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ABSTRACT

On seven dates during the summer and fall of 1987, stream-type chinook salmon juveniles (8-10 g average weight) were placed in marine net-pens with access to a low-salinity lens, to be tested for seawater tolerance. Overwinter survival averaged 72 % (range 27-87%). Plasma concentrations of sodium for fish challenged to 30% salinity (full-strength) seawater for 24 hours were above critical (170 mmol/l) on all but the late June challenge date. Plasma concentrations of sodium for fish challenged to 30% salinity with access to a low-salinity lens were below critical for all dates tested. Survival to mid-May 1988 and overwinter mortality rate were not correlated with date of entry into the net-pens or with plasma sodium concentrations 24 hours after entry. The juvenile chinook salmon demonstrated the ability to acclimate, survive, and grow in an environment of predominantly full-strength seawater throughout most of the year when access to a low-salinity environment was provided. Culture of juvenile chinook salmon in marine net-pens with a low-salinity lens might be a useful rearing strategy for salmon hatcheries with access to suitable marine sites.

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INTRODUCTION

Many hatcheries in Southeast Alaska supplement traditional freshwater smolt production by using marine net-pens to produce juveniles for release (Heard and Crone 1976, Martin and Wertheimer 1987, Martin et al. in prep.). Survival during culture in marine net-pens, however, has varied greatly and may be related to the seawater adaptability (osmocompetence) of the juveniles when introduced into the saline environment of a marine net-pen (McGee 1985). For the enhancement program in Southeast Alaska to maximize the production of chinook salmon, the relationship between time of seawater entry and survival to release of juvenile chinook salmon (Oncorhynchus *tshawytscha)* cultured in marine net-pens must be understood.

The successful osmotic transition of juvenile salmon from fresh water to seawater is related to time of seawater entry, size at entry, salinity during transition, photoperiod exposure, and stock. Normally wild, yearling (stream-type) chinook salmon enter seawater between early April and late June throughout most of their North American range (Healey 1991). Seawater adaptability of juvenile salmon reared in hatcheries has been related to changes in gill sodium-potassium ATPase activity and blood concentrations of thyroid hormones, and follows a seasonal pattern that normally peaks in spring (Zaugg and McLain 1972, Dickhoff et al. 1978, Lasserre et al. 1978, Ewing et al. 1979). On entering seawater, wild yearling chinook salmon generally are 68-134 mm in fork length (Healey 1991), or about 5-25 g. Larger juveniles are usually more osmocompetent than smaller individuals in full-strength seawater (> 30% salinity) (Mahnken et al. 1982). Wild juvenile chinook salmon generally migrate from fresh water into a complex environment of mixed salinities normally found in river mouths or nearshore marine areas. They can adapt more readily to high-salinity environments when allowed a short period to acclimate in intermediate-salinity environments (Wagner et al. 1969, Kepshire and McNeil 1972, Gould et al. 1985).

Most research on seawater adaptability has focused on the measurement of various physiological parameters (e.g., gill sodium-potassium ATPase activity and blood thyroxine concentration) of fish still in fresh water and the subsequent indexing of those parameters to performance after static challenges to salinities greater than 28 % . Some researchers then measure concentrations of sodium in the blood plasma and survival after 24 hours to determine osmocompetence (for reviews, see: Folmar and Dickhoff 1980, Wedemeyer et al. 1980). Rarely, longer-term growth is used as a measurement of osmocompetence (Clarke and Shelbourn 1982, Mahnken et al. 1982, Gould et al. 1985).

Normal testing environments are usually homogeneous, with a specific salinity, and thus are not hydrographically representative of the complex, layered-salinity environment of most estuaries (Powers 1963, Officer 1976). Upon ocean entry, most wild and enhanced juvenile chinook salmon encounter a situation that allows them to move freely between low- and high-salinity environments; this is significantly different from the environments they have traditionally been tested in. Nevertheless, researchers have given little attention to the seasonal changes in the adaptability of juveniles to an environment that allows access to low salinities and to the effects of size on this ability.

Some stocks of chinook salmon migrate seaward outside the normal migration period (Healey 1991). Juveniles might be able to adapt to a primarily marine environment throughout the year if they have access to a low-salinity zone. The correlations observed between timing of seaward migration, gill sodium-potassium ATPase, and blood thyroxine concentration may be related more to the enhanced probability of survival due to some other factor (e.g., increased seasonal food availability or predator avoidance) than to the ability to adapt to seawater under natural conditions. Variation in survival during culture of juvenile chinook salmon in marine net-pens may be related to variations in time of seawater entry, size at seawater entry, the salinity of pen environments, or other factors.

In this experiment we challenged unacclimated, underyearling stream-type juvenile chinook salmon to a full-strength seawater environment that allowed access to a low-salinity lens. We evaluated osmocompetence by measuring plasma sodium concentration and survival after 24 hours of exposure to full-strength seawater, in juveniles with and without access to a low-salinity lens. We evaluated general seawater adaptability of juveniles with access to a low-salinity lens by measuring survival and growth in marine net-pens until the time of normal seawater entry in mid-May.

