Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 2131 - 2152 (2024)
www.ejabf.journals.ekb.eg

Comparative Study on Genetic Variation and Nutritional Value of Freshwater Crayfish *Procambarus calrkii* in the River Nile, Egypt

Saeed M.*[1], Kilada R. [2], Saad A.A.[1], Mehanna S.F. [3], Abeer S. A[4], Khalil M.T. [1]

[1]Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt
[2]Department of Biological Sciences, University of New Brunswick (Saint John), Canada
[3]Fisheries Division, National Institute of Oceanography and Fisheries (NIOF), Suez and Aqaba Gulfs Branch, Suez, Egypt
[4]Department of Biochemistry, Animal Health Research Institute, Giza, Egypt

***Corresponding Author: dr_mohamed_2012@hotmail.com**

ARTICLE INFO

Article History:
Received: July 18, 2024
Accepted: Dec. 15, 2024
Online: Dec. 28, 2024

Keywords:
Procambarus clarkii,
Freshwater crayfish,
River Nile,
Nutritional value,
Genetic variation,
COI

ABSTRACT

Since its introduction in the early 1980s, the invasive freshwater crayfish *Procambarus clarkii* has flourished in the River Nile. Its distribution now spans from the Delta region and Giza in the north to Aswan in the south. The economic importance of this species in Egypt has increased over the past decade, as reflected in the significant rise in the export of processed and live animals to China and the USA. Crayfish processing factories along the River Nile heavily depend on fisheries targeting this species, further enhancing its economic value in Egypt. The present study aimed to assess the genetic variation in the COI gene of *P. clarkii* from the Giza and Aswan populations in comparison to native USA populations. Isolates collected from Giza were closely related to *P. clarkii*, with a similarity index of 99.41%, while isolates from Aswan were also closely related, with a similarity index of 99.26%. Aswan isolates are derived from the Giza isolates. Egyptian isolates show the closest relationship to Chinese isolates, both of which are descended from the United States isolates. Furthermore, the nutritional composition of the flesh was evaluated during different fishing seasons to assess its nutritional quality in the two locations. The levels of lipids and proteins were significantly higher in Aswan than in Giza, in both sexes and during the summer season. This suggests that the flesh from the southern regions of Egypt is of a higher quality.

INTRODUCTION

The freshwater crayfish *Procambarus clarkii*, a member of the family Cambaridae, is native to the southern and central states of the United States. Additionally, it has been introduced to every continent except Antarctica and Australia **(Loureiro *et al.*, 2015; Oficialdegui *et al.*, 2019)**. This species is suited for commercial exploitation due to a variety of life history characteristics, such as high fecundity, polytrophism, behavioral adaptability, rapid growth, and resistance to illnesses and harsh climatic conditions **(Huner & Lindqvist, 1995; Barbaresi & Gherardi, 2000; Hazlett *et al.*, 2003)**. Additionally, it has adapted effectively to

living in regions where the level of shallow waters fluctuates significantly throughout the year, often burrowing to survive **(Huner & Barr, 1984)**.

P. clarkii was introduced into Egyptian aquatic habitats for aquaculture in the early 1980s from the USA. It has spread throughout all freshwater environments, such as marshes, bonds, and streams **(Ibrahim *et al.*, 1995; Rawi, 1995; Tolba, 2017)**. It has spread quickly throughout Egypt's aquatic ecosystems, extending from the Giza Governorate to the whole Delta region in the north and Aswan Governorate in the south **(Ibrahim *et al.*, 1995; Rawi, 1995)**.

High genetic diversity results from invasive species' ability to avoid inbreeding and to adapt well to new environments **(Keller & Waller, 2002; Yue *et al.*, 2010)**. The red swamp crayfish, for example, is one of the most successful invasive species and has a significant amount of genetic diversity due to traits like rapid migratory and breeding rates **(Yue *et al.*, 2010; Liu *et al.*, 2013)**. Molecular markers, including AFLP, RAPD, SSR, and mtDNA, are used frequently to evaluate genetic diversity **(Powell *et al.*, 1996; Zhai *et al.*, 2019)**.

Due to advances in data analysis and sequencing, mtDNA has gained popularity; however, it is susceptible to the effects of genetic drift **(Filipová *et al.*, 2011)**. Maternal inheritance, resistance to genetic recombination, fast evolution, and the availability of effective PCR primers are some of the characteristics of mtDNA as a genetic marker **(Simon *et al.*, 1994; Hebert *et al.*, 2004)**. Consequently, mtDNA is considered as potent instrument for researching molecular diversity and population genetic structure **(Xu *et al.*, 2009)**.

One of the mitochondrial genes with a moderate rate of evolution and a strong intra- and interspecies evolutionary signal is cytochrome oxidase subunit I. Due to these characteristics, COI has frequently been used to conduct research on phylogeography, evolution, and genetic variants, as well as to settle taxonomic disputes in a variety of animal groups **(Avise, 2000; Cao & Wu, 2019)**. Scientists realized how useful these COI primers were for more comprehensive, systematic research on metazoan invertebrates, such as coelomate protostomes and deuterostomes, acoelomates, and pseudocoelomates **(Brown, 1985)**.

The genetic diversity of various *P. clarkii* populations has spread worldwide, both in its native and introduced habitats, based on different detection methods at various time points **(Barbaresi *et al.*, 2003; Yue *et al.*, 2008; Zhu & Yue, 2008; Yue *et al.*, 2010; Ibrasheva, 2011; Torres & Álvarez, 2012; Liu *et al.*, 2013; Zhu *et al.*, 2013; Paulson & Martin, 2014; Quan *et al.*, 2014; Radwan *et al.*, 2014; Jiang *et al.*, 2015; Huang *et al.*, 2017; Liu & Zhou, 2017; Vella *et al.*, 2017; Almerão *et al.*, 2018; Yi *et al.*, 2018; Oficialdegui *et al.*, 2019; Liu *et al.*, 2020; Zhong *et al.*, 2020; Dörr *et al.*, 2021; Guo *et al.*, 2022)**.

P. clarkii is an economic crustacean species. Its tremendous fecundity, diverse feeding preferences, rapid growth, and ease of breeding have made it a valuable aquatic resource. Over the past few years, freshwater crayfish production and consumption have skyrocketed worldwide, with China currently leading the world in crayfish production **(Rodríguez-Estival *et al.*, 2019)**. Chinese customers greatly esteem crayfish due to their distinct flavor, texture, and high nutritional content

(Souty-Grosset *et al.*, 2016; Li *et al.*, 2021; Zhou *et al.*, 2021). As the consumer market grows, crayfish processing also expands quickly **(Zhu *et al.*, 2021)**.

Regardless of this negative side of the occurance of *P. clarkii* in the Nile water, it can still be a source of food in Egypt and internationally **(Hunner, 1988; Huner *et al.*, 1988; Huner & Barr, 1991)**. The same authors discovered that, depending on size, age, and whether or not the chelae muscles are healed, the yield of its abdominal muscle varies between 10 and 40% of the total body mass. As a result, it may be regarded as a novel, inexpensive food source that is a favorite aquatic supper in Egypt and other countries **(Ibironke *et al.*, 2018)**.

The flesh of the crayfish acts as a source of protein, and it contains more phosphorus than freshwater fish. Moreover, its exoskeleton can be used as forage for animals due to the presence of iron, fats, and carbohydrates. Furthermore, the young crayfish can be used as bait in fish hunting **(Mona *et al.*, 2000a)**.

Hamdi and Zaghloul (2006) evaluated *P. clarkii* as a cheap source of human diet and males yielded more flesh than females. They also found that its muscles contained a high protein content and essential minerals which were cheaper than those in some marine shrimps.

In 2009, two studies were performed on the nutritional content of *P. clarkii* in Egypt. According to the first study's analysis of the crayfish abdomen samples' chemical composition, the mean amounts of total protein, fat, and ash were 13.88, 1.76, and 1.52%, respectively, for dry weight **(Zaglol & Eltadawy, 2009)**. Rendering to the second study, dry crayfish meat had the following percentages: 79, 40.9, 42, and 13.1% for moisture, ash, protein, and fat, respectively **(Ibrahim & Khalil, 2009)**.

