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CHANGES IN TISSUE CHLORIDE AND PHYSIOLOGICAL ACTIVITY OF THE BRACKISH-WATER BIVALVE, *CORBICULA JAPONICA*, IN RESPONSE TO VARIATIONS IN SALINITY

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I. Introduction

The brackish-water bivalve, *Corbicula japonica*, lives in shallow estuarine waters, inlets and brackish lakes. In general, the adult clam is buried on the bottm surface with both their siphons protruding. However, in winter, this animal is found in bottom materials at depths ranging from 4 to 10 cm layer.

In Zyusan-gata Inlet, as already reported in a previous paper (Fuji, 1955), the chlorinity of water shows a wide variation in different seasons. The pattern of seasonal chlorinity variation in this inlet is shown in Figure 1, data having been obtained from daily measurements on chlorinity in the coastal observation. Figure 1 shows that from late spring to middle winter the chlorinity varied between the approximate limits of 0.5 and 1.5% Cl; but after this period to early spring it approaches nearly zero. It is conceivable that the above pattern of chlorinity variation has a similar tendency at the innermost parts of the inlet. Change of chlorinity in bottom water forms a controlling factor in the survival of the animal habitant, in this inlet, associated with other environmental factors. Especially, it possesses more high importance than the others because of its wide and violent variation. The brackish-water habitat is chracterized by marked salinity variation, and in attempting to analyse the physiological adaptations of brackish-water

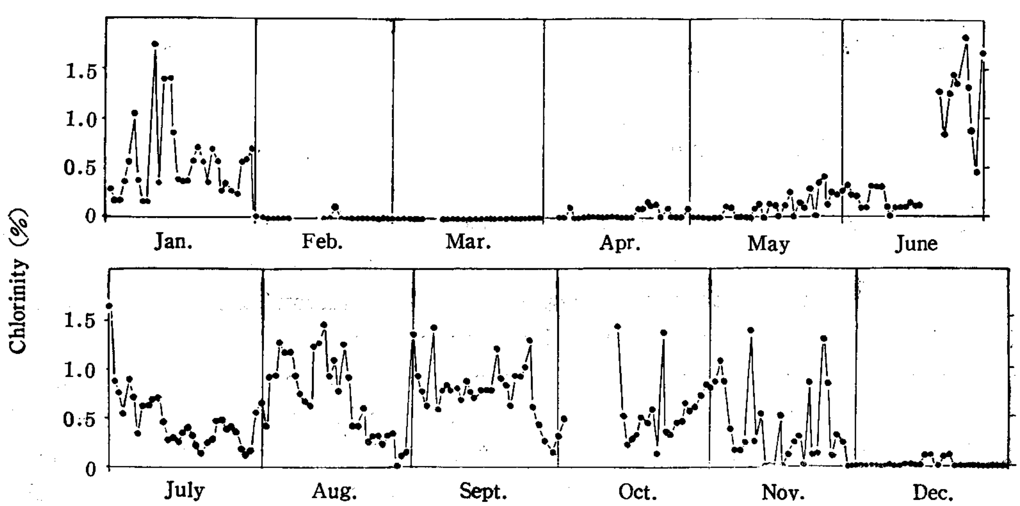

Fig. 1. Pattern of annual chlorinity variation in Zyusan-gata Inlet

animals to their habitat, it is neccessary to consider the extent of salinity variation, which varies from over 1.5% Cl. down to nearly fresh water.

It has been pointed out by many researchers that the salinity change in surrounding water exerts physiological and ecological influence on the marine and fresh water animals. In marine bivalves, Fox (1941) demonstrated that the adult California mussel was able to adjust itself to a considerable range of salinities; Hopkins (1936) reported influences of heterosmotic environments on the shell movements and water filtration of Japanese oyster. In brackish-water animals, Beadle (1931) investigated changes in water content and respiratory activity of *Nereis diversicolor* and *N. cultrifera*. Nomura (1930) pointed out that the interstitial salinity in bottom material plays a controlling role with the animal habitants.

The present study was designed to determine the physiological influence of chlorinity change on the clam from the ecological viewpoint. In this paper are reported the results of field and experimental observations showing variability in the shell movements, water filtration and tissue chloride of the clam in responce to the widely differing chlorinity conditions in company with chloride variations in overlying water and interstitial water in Zyusan-gata Inlet.

Before proceeding further, the author wishes to express his cordial thanks to Prof. T. Tamura of the Faculty of Fisheries, Hokkaido University, for his guidance throughout the work. The author is also indebted to Assist. Prof. H. Ohmi of the same Faculty, for his many valuable advices.

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II. Field observations on the chloride contents in bottom water, interstitial water and clam tissues

In the brackish lakes or estuaries, Asahina (1942), Nomura *et al.* (1955) and Smith (1955, 1956) have specified that the chloride concentration of interstitial water in bottom material has an important function as a controlling factor on the survival of benthic animals.

