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DETERMINATION OF SMOLT STATUS IN JUVENILE ANADROMOUS RAINBOW TROUT AND CHINOOK SALMON[1]

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Abstract - Gill sodium, potassium-activated adenosine triphosphatase (Na^+,K^+-ATPase) activity was used as a quantitative measure of the progress of smoltification in non-native populations of salmonines that are stocked into Minnesota waters of Lake Superior. Because olfactory imprinting occurs concurrently with smoltification, it is important for fisheries managers to know when smoltification occurs. Stocking before smoltification is critical to fisheries that depend on homing to particular locations. Readiness to smolt cannot be reliably assumed from superficial criteria such as coloration, length, weight, condition factor, water temperature, date, or emigration behavior in general. ATPase activity assay is a useful indicator of smoltification, but the assay is complex, uses perishable reagents, and requires specialized equipment, making it impractical for intermittent use on a small scale. Therefore, the relationships between ATPase activity and the superficial criteria were evaluated for use with local populations. ATPase activity levels were tested in chinook salmon, and in steelhead and Kamloops strains of anadromous rainbow trout during hatchery residency up until the time of stocking. These species/strains were then tested after stocking and recapture to determine whether stocking stimulated smoltification. Steelhead that had been stocked as fry in French River or spawned naturally in Knife River were also tested when they were captured as emigrants in smolt traps located in these rivers. The ATPase activity level that distinguished smolts from non-smolts was determined to be 11 μmol $P_i \cdot (\text{mg protein})^{-1} \cdot h^{-1}$ for chinook salmon, and 10 μmol $P_i \cdot (\text{mg protein})^{-1} \cdot h^{-1}$ for all groups of anadromous rainbow trout tested. Threshold sizes above which smolting occurred were determined from plots of ATPase activity versus fork length, weight, and body depth. Results indicated that 91% of chinook salmon were

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stocked at sizes that exceed the threshold size for the onset of smoltification, but most held at temperatures below 9°C were not in smolt condition. Many chinook salmon held above 9°C were in smolt condition. All yearling rainbow trout were stocked at sizes above the threshold for smolting, and some had elevated ATPase levels indicating smoltification. These fish may have been partially imprinted on hatchery water which flows into French River, which may have affected subsequent imprinting in other locations. Fry-stocked and wild steelhead that emigrated before age 2 were not smolts (and therefore not imprinted), and may be emigrating simply as a result of natural instream movement. Smolting steelhead emigrants were found on nearly all spring sampling dates in the French and Knife rivers, but no smolts were found in fall samples from French River. Size thresholds (fork length, weight, or body depth) may be used to determine the onset of possible smoltification in these populations of hatchery or stream-reared fish. ATPase activity levels showed statistically significant differences between parr, intermediate, and silver colorations for most species/strains examined, but no external feature (color, condition factor, temperature, date) of an individual fish was a reliable indicator of smolt status. Stocking into a river significantly increased ATPase activity levels of hatchery chinook salmon and Kamloops, but decreased ATPase activities in steelhead (that had a mean ATPase activity above the threshold for smolting prior to stocking). Development of a stocking protocol that insures imprinting to remote locations, while maintaining the lowest mortality rates may not be an achievable goal for anadromous stocks in this region. Harsh stream conditions, low carrying capacity, weather conditions that dictate sub-optimum stocking strategies, and short stream lengths limit the ability to maximize imprinting. Partially imprinted fish stray more, increasing the likelihood of spawning overlap between hatchery Kamloops and naturalized steelhead strains. Maintaining chinook salmon for the French River at or above 10°C, and those to be stocked in other locations below 9°C, and stocking in May or June when river temperatures are above 10°C may maximize imprinting in desired locations. Stocking the larger steelhead and Kamloops in French River, and smaller individuals in other streams in May and June may maximize survival of broodstock and homing to desired locations.

Introduction

Olfactory imprinting in anadromous species occurs primarily during parr-smolt transformation, or smoltification, although salmon may also remember odors experienced before that stage (Hasler and Scholz 1983; Morin and Døving 1992; Quinn 1993; Pascual et al. 1995; McCormick et al. 1998). Therefore, stocking before smoltification is critical to fisheries that depend on homing of spawning adults to a specific tributary. Smoltification consists of a variety of morphological, behavioral, and biochemical changes that transform the dark banded, bottom-dwelling parr that can only live in fresh water into a pelagic, more slender, silvery smolt that can tolerate salt water (Hoar 1976; Folmar and Dickhoff 1980; Langdon and Thorpe 1985; McCormick et al. 1998). Most studies of smoltification have involved populations that migrate to saline environments. For these fish, smoltification is critical for survival, in part because of the physiological changes that increase salinity tolerance (Wedemeyer et al. 1980; Folmar and Dickhoff 1981). Concurrent changes in hormones, metabolism, feeding behavior, schooling behavior, olfactory imprinting, and coloration may also play a role in survival unrelated to salinity, which could be important for anadromous species even in landlocked locations.

Chinook salmon *Oncorhynchus tshawytscha* and two strains of anadromous rainbow trout *O. mykiss* are stocked into Minnesota tributaries to Lake Superior, where they were introduced to provide a shore fishery and an alternative to lake trout fishing. Both species are native to the Pacific coast where they emigrate to the ocean as juveniles to grow and mature, but their life history patterns are similar in the freshwater environment of Lake Superior and its tributaries. Chinook salmon (introduced in the 1970s) and the steelhead strain rainbow trout (introduced in the late 1800s) have become naturalized in Lake Superior, but feral brood stock of both species, are captured annually for propagation at the French River Coldwater Hatchery. Feral brood stock of a hatchery strain of rainbow trout known locally as Kamloops are also used for hatchery propagation. The hatchery-reared chinook salmon and rainbow trout are stocked in selected rivers along Minnesota's Lake Superior shoreline with the intent that they will imprint and subsequently return to spawn at these locations.

Salinity tolerance is obviously irrelevant for fish in the Laurentian Great Lakes, but the physiological changes that prepare them for a saline environment persist and provide a means of pinpointing smoltification. The activity level of gill sodium, potassium-activated adenosine triphosphatase (Na^+,K^+-ATPase), which is strongly correlated with salinity tolerance, can be used as a quantitative measure of the progress of smoltification in anadromous salmonines (Zaugg 1982; Boeuf and Prunet 1985; McCormick et al. 1987; Johnson et al. 1991; Schrock et al. 1994; see Appendix 1). The chemical assay is recommended because readiness to smolt cannot be reliably assumed from superficial criteria such as coloration, size, condition factor, or emigration behavior, and some characteristics vary with species, population, or locale. Skin silvering that was quantitatively measured with a video analysis system was been found to be statistically significant for some but not for all groups of fish tested, and the method worked better for steelhead than for chinook salmon (Haner et al. 1995). Beeman et al. (1995) found that truss measurements taken from photographs of steelhead could be used to determine smolt status, but measurements varied between rearing locations, and large sample sizes were required for statistical significance. Although coloration, size, condition factor, and behavior are generally imprecise measures of the peak of smoltification (Zaugg and McLain 1972; Soivio et al. 1988; Virtanen et al. 1991), they may be applicable for local populations if confirmed by correlation with gill Na^+,K^+-ATPase activity (hereafter called "ATPase activity"). The superficial characteristics would be particularly useful for predicting the timing of smoltification in the hatchery or stream-reared stocks, where facilities and budgets are inadequate to support the chemical assay on a routine basis.

Prolonged rearing, along with unnatural temperatures, flow, photoperiod, crowding, and disease treatments in hatcheries can cause the smolt transformation to be accelerated, abbreviated, delayed or reversed (Wedemeyer et al. 1980). For example, steelhead are particularly sensitive to temperature-accelerated growth which may inhibit smoltification, especially above 13°C (Wedemeyer et al. 1980). Normal ATPase development can be suppressed by crowding stress in juvenile chinook salmon (Strange et al. 1978) and coho salmon (Sower and Fawcett 1991). Photoperiod cycle can synchronize the development of smolt characteristics and migratory behavior, and prolonged exposure to a long photoperiod can inhibit growth and smolting in chinook salmon (Ewing et al. 1979), and steelhead (Wagner 1974).

Despite potential negative effects of prolonged hatchery rearing on imprinting and smoltification, there is public pressure to rear steelhead to yearling rather than fry size, and to rear all hatchery stocks to the largest possible sizes before stocking. There is a common perception that larger stocked yearlings survive better than smaller yearlings, although data are inconclusive and difficult to test due to straying (Schreiner et al., in preparation). Steelhead fry are typically stocked above natural barriers in tributary streams at swim-up (Table 1). Steelhead yearlings, Kamloops yearlings and chinook salmon young-of-the-year (y-o-y) are stocked in lower reaches of tributary streams in the spring at "smolt size", presuming that they will imprint before they emigrate, which often occurs in less than one week (Table 1). The actual physiological state of the "smolt size"

Table 1. Anadromous species/strains stocked into Minnesota tributaries of Lake Superior.

Size at stocking[a]	Rivers where stocked	Dates when stocked	Approximate locations where stocked
		Chinook salmon	
Young-of-year ("smolt size")	Lester River	mid-June	Strand Rd, 8.3 km upstream from the river mouth.
	French River	mid-late June	100 m upstream from the river mouth.
	Cascade River	early June	6 km upstream from the river mouth; or into Lake Superior at mouth of creek if water levels very low and warm.
	Baptism River	early June	In the last 13 years, they have been stocked 4 times at Eckbeck Campground, 6 km upstream from the lake, twice in the town of Finland about 14 km upstream from the lake, and (due to low flows) 7 times into Lake Superior from 1 km to 6 km southwest of the river mouth.
		Steelhead	
Fry	French River[b]	mid-June to early July	At various locations up to 13 km upstream from the river mouth.
Yearling ("smolt size")	French River	mid-May to early June[c]	100 m upstream from the river mouth.
	Knife River	mid-May to early June[c]	Below the fish trap, 1.2 km up from the river mouth.
	Silver Creek	late April to mid-May	100 m upstream from the river mouth at Highway 61.
	Gooseberry River	late April to mid-May	In Gooseberry Park, 0.7 km upstream from the river mouth.
		Kamloops	
Yearling ("smolt size")	Lester River	mid-late May	Strand Rd., 8.3 km upstream from the river mouth.
	Chester Creek	mid-late May	Chester Bowl, 2.3 km upstream from the creek mouth; or into Lake Superior at mouth of creek if water levels are very low.
Yearling ("post-smolts")	"French River"	mid-May to mid-August	Bluebird Landing in Lake Superior, about 4 km northeast of the mouth of the French River.

[a] Stocking of chinook salmon young-of-year and most rainbow trout yearlings is done at night when possible, to minimize bird predation. Large Kamloops are stocked at Bluebird landing during daylight, as their size and location render them less vulnerable to bird predation.

[b] If fry are available, they may be stocked in various other streams, in even or odd years (Schreiner 1995).

[c] A few steelhead yearlings were stocked in August and September in 1990 and 1991, but returns were poor.

fish is uncertain, since many attain a silvery color during their hatchery residence.

Kamloops y-o-y are sorted by size in September-October, and the different sizes are reared separately (with some mixed sizes in one tank due to space constraints). The following spring, the larger yearlings are transported to Chester Creek and Lester River for stocking, and the smaller individuals are retained for further rearing. Imprinting in the hatchery is expected for these longer-reared fish, and they are later stocked at Bluebird Landing rather than in French River. Past experience has shown that when these stocks are placed directly into French river, the smaller individuals remain for some time and bird predation is high. French River receives the hatchery outflow, so Kamloops broodstock taken from French River are thus fish which imprinted in the hatchery prior to stocking. Steelhead y-o-y are also sorted by size in September, but space constraints prohibit separate rearing of the different size classes for the entire duration of their hatchery residence.

Adverse environmental conditions unavoidably dictate stocking strategies in Minnesota's tributary streams in some years. Managers need to know the fishes' smolting status to evaluate the consequences of various stocking procedures. When river flows are very low, "smolt size" chinook salmon or Kamloops may be stocked into river mouths to reduce bird predation and provide access to Lake Superior. Some of these fish have even been stocked directly into the lake near river mouths when low flows and gravel bars restricted passage between rivers and the lake. These stocking strategies are implemented to increase initial survival, and the fish tend to emigrate within a few days. It is unknown, however, whether these fish have already smolted and imprinted to hatchery water, whether they emigrate before imprinting, or whether they smolt and imprint immediately after stocking.

Chinook salmon, steelhead, and Kamloops routinely stray to other rivers along the Minnesota shoreline and beyond, suggesting that they partially imprint in the hatchery, or fail to imprint well at their stocking sites. From 1991 to 1999, 58% of the steelhead yearlings stocked in Knife River returned to French River, while less than 1% of those stocked into French River strayed to Knife River (Schreiner et al., in preparation). Based on adult returns of chinook salmon to French River from 1991 and 1992 year classes, only about one-third had been stocked in the French River, while two-thirds had been stocked elsewhere along Minnesota's shoreline (Jones and Schreiner 1997). Chinook salmon stocked by each state or province surrounding Lake Superior are routinely caught in spawning runs in each of the other jurisdictions (Peck et al. 1999), indicating a high degree of straying.

Rainbow trout and chinook salmon stocks in Minnesota waters of Lake Superior have been declining in recent years, and factors contributing to this decline are under investigation (Schreiner 1995). Preliminary findings show that 8-10% of the smolts produced from fry-stocked steelhead return to French River as adults, while less than 1% of the stocked yearling steelhead return as adults (D. Schreiner, Lake Superior Area Fisheries, personal communication). Lack of olfactory imprinting to appropriate natal (or stocking) streams is one possible reason for declining returns. Another possibility is that straying of rainbow trout strains might enable intraspecific spawning between steelhead and Kamloops, producing eggs or offspring with reduced viability (Negus 1999).

Zaugg and McLain (1972) found that keeping steelhead in hatchery ponds for more than one year may result in a reduced number of true smolts capable of migrating. Stocking outside of the normal season for smolting can also increase straying (Wagner et al. 1963; Quinn 1993), which can result in increased genetic mixing between wild and hatchery stocks, thus reducing genetic differences essential for their fitness (Hindar et al. 1991; Quinn 1993; Pascual et al. 1995). Tracking the onset of smoltification in hatchery juveniles could enable managers to determine the most appropriate times to stock, and avoid prolonged rearing.

Determination of the smolt status of steelhead emigrants (naturally spawned or stocked as fry) may help to explain the cause of early emigration, help to explain the fate of these fish, assist in evaluation of habitat improvements, and assist in setting optimum stocking densities for fry. Steelhead stocked as fry into tributary streams emigrate at a variety of ages, sizes, and colorations. While some emigrants are large and silvery like smolts, others are small and still retain parr marks. Premature emigration due to intraspecific competition and self-thinning (Grant 1993; Marschall and Crowder 1995) may explain their failure to survive or return at spawning time. Early emigration could also be caused by displacement due to high water flows, which is beyond our control, or early smolting, which would implicate some genetic factor.

The objectives of this study were to: 1) evaluate the readiness to smolt in hatchery chinook salmon, and steelhead and Kamloops strains of rainbow trout; 2) determine whether smoltification is stimulated when fish are stocked into a stream; 3) identify the smolt status of emigrating steelhead captured in smolt traps; 4) correlate ATPase activity with coloration, size, condition, water temperature, and date in hopes of finding alternate indicators of smoltification applicable to local stocks; and 5) recommend rearing or stocking strategies that maximize imprinting at intended locations.

Study Area

I tested chinook salmon, and steelhead and Kamloops strains of rainbow trout being reared at the French River Coldwater Hatchery located near the mouth of French River (Figure 1). The hatchery uses Lake Superior water for rearing, but the outflow water enters French River about 100 m upstream from the mouth. The hatchery-reared stocks were destined for various Lake Superior tributaries, where they were expected to imprint and provide fishing opportunities during spawning runs (Table 1, Figure 1). I also tested fry-stocked steelhead from the French River and wild (naturally spawned) steelhead from the Knife River that were captured in smolt traps located in these rivers. The French River smolt trap is located about 0.5 km from the mouth, and the Knife River smolt trap is about 1 km from the mouth of the river.

Minnesota's shoreline of Lake Superior contains 54 tributary rivers or streams with habitat for anadromous species (MNDNR 1992). Knife River is the only river accessible to anadromous fish for its entire 113 km length. The remaining 53 streams total about 62 km of habitat, but 25 of these streams have marginal habitat due to their small size. A few other streams with headwaters in Minnesota drain into Lake Superior via a Wisconsin tributary. The steep landscape in Minnesota's "North Shore" region of Lake Superior forms a barrier to upstream migration of fish in most streams, with waterfalls that often occur only a few meters upstream from the lake. In general, the streams have little groundwater input, and are subject to high spring runoff and widely fluctuating flows. Cold winter temperatures can cause anchor ice, ice dams, and dry stream beds in some locations in some years. Warm, dry weather can severely reduce flows and heat the water, and heavy rainfall or snow melt can rapidly increase flows to flood stage. These streams have low productivity and a limited carrying capacity for fish. Brook trout *Salvelinus fontinalis* inhabit the headwaters of most streams, and protection of these populations is intended whenever the stocking of other species is considered. Below this region and above the barriers there are reaches that are seasonally thermally marginal for brook trout, but contain a few brown trout *Salmo trutta*, sculpins *Cottus* spp. and minnows. These reaches are stocked with steelhead fry which remain there until ready to emigrate (Figure 1; Table 1). Chinook salmon y-o-y and rainbow trout yearlings are also stocked into streams at "smolt size". To reduce the probability of interbreeding with wild stocks of steelhead, Kamloops stocking has been restricted to three tributaries (Lester River, Chester Creek, and French River) near the city of Duluth since 1992 (MNDNR 1992; Table 1; Figure 1).