Nutritional value of *P. clarkii* flesh was discussed by various authors in Egypt and all over the world **(Huner *et al.*, 1988; Elmossalami & Emara, 1999; Mona *et al.*, 2000b; Amine *et al.*, 2008; Zaglol & Eltadawy, 2009; El-Kholie *et al.*, 2012; El-Sherif & Abd El-Ghafour, 2015; Abd-Elgawad *et al.*, 2018; Li *et al.*, 2021; Farrag *et al.*, 2022; Yang *et al.*, 2023; Zhang *et al.*, 2023)**.

The present study aimed to assess the genetic variation in the mitochondrial cytochrome oxidase subunit I gene (COI) of *P. clarkii* by comparing samples from Giza and Aswan populations. The primary goal was to assess the genetic diversity between these two populations and to compare them to the native populations of *P. clarkii* from China and the USA. Additionally, the study evaluated the nutritional value of *P. clarkii* flesh (muscle) in both sexes during different fishing seasons to determine the meat quality of populations from Giza and Aswan.

MATERIALS AND METHODS

1. Study area and sampling

The River Nile flows into the Mediterranean Sea after emerging from Lake Victoria in central Africa, covering a total surface area of 3,350,000km² and a total length of 6,671km. The Egyptian sector of the Nile is about 1,532km long and has a

surface area of approximately 769,330km². The main course of the Nile extends for about 950km from the Aswan High Dam to Cairo (Fig. 1).

Crayfish samples were collected from two landing sites; Giza on the north of the Nile (29° 48´ N, 31° 16´E) and Aswan on on the south of the Nile (24° 58´ N, 32° 52´ E), during the summer and autumn of 2022 (Fig. 1).

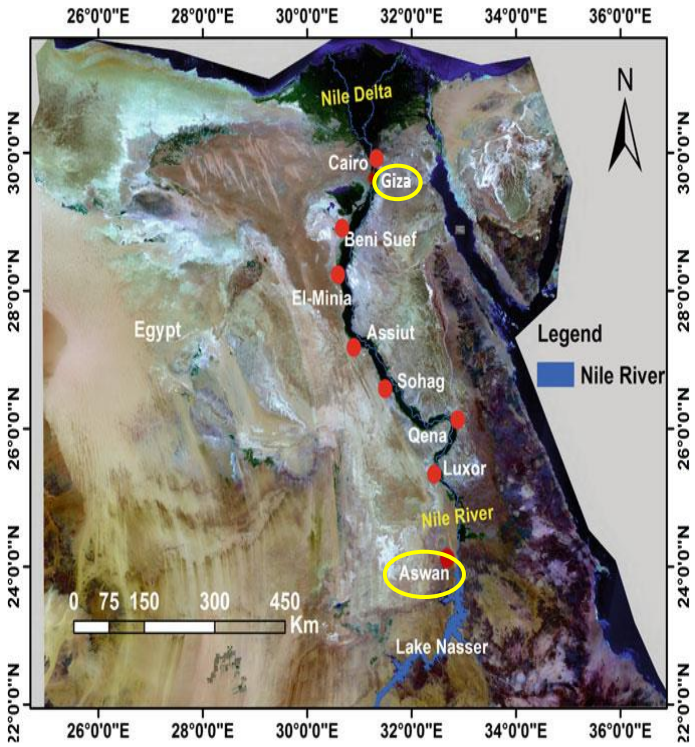

Fig. 1. Egypt map showing 2 study sites along the River Nile **(El-Rawy *et al.*, 2022)**

2. Genetic variation

2.1 DNA extraction

Eight *P. clarkii* specimens were collected in September 2021, with four from Giza and four from Aswan. The specimens were euthanized by freezing and then dissected to obtain eight fresh abdominal muscle samples. The muscle samples were immediately placed on ice to preserve the integrity of the DNA. To homogenize the tissue samples, 250µl of lysis buffer (100 mM Tris-HCl, 1.4M NaCl, 20 mM EDTA, and 10% SDS) was used. The specimens were then homogenized in CTAB extraction buffer (200 mM Tris-HCl, 1.4M NaCl, 20 mM EDTA, CTAB powder, and 2% β-mercaptoethanol), with 25µl of 20mg/ ml proteinase K added to break down cellular structures and release DNA. The homogenates were incubated for 2–3 hours at 65°C in a water bath. Chloroform-isoamyl alcohol (24:1) was used to purify the DNA, and isopropanol was added to precipitate the DNA. The DNA-containing aqueous phase was carefully transferred to a fresh tube. Isopropanol was added again to further precipitate the DNA, and the mixture was centrifuged to form a visible DNA pellet at the bottom of the tube. The DNA pellet was washed with ethanol to remove any

residual contaminants, then air-dried. Finally, the dried DNA pellet was re-suspended in sterile, DEPC-treated water and stored at -20°C until further use.

2.2 Polymerase chain reaction

The target region, approximately 700bp of the mitochondrial cytochrome c oxidase subunit I (COI) gene, was amplified using the primers described by **Folmer *et al.* (1994)**. **LCO 1490 [F]:** 5'- GGTCAACAAATCATAAAGATATTGG -3'
HCO 2198 [R]: 5'- TAAACTTCAGGGTGACCAAAAAATCA -3'

The PCR reaction was conducted in an Applied Biosystems thermal cycler. A total volume of 25µl was used to prepare the PCR reaction mixture, which included 12.5µl of 2X Master Mix, 1.5µl each of forward and reverse primers, 2.5µl of template DNA, and 7.5µl of nuclease-free water.

The PCR thermal cycling conditions were as follows: an initial denaturation step for 5 minutes at 94°C, followed by 35 cycles of 30 seconds at 94°C, 60 seconds at 60°C, and 60 seconds at 72°C, with a final extension of 7 minutes at 72°C.

2.3 Gel electrophoresis

1.5g of glass was dissolved in 100ml of 1x TAE buffer in a microwave, and then 3µl of ethidium bromide dye was added to create a 1.5% agarose gel. Molten agarose was poured onto tray with comb allowing it to solidify. Gel was transferred into electrophoresis chamber filled with buffer. After solidification, the comb was removed forming wells. DNA leader and PCR products were loaded into wells. 100 volt was applied across the gel for 50min. Nucleic acids in gel were visualized under UV light [Spectroline ^{TM}] as orange bands. Estimation of obtained band size was judged by comprising it with DNA leader.

2.4 Sequencing and analysis of COI gene

PCR products were purified using the Qiagen extraction kit according to the manufacturer's instructions before being submitted for DNA sequencing. The Macrogen facility in Korea employed the BigDye™ Terminator sequencing kit [ABI Applied Biosystems] to analyze the amplified fragments.

The resulting mitochondrial COI gene sequence was analyzed, assembled, and compared with all available sequences in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST), with the BioEdit software version 7.2.5. The nucleotide sequences were uploaded to GenBank. Multiple sequence alignments were performed using ClustalW. Gaps were reduced, and the alignment was visually verified using BioEdit 7.2.5.

2.5 Phylogenetic analysis

The mitochondrial COI gene sequences of *P. clarkii* samples were aligned and compared with the closely related sequences retrieved from the GenBank database of NCBI. Phylogenetic analysis of all isolates was carried out using MEGA version 7.0

using the neighbor-joining method with 1000 bootstrap replications **(Tamura *et al.*, 2007)**. The P-distance of nucleotide difference was used to construct Phylogenetic tree **(Saitou & Nei, 1987)**.

3. Nutritional value

The assessment of the nutritional composition of *P. clarkii* flesh is crucial for determining the meat quality obtained from populations in Giza and Aswan. This study aimed to compare key nutritional components, specifically crude protein, crude lipid, and ash contents, between crayfish populations in these two regions

To facilitate a comparison, crayfish specimens were systematically collected from both Giza and Aswan during summer and the early autumn of 2022. The selection of these seasons is strategic, aligning with the commercial fishing seasons for *P. clarkii* in the River Nile in Egypt, while commercial fishing was temporarily suspended from October to April. This suspension is in line with the crayfish spawning seasons, occurring in late autumn and late mid-spring. The careful consideration of these seasonal factors ensures that the collected specimens are representative of the optimal harvesting periods, allowing for a meaningful and accurate assessment of the nutritional value of *P. clarkii* in Giza and Aswan. Note that each parameter was determined in 5 specimens per season in each location.

