
Research Paper

**A Comparative Study on the Blood Osmolality of the Mud Crab
(*Scylla serrata*) and the Blue Swimming Crab (*Portunus pelagicus*)
Exposed to Different Salinities: A Case Study for
the Topic “Osmotic Regulation” in High School Biology**

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Blood osmolality of the mud crab (*Scylla serrata*) and blue swimming crab (*Portunus pelagicus*) was examined 0, 1, 3, 6, 9, 12, 24, 48, 72 and 96 hrs after transferred from seawater of salinity of 30 ppt to salinities of 5, 10, 15, 20, 25, 30 (control), 35 and 40 ppt at 25°C. Blood osmolality of both crab species reached to constant levels within 72 hrs. *S. serrata* survived for 96 hrs in all salinities tested whereas *P. pelagicus* survived for 96 hrs in salinities of 15, 20, 25, 30, 35 and 40 ppt but died 12 hrs after transferred into salinities of 5 and 10 ppt. Thus, the salinity ranges over which osmoregulation was performed efficiently were 5 - 40 ppt for *S. serrata* and 15 - 40 ppt for *P. pelagicus*. The results showed that *S. serrata* is a strong hyperosmotic regulator whereas *P. pelagicus* is an ordinary osmoconformer. This study could probably provide a laboratory model for teaching of osmotic regulation system for high school biology.

Keywords: blood osmolality, blue swimming crab, laboratory model, mud crab, Portunus pelagicus, Scylla serrata

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Introduction

“Osmotic regulation” or “Osmoregulation” is the regulation of water amount and ion concentrations in the body. Keeping this regulation precise is critical in maintaining life. Animals in marine environment can survive in media of different salinities and may be good osmoregulators depending on the salinity range. Most marine crustaceans have body fluids that are isosmotic with the medium in which they live. When the concentration of the medium changes, an animal may respond to the changes in one of the following two ways: One is simply allow the

osmotic concentration of its body fluids to correspond to that of the medium, thus remaining isosmotic with the medium; such an animal is called an “osmoconformer”. The other is to maintain or regulate its osmotic concentration at a certain level in spite of external concentration changes, such an animal is called an “osmoregulator”.

Changes in salinity may disrupt the osmotic balance of decapod crustaceans (Lignot *et al.*, 2000). In readjusting the osmotic concentration, they may have to expend considerable quantities of energy. Several studies have shown that vari-

ous species of the shore crabs have remarkably well developed abilities of osmotic regulation, allowing them to withstand exposure to media ranging from 10 to 200% seawater (SW) (Graszynski and Bigalke, 1986). Some crabs can keep their blood composition appreciably hyperosmotic relative to the medium when acclimated to low salinities (De Vries *et al.*, 1994). Exposure of crabs to low salinity is clearly detrimental to osmoregulatory ability (Bamber and Depledge, 1997), for example, *Callinectes sapidus* and *C. similis* exhibit a decline in hemolymph osmolality at low salinities (Guerin and Stickle, 1997) and *Rhithropanopeus harrisii* is a hyperosmotic regulator at salinities below 24 ppt and become hypoosmotic at higher salinities (Diamond *et al.*, 1989). Mud fiddler crabs were shown to be hyperosmoregulator in 10% SW and 50% SW and to be hypoosmoregulator in 150% SW and 200% SW (Holliday, 1985). *Hemigrapsus sanguineus* exhibit strong hyperosmoregulation in the range of 50% SW - 75% SW (Watanabe, 1982).

The mud crab (*Scylla serrata*) and the blue crab (*Portunus pelagicus*) are native throughout the Indo-West Pacific region (Xiao and Kumar, 2004), however their natural habitats are different. *S. serrata* is an estuarine-adapted crab found in mangrove swamps, whereas *P. pelagicus*, also known as sand crabs inhabits in shallow coastal water area. Due to the differences in the natural habitats of *S. serrata* and *P. pelagicus*, both species are thus likely to encounter different salinities. Since salinity is one of the important environmental factors used to test physiological response of aquatic animals. The response of both species of crab to fluctuations in salinity may be described by determining those species' ability to regulate their osmotic concentration.

