ビワマスとアマゴの浸透圧調節能の季節的変化

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Seasonal Changes in Hypoosmoregulatory Ability of Biwa Salmon *Oncorhynchus rhodurus* and Amago Salmon *O. rhodurus**1

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Seasonal changes in hypoosmoregulatory ability of hatchery-reared biwa salmon *Oncorhynchus rhodurus* and amago salmon *O. rhodurus* were examined by following changes in plasma Na^+ concentration after transfer to 70% seawater. The landlocked biwa salmon lose parr marks and become silvery bodied (smolt) underyearling during early summer. They showed the maximum hypoosmoregulatory ability from underyearling May to July, when they transformed from parr to smolt and migrated to the lake. However, the smolt did not show increased seawater adaptability as compared with parr in underyearling May, and the ability decreased markedly in autumn and winter. There was no essential difference in seawater adaptability between smolt and dark parr or yearling parr. On the other hand, amago salmon, which smoltify and go down stream to the sea in late autumn and early winter, showed better seawater adaptability than biwa salmon throughout all developmental stages examined. Especially, the smolts during the period from November to January showed good osmoregulatory ability. The poor hypoosmoregulatory ability of biwa salmon as compared with amago salmon may be related to their lacustrine nature and a long period passed since this species was landlocked in Lake Biwa.

Introduction

Many salmonid fishes maigrate to the sea after remaining for some period in their natal rivers, and their migratory season, age and body size vary in accordance with each species. Pacific salmon, chum salmon *Oncorhynchus keta* and pink salmon *O. gorbuscha*, go down to the sea as alevins soon after yolk absorption, whereas the seaward migration of other species generally occur in spring between ages 1^+-3^+. Physiological studies revealed that they acquire seawater adaptability and preference maximally at or near their migratory season. Morphologically, anadromous salmonids transform from parr to silvery smolt prior to their seaward migration, usually in spring.[1-3]

Biwa salmon *O. rhodurus* is indigenous to Lake Biwa located in Mainland of Japan, and considered as a lacustrine form of amago salmon *O. rhodurus* which have a limited distribution in coastal sea and rivers of the southwestern Pacific side of Japan.[4-6] Biwa salmon transform from parr to silvery smolt and go downstream into the lake in underyearling spring and early summer.[7] After spending 3–5 years in the lake, they ascend the rivers discharging into the lake to spawn in autumn. A part of amago salmon, on the other hand, smoltify and migrate downstream about one year after hatching to the coastal sea in autumn and winter, and return to the river after half a year.[8,9] Some of the amago salmon population remain in the stream as parr and mature as yearling.

In spite of many ecological differences between biwa and amago salmon, little has been studied on the development of seawater adaptability. The present study was undertaken to examine the seasonal changes in the hypoosmoregulatory ability of biwa and amago salmon reared in the pond with reference to growth, maturation and smoltification.

Materials and Methods

Biwa and amago salmon were reared in running spring fresh water (12±1°C) under natural photoperiod in Samegai Trout Farm. These fish were from the stocks which had originated from Lake Biwa (biwa salmon) and Gifu Prefecture (amago salmon), respectively. They were fed a

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Table 1. Body weight and GSI of biwa and amago salmon used in the experiments

