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Citation	北海道大學水産學部研究彙報, 29(2), 110-117
Issue Date	1978-06
Doc URL	<a href="http://hdl.handle.net/2115/23636">http://hdl.handle.net/2115/23636</a>
Type	bulletin (article)
File Information	29(2)_P110-117.pdf



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## Water Movement and Alkaline Phosphatase Activity in the Intestine of Sticklebacks Adapted to Seawater or Freshwater

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### Abstract

Changes of water movement from mucosa to serosa in the isolated intestine and the intestinal alkaline phosphatase (AL-Pase) activity were investigated in sticklebacks transferring freshwater adapted land-locked fish to seawater or seawater adapted anadromous fish to freshwater. There was a close correlation between changes of water movement and AL-Pase activity, both increased by seawater transfer and decreased by freshwater transfer. The anadromous fish showed a higher AL-Pase activity even after transfer to freshwater than that augmented in the land-locked fish after seawater transfer. In the land-locked fish, muscular water contents and body weight decreased after seawater transfer and recovered gradually. The anterior half of intestine showed a higher AL-Pase activity than the posterior half in freshwater fish, but a greater rate of increase of activity was observed in the posterior half by transferring the fish to seawater.

### Introduction

Marine teleosts ingest seawater and absorb water through the intestine to compensate the osmotic water loss from the body surface<sup>1)</sup>. In eels, it has been reported that the rate of intestinal water absorption is particularly high during the first week of seawater adaptation<sup>2-3)</sup>, and that the activity of alkaline phosphatase (AL-Pase) and  $\text{Na}^+\text{-K}^+\text{-ATPase}$  of the intestine show marked increases with the enhanced water uptake<sup>4-6)</sup>. On the 7th day in seawater, the  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity was about 2.5 times that of freshwater eels<sup>5)</sup>, whereas the AL-Pase activity was about 5.0 times<sup>7-8)</sup>, therefore, the intestinal AL-Pase was suggested to play an important role in water uptake in euryhaline or marine teleosts<sup>9)</sup>.

The present study is aimed to make clear the correlation for a longer period between water transport ability and AL-Pase activity in the intestine of the three-spined stickleback of both land-locked and anadromous forms. Changes of the AL-Pase activity and water movement through the intestinal wall were observed when transferring the fish from freshwater to seawater or *vice versa*.

### Materials and Methods

Both the land-locked and anadromous forms of the threespined sticklebacks (*Gasterosteus aculeatus* L.) were used as materials. Land-locked fish were collected

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from salmon culture ponds of Mori Branch of Hokkaido Fish Hatchery and kept in an aquarium with circulating aerated freshwater at 15°C. For the seawater adaptation experiment, the fish were transferred directly to another aquarium with circulating natural seawater. Anadromous sticklebacks were captured along the coast near Hakodate and maintained in an aquarium with circulating seawater at 15°C. For the freshwater adaptation experiment, the fish were transferred directly to freshwater of the same temperature. In both experiments they were sacrificed by decapitation after pre-determined adaptation periods. The intestine was quickly dissected out and AL-Pase activity and water transport were measured as described below. In some land-locked fish, the intestine was divided into the anterior and the posterior halves at the twisted mid-portion and each AL-Pase activity was measured separately. A piece of muscle near the dorsal spines was scraped off and used for a determination of the water content.

The isolated intestine was washed with Ringer's solution. After blotting with filter paper, it was immersed in an ice-cold 0.25 M sucrose solution and homogenized with a blender. The homogenate was used as enzyme solution.

The AL-Pase activity was measured with the homogenates by the method of Bessey *et al.*<sup>10)</sup> using p-nitrophenyl phosphate disodium (Wako Pure Chemical Ind., Tokyo) as substrate. Liberated p-nitrophenol (p-NP) was determined with a spectrophotometer (Hitachi 139), at wavelength 410 nm. Total proteins of the enzyme solution were estimated by the method of Gornall<sup>11)</sup> and the enzyme activity was expressed as  $\mu$ moles p-NP/mg protein/hr.

