COLUMBIA RIVER SALMONID OUTMIGRATION:

McNARY DAM PASSAGE AND ENHANCED SMOLT QUALITY

Completion Report

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EXECUTIVE SUMMARY

We evaluated the effects of the McNary Dam transportation system on emigrating fall and spring chinook smolts using physiological indices of stress (e.g., plasma cortisol, hepatic glycogen, leucocrit, interrenal cell nuclear diameter) and performance tests (e.g., saltwater challenge, secondary stress challenge, disease resistance). We also conducted controlled experiments in a hatchery environment to characterize the fishes' physiological responses to stress to allow a basis for judging the nature of the stress experienced by smolts in the system at McNary Dam. Based on these studies, we concluded that:

- Juvenile fall chinook were stressed, apparently in a cumulative manner, by elements of the collection system at McNary Dam.
- Changes in the collection system between 1982 and 1983 decreased the total stress experienced by fall chinook collected.
- There were seasonal variations in some physiological responses to stress, probably the result of changes in the environment.
- Optimum length of time for fall chinook to recover from the stresses of collection was 12 to 48 h.
- Fish collected during the day under artificially darkened conditions were not as stressed as fish collected under sunlight, and they recovered faster.
- The maximum raceway density of 0.5 lbs·gal^{-1} was not excessive.
- Fall chinook which were anesthetized, handled, and marked were no more stressed than fish which just went through the collection system but require at least 24 h to recover from marking before transport or liberation.
- Pre-anesthetization of fall chinook entering the marking facility appeared to attenuate the degree of stress to which fish were subjected during anesthetization and handling.
- Loading fish into either barge or truck was the most stressful event in the transportation procedure; however, unloading and liberating needs to be evaluated.
- The transport vehicles were not measurably stressful and the fish showed some recovery from the stress of loading while enroute.
- The maximum loading density of 0.5 lb·gal^{-1} for trucks was not excessive.

TABLE OF CONTENTS

GOAL AND OBJECTIVES

The ultimate goal of this study, as originally stated, was to increase yield (catch and escapement) of salmonids in the Columbia River. Our principal objectives were (1) evaluate the McNary Dam smolt bypass and collection facility; and (2) propose and evaluate methodologies that minimize stress caused by bypass, collection, and handling. These objectives were accomplished by testing the following operational objectives and null hypotheses:

1. Evaluate the stress imposed by passage and collection at McNary Dam.

   1a. Ho: Dam passage and collection is not stressful and certain features (e.g., bar-sorters) encountered by migrants at dams are not more stressful than other features of the bypass and collection system.

   1b. Ho: Recovery time after dam passage and collection is not necessary to ensure optimum smolt quality and performance at the time of loading for transportation or at the time of release.

2. Evaluate the cumulative stress imposed by dam passage, collection, and transportation.

   2a. Ho: Fish transported after collection at dams are not more stressed than those either transported directly from hatcheries or allowed to migrate after dam passage.

3. Evaluate the effects of anesthetics, handling, and marking on smolts collected at McNary.

   3a. Ho: Anesthetized and handled fish are not more stressed than fish bypassed or collected at dams.

3b. Ho: Recovery period for optimum performance for fish anesthetized at dams is not longer than the time needed to regain swimming activity.

4. Evaluate means of alleviating the severity of stress from bypass, collection, handling, and transportation.

4a. Ho: Severity of stress does not differ during the course of the smolting cycle.

4b. Ho: Loading density at McNary holding facilities is not important in determining performance of fish.

4c. Ho: The density at which fall chinook are transported from McNary Dam to Bonneville Dam is not important in determining performance of fish.

INTRODUCTION

The construction of hydroelectric dams on the Columbia River and its main tributary, the Snake River, has greatly altered this ecosystem and affected the anadromous salmonids in the system. Dams have reduced the river flow (Raymond 1979, Bentley and Raymond 1976), increased water temperature (Bentley and Raymond 1976), and increased gas supersaturation (Ebel and Raymond 1976). Completion of the last four dams on the Columbia and Snake Rivers resulted in a two- to three-fold increase in the time required for salmonid smolts to migrate downriver (Raymond 1968, 1969, 1979), and it has been hypothesized that this has increased the exposure to predation and pathogens and has increased residualism among smolts (Bentley and Raymond 1976). Moreover, environmental modifications in the Columbia River system have been beneficial to native and exotic predators (Stainbrook 1983; Maule and Horton 1984; Gray et al. 1984) and, perhaps, competitors (Hjort et al. 1981). These factors have resulted in reduced survival of salmonids (chinook salmon and steelheads) migrating from the upper Snake River, such that in years of very low water flow, survival to The Dalles Dam may be as low as 5% (Raymond 1979). Ultimately, this reduced survival of emigrants is reflected in as much as a 10-fold reduction in percent of emigrants returning as adults (Raymond 1979).

Columbia River hatcheries have increased production as part of a strategy to increase numbers of returning adults. In the mid-1960's, the annual Columbia River system smolt migrations were estimated to be 3 to 5 million fish of wild origins (Raymond 1979). In 1984, more than 23 million smolts, mostly hatchery production, passed McNary Dam (Koski et al. 1985).

Various management strategies have been aimed at modifying the dams to eliminate gas supersaturation problems, divert fish away from turbine intakes, and bypass fish downstream of the dam. Additionally, smolts are now collected at the dams, transported in trucks or barges, and released below Bonneville Dam, the last downstream barrier on the Columbia River (Delarm et al. 1984). Transportation of some salmonid species has shown positive results when compared to non-transported migrants, but the percent of smolts returning as adults remains low (Park et al. 1983).

The collection and transportation of smolts places physiological demands on the fish which may result in reduced fitness to perform activities necessary for survival (Schreck 1981). In this study, we evaluated the collection and transportation system at McNary Dam (Fig. 1) to determine which elements of the system were the most stressful to emigrating salmonids, and proposed methodologies to minimize the impact of those stressful elements. Our conceptual framework in the study was based on the model proposed by Schreck (1981, 1982) in which an organism's ultimate capacity to perform physiological tasks, or performance capacity, is determined by the environment and stress, which can act physiologically and psychologically to reduce performance capacities. For example, as smolts migrate downstream, they have individual abilities or capacities to resist disease or avoid predators. When these fish encounter the stresses of a hydroelectric dam, their ability to perform these tasks, or performance capacity, may be reduced.

Figure 1. The mid and lower Columbia and Snake Rivers.

In this study, we collected emigrating salmonids from various points within the collection and transportation system and monitored various physiological indices of stress (e.g., plasma cortisol, hepatic glycogen, among others). At the same time, we subjected fish to several secondary challenges (i.e. saltwater, secondary stress, disease) to measure their actual performance capacities. Thus we describe the fishes' physiological status and the effects of various elements in the system on the ability of the fish to perform tasks relevant to survival.

GENERAL METHODS

During the course of this study, methods and experimental design were changed to fit the needs of the particular investigation. However, certain aspects of the methods were the same throughout the study and these methods are presented here.

Clinical Indices of Stress

Sample collection. Whenever fish were collected for plasma or tissue samples, they were immediately transferred by dipnet into a bucket containing 200 $mg \cdot L^{-1}$ tricaine methanesulfonate (MS-222). It has been shown that at this level, MS-222 does not significantly alter plasma cortisol levels (Strange and Schreck 1978) or other physiological variables (Black and Conner 1964; Houston et al. 1971). This method has been successfully used for rainbow trout (Barton et al. 1980) and was verified by Barton (unpublished data) for juvenile fall chinook by placing fish in 200 $mg \cdot L^{-1}$ MS-222 and serially sampling them through 30 min in one experiment and 45 min in another. There were no systematic differences in plasma cortisol in either experiment.

As soon as fish were immobilized in MS-222, we severed the caudal peduncle and collected blood in heparinized 0.25 ml capillary tubes. Blood samples were centrifuged, and the plasma was removed and stored at -20 C. Additional blood was taken for hematocrit and leucocrit determination (McLeay and Gordon 1977), and blood smears were made for blood cell counts. We also removed livers which were stored in cool 30% KOH, and head kidneys which were stored in 10% phosphate-buffered formaldehyde.

Sample analyses. Hematocrit and leucocrit were recorded at the site. Blood smears were air dried, fixed with 95% ETOH and, at a later date, stained with Giemsa stain. Following the method of Weinreb (1958), we examined the smears at 1000x magnification and counted the number of white blood cells (WBC) found within the area of 300 erythrocytes. WBC identification was based on descriptions of Anderson (1974) and Yasutake and Wales (1983). Thawed plasma was assayed for cortisol using a radioimmunoassay (Redding et al. 1984), glucose using the O-toluidine method (Hyvarinen and Nikkila 1962 as cited by Wedemeyer and Yasutake 1977) and lactate using a fluorimetric, enzyme reaction (Passonneau 1974). Hepatic glycogen was assayed by a phenol-sulfuric acid method (Montgomery 1957) that was previously modified and verified in our laboratory for salmonids. Dimensions of interrenal cell nuclei were determined from photomicrographs of Harris' hematoxylin and eosin-stained, six-micron-thick sections. The slides were viewed using a fixed-position projector and screen such that the interrenal cells were projected at 1,769x magnification. Length and width measurements of 20 nuclei were averaged to get the score for each fish. Proximate analysis of whole-fish body composition was obtained by drying whole fish and determining fat content with a Goldfisch ethanol fat extractor, and ash content by incineration at 500 C in a muffle oven; an estimate of protein was obtained by subtracting fat and ash from dry weight. Plasma and gill filaments were taken during each sampling period. As indices of smoltification, we determined plasma thyroxine using the radioimmunoassay method of Dickhoff et al. (1978); gill Na-K ATPase activity was determined for us by Dr. W. Zaugg, National Marine Fisheries Service, Cook, Washington.

Performance Challenges

Osmoregulatory challenge. During each sampling period in 1982, 1983, and 1984, fish at McNary Dam and those transported to Bonneville Dam were collected and exposed to sublethal osmoregulatory challenge. Normal plasma Na levels of smolts in fresh water are between 140-150 meq$\cdot L^{-1}$. When a smolt is put in salt water, plasma Na increases to as high as 210 meq$\cdot L^{-1}$. The smolt's ability to decrease plasma Na is a measure of that fish's osmoregulatory capacity which will decrease if the fish is under stress. In 1982, fish were rapidly transferred from the site of collection to buckets containing salt water (Marine Environments, Inc.). The fish and salt water were then poured into 38 L aquaria, also containing salt water. A salt concentration of 15.0 ± 1.0 parts per thousand was selected after a bioassay established this salt level as being tolerable by the salmon. Compressed oxygen was bubbled into each aquarium. The aquaria were in a flow-through water bath so that ambient river temperature was maintained. After 24 h in salt water, all fish were bled, and plasma was collected and stored at -20 C. Total plasma sodium (meq. L^{-1}) was assayed with a flame photometer. In 1983 the SW challenges were changed, in that spring chinook or fall chinook were held in dark plastic buckets containing 20.0 ± 1.0 ppt salt water, and the challenges ended after 18 h. These changes were made to eliminate the transfer procedure, increase the osmotic challenge, and reduce the time allowed for recovery. Samples collected in 1983 and 1984 were assayed using a Na-K analyzer.

Secondary stress challenge. The secondary stress consisted of capturing fish in a dipnet and holding them out of the water for 30 s. We do not believe that this is a stress that smolts would normally encounter, but rather we used it as a uniform challenge to help clarify the degree of stress previously encountered by the fish. We used this same standardized stress in our stress characterization studies. During 1982, the secondary stress test was conducted on three groups of fish which had been in a raceway for 0, 1 or 7 d, and fish which had been transported to Bonneville Dam. During each sampling period in 1983 and 1984, we challenged fish which had been in the raceway for less than 4 h, fish from the gatewell, and fish transported to Bonneville Dam. In 1984, we also secondarily stressed fish which had been in the raceway for 24 h. In each instance, after the stress, the fish were released into 100 L tanks with flow-through water and were serially sampled through 24 h.

Swimming performance. In 1984, we conducted experiments designed to assess the effects of collection and transport on the swimming ability of juvenile fall chinook (see Stress and Swimming Performance for a detailed description of apparatus). Swimming tubes were used to measure fatigue time--the length of time that a fish can maintain a given swimming speed. Replicate groups of fish were removed from raceways at various times following collection at McNary Dam and placed into duplicate swim tubes. Following a 10-min acclimation period with minimal flow through the tubes, water velocity was increased to 25 $cm \cdot s^{-1}$ for 30 min. Water velocity was then increased to 60 $cm \cdot s^{-1}$ and the time at which each fish stopped swimming was noted. Similar experiments were performed with fish held in tanks following transport to Bonneville Dam.

Hatchery and Emigrating Fish as Controls

On May 28, 1982, approximately 150 fall chinook from Priest Rapids Hatchery were transported to McNary Dam and were placed in a large metal tank (ca. 0.6 x 0.6 x 1.8 m) with flow-through river water (see: Disease Challenge for details of the transportation). An additional 150 fish were held in a 1.2 m circular fiberglass tank with flow-through water at Bonneville Dam. These fish were to serve as controls, to establish baseline resting levels of the clinical indices of stress and baseline performance capacities for the challenge tests. After being held for 2 weeks, the fish appeared to be in worse condition than fish coming through the collection system. Daily mortalities were high and many fish had fungus. Approximately 2 weeks prior to our sampling in July and August 1982, 150 fall chinook were removed from the holding raceway at McNary Dam and held in conditions as described above. An additional 150 fish were removed from a transport truck at Bonneville Dam and also held. We reasoned that in 2 weeks these fish would acclimate to the holding facilities and could serve as controls for the June and July sampling. These fish also suffered high mortalities and obviously did not adapt to captivity. We did obtain plasma and tissue samples from these fish and assayed these for clinical indices of stress. The results confirmed that these fish did not acclimate. Those results are not included in this report so as not to confuse the results of the other sampling. Similarly, fall chinook from Priest Rapids Hatchery were transported to the OSU Marine Science Center to be controls for the disease challenge and seawater growth experiments. Although these fish were used in the experiments, their very poor quality and survival, independent of the experiments, negates their value as controls.

Statistical Analyses.

We performed one-way and two-way analyses of variance (ANOVA) and then made multiple comparisons using Fisher's Least Significant Difference test (LSD test) to identify those pairs of data points which were significantly different at the $P < 0.05$ level (Nie et al. 1975). We also compared linear regressions of time course data (Neter and Wasserman 1974); however, this type of analysis had limited application as most of the curves could not be linearly transformed without significant reduction in correlation coefficient.

SYSTEM EVALUATION

Experimental Rationale and Methods

Collection facility. The McNary Dam collection system is intended to divert emigrating smolts away from the turbines and shunt them to the downstream side of the dam. Here they are either returned immediately to the river, or held prior to being transported and released below Bonneville Dam. Fish encountering the dam are diverted into turbine intake gatewells by submersible traveling screens (Fig. 2). From the gatewell, fish are shunted through sluiceways and pipes with various water velocities and pressures to the downstream side of the dam. Included in this system is a vertical pipe approximately 12-m long by 0.5 m diameter. On the downstream side of the dam, fish surface in an upwelling-box, cross a perforated, stainless steel plate which reduces water volume, and encounter a bar-sorter which is spaced such that smolts pass between the bars while adult fish and trash continue to the end of the bars and return to the river. The bars are positioned just below the water level of the encompassing metal box (ca. 5,800 L). Fish voluntarily move from this box to another system of pipes and sluiceways through which they can be diverted to a raceway, the subsampling system (see below), or back to the river. Modifications of specific portions of this system occurred between 1982 and 1983 smolt emigrations and are outlined in Delarm et al. (1984).

Fall chinook smolts were collected at McNary Dam June 14-24, July 7-16, August 2-11, 1982; June 14-19, July 7-13, August 1-4, 1983; and June 12-15, July 10-14, August 7-10, 1984. Spring chinook were sampled during May 3-6 and May 23-26, 1983. We monitored changes in degree of smoltification and

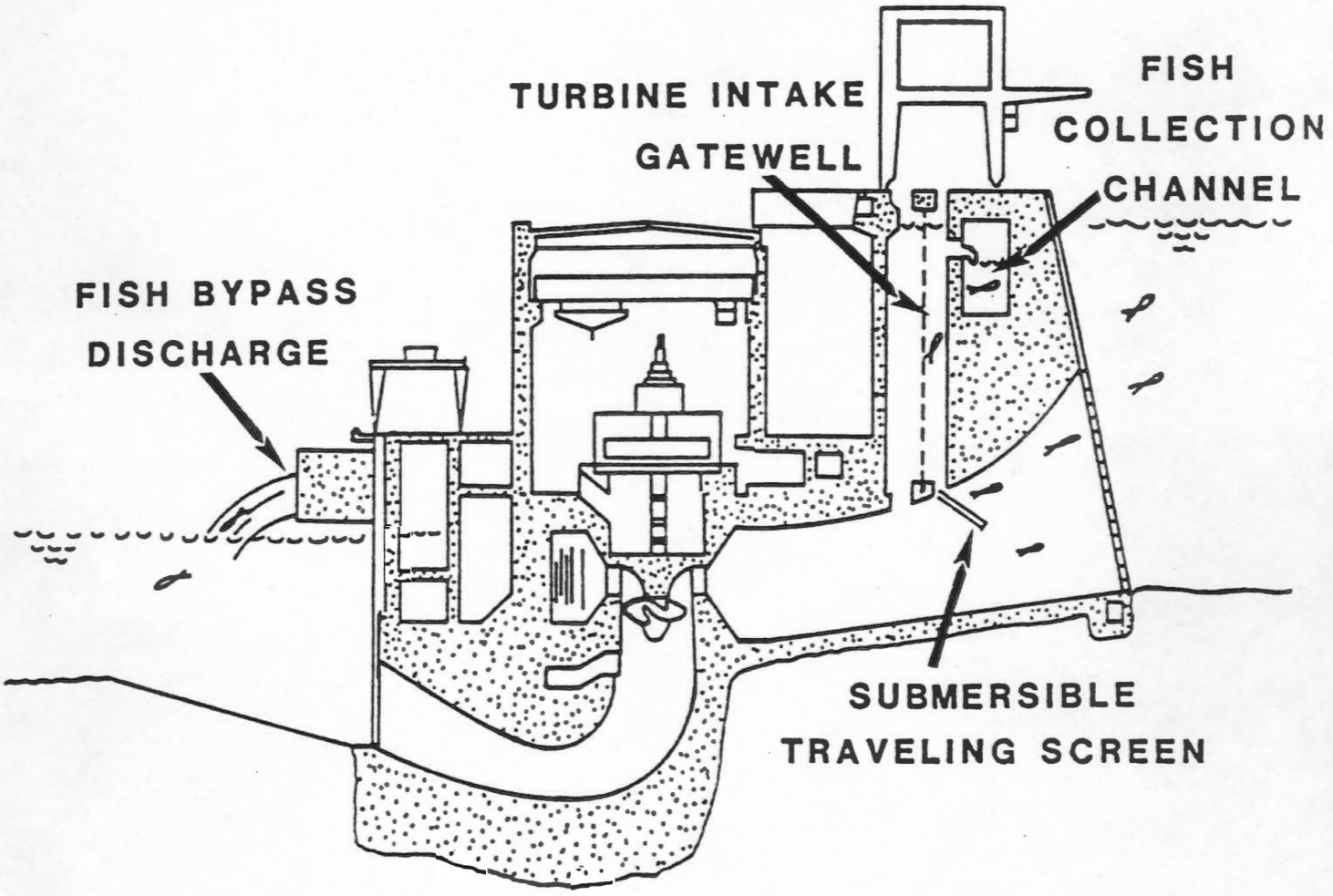

Figure 2. Cross section through a typical dam powerhouse, showing various elements of the fish bypass system (from: Smith and Wold 1982).

changes in the environment. However, we did not monitor place of origin, i.e. stock differences, of fish sampled. During each sampling period, we collected plasma and tissue samples to monitor clinical indices of stress and we measured smolts' performance in challenge tests. The first place we collected smolts was the turbine intake gatewell, using the gatewell dip-basket technique described by Bentley and Raymond (1968). At the gatewell, the fish have been minimally manipulated by the system. After the fish passed to the downstream side of the dam, they were collected just before the bar-sorter, as they entered the raceway, and at various time points after they entered the raceway. In 1982 we sampled fish after they had been in the raceway for 1, 2, 4, and 8 d. Based on our 1982 results and to examine the short-term effects of holding fish in the raceway, in 1983 and 1984 we sampled fish which had been held for 1, 3, 6, 12, 24, 48, and 72 h. In order to conduct valid tests, the raceways needed to be filled to normal operational fish density, but it was necessary that all fish entered the raceway at approximately the same time so that we would know how long a given fish had been in the raceway. For example, during the early and late portions of the runs, it took over 24 h to fill a raceway to a density of 0.25 $lb \cdot gal^{-1}$ (0.03 $kg \cdot L^{-1}$) which is one-half the maximum allowable limit. To solve this problem, in 1982 we loaded as many fish as possible for a maximum of 4 h, at which time we crowded the fish to one end of the raceway and put a barrier screen in the raceway. This proved unsatisfactory because it required additional manipulation of the fish, and we had mechanical problems with the barrier screen. In 1983, we constructed a 3 x 6 x 10 ft (0.9 x 1.8 x 3.0 m) livecage of PVC pipe covered on all sides but the top with 0.125-inch (3.17 mm) knotless nylon mesh

netting. When suspended in the raceway, the livecage encompassed approximately 790 gal (3,000 L) of water, and fish were collected directly into it via the established collection system. The livecage solved the problems encountered with the mechanical crowding procedure and was used again in 1984.

As another indication of degree of stress encountered in the system, in 1983 and 1984 we examined recovery rates of fish collected at the various sampling points. Fish taken from before the bar-sorter and the raceway were held in small (ca. 25 L), dark plastic buckets furnished with continuous flow-through river water, 12 fish per bucket. Large (ca. 100 L), dark plastic tanks with flow-through river water, 100 fish per tank, were used for fish taken from gatewell and transport vehicles (see: Transport Evaluation) as it was necessary to collect a large number of fish at one time at these stations. Fish thus collected were serially sampled through 24 h.

Raceway density evaluation. It has been shown that the density at which fish are held can have physiological consequences (Fagerlund et al. 1981; Patino and Schreck, unpublished data); in fact, holding fish at extremely high densities (i.e., confinement in a dipnet or small livecage) has been used as a chronic stressor (Strange and Schreck 1978; Barton et al. 1980). In addition to our regular raceway sampling, during August 12-14, 1982, we examined several raceway densities at McNary Dam to determine the optimal density to hold emigrant fall chinook (Objective 4b). We had intended to repeat this in July 1983; however, the vagaries of the fish run prohibited this replication (i.e., too many fish in the system in July and too few in August). As has been indicated, it was difficult to obtain enough fish

through the collection facility on any single day to adequately assess the effects of various raceway loading densities. During the August 1982 tests, we simulated various densities by first loading the raceways to a given weight of fish and then crowding them so that the fish weight available to raceway volume was either 0.50, 0.25, or 0.13 $lb \cdot gal^{-1} \cdot min^{-1}$ (0.06, 0.03, 0.015 $kg \cdot L^{-1} \cdot min^{-1}$), 0.5 $lb \cdot gal^{-1} \cdot min^{-1}$ being the maximum production value in use. Since up to 24 h were required to fill the raceways, fish at the time of crowding had been in the raceway from 0 to 24 h. We designated these samples as Crowded, t = 0. The fish were also sampled 24 h later (t = 24 h).

Darkened collection facility. During July 15-20, 1984, we studied the effects of darkening the elements of the McNary Dam collection facility. Our experimental design was aimed at testing the hypothesis that darkening the collection facility would reduce the severity of the collection stress or hasten recovery from the stress. To this end, we did paired comparisons of the system with the upwelling box, bar-sorter, and raceway covered to eliminate as much light as possible (covered treatment) versus the system exposed to sunlight (exposed treatment).

We shaded the upwell box and bar-sorter by laying a double thickness of black, 1/4-inch netting over three sides of the guardrail which surrounds the structure. We then covered the top and fourth side of the structure with 4-mil black plastic which was attached to PVC plastic frames and laid across the guardrails. This system allowed us to shift easily from the covered to exposed mode and did not interfere with Corps of Engineers personnel monitoring the system. We covered the flume between the

bar-sorter and raceway with black plastic or plywood which remained in place throughout all tests. Alternate raceways were covered with black plastic. Fish were loaded into a raceway for a maximum of 2 hours and, after we covered or uncovered the upwell box and bar-sorter, fish were immediately shifted to the other treatment. This resulted in some fish entering the system under one treatment, but being held in a raceway of the opposite treatment. This was unavoidable as we wanted to maintain comparable starting times for the paired raceways. Moreover, we believe that the number of fish treated in this manner was minimal, as fish were entering the system at a high rate (ca. 10,000 fish$\cdot$hour^{-1}). Fish were sampled as they fell into the raceway, after being in the raceway 1, 3, 6, and 11 hours and just before being loaded into a truck for transport to Bonneville Dam. All paired comparisons were replicated four times and each began as early in the day as possible (i.e. 0600 to 0800 hours).

Anesthetic, handling, and marking. The primary tools on the Columbia River to study emigration patterns and to evaluate the efficacy of management practices, such as transporting smolts, is the marking of smolts by cold-brand and the insertion of coded wire tags (CWT) into smolts' snouts. Marking fish requires that they be anesthetized and handled, and it is assumed that upon release these fish behave and survive similarly to the general smolt population. In order to determine if anesthetized and marked fish are stressed more or less than fish just collected at the dam (Objective 3a) and to determine optimum time for recovery from the marking procedures (Objective 3b), we investigated this system during July 12-14, 1982, and July 10-14, 1983. As indicated in the description of the collection facility, after fish have passed below

the bar sorter, a subsample can be taken for marking. From the bar-sorter, fish passed along a sluiceway to a large, stainless steel box (ca. 7,000 L). The water in this box flushed the fish through electronic counters and into a larger, aluminum holding tank (ca. 13,750 L). Each day, the fish accumulated during the previous 24 h were crowded and then dipnetted from the holding tank and passed, via a stainless steel chute into an anesthetic bath containing MS-222 at a concentration of approximately 50 $mg \cdot L^{-1}$. Workers examined the anesthetized fish and recorded species, degree of descaling, and presence of brands. Unmarked, non-descaled fish (fall chinook at the time of our study) were shunted to the marking stations in a flow of anesthetic-laden water. At the marking station, workers clipped off the fish's adipose fin, applied a freeze brand, and inserted a CWT. Marked fish were shunted in fresh water to a raceway or directly into a transport-truck tank. We collected fish from the holding tank, the anesthetization bath, and after they were fully marked (adipose fin clipped, branded, and CWT). In 1982 we held fully marked fish in a large plastic tank (ca. 100 L) with flow-through water, and sampled them after 24 and 48 h to determine recovery rates. In 1983 we collected fish which had been fully marked with a brand and CWT and fish which had received a clipped adipose fin and brand, but not a CWT. These groups were held in similar plastic tanks and were serially sampled through 72 h.

Pre-anesthetization study. During July 1984, we conducted experiments designed to investigate the potential benefits of exposing collected fish to an anesthetic bath before entering the marking facility described above. The Corps of Engineers built a stainless steel pre-anesthetization box which fit into one section of the larger holding tank. One side of this box was

perforated so that excess water would flow out of the box. Smolts were diverted from the electronic counters to the pre-anesthetization box via a 15-cm diameter flexible hose. After approximately 100 fish were collected (2-3 minutes), the box was lifted partially out of the water to reduce the contained volume, and MS-222 was added to a final concentration of approximately 50 $mg \cdot L^{-1}$. After the fish were obviously anesthetized (i.e. displayed loss of equilibrium), they were transferred to the marking shed via dipnet and examined for marks in the normal manner. After this examination, the fish were put into a large (100 L) opaque, plastic tank with flow-through river water, from which they were serially sampled through 24 h to estimate recovery. Fish used as controls were collected in the holding tank, transferred by dipnet to the marking shed, anesthetized, examined for marks, and then held in a large opaque plastic tank and serially sampled through 24 h. This experiment was repeated on three successive days.

Results and Discussion

Collection facility. Smolts passing through the collection facility are stressed, apparently in a cumulative manner, by the elements of the system. In 1982, plasma cortisol levels increased as fish progressed through the system, until some time after they reached the raceway where the cortisol levels peaked and returned to relatively low levels within 4 d (Fig. 3). Plasma cortisol levels in fish held for 8 d in the early run were elevated (Fig. 3), indicating that holding fish beyond 4 d might be counter-productive. The late-run fish had significantly higher cortisol levels in the gatewell, pre-sorter, and early raceway samples which we attributed

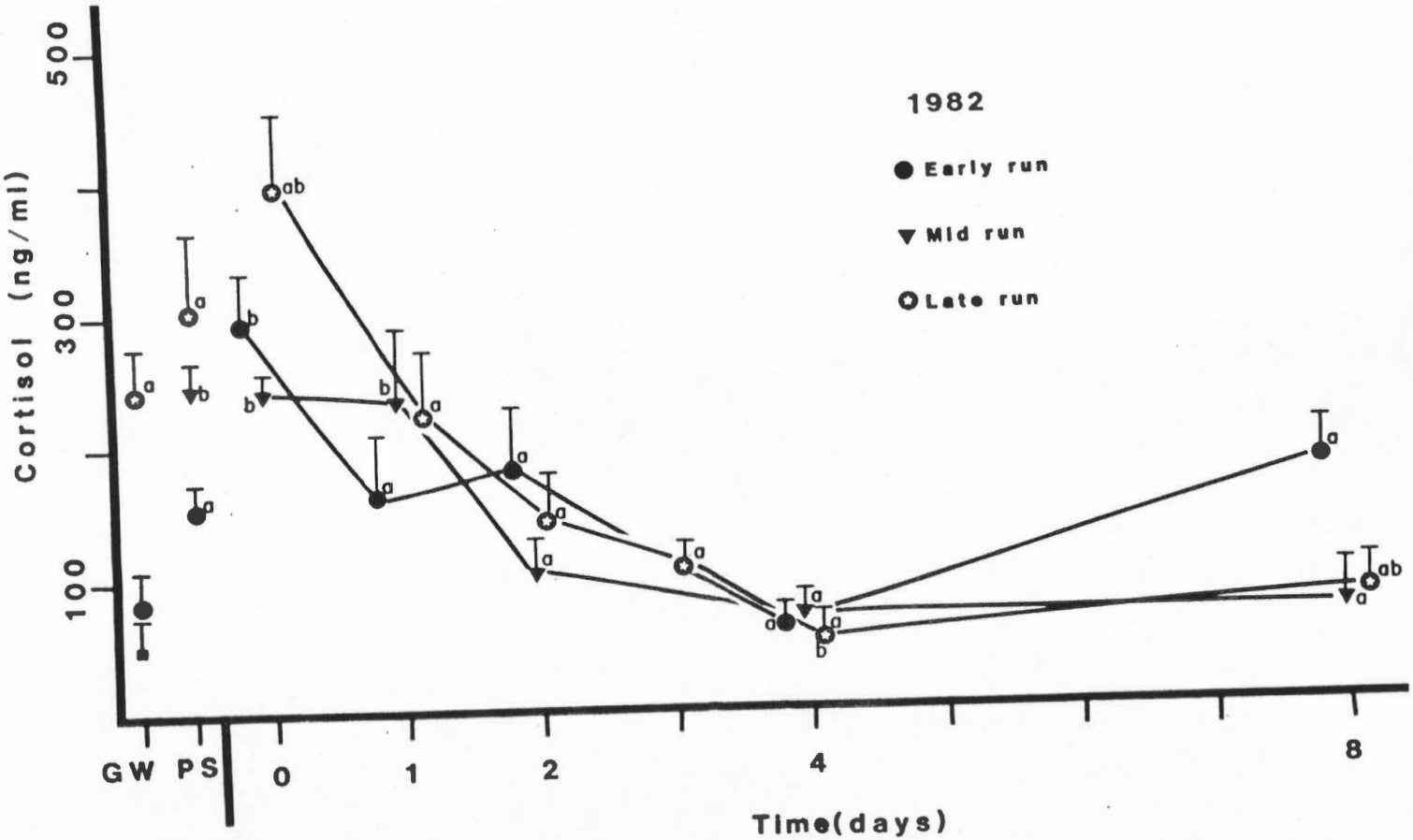

Figure 3. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS) and during 8 d of raceway recovery. Sampling was conducted on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points represent mean + SE for n = 10 to 13, except GW for June sample, n = 5. Values marked (a) are significantly different from Time = 0 of same line; values marked (b) are significantly different from gatewell gatewell, t = 0 ($P < .05$, LSD tests).

to the large number of adult American shad in the collection system. Water temperature was high during the late run (21-22 C); however, our characterization studies show that acclimation temperature does not influence plasma cortisol dynamics (see Acclimation temperature and stress). During the 1983 late run, we were unable to sample smolts from the gatewell because of the large numbers of shad present relative to few smolts. However, our results from other parts of the system (Figs. 4-6) did not show elevated cortisol in the late 1983 sampling, even though water temperature was elevated. Furthermore, smolts collected from all parts of the collection facility during 1984 (Figs. 7-9) did not exhibit plasma cortisol elevations of the magnitude seen in 1982.

The peak plasma cortisol levels of fall chinook sampled in 1982 are generally higher (300-400 $ng \cdot ml^{-1}$) than the levels in fish sampled directly from the system or raceway (i.e. not from tanks) in 1983 and 1984 (150-175 $ng \cdot ml^{-1}$). This may reflect the change in our raceway holding procedure (i.e., mechanical crowding vs. livecage) for the latter two years; however, the peak values in 1982 were in fish sampled as they entered the raceway and were not affected by holding regimen. The magnitude of the plasma cortisol elevation appears to have an effect on the time required for plasma cortisol to return to pre-stress levels (recovery time). Based on a pool of plasma cortisol levels in fish from the gatewell, we defined plasma cortisol levels of approximately 70 $ng \cdot ml^{-1}$ or less as the baseline for fish in the system. When plasma cortisol levels were highest (300-400 $ng \cdot ml^{-1}$), it took between 2-4 d for them to return to the baseline (Figs. 3-4); however, when the peak levels were lower (150-200 $ng \cdot ml^{-1}$), plasma cortisol returned to levels not

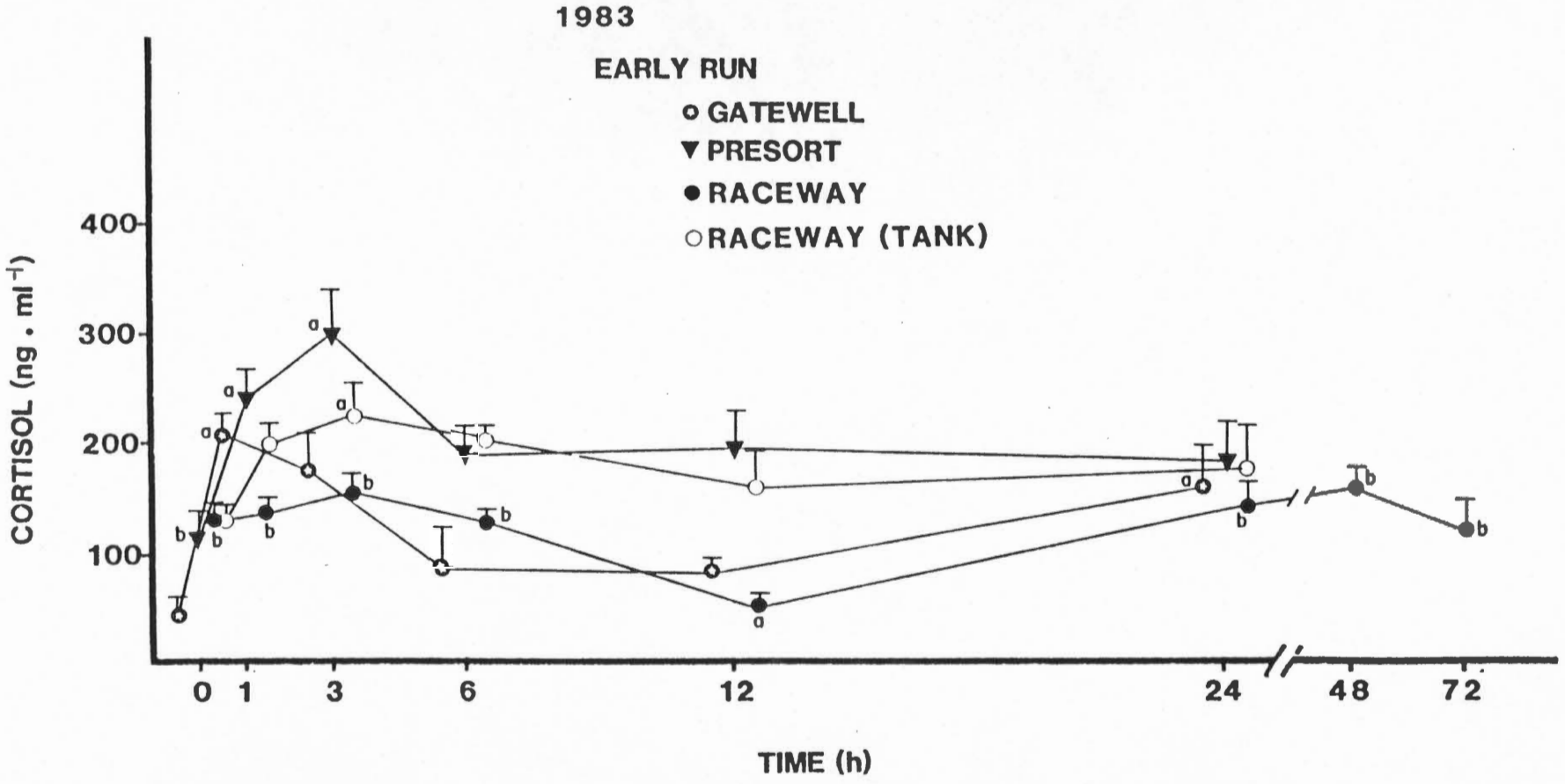

Figure 4. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell, before passing the bar-sorter (presort) and raceway. Fish from the gatewell and presort were held in large, opaque plastic tanks (ca. 100L) and serially sampled through 24 or 72 h during June 14-19, 1983. All points represent the mean + SE for 6 to 12 fish. Values marked (a) are significantly different from gatewll Time = 0 of same line; values marked (b) are significantly different from gatewell, t = 0 ($P < .05$, LSD tests).

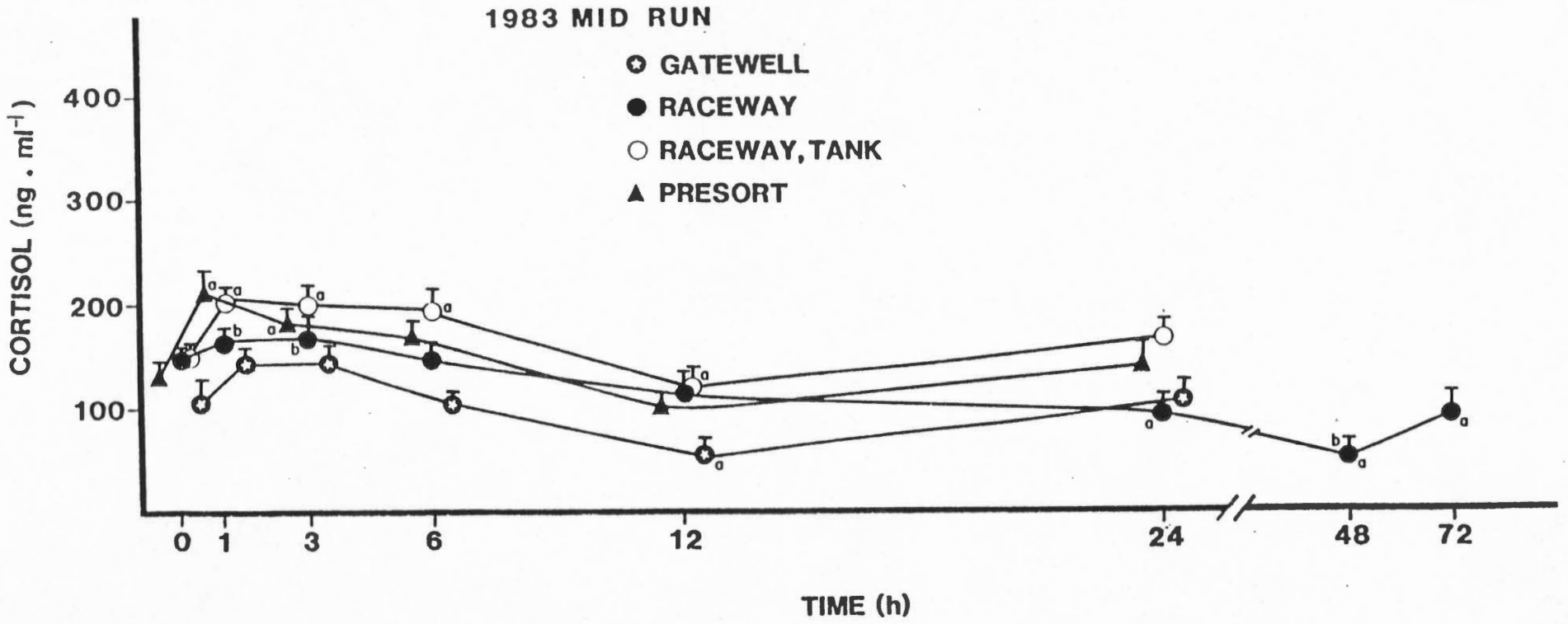

Figure 5. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell, before passing the bar-sorter (presort) and raceway. Fish from the gatewell and preseort were held in large, opaque plastic tanks (ca. 100L) and serially sampled 24 and 72 h during July 7-13, 1978. All points represent the mean + SE for 10 to 12 fish. Values marked (a) are significantly different from gatewell Time = 0 of same line; values marked (b) are significantly different from gatewell, t = 0 ($P < .05$, LSD tests).

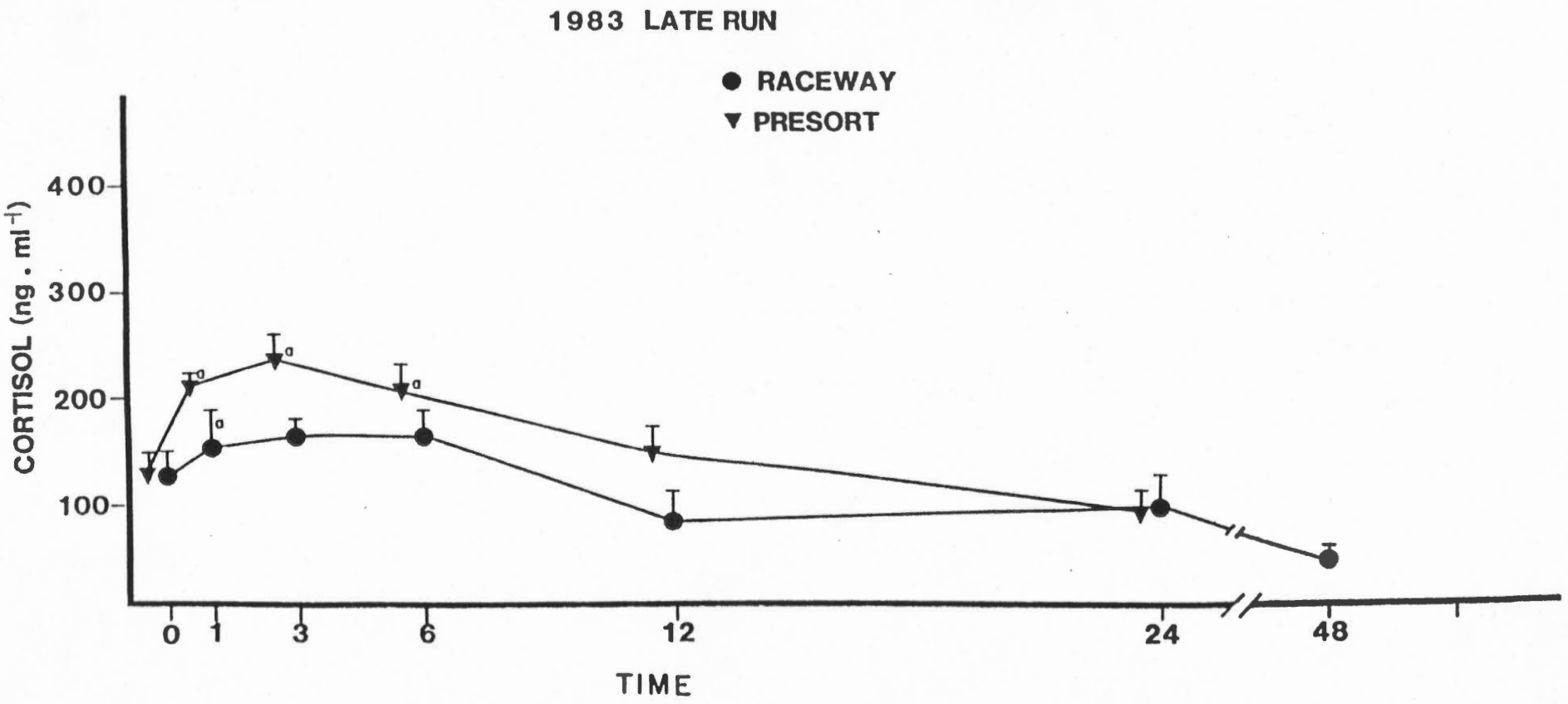

Figure 6. Plasma cortisol levels of juvenile fall chinook salmon sampled from the McNary Dam, before passing the bar-sorter (presort) and raceway. Fish from the presort were held in large, opaque plastic tanks (ca. 100L) and serially sampled through 24 or 72 h during August 1-4, 1983. All points represent the mean + SE for 6 to 12 fish. Values marked (a) are significantly different from T = 0 of same line ($P < .05$, LSD test).

