THE EFFECT OF HYDROGEN ION CONCENTRATION AND SALINITY
OF WATER ON EXTRUDING RESPONSE AND SURVIVAL OF
ONCOMELANIA NOSOPHORA, THE VECTOR SNAIL OF
SCHISTOSOMA JAPONICUM IN JAPAN* **

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Oncomelania quadrasi, the vector snail of *Schistosoma japonicum* in the Philippines, is said to have been found in waters ranging from pH 6.2 to pH 7.8 and on soils ranging from pH 4.6 to pH 7.2 (Pesigan *et al.*, 1958). On the pH range conditioning the activity and survival of *O. nosophora*, however, very few reports are available.

As to the effect of sea water on the survival of *Oncomelania* snails, Muto *et al.* (1915) and Nagao *et al.* (1918) noted that when snails were submerged into sea water experimentally all of them died within 49 hours and 4 to 6 days, respectively. Practically, the habitat of *O. nosophora* in Suzunuma district of Shizuoka Prefecture was flooded with sea water in July 1917 and as a result of this almost all snails were found dead (Muto *et al.*, 1918). The present report gives data concerning the effect of pH and salinity on the extruding response and survival of *O. nosophora*.

MATERIALS AND METHODS

Snails: *Oncomelania nosophora* used in this experiment were collected from the suburbs of Kofu City in Yamanashi Prefecture. Up to the commencement of experiment they were kept off the water for two or three days in the laboratory. Only snails which were actively moving when placed in dechlorinated water were selected for the experiment.

pH: Sixteen series of water with various pH values, namely 2.2, 2.7, 2.9, 3.3, 3.5, 4.0, 4.2, 5.6, 7.2, 7.5, 8.9, 9.2, 10.0, 10.4, 11.1 and 11.3, were produced by the addition of different amounts of N/10 HCl or N/10 NaOH into distilled water. During the experiment the pH value was measured three times, at the beginning, in the middle and at the termination of the experiment, by means of Hitachi pH Meter. The pH value during the period of experiment varied by only ± 0.1 from the starting pH.

Salinity: To make the experimental condition as simple as possible, Herbst's artificial sea water (5/9M NaCl 100, 5/9M KCl 2.1, 10/27M CaCl₂ 3.4 and 10/27M MgCl₂ 15.9) was used. The pH value in this mixture varied between 6.2 and 6.4.

Observations: (1) Extruding response: When a snail was put into water, it opens the operculum, initially stretches out the foot gradually and the head with the tentacles later. The grade of the response was classified "+" when they opened the opercula and stretched the feet and heads: "±" when they only slightly opened the opercula: and "-" when their opercula

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remained closed. In this paper, however, the response such as “ $\pm$ ” will be included in “—” to facilitate understanding of the general feature of the phenomena observed. The response was measured by the percentage of snails which responded.

(2) Survival: At the conclusion of exposure all snails were rinsed with distilled water, laid in dechlorinated water for 24 hours and then examined for survival or death. Snails were judged as dead when they showed no movement after their transfer into water. Those which showed no movement were dissected afterwards to confirm their death.

RESULTS

1. Extruding Response in Various pH Values.

Thirty snails were put into a reagent bottle (wide mouth), 10 cm in diameter and 15 cm in height, containing 750 cc of water with various pH values, and their extruding responses were observed. The temperature during the period of observation ranged from 24° to 26°C. Fig. 1 shows the results of the observation. As early as one hour after placing snails into water, all snails showed the extruding response in each series between pH 4.0 and 10.0. At pH 10.0, however, the number of snails which responded decreased slightly with the lapse of time and six hours later, only 25 or 83% of the

Fig. 1. The extruding response of *O. nosophora* in waters of various pH values (24°C–26°C).

snails showed the response. At pH 10.4, one hour later, 26 or 87% of the snails showed the response, but afterwards began to pull in their head, and two hours later, the percentage of the number of snails which responded varied slightly from 30% to 40%. At pH 3.5 and 3.3, the percentage was 17% and 13%, respectively after two hours. Such a state of the response continued for the following six hours. In acid waters such

as below pH 2.9 and in alkaline waters such as above pH 11.1 no extruding response was observed at all. Fig. 2 shows the percentage of the number of snails which responded in various pH values after 6 hours.

