INFULENCE OF CONTAMINATED SEDIMENTS ON JAPANAESE HORSESHOE CRAB

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ABSTRACT
To examine the present and old sediment qualities, 1.5m-sediment core sample was obtained from the bottom of Kasaoka bay in Japan and it was divided into two parts, i.e., top (upper 50 cm) and bottom (lower 50cm) parts. These core sediments and other surface sediments obtained from different part of the bay were used for the breeding experiments of horseshoe crab larvae. Thirty larvae were used in each experiment. The death rate of the larvae varied with the degree of contamination of sediments with hazardous organic compounds, such as *Tributyltin*,TBT and *Triphenyltin* ,TPT , heavy metals, etc.

RÉSUMÉ
Afin d'examiner les caractéristiques des sédiments actuels et anciens, un échantillon de 1.5 m provenant du fond de la baie Kasaoka au Japon a été divisé en deux parties, c'est-à-dire le haut (50cm) et le bas (50cm). Les sédiments obtenus à la surface et au milieu de différents endroits dans la baie ont été utilisés pour les expériences des larvées du crabe Horseshoe. Trente (30) larvées ont été utilisées pour chaque expérience. Le taux de mortalité des larvées variait selon le degré de contamination des sédiments par des composés organiques nocifs, tels que le *Tributyltim*, TBT et le *Triphenyltin,* TPT, les métaux lourds, etc.

1. INTRODUCTION

A seed of Japanese horseshoe crab, which is one of the four species of horseshoe crabs living in the world, continues to preserve the seed for a period of two hundred million years (see Figure 1). However, the Japanese horseshoe crab is nearing the crisis of annihilation due to a rapid environmental change. The Japanese horseshoe crab is on the red list published by the ministry of the environment of Japan, as well as other precious species.

There are several breeding areas and habitat of Japanese horseshoe crab in Seto Inland Sea and some other parts of Japan. Kasaoka Bay, which located in the middle part of Seto Inland Sea, was designated to a natural monument in 1928, because it was the northern limit of the breeding area and habitat.

The rapid change of environment in Kasaoka Bay was initiated by the reclamation of the bay, started in 1967. The bay was, therefore, changed from the open bay to a narrow waterway, as shown in Figure 2. The reclamation of Kasaoka Bay has decreased the habitat of the horseshoe crabs. At present, life and agricultural wastewater is drained into Kasaoka bay through the existing rivers and drains.

Kasaoka-city releases a large number of horseshoe crab larvae every year for the proliferation of horseshoe crabs. In fact, more than two hundred thousand larvae have released into Kasaoka bay since 1975. However, until now, the larvae were seldom found in the bay. Therefore, Kasaoka-city made investigation twice to know the reason why the larvae could not live. The reports concluded that the waves induced by high-speed boats gave bad influence on the habitat. It was also commented that polluted sediments and seawater in some parts of Kasaoka bay damaged the horseshoe crabs and their embryos (Itow, 1994). It may be also possible that the contaminants of sediments damage to the horseshoe crabs, because they inhabit in muddy sediments.

The aim of this study is to investigate the sediments in Kasaoka bay, and to examine the sediment qualities from the breeding experiments using the horseshoe crab larvae.

Figure 1. Adult Japanese horseshoe crab.

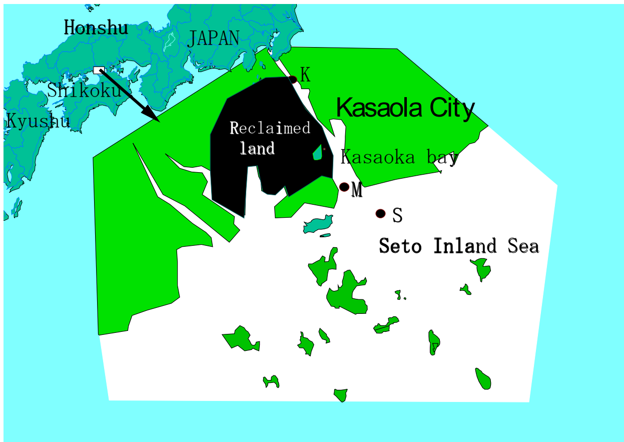


Figure 2. Sampling sites of sediments in Seto Inland Sea.

