コイ筋原繊維Ca2+-およびMg2+-ATPaseの諸性質に及ぼす棲息水温の影響

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著者名	三嶋,敏雄 横山,哲夫 矢野,健治 槌本,六良
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The Influence of Rearing Water Temperature on the Properties of Ca^{2+}- and Mg^{2+}-ATPase Activity on Carp Myofibril

Tosio Misima,*1 Tetsuo Yokoyama,*1 Kenji Yano,*2
and Mutsuyosi Tsuchimoto*2

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The effects of habitat temperature on the temperature dependence, Michaelis constants (*Km*), and maximum velocity (V_{max}) of myofibrillar Ca^{2+}- and Mg^{2+}-ATPase were experimentally studied using carps reared at 10°C (group L) and 30°C (group H) for different lengths of time. The temperature dependences of Mf Ca^{2+}- and Mg^{2+}-ATPase rose in both groups L and H with a longer rearing period, although the degree of increase differed for Mf Ca^{2+}- and Mg^{2+}-ATPase. The degree in Mf Ca^{2+}-ATPase was larger in group L than in group H, while the degree in Mf Mg^{2+}-ATPase was smaller in group L than in group H. *Km* values of both Mf Ca^{2+}- and Mg^{2+}-ATPase were smaller slightly in group L than in group H at reaction temperatures of 5°C to 30°C in the former, and 10 to 20°C in the latter. The *V*max level of Mf Ca^{2+}-ATPase was higher in group L than in group H at 30°C, while the opposite was observed at 10°C. The *V*max level of Mf Mg^{2+}-ATPase, however, was higher in group L than in group H at all reaction temperatures from 5°C to 30°C. We consider that these findings in Mf Mg^{2+}-ATPase indicate the existence of an efficient mechanism for the production of energy in fishes living in low habitat temperatures.

The body temperature of fish differs according to habitat water temperature, coinciding almost exactly with the habitat temperature. Fishes living in low water temperatures such as 0°C, however, can be just as active in swimming, feeding and growing as fishes living in higher water temperatures.

This is a subject of study attracting the interest of many researchers. Some studies referred to myosin ATPase [EC 3.6.1.3.] in fish, and demonstrated that the relative thermostability of the Ca^{2+}-or Mg^{2+}-ATPase activity was markedly higher in mammals than in fish, and that of fish tended to be higher in fishes living in high temperatures than in those living in low temperatures.[1-7] We also studied the relative thermostability of Mf Ca^{2+}-ATPase (K_D) in many demersal fishes caught in six areas ranging from tropical waters of 28°C (the water temperature measured on the bottom of the sea) to cold waters of 2°C.

As a result, we reported that the mean value of thermostability for fishes from a certain fishing ground tended to be higher with a higher habitat temperature, and the same influence of habitat temperature was seen for same species of fish living in two different habitat temperatures.[8] The same influence of habitat temperature was seen for carp reared in two different temperatures, at 10°C and at 30°C.[8] These observations indicated that the properties of Mf Ca^{2+}- and Mf Mg^{2+}-ATPase activity might differ with the habitat temperature.

Johnston *et al.*[10] reported that the free energy in thermodynamic activation parameters of Mf Mg^{2+}-ATPase was approximately equal among fishes living in different habitat temperatures. They speculated that the change occurring easily in low temperatures might also occur in Mf Mg^{2+}-ATPase of fishes living in a low water temperature. In support of Johnston's hypothesis, Hashimoto *et al.*[7] reported the same observation using thermodynamic activation parameters estimated from the corresponding Arrhenius plots of thermostability (K_D) on Mf Ca^{2+}-ATPase in fish.

Though these findings have obtained, there have been few reports studing in detail the influence of habitat water temperature on properties of Ca^{2+}-ATPase and Mg^{2+}-ATPase activity on myofibril.

*1 Laboratory of Marine Machinery, Instrument, and Engineering Materials, Graduate School of Marine Science and Engineering, Nagasaki University, Nagasaki 852, Japan (三嶋敏雄, 横山哲夫: 長崎大学海洋生産科学研究科).

*2 Laboratory of Fishery Nutritional Sciences, Faculty of Fisheries, Nagasaki University, Nagasaki 852, Japan (矢野健治, 槌本六良: 長崎大学水産学部).

In order to clarify the biological mechanism of adaptation to habitat water temperature in myosin ATPase activity of fish, we report the changes of the Michaelis constant (*Km*), the reaction velocity, and the temperature dependence of a reaction with the change of habitat water temperature by Mf Ca^{2+}-ATPase and Mf Mg^{2+}-ATPase, using carp reared at 10°C and 30°C.

Experimental Methods

Fish samples

We used the common carp *Cyprinus carpio* in the present study because it is easy to rear in a wide range of temperatures. A total of 17 fish, 25～30 cm in length, were preliminarily reared in a tank at 18°C for one week. They were then divided into two groups to be reared separately at two experimental temperatures, 10°C or 30°C. The temperature was either lowered or raised from the preliminary to experimental level over a period of three days. The fish were fed daily with pelleted carp food in sufficient quantities.

Several fish were taken from each group after one, two and five months. Details are shown in Table 1. They were killed by cutting the hindbrain, and then used as samples to prepare myofibrils.