Fig. 2. The summary of extruding response of *O. nosophora* in various pH values 6 hours after placing in water (24°C-26°C).

2. Survival in Various pH Values.

The apparatus used in this experiment was the same as that used in the previous section except the following point: before placing snails into waters of various pH values, twenty snails were put into a pouch made of gauze to prevent them from creeping out of water. In each pH series 3 pouches or 60 snails were used. After 24, 48 and 72 hours' application snails in each pH series were examined for their death. The temperature during the period of observation ranged from 24.5 to 27.0°C. The results are shown in Fig. 3. Twenty four hours later, as seen in the figure, the mortality of snails placed into acid waters such as pH 2.9, 2.7 and 2.3 and alkaline ones such as pH 10.4, 11.0 and 11.3 was less than 30%; and 72 hours later, the mortality rose to 50% at pH 2.9, 35% at pH 2.7, 95% at pH 2.3, 55% at pH 10.4, 35% at pH 11.0 and 80% at pH 11.3. Fig. 4 shows the summary of mortality of snails in waters of various pH values after 72 hours. No snails were found dead, within 72 hours, in each series between pH 3.3 and 9.2.

3. Extruding Response in Different Concentrations of Sea Water.

Twenty snails were put into the same bottle as used in the previous sections containing 750 cc of different concentrations of sea water, and their extruding responses were observed one hour after the exposure. The temperature during the period of experiment was $18 \pm 1^\circ\text{C}$. Fig. 5 shows the results of the observation. As seen in this figure, all snails showed the extruding response in concentrations of sea water less than 30%,

Fig. 3. The mortality of *O. nosophora* in waters of various pH values (24.5°C-27.0°C).

Fig. 4. The summary of mortality of *O. nosophora* in waters of various pH values 72 hours after placing (24.5-27.0°C).

whereas no such response was observed in sea water more than 31.7%. The threshold concentration of sea water to induce the response, therefore, exists between 30% and 31.3%.

Fig. 5. The summary of extruding response of *O. nosophora* in different concentrations of artificial sea water 1 hour after exposure ($18 \pm 1^\circ\text{C}$).

4. Survival in Different Concentrations of Sea Water.

Twenty snails placed in a gauze pouch were put into a bottle containing 750 cc of sea water of different concentrations. After 2, 4 and 6 days, the snails in each concentration were examined for viability. The temperature during this experiment was $18-25^\circ\text{C}$. The results are shown in Fig. 6. Two days later, as seen in the figure, the mortality of snails being put into 100% sea water was less than 15%; and 6 days later, the mortality rose to 90% in 100% sea water, 80% in 75%, 50% in 50% and 5% in 25% sea water. No snails were found dead, within 6 days, in 10% sea water.

5. Effect of Salinity on Extruding Response and Survival.

As already mentioned above, all snails showed the extruding response in concentrations of sea water less than 30%. From the viewpoint of osmotic pressure, therefore, all snails should show the response in aquatic solutions of M/8 NaCl, M/8 KCl, M/12 CaCl_2 and M/12 MgCl_2 . To know whether the effect was caused by a salt or an ion the following experiment was performed in the single salt solution at first and then in the mixture of them.

The apparatus and the methods used in this experiment were essentially the same as those used in the previous section. The mixtures of single salt solutions were prepared in the same portions as they are in artificial sea water. The composition of the various

Fig. 6. The mortality of *O. nosophora* in different concentrations of artificial sea water (18–25°C).

solutions used in this experiment is shown in Fig. 7. The observations were made after one hour for the extruding response and after 72 hours for the survival. The temperature during the period of experiment ranged between 18°C and 22°C. The results are summarized in Fig. 7. The rates of the response in four single salt solutions, as seen in the figure, were 0% in M/8 NaCl, 0% in M/8 KCl, 5% in M/12 CaCl₂ and 15% in M/12 MgCl₂. Such an inhibitory effect of NaCl on the response, however, was decreased by the addition of other salts, especially of CaCl₂. The highest rate of the

Fig. 7. The extruding response and mortality of *O. nosophora* in various salt solutions (18–22°C).

response was found in the mixture solutions of four salts, NaCl, KCl, CaCl₂ and MgCl₂.