2. SITE LOCATIONS AND SEDIMENTS

To perform the breeding experiments of the larvae of horseshoe crabs, sediment core samples were obtained from two sites of Kasaoka bay. At the same time, sediments were obtained from the surrounding area to compare the sediment qualities (Fukue, et.al., 2003). The core samples were obtained with a gravity box core sampler. The length of the corer was 1.5 meter. The sampling positions are indicated as K , M and S in Figure 2. Sites K locates behind Kasaoka bay, and Site M locates at the mouth of Kasaoka bay. Site S locates at the offshore of Kasaoka bay. The core sample obtained from Site K was divided into two parts, i.e., top (upper 50 cm) and bottom (lower 50cm) parts, to distinguish into Ka and Kb, respectively. The samples obtained from Sites K and M were subjected to the breeding experiments.

3. PROPERTIES AND CONTAMINATION OF SEDIMENTS

All the sediments obtained are silty-clay. The specific gravity of the samples ranges between 2.6 and 2.73. The contents of organic matter are relatively low. To investigate the contamination of the sediments, the concentrations of eleven elements, i.e., Al, Ba, Cd, Co, Cu, Fe, Mn, Ni, Pb, Ti and Zn, were measured using ICP analysis. The concentration of phosphorus was measured using the atomic absorption analysis. The concentrations of organotin compounds were measured with GC/MAS. The leaching tests of heavy metals on the sediment samples were also performed.

Table 1 shows the concentrations of some heavy metals (See Fukue et al, 2003), phosphorus, aluminum, TBT and TPT. The ADI for TPT established by FAO/WHO is 0.5 µg/kg/day. The comparison of TBT and TPT concentrations between the three sites is shown in Figures 3 and 4. From the figures, it is obvious that the K sediments are considerably contaminated with TBT and TPT. Though the

Table 1 Contaminants in the sediments.

Site / elements	Ka (surface)	Kb (0.5-1m)	M (surface)	S (surface)
Al (g/kg)	81.3	77	67.7	81.6
P (mg/kg)	636	399	196	426
Ti (g/kg)	3.4	3	2.8	3
Mn (mg/kg)	1647	1005	1063	1399
Fe(g/kg)	29.8	25.2	26.8	31.3
Cu(mg/kg)	46.8	22.8	40.1	45.6
Zn(mg/kg)	291.0	172.4	154.3	277.9
Pb(mg/kg)	128.0	95.0	54.4	42.6
TBT(µg/kg)	90	0.3	16	13
TPT(µg/kg)	150	0.1	0.8	1.1

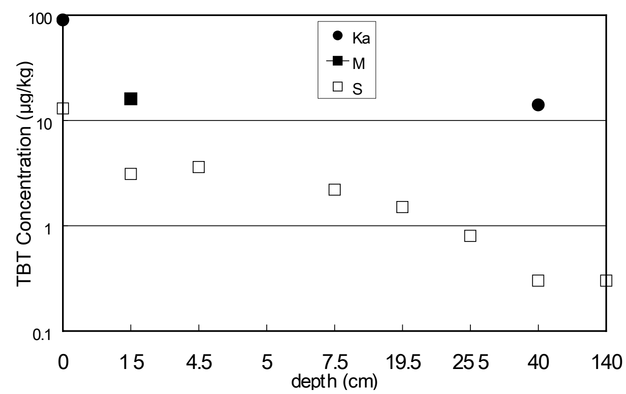


Figure 3. TBT concentrations in the investigation sites.

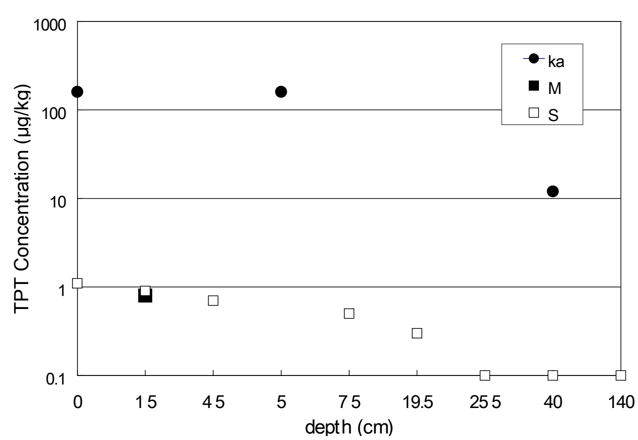


Figure 4. TPT concentrations in the investigation sites.

concentrations of the Kb sediments were not measured, it is assumed to be nearly background value, because of the depth. Note that TBT and TPT are not an old case so that it was used.