Preparation of myofibrils

We followed the method of Kato *et al.*[11] in preparation of myofibrils. White muscle adjacent to the dorsal fin was taken and homogenized for 3 min in 40 m*l* of 0.1 M-KCl 40 mM-Tris-HCl Buffer, pH 7.0, 1% Triton X-100. The homogenate was centrifuged for 15 min at 3000 rpm. 70 m*l* of the same buffer except Triton X-100 was added to the residue with stirring, which was then centrifuged for 7 min at 3000 rpm. Redispersion and centrifugation were repeated several times.

Well-washed myofibrils were filtered through a layer of gauze to remove connective tissue and unbroken residues. These procedure were carried out with ice-cold. The concentration of myofibrils in the suspension was colorimeterically monitored by the biuret method and adjusted to approximately 0.6 mg/m*l* by diluting with 0.1 M KCl 40 mM-Tris-HCl Buffer, pH 7.0. The prepared myofibrils were stored on ice and all of ATPase assays were finished within 8 h after the preparation.

ATPase activity

We followed the method of Kato *et al.*[11] in Mf Ca^{2+}- and Mf Mg^{2+}-ATPase assay. In experiment 1, the homogenate of myofibrils was pipetted into test tubes in 0.5 m*l* portions, and then 4 m*l* of

Table 1. Details concerning fish specimens used in experiment 1 and 2, in carps reared at two temperatures, 10°C (group L) and 30°C (group H), for periods of 1 month, 2 months, and 5 months

Experiment	Carps reared at 10°C		Carps reared at 30°C	
	Specimen number	Rearing period (months)	Specimrn number	Rearing period (months)
Experiment 1	No. 1	one	No. 11	one
Ca^{2+}-ATPase	2	five	12	five
	3	five	—	
Mg^{2+}-ATPase	No. 1	one	No. 11	one
	4	two	13	two
	5	five	14	five
Experiment 2	No. 6	one	No. 15	one
Ca^{2+}-ATPase	7	〃	16	〃
	8	〃	17	〃
	9	〃	18	〃
Mg^{2+}-ATPase	No. 5	five	No. 14	five
	10	〃	19	〃
Total	10		9	

In experiment, the relative activity of Ca^{2+}- and Mg^{2+}-ATPase was measured in ATP concentration with a much lower velocity than Vmax in experiment 2.

Group L were No. 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

Group H were No. 11, 12, 13, 14, 15, 16, 17, 18, and 19.

0.1 M KCl, 25 mM Tris-maleate Buffer (pH 7.0) and 5 mM $CaCl_2$ (for Mf Ca^{2+}-ATPase) or 1 mM $MgCl_2$ (for Mf Mg^{2+}-ATPase) were added. After Myofibrils in the reaction media were preincubated till it became an assay temperature, the reaction was initiated by the addition of 0.5 m*l* of ATP. Five kinds of ATP concentrations (0.04, 0.05, 0.07, 0.1, and 0.2 mM) were used, and the acurate concentrations were determined by analysis, is described later. The reaction temperatures were 30, 25, 20, 15, 10, and 5°C. The reaction periods were 0, 1, 3, and 5 min at 30°C of reaction temperature, 0, 2, 4, and 6 min at 25°C, 0, 2, 4, and 7 min at 20°C, 0, 3, 6, and 9 min at 15°C, and 0, 4, 8, and 13 min at 10°C and 5°C in Mf Ca^{2+}-ATPase. The reaction periods were 0, 0.5, 0.75, 1, and 1.25 min at 30°C, 25°C, and 20°C, 0, 0.5, 0.75, 1, 1.25, and 1.5 min at 15°C, 0. 0.5, 0.75, 1, 1.5, and 2 min at 10°C, and 0, 0.5, 0.75, 1.25, 2, and 2.5 min at 5°C in Mf Mg^{2+}-ATPase. These reaction periods were determined not to cause the slowing down of reaction velocity by the decrease of substrate concentration. The reactions were terminated using 1 m*l* of 40% PCA at each reaction period.

We followed the method of Ehira *et al.*[12] for extraction of ATP and ADP. The extracted specimens were analyzed by our method using high-performance liquid chromatography.[13] We used μBondasphere 5 μ C18–100 Å purchased from Waters Ltd. as the column.

As Mf Ca^{2+}- and Mf Mg^{2+}-ATPase activity showed a first-order reaction, the initial velocity was calculated from next formula.[14]

$v=2.303\times So\times\phi$ (So; initial substrate concentration)
(ϕ; slope of regression line between time and log ATP concentration)

But since Mf Mg^{2+}-ATPase showed initial burst at an early stage of the reaction, the activity was calculated from the steady state thereafter.[15] ATPase activity was expressed as the amount of ATP released in a unit time (μmol/min) for 1 mg of myofibrillar protein. *Km* and V_{max} (presumption V_{max}) were determined from the activity in low ATP concentration by Lineweaver-Burk plot. In experiment 2, the reaction velocity in saturated substrate concentration (measurement *V*max) was carried out in the reaction media containing 1 mM ATP and 0.6 M KCl (for Mf Ca^{2+}-ATPase), or 1 mM ATP and 0.1 M KCl (for Mf Mg^{2+}-ATPase). Although the other media were the same as in experiment 1, the activity was measured from Pi released.[16]

Results

Mg^{2+}-ATPase Kinetics of Myofibrils

Common logarithm value of the ATP concentrations against time were shown in Fig. 1. The ATPase assay was carried out at 30°C. The decrease of common logarithm value of ATP concentration from 0 min to 0.5 min were larger than that from 0.5 min to 1.25 min in all ATP concentrations. This apparent decrease at early stage was regarded as initial burst of Mf Mg^{2+}-ATPase. The decrease of common logarithm value of ATP concentration from 0.5 min to 1.25 min indicated the straight lines conforming a first-order reaction in all substrate concentration. In other reaction temperature, the reaction time were established as showing the straight line. Mf Mg^{2+}-ATPase velocity was calculated from the slope of regression line between time and common logarithm value of ATP conentration, and Mf Ca^{2+}-ATPase velocity was also obtained similarly, although Mf Ca^{2+}-ATPase didn't show initial burst.

