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Comparative Analysis of Na^+/K^+-ATPase and PEPCK Gene Fragments in *Metapenaeus affinis* from Contrasting Salinity Environments in Southern Iraq

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ABSTRACT

This study investigated the presence and partial sequence variation of two functional genes, sodium-potassium ATPase alpha-subunit (Na^+/K^+-ATPase) and phosphoenolpyruvate carboxykinase (PEPCK), in *Metapenaeus affinis* populations inhabiting contrasting salinity environments in Basrah Governorate, southern Iraq. Representative environments included Al-Musahab Marsh, a low-salinity environment (9.8 ppt), and Khor Al-Zubair Channel, a hypersaline marine environment (50.4 ppt). Morphological taxonomy confirmed that the examined specimens belonged to *M. affinis*. Genomic DNA was extracted from gill tissues processed separately for each individual sample. Specific primers were newly designed using genomic sequences of *M. affinis* retrieved from the NCBI GenBank database as reference templates (Accession No. MT176267.1 for Na^+/K^+-ATPase and FJ441195.1 for PEPCK), and primer specificity was validated *in silico* using NCBI Primer-BLAST. Gradient PCR identified optimal annealing temperatures of 56.9 °C for Na^+/K^+-ATPase and 55.3 °C for PEPCK. Conventional PCR successfully amplified partial fragments of 679 bp and 438 bp for Na^+/K^+-ATPase and PEPCK, respectively. Bidirectional Sanger sequencing and pairwise sequence alignment using NCBI-BLAST revealed high sequence conservation between the two ecotypes, with 99% identity for Na^+/K^+-ATPase and 97% identity for PEPCK. These results provide preliminary baseline genetic data for osmoregulatory and metabolic loci in local shrimp populations. However, the amplified genomic fragments should be interpreted as evidence of gene presence and sequence similarity rather than direct evidence of differential gene expression or functional adaptation. Further transcriptomic and enzymatic analyses are required to evaluate the adaptive role of these genes under salinity stress.

INTRODUCTION

Crustaceans are high-value components of global fisheries and aquaculture production and make a substantial contribution to the international seafood market **(Roy *et al.*, 2010; FAO, 2022)**. The penaeid shrimp *Metapenaeus affinis* is an ecologically and economically important species that supports local fisheries in the shallow marine coastal waters and muddy or sandy estuarine habitats of southern Iraq, including the Shatt Al-Arab region **(Holthuis, 1980)**. This region is characterized by sharp salinity gradients, ranging from low-salinity marsh habitats, such as Al-Musahab Marsh (9.8 ppt), to hypersaline marine environments, such as Khor Al-Zubair Channel (50.4 ppt). Euryhaline crustaceans depend on efficient osmoregulatory and metabolic mechanisms to occupy such variable habitats. Therefore, this study focuses on two functional genes in gill tissues: Na^+/K^+-ATPase, a key ion-transport enzyme involved in osmoregulation, and PEPCK, a rate-limiting enzyme involved in cellular energy metabolism under environmental stress **(Asaro *et al.*, 2023)**.

Evaluating these loci provides reference molecular data for monitoring *M. affinis* populations facing increasing salinization pressures in southern Iraq.

Recent research from southern Iraq has also emphasized that Khor Al-Zubair supports distinct macroinvertebrate assemblages under intertidal environmental conditions, highlighting the relevance of site-specific molecular and ecological monitoring in this region **(Ali *et al.*, 2025)**.

This species has high ecological plasticity, allowing individuals to move between marine and estuarine environments during their life cycle **(Motoh, 1981)**. Spawning occurs in offshore marine waters, after which pelagic post-larvae migrate into brackish estuaries and marshlands that function as nursery habitats before recruitment of adults back to marine areas. The species can tolerate wide changes in salinity and temperature **(Staples & Vance, 1986)**. As a benthic omnivore feeding on detritus and microorganisms, *M. affinis* occupies an important position in estuarine food webs, contributing to energy transfer and serving as prey for teleost fishes and larger aquatic predators **(Staples & Vance, 1986)**. Its natural distribution extends across the Indo-West Pacific, where it inhabits shallow coastal waters, continental shelves, and tidal estuaries in the Arabian Gulf, India, Southeast Asia, and northern Australia **(Grey *et al.*, 1983)**.

Genetic studies provide important tools for characterizing physiological and adaptive responses of marine organisms to environmental change, especially salinity, which is one of the major factors affecting aquatic life. This is particularly relevant for shrimps such as *M. affinis*, which move between marine and semi-riverine environments and experience variable salinities throughout their life cycle **(Zhao *et al.*, 2015)**. Molecular approaches that assess gene presence and partial sequence variation at the genomic DNA level can help identify candidate genes associated with vital processes, including ion transport, metabolic regulation, and stress responses **(Farhadi *et al.*, 2023)**. A clearer understanding of these mechanisms can support water-resource management and aquaculture planning **(Kaeodee *et al.*, 2011)**. Such molecular markers may also help evaluate shrimp population resilience and inform selective breeding programs under variable salinity and environmental stress conditions **(Li *et al.*, 2020)**.

Salinity is a major environmental factor regulating the distribution of aquatic organisms and directly affects key physiological processes, including ion and fluid balance **(Kültz, 2015)**. Osmotic balance is closely linked to salinity and influences respiration, growth, metabolism, and reproduction. Consequently, aquatic organisms activate molecular and cellular regulatory mechanisms to maintain homeostasis under changing salinity conditions **(Romano & Zeng, 2012)**. Salinity adaptation has been associated with changes in the expression of stress-responsive genes involved in ion transport and cellular metabolism **(Li *et al.*, 2020)**. When organisms move between low- and high-salinity environments, osmotic imbalance triggers complex physiological responses collectively known as osmoregulation **(Kültz, 2015)**.

In euryhaline crustaceans, active ion transport across the gill epithelium is primarily mediated by specialized ionocytes, or mitochondrion-rich cells, which allow osmotic homeostasis under variable salinity conditions **(McNamara *et al.*, 2015)**. These cells contain extensive membrane infoldings and abundant mitochondria that supply ATP for active transport. In low-salinity environments, Na^+ and Cl^- uptake against steep electrochemical gradients depends largely on Na^+/K^+-ATPase located in the basolateral membrane **(Lucu & Towle, 2003)**. By hydrolyzing ATP, this enzyme exports Na^+ to the hemolymph and imports K^+, creating electrochemical gradients that support ion uptake through apical channels. Therefore, Na^+/K^+-ATPase is one of the most important

ion-transport proteins in crustaceans, and its gene expression is known to respond to salinity changes **(Shekhar *et al.*, 2014)**.