It is understood that organotin compounds are extremely harmful for some creatures. In marine environments, the organotin was dissolved from the paint that was used for ships. Many researchers pointed out that a very low concentration of TBT causes an abnormal generative organ of some kind of spiral shells. It was found that the thickness of contaminated sediments with TBT and TPT is around 50 cm (Fukue, et.al., 2001). The contaminated thickness of sediments decreases with a distance from the coastal lines.

The leachates from the sediments showed a very low concentration of heavy metals and other hazardous substances. This is because the sediments have already been washed during the time of deposition (Fukue et al, 2003). And also, the sediments have been submerged in the seawater for a long time.

The Pb concentration in Site Ka is 128 mg/kg and is approximately six times greater than the background value, i.e., 20 mg/kg (Fukue et al, 2003). For other heavy metals, a similar trend can be seen. Considering that the background values of organotins are almost zero, the concentrations of TBT and TPT for Ka sediments are extremely high.

From Table 1, the concentration with respect to each element is in the following orders.

With concentration of P
Ka>S>Kb>M
With concentration of Cu
Ka>S>M>Kb
With concentration of Pb
Ka>Kb>M>S
With concentration of TBT
Ka>M>S>Kb
With concentration of TPT
Ka>S=M>Kb

The results obviously show that Ka sediments show the worst quality of the three types of sediments with respect to contamination. The elements and compounds considered here don't necessarily damage the horseshoe crabs. However, there are other hazardous substances, such as ammonium, sulfides, etc., contained in the sediments, and their concentrations may show a similar trend shown above.

4. BREEDING EXPERIMENTS

The breeding experiments of first-instar larvae were made using the sediment samples, i.e., Ka, Kb and M sediments. The sediment samples were put into containers, respectively. Seawater filtered by sands was poured in the containers. Then, thirty first-instar larvae were put in the each container, as shown in Figure 5. Commercially available small shrimps were used as the bait. The bait was usually given once in 3 days. Occasionally, they were kept for a week without bait. A half volume of seawater was exchanged when the bait was given.

The breeding experiment was performed for 535 days. The death rate during the experiments is shown in Figure 6. At

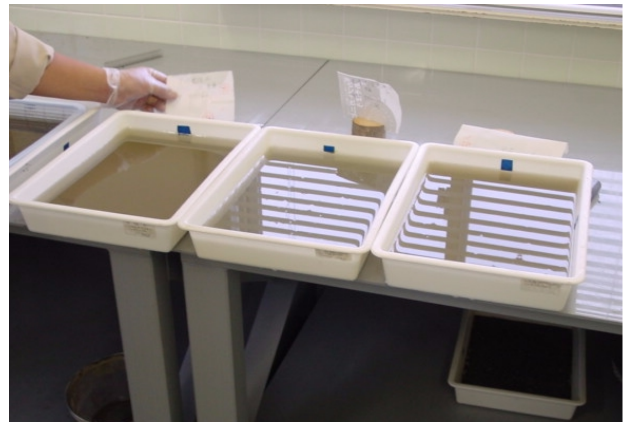


Figure 5. Breeding experiments of horseshoe crabs.

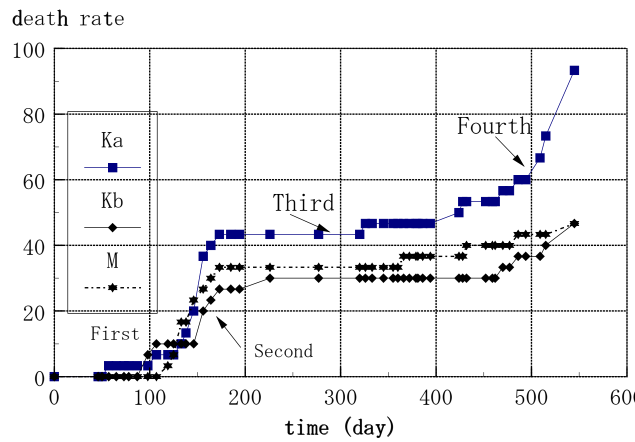


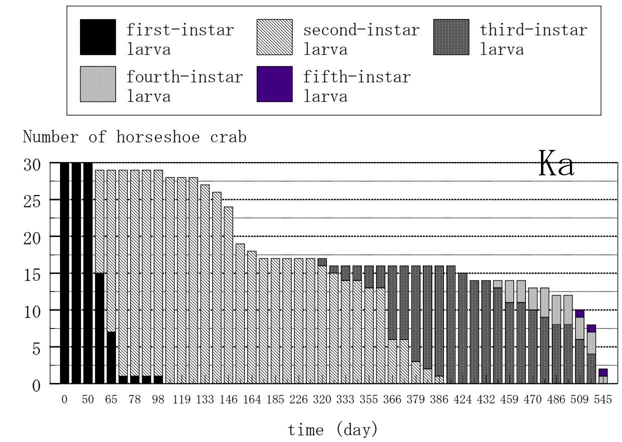
Figure 6. Death rates of larvae for different sediments.