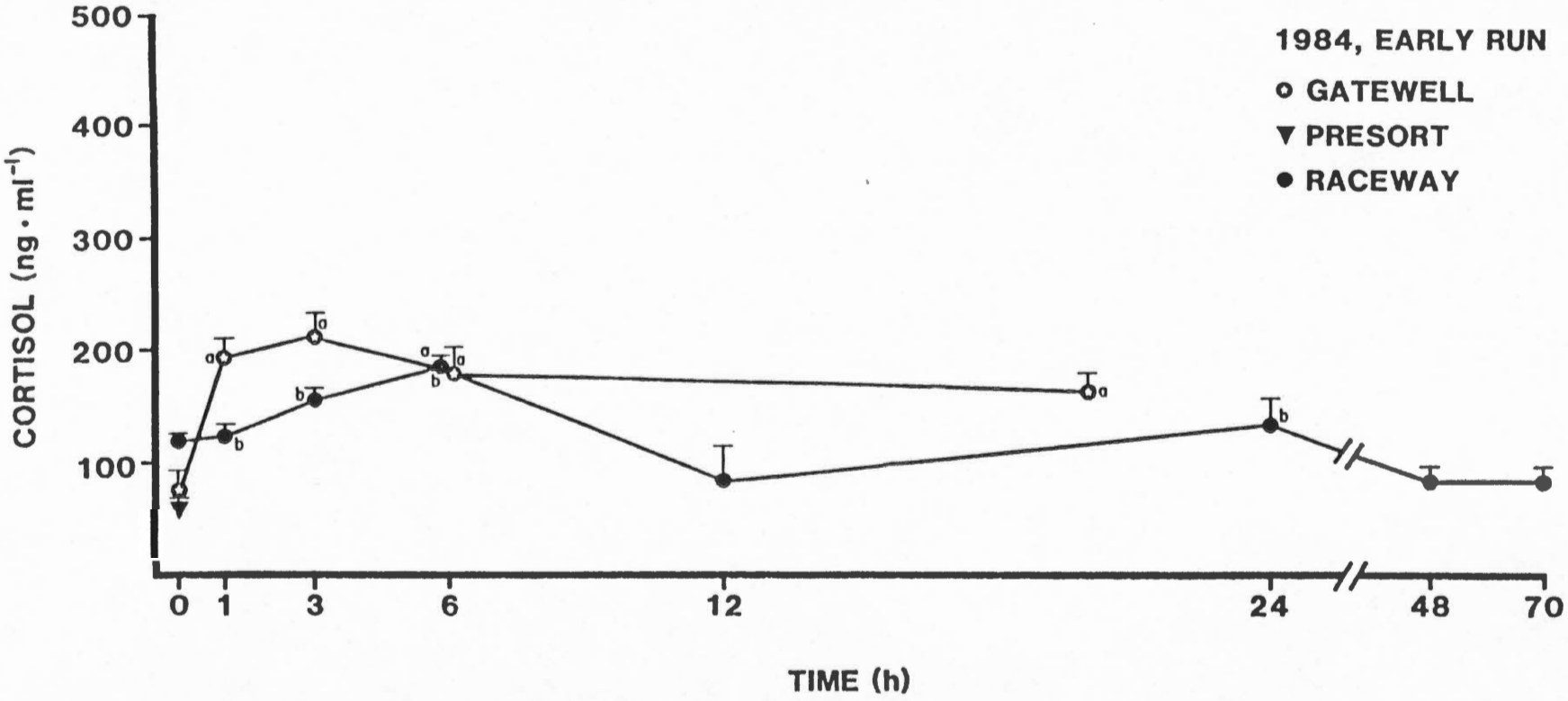

Figure 7. Plasma cortisol levels in juvenile fall chinook salmon sampled from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and raceway. Sampling was conducted during June 12-15, 1984. Fish from the gatewell were held in large, opaque plastic tanks (ca. 100 L) and serially sampled through 20 h. All points represent the mean + SE for 7 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line; values marked (b) are significantly different from gatewell Time = 0 ($P < .05$, LSD tests).

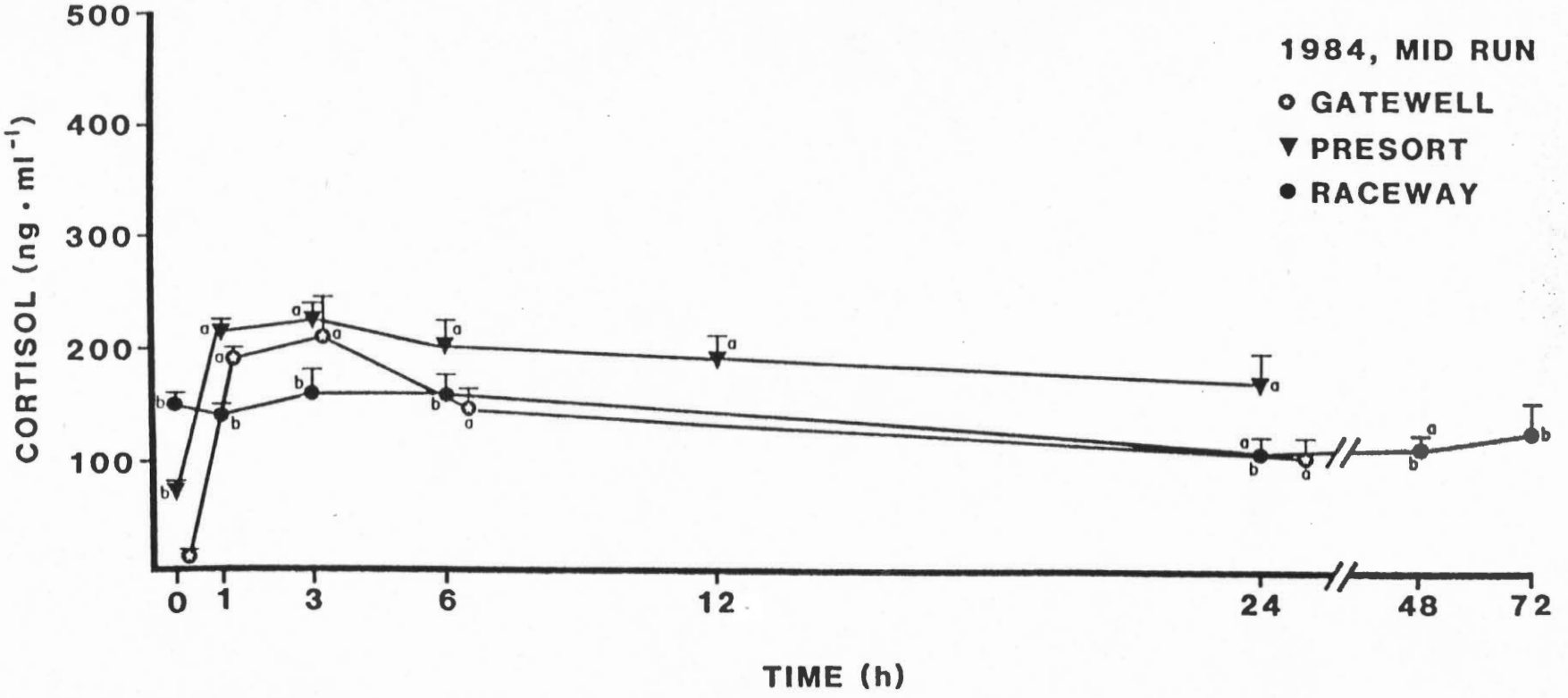

Figure 8. Plasma cortisol levels in juvenile fall chinook salmon sampled from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and raceway. Sampling was conducted during July 10-14, 1984. Fish from the gatewell and presort were held in large, opaque plastic tanks (ca. 100 L) and serially sampled through 24 h. All points represent the mean + SE for 10 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line; values marked (b) are significantly different from gatewell Time = 0 ($P < .05$, LSD tests).

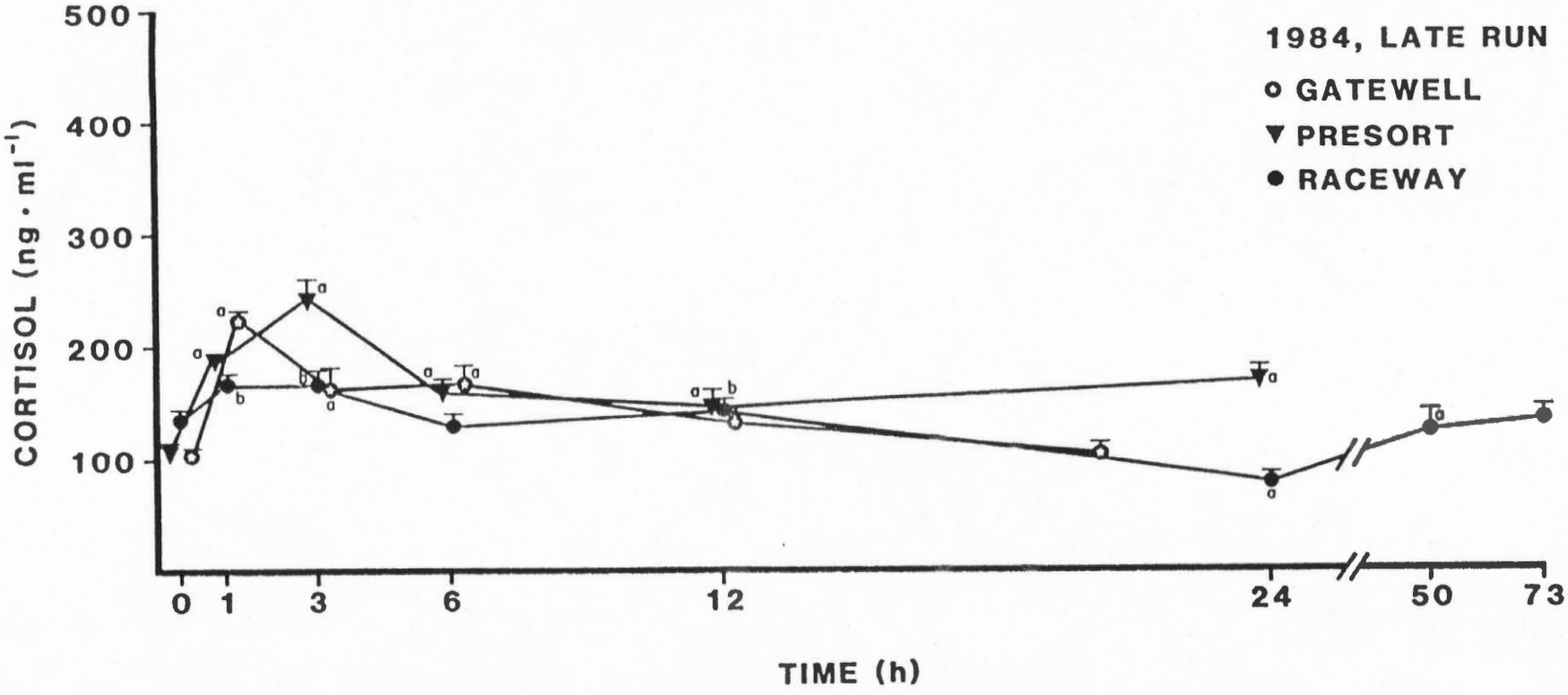

Figure 9. Plasma cortisol levels in juvenile fall chinook salmon sampled from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and raceway. Sampling was conducted during August 7-10, 1984. Fish from the gatewell and presort were held in large, opaque plastic tanks (ca. 100 L) and serially sampled through 24 h. All points represent the mean + SE for 10 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line; values marked (b) are significantly different from gatewell Time = 0 ($P < .05$, LSD tests).

significantly different ($P < 0.05$; LSD test) from the baseline within 12-48 h after fish entered the raceway (Figs. 4-9).

While there were no consistent patterns in absolute plasma cortisol response through the smolt runs, in all but one sampling period the plasma cortisol of fish sampled just before the bar-sorter was significantly greater ($P < 0.05$, LSD test) than plasma cortisol in fish from the gatewell. Furthermore, plasma cortisol in fish sampled as they entered the raceway was significantly greater ($P < 0.05$, LSD test) than in fish from the bar-sorter in most of the sampling periods. Thus, the cumulative nature of the stresses imposed by the individual elements in the system is demonstrated in most sampling periods. In 1982, the highest absolute cortisol values and greatest changes in plasma cortisol were from the late-run fish (Fig. 3). In 1983 the highest values occurred during the early run (Fig. 4); and in 1984, the highest values occurred during the late run (Fig. 9) while the greatest changes were observed in the mid-run (Fig. 8). This variability could be the result of variation in the environment or the fish. We found no differences in weight, length (Fig. 10), gill Na-K ATPase, or thyroxine (Figs. 11-12) between 1982 and 1984 which could account for this variability. Water temperature and river velocity are two environmental factors which showed variability during the three years (Table 1); however, there does not appear to be a correlation between these environmental factors and variability in the plasma cortisol response (also see: <u>Acclimation temperature and stress</u>).

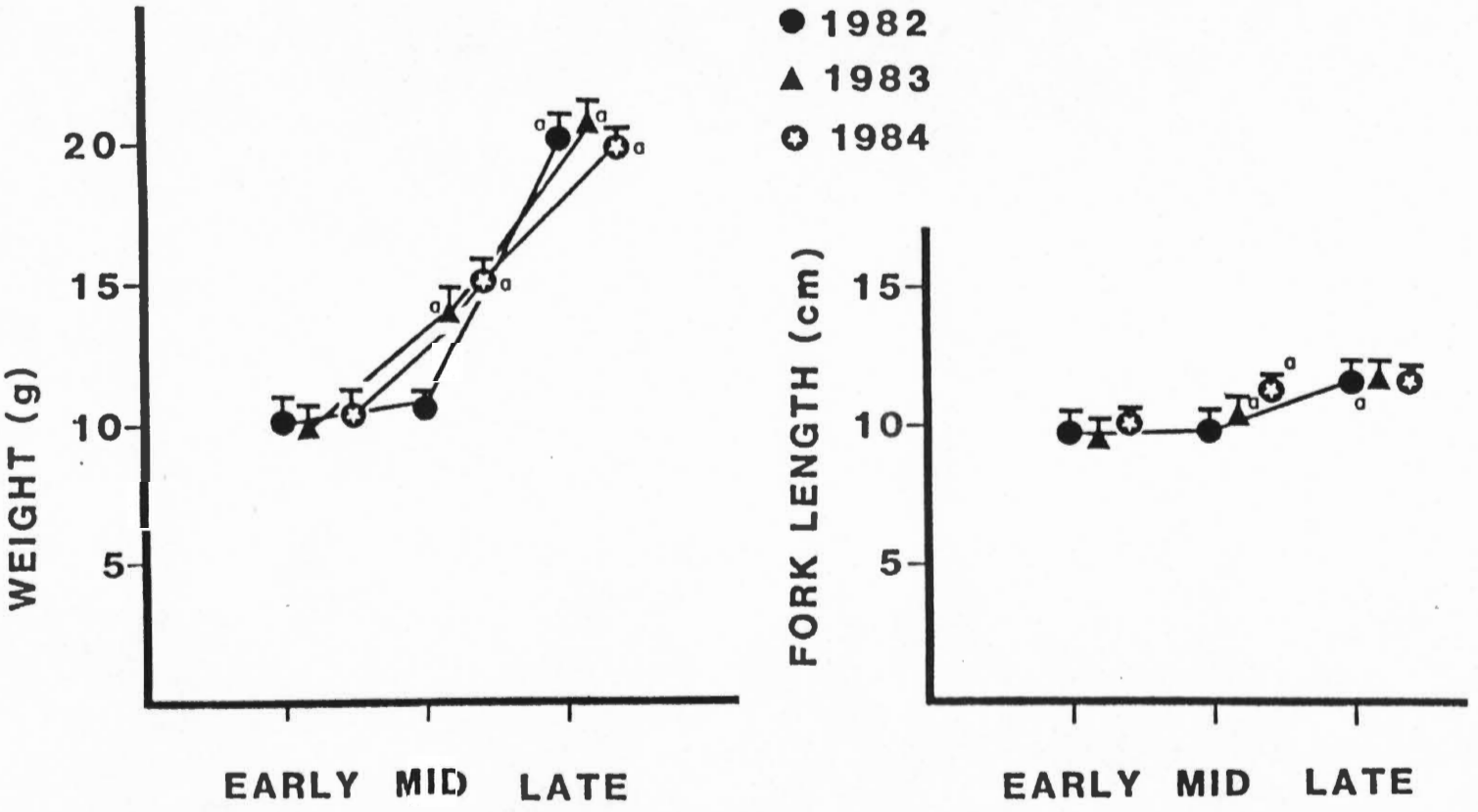

Figure 10. Weights and fork lengths of juvenile fall chinook salmon collected at McNary Dam on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982, and at similar times in 1983 and 1984. All points represent the mean + SE for 100 fish. Values marked (a) are significantly different from other values of the same year ($P < .05$, LSD test).

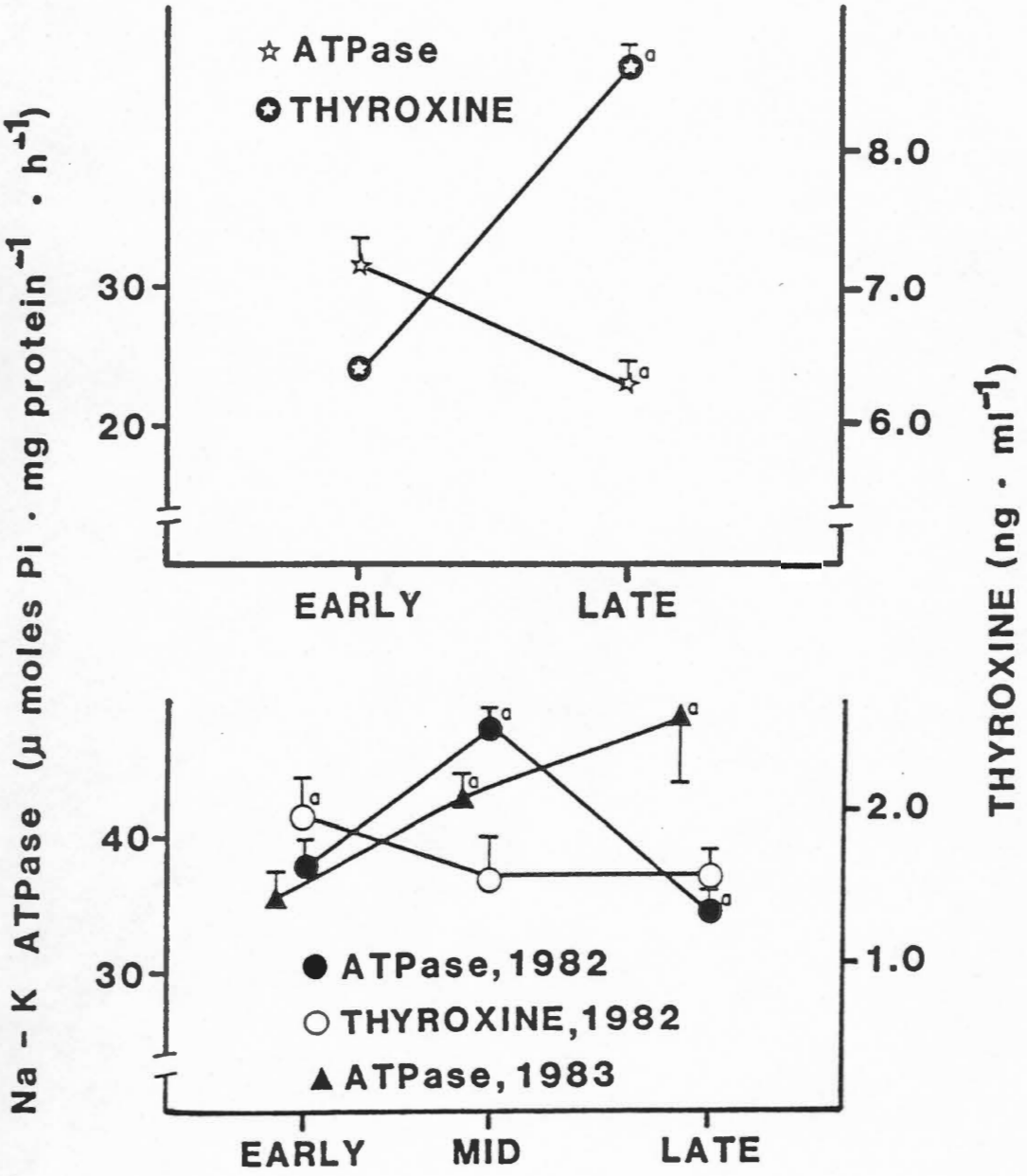

Figure 11. Indices of smoltification (Na-K-ATPase activity and plasma thyroxine levels) for juvenile fall chinook salmon collected at McNary Dam on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982, and similar times in 1983 (bottom); and for juvenile spring chinook salmon collected at McNary Dam on May 3-6 (early) and May 23-26 (late), 1983 (top). All points represent means + SE for 15 to 30 fish. (Na-K-ATPase activity was assayed by Dr. W. Zaugg, NMFS, Cook, Washington.) a = significantly different from all similar variables of the same year ($P < .05$, LSD test).

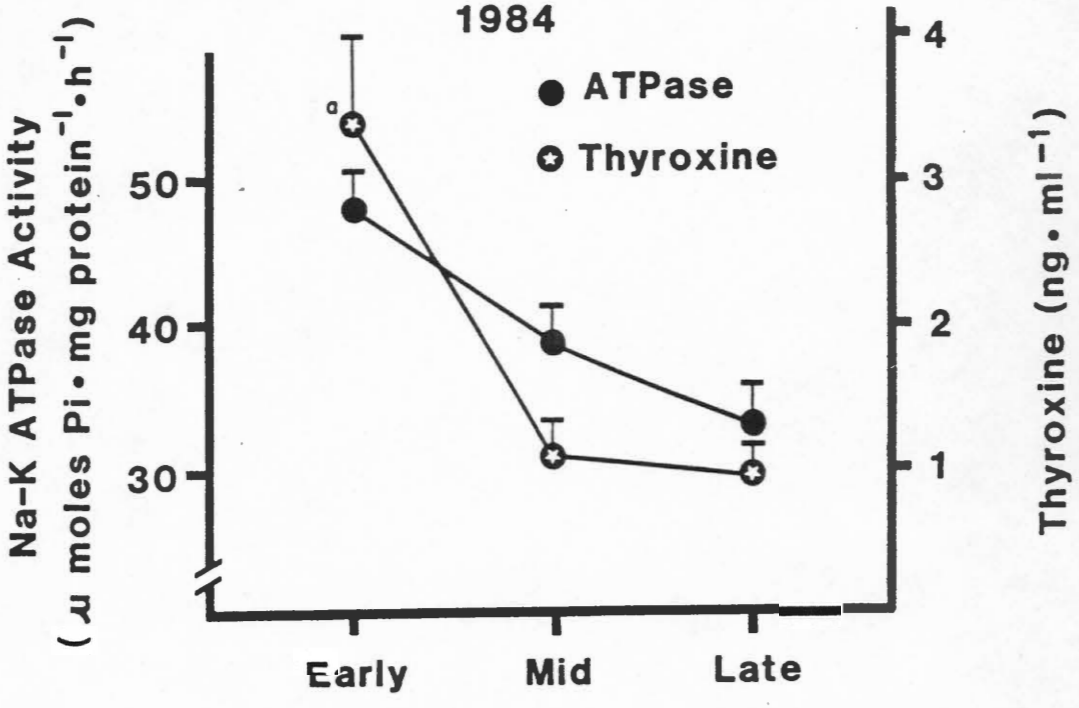

Figure 12. Indices of smoltification (Na-K ATPase activity and plasma thyroxine levels) for juvenile fall chinook salmon collected at McNary Dam during June 12-15 (early), July 10-14 (mid), and August 7-10 (late), 1984. All points represent the mean + SE for 20 fish. Values marked (a) are significantly different from other values of the same year ($P < .05$, LSD test). (Na-K ATPase activity was assayed by Dr. W. Zaugg, NMFS, Cook, Washington.)

Table 1. Columbia River flow, water temperature, and percent of river flow going over spillways at McNary Dam during sampling periods in 1982, 1983, and 1984.

	Flow ($X10^6$ cfs)	Spill (%)	Water Temp. (° C)
1982, June	400 ± 8	52	16
July	304 ± 8	39	18
August	187 ± 8	0	21
1983, May	305 ± 11	45	13
June	269 ± 9	26	15
July	202 ± 8	0	17
August	173 ± 4	0	20
1984, June	327 ± 12	45	15
July	207 ± 5	22	17
August	151 ± 7	0	21

There was no significant change in interrenal nuclear diameters in fall chinook collected at McNary in 1982 (Fig. 13), indicating that the stresses encountered at the dam are not of a long enough duration to cause chronic mobilization of cortisol synthetic mechanisms. Interrenal cells actively manufacturing cortisol have a hypertrophied nucleus as the result of RNA synthesis. In acute stress, however, cortisol will be released without fully mobilizing the synthetic process.

Hepatic glycogen, which may be converted to glucose as an energy source during stress, was highly variable in fall chinook. In 1982, hepatic glycogen tended to decrease in fish collected in the system and through 4 d in the raceway when glycogen levels were barely detectable (Fig. 14). The 1983 sampling extended through 3 d, and despite variability, it appears that glycogen is decreasing through the holding period (Fig. 15). The glycogen levels in fish at the dam are close to the levels in hatchery fish after 20 d of fasting (see: Nutrition and Stress), and the high variability is, speculatively, the result of some smolts not feeding after release from the hatchery. Subjectively, we recall many more empty stomachs than full stomachs in fish dissected at the dam.

Smolts collected in 1984 from the gatewell and presort and held in tanks had increases in plasma glucose (ca. 200 mg•100 ml^{-1}) for up to 12 h after collection (Figs. 16-18). However, fish held in the raceway exhibited peak plasma glucose levels of 75-150 mg•100 ml^{-1} with few statistically significant changes through 72 h. Acclimated, well-fed hatchery fall chinook have baseline levels of 50-80 mg•100 ml^{-1} (see: Stress Characterization). Fish which were not fed for 20 d did not show as great an increase in plasma glucose as well-fed fish when stressed (see:

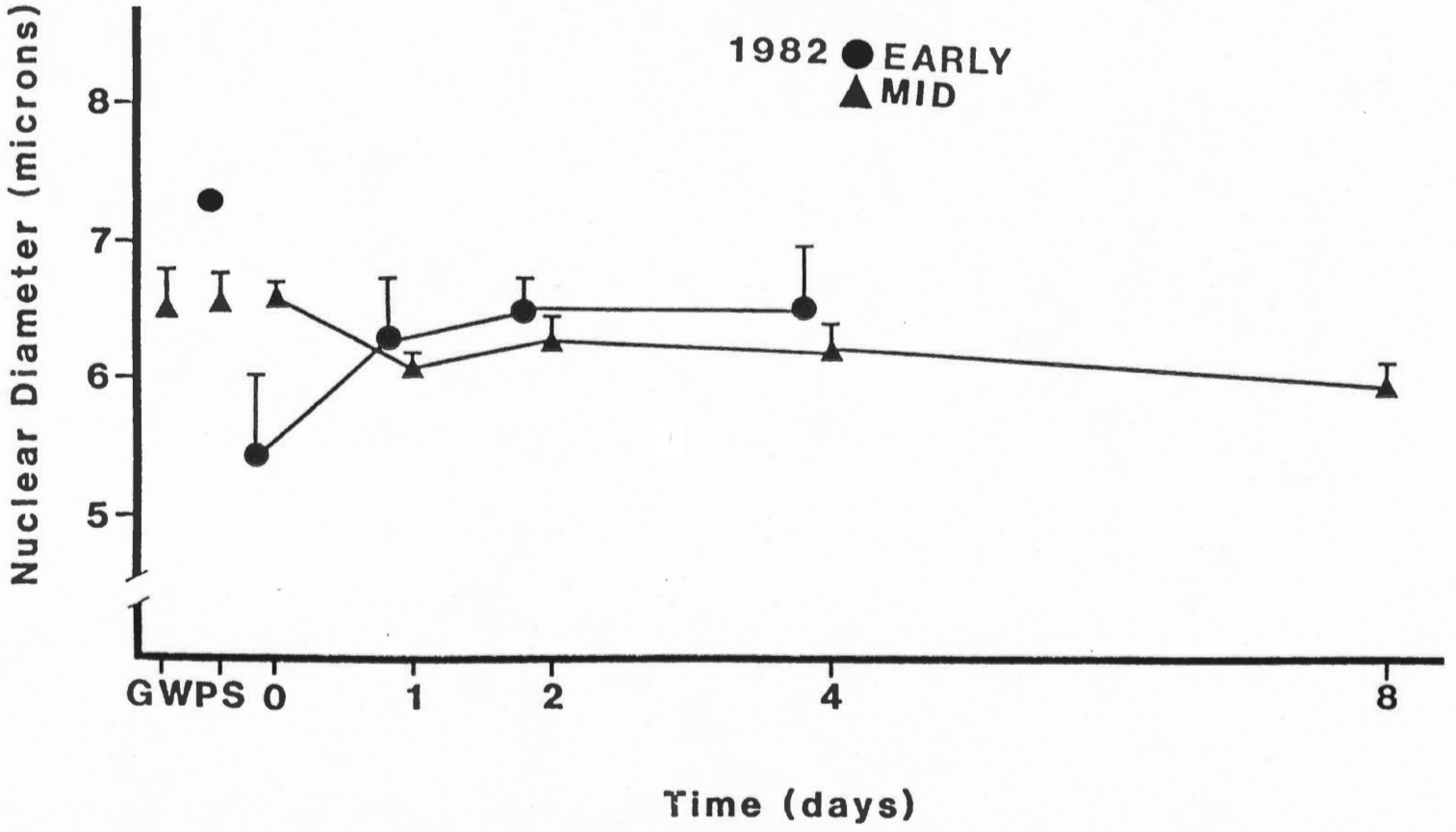

Figure 13. Interrenal cell nuclear diameters of juvenile fall chinook salmon collected from the gatewell (GW), before the fish pass the bar-sorter (PS), and for up to 8 d in a raceway at McNary Dam. Samples were collected June 14-24 (early) and July 7-16 (mid), 1982. All points are the means + SE for 4 to 6 fish.

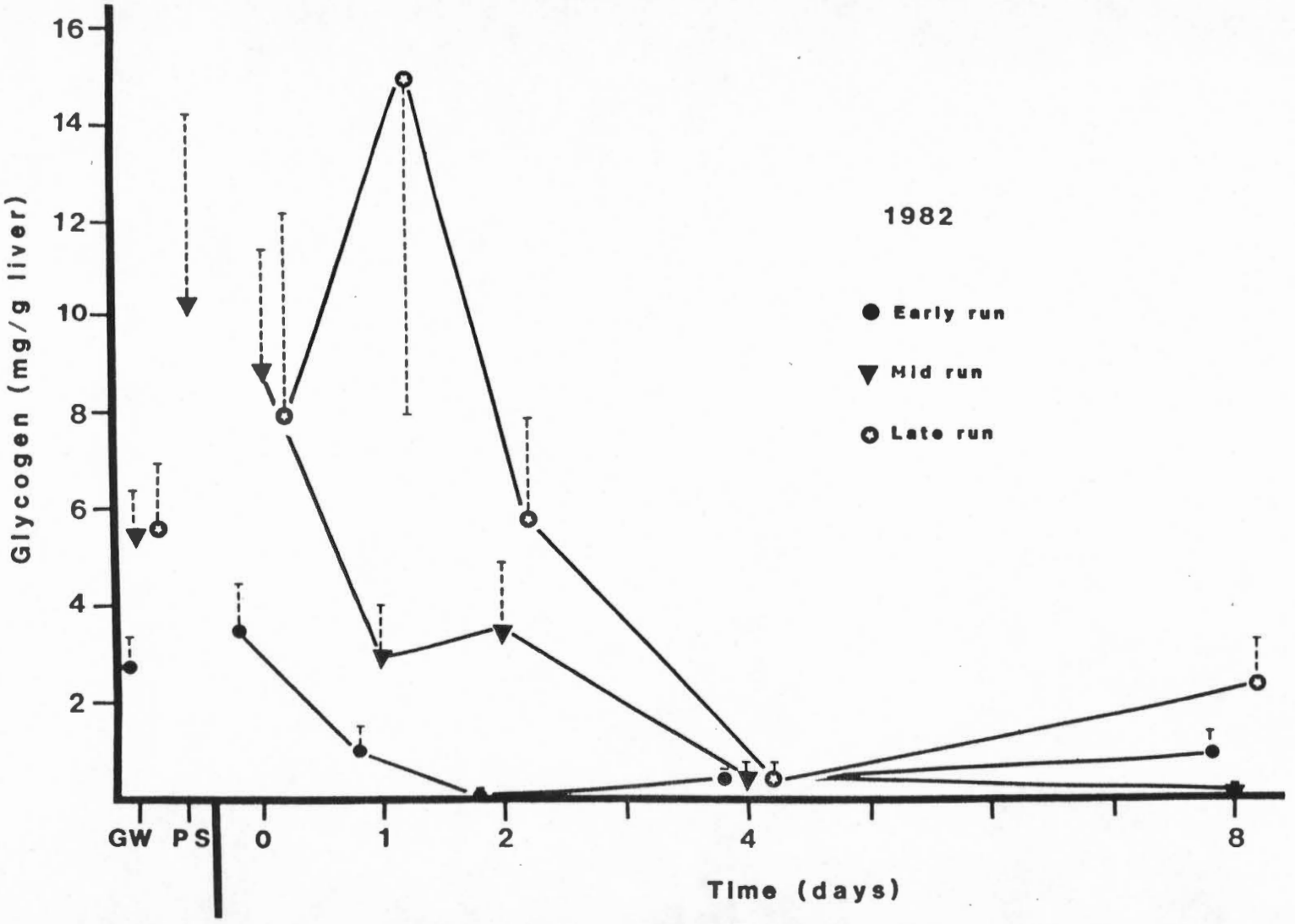

Figure 14. Hepatic glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS), and during 8 d of raceway recovery. Sampling was conducted on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points represent mean + SE for n = 5 or 6.

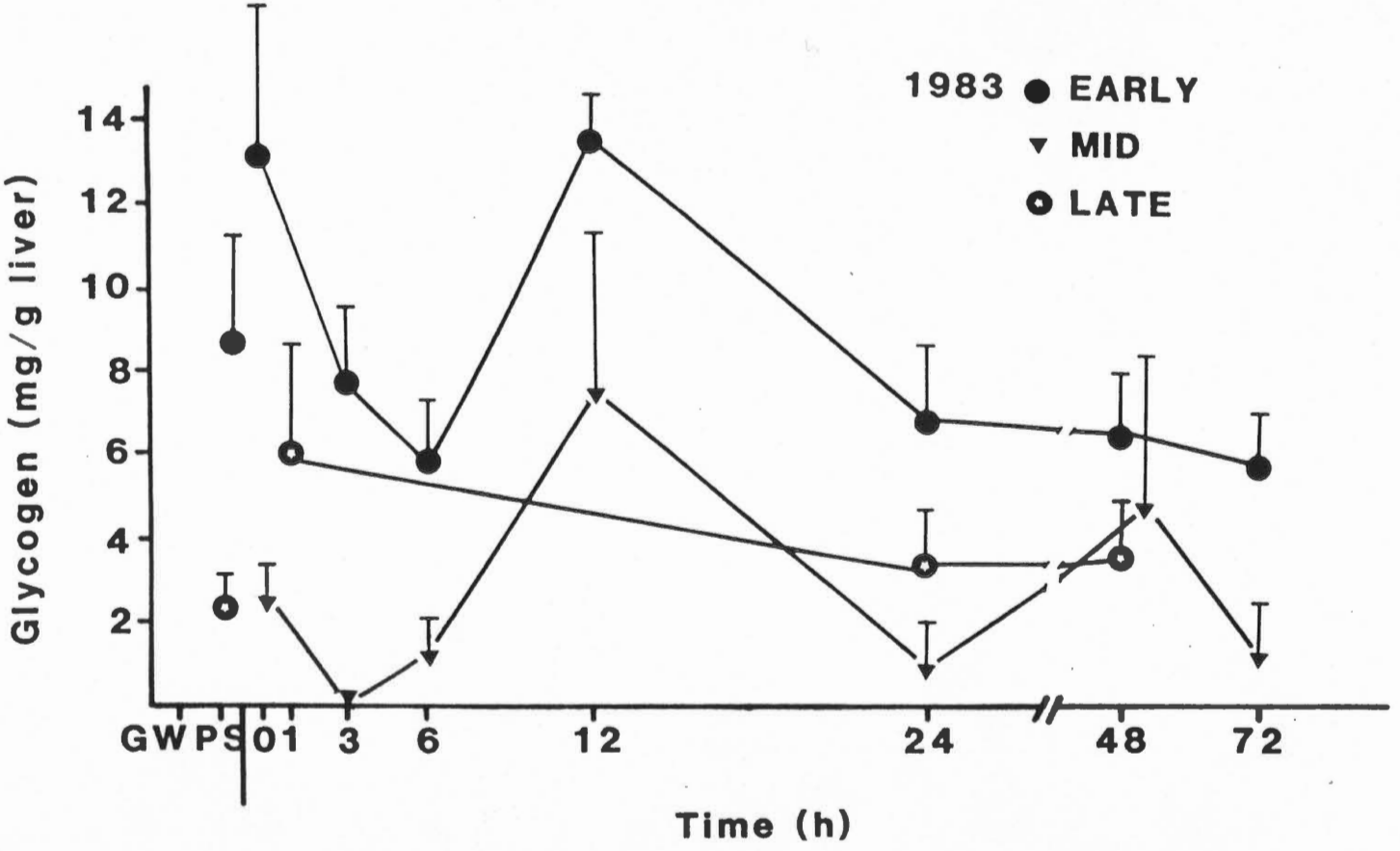

Figure 15. Hepatic glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS) and during 8 d of raceway recovery. Sampling was conducted on June 14-19 (early), July 7-13 (mid), and August 1-4 (late), 1983. All points represent mean + SE for n = 5 or 6.