Metabolic pathways in gill cells are closely linked to ion-transport processes because osmoregulation requires substantial energy. Enzymes such as PEPCK may support energy metabolism and bicarbonate supply for ion-exchange processes during environmental stress **(Henry *et al.*, 2012)**. PEPCK is a key rate-controlling enzyme in gluconeogenesis and connects intermediary metabolism with cellular energy balance. Its encoding gene is located in the nuclear genome and is expressed in several tissues, particularly in metabolically active organs and their crustacean equivalents **(Li *et al.*, 2019)**. Euryhaline crustaceans exposed to steep salinity gradients must maintain osmotic balance while meeting high ATP demands, making PEPCK-regulated metabolic pathways relevant to salinity stress responses **(Li *et al.*, 2020)**.

Conventional polymerase chain reaction (PCR) was applied to screen gDNA and evaluate the presence of this metabolic marker in the understudied penaeid shrimp *M. affinis*. PCR-based assays are highly specific because target DNA segments are amplified using defined primers, thereby validating the physical presence of selected genomic fragments **(Spens *et al.*, 2017)**. PCR remains one of the most accessible tools for investigating molecular diversity in non-model organisms **(Porter & Hajibabaei, 2018)**. Although *M. affinis* is ecologically and economically important, genomic resources for its functional genes remain limited **(Rahi *et al.*, 2019)**. It should be emphasized that amplification and sequencing of partial PEPCK and Na^+/K^+-ATPase fragments at the genomic DNA level confirm gene presence and sequence similarity only. Without downstream transcriptomic, expression, or enzymatic data, genomic fragments alone cannot be interpreted as evidence of habitat-specific salinity adaptation or differential physiological response.

This study aims to investigate the presence and partial sequence diversity of two functional genes, Na^+/K^+-ATPase and PEPCK, in *M. affinis* from representative salinity environments in southern Iraq. The specific objectives are to design gene-specific primers based on homologous penaeid sequences, optimize and perform conventional PCR amplification using gill-tissue gDNA, generate partial gene fragments through bidirectional Sanger sequencing, and compare the resulting sequences using pairwise alignment. The study therefore establishes preliminary baseline genetic data for these loci in local *M. affinis* populations.

MATERIALS AND METHODS

Study Area and Sampling

Shrimp samples were collected from two natural environments with contrasting salinity levels in Basrah Governorate, southern Iraq. Sampling was conducted during mid-November 2025 to maintain environmental consistency and reduce seasonal variation in physiological properties. The sampling sites were Al-Musahab Marsh (30°39′22.0″N, 47°39′39.8″E), representing a low-salinity environment influenced by Shatt Al-Arab water-quality changes **(Hassan, 2025)**, and Khor Al-Zubair Channel (47°51′49.6″E, 30°13′30.1″N), representing a hypersaline marine environment with high salinity and relatively stable ionic composition and electrical conductivity **(Ali *et al.*, 2025)**.

Shrimp samples were collected using a bottom trawl net measuring 15 × 3 × 1 m (length × width × height) with a mesh size of 5 mm. The net was towed by a fishing boat for a fixed duration of 20 min at each sampling station. After retrieval, shrimp specimens were collected from the catch. A total of 30 individuals were obtained, with 15 specimens from each sampling site. Specimens

were selected within a uniform size range (14–18 cm total length) to minimize age- and size-related physiological variation. An approximately 1:1 sex ratio was maintained, consisting of 15 males and 15 females in total: eight males and seven females from Al-Musahab Marsh, and seven males and eight females from Khor Al-Zubair Channel. All selected specimens were verified as mature to reduce confounding metabolic and osmoregulatory variation associated with juvenile development. Immediately after collection, samples were transported to the Biotechnology Laboratory, Department of Natural Marine Science, College of Marine Sciences, University of Basrah. Salinity, temperature, pH, total dissolved solids (TDS), and dissolved oxygen (DO) were measured during sampling using a calibrated multiparameter meter (HORIBA, Japan). Three replicate readings were taken for each parameter at each sampling site. Gill and muscle tissues were sampled using sterile dissecting instruments and placed in sterile 1.5 mL tubes containing 95% ethanol, then stored at -20 °C until DNA extraction.

Morphological Characteristics

Collected shrimp specimens were identified and classified based on morphological and anatomical characteristics using precise diagnostic body features. Visual examination was used to evaluate body-surface texture, hair distribution, and rostrum structure. For definitive taxonomic validation, anatomical sexual dimorphism was examined carefully. In male specimens, the fifth thoracic leg was inspected for the presence of a proximal notch and a twisted chitinous tubercle on the merus, together with the shape of the medial projection of the petasma. In female specimens, the longitudinal structure, grooves, and lateral margins of the anterior plate of the thelycum were examined. Color patterns and morphometric measurements were also recorded for both sexes. Specimens were photographed with scale bars. Species confirmation was achieved by comparing the observed anatomical and morphometric characters with the standard taxonomic descriptions of **Josileen and Pillai (2013)**. Molecular species identification was not performed and is acknowledged as a limitation of the study.

Genomic DNA Extraction

Genomic DNA was extracted from gill tissues of each specimen using the Genomic DNA Mini Kit for tissue (Geneaid, Taiwan) according to the manufacturer's instructions, with minor modifications to improve efficiency. Approximately 20–25 mg of tissue was homogenized and placed in a 1.5 mL microcentrifuge tube. Then, 200 µL of GST Buffer and 20 µL of Proteinase K were added, and samples were incubated at 60 °C overnight until complete tissue lysis. Samples were centrifuged at 14,000 rpm for 1 min, after which 200 µL of GSB Buffer was added and mixed thoroughly. Subsequently, 200 µL of absolute ethanol was added and mixed, and the solution was transferred to a GS Column. The column was centrifuged at 14,000 rpm for 1 min to allow DNA binding to the silica membrane. Washing steps were performed using W1 Buffer and Wash Buffer to remove impurities, proteins, and residual salts. Finally, DNA was eluted with 50 µL of Elution Buffer and stored at -20 °C until PCR amplification.

Genomic DNA Electrophoresis

To assess DNA integrity, electrophoresis was performed using a 1% agarose gel (Promega, USA) dissolved in 1× Tris-borate-EDTA (TBE) buffer (MarLiJu, Korea) and stained with DNA Green Viewer. Six microliters of DNA were mixed with two microliters of loading dye and loaded into the gel. Electrophoresis was performed at 65 mA and 95 V for 30 min **(Lee *et al.*, 2012)**. The gel was visualized using a UV-light gel transilluminator (LABGIC, China).

DNA Quality and Quantity Assessment Spectrophotometry

DNA concentration and purity were measured using a NanoDrop spectrophotometer (IMPLEN, Germany) by reading absorbance at 260 and 280 nm. Only biological replicates with acceptable 260/280 ratios between 1.8 and 2.0 were used for PCR amplification, whereas samples outside this range were excluded from PCR reactions.