The isolated intestine was filled with Ringer's solution containing 128 mM NaCl, 2.8 mM KCl, 1.8 mM CaCl<sub>2</sub> (buffered pH 8.0 with 10 mM Tris-HCl). The filled sac was lightly blotted on filter paper, weighed and placed in a flask containing 50 ml of Ringer's solution. The incubation was made at 15°C for 2 hrs. At the end of incubation, the intestine was weighed again. Net water movement from mucosa to serosa was expressed as percentage of the weight loss to the initial weight.

## Results

When land-locked sticklebacks were introduced into seawater, the water movement changed, as shown in Figure 1. Enhancement of the water movement was observed on the 1st day after transfer. The maximum value was obtained on the 7th day followed by a slight decrease leading to a constant level. When the fish were put back again in freshwater on the 7th day, the rate of water movement dropped rapidly. It reached the initial level on the 3rd day after returning to freshwater. In accordance with the transfer from freshwater to seawater, the intestinal AL-Pase activity increased gradually (Fig. 2). The maximal value (2.00  $\mu$ mole p-NP/mg protein/hr) was observed on the 7th day showing about twice the initial value (1.09  $\mu$ moles p-NP/mg protein/hr). After 3 weeks, the activity dropped to a stable level (1.40  $\mu$ moles p-NP/mg protein/hr). In the case of readaptation to freshwater on the 7th day, the enzyme activity showed a hasty decrease. Around the 3rd day after the return to freshwater, it approached the initial level and maintained a stable value thereafter. There was a distinct correlation between the AL-Pase activity and water movement ( $r=0.84$ ,  $P<0.01$  in

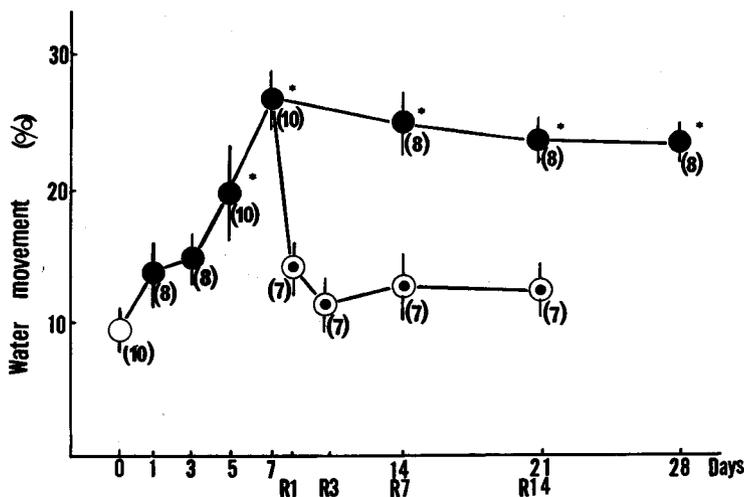


Fig. 1. Changes in water movement in isolated intestines of land-locked sticklebacks after transfer from freshwater to seawater (●) and return to freshwater (○). The vertical line indicates a standard error at each point. The number of animals tested are given in parentheses. \*Significantly different from the initial (○) at 5 per cent level.

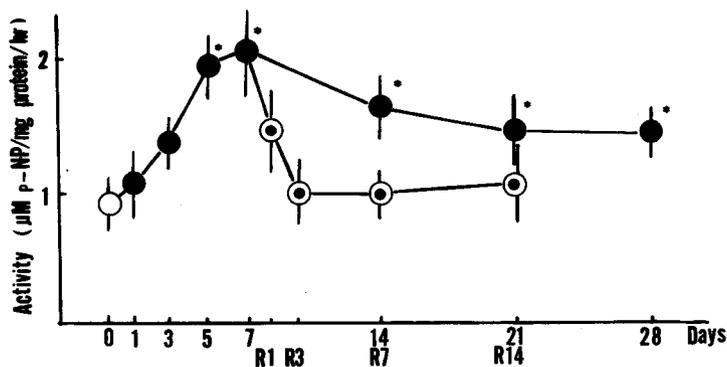


Fig. 2. Changes in activity of intestinal AL-Pase of land-locked sticklebacks after transfer from freshwater to seawater (●) or return to freshwater (○). Each point represents Mean±S.E. of 10 or 7 fish.

