designed the feeds and the feed trial and analyzed the data. J.G. performed statistical analyses and growth predictions. J.L. and A.K.M. performed the chitin extraction. F.B. and F.L. conceived the study. S.T. and F.L. wrote the paper with input from the other authors. All authors read and approved the final manuscript.

Competing interests

K.M. and F.B. are employees of Evonik, F.L. received consultation fees from Evonik. S.T., J.G., J.L. and A.K.M declare no potential conflict of interest.

Figure legends

Figure 1. Size, weight and chitin content of marbled crayfish. (A) A wild-caught marbled crayfish with a total length of 12.6 cm and (B) a weight of 49.11 g. (C) Size and weight data for a total of 1,537 animals. The figure shows animals > 76 mm (filled circles); grey: animals <20 g; black: animals >20 g. Open circles: Size was measured, weight was estimated using regression analysis (red line) from the data where both measurements where available; grey: animals <20 g; blue: animals estimated >20 g. (D) Comparison of chitin content in marbled crayfish (*P. virginalis*) shells and shrimp (*L. vannamei*) shells. An unpaired two-tailed t-test showed that the difference between the two groups is significant (p=0.0016).

Figure 2. Comparison of amino acid profiles for the development of tailored feeds. Bars show relative amino acid levels for marbled crayfish (blue), control feed (orange) and tailored feeds (purple). All profiles are normalized to lysine (100%).

Figure 3. Animal survival in the feed trial. Kaplan-Meier plot showing the survival probability for animals of all groups. The overall survival probability is 52%, taking in consideration that animals were removed for molecular analysis.

Figure 4. Weight gains for different feeds. Box plots show the weight per group and week. (A) Comparison of the control feed (D1) with the matched tailored feed (D3). The tailored feed D3 shows a higher weight gain compared to D1. (B) Comparison of all tailored feeds (D2-D5). D2 shows the lowest weight gain over time. D3 and D5 have the highest weight gain, but for D5 the weight gain is stronger at the beginning of the trial until week 9. From week 9 till the end of the trial D3 shows the highest weight gain. Both groups have almost the same weight gain after 13 weeks with 2.81 g/animal for D3 and 2.63 g/animal for D5.

Figure 5. The influence of feed additives on the pigmentation of the marbled crayfish. (A) Animal fed with a carotenoid-free version of D5. (B) Animal fed with astaxanthin-supplemented D5. (C) Animal fed with control feed (D1).

Table 1. Whole-body amino acid profile and requirements of adolescent crayfish predicted using a factorial modeling approach.

Amino acids	**Whole-body AA profile in % (dry matter basis)**	**Calculated EAA requirements in % feed total (digestible)**	**Ratio to Lys%**
Met	0.75	0.64 (0.51)	40
Cys	0.49	0.46 (0.37)	29
M+C	1.24	1.10 (0.88)	69
Lys	2.48	1.60 (1.36)	100
Thr	1.59	1.27 (1.08)	79
Trp	0.41	0.33 (0.28)	20
Arg	3.20	2.12 (1.81)	132
Ile	1.60	1.06 (0.90)	66
Leu	2.66	1.77 (1.50)	110
Val	1.75	1.16 (0.99)	72
His	1.00	0.80 (0.68)	50
Phe	2.03	1.35 (1.15)	84
Gly	2.02		
Ser	1.56		
Pro	1.58		
Ala	2.05		
Asp	3.78		
Glu	5.59		
Crude protein	41.97		

Table 2. Overview of the feeds that were compared in this trial.

Feed	**Features**
D1	Established standard (NovoPleco, 0.52% Met)
D2	Tailored. Met deficient (0.45% Met)
D3	Tailored. Met equivalent to standard (0.52% Met)
D4	Tailored. Met according to aa profile (0.6% Met)
D5	Tailored. Met surplus (0.7% Met)

Table 3. Mass gains and feed conversion ratios (FCR) of crayfish fed different diets over 90 days.

