アメマスの塩分耐性とそれにおよぼす水温の影響

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Seawater Tolerance of White-spotted Charr (*Salvelinus leucomaenis*) Related to Water Temperature

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Abstract

Seawater tolerance of white-spotted charr derived from southwestern Hokkaido, northern Japan, was examined by 24-hour seawater challenge tests. Seawater tolerance of charr (1+) did not change seasonally. High water temperature (>16℃) restrained osmoregulation but low temperature (<4℃) did not.

Keywords : white-spotted charr, *Salvelinus leucomaenis*, seawater challenge test, serum Na^+, seawater tolerance

Introduction

White-spotted charr (*Salvelinus leucomaenis*) are distributed in far east Asia (Chereshnev, 1991), and their anadromous populations occur in Hokkaido, northern Japan (Kawanabe, 1989; Goto, 1991). Timing of upstream and/or downstream migration of this species varies among the northward, eastward, and southwestward of Hokkaido (Gritsenko and Churikov, 1976; Yamamoto and Nakano, 1996; Shibata, 1938; Takami, 1995). However, the factor influencing these behavior is unknown. In general, seawater tolerance in salmonids shows typical seasonal changes (Hoar, 1976). Seawater tolerance of Arctic charr (*S. alpinus*) fluctuates seasonally and is thought to affect their migratory habits (Finstad et al.,1989). Low and high seawater temperatures also influence hypo-osmoreguratory capacity (Gordon, 1959; Blackburn and Clarke, 1987; Finstad et al., 1988; Johnsson and Clarke, 1988; Sigholt and Finstad, 1990). Seasonal migration patterns of salmonids are different among several stocks (Thorpe, 1988; Mayama et al.,1989). Therefore, regional variation in the migration patterns of anadromous white-spottd charr should be determined by climatic and/or biological factors. The aim of this paper is to investigate seasonal changes in and thermal effects on the seawater tolerance of anadromous white-spotted charr obtained from southwestern Hokkaido.

Materials and Methods

Fish

White-spotted charr (1+) used in the present study were derived from wild parents (anadromous females × stream-resident males) that were captured in the Ken-ichi (N42° 07', E140° 01') and the Furuu Rivers (N43° 08', E140° 26') in southwestern Hokkaido. Anadromous adults (2+) were also used for the experiment of low temperature effects on seawater tolerance.

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These fish were derived from wild parents (anadromous females × anadromous and stream-resident males) from the Furuu River and had smoltified in their second spring.

the fish were reared in freshwater at a constant water temperature (7-8℃) under a natural photoperiod being fed dry pellets in the Makkari Research Branch, Hokkaido Fish Hatchery, until experiments. The fish had been reared in 250-liter FRP tanks (L140×W60×D50 cm) in their first and second years and in a 1.5-metric-ton FRP circular tank in their third year. Food supply was stopped for 3 days before the experiments.

Experimental Tanks

Two 150-liter aquarium tanks (L75×W45×D45 cm) made of plexiglas® were used for the freshwater and seawater tests. Freshwater (7-8 ℃) was flowed into the aquarium for freshwater control. The freshwater, that overflowed outside the aquarium, circulated around the seawater aquarium to stabilize the inside temperature. The seawater was recirculated and cleaned by a filter (SUISAKU Jumbo S) with an air pump. The temperatures in the aquaria fluctuated a little seasonally (freshwater: 7.1-8.2, seawater: 6.7-8.7℃).

Maximally 8 sets of 200-liter FRP tanks (L90×W60×D40 cm) were used for seawater and freshwater tests to examine the thermal effects on seawater tolerance. In each tank, a filter (SUISAKU Jumbo S) with an air pump, a cooler (IUCHI model 200TN), and/or two heaters (NISSO 26605, 150W) with thermostats (OMRON model E5LD and/or NISSO MX-500 27100) were set to maintain the water temperature. Packed ice was also used to maintain water temperature at 1 ℃. Temperature was controlled within ±0.5℃ for the target temperature. The pH of the water varied between 6.8 and 7.4.

Seawater

Seawater was obtained from the coasts of southwestern Hokkaido. Salinity by a refractmeter (IUCHI model S-10) was maintained at 33-34 $g \cdot l^{-1}$.