METHODS

The site of the experiment was the Alaska Fisheries Science Center's Little Port Walter Biological Research Station on Baranof Island in Southeast Alaska (Fig. 1). Coded-wire-tagged adult chinook salmon, from parents originating from a single transplant of eggs from the Chickamin River in southern southeastern Alaska in 1976 (Hard et al. 1985), were spawned at the Sashin Creek weir at Little Port Walter in August 1986. Various lots of eggs were held in ambient, heated, or refrigerated water to produce juveniles weighing 8-10 g at seven net-pen entry periods in 1987 (13 June; 11 July; 27 Aug.; 15, 16 Sept.; 3, 4 Oct.; 17, 18 Oct.; 6, 7 Nov.). Photoperiod history was different for each of the three summer trial dates: fish for the June entry date were started on food in late November; July fish were started in mid-December; August fish were started in early April. Fish used for the four fall entry dates were started in mid-May. Size for the fall entry date fish was controlled by manipulation of feeding rates early in summer to produce four populations that were fed at excess levels the final month of freshwater rearing.

During each entry period, juveniles were placed into a 3.7 x 3.7 x 3.7 m polyester net-pen of 9.5-mm mesh with an effective rearing volume of 46.5 m^3. Each entry period had a separate pen or pens, and all pens were surrounded by a Hypalon (rubberized, waterproof material) barrier that extended 1.5 m downward from the water surface (Fig. 2). The barrier was supported by an aluminum and wood framework extending downward from the flotation collar. About 20 l/min of fresh water was introduced into the surface of each pen, creating a l-m-deep lens of low salinity (artificial freshwater lens system [AFLS]). To keep predators out, a l-m-high fence of 25-mm-mesh nylon webbing was placed around the pens and bird netting was placed over the pens. In addition, a standard 12-volt electric fence was placed around the pens to discourage mink *(Mustella* vison) and river otter *(Lutra* canadensis). Salinity and temperature were measured weekly with a Beckman salinometer.

A 510 1 fiberglass tank containing seawater was used for the 24-hour exposure trials; 20 1/min of seawater was pumped from below 2-m depth of the bay, where salinities ranged from 29.5 to 30.5% throughout the trials. Fish density in the tank averaged 1.1 g/l.

During each net-pen entry period, the juveniles were anesthetized with MS-222 and marked by implanting a coded-wire tag in the snout and removing the adipose fin. The juveniles were then moved directly into net-pens in the AFLS, and a subsample of 45-160 fish was placed into the full-strength seawater container. For each of the three summer entry periods, 560 fish were placed into net-pens in the AFLS; fish density averaged 0.1 g/l. For each of the four fall entry periods, 7,560 fish were placed into net-pens in the AFLS; fish density averaged 1.7 g/l. To replicate overwinter survival, an additional lot of 7,500 fish was placed in net-pens in a separate AFLS on each of the four fall entry dates. Smaller lots of fish were available in summer because of the limited amount of heated water available for accelerated rearing. Juveniles were bulk-weighed in lots of 300-400 to determine average weight at entry. All fish were fed BioDiet fish food at the manufacturer's specifications throughout the experiment. In mid-May 1988, the survivors in each pen were counted by hand and 100 fish from each net-pen were individually weighed and measured.

Sixty fish from the AFLS and all live fish (up to 60) from the full-salinity container were removed 24 h after entry for each period (for some entry periods, fewer than 60 fish were available for analysis from the full-salinity groups because of mortality). They were killed by a quick blow to the head, and the caudal peduncle. was severed. Blood for plasma sodium analysis was collected from the caudal artery with a micro-hematocrit tube treated with ammonium-heparin. We collected 0.1 ml of whole blood from each fish and pooled the blood from three fish. The sample was then centrifuged for 3 minutes, and the plasma was removed, sealed, and stored at -20°C for later analysis. Plasma samples were processed on an Orion Model 1020 blood sodium analyzer. Machine readings were multiplied by 0.941 to make readings comparable with flame photometric values using a conversion chart (Orion Research Inc. 1983) and with previously reported serum protein and lipid values for chinook salmon juveniles (Woo et al. 1978).

On the June and July entry dates, plasma sodium concentration was also measured in 60 fish removed directly from the freshwater raceway and anesthetized, tagged, and returned to the freshwater raceways for 24 hours, as freshwater controls.

Overwinter survival for each entry period was determined by dividing the number of live fish in the spring by the number placed in the net(s). Overwinter mortality rate (r) was calculated to compensate for the differing number of days of culture by r = 1n (overwinter survival)/days of culture.

Linear regression analysis was used to compare 24-hour plasma sodium concentrations with total overwinter survival and with daily overwinter mortality rate in juveniles challenged to full-strength seawater and in those exposed to the AFLS (four regressions). Linear regression analysis was also used to compare total overwinter survival and daily overwinter mortality rate with date of AFLS entry (two regressions). Two-way analysis of variance was used to examine the interaction of date of entry with plasma sodium levels of juveniles exposed to seawater or in the AFLS.