The purpose of the present study is to determine the capability of *S. serrata* and *P. pelagicus* to regulate osmotic concentrations in

the blood, when they are transferred from 30 ppt to 5, 10, 15, 20, 25, 30 (control), 35 and 40 ppt in the laboratory. This will provide an understanding of the problem of how these species responds osmotically to variable salinities.

Materials and Methods

Maintenance of Crabs

Adult males of the mud crab *S. serrata* (with average weight of 83 ± 5 g and carapace length of 116 ± 18 mm) and the blue crab *P. pelagicus* (with average weight of 56 ± 7 g and carapace length of 106 ± 13 mm) were obtained from a private farm, Chantaburi province and from fishermen at Bangsaen, Chonburi province, Thailand, respectively. The crabs were transported to the laboratory in Department of Aquatic Science, Faculty of Science, Burapha University, and maintained in fiberglass tanks with a circulating seawater system at temperature of 25°C and salinity of 30 ppt under natural dark-light cycle. Seawater was changed every a few days for duration of the experimental period. The crabs were acclimated for at least one week prior using in the experiments and fed every day with pieces of mussel. But they were not fed 48 hrs before and during the experimental periods. Only crabs in the intermolt stage were selected for the study.

Experimental media

Seawaters of different salinities were used as test media for osmotic regulation studies. The normal seawater was 30 ppt in salinity which equivalent to 943 mOsm kg^{-1} . Concentrated seawaters (35 and 40 ppt) were obtained by evaporating the natural seawater. Diluted seawaters (5, 10, 15, 20 and 25 ppt) were prepared by dilution of natural seawater with distilled water. Osmolalities of experimental media are shown in Table 1.

Table 1 Osmolalities of experimental media

Salinity (ppt)	5	10	15	20	25	30	35	40
Osmolality (mOsm kg ⁻¹)	157	314	471	629	786	943	1100	1257

Measurement of blood osmolality

Forty-eight crabs of each species (*S. serrata* and *P. pelagicus*) were transferred individually from the holding tanks to a glass chamber containing 2 liter of each test medium. Individual chamber was aerated throughout the experiment. There were eight treatments (salinity levels of 5, 10, 15, 20, 25, 30, 35 and 40 ppt) and each treatment was conducted in six replicates with one crab in each chamber.

The osmolality of seawater and the blood osmolality of *S. serrata* and *P. pelagicus* were measured at 1, 3, 6, 9, 12, 24, 48 and 96 hrs after transferring the crabs into each experimental salinity. A 10 µl of blood sample was drawn from each crab by puncturing the membrane at the base of the fifth walking leg with a 26g needle. The osmolality of blood sample was read in an osmometer (Wescor Vapor Pressure Osmometer model 5520) which had been calibrated with standard solutions of 100, 290 and 1000 mOsm kg⁻¹.

Data analysis

Blood osmolalities of crabs were expressed as mean ± S.D. Data were analyzed by using SPSS (Statistical Package for the Social Sciences) computer program. Differences in blood osmolality after abrupt salinity changes were compared using a one-way analysis of variance to estimate the time when steady-state values were reached. If statistically significant differences were indicated at 0.05 level, then subsequent multiple comparisons of means among treatments were performed using the Scheffe pairwise comparisons method. If the blood osmolality value at a certain point in time was the same as that at later times, then the blood con-

sidered to have reached a steady state at that point (in time). The relationships between mean blood osmolality and medium osmolality were plotted to estimate the isosmotic crossover values, where blood osmolality = medium osmolality.

Results**Blood osmolality of *Scylla serrata***

Changes in blood osmolality were observed in the crabs immediately after transferred from 30 ppt to different salinities. The blood osmolalities of *S. serrata* in relation to time are depicted in Figure 1. *S. serrata* survived and their blood osmolalities reached constant levels within 72 hrs in all test salinities. At 72 hr, the mean blood osmolality in the control crabs (30 ppt) was 958±23 mOsm kg⁻¹. For crabs, 72 hrs after transferred from 30 ppt to 5, 10, 15, 20, 25, 35 and 40 ppt, blood osmolalities stabilized at 743±54, 762±27, 777±28, 803±13, 849±24, 1077±10 and 1221±42 mOsm.kg⁻¹, respectively. The blood osmolality of *S. serrata* as a function of medium osmolality is given in Figure 2. The isosmotic point was approximately 1000 mOsm kg⁻¹ which is equivalent to the salinity of 31.8 ppt.