3.1. Determination of crude protein contents

The crude protein content of the experimental diets was measured by Dumas's method using Tru Spec Nitrogen determinator **(AOAC, 2016a)** as follows:

The principle of this method is based on three phases, during the analysis cycle, namely: purge, combust, and analyze phases. Firstly, *P. clarkii* flesh samples were homogenized, ground, and then encapsulated. During the purge phase, the encapsulated sample was placed in the loading head, peeled, and purged of any atmospheric gases that had entered during the sample loading. In the combust phase, the sample was dropped into a burning furnace at 950°C and was flushed with oxygen for accelerating and complete combustion. For additional oxidation and particle removal, the combustion products were sent through a secondary furnace set at 850°C. A two-stage thermoelectric cooler and an extra furnace filter were employed to remove moisture. After that, the combustion gasses were gathered in the ballast, a collection vessel. Oxygen was fed into the ballast and combined with the combustion gasses during the analyze phase. After that, a 3ml aliquot loop was used to purge the ballast's homogeneous combustion gasses. Once the gases had equilibrated, they were transferred to a helium carrier flow, swept through hot copper to remove oxygen, change nitrogen dioxide (NO_2) to molecular nitrogen (N_2), and then flowed through a Lecosorb and anhydrone to remove carbon dioxide (CO_2) and water (H_2O), respectively. A thermal conductivity cell was used to determine the nitrogen content. The final result was represented as a weight percentage. The crude protein was calculated via the multiplication of the nitrogen content by a conventional factor of 6.25.

3.2. Determination of crude lipid contents

The crude lipid content in flesh specimens of crayfish was determined according to the method of the **AOAC (1995)** as follows:

Two parts from the sample (0.5-1g) were taken. The two parts were placed in two weighed ashless filter paper in a soxhelet apparatus (500ml capacity). Petroleum ether (60-80 °C) was used as a fat solvent, and the extraction period ranged between 16-18 hours. The insoluble residue for each sample was dried in the oven at 80ºC for 4 hours, and the constant weight was recorded. The difference in weight gave the amount of extract of the present lipids, which was calculated as a percentage.

3.3 Determination of ash contents

The ash content of the flesh samples of *P. clarkii* was determined according to the method of **AOAC (1995)** by using a muffle furnace. Part of the fine powder of the samples (1g) was put in a weighed porcelain crucible and incinerated in the muffle furnace at 550°C for 7 hours. The crucible containing the ash was placed in a desiccator and was then weighed. The residue remaining in the crucible indicates the amount of ash present in the flesh sample.

4. Statistical analysis

Statistical analysis was conducted by one-way ANOVA test to determine if there were significant differences in mean values of protein, lipids, and ash contents in the flesh of *P. clarkii* between locations, seasons, and sexes. IBM SPSS Statistics 27.0.1 software was used for all statistical analyses.

RESULTS AND DISCUSSION

1. Genetic variation

The nucleotide sequence of the mitochondrial COI gene from eight isolates was subjected to nucleotide BLAST analysis using the NCBI database. Four isolates collected from Giza were closely related to *P. clarkii* with a similarity index of 99.41% while other isolates collected from Aswan were closely related to *P. clarkii* with a similarity index of 99.26%. The sequences of all isolates were submitted in GenBank as *P. clarkii* isolate PC-Gi-1, PC-Gi-2, PC-Gi-3, PC-Gi-4, PC-As-5, PC-As-6, PC-As-7, PC-As-8 with accession numbers OR016165.1, OR016166.1, OR016167.1, OR016168.1, OR018993.1, OR018994.1, OR018995.1, and OR018996.1, respectively.

The present results were supported by phylogenetic analysis using the neighbor-joining tree method with 1000 bootstrap replications compared with isolates collected from China and the United States (Fig. 2). Phylogenetic analysis indicated that the isolates from Egypt were placed within the *P. clarkii* clade. The Aswan isolates are originated from the Giza isolates. The Egyptian isolates are most closely related to those from China, and both are derived from the United States isolates. All isolates showed a reduced genetic variation compared to the native isolates.

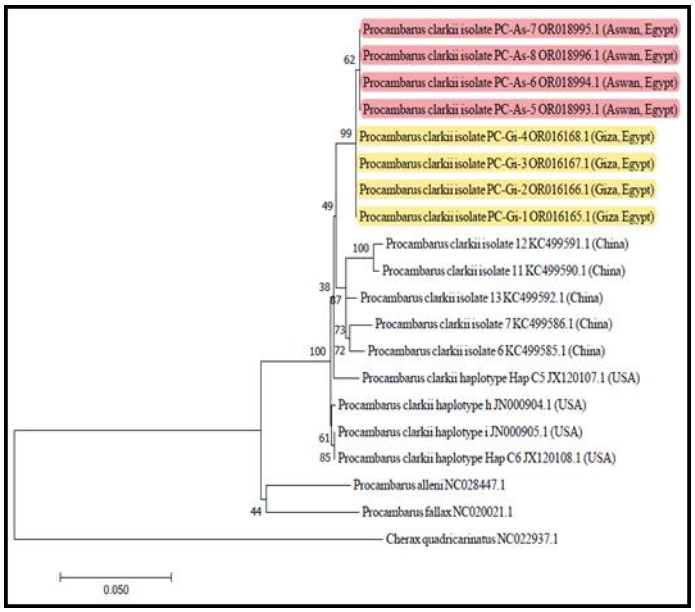

Fig. 2. COI gene sequence of local isolates of *P. clarkii* and its relatives: a neighbor-joining phylogenetic tree. Bootstrap values retrieved from 1000 trees are represented by the numbers on the nods. A 5% nucleotide substitution is shown by the bar. The current isolates' accession numbers were highlighted.

One of the primary studies to assess the genetic variation of *P. clarkii* is that of **Busack (1988)**. They used enzyme electrophoresis to investigate nine populations in the southeastern United States, spanning from Illinois to Texas, which represents the species' natural range. Busack's findings revealed little variation between populations, which was believed to be due to the species' likely recent emergence in the late Miocene, when much of its present native range was formed. In both the current study and that of **Busack (1988)**, little genetic variation was identified, regardless of the number of organisms introduced, based on the genetic similarity of populations from the native range.

It is noteworthy that even after more than 80 years, the exotic populations from east-central China have not significantly differentiated from native populations of *P. clarkii* **(Yue *et al.*, 2010)**. No populations were recorded in Europe after more than 35 years of invasion **(Barbaresi *et al.*, 2007)**.

Using microsatellites, **Yue *et al.* (2010)** found a "significant heterozygote deficit" in six *P. clarkii* populations they examined in China, including one that was the original population introduced in 1929. The authors suggested that recent bottlenecks are the cause of these populations' reduced genetic diversity.

Barbaresi *et al.* (2007) acquired 16S and COI mitochondrial gene sequences of *P. clarkii* from 12 populations in Italy, Spain, France, Portugal, and Switzerland. The 16S sequences showed only one haplotype with no variation, while six haplotypes were detected for COI, with one haplotype found in 10 of the 12 populations.

In the latter case, however, all of the haplotypes were highly similar, with the exception of one or two nucleotide alterations. In the present study, little genetic variation was detected. Isolates collected from Giza and Aswan were closely related to *P. clarkii*, with similarity indices of 99.41 and 99.26%, respectively. Egyptian isolates were most closely related to Chinese isolates, and both of these groups were more closely related to the United States isolates.

In contrast to the results described above, **Barbaresi *et al.* (2003)** found an "unexpectedly high" level of genetic variation in *P. clarkii* populations from Portugal and Italy using RAPD markers, linking this pattern to repeated importing into the same regions. According to the previously discussed studies, and unlike the findings reported here, *P. clarkii* shows less genetic variation both within its native range and across a variety of introduced regions.

Oficialdegui et al. (2019) found the highest haplotype diversity of *P. clarkii* in its native range in the USA. The majority of haplotypes found in other invaded areas around the world also appeared in Louisiana but not in other native populations of the USA or Mexico (Fig. 3). This pattern is likely linked to the commercial exploitation of this species in Louisiana **(Gary, 1975; Alford *et al.*, 2017)**.