Methods

At the time of sampling, each fish was anesthetized, and gill filament samples were excised using techniques described by McCormick (1993) and Schrock et al. (1994). Each sample was placed in SEI (sucrose, disodium EDTA, imidazole) buffer solution, numbered, and frozen in liquid nitrogen within one-half hour. The samples were later transferred to a -80°C freezer until analysis. Each fish was weighed (g), measured [fork length (FL), total length (TL), and body depth] (mm), and categorized by body coloration (Parr, Intermediate, or Silver). The "Parr" designation meant that all parr marks were visible. The "Intermediate" designation meant that parr marks closest to the head were gone, but marks were still visible near the caudal fin. "Silver" meant that all parr marks were gone, or those remaining near the caudal fin were extremely pale. Categorization was done indoors or out of direct sunlight to reduce reflection that could

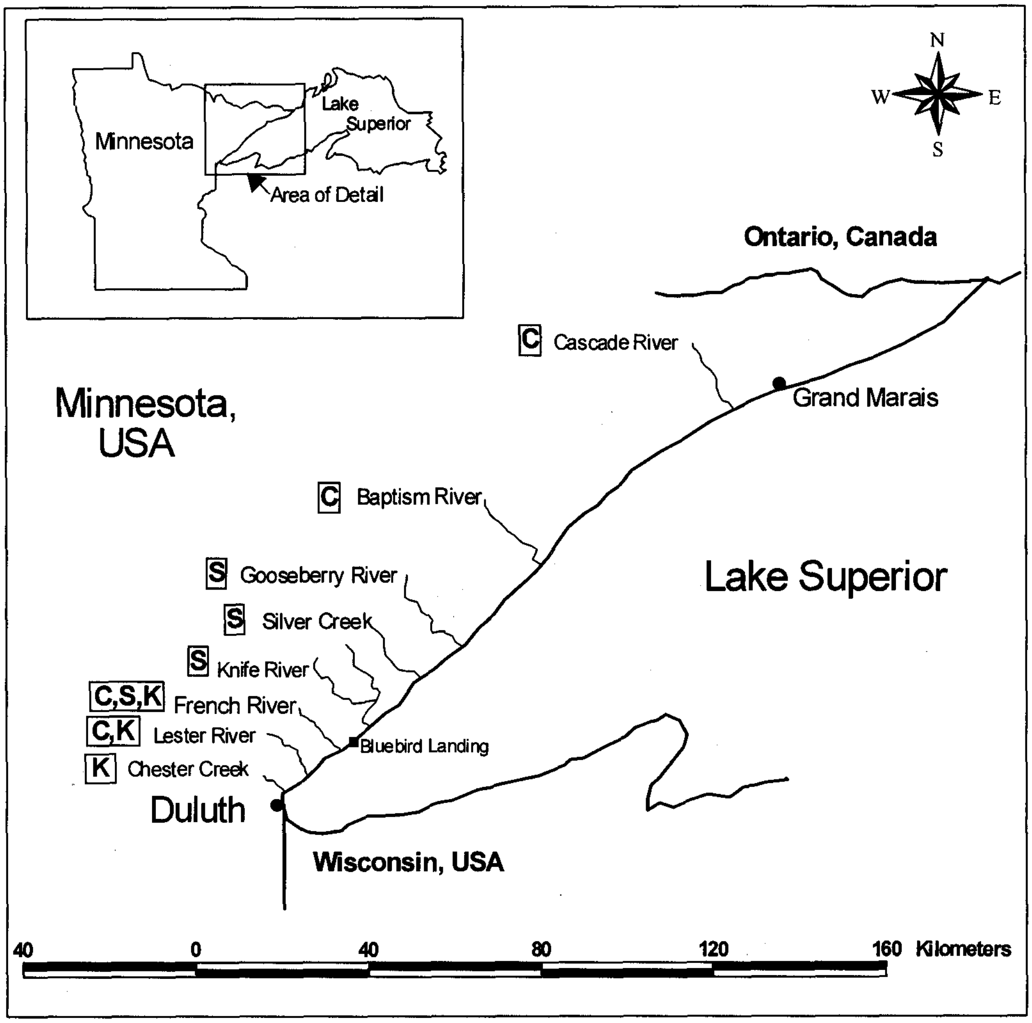

Figure 1. Minnesota's Lake Superior shoreline, known as the North Shore, including tributaries into which anadromous species are stocked. Letters in boxes preceding river names indicate species/strains stocked into those rivers: C=chinook salmon, S=steelhead, K=Kamloops. All anadromous fish stocked into Minnesota waters are reared at the French River Coldwater Hatchery.

affect perceived body color. Water temperatures in the river and in the hatchery were continually monitored. All live fish were released after sampling.

Gill filament samples were prepared following the protocol of Schrock et al. (1994) with a few modifications described here. Samples were stored and processed in 2 ml Bio-Stor skirted vials. Eighteen samples were processed at a time, because that was the capacity of the centrifuge, and the capacity of each microwell plate when samples were replicated. The samples were extracted in SEI only, eliminating the solution containing deoxycholate, a detergent additive which was found to be unnecessary. Size of each sample was judged visually as soon as possible during thawing, and samples were kept in an ice bath between each step. After grinding the samples with a Tissue Tearor[2], they were centrifuged for eight minutes at 2,000 relative centrifugal force (RCF). The supernatant was gently pipetted off (not poured off) to insure that the pellet was not disturbed. Chilled SEI was then added dropwise, based on sample size as follows: extra-small - 5 drops; small - 6 to 7 drops; medium - 12 drops; large - 20 drops. Each vial was vortexed on the high setting for 10 seconds to disperse the pellet. Samples were sonicated for 4-5 continuous seconds using a Sonics and Materials Inc. Vibra Cell 50 Watt Ultrasonic Processor[2] set at Output=20. Samples were then centrifuged for 10 minutes at 12,000 RCF, and 150 - 200 μl of the enzyme preparation supernatant was transferred to labeled culture tubes. Replicates of each sample, references, and standards were included in each microassay plate, to reduce the probability of incorrect values due to pipetting errors. Values of the replicates were averaged.

The evaluation procedure consisted of two assays: one to measure the amount of inorganic phosphate (P_i) liberated by the action of ATPase; and one to measure the amount of protein (see Appendix 1). ATPase activity was reported as μmol $P_i \cdot (\text{mg protein})^{-1} \cdot h^{-1}$. The phosphate assay followed the procedure of Schrock et al. (1994). The Bio-Rad protein assay, adapted from a method by Bradford (1976) was substituted for the modified Lowry et al. (1951) protein assay used by Schrock et al. (1994) because it was simpler, accurate, repeatable, and required only one reagent with a longer shelf life. All phosphate and protein samples were read at 590 nm on a Biolog Microstation[2] 96-well plate reader.

ATPase activities were plotted versus fork length (mm), weight (g), body depth (mm), condition factor [calculated as: $10^5(\text{weight/fork length}^3)$], and sampling date, with body color distinguished by different symbols. Only FL was used in analyses, because it was a more consistent measurement than TL for hatchery fish due to obvious fin erosion, as demonstrated by the larger differences between FL and TL in stream-reared versus hatchery-reared fish (Appendix 2). Mean water temperature was included in plots of date versus ATPase activity, and maximum water temperature was included for stream-reared fish. From these plots, I determined the threshold activity levels that distinguished non-smolts from smolts. Recommended guidelines for non-smolting juveniles were 0 μmol to about 10-12 μmol $P_i \cdot (\text{mg protein})^{-1} \cdot h^{-1}$, and for smolting juveniles were 12 - 15 μmol $P_i \cdot (\text{mg protein})^{-1} \cdot h^{-1}$ or higher (R. Schrock, USGS, Western Fisheries Research Center, personal communication), with the understanding that these levels will vary by species, procedure, and laboratory where tested. Threshold sizes at which ATPase activities increased abruptly (indicating the onset of smoltification) were also discerned from plotted data.

To evaluate the actual smolt condition of hatchery stocks (Objective 1), ATPase activity was measured in chinook salmon, steelhead, and Kamloops during hatchery rearing to "smolt size" (Table 2). Hatchery-reared chinook salmon achieved "smolt size" as y-o-y, while steelhead and Kamloops were reared to yearling stage. Ordinarily, 36 samples were taken from each species/strain on each sampling date. Hatchery year classes

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[2] Reference to trade names does not imply endorsement by the Minnesota Department of Natural Resources.

Table 2. Populations sampled for gill ATPase activity.

Species or strain	Rearing location	Number of samples taken	Number of assays used[a]	When samples were taken
Chinook salmon	hatchery	370	242 (65%)	Periodically up to the day of stocking
Steelhead	hatchery	390	343 (88%)	Periodically up to the day of stocking
Kamloops	hatchery	495	459 (93%)	Periodically up to the day of stocking
Chinook salmon,	hatchery	169	98 (58%)	At time of recapture in French River smolt trap after stocking about 1 km upstream.
steelhead,		38	38 (100%)	
and Kamloops		48	36 (75%)	
Steelhead emigrants (stocked as fry)	French River	491	368 (75%)	At time of capture in French River smolt trap
Wild steelhead	Knife River	166	122 (73%)	At time of capture in Knife River smolt trap

[a] Some samples were eliminated from final analysis due to small size or some problem during assay.

included were: chinook salmon - 1997, 1998 and 1999; steelhead - 1996 and 1997; and Kamloops - 1996, 1997 and 1998. The 1997 year class of chinook salmon was reared with two different thermal regimes: normal rearing temperatures of 5-7°C; and accelerated growth temperatures of 10-12°C. Eighteen samples of chinook salmon from each temperature regime were taken on each sampling date in 1997. Chinook salmon rearing temperatures in 1998 were also somewhat elevated to speed growth, and rearing temperatures in 1999 resembled the normal growth temperatures of 1997. Threshold sizes and ATPase activities of these stocks were examined to determine whether smolting (indicated by an elevation in ATPase activity above threshold values) was occurring during hatchery residency.

To determine whether smoltification is stimulated by placement in flowing water (Objective 2), samples were taken from chinook salmon, steelhead, and Kamloops just before stocking for comparison with samples taken after recapture in the French River smolt trap (Tables 2 and 3). No individuals were sampled more than once; a subsample of the hatchery fish were stocked about 1 km above the smolt trap so they could be recaptured, and fish remaining in the hatchery were sampled on the same day to represent pre-stocked fish. Fish sizes were plotted versus ATPase activities before stocking and after recapture. Mean ATPase activities before and after stocking were compared using *t* tests to determine if smolting status differed between the two groups.

To determine the actual smolt status of stream-reared steelhead emigrants (Objective 3), steelhead that had been stocked as fry in French River or naturally reproduced in Knife River were sampled when they were captured in smolt traps located in these rivers. Samples were taken at these locations during periods when at least 3-5 fish per day were being trapped. Up to 36 fish were sampled per day, omitting fish less than 80 mm FL (which were difficult to sample non-lethally, and were always parr) when large numbers were captured. Threshold sizes and ATPase activities of the two groups of stream-reared fish were examined and compared to determine the presence of smolting.

Plots of ATPase activity versus condition factor, date, body color, and water temperature were used to determine correlations between these factors (Objective 4). ATPase activities of the three color categories of each species/strain were analyzed using analysis of variance (ANOVA) with posthoc Bonferroni pairwise comparisons. Threshold sizes of hatchery-reared and stream-reared rainbow trout strains were compared to determine whether the onset of smoltification differed between habitats. Finally, stocking methods, habitat options, and related literature were evaluated to formulate management recommendations to maximize imprinting, survival, and perpetuation of these species/strains (Objective 5).

Table 3. Samples taken just before stocking, and after stocking and recapture in the French River. Recapture of emigrating fish stocked in spring 1999 was curtailed when a severe flood interrupted trap operation on 4 July. ATPase activity is reported in μmol P_i·(mg protein)$^{-1}$·hr^{-1}.

Species or strain	Number sampled before stocking	Number stocked	Number recaptured	Stocking date	Mean ATPase activity before stocking	Mean ATPase activity after stocking
Chinook salmon	-	100	98 (98%)	14 May 1997		9.1
	39	50	27 (54%)	8 June 1998	7.4	10.0
	36	50	16 (32%)	17 May 1999	6.5	13.0
	-	50	28 (56%)	26 May 1999		13.2
Steelhead	36	50	38 (76%)	1 June 1998	12.4	8.3
Kamloops	36	50	41 (82%)	18 May 1999	9.2	10.6
	36	50	7 (14%)	28 June 1999	8.0	10.1

Table 4. ATPase activities and sizes that defined the threshold of smolting for chinook salmon and various groups of anadromous rainbow trout.

Species or strain	Rearing location	ATPase activity[a]	Fork length (mm)	Weight (g)	Body depth (mm)	Approximate number per pound[b]
Chinook salmon	hatchery	11	71	4	15	100
Steelhead	hatchery	10	150	40	30	10
Kamloops	hatchery	10	150	40	30	10
Fry-stocked steelhead	French River	10	125	20	22	
Wild steelhead	Knife River	10	125	20	22	

[a] (μmol P_i·(mg protein)$^{-1}$·hr^{-1})

[b] "Number per pound" is a frequently used quantity in the hatchery. Because these fish cannot be blotted effectively when held in a net, and because the threshold weight is a conservative estimate for the low end of smolting, the number of fish has been reduced slightly to provide a rough guideline for hatchery managers.

Results

ATPase activity was analyzed in over 2,000 samples (Table 2). Non-lethal samples of sufficient size were difficult to obtain from fish smaller than 80 mm FL. Steelhead and Kamloops (80-350 mm FL) suffered little immediate or delayed mortality. Chinook salmon (57-65 mm FL) suffered higher mortality at the time of sampling, and even the largest individuals were fairly fragile. Gill filament sample sizes varied, and there was a tendency to take an inadequate amount of gill filament from the smaller fish. Samples with values that fell below the standards (that were run with each assay) were eliminated. A number of assays were also eliminated because exceedingly low values were traced to spoilage of the ATP reagent.

Hatchery stocks

An ATPase activity of 11 μmol P_i·(mg protein)$^{-1}$·hr^{-1} was determined to be the threshold for smolting in chinook salmon (Table 4; Figure 2). Some of the smallest chinook salmon had ATPase activities close to this value, most parr-colored chinook salmon had ATPase activities less than or equal to this value, and an abrupt increase above this value occurred in some fish beyond a particular size. Threshold sizes for the onset of smoltification were determined from data of all year classes combined. There appeared to be a second abrupt increase in ATPase

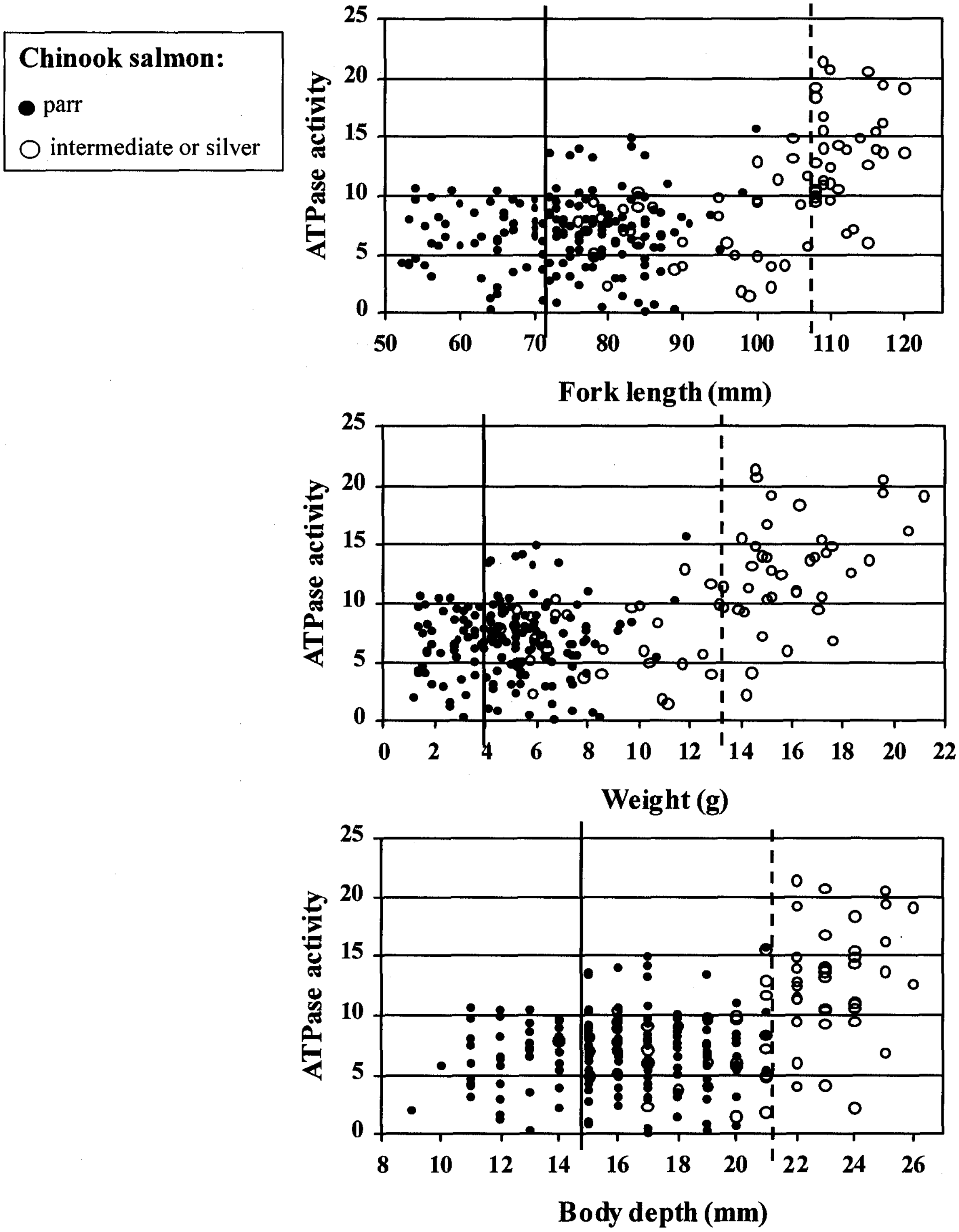

Figure 2. Chinook salmon ATPase activity levels versus fork length, weight, and body depth. Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly. Dashed lines delineate sizes at which a second abrupt increase in ATPase levels is seen.

activities above 15 µmol P_i·(mg protein)$^{-1}$·hr^{-1} (Figure 2). Separating the data by year and thermal regime revealed that the largest of the accelerated growth fish in 1997 attained the highest ATPase levels, and only these fish attained levels above 15 µmol P_i·(mg protein)$^{-1}$·hr^{-1} in the hatchery (Figures 3 and 4). Evidence of smolting was seen primarily in chinook salmon reared at or above 9°C.