It is said that the different particle compositions of bottom material make a difference in the interstitial chlorinity profiles, and so, in the muddy bottom, even if the chloride concentration in bottom water is suddenly changed in some degree, the interstitial chlorinity is still kept in its initial concentration, while in the sandy bottom —— such as the clam fields in Zyusan-gata Inlet —— it is unstable (Asahina, 1942).

Field observations were carried out to survey the correlation of chloride concentration between bottom water, interstitial water and clam tissues in June, September and

December. Samplings were repeated four times per area in each month. In order to observe the chlorinity profiles th following operations were carried out at the designed six areas. Water from bottm layer at 1 cm above the bottom was sampled by a syringe of about 25 ml capacity and the chlorinity was determined by means of Mohr or Vorhard method. The bottom material was collected in tubular forms by a steel tube, and was divided into four parts of 0–3, 3–6, 6–9 and 9–12 cm in depth. The bottom samples in each part were brought to laboratory in a rubber stoppered glass tube. In the laboratory 30–40 gram samples were spooned out from each tube into small beakers and weighed. Samples were then dried to constant weight in an electric oven at 105±3°C. The loss of weight represents the water contents of each sample. Into each beaker was pipetted 50 ml of distilled water, each dried sample was crushed with a glass rod, the samples were covered to prevent evaporation, and were each beaker were removed 15 ml of supernatant water by a pipetts, and the samples were titrated with silver nitrate. From the chloride value obtained on the 15 ml and the original water content as obtained by drying, the chloride contents of each bottom sample were calculated.

The flesh of clams was prepared for chloride analysis in the following way. The clam was removed from its valves as rapidly as possible. The flesh was blotted on absorbent paper to remove most of the adhering water, then rinsed briefly in 95% alcohol in order to remove most of the remaining water from body surface and gill capillaries. After weighing, chloride analysis of the whole tissues was done by the method of Sunderman & Williams (1931, 1933).

The result of field observations was summarized in Figure 2. In June and September the chloride concentrations in bottom water were higher than that in interstitial water in the surface layer of substratum, while in December they were vice versa in the areas which are situated in the vicinity of a narrow strait connecting with the Japan Sea (stations of Nakazima, Okinose and Shidoshomae); but in the estuarine areas (stations of Yamada, Iwaki and Imaizumi) the chloride in interstitial water had a lower content than that in overlying water in each season.

As shown in Figure 1, the chlorinity of bottom water is nearly zero during winter to spring, but between summer and autumn it has relatively higher value. Accordingly, it may be considered that the interstitial water in surface layer of bottom material has still continued to hold a comparatively high content of chloride. Although the variation of interstitial chlorinity is influenced not only by potentiality of permeation but also by degree of difference in chlorinity between the overlying water and interstitial water, in regard to such variation it may be assumed that under conditions of seasonal variation in the chlorinity of bottom water at each of the given points in the inlet the chlorinity of the interstitial water would be gradually shifted in corresponding fashion. The value of interstitial chlorinity indicates a seasonal variation in the surface layer of

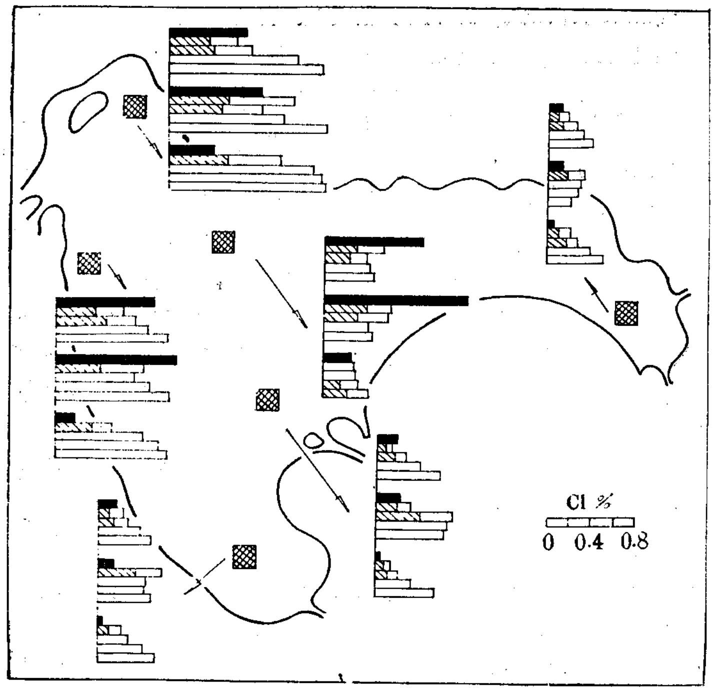

Fig. 2. Comparison of chlorinity profiles in bottom water, interstitial water and clam tissue

