

# Mud crab *Decapoda Scylla serrata* glutamate dehydrogenase: molecular cloning, tissue expression and response to hyposmotic stress

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**Abstract** Glutamate dehydrogenase (GDH) is an important enzyme in the regulation of osmotic balance in aquatic organisms. In this study, the full-length cDNA of GDH from mud crab *Decapoda Scylla serrata* was cloned and expressed in *Escherichia coli*. The recombinant protein was purified and characterized by SDS-PAGE, Western blotting and mass spectrometry. The expression of GDH in various tissues and its response to hyposmotic stress were investigated. The results showed that GDH was widely distributed in various tissues, with the highest expression in the gills. The expression of GDH in the gills increased significantly after exposure to hyposmotic stress, indicating that GDH plays a role in the regulation of osmotic balance in mud crab.

The first step in the process of creating a new product is to identify a market need. This involves conducting market research to understand the preferences and behaviors of potential customers. Once a need is identified, the next step is to develop a concept that addresses this need. This concept should be innovative and differentiated from existing products in the market.

After developing the concept, the next step is to create a prototype. This allows the company to test the feasibility of the product and gather feedback from potential users. The prototype should be functional and represent the key features of the final product. Based on the feedback received, the company can make necessary adjustments to the design and functionality.

Once the prototype is refined, the next step is to conduct a pilot test. This involves producing a small batch of the product and distributing it to a select group of customers. The purpose of the pilot test is to evaluate the product's performance in a real-world setting and gather valuable feedback from actual users. This feedback can be used to make further improvements to the product.

Finally, once the product has been thoroughly tested and refined, the company can proceed with full-scale production. This involves manufacturing the product in large quantities and distributing it to the market. The company should continue to monitor the product's performance and customer feedback to ensure ongoing success and make any necessary updates or improvements.

(HE)  $\alpha$  H

*GDH*  $\rightarrow$   $\text{NAD}^+$   $\rightarrow$   $\text{NADH}$   $\rightarrow$   $\text{M}^+$   $\rightarrow$  *Litopenaeus vannamei*

[illegible]

*Scylla paramamosain*

*GDH*  
*GDH*  
*S. paramamosain*

## Materials and methods

H<sub>1</sub> ± S. paramamosain H<sub>1</sub>  
 ( ± ) H<sub>1</sub>  
 Ruditapes philippinarum

## Methods

The diagram shows a 2D lattice of particles. Particles are represented by small circles with a cross inside. They are arranged in a grid. Labels include (M, E) for a pair of particles, (M) for a single particle, and (E) for another particle. Arrows indicate interactions or movement between particles.

The diagram illustrates the experimental design. It features a central box labeled 'H' (Healthy) at the top. Below it, a box labeled 'μ' (Microbiome) is connected to 'H' by a vertical line. To the right of 'μ', a box labeled 'GDH' (Glucose Dehydrogenase) is connected by a horizontal line. Below 'GDH', a box labeled 'M' (Microbiome) is connected by a vertical line. To the left of 'M', a box labeled 'GDH' is connected by a horizontal line. The entire diagram is enclosed in a large box labeled 'GDH'.