Blood osmolality of *Portunus pelagicus*

Changes in blood osmolality were observed in the crabs immediately after transferred from 30 ppt to different salinities. The blood osmolalities of *P. pelagicus* in relation to time are depicted in Figure 3. *P. pelagicus* survived in media ranging from 15 ppt – 40 ppt. At 5 and 10 ppt, the blood osmolalities of crabs rapidly decreased and the crabs could not survive after 12 hrs of exposure. At 15, 20 and 25 ppt, the blood

osmolalities of crabs decreased slowly and then became constant after 72 hrs. The blood osmolalities of crabs at 72 hrs in 15, 20 and 25 ppt seawater were 627 ± 24 , 757 ± 24 and 841 ± 23 mOsm kg^{-1} in 15, 20 and 25 ppt at 72 h, respectively. In the salinity of 30 ppt, the blood osmolality of the crab was 969 ± 28 mOsm kg^{-1} at 72 hr of exposure time. In 35 and 40 ppt, blood osmo-

lality increased slowly and became constant at 1105 ± 19 and 1252 ± 32 mOsm kg^{-1} after 72 hrs respectively. The blood osmolality of *P. pelagicus* as a function of medium osmolality is given in Figure 4. The isoosmotic point was approximately 1100 mOsm kg^{-1} which is equivalent to the salinity of 35 ppt.

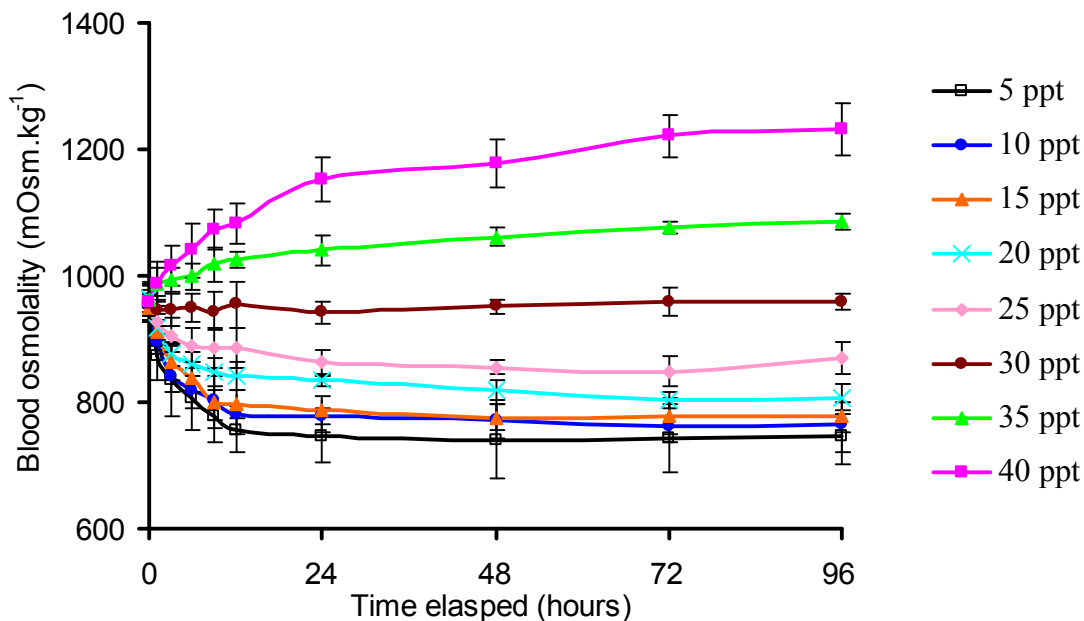


Figure 1 Changes in the blood osmolality of *Scylla serrata* when the crabs were subjected to different salinity levels for different time period at 25°C (n=6)

