Effect of high alkalinity on shrimp gills: histopathological alternations and cell specific responses

Qianqian Ge[1], Jiajia Wang[1,2], Jitao Li[1,2], Jian Li[1,2]*

[1]Laboratory for Marine Fisheries Science and Food Production Processes, Pilot National Laboratory for Marine Science and Technology (Qingdao)

[2]Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, 266071, China

Introduction

High alkalinity stress was considered as a major risk factor for aquatic animals surviving in saline-alkaline water. However, few information exists on the effects of alkalinity stress in crustacean species or how this stressor affects the gill. The present study evaluated the effect of alkalinity stress in *Exopalaemon carinicauda* to determine changes in homeostasis and gill microstructure, and explore the heterogeneity response of gill cells by single-cell RNA sequencing (scRNA-seq). It was shown that high alkalinity stress resulted in a remarkable increased in the hemolymph osmolality and pH. For the gills, the pillar cells showed more symmetrical arrangement and longer lateral flanges, and the nephrocytes showed larger vacuoles after alkalinity stress compared with the control group. ScRNA-seq results showed that alkalinity stress leaded to decreased proportion of pillar cells and increased proportion of nephrocytes significantly. The differentially expressed genes (DEGs) related to ion transport, especially acid-base regulation, such as V(H^+)-ATPases and carbonic anhydrases, were down-regulated in pillar cells and up-regulated in nephrocytes. Furthermore, pseudotime analysis showed that some nephrocytes transformed to perform ion transport function in response to alkalinity exposure. Notedly, the positive signals of carbonic anhydrase were obviously observed in the nephrocytes after alkalinity stress. These results indicated that the alkalinity stress inhibited the ion transport function of pillar cells, but induced the active role of nephrocytes in alkalinity adaptation. Collectively, our results provided the new insight into the cellular and molecular mechanism behind the adverse effects of saline-alkaline water and the saline-alkaline adaption mechanism in crustaceans.

Results

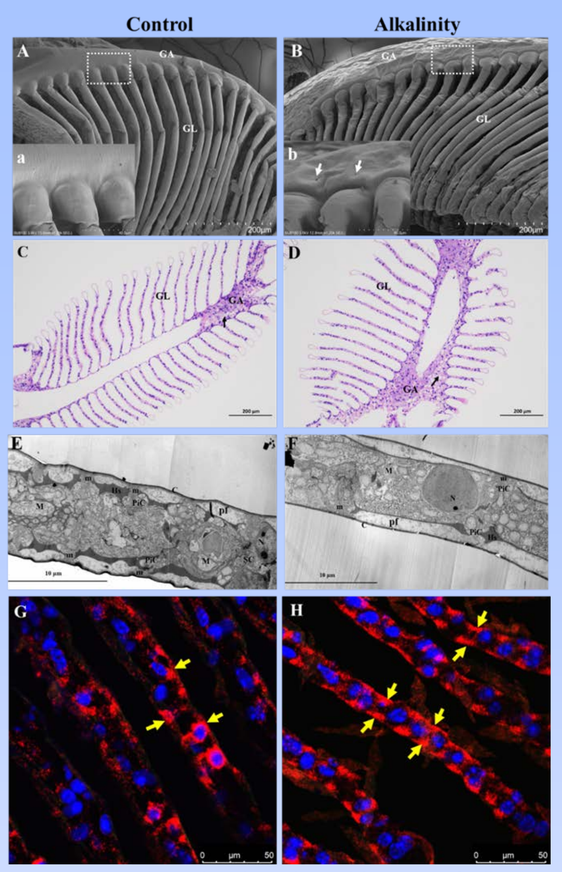


Figure 1. Histological and cytological features of gill *in E. carinicauda*. The gill filament surface in the control group (A) and alkalinity group (B). The gill histopathology in the control group(C) and alkalinity group (D). Ultrastructure of transverse sections of gill lamellae in the control group (E) and alkalinity group (F). The CAc location in the pillar cells of control group (G) and alkalinity group (F).