Serum Na^+ Concentration

Experimental fish were taken with a dip net from the tanks and held with a paper towel. Blood samples were immediately collected from the caudal vessels using a plastic syringe (TERUMO, 1mℓ, 25G×1"), and allowed to clot at room temperature for 0.5-1 hour. Anadromous adults (2+) were killed by a blow to the head before the blood collection because of their large size. The fish were then measured in length, weighed, and classified into immature parr, smolt, and stream-resident maturing male by body colorations (Hoar, 1976; Folmar and Dickhoff, 1980) and/or gonad development (Blackett, 1968, cited in Takashima et al., 1995). Sera were separated by centrifugation (HITACHI SCT-15B) at 3000 rpm (750G) for 10 minutes and stored in a sample cup (Eppendolf 3810, 1.5mℓ) at−85℃ until analysis. Serum was diluted 1001 times (v/v) with ultra pure water then serum Na^+ concentration was measured by an atomic absorption spectrophotometer (HITACHI model Z-6000). No fish died during the seawater challenge tests.

Changes in Serum Na^+ Concentration

In late July 1996, reared fish were randomly selected from a 250-liter tank and directly transferred into fresh- and seawater in the 150-liter aquaria. Blood samples of each 6 fish were collected from each aquarium at intervals of 0, 6, 12, 24, 48, 72, and 96 hours after. A total of 84 blood samples were then collected from the fresh- and seawater. Maximum fish density was 12 $g \cdot l^{-1}$ in the experimental tanks. The used fish included parr, smolts, and stream-resident

males (fish size, mean ± *SD*, n=84:fork length FL:19.6 ± 1.5 cm, body weight BW:77 ± 19 g). The smolts had smoltified in their second spring.

Seawater Tolerance

In late July and early August 1996, fish were randomly selected from the rearing tank including parr, smolts, and stream-resident males, and were transferred directly into fresh- and seawater. Blood samples were collected 24 hours after the direct transfer. Each of the 37 samples was obtained from fresh- and seawater. Maximum fish density was 6 g • l^{-1} (fish size for the seawater test, mean ± *SD*,n=37, FL:19.2±1.7 cm, BW:70±19 g).

Seasonal Changes

Experiments were conducted four times early in August, October 1996, January, and March 1997. Each of the 6 fish was transferred directly into fresh- and seawater from the rearing tank, and blood samples were collected 24 hours after the direct transfer. A total of 48 blood samples were then obtained from fresh- and seawater. Maximum fish density was 8 g • l^{-1} (fish sizes, mean±*SD*,n=12:August 1996, FL:20.8±1.4, BW:94±20; March 1997,25.2±2.6 cm, 192±60 g). For the tests, smolts were selected as possible but samples included 7 stream-resident maturing males.

High Temperature Effects

In early and mid-August 1996, 8 groups of each 6 individuals were transferred into fresh- and seawater (8, 16, 20, and 22℃) in the 200-liter FRP tanks. Before the test, the fish of each temperature group were previously acclimated as follows; water temperature was raised from 8 ℃ to each target temperature level at the rates of 4℃ • d^{-1} up to 20℃ and 2℃ • d^{-1} for 20-22℃ and maintained at each target level for 24 hours. Blood samples of all 6 fish were collected from the fresh- and seawater at the 4 different temperature levels 24 hours after the transfer. At 20 ℃ in freshwater, only 5 samples were collected. Size (mean±*SD*) of the fish used was FL 20.8± 1.2 cm and BW 90±19 g (n=48), and maximum fish density was 3 g • l^{-1}. For the tests, smolts were selected as possible but samples included 12 stream-resident maturing males.

Low Temperature Effects

In late December 1996 and early January 1997, 6 groups of each 6 individuals were transferred into fresh- and seawater at 7, 4, and 1℃ in the 200-liter FRP tanks. Before the test, the fish of each temperature group were previously acclimated as follows; water temperature was decreased from 7℃ to each target temperature level at a rate of 3℃ • d^{-1} and maintained at each target level for 24 hours. Blood samples of each 6 fish were then collected from the fresh- and seawater at the three different temperatures 24 hours after the transfer. All the fish used had smoltified. Size (mean±*SD*) of the fish used was FL 22.3±1.4 cm and BW 116±24 g (n=36), and maximum fish density was 4 g • l^{-1}

In mid-January 1997, 4 groups of each 6 anadromous adults were transferred into the 200 -liter FRP tanks (seawater at 7, 4, and 1℃; freshwater at 7℃). These fish were previously acclimated by the same method as the above before the test. Blood samples of each 6 fish were collected from seawater at 7, 4, and 1℃, and freshwater at 7℃, respectively, 24 hours after the fransfer. Size (mean±*SD*) of the fish used was FL 37.1±2.5 cm, BW:564±126 g (n=24), and maximum density was 17 g • l^{-1}.