Overwinter growth rates were calculated by r = ln(w,lw,)lt, where r is equal to percent body weight gain per day, w_1 is equal to the average weight of a fish in the population at the end of culture,. w,, is equal to the average weight of a fish in the same population at seawater entry, and t equals the number of days of culture.

Initial data analysis revealed patterns within the 24-hour plasma sodium data that were further examined by linear regression of plasma sodium concentration by time of entry for both environments and by analysis of covariance of those data.

Data caveats were 1) If a substantial number of smaller fish of a given population died, then the actual overwinter growth rate could be overestimated because smaller juveniles are generally less osmocompetent than larger ones. Comparisons of differences in growth rates between entry periods are limited because water temperature changes seasonally and affects growth rate. 2) Mortality influenced 24-hour plasma sodium concentrations of fish in full-strength seawater. Substantial mortality occurred on two entry dates. Survivors probably had lower plasma sodium concentration than those that died, resulting in artificially low concentrations for those entry dates; these results should be viewed as minimums. 3) Although we attempted to maintain similar sizes of fish for all entry periods, some variability occurred. Entry sizes were smallest in mid-September (8.0 g) and in mid-October (8.6 g), which could have resulted in higher plasma sodium concentrations than in fish 1.5-2.0 g larger. 4) Two weeks after seawater entry, an outbreak of *Vibrio anguillarum* was diagnosed in the July entry group, resulting in substantial mortality.

RESULIS

Salinity in the AFLS remained relatively constant, averaging 30% between 1.5 and 4.0 m depths. The upper 1 m of the AFLS varied most: the average surface salinity was less than 5 % and salinity at 1 m averaged 8 % (Fig. 3). Freshwater temperatures varied normally: a high of 15°C in late July and a low of 1.5ºC in February (Fig. 4). Seawater temperature varied substantially less: a maximum of 11.5ºC in July and a minimum of 4.5 ºC in January.

For all entry periods, 24-hour plasma sodium concentrations were higher in juveniles in full-strength seawater than in those in the AFLS (Fig. 5). Plasma sodium concentrations were below 170 mmol/l for all entry periods for the juveniles in the AFLS and above 170 mmol/l for all entry periods except mid-June for those in full-strength seawater. Plasma sodium concentrations were lowest in mid-June and highest in mid-October for both environments.

Overwinter survival in the AFLS averaged 72% (range, 27-87%) (Table 1). Survival was lowest for juveniles placed in net-pens in July and highest for those placed in August. Compensation for length of culture in the AFLS confirmed the survival pattern: mid-July had the highest mortality rate and late August had the lowest.

In the AFLS, 24-hour survival after entry was 100% for each entry period. In full-strength seawater, 24-hour survival was lowest (8%) for the mid-September entry and highest (100%) for the mid-June, late August, and early October entries.

Overwinter growth in the AFLS was good: fish in all groups at least doubled their initial weight. Growth rates ranged from an average of 0.98% body weight gain per day for the summer entry groups to an average of 0.38% for the fall entry groups.

Use of the AFLS by the juveniles varied seasonally and diurnally. During the day in summer and fall, the fish schooled and swam in a circle in the pens at the interface of the fresh and saltwater layers, whereas in winter and spring they remained in the seawater near the bottom of the pens. During darkness, the fish tended not to school and distributed throughout both fresh and seawater layers. In all seasons, the fish moved into the freshwater layer for feeding.

Plasma sodium concentration after 24 hours in juveniles in each environment was not related to overwinter survival or overwinter mortality rate. Mean plasma sodium concentration was not significantly related to overwinter survival (seawater $r^2 = 0.135$, $P = 0.267$; AFLS $r^2 = 0.096$, $P = 0.355$) or overwinter mortality rate (seawater $r^2 = 0.016$, $P = 0.709$; AFLS $r^2 = 0.002$, $P = 0.897$) within either treatment.

Survival, as determined by total overwinter survival or daily overwinter mortality rate, was not related to date of seawater entry. Date of net-pen entry was not significantly related to total overwinter survival ($r^2 = 0.251$, $P = 0.117$) or overwinter mortality rate ($r^2 = 0.119$, $P = 0.299$).

Two-way analysis of variance on 24-hour plasma sodium concentrations in juveniles in either seawater only or the AFLS over the three summer and four fall entry dates indicated significant differences ($F_{1,222} = 641.55$, $P < 0.001$) between treatments. Juveniles in the AFLS had substantially lower plasma sodium concentrations (Table 1, Fig. 5). The two-way analysis also revealed a significant effect of time across treatment ($F_{6, 222} = 56.69$, $P < 0.001$): for both treatments, plasma sodium concentrations were lower in June and higher in the fall.