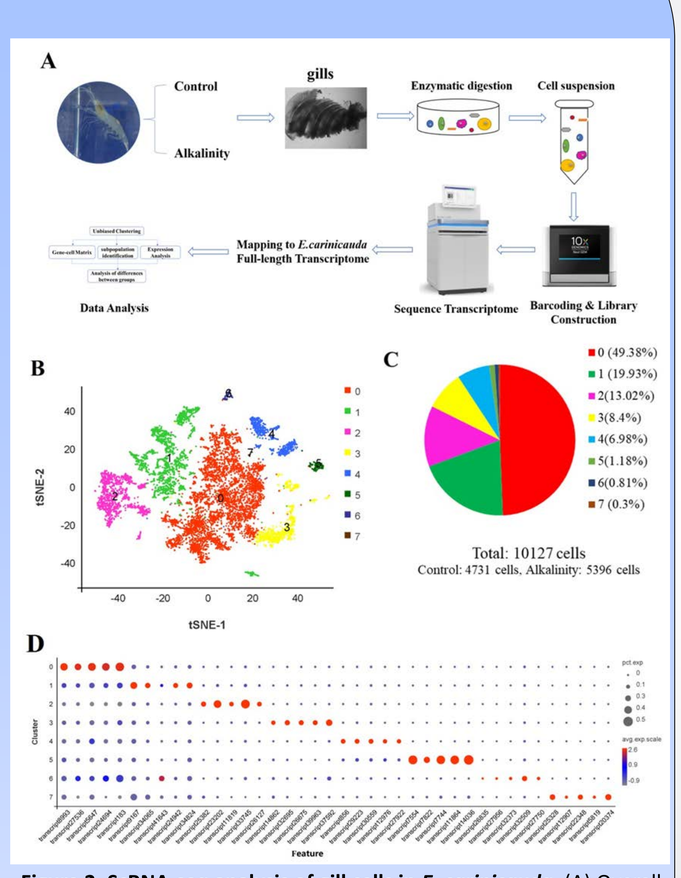


Figure 2. ScRNA-seq analysis of gill cells in *E. carinicauda*. (A) Overall workflow for cell sorting and single-cell data analyses. (B) The t-SNE nonlinear clustering of gill cells in *E. carinicauda*. (C) Number of cells in each cluster and their proportional distribution in the total gill dataset. (D) Top 5 DEGs (x axis) identified in each cluster (y axis). Dot size represents the fraction of cells in the cluster that express the gene; intensity indicates the mean expression (Z-score) in expressing cells, relative to other clusters.

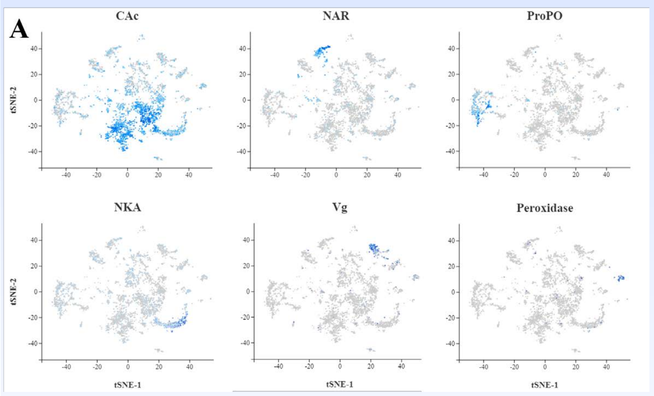
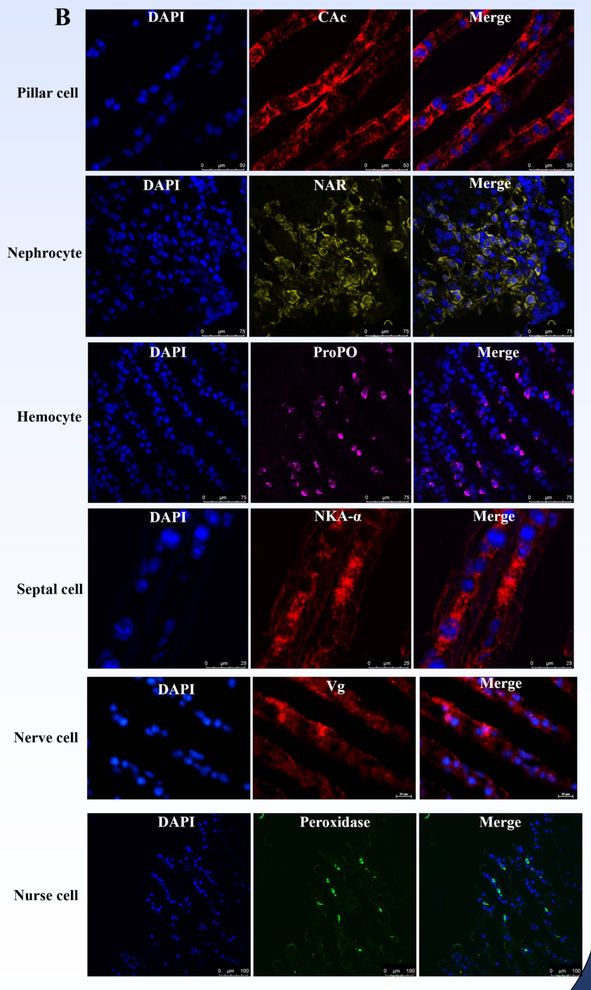