Results

Changes in Serum Na^+ Concentration

Serum Na^+ concentrations in seawater were

higher than those in freshwater (2-way factorial ANOVA, $df=1$, $F=91.5$, $p<0.0001$). The serum Na^+ levels in seawater were significantly different among the various exposure times (Kruskal-Wallis test, $df=6$, $H=22.73$, $p=0.0009$). The Na^+ levels increased until 48 hours after the transfer, and thereafter remained at 160-170 mmol $\cdot$ l^{-1} (Fig. 1). Seventy-two and 96 hours after the transfer, Na^+ of some fish (1 parr and 2 maturing males) exceeded 170 mmol $\cdot$ l^{-1}. Fork lengths of the fish were 16-23 cm and slightly differed among the exposure times (2-way factorial ANOVA, $df=6$, $F=2.54$, $p=0.03$).

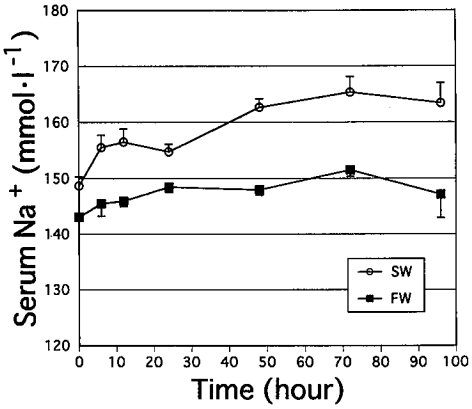

Fig. 1 Temporal changes in serum Na^+ concentrations in white-spotted charr (1+) exposed to seawater (8 ℃, 34 g $\cdot$ l^{-1}). Values are mean$\pm SE$, $n=6$.

Seawater Tolerance

Serum Na^+ concentrations 24 hours after the seawater transfer were significantly higher than those in freshwater (mean$\pm SE$, seawater: 150.1 $\pm$0.9, freshwater: 139.5$\pm$1.2; Mann-Whitney U-test, $U=196.5$, $p<0.0001$) but almost less than 160 mmol $\cdot$ l^{-1}. There was no significant difference in serum Na^+ concentrations in seawater among parr, smolts, unidentified females, immature, and maturing males (Kruskal-Wallis test, $df=4$, $H=3.1$, $p=0.54$; Table 1). Sizes of these 5 groups differed significantly, and immature parr were slightly smaller (Kruskal-Wallis test, $df=4$, $H=9.8$, $p=0.04$). There was no significant correlation between individual fish sizes of the 5 groups and serum Na^+ concentration levels in seawater (Spearman's rank correlation, $n=37$, $\rho=-0.02$, $p=0.90$; minimum length 14.2 cm; Fig. 2).

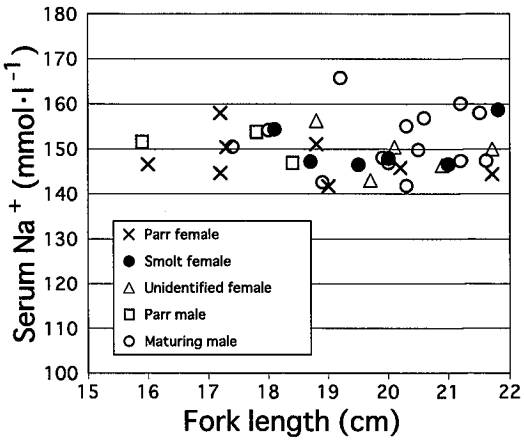

Fig. 2 Relationships between fish sizes and serum Na^+ concentrations in white-spotted charr (1+) exposed to seawater (8℃, 34 g $\cdot$ l^{-1}) for 24 hours.