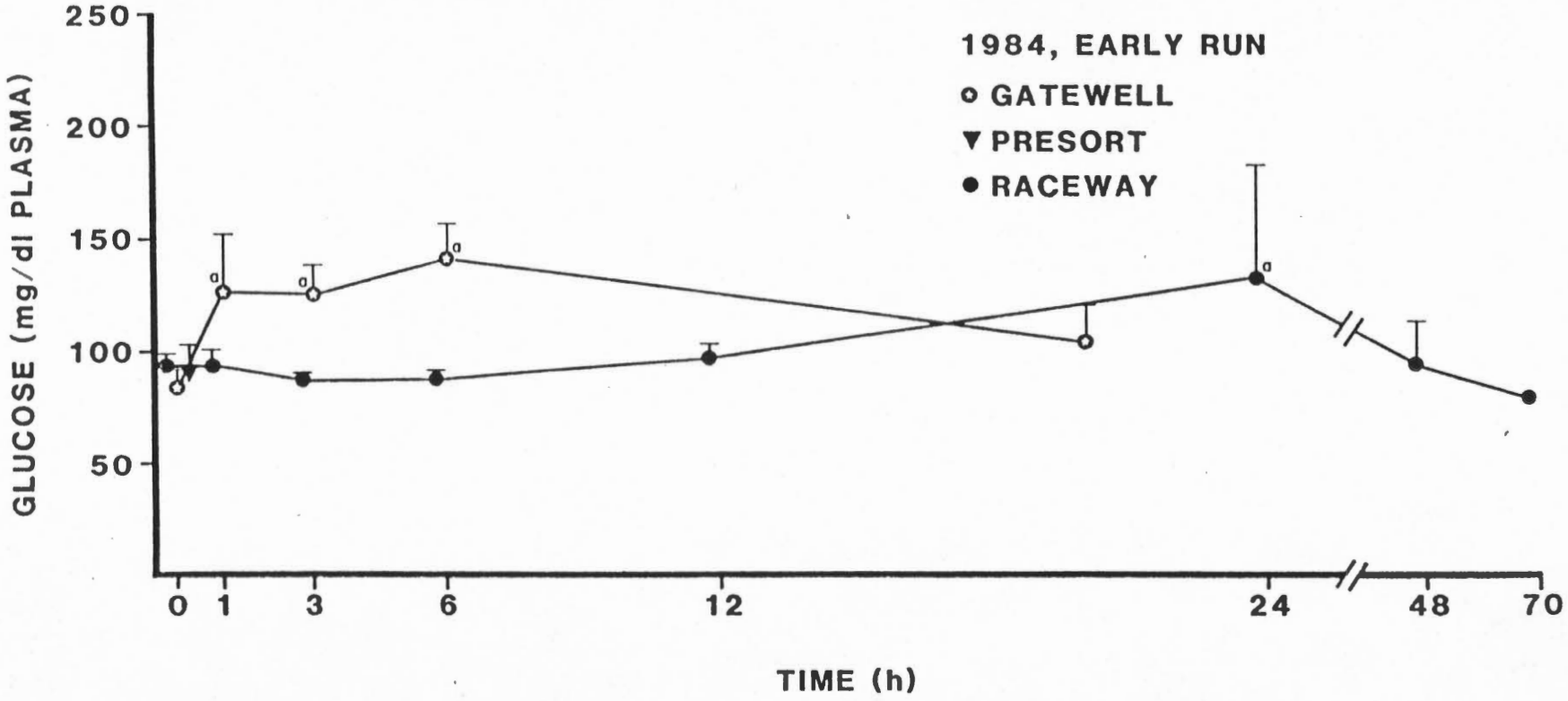

Figure 16. Plasma glucose levels in juvenile fall chinook salmon taken from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and the raceway; and fish sampled after various times in the raceway and in large plastic buckets with flow-through water. Samples were collected during June 12-15, 1984; all points represent the mean + SE for 4 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

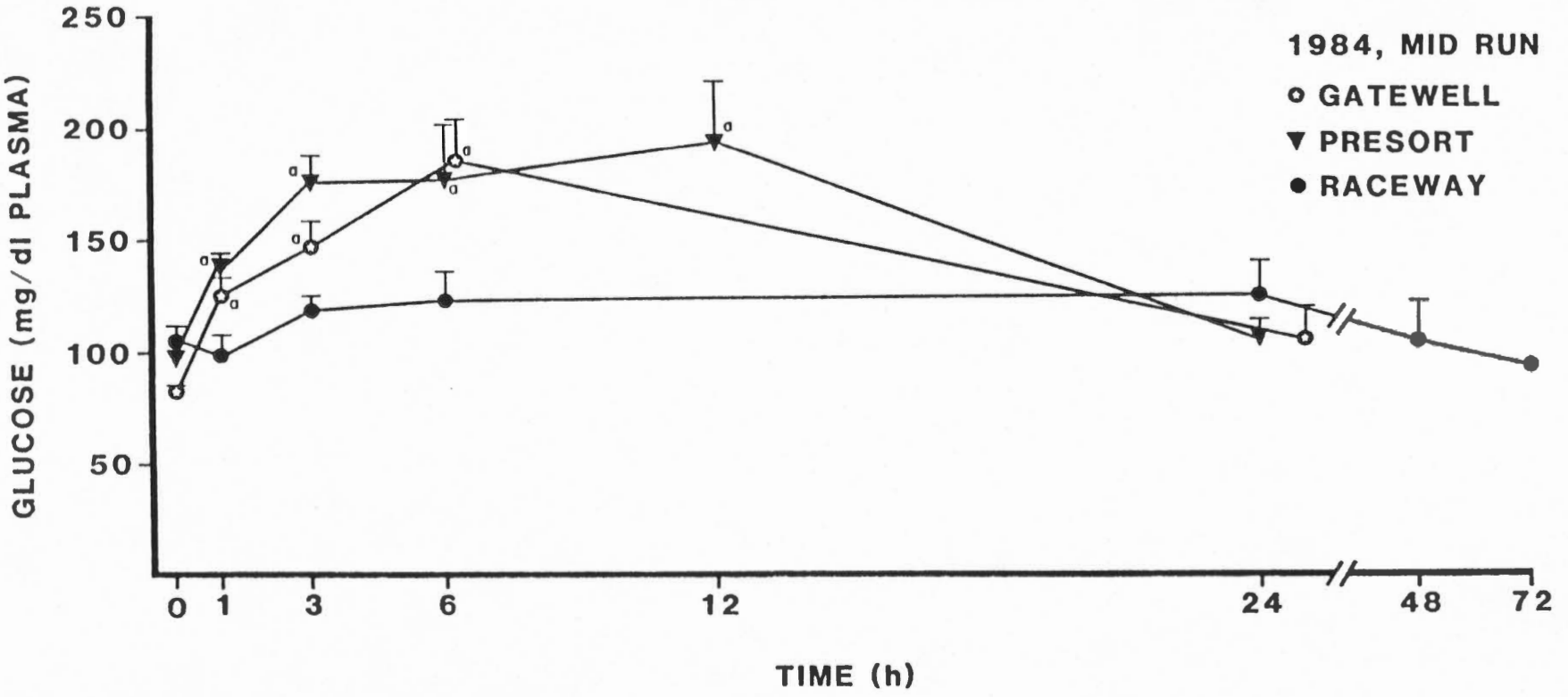

Figure 17. Plasma glucose levels in juvenile fall chinook salmon taken from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and the raceway; and fish sampled after various times in the raceway and in large plastic buckets with flow-through water. Samples were collected during July 10-14, 1984; all points represent the mean + SE for 4 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

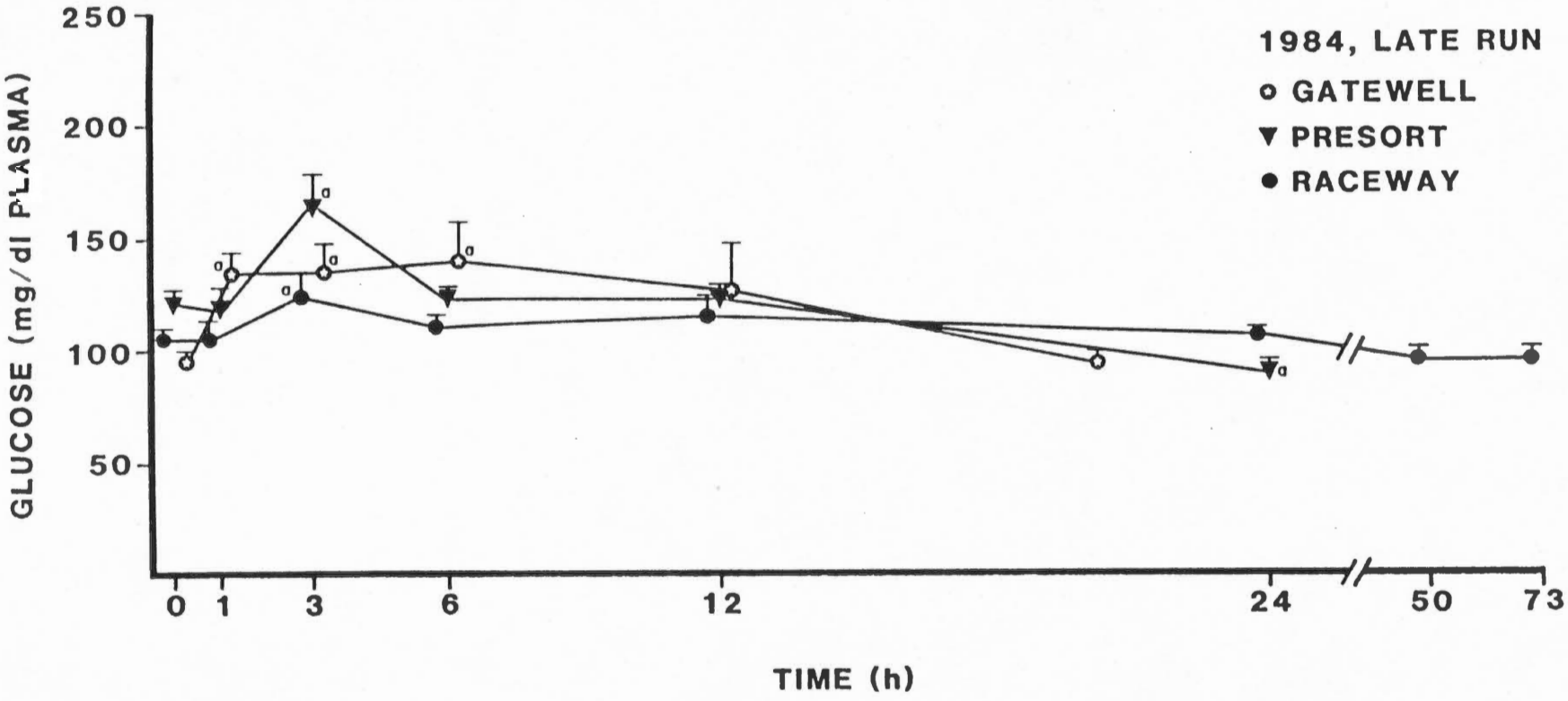

Figure 18. Plasma glucose levels in juvenile fall chinook salmon taken from the McNary Dam gatewell, just before crossing the bar-sorter (presort), and the raceway; and fish sampled after various times in the raceway and in large plastic buckets with flow-through water. Samples were collected during August 7-10, 1984; all points represent the mean + SE for 9 to 13 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

Nutrition and Stress Response). The high plasma glucose levels of fish collected from the gatewells and presort positions and held in tanks, preclude the possibility that the fish in the raceways were unable to mobilize glucose because of nutritional state. It appears that the stresses of the collection system did not result in a complete mobilization of glucose.

Hematocrits of fall chinook had little variability at McNary Dam in 1982 (Fig. 19); mean values remained close to 50% throughout the sampling. In 1983 and 1984, however, the hematocrits were lower (ca. 40-45%) in samples from the gatewell and presort, and increased in samples from the raceway, especially in the 1983 midrun sample (Figs. 20-21). Leucocrits did not show a consistent pattern of response in fall chinook (Figs. 19, 21, and 22). However, the numbers of WBC relative to numbers of erythrocytes on blood smears was significantly depressed 24-48 h after fall chinook entered the raceway (Figs. 22-24). The reduction in WBC may be the result of increased plasma cortisol (Figs. 3-9) which can have cytolytic or redistributional effects on the WBC (Baxter 1976).

Proximate analysis of body composition revealed an increase in percent fat and a decrease in percent moisture in fall chinook as the run progressed in 1982 (Fig. 25). However, there were no detectable changes in body composition which could be attributed to the stress of collection or transportation.

Osmoregulatory ability, as measured by plasma Na levels after a sublethal saltwater challenge, decreased in fall chinook as the runs progressed (Figs. 26-28), probably as a result of increasing water temperatures. There was also a significant decrease in osmoregulatory

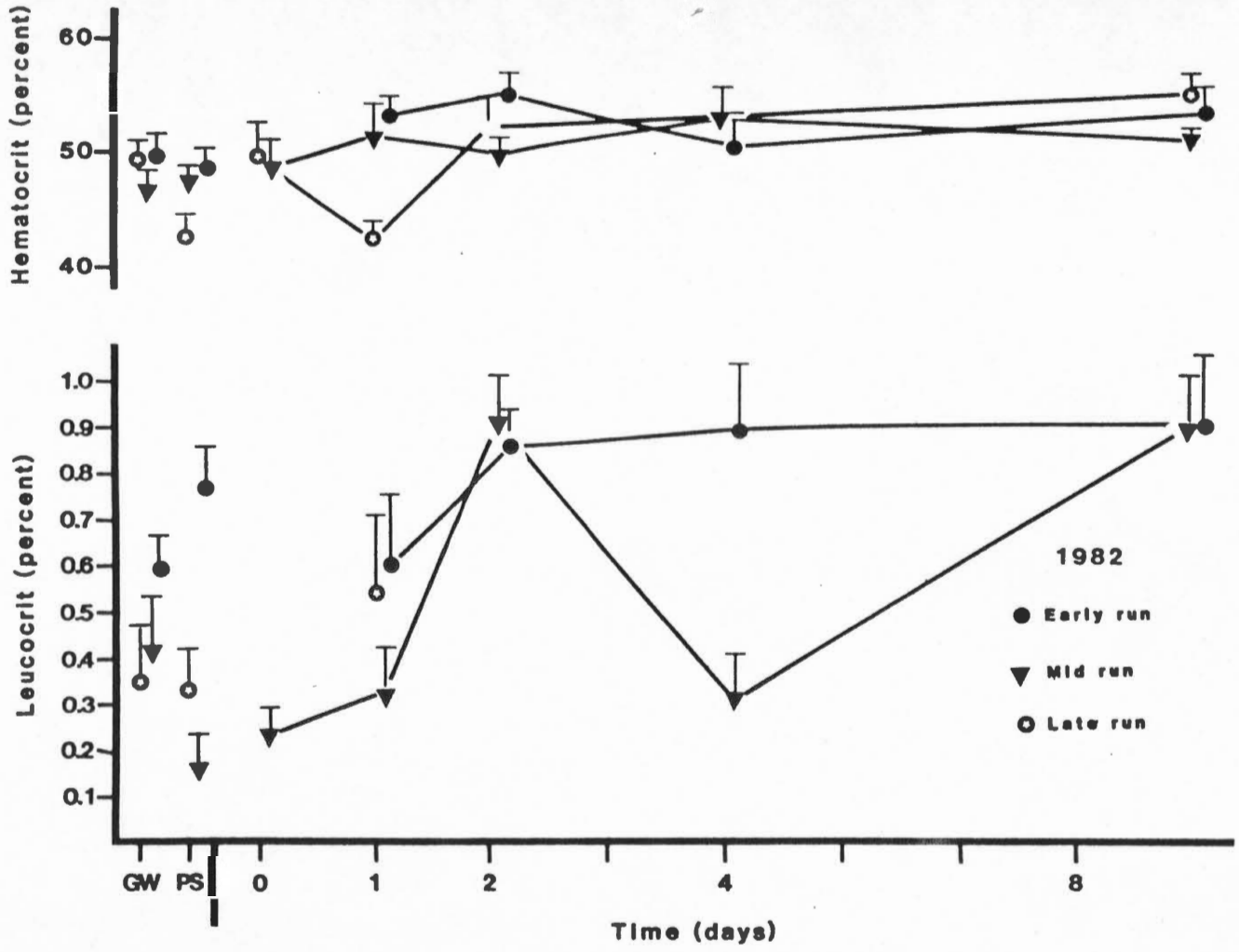

Figure 19. Leucocrit and hematocrit values (mean + 1 SE) for juvenile fall chinook salmon sampled from the gatewell (GW) and pre-sorter (PS) and through 8 d of recovery at McNary Dam. Each point represents 6 fish taken on June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982.

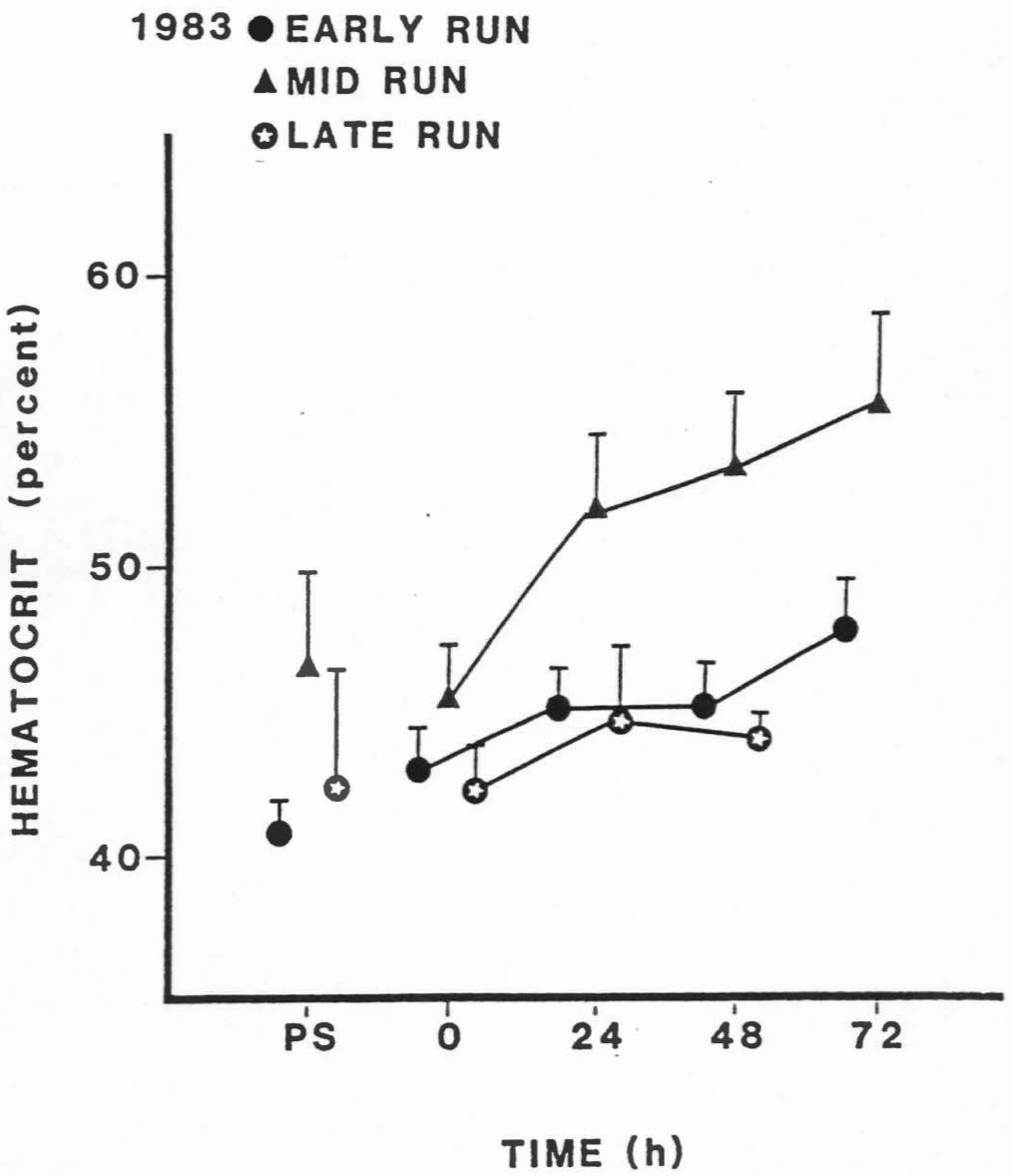

Figure 20. Hematocrit values for juvenile fall chinook salmon collected before they cross the bar-sorter (PS) and during various times in the raceway at McNary Dam during June 14-19 (early), July 7-13 (mid), and August 1-4 (late), 1983. All points are the means + SE from 6 fish.

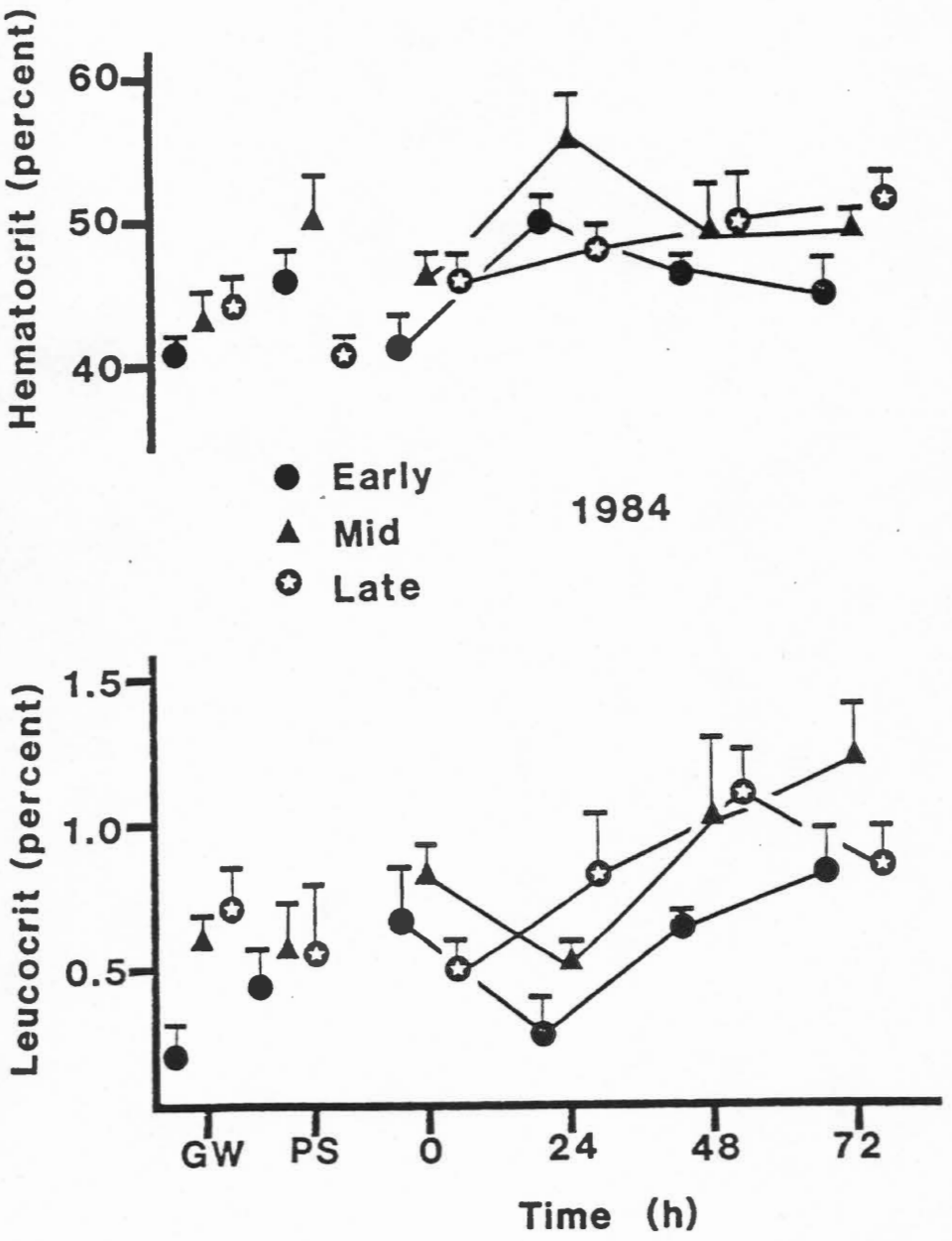

Figure 21. Hematocrit and leucocrit values for juvenile fall chinook salmon collected from the gatewell (GW), just before crossing the bar-sorter (PS), and at various times in the raceway at McNary Dam during June 12-15 (early), July 10-14 (mid), and August 7-10 (late), 1984. All points represent the mean + SE for 6 fish.

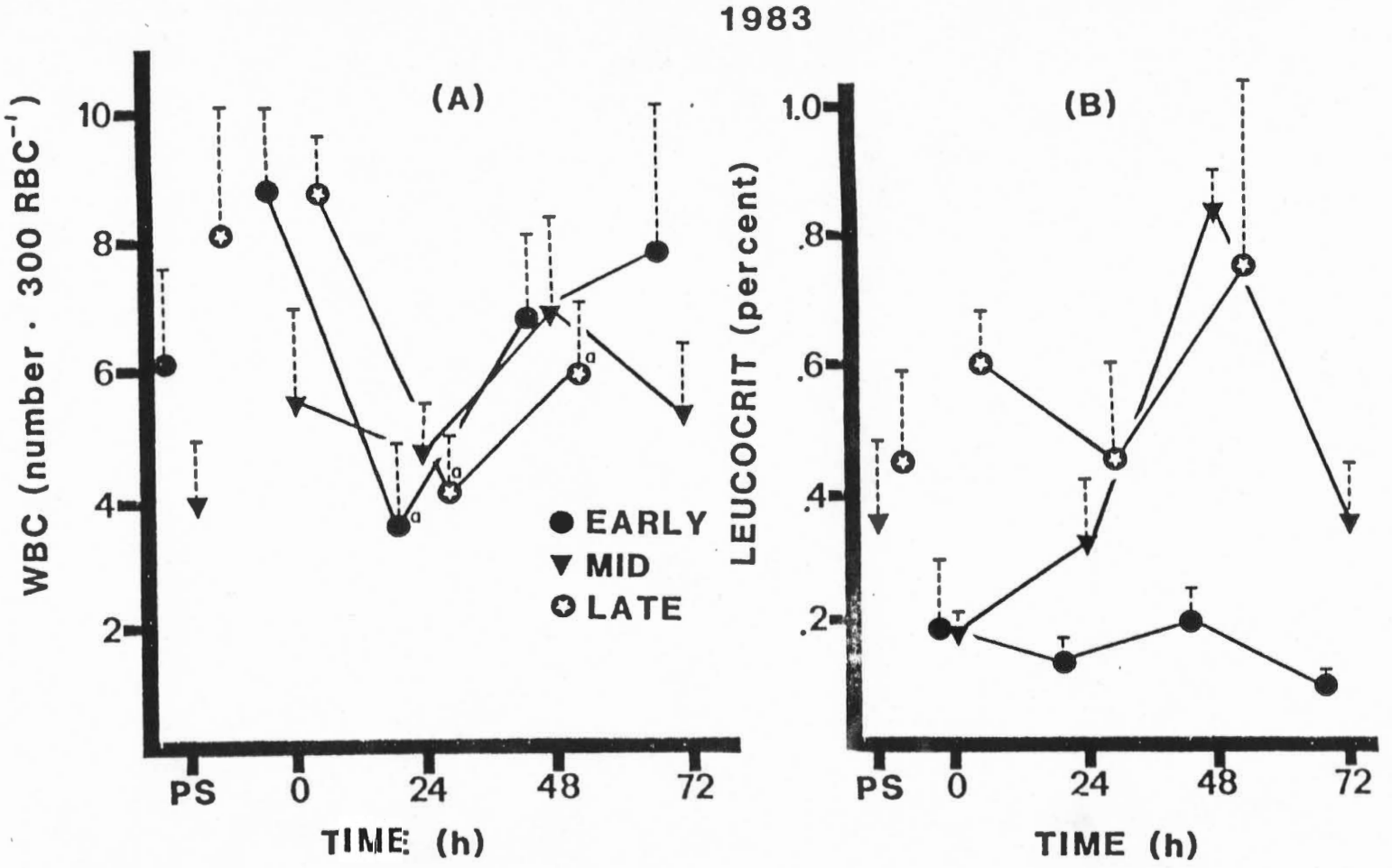

Figure 22. White blood cell (WBC) counts (A) and leucocrit values (B) for outmigrating fall chinook salmon sampled from just before the bar-sorter (PS) and after various recovery times in the raceway. WBC points are the means + 1 SE of the average of two replicated counts of the number of WBC's among 300 erythrocytes (RBC) on blood smears from 6 fish. Leucocrits are means + 1 SE for the same 6 fish. Points marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

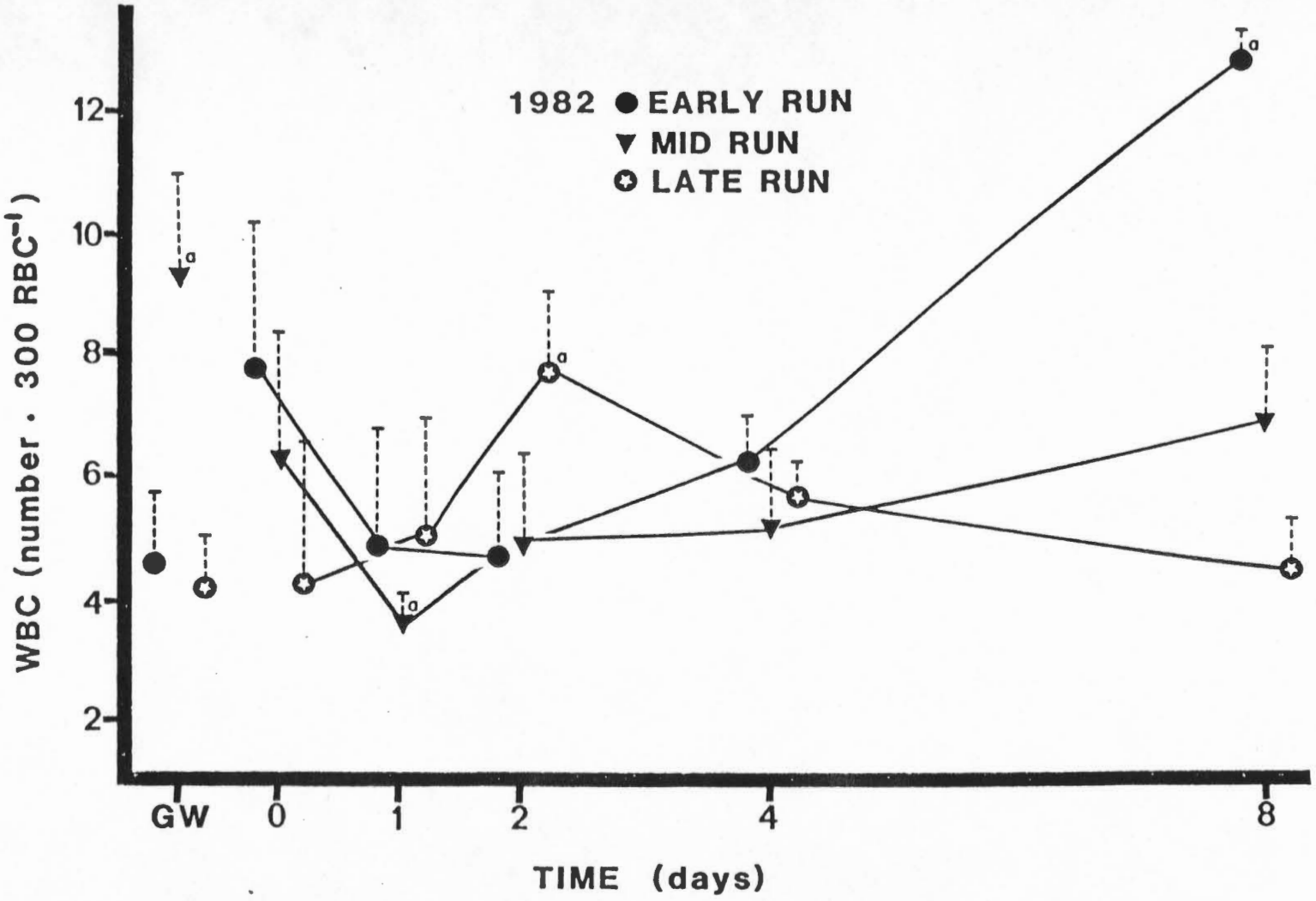

Figure 23. White blood cell (WBC) counts for outmigrating fall chinook salmon collected from the gatewell (GW) and after various recovery times in the raceway at McNary Dam. Samples were collected June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. All points are means + SE of the average of two replicate counts of the number of WBC's among 300 erythrocytes (RBC) on blood smears from 6 fish. Points marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

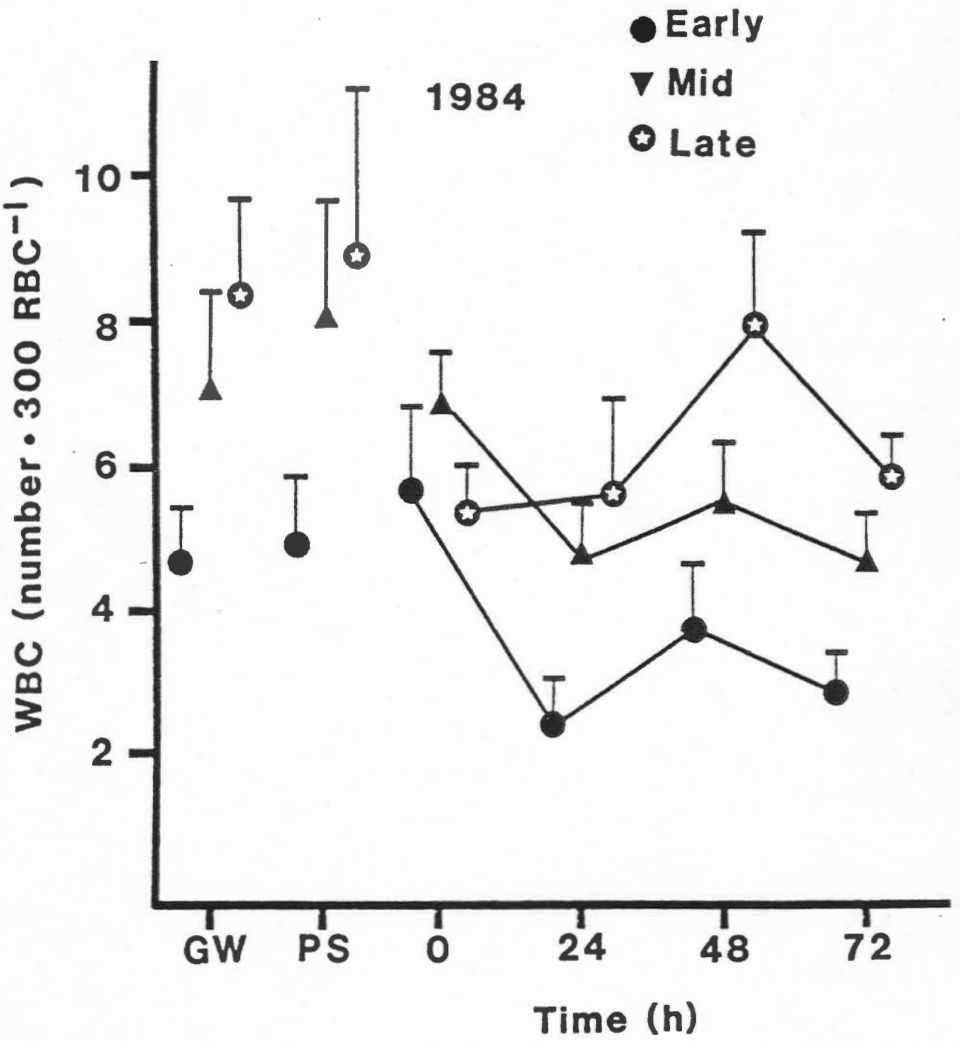

Figure 24. White blood cell (WBC) counts for juvenile fall chinook salmon collected from the gatewell (GW), just before crossing the bar-sorter (PS), and at various times in the raceway at McNary Dam during June 12-15 (early), July 10-14 (mid), and August 7-10 (late), 1984. WBC values are the mean + SE of the average of two replicate counts of the number of WBC's among 300 erythrocytes (RBC's) on blood smears from 6 fish.

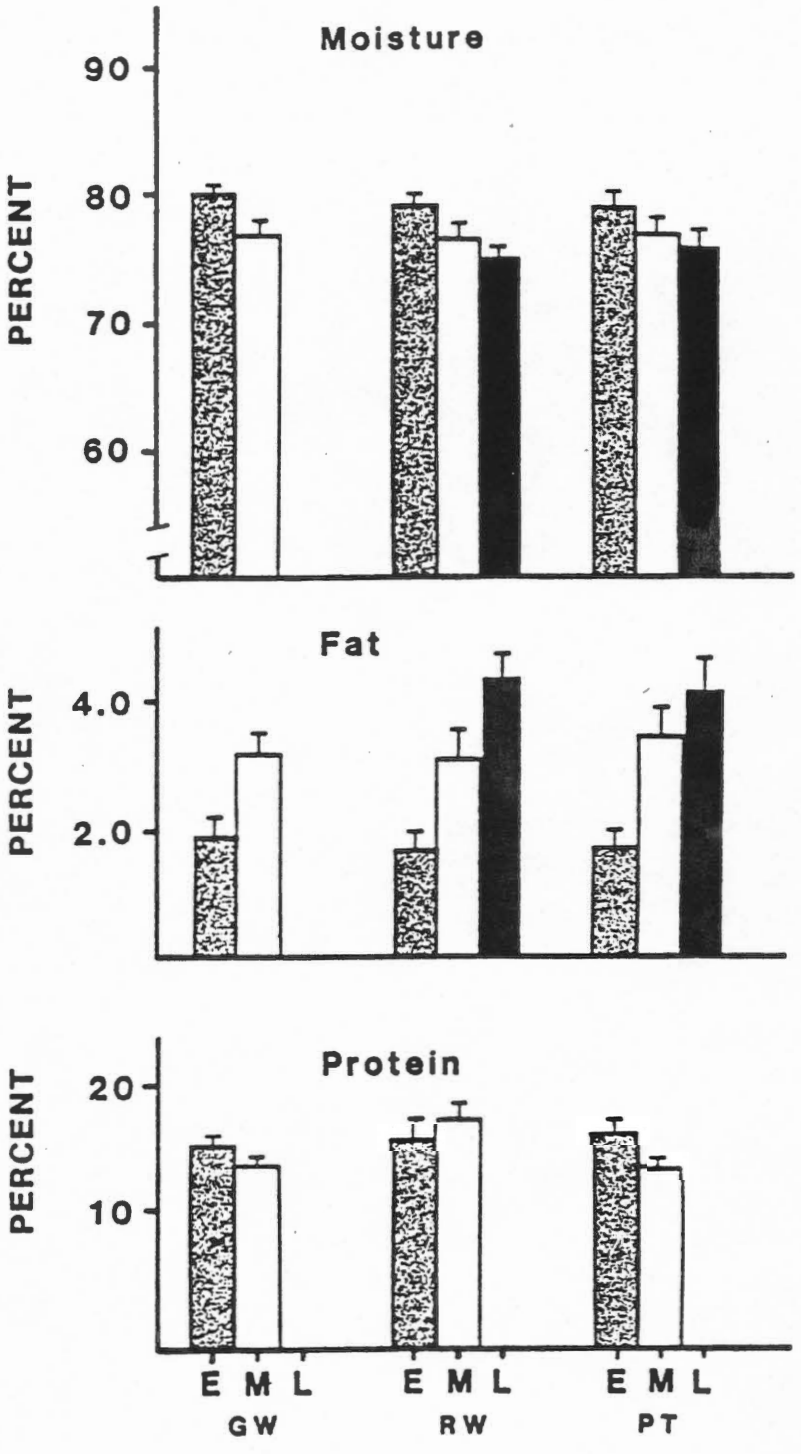

Figure 25. Results of proximate analysis of whole body constituents of juvenile fall chinook salmon collected during June 14-24 (early), July 7-16 (mid), and August 2-11 (late), 1982. Fish were taken from the gatewells (GW) and just before they entered a raceway (RW) at McNary Dam, and after transport to Bonneville Dam (PT). All bars represent the means + SE for 6 fish.

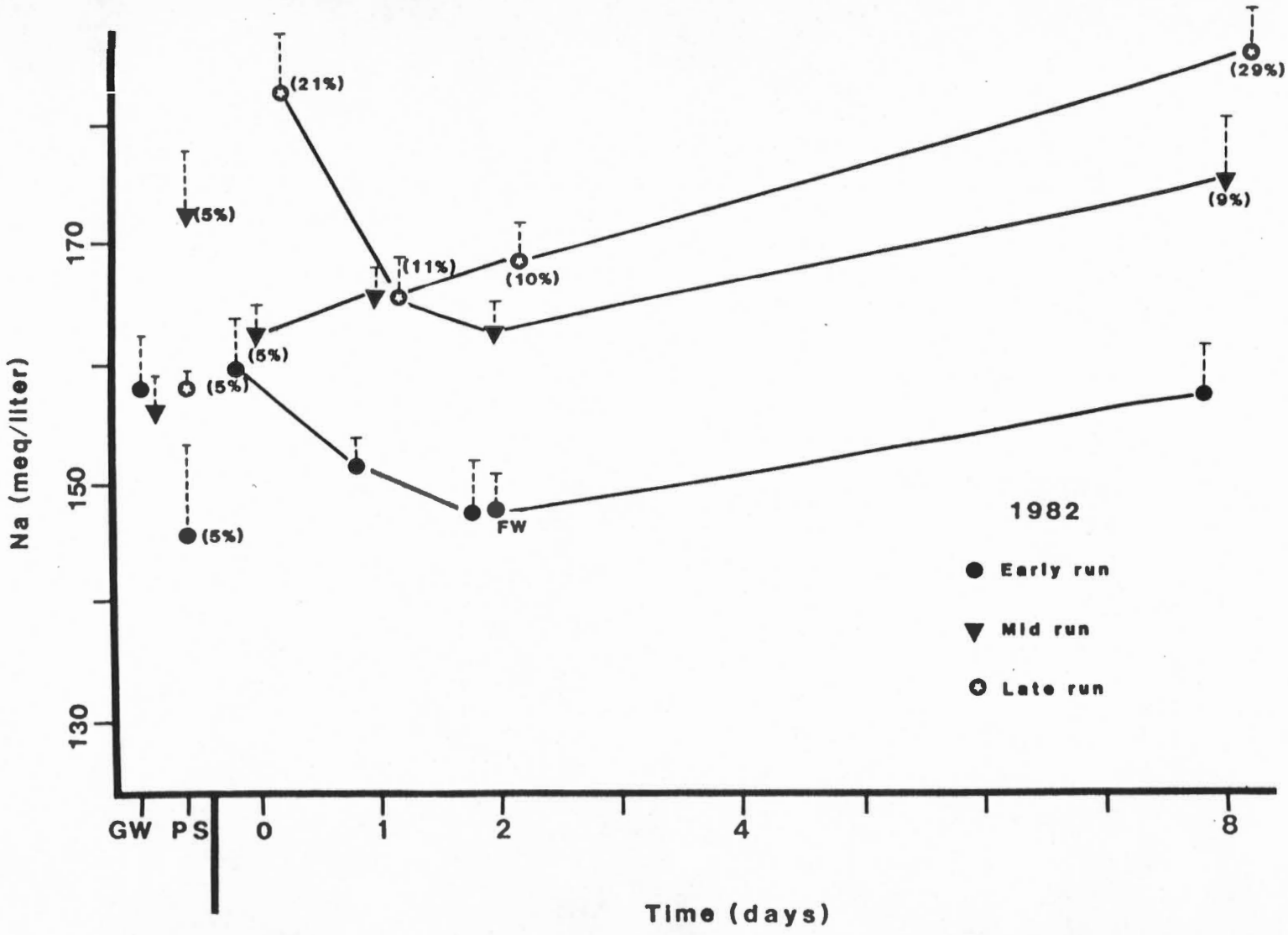

Figure 26. Plasma Na levels (mean + SE) of fall chinook salmon, 24 h after being put in 15 parts per thousand salt water or fresh water (FW). Fish were taken from the McNary Dam gatewell (GW) before passing the bar-sorter (PS) and during 8 d of recovery in the raceway after collection. Sampling was completed June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points are duplicates of 10 fish each; percent mortality is in parentheses.

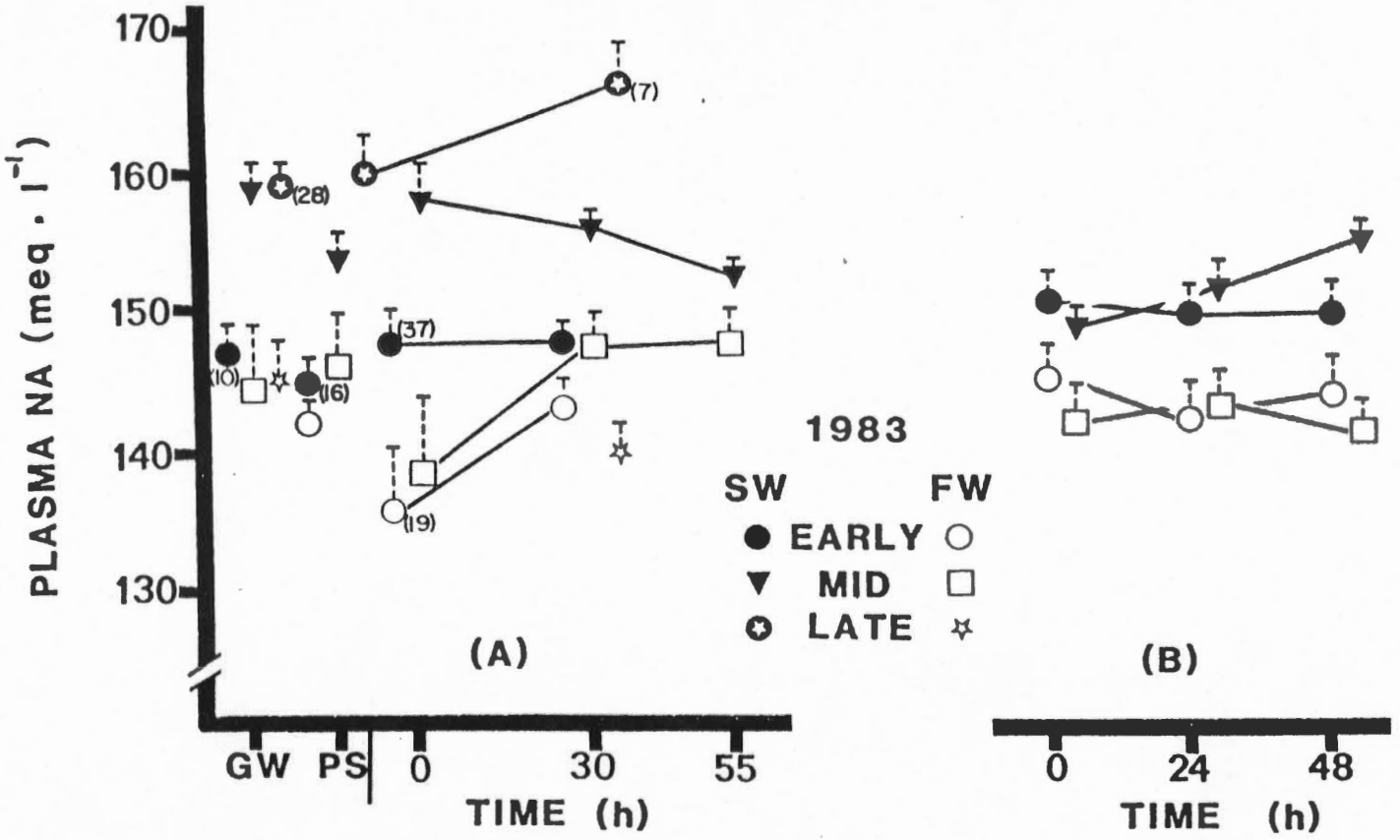

Figure 27. Plasma Na levels of outmigrant fall chinook salmon, 18 h after being put in 20 parts per thousand salt water (SW) or fresh water (FW). Fish were taken from McNary Dam gatewell (GW), just before the bar-sorter (PS) and after various recovery times in the raceway (A), and after various recovery times following transport to Bonneville Dam (B). All points are the mean + SE of replicate 10-fish groups challenged June 13-18 (early), July 7-16 (mid), and August 2-5 (late), 1983. Percent mortalities are in parentheses.