In this figure, the histograms of black area (■), white area (□), and shadowed area (▨) indicate the chloride content in bottom water, interstitial water and clam tissue respectively. The three groups of histograms in each areas, indicate downwards the results of chloride profiles in June, September and December respectively, and in each group the interstitial chlorinity profile is downwards divided into four parts, each 3 cm in depth. Each histogram indicates the average value of four times analysis.

bottom material, while 9–12 cm layer in each area the value of chloride content shows a stable situation with no significant seasonal shift. It is strongly suggested that, in relatively deeper layer (9–12 cm depth), the value of interstitial chlorinity may arrive at a nearly stable level because of poor permeation or of secretion of chloride between the overlying water and interstitial water. Although there can be seen a relationship to some extent between chloride contents of clam tissues and that of overlying water in each area and season, no parallel values were seemingly observable. And it is a very interesting phenomenon that the ratio between grams Cl per 100 grams wet tissues and per 100 ml interstitial water maintain an approximate value of 1:2.

From the above field observations is derived the following suggestion. The chloride variation in clam tissues is more strongly affected by the interstitial chlorinity which resulted from the chloride exchange between the overlying water and interstitial water, although it may be directly influenced by the chloride of overlying water to some degree. At any rate, it is obvious that the chlorinity of interstitial water reflects in some degree the chlorinity of the water that has lain above the bottom; the profiles of the interstitial chlorinity were slowly shifted with seasonal variation of the chlorinity of the overlying water, and the tissue chloride of clams varies within a considerable range in proportion to the chloride contents of the external medium in each area.

III. Change in tissue chloride of clam in response to varying salinities

The results obtained from the above field observations have revealed some unknown items of information on the progreess of chloride variation of clam tissue under widely differing salinity conditions. In the following experiments, when chloride concentration of the environmental water is prepared with a sudden and gradual change, the progress of the chloride variation of clam tissues is traced at each designed period.

Experiments were not begun until the clam had been kept in the aquaria for at least two days, under the condition of constant illumination by lighting with a 100 watt tungsten lamp. Most of the clams were 18–22 mm in shell height. Both sexes were employed. In all aquaria the water was continuously circulated and aerated by streams of air bubbles. Dilute sea water was prepared by mixing well water (0.03–0.02% Cl) with stock sea water (1.8% Cl). The water temperature was adjusted at 18±0.5°C by means of a thermostat.

(1) *Change in tissue chloride following sudden alteration in dilute sea water of varying chloride concentrations*

For alterations of expeimental water the fresh water in aquaria is removed at a known quantities and equal quantities of sea water is added without any disturbance on the clam. The added sea water is sufficiently mixed by air bubbles within a few minutes, then it showed the designed chloride concentrations. Tissue chloride measurement was made on groups of five or seven clams taken as a unit in each designed period.

The course of adjustment is presented in Figure 3. Each vertical bar in the figure represents the standard error of four or five determinations. In dilute sea water of 0.4 and 0.8% Cl the chloride content of the clam tissue increases rapidly within the first 8–12 hours. After this period the tissue chloride shows little variation throughout the 150 hours of the experiment, and the tissue chloride represents approximately 0.2 and 0.4% Cl in wet tissues respectively. All animals which were kept in 0.4 and 0.8% Cl for 150 hours were opening their valves, projecting both their siphons and thus were assumed to be normal, but none of the clams in the water containing 1.1 and 1.4% Cl appeared normal. Gradual increment of tissue chloride continues throughout the 70 hours or more in dilute sea water of 1.1 and 1.4% Cl; this phenomenon is detected more distinctly in 1.4% Cl than in 1.1% Cl water.

On the other hand, in fresh water the tissue chloride has no significant variation being maintained in 0.028 to 0.018% Cl throughout 150 hours of the experiment.

A second step of experiments was run to determine the variation of tissue chloride in fresh water after 150 hours in various degrees of dilute sea water. The curve of change in chloride content is plotted on the right side of Figure 3. During the first 4