For all rainbow trout tested, an abrupt increase in ATPase activity above 10 µmol P_i·(mg protein)$^{-1}$·hr^{-1} occurred at about the same size that some color change was first apparent, so this activity level was interpreted as the threshold of smolting activity (Table 4; Figure 5). Fish size at onset of smolting appeared to depend upon rearing location. Steelhead and Kamloops reared in the hatchery began to exhibit elevated ATPase activity levels at larger sizes than emigrants reared in the stream (Figures 5-7). Elevated ATPase levels were rare in "very large" hatchery steelhead and Kamloops over 260 mm FL, 200 g, and 60 mm body depth (Figures 5-8).

At the time of stocking, 91% of the chinook salmon and 100% of steelhead and Kamloops exceeded threshold size for the onset of smolting (Figures 9 - 11). While less than 1% of the hatchery steelhead exceeded any of the "very large" size measurements at the time of stocking, between 20% and 36% of the Kamloops exceeded at least one of these "very large" measurements at the time of stocking (Figures 10 and 11).

Mean ATPase activity increased significantly ($P<0.05$) in chinook salmon and Kamloops after stocking and recapture, but decreased significantly ($P<0.05$) after stocking and recapture in steelhead (Table 3; Figures 9-12). Elevated ATPase activities were found in chinook salmon above threshold sizes, and throughout the size ranges of steelhead and Kamloops just before stocking and after recapture, but not all recaptured fish had elevated levels (Figures 9-12). Most of the stocked chinook salmon emigrated immediately after stocking, and the highest and lowest ATPase levels were measured in those recaptured first (Figure 12). Most of the later recaptures apparently remained in the stream until they were smolting (indicated by elevated ATPase activities). The largest number of chinook salmon with ATPase levels below the threshold of smolting were recaptured one day after stocking at low temperatures (below 9°C). Emigration by stocked steelhead and Kamloops was somewhat more gradual, and no relationship between water temperature and ATPase level was apparent.

Color determination of fish was subjective, and there was a continuous gradation in color change. ATPase activities differed significantly ($P<0.05$) between the three color categories of each species/strain, except between Kamloops intermediate and silver categories. In all other cases, parr-colored fish had the lowest and silver-colored fish had the highest ATPase activities (Table 5). Chinook salmon were especially difficult to classify, as parr marks appeared or disappeared depending on the angle of viewing. Therefore, the "intermediate" and "silver" classifications were combined in plots of chinook salmon data.

There was no clear relationship between ATPase activity and condition factor of chinook salmon (Figure 13). While the condition factors of the different groups of rainbow trout differed, no relationship to ATPase activity was evident (Figure 14). The condition factor of stocked and recaptured steelhead and Kamloops tended to be lower than their condition prior to stocking (Figure 15).

Elevated ATPase activities were measured in chinook salmon reared at warmer temperatures in 1997 and 1998 on most dates from April until stocking in June (Figure 16). Elevated ATPase values were rare in chinook salmon reared at lower temperatures in 1997 and 1999. Elevated ATPase activities were measured in some hatchery steelhead from early March until stocking in late May (Figure 17), and in hatchery Kamloops from early March through mid July (Figure 18).

Stream-reared steelhead

Fry-stocked and wild steelhead appeared to have the same threshold ATPase

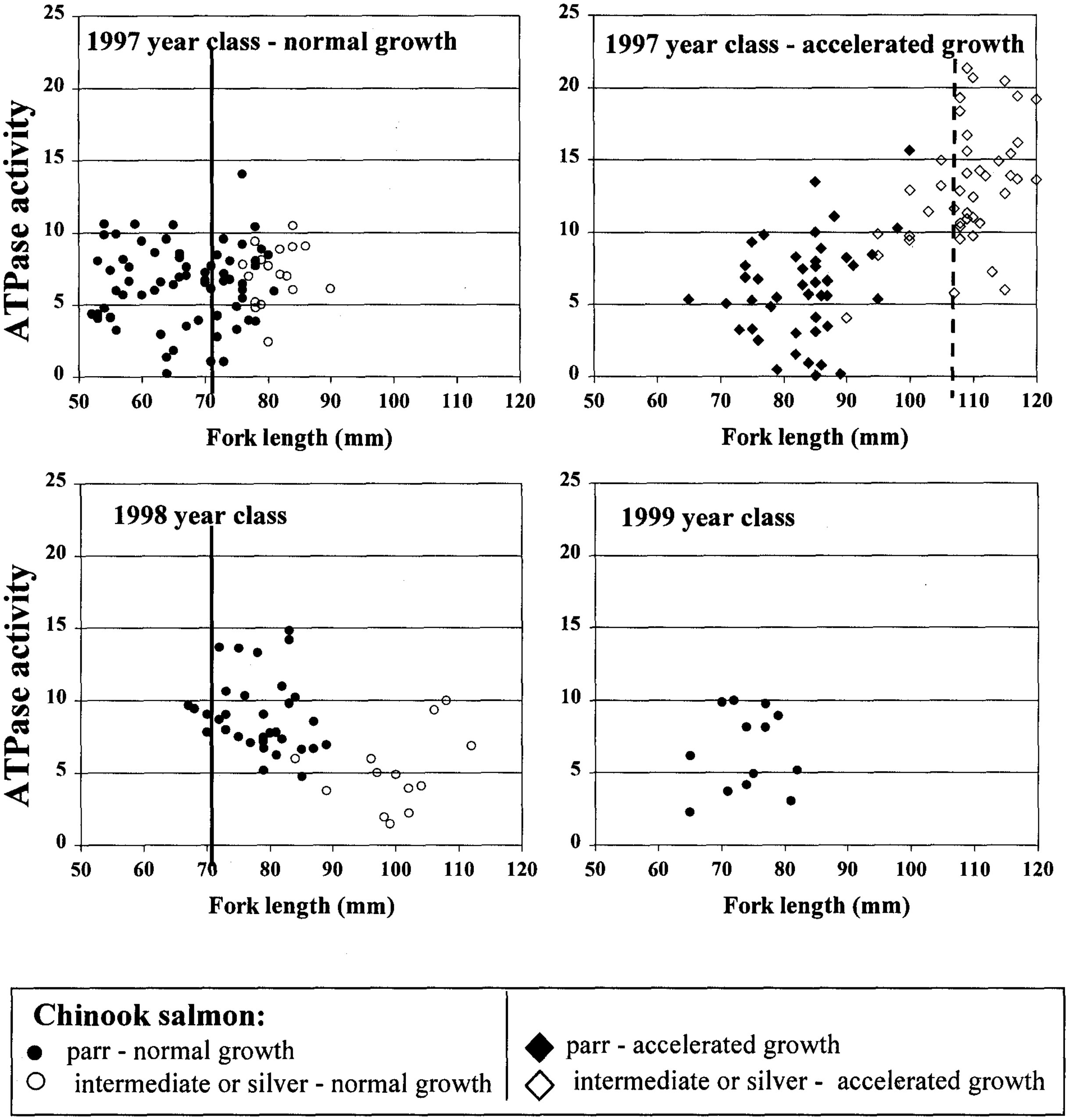

Figure 3. ATPase activity levels versus fork length of different year classes of chinook salmon. Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

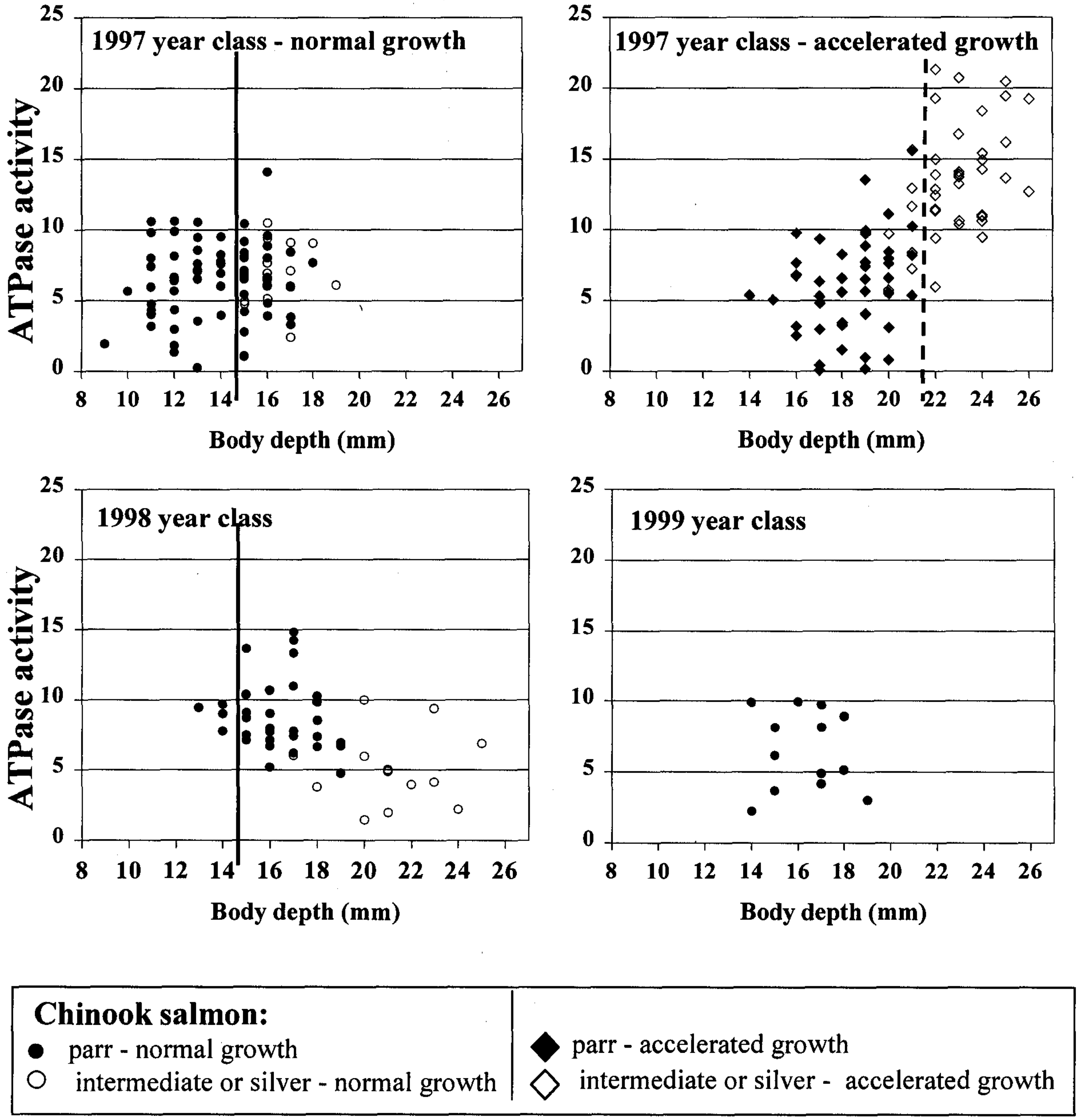

Figure 4. ATPase activity levels versus body depth of different year classes of chinook salmon. Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

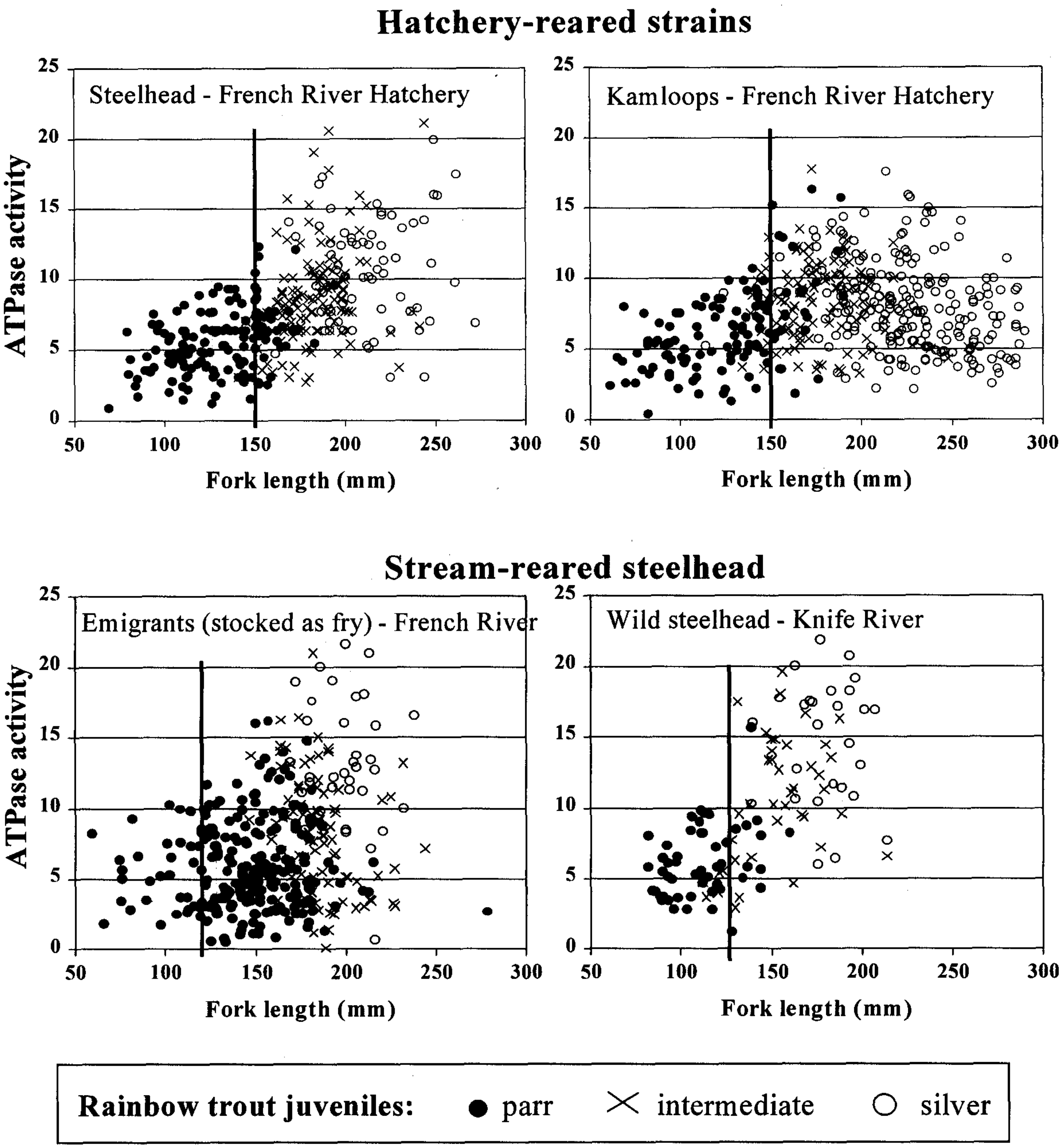

Figure 5. ATPase activity levels versus fork length of anadromous rainbow trout populations, including two strains reared in the hatchery, and two groups reared in streams. Note that the fork length scale does not extend high enough to include all Kamloops (see Figure 8). Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

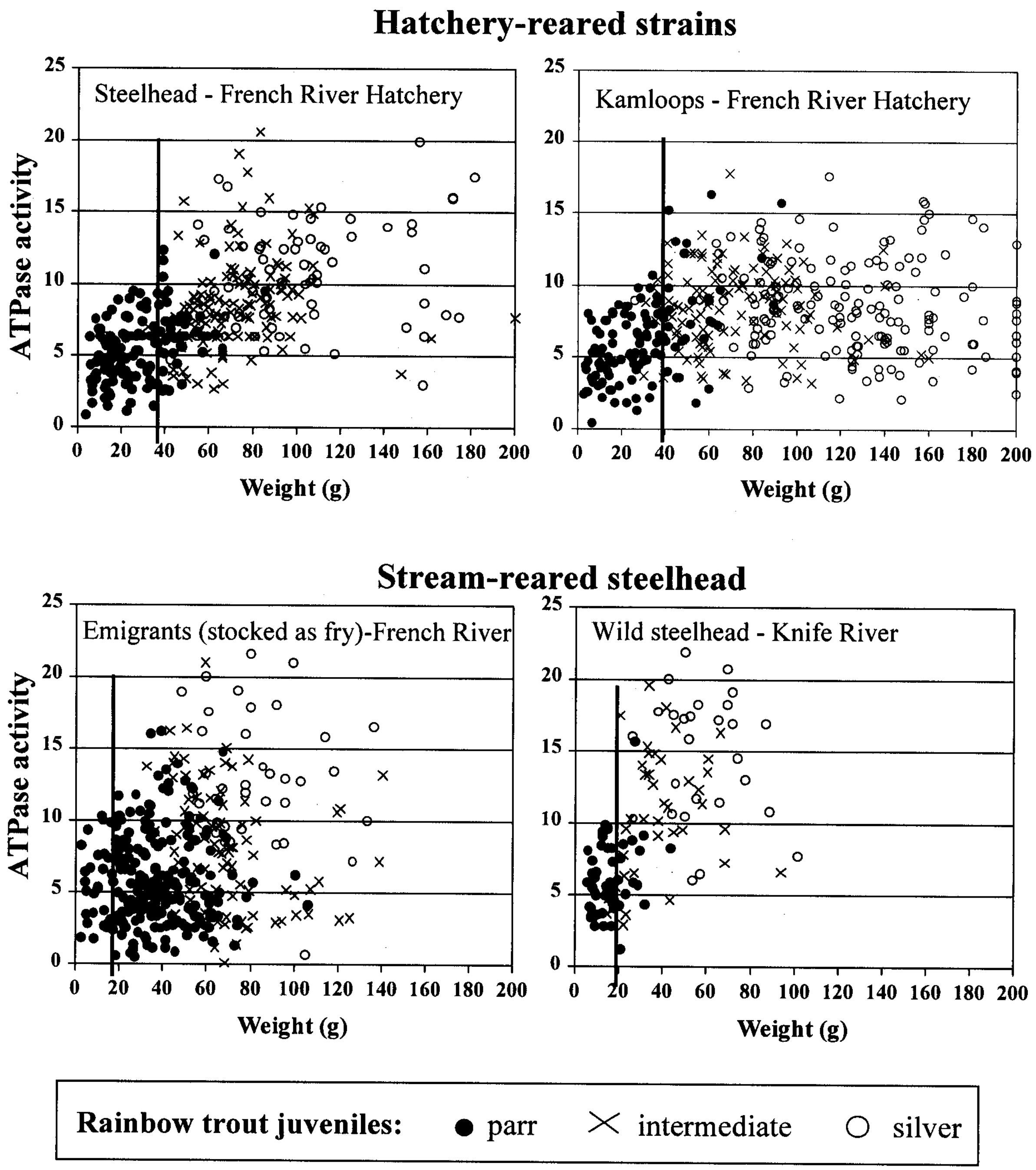

Figure 6. ATPase activity levels versus weight of anadromous rainbow trout populations, including two strains reared in the hatchery, and two groups reared in streams. Note that the weight scale does not extend high enough to include all Kamloops (see Figure 8). Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