The question of how such a species could become a successful invader remains, given the limited genetic variation discovered for *P. clarkii* in this and earlier studies. A recent analysis suggests that many introduced species have experienced genetic bottlenecks but have still managed to establish and thrive in new habitats **(Dlugosch & Parker, 2008)**.

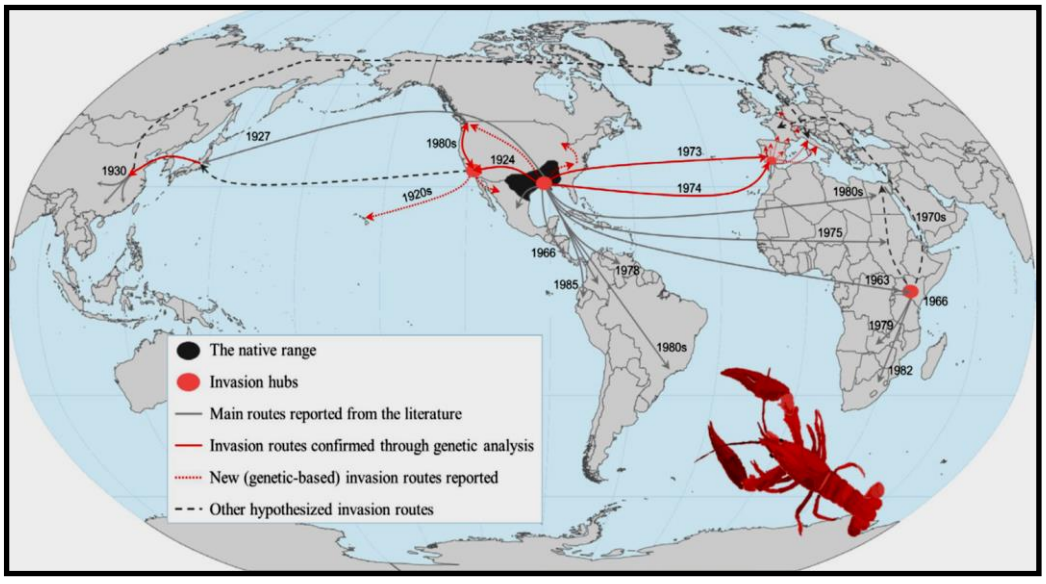

Fig. 3. The global invasion routes of the red swamp crayfish, *Procambarus clarkii*, based on mitochondrial DNA **(Oficialdegui *et al.*, 2019)**

2. Nutritional value

The values of these parameters for the fishing season in the two locations are presented in Table (1). The one-way ANOVA test was conducted to determine if

there were significant differences in the nutritional value of crayfish from Giza and Aswan (Table 2).

In Giza, fresh crayfish meat had the following constituents in summer for males and females, respectively, on a wet weight basis: crude protein 14.28, 17.11%; fat 4.37, 4.69%; and ash 1.58, 1.53%. In autumn, the values were: crude protein 11.66, 12.89%; fat 3.26, 3.55%; and ash 1.67, 1.61% for males and females, respectively (Table 1).

In the Giza region, the percentages of crude protein and crude lipids were significantly higher in females compared to males in each season. When considering the seasons, both sexes displayed significantly higher values for these two parameters in summer compared to autumn. Additionally, the percentages of ash content were significantly higher in males than in females during the same season, and also higher in both sexes in autumn than in summer (Fig. 4A).

In Aswan, the flesh of *P. clarkii* had the following constituents in summer for males and females, respectively, on a wet weight basis: crude protein 17.3, 23.4%; fat 4.57, 5.08%; and ash 1.12, 0.95%. In autumn, the values were: crude protein 12.51%, 14.37%; fat 4.23%, 4.51%; and ash 1.21%, 1.13% for males and females, respectively (Table 1).

Similar to the observations in Giza, the percentages of protein and lipids in Aswan exhibited analogous patterns. The values for both parameters were higher in females compared to males in each season, and there was a noticeable increase in both sexes during the summer compared to the autumn. The percentage of ash content was significantly higher in male samples compared to females in both summer and autumn. Moreover, the percentage of ash was significantly higher in both sexes in autumn than in summer (Fig. 4B).

When comparing the data from the two locations, Giza and Aswan, it is evident that the levels of crude protein and crude lipids were significantly higher in Aswan than in Giza in both sexes and during the summer season. This observation suggests a higher quality of flesh from the southern regions of Egypt. This conclusion is further supported by the lower ash contents in Aswan samples, confirming the superior quality of flesh for consumption (Fig. 4C, D).

The chemical composition and nutritional values of fish and shellfish vary greatly depending on a number of factors, such as species, nutritional level, diet, harvest season, catch location, and environmental conditions **(Tanakol *et al.*, 1999; Guillaume, 2001; Berge *et al.*, 2004; Bayissa, 2021)**.

Various types of shellfish are harvested globally for human consumption. The consumable parts mainly consist of the muscular portions, which are considered a

source of high-quality nutritional protein. Consequently, the findings of the present study confirm that *P. clarkii* is an excellent food source, owing to its high protein levels and satisfactory fat content. This makes *P. clarkii* a valuable source of animal protein **(Huner & Barr, 1991; Soliman *et al.*, 1998)** for the Egyptian population, many of whom suffer from malnutrition.

Table 1. Percentages of protein, lipid, and ash content in the flesh of red swamp crayfish *P. clarkii* during fishing seasons in Giza and Aswan (n=5 for each value)

Location	Season	Sex	Protein			Lipids			Ash		
			%	SD	SE	%	SD	SE	%	SD	SE
Giza	Summer	M	14.28	0.192	0.086	4.37	0.101	0.045	1.58	0.110	0.049
		F	17.11	0.143	0.064	4.69	0.109	0.049	1.53	0.084	0.037
	Autumn	M	11.66	0.096	0.043	3.26	0.110	0.049	1.67	0.094	0.042
		F	12.89	0.188	0.084	3.55	0.084	0.037	1.61	0.109	0.049
Aswan	Summer	M	17.3	1.592	0.712	4.57	0.073	0.032	1.12	0.079	0.034
		F	23.40	0.258	0.115	5.08	0.192	0.086	0.95	0.07	0.031
	Autumn	M	12.51	0.057	0.025	4.23	0.233	0.104	1.21	0.074	0.033
		F	14.37	0.145	0.065	4.51	0.143	0.064	1.13	0.083	0.037

Table 2. Two–way ANOVA to show the difference in biochemical composition of *P. clarkii* between locations, sexes, and seasons

Parameter	Source	F ratio	*P*-values
Protein	location	248.489	< 0.001
	season	782.283	< 0.001
	sex	264.973	< 0.001
Fat	location	199.663	< 0.001
	season	315.161	< 0.001
	sex	62.017	< 0.001
Ash	location	310.938	< 0.001
	season	15.900	< 0.001
	sex	9.840	0.004

