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# *Cherax destructor* (Clark, 1836) and *Cherax quadricarinatus* (von Martens, 1868): Biochemical parameters and preliminary analysis of food quality

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ABSTRACT

The breeding of *Cherax spp*., initially conducted in Australia, has aroused interest in Europe and Italy over the past two decades. The use of these species in aquaculture has led to the study of their nutritional properties to evaluate the commercial potential and to identify biochemical haemolymphatic parameters which may be of use when monitoring the health status of farmed animals. Moisture, ash, lipid, protein and fatty acid contents of the abdomen muscle of *Cherax destructor* and *Cherax quadricarinatus* were evaluated in this study and compared with other crustacean species. Haemolymphatic levels of glucose, triglycerides, cholesterol and lactate dehydrogenase were also measured. The two species did not show significant differences in nutritional or biochemical haemolymphatic parameters (except for glucose) and were found to possess good nutritional values for human consumption.

## 1. Introduction

Marine and freshwater animals play an important role in a range of contexts. They act as environmental bioindicators (Chiaramonte et al., 2020; Mauro et al., 2021; Mauro et al., 2022a), a valuable source of bioactive molecules (Mauro et al., 2020; Mauro et al., 2022b; Luparello et al., 2022; Punginelli et al., 2022), in addition to being a source of food for human consumption (Bilgin and Fidanbaş, 2011; Abdel-Salam, 2013; Kampouris et al., 2021; Wang et al., 2021). This latter is of particular importance given the continual increase in the human population. Demand for animal protein is also growing and aquatic species are an important source of essential nutrients, including proteins, fatty acids, vitamins (Reames, 2012) and trace elements (Tacon and Metian, 2013). However, this increase in demand may also lead to overexploitation of natural populations with serious consequences on biodiversity (Madsen et al., 2011). Aquaculture is not only an important source of food but also reduces the exploitation of natural environments and provides animals for sport fishing, scientific research, conservation and the reintroduction of species, and ornamental trade (Calado et al., 2017). The aquaculture sector is key for future development; however, a number of issues currently hinder its growth and sustainability. Limiting factors include disease and parasite proliferation, and stress (due to farming conditions, population density and poor quality of 'waterfall', etc.) which often influence the health status of farmed animals (Madsen and Stauffer, 2024; Sepúlveda et al., 2004; Murray and Peeler, 2005; Shafiq et al., 2023). Given the importance of successfully keeping animals in aquaculture facilities, researchers have evaluated status during reproduction, larval rearing, transportation and growth in *Penaeus vannamei,* for example, (developing protocols based on four of the five animal welfare domains: nutrition, environment, health and behavior (Pedrazzani et al., 2023). Gustafson et al. (2005), when studying *Elliptio complanata*, demonstrated how hemolymph (the circulatory fluid that transports nutrients, respiratory gases, enzymes, metabolic wastes and toxic substances throughout the body of freshwater invertebrates) can provide important information for assessing the health of animals or populations. Other researchers have also evaluated the effects of different types of stress (e.g., pathogen infection, parasitic dinoflagellate) on the haemolymphatic parameters of farmed invertebrates (Li et al., 2015; Kong et al., 2023). Crustaceans are a dominant and diverse group of aquatic fauna and include organisms with considerable

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ecological importance (such as copepods, water fleas, krill and mysids) and species with high economic value in aquaculture (shrimp, lobster, prawns and crabs) (Martin and Davis, 2001; FAO, 2020).