Activity of Mf Ca^{2+}-ATPase at low ATP concentration

Changes in the activity of Mf Ca^{2+}-ATPase with the reaction temperature were shown in Fig. 2 by ATP concentrations in No. 1 (group L) and No. 11 (group H). The activity in each of the low ATP concentrations near *Km* decreased exponentially with a lower reaction temperature both in

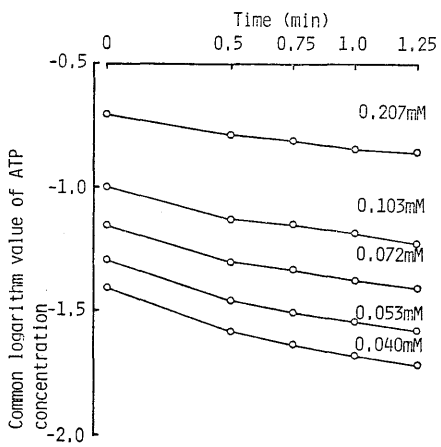

Fig. 1. The change of ATP hydrolysis in each ATP concentration on Mf Mg^{2+}-ATPase. The numbers in the figure represent ATP concentrations.

No. 1 (group L) and No. 11 (group H), reared at 10°C and 30°C for one month respectively. The degree of the decrease was greater in group L (No. 1) than in group H (No. 11). The ranking of the activity in similar ATP concentrations also differed between group L (No. 1) and group H (No. 11) by the reaction temperatures, showing that at 5°C and 10°C group L (No. 1) was lower than group H (No. 11),while at 30°C it was higher than group H.

Consequently, the ranking crossed close to the reaction temperature of 20°C. The crossing of the ranking, however, was not observed between either No. 2 or No. 3 fish of group L and No. 12 fish of group H reared for five months, showing that the ranking was higher in group L than in group H at all reaction temperatures, and that the degree of the change was greater in group L (No. 2 and No. 3) than in group H (No. 12) similar to the result between No. 1 and No. 11 (Fig. 2).

The change of Mf Ca^{2+}-ATPase activity in accordance with the reaction temperature suggested that the two related logarithmic functionally with each other. The Arrhenius plots for Mf Ca^{2+}-ATPase were shown in Fig. 3. Although the activity was different largely by ATP concentrations, the slopes of the regression lines tended to be quite similar by specimens. The mean and the standard deviation of the slopes were shown by specimens in Fig. 4. The standard deviation of each specimen was very small, indicating that the slopes of different ATP concentrations were quite similar among specimens.

Comparing the mean values of slopes among fish specimens, the slopes of specimens reared for one month were significantly smaller than those of specimens reared for five months both in groups L and H, and the slopes were significantly larger in group L than in group H. These results suggested that the temperature dependence of Mf Ca^{2+}-ATPase activity in low ATP concentrations changed according to rearing water temperature and rearing period.

Michaelis constants (Km) of Mf Ca^{2+}-ATPase

The change in *Km* of Mf Ca^{2+}-ATPase with

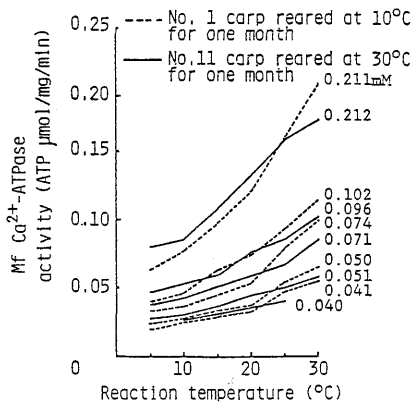

Fig. 2. The changes in the activity of Mf Ca^{2+}-ATPase on two carps reared at 10°C and 30°C by low ATP concentrations. The numbers in the figure represent ATP concentrations.

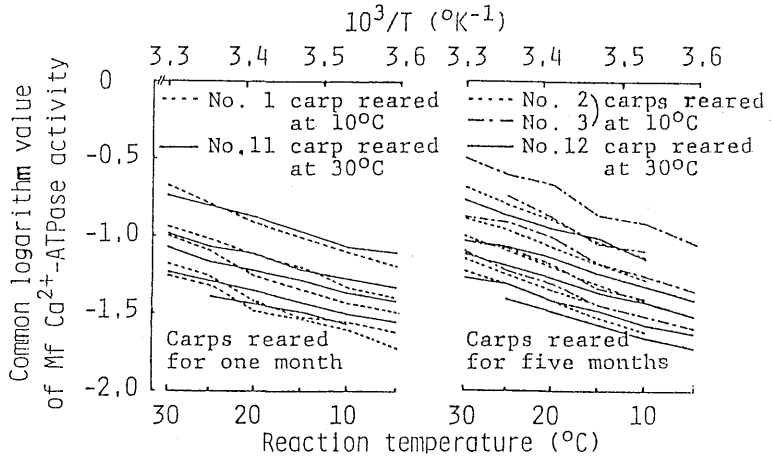

Fig. 3. Arrhenius plot showing changes in the activity of Mf Ca^{2+}-ATPase with the reaction temperature by the specimens of carps having a different rearing water temperature and rearing period. The dotted or broken and solid lines in the figure represent group L fish and group H fish on month 1 or month 5, respectively, while the five lines of the same symbol represent different ATP concentrations within a range of 0.04 to 0.2 mM near *Km* value.