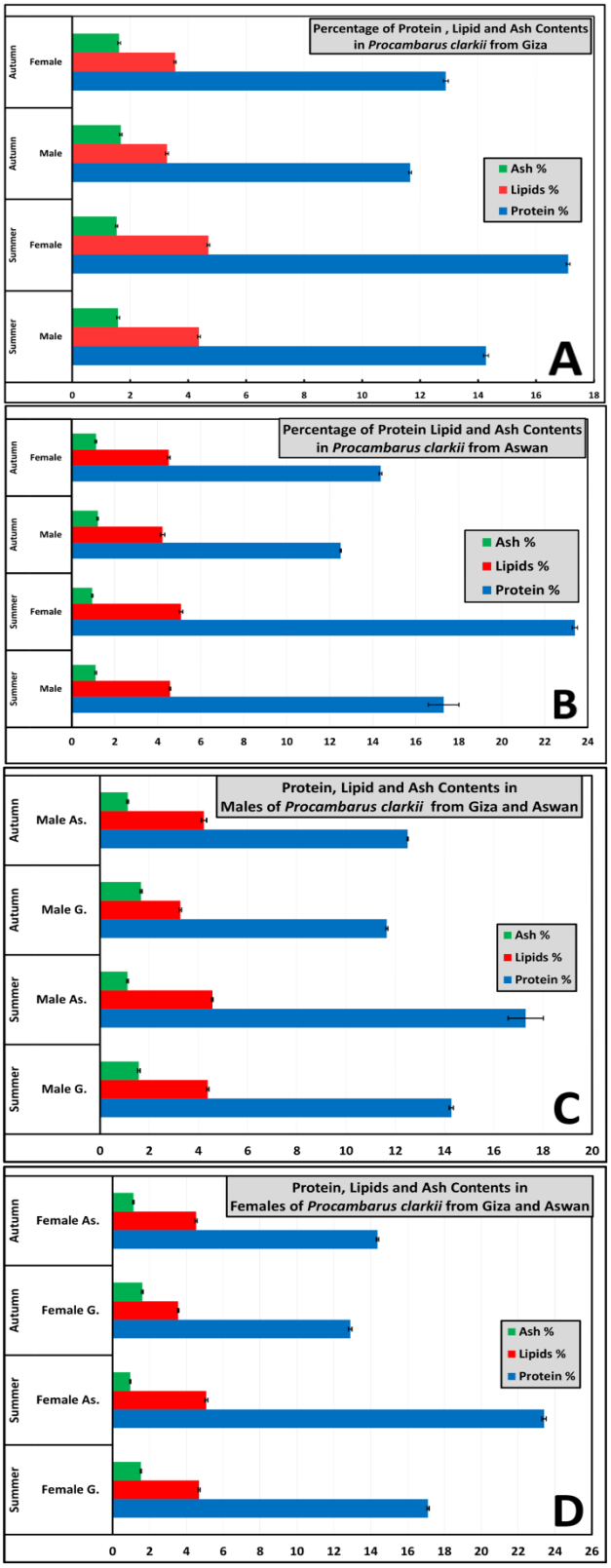

Fig. 4. Percentage of protein, lipid, and ash values in the flesh of *P. clarkii* during the fishing season in summer and autumn. **(A)** Giza. **(B)** Aswan. **(C)** Males in Giza and Aswan. **(C)** Females in Giza and Aswan

Elmossalami and Emara (1999) analyzed the chemical composition of *P. clarkii* meat harvested from riverbanks in Cairo and Giza. They found that the mean values for crude protein, crude fat, and ash contents were 15.6, 0.59, and 1.51%, respectively. These findings align with the present study, particularly in terms of crude protein values during summer in Giza, which were lower than those in Aswan in summer but higher than those in autumn in both locations. Regarding ash content, this study concurs with the values from Giza and surpasses those from Aswan. However, in terms of fat content, the values from the present study were significantly lower than those observed in **Elmossalami and Emara**'s **(1999)** study.

Mona *et al.* (2000b) investigated the nutritive value of *P. clarkii* samples collected from El-Agezy drain in Tanta City, Gharbia Governorate. They reported the following protein, fat, and ash levels for both males and females: 15.6, 23.5% for protein; 4.1, 1.8% for fat; and ash contents of 5.4, 5.2%. The protein content in males aligns with the corresponding value in our study during summer. Additionally, the protein content in females is comparable to that of females collected during the summer in Aswan in the present study. Regarding fat content, the values from this study correspond with our study on females but are notably lower than those recorded for males in all instances. The ash content values in this study are approximately three times higher than those found in our study, both for males and females.

Amine *et al.* (2008) investigated the chemical composition of the red swamp crayfish meat sourced from various markets in Alexandria, Egypt. Their findings revealed protein percentages ranging from 14.9 to 22.66%, fat from 0.84 to 1.33%, and ash from 1.064 to 1.8%. The protein and ash content ranges in their study align with our results, while the fat content range is notably lower than what we observed.

El-Kholie *et al.* (2012) conducted a study on the chemical composition of crayfish flesh collected from the River Nile between October and December 2010. They reported protein, lipid, and ash contents of 19.77, 1.99, and 1.45%, respectively. The protein content in their study is higher than all values observed in the present study, except for the protein content in females collected during the summer in Aswan, which was 23.4%. The fat content is lower than that recorded in the present study, but the ash content is similar to our findings.

El-Sherif and Abd El-Ghafour (2015) performed a study on the chemical composition of crayfish flesh collected from El-Kanater fish market, Egypt. The reported percentages, for protein, lipid, and ash contents, of 15.22, 1.29, and 1.18%, respectively. The protein content aligns with that of specimens collected in the summer in our study. However, the fat content is lower than what we observed, while the ash content corresponds with our findings.

Outside Egypt, several studies have explored the nutritional composition of *P. clarkii*. For example, **Huner and Barr (1991)** examined crayfish in Louisiana, USA, revealing protein, lipid, and ash contents of 17.13, 2.83, and 1.05%, respectively. In Shanghai, China**, Li *et al.* (2021)** assessed the nutritional quality of *P. clarkii* muscle cultivated using a commercial diet and biofloc technology. Their findings indicated protein values of 19.48 and 19.52%, lipid values of 0.89 and 0.91%, and ash values of

1.41 and 1.39% for specimens reared on the commercial diet and biofloc, respectively.

Zhang *et al.* (2023) compared the nutritional qualities of *P. clarkii* meat from aquaculture ponds, rice fields, and wild environments in Jiangsu Province, China. They found that the percentage of crude protein was 15.5% in rice field specimens, 17.91% in wild-caught specimens, and 18.95% in aquaculture pond specimens. They also observed that the lipid contents in specimens from rice fields and ponds were 1.1 and 1.19%, respectively, which were higher than those in wild samples. Finally, they reported that the highest ash level, 1.65%, was found in pond specimens. These findings, influenced by diverse environmental conditions, food composition, and growth periods **(Miao *et al.*, 2020)**, suggest that crayfish in cultivated ponds exhibit accelerated growth and increased body mass at harvest **(Xiong *et al.*, 2020)**. Pond and rice-field crayfish are fed various diets, including compound feed, minced meat, and plant-based feed such as wheat and rice bran, as well as vegetable leaves **(Zou & Zhang, 2015)**. In contrast, wild crayfish do not receive artificial feeding, which causes variations in the chemical composition of crayfish meat.

In the present study, the values for crude protein and crude lipid were significantly higher in Aswan than in Giza for both sexes and during the summer season. In both locations, females exhibited higher values than males (Tables 1, 3). **Fernandes *et al.* (1994)** studied *P. clarkii* in the River of Portugal and found considerable seasonal variation in total lipid levels. Lipid levels increased in spring, declined in the summer, and remained stable throughout winter. As a result, *P. clarkii* does not accumulate large amounts of food for use during winter. Furthermore, the reproductive cycle of the species, rather than its dietary cycle, is linked to seasonal variations in total lipid levels. Seasonal variations in protein levels were minimal and did not appear to be related to nutritional metabolism. The authors also noted that the nutritive content was higher in females than in males. Additionally, **Schirf *et al.* (1987)** reported that winter food deprivation in *P. clarkii* resulted in a significant decrease in muscle lipid and protein contents.

It is well known that protein levels can influence the gonadal development of fish and crayfish. Higher protein and lipid levels in females of *P. clarkii* in the present study may be attributed to the demands of ovarian development. **Lu *et al.* (2020)** reported that higher protein contents in females of *P. clarkii* are essential for the development of oocytes, especially in the late stages. For instance, **Gunasekera *et al.* (1995)** reported that a high-protein diet promoted earlier sexual maturation in the Nile tilapia (*Oreochromis niloticus*), while a low-protein diet (10% protein) delayed oocyte growth and resulted in fewer eggs or changes in their chemical composition. The American lobster (*Homarus americanus*) relies primarily on protein for reproduction, and a high-protein diet facilitated the development of its gonadal organs. Rodríguez-**González *et al.* (2009)** investigated the effects of changing protein levels on gonadal growth in the red claw crayfish (*Cherax quadricarinatus*) and

found that high protein levels resulted in elevated protein content in the hepatopancreas, which was subsequently made available for gonadal development. **Rodríguez-González *et al.* (2014)** further noted that protein content in the female red claw crayfish influenced the growth and survival of their juveniles significantly.