Farmed crustaceans are a significant component of future protein production in the aquaculture sector (Bondad-Reantaso et al., 2012). Freshwater species of particular importance for crustacean farming are *Cherax quadricarinatus* (Australian yabby) and *Cherax destructor,* (common yabby) (Ghanawi and Saoud, 2012; Zheng et al., 2019). Both species are native to Australian watersheds (Zheng et al., 2020) are farmed more easily than other marine species (Ghanawi and Saoud, 2012; Zheng et al., 2019; Nguyen et al., 2016). They possess rapid growth and high survival rates (McCormack, 2014), and display remarkable adaptation abilities even to a range of temperatures (McCormack, 2014; Garcıa-Guerrero et al., 2003). These qualities have led to their success in aquaculture and aquarium keeping in many countries around the world (Mauro et al., 2022a; Rodgers al., 2006; Xie et al., 2010). Scientists have now studied the effects of food supplements in both species (Sang et al., 2011; Mac Loughlin et al., 2016; Chen et al., 2020), changes in haemolymphatic parameters following molting (Van Mai and Fotedar, 2018), the effects of pollutants (Pham et al., 2017; Stara et al., 2018), their acoustic signals and behavior (De Vita et al., 2023), and have also evaluated hemolymphatic parameters to establish the health status of the animals (Mauro et al., 2022a). Only Tee et al. (2022) and Jones (1990) have analyzed the nutritional properties and the biology of *C. quadricarinatus* meats to date, and no one appears to have studied the nutritional properties of the congeneric species C. destructor. One aim of this study was to supplement the study by Mauro et al., (2022a) on biochemical health status parameters, including new information on glucose, triglycerides, cholesterol and lactate dehydrogenase in both species. The mobilization of glucose from body reserves is a fundamental physiological process and glucose levels in the hemolymph of decapod crustaceans allows us to detect not only day/night rhythmicity ( Sathyanandam et al., 2008) but also physiological response to stress conditions (e.g., increased levels in cells due to exposure to air), a major concern in aquaculture as mentioned earlier (Santos et al., 2001; ). Triglycerides are part of the circulating neutral lipids of crustaceans and are catabolized preferentially during fasting, unlike polar lipids. They are thus of fundamental importance in the evaluation of the nutritional status of these animals as triglycerides could constitute a good source of energy (Silva-Castiglioni et al., 2016). Cholesterol is a significant sterol in crustaceans and is present in both cells and the hemolymph (both in free form and combined with fatty acids). Crustaceans are unable to synthesize cholesterol and, therefore, its presence in their diet is essential for growth and survival. Furthermore, the cholesterol requirement is species-specific in crustaceans and may depend on dietary sources of protein and lipids. Cholesterol is essential for the synthesis of ecdysteroids and sesquiterpenoids, a class of hormones which regulate molting and reproduction. Lactate dehydrogenase is critical for anaerobic glycolysis and produces lactate from pyruvate. It is an enzyme which is expressed in a tissue-specific manner during hypoxia (Mauro et al., 2022a) and thus plays a fundamental role as a biomarker in the evaluation of any given infection process (e.g., White Spot Syndrome Virus) which happens to compromise glycolysis (thereby causing an increase in the use of glucose and lactate accumulation in hemocytes) (Zheng et al., 2020; Rodgers et al., 2006; Hernández-Palomares et al., 2018). Another, goal of this study was to provide a preliminary characterization of the nutritional properties of these two species by analyzing levels of proteins, lipids, ash, moisture and fatty acids. Our hypotheses envisaged good nutritional qualities of the meat of these species and the absence of any significant differences in the haemolymphatic parameters evaluated. However, the information obtained from this study would allow us to create a baseline of information useful both for the management practices of animals (feeding and maintenance conditions) and the monitoring of their health status. The results obtained could be extremely important in providing a complete range of haemolymphatic parameters for these organisms in the absence of stress conditions, thus providing a fundamental tool for monitoring their health status. Furthermore, evaluation of the nutritional properties would allow us to better understand the nutritional and economic potential for human consumption, an aspect which requires the constant search for new products with adequate and beneficial nutritional characteristics.

## 2. Materials and methods

### 2.1. Animals

To evaluate the haemolymphatic biochemical parameters and food quality of two *Cherax spp.,* 45 animals were used for each species: *C. quadricarinatus* (weight 56.16 ± 7 g and total length 10.90 ± 0.82 cm) and *C. destructor* (weight 53.56 ± 4 g and length 8.90 ± 1 cm). The animals were supplied by the yabby aquaculture facility located in Eastern Sicily, in the town of 'Fiumefreddo di Sicilia' (Catania - Italy). 45 individuals of each species were divided randomly into three groups (three replicas, 15 individuals in each), placed in three different tanks (rectangular, 80 L each) and acclimatized for two weeks at a constant temperature (21 ± 1 °C) and continuous aeration ($O_2$ > 5.0 mg/l). The animals were fed once a day at the same time in the afternoon using commercial feed produced by a Veronesi company (chemical composition shown in the Table 1); quantities were based on live weight percentages (2 %). Uneaten feed and feces were removed daily and feeding was stopped 24 h before final sampling.

### 2.2. Samples

Samples of hemolymph and abdomen muscle were obtained from every individual of both the *Cherax spp.* The animals were anesthetized individually in ice for 10 min to collect hemolymph from each individual (using a 21-gauge needle inserted into the pericardial sinus at the base of the first abdominal segment). Hemolymph from each individual was sampled rapidly without the use of anticoagulant (which can negatively affect the biochemical results) and centrifuged immediately at 800 g for 10 min at 4 °C to separate the cells from the cell-free fluid. This latter was used for the evaluation of glucose, lactate dehydrogenase, cholesterol and triglyceride levels. Abdominal muscle sampling was performed on each individual at the end of the hemolymph sampling using protocols developed by Barrento et al., (2008), (2009a). A few modifications were made to the protocols, such as individuals being kept in refrigerated conditions (for at least one hour) to decrease their metabolism before being euthanized. The abdominal muscle was individually sampled, cleaned from viscera and weighed. To obtain sample quantities suitable for food quality evaluation, three muscle pools were created for each species using 15 individuals in each pool. Samples were frozen at −80° C, freeze dried to obtain a powder and stored at −20° C until subsequent analyses.