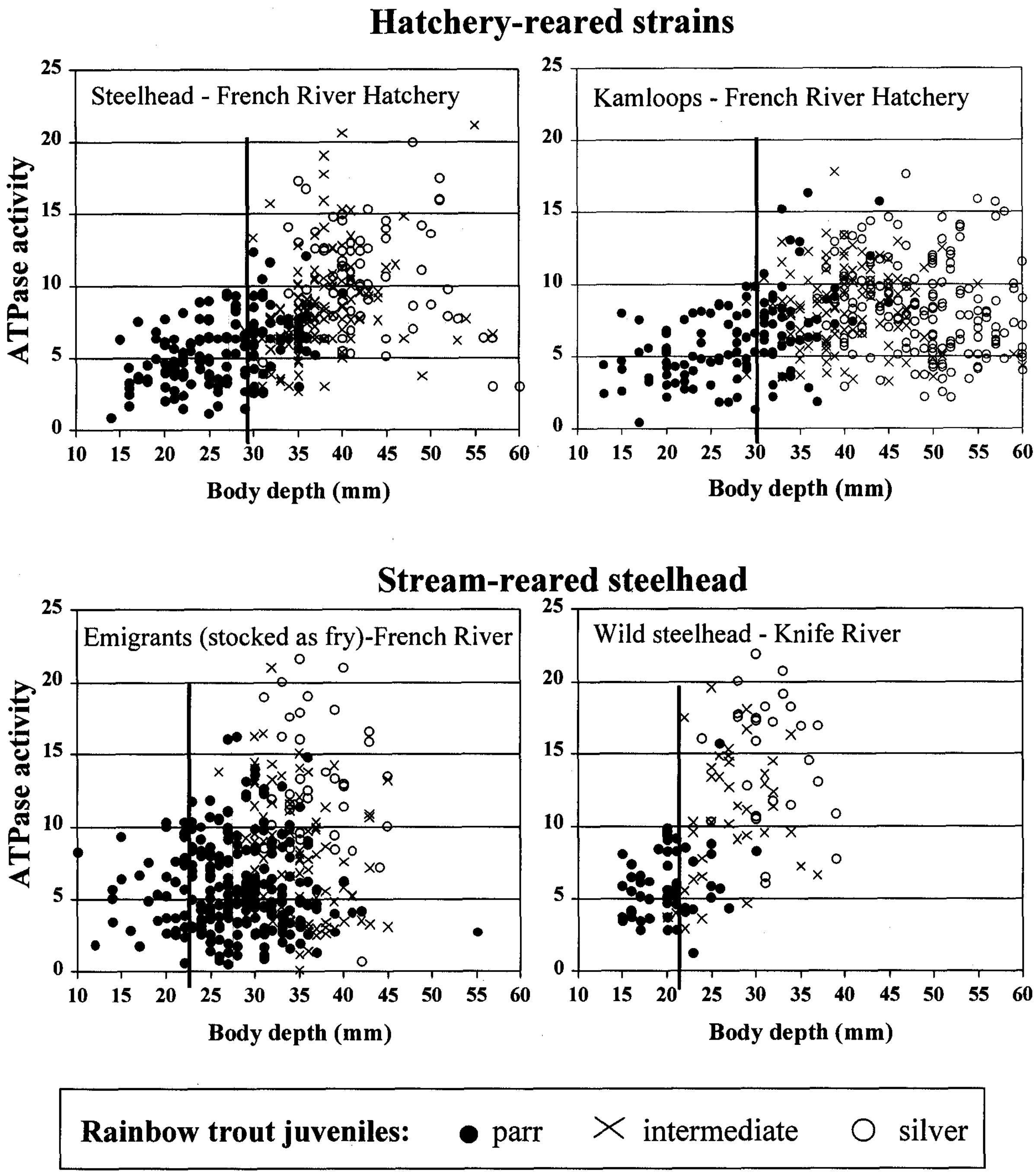

Figure 7. ATPase activity levels versus body depth of anadromous rainbow trout populations, including two strains reared in the hatchery, and two groups reared in streams. Note that the body depth scale does not extend high enough to include all Kamloops (see Figure 8). Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

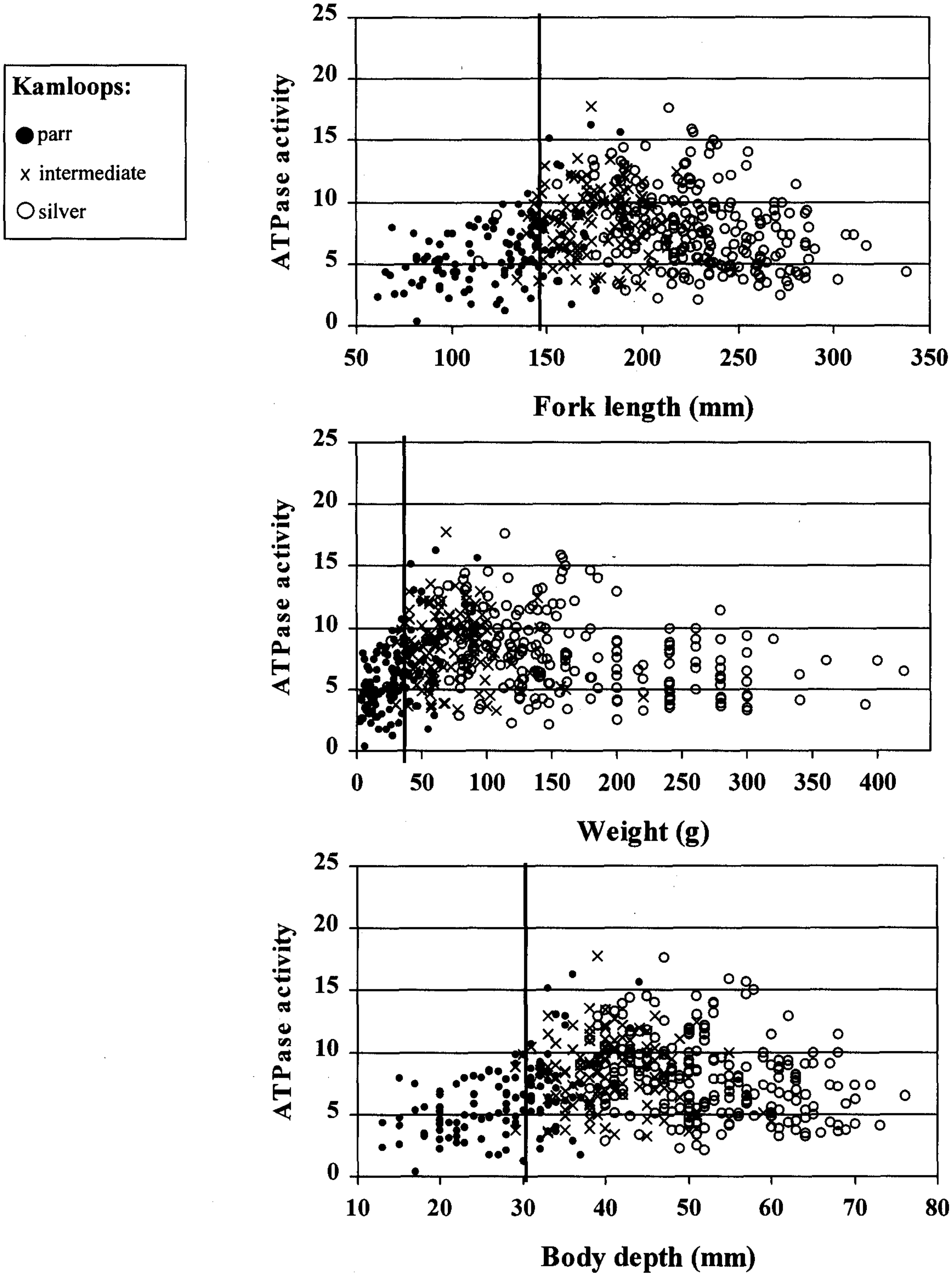

Figure 8. Kamloops ATPase activity levels versus full range of fork lengths, weights, and body depths. Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

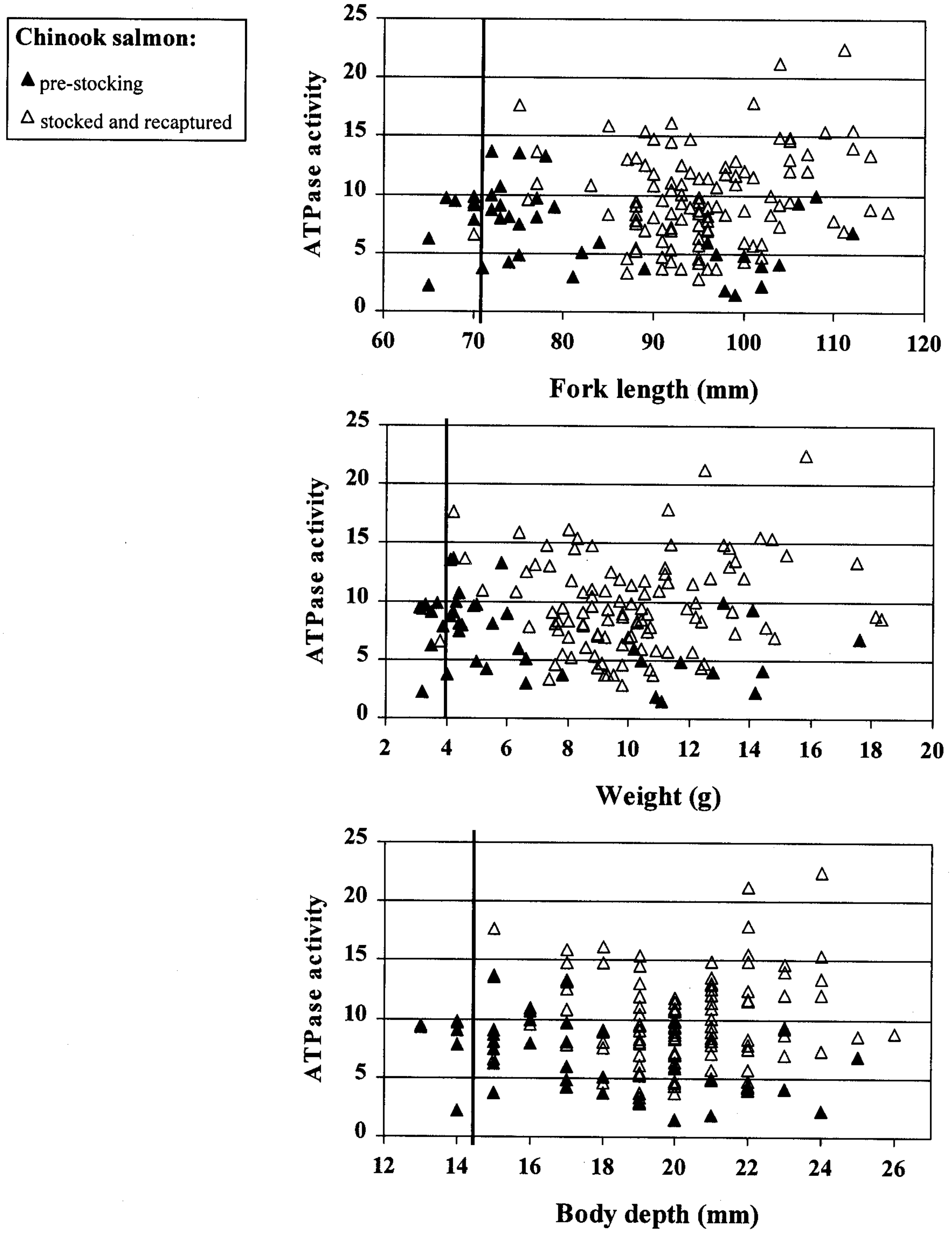

Figure 9. ATPase activity levels measured in chinook salmon just before stocking, and after stocking and recapture in the French River smolt trap. Vertical lines delineate threshold sizes at which ATPase levels appear to rise abruptly.

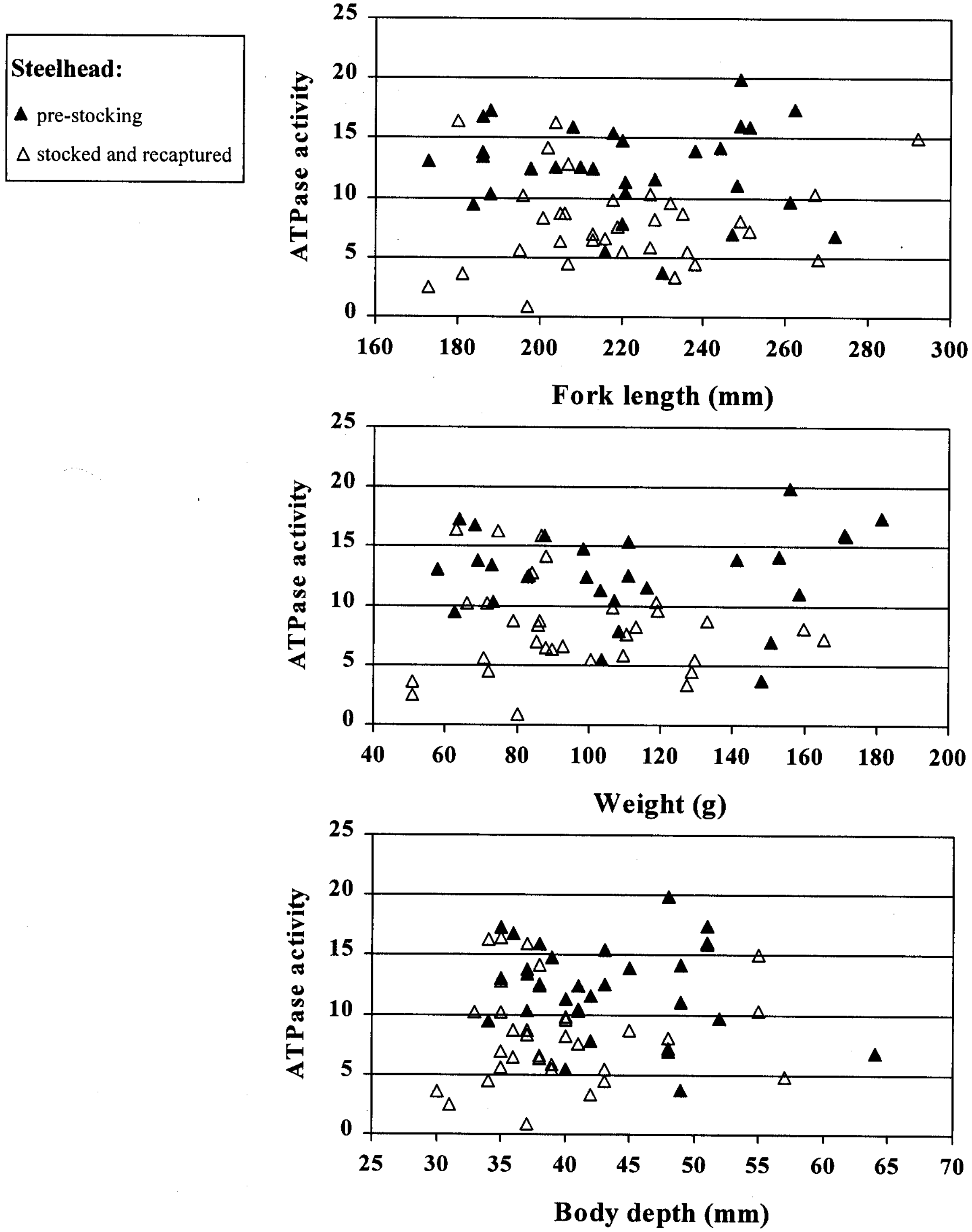

Figure 10. Steelhead ATPase activity levels versus fork length, weight, and body depth measured in hatchery yearlings just before stocking, and after stocking and recapture.