Further analysis of the effect of entry date on 24-hour plasma sodium concentration by linear regression indicated significant positive relationships between date of entry and plasma sodium concentration (AFLS $r^2 = 0.498$, $P < 0.001$; seawater $r^2 = 0.441$, $P < 0.000l$), which indicates a trend of generally increasing plasma sodium concentration over time. The analysis of covariance indicated significantly different slopes $F_{6,222} = 18.58$, $P < 0.001$) and intercepts ($F_{6,222} = 35.90$, $P < 0.000l$), with a proportionately greater change over time and a substantially higher intercept (153 mmol/l vs. 135 mmol/l) for the fish exposed to full-strength seawater.

DISCUSSION

Our results indicate that juvenile stream-type chinook salmon weighing 8-10 g can adapt to a high-salinity marine environment throughout much of the year when low salinity water is available. This finding appears to contrast with the seasonality of osmo-competence indicated by previous measurements of gill sodium-potassium ATPase activity of juvenile chinook salmon (Ewing et al. 1979) and blood thyroxine concentrations (Dickhoff et al. 1982) and by previous indirect measurement of timing of osmocompetence and normal smolt migration (Wagner et al. 1969).

Plasma sodium concentrations and survival after 24 hours of exposure to full-strength seawater indicated generally deteriorating osmocompetence from late June through early November. This result supports the evidence of Dickhoff et al. (1982) and Gould et al. (1985) of periodicity in osmocompetence upon direct exposure to full-strength seawater. Wagner et al. (1969) showed an opposite trend, with increased 30-day survival from spring through late winter of juvenile chinook salmon exposed to full-strength seawater; however, those juveniles grew so rapidly throughout the experiment that the effects of size confounded the influence of time on osmocompetence. Photoperiod history is known to have a substantial effect on osmoregulatory ability in stream-type chinook salmon (Clark et al. 1989, 1992). The good seawater tolerance shown by the June and, to a lesser degree, the July entry fish probably relates to their short day-exposure history.

Fish in the AFLS had some seasonal increase in sodium concentration; however, sodium concentrations were generally well within the osmocompetent range and the lack of 24-hour mortality in all entry periods confirms their osmocompetence.

Plasma sodium concentration and short-term mortality can be important in determining osmocompetence and swimming performance (Brauner et al. 1992) at that time. However, they may not be useful predictors of longer-term growth and survival of fish released into the natural environment or of captive fish in an AFLS. For fish in an AFLS until May, we found no significant relationship between 24-hour plasma sodium concentrations, total survival, or overwinter mortality rate up to release several months later. All populations at least doubled their average weight over winter, indicating no significant impairment of growth. In contrast, Clarke and Shelbourn (1982) found an inverse relationship between growth and 24-hour plasma sodium concentrations in juvenile coho salmon *(Oncorhynchus kisutch)* reared without a freshwater lens.

Elevated gill ATPase activity and increased thyroxine concentrations in juvenile salmon in fresh water are related to seaward migration and, ultimately, seawater adaptation. However, they may not indicate whether juvenile salmon, particularly chinook, can adapt osmotically to a marine existence during much of their normal freshwater development, when the salinity regime of their environment is more representative of the natural, layered-salinity environment common in estuaries. Access to a low-salinity environment appears to allow juveniles that are not generally osmocompetent to survive and grow in a high-salinity environment by periodically using the lens for osmotic relief.

Our results indicate that 8-10 g stream-type chinook salmon can be cultured successfully overwinter in marine net-pens with an artificial freshwater lens. The timing of AFLS entry is highly flexible, but problems associated with summer entry (e.g. disease) indicate the fall entry period is most suitable. High rates of overwinter survival should be possible at almost any marine site with access to small amounts of fresh water. Although the juveniles produced with the AFLS appeared normal at release, the return of adults to the hatchery and their presence in harvests will be the final proof.

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Table 1 .--Culture information for juvenile chinook salmon in 30% salinity seawater or seawater with an artificial freshwater lens (AFLS). FW = fresh water; SD = standard deviation.

Entry period	Treatment	Mean weight (g) Initial	Mean weight (g) Final	Plasma sodium concentration (mmol/l) Mean	Plasma sodium concentration (mmol/l) SD	Plasma sodium concentration (mmol/l) Number of samples	Overwinter survival (%)	Overwinter mortality rate (%)	24-h survival (%)	Mean daily growth rate (%)
Mid-June	FW control	9.6	—	137.8	2.36	19	—	—	100.0	—
	30‰	9.6	—	145.3	4.09	15	—	—	100.0	—
	AFLS	9.6	251.0	139.6	2.43	19	67.8	0.12	100.0	1.00
Mid-July	FW control	10.4	—	144.4	4.14	19	—	—	100.0	—
	30‰	10.4	—	172.9	10.05	19	—	—	89.4	—
	AFLS	10.4	285.0	140.7	4.34	19	26.8	0.44	100.0	1.13
Late August	30‰	10.6	—	183.1	22.41	20	—	—	100.0	—
	AFLS	10.6	85.9	144.9	2.04	20	87.2	0.05	100.0	0.84
Mid-September	30‰	8.0	—	206.7	9.61	3	—	—	8.3	—
A	AFLS	8.0	24.9	142.1	2.59	20	78.3	0.10	100.0	0.46
B	AFLS	8.0	22.3	—	—	—	76.0	0.12	100.0	0.41
Early October	30‰	9.2	—	192.1	12.24	19	—	—	100.0	—
A	AFLS	9.2	17.1	149.9	5.21	18	68.9	0.17	100.0	0.36
B	AFLS	9.2	20.0	—	—	—	69.4	0.17	100.0	0.39
Mid-October	30‰	8.6	—	213.6	19.04	10	—	—	51.7	—
A	AFLS	8.6	19.7	161.7	6.34	19	76.2	0.13	100.0	0.36
B	AFLS	8.6	18.2	—	—	—	71.7	0.16	100.0	0.34
Early November	30‰	9.5	—	193.6	16.55	16	—	—	91.9	—
A	AFLS	9.5	18.3	152.0	6.37	19	80.8	0.11	100.0	0.35
B	AFLS	9.5	18.3	—	—	—	86.4	0.08	100.0	0.35