	Carps reared at 10°C			Carps reared at 30°C	
Specimen number	No. 1	No. 2	No. 3	No.11	No.12
Rearing period (months)	one	five	five	one	five
n	5	5	5	5	5
Mean ±S.D.	-0.161 ±0.009	-0.180 ±0.009	-0.183 ±0.016	-0.115 ±0.011	-0.138 ±0.009

11.906 p<0.01	0.065 N.S.	59.040 p<0.001	13.005 p<0.01	
6.566 p<0.05	110.783 p<0.001	28.020 p<0.001		
54.316 p<0.001	55.695 p<0.001			
16.329 p<0.01				

Fig. 4. The mean value and the standard deviation of the slopes in fish specimens calculated from the Arrhenius plot between the reaction temperature (10^3/T) and the Mf Ca^{2+}-ATPase activity (common logarithm value) on each substrate concentration. The figures show F_0 values and significance levels of F-test on the difference in the means of slope for each combination of two specimens.

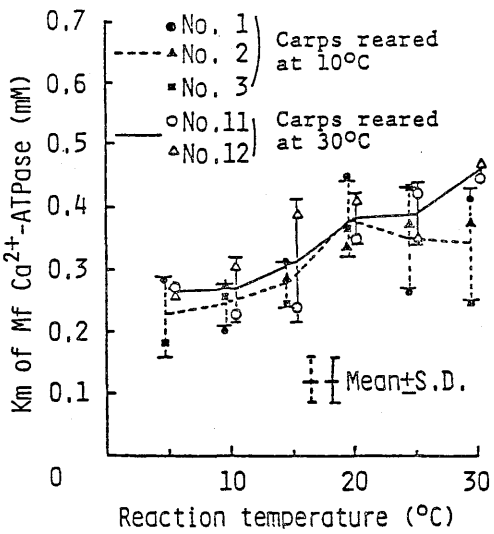

Fig. 5. The change in Km of Mf Ca^{2+}-ATPase with the reaction temperature. The dotted and solid lines in the figure represent the mean levels at month 1 and month 5 combined in groups L and H fish, respectively.

the reaction temperature was shown in Fig. 5. The mean values of Km decreased significantly ($p<0.001$) with a lower reaction temperature in both groups L and H (by analysis of variance). In other words, the enzyme-substrate affinity of Mf Ca^{2+}-ATPase became stronger with a lower reaction temperature. Comparing the mean value of Km between group L and group H, group L was significantly smaller ($p<0.05$) than group H (by analysis of variance), but the difference was very small. Therefore the enzyme-substrate affinity of Mf Ca^{2+}-ATPase was stronger slightly in group L than in group H.

V_{max} of Mf Ca^{2+}-ATPase

The change in presumption V_{max} of Mf Ca^{2+}-ATPase with the reaction temperature was shown in Fig. 6. The levels of presumption V_{max} decreased exponentially with a lower reaction temperature in groups L and H and in all fish specimens. Comparing the mean value of presumption V_{max} between groups L and H, group L showed a tendency to be larger than group H in high reaction temperatures from 20°C to 30°C, although at 5°C it showed the opposite tendency. The slope of the regression line calculated by Arrhenius plots was larger slightly in group L than in group H. Consequently, the temperature dependence of Mf Ca^{2+}-ATPase was larger slightly in group L than in group H. This tendency of presumption V_{max} coincided closely with the result of reaction velocity in low ATP concentration near Km shown in Figs. 3 and 4.

The levels of the measurement V_{max} were shown in Fig. 7. This was the result of specimens reared at 10°C and 30°C for one month. Two regression lines of group L and group H crossed at the reaction temperature of 25°C, and the slopes were significantly larger ($p<0.001$) in group L than in group H. These tendencies agreed with the results of the reaction velocity in low ATP concentrations near Km shown in Figs. 2 and 4.