Primer Design

Because pre-existing specific primers for the target genes of *M. affinis* were unavailable, new primers were designed using genomic sequences of *M. affinis* retrieved from the NCBI GenBank database as reference templates (Accession No. MT176267.1 for Na^+/K^+-ATPase and FJ441195.1 for PEPCK). Multiple sequence alignment using Clustal Omega was performed before primer design to identify conserved exonic regions and stable consensus sequences. Regions showing high variability or potential SNPs were avoided to ensure stable annealing across biological replicates. Primers were designed using IDT PrimerQuest according to the following criteria: primer length of 18–25 bp, GC content of 40–60%, melting temperature of 55–60 °C, and absence of hairpins or primer dimers. NCBI Primer-BLAST and the NCBI Standard Nucleotide BLAST tool were used to evaluate primer specificity and reduce the likelihood of non-specific amplification.

PCR Gene Amplification

Before the main amplifications, gradient PCR experiments were conducted using selected samples in a DLAB thermocycler (USA) to determine the optimal annealing temperature (Ta) for each primer pair. Each gradient reaction was performed in a final volume of 25 µL containing 12.5 µL of GoTaq Green Master Mix (Promega, USA; containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer), 2 µL of each forward and reverse primer (10 pmol; final concentration 0.4 µM), 3 µL of gDNA template, and 5.5 µL of nuclease-free water. Initial denaturation and extension temperatures were set at 95 °C and 72 °C, respectively, while Ta was tested across a gradient range of 48–62 °C. Amplicons were evaluated using agarose gel electrophoresis to identify the Ta that maximized amplification efficiency and minimized non-specific binding. After optimization, PCR amplification for all samples was conducted using the same master-mix composition, primer concentrations, and reagent volumes. The optimized cycling program consisted of initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 60 s, annealing at the optimized Ta for 60 s, and extension at 72 °C for 60 s; followed by final extension at 72 °C for 5 min. A negative control containing nuclease-free water instead of gDNA was included in each PCR run to detect contamination. Because positive-control DNA for the target genes of *M. affinis* was unavailable, amplification of the *E. coli* 16S rRNA gene using universal bacterial primers (27F and 1242R) was included as a procedural amplification control.

Electrophoresis of PCR Products

Gradient and conventional PCR products were analyzed using 1.5% agarose gels dissolved in 1× TBE buffer and stained with DNA Green Viewer. Five microliters of PCR product were loaded into each well with a 100+ bp DNA marker (Bioneer, Korea) to determine amplicon size. Electrophoresis was performed at 65 mA and 90 V for approximately 45 min **(Lee *et al.*, 2012)**, and gels were visualized using a UV-light gel transilluminator.

Sequencing and Sequence Analysis Procedures

Following successful amplification of the target genes, purified PCR products (679 bp for Na^+/K^+-ATPase and 438 bp for PEPCK) were bidirectionally sequenced using Sanger sequencing

by Macrogen, South Korea. Forward and reverse primers were used as sequencing primers. Raw chromatogram files were inspected manually using FinchTV (version 1.4.0; Geospiza Inc., USA), and low-quality bases at the 5′ and 3′ termini were trimmed. BioEdit softwareee (version 7.2, USA) was used to generate consensus sequences and perform multiple alignments. Forward and reverse reads were aligned to generate consensus sequences for each biological replicate from each site.

RESULTS

Measurement of Environmental Factors

Environmental parameters measured at Al-Musahab Marsh and Khor Al-Zubair Channel are presented in Table (1). Salinity and TDS were markedly lower at Al-Musahab Marsh than at Khor Al-Zubair Channel, confirming the contrasting salinity conditions of the two sampling environments. Temperature and pH values were moderate at both sites.

Table 1. Mean values of environmental factors measured at Al-Musahab Marsh and Khor Al-Zubair Channel, southern Iraq.

Environmental Factor	Sampling sites	
	Al -Masahab Marsh	Khor Al- Zubair Channel
Temperature (ºC)	30.7±2.03	27.6±1.65
Salinity (ppt)	9.8±1.10	50.4±3.24
pH	7.9±0.20	7.5±0.24
TDS (g/L)	9.4±0.81	40.0±2.37
DO (mg/L)	7.2±1.00	9.7±1.76

Morphological Taxonomic Identification

Morphological examination confirmed that all diagnostic characters matched the approved taxonomic descriptions of *M. affinis* (Fig. 1). A total of 30 adult specimens were examined and measured: 15 from Al-Musahab Marsh (eight males and seven females) and 15 from Khor Al-Zubair Channel (seven males and eight females). The specimens were characterized by an elongated, semi-transparent, light-brown body that was entirely pubescent (Table 2). The rostrum was elongated and straight, with 7–10 dorsal teeth and one ventral tooth. Critical generic and specific landmarks were clearly observed, including carapace shape, lateral spine placement, pleopod and pereiopod morphology, and sex-specific reproductive structures. In males, the fifth thoracic leg showed a strongly developed proximal notch, a helically oriented chitinous tubercle on the merus, and a crescent-like medial projection on the petasma. In females, the thelycum was characterized by a longitudinal anterior plate with a deep midline groove and elevated lateral margins forming two parallel crests. Morphometric measurements showed a total-length range of 14.0–18.6 cm, with a maximum total length of 18.6 cm in females and 14.6 cm in males.

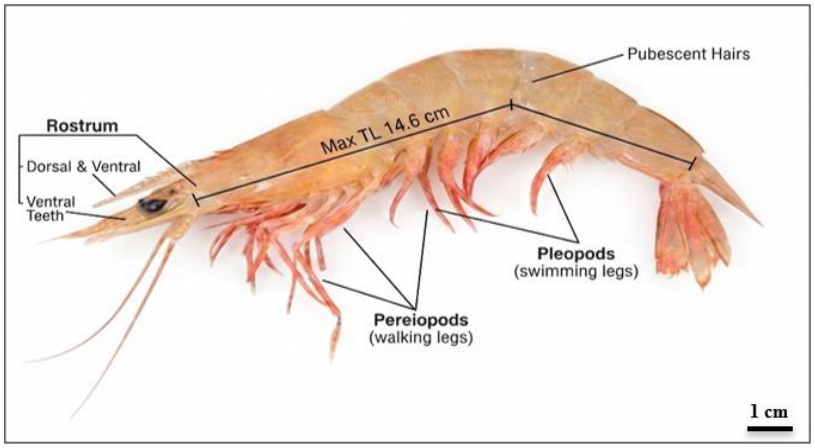

Fig. 1. Morphological characteristics of the shrimp *M. affinis* collected from the Iraqi environment (1 cm scale bars).

Table 2. Diagnostic features of collected *Metapenaeus affinis* specimens versus taxonomic reference keys.