Seasonal Changes

Serum Na^+ concentrations both in fresh- and seawater did not fluctuate seasonally and

Table 1 Serum Na^+ concentrations in 24-hour seawater challenge tests for white-spotted charr. Data are mean $\pm$ *SD* for the fork lengths and mean $\pm$ *SE* for the Na^+ concentrations

Sex	Female			Male		
Group	Parr	Smolt	Unidentified	Parr	Maturing	Total
n	8	6	5	3	15	37
Fork length (cm)	18.4$\pm$1.9	19.9$\pm$1.4	20.2$\pm$1.1	17.4$\pm$1.3	20.0$\pm$1.2	19.5$\pm$1.6
Serum Na^+(mmol $\cdot$ l^{-1})	147.8$\pm$1.8	150.2$\pm$2.1	149.2$\pm$2.2	150.8$\pm$2.0	151.5$\pm$1.7	150.1$\pm$0.9

were less than 170 mmol • l^{-1} level in the seawater (2-way factorial ANOVA, between months: $df=3$, $F=0.49$, $p=0.69$; Fig. 3). Throughout the experiment period, there was no difference in serum Na^+ concentrations between the smolts ($n=21$) and the mature males ($n=3$) (Mann-Whitney U-test, $U=16.0$, $p=0.18$).

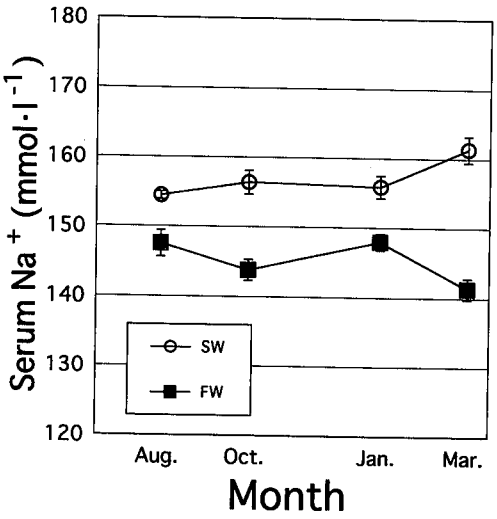

Fig. 3 Seasonal changes in serum Na^+ concentrations in white-spotted charr (1+) exposed to seawater (8℃, 34 g • l^{-1}) for 24 hours. Values are mean ± *SE*, $n=6$.

High Temperature Effects

Averages of serum Na^+ concentrations in seawater exceeded 160 mmol • l^{-1} at 16℃ and showed higher levels at 20 (>170) and 22℃ (>180 mmol • l^{-1}) (Fig. 4). Serum Na^+ concentrations in seawater markedly differed among the different temperature levels (Kruskal-Wallis test, $df=3$, $H=17.65$, $p=0.0005$). Fork lengths of the fish tested did not differ among the temperatures or between the fresh- and seawater (2-way factorial ANOVA, $p>0.41$).

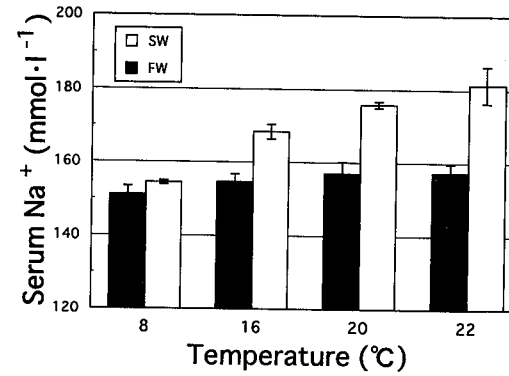

Fig. 4 Serum Na^+ concentrations in white-spotted charr smolts (1+) exposed to seawater (34 g • l^{-1}) at 8,16,20, and 22℃ for 24 hours in summer. Values are mean ± *SE*, $n=6$ except 20℃ fresh water ($n=5$).

Low Temperature Effects

Serum Na^+ concentrations in seawater significantly differed among the temperature levels of 7,4, and 1℃ (Kruskal-Wallis test, $df=2$, $H=7,81$, $p=0.02$). However, the Na^+ levels were less than 160 mmol • l^{-1} at any temperature, and it cannot be observed that Na^+ levels increase at lower temperatire (Fig. 5). Fork lengths of the fish tested did not differ among the temperatures or between the fresh- and seawater (2-way factorial ANOVA, $p>0.17$).

