アカテガニの生理・生態学的な研究

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川村龍矢，鈴木信雄

〒927-0553 鳳珠郡能登町小木 金沢大学 環日本海域環境研究センター 臨海実験施設

Ryoya KAWAMURA, Nobuo SUZUKI: Physiological and ecological study of red clawed crab, *Chiromantes haematocheir*

Background

The red-clawed crab *Chiromantes haematocheir* (DE HAAN) (Figure 1) is a crustacean belonging to the decapod family Crassulaceae, and inhabits coasts and forests from mainland to southwest Islands in Japan. Adults of the Red-clawed crab live in forests, but they have a unique life history in which they temporarily live in the sea during the growth process from the zoea and megalopa stages until they become juvenile crabs. On the other hand, Tsukumo Bay in Noto Town, Ishikawa Prefecture has a long coastline and a drowning valley topography with forests near the sea. Therefore, in this study, I focused on the physiological and ecological characteristics of the larvae of the Red-clawed crab inhabiting Tsukumo Bay, Noto Peninsula.

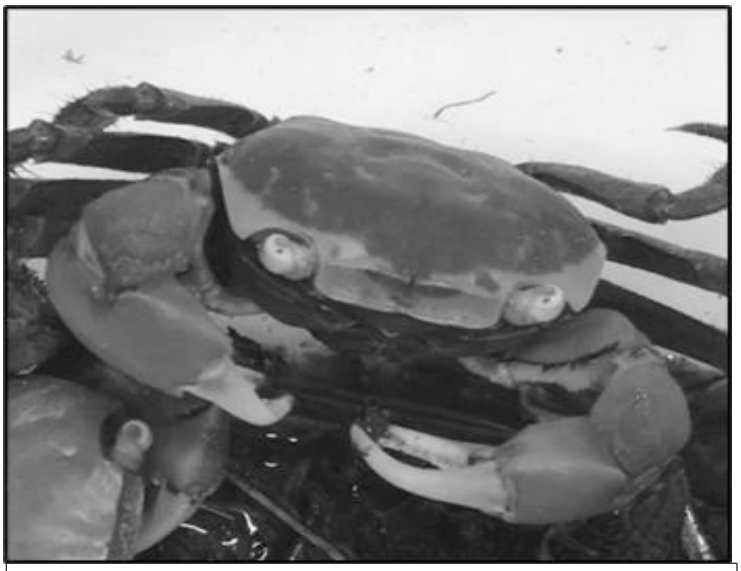

Figure 1.
Photograph of male red-clawed crab, *Chiromantes haematocheir* collected in the coastal forests of Tsukumo bay.

Methods

I collected female red-clawed crabs that laid eggs on the coast of Tsukumo Bay, Noto Peninsula. Zoea larvae were obtained by artificially letting offspring of the crabs. Subsequently, the zoea larvae were placed in a petri

dish, and artificially reared by feeding S-type rotifers, until megalopa larvae were reared. Larvae were sampled during the rearing period and placed in RNAlater for cryopreservation. Total RNA was extracted from the sampled larvae, reverse transcription was performed, and qPCR was performed to analyze the gene expression of the red-clawed crab larvae.

Expression analysis used Na^+/K^+-ATPase: *nka*, which is known to be involved in osmotic regulation. The sequence of *nka* was determined by RNAseq analysis. On the other hand, research on rhythm was also carried out along with ecological surveys. I set up a trap in Tsukumo Bay and investigated the return pattern of megalopa larvae in comparison with the tidal rhythm. To further investigate the intrinsic rhythm, the clock genes *per1* and *timeless* were sequenced from RNAseq data and expressed by qPCR.

Results and Discussion

In this study, I first focused on the osmoregulatory function. The salt tolerance of newly released zoea larvae in captivity was investigated, and it was found that the zoea larvae immediately adapted into hypersaline environment after hatching, and that the salinity range of 9 to 33 PSU does not affect their survival. Therefore, I investigated the changes in *nka* expression levels during the developmental process from zoea larvae to megalopa larvae by qPCR method. The expression level of *nka* tended to be lower in megalopa larvae than in zoea larvae. It is expected that megalopa larvae have reduced adaptability to seawater, and may be related to megalopa larvae returning to shore.

On the other hand, traps for megalopa larvae were set up at multiple locations in Tsukumo Bay, and the patterns of megalopa larvae returning to the shore were investigated. Therefore, I focused on the rhythm of red-clawed crab larvae and examined it using multiple clock genes. As a result, it was found that zoea larvae have a 24-hour cycle expression rhythm for *per1* and *timeless*, suggesting that zoea larvae have an intrinsic rhythm. In the future, I will be planning to investigate the relationship between tidal rhythms and changes in the expression of clock genes.

From the above, it was found that there was a change in the osmoregulatory function during the larval stage of the red-clawed crab, and that this change was accompanied by a change in the expression level of *nka*, which is involved in osmoregulation. In addition, it is possible that the endogenous rhythm of red-clawed crab larvae is related to the observed return to shore in the spring tide of megalopa larvae.

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