Diagnostic Body Feature	Observed Traits in Collected Specimens	Reference Keys for *M. affinis* (Josileen & Pillai, 2013)
Rostrum Length and Teeth	Straight, reaching slightly beyond the antennular peduncle; 7–10 dorsal teeth, 1 ventral tooth	Rostrum with 7–11 dorsal teeth and 1 ventral tooth
Pubescence	Body surface covered with fine, dense, pubescent hairs	Body entirely pubescent
Petasma (Male)	Median anterior projection crescent-shaped; distomedian lobes form small outward-turned flaps	Symmetrical, with a characteristic medial projection capping the distal margin
Thelycum (Female)	Anterior plate longitudinally grooved with elevated parallel lateral margins	Seminal receptacle structure featuring an anterior median plate flanked by prominent lateral ridges

DNA Integrity Verification

The quality of extracted gDNA was assessed using 1% agarose gel electrophoresis stained with DNA Green Viewer. Clear DNA bands were observed in the analyzed samples (Fig. 2), indicating good DNA integrity and limited degradation.

Assessment of purity and concentration of extracted genomic DNA:

NanoDrop measurements showed that the extracted gDNA generally had suitable concentrations for molecular analysis. The 260/280 absorbance ratios are presented in Table (3). Samples with purity values outside the acceptable range were excluded from subsequent PCR amplification.

Fig. 2. Agarose gel electrophoresis (1.0% agarose, 65 mA, 95 V for 30 min) of extracted gDNA from *M. affinis* isolates. Lanes 1-8: samples of low-salinty environment (FR1–FR8); Lanes 9-16: samples of hypersaline marine environment (MR1–MR8). The gel illustrates the presence of the extracted gDNA from different shrimp samples.

Table 3. Concentration (ng/µL) and purity of DNA extracted from shrimp samples collected from Al-Musahab Marsh and Khor Al-Zubair Channel, southern Iraq.

NO.	Fresh water samples			Marine water samples		
	Sample ID	DNA concentration (ng/µl)	DNA purity	Sample ID	DNA concentration (ng/µl)	DNA purity
1.	FR1	114	1.93	MR1	88	1.94
2.	FR2	198	1.96	MR2	68	1.78
3.	FR3	167	2.02	MR3	101	1.95
4.	FR4	161	1.94	MR4	121	1.92
5.	FR5	141	1.60	MR5	80	1.84
6.	FR6	367	1.77	MR6	118	1.93
7.	FR7	112	2.04	MR7	227	1.82
8.	FR8	68	1.83	MR8	113	1.72
9.	FR9	91	1.74	MR9	194	1.92
10.	FR10	234	1.97	MR10	72	2.01
11.	FR11	320	1.91	MR11	144	2.00
12.	FR12	156	2.18	MR12	138	1.96
13.	FR13	163	1.90	MR13	89	1.72
14.	FR14	97	2.16	MR14	70	1.62
15.	FR15	151	1.99	MR15	122	2.13

Primer Design

Specific primers for *M. affinis* were newly designed using genomic sequences retrieved from the NCBI GenBank database as reference templates: MT176267.1 for Na^+/K^+-ATPase and

FJ441195.1 for PEPCK (Table 4). Primer specificity was validated and PCR conditions were optimized by gradient PCR (Fig. 3). BLAST analysis of the Na^+/K^+-ATPase primers showed high-identity alignment with decapod crustacean Na^+/K^+-ATPase sequences, while PEPCK primers produced stringent full-length alignments with penaeid PEPCK sequences. Gradient PCR identified stable annealing across biological replicates at 56.9 °C for Na^+/K^+-ATPase, producing a distinct 679 bp amplicon, and 55.3 °C for PEPCK, producing a well-defined 438 bp amplicon. These optimized temperatures produced clear, quantifiable bands on 1.5% agarose gels stained with DNA Green Viewer and were used for subsequent amplification.

Table 4. Primers designed for Na^+/K^+-ATPase and PEPCK genes used in PCR amplification.

Primer name	Primer Sequence (5` - Oligo Seq - 3`)	Primer length (bp)	T_m (°C)	GC% content	Product size (bp)
Forward *Na+/K+-ATPases*	GACTTTGGGATCCACATCTACC	22	60.7	50%	679
Reverse *Na+/K+-ATPases*	CATAGACATCAGACCCACGAAG	22	59.9	50%	
Forward *PEPCK*	GTCCTCTCCAACACCATCTTC	21	61.1	52%	438
Reverse *PEPCK*	TTTCAGGTAGTGTCGGAAGTTATAG	25	57.8	40%	

Conventional PCR for Gene Amplification

After optimization by gradient PCR, conventional PCR was performed to amplify the target genes in *M. affinis* samples. Of the 30 adult specimens collected, gDNA was extracted from all individuals and assessed for purity. Based on the strict 260/280 quality-control threshold of 1.8–2.0, seven samples were excluded because of suboptimal purity, leaving 23 high-quality biological replicates for amplification. For Na^+/K^+-ATPase, all 23 qualified samples produced clear, reproducible bands at the expected size of 679 bp under the optimized annealing temperature of 56.9 °C. Similarly, all 23 qualified samples yielded distinct PEPCK bands at the expected size of 438 bp under the optimized annealing temperature of 55.3 °C. Therefore, 100% of the qualified biological samples were successfully amplified for both target genes. Representative amplification results are shown in Fig. (4).

Sequence Alignment of Na^+/K^+-ATPase and PEPCK Genes

Following sequencing, raw reads were edited, and low-quality bases at the 5′ and 3′ ends were trimmed using FinchTV. BioEdit was used to generate consensus sequences and perform multiple alignments. For low-salinity samples, the NCBI-BLAST pairwise alignment of the partial Na^+/K^+-ATPase sequence (Fig. 5) showed 96% sequence identity (593/616 identical nucleotides; 0/616 gaps) with the NCBI reference strain *M. affinis* BP32 (Accession No. MT176267.1). The PEPCK sequence (Fig. 6) showed 98% identity (372/380 identical nucleotides; 1/380 gap) with the NCBI reference *M. affinis* isolate (Accession No. FJ441195.1). Table (5) summarizes the accession number, query length, sequence identity, query coverage, E-value, and gaps for each target gene.

Table 5. BLAST alignment summary for *Na⁺/K⁺-ATPase* and *PEPCK* genes from Riverine (Al-Musahab Marsh) *M. affinis* specimens.