On the other hand, the mortalities of snails in the four single salt solutions were found to be higher than those in the mixture of them of the same concentration. The lowest mortality was found in the mixture solution of four salts. Thus, the toxic effect of NaCl on snails was decreased by the addition of the other salts, especially of CaCl₂.

DISCUSSION

According to WHO Technical Report Series No. 120 (1958), *Bulinus truncatus*, *Biomphalaria adowensis* and *Australorbis glabratus*, schistosoma-bearing snails, can be bred between pH 4 and pH 10. As already seen in Figs. 2 and 4, *O. nosophora* has also a wide limit of tolerance to pH. Although laboratory data cannot safely be transposed to field situations, the present study suggests that *O. nosophora* has rather a wide range of pH for its distribution.

Muto *et al.* (1915) and Nagao *et al.* (1918) stated that when snails were submerged into sea water experimentally all of them died within 49 hours and 4 to 6 days, respectively. The data in Fig. 6, however, indicate that when snails were submerged into 100% sea water 10% of them still survived in it for 6 days. In the experiments of Muto *et al.* and Nagao *et al.* natural sea water was used, whereas artificial sea water was used in the present study to make the experimental condition as simple as possible. It is said that in case natural sea water is used for laboratory experiment it should be decayed before use by placing it in the laboratory for a long time because it contains various organic matters. However, no description on this point is available in previous works mentioned. In addition, no information is available about the temperature in their papers. The wide difference among Muto's, Nagao's and the present author's results may be ascribed either to the use of different sea waters or to the application of different temperatures during the period of experiment.

Single salt solutions, M/8 NaCl, M/8 KCl, M/12 CaCl₂ and M/12 MgCl₂, as seen in Fig. 3, have high inhibitory and toxic effects on the extruding response and survival of snails. The inhibitory and toxic effects of NaCl, however, were decreased by the addition of the other salts, namely KCl, CaCl₂ and MgCl₂. The possibility that Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺ ions have antagonistic action to one another on the activity and survival of snails must be admitted. It is suggested that high salinity such as sea water has an important role in regulating the activity of snails and in limiting their distribution.

SUMMARY

To know the effect of pH and salinity on the extruding response and survival of *O. nosophora*, several experiments were carried out and the results are summarized as follows:

1. All snails showed the extruding response in waters ranging from pH 4.0 to 9.2.
2. No snails were found dead, within 72 hours, in waters ranging from as low as pH 3.3 to as high as pH 9.2.
3. The threshold concentration of sea water to induce the extruding response of snails exists between 30% and 31.3%.
4. The mortalities of snails submerged into 100%, 75%, 50%, 25% and 10% sea water for 6 days were 90%, 80%, 50%, 5% and 0%, respectively.

5. Na^+ , K^+ , Ca^{++} and Mg^{++} ions have an antagonistic action to one another on the extruding response and survival of snails.

6. High salinity such as sea water has an important role in regulating the activity of snails and in limiting their distribution.

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REFERENCES

- MUTO, M. & USAMI, K. (1915): An opinion on the prevention of *Schistosomiasis japonica* in Shizuoka Prefecture. "Tokyo Iji Shinshi", 1937, 1907-1910 (text in Japanese).
- MUTO, M. & USAMI, K. (1918): On the effect of sea water on *Oncomelania nosophora*, in a flooded district of Shizuoka Prefecture. "Chuo Igaku Zasshi", 25, 81-89 (text in Japanese).
- NAGAO, M. & KATO, S. (1918): The exterminatory measures of *Oncomelania nosophora*, an intermediate host of *Schistosoma japonicum*. "Nihon Shokakigakkai Zasshi", 17, 25-42 (text in Japanese).
- ODUM, E. P., (1953): Fundamentals of ecology. W. B. Saunders Company, Philadelphia.
- PESIGAN, T. P., HAIRSTON, N. G., JAUREGUI, J. J., GARCIA, E. G., SANTOS, A. T., SANTOS, B. C. & BESA, A. A. (1958): Studies on *Schistosoma japonicum* infection in the Philippines 2. The molluscan host. Bull. World Health Org., 18, 481-578.
- W. H. O. (1957): Technical report series, No. 120.