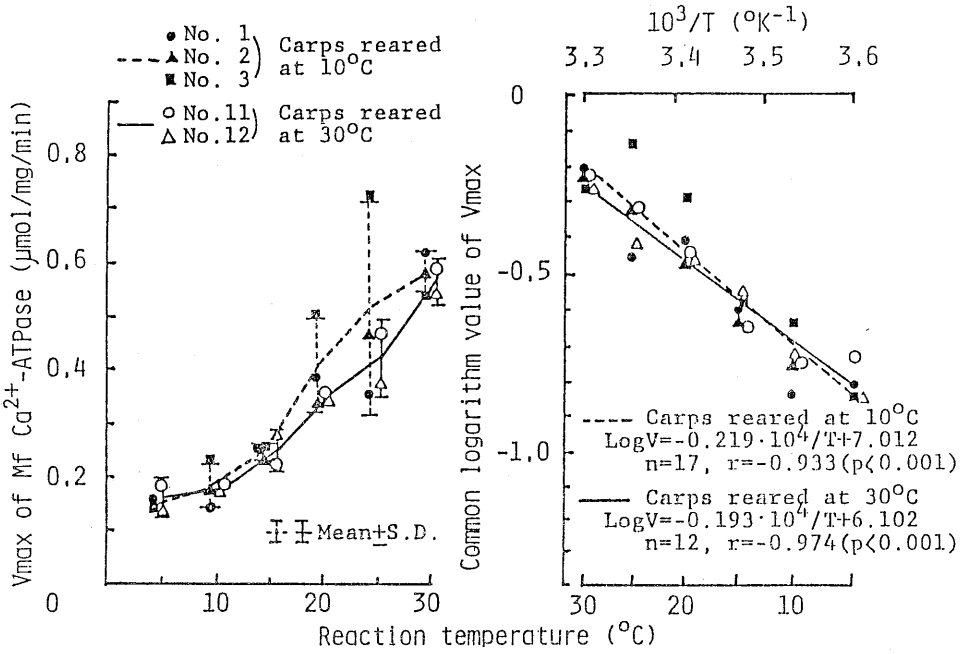

Fig. 6. The change in presumption V_{max} of Mf Ca^{2+}-ATPase with the reaction temperature. The left side figure represents the relationship between the reaction temperature and presumption V_{max} level, and the dotted and solid lines represent the regression lines of groups L and H fish.

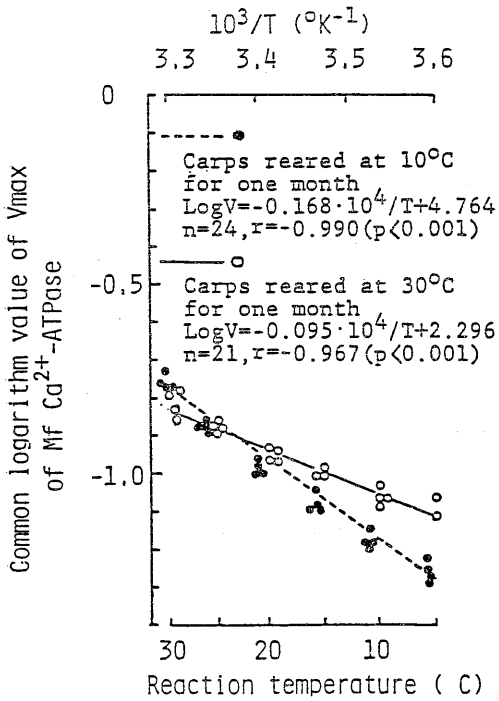

Fig. 7. The change in measurement V_{max} of Mf Ca^{2+}-ATPase with the reaction temperature in Arrhenius plot. This measurement V_{max} was measured in groups L and H fish at month 1. The dotted and solid lines represent the regression lines of groups L and H fish, respectively.

Activity of Mf Mg^{2+}-ATPase at low ATP concentration

Although the change of Mf Mg^{2+}-ATPase activity with the reaction temperature was not shown in the figures, the activity decreased exponentially with a lower reaction temperature both in groups L and H in the same manner as the change found in Mf Ca^{2+}-ATPase activity. The Arrhenius plots weer shown in Fig. 8. The relationship between $10^3/T$ and common logarithm of the reaction velocity took the form of two connected straight lines changing direction near 10°C in almost all the ATP concentrations of all specimens. The slope of the straight lines tended to be smaller from a reaction temperature of 30°C to around 10°C than in that from reaction temperatures under 10°C. In this paper we described the results of the former straight line including the two rearing temperatures.

Comparing the activity of Mf Mg^{2+}-ATPase between groups L and H (Fig. 8), the difference of the activity was not apparent during the one month of rearing. But in longer rearing periods of two and five months the ranking of the activity was much higher in group L than in group H in each of the low ATP concentrations near *Km*. Among the rearing periods, the ranking of the

activity increased with a longer rearing period both in groups L and H, although the degree of increase tended to be much larger in group L than in group H.

The five straight lines from each fish showed a tendency to be parallel although the levels varied greatly by ATP concentrations. The mean slope value and the standard deviation of the five straight lines were shown in Fig. 9 by specimens. The standard deviation of each specimen was very small, indicating that the slopes in the five ATP concentrations were very similar. Comparing the mean slope value among six specimens, the mean slope value showed a tendency to be larger with a longer rearing period both in groups L and H, and the difference was significant ($p<0.05$) except on two occasions in the one-month, two-month and five-month periods. This tendency