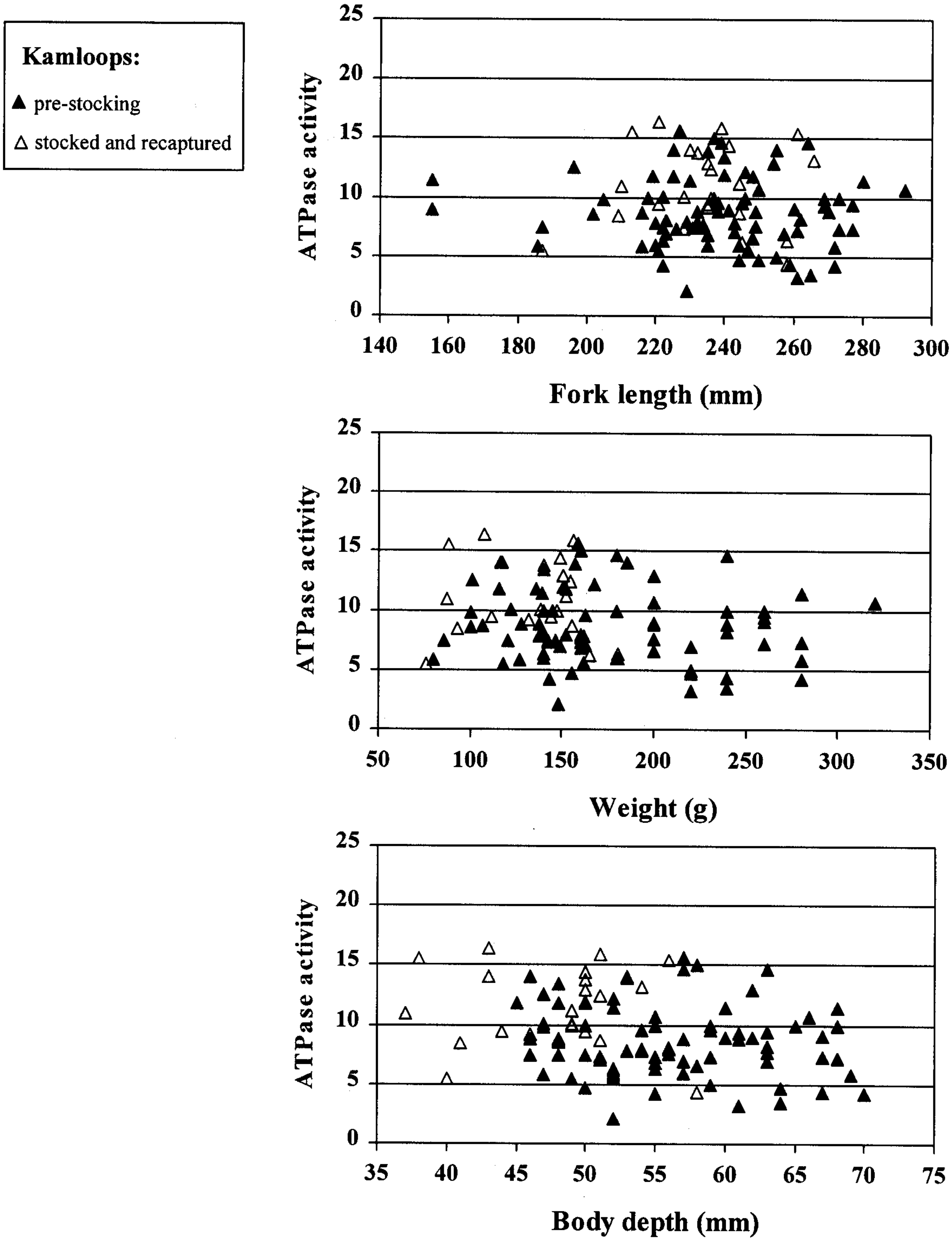

Figure 11. Kamloops ATPase activity levels versus fork length, weight, and body depth measured in hatchery yearlings just before stocking, and after stocking and recapture.

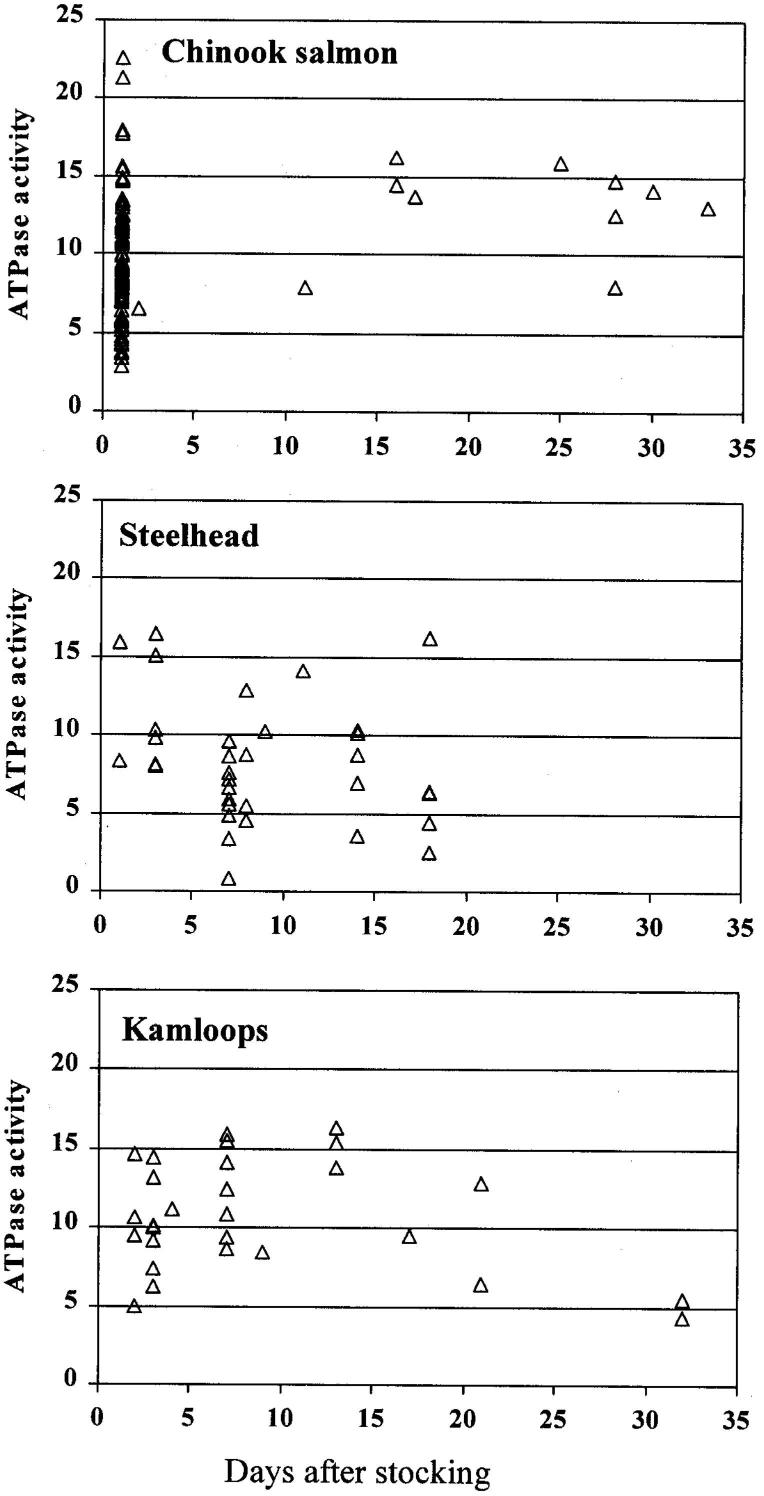

Figure 12. Travel times and ATPase activity levels measured in stocked and recaptured chinook salmon young-of-the-year, steelhead yearlings, and Kamloops yearlings in French River.

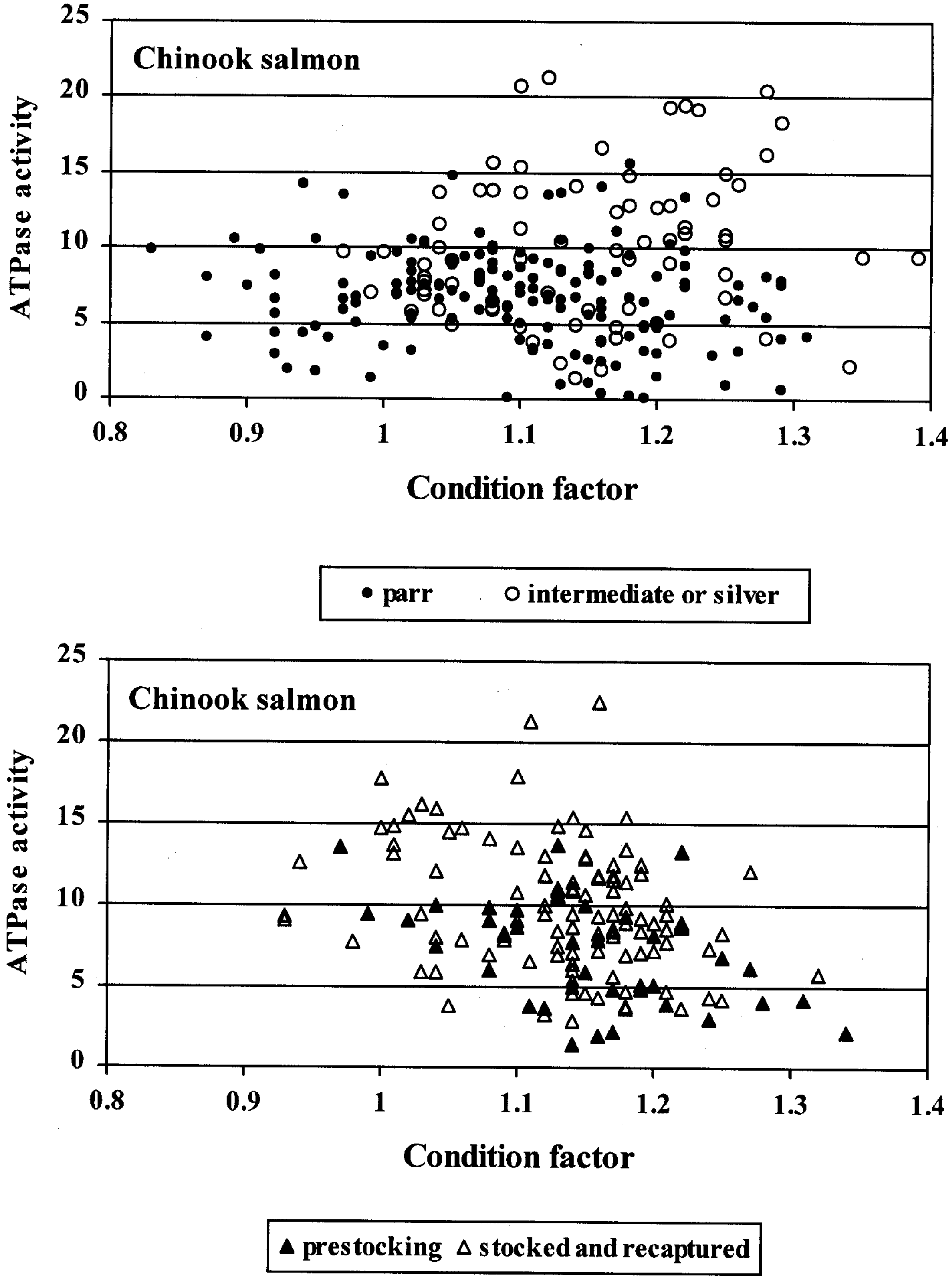

Figure 13. Chinook salmon ATPase activities versus condition factor in the hatchery; and in young-of-the-year fish just before stocking, and after recapture in the French River smolt trap.

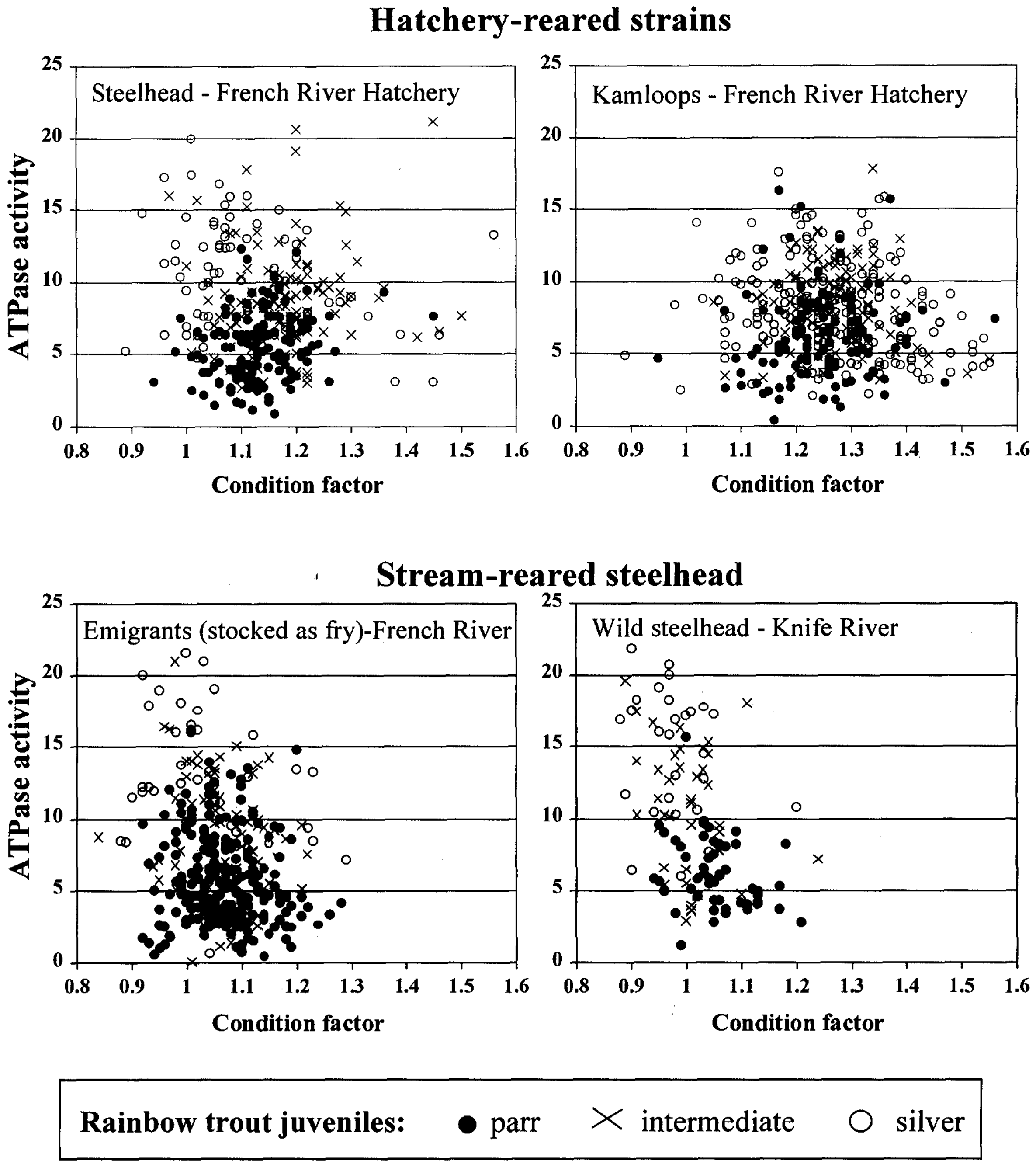

Figure 14. ATPase activity levels versus condition factor of anadromous rainbow trout populations, including two strains reared in the hatchery, and two groups reared in streams.

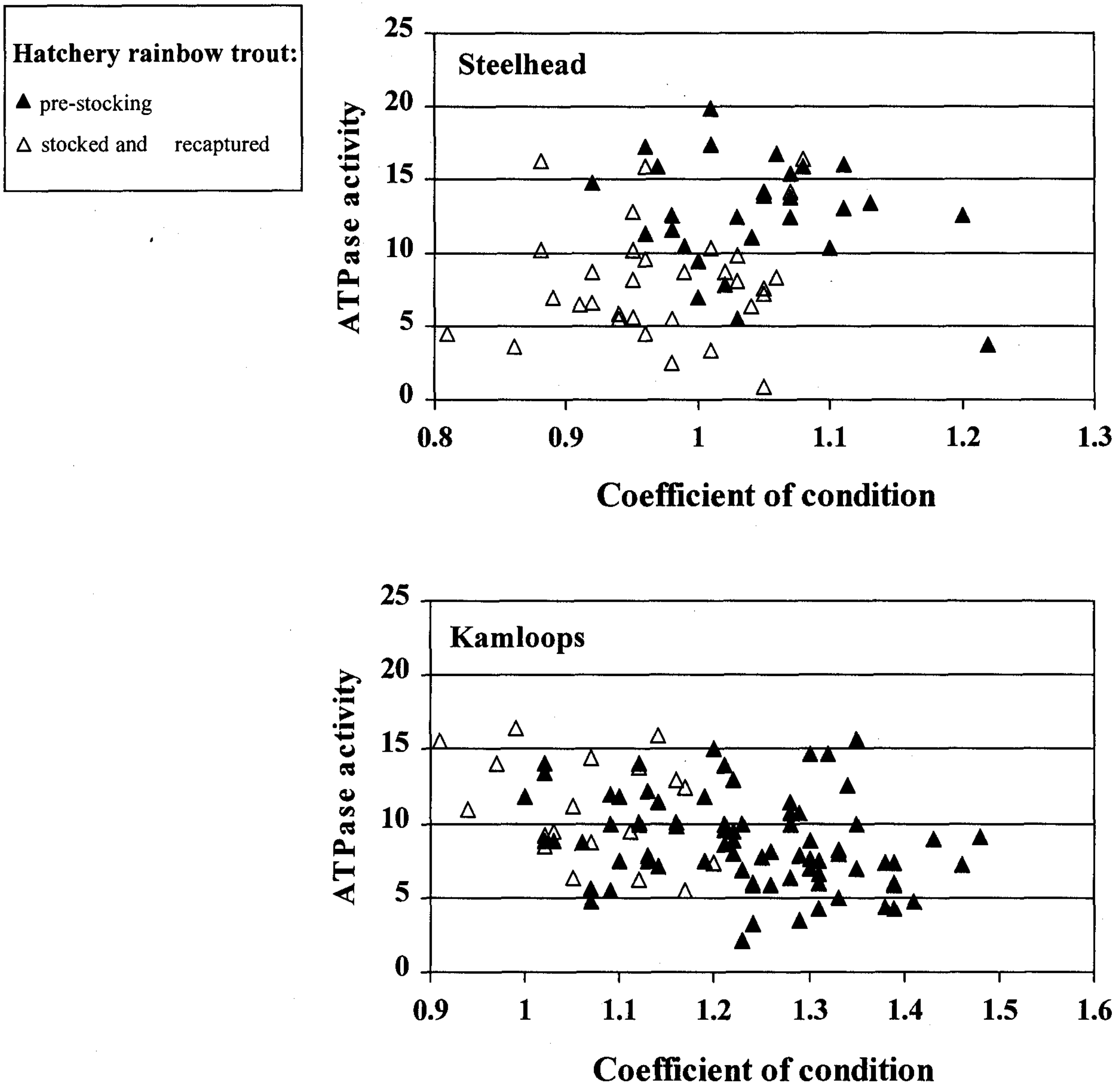

Figure 15. ATPase activity levels versus condition factor of steelhead and Kamloops yearlings just before stocking, and after stocking and recapture.