**Table 1**
Chemical composition (g/100 dry matter) of commercial feed.

| Granulometry (mm) | 0.6 |
|---|---|
| Dry matter | 94.0 |
| Crude Protein (%) | 34.60 |
| Ether extract (%) | 8.60 |
| Cellulose (%) | 1.60 |
| Ash (%) | 11.30 |
| Total carbohydrates (%) | 32.40 |
| Vitamin C (mg/kg) | 500 |
| Vitamin E (mg/kg) | 400 |
| Energy (kj/g) | 17.1 |

### 2.3. Biochemical, chemical analysis and fatty acid composition

Levels of glucose, lactate dehydrogenase, cholesterol and triglycerides were evaluated on the cell-free samples of each individual of both *Cherax spp.* using an Accutrend Plus instrument (Roche) according to the manufacturer's instructions. Photometry reflection was performed using Accutrend test strips (with 15 samples) specific for each parameter and for each individual. Moisture, ash, crude protein and lipid contents were determined to find the proximate compositions of the abdominal muscle according to procedures from the Association of Official Analytical Chemists (AOAC, 2012). Fatty acids (FA) were extracted according to methods developed by O'Fallon et al. (2007): C23:0 (Sigma-Aldrich) using as internal standard (0.5 mg/g freeze-dried sample) was used for FA quantification. Each sample (1 μL) was injected into an HP 6890 gaschromatography system equipped with a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA, USA). The separation and identification of each FA was performed as described by Alabiso et al. (2020). Fish lipid quality (FLQ) and Hypocholesterolemic/hypercholesterolemic ratio (H/h) were evaluated with the respective formulas reported by Chen and Liu (2020):

$$FLQ = 100 \times (C22:6\ n\text{-}3 + C20:5\ n\text{-}3)/\Sigma FA$$

and

$$HH = (cis\text{-}C18:1 + \Sigma PUFA)/(C12:0 + C14:0 + C16:0)$$

### 2.4. Statistical analysis

Animal biochemical parameters and food chemical and fatty acid profiles were statistically analyzed using SAS 9.2 software MIXED procedure (SAS Institute Inc., Campus Drive Cary, NC, USA). *Cherax spp.* (2 levels: *C. quadricarinatus* and *C. destructor*) represented the fixed factor and muscle pool (3 levels as replicates) was the random factor used as the error term in the mixed model. When the effect of species was significant ($p \leq 0.05$), means were compared using p-values adjusted according to multiple comparison Tukey-Kramer. All data are presented using the mean ± standard deviation.

## 3. Results

Biochemical results obtained on cell-free samples of both *Cherax spp.* are shown in Fig. 1. Significantly lower glucose levels were observed in *C. quadricarinatus* compared to *C. destructor* (Fig. 1A). Non-significant differences were observed for lactate dehydrogenase and triglycerides between the species (Fig. 1B, D), slightly higher in *C. quadricarinatus* than *C.destructor,* likewise minimally lower cholesterol levels were observed in *C. destructor* respect to *C. quadricarinatus* (Fig. 1C).

Regarding food quality, Table 2 shows the chemical gross composition of the two *Cherax spp.* The percentages of moisture, crude protein,

**Table 2**
Chemical gross composition (g/100 g) of abdomen muscles in *C. destructor* and *C. quadricarinatus*.

| Parameter | *Cherax destructor* | *Cherax quadricarinatus* |
|---|---|---|
| Moisture | 82.6 ± 4.10 | 81.0 ± 2.15 |
| Crude protein | 16.0 ± 2.15 | 17.17 ± 3.54 |
| Lipids | 0.47 ± 0.01 | 0.50 ± 0.03 |
| Ashes | 0.99 ± 0.15 | 1.30 ± 0.18 |