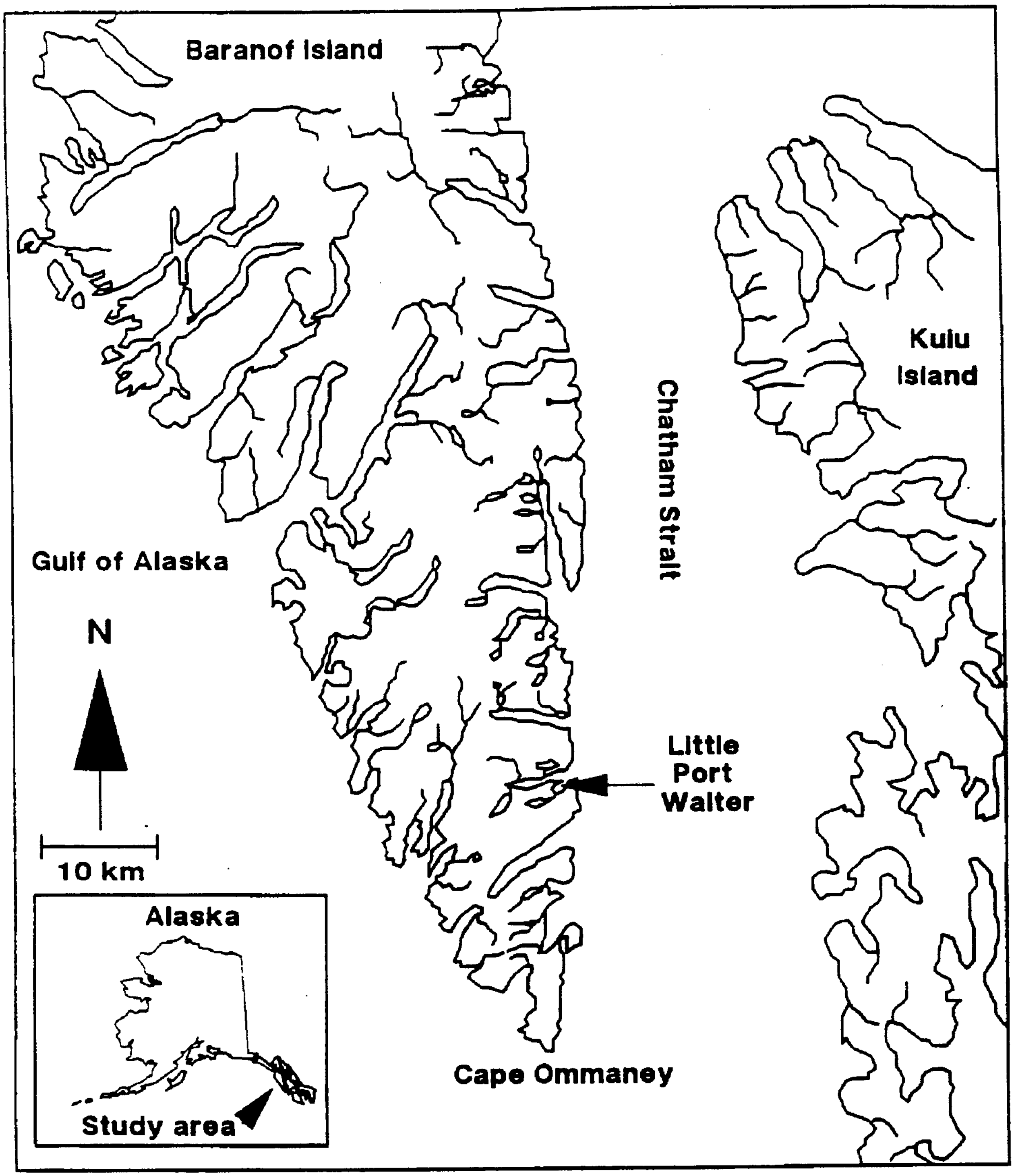

Figure l.--Location of National Marine Fisheries Service Little Port Walter Biological Research Station, Baranof Island, Alaska.