Age	Date	Phase	Biwa salmon			Amago salmon		
			Body weight*1	GSI*2	N	Body weight	GSI	N
	Feb. 15–24	Parr	0.59±0.03		75	0.46±0.02		72
	Mar. 12–21	Parr	1.74±0.05		70	1.57±0.04		69
	Apr. 17–19*3	Parr	3.15±0.15		20	2.84±0.18		19
	May 15–31	Parr	6.93±0.21		70	7.74±0.29		70
		Parr				11.38±0.65		20
	Jun. 13–14*3	Smolt	9.94±0.42		20			
		D. Parr*4	14.00±0.58		20			
		Parr				24.69±0.90		70
	Jul. 14–31	Smolt	14.96±0.40		70			
		D. Parr	28.24±0.98		70			
		Parr				31.42±3.03	0.34±0.08	10
0+	Aug. 11–13*3	Smolt	20.44±0.83	0.06±0.01	20			
		D. Parr	40.70±1.61	1.43±0.34	20	35.28±2.38	1.57±0.39	10
		Parr				43.68±1.08	0.15±0.02	69
	Sep. 24–Oct.8	Smolt	29.16±2.67	0.07±0.01	70			
		D. Parr	51.94±1.58	6.19±0.55	69	59.76±1.94	4.11±0.51	70
		Smolt	32.48±2.19	0.10±0.04	21	53.88±2.29	0.15±0.02	21
	Oct. 25–28*3	D. Parr	52.20±3.11	3.55±0.42	20	58.64±3.24	5.61±1.81	20
		Smolt	31.41±0.86	0.05±0.01	73	58.92±1.84	0.13±0.01	69
	Nov. 9–30	D. Parr	45.03±1.47	3.84±0.44	69	58.34±2.00	3.81±0.88	70
		Smolt	36.46±2.15	0.10±0.01	20	82.54±5.14	0.16±0.02	20
	Dec. 10–11*3	D.Parr	49.90±1.92	3.98±0.36	20	81.48±4.86	2.90±1.65	20
		Smolt	35.28±0.88	0.13±0.02	70	95.81±3.23	0.22±0.03	70
	Jan. 22–31	D. Parr	42.32±1.52	1.59±0.35	70	74.32±2.22	0.28±0.03	70
1+		Smolt	35.99±1.41	0.11±0.03	20	98.67±7.84	0.24±0.05	20
	Feb. 20–26*3	D. Parr	40.00±1.97	0.34±0.20	18	83.34±4.00	0.24±0.10	19
		Parr				90.12±3.96		70
	Mar. 12–25	Smolt	38.24±1.17		71	109.02±4.28		69
		D. Parr	52.70±2.05		69			
		Parr	58.39±5.90		20	62.81±3.20		20
	Apr. 18–20*3	Smolt	33.11±1.29		20	70.70±4.36		21
		Parr	53.84±2.16		70	72.81±2.34		70
	May 15–Jun. 1	Smolt	36.86±1.20		71			
		Parr	76.18±6.07		20	91.50±4.55		20
	Jun. 13–14*3	Smolt	39.33±1.53		20			
		Parr	95.63±4.21	0.54±0.11	70	110.96±4.01	1.06±0.23	69
1+	Jul. 14–25	Smolt	49.01±1.75	0.10±0.02	69			
		Parr	100.45±6.64	1.89±0.41	20	118.35±9.03	2.23±0.56	19
	Aug. 12–13*3	Smolt	62.37±3.13	0.12±0.01	19			
		Smolt	90.78±8.46	0.15±0.03	69			
	Sep. 18–28	D. Parr	145.09±6.45	8.08±1.02	70	140.56±7.34	6.72±0.70	70
	Oct. 26–27*3	Smolt	67.51±3.36	0.12±0.02	20	*5		
	Nov. 23–30	Smolt	53.06±1.83	0.11±0.03	70			
	Dec. 10–11*3	Smolt	63.05±4.65	0.31±0.04	20			
	Jan. 22–31	Smolt	62.58±2.62	0.21±0.04	70			
2+	Feb. 25–26*3	Smolt	79.37±5.68	0.18±0.05	20			
	Mar. 12–25	Smolt	73.96±2.72		70			

*1 Mean body weight±SEM (g).
*2 GSI=gonad weight(g)×100/body weight(g).
*3 24 h seawater challenge period.
*4 D. Parr; dark parr.
*5 All the amago salmon died after spawning in 1+ October.

commercial trout diet twice daily. They were divided into three groups; parr, smolt (silvery body color) and dark parr (precociously mature male and yearling mature specimens) based on visual criteria as described by Fujioka.[10] Experiments were carried out during underyearling February to yearling September (amago salmon) or to March of 2^+ year (biwa salmon) in 1984–1986 (Table 1).

Hypoosmoregulatory ability was evaluated by following the time course change of plasma Na^+ levels after direct transfer of the fish from fresh water (12±1°C, Na^+: 0.07 mM) to 70% artificial seawater (SW) (10–14°C, Na^+: 290–310 mM, Jamarin Lab.). 50 *l* aquaria were used for 10–12 fish. 70% SW was chosen, because biwa salmon died within two days after direct transfer to 100% seawater.[11] The water was aerated, filtered through activated charcoal and changed every other day. Plasma samples were taken from 5–11 fish 24 h after the transfer to 70% SW monthly, and from 4–12 fish after 3, 6, 8, 24 h and 3, 7 days bimonthly. The hypoosmoregulatory ability of juveniles 4 or 6 months after hatching was also examined. They were acclimated to 70% SW for 7 days, and plasma samples were taken 1 and 3 days after the transfer to 100% SW (12±14°C, Na^+: 408–425 mM).