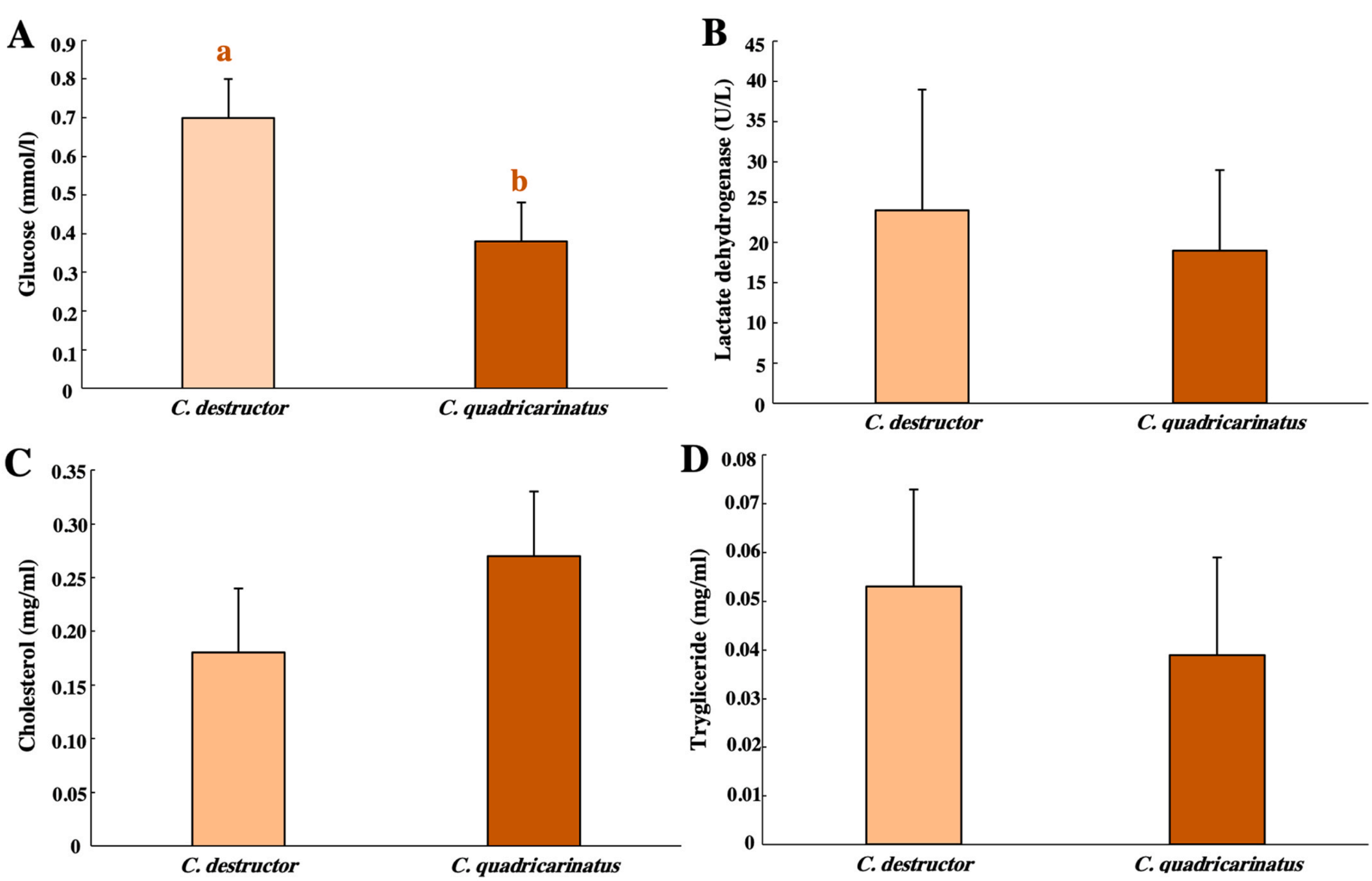


**Fig. 1.** Biochemical parameters obtained in cell-free samples of *C. quadricarinatus* and *C. destructor*. **A)** Glucose level a, b = $p < 0.01$.; **B)** Lactate dehydrogenase level. **C)** Cholesterol level; **D)** Trygliceride level. All parameters were expressed as a mean ±SD.

lipids and ashes were found to be similar in the two species.

Table 3 lists the fatty acid composition of *Cherax spp*. abdomen muscle. Higher fatty acid percentages were found for oleic acid, palmitic acid, eicosapentaenoic acid, stearic acid, linoleic acid and arachidonic acid, and lower values for petroselinic and pentadecanoic acid. Table 4 shows the fatty acid profile of *Cherax* muscle, clustered by category and two nutritional indices. Significantly higher amounts of saturated fatty acids (SFA) were found in the *C. destructor* species due to a greater palmitic and arachidonic acid content, while significantly lower quantities of monounsaturated fatty acids (MUFA) were found as a result of a lower oleic and palmitoleic acid content. The omega 6 fatty acid content was also significantly higher in *C. destructor*, mainly as a consequence of a greater arachidonic and docosapentaenoic acid content; this result also affected the relative composition of omega 6 and omega 3 fatty acids in the samples.

## 4. Discussion

### 4.1. Biochemical analysis in hemolymph

It is known in literature that hemolymph analysis is an extremely important tool in the study of physiological or biochemical changes due to stress conditions in aquatic organisms (Digilio et al., 2016; Faggio et al., 2016; Wang et al., 2019). The importance of using hemolymph in farmed crustaceans to assess health should be adequately considered. Hemolymph is the circulatory fluid, a vital medium for transporting nutrients, respiratory gases, enzymes, metabolic wastes, and other essential substances.

Analysis of the hemolymph provides a valuable source of information on the physiological conditions of crustaceans as changes in hemolymph parameters can indicate a range of aspects regarding health (pathogenes, stress responses, immune function, metabolic activity) and general well-being. Furthermore, hemolymph analysis can help in the early detection of diseases or abnormalities in farmed crustaceans.