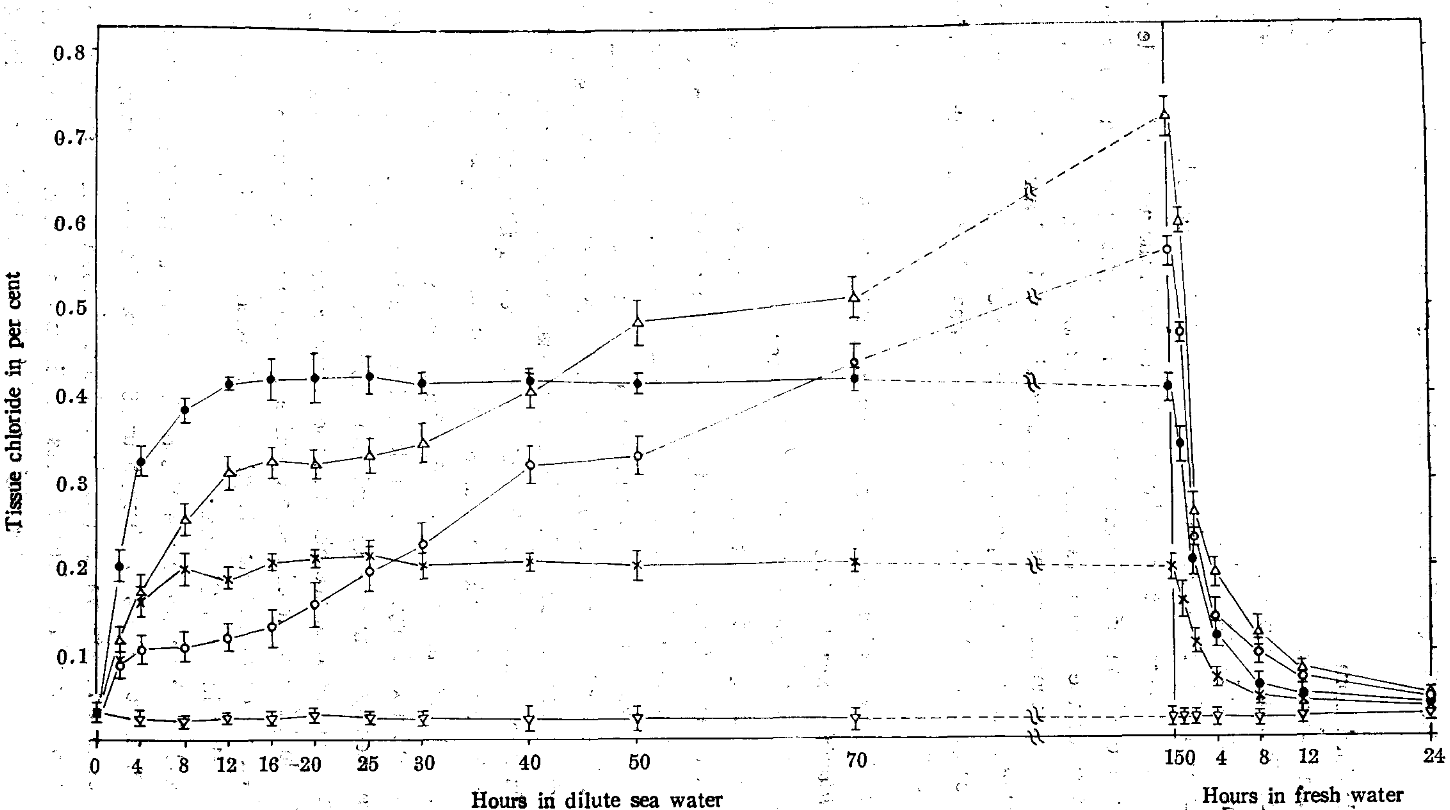

Fig. 3. Changes in tissue chloride of clam following sudden alteration of chlorinity in surrounding water Vertical lines indicates plus or minus of the standard error of the mean. The symbols indicate as follows : ▽—▽ fresh water (0.028% Cl), ×—× 0.4% Cl, ●—● 0.8% Cl, ○—○ 1.1% Cl, △—△ 1.4% Cl.

hours this value decreases quickly. After this period the gradual loss of chloride continues until 24 hours or more, whenafter the chloride content varies 0.028–0.042% Cl. The chloride loss continues with approximately the same tendency in every case, although the decrease in tissue chloride is slightly rapid in lower content as compared with that in the higher one.

(2) *Chloride variation shown by the clam in gradually altered chlorinity of environmental water*

The gradual alteration of the environmental water was brought about in the following manner. From an aquarium a known quantity of fresh water was withdrawn, discarded and replaced by an equal quantity of sea water without any disturbance to the clams at 12 hour intervals. These alterations brought about a gradual rise from the initial value to 0.2% Cl in the next solution, during the experimental period. When the experimental solution attained to 1.8% Cl, above treatments for gradual rise of the water were performed in opposite direction until the solution returned to fresh water condition.