Target Gene	Accession No.	Query Length (bp)	Sequence Identity	Query Coverage	E-value	Gaps
Na⁺/K⁺-ATPase	MT176267.1	621	96%	99%	0.0	0%
PEPCK	FJ441195.1	385	98%	99%	0.0	0%

```
Query: None Query ID: lcl|Query_391459 Length: 621

>Metapenaeus affinis isolate BP32 sodium-potassium ATPase alpha subunit (NaK) gene, partial CDs
Sequence ID: MT176267.1 Length: 723
Range 1: 46 to 661
Score:1014 bits(549), Expect:0.0,
Identities:593/616(96%),  Gaps:0/616(0%), Strand: Plus/Plus

Query  1    ACCCWCACACAGAACCGTATGACAGTAGCRCATATGTGGTTTGACAATACCATCATTGAA  60
            |||| ||||||||||||||||||||||| |||||||||||||||||||||||||||||||
Sbjct  46   ACCCTCACACAGAACCGTATGACAGTAGCACATATGTGGTTTGACAATACCATCATTGAA  105

Query  61   GCCGATACATCAGAAGATCAATCTGGCTGCCAGTATGACAAAACATCTCAAGGTTGGAAG  120
            || |||||||||||||||| |||||||||||||||||| ||||||||||||||||||||
Sbjct  106  GCTGATACATCAGAAGATCAGTCTGGCTGCCAGTATGACAAGACATCTCAAGGTTGGAAG  165

Query  121  GCACTGTCAAGAATTGCTGCCCTCTGCAATCGTGCTGAATTCAAGACTGGTCAGGATTCT  180
            |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  166  GCACTGTCAAGAATTGCTGCCCTCTGCAATCGTGCTGAATTCAAGACCGGTCAGGATTCA  225

Query  181  GTTCCCATCCTAAAGCGTGAAGTAAACGGTGACGCTTCTGAAGCTGCCTTGTTGAAGTGT  240
            ||||||||| |||| |||||||||||||||| |||||||||||||||||||||||||||
Sbjct  226  GTTCCCATCTTAAAACGTGAAGTAAACGGTGATGCTTCTGAAGCTGCCTTGTTGAAGTGT  285

Query  241  GTAGAATTAGCCTGTGGTGATGTAAAGGGCTGGCGTGCACGCAACAAGAAGGTTTGTGAA  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  286  GTAGAATTAGCCTGTGGTGATGTAAAGGGCTGGCGTGCACGCAACAAGAAGGTATGTGAA  345

Query  301  ATTCCATTCAACTCTACAAACAAGTATCAAGTATCCATCCATGAAACTGAAGATAAGAAC  360
            |||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  346  ATTCCATTCAACTCTACAAACAAGTATCAAGTATCCATCCACGAAACTGAAGATAAGAAC  405

Query  361  GACCCACGATACCTTCTTGTGATGAAGGGAGCCCCAGAGAGGATCCTGGAGCGTTGCTCT  420
            ||||||||||||||| |||||||||||||||||| |||||||||| || |||||||||
Sbjct  406  GACCCACGATACCTTGTTGTGATGAAGGGAGCCCCTGAGAGGATCCTAGAACGTTGCTCT  465

Query  421  TCTATCTACATCAATGGAGAGGAAAAGCCCCTTGATGAAGAAATGAAAGAAGCTTTCAAC  480
            |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  466  TCTATCTACATCAATGGAGAGGAAAAGCCCCTCGATGAAGAAATGAAAGAAGCTTTCAAT  525

Query  481  AATGCCTACCTGGAATTGGGTGGTCTCGGAGAACGTGTGCTTGGTTTCTGTGACTACATG  540
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  526  AATGCCTACCTGGAATTGGGTGGTCTCGGAGAACGTGTGCTTGGTTTCTGCGACTATATG  585

Query  541  CTGCCAACTGACAAATACCCTCTTGGATACCCCTTCGATGCTGATTCTGTAAACTTCCCA  600
            ||||||||||||||| |||||||||| |||||||||||||||||||||||||||||||||
Sbjct  586  CTGCCAACTGACAAGTACCCTCTTGGTTACCCCTTCGATGCTGATTCTGTAAACTTCCCA  645

Query  601  GTCCATGGTCTGCGCT  616
            || ||||||||||||||
Sbjct  646  GTTCATGGTCTGCGCT  661
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Fig. 5. BLAST analysis and partial alignment of the *Na⁺/K⁺-ATPase* gene sequence of *M. affinis* in a low-salinty environment compared to the NCBI-registered *M. affinis* BP32 isolate.

```
Query: None Query ID: lcl|Query_5330657 Length: 385


>Metapenaeus affinis phosphoenolpyruvate carboxykinase (PEPCK) gene, partial CDs
Sequence ID: FJ441195.1 Length: 553
Range 1: 91 to 469

Score:660 bits(357), Expect:0.0,
Identities:372/380(98%),  Gaps:1/380(0%), Strand: Plus/Minus

Query  1    TGATGACCTTTGCTTTGTGCTCAGCCGCGGCAGTCGCCTCGGACCTCATGGCCGCTCCTA  60
            |||||||||| ||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  469  TGATGACCTTAGCTTTGTGCTCAGCCGCGGCAGTCGCCTCGGATCTCATGGCCGCTCCTA  410

Query  61   CCAAGACGCCATGCTTCCAGTCGAAAGCCTCGTAGATGAGAGGAACTCCCTCGGGACGTC  120
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  409  CCAGGACGCCATGCTTCCAGTCGAAAGCCTCGTAGATGAGAGGAACTCCCTCGGGACGTC  350

Query  121  TTCCTCCAAAGAGGATGGCAGAGATGGGTACACCCTTAGGGTCTTCCCACGCTGGGTCGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  349  TTCCTCCAAAGAGGATGGCAGAGATGGGTACACCCTTAGGGTCTTCCCACGCTGGGTCGA  290

Query  181  TGATGGGACACTGTCCAGCTGGCGTACAGAATCGAGAGTTGGGATGAGCAGCTGGTTTAC  240
            |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  289  TGATGGGACACTGTCCAGCTGGCGTACAGAATCGAGAGTTGGGATGGGCAGCTGGTTTAC  230

Query  241  CCGACTCTGGGCTCCAGTTGGCATCTCCGAGCCAAGAGGTGATGCTCACATTGTTGGGGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  229  CCGACTCTGGGCTCCAGTTGGCATCTCCGAGCCAAGAGGTGATGCTCACATTGTTGGGGG  170

Query  301  TTTCCTTCTCCAGGCCTTCCCAGAAGACGCCTCCGTCGCTCGTTTTGGCAACGTTGGTGA  360
            ||||||| |||||||||||||||||| |||||||||||| ||||||||||||||||||||
Sbjct  169  TTTCCTTYTCCAGGCCTTCCCAGAAGAYGCCTCCGTCGCTAGTTTTGGCAACGTTGGTGA  110