**Table 3**
Fatty acid composition (% of identified FAME) of abdomen muscles in *Cherax spp*.

| Fatty acids | Lipid number | *Cherax destructor* | *Cherax quadricarinatus* |
|---|---|---|---|
| Miristic acid | C14:0 | 2.02 ± 0.32 | 1.79 ± 0.25 |
| Pentadecanoic acid | C15:0 | 0.34 ± 0.10 | 0.24 ± 0.18 |
| Palmitic acid | C16:0 | 16.85 ± 3.50[a] | 15.22 ± 5.00[b] |
| Eptadecanoic acid | C17:0 | 0.80 ± 0.45 | 0.53 ± 0.30 |
| Stearic acid | C18:0 | 8.06 ± 0.09 | 8.13 ± 0.04 |
| Arachidonic acid | C20:0 | 0.73 ± 0.30[a] | 0.47 ± 0.45[b] |
| 10-pentadecanoic acid | C15:1 | 1.04 ± 0.19 | 0.93 ± 0.13 |
| 10-transpentadecenoic acid | C15:1 T | 0.93 ± 0.15 | 1.04 ± 0.20 |
| Palmitoleic acid | C16:1 | 3.04 ± 1.20[b] | 3.99 ± 1.02[a] |
| 10-eptadecanoic | C17:1 | 0.54 ± 0.08 | 0.51 ± 0.06 |
| Petroselinic acid | C18:1 C6 n12 | 0.14 ± 0.01 | 0.14 ± 0.05 |
| Oleic acid | C18:1 C9 n9 | 18.85 ± 3.50[b] | 20.79 ± 2.02[a] |
| Vaccenic acid | C18:1C11 | 3.19 ± 0.32 | 2.97 ± 0.25 |
| Linoleic acid | C18:2 n6 LA | 7.64 ± 1.02[b] | 8.57 ± 0.98[a] |
| Alfa-linoleic acid | C18:3 n3 ALA | 1.58 ± 0.08 | 1.52 ± 0.10 |
| 11–14 C-eicosadienoic acid | C20:2 n6 | 1.45 ± 0.30 | 1.24 ±0.51 |
| Eicosatrienoic acid | C20:3 n3 | 0.21 ± 0.05 | 0.18 ± 0.08 |
| Arachidonic acid | C20:4 n6 | 6.72 ± 2.50[a] | 5.60 ± 3.01[b] |
| Eicosapentaenoic acid | C20:5 n3 EPA | 18.14 ± 5.01 | 18.51 ± 4.21 |
| Nervonic acid | C24:1 n9 | 0.23 ± 0.08 | 0.27 ± 0.06 |
| Docosaesaenoic acid | C22:6 n3 DHA | 1.04 ± 0.06 | 0.99 ± 0.07 |
| Docosatetraenoic acid | C22:4 n6 | 0.40 ± 0.16[b] | 0.80 ± 0.20[a] |
| Docosapentaenoic acid | C22:5 n6 | 3.91 ± 0.99[a] | 2.95 ± 1.01[b] |

On the row: a, b = $p < 0.05$.

**Table 4**
Fatty acid profile (% of FAME) and health index of abdomen muscles in *Cherax spp*.

| Fatty acids | Lipid number | *Cherax destructor* | *Cherax quadricarinatus* |
|---|---|---|---|
| ∑ saturated | SFA | 28.80[a] | 26.32[b] |
| ∑ monounsaturated | MUFA | 27.96[b] | 30.64[a] |
| ∑ polyunsaturated | PUFA | 41.09 | 40.36 |
| ∑ unsaturated | UFA | 69.05[b] | 71.00[a] |
| ∑ PUFA / ∑ SFA | | 1.43 | 1.53 |
| ∑ ω3 | | 20.97 | 21.20 |
| ∑ ω6 | | 20.12[a] | 19.16[b] |
| ∑ ω6 / ∑ ω3 | | 0.95[a] | 0.90[b] |
| FLQ[1] | | 19.18 | 19.50 |
| H/h[2] | | 3.35 | 3.78 |

On the row: a, b = $p < 0.05$. [1] FLQ: Fish lipid quality 100 × (C22:6 n-3 + C20:5 n-3)/ΣFA.
[2] H/h: Hypocholesterolemic/hypercholesterolemic ratio (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0)