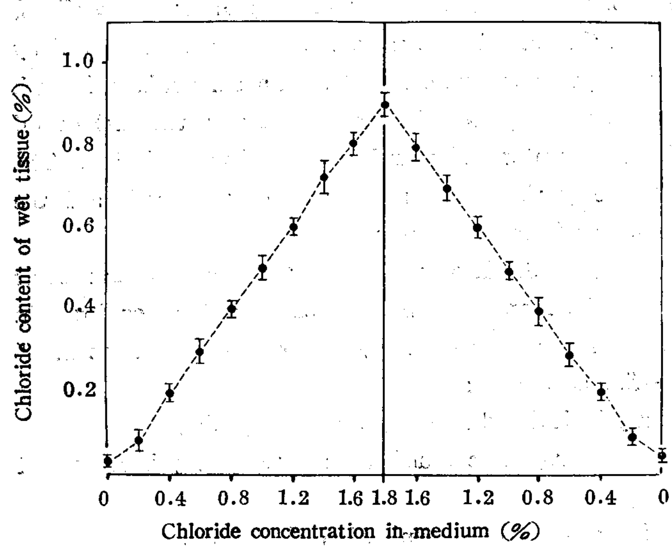

Fig. 4. Chloride variation of the clam tissue in gradually altered chlorinity of environmental water

Vertical lines cover the range of the standard error of the mean.

Figure 4 shows the results of chloride analysis. It is clearly illustrated from the foregoing experiments that the maintenance of an approximately constant ratio of 2:1 occurs between the chloride concentration in the surrounding media and the tissue chloride of clam immersed therein as is noted by the straight line between nearly zero and 1.8% Cl.

According to Fox (1941) the tissue chloride of the adult California mussel shows no significant difference under the constant chloride conditions all the year round; and mature males show slightly higher chloride contents than the mature females do. Although no attempts were made to confirm these facts in the clam, however, it will be of no significance in respect to the purpose of this work.

IV. Relation between chloride concentrations of experimental media and physiological activity of clams immersed therein

In lamellibranch mollusks the process of feeding is regulated by three factors: function of the ctenidial cilia, activity of the adductor muscles attached to shells and openness of inhalent siphon or pore. It is easily presupposed that the variation of tissue chloride may cause some signs of disturbance in th physiological activity of the animals. The following experiments were made to determine how the physiological activity changes with the chloride variation of clam tissue. In this experiment as an available measuring method of physiological activity use was made of shell movements and water filtration, which reflected all factors which may influence the calms. The experimental conditions, such as illumination, pH and temperature, were similarly adjusted as described in the former section.

(1) *Shell movements of clams in various degrees of dilute sea water*

Records of shell movements were made on smoked paper of clock-work kymograph which were geared to make one revolution in 24 hours. For an analysis of the recordings, the area between the record line and the base line (complete closure) were calcuated by recording on section paper. These experiments were repeated on six individuals in each designed dilute sea water. Response was expressed by the percentage between the total area enclosed per 12 hours in the fresh water and that in each designed solution. The term "shell movements" indicates only the actual movements of the shells and the distance apart of valves is expressed as "degree of openness".

Typical records are shown in Figure 5. In general, from the kymographic records the shell movements of clams may be represented by five shell positions or forms in accord with the increment of chloride concentration ranging from fresh water to 1.8% Cl. The five forms can be classified as follows:

Form I : Shell movements rhythmical and regular, complete opening

Form II : Shell movements more rhythmical and violent, temporary increment of openness

Form III : Shell movements irregular, gradual diminution of openness

Form IV : Stationary or occasional shell movements, but opening

Form V : No shell movements, complete closure or narrow opening of the shell for almost entire duration of the experiments

When the experimental solutions are immediately converted from fresh water to salt water, in 0.4% Cl, shell movement tracing showed Form II during first 1 or 2 hours, but after this period the movement returned to Form I. In 0.8 and 0.95% Cl the shape of movements represented Form II and was followed by Form III; after between 5 and 10 hours it then returned to Form I. Nevertheless, in the dilute sea water of 1.1% Cl