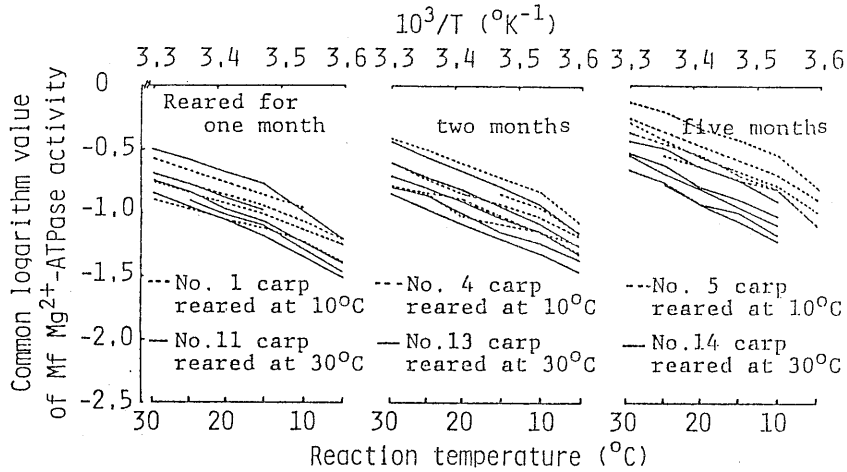

Fig. 8. The Arrhenius plots showing changes in the activity of Mf Mg^{2+}-ATPase with the reaction temperature by the specimens of carps having a different rearing water temperature and rearing period. The left, middle, and right sides in the figure show the results on month 1, month 2 and month 5, respectively. The dotted and solid lines in the figure represent group L fish and group H fish, and the five lines of the same symbol in the same side represent different ATP concentrations with in a range of 0.04 to 0.2 mM near *K*m value.

	Carps reared at 10°C			Carps reared at 30°C		
Specimen number	No. 1	No. 4	No. 5	No.11	No.13	No.14
Rearing period (months)	one	two	five	one	two	five
n	4	5	5	5	5	5
Mean	-0.156	-0.175	-0.185	-0.178	-0.199	-0.220
±S.D.	±0.012	±0.007	±0.022	±0.017	±0.007	±0.015
	9.544 p<0.05	0.973 N.S.	0.356 N.S.	6.944 p<0.05	7.760 p<0.05	
		0.550 N.S.	0.124 N.S.	1.771 N.S.	17.476 p<0.01	
			4.850 N.S.	31.357 p<0.001	8.243 p<0.05	
				54.535 p<0.001	37.415 p<0.001	
					48.436 p<0.001	

Fig. 9. The mean value and the standard deviation of the slopes in fish specimens calculated from the Arrhenius plot between the reaction temperature ($10^3/T$) and the Mf Mg^{2+}-ATPase activity (common logarithm value) on each ATP concentration. The figures show F_0 values and significant levels of the *F*-test on the difference in the means of slope for each combination of two specimens.

was similar to the result of Mf Ca^{2+}-ATPase activity shown in Fig. 4. For the rearing temperature, the mean slope values between two specimens in the same rearing period were significantly smaller ($p<0.05$) in group L than in group H except between specimens reared for one month. This tendency was completely contrary to the result in Mf Ca^{2+}-ATPase shown in Fig. 4. These results suggested that the temperature dependance of Mf Mg^{2+}-ATPase activity was influenced by the rearing temperature, and that the degree of the influence was smaller in group L than in group H.

Michaelis constants (Km) of Mf Mg^{2+}-ATPase

The change in *Km* of Mf Mg^{2+}-ATPase with the the reaction temperature was shown in Fig. 10. The change in *Km* with lower reaction temperature showed different tendencies in groups L and H. Group L remained almost unchanged (in specimens reared for five months) or decreased slightly (in specimens reared for one and two months), while group H increased in a range from 30°C to around 10°C and decreased under 10°C.

Km levels between groups L and H were significantly different ($p<0.05$ by analysis of variance) with regard to reaction temperatures, although the difference was small, and the difference varied in terms of the reaction temperature and the rearing period. In other words, enzyme-substrate affinity was stronger slightly in group L than in group H, and the tendency was apparent particularly in the reaction temperature ranging from 20°C to near 10°C.

The maximum velocity of Mf Mg^{2+}-ATPase

Changes with the reaction temperature in presumption *V*max of Mf Mg^{2+}-ATPase, which were determined from the activity in low ATP concentration near *Km* by Lineweaver-Burk plots, is shown in Fig. 11. The parameters of the regression line and the coefficient of correlation, which were calculated between the reaction temperature and *V*max value by groups and by specimens, were shown in Table 2. As the slopes of regression lines tended to be greater from 10°C to 5°C than from 30°C to 10°C, the parameters and the coefficient of correlation in Table 2 were shown with the results from 30°C to 10°C.

Comparing the level of V_{max} between groups L and H in fish reared for one month (Fig. 11), the level was lower in group L at a reaction temperature of 10°C or 15°C than in group H, while in fish reared for two months the level tended to be the same between groups L and H or higher in group L at a reaction temperature of 30°C to 10°C than in group H. Fish reared for five months, however, showed the opposite tendency. The level was extremely higher in group L than in group H. In comparing the *V*max level among rearing periods, the *V*max level in group L tended to be distinctly higher with longer rearing periods, although in group H this tendency was only slight. These results corresponded to the results of the reaction velocity at low ATP concentration (Fig. 8). In addition, the V_{max} level of Mf Mg^{2+}-ATPase was almost the same as that of Mf Ca^{2+}-ATPase shown in Fig. 6.