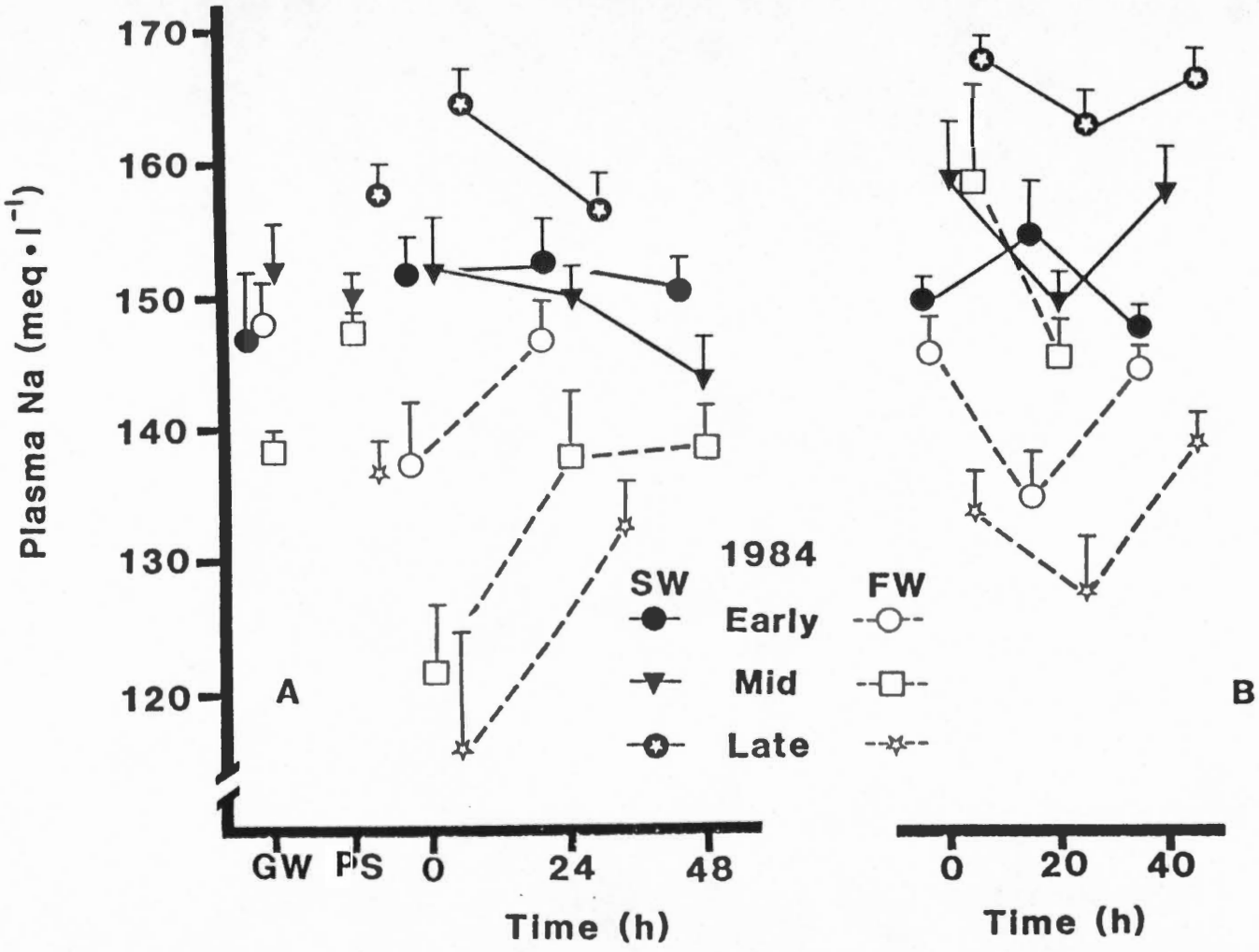

Figure 28. Plasma Na levels of juvenile fall chinook salmon 18 h after being put in 20 parts per thousand salt water (SW) or fresh water (FW). Fish were taken from the McNary Dam gatewell (GW), just before crossing the bar-sorter (PS), and after various times in the raceway (A), and after various times following transport to Bonneville Dam (B). All SW points represent the mean + SE for two replicate groups of 8 to 10 fish; FW points represent the mean + SE for 7 to 14 fish challenged June 12-15 (early), July 11-14 (mid), and August 8-10 (late), 1984.

ability of fish held in the raceways for 8 d (Fig. 26). The plasma Na levels of fish placed in fresh water during 1983 and 1984 also show the decrease in osmoregulatory ability as the runs progressed (Figs. 26-27). These fish were not challenged by the fresh water, but the fact that plasma Na levels decreased significantly in fish collected as they entered the raceways (Time = 0) indicates that the stress of the collection system reduced their ability to maintain a constant plasma osmolality.

The secondary stress test demonstrated the cumulative effects of the stresses fish encounter in the collection facility. Plasma cortisol reached progressively higher levels and required a longer time to return to pre-stress levels in fish which had been in the raceway for 0 to 20 h when compared to fish transported to Bonneville Dam or fish that had been in the raceway for 7 d (Fig. 29). Also of note is that fish in the raceway for a short time and fish transported to Bonneville had the same pre-stress plasma cortisol titers (ca. 200-250 $ng \cdot ml^{-1}$), but the plasma cortisol increase in fish which had been in the raceway for a short time was twice that of transported fish (Fig. 29). This indicates that even though fish had comparable plasma cortisol levels, their response to another stress was affected by the length of time since the previous stress (see: Multiple stress). Cumulative effects of stress were also seen in fall chinook subjected to the secondary stress in 1983 (Fig. 30); however, the peak cortisol levels were considerably lower than in 1982 (ca. 375 vs. 1050 $ng \cdot ml^{-1}$). In 1984, plasma cortisol levels in secondarily stressed fish (Figs. 31-33) were similar to those in fish from 1983; however, peak values were all very much the same (ca. 275-300 $ng \cdot ml^{-1}$) independent of where or when the fish were collected. Although the differences are not

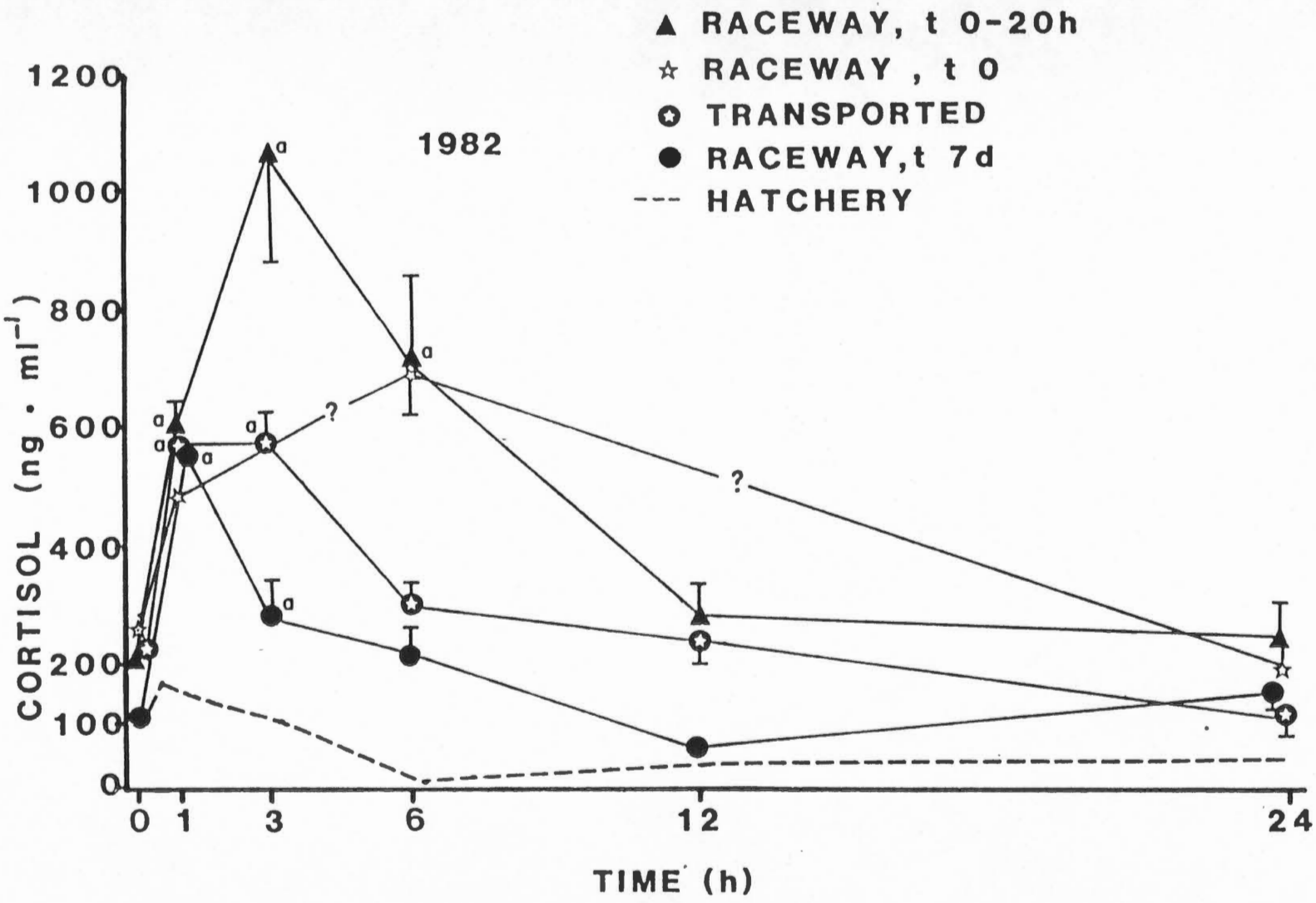

Figure 29. Plasma cortisol levels of outmigrating fall chinook salmon from McNary Dam, subjected to a 30-s handling stress by suspension out of water in a dipnet. Fish were taken from just before entry into a raceway, after 0 to 20 h, and 7-d raceway recovery, and after transport to Bonneville Dam. For comparison, results for hatchery, laboratory-acclimated fish subjected to the same 30-s handling stress are included. Points are mean + SE for 6-13 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

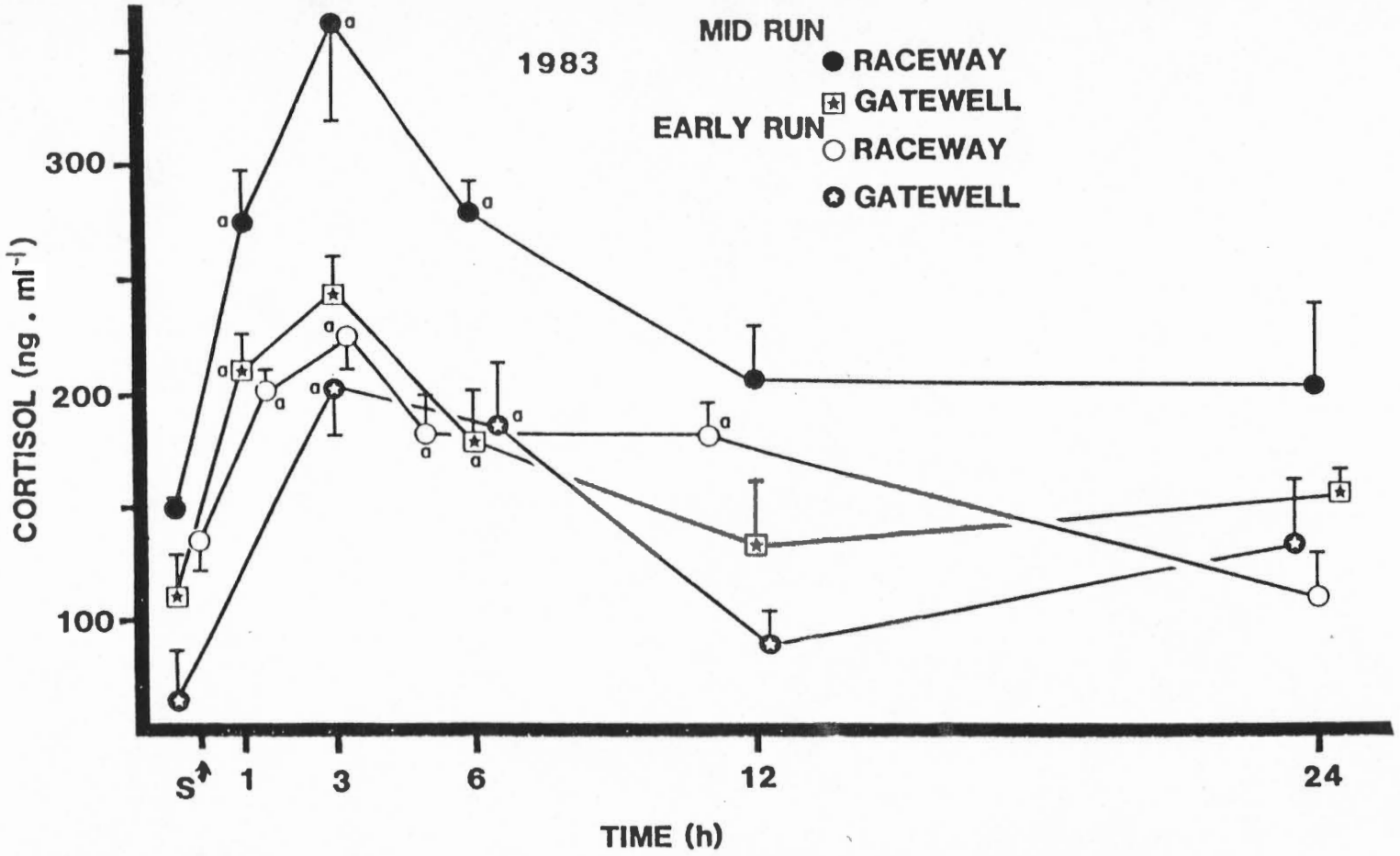

Figure 30. Plasma cortisol levels in fall chinook salmon collected from the gatewell or raceway at McNary Dam, held in the air in a dipnet for 30 s, and released into a tank (ca. 100L) with flow-through water, and sampled through 24 h. Sampling was completed during June 14-19 (early run) and July 7-13 (mid run), 1983. All points are the mean + SE of 10 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

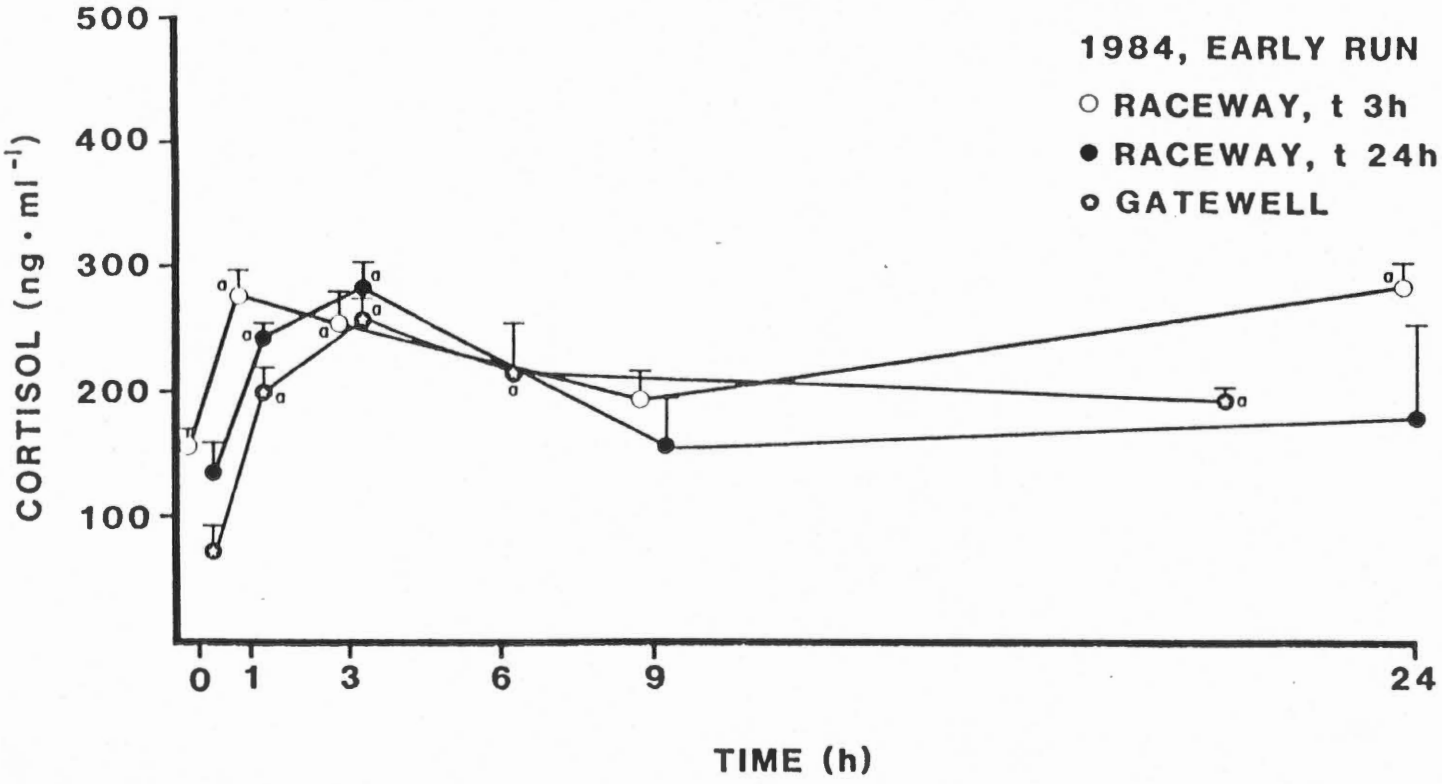

Figure 31. Plasma cortisol levels in juvenile fall chinook salmon taken from the gatewell and raceway at McNary Dam, suspended in the air in a dipnet for 30 s, and allowed to recover in large plastic buckets with flow-through water. Samples were collected during June 12-14, 1984; all points represent the mean + SE for 7 to 10 fish except for gatewell t = 20 h and raceway (t24h) t = 24 h where n = 3. Values marked (a) are significantly different from T = 0 of same line ($P < .05$, LSD test).

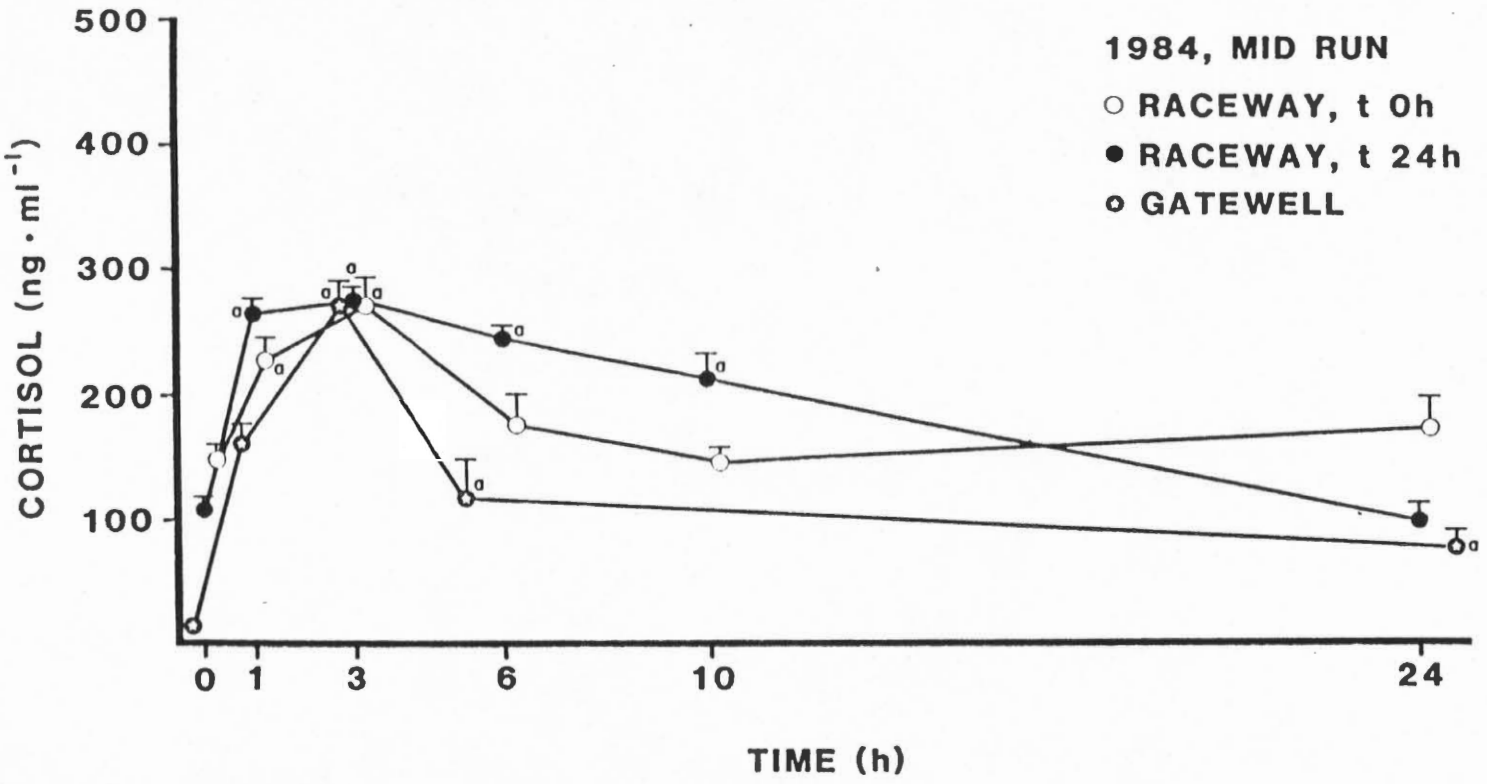

Figure 32. Plasma cortisol levels in juvenile fall chinook salmon taken from the gatewell and raceway at McNary Dam, suspended in the air in a dipnet for 30 s, and allowed to recover in large plastic buckets with flow-through water. Samples were collected during July 10-14, 1984; all points represent the mean + SE for 8 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

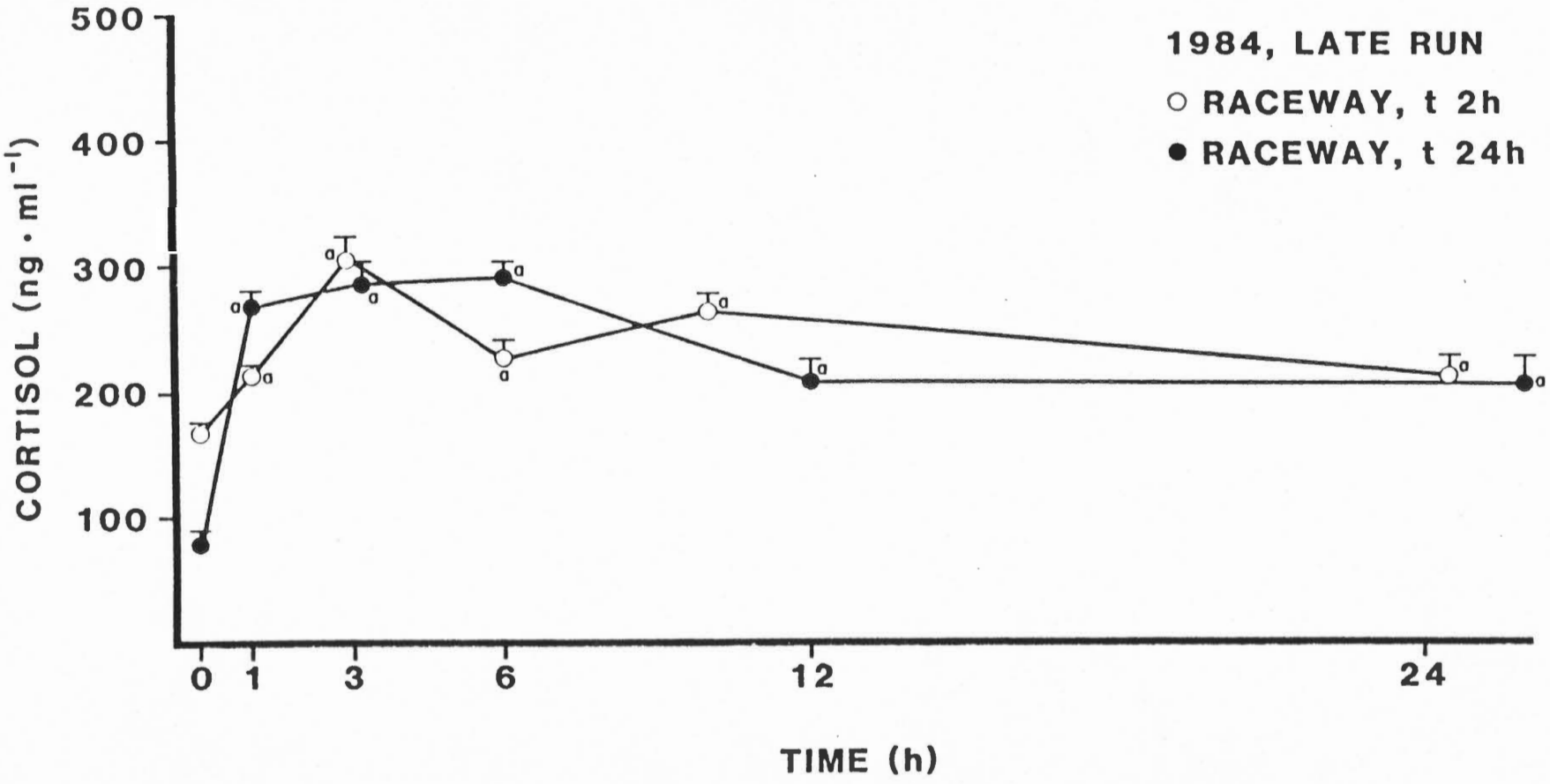

Figure 33. Plasma cortisol levels in juvenile fall chinook salmon taken from the raceway at McNary Dam, suspended in the air in a dipnet for 30 s, and allowed to recover in large plastic buckets with flow-through water. Samples were collected during August 7-9, 1984; all points represent the mean + SE for 10 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

statistically significant, fish taken from the gatewell generally had lower plasma cortisol than did fish from the raceway (Figs. 31-33). Plasma lactate was assayed for fall chinook which were secondarily stressed in 1982; however, there was no pattern of treatment effects (Fig. 34).

The results of the swim tube experiments performed at McNary Dam in 1984 suggest that swimming ability is impaired in fish immediately after they have gone through the collection facility (Table 2). Although there is considerable variability in the absolute values between runs, it appears that less time is required to fatigue fish which were collected at the bar-sorter or upon entering the raceway than fish taken from the gatewell. The increase in time to fatigue after 24-48 h indicates that the impairment is temporary, but that some time is required to recover swimming ability. Laboratory work indicates that stress can also reduce fishes' ability to respond to an adverse stimulus (see: Stress and Swimming Performance). This could be critical when recently-bypassed smolts need to avoid predators or physical obstructions in the environment.

Spring chinook smolts were also stressed by the collection system as evidenced by their plasma cortisol responses (Figs. 35-36) which paralleled those of fall chinook sampled in 1983 (Figs. 4, 5, and 6). The spring chinook responded to the stresses of the collection system in a cumulative manner as shown by the increasing plasma cortisol levels in fish as they move through the system (Figs. 35-36). Cortisol levels of spring chinook also returned to baseline (i.e., < 100 ng$\cdot$ml^{-1}) within 24 h. Hepatic glycogen in spring chinook (Fig. 37) was consistently lower than levels in hatchery fish (fall chinook) fasted for 20 d, but did not reflect the continuing decrease which was seen with some fall chinook (Figs. 14-15).

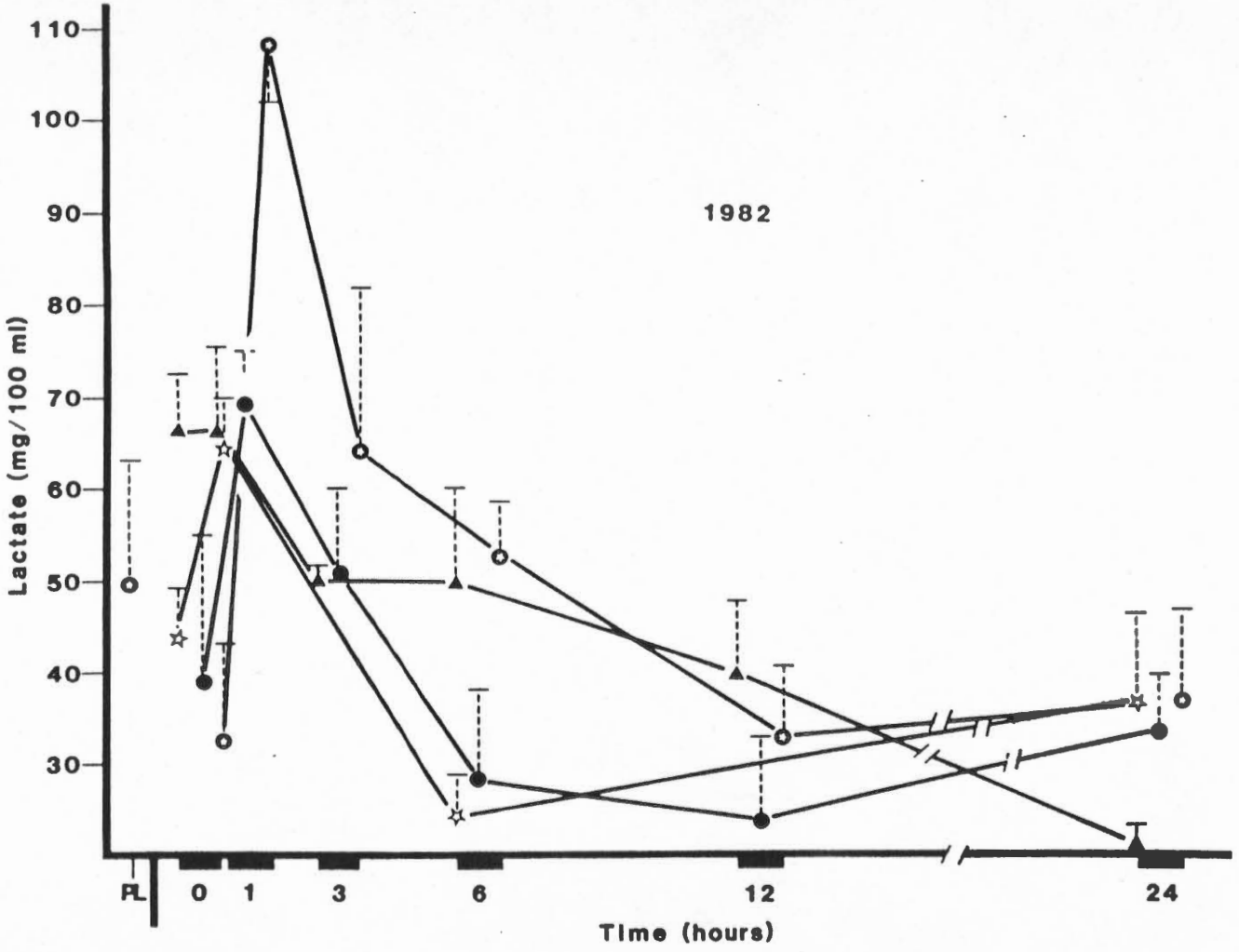

Figure 34. Plasma lactate levels (mean + 1 SE) of outmigrant fall chinook salmon subjected to a 30-s suspension out of water in a dipnet. Fish were taken from just before entry into a raceway (☆), after 0-20 h (▼) and 7-d (●) recovery, and after being loaded into a truck (PL) and transported to Bonneville Dam (✪). All points represent 3 fish.

Table 2. Geometric means of fatigue times (minutes) of juvenile fall chinook salmon taken from the gatewell, just before crossing the bar-sorter (presort), and after various times in a raceway (RW) at McNary Dam.

Sample	Early Run June, 1984	Mid-Run July, 1984	Late Run August, 1984
Gatewell	[a]	2.10	[a]
Presort	[a]	0.24	0.17
RW t=0	0.06	0.66	0.08
RW t=24	1.80	0.83	0.29
RW t=48	4.60	4.90	0.61

[a]Measurements not made

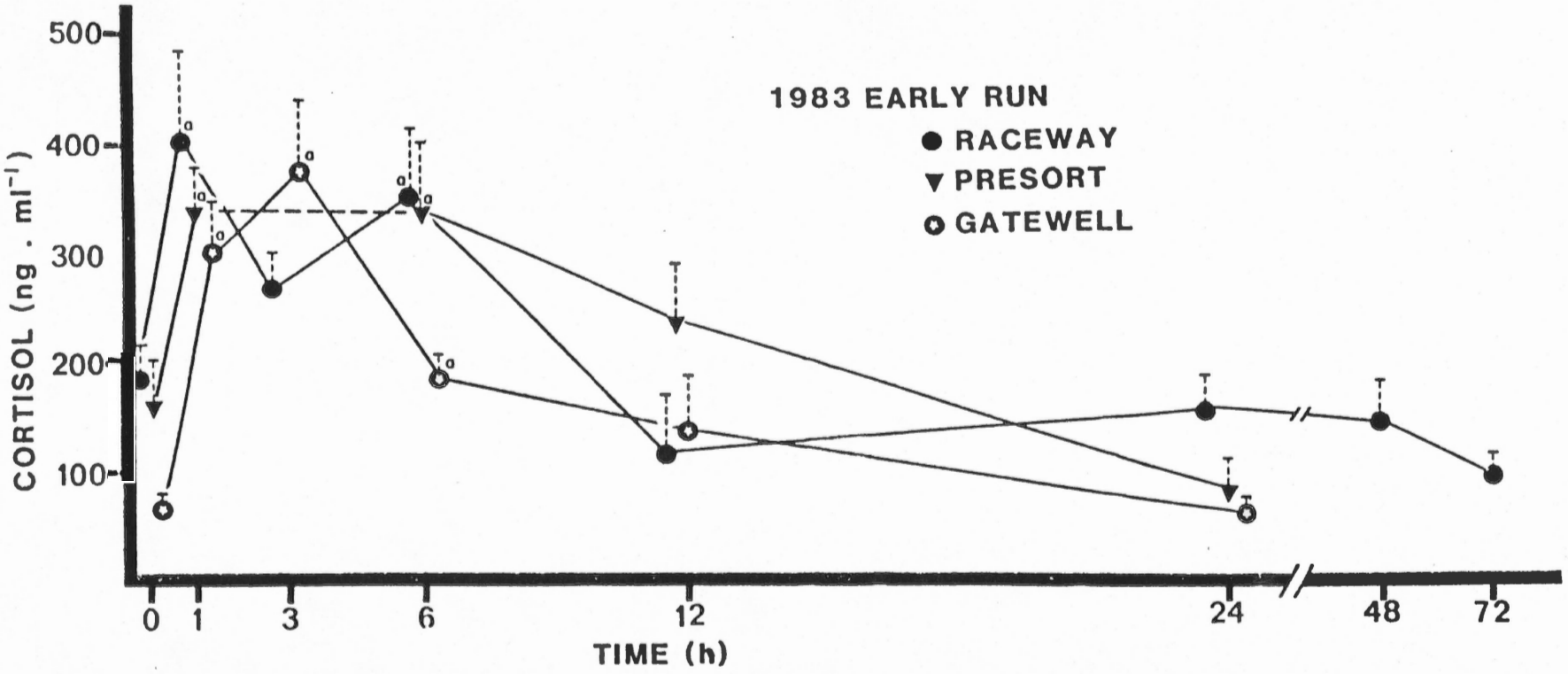

Figure 35. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell, just before the bar-sorter (presort) and the raceway, and after various recovery times in the raceway and in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 3-6, 1983. All points are the mean + 1 SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

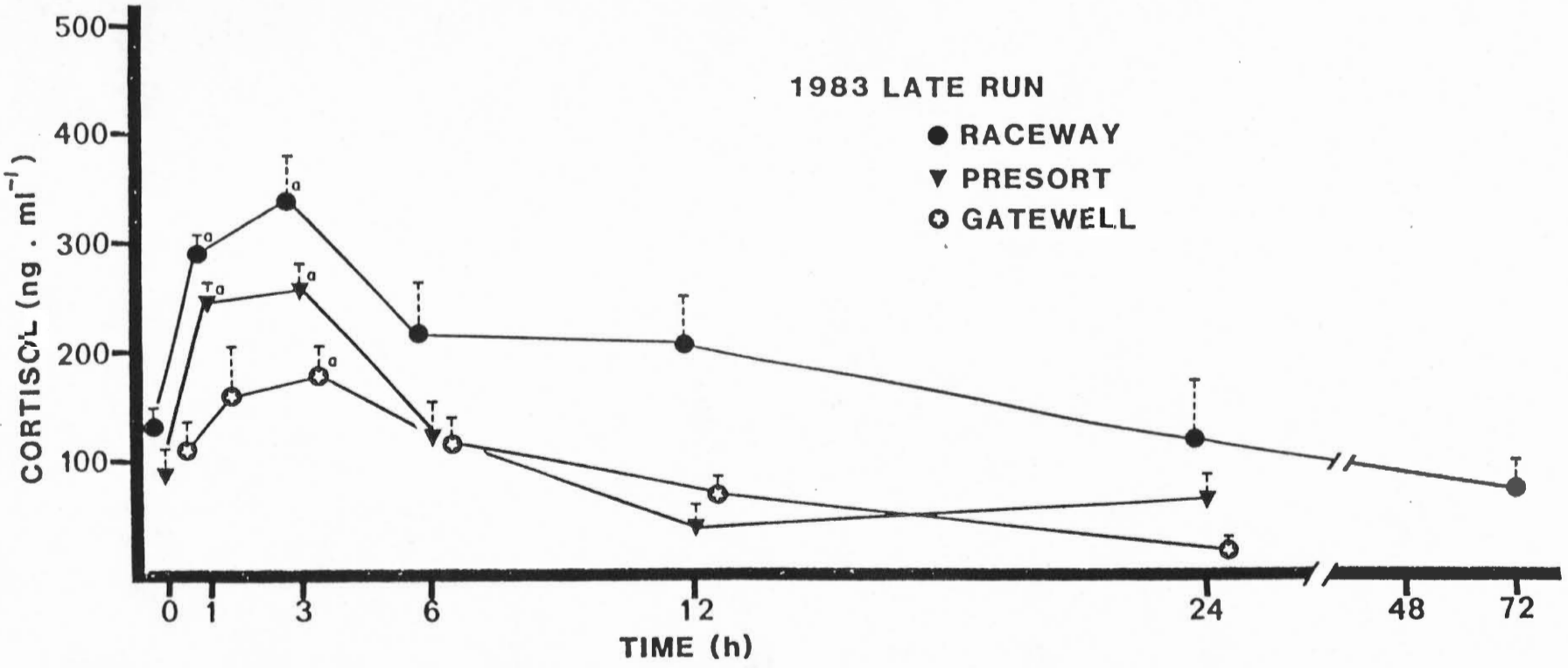

Figure 36. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell, just before the bar-sorter (presort) and the raceway, and after various recovery times in the raceway and in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 23-27, 1983. All points are the mean +1 SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

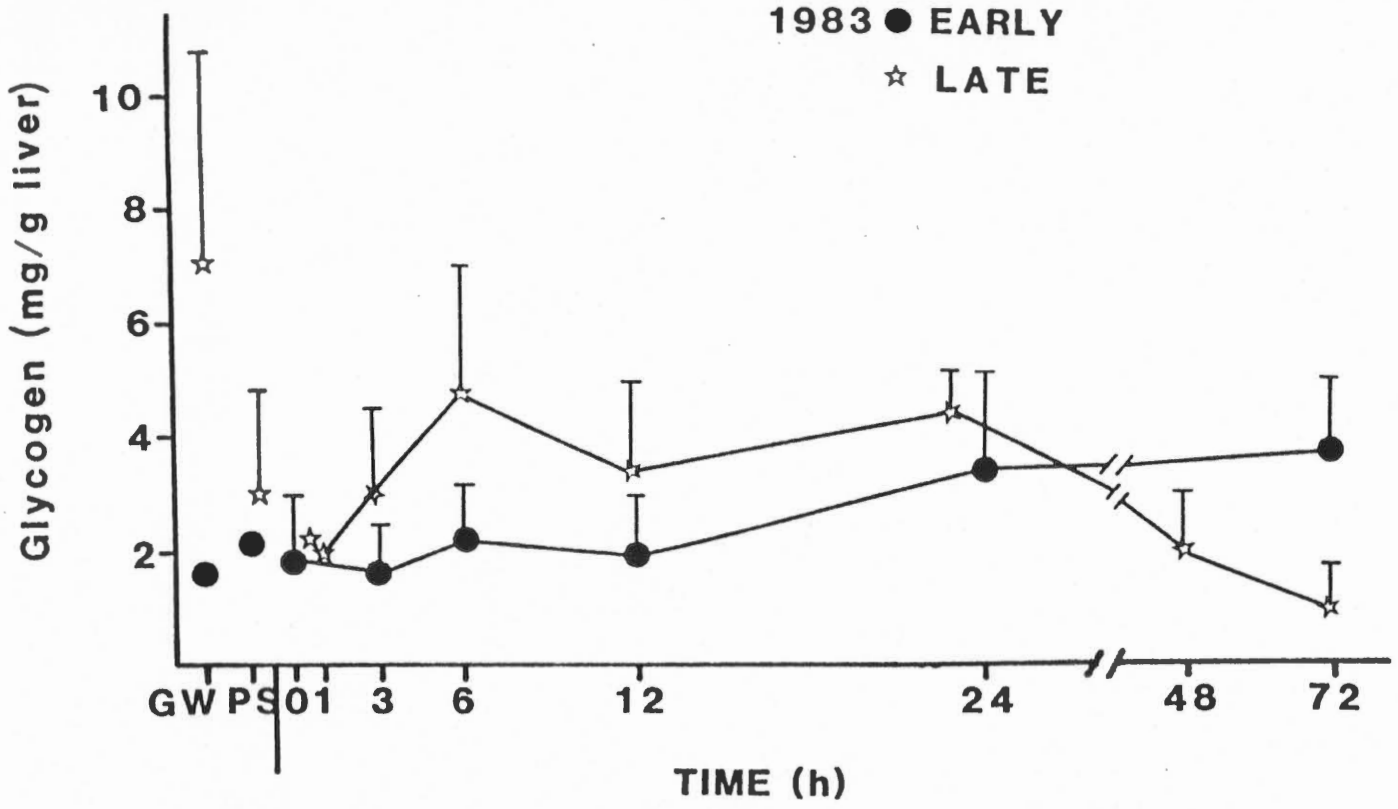

Figure 37. Hepatic glycogen levels in juvenile spring chinook salmon sampled from the McNary Dam gatewell (GW), before passing the bar-sorter (PS) and during 72 h of recovery in a raceway. Sampling was conducted during May 3-6 (early) and May 23-27 (late), 1983. All points are mean + SE of 4 to 6 fish.

This is consistent with results for fasted fish (see: Nutrition and Stress), and indicates that hepatic glycogen can be depressed only so far before other energy reserves are activated (i.e., lipids and muscle glycogen). Initial plasma glucose levels in spring chinook were significantly higher in early-run fish (Fig. 38; ca. 75 $mg \cdot dl^{-1}$) than in late-run fish (Fig. 39; ca. 35 $mg \cdot dl^{-1}$). These variations were probably the result of different histories (i.e., different origin and nutrition) of fish passing the dam at the different times. The stresses of the collection system caused increases in plasma glucose to approximately the same level in early-and late-run spring chinook, levels which were comparable to those of fasted fish subjected to a single 30-s stress (see: Nutrition and Stress). Hematocrit and leucocrit values of spring chinook smolts showed considerable variability and no consistent patterns between runs (Fig. 40). WBC counts (Fig. 41) were also variable, but there appeared to be a depression in relative numbers of WBC between 0 to 48 h after the fish reached the raceway, similar to that found in fall chinook (Figs. 22 and 23).