Figure 3. Overview of the gill cell clusters in *E. carinicauda*. (A) t-SNE plots showing expression of marker genes in cluster 0-5. The gene expression level is color coded. (B) FISH image showing the location of marker genes in different cell types in the gill filament.



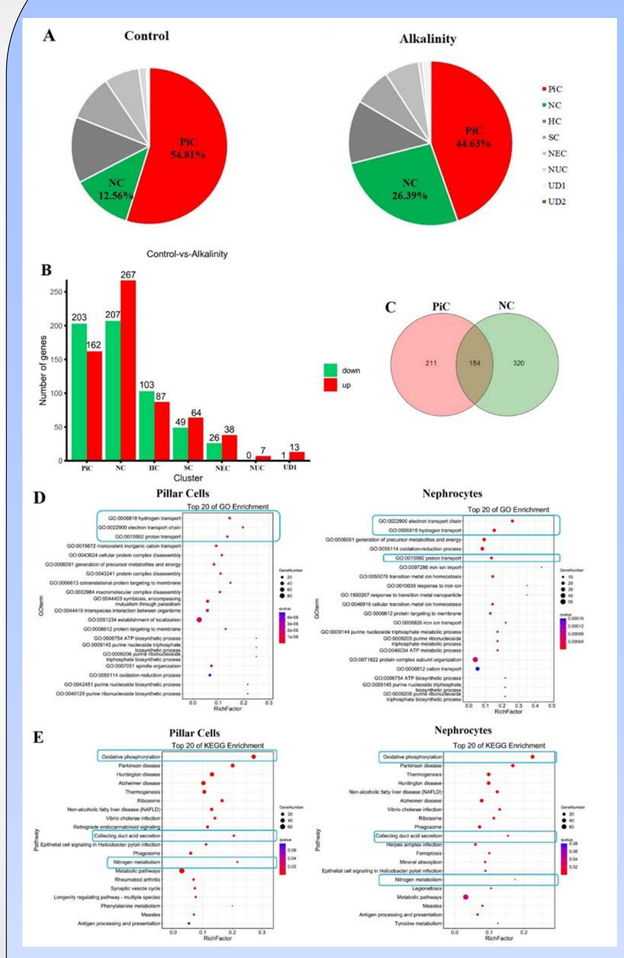


Figure 4. Gene expression characteristics of pillar cells and nephrocytes after alkalinity stress. (A) Pie charts of 8 clusters identified from gill in control and alkalinity stress group by t-SNE analysis. UD1: Undefined cell 1, UD2: Undefined cell 2. (B) Down- and up-regulated DEG numbers in each cell type of gills after alkalinity stress. (C) Venn diagram analysis of DEGs in pillar cells and nephrocytes after alkalinity stress. (D) GO enrichment of DEGs in pillar cells and nephrocytes. (E) KEGG enrichment of DEGs in pillar cells and nephrocytes.