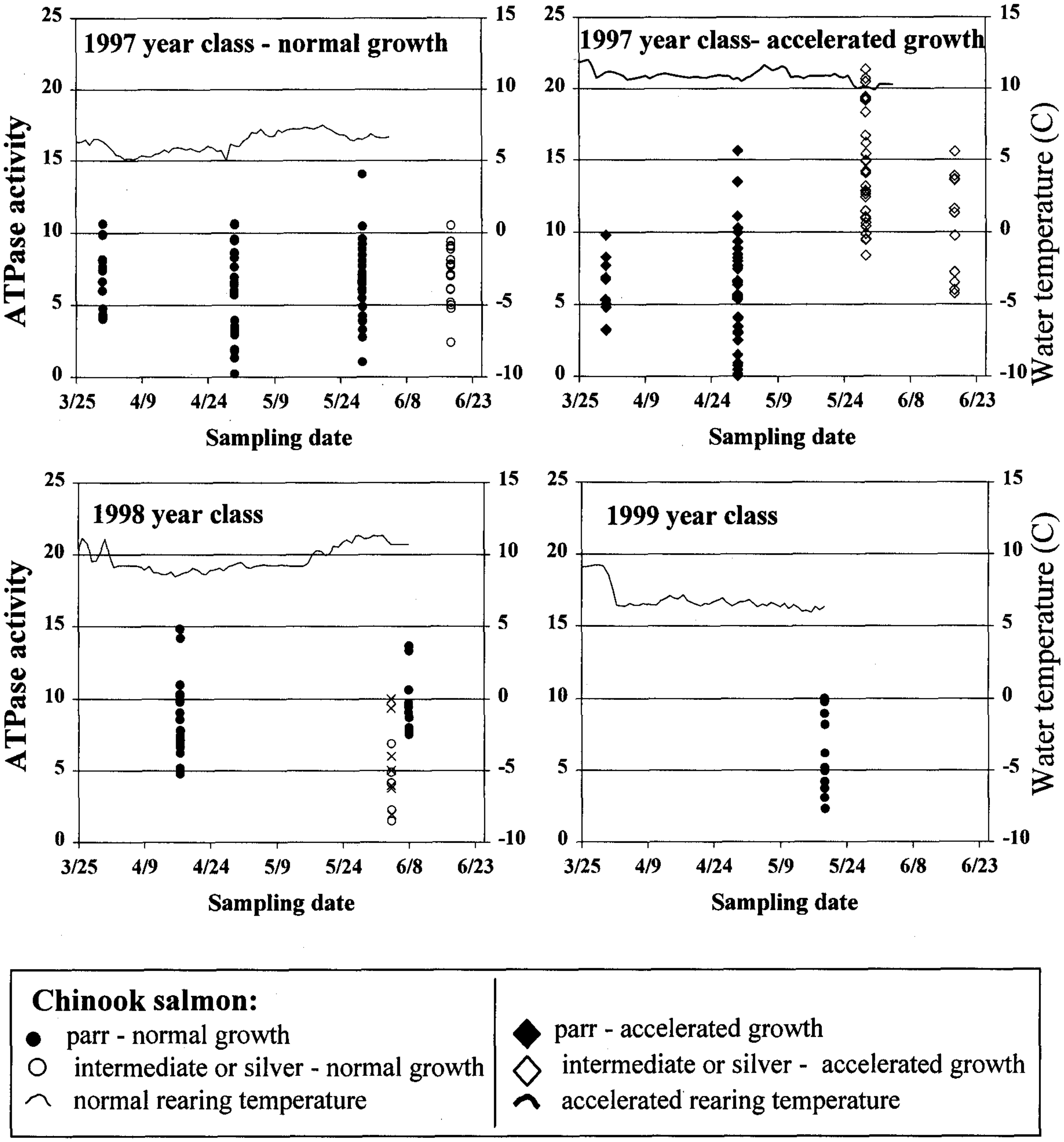

Figure 16. ATPase activity levels measured in chinook salmon in the hatchery on various dates, also showing rearing temperatures. Note that temperatures were not recorded in 1997 after early June, as most chinook salmon were stocked by that time. The last group sampled that spring had been held for two weeks at the Duluth Area Fisheries Headquarters in a tank with a mixture of French River water and Lake Superior water, maintained at approximately 10-12°C.

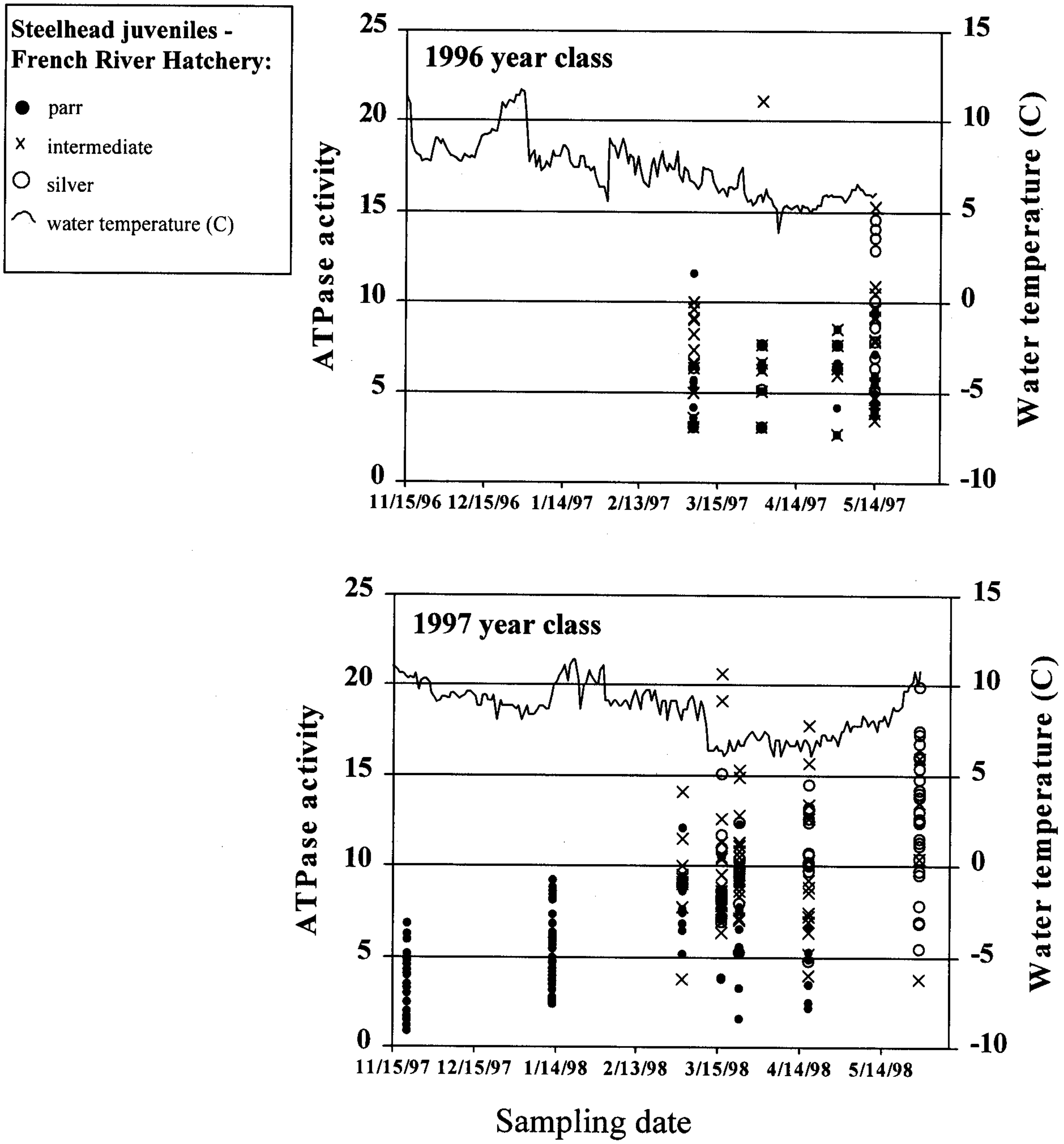

Figure 17. ATPase activity levels measured in hatchery steelhead on all sampling dates, also showing rearing temperatures.

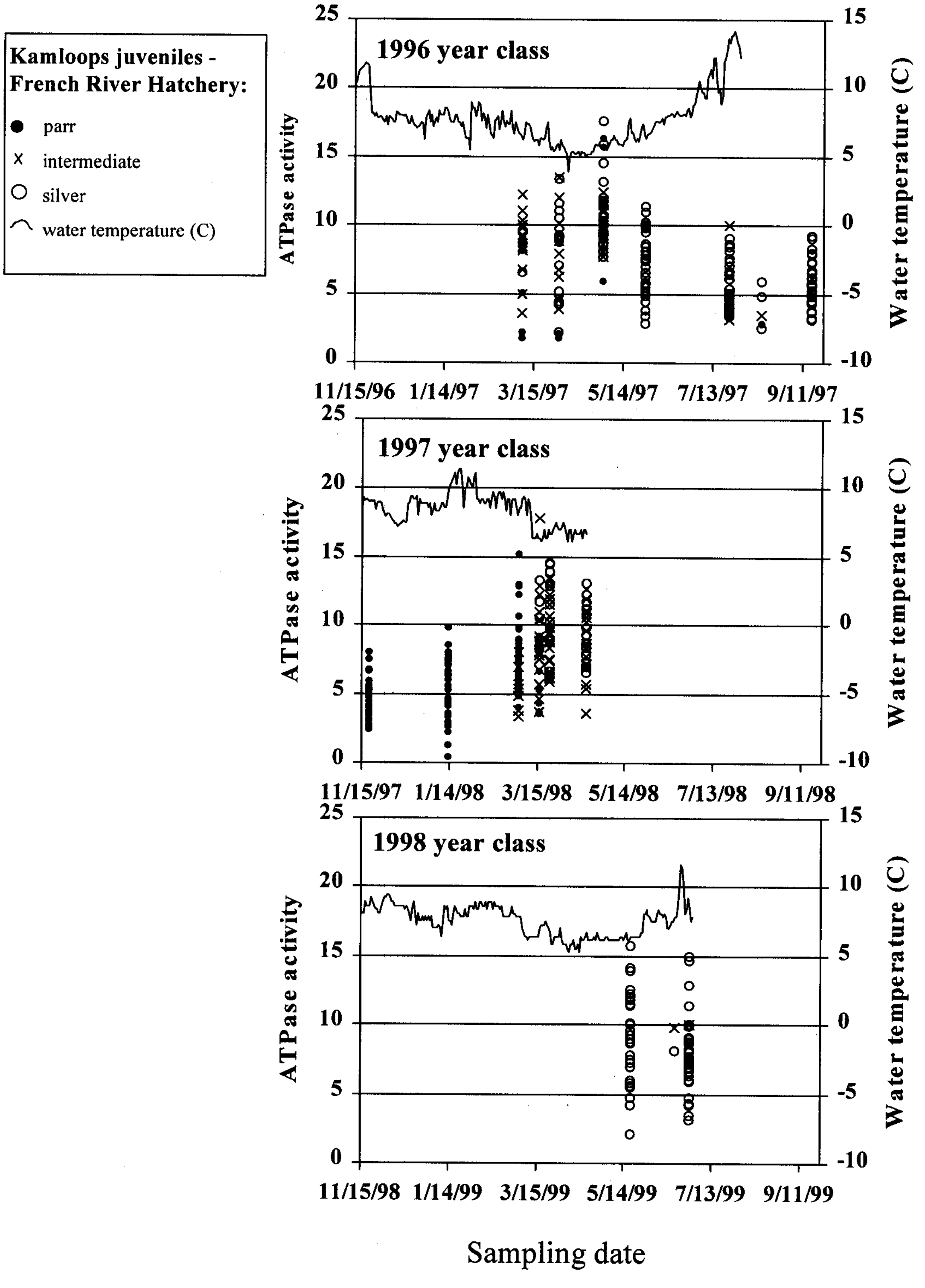

Figure 18. ATPase activity levels measured in hatchery Kamloops on all sampling dates, also showing rearing temperatures.

Table 5. Mean ATPase activities (N; Standard Deviation) in different color phases of each species/strain tested.

Species or strain	Rearing location	Color phase Parr	 Intermediate	 Silver
Chinook salmon	hatchery	6.9 (158; 3.1)	9.2 (111; 3.9)	10.9 (71; 4.7)
Steelhead	hatchery	5.6 (148; 2.4)	9.4 (129; 3.6)	10.5 (103; 3.9)
Kamloops	hatchery	6.2 (123; 2.9)	8.4 (116; 2.8)	8.2 (255; 3.3)
Steelhead emigrants (stocked as fry)	French River	5.8 (239; 3.1)	8.2 (89; 4.2)	12.5 (38; 4.6)
Wild steelhead	Knife River	5.9 (53; 2.5)	10.6 (41;4.4)	14.7 (27; 4.4)

activity (10 μmol P_i·(mg protein)$^{-1}$·hr^{-1}) for the onset of smoltification as hatchery steelhead and Kamloops (Table 4). Threshold sizes were smaller for these stream-reared emigrants, however, than for the hatchery fish (Figures 5-7). Only one fry-stocked steelhead emigrant exceeded 260 mm FL, the size above which little smolting was seen in hatchery fish, and no wild steelhead emigrants approached this size (Figures 5-7). Fry-stocked steelhead emigrants captured in the French River smolt trap, and wild steelhead emigrants captured in the Knife River smolt trap showed elevated ATPase activities on most dates sampled in the spring, but no elevated activities were found in the fall (Figures 19-21). The highest ATPase activity levels were seen in the stream-reared fish in May, and no clear relationship was seen between ATPase activity levels and water temperature (Figures 19-21).

Discussion

Hatchery stocks

The ATPase assay is an involved and exacting procedure requiring specialized equipment not ordinarily found in a fisheries management office. Reagents were perishable, and techniques required practice for accuracy and timing. For these reasons, I used the technique to define other indicators that will assist Minnesota fisheries managers in pinpointing the onset of smoltification in local populations.

The gradual, or two-step increase in gill ATPase activity with increasing size of chinook salmon in this study is not a unique observation. Ewing et al. (1979) found that larger chinook salmon usually had higher gill ATPase activities, with the highest levels seen in fish over 100 mm FL, as I found in this study. Hoar (1976) found that chinook salmon, unlike other salmonids, appeared to acquire salinity resistance gradually without sharp increases associated with smoltification. Ewing et al. (1979) suggested an 80 mm FL threshold as the minimal size at which chinook salmon could respond to photoperiod inducement of increased ATPase activity, while I observed elevated ATPase activities in chinook salmon as small as 71 mm FL in this study.

Hatchery-reared steelhead and Kamloops displayed elevated ATPase activity levels at larger threshold sizes than stream-reared fish, but factors that may affect smoltification such as crowding and disease treatments cannot be completely avoided in a hatchery. Rearing temperatures at the French River Coldwater Hatchery are kept below 13°C (Figures 16-18), a temperature reported to inhibit steelhead smoltification (Wedemeyer et al. 1980). In contrast, stream temperatures frequently exceeded 13°C during the spring emigration period (Figures 19 and 21). Even chinook salmon reared at "elevated" temperatures were held between 10-12°C (Figure 16), which is reported to be favorable for growth and survival of this species (Beckman et al. 1999). Steelhead and most chinook salmon were stocked during times of natural emigration, but some Kamloops were held well beyond this period.