Osmoregulatory capacity, as measured by saltwater challenges in spring chinook, was better in the late run than in the early run (Fig. 42), which may reflect the increased size of smolts (from 21.0 ± 1.0 to 25.4 ± 0.6 g) without a significant increase in water temperature (Table 1). Inexplicably, the osmoregulatory capacity of spring chinook taken from the gatewell (the sampling station we assumed was the least stressful) was reduced compared to fish from other parts of the collection system (Fig. 42). Response of spring chinook smolts to a secondary stress further illustrated the cumulative nature of stresses in the collection system (Fig. 43). Fish which were taken from the gatewell or after 1 h in the raceway had the same

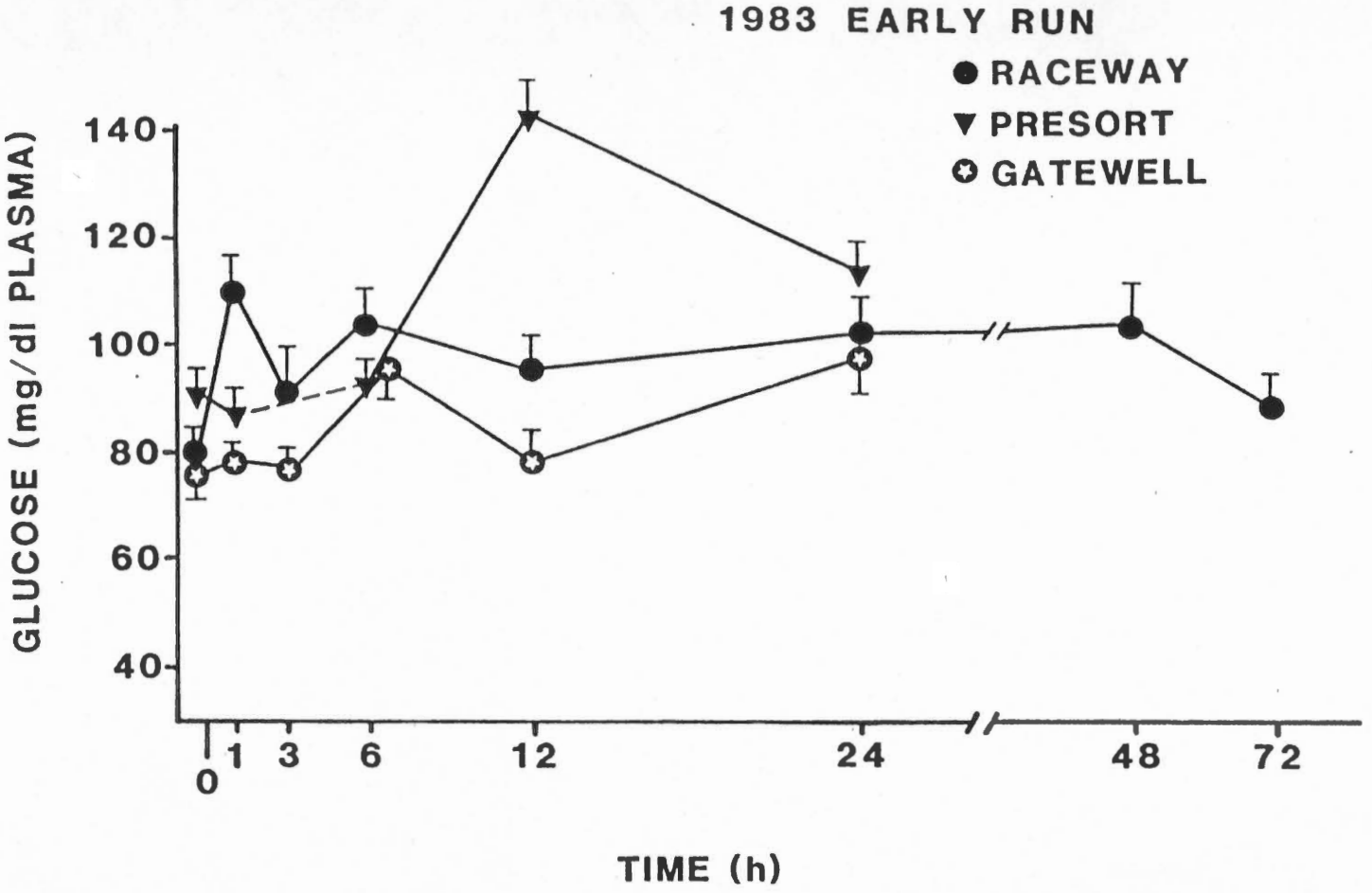

Figure 38. Plasma glucose in juvenile spring chinook salmon taken from the McNary Dam gatewell, just before the bar-sorter, and the raceway; and after various recovery times in the raceway and in plastic tanks with flow-through water. Points are mean + SE of 10 to 12 fish collected during May 3-6, 1983.

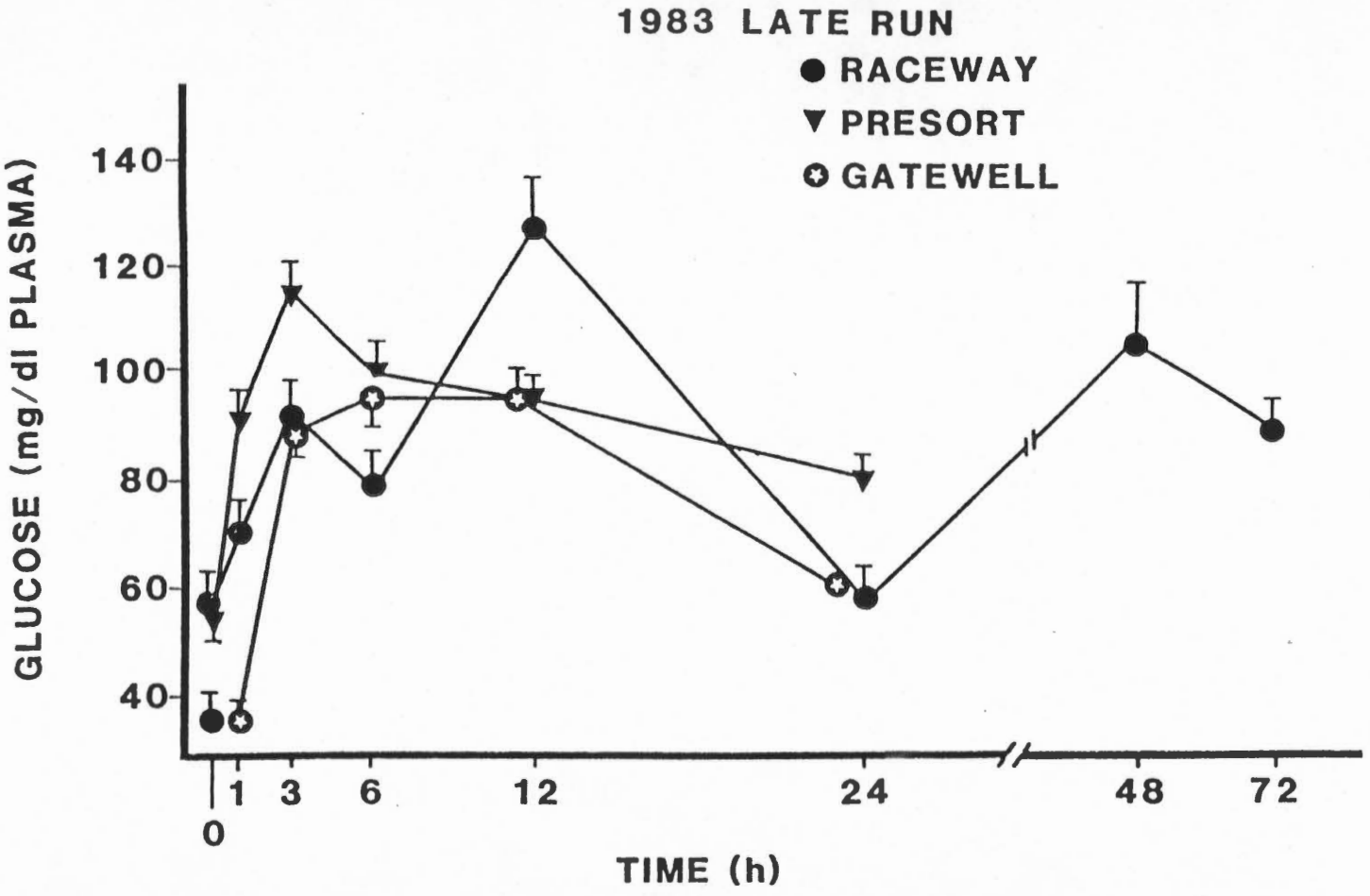

Figure 39. Plasma glucose in juvenile spring chinook salmon taken from the McNary Dam gatewell, just before the bar-sorter, and the raceway; and fish sampled after various recovery times in the raceway and in plastic tanks with flow-through water. Points are mean + SE of 10 to 12 fish collected during May 23-27, 1983.

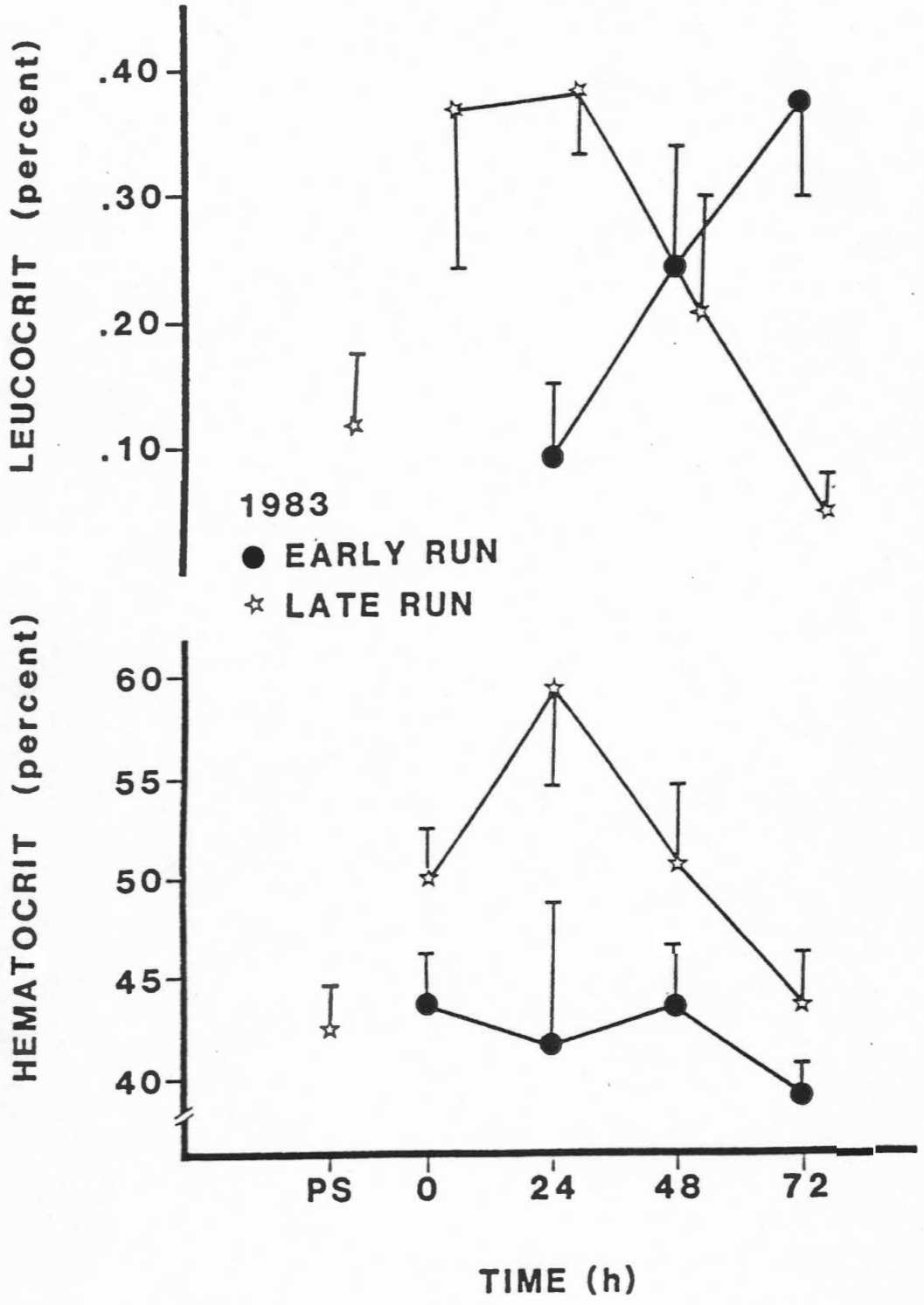

Figure 40. Leucocrit and hematocrit values in juvenile spring chinook salmon collected from McNary Dam during May 3-6 (early run) and May 23-27 (late run), 1983. Fish were sampled just before the bar-sorter (PS) and through 72 h of recovery in a raceway. Points are the means + SE of 6 fish.

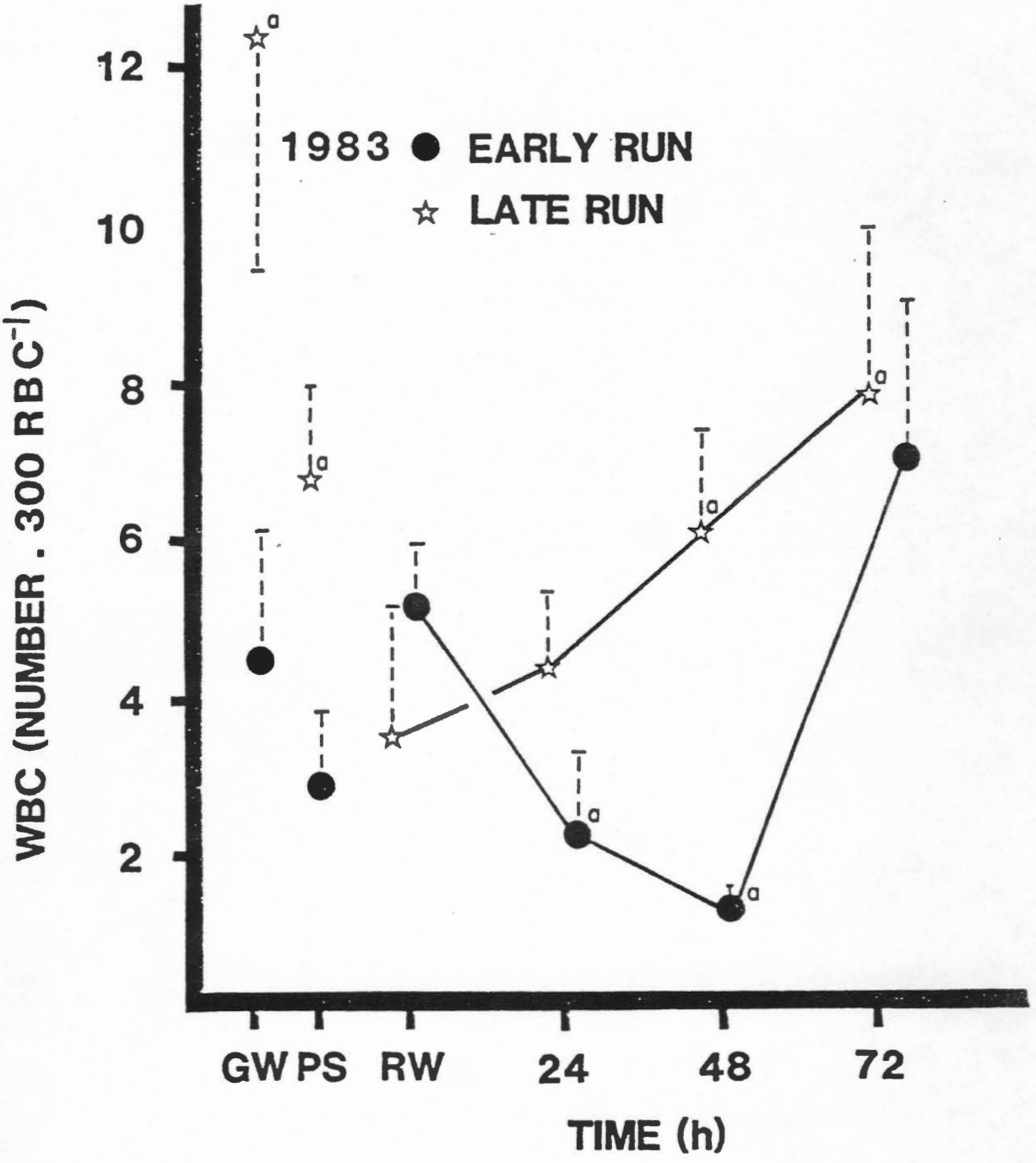

Figure 41. White blood cell (WBC) counts for outmigrating spring chinook salmon collected from the gatewell (GW), just before the bar-sorter (PS), just before entering the raceway (RW), and after various recovery times in the raceway. Samples were collected during May 3-6 (early run) and May 23-27 (late run), 1983. All points are the means + SE of the average of two replicate counts of the number of WBC's among 300 erythrocyte (RBC) on the blood smears from 6 fish. Points marked (a) are significantly different from RW of same line ($P < .05$, LSD test).

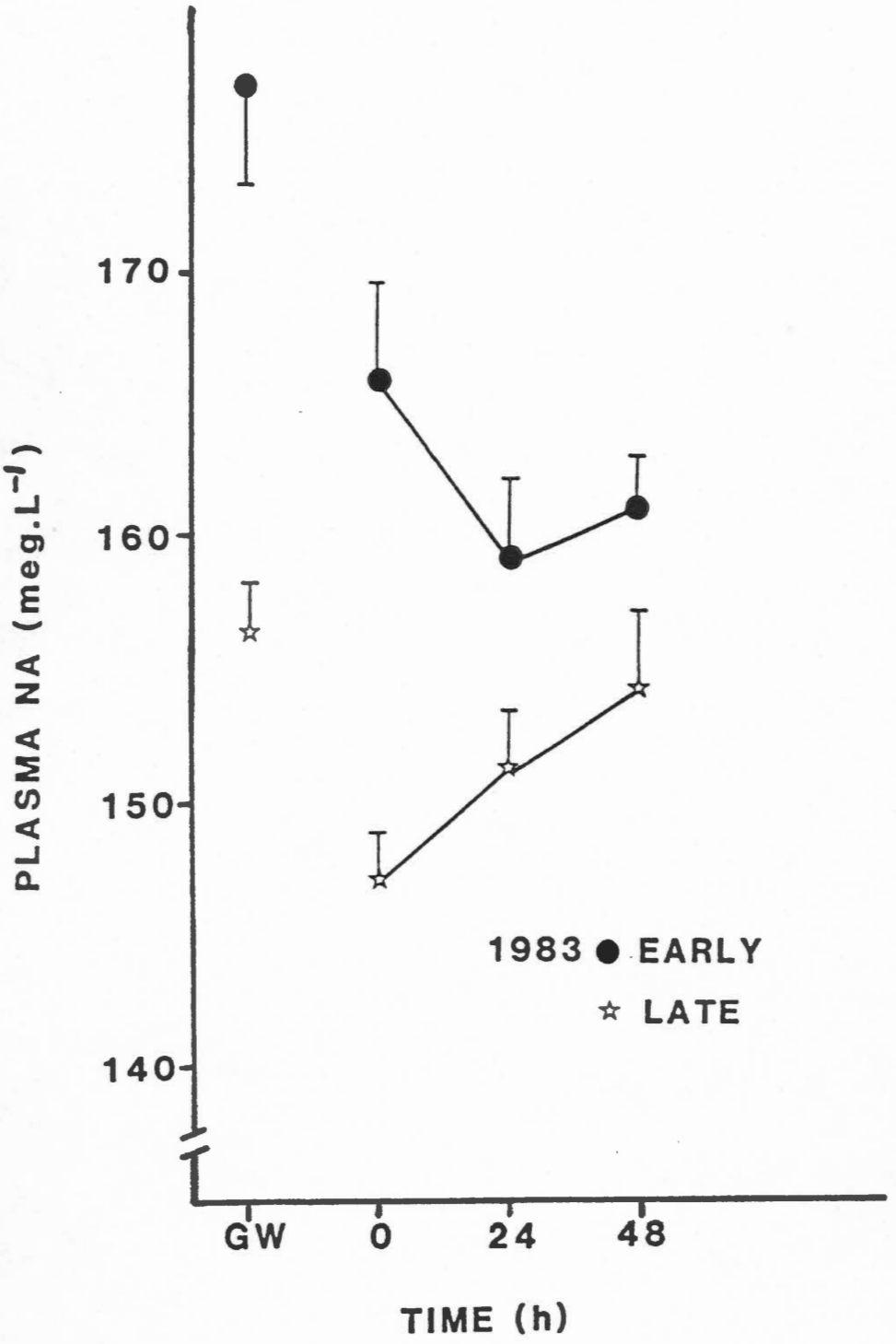

Figure 42. Plasma Na levels in juvenile spring chinook salmon, 18 h after being put in 17 parts per thousand salt water. Fish were taken from McNary Dam gatewell (GW) after various recovery times in the raceway. All points are the mean + SE of replicate 10-fish groups challenged May 3-6 (early) and May 23-27 (late), 1983.

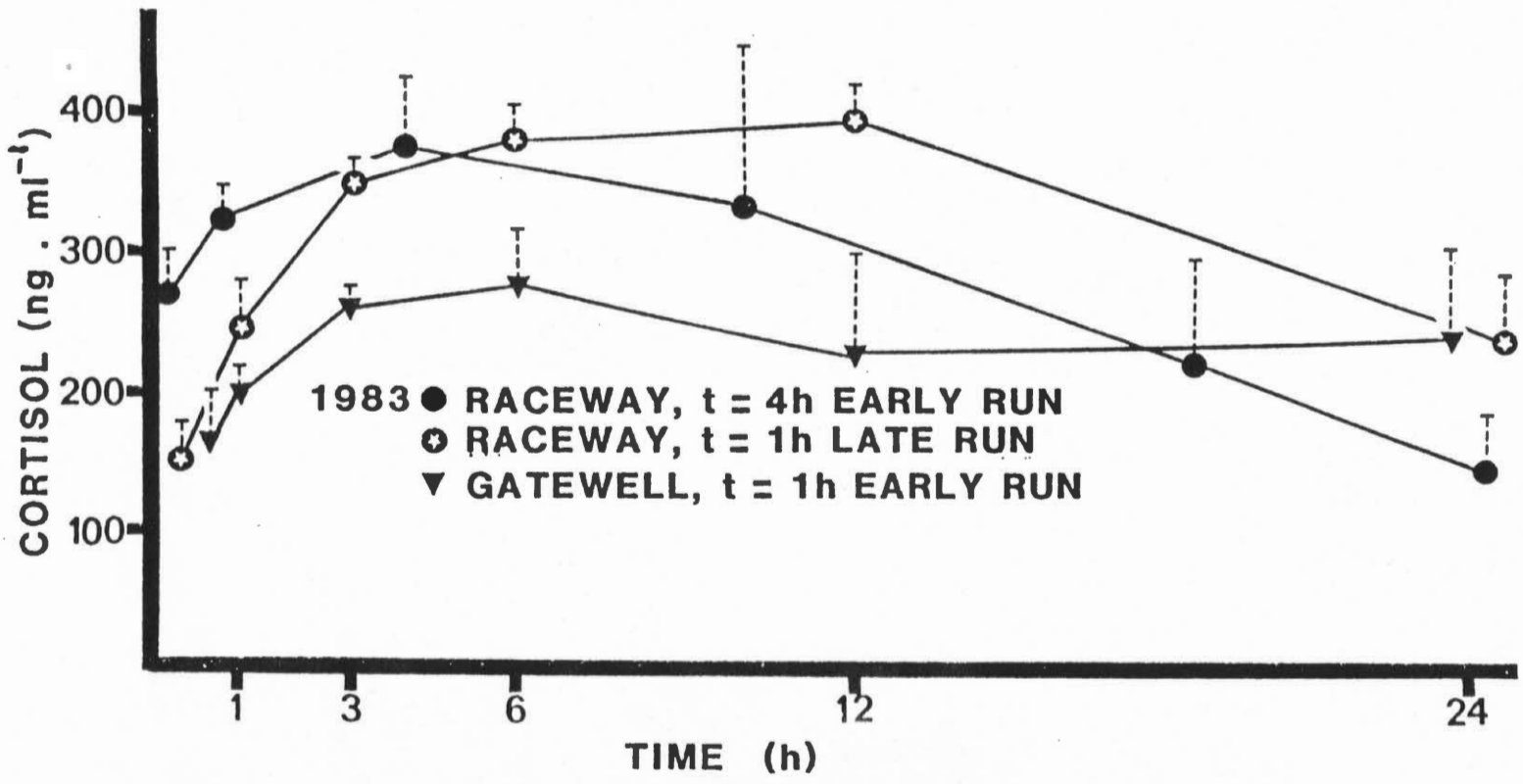

Figure 43. Plasma cortisol levels for outmigrating spring chinook salmon taken from the gatewell and the raceway, and suspended in the air in a dipnet for 30 s and allowed to recover in plastic buckets with flow-through water. Samples were collected at McNary Dam, May 3-5 (early) and 23-27 (late), 1983. All points are means + SE of 10 to 12 fish.

mean plasma cortisol levels prior to the stress challenge (ca. 150 $ng \cdot ml^{-1}$). However, after the stress challenge the plasma cortisol of fish from the gatewell peaked at approximately 275 $ng \cdot ml^{-1}$, while levels in fish from the raceway peaked at approximately 410 $ng \cdot ml^{-1}$, indicating that fish from the raceway were sensitized by the stress of the collection system. Moreover, the system elicited a maximum plasma cortisol increase from spring chinook. That is, the plasma cortisol levels of spring chinook which had gone through the system and were secondarily stressed (Fig. 43) were not greater than the plasma cortisol levels of fish which had gone through the system but were not secondarily stressed (Figs. 35 and 36). By comparison, secondarily stressed fall chinook had plasma cortisol levels which were considerably greater than those of fish from the system (Figs. 3-9 and 29-33). Furthermore, plasma cortisol levels in fall chinook were generally reduced 12 h after the secondary stress (Figs. 29-32) while cortisol response in spring chinook remained elevated beyond 12 h (Fig. 43). This may indicate that spring chinook are more sensitive than fall chinook to the system or that factors in the system, such as the presence of steelhead trout, make the system more stressful to the spring chinook.

Raceway density evaluation. Loading raceways with fall chinook to the established maximum of 0.5 $lbs \cdot gal^{-1}$ did not appear to have serious long-term effects on the fish, as judged by plasma cortisol levels (Fig. 44). It appeared that increasing density from 0.13 to 0.25 to 0.5 $lbs \cdot gal^{-1}$ caused an increase in initial plasma cortisol levels, but most cortisol levels were significantly reduced after 24 h. The initial plasma cortisol response of fish held in the raceway at 0.5 $lbs \cdot gal^{-1}$ was highest in fish sampled in the late run followed by the mid-run and early run during

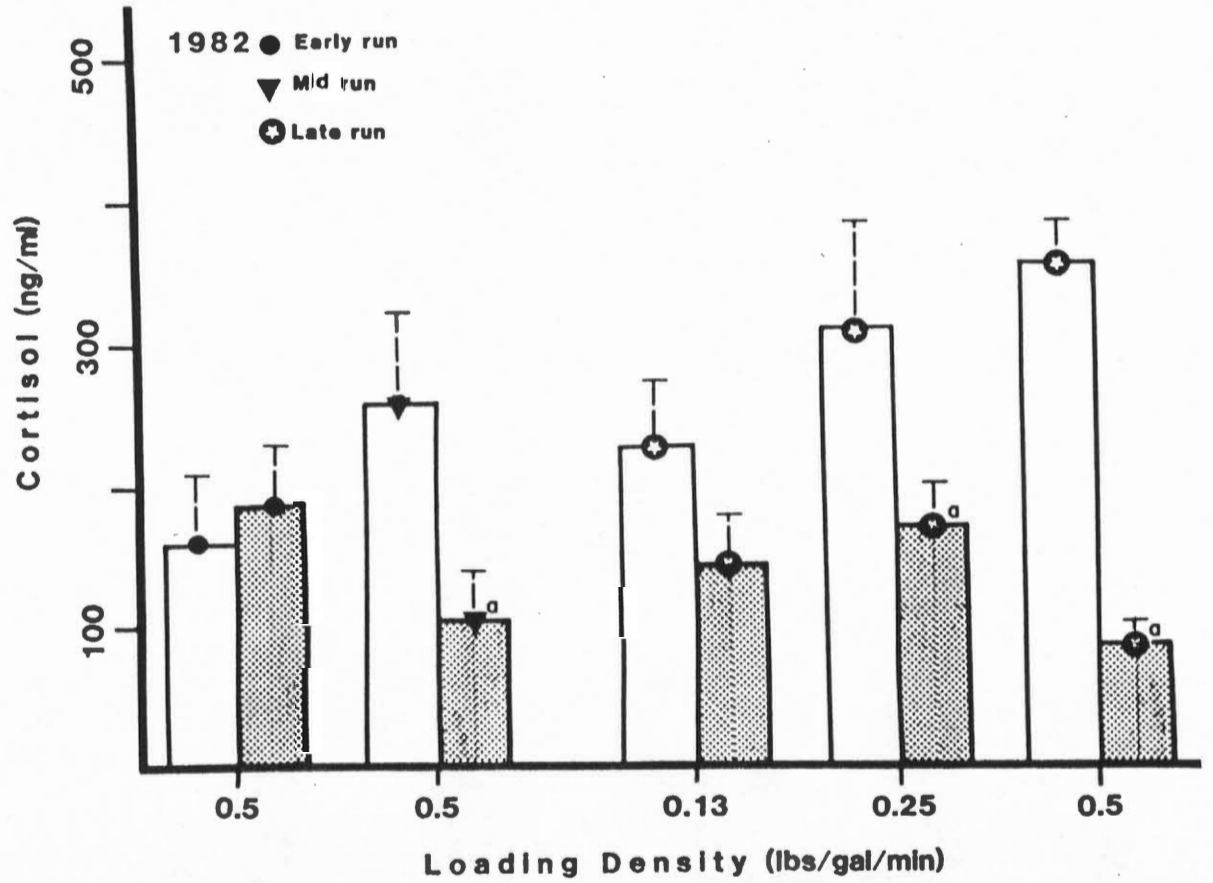

Figure 44. Plasma cortisol levels of juvenile fall chinook salmon collected at McNary Dam and allowed to recover in raceways at various loading densities. Samples were taken after raceways were loaded and fish crowded to appropriate density (clear bars) and 24 h after crowding (stippled bars). Points are means + SE for n = 11 to 13. Points marked (a) are significantly different from values of fish immediately after they were crowded to appropriate density ($P < .05$, LSD test).

1982 (Fig. 44), indicating that there may have been some environmental factor or predisposing factor in the fish which affected the cortisol response to stress as the run progressed. During both the mid-run and late run, 1983, plasma cortisol levels were approximately 150 $ng \cdot ml^{-1}$ in fall chinook smolts as they entered the raceway, and approximately 100 $ng \cdot ml^{-1}$ after the fish were held at 0.5 $lbs \cdot gal^{-1}$ for 24 h (Figs. 5 and 6), suggesting that there was no difference in the response to maximum loading density through the 1983 run. Sampling techniques were different between 1982 and 1983 and so the data are not comparable between years. Spring chinook smolts were held at 0.5 $lb \cdot gal^{-1}$ during both sampling periods in 1983. During the early portion of the run, smolts entering the raceway had plasma cortisol levels of approximately 190 $ng \cdot ml^{-1}$ as compared to approximately 160 $ng \cdot ml^{-1}$ during the late run (Figs. 35 and 36). After 24 h in the raceway, cortisol levels were approximately 140 and 130 $ng \cdot ml^{-1}$ in early-run and late-run fish, respectively (Figs. 35 and 36).

The elevated initial plasma cortisol levels in fall chinook sampled during 1982 were undoubtedly caused by the sampling procedure which required crowding the fish into one end of the raceway prior to taking the sample of fish. However, the important point is that throughout the three years of sampling, mean plasma cortisol levels were reduced after 24 h regardless of the loading density. Thus, there appears to be no advantage in holding smolts at less than 0.5 $lbs \cdot gal^{-1}$ prior to transport.

Darkened collection facility. In modifying the collection facility, we were successful in reducing the amount of light to which fish were exposed between surfacing at the upwell box and entering a raceway. Shading the upwell box and bar-sorter with black netting and plastic effected a 20-fold

reduction in light intensity; covering raceways with black plastic resulted in light levels reduced at least 100-fold (Table 3).

Darkening the collection facility appears to have had a significant effect in reducing the stress and hastening the recovery experienced by juvenile fall chinook (Fig. 45). Regression and variance analyses indicate that plasma cortisol levels were significantly reduced ($F = 8.63$ and 15.51 for regression and ANOVA, respectively; $P < .001$ for both) in fish collected when the system was covered to exclude light compared to fish collected under normal, exposed conditions. A multiple comparisons analysis (Least Significant Difference, LSD test) indicates that plasma cortisol titers were not elevated in fish from the covered treatment, but were significantly lower ($P < .05$) in fish held in covered raceways for 6 h. In contrast, fish collected under normal, exposed conditions demonstrated signficant elevations ($P < .05$) in plasma cortisol levels within 3 h of entering a raceway; these titers were greater than titers in fish from the covered raceway for at least 11 h after collection (Fig. 45).

In view of our studies with multiple stresses (see: STRESS CHARACTERIZATION STUDIES), we interpret the results of this experiment to mean that darkening the collection facility eliminated some stressful element(s). It is possible that (1) fish were stressed by the bright sunlight normally encountered as they surfaced at the upwell box and to which they were exposed during their stay in a raceway, and/or (2) fish were stressed after reaching the raceway through the visual perception of their surroundings, i.e. the crowding and general activity characteristic of social interaction. We believe that further experiments are warranted in order to explore management strategies which might optimize the benefits gained by darkening the collection facility.

Table 3. Light intensity (Einsteins$\cdot m^{-2} \cdot sec^{-2}$) at the water surface during normal McNary Dam fingerling facility operations (Exposed) and with shaded upwell box and bar-sorter (Shaded) and covered raceway (Covered). Measurements were taken in the morning, afternoon, and after sunset on July 16, 1984.

Conditions	Exposed	Shaded	Covered
0800h, clear sky	1.6×10^2	2.8	1.3
1500h, scattered high clouds	5.7×10^2	2.7×10^1	2.0
2200, dark[a]	2.5	b	c

[a]with normal operations, artificial lighting

[b]reading not taken

[c]below level of detection

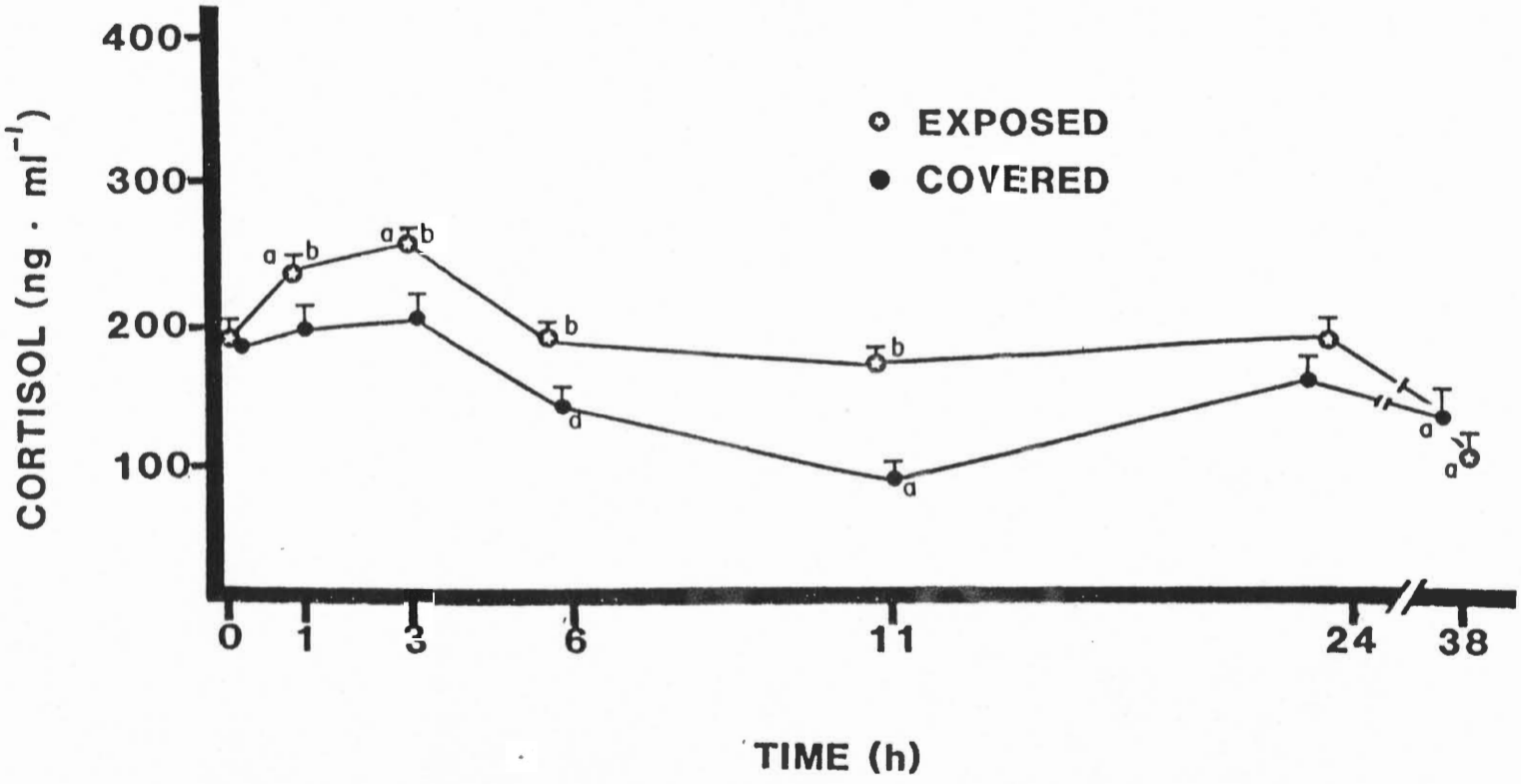

Figure 45. Plasma cortisol of juvenile fall chinook salmon collected at McNary Dam under normal operations (exposed), and with the elements of the fingerling facility covered to exclude as much light as possible (covered), during July 15 to 20, 1984. Values are means + 1 SE for four pooled replicates; total sample sizes are 30 to 40 fish. Regression analysis and analysis of variance indicate that the treatments are significantly different ($F = 8.63$ and 15.51, respectively: $P < 0.001$). Points labelled (a) are significantly different from Time = 0 of same line, and points labelled (b) are significantly different for the other treatment of the same time ($P < 0.05$, Least Significant Difference test).

Anesthetic, handling, and marking. Plasma cortisol values were significantly higher in fish sampled from the marking facility at McNary Dam in 1982 as compared to 1983 (Fig. 46). After initial examination of the 1982 results, we speculated that the increase in plasma cortisol between 24 and 48 h might have been the result of an inflammatory or other response to the presence of the CWT in the fish's snout. The 1983 results failed to confirm this and, in fact, it appeared that fish with a CWT were no more stressed than fish which were only branded. Moreover, within 12 h, fall chinook plasma cortisol had declined to pre-mark levels and remained low for at least 72 h. It appears that marked fish were not stressed more than fish which went through the collection system without being handled, but that at least 12 h was required for recovery. This does not address the response of smolts to additional stresses of being loaded into a transport vehicle and released into the river. That is, we do not know if these marked fish are any more or less susceptible to additional stress than non-marked fish (see: Multiple stress).

Pre-anesthetization study. The use of pre-anesthetization appears to have had a positive effect on moderating the stress encountered by smolts entering the marking shed (Fig. 47). Regression and variance analyses indicate that pre-anesthetized fish had significantly lower plasma cortisol levels ($F = 4.80$ and 6.65 for regression and ANOVA, respectively; $P < .02$ for both) than control animals. Multiple comparisons of mean values (LSD test) indicates that plasma cortisol levels were elevated in both treatment and control fish after one hour. However, fish which were pre-anesthetized had significantly lower ($P < .05$) plasma cortisol levels

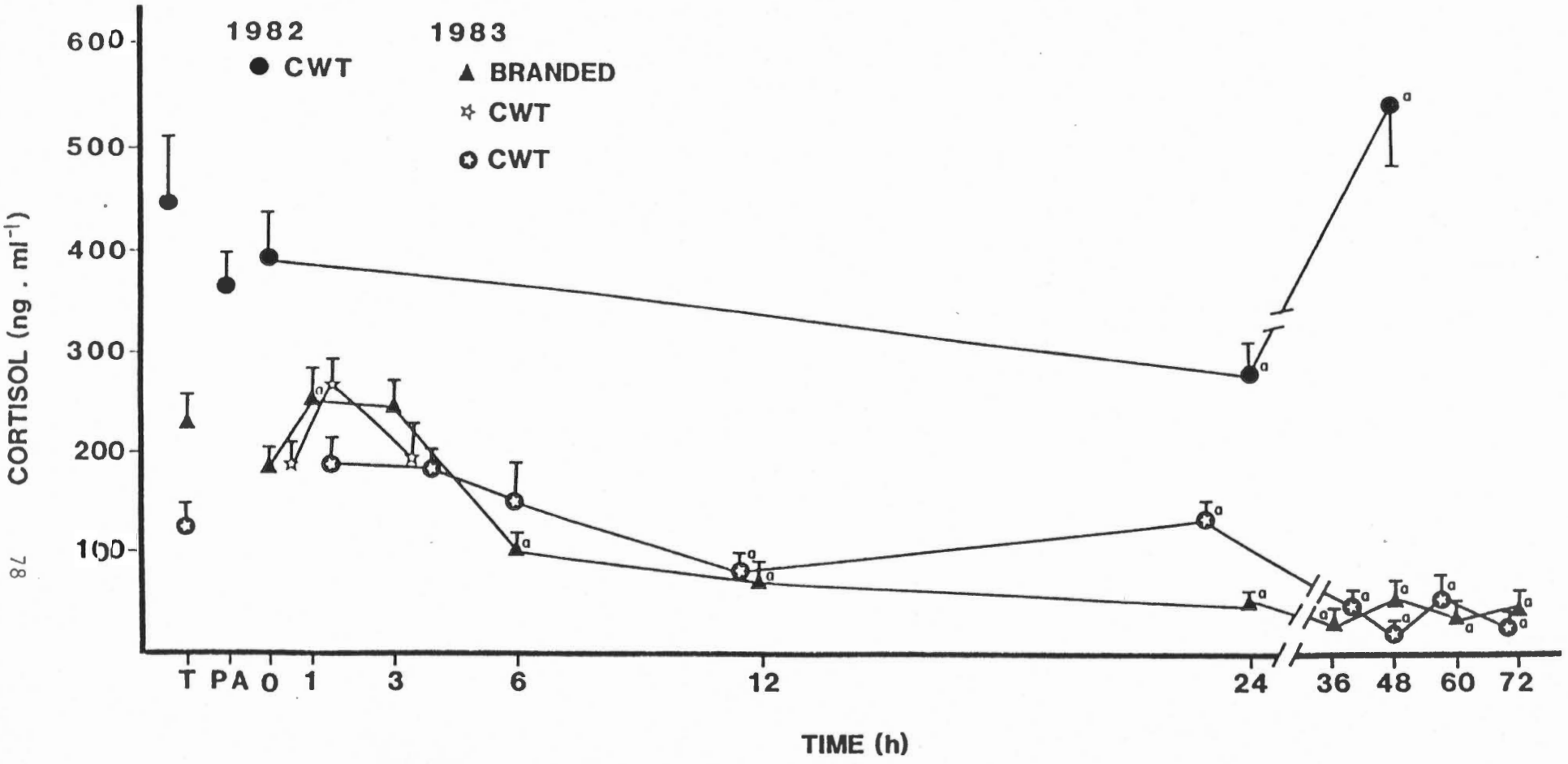

Figure 46. Plasma cortisol levels in juvenile fall chinook salmon collected from the marking facility at McNary Dam during July 14-22, 1982, and July 7-13, 1983. Fish were sampled from the holding tank (T), and after they were anesthetized (PA), and after they were fin-clipped and cold-branded (BRANDED), and after they were branded and had coded wire tags inserted into their snouts (CWT). Branded and CWT fish were also collected, held in large plastic tanks (ca. 100L) with flow-through water, and serially sampled through 72 h. All points are the means + SE of 10 to 12 fish. Points marked (a) are significantly different from T = 0 of same line ($P < .05$, LSD test).