Query  361  AGATGGTGTTGGGAGAGGAC  380
            |||||||||||| |||||||
Sbjct  109  AGATGGTGTTGG-AGAGGAC  91
```

Fig. 6. BLAST analysis and partial alignment of the *PEPCK* gene sequence of *M. affinis* in a low-salinty environment compared to the NCBI-registered *M. affinis* isolate.

In the hypersaline marine ecotype from Khor Al-Zubair Channel, the partial Na^+/K^+-ATPase sequence (Fig. 7) showed 96% identity (600/622 identical nucleotides; 0/622 gaps) with the NCBI reference strain *M. affinis* BP32 (Accession No. MT176267.1). The partial PEPCK sequence (Fig. 8) showed 97% sequence identity (392/406 identical nucleotides; 1/406 gap) with the NCBI reference *M. affinis* isolate (Accession No. FJ441195.1). These alignment results are summarized in Table (6).

Table 6. BLAST alignment summary for *Na⁺/K⁺-ATPase* and *PEPCK* genes from marine (Khor Al-Zubair Channel) *M. affinis* specimens.

Target Gene	Accession No.	Query Length (bp)	Sequence Identity	Query Coverage	E-value	Gaps
Na⁺/K⁺-ATPase	MT176267.1	639	96%	97%	0.0	0%
PEPCK	FJ441195.1	407	97%	100%	0.0	0%

```
Query: None Query ID: lcl|Query_4295027 Length: 639


>Metapenaeus affinis isolate BP32 sodium-potassium ATPase alpha subunit (NaK) gene, partial CDs
Sequence ID: MT176267.1 Length: 723
Range 1: 47 to 668

Score:1029 bits(557), Expect:0.0,
Identities:600/622(96%),  Gaps:0/622(0%), Strand: Plus/Plus

Query  1    CCCTSACACAGAACCGTATGACAGTAGCACATATGTGGTTTGACAATACCATCATTGAAG  60
            |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  47   CCCTCACACAGAACCGTATGACAGTAGCACATATGTGGTTTGACAATACCATCATTGAAG  106

Query  61   CCGATACATCAGAAGATCAATCTGGCTGCCAGTATGACAAAACATCTCAAGGTTGGAAGG  120
            | ||||||||||||||||| |||||||||||||||||| ||||||||||||||||||||
Sbjct  107  CTGATACATCAGAAGATCAGTCTGGCTGCCAGTATGACAAGACATCTCAAGGTTGGAAGG  166

Query  121  CACTGTCAAGAATTGCTGCCCTCTGCAATCGTGCTGAATTCAAGACTGGTCAGGATTCTG  180
            |||||||||||||||||||||||||||||||||||||||||||||| |||||||||||| |
Sbjct  167  CACTGTCAAGAATTGCTGCCCTCTGCAATCGTGCTGAATTCAAGACCGGTCAGGATTCAG  226

Query  181  TTCCCATCCTAAAGCGTGAAGTAAACGGTGACGCTTCTGAAGCTGCCTTGTTGAAGTGTG  240
            |||||||| |||| |||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  227  TTCCCATCTTAAAACGTGAAGTAAACGGTGATGCTTCTGAAGCTGCCTTGTTGAAGTGTG  286

Query  241  TAGAATTAGCCTGTGGTGATGTAAAGGGCTGGCGTGCACGCAACAAGAAGGTTTGTGAAA  300
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  287  TAGAATTAGCCTGTGGTGATGTAAAGGGCTGGCGTGCACGCAACAAGAAGGTATGTGAAA  346

Query  301  TTCCATTCAACTCTACAAACAAGTATCAAGTATCCATCCATGAAACTGAAGATAAGAACG  360
            |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  347  TTCCATTCAACTCTACAAACAAGTATCAAGTATCCATCCACGAAACTGAAGATAAGAACG  406

Query  361  ACCCACGATACCTTCTTGTGATGAAGGGAGCCCCAGAGAGGATCCTGGAGCGTTGCTCTT  420
            |||||||||||||| |||||||||||||||||||| |||||||||| || ||||||||||
Sbjct  407  ACCCACGATACCTTGTTGTGATGAAGGGAGCCCCTGAGAGGATCCTAGAACGTTGCTCTT  466

Query  421  CTATCTACATCAATGGAGAGGAAAAGCCCCTTGATGAAGAAATGAAAGAAGCTTTCAACA  480
            |||||||||||||||||||||||||||||||| |||||||||||||||||||||||||| |
Sbjct  467  CTATCTACATCAATGGAGAGGAAAAGCCCCTCGATGAAGAAATGAAAGAAGCTTTCAATA  526

Query  481  ATGCCTACCTGGAATTGGGTGGTCTCGGAGAACGTGTGCTTGGTTTCTGTGACTACATGC  540
            |||||||||||||||||||||||||||||||||||||||||||||||||| ||||| ||||
Sbjct  527  ATGCCTACCTGGAATTGGGTGGTCTCGGAGAACGTGTGCTTGGTTTCTGCGACTATATGC  586

Query  541  TGCCAACTGACAAATACCCTCTTGGATACCCCTTCGATGCTGATTCTGTAAACTTCCCAG  600
            |||||||||||||| |||||||||| |||||||||||||||||||||||||||||||||
Sbjct  587  TGCCAACTGACAAGTACCCTCTTGGTTACCCCTTCGATGCTGATTCTGTAAACTTCCCAG  646

Query  601  TCCATGGTCTGCGCTTCGTGGG  622
            | ||||||||||||||||||||
Sbjct  647  TTCATGGTCTGCGCTTCGTGGG  668
```

Fig. 7. BLAST analysis and partial alignment of the *Na⁺/K⁺-ATPase* gene sequence of *M. affinis* in a marine environment compared to the NCBI-registered *M. affinis* BP32 isolate.

```
Query: None Query ID: lcl|Query_7109777 Length: 407


>Metapenaeus affinis phosphoenolpyruvate carboxykinase (PEPCK) gene, partial CDs
Sequence ID: FJ441195.1 Length: 553
Range 1: 124 to 528

Score:686 bits(371), Expect:0.0,
Identities:392/406(97%),  Gaps:1/406(0%), Strand: Plus/Plus

Query  1    AAAACRAGGCGACGGAGGCGTCTTCTGGGAAGGCCTGGAGAAGGAAACCCCCAACAATGT  60
            ||||| | |||||||||||| |||||||||||||||||| ||||||||||||||||||||
Sbjct  124  AAAACTA-GCGACGGAGGCRTCTTCTGGGAAGGCCTGGARAAGGAAACCCCCAACAATGT  182

Query  61   GAGCATCACYTCTTGGCTGGGAGATGCCAACTGGAGCCCAGAGTCGGGTAAACCAGCTGC  120
            ||||||||| |||||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  183  GAGCATCACCTCTTGGCTCGGAGATGCCAACTGGAGCCCAGAGTCGGGTAAACCAGCTGC  242