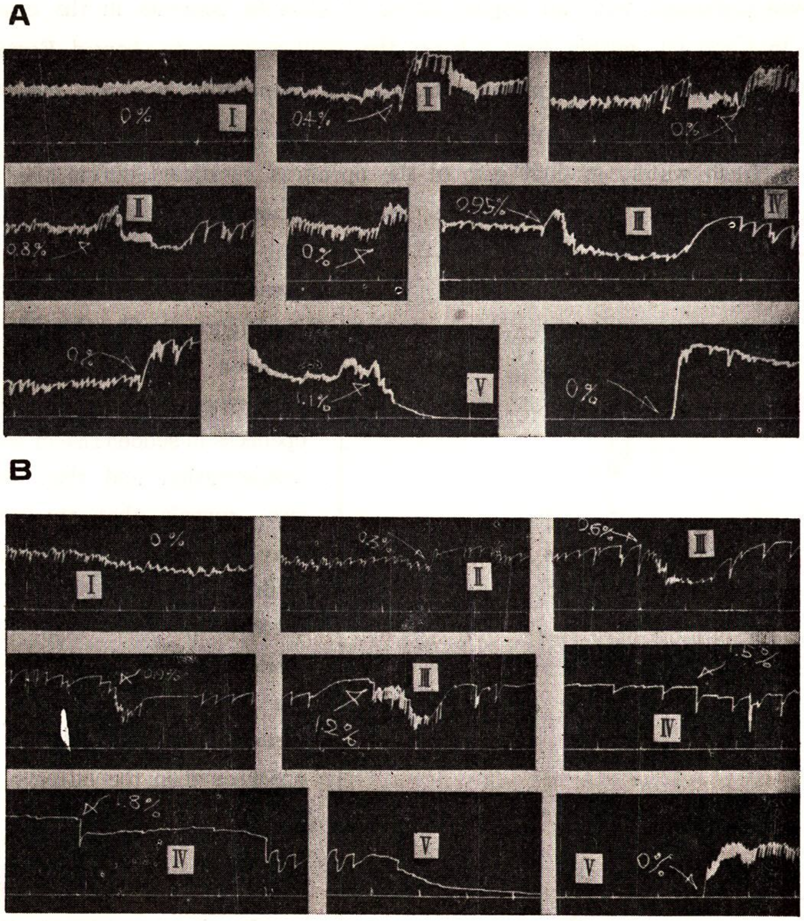

Fig. 5. Typical records of "shell movements" of clam when the environmental water increased suddenly (A) and gradually (B) from fresh water to various dilutions of sea water

Roman numbers in this figure indicate five shell movement forms. Time signals on the base line (complete closure) are marked at one hour intervals.

the addctor muscle delineated furious movements following the change of environmental solution from fresh water; the gradual decrease of openness (Form III) continued a few hours, finally arrived at Form V. Such tendency become more evident in 1.4% Cl.

On the other hand, when the chloride concentration of solution varying between fresh water and nearly straight sea water (1.8% Cl) was gradually increased, shell movements of clam showed a considerable difference. The typical examples of shell movements are shown in Figure 5 B. No significant differences were traced between the shell movements in 0.3% Cl and those in fresh water, while in 0.6, 0.9 and 1.2% Cl shell movements drew Form II, followed by Form III. In these concentrations the duration of Form III activity was prolonged with an augmentation of chloride contents in the experimental solution. In 1.5% Cl, within first 3 hours the shell movements showed Form IV, and in 1.8% Cl there appeared no movements, narrow opening or complete closure of the shell (Form V) during same period. When the experimental media were suddenly converted to fresh water, gradual gain of the openness continued during the first few hours in company with violent movements, and then position arrived at Form I.

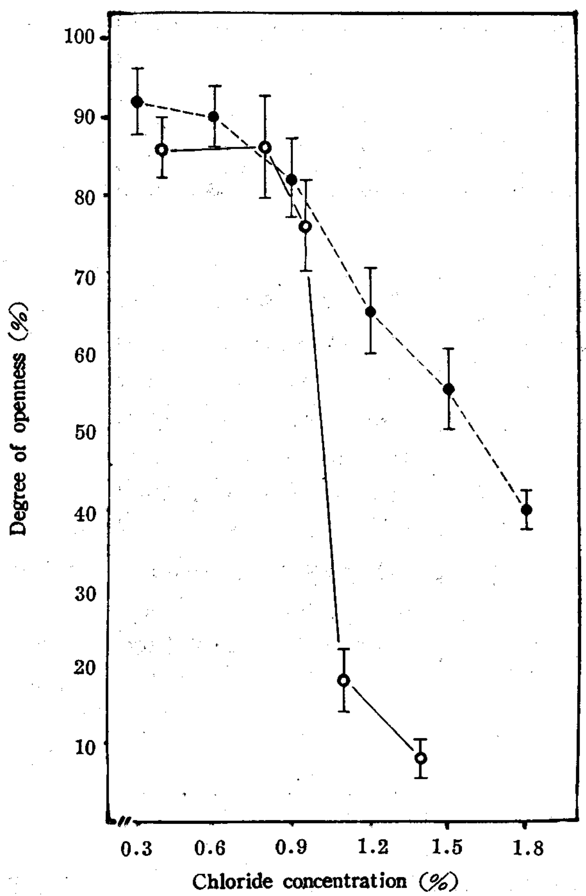

Fig. 6. Showing the "degree of openness" of valves in various chlorinity
Full line represents the values in case of sudden increase in various chlorinities, and broken line represents gradual increase. The vertical lines indicate plus or minus of the standard error of the mean.

The results of analysis of the kymograph recordings is summarized in Figure 6. In chloride contents between fresh water and 1.0% Cl, a comparison between the degree of openness in sudden rise of the chloride concentration and that in gradual rise, reveals that the similarities between the two cases are more striking than the differences and there is no significant difference, while in above 1.1% Cl concentration the difference is significant, the former more abrupt decrease of openness than the latter.