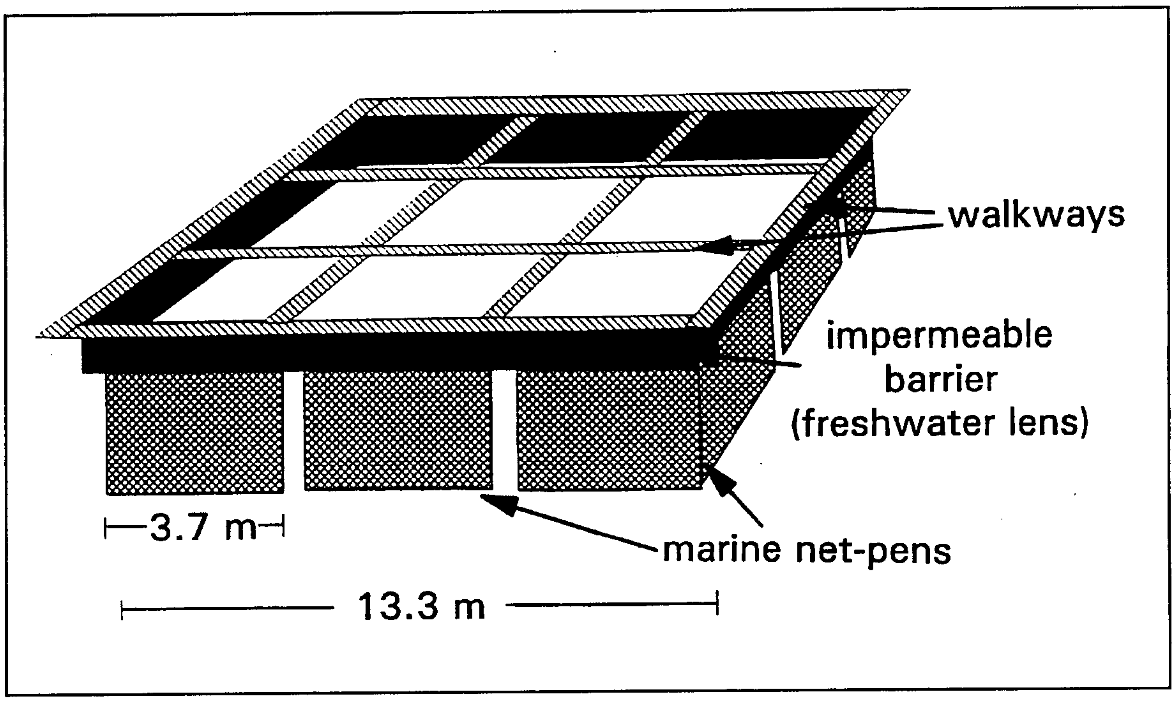

Figure 2.--Schematic diagram of an artificial freshwater lens system with space for nine 3.7 m X 3.7 m net-pens.