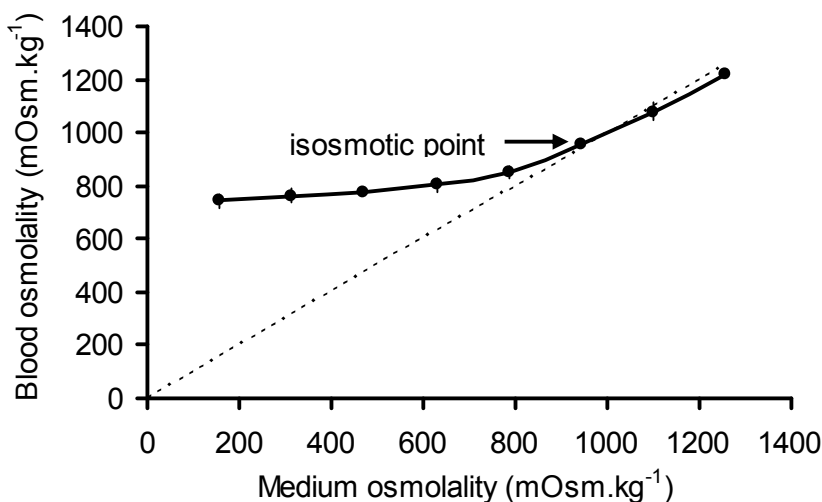


Figure 2 The relationships between the blood osmolality of *Scylla serrata* and the medium osmolality

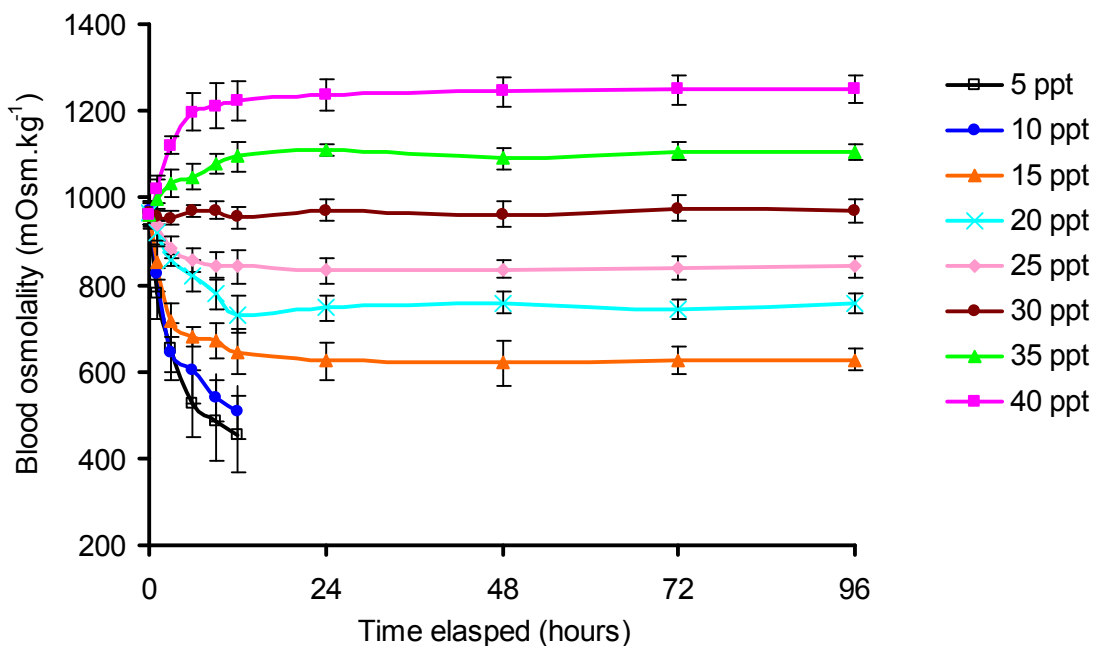


Figure 3 Changes in the blood osmolality of *Portunus pelagicus* when the crabs were subjected to different salinity levels for different time period at 25°C (n=6)