(2) *Change of water filtration in various dilution sea water*

In this experiment the water filtration of clam was measured by the rate of removal of particles in suspension of acid earth. Stock suspension was prepared by following treatments. Seven grams acid earth was added to one liter fresh water, the sample was mixed by stirring, and was allowed to stand for 2.5 hours, finally the suspension of acid earth was decanted without any disturbance of the precipitates. Suspension in experimental water was prepared from the stock fresh water suspension. A dilution of one part of stock suspension with ten parts of salt water was used. Such preparations have an approximately 90 mg weight per liter in initial concentrations of the acid earth, and the particles of most usual size were 2–6μ in diameter. In the experimental solution containing various chloride concentrations, the acid earth was mixed by means of continuous air bubbles for aeration to form a stable suspension within at least the first 3 hours. During the experiments pH values varied betwwen 7.0 and 8.3. Measuring of water filtration on groups of 17 adult clams (18–22 mm in shell height) taken as a unit began after 12 hours in the water of known chloride contents.

The rate of filtration was computed from the formula given by Jørgensen (1949). This is

$$P_t = P_o \quad exp \left(- \frac{M}{m} \cdot t \right)$$

in which M is the value of suspension in liters, m is the quantity of water filtered in liters per hour, while P_o and P_t are the concentrations of the suspended material at the initial time and after t hours respectively. The concentrations of acid earth were determined colorimetrically by means of a photoelectric colorimeter. It was found that the light absorption, expressed as a function of the concentrations of acid earth, followed Beer's law. In these experiments, therefore, the proportion of particles removed from acid earth suspension could be directly determined from the corresponding decrease in light absorption.

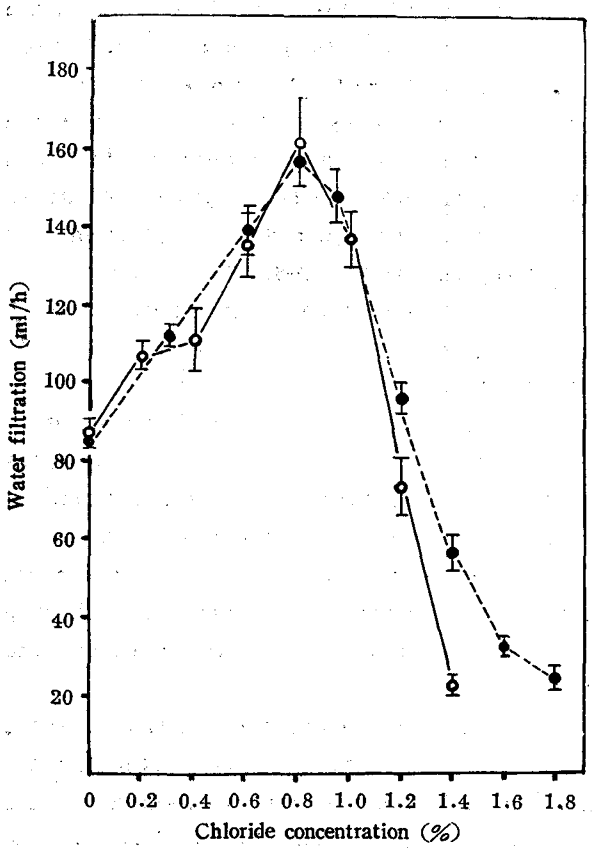

Fig. 7. Average values of water filtration in various dilution sea waters
Each symbol and vertical bar is the same as those in Figure 7.

The average values of water filtration per individual adult clams per hour are shown in Figure 7. Difference between the average of the filtration in case of sudden rise of salinity and that in gradual rise is not recognized, the augmentation of pumping is measured by the corresponding increase of chloride concentration ranging between fresh water and 0.8% Cl. In the chloride concentrations above 1.0%, an average value of filtration in sudden rise is slightly inferior to that in gradual rise, but the difference is significant.

From these obtained results, it is suggested that the influences on salinities are much stronger upon the function of ctenidial cilia than on that of adductor muscle, in chlorinity of under 1.1%. On the other hand, in the solution above 1.1% Cl, the rhythmical movements of shell decline, and diminution of openness begins with this concentration. Moreover, decrease of water filtration is conspicuously displayed under such chloride concentrations. In conclusion, frcm these experiements it may be stated that several signs of disturbance in physiological activity of the clam were caused in water of chloride contents above 1.1% Cl.