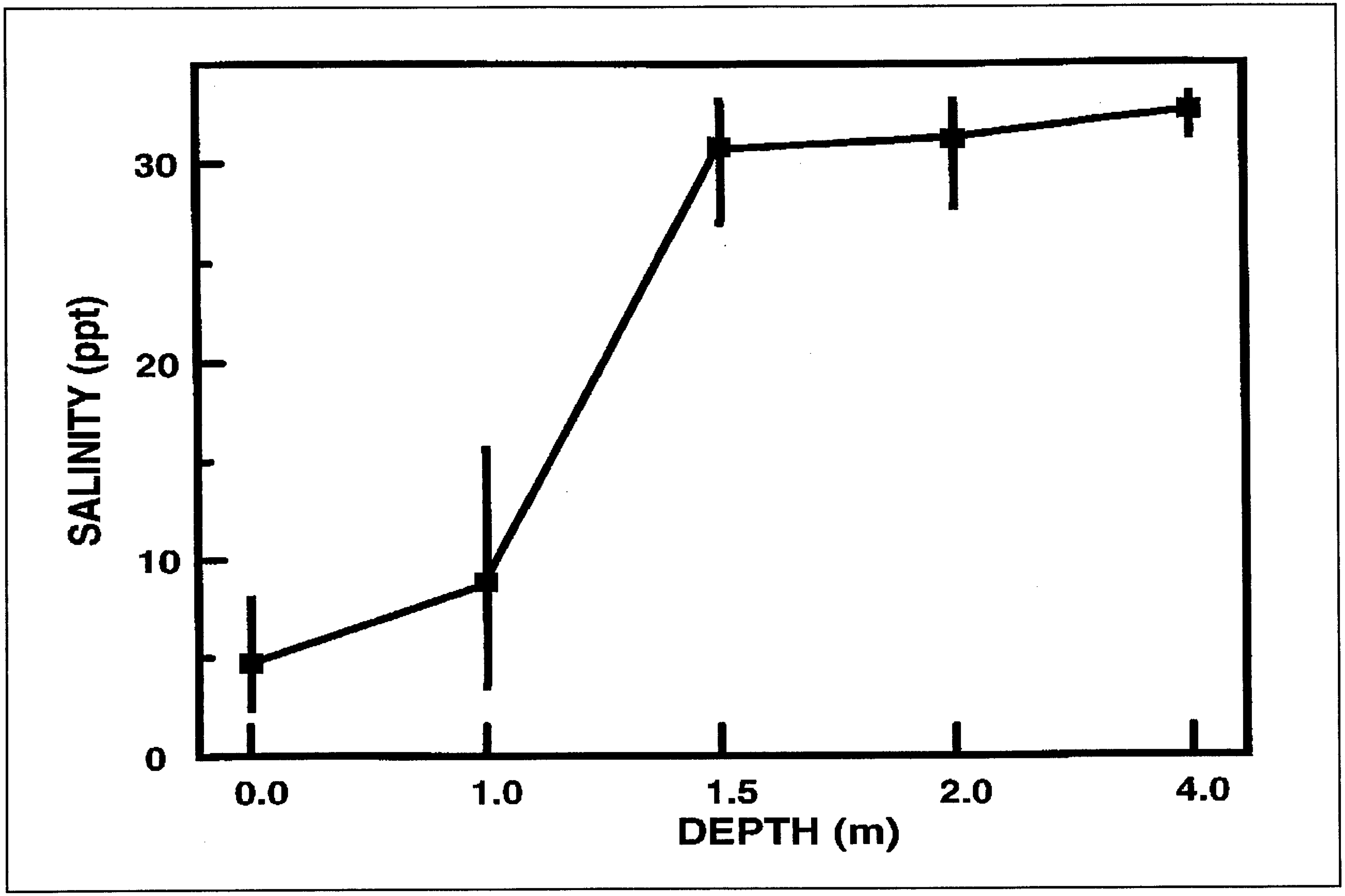

Figure 3.--Mean salinity (±SD) by depth from October 1987 to May 1988 for marine net-pens with an artificial freshwater lens.