\* Significantly different from the initial (○) at 5 per cent level.

seawater adapted land-locked fish;  $r=0.89$ ,  $P<0.01$  in readapted freshwater adapted land-locked fish).

When seawater adapted anadromous sticklebacks were transferred to freshwater the AL-Pase activity and water transport ability of the intestine changed, as shown in Figs. 3 and 4. The water movement across the intestine showed a decrease toward the 3rd day and after that maintained a settled value (Fig. 3). Also, the AL-Pase activity was reduced up to the 3rd day after transfer and maintained a

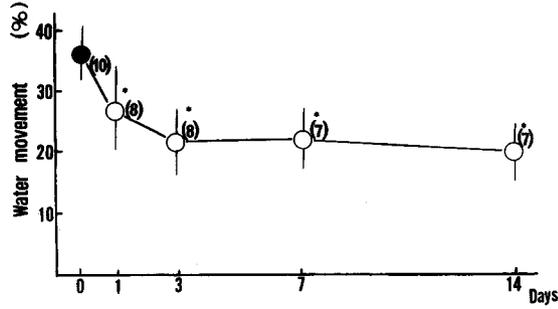


Fig. 3. Changes in water movement in isolated intestines of anadromous sticklebacks after transfer from seawater to freshwater. Each point represents Mean  $\pm$ S.E. The number of fish tested are given in parentheses.

\* Significantly different from the initial (●) at 5 per cent level.

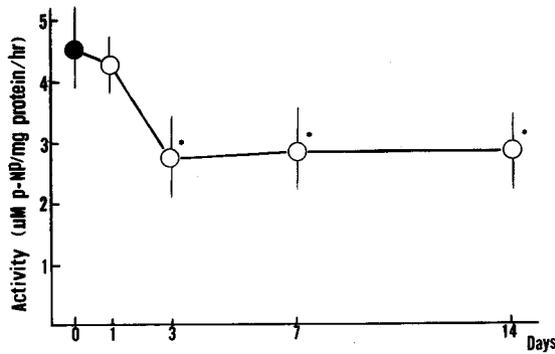


Fig. 4. Changes in activity of intestinal AL-Pase of anadromous sticklebacks after transfer from seawater to freshwater. Each point represents Mean  $\pm$ S.E. of 10 or 7 fish.

\* Significantly different from the initial (●) at 5 per cent level.

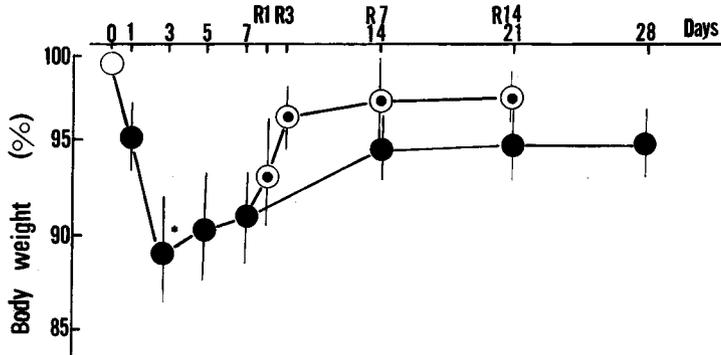


Fig. 5. Changes in body weight of land-locked sticklebacks following transfer from freshwater to seawater (●) or return to freshwater (○). Each point represents Mean  $\pm$ S.E. of 15 or 7 fish.