Feed	Total mass gain	Mean weight gain per animal	Mean feed intake per animal	FCR
D1	4.36	1.43 g	3.62 g	2.41
D2	6.83	0.97 g	3.29 g	3.35
D3	9.61	2.81 g	3.81 g	1.38
D4	8.15	1.56 g	3.11 g	2.22
D5	10.11	2.63 g	4.29 g	1.72

FCR comparisons: D1:D3 p=0.041, D2:D3 p=0.003, D2:D5 p=0.012

Figure 1

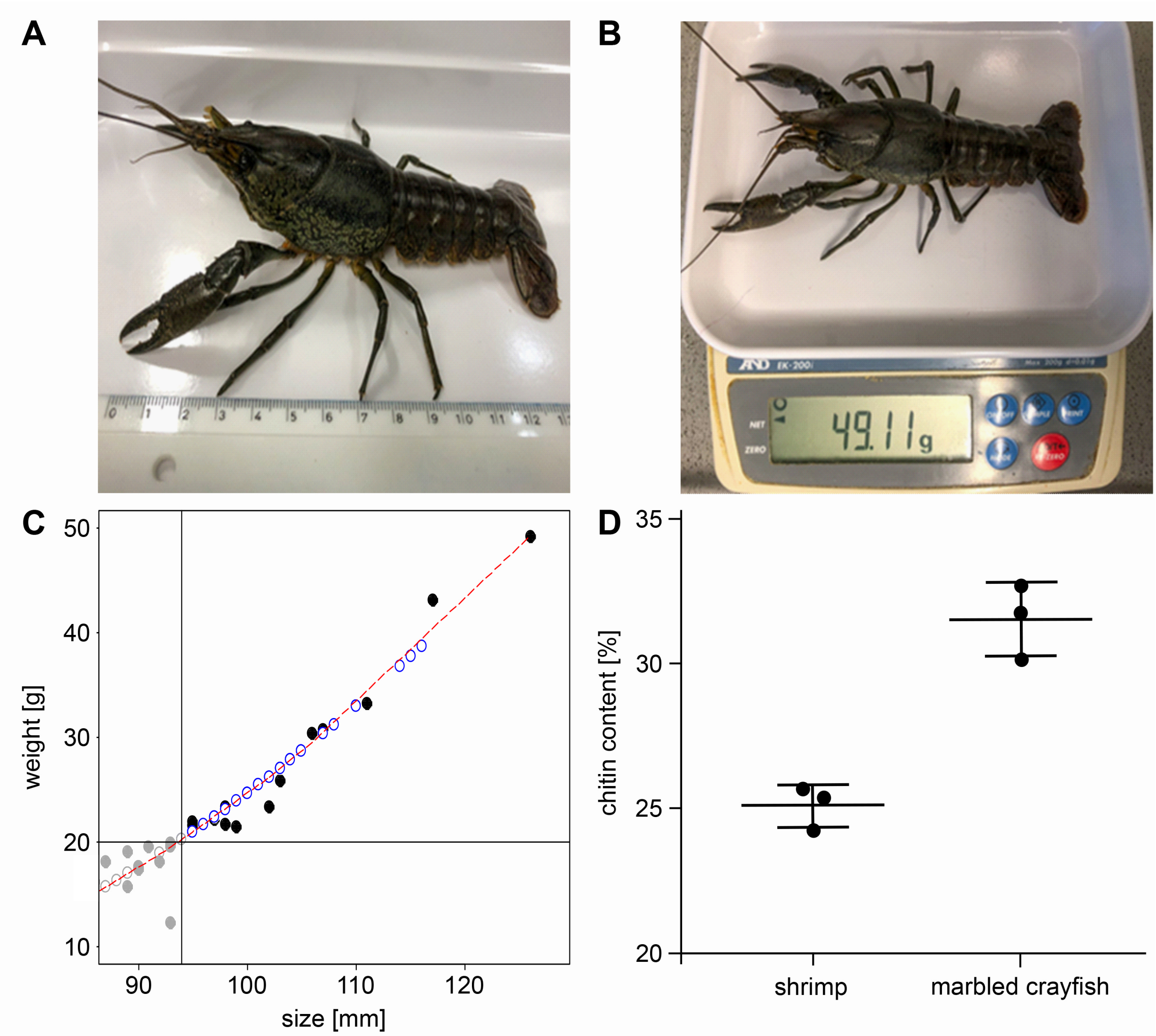
A
B
49.11 g
C
weight [g]
size [mm]
50
40
30
20
10
90
100
110
120
D
chitin content [%]
35
30
25
20
shrimp
marbled crayfish