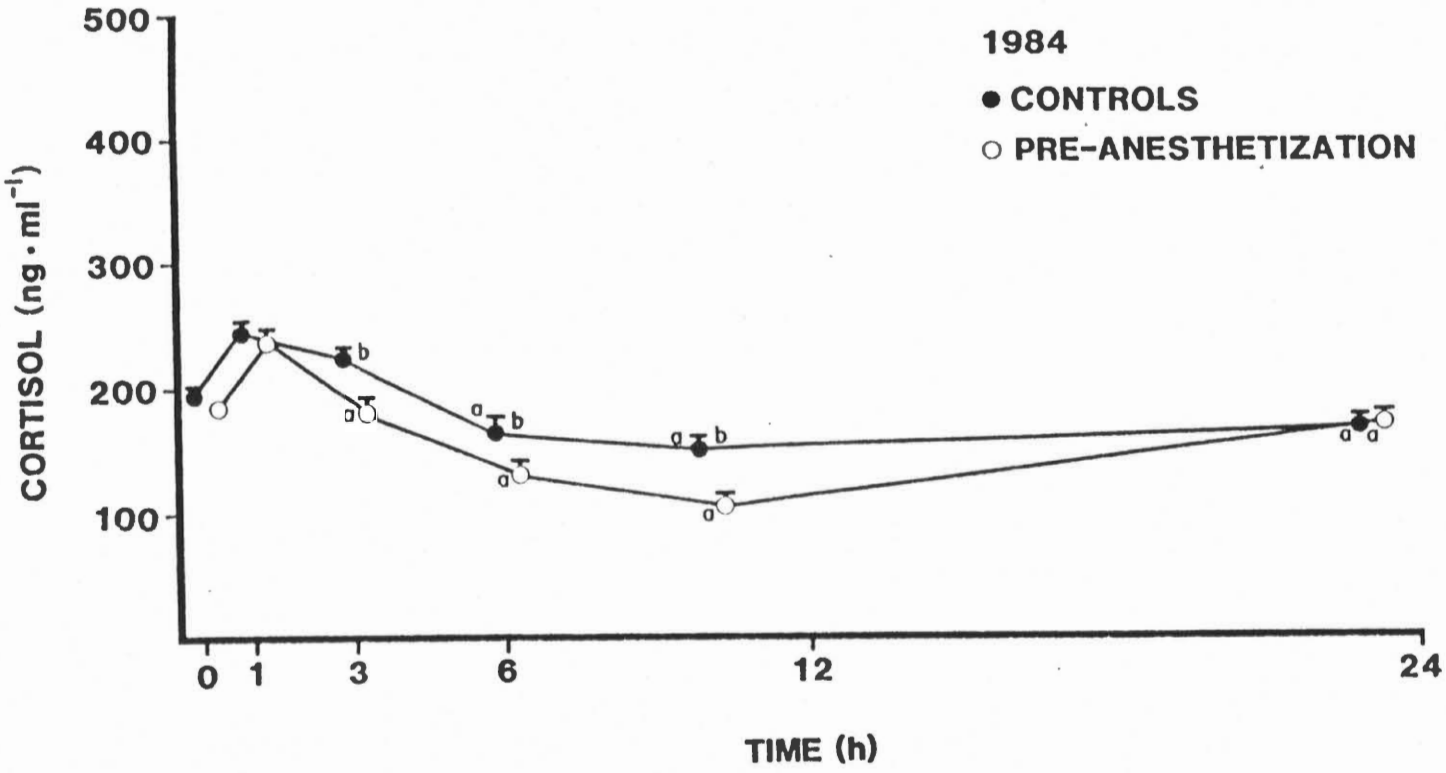

Figure 47. Plasma cortisol of juvenile fall chinook salmon sampled at McNary Dam after being collected, dipnetted, anesthetized and examined for marks under normal operating protocol (controls), and similar fish which were anesthetized before being dipnetted (pre-anesthetization). Values are means + 1 SE for three pooled replicates; total sample sizes are 25 to 30 fish. Regression analysis and analysis of variance indicate that the treatments are significantly different ($F = 6.65$ and 4.80; $P < 0.02$). Points labelled (a) are significantly different from Time = 1 h of the same line, and points labelled (b) are significantly different from the other treatment of the same time ($P < 0.05$, least Significant Difference test).

at 3, 6, and 12 hours. We were surprised to find differences in the treatments because the experimental system used to collect fish into the pre-anesthetization box caused considerable water turbulence. For this reason, we anticipated that pre-anesthetized fish might be more stressed than control fish independent of pre-anesthetization effects. The fact that there was a positive effect using this experimental system suggests that a system in which smolts enter the pre-anesthetization box of their own volition, as is used at Snake River collection facilities, may result in even greater benefits. We believe that the anesthetization of fish before they are handled has management application. Furthermore, the use of a pre-anesthetization box in conjunction with a darkened collection system might prove to be optimal in terms of alleviation or avoidance of stress.

TRANSPORT EVALUATION

Experimental Rationale and Methods

Truck transport. The U.S. Army Corps of Engineers maintains a fleet of fish transportation tank trucks. The tanks had a maximum capacity of 3,500 gal (13,250 L) and could be divided into three compartments of 25, 25, and 50% of the total volume. Water temperature was controlled by a refrigeration unit, and 5-6 $L \cdot min^{-1}$ of bottled oxygen was bubbled through the water, a rate which previously had been determined to maintain oxygen at or near saturation (Brad Eby, U.S. Army Corps of Engineers, personal communication). Fish which had been in the raceways for a maximum of 48 h, were loaded into the tanks by crowding them to one end of the raceway into a sluiceway which emptied into the tank, a drop of approximately 1 to 2 m. After the 3- to 4-h trip to Bonneville Dam, the fish were released back to the river via a port at the rear of the tank. In 1982, fish were flushed out of this port and into a long tube (ca. 100 x 0.5 m) which emptied at the water surface downstream of the Bonneville Dam first powerhouse. In 1983 and 1984, truck-transported fish were released directly into the river at Dalton Point boat ramp, approximately 21 km below Bonneville Dam.

In order to examine the effects of the transportation procedures (Objective 2a) and to determine the optimal, if any, recovery time for transported fall chinook smolts (Objective 1b), we collected fish after truck transport to Bonneville Dam during June 16-24, July 14-22, and August 2-10, 1982; and June 15-18 and August 16-18, 1983. For this evaluation, we collected fish which had been in a raceway for 48 h, fish which had just been loaded into the truck, and fish immediately after

transport to Bonneville Dam. We also removed fish from the trucks at Bonneville Dam, held them in 1.2 m circular fiberglass tanks with flow-through river water, and monitored recovery rates through 8 d (1982). In 1983, fish were held in large, dark plastic tanks and were serially sampled through 72 h.

Disease challenge of transported fish. Fall chinook salmon were transported from the raceways at McNary Dam to the Oregon State University Mark O. Hatfield Marine Science Center, Newport, Oregon, on June 17, July 8, and August 9, 1982. For each transport run, about 500 fish were netted from the raceway in the early morning and carried in water buckets to the transport truck which had a water tank with 757-L capacity and water recirculation system for aeration. Each transport run lasted 8 to 9 h (distance about 645 km). Water temperature was maintained at ambient river temperature (± 1.0 C) by adding non-chlorinated ice, as needed. At the Marine Science Center, 300 fish were equally distributed among twelve 0.61-m circular tanks. Six of the tanks held fresh water throughout the experiment and the other six were changed to saltwater when all tanks were inoculated with 8.5 ml of Vibrio anguillarum serotype I in trypticase soy broth. The final concentrations of V. anguillarum were 1.4 to 2.0×10^5 cells per 1.0 ml of aquarium water as determined by plate count. In an effort to determine post-transport recovery rates, we exposed fish in replicate saltwater and freshwater tanks to V. anguillarum within 1 h of arrival, and two replicate sets of freshwater and saltwater tanks were exposed 1 d after transport and 8 d after transport. Exposure was accomplished by shutting off the water supply for 30 minutes and pipetting the prepared V. anguillarum culture into each tank. The number of

mortalities in each tank were noted daily from the beginning of the experiment until 13 d after exposure to V. anguillarum.

The percent mortality and mean of the times to death (MTD) were calculated for each tank with the branded and transported fish considered separately.

$$\text{MTD} = \frac{\text{(Mortalities/day) (days from challenge)}}{\text{total mortalities during challenge}}$$

$$\text{Percent mortality} = \frac{\text{number dead during challenge}}{\text{total number of fish at start of challenge}}$$

Fish that died before exposure to V. anguillarum were not included in the calculations. When fish in two tanks experienced equal mortality, but the MTD of fish in one was significantly higher than in the second, we concluded that fish in this second tank were better able to resist the lethal effects of the disease.

Seawater growth and survival. Growth and survival studies were conducted on fall chinook salmon transported from McNary Dam to the Marine Science Center in the same truck as that used to transport fish for the V. anguillarum challenge (June 17, July 8, August 9, 1982). Upon arrival at the Marine Science Center, the fish were netted from the truck and placed in two 0.91-m circular seawater tanks. The fish were fed Oregon Moist Pellets daily to satiation. Dead fish were removed and recorded daily for a 15-d period.

We did not want the stress of anesthetization and handling to interfere with this growth experiment, so the initial weights and lengths were not

taken from the actual fish used in the experiments. For the June experiment, we used the mean weights and lengths of fish collected at McNary Dam (n = 76), and in July and August initial weights and lengths were determined from a subsample of fish (n = 30) taken from the transport tank but not used in the growth experiment. The mean daily minimum and maximum water temperatures during the study were 12.0 and 16.0 C, respectively, in June and August, and 13.5 and 17.2 C, respectively, in July. Salinity was 32.5 ± 0.5 parts per thousand throughout the studies.

At the end of the 15-day growth period, fish from the experimental growth tanks were anesthetized, measured, and weighed. In August, the weighed fish were returned to their tanks, and after another 15 d, were again weighed and measured. In late July we obtained fall chinook from Trask Fish Hatchery, Tillamook, Oregon, as controls for the August seawater growth experiment.

Transported fish density. The environmental conditions during transportation of fish can have an effect on physiological indices of stress and on survival (Specker and Schreck 1980; Barton and Peter 1982). One aspect of this environment is the density of fish during transport. To investigate whether or not transport densities had differential effects on fall chinook smolts (Objective 4c), we: 1) evaluated data obtained from the regular transport runs which were monitored in 1982 and 1983; 2) during August 8-9, 1982, we compartmentalized an Army Corps of Engineers truck and loaded the compartments with fish to either 0.1, 0.5, or 0.8 $lb \cdot gal^{-1}$ (0.01, 0.06, or 0.10 $kg \cdot L^{-1}$); 3) during August 5-10, 1982, we used a small pickup truck-mounted transport tank to simulate the large truck transport. The capacity of the small tank was 200 gal (757 L), water temperature was

controlled by a refrigeration unit, and oxygen was maintained at 11 ± 1 parts per million by bottled oxygen. In all transport trials, fish which had been in a raceway for a maximum of 48 h were sampled after they were loaded into a transport vehicle, and again after a 3- to 4-h transport.

Day versus night truck transport. During July 1984, fall chinook salmon held at McNary Dam in raceways which were either exposed to ambient light conditions or covered with black plastic (see: Darkened collection facility) were used in an experiment designed to compare day and night truck transport. After fish had been in raceways for approximately 24 or 38 h, they were loaded into Corps of Engineers trucks and transported to Bonneville Dam during the day or at night, respectively. We originally intended to turn off some or all of the lights at the fingerling facility during the night loading; however, lightmeter readings for normal (lights on) operations were comparable to those recorded at the water surface of raceways covered with black plastic under sunlight (Table 3). Therefore, the lights were left on in the interest of safety. It was necessary to remove black plastic from covered raceways approximately 15 minutes before loading fish in order to remove trash from these raceways. All of the fish from an exposed raceway were put in one compartment of the truck (50% of total volume) and the fish from the paired, covered raceway were put into the other compartment. Fish density in all transport vehicles was between 0.18 and 0.25 $lb \cdot gal^{-1}$. Two trucks of fish were transported in the morning (ca. 0800 hours) and two at night (ca. 2200 hours). Samples were taken before and after fish were loaded into trucks and immediately upon arrival at Bonneville Dam. We removed fish from each compartment and held them in plastic tanks (ca. 100 L) with flow-through water from which they were sampled after 1, 3, 6, 12, and 24 hours.

Barge transport. The Corps of Engineers has two types of barges for transporting fish. The large, newer barges have four holding tanks, each of which hold a maximum of 5 lbs of fish$\cdot$gal$^{-1}\cdot$min^{-1} (2.9 kg$\cdot$L^{-1}min^{-1}). Each hold is equipped with a diesel-powered pump which is capable of pumping 2400 gallons (91,200 L) of river water each minute. Thus, the maximum capacity for the barge is 48,000 lbs (21,818 kg) of fish (ca. 1.4 million fall chinook). The older barges are basically the same as the new barges; however, they have three holds with total capacity of 24,000 lbs. of fish. The old barges are only used when large numbers of smolts are collected; we did not collect fish from these during any of our sampling periods. During the major portion of the 1983 and 1984 fall chinook emigrations, collected fish were transported by barge. During July 14-22, 1983, and June 12-15, July 11-13 and August 8-10, 1984, we investigated the effects of barge transport on fall chinook smolts. Fish were collected before and after being loaded into a barge, during the barge transport trip, and upon arrival at Bonneville Dam. Fish were also removed from the barge at Bonneville and held in dark, plastic tanks with flow-through water and serially sampled through 72 h.

Results and Discussion

Truck transport. The overall transportation protocol was stressful to fall chinook smolts. However, the fish were stressed during the processes of loading and, perhaps, unloading but were not stressed by being in the transport vehicle. There were two trends clearly indicated in the 1982 and 1983 plasma cortisol results (Figs. 48-49). First, plasma cortisol levels increased between fish in the raceway and immediately after they were loaded

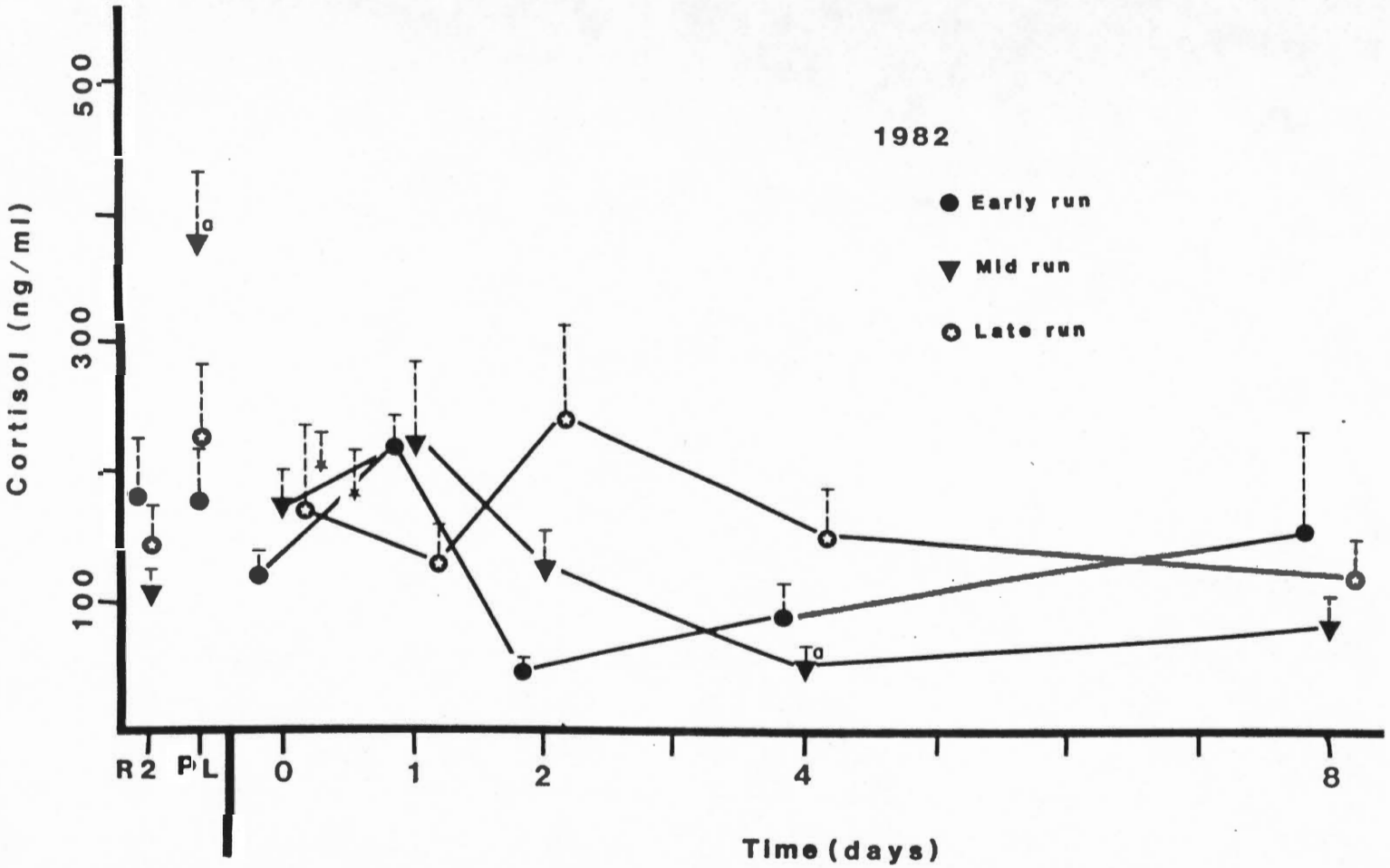

Figure 34. Plasma cortisol levels of juvenile fall chinook salmon sampled from McNary Dam after 2 d of recovery in the raceway after collection (R2) and after loading into transport truck (PL), and during 8 d of post-transport recovery at Bonneville Dam. Samples were taken June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. On August 20, 1982, fish were sampled from a transport truck and after release (★). All points represent mean + SE for n = 11 to 14.

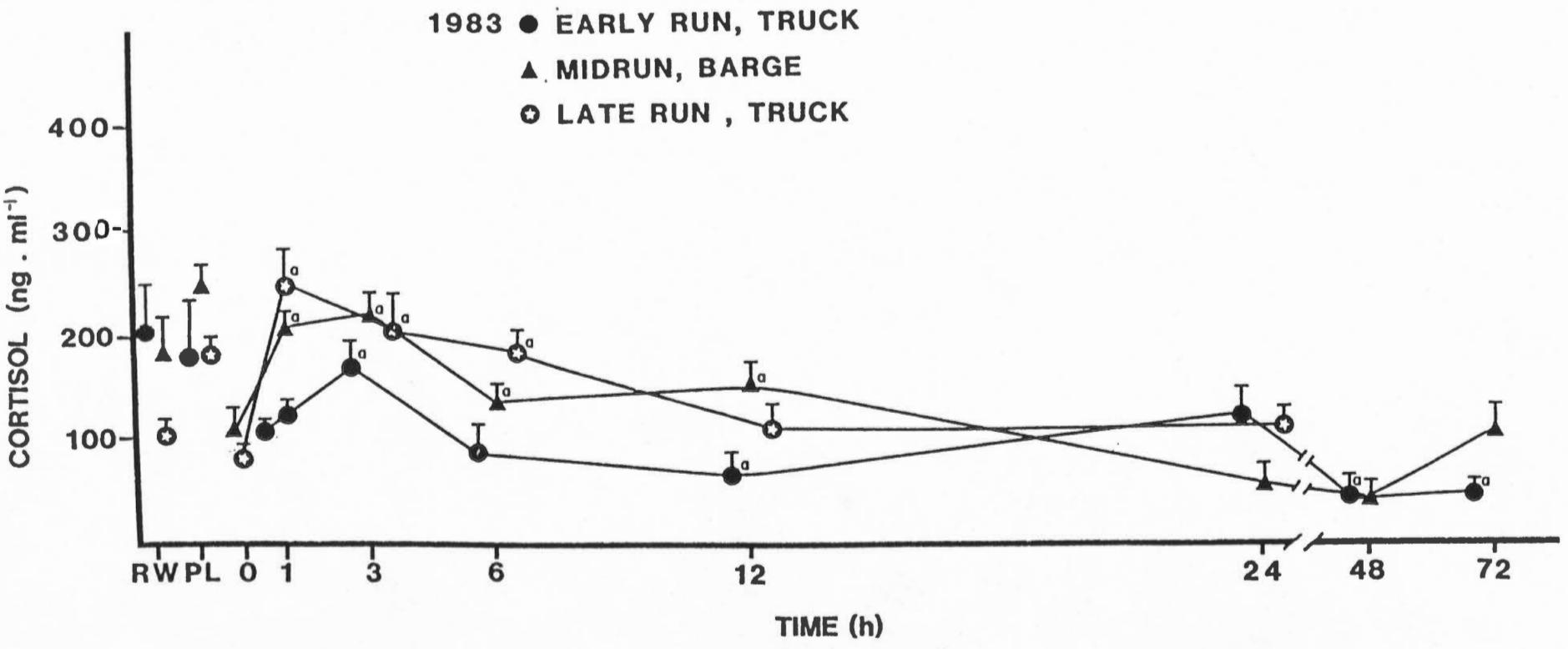

Figure 49. Plasma cortisol of juvenile fall chinook salmon sampled from a raceway at McNary Dam just prior to being loaded into a truck or barge (RW), after being loaded onto a truck or barge (PL), and after a 3- to 4-h truck transport or 15- to 16-h barge transport to Bonneville Dam. Fish were also removed from the transport vehicle, held in plastic buckets (ca. 100L) with flow-through water, and serially sampled through 72 h. All points are means + SE of 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of the same line ($P < .05$, LSD test).

into a truck (or barge; see Fig. 59). Second, there was a net decrease in plasma cortisol levels between the time fish were loaded into a truck (or barge; Fig. 59) and after 3 to 4 h transit to Bonneville Dam. There were no significant differences in plasma cortisol levels of fall chinook removed from the truck at Bonneville Dam at different times of the run in 1982 (Fig. 48) or 1983 (Fig. 49). The highest plasma cortisol levels were comparable between years, but the 1982 data indicated that levels peaked 1 d after transport. The 1983 plasma cortisol levels reached a maximum within 3 h and were reduced 24 h after fish were removed from the truck. We believe that this difference reflected conditions in the holding facilities and is not indicative of differences in transportation. It appears that the plasma cortisol dynamics of fish removed from a transport truck at Bonneville Dam are best described by the 1983 results (Fig. 49), in that cortisol levels increase to a peak within 3 to 6 h and then decrease to baseline levels within 24 h. This same pattern is seen in the other trucked or barged fish (see: Day versus night truck transport and Barge transport).

Interrenal cell nuclear diameters of transported fish did not change significantly during 4 to 8 d after transport (Fig. 50). Liver glycogen of fish transported by truck (Fig. 51) was generally lower than that of fish sampled at McNary Dam (Figs. 14 and 15) and declined to almost negligible levels within 8 d. Hematocrit values of transported fall chinook (Fig. 52) were more variable than those of fish at McNary Dam (Fig. 19). The 1983 results were unusual in that there was almost a 10% difference in hematocrits of fish prior to transport comparing early run to mid-run (Fig. 53). However, in all of the hematocrit data (Figs. 19, 20, 52, and 53) after some recovery, the values are approximately 50%. The relative

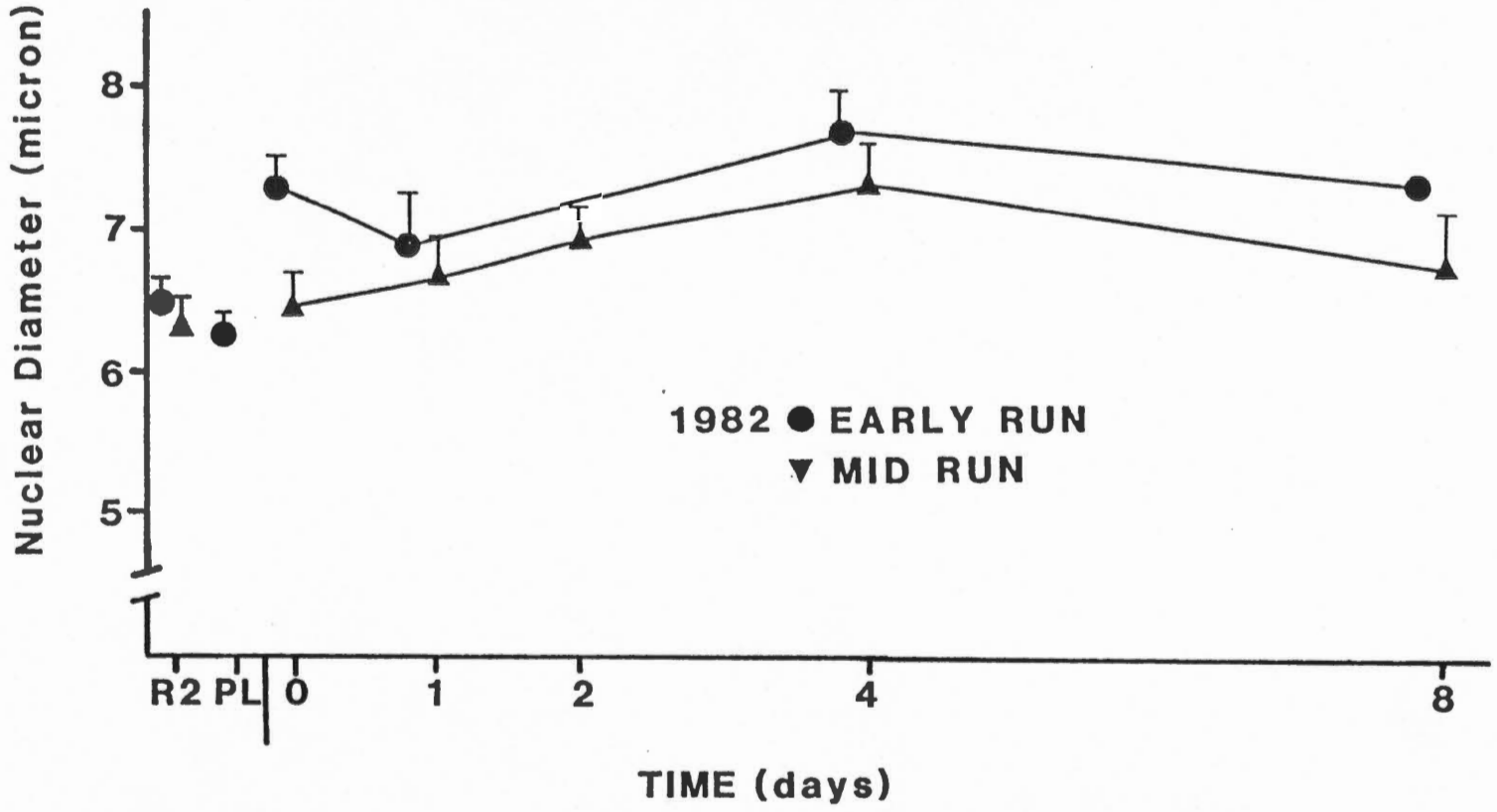

Figure 50. Interrenal cell nuclear diameters of juvenile fall chinook salmon collected after they had been in a raceway at McNary Dam for 2 d (R2), after the fish had been loaded into a transport truck (PL), and for up to 8 d after transport to Bonneville Dam. Samples were collected June 14-20 (early) and July 7-16 (mid), 1982. All points are the means + SE for 4 to 6 fish.

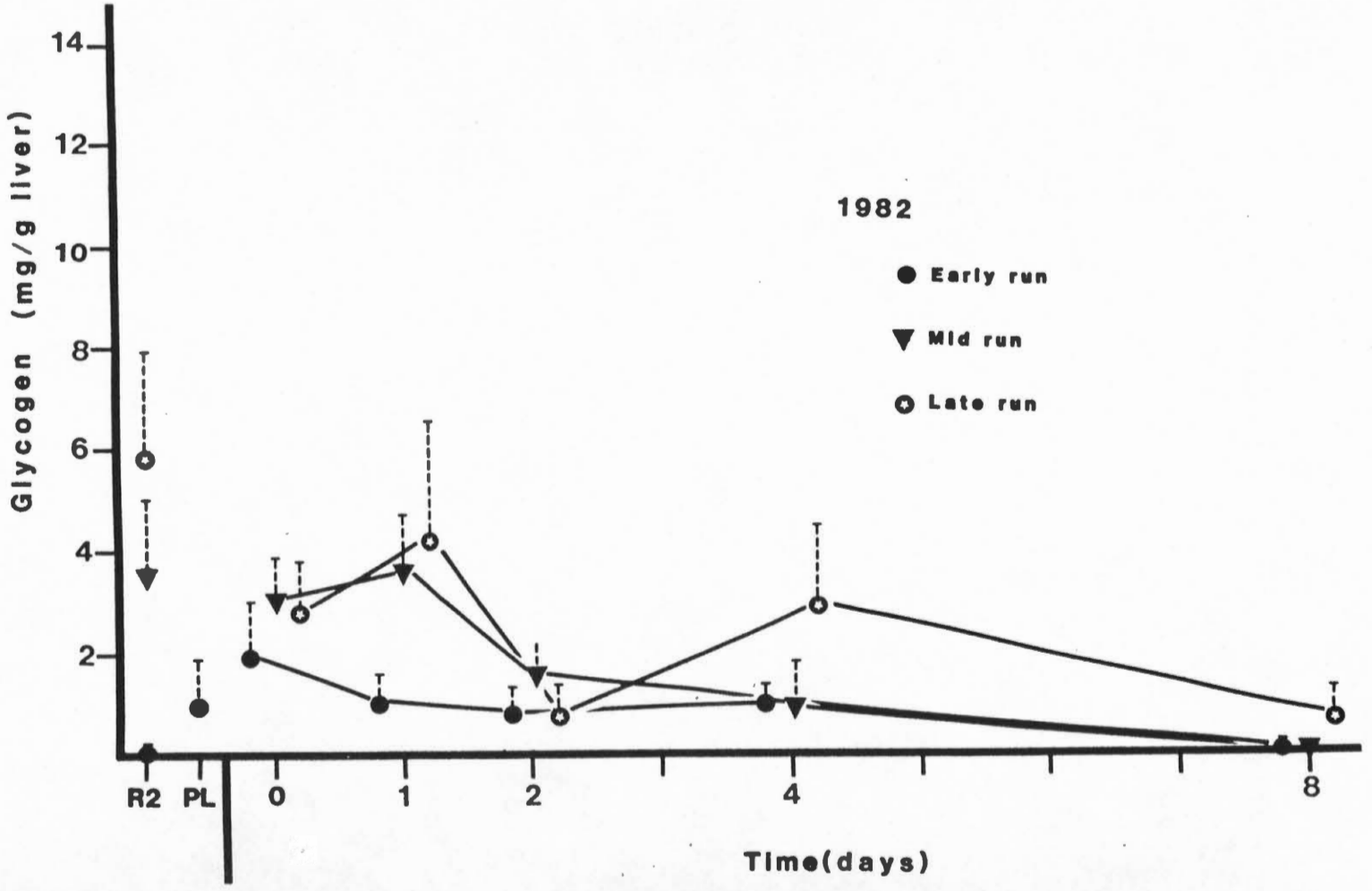

Figure 51. Liver glycogen levels of juvenile fall chinook salmon sampled from the McNary Dam after 2 d of recovery in the raceway (R2), after loading into transport truck (PL), and during 8 d of post-transport recovery at Bonneville Dam. Samples were taken June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. All points represent mean + SE for n = 5 or 6.

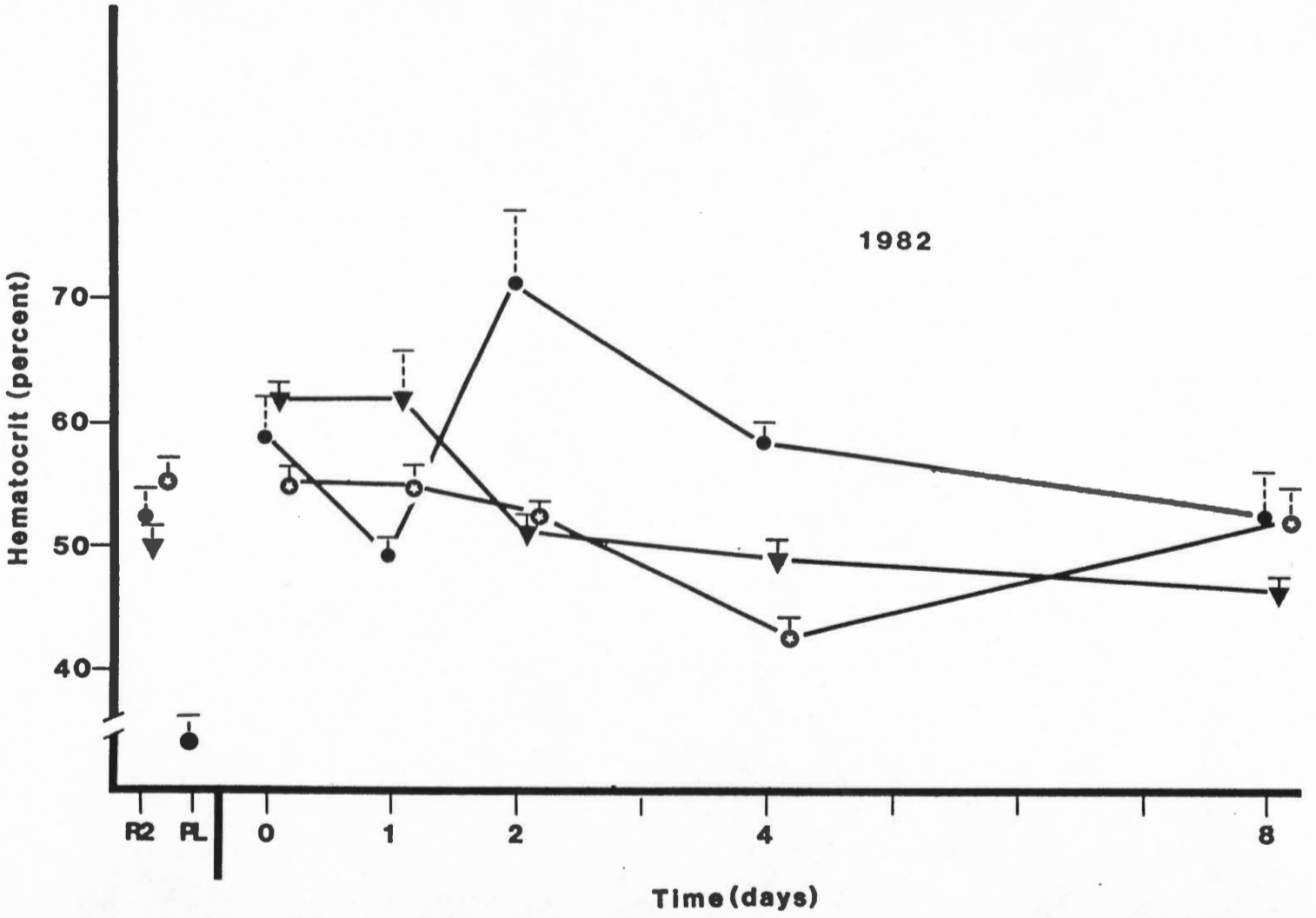

Figure 52. Hematocrit values (mean + SE) for outmigrant fall chinook salmon sampled after 2 d recovery in a raceway (R2), after being loaded into a truck (PL) at McNary Dam, and through 8 d of recovery after being transported to Bonneville Dam. Each point repreents 6 fish taken on June 16-24 (●), July 14-22 (▼), and August 2-10 (✪), 1982.

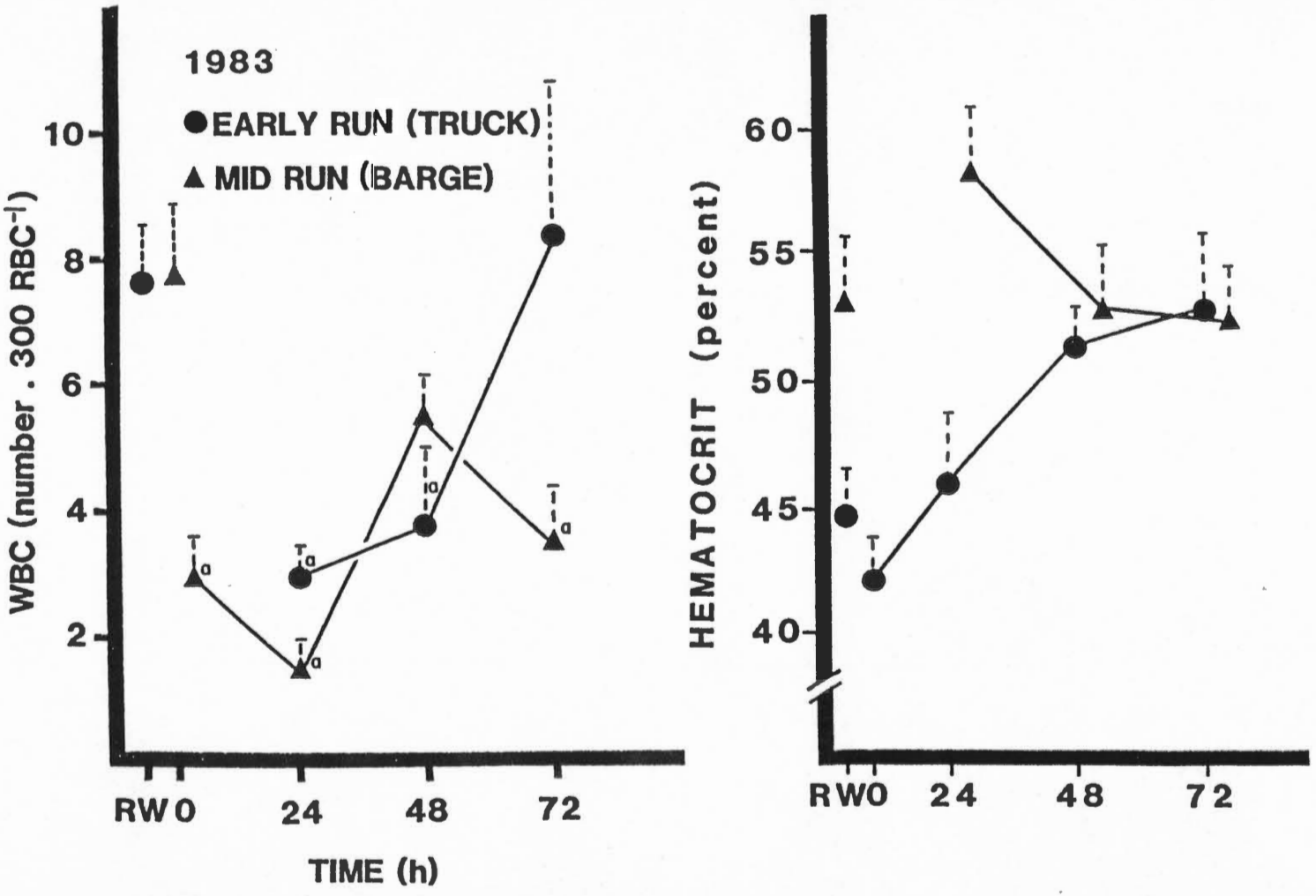

Figure 53. White blood cell (WBC) counts and hematocrit values in juvenile fall chinook salmon collected from a raceway at McNary Dam just prior to being loaded into a transport vehicle (RW) and for up to 72 h after being transported to Bonneville Dam. Sampling was done during June 14-24 (early run) and July 7-16 (mid run), 1983. All points are the means + SE for 5 to 6 fish. WBC counts are the average of two replicate counts of the number of WBC among 300 erythrocytes (RBC) on blood smears. Points marked (a) are significantly different from raceway values with the same symbol ($P < .05$, LSD test).

numbers of WBC in fish transported to Bonneville Dam do not exhibit any strong trends, but it appears that WBC number increases through time after transport (Figs. 53 and 54).

Saltwater challenges of fall chinook transported in 1982 indicated that osmoregulatory ability was reduced in fish as the run progressed and the longer period of time that fish were held after transport (Fig. 55). There is some indication that mid-run fall chinook were better able to osmoregulate than fish from other portions of the run as this group was the only group which grew during the 15 d saltwater growth experiment at the Marine Science Center. In 1983, there was no difference in osmoregulatory ability of early-run fish transported by truck compared to mid-run fish transported by barge (Fig. 27), which confirmed our conclusion based on plasma cortisol that there was no difference between truck and barge transport (see: Barge transport). The secondary stress challenge of truck-transported fall chinook resulted in a three-fold greater maximum plasma cortisol response in 1982 than in 1983 (Figs. 29 and 56); however, in both years, within 6 h plasma cortisol levels declined to levels equivalent to those of fish prior to transport and prior to the secondary stress.

Disease challenge of transported fish. In general, fish allowed 1 d of recovery after transport from McNary Dam resisted Vibrio anguillarum longer than did fish exposed immediately after transport, or after 8 d of recovery (Table 4). These results parallel the 1982 saltwater challenge data, which indicated a decreased ability to osmoregulate after fish had been held 7 or 8 d (Figs. 26 and 55). The stress of transport and handling could explain the decreased ability to withstand V. anguillarum initially. Prolonged holding of migrating fish may also have caused increased stress, which decreased the fishes' ability to resist V. anguillarum.

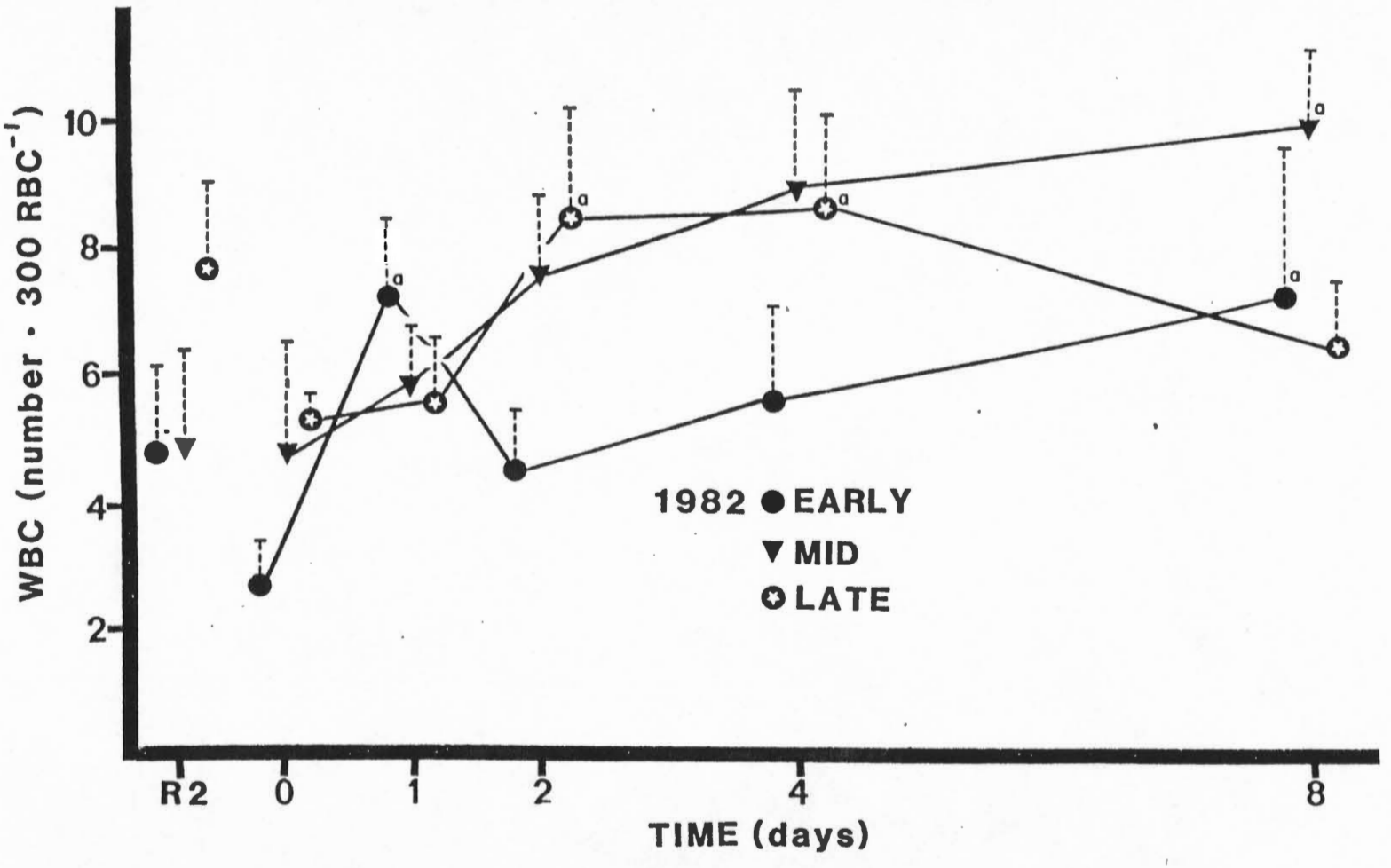

Figure 54. White blood cell (WBC) counts of juvenile fall chinook salmon collected after being in a raceway at McNary Dam for 2 d (R2), after transport, and for up to 8 d after transport to Bonneville Dam. Sampling was during June 16-24 (early), July 14-22 (mid), and August 2-10 (late), 1982. All points are means + SE of the average of two replicate counts of the number of WBC's among 300 erythrocytes (RBC) on blood smears of 6 fish. Points marked (a) are significantly different from those of Time = 0 of same line ($P < .05$, LSD test).