the beginning of the experiments, the larvae little died, as shown in Figure 6. It can be seen that the death rates consist of four stages. The first stage shows a very low death rate until 100 days. The second stage is described as an abrupt increase in the death rate form 100 to 170 days. The third stage is described as a stable condition, in which a number of dead bodies are very little. This stage ranges from 170 to 400 days. After 400 days, the death rate increased again. This stage is the fourth stage. At this stage, only one larva remained for the Ka sediments, while 16 larvae are still alive in the Kb and M sediments. The sediments in this stage were obviously changed from initial states, when fungi were found on the surface of the sediments used for the experiments.

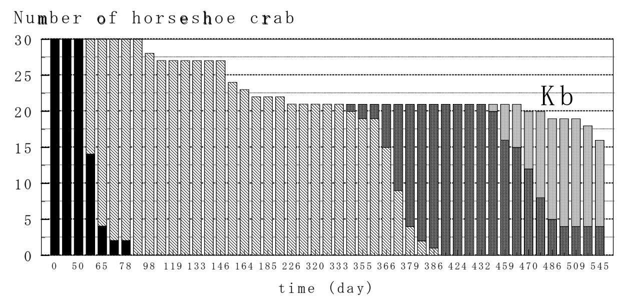
After 400 days, the death rates are 44 % for the Ka sediments, 27 % for the M sediments, and 25 % for the Kb sediments. Note that the Kb sediments older than the Ka sediments for the same site. Therefore, the result implies that the younger sediments are more contaminated than the older sediments, and that the contamination damaged the larvae of horseshoe crabs. At present, there is no proof which substance more affects the death rate of the larvae. It may be due to the compound contamination.

Though it is important to know what is a serious substance, many breeding experiments are required to understand it. If sediments are removed by dredging, the species and concentration of the substances become less important. In this case, the dredged sediments have to be disposed properly.

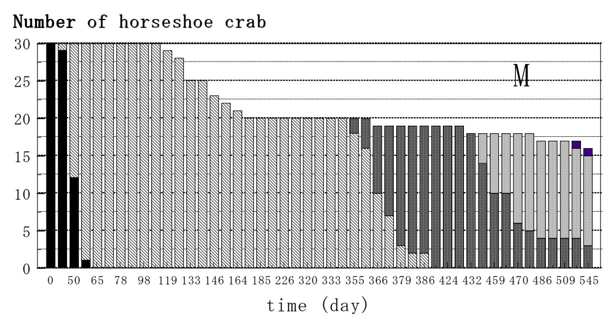
The experimental results are also shown in Figure 7. The horseshoe crabs repeated molting to grow up. A number of molting is also shown in Figure 7. From the Figure 7, it is seen that the death rate is the highest for second-instar larvae. This trend accords with previous experience that the second-instar larvae are the most sensitive to environment. Most of the remained second-instar larvae continue to live and become third-instar larvae, as seen in Figure 7. At 400 days, all the remained larvae are third-instar larvae. During the experiments, one of the larvae molted five times for the Ka and M sediments, respectively. The number of the molting seems to be independent of the death rate.



a) Molting of larvae for Ka sediments.



b) Molting of larvae for Kb sediments.



c) Molting of larvae for M sediments.

Figure 7. Breeding experimental results using different sediments.

5. CONCLUSIONS

Sediment quality in Kasaoka bay, designated to a natural monument, was examined by breeding experiments of the Japanese horseshoe crabs. The chemical analysis showed that the sediments were contaminated with heavy metals and oraganotin compounds. The surface sediments with a thickness of 50 cm were more contaminated than the deeper sediments.

The breeding experiments of the Japanese horseshoe crabs were made using different sediments obtained from Kasaoka bay. The experimental results showed that the higher degree of contamination provide a higher death rate of horseshoe crabs.

The horseshoe crab, which has preserved the seed for a period of two hundred million years, can't live any more in Kasaoka bay. One of the reasons of this is the contamination of the sediments. Therefore, it is required to remove or remediate the contaminated sea bottom.

ACKNOWLEDGEMENT

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