Blood was collected from the caudal artery of the fry weighing less than about 10 g as described by Iwata *et al.*,[12] and from the caudal vessels with a syringe needle in the larger fish. Plasma was separated by centrifuging for 10 min at room temperature. Plasma Na^+ concentration was measured by atomic absorption spectrophotometry (Hitachi 180–50, Nippon Jarrell-Ash AA-860).

Data were presented as mean±SE. Duncan's new multiple range test was used to assess the significance of seasonal changes in plasma Na^+ levels. Student's test or Cochran-Cox test was used to compare the plasma sodium levels between fresh-water and seawater.

Results

Seasonal Changes in Plasma Na^+ Levels 24 h after Transfer from Fresh Water to 70% SW

Plasma Na^+ concentrations of biwa salmon in fresh water varied in the range between 135–168 mM (Fig. 1). When they were transferred to 70% SW, plasma Na^+ levels increased significantly ($P<0.01$) by 30–80 mM, or above 200 mM in most cases, 24 h after the transfer. There was no

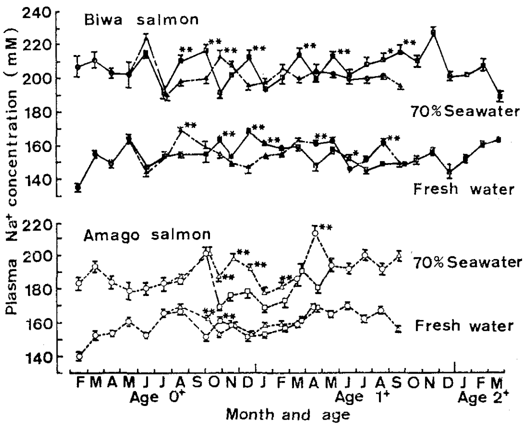

Fig. 1. Seasonal changes in plasma Na^+ concentrations of biwa and amago salmon 24 h after the transfer from fresh water to 70% seawater. Vertical bars represent standard errors of the means (n=5–11). *, ** Significantly different from dark parr or parr at $P<0.05$ and $P<0.01$, respectively.
Biwa salmon ●: parr, ■: smolt, ▲: dark parr.
Amago salmon ○: parr, □: smolt, △: dark parr.

seasonal variation in either the plasma Na^+ levels in fresh water or those 24 h after the transfer to 70% SW, although both levels fluctuated markedly each month.

The plasma Na^+ levels of amago salmon in fresh water were about the same as those of biwa salmon (Fig. 1). The levels in 70% SW increased significantly ($P<0.01$) to about 170–200 mM 24 h after the transfer to 70% SW, but they were about 20 mM lower than in biwa salmon. Especially, the plasma Na^+ levels of smolts 24 h after the transfer to 70% SW between October and February (except for January) were fairly low (168–176 mM) and were significantly ($P<0.01$) lower than those in dark parr of the same season. A significant ($P<0.01$) increase was seen in the levels of smolts in yearling during March and April from that in January.

Changes in Plasma Na^+ Levels During Acclimation to 70% SW

Changes in plasma Na^+ concentrations of biwa and amago salmon parr after the transfer to 70% SW are summarized in Fig. 2A-C. In Feburuary, biwa salmon parr did not survive for more than 4 days in 70% SW, whereas in March, 90% of them survived for 7 days (Table 2). In May, the plasma Na^+ level returned to freshwater-adapted level