With regard to the change of the preseumption V_{max} with the reaction temperature, the slope tended to be larger with longer rearing period both in groups L and H (Table 2). This tendency was the same as the result of the reaction velocity

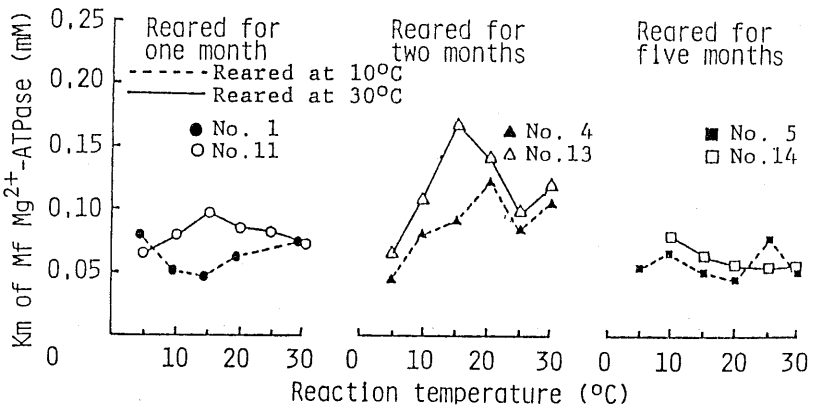

Fig. 10. The change in *K*m of Mf Mg^{2+}-ATPase with the reaction temperature. The dotted and solid lines in the figure represent the mean levels at month 1, month 2 and month 5 combined in groups L and H fish, respectively.

at low ATP concentration shown in Fig. 9. However, comparing the slopes between groups L and H by the rearing periods, the slope was not smaller in group L than in group H except in fish reared for five months, differing from that at low ATP concentration in Fig. 9.

The results of the measured V_{max} were shown in Fig. 12. The V_{max} levels were higher in group L than in group H at all reaction temperatures, and the change of the V_{max} levels with the reaction temperatures and the slope was smaller in group L than in group H. These results agreed well with that of the persumption V_{max} in fish reared for five months (Table 2) and that at low ATP concentrations (Fig. 9).

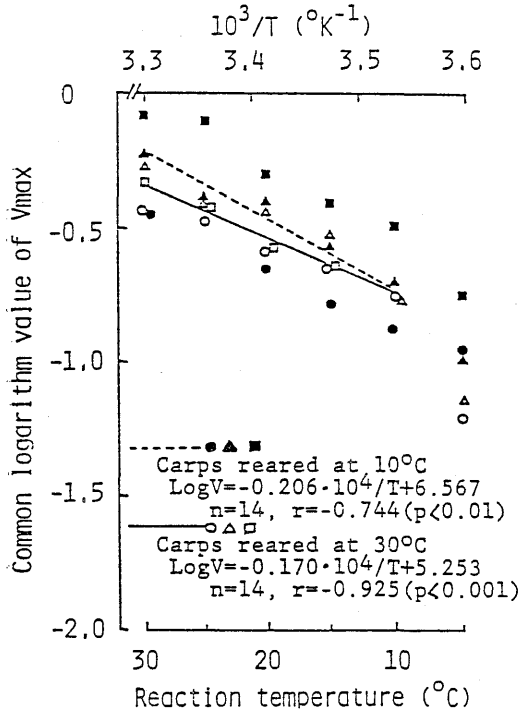

Fig. 11. The change in presumption V_{max} of Mf Mg^{2+}-ATPase with the reaction temperature in Arrhenius plot. The dotted and solid lines in the figure represent the regression lines of groups L and H fish.

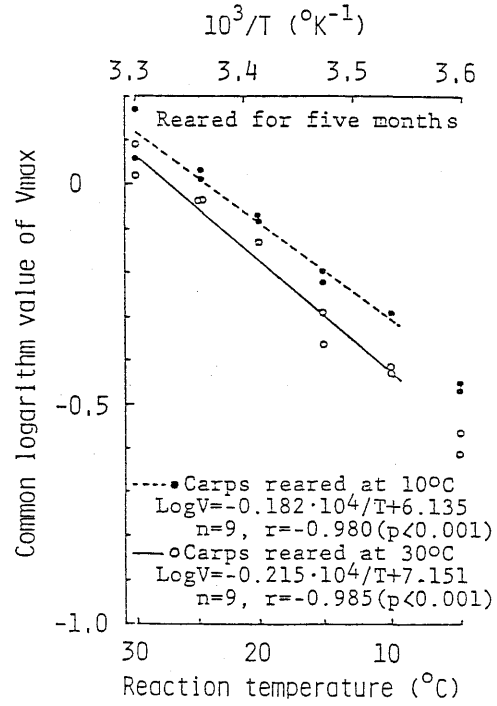

Fig. 12. The change in measurement V_{max} of Mf Mg^{2+}-ATPase with the reaction temperature in Arrhenius plot. This measurement V_{max} was measured in groups L and H fish at month 5. The dotted and solid lines represent the regression lines of groups L and H fish, respectively

Table 2. The parameters of the regression line and the coefficient of correlation

Rearing temp. (°C)	Rearing period (months)	Specimen number	n	Slope*	Intercept*	r*
10	one	No. 1	4	−0.186	5.756	−0.998**
	two	4	5	−0.195	6.211	−0.983**
	five	5	5	−0.232	7.676	−0.863
		Total	14	−0.206	6.567	−0.744**
30	one	No. 11	4	−0.134	3.996	−0.988*
	two	13	5	−0.185	5.825	−0.961**
	five	14	5	−0.291	9.437	−0.937*
		Total	14	−0.170	5.253	−0.925***

* calculated between the reaction temperatures and presumption V_{max} value in Mf Mg^{2+}-ATPase by groups and by rearing periods. The asterisks show the significance levels