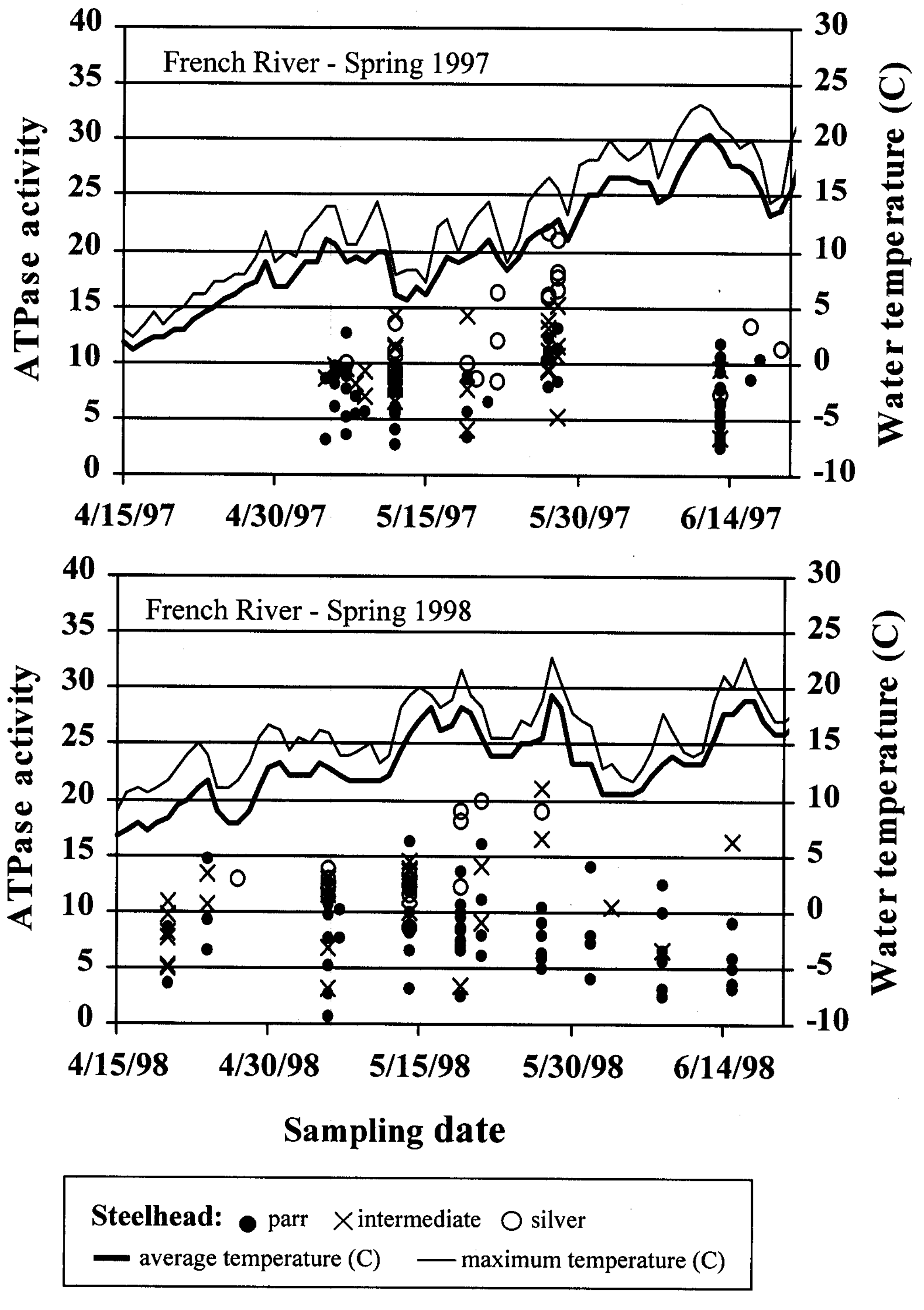

Figure 19. ATPase activity levels measured in steelhead (stocked as fry) emigrating in spring in French River, also showing daily average and maximum river temperatures.

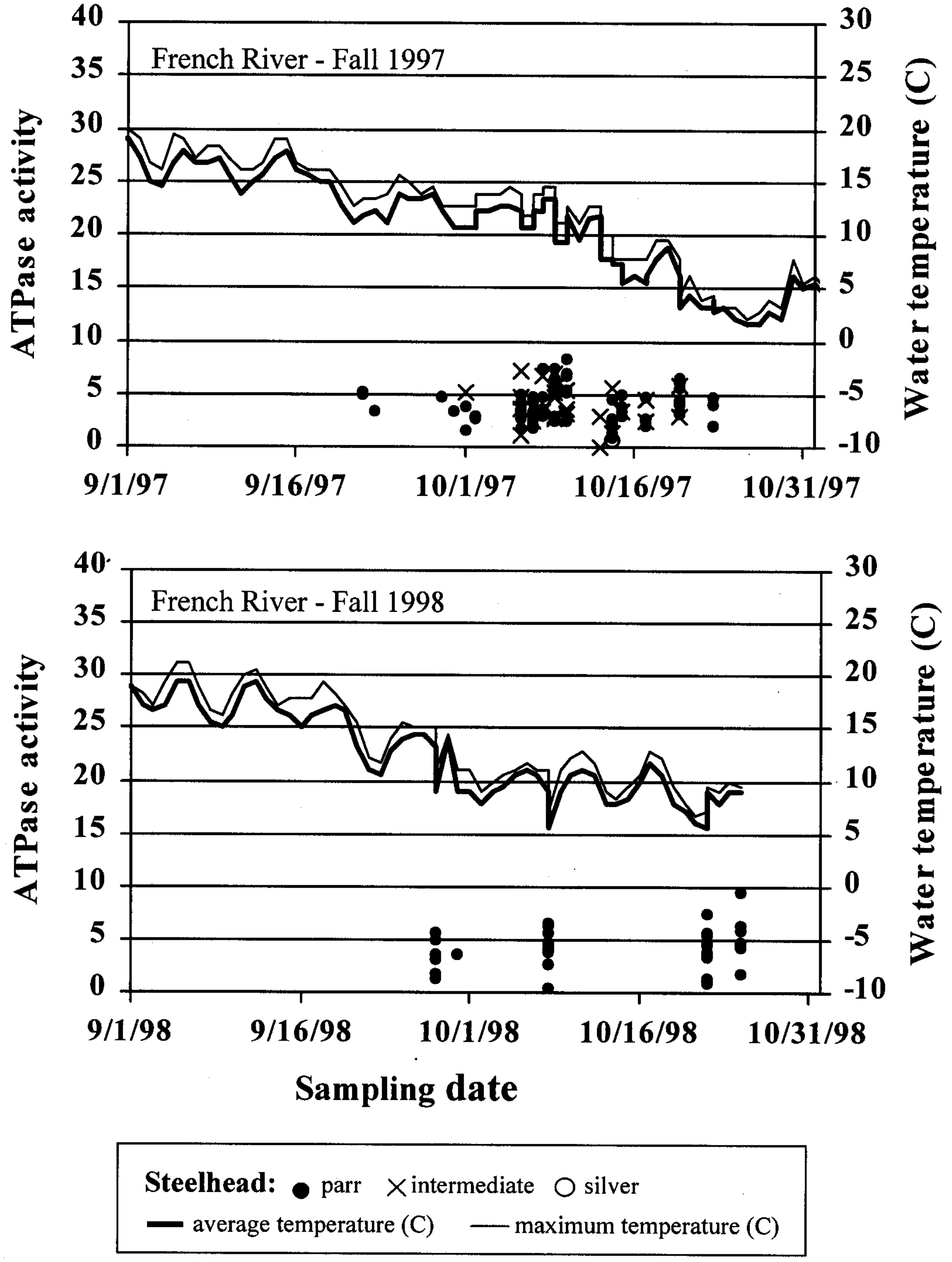

Figure 20. ATPase activity levels measured in steelhead (stocked as fry) emigrating in fall in French River, also showing daily average and maximum river temperatures.

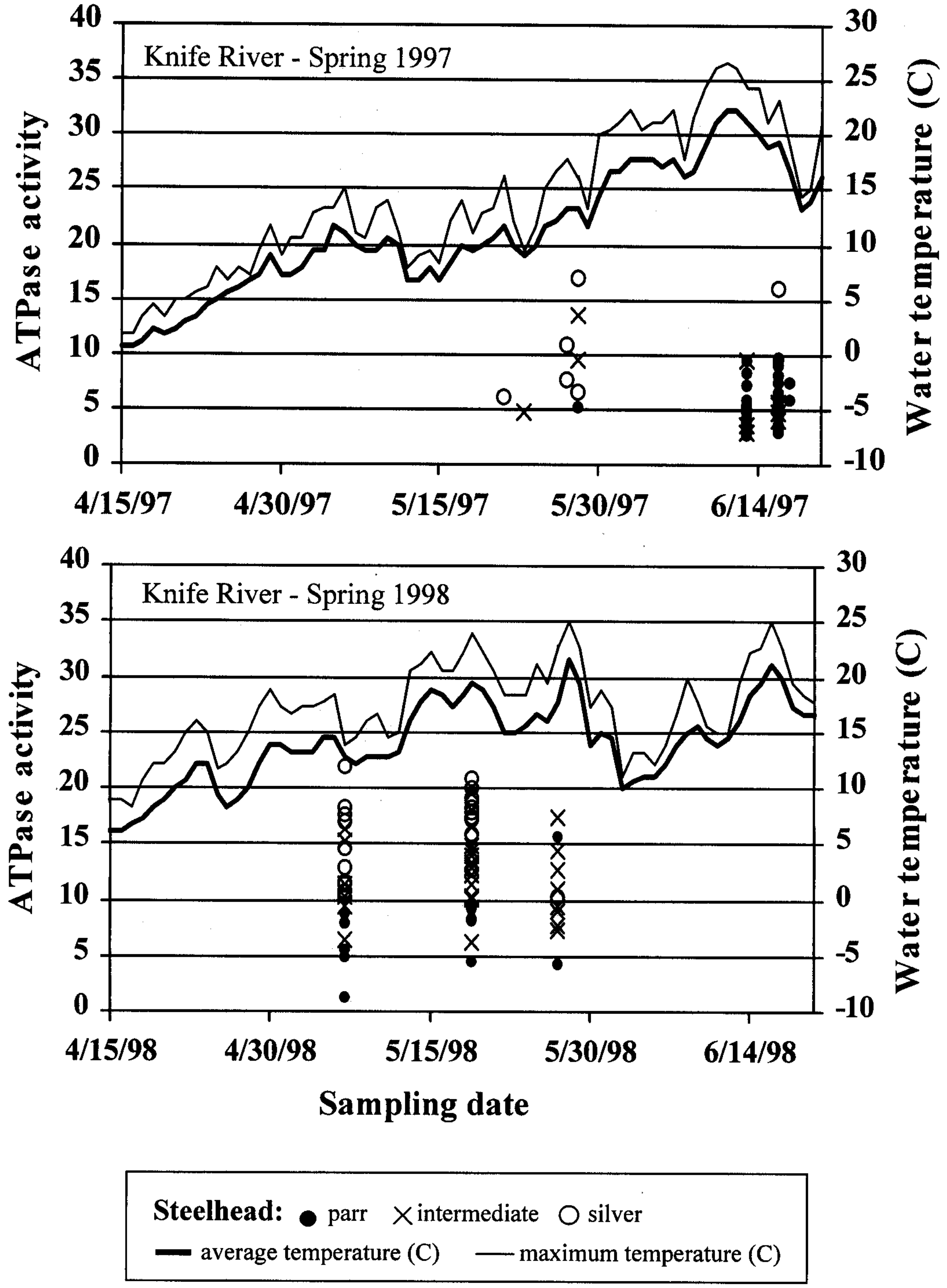

Figure 21. ATPase activity levels measured in wild steelhead emigrating in spring in Knife River, also showing daily average and maximum river temperatures.

Although mean ATPase activity differed significantly between each color category for all species/strains except Kamloops, color change was not a precise indicator of smolting for individual fish. Most rainbow trout above the threshold sizes had some color change (intermediate or silver), but all three colorations were found in smolting fish (indicated by elevated ATPase activities) of all species/strains. Threshold size (particularly FL) is a better guide than color for predicting the onset of smoltification in hatchery and stream-reared stocks in Minnesota.

Condition factor does not appear to be a good predictor of smolting in any of the Minnesota stocks tested (Figures 13 - 15). Stocked and recaptured rainbow trout and chinook salmon in this study had lower weights and condition factors than fish just before stocking, but weight loss immediately after stocking is not unusual for hatchery fish that have never foraged independently. Beckman et al. (1999) concurs that condition factor changes can occur for a variety of reasons, and are not a good indicator of smolt status. Condition factor was negatively correlated with ATPase activity for some steelhead in Washington and Oregon (Fessler and Wagner 1969; Zaugg and McLain 1972; Beeman et al. 1995; Tipping et al. 1995; Tipping and Byrne 1996), but a cause and effect was not demonstrated. Tipping and Byrne (1996) found that reducing feed caused faster emigration of hatchery-reared steelhead, although no chemical analyses were done to prove that the emigrants were true smolts. Self-thinning (Grant 1993) could have been a factor if the fish perceived that the environment was insufficient to support them. In fact, fasting has been found to inhibit elevation of gill ATPase in Atlantic salmon (Virtanen and Soivio 1985).

Hatchery imprinting or sequential imprinting may explain the high level of straying by stocked steelhead and chinook salmon in Minnesota waters. All yearling steelhead and Kamloops, and 91% of the y-o-y chinook salmon exceeded threshold sizes for smolting at the time of stocking, which suggests that these fish may have partially imprinted to hatchery water. Pascual et al. (1995) suggested that emigrating downstream during the appropriate season and physiological state are important to smolt transformation and imprinting, but fish may also remember odors experienced prior to this time. Maturing salmon tend to reverse the sequence of their outward migration as juveniles. According to Quinn et al. (1989), displaced salmon return first to the odors of their release site, and will continue to the rearing site (hatchery) if its odors can be detected. If there is no stimulus for further migration once they reach the release site, the fish will remain there (Dittman and Quinn 1996). Beckman et al. (1999) determined that promoting smoltification while chinook salmon were in the hatchery was important for post-release survival, but these fish (in a Columbia River tributary) were not being transported for imprinting in a remote location, they had to migrate hundreds of kilometers downstream, and salinity tolerance was imperative. Wagner et al. (1963) also found that hatchery-reared steelhead exhibited the highest survival as returning adults when released at 160 mm FL or longer (well within the range of smolt size determined in this study), but these fish were also not being transported to a remote location, and had ample emigration distance in which to smolt and imprint before reaching the ocean.

The issue of straying is important where there is a desire to maintain spatial segregation between naturalized and hatchery stocks. Some level of straying is natural in anadromous populations, and provides a means for expansion of habitat range. However, hatchery rearing and release techniques can sometimes increase straying, increasing the potential for interactions among hatchery and wild stocks, and the potential for substantial genetic interchange among populations (Quinn et al. 1991; Pascual et al. 1995). Although Kamloops stocking has been restricted to Lester River, Chester Creek, and French River to reduce the spatial overlap with steelhead during spawning runs, this strategy may be less effective than desired. Spatial overlap at French River is not considered a problem, however, since spawning habitat is practically nonexistent, and recapture of feral adults at this location is essential for hatchery production.

Chinook salmon released into the Columbia River during low flows and high temperature produced the lowest straying rates, possibly because fish stayed in the rivers longer, imprinting was facilitated at high temperatures, or the odors for olfactory imprinting were more concentrated at that time (Pascual et al. 1995). However, when flows are low in Minnesota tributaries, fish may be trapped in the stream by gravel bars, and mortality can be high due to bird predation and warm temperatures. The conspicuous mortalities associated with this type of situation create very poor public relations for the Department of Natural Resources, so stocking directly into Lake Superior is preferable at these times.

Exposure to stream water alone may not be sufficient for imprinting. Exposure to flowing water and undergoing the actual migratory experience are most effective for imprinting (Beeman et al. 1995; Pasqual et al. 1995; Dittman et al. 1996). The chinook salmon sampled on 18 June 1997 had been held in a tank of French River water for two weeks to increase their exposure to imprinting odors in a protected environment. ATPase activity levels measured on that date tended to be lower than those measured in the hatchery just 19 days earlier, indicating a reduction in smoltification, and bringing into question the efficacy of this procedure. Stress from being moved, crowding, or reduced flow may have contributed to the reduced ATPase levels. Prior imprinting in the hatchery would also predispose them to return to French River, any additional imprinting could only help, and the benefits of a shorter exposure to flowing water in the mouth of the river at an earlier (and possibly more appropriate) date are debatable. Returns of adults from 2000-2002 may reveal whether the 1997 year class had superior survival or imprinting.

Smoltification is transient, and fish retained in a hatchery for some weeks after the usual time of migration, or after the beginning of physiological changes can revert to pre-smolt condition (Fessler and Wagner 1969; Zaugg and McLain 1972; Hoar 1976; Pascual et al. 1995). This reversion to pre-smolt condition was apparent in nearly all hatchery rainbow trout in this study larger than 260 mm FL, 200 g, and 60 mm body depth (Figures 5-7). Not surprisingly, all but one stream-reared fish sampled emigrated at smaller sizes. Stream-reared steelhead emigrated primarily from early May through June, so this may be the most appropriate time to stock fish. Emigration timing by wild chinook salmon in Minnesota waters is unknown, but data from the Brule River in Wisconsin show that most smolt as y-o-y in May and June (DuBois and Pratt 1994). This is also the time hatchery stocks are planted in Minnesota, so continuation of this practice is recommended.

Smoltification was stimulated to some extent by stocking in chinook salmon and Kamloops, but not in steelhead. As steelhead was the only species/strain with mean ATPase activity above the threshold of smoltification prior to stocking, their physiological state was apparently different, and they may have handled the stress of stocking differently. The extremely short (1 km) distance between stocking and recapture locations provided a minimal trial of stocking response. Most (93%) of the chinook salmon that were stocked and recaptured had emigrated immediately after stocking, and 50% of the steelhead and Kamloops yearlings that were stocked and recaptured had emigrated within one week. The rapid emigration of some pre-smolts along with smolting chinook salmon may have been a schooling behavior, while other pre-smolts remained in the stream until smoltification. Fish that were not recaptured after stocking in this study (excluding Kamloops stocked in June 1999 prior to the flood) may have died or residualized upstream, neither of which is a desired outcome.

Seelbach et al. (1994) suggested that minimizing straying by stocking upstream outweighs the costs of higher mortality, when the goal is to create fisheries in specific locales. Seelbach and Miller (1993) found that hatchery steelhead "smolts" (mean length 195 mm TL) stocked 5.5 km upstream in the Huron River (a Michigan tributary to Lake Superior) emigrated quickly (50% within 8 days) and strayed widely. Stocked parr suffered high mortality in tributaries to Lake Michigan, smaller stocked "smolts" (a designation based on appearance) suffered higher mortality than larger "smolts", and "smolts" stocked farther upriver had higher mortality than those stocked into river mouths (Seelbach et al. 1994). However, "smolts" stocked into river mouths strayed much more than those stocked upriver (as far as 240 km).