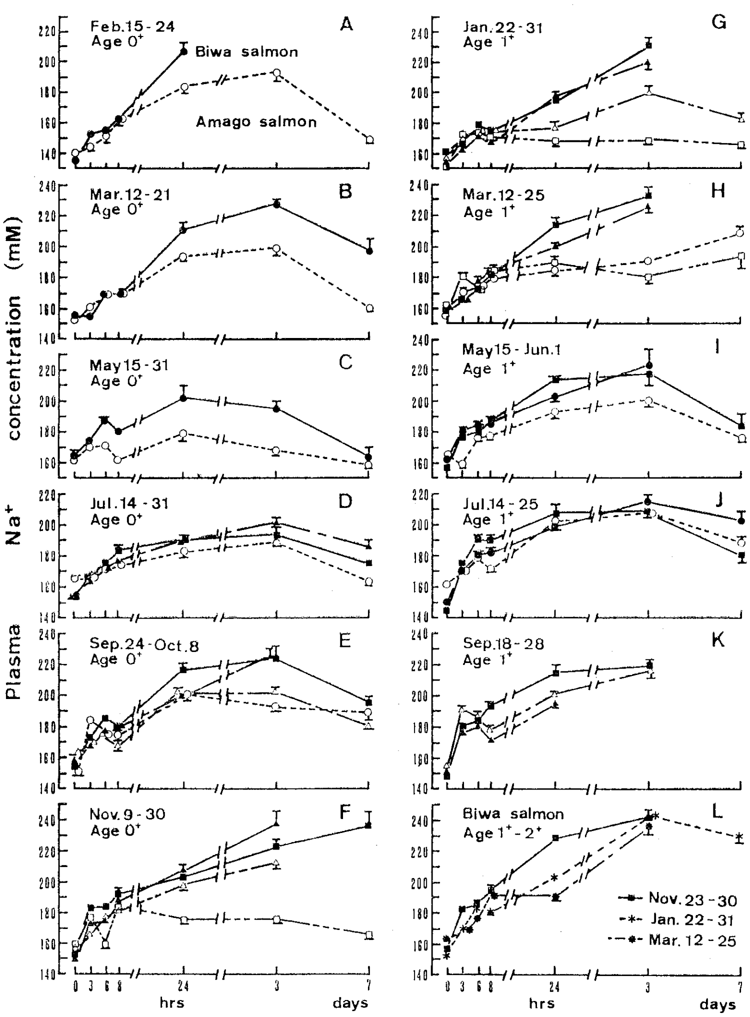

Fig. 2. Changes in plasma Na^+ concentrations of biwa and amago salmon after the transfer from fresh water to 70% seawater. Vertical bars represent standard errors of the means (n=4–12).
Biwa salmon ●: parr, ■: smolt, ▲: dark parr.
Amago salmon ○: parr, □: smolt, △: dark parr.

after 7 days in 70% SW, indicating high hypoosmoregulatory ability. On the other hand, amago salmon parr of the same age maintained lower plasma Na^+ levels than biwa salmon parr during acclimation to 70% SW. In Feburuary, the mortality of the fish was 45% during the 7 day experiment (Table 2). The plasma Na^+ levels returned to original freshwater-levels after 7 days, and no mortality was seen in March and May.

Biwa salmon parr transformed to silvery bodied (smolt) or dark parr (precocious male) in underyearling June, whereas amago salmon parr changed to smolt and dark parr from August to October. As shown in Fig. 2D-H, plasma Na^+ levels of biwa smolt and dark parr in July were maintained relatively low (less than 193 mM) in 70% SW. During the period from September to March, however, their hypoosmoregulatory ability seem to have decreased. From November to March, no difference was seen between smolt and dark parr in the hypoosmoregulatory ability or mortality. Changes in plasma Na^+ concentrations of amago parr in 70% SW in July were essentially the same as those in March and May. A plasma Na^+ level after the transfer was maintained slight high in September. From November to March, the values in 70% SW of amago smolts were maintained at lower levels than those of biwa salmon. Especially, the smolts in January showed best hypoosmoregulatory ability, with a peak

Table 2. Percent survival of biwa and amago salmon in 70% SW at 7 days after the transfer

Age	Month*	Biwa salmon			Amago salmon		
		Parr	Smolt	Dark parr	Parr	Smolt	Dark parr
0+	February	0			55		
	March	90			100		
	May	100			100		
	July		100	80	100		
	September		100	0	100		100
	November		42	0		100	20
1+	January		18	10		100	100
	March		8	33	50	55	
	May	30	90		100		
	July	40	44		80		
	September		20	10			10
	November		0				
2+	January		90				
	March		10				

* Date are shown in Table 1.

level of only 173 mM after 6 h. Amago dark parr in underyearling September (before spermiation; GSI: 4.11) and January (after spermiation; GSI: 0.28) showed similar seawater adaptability to parr or smolt. However the fish in November (active spermiation) was unable to adjust the plasma Na^+ level to 70% SW.

Plasma Na^+ concentrations of biwa smolt and parr 1 and 3 days after the transfer to 70% SW in yearling May and July were higher than 200 mM (Fig. 2 I, J). Their hypoosmoregulatory ability decreased further from yearling September to March (Fig. 2 K, L). In amago parr and dark parr, on the other hand, the Na^+ concentrations after the transfer to 70% SW in yearling May were maintained lower than those of biwa salmon, but the changes in plasma Na^+ concentrations in July and September (mature fish) showed the similar pattern to those of biwa salmon.