V. Discussion

The factors which influence the physiological activity of clams, for example, Cl regulation, shell movements and water filtration can be stated to be such physico-chemical conditions as pH value, water temperature and light intensity with the exception of salinity. Recently, in the freshwater mussel, *Hyridella australis*, Hiscock (1950) has reported that within temperature range 17 to 27°C, there was evidenced no well-defined influence on shell movements or on the degree of openness, whereas, the light intensity showed a marked influence. In the present study, water temperature and light intensity were constantly adjusted during the course of experimets, and as shown in Table 1, variation of tissue chloride, degree of openness and water filtration have been recognized to be insignificant within the pH range of 7.0–8.3.

The variations in tissue chloride may have resulted not only from the permeation or secretion of salts, but also from the loss or gain of water. If such chloride exchange proceeds throughout all the semi-permeable tissue of clams, it is a very interesting phenomenon that the maintenance of a ratio of roughly 1:2 between grams per 100 gram wet tissues and grams Cl per 100 ml salt water is noted as a straight line within the chlorinity range of 0.03 and 1.8%, and that in this respect it is similar to the result obtained in California mussel by Fox (1941). Although none of these experiments deals with the actual mechanism involved in osmo-regulation and physiological activity of clams, the maintenance of a constant ratio between internal and external chloride may result from neural activities which have some means of controlling movement of water and salt through the semi-permeable tissue of clam.

In view of the fact that the duration of remarkable increment of tissue chloride after change from fresh water to sea water coincides with the duration showing the pattern of shell positions of Form II or III in weaker chloride water (0.3–0.9% Cl), the violent movements of shell recorded are reasonably presumed to be the result of Cl exchange between internal and external water.

In summarizing the above field and experimental observations, it is recalled that the adult clam is able to adjust itself, with accompanying changes in tissue chloride, to a

Table 1. Influence of different pH value upon tissue chloride and physiological activity of clams in dilute sea water
In the course of the experiments, water temperature is 18±0.5°C and chlorinity of water is adjusted in 0.4% Cl.

(A) Tissue chloride

pH	Chloride contents (% Cl)			
	0 hour	4 hours	12 hours	24 hours
7.0	0.039	0.125	0.190	0.194
	0.032	0.128	0.189	0.210
	0.040	0.133	0.211	0.193
	0.037	0.128	0.197	0.199
	0.034	0.119	0.197	0.200
8.3	0.040	0.115	0.161	0.193
	0.040	0.145	0.152	0.194
	0.037	0.126	0.166	0.202
	0.039	0.129	0.181	0.196
	0.034	0.119	0.197	0.203

$F_0 = 1.46 < F_{(0.05)} = 216$ (insignificant)

(B) Degree of openness

pH	Openness (mm^2)					
	1st	2nd	3rd	4th	5th	6th
7.0	1660	1320	1570	1880	1490	1590
8.3	1710	1220	1510	1800	1550	1470

$F_0 = 1.55 < F_{(0.05)} = 6.61$ (insignificant)

(C) Water filtration

pH	Filtration (ml/h)			
	1st	2nd	3rd	4th
7.0	142	96	121	117
8.3	131	112	90	121

$F_0 = 1.46 < F_{(0.05)} = 216$ (insignificant)

considerable range of salinities in its habitats in Zyusan-gata Inlet as well as laboratory. Accordingly, even if this animal were exposed to a foreign environment, suddenly changing from fresh water to sea water, it may tolerate the change for a considerable period in the extreme case. Such euryhalinous character of this animal may result not only from the adaptability of osmo-regulation furnished by itself, but also from the function of interstitial water which mollifies any sudden change of salinity.

It is indubitable that the salinity variation in surrounding water is not the only factor responsible for the physiological activity of *Corbicula japonica*, but it may be suggested that the influence of salinity change upon the sensitivity of adult and larval clam provides one helpful clue in further investigation on various physiological and ecological problems.

VI. Summary

1. The results of field observation indicate that the chloride variations of clam tissue were affected more strongly by the interstitial chloride in bottom material than by the direct influence of the overlying water.

2. When the experimental water was changed suddenly from fresh water to various degrees of dilute sea water, the tissue chloride showed significant changes in laboratory experiments. The chloride increased markedly during the first 8–12 hours, but after this period the values of tissue chloride attained to a constant level showing an approximate value of 1:2 in ratio between grams Cl per 100 grams wet clam tissue and per 100 ml environmental water, in 1.1% Cl or under, however, in the chloride concentrations above 1.1% Cl, the chloride variation showed a considerable difference as compared with above example, and the gradual increment of tissue chloride continued during the period of 150 hours.

3. If chloride content is gradually changed, a roughly constant ratio of 1:2 is demonstrated between the tissue chloride of clam and the chloride content in surrouding media ranging from nearly zero to 1.8% Cl.

4. Shell movements and water filtration of clams were adopted as an available method for measuring their physiological activity. From these experiments, it is suggested that several signs of disturbance in physiological activity of the clam were caused under the condition of chloride concentrations above 1.1 or 1.2%.

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