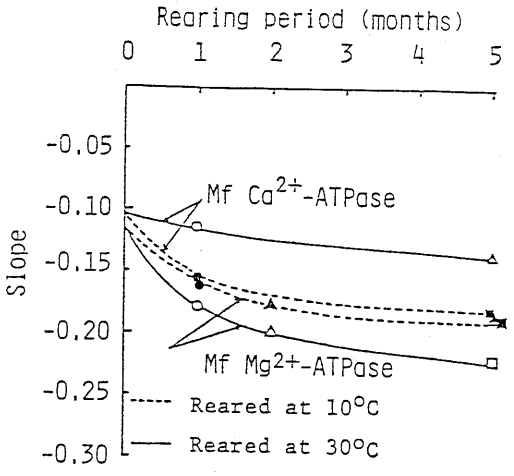

Fig. 13. The relationship between the rearing period and the temperature dependence of Mf Ca^{2+}- or Mg^{2+}-ATPase, the slope of the regression line, which was calculated from the Arrhenius plot between the reaction temperature and Mf Ca^{2+}- or Mg^{2+}-ATPase activity. The dotted and solid lines in the figure represent the mean levels of groups L and H fish, respectively. The zero month of the rearing period, month 0, shows the result in carps reared preliminarily at 18°C for one week.

Discussion

The temperature dependences of a reaction on Mf Ca^{2+}- and Mg^{2+}-ATPase tended to be larger with longer rearing periods in both groups of carp reared at 10°C (group L) and 30°C (group H). Therefore the temperature dependence of a reaction in carps reared preliminarily at 18°C (month 0) was smaller than that of group L and group H (Fig. 13). These results suggested that the temperature dependences of a reaction in Mf Ca^{2+}- and Mg^{2+}-ATPase did not always change in proportion to the increasing and decreasing of the rearing water temperature, and that the smallest level was between rearing water temperatures of 10°C and 30°C. Furthermore, the results correspond closely with findings that the human basal metabolism is smallest at an environmental temperature of 24°C and increases below or above 24°C. The activity in Mf Ca^{2+}- and Mg^{2+}-ATPase also increased in proportion to the temperature dependence of a reaction, it was considered that the carp basal metabolism might be smallest at a habitat water temperature near 24°C in the same way as it is in humans. In relation to this finding, Heap *et al.*[17] reported that Mf Mg-ATPase activity of carps reared at 10°C and 28°C changed till 4 weeks, and were with steady state from 4 weeks to 10 weeks. Though they didn't rear for long period as five months, their finding tended to differ from our result a little. Therefore it was considered the influence of rearing period in Mf ATPase need to study more to details.

On the one hand, the change of temperature dependence of a reaction in Mf Ca^{2+}-ATPase with the rearing periods was larger in group L than in group H. On the other hand, however, that in Mf Mg^{2+}-ATPase was smaller in group L than in group H (Fig. 13). In this connection, Johnston *et al.*[1] reported that the temperature dependence of a reaction in Mf Mg^{2+}-ATPase was smaller in cod living in a frigid zone than in tilapia living in a tropical zone, and Penny *et al.*[18] reported the same finding in that of gold fish reared at different water temperatures. Both of these findings corresponded with our result in Mf Mg^{2+}-ATPase.

The change in Mf Ca^{2+}-ATPase, however, has not been reported previously. The fact that the influence of rearing water temperature on temperature dependence of a reaction was different between Mf Ca^{2+}- and Mg^{2+}-ATPase is very interesting, but the physiological and biological meaning of the difference is not clear.

The *Km* values of both Mf Ca^{2+}- and Mg^{2+}-ATPase were smaller slightly in group L than in group H at reaction temperatures of 5°C to 30°C in the former and 10°C to 20°C in the latter. In other words, these enzyme-substrate affinites were larger slightly in group L than in group H. Hochachka *et al.*[19-21] and Somero *et al.*[22] also reported that *Km* values of acetylcholine esterase, isocitrate dehydrogenase, and pyruvate kinase in fishes were different by the habitat water temperature. These reports suggest that the change of *Km* is very important for adaptation to habitat water temperature. *Km* of Mf Ca^{2+}- and Mg^{2+}-ATPase in carps reared at 10°C were smaller slightly than that reared at 30°C. This difference might have an meaning for adaptation to habitat water temperature.

In addition, the molecular mechanism causing this difference is an important subject. The above findings suggest that myosin ATPase may has a flexible structure in a high order and can react easily on substrate at low habitat temperatures. These facts also may support the previous findings that the thermostabilities of Mf Ca^{2+}- and Mg^{2+}-ATPase are smaller in fishes living in low habitat

temperature than in fishes living in high habitat temperature. On the other hand, it has known that some enzymes are not influenced, or have an reverse influence by habitat temperature.[19] These deferences by enzyme might also have the important meaning for adaptation in fish.

We consider the increases of enzyme-substrate affinity and the change of temperature dependence and the level at *V*max on Mf Mg^{2+}-ATPase in carps reared at 10°C to be useful in the production of energy at low habitat water temperatures. These differences in enzyme might show the importance in physiological. The results of our study, which used carps living in a wide range of habitat water temperatures, may provide an important suggestion as to how fishes living in widely differing temperatures adapt to the habitat and conserve life in a very low habitat water temperature.

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