Figure 2

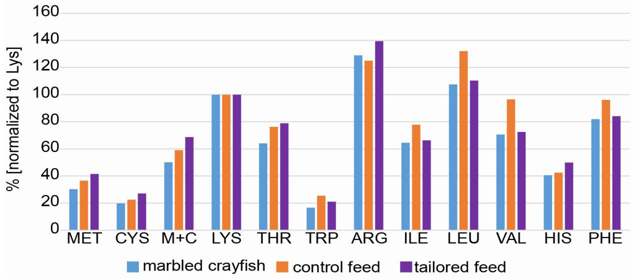

Figure 3

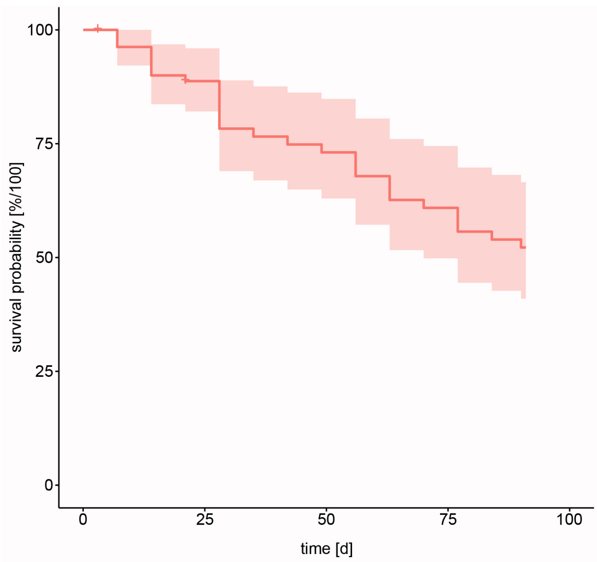

Figure 4

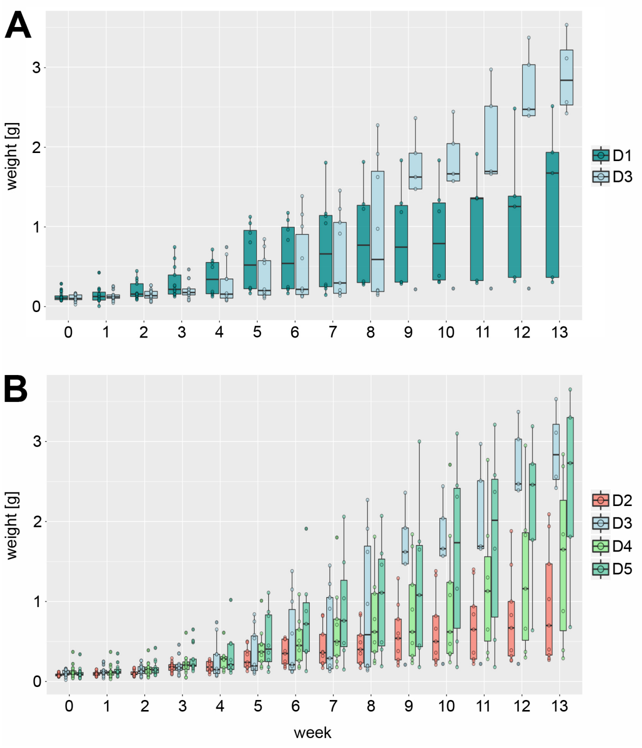

Figure 5