Values in adults (2+) were also low (<160 mmol • l^{-1}) and did not differ significantly

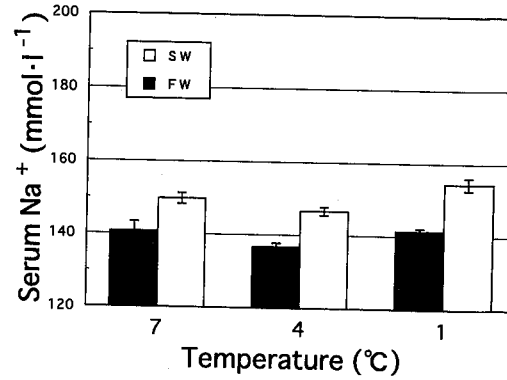

Fig. 5 Serum Na^+ concentrations in white-spotted charr smolts (1+) exposed to seawater (34 g • l^{-1}) at 7, 4, and 1℃ for 24 hours in winter. Values are mean ± *SE*, $n=6$.

among the seawater temperature levels of 7, 4, and 1℃ (Kruskal-Wallis test, $H=4.44$, $p=0.11$; Fig. 6). There was no difference in fork length of the adults among the test groups (1-way ANOVA, $df=3$, $F=0.09$, $p=0.97$).

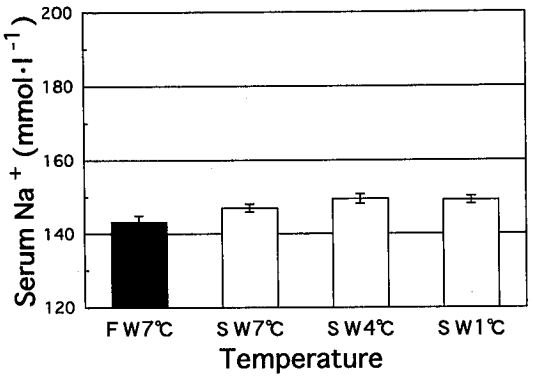

Fig. 6 Serum Na^+ concentrations in anadromous white-spotted charr adults (2+) exposed to freshwater (7℃) and seawater (34 g • l^{-1}, 7, 4 and 1℃) for 24 hours in winter. Values are mean ± *SE*, $n=6$.

Discussion

In northern Hokkaido, white-spotted charr mainly transform to smolt at 2-4 years of age and about 15 cm in fork length (Yamamoto and Nakano, 1996). In the present study, some individuals larger than 15 cm smoltified at 1+ year. In Arctic charr, hypo-omoregulatory capacity of one or two-year-old smolts, which were produced in a hatchery, was slightly lower than that of relatively older wild smolts (Finstad and Heggberget, 1995). In white-spotted charr, however, seawater tolerance seems to be displayed not only by smolts but also parr and stream-resident maturing males larger than 15 cm at 1+ year throughout a year. This is because the serum Na^+ concentrations in the 24-hour seawater challenge tests were less than 170 mmol • l^{-1} (Blackburn and Clarke, 1987). Although fluctuations in Na^+ levels after > 24 hours in seawater have not yet been examined for smolts and parr separately, their mean Na^+ levels were adequately low (< 170 mmol • l^{-1}) for 96 hours (Fig. 1) as well as those of Arctic charr having sufficient seawater adaptability (Finstad et al, 1989). Gorie (1996) revealed that fluvial 0+ *S. leucomaenis* (about 15 cm and 35 g in size) possess seawater tolerance in winter.

It was reported that high water temperature influences seawater tolerance of salmonids (Gordon, 1959; Johnsson and Clarke, 1988). Johnsson and Clarke (1988) discussed that the high temperature effect may be caused by interactive effects of high metabolism and of temperature dependent activity of gill Na / K-ATPase. In the case of stream-resident white-spotted charr, upper limit of preferred temperature range is 24 ℃ in freshwater (Takami et al., 1997). However, white-spotted charr were thought to have decreased seawater tolerance at high temperatures. At 20 (> 170) and 22℃ (> 180 mmol • l^{-1} in serum Na^+), the tested charr obviously reduced the adaptability to seawater. Migrational return of Arctic charr is thought to be affected by the seasonal changes in hypo-osmoreguratory capacity (Finstad et al., 1989). A reduction of this capacity in charr might result in the return to freshwater. In southwestern Hokkaido, anadromous white-spotted charr migrate to the sea in April and return in June while some charr emigrate to the sea in autumn and overwinter there (Takami, 1995). The other populations migrate upstream in later seasons of summer and autumn in northern and eastern Hokkaido (Yamamoto and Nakano, 1996; Shibata, 1938). In summer, surface sea temperature is > 20℃ in southwestern Hokkaido but < 20℃ in northern or eastern regions (Fig. 7). Therefore, the return to freshwater of white-spotted charr might be triggered by thermal reductions of hypo-osmoreguratory capacity.