Query  121  TCATCCCAACTCTCGATTCTGTACGCCAGCTGGACAGTGTCCCATCATCGACCCAGCGTG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  243  CCATCCCAACTCTCGATTCTGTACGCCAGCTGGACAGTGTCCCATCATCGACCCAGCGTG  302

Query  181  GGAAGACCCTAAGGGTGTACCCATCTCTGCCATYCTCTTTGGAGGAAGACGTCCCSARGG  240
            |||||||||||||||||||||||||||||| |||||||||||||||||||| | ||
Sbjct  303  GGAAGACCCTAAGGGTGTACCCATCTCTGCCATCCTCTTTGGAGGAAGACGTCCCGAGGG  362

Query  241  GGTTCCTCTCATCTACGAGGCTTTCGACTGGAAGCATGGCGTCYTGGTAGGAGCGGCCAT  300
             |||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  363  AGTTCCTCTCATCTACGAGGCTTTCGACTGGAAGCATGGCGTCCTGGTAGGAGCGGCCAT  422

Query  301  GAGRTCCGAGGCGACTGCCGCGGCTGAGCACAAAGCAAAGGTCATCATGCATGACCCCTT  360
            ||| |||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  423  GAGATCCGAGGCGACTGCCGCGGCTGAGCACAAAGCTAAGGTCATCATGCATGACCCCTT  482

Query  361  CGCCATGCGCCCCTTCTTCGGCTATAACTTCGGACACTACCTGAAA  406
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  483  CGCCATGCGCCCCTTCTTCGGCTATAACTTCGGACACTACCTGAAA  528
```

Fig. 8. BLAST analysis and partial alignment of the *PEPCK* gene sequence of *M. affinis* in a marine environment compared to the NCBI-registered *M. affinis* isolate.

Comparative sequence analysis of partial Na⁺/K⁺-ATPase and PEPCK genes between low-salinity and hypersaline marine *M. affinis* isolates revealed minor nucleotide variation between sampling sites. Representative individuals from both environments were compared using one representative sequence per habitat. Alignment of the Na⁺/K⁺-ATPase gene showed very high genetic conservation, with 99% identity (609/610 bp; 0% gaps) (Fig. 9), suggesting strong structural conservation of this ion-transport locus across contrasting salinities. In contrast, the PEPCK gene showed 97% identity (345/355 bp; one gap at position 8 in the low-salinity isolate) (Fig. 10). These sequence patterns provide baseline data for the studied functional genes across the two environments. However, phylogenetic analysis, population-level sampling, and tests for selection are required before any microevolutionary divergence or adaptive differentiation can be confirmed.

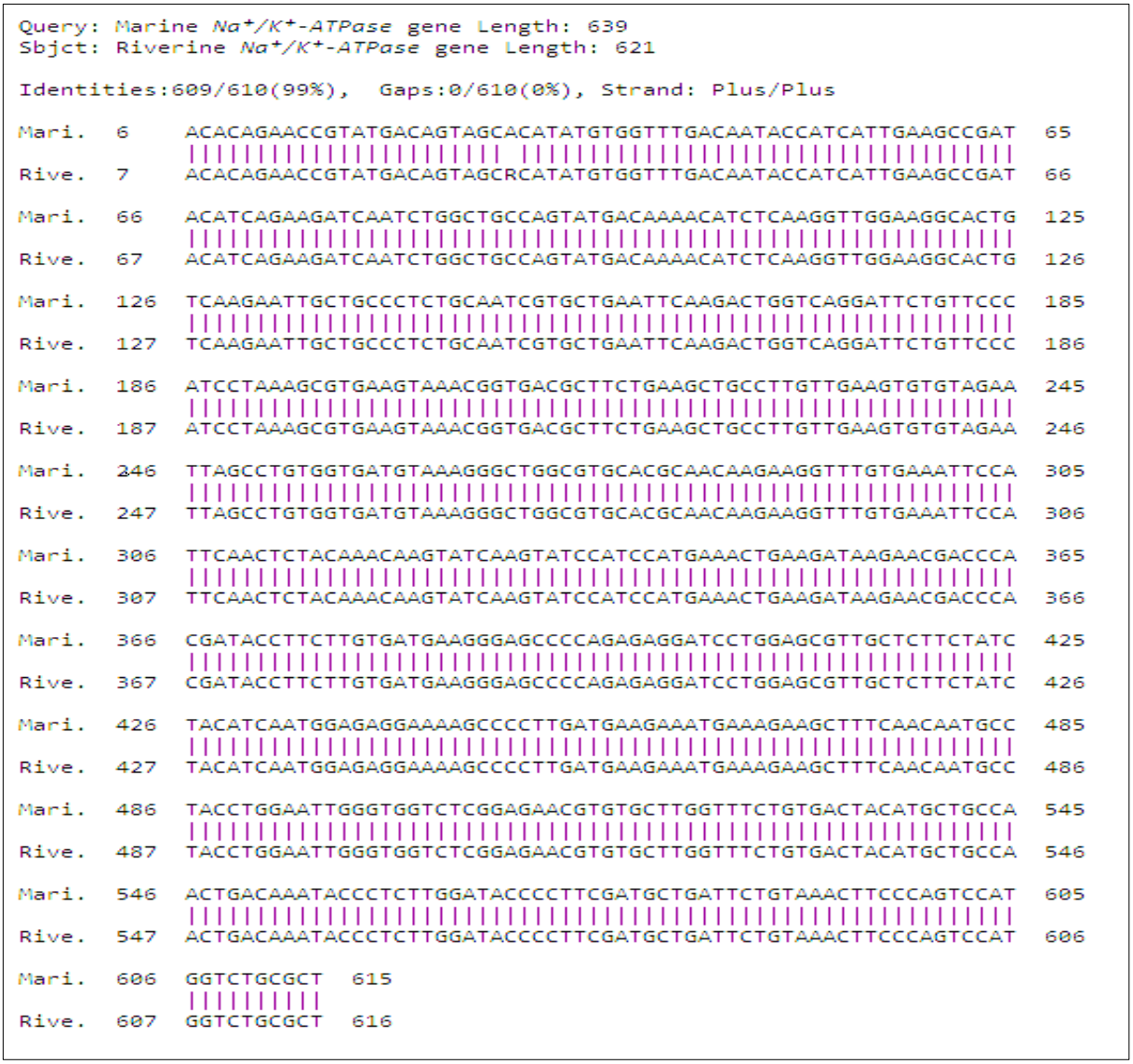

Fig. 9. BLAST analysis and partial alignment of the *Na⁺/K⁺-ATPase* gene sequence of *M. affinis* in a marine environment compared to the *M. affinis* in a low-salinty environment.