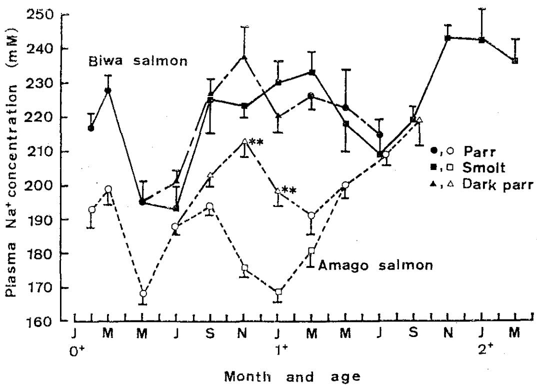

Fig. 3. Seasonal changes in plasma Na^+ concentrations of biwa and amago salmon 3 days after the transfer from fresh water to 70% seawater (n=3–11). ** significantly different between smolt and dark parr at $P<0.01$.

Seasonal Changes in Plasma Na^+ Levels 3 Days after Transfer from Fresh Water to 70% SW

Fig. 3 shows seasonal changes in plasma Na^+ concentrations 3 days after the transfer to 70% SW, since maximum increase was observed 3 days after the transfer to 70% SW (Fig. 2). Significance ($P<0.01$) of seasonal changes in the plasma Na^+ levels were observed in both biwa and amago salmon. In biwa salmon, the levels were higher than 210 mM except for underyearling between May and July. In amago salmon, on the other hand, minimum levels were seen in underyearling in May (parr) and yearling in January (smolt), and the same levels were attained as in biwa salmon in yearling between July and September.

Changes in Plasma Na^+ Levels after Transfer of the Fish Acclimated to 70% SW to 100% SW

When biwa salmon parr (3.2 g) acclimated to 70% SW for 7 days were transferred to 100% SW in April, plasma Na^+ levels increased significantly ($P<0.01$) from 205 mM to 250 mM after 24 h, and they died within 72 h (Fig. 4A). Amago salmon parr (2.3 g), on the other hand, regulated the plasma Na^+ levels better. Their plasma Na^+ concentrations in creased significantly ($P<0.01$) from 172 mM to 204 mM 24 h after the transfer to 100% SW. However, only 4 out of 18 fish survived in 100% SW for 72 h. The plasma Na^+ level

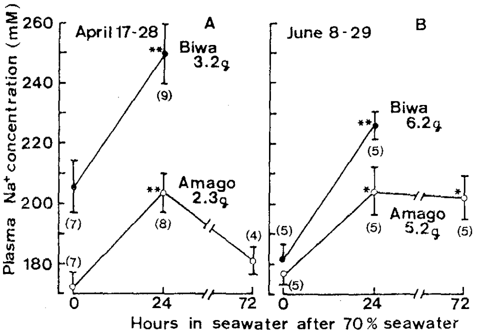

Fig. 4. Changes in plasma Na^+ concentrations of biwa and amago salmon after the transfer from 70% seawater to 100% seawater. Vertical bars represent standard errors of the means (n=4–11). *, ** significantly different from the 0 h value, at $P<0.05$ and $P<0.01$, respectively.

of survived fish was 181 mM. When biwa smolt (6.2 g) were transferred from 70% SW to 100% SW in June, plasma Na^+ concentrations increased significantly ($P<0.01$) from 182 mM to 226 mM after 24 h, and all of them died within 3 days (Fig. 4B). In amago salmon parr (5.2 g), plasma Na^+ levels increased significantly ($P<0.05$) from 177 mM to 204 mM 24 h after the transfer, and the same level was maintained until day 3. All the fish survived for 3 days in 100% SW, but died after 4 days.

Discussion

As shown in the present study, biwa salmon did not develop enough hypoosmoregulatory ability to adapt to full-strength seawater from underyearling February to March of 2^+ year. The maximum ability was observed during underyearling May and July, when they transformed from parr to silvery bodied (smolt) and migrated to the lake. Even then, they required 7 days to acclimatise to 70% SW. Thereafter the ability of smolt to adapt to 70% SW decreased markedly, and no difference was seen between smolt and precociously mature parr. On the other hand, seawater adaptability of amago salmon was relatively greater than that of biwa salmon during all developmental stages examined. Especially, the smolt in November and January showed maximum osmoregulatory ability, in accordance with their migratory season as reported previously.[4] Kubo[13] and Gorbman *et al.*[14] described morphological characteristics which developed during parr-smolt transformation in masu and coho salmon, and characterized the final stage of smolt as the body silvering and intense darkening of the dorsal and caudal fin margin. Kubo[13] demonstrated that seawater adaptability of masu salmon smolt was imcomplete prior to the final stage. Biwa salmon smolt did not show blackening of dorsal fin tip as other species.[10] Although the physiological significance of darkening of fin margin is not known, poor hypoosmoregulatory ability of biwa salmon smolt seems to be relative to incomplete smoltification.