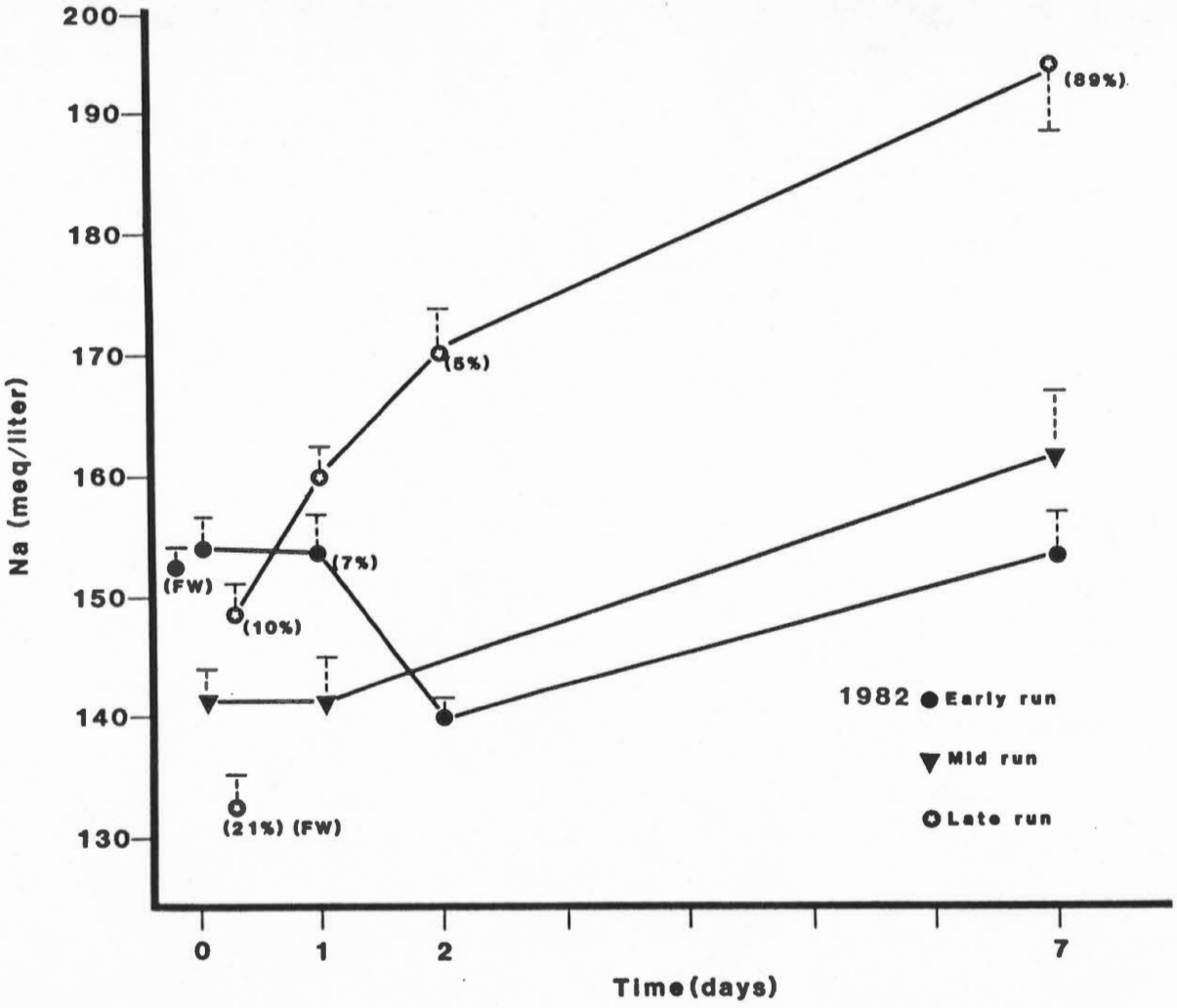

Figure 55. Plasma Na levels (mean + SE) of outmigrant fall chinook salmon sampled after transport from McNary Dam and during 8 d of recovery at Bonneville Dam. All points are duplicates of 10 fish which were sampled 24 h after being put in 15 parts per thousand salt water or fresh water (FW) during June 16-24 (early run), July 14-22 (mid run), and August 2-10 (late run), 1982. Percent mortality is in parentheses.

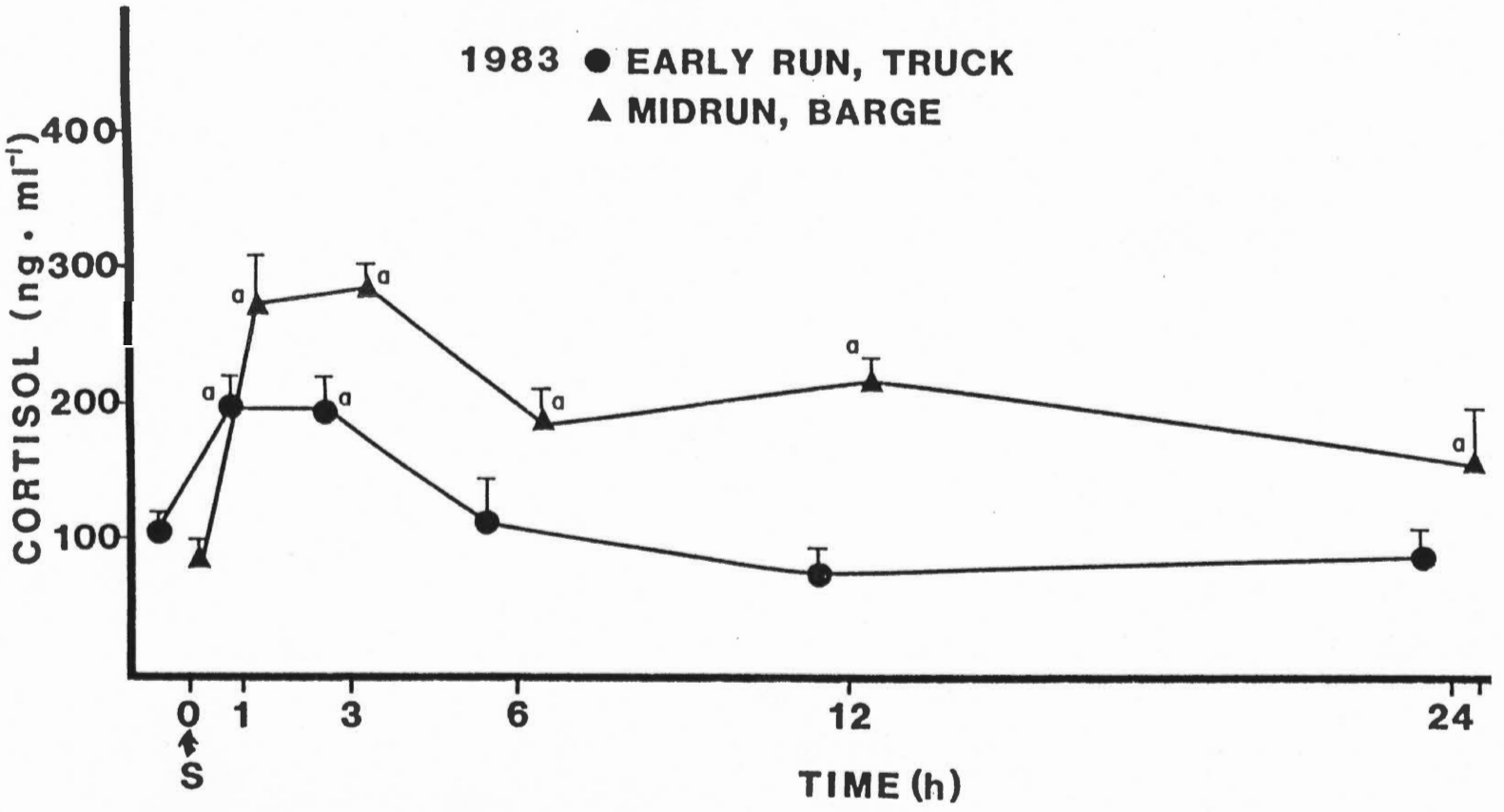

Figure 56. Plasma cortisol levels in juvenile fall chinook salmon which were transported by truck or barge from McNary Dam to Bonneville Dam, and then secondarily stressed by being held in a dipnet out of water for 30 s. Fish were then held in a plastic tank (ca. 100L) and serially sampled through 24 h. All points are means + SE for 10 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

Table 4. Mean of the time to death (MTD) = $\frac{(\text{mortalities} \cdot \text{day}^{-1})\ (\text{total days} = 113)}{\text{total mortalities}}$ and percent total mortalities for juvenile fall chinook exposed to Vibrio anguillarum in fresh water (FW) or salt water (SW) after transport from the McNary Dam fingerling collection facility, 1982. A and B are replicate trials. Numbers of fish at the time of the Vibrio challenge are in parentheses.

Dates	Post-transport Days to Challenge	FW (A) MTD	FW (A) % Mortality	FW (B) MTD	FW (B) % Mortality	SW (A) MTD	SW (A) % Mortality	SW (B) MTD	SW (B) % Mortality
Early Run									
June 17 -	0	3.76	65(26)	4.12	62(26)	4.10	91(23)	3.52	100(27)
July 8	1	8.62	62(26)	6.67	50(18)	6.10	79(24)	6.67	84(25)
	8	5.73	69(32)	3.76	71(24)	6.67	90(10)[a]	6.05	86(22)[a]
Mid-Run									
July 9 -	0	4.79	96(25)	8.11	93(30)	4.10	75(24)	3.81	64(25)
July 30	1	6.96	100(25)[a]	5.70	100(24)	4.07	65(23)	4.92	54(24)
	8	2.30[b]	100(10)[a]	1.76[b]	100(17)[a]	3.17	57(21)	4.80	83(17)[a]
Late Run									
Aug. 9 -	0	3.59	76(29)	5.17	93(28)	3.87	96(25)	4.70	89(26)
Aug. 31	1	4.36	76(29)	7.56	86(29)	6.43	78(27)	5.82	74(27)
	8	3.26	74(27)	2.56	93(29)	2.70	95(17)[a]	- [a]	

[a] pre-challenge mortalities $\geq$ 18%

[b] 100% mortality before day 13 of challenge

Seawater growth and survival. The lengths and weights of transported fish introduced into sea water changed very little in any of three trials (Table 5). The only statistically significant changes were reductions in weight during the early and late portions of the run; however, fish in the mid-run test did show some growth, and the acclimated hatchery fish showed significant growth in length and weight during the 30 d test period (Table 5). During tests, the transported fish ate little or no food in comparison with the hatchery fish. This failure to eat can account for the loss in weight of the transported fish.

Mortality was very high in fish acclimated to the experimental setting (ca. 30% after 15 d, and 48 to 71% after 30 d) and in fish transported from McNary Dam (0 to 39% after 15 d, and 42 to 68% after 30 d). However, the mean of the time to death (MTD) was earlier in transported fish (1.0 to 2.6 d) when compared to acclimated fish (MTD = 8.4 to 9.4 d), suggesting osmoregulatory failure in the transported fish. There were no mortalities among the fish transported in July which is the time of peak abundance of emigrating fall chinook on the Columbia River (Basham et al. 1983). Na-K ATPase was significantly higher during this time (Fig. 11), and fish transported to Bonneville Dam during the mid run and challenged with salt water immediately upon arrival were better able to regulate plasma Na levels than the early-or late-run fish (Fig. 55). All of this suggests that fall chinook smolts emigrating in the mid-run were at an osmoregulatory optimum for entry into sea water after transportation.

Transported fish density. Loading density did not have a discernible effect on plasma cortisol levels in fall chinook immediately after they were loaded into Corps of Engineers' trucks (Fig. 57). Again, plasma cortisol levels

Table 5. Fork length (FL), weight, condition factor ($K = \frac{g \times 100}{cm^3}$), percent weight change and mortality of fall chinook transported from McNary Dam fingerling facility and introduced into seawater at the Marine Science Center for 15 or 30 days. A and B are replicate trials.

Dates	Treatment		Final N	FL (cm) (mean±SE)	Weight(g) (mean±SE)	K	Weight change(%)	Mortality %	Mortality MTD[b]
Early run									
June 18	Transported	Initial	76	9.4±0.2	9.7±0.4	1.0			
Jul 2	15 day	A	52	9.3±0.2	8.2±0.4[a]	1.0	(-15.5)	39	1.0
		B	40	9.5±0.2	8.7±0.6	1.0	(-10.3)	39	1.1
Mid-run									
Jul 8	Transported	Initial	30	9.4±0.2	8.2±0.7	1.0			
Jul 23	15 day	A	44	9.7±0.2	9.5±0.4	1.0	15.9	0	
		B	45	9.6±0.2	9.1±0.4	1.0	11.0	0	
Late run									
Aug 9	Transported	Initial	30	11.7±0.1	18.7±0.6	1.2			
Aug 24	15 day	A	41	11.4±0.3	16.7±0.5[a]	1.1	(-10.7)	10	2.5
		B	45	11.6±0.1	16.6±0.5[a]	1.1	(-10.2)	11	2.5
Sept 9	30 day	A	13	11.9±0.2	16.5±1.0	1.0	(-9.6)	68	
		B	26	11.6±0.2	15.7±0.7[a]	1.0	(-16.0)	42	
Aug 9	Acclimated[c]	Initial	30	9.4±0.1	9.6±0.4	1.2			
Aug 24	15 day	A	14	9.7±0.3	10.0±0.7	1.1	4.2	30	9.4
		B	21	9.6±0.2	10.2±0.7	1.2	6.3	33	8.4
Sept 9	30 day	A	6	10.8±0.4[a]	14.8±2.0[a]	1.2	54.2	71	
		B	11	10.3±0.4[a]	12.7±1.7[a]	1.2	32.3	48	

[a] Value significantly different from initial value ($P>.01$, t-test).

[b] Mean of the time to death = $\frac{(\text{mortalities} \cdot \text{day}^{-1})\ (\text{total days} = 15)}{\text{total mortalities}}$

[c] Juvenile fall chinook obtained from Trask Fish Hatchery, Tillamook, Oregon on July 23, 1982 and held at MSC in freshwater and switched to seawater on August 9.

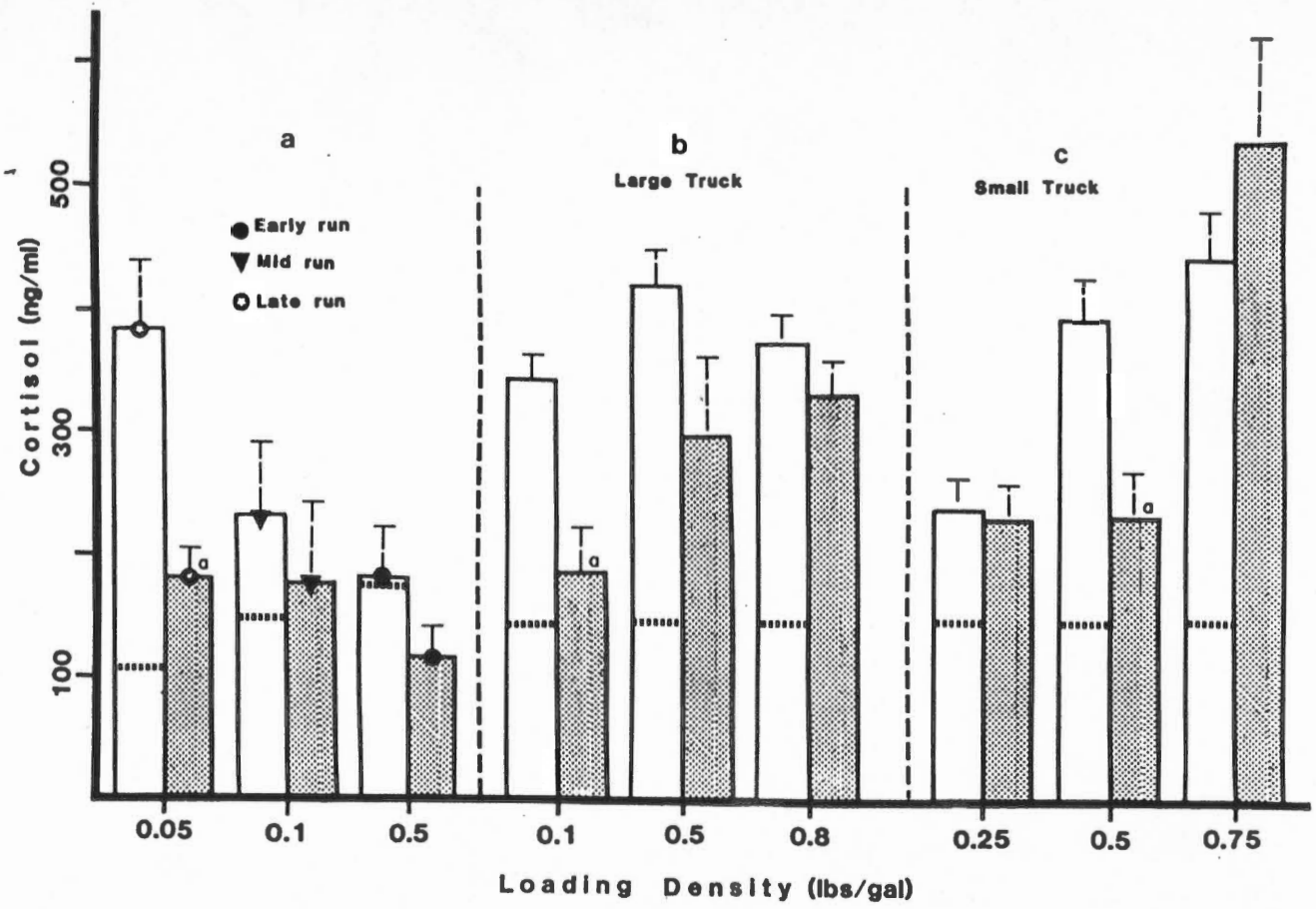

Figure 57. Plasma cortisol levels (mean + SE) of juvenile fall chinook salmon immediately after being loaded into a transport truck at McNary Dam (clear bars) and after 3-4 h of transport to Bonneville Dam (stippled bars). Sections of the figure represent (a) fish sampled from normal transportation operations throughout the season, (b) late-run fish loaded at various densities in a single truck partitioned into three cells, and (c) late-run fish loaded into a small, 200-gallon pickup truck mounted tank. Dashed line in clear bars are cortisol levels of fall chinook after 2 day of recovery in the raceway after collection at McNary Dam. All sample sizes are 11 to 14 fish. Values marked (a) are significantly different from values of fish immediately after being loaded ($P < .05$, LSD test).

were lower after 3 to 4 h of transport than immediately after fish were loaded into the truck; however, it appears that the absolute reduction was greatest at low densities. For example, late-run fish were transported at 0.05 lbs$\cdot$gal^{-1}, and plasma cortisol decreased from approximately 390 ng$\cdot$ml^{-1} to 190 ng$\cdot$ml^{-1}, a reduction of 200 ng$\cdot$ml^{-1} (Fig. 57). During the mid-run, plasma cortisol in fish transported at 0.5 and .08 lbs$\cdot$gal^{-1} decreased by 125 and 40 ng$\cdot$ml^{-1} (Fig. 57), respectively, even though the plasma cortisol levels immediately after loading were approximately the same, 375 to 425 ng$\cdot$ml^{-1}. These results also indicate that the use of the small transport truck does not satisfactorily simulate the environment of the Corps of Engineers' truck. Although plasma cortisol levels are similar in fish immediately after they were loaded on the small truck, the reduction in cortisol levels is not comparable to that in fish transported in the Corps of Engineers' truck. In fact, at the highest density in the small truck, there was an increase in plasma cortisol in fish during transport (Fig. 57).

Day versus night truck transport. In the day versus night truck transport experiment, there were four treatment groups (Fig. 58), each of which was duplicated. Comparisons of the linear regressions of the rate of reduction of plasma cortisol 1 h after arrival at Bonneville Dam reveals that there was no significant difference between night transported fish from the exposed or covered raceway treatment ($P < 0.50$; $F = 1.07$). There was a significant difference between day transported fish from the exposed or covered recovery treatments ($P < 0.05$; $F = 4.08$); however, we believe that this is a result of mistakenly putting twice as many fish in the holding tank for one of the day-transported, covered raceway groups. If that

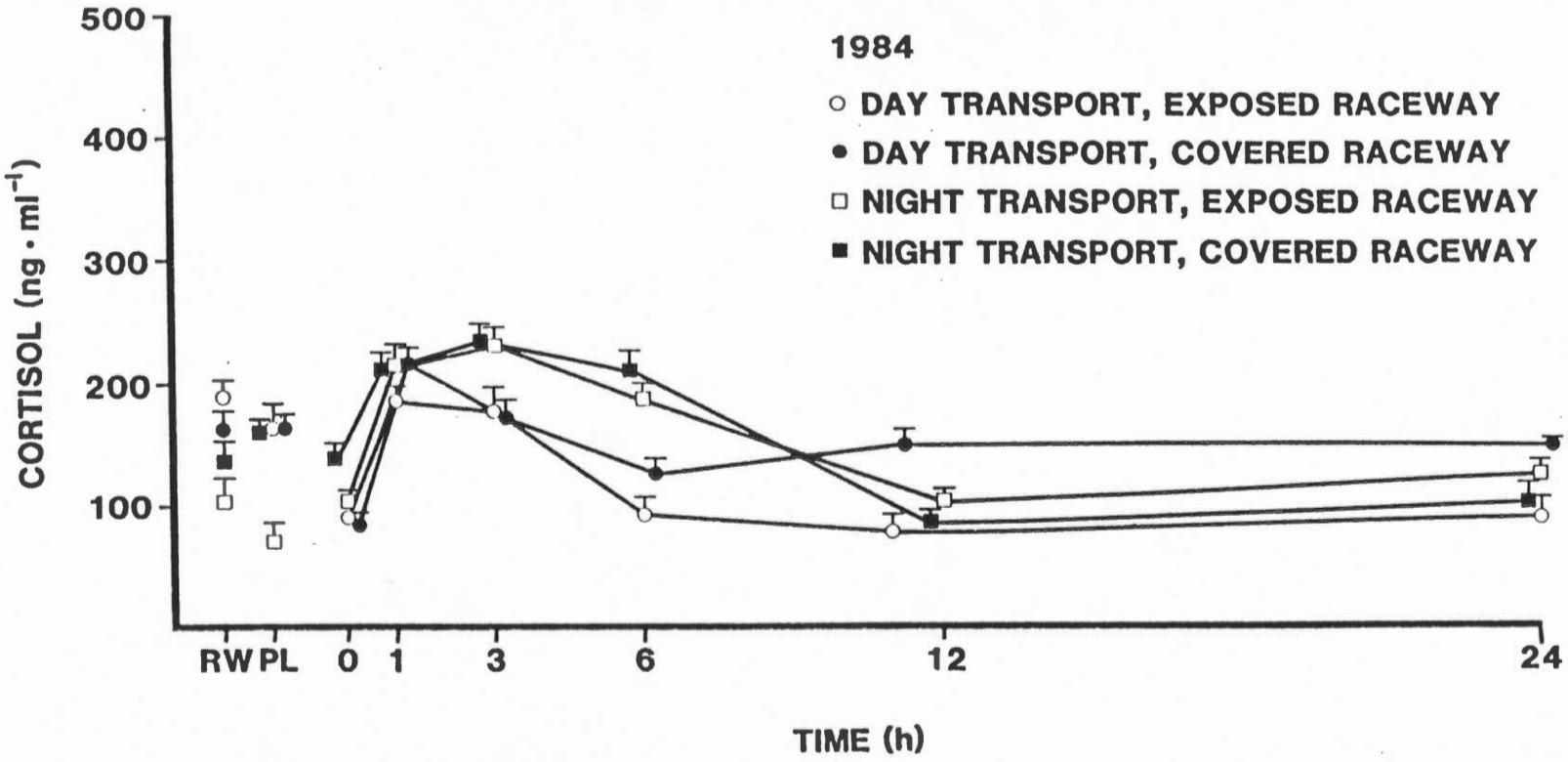

Figure 58. Plasma cortisol levels in juvenile fall chinook salmon collected at McNary Dam under normal conditions (exposed raceways) or when the upwell box and bar-sorter were shaded and the raceway covered with black plastic (covered raceways), and subsequently transported by truck to Bonneville Dam either at night or during the day. At Bonneville, fish were held in large, opaque plastic tanks with flow-through water and sampled over 24 h. Fish density in all transport vehicles was between 0.18 and 0.25 lb·gal^{-1}. All points represent the mean + SE of duplicate groups of 10 to 12 fish.

replicate were removed, there would be no significant difference in the remaining three replicated day-transported fish. Linear regression of day transport is significantly different from night transport ($P < 0.001$; $F = 12.98$). Paired comparisons of day versus night revealed that the treatments were significantly different at 3 and 6 h after arrival, while night transported fish were significantly reduced 12 h after arrival ($P < 0.05$; LSD test). These results are just the opposite of what we expected. The transported fish had been in the raceways for differing lengths of time (24 h and 38 h for day and night, respectively) and perhaps this biased the plasma cortisol response. Before concluding that day transport is less stressful than night transport, we believe that tests with fish in the raceways for comparable times should be made. Furthermore, these fish were loaded into trucks shortly after dawn and dusk, and tests of fish transported beginning at noon and midnight might be more indicative of treatment effects.

Barge transport. The plasma cortisol dynamics of fish removed from the barge at Bonneville Dam during the 1983 mid run and throughout 1984 were very similar to those of fish trucked to Bonneville Dam during the early and late runs of 1983 (Figs. 49 and 59). In fact, plasma cortisol levels in fish sampled from the barge while enroute were reduced to pre-load levels within 3 h after the fish were loaded into the barge (Fig. 60), indicating that recovery from the stress of being loaded into the barge was similar to the recovery from being loaded into the truck.

Plasma glucose levels were elevated in fish sampled from the barge enroute during the early and mid runs of 1984, but in both cases returned to

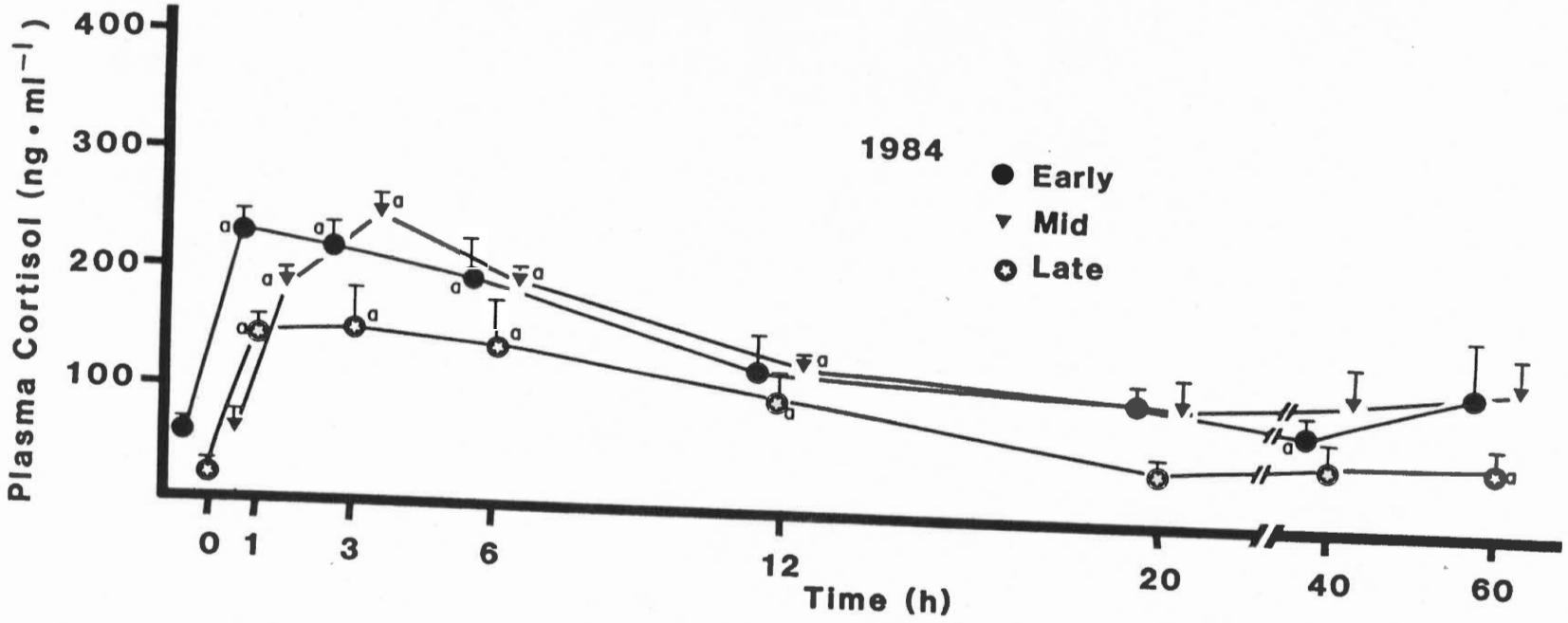

Figure 59. Plasma cortisol levels in juvenile fall chinook salmon transported by barge from McNary Dam to Bonneville Dam where they were held in large, opaque plastic tanks with flow-through water. Sampling was conducted during June 12-15 (early), July 11-13 (mid), and August 8-10 (late), 1984; all points represent the mean + SE for 9 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

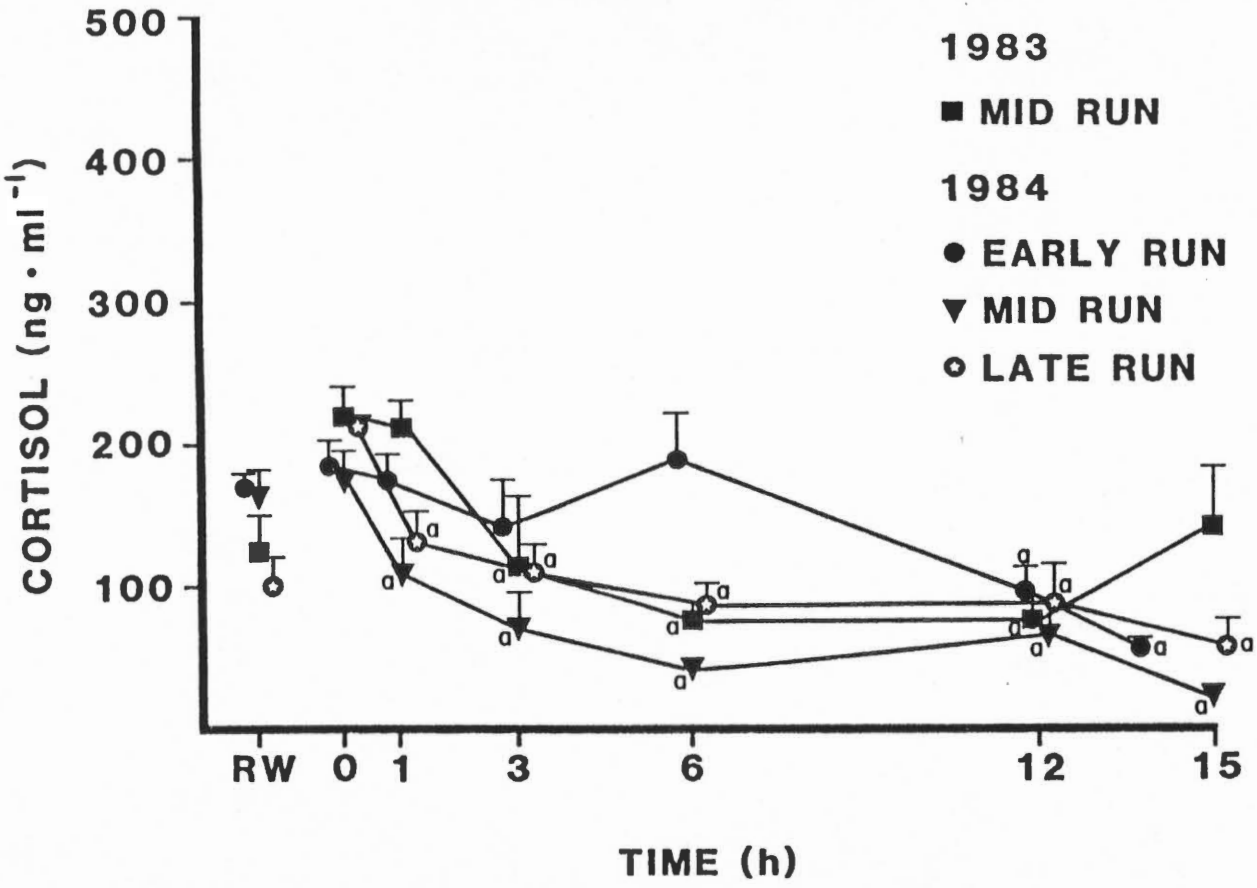

Figure 60. Plasma cortisol levels in juvenile fall chinook salmon collected from a McNary Dam raceway just before being loaded into a transport barge (RW) and throughout transport to Bonneville Dam on July 9, 1983 and June 12 (early), July 10 (mid), and August 7 (late), 1984. All points represent the mean + SE for 10 to 12 fish. Points marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

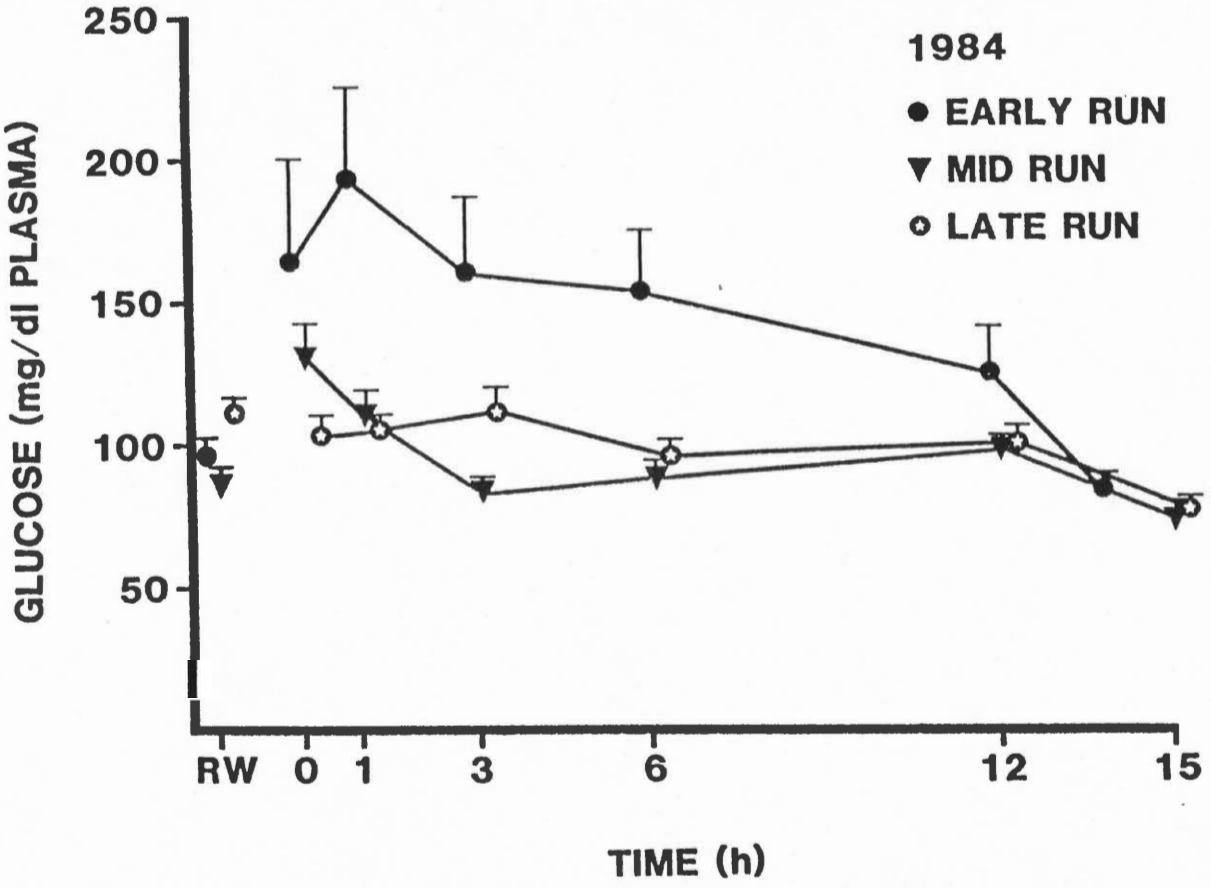

Figure 61. Plasma glucose levels in juvenile fall chinook salmon collected from a McNary Dam raceway just before being loaded into a transport barge (RW) and throughout transport to Bonneville Dam on June 12 (early), July 10 (mid), and August 7 (late), 1984. All points represent the mean + SE for 10 to 12 fish.

pre-load levels before reaching Bonneville Dam (Fig. 61). Plasma glucose levels measured in fish held at Bonneville Dam following barge transport in 1984 were highly variable and show no consistent trend from run to run (Fig 62).

There were no diffences in hematrocrit or WBC numbers that could be attributed to the different transport vehicles (Figs. 53 and 63), nor was there a difference in osmoregulatory ability between trucked and barged fish (Figs. 27b-28b). However, fish transported by barge in 1983 (Fig. 56) and 1984 (Figs. 64 and 65) had higher plasma cortisol levels in response to the secondary stress challenge than did fish transported by truck in 1983 (Fig. 56). Fish barged to Bonneville Dam and held in large tanks for 18 h before being subjected to a secondary stress recovered twice as fast as fish subjected to secondary stress upon arrival (Figs. 64 and 65). While this observation alone may suggest that a holding period between unloading transport vehicles and releasing emigrating smolts below Bonneville Dam might be beneficial, the results of the swim tube experiments fail to support such a conclusion. In fact, the swimming performance of the fish held for 20 h post-transport appears to be depressed when compared to new arrivals (Table 6).

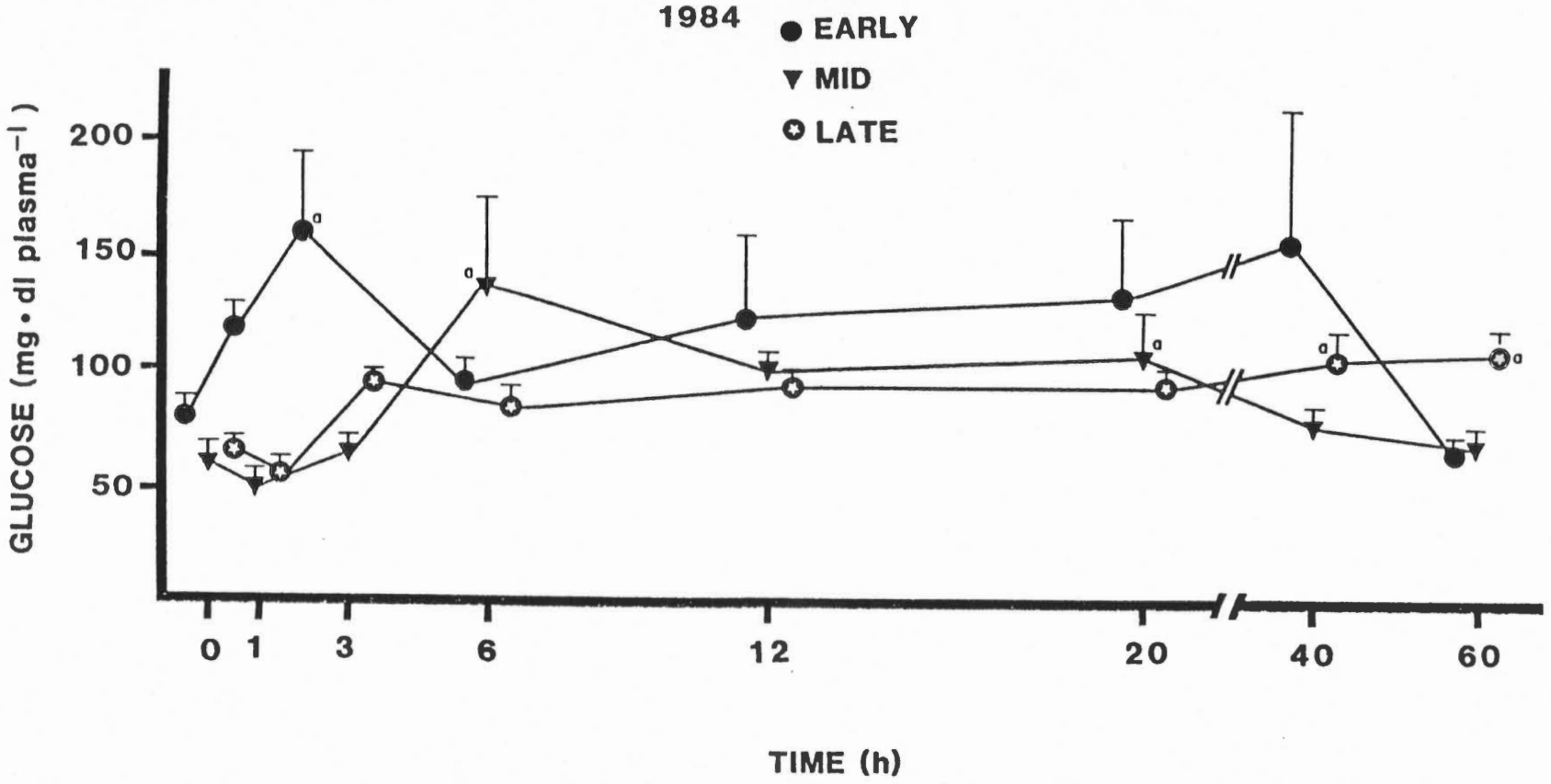

Figure 62. Plasma glucose levels in juvenile fall chinook salmon transported by barge from McNary Dam to Bonneville Dam where they were held in large, opaque plastic tanks with flow-through water. Sampling was conducted during June 12-15 (early), July 11-13 (mid), and August 8-10 (late), 1984; all points represent the mean + SE for 9 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

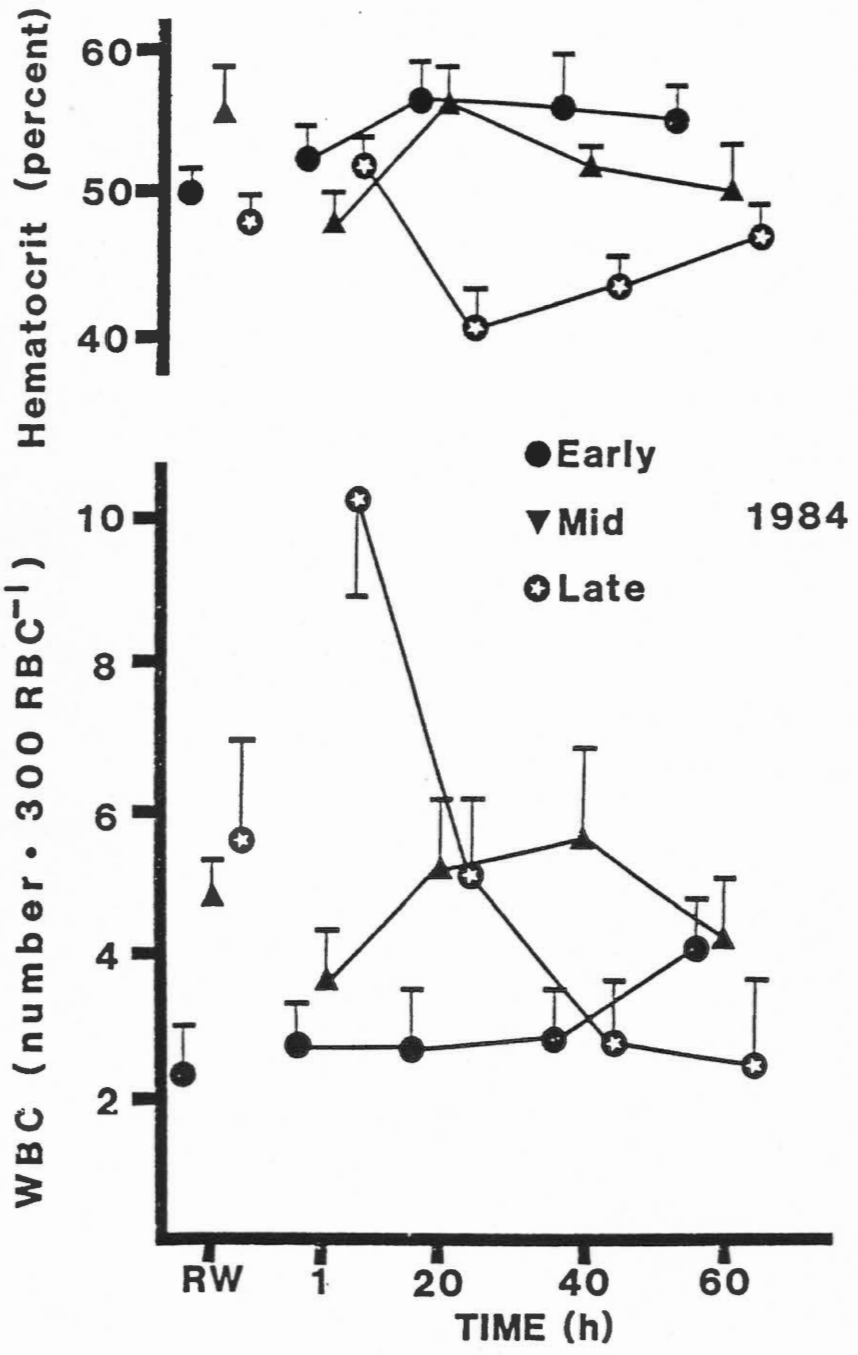

Figure 63. White blood cell (WBC) counts and hematocrit values for juvenile fall chinook salmon collected from a raceway at McNary Dam just prior to being loaded into a barge (RW) and for up to 60 h after being transported to Bonneville Dam. Sampling was conducted during June 11-15 (early), July 10-14 (mid), and August 7-10 (late), 1984. All points represent the mean + SE for 6 fish. WBC counts are the average of two replicate counts of the number of WBC's among 300 erythrocytes (RBC's) on blood smears.