Hemolymph analysis provides a non-invasive and efficient method to monitor the health status of farmed shellfish in aquaculture environments. Mauro et al. (2022a) recently evaluated a number of haemolymphatic parameters in *C. quadricarinatus* and *C. destructor* to provide a useful tool when monitoring *Cherax spp.,* health in fish farms. Glucose, triglyceride, cholesterol and lactate dehydrogenase levels were evaluated to further knowledge in these *Cherax* species. Some authors point out that the development of new analyses is needed. Changes in glucose levels could be useful to not only signal stress conditions and alterations in homeostasis but also the onset of mechanisms used to restore balance through energy stored in tissues (Lu et al., 2015). Hyperglycemia is known to be a physiological response shown by aquatic animals under stressful or unfavorable environmental conditions (Durand et al., 2000; Speed et al., 2001; Celi et al., 2013; Nicosia et al., 2014; Vazzana et al., 2016). Hyperglycemia in crustaceans occurs due to various stress factors (temperature, salinity, oxygen levels, pollution, handling stress, or exposure to toxins) that can disrupt normal metabolic processes, causing an increase in glucose concentrations in the hemolymph (Smith et al., 2018). Such conditions trigger the activation of hormonal pathways that regulate glucose metabolism, and, in the case of crustaceans, conditions which may release stress hormones. These hormones, such as hyperglycaemic hormones or molt-inhibiting hormones, play a crucial role in modulating glucose levels by stimulating glycogenolysis and gluconeogenesis (Durand et al., 2000; Lorenzon et al., 2002; Santos et al., 2001; Bergmann et al., 2001; Toullec et al., 2002). Moreover, prolonged exposure to elevated glucose levels can compromise immune function, osmoregulation, reproductive processes, and increase susceptibility to disease (Smith et al., 2018) in crustaceans. In our study, haemolymphatic glucose levels in the two *Cherax* species (not subjected to stress conditions) were provided. Our results showed significantly lower glucose levels in *C. quadricarinatus* than in *C. destructor*. Furthermore, glucose levels obtained in *C. destructor* were similar to those reported by Morris et al. (2005) and Stara et al. (2019), although found to differ from those reported in other crustacean species (Celi et al., 2013; Banaee et al., 2019). In contrast, glucose levels in *C. quadricarinatus* were found to be higher than those reported by other authors in the same species (Prymaczok et al., 2008). Lactate dehydrogenase is an oxidoreductase enzyme that plays an important role in cellular glycolysis, converting pyruvate into lactate. It is one of the most important enzymes as it is involved in the anaerobic metabolism of carbohydrates in stress conditions. Changes in levels of this enzyme (such as an increase) are indicative of some kind of physiological response, the enactment of mechanisms for energy supply or conditions of hypoxia and cell damage. This is confirmed by Stara et al. (2019), Bhavan and Geraldine (2001) Bhavan et al. (2008) and Banaee et al. (2019), who evaluated enzymes in the hemolymph of *C. destructor*, *Macrobrachium malcolmsonii* and *Astacus* *leptodactylus* when exposed to stress conditions. Some authors have analysed the levels of lactate dehydrogenase in both the *Cherax* species used in our study (Banaee et al., 2019; Morris et al., 2005; Stara et al., 2019; Prymaczok et al., 2008; Bhavan and Geraldine, 2001; Ellis and Morris, 1995; Morris and Callaghan, 1998); no statistically significant differences were apparent from our results between the two species, although values were found to differ from those reported in literature (Banaee et al., 2019). This could depend on the type of analysis conducted, as other authors carried out enzymatic evaluations on plasma and not on serum, as we did. Our results for cholesterol and triglyceride levels showed opposite trends in *Cherax spp*. Cholesterol levels were slightly higher in *C. quadricarinatus,* although differences were not significant. Triglycerides, however, were greater in *C. destructor*. The evaluation of these parameters is important as they constitute important biomarkers for lipid metabolism alterations (Guan et al., 2016). Their consumption in animals subjected to stressful conditions can be indicative of metabolic changes, greater demand for energy, increases in the activity of lipase (responsible for the breakdown of lipids), or damage to haemocytes (Calow, 1991; Fu et al., 2007; Shi et al., 2015; Olsvik et al., 2015; Abd El-Atti and Saied, 2018; Wu et al., 2020). The results obtained in our study were different from those reported in literature regarding the same species or other crustacean species, although other studies confirmed that cholesterol levels are higher in *C. quadricarinatus* (Banaee et al., 2019; Wu et al., 2020; Safaeian et al., 2020). Cholesterol levels in hemolymph depend on diet, as crustaceans are unable to synthesize cholesterol. Higher levels found in *C. quadricarinatus* compared to *C. destructor* (although not significant, in agreement with other authors) could be simply due to a species-specific difference. Cholesterol is essential for the synthesis of other hormones and knowledge of their levels is essential to understand the molting and reproduction steps of these animals (Kumar et al., 2018). Finally, regarding the biochemical results in particular, the significantly lower glucose levels in *C. quadricarinatus* hemolymph could be due to the experimental temperature used. The crayfish of both species were held at 21 °C. This is an optimal temperature for *C. destructor* but not for *C. quadricarinatus,* which prefers temperatures ranging from 23 to 28 °C (Jones, 1990, 1995). This is likely to have impacted the metabolic activity, with probable effects on hemolymph and tissue biochemistry. Notwithstanding, our study provided base levels for these parameters for the two Cherax spp. Combined with those obtained by Mauro et al. (2021); Table 5), our results give a general picture of the different hemolymphatic parameters for these two species and provide a useful tool for successful monitoring in aquaculture facilities.

**Table 5**
Biochemical parameters evaluated in *C. destructor* and *C. quadricarinatus* hemolymph in this study and by Mauro et al. (2021).

| | *Cherax destructor* | *Cherax quadricarinatus* |
|---|---|---|
| Glucose (mmol/l) | 0.70 ±0.12 | 0.37 ±0.12 |
| Lactate dehydrogenase (U/L) | 24.28 ±14.49 | 18.2 ±10.23 |
| Cholesterol (mg/ml) | 0.17 ±0.06 | 0.27 ±0.05 |
| Triglyceride (mg/ml) | 0.05 ±0.02 | 0.04 ±0.02 |
| Total Haemocyte Count | $1.568\times10^3$ ± $560\times10^3$ | $1.678\times10^3$ $\pm707\times10^3$ |
| Esterase in cell (U/μg protein) | 0.050 ±0.016 | 0.044 ±0.017 |
| Alkaline phosphatase in cell (U/μg protein) | 0.083 ±0.036 | 0.096 ±0.043 |
| Esterase in cell-free (U/μg protein) | 0.009 ±0.004 | 0.010 ±0.004 |
| Alkaline phosphatase in cell- free fluid (U/μg protein) | 0.014 ±0.004 | 0.016 ±0.004 |
| Osmolality (mOsm) | 448 ±51.41 | 409 ±18.75 |
| pH | 7.66 ±0.09 | 7.56 ±0.10 |
| Total Protein (μg/ml) | 3409 ±1276 | 24,554 |