Stream-reared steelhead

Stream-reared steelhead displayed elevated ATPase activity levels at smaller threshold sizes than hatchery-reared steelhead or Kamloops. Color was not a precise indicator of smolting for individual stream-reared steelhead, although most silver-colored fish were smolts. Elevated ATPase activities were found in fish of all three colorations. Condition factor was also not a good predictor of smoltification. The threshold length for smolting steelhead in French and Knife Rivers was equivalent to the length that differentiates age 1 from age 2 fish in May in these rivers (Olsen et al. 2000; Spurrier et al. 2000a), suggesting that smolting emigrants were at least age 2. These results eliminate early smolting as an explanation for the occurrence of small emigrants. Capture of emigrants in the Knife River trap has been significantly correlated with lower flows, and captures are rare during spate flows, so high water does not provide an explanation for most premature emigration (Morse and Olsen 1999).

Early emigrants of fry-stocked and wild steelhead in Minnesota's tributary streams have poor survival. Scale samples taken from steelhead emigrants (originally stocked as fry) in the French River from 1995 to 1999 revealed that about 81% were under age 2, whereas only about 10% of adults returning from 1992 to 1998 had emigrated before age 2 (Olsen et al. 2000). Scale samples taken from wild steelhead emigrants in the Knife River from 1997 to 1999 revealed that approximately 86% were under age 2, whereas only about 16% of the adults returning from 1996 to 1998 had emigrated before age 2 (Spurrier et al. 2000a). Most adults had emigrated at age 2 in both rivers. Because high numbers of age 1 emigrants are captured in both the French River and Knife River smolt traps, they would appear to be a natural occurrence in this area, and not simply the result of over-stocking. Hassinger et al. (1974) noted similar, but less severe mortality in early emigrants from Kimball and Kadunce creeks (located about 13 km northeast of Grand Marais, Minnesota), where 31% of wild steelhead emigrated before age 2, while only 12% of spawning adults had emigrated before age 2. Even in the Brule River, a larger more productive Wisconsin tributary to Lake Superior, wild steelhead showed a similar pattern with 59% emigrating before age 2, while only 3% of the returning adults had emigrated before age 2 (Scholl et al. 1984). Premature emigration is not universal, as only 5% of steelhead in a large (over 65 km) Oregon river emigrated before age 2, similar to the emigration rates determined from returning adults (Wagner et al. 1963). Seelbach (1993) also found few emigrants under age 2 in the Little Manistee River, a Lake Michigan tributary about 107 km long with abundant groundwater, stable flows, and limited spawning habitat (which would limit the density of parr). Juvenile steelhead evidently remained in these Oregon and Michigan rivers until they imprinted and smolted, with little premature emigration.

Self-thinning due to intraspecific competition (Grant 1993; Marschall and Crowder 1995) has been suggested as a reason for premature emigration in North Shore streams, but this concept does not fully explain downstream movement by these steelhead parr. The self-thinning theory implies that the streams are being stocked or naturally recruited above their carrying capacity for age 1 parr. However, the number of age 1 emigrants captured at a trap on Little Knife River (a branch of Knife River) is directly correlated with the number of age 2 emigrants from the same year class (Spurrier et al. 2000b; Figure 22), suggesting that when conditions permit better survival to age 1, more fish are likely to survive to age 2. If these year classes were approaching carrying capacity for age 1 fish, one would expect similar numbers of age 2 emigrants each year despite fluctuations in age 1 emigrants. Leider et al. (1986) found that many pre-smolt steelhead in a tribu-

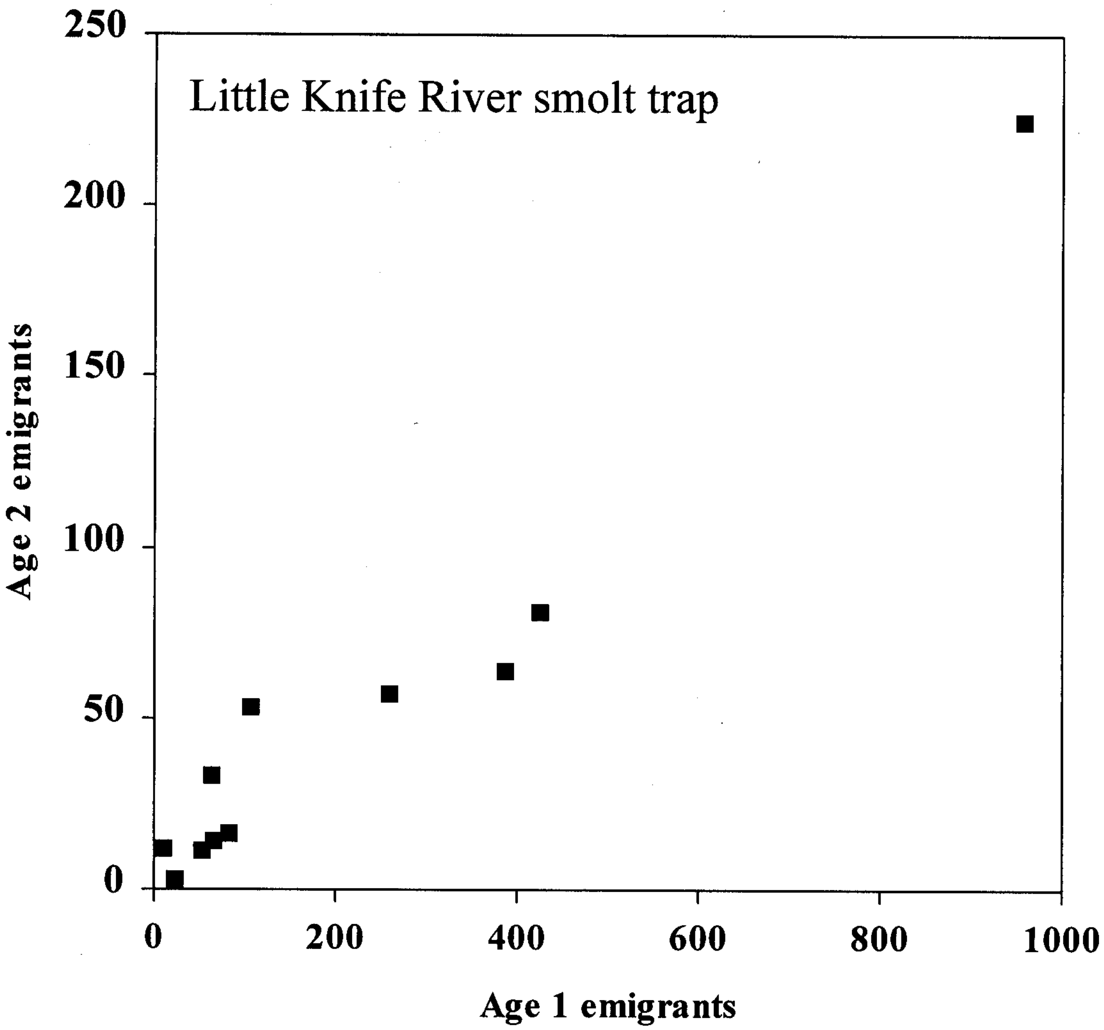

Figure 22. Wild steelhead emigrants captured in the Little Knife River smolt trap. Each point represents fish from a single year class, from 1987 to 1997 (Duluth Area Fisheries file data).

tary of the Kalama River system in Washington moved down to the main stem, some of these fish later moved back up the tributary, but many stayed in the main stem until smolting the following year. Thus, survival of a majority of the steelhead appeared to depend on availability of suitable rearing environments in downriver areas (Leider et al.1986). Close and Anderson (1992) found that even age 0 parr migrated downstream as much as 5-10 km after being stocked as fry in North Shore streams. Minnesota's tributaries are relatively short and have high gradients which would preclude upstream movement by parr. I speculate that natural in-stream movement results in the premature emigration of naive parr, these fish quickly reach the cold, predator inhabited waters of Lake Superior at a very edible size, and prospects for survival are poor.

General conclusions

Minnesota's Lake Superior tributaries are not native habitat for anadromous Pacific salmonids, and can be inhospitable for these species. In years with favorable temperatures and flows, growth and survival of stocked or wild fry can produce good year classes. Ideal stocking densities can be calculated, and watershed improvements may provide some benefits in terms of carrying capacity, but natural weather events are beyond our control and have overriding impacts on fish survival in the streams. Low productivity in the streams limits carrying capacity at the best of times, and the cold unproductive waters of Lake Superior are no less formidable. Rainbow trout survive here at the edge of their thermal tolerance (Pauley et al. 1986; Wismer and Christie 1987), and predation and competition in Lake Superior may be intense (Negus 1995). Tributary streams are extremely short for sufficient imprinting of stocked "smolts size" fish, and holding the fish in the hatchery beyond their natural smolting period increases straying. Simply put, habitat and hatchery options are limited, and each stocking strategy has its tradeoffs.

A summary of the conclusions for each of my initial objectives follows: 1) Steelhead, Kamloops, and some chinook salmon are currently held in the French River Coldwater Hatchery beyond the onset of smoltification. Evidence of smolting was seen primarily in chinook salmon reared at or above 9°C. Some rainbow trout (particularly Kamloops) are even held past the period of smoltification and regression. 2) Some smoltification was stimulated by placement of chinook salmon and Kamloops in a flowing stream, but steelhead (already displaying elevated ATPase levels) did not show a similar response. 3) Emigrating steelhead (fry-stocked or wild) do not smolt before age 2 and many are emigrating prematurely. 4) No external feature can be used to verify smoltification of individual fish with absolute certainty, but threshold sizes, coloration, and time of year can bracket when smoltification is imminent. 5) This study and the accompanying literature review helps to explain the fate of premature emigrants and the extent of straying by anadromous species in Minnesota waters. While habitat is limiting, some management and stocking strategies have been derived that should improve imprinting in desired locations.

Management implications

Several of the concepts and stocking practices outlined here have been a part of fisheries management on Minnesota's North Shore in recent years, but current data justify and reinforce their importance and consistent application.

1) No single external feature can be used to verify that an individual fish is smolting on any given day. However, fish above threshold sizes, having intermediate or silver coloration, in spring (April - June) probably are, have, or will undergo smoltification within a short period of time, and imprinting may be underway. Fish that fit these criteria should be stocked in the location to which imprinting is desired, especially if that location is not French River.

2) Survival of larger stocked yearlings is not always superior to survival of smaller yearlings, and imprinting to locations away from the hatchery may be compromised in

larger fish; thus return to the fishery is uncertain. Greater returns of larger post-smolt fish are seen at French River, since it receives the hatchery outflow to which the fish are partially imprinted. Other locations may not experience similar returns when large fish are stocked.

3) Hatchery chinook salmon, steelhead, and Kamloops should be stocked in May and June, to mimic the natural timing of smoltification. Many are presently stocked during this period. Stocking before the first of May is precluded by ice conditions, high spring runoff, and the rainbow smelt spawning run which must be avoided to prevent bycatch of smolts by dipnetters.

4) Fish stocked at locations other than French River should be placed some distance upstream from the mouth to allow imprinting, and some flexibility in stocking times is needed to avoid extremely low water periods. Stocking below headwater areas where brook trout reside is recommended to avoid negative impacts on resident populations. In streams that contain steelhead y-o-y, stocking of "smolt size" fish at the lower end of nursery areas is necessary to avoid competition with, or predation on the y-o-y. Apparently an emigration distance of as little as 1 km can be enough to stimulate smoltification in some fish, so relatively short distances may be better than stocking in river mouths. Fish stocked into French River cannot be released until the spring spawning run of steelhead and Kamloops is completed, so that smolts do not interfere with seining for brood stock.

5) Chinook salmon smoltification appears to be influenced by temperature to some extent. Increasing temperature early in the rearing process should help to bring 100% of the y-o-y to the threshold size of 71 mm FL by May. Chinook salmon above threshold sizes that will be stocked in locations other than French River should be maintained at 5-7°C to reduce imprinting in the hatchery. Placement at several locations or on several days from May through June may reduce immediate mass-emigrations of non-smolts along with smolts. Stocking into water at or above 10°C is recommended to enhance smoltification and imprinting at that time. Increasing the rearing temperature to about 10-12°C on chinook salmon to be stocked in French River is recommended to stimulate smoltification, which could improve imprinting for these fish.

6) Smaller steelhead should be stocked at locations other than French River in May, and larger fish at French River in June. These yearlings could be graded prior to stocking to separate smaller fish from larger fish, or the extra month of rearing may be sufficient to increase growth and imprinting by French River stocks.

7) Based on the current size structure of stocked Kamloops yearlings (with all FL exceeding the threshold for smolting), the smaller fish should be stocked at locations other than French River in May to improve imprinting, and the larger fish should be stocked at French River or Bluebird Landing in June. Kamloops stocked at Bluebird Landing should be well above the threshold size for smolting to promote imprinting in the hatchery. If the fish destined for French River/Bluebird Landing are all large, stocking directly into French River may be possible, as the large fish are more likely to emigrate immediately. This could reduce straying to Sucker River, which is located within 1 km of Bluebird Landing.

8) Enhancing wild stocks and reducing hatchery residence time for propagated fish is advisable due to inherent disadvantages of hatchery rearing. Managers should further evaluate the present mixed strategy of stocking both steelhead fry and yearlings. Stocking of steelhead fry offers a better chance for imprinting than stocking larger sizes, although instream mortality is obviously higher for fry. Stocking fry as far upstream as possible (but below headwater brook trout habitat), would provide more opportunity for instream movement, which is nearly all downstream, before they encounter Lake Superior.

9) Upstream stocking of very large (greater than 260 mm FL, 200 g, or 60 mm body depth) steelhead or Kamloops should be avoided, as these fish may have already smolted, and may tend to residualize (remain in the stream) without resuming smoltification.

10) Straying by chinook salmon reduces fishing opportunities at destined locations

during spawning runs, but has little effect on the trolling fishery. Natural reproduction in Minnesota tributaries by chinook salmon is probably quite limited by lack of habitat, but is widespread in other parts of the lake. These stocks have only inhabited Lake Superior since the 1970s, so preservation of a differentiated wild stock is not yet a recognized management goal.

11) Straying by Kamloops and steelhead yearlings not only reduces fishing opportunities at destined locations, but increases the risk of mixing between hatchery and naturalized strains. As our ability to reduce straying is limited, the risk of Kamloops hybridization with the wild steelhead population must be weighed in future management decisions.

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Appendix 1

The role of Na^+-K^+-ATPase activity in physiological adaptation to saltwater:

The concentration of Na^+ and Cl^- ions in the blood plasma of fish is intermediate to the concentration found in freshwater and seawater, so their bodies must constantly work to maintain this difference in concentration. Much of the passive diffusion and active transport involved in maintaining the proper concentration of ions takes place in the gills. When anadromous species migrate downstream in their native coastal habitat, gill chloride cells enlarge and proliferate, which is associated with an increase in the activity of Na^+-K^+-ATPase. These specialized chloride cells contain the Na^+-K^+-ATPase system which actively transports Na^+ out of the cell in exchange for K^+ (Moyle and Cech 1982). The total ionic concentration in the plasma must be maintained at about one-third that of seawater, but several factors work against that lower concentration. Gills have a relatively high permeability to monovalent ions, so Na^+ and Cl^- move passively from seawater into the plasma. Also, water diffuses passively from plasma into the environment. Seawater is ingested to replace lost water, but additional dissolved ions are absorbed in the intestine along with the ingested water. Thus excretion of ions through the gills is necessary to maintain a reduced plasma concentration, since teleostean kidneys are unable to form a urine more concentrated than the blood (Moyle and Cech 1982). This need to excrete monovalent ions in seawater is a marked change from the situation in freshwater, which has a much lower ion concentration than blood plasma. In freshwater, small ions such as Na^+ and Cl^- are continually lost to the environment by diffusion across the gill epithelia, and more ions are lost in large volumes of dilute urine that is produced to expel excess water that diffuses passively into the gills. Specialized chloride cells containing Na^+-K^+-ATPase on the gill filaments actively transport Na^+ and Cl^- into the plasma, but these cells are less numerous than the chloride cells in marine fish that transport ions out of the plasma (Moyle and Cech 1982). Changes in habitat from freshwater to saltwater require adaptive changes in ion-regulatory abilities, which can be recognized through increased Na^+-K^+-ATPase activity in the gills. Despite the fact that anadromous rainbow trout and chinook salmon living in Lake Superior and its tributaries never encounter seawater, the native physiological adaptations of these species persist.

An explanation of the assay for ATPase activity:

ATPase hydrolyzes adenosinetriphosphate (ATP) to liberate inorganic phosphorus (P_i). The activity of Na^+,K^+-activated ATPase in gill filaments is demonstrated by adding ATP to the prepared tissue in a solution containing Na^+ and K^+, and letting the hydrolysis occur for 10 minutes at 37°C. The evaluation procedure then consists of two assays: one to measure the liberated P_i, and one to measure protein levels which indicates sample size. A precisely measured amount of gill filament is not critical, because the results of this evaluation are reported as a rate: amount of liberated P_i per unit protein per hour. There are several types of ATPase, and the one of interest is sensitive to the presence of ouabain, which is an inhibitor for the active transport mechanism in cells. Thus the P_i assay plate tests aliquots of each sample in two types of buffer, one without ouabain (which measures ouabain sensitive plus ouabain insensitive activity) and one with ouabain (which measures ouabain insensitive activity). The ouabain sensitive activity is calculated as the difference between the two measured activities. The ouabain sensitive activity of ATPase is reported as $\mu mol\ P_i \cdot (mg\ protein)^{-1} \cdot h^{-1}$.

Appendix 2

Mean differences (in mm) between fork length and total length of all fish sampled, within different ranges of fork length.

		Fork length range				
Species or strain	Rearing location	50-100mm	100-150mm	150-200mm	200-250mm	>250mm
Steelhead	hatchery	4	6	8	9	8
Kamloops	hatchery	4	6	8	8	9
Steelhead emigrants (stocked as fry)	French River	6	9	12	13	17
Wild steelhead	Knife River	7	9	12	14	

		Fork length range			
		50-75mm	75-100mm	100-125mm	>125mm
Chinook salmon	hatchery	6	8	9	11

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