\* Significantly different from the initial (○) at 5 per cent level.

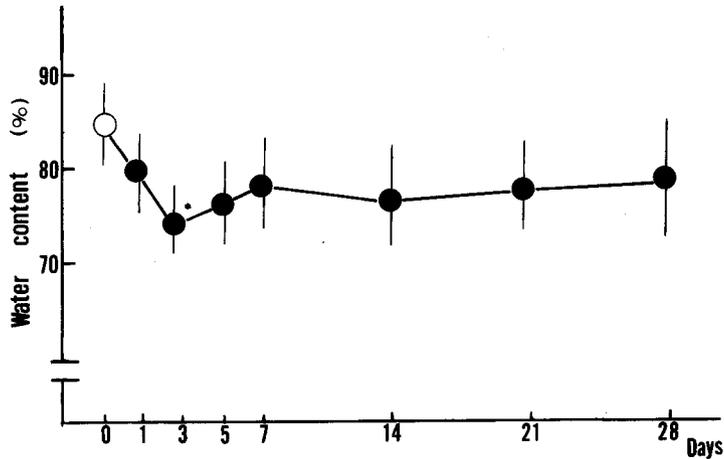


Fig. 6. Changes in water contents of muscle of land-locked sticklebacks after transfer from freshwater to seawater (●). Each point represents Mean±S.E. of 10 or 8 fish.  
\* Significantly different from the initial (○) at 5 per cent level.

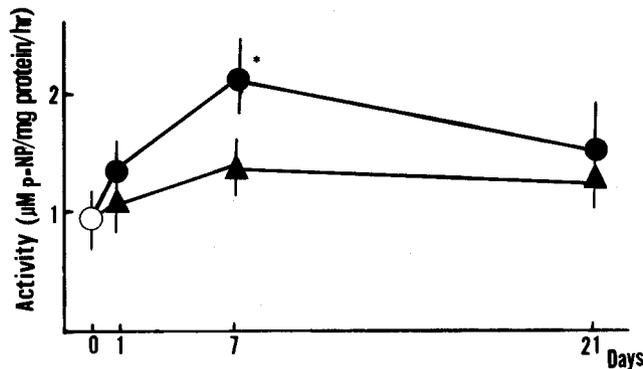


Fig. 7. Changes in activity of AL-Pase of land-locked sticklebacks after transfer to hyperosmotic seawater, 750 mosm/l (●), or isosmotic seawater, 350 mosm/l (▲). Each point represents Mean±S.E. of 10 or 7 fish.  
\* Significantly different from the initial (○) at 5 per cent level.

constant level, about 3.00  $\mu$ moles p-NP/mg protein/hr. There was a strict correlation between these two parameters ( $r=0.94$ ,  $P<0.05$ ).

The above-mentioned changes affected the body weight and the water content of muscle which dropped up to the 3rd day, then recovered (Figs. 5 and 6). When the fish were returned from seawater to freshwater on the 7th day, the recovery was more rapid. Similarly, the water content of muscle decreased up to the 3rd day after the transfer to seawater and subsequently restored a constant level (about 80%) after the 7th day. A firm correlation was observable between the changes in body weight and water content of muscle ( $r=0.87$ ,  $P<0.05$ , Figs. 5 and 6).

Table 1. Comparison of AL-Pase activity between the anterior and the posterior halves of the intestines of freshwater adapted and seawater adapted land-locked sticklebacks. Each value represents Mean  $\pm$  S.E. of 7 fish.