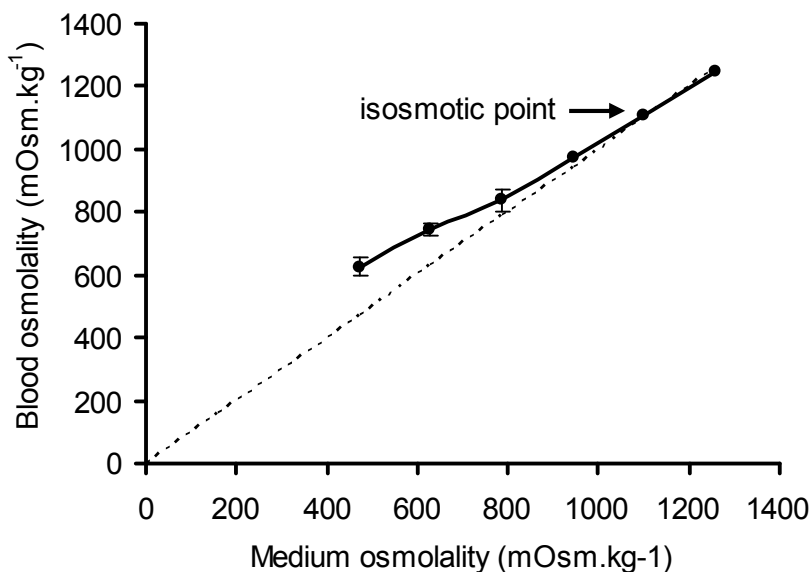


Figure 4 The relationships between the blood osmolality of *Portunus pelagicus* and the medium osmolality

Discussion

The osmoregulatory ability of aquatic animals can be determined by measuring the blood osmolality under various salinity conditions (Lignot *et al.*, 2000) and comparing with the osmolality of the medium. The present study

showed that *S. serrata* is hyperosmoregulator in a medium below 1000 mOsm kg⁻¹ and become an osmoconformer in a medium above that level. The similar finding was obtained by Davenport and Wong (1987) who reported that *S. serrata* is a hyperosmoregulator in salinities lower than

those of the open sea, and becomes an osmoconformer at a medium osmolality greater than 900 mOsm kg⁻¹ at 28-30°C. The blue crab *C. sapidus* (Tagatz, 1971) and the mud crab *Panopeus herbstii* (Blasco and Forward, 1988) also regulate their blood osmolalities hyperosmotically at salinities lower than 28 ppt and become osmoconformers at salinities higher than 28 ppt. *S. serrata* is a large portunid crab, which matures and spawns in seawater, spends post-larval and juvenile phases in brackish water, and then returns to the sea as a pre-adult. Thus, this species is likely to encounter fluctuation of salinity in natural habitat.

Comparing with the *S. serrata*, blood osmolality of *P. pelagicus* decreased with the reduction of salinity and exhibited positive linear relationship with the medium osmolality. *P. pelagicus* was observed to be osmoconformer. Similar results have been reported for some penaeid prawns (Chen and Lin, 1994; Sang and Fotedar, 2004; Setiarto *et al.*, 2004) and other portunid crabs (Guerin and Stickle, 1997). *P. pelagicus* is found in coastal marine and estuarine waters throughout the Indo-West Pacific (Kailola *et al.*, 1993), China, Japan, Philippines, Australia, Thailand and East Africa. In Thailand, *P. pelagicus* is mainly found along the Gulf of Thailand, in the estuary areas and the Andaman Sea. This crab species lives in a wide range of inshore and continental shelf areas, including sandy, muddy, algal and seagrass habitats, from the intertidal zone to a depth of at least 50 m (Edgar, 1990). It usually lives under stones and seaweeds in rock pools. However, in some areas it is found living on sandy bottoms, using the swimming paddles to excavate a burrow to hide.

The results of this study demonstrated that *P. pelagicus* is a stenohaline species and sufficiently sensitive to environmental changes such as salinity. *P. pelagicus* can tolerate only a narrow salinity range which makes difference from

S. serrata, being euryhaline crabs that can by definition tolerate a wide range of salinities. In laboratory, *P. pelagicus* showed to be an osmoconformer or a poor osmoregulator, which does not enable them to cope with a low salinity level less than 15 ppt. This characteristic stands in contrast with *S. serrata* which is a good osmoregulator and can survive in media of salinity range from 5 to 40 ppt. Salinity can have an immediate and significant effect on survival and growth of *P. pelagicus*. It has been suggested that salinities closer to the isosmotic point result in decreased metabolic demands and, therefore, increased growth, since the crab would be expending the least energy in doing osmotic work (Panikkar, 1968). The suitable salinity range has significant implications for aquaculture.

This study could also probably be used as laboratory model for teaching of osmotic regulation system for high school biology project or laboratory on the topic "Osmotic regulation," since there are several practical benefits of using crustaceans in teaching lab: 1) they are less expensive than vertebrates, 2) their use does not require approval of animal-care communities.

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