## Results

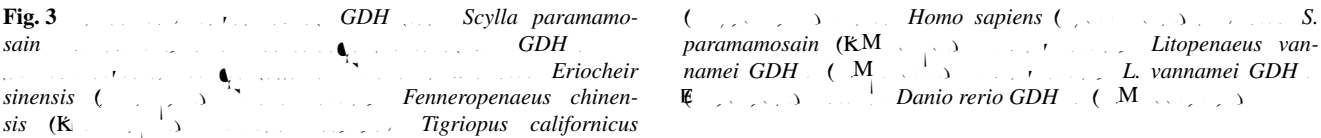
## H

GDH *S. paramamosain*

GDH *S. paramamosain*

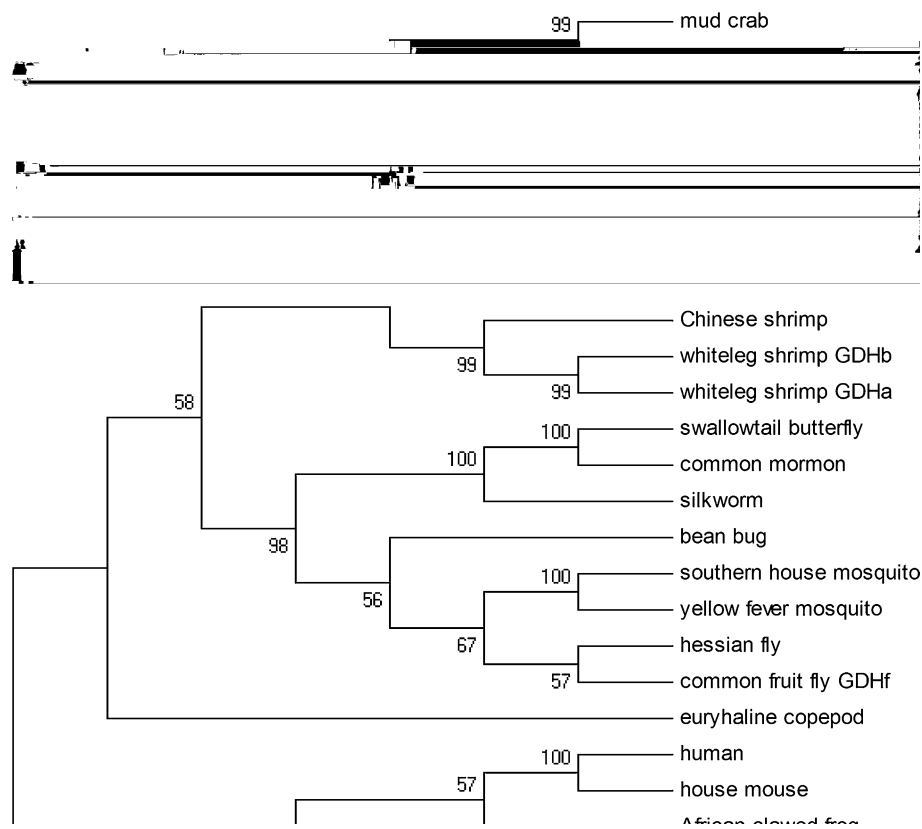
GDH  
% *E. sinensis*  
*L. vannamei* GDH *Fenneropenaeus chinensis* *L.*  
*vannamei* GDH ( )  
( )  
GDH *S. paramamosain*  
*S. paramamosain*  
*E. sinensis* *L. vannamei* *F. chinensis*

*GDH* is a dimeric enzyme composed of two identical subunits, each of which contains a zinc-dependent catalytic domain and a non-catalytic domain. The catalytic domain is responsible for the conversion of L-glutamate to L-glutamine, while the non-catalytic domain is involved in the regulation of the enzyme activity. The enzyme is encoded by the *GDH* gene, which is located on chromosome 10p13. The enzyme is expressed in various tissues, including liver, kidney, and brain. In the liver, *GDH* is primarily expressed in the cytoplasm of hepatocytes, while in the kidney, it is expressed in the proximal tubules. In the brain, *GDH* is expressed in the cytoplasm of neurons and glial cells. The enzyme is involved in the regulation of the urea cycle, which is a metabolic pathway that converts ammonia into urea. In the liver, *GDH* is involved in the regulation of the urea cycle by converting L-glutamate to L-glutamine, which then enters the urea cycle. In the kidney, *GDH* is involved in the regulation of the urea cycle by converting L-glutamate to L-glutamine, which then enters the urea cycle. In the brain, *GDH* is involved in the regulation of the urea cycle by converting L-glutamate to L-glutamine, which then enters the urea cycle.