### *4.2. Abdomen muscle chemical analysis*

Shrimp meat is known to be rich in both organic and inorganic constituents; the main constituents being proteins, amino acids, carbohydrates, lipids and minerals (Abulude et al., 2006; Bhavan et al., 2010). Moisture, ash, lipid and crude protein levels are, therefore, important indicators when evaluating the physiological state of an organism and the food potential of the meat (Bhavan et al., 2010; Dempson et al., 2004). These parameters can change when comparing different species or individuals of different size, sex and feeding season (Barrento et al., 2009a; Rosa and Nunes, 2003; Pulina et al., 2017). Ash, crude protein, moisture and lipid levels of *Cherax spp.* muscle were analysed in our study and the results were compared with data reported in literature on both fresh and saltwater species typically used in aquaculture. Our results showed lower ash content in *C. quadricarinatus* compared to *C. destructor,* although differences were not significant. Muscle mineral content is correlated with the maintenance of an adequate acid-base balance and supports colloidal systems in crustaceans (Gunalan et al., 2013). The ash content in the *Cherax spp* we investigated is probably related to the phenomena of biomineralization and calcification, which occurs in crustaceans when synthesizing the new exoskeleton (Luquet, 2012).). In contrast, Jones (1990) reported higher ash levels in *C. quadricarinatus* compared to our results and this could be due to different feed used. Moisture levels were found to be similar in *Cherax spp.* and in agreement with the results reported in literature on the same specie (Jones, 1990). Moisture content in muscle tissue is related to mechanisms of osmoregulation in many crustacean species and provides the product with good texture (Chand et al., 2015). This characteristic helps the animals to adapt to different water salinity conditions, ensuring adequate growth. The two Cherax spp. investigated seemed to show similar osmoregulation and comparable levels of muscle moisture. Crude protein levels were lower in *C. destructor* than in *C. quadricarinatus*, although not to a significant degree. Crude protein was higher in both species compared to levels reported by Jones (1990) on the same species and similar to levels reported by other authors for *H. gammarus, H. americanus, E. sinensis, P. clarkii, P. trituberculatus*, *C. pagurus*, *P. elephas* (Barrento et al., 2009b; Kampouris et al., 2021; Banaee et al., 2019; Shao et al., 2014; Li et al., 2021; Maulvault et al., 2012; Yuan et al., 2020). The ability of the species to transform feed proteins into muscle proteins is important in the case of breeding Cherax,. Both species investigated, given their high protein content, represent good protein sources for human nutrition. Lipid levels in both *Cherax spp*. were highly comparable and also similar to other species, such as *H. gammarus*, *P. elephas* and *P. aztecus* (Kampouris et al., 2021; Banaee et al., 2019). However, they were found to be lower compared to *H. americanus*, *P. trituberculatus*, *E. sinensis*, *C. pagurus*, *P. clarkii* and *M. rosembergii* (Banaee et al., 2019; Bhavan et al., 2010; Shao et al., 2014; Li et al., 2021; Maulvault et al., 2012; Yuan et al., 2020). Lipid plays a key role in various biochemical processes in the metabolism of crustaceans. Moreover, lipid levels were lower than those obtained by Jones (1990) in the same species. In general, the meats of the two *Cherax* species showed excellent nutritional composition, both when compared with other aquatic species typically used for human nutrition and when compared with beef, pork and poultry meats. The *Cherax* species provided, in fact, a higher protein and lower fat content (Pulina et al., 2017) than these latter meats. Characterization of the two *Cherax spp*. from a chemical and nutritional point of view is important also in consideration of the limited literature on the topic.

### *4.3. Abdomen muscle fatty acid composition*

Muscle tissue has been shown in literature to provide the most stable composition as less exposed to seasonal variations (Ying et al., 2006). It is the main storage site for proteins which are characterized by a fat content composed mainly of lipids, in particular, phospholipids (Muriana et al., 2006; Chanmugam et al., 2006; Joint FAO/WHO/UNU, 2007;