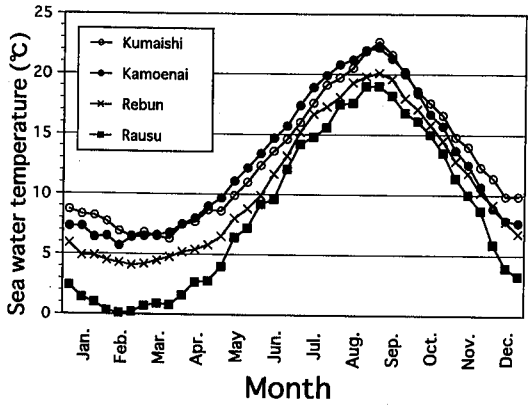

Fig. 7 Ten day changes in surface sea temperature off coasts of Kumaishi and Kamoenai (where the Ken-ichi and Furuu Rivers are located, respectively), southwestern Hokkaido, Rebun (N45°38', E 141°24'), northern Hokkaido, and Rausu (N44°03', E145°12'), eastern Hokkaido, averaged from 1992 to 1994. Data are obtained from the *Data Book of Temperature in Aquaculture Grounds*, No.25 (1996) (Hokkaido Aquaculture Development Authority, Sapporo).

In contrast, it is thought that low water temperature did not restrain the osmoregulation in white-spotted charr. In northern or eastern regions, white-spotted charr generally overwinter in freshwaters (Shibata, 1938; Gritsenko and Churikov, 1976; Yamamoto and Nakano, 1996), and sea-overwintering charr occur only in southwestern Hokkaido (Takami, 1995). The Ken-ichi and the Furuu River populations which were used in the present experiments also overwinter at sea (Takami, 1995; Aoyama, 1997). Winter sea temperature in southwestern Hokkaido (>6℃) is higher than those in northern and eastern regions (<4℃) (Fig. 7). Sea-overwintering charr are known to grow rapidly with active feeding (Takami et al., 1996). Although it was reported that low temperature affects seawater tolerance in rainbow trout (*Oncorhynchus mykiss*) (Finstad et al., 1988) and Atlantic salmon (*Salmo salar*) (Sigholt and Finstad, 1990), white-spotted charr seem to have high seawater tolerance at 4 and 1℃. As well as anadromous Arctic charr (Grainger, 1953) and Dolly Varden (*Salvelinus malma*) (Armstrong, 1974, 1984), white-spotted charr do not over winter in the sea in relatively colder regions. This may be because their seawater tolerance differs from that of the southwestern ones, or because prey environments and/or predators influence their growth and/or mortality there.

In the present study, seawater tolerance of white-spotted charr are mainly evaluated by the serum Na^+ concentration level of the 24-hour seawater challenge tests. To elucidate physiology in relation to migration behavior of this species, study on more factors, osmolality, muscle water content, chlorine, magnesium, and gill Na/K-ATPase in several exposure times, should be needed.

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アメマスの塩分耐性とそれにおよぼす水温の影響

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海水チャレンジテストによってアメマスの塩分耐性を調べた。アメマス満1歳魚（15cm以上）の海水投入24時間後の血中ナトリウムイオン濃度は，スモルト，パー，河川残留型成熟雄いずれも低く，また季節変化もみられなかった。したがってアメマスは満1歳で成熟や季節に関係なく，充分な塩分耐性を持つことが示唆された。水温と海水投入24時間後の血中ナトリウムイオン濃度との関係から，アメマスの浸透圧調節能は水温1℃，4℃といった低水温では影響を受けないが，16℃以上の高水温で影響が現れ，20℃以上では浸透圧調節能がかなり低下することが明らかとなった。これらの結果から降海したアメマスが初夏に河川に遡上するのは，海水適応能の季節変化に起因するのではなく，海水温の上昇により浸透圧調節能が低下するためと考えられた。