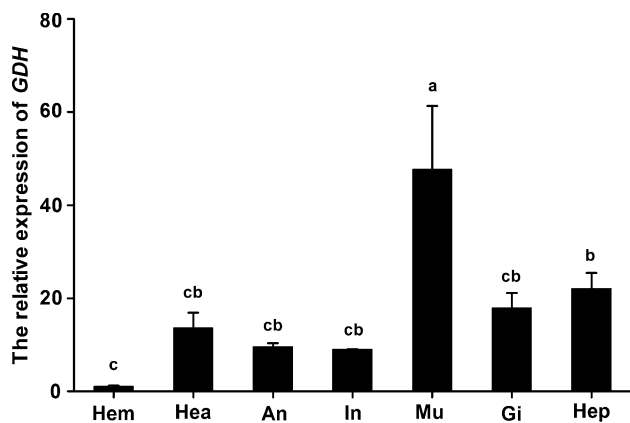
*sinensis* (Kishinouye, 1901) *Fenneropenaeus chinensis* (Kishinouye, 1901) *Tigriopus californicus*

[illegible]

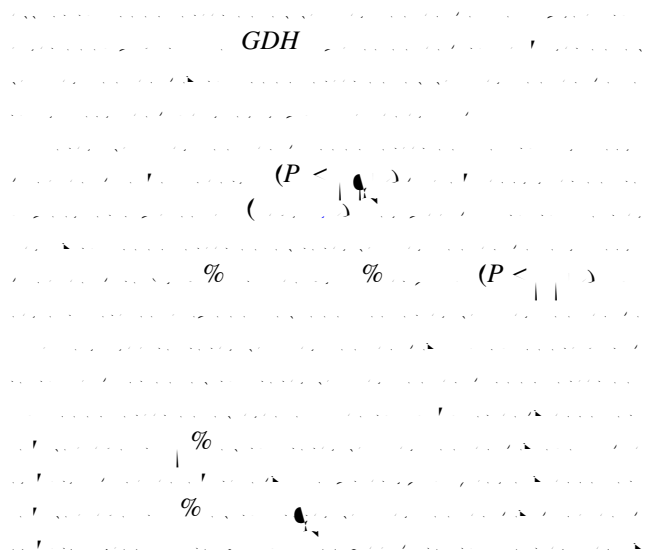


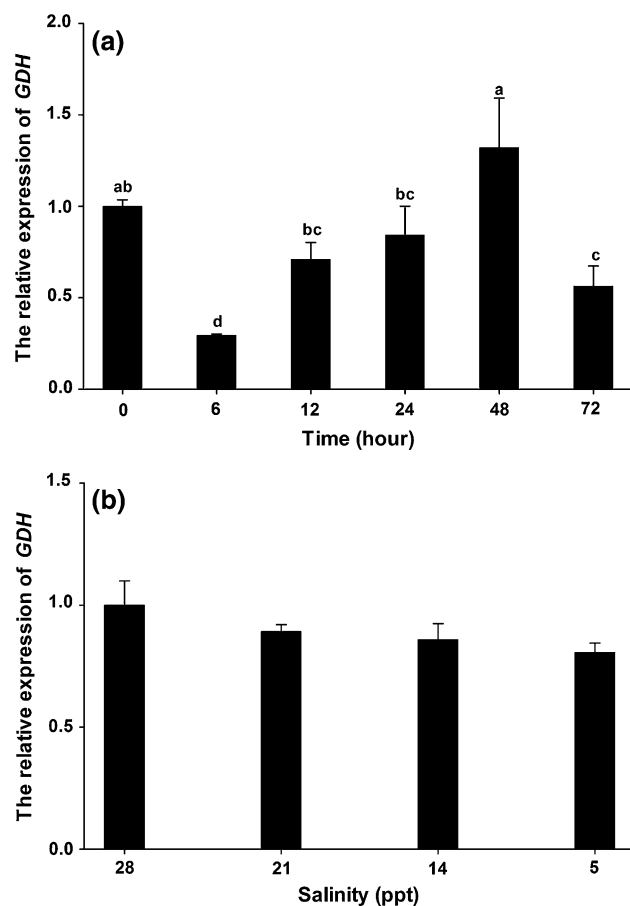
**Fig. 4** Phylogenetic tree of GDH protein sequences from various species. The tree is rooted with the African clawed frog (*Xenopus laevis*) as the outgroup. The species included are: *Scylla paramamosain*, *Chinese shrimp*, *whiteleg shrimp GDHb*, *whiteleg shrimp GDHa*, *swallowtail butterfly*, *common mormon*, *silkworm*, *bean bug*, *southern house mosquito*, *yellow fever mosquito*, *hessian fly*, *common fruit fly GDHf*, *euryhaline copepod*, *human*, *house mouse*, and *African clawed frog*. Bootstrap values are indicated at the nodes.