Chum and pink salmon alevins and fry are reported to survive after the direct ransfer to 100% seawater from 60 days after hatching.[15,16] In the present study, a half of amago salmon fry weighing 0.5 g adapted to 70% SW, and a part of the fry weighing 2.3 g pre-adapted to 70% SW were able to survive in 100% SW. The development of the seawater adaptability in amago salmon fry resembles to that in closely related masu salmon. Masu salmon fry 120 days after hatching (2.1 g) survived in 75% SW, and some survived in 100% SW for 12 days.[17] In the present study, biwa salmon fry weighing 6.9 g adapted to 70% SW within a week, 5 months after hatching, having to reach a greater age to acquire adaptability to 70% SW than in amago and masu salmon. Fujioka[11] showed better seawater tolerance of eyed egg of amago salmon than those of biwa salmon, indicating slower and incomplete development of seawater adaptability in biwa salmon compared with amago and masu salmon.

Blackburn and Clarke[18] reported that 24 h challenge period is best for detecting differences in short term seawater adaptability in juvenile salmonids since plasma Na^+ level is then at or near the maximum. In juvenile biwa and amago salmon, however, maximal increase in plasma Na^+ concentration was observed 3 days after the transfer except for amago smolts. Therefore, the estimation of seawater adaptability by 24 h challenge to 70% SW may not be adequate in these species. Thus, changes in plasma Na^+ levels after 3 days appear to indicate clearly the seasonal pattern of seawater adaptability; the ability of biwa salmon increased slightly in underyearling spring and early summer, and in amago salmon hypoosmoregulatory ability developed markedly in underyearling spring, late autumn and winter.

Temporal changes in the development in seawater adaptability were observed in steelhead trout *salmo gairdneri*, masu and sockeye salmon *O. nerka*,[13,19,20] although they were not so remarkable in chum salmon.[21] Conte and Wagner[19] pointed out that the regression of osmotic and ionic regulatory system could be of ecological importance to the species; its usefulness lies in preventing animals from pursuing seaward movement during unfavorable conditions. A part of smoltified amago salmon migrate into the coastal sea from late autumn to early winter,[22] coincided with the period of the best hypoosmoregulatory performance. The seasonal changes in seawater adaptability seems to be critical for amago salmon, since the temperature of coastal seawater is suitably low only during winter.[22]

In this study, mature biwa and amago salmon showed decrease in seawater adaptability, especially during spawning season. McCormick and Naiman[23] reported that in anadromous brook trout *Salvelinus fontinalis*, salinity tolerance of mature males reached the lowest point in autumn when gonadosomatic index was high. Sex steroids are known to inhibit smoltification and also to decrease seawater adaptability in several salmonids.[24-26] Although chum salmon maintains good osmoregulatory ability throughout their life, they also lose hypoosmoregulatory ability in seawater after completion of gonadal maturation.[27,28] These results indicate that sex steroids may act inhibitorily in the hypoosmoregulatory system. In biwa salmon, however, both smolt and dark parr exhibited marked decrease in seawater adaptability during autumn and early spring. Further studies are needed to clarify the relation between osmoregulatory ability and sexual maturation.

The poor hypoosmoregulatory ability of biwa salmon as compared with amago salmon may be related to their lacustrine nature. However, Kaeriyama *et al.*[20] reported that landlocked sockeye salmon maintain good seawater adaptability. Foerster[29] indicated that sea-run sockeye salmon can develop from landlocked population. According to Burton and Idler,[30] landlocked Atlantic salmon *S. salar* show signs of smoltification, and a part of them survived in full strength seawater for 42 days. These lacustrine forms are considered to be derived from anadromous populations in post glacial times.[31] On the other hand, land-locked ayu *Plecoglossus altivelis* in Lake Biwa seem to have diverged from the anadromous form,[32] and are considered to have inhabited in the lake at least before the last glacial ages.[33] According to Azuma,[34] the landlocked ayu seem to have gradually lost the ability in diadromy. However, they seem to have similar hypoosmoregulatory ability to the anadromous form at least in juvenile fish.[35] Loss of seawater adaptability in biwa salmon may be related to the longer landlocked period than the lacustrine form of sockeye or Atlantic salmon.

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