Table 3. Percentage of crude protein, crude lipid, and ash contents in flesh of *P. clarkii* from different studies in Egypt and other countries

Location	Source	Protein %	Lipids %	Ash %	Author
Giza, Egypt	Summer, Male	14.28	4.37	1.58	Present study
Giza, Egypt	Summer, Female	17.11	4.69	1.53	
Giza, Egypt	Autumn, Male	11.66	3.26	1.67	
Giza, Egypt	Autumn, Female	12.89	3.55	1.61	
Aswan, Egypt	Summer, Male	17.3	4.57	1.12	
Aswan, Egypt	Summer, Female	23.4	5.08	0.95	
Aswan, Egypt	Autumn, Male	12.51	4.23	1.21	
Aswan, Egypt	Autumn, Female	14.37	4.51	1.13	
Giza, Egypt	Combined sexes	15.6	0.59	1.51	Elmossalami 1999
Tanta, Egypt	Male	15.6	4.1	5.4	Mona *et al.*, (2000b)
Tanta, Egypt	Female	23.5	1.5	5.2	
Alexandria, Egypt	Combined sexes	14.9 to 22.66	0.84 to 1.33	1.06 to 1.8	Amine *et al.*, (2008)
Egypt	Combined sexes	19.77	1.99	1.45	El-Kholie *et al.*, (2012)
El-Kanater, Egypt	Combined sexes	15.22	1.29	1.18	El-Sherif and Abd El-Ghafour, (2015)
Louisiana, USA	Combined sexes	17.13	2.83	1.05	Huner and Barr, (1991)
Shanghai, China	Feed on Commercial diet	19.48	0.89	1.41	Li *et al.*, (2021)
Shanghai, China	Feed on Biofloc	19.52	0.91	1.39	
Jiangsu, China	Aquaculture Pond	18.95	1.19	1.65	Zhang *et al.*, (2023)
Jiangsu, China	Rice field	15.5	1.1	-	
Jiangsu, China	Wild caught	17.91	-	-	

CONCLUSION

This study analyzes mitochondrial COI gene variations in eight *P. clarkii* samples from Egypt. Four isolates from Giza showed a 99.41% similarity to native *P. clarkii* from the USA, while those from Aswan showed a 99.26% similarity. Biochemical constituents fluctuated during the fishing seasons in Giza and Aswan, with higher concentrations of crude protein and fat in Aswan. This suggests that the flesh quality in southern Egypt is superior. Future research should focus on assessing

genetic variability among different crayfish populations and investigating seasonal changes in the chemical composition of *P. clarkii* flesh across different Egyptian governorates.

REFERENCES

Abd-Elgawad, A.I.; Qassem, A.E. and Ghoneim, G.A. (2018). Preparation untraditional burger by using crayfish (*Procambarus clarkii*). *J. Food Dairy Sci.,* **9**(7)**:** 245-250.

Alford, A.B.; Kaminski, R.M.; Grado, S.C.; D'Abramo, L.R. and Avery, J.L. (2017). Harvest of crayfish as an ecosystem service of wetlands compared to production systems with planted forage. *Aquac. Econ. Manag.,* **21**(2)**:** 295-313.

Almerão, M.P.; Delaunay, C.; Coignet, A.; Peiró, D.F.; Pinet, F. and Souty-Grosset, C. (2018). Genetic diversity of the invasive crayfish *Procambarus clarkii* in France. *Limnol.,* **69:** 135-141.

Amine, T.M.; Aiad, A.S. and Abu El-Nile, M. (2008). Evaluation of Chemical Quality and Nutrition Value of Fresh Water Cray Fish (*Procambarus clarkii*). *J. High Inst. Public Health,* **38:** 1-13.

AOAC (1995). Official Methods of Analysis of the Association Official Analytical Chemists, vol. 1. 16th edition. . *Association of Official Analytical Chemists* **vol. 1. AOAC, Arlington, USA. 1298 pp.**

AOAC (2016a). Dumas method no. 968.06. In: ″Official Methods of Analysis″. 20th edition. . *Association of Official Analytical Chemists* **Latimer, G.W.Jr. (ed.). AOAC, INC. Suite, Rockville, Maryland 2850-3250, USA. Chapter 4.**

Avise, J.C. (2000). *Phylogeography: the history and formation of species*. Harvard university press.

Barbaresi, S. and Gherardi, F. (2000). The invasion of the alien crayfish *Procambarus clarkii* in Europe, with particular reference to Italy. *Biol. Inv.,* **2:** 259-264.

Barbaresi, S.; Fani, R.; Gherardi, F.; Mengoni, A. and Souty-Grosset, C. (2003). Genetic variability in European populations of an invasive American crayfish: preliminary results. *Biol. Inv.,* **5:** 269-274.

Barbaresi, S.; Gherardi, F.; Mengoni, A. and Souty-Grosset C. (2007). Genetics and invasion biology in fresh waters: a pilot study of *Procambarus clarkii* in Europe. *Biol.inv. inland waters: Profiles, distribution, threats***:** 381-400.

Bayissa, T.N. (2021). Impact of species and environment on the distribution of nutrients in fish. PhD thesis, Ghent University.

Berge, G.M.; Ruyter, B. and Åsgård, T. (2004). Conjugated linoleic acid in diets for juvenile Atlantic salmon (*Salmo salar*); effects on fish performance, proximate composition, fatty acid and mineral content. *Aquac.,* **237**(1-4)**:** 365-380.

Brown, W.M. (1985). The mitochondrial genome of animals. In "Molecular Evolutionary Genetics"(RJ MacIntyre, Ed.). *Plenum Press, New York. Clary, DO, and Wolstenholme, DR (1985). The mitochondrial DNA molecule of*

Drosophila yakuba: Nucleotide sequence, gene organization, and genetic code. J. Mol. Evol., **22:** 252-271.

Busack, C.A. (1988). Electrophoretic variation in the red swamp (*Procambarus clarkii*) and white river crayfish (*P. acutus*)(Decapoda: Cambaridae). *Aquac.,* **69**(3-4)**:** 211-226.

Cao, L. and Wu, K. (2019). Genetic diversity and demographic history of globe skimmers (Odonata: Libellulidae) in China based on microsatellite and mitochondrial DNA markers. *Sci. rep.,* **9**(1)**:** 8619.

Dlugosch, K.M. and Parker, I.M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol. Resources,* **17**(1)**:** 431-449.

Dörr, A.J.M.; Scoparo, M.; Cardinali, I.; La Porta, G.; Caldaroni, B.; Magara, G.; Pallottini, M.; Selvaggi, R.; Cenci-Goga, B. and Goretti, E. (2021). Population ecology and genetic diversity of the invasive alien species *Procambarus clarkii* in Lake Trasimeno (Italy). *Biol.,* **10**(10)**:** 1059.

El-Kholie, E.M.; Khader, S.A. and Abdelreheem, M.A.T. (2012). Chemical, physical, microbiological and quality attributes studies on River Nile crayfish. *Afr. J. Biotech.,* **11**(51)**:** 11262-11270.

El-Rawy, M.; Fathi, H.; Abdelrady, A. and Negm, A.M. (2022) Environmental impacts of treated wastewater contaminates on groundwater quality in the Nile River valley, Egypt. In *Sustainability of Groundwater in the Nile Valley, Egypt.* Springer, 91-120.

El-Sherif, S.A.E. and Abd El-Ghafour, S. (2015). Nutritive value of canned River Nile Crayfish (*Procambarus clarkii*) products. *Egypt. J. Aquat. Res.,* **41**(3)**:** 265-272.

Elmossalami, M.K. and Emara, M.T. (1999). Safety and quality of fresh water crayfish *Procambarus clarkii* in the river Nile. *Food Nahrung,* **43**(2)**:** 126-128.

Farrag, M.S.; El-Geddawy, M.A. and Ahmed, S.A. (2022). More evidences for the nutritional quality and future exploitation of the invasive crayfish *Procambarus clarkii* (Girard, 1852) from the River Nile, Egypt. *Egypt. J. Aquat. Res.,* **48**(2)**:** 151-156.

Fernandes, M.A.S.; Mendonça, M.I.R.; Marques, J.C. and Madeira, V.M. (1994). Seasonal changes in the biochemical composition and energy content of the red swamp crayfish *Procambarus clarkii* (girard) in the lower Mondego river valley, Portugal. . *J. Crust. Biol.,* **14(4):** 736-743.