*Mayetiola destructa* (K), *Homo sapiens* (M), *Mus musculus* (M), *Oncorhynchus mykiss* (M), *Monopterus albus* (M), *Carassius auratus red var* (M), *Bombyx mori* (M), *Culex quinquefasciatus* (M), *S. paramamosain* (KM), *Papilio xuthus* (K), *Litopenaeus vannamei* (M), *L. vannamei GDH* (K), *Misgurnus anguillicaudatus* (M), *Aedes aegypti* (M), and *Danio rerio GDH* (M).



**Fig. 5** Relative expression of GDH in *Scylla paramamosain* Hem, Hea, An, In, Mu, Gi, and Hep. Bars represent standard deviation. Letters above bars indicate significant differences ( $P < 0.05$ ).





**Fig. 6 a** *GDH* *Scylla paramamosain* **b**

H *S. paramamosain* Bars

E M different letters

(n = 3, P < 0.05)

[illegible]

$$(P \leq \frac{1}{1000})$$

$$(P \leq \frac{1}{1000})$$

$$(P \leq \frac{1}{1000})$$

$$(P \leq \frac{1}{1000})$$

## Discussion

**H**

**H**

*S. paramamosain*

*S. paramamosain*

*Macrobrachium amazonicum*

*L. vannamei*

*Scylla serrata*

GDH

GDH,

*S. paramamosain*

*Tigriopus californicus*, *L. vannamei*, *E. sinensis*

GDH,

**Table 2** *Scylla paramamosain* (Moulson and Forster) (Decapoda, Scyllidae) (continued)

[illegible]



$$n = 1, P = 1 \quad (n = 1, P = 1)$$

— — — — —

# H

M  
 H  
 M  
 S. paramamosain  
 L. vannamei E. sinensis  
 GDH  
 (P < ) ( < )  
 GDH L. vannamei  
 + K<sup>+</sup>  
 GDH E. sinensis  
 H T. californicus GDH  
 T. californicus S.  
 paramamosain T. californicus  
 GDH T. californicus  
 S. paramamosain  
 GDH  
 H  
 E. sinensis, GDH  
 H  
 GDH  
 S. paramamosain  
 H GDH  
 paramamosain  
 GDH

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$\text{GDH}$   
*S. paramamosain*  
 $\text{GDH}$   
 $\text{GDH}$  *S. paramamosain*  
*S. paramamosain*  
 $\text{GDH}$   
*S. paramamosain*

[illegible]

The first two terms in (1) are the kinetic energy of the fluid and the kinetic energy of the solid, respectively. The third term is the potential energy of the fluid, and the fourth term is the potential energy of the solid. The fifth term is the work done by the external forces on the solid. The sixth term is the work done by the external forces on the fluid. The seventh term is the work done by the external forces on the interface. The eighth term is the work done by the external forces on the boundary. The ninth term is the work done by the external forces on the volume. The tenth term is the work done by the external forces on the surface. The eleventh term is the work done by the external forces on the volume. The twelfth term is the work done by the external forces on the surface. The thirteenth term is the work done by the external forces on the volume. The fourteenth term is the work done by the external forces on the surface. The fifteenth term is the work done by the external forces on the volume. The sixteenth term is the work done by the external forces on the surface. The seventeenth term is the work done by the external forces on the volume. The eighteenth term is the work done by the external forces on the surface. The nineteenth term is the work done by the external forces on the volume. The twentieth term is the work done by the external forces on the surface.

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Figure 1 consists of nine scanning electron micrographs arranged in a 3x3 grid. The top row shows the surface of *Macrobrachium olfersii*, characterized by a relatively smooth texture with some fine ridges. The middle row shows the surface of *Crassostrea gigas*, which exhibits a more pronounced, wavy, and ridged texture. The bottom row shows the surface of *Litopenaeus vannamei*, which has a highly textured, pitted, and irregular surface. Each micrograph includes a scale bar in the bottom right corner, representing 100 micrometers.