New, 1986). Lipids play an important role in maintaining the integrity of cellular membranes and are a vital source of energy produced through metabolism (New, 1986; New et al., 2008; Ricardo et al., 2003). We also know that shrimp muscle contains lower lipid levels and is thus preferred by consumers (Bell and Sargent, 2003; Bhavan, 2009). In this study, fatty acid content was evaluated and differing amounts were found for the two *Cherax* species. The presence of SFA, MUFA and PUFA were detected. Saturated and monounsaturated FAs are an important source of energy, especially during larval development (Floreto et al., 2000). Polyunsaturated FAs (e.g., EPA and DHA), in contrast, are important structural components of cell membranes (Hird, 1986) and are essential for maturation, reproduction and molting (Read, 1981). The values we found in the muscle of these species were similar to those observed by other authors in the muscle of other aquaculture species, such as *C. pagurus, M. rosenbergii, P. elephas, O. aztecus* (Kampouris et al., 2021; Banaee et al., 2019; Bhavan et al., 2010). Furthermore, other authors have shown that the use of fatty acids in the diet of various aquaculture invertebrate species had significant effects on growth and survival rates (Bell and Sargent, 2003; Read, 1981; Sargent et al., 1999). According to literature, the presence of PUFA (e.g., linoleic), EPA and DHA indicated good levels of growth, survival and tolerance to possible stress conditions (Watanabe et al., 1989; Watanabe, 1993). Arachidonic acid levels were also satisfactory; this is a precursor of the hormone prostaglandin, which is essential for reproduction (Bell and Sargent, 2003; Tamaru and Ako, 2000; Tamaru et al., 1997). In general, and specifically for both species, lower quantities of saturated compared to unsaturated fatty acids were found. This is a positive result as it is known that saturated fatty acids have a less favorable effect on human health. Greater quantities of arachidonic and, above all, palmitic fatty acids determined an increase in the amount of SFA in *C. destructor*. The values of these two SFAs were in line with those found by Li et al. (2011) in freshwater shrimps. The most abundant monounsaturated fatty acids were palmitoleic and, above all, oleic, both of which were found in significantly greater quantities in *C. quadricarinatus*. From a nutritional point of view, MUFAs exert multiple effects on human health; indeed, there is ample reference in literature to indicate that MUFAs reduce the risk of contracting cardiovascular and/or inflammatory diseases (Liu et al., 2021). The omega 6 fatty acid content also differed between the two species in the study. The abundance of arachidonic acid, in particular in *C. destructor,* led to significantly greater omega 6 levels for this species. Arachidonic acid levels were similar to those of Giant freshwater prawn found by Li et al. (2011). The omega-6/omega-3 ratio was lower as a consequence, and, therefore, more favorable for *C. quadricarinatus*. However, it should be noted that this ratio was significantly lower for both species in the study than the threshold value of 5 indicated by the WHO/FAO (Di Grigoli et al., 2022). In general, it is worth mentioning that from a nutritional point of view both species showed appreciable PUFA content, primarily composed of EPA and, to a lesser extent, LA. PUFAs are thought to have a more powerful lowering effect on hypertriglyceridemia and cholesterol than SFAs (Prato et al., 2018), and EPA helps prevent and treat some diseases of the cardiovascular system (Shahidi and Ambigaipalan, 2018). The fatty acids EPA and DHA belong to the n-3 fatty acid category are considered a dietary essential (González-Félix et al., 2002) for adequate growth and development during human life. These fatty acids cannot be synthesized easily by humans and thus their intake through nutrition is considered essential. In general, n-3 fatty acids also have anti-inflammatory properties and many other key health benefits. Higher FLQ and H/h indices generally indicate better quality of the dietary fat source. Although the different acid composition found in the muscle of the two species in our study did not significantly influence the nutritional indices FLQ and H/h, these indices were deemed to be *high* for both species and higher than various other food sources considered by Chen et al. (2020). The data obtained highlight the good nutritional properties of these two *Cherax spp.* for the human diet. The characterization of the fatty acid composition of the two Cherax species is, in our opinion, important, also given the limited information currently found in literature. It provides a contribution to knowledge of particular significance as we know that the fatty acid composition and relative ratios of food sources significantly affect human health. Their anti-inflammatory and anticoagulant properties are essential in pregnancy and help reduce the risk of various diseases, such as arteriosclerosis, cardiovascular disease and strokes (Von-Schacky et al., 1999; Christensen et al., 2001).

Based on the results obtained, the Cherax species we investigated can be considered a good source of essential and healthy fatty acids. This information is useful both for consumers, who are increasingly looking for suitable dietary products, and for producers to boost their product.

## 5. Conclusions

The demand for new, high quality foods with good nutritional value is constantly growing. Haemolymphatic sampling and the evaluation of biochemical parameters is one of the most reliable methods for the clear, periodic and timely monitoring of an animal's state of health. In this study, parameters were broadened to provide a more complete panel of essential biomarkers in the evaluation of the health conditions of these organisms. To ensure the economic feasibility of these two species for aquaculture, it is essential to provide consumers with the nutritional content. Both species showed similar and satisfactory contents of minerals, lipids, proteins and moisture, when compared to other successfully marketed aquatic species. Studies like this should also be carried out on other species used in aquaculture. This would make it possible to improve the sustainability of farms, their yield and the state of health of the animals. Moreover, the study presented can be further amplified by analysing the fatty acid profile of the meat, for example, which could have significant potential. A more in-depth analysis of the other component (amino acids, for example), recognized as an important food trait, is of interest for future studies.

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## CRediT authorship contribution statement

**Giuseppe Maniaci:** Investigation, Data curation. **Marialetizia Ponte:** Methodology, Investigation, Data curation. **Manuela Mauro:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mirella Vazzana:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Antonino Di Grigoli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Investigation, Formal analysis, Conceptualization. **Marco Auculeo:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Angelica Listro:** Methodology. **Claudio Gargano:** Methodology. **Paola Bellini:** Methodology. **Pietro Chirco:** Methodology, Investigation. **Vincenzo Arizza:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Conceptualization. **Lucie Branwen Hornsby:** Writing – review & editing, Writing – original draft. **Giampaolo Badalamenti:** Methodology.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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