Filipová, L.; Lieb, D.A.; Grandjean, F. and Petrusek, A. (2011). Haplotype variation in the spiny-cheek crayfish Orconectes limosus: colonization of Europe and genetic diversity of native stocks. *J. N. Am. Benthol. Soc.,* **30**(4)**:** 871-881.

Folmer, O.; Black, M.; Hoeh, W.; Lutz, R. and Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.,* **3**(5)**:** 294-299.

Gary, D.L. (1975).The geography of commercial crayfish ponds in south Louisiana. *J. Fresh. Crayfish*, **2:** 117-124.

Guillaume, J. (2001). *Nutrition and feeding of fish and crustaceans*. Vol. 408. Springer Verlag, 2001.

Gunasekera, R.M.; Shim, K.F. and Lam, T.J. (1995). Effect of dietary protein level on puberty, oocyte growth and egg chemical composition in the tilapia, *Oreochromis niloticus* (L.). *Aquac.,* **134**(1)**:** 169-183.

Guo, X.; Liu, G.; Zhou, Y.; Wei, W.; Li, Z.; Zhou, L.; Wang, Z.W. and Gui, J. (2022). Genetic Diversity Evaluation and Population Structure Analysis of Red Swamp Crayfish (*Procambarus clarkii*) from Lakes and Rice Fields by SSR Markers. *Fish.* **7**(4)**:** 142.

Hamdi, S.A.H. and Zaghloul, K.H. (2006). Evaluation of the crawfish *Procambarus clarkii* as a cheaper source of human diet in comparison with two marine shrimps in Egypt. *J. Egypt. Ger. Soc. Zool.,* **50**(D)**:** 153.

Hazlett, B.A.; Burba, A.; Gherardi, F. and Acquistapace, P. (2003). Invasive species of crayfish use a broader range of predation-risk cues than native species. *Biol. Inv.,* **5:** 223-228.

Hebert, P.D.N.; Penton, E.H.; Burns, J.M.; Janzen, D.H. and Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. *Proc. Nation. Acad. Sci.,* **101**(41)**:** 14812-14817.

Huang, J.; Tang, S.; Cai, F.; Lin, Y. and Wu, Z. (2017). Microsatellite evidence of dispersal mechanism of red swamp crayfish (*Procambarus clarkii*) in the Pearl River basin and implications for its management. *Sci. rep.,* **7**(1)**:** 8272.

Huner, J.V. and Barr, J.E. (1984). Red swamp crawfish: Biology and exploitation. Louisiana sea grant college program. Center for Wetland Resources, Lousiana State Univ., 1984.

Huner, J.V.; Lindqvist, O.V. and Könönen, H. (1988). Comparison of morphology and edible tissues of two important commercial crayfishes, the noble crayfish, Astacus astacus Linné, and the red swamp crayfish, *Procambarus clarkii* (Girard)(Decapoda, Astacidae and Cambaridae). *Aquac.,* **68**(1)**:** 45-57.

Huner, J.V. and Barr, J.E. (1991) *Red swamp crawfish: biology and exploitation*. Louisiana sea grant college program. Center for Wetland Resources, Lousiana State Univ., 1991.

Huner, J.V. and Lindqvist, O.V. (1995). Physiological adaptations of freshwater crayfishes that permit successful aquacultural enterprises. *Am. Zool.* **35**(1)**:** 12-19.

Hunner, J.V. (1988). Comparison of the morphology and meat yields of red swamp crawfish and white river crawfish. *Crawfish Tales*, **7**(2)**:** 29-30.

Ibironke, S.I.; Adepeju, A.B.; Otutu, O.; Oyedele, D.S. and Esan, Y.O. (2018). Nutritional evaluation and comparison study of seafoods such as fish and crayfish supplement dietary. *Food Process. Tech.,* **1**(6)**:** 75-78.

Ibrahim, A.M.; Khalil, M.T. and Mubarak, F.M. (1995). On the feeding behavior of the exotic crayfish *Procambarus clarkii* in Egypt and its prospects in the biocontrol of local vector snails. *J. Union Arab. Biol.,* **4:** 321-340.

Ibrahim, A.M. and Khalil, M.T. (2009). *The red swamp crayfish in Egypt.* 1st edn. Publication of the Center of Research and Studies of Protectorates, Ain Shams University, Cairo, Egypt.

Ibrasheva, D. (2011). Phylogenetic Analysis of Freshwater Crayfish of Massachusetts: The genus *Procambarus*. PhD thesis, *Worcester polytechnic inst*.

Jiang, H.; Qian, Z.; Lu, W.; Xing, Z.; Yu, H. and Li, J. (2015). Microsatellite marker identification from transcriptome derived sequences of the red swamp crawfish, *Procambarus clarkii. Conserv. genet. res.,* **7:** 729-731.

Keller, L.F. and Waller, D.M (2002). Inbreeding effects in wild populations. *Trends ecol. evol.,* **17**(5)**:** 230-241.

Li, J.; Huang, J.; Li, C.; Zhang, Y.; Wang, Y.; Hou, S.; Cheng, Y. and Li, J. (2021). Evaluation of the nutritional quality of edible tissues (muscle and hepatopancreas) of cultivated *Procambarus clarkii* using biofloc technology. *Aquac. Rep.,* **19:** 100586.

Liu, F.; Qu, Y.; Geng, C.; Wang, A.; Zhang, J.; Li, J.; Chen, K.; Liu, B.; Tian H. and Yang W. (2020). Analysis of the population structure and genetic diversity of the red swamp crayfish (*Procambarus clarkii*) in China using SSR markers. *Elect. J Biotech.,* **47:** 59-71.

Liu, G.; Zhou, L.; Li, X. and Lu, D. (2013). Population genetic structure of the invasive red swamp crayfish in China revealed by ITS1 variation. *Biochem. genet.,* **51:** 841-852.

Liu, G. and Zhou, L. (2017). Population genetic structure and molecular diversity of the red swamp crayfish in China based on mtDNA COI gene sequences. *Mitochond. DNA Part A,* **28**(6)**:** 860-866.

Loureiro, T.G.; Anastácio, P.M.; Araujo, P.B.; Souty-Grosset, C. and Almerão, M.P. (2015). Red swamp crayfish: biology, ecology and invasion-an overview. *Nauplius* **23:** 1-19.

Lu, X.; Peng, D.; Chen, X.; Wu, F.; Jiang, M.; Tian, J.; Liu, W.; Yu, L.; Wen, H. and Wei, K. (2020). Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (*Procambarus clarkii*). *Aquac. Rep.,* **18:** 100542.

Miao, S.; Han, B.; Li, J.; Hu, J.; Wan, W.; Sun, L. and An, Z. (2020). Effects of dietary protein level on the growth performance, feed utilization and immunity of red swamp crayfish *Procambarus clarkia*. *Aquac. Rep.,* **18:** 100540.

Mona, M.; Geasa, N. and Sharshar, K. (2000a). Chemical composition of freshwater crayfish (*Procambarus clarkii*) and its nutritive value. *Egypt. J. Aquat. Biol. and Fish.,* **4**(1)**:** 19-34.

Mona, M.; Geasa, N. and Sharshar, K. (2000b). Chemical composition of freshwater crayfish (*Procambarus clarkii*) and its nutritive value. *Egypt. J. Aquat. Biol. and Fish.,* **4**(1)**:** 19-34.

Oficialdegui, F.J.; Clavero, M.; Sánchez, M.I.; Green, A.J.; Boyero, L.; Michot, T.C.; Klose K.; Kawai, T. and Lejeusne, C. (2019). Unravelling the global invasion routes of a worldwide invader, the red swamp crayfish (Procambarus clarkii). *Fresh. Biol.***64**(8)**:** 1382-1400.

Paulson, E.L. and Martin, A.P. (2014). Discerning invasion history in an ephemerally connected system: landscape genetics of *Procambarus clarkii* in Ash Meadows, Nevada. *Biol. Inv.* **16:** 1719-1734.

Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breeding* **2:** 225-238.

Quan, A.S.; Pease, K.M.; Breinholt, J. W. and Wayne, R.K. (2014), Origins of the invasive red swamp crayfish (*Procambarus clarkii*) in the Santa Monica mountains. *Aquat. Inv.* **9**(2).