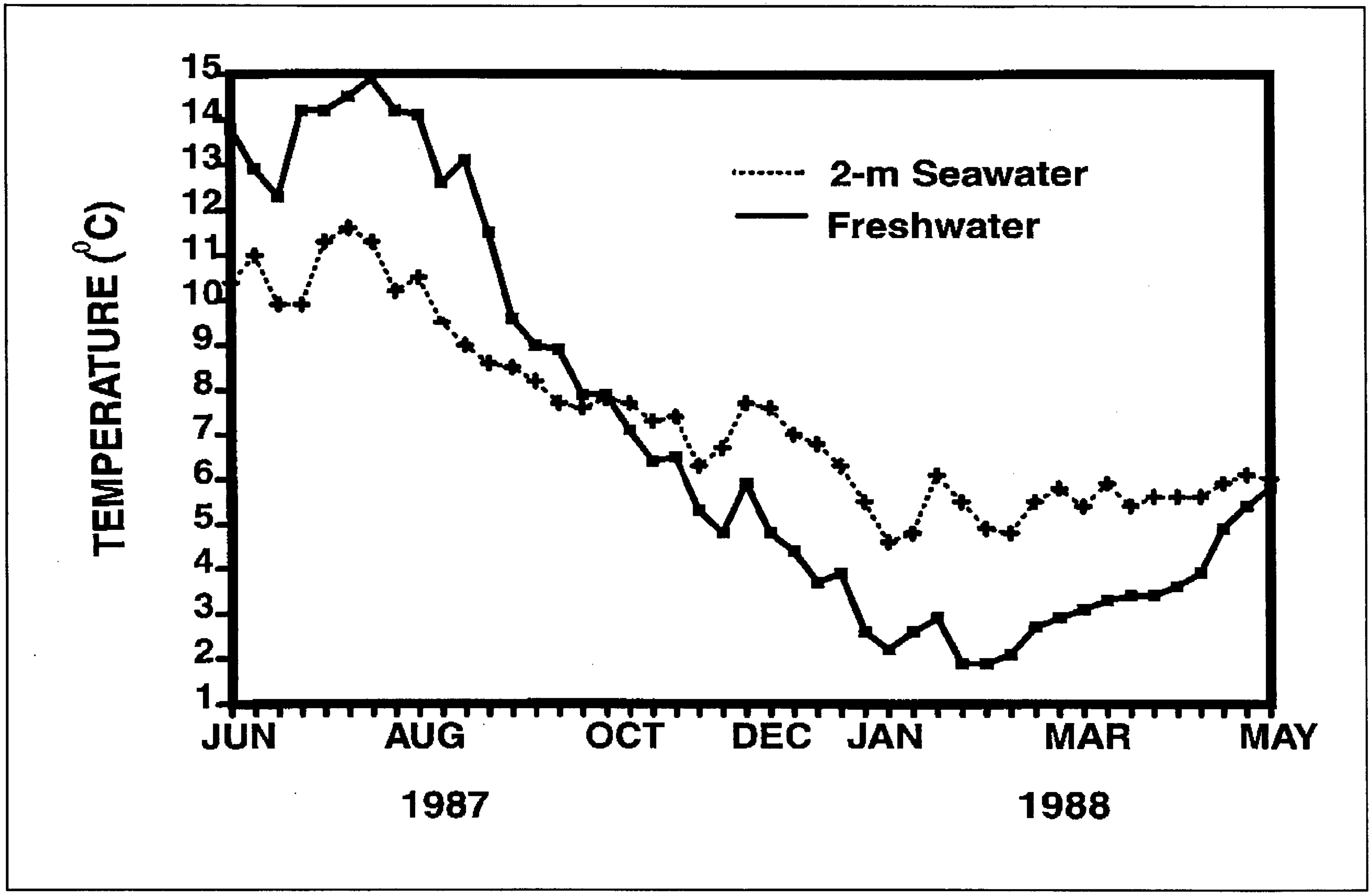

Figure 4.--Weekly mean freshwater and 2-m depth seawater temperatures from late June 1987 through early May 1988 at Little Port Walter, southeastern Alaska.

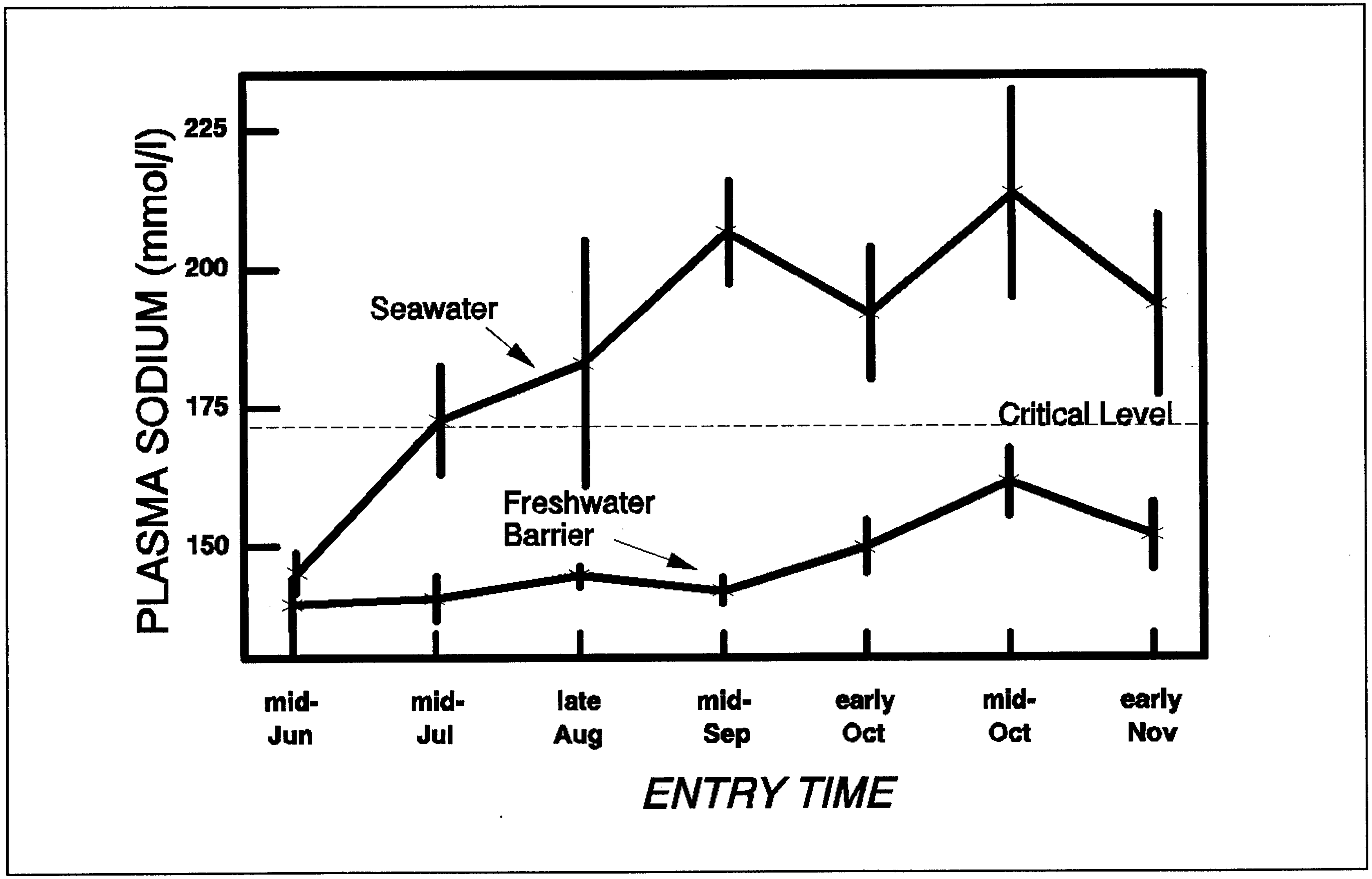

Figure 5.--Mean 24-h (+SD) plasma sodium concentrations for juvenile chinook salmon exposed to full-strength seawater or seawater with a freshwater lens. Dashed line represents maximum levels for osmocompetent juveniles.

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