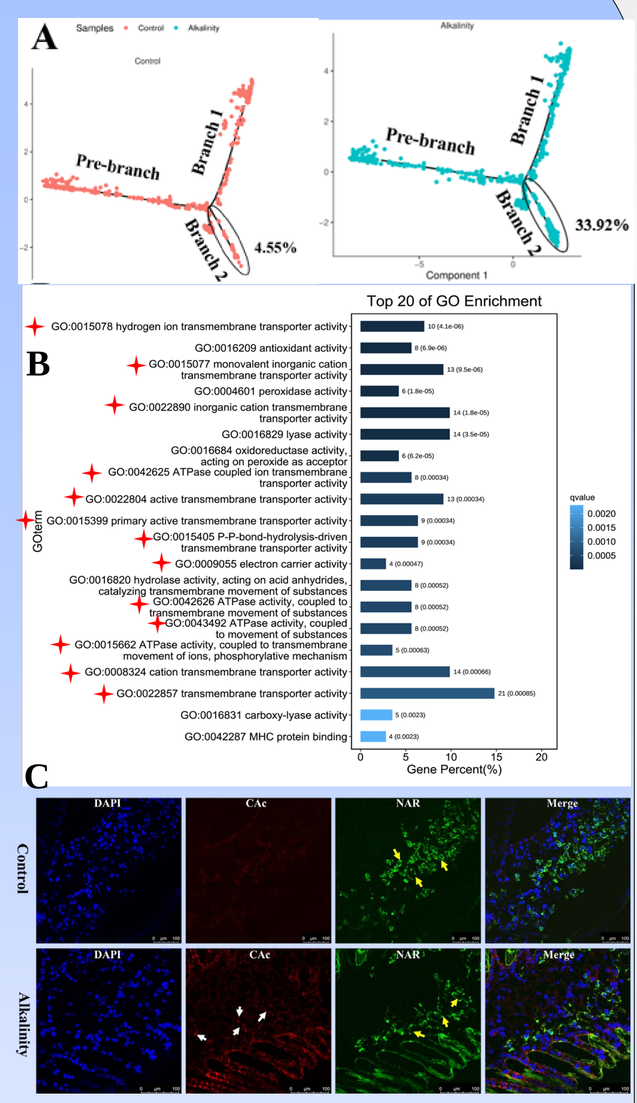
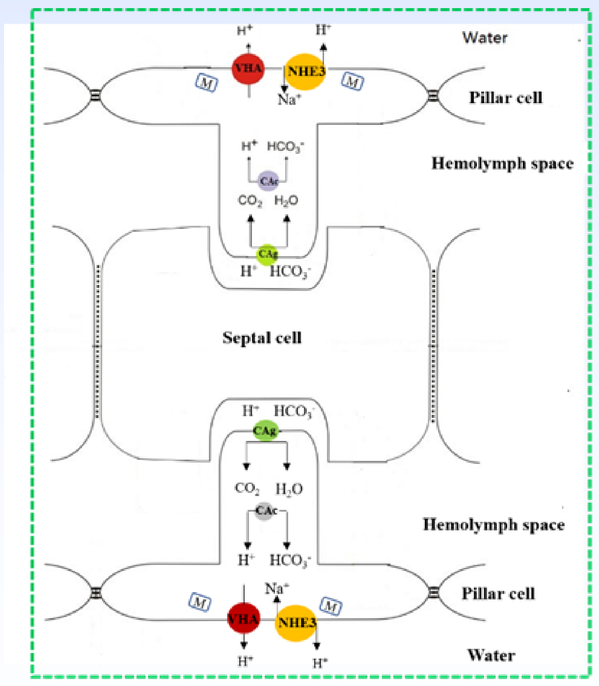


Figure 5. Pseudotime analysis of nephrocytes. (A) Pseudotime analysis of DEGs in nephrocytes. (B) GO analysis of DEGs in Branch 2. (C) Changes of CAc (white arrow) and NAR (yellow arrow) in the nephrocytes of gill axis after alkalinity stress.



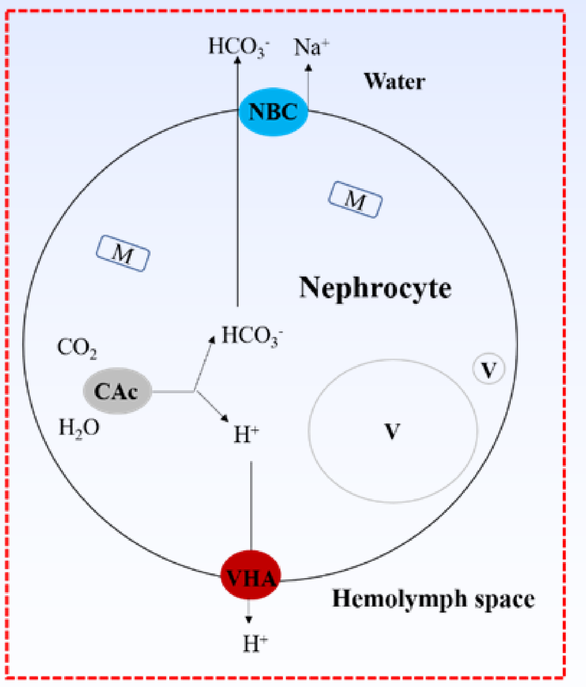


Figure 6. Hypothetical model of ion transport in gill cells of E. carinicauda after alkalinity stress according to the previous study with modification. The expression of ion transport related genes in the green box are down-regulated and the expression of ion transport related genes in the red box are up-regulated.

Contacts

Jian Li: lijian@ysfri.ac.cn

Qianqian Ge: ge2682@126.com