Intestine	$\mu$ M p-NP/mg protein/hr		
	Freshwater	Seawater	
Anterior half	0.66 $\pm$ 0.11	1.08 $\pm$ 0.18	P<0.05
Posterior half	0.43 $\pm$ 0.08	0.95 $\pm$ 0.18	P<0.01

The effect of osmolarity of ambient water on the intestinal AL-Pase activity was examined by transferring land-locked sticklebacks to diluted seawaters (Fig. 7). The AL-Pase activity of fish in isosmotic seawater (350 mosm/l) was the same as that of the initial value of freshwater adapted land-locked fish, while an increase of the activity was observed in fish transferred to hyperosmotic seawater (750 mosm/l).

The difference of the AL-Pase activity between the anterior and the posterior halves of intestine is shown in Table 1. The AL-Pase activity of the anterior half was 1.5 times higher than that of the posterior half in freshwater adapted land-locked fish. In seawater adapted land-locked fish for 10 days, however, the activities of both halves became approximately identical by a higher rate of increase in the posterior half than in the anterior half.

### Discussion

A close correlation was clearly observed between the changes of the AL-Pase activity and the water transport of the intestine of sticklebacks transferred from freshwater to seawater or *vice versa*. The body weight and water content of muscle changed in accordance with the changes of these intestinal factors. This strongly suggests that the intestinal AL-Pase is intimately involved in the water regulation of body transporting water through the intestinal wall.

It was observed that both the AL-Pase activity and the water transport decreased gradually after one week adaptation in seawater. Therefore, at the beginning of seawater adaptation the intestine seemed to work instantly prior to other osmoregulatory organs such as the kidney and the gills. On the other hand, when seawater adapted fish were introduced into freshwater, both the AL-Pase activity and the water transport dropped rapidly up to the 3rd day and settled at a constant level thereafter. This shows that the fish immediately stop water uptake through the intestine in freshwater, probably following a rapid reduction of drinking rate<sup>12)</sup>. The anadromous fish transferred to freshwater, however, showed a still higher activity of AL-Pase (about 3  $\mu$ moles p-NP/mg protein/hr) compared with even the maximum value (about 2  $\mu$ moles p-NP/mg protein/hr) of the land-locked fish kept in seawater. This would indicate that the net decrease or increase of AL-Pase activity found in these fish corresponds to a part that is responsible for a change of water transport ability. The water transport ability in anadromous fish in freshwater lowered to a level (about 25%) that was almost the same as the land-locked fish in seawater. As this was obtained by

*in vitro* experiments, it would not seem to show that the anadromous fish in freshwater actually absorb as much water *in vivo* as do the land-locked fish in seawater.

An activation of the intestinal AL-Pase was brought about by rinsing the fish in hyperosmotic (about 70%) seawater but not in the isosmotic (about 30%) seawater. Therefore, what seems to stimulate the intestinal AL-Pase activity is hyperosmoticity of the ambient water. How does a hyperosmotic ambient water trigger the elevation of the enzyme activity is not known. Only Oide<sup>5)</sup> showed in freshwater eels that intestinal AL-Pase was not activated by the transfer to seawater after hypophysectomy, and she suspected an involvement of ACTH in the mechanism.

The intestinal water movement must be a solute-linked water flow that is dependent on the active transport of sodium chloride<sup>13)</sup>. Although the function of AL-Pase involved in water movement has been poorly understood, Na<sup>+</sup>-K<sup>+</sup>-ATPase is well known membrane-bound enzyme which participates in sodium transport in various cells<sup>14-15)</sup>. The function of AL-Pase in the intestinal osmoregulation mechanism and its difference from Na<sup>+</sup>-K<sup>+</sup>-ATPase should be further investigated based on the physico-chemical nature of this enzyme system which changes its activity with environmental salinity changes.

The posterior half of the intestine of seawater transferred sticklebacks showed a higher rate of increase in AL-Pase activity than the anterior half. Utida<sup>9)</sup> could not observe such a difference in the intestine of rainbow trout. Although it was not possible to determine the water transport of both halves separately in correlation with the changes of the enzyme activity, there would be functional differentiation as to the ability of ion and water transport between the anterior and the posterior intestines.

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