Radwan, H.A.; Hassan, A.M.; El-Aziem, S.H.A. and Abbass, A.A. (2014). Cytogenetic characterization and genetic variations between freshwater crayfish *Procambarus clarkii* in Egypt. *Res. J. Pharm. Biol. Chem. Sci.,* **5**(2)**:** 1801-1816.

Rawi, M.S. (1995). Toxicological and physiological characteristics of Aluminum in some freshwater molluscs and crustaceans. *Proc. Zool. Soc. AR Egyp.,* **24:** 224-243.

Rodríguez-Estival, J.; Morales-Machuca, C.; Pareja-Carrera, Je.; Ortiz-Santaliestra, M.E. and Mateo, R. (2019). Food safety risk assessment of metal pollution in crayfish from two historical mining areas: Accounting for bioavailability and cooking extractability. *Ecotox. Environ. Safety,* **185:**109682.

Rodríguez-González, H.; Hernández-Llamas, A.; García-Ulloa, M.; Racotta, I.S.; Montoya-Mejía, M. and Villarreal, H. (2014). Effect of protein and lipid levels in diets for female red claw crayfish *Cherax quadricarinatus* on quality of offspring (juvenile), with emphasis on growth performance, biochemical composition and stress resistance to low oxygen, high ammonia and salinity. *Aquac. Nutr.,* **20**(5)**:** 557-565.

RodrÍguez-González, H.; Villarreal, H.; García-Ulloa, M. and Hernández-Llamas, A. (2009). Evaluation of practical diets containing different protein levels on gonad development of female redclaw crayfish *Cherax quadricarinatus*. *Aquac. Nutr.,* **15**(4)**:** 347-355.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.,* **4**(4)**:** 406-425.

Schirf, V.R.; Turner, P.; Selby, L.; Hannapel, C.; De La Cruz, P. and Dehn, P.F. (1987). Nutritional status and energy metabolism of crayfish (*Procambarus clarkii*, Girard) muscle and hepatopancreas. Comparative Biochemistry and physiology. *Compar. Physiol.,* **88(3):** 383-386.

Simon, C.; Frati, F.; Beckenbach, A.; Crespi, B.; Liu, H. and Flook, P. (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals Entomol. Soc. Am.,* **87**(6)**:** 651-701.

Soliman, G.N.; El-Assal, F.; El-Deen, M.S. and Hamdi, S.A.H. (1998). The reproductive biology of the red swamp crayfish *P. clarkii* (Girard, 1852)(Decapoda: Cambridae) in the River Nile, Egypt. *Egypt. J. Zool.,* **30:** 311-325.

Souty-Grosset, C.; Anastacio, P.M.; Aquiloni, L.; Banha, F.; Choquer, Justine; Chucholl, C. and Tricarico, E. (2016). The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-being. *Limnol.,* **58:** 78-93.

Tamura, K.; Dudley, J.; Nei, M. and Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.,* **24**(8)**:** 1596-1599.

Tanakol, R.; Yazici, Z.; Şener, E. and Sencer, E.(1999). Fatty acid composition of 19 species of fish from the Black Sea and the Marmara Sea. *Lipids,* **34**(3)**:** 291-297.

Tolba, R. (2017). The red swamp crayfish *Procambarus clarkii* (decapoda; cambaridae) as bio-indicator for otal water quality including Cu and Cd pollution. *Egyp. Soc. Develop. Fish.*

Torres, E. and Álvarez, F.(2012). Genetic variation in native and introduced populations of the red swamp crayfish *Procambarus clarkii* (Girard, 1852)(Crustacea, Decapoda, Cambaridae) in Mexico and Costa Rica. *Aquat. Inv.* **7**(2).

Vella, N.; Vella, A. and Mifsud, C.M. (2017). First scientific records of the invasive Red Swamp Crayfish, *Procambarus clarkii* (Girard, 1852)(Crustacea: Cambaridae) in Malta, a threat to fragile freshwater habitats. *Nat. Engin. Sci.,* **2**(2)**:** 58-66.

Xiong, B.; Xu, T.; Li, R.; Johnson, D.; Ren, D.; Liu, H.; Xi, Y. and H., Y. (2020). Heavy metal accumulation and health risk assessment of crayfish collected from cultivated and uncultivated ponds in the Middle Reach of Yangtze River. *Sci. Total Environ.* **739:** 139963.

Xu, Shuhua; Yin, X.; Li, S.; Jin, W.; Lou, H.; Yang, L.; Gong, X.; Wang, H.; Shen, Y. and Pan, X. (2009). Genomic dissection of population substructure of Han Chinese and its implication in association studies. *Am. J. Human Genet.,* **85**(6)**:** 762-774.

Yang, B.; Zhang, Y.; Jiang, S.; Lu, J. and Lin, L. (2023). Effects of different cooking methods on the edible quality of crayfish *(Procambarus clarkii*) meat. *Food Chem. Advances,* **2:** 100168.

Yi, S.; Li, Y.; Shi, L.; Zhang, L.; Li, Q. and Chen, J.(2018). Characterization of Population Genetic Structure of red swamp crayfish, *Procambarus clarkii*, in China. *Sci. rep.,* **8**(1)**:** 5586.

Yue, G.H.; Li, J.; Bai, Z.; Wang, C.M. and Feng, F. (2010). Genetic diversity and population structure of the invasive alien red swamp crayfish. *Biol. Inv.* **12:** 2697-2706.

Yue, G.H.; Wang, G.L.; Zhu, B.Q.; Wang, C.M.; Zhu, Z.Y. and Lo, L.C. (2008). Discovery of four natural clones in a crayfish species *Procambarus clarkii*. *Inter. J. Biol.Sci.,* **4**(5)**:** 279.

Zaglol, N.F. and Eltadawy, F. (2009). Study on chemical quality and nutrition value of fresh water crayfish (*Procambarus clarkii*). *J. Arab. aquac. soc.,* **4**(1)**:** 1-18.

Zhai, D.D.; Li, W.J.; Liu, H.Z.; Cao, W.X. and Gao, X. (2019). Genetic diversity and temporal changes of an endemic cyprinid fish species, *Ancherythroculter nigrocauda*, from the upper reaches of Yangtze River. *Zool. Res.,* **40**(5)**:** 427.

Zhang, Y.; Yang, B.; Yang, L.; Jiang, S.; Lu, J. and Lin, L. (2023). Comparison of the nutritional qualities of the pond, rice-field and wild crayfish (*Procambarus clarkii*) meat. *Food Chem. Adv.,* **2:** 100272.

Zhong, Y.; Tang, Z.; Huang, L.; Wang, D. and Lu, Z. (2020). Genetic diversity of *Procambarus clarkii* populations based on mitochondrial DNA and microsatellite markers in different areas of Guangxi, China. *Mitoch. DNA Part A* **31**(2)**:** 48-56.

Zhou, M.; Wu, Q.; Wu, H.; Liu, J.; Ning, Y.; Xie, S.; Huang, W. and Bi, X. (2021). Enrichment of trace elements in red swamp crayfish: Influences of region and production method, and human health risk assessment. *Aquac.,* **535:** 736366.

Zhu, B.F.; Huang, Y.; Dai, Y.G.; Bi, C.W. and Hu, C.Y. (2013). Genetic diversity among red swamp crayfish (*Procambarus clarkii*) populations in the middle and lower reaches of the Yangtze River based on AFLP markers. *Gen. Mol. Res.,* **12**(1)**:** 791-800.

Zhu, L.; Yuan, G.; Jiang, X.; Zhang, J.; Zhao, X.; Pei, C.; Li, L. and Kong, X. (2021). Molecular characterization of Rab5 and Rab6, and their involvement in innate immunity in red swamp crayfish *Procambarus clarkii*. *Aquac.* **533:** 736085.

Zhu, Z.Y. and Yue, G. H. (2008), Eleven polymorphic microsatellites isolated from red swamp crayfish, *Procambarus clarkii*. *Mol, Ecol, Resources* **8**(4)**:** 796-798.

Zou, Y.M. and Zhang, C.X. (2015). Comprehensive breeding technology of crawfish in rice field. In.: Chemical industry press, Beijing (in Chinese).