```
Query: Marine PEPCK gene Length: 407
Sbjct: Riverine PEPCK gene Length: 393

Identities:345/355(97%),  Gaps:1/355(0%), Strand: Plus/Minus

Mari.  1    AAAACRAGGCGACGGAGGCGTCTTCTGGGAAGGCCTGGAGAAGGAAACCCCCAACAATGT  60
            ||||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Rive.  354  AAAACGA-GCGACGGAGGCGTCTTCTGGGAAGGCCTGGAGAAGGAAACCCCCAACAATGT  296

Mari.  61   GAGCATCACYTCTTGGCTGGGAGATGCCAACTGGAGCCCAGAGTCGGGTAAACCAGCTGC  120
            ||||||||| |||||||| ||||||||||||||||||||||||||||||||||||||||||
Rive.  295  GAGCATCACCTCTTGGCTCGGAGATGCCAACTGGAGCCCAGAGTCGGGTAAACCAGCTGC  236

Mari.  121  TCATCCCAACTCTCGATTCTGTACGCCAGCTGGACAGTGTCCCATCATCGACCCAGCGTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Rive.  235  TCATCCCAACTCTCGATTCTGTACGCCAGCTGGACAGTGTCCCATCATCGACCCAGCGTG  176

Mari.  181  GGAAGACCCTAAGGGTGTACCCATCTCTGCCATYCTCTTTGGAGGAAGACGTCCCSARGG  240
            |||||||||||||||||||||||||||||||| |||||||||||||||||||| | ||
Rive.  175  GGAAGACCCTAAGGGTGTACCCATCTCTGCCATCCTCTTTGGAGGAAGACGTCCCGAGGG  116

Mari.  241  GGTTCCTCTCATCTACGAGGCTTTCGACTGGAAGCATGGCGTCYTGGTAGGAGCGGCCAT  300
             |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Rive.  115  AGTTCCTCTCATCTACGAGGCTTTCGACTGGAAGCATGGCGTCTTGGTAGGAGCGGCCAT  56

Mari.  301  GAGRTCCGAGGCGACTGCCGCGGCTGAGCACAAAGCAAAGGTCATCATGCATGAC  355
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Rive.  55   GAGGTCCGAGGCGACTGCCGCGGCTGAGCACAAAGCAAAGGTCATCATGCATGAC  1
```

Fig. 10. BLAST analysis and partial alignment of the *PEPCK* gene sequence of *M. affinis* in a marine environment compared to the *M. affinis* in a low-salinty environment.

DISCUSSION

In the present study, partial sequence data for two functional genes, Na⁺/K⁺-ATPase and PEPCK, were successfully amplified from gDNA and compared between *M. affinis* specimens collected from two contrasting salinity environments in southern Iraq. Morphological taxonomy validated the species identity, and newly designed primers consistently amplified PCR products of 679 bp and 438 bp, respectively. NCBI-BLAST pairwise sequence alignments showed high sequence conservation between the low-salinity ecotype from Al-Musahab Marsh (9.8 ppt) and the hypersaline marine ecotype from Khor Al-Zubair Channel (50.4 ppt). The local ecotypes showed 99% sequence identity for the Na⁺/K⁺-ATPase locus and 97% identity for PEPCK.

These data provide baseline information on genetic variation within functional gene regions across contrasting environments and confirm the presence and structural stability of the target loci in the studied species **(Kaeodee *et al.*, 2011)**. This represents one of the first molecular reports documenting these specific genomic fragments in *M. affinis* from southern Iraq and provides a foundation for further genomic investigations. The appearance of bands at the expected molecular sizes indicates the efficiency of the newly designed primers and the integrity of the extracted DNA

(Farhadi *et al.*, 2023). However, assessment of actual gene expression requires RNA-based approaches, such as quantitative PCR (qPCR), as a subsequent step.

Na^+/K^+-ATPase is a vital gene associated with osmotic regulation. It encodes a membrane enzyme responsible for transporting sodium and potassium ions across cell membranes and maintaining ionic balance, especially in marine crustaceans inhabiting variable-salinity environments **(Lucu & Towle, 2003)**. PEPCK is involved in gluconeogenesis and energy metabolism and is considered an important indicator of metabolic responses to environmental stress **(Shekhar *et al.*, 2013)**. The successful amplification of Na^+/K^+-ATPase in shrimp from both environments highlights its fundamental role in osmoregulation in euryhaline crustaceans **(Wang *et al.*, 2002)**. This finding agrees with previous studies showing that marine crustaceans rely on the sodium-potassium pump to cope with salinity variation, particularly in the gills, which are the main site of ion exchange **(Towle & Weihrauch, 2001; Zhang *et al.*, 2025)**.

For PEPCK, changes in expression or activity have been associated with increased energy demands under unstable environmental conditions, because crustaceans require additional energy to support osmoregulation, respiration, and locomotion **(Li *et al.*, 2014)**. Therefore, future expression-based analyses of PEPCK may help determine whether shrimp from one environment experience greater metabolic stress than those from the other. Potential environmental drivers may include salinity, dissolved oxygen, temperature, water chemistry, organic or mineral pollutants, biomass productivity, and climatic conditions. These factors can influence gene expression in aquatic organisms, particularly genes involved in metabolism and osmoregulation **(Freire *et al.*, 2008; Romano & Zeng, 2012)**.

Sequence alignment of the partial Na^+/K^+-ATPase and PEPCK fragments demonstrated high conservation between shrimp specimens from Khor Al-Zubair Channel and Al-Musahab Marsh. This sequence homology suggests structural stability of these osmoregulatory and metabolic loci and indicates that the populations inhabiting contrasting salinity environments share highly conserved genetic architecture in the specific regions examined. Nevertheless, because this study analyzed partial genomic fragments only, the results should not be overinterpreted as evidence of functional adaptation. Broader population sampling, full-length gene sequencing, expression analysis, and enzymatic assays are needed to clarify the functional significance of these loci under salinity stress.

CONCLUSION

The findings of the present study confirm the presence and structural conservation of Na^+/K^+-ATPase and PEPCK gene fragments in *M. affinis* across contrasting aquatic environments in southern Iraq. These molecular data provide preliminary baseline markers for evaluating local shrimp populations inhabiting variable salinity conditions. The amplified genomic fragments demonstrate high sequence similarity between low-salinity and hypersaline marine specimens, particularly for Na^+/K^+-ATPase. However, the results should be interpreted as evidence of gene presence and partial sequence conservation rather than direct proof of differential expression or functional adaptation. Future studies should integrate larger sample sizes, full-length gene sequencing, qPCR-based expression analysis, and physiological assays to clarify the role of these genes in salinity tolerance. Such information will be valuable for ecological monitoring, shrimp-habitat conservation, water-resource management, and aquaculture planning under increasing salinization and climate-change pressures in Iraqi aquatic systems.

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