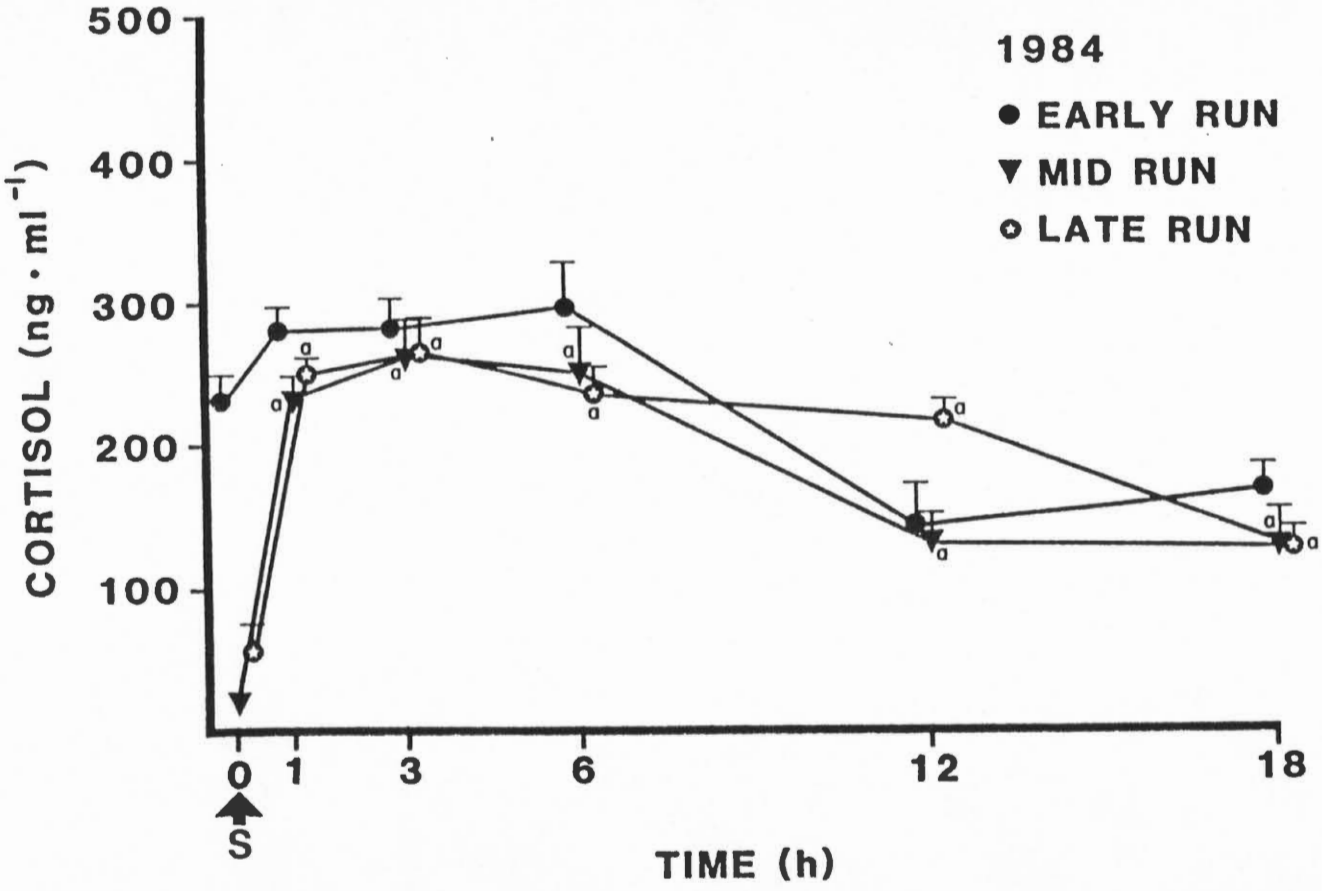

Figure 64. Plasma cortisol levels in juvenile fall chinook salmon transported by barge from McNary Dam to Bonneville Dam and then secondarily stressed (arrow) by being suspended in the air for 30 s. Fish were then held in large, opaque plastic tanks with flow-through water and sampled over 18 h. Sampling was conducted on June 12 (early), July 11 (mid), and August 8 (late), 1984; all points represent the mean + SE for 9 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

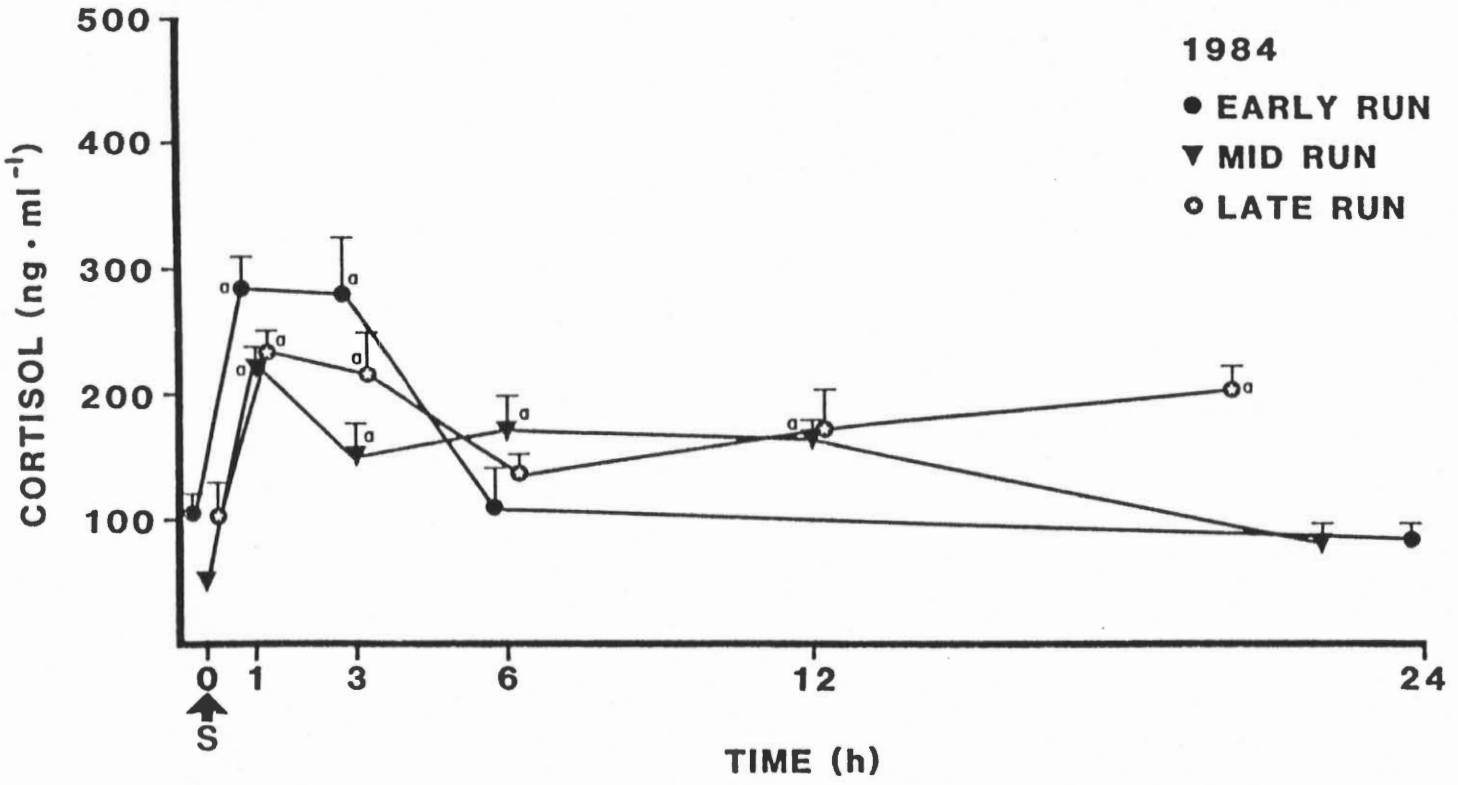

Figure 65. Plasma cortisol levels in juvenile fall chinook salmon transported by barge from McNary Dam to Bonneville Dam, held in large, opaque plastic tanks for 18 h, and then secondarily stressed (arrow) by being suspended in the air for 30 s. Fish were then returned to plastic tanks and sampled over 24 h. Sampling was conducted on June 13 (early), July 12 (mid), and August 9 (late), 1984; all points represent the mean + SE for 9 to 12 fish. Values marked (a) are significantly different from Time = 0 of same line ($P < .05$, LSD test).

Table 6. Geometric means of fatigue times (minutes) of juvenile fall chinook salmon held for various times following barge transport from McNary Dam to Bonneville Dam.

Time (h) post-transport	Early Run June, 1984	Mid-Run July, 1984	Late Run August, 1984
0-3	[a]	1.70	6.40
20	2.80	0.76	1.70
40	2.70	0.90	1.60
60	[a]	1.70	[a]

[a]measurements not made

Multiple Stresses

Experimental design, results, and discussion. We conducted laboratory experiments to determine physiological responses to standardized multiple stresses. These permitted a better understanding of physiological responses to repeated stresses which a fish might encounter in various elements of the collection system, and assisted in interpretation of the clinical indices of stress data obtained from fish at McNary Dam and after transport. Juvenile fall chinook salmon obtained from Trask Hatchery, Tillamook, Oregon, were held under constant conditions (i.e., fed ca. 1.5% initial body wt$\cdot d^{-1}$; water temperature ca. 12.5 C) at the Oregon State University Smith Farm facility in Corvallis. In the experiments, tank acclimated fish (FL 11.5 cm, wt 16.6 g; 2 wk in tanks at 4$\cdot$L min^{-1} inflow) were exposed to the standardized handling stress consisting of dip-netting the fish from the tank and suspending them in the air for 30 s before returning them to an identical tank. Experiments were conducted with 6 fish each from duplicate tanks and the results were pooled (n = 12).

The physiological responses to a single standard stress were determined at 0, 0.5, 1, 3, 6, 12, and 24 h after the 30-s stress. Then, to determine effects of subsequent stresses, fish were stressed twice with a 3-h interval between, and another group of fish was stressed three times at 3-h intervals. In another series of experiments, fish were subjected to two 30-s handling stresses spaced 1, 3, or 12 h apart, and then serially sampled over 24 h. At each monitoring time, plasma and tissue samples were collected for later analysis of plasma cortisol, glucose and lactate, and hepatic glycogen.

Plasma cortisol in juvenile chinook reached a maximum of 182 $ng \cdot ml^{-1}$ in 0.5-3 h, and returned to control levels within 6 h when the fish were exposed to a single acute, but severe, 30-s handling stress (Fig. 66). In fish subjected to a second and third identical handling stress 3 h and 6 h after the initial handling stress, cortisol response followed the same pattern, but peak levels of 296 and 476 $ng \cdot ml^{-1}$, respectively, were additive upon prior responses. In all cases, cortisol declined to control levels within 24 h (Fig. 66). Similarly, levels of plasma glucose were additive upon prior responses. Peak levels of glucose occurred 3-6 h after application of the final stress, and were 83.8, 133.5 and 204.2 $mg \cdot 100\ ml^{-1}$ for the single stress, two handling stresses and three stresses spaced 3 h apart, respectively (Fig. 67). Plasma lactate levels rose rapidly to 51.0 $mg \cdot 100\ ml^{-1}$ within 0.5 h after a single stress; lactate levels exhibited a similar response to a second stress at 3 h, but remained high for a longer period of time (Fig. 68). Plasma lactate levels after the initial stress, and the second stress in the triple-stress experiment were not consistent with the previous responses. The response to the third stress at 6 h was similar in pattern to that from a single stress, with the absolute peak response of 76.3 $mg \cdot 100\ ml^{-1}$ occurring at 0.5 h (Fig. 68). In fish subjected to the second stress at 3 h and fish subjected to the second and third acute stresses at 3 h and 6 h, minimum liver glycogen levels occurred at 6 h after the initial stress (Fig. 69). In both groups, liver glycogen decreased from greater than 30 $mg \cdot g^{-1}$ to less than 6 $mg \cdot g^{-1}$. However, fish that were subjected to the single initial handling stress did not demonstrate any consistent changes in liver glycogen (data not shown).

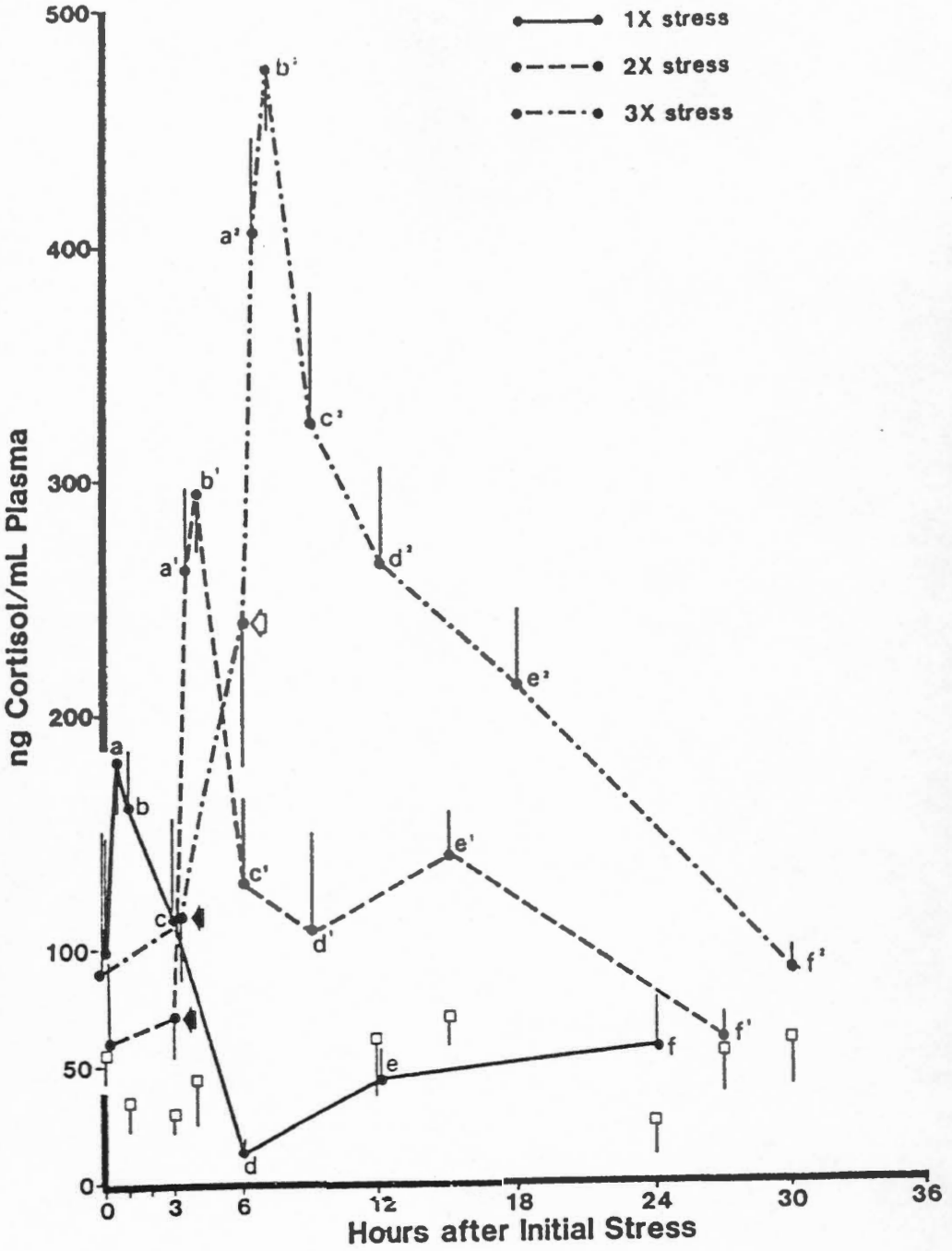

Figure 66. Plasma cortisol (ng/mL+/-SE) in juvenile fall chinook salmon subjected to a single (1X) 30-s handling stress, or to two (2X) or three (3X) 30-s handling stresses spaced 3 h apart. Sample sizes of n=12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h: $\underline{a \quad a^1} < a^2$ 6 h: $\underline{d \quad d^1} < d^2$

1 h: $b < b^1 < b^2$ 12 h: $e < e^1 < e^2$

3 h: $\underline{c \quad c^1} < c^2$ 24 h: $\underline{f \quad f^1 \quad f^2}$

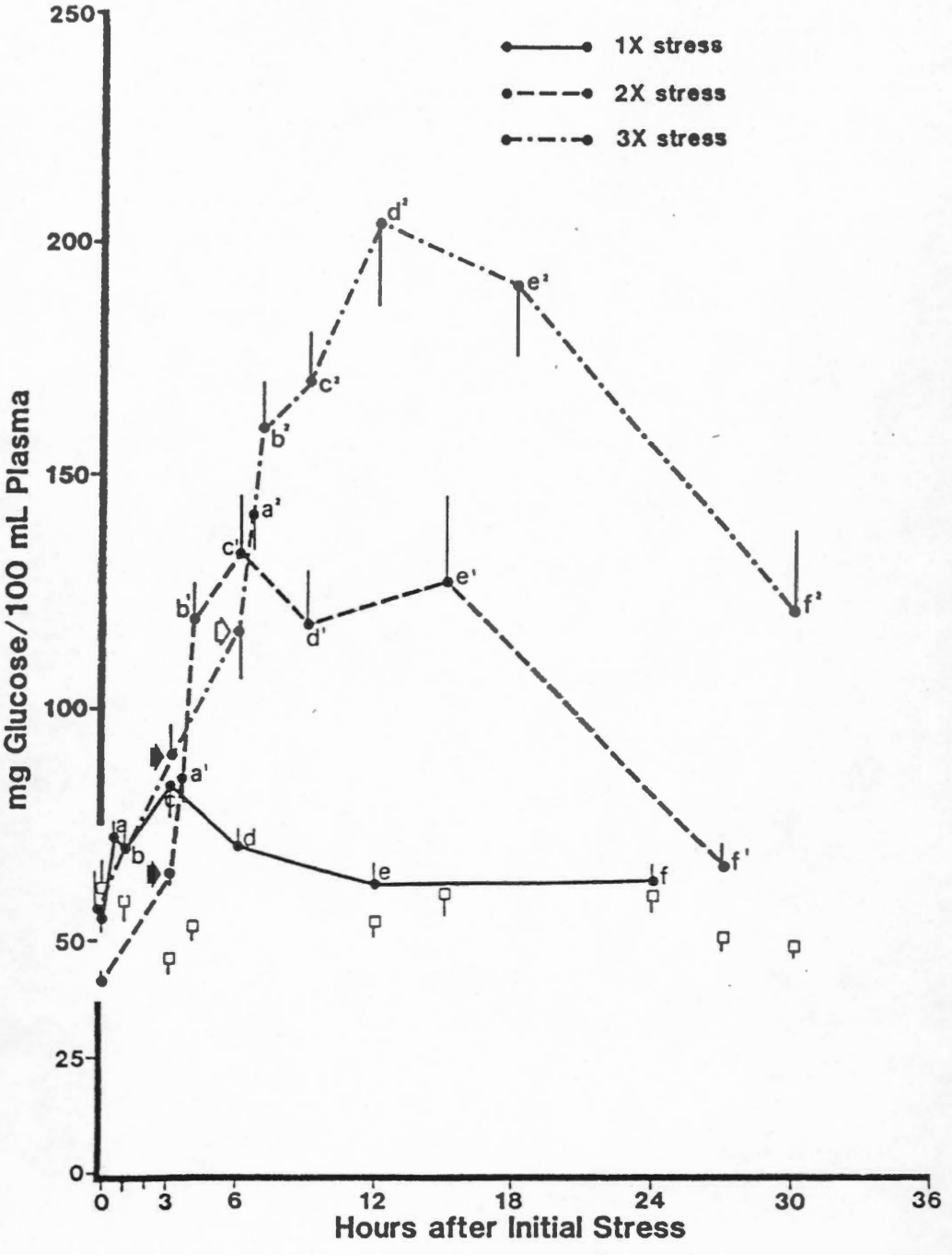

Figure 67. Plasma glucose (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to a single (1X) 30-s handling stress, or to two (2X) or three (3X) 30-s handling stresses spaced 3 h apart. Sample sizes of n = 12 represent pooled data for duplicate tanks. Open squares are for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h: $\underline{a \quad a^1} < a^2$ 6 h: $d < d^1 < d^2$

1 h: $b < b^1 < b^2$ 12 h: $e < e^1 < e^2$

3 h: $c < c^1 < c^2$ 24 h: $\underline{f \quad f^1} < f^2$

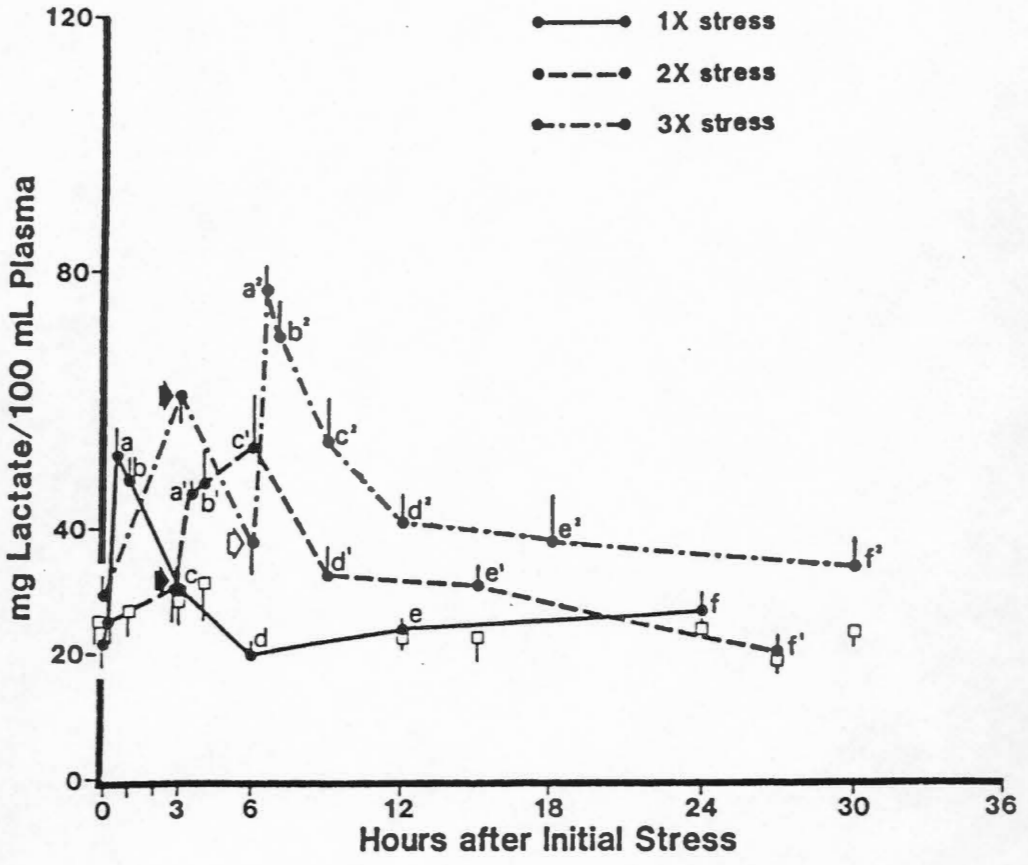

Figure 68. Plasma lactate (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to a single (1X) 30-s handling stress, or to two (2X) or three (3X) 30-s handling stresses spaced 3 h apart. Sample sizes of n = 9-12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h: $\underline{a^1 \quad a} < a^2$

1 h: $\underline{b^1 \quad b} < b^2$

3 h: $\underline{c \quad c^1 \quad c^2}$

6 h: $d < \underline{d^1 \quad d^1}$

12 h: $\underline{e \quad e^1 \quad e^2}$

24 h: $\underline{f^1 \quad f \quad f^2}$

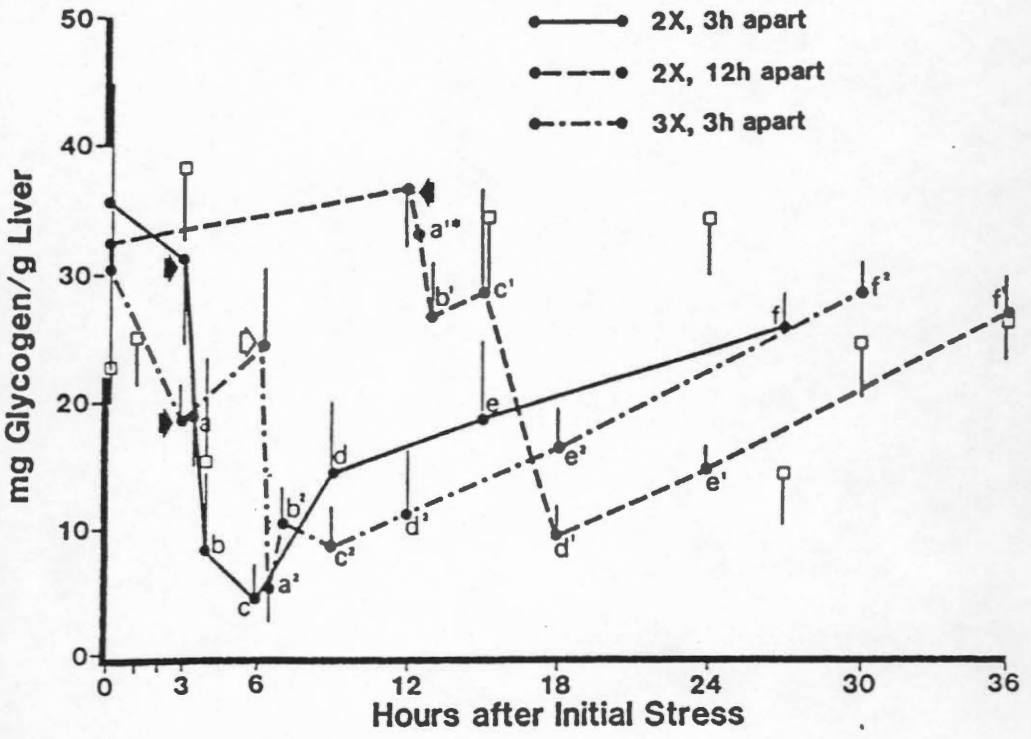

Figure 69. Liver glycogen (mg/g +/-SE) in juvenile fall chinook salmon subjected to two (2X) 30-s handling stresses spaced either 3 h or 12 h apart, or to three (3X) 30-s handling stresses spaced 3 h apart. Sample sizes of n = 6 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h:	a^2	<	a	
1 h:	b		b^2	< b^1
3 h:	c		c^2	< c^1
6 h:	d^1		d^2	d
12 h:	e^1		e^2	e
24 h:	f		f^1	f^2

(*Value of a^1 is an estimate using body weights x 1% since individual liver weights were not available for that sample.)

Fish stressed twice, 1 h apart, had cortisol levels similar to fish stressed twice, 3 h apart; the maximum level of 379 $ng \cdot ml^{-1}$ was cumulative upon the plasma cortisol level of 201 $ng \cdot ml^{-1}$ from the earlier stress (Fig. 70). However, when fish were allowed 12 h to recover, a second stress elicited a much greater relative response in plasma cortisol, i.e., from 64 to 378 $ng \cdot ml^{-1}$ in 0.5 h (Fig. 70). This increased response to a second stress was not evident for plasma glucose, where levels were the same after the second stress independent of the time since the first stress; peak levels after the second stress were 135, 134, and 117 $mg \cdot 100\ ml^{-1}$ for 1 h, 3, and 12 h recovery periods, respectively (Fig. 71). Plasma lactate levels after a second stress were similar to those observed for plasma cortisol. That is, after a 12-h recovery period between stresses, there was a much greater increase in plasma lactate to a maximum level of 98.9 $mg \cdot 100\ ml^{-1}$ in 0.5 h, than there was to a second stress after a 1-h or 3-h recovery period (Fig. 72). When two or three stresses were applied, each separated by 3 h, the minimum liver glycogen levels occurred at 6 h after the initial stress regardless of the application of the second and third stresses. However, the minimum liver glycogen level of 9.5 $mg \cdot g^{-1}$ occurred at 6 h after the second stress when the two stresses were applied 12 h apart (Fig. 69).

These results, particularly for cortisol and glucose, support our hypothesis that certain physiological responses to stress in fish are cumulative. The recovery time between stresses may affect both magnitude and timing of responses to multiple stresses such as changes in cortisol, lactate and glycogen, and that recovery from a single acute stress may take considerably longer than corticoid and glycemic dynamics would indicate.

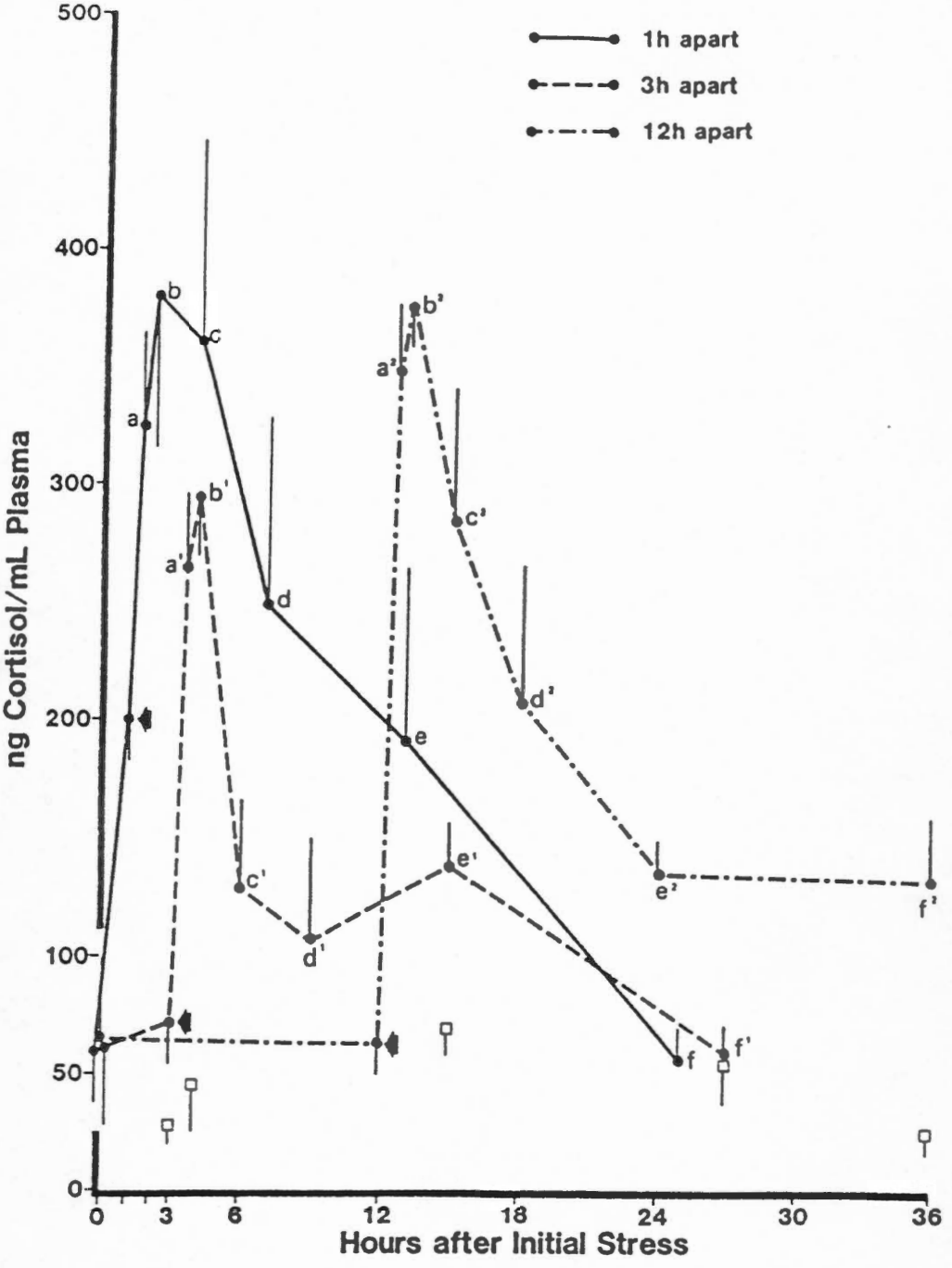

Figure 70. Plasma cortisol (ng/mL +/-SE) in juvenile chinook salmon subjected to two 30-s handling stresses spaced 1, 3, and 12 h apart. Arrows indicate time of second stress. Sample sizes of n = 12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h: $\underline{a^1 \quad a \quad a^2}$

1 h: $\underline{b^2 \quad b^2 \quad b}$

3 h: $\underline{c^1 < c^2 \quad c}$

6 h: $\underline{d^1 \quad d^2 \quad d}$

12 h: $\underline{e^2 \quad e^1 \quad e}$

24 h: $\underline{f \quad f^1 < f^2}$

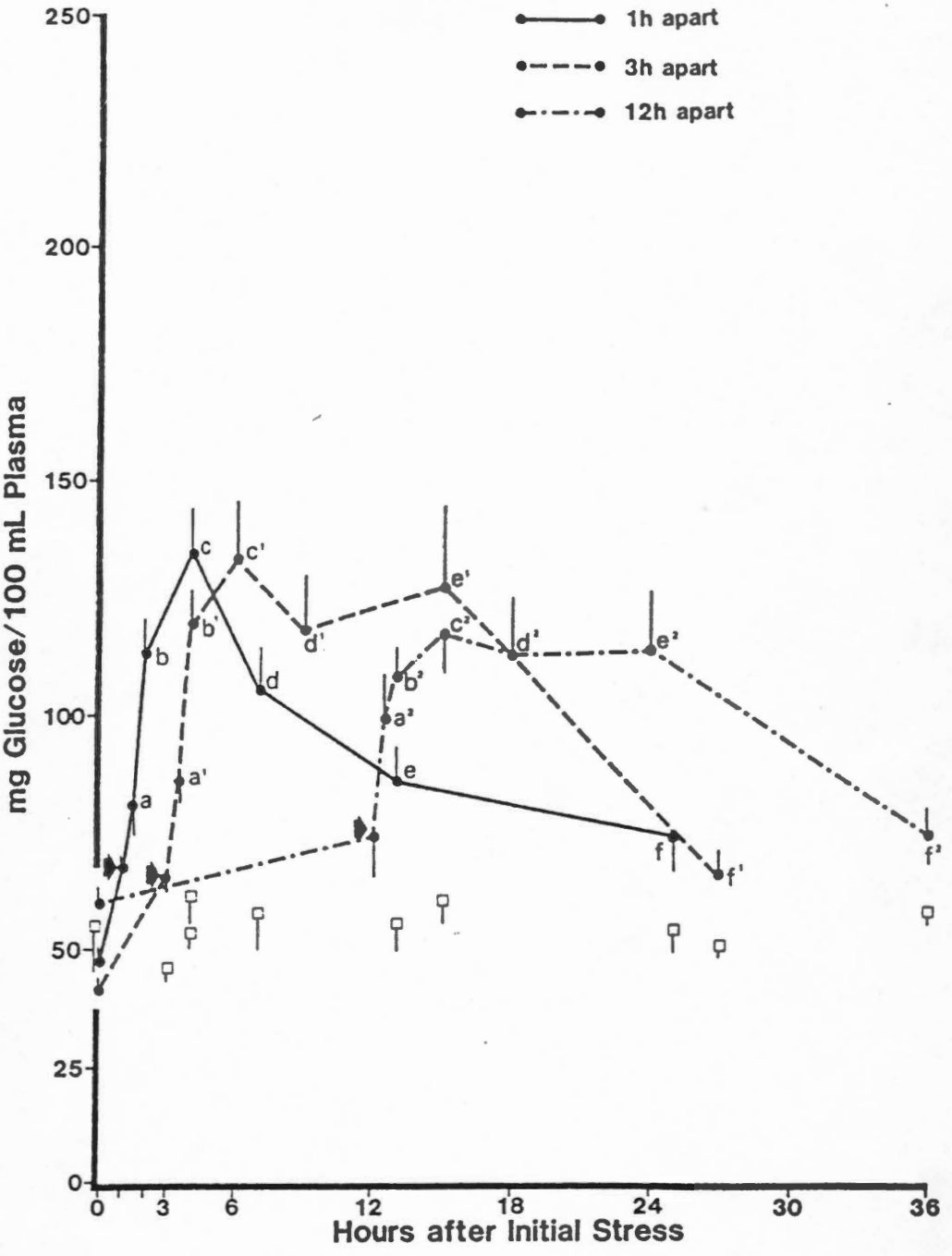

Figure 71. Plasma glucose (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to two 30-s handling stresses spaced 1, 3, or 12 h apart. Arrows indicate time of second stress. Sample sizes of n = 12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of the final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h:	a	a^1	a^2		6 h:	d	d^2	d^1
1 h:	b^2	b	b^1		12 h:	e	e^2	e^1
3 h:	c^2	c^1	c		24 h:	f^1	f^2	f

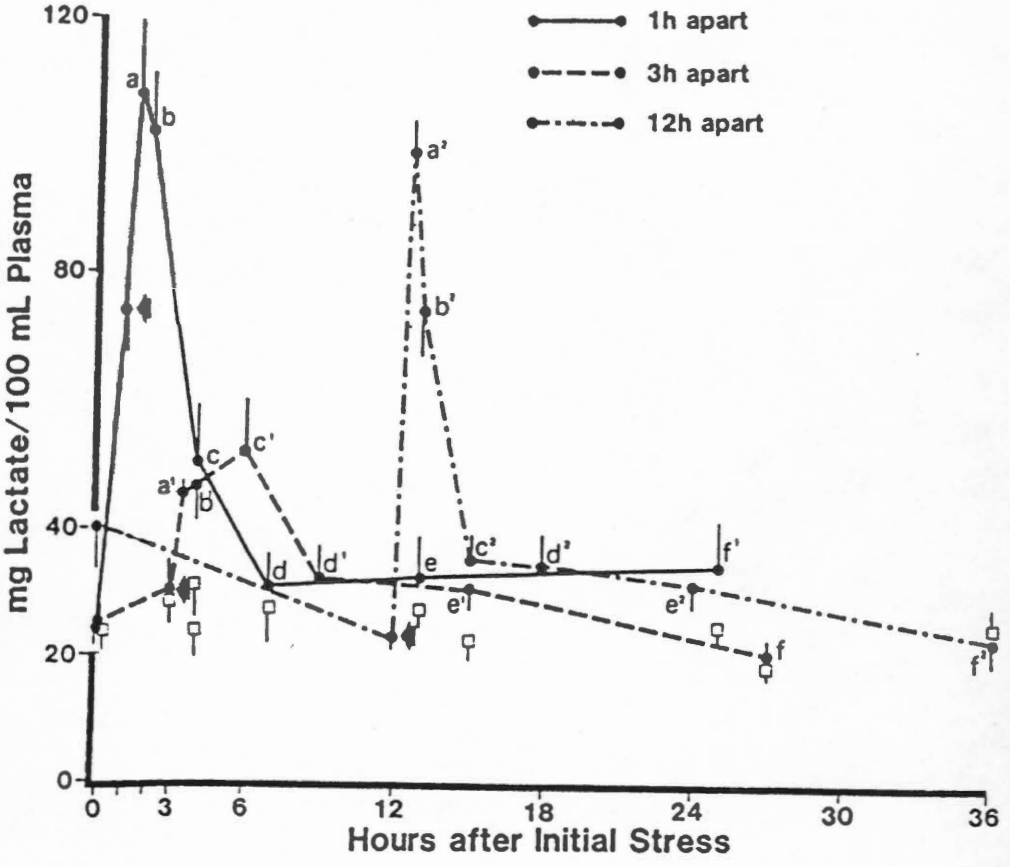

Figure 72. Plasma lactate (mg/100 mL +/-SE) in juvenile fall chinook salmon subjected to two 30-s handling stresses spaced 1, 3, or 12 h apart. Arrows indicate time of second stress. Sample sizes of n = 9-12 represent pooled data for duplicate tanks. Open squares are values for unstressed controls. Mean values for specific time points after application of final stress, as designated by letters on the figure, are ranked as follows from left to right in order from lowest to highest; means not significantly different are underlined (Duncan's new multiple-range test at 5%):

0.5 h:	a^1	< a^2		a
1 h:	b^1	< b^2	<	b
3 h:	c^2		c	c^1
6 h:	d		d^1	d^2
12 h:	e^1		e^2	e
24 h:	f^1		f^2	f

This could be an important factor to consider when making recommendations about optimum allowable recovery time for fish which have been subjected to physical disturbances, such as they encounter during the collection and transportation procedures. Research is being continued in this area to determine the length of this apparent post-stress "sensitive" period to additional stresses.

Acclimation Temperature and Stress

Experimental design, results, and discussion. Water temperature at McNary Dam varies considerably during the smolt emigration (Table 1). It has been shown that water temperature has a profound influence on many physiological functions in fish (e.g., Brett 1979, O'Neill 1980) including their physiological responses to stress (Umminger and Gist 1973; Strange et al. 1977). We examined the effect of acclimation temperature upon the magnitude of physiological responses, and subsequent recovery, resulting from an acute stress to determine if the fish react variously to the collection system due to environmental factors.

Juvenile fall chinook salmon were acclimated to three experimental temperatures (7.5 ± 0.5 C, 12.5 ± 0.5 C, and 21.0 ± 0.5 C) using programmable, cam-operated pneumatic temperature controllers (Golden 1974). In two groups of duplicate tanks of fish acclimated to 12.5 C, temperature was either continuously increased or decreased by 1 $C \cdot d^{-1}$ until the desired temperature was reached, after which the fish were acclimated at this temperature for an additional 2 wk. A third group of fish in duplicate tanks remained at 12.5 C. These temperatures were selected because 21.0 C is close to the maximum encountered by juvenile fall chinook salmon during

outmigration in the Columbia River; 12.5 C was the ambient rearing temperature for the experimental fish, and 7.5 C was the lowest temperature possible with the apparatus.

Throughout the acclimation period and the experiment, fish were held in 350-L tanks at a density of ca. 6 $g \cdot L^{-1}$, having an inflow of 10 $L \cdot min^{-1}$ aerated well water. Up to, but not during, the experiment, the fish were fed daily with Oregon Moist Pellets at ca. 1.5% body weight. After acclimation at the final temperature, fish were subjected to the standard 30-s handling stress and returned to the tank. Plasma samples for cortisol and glucose were obtained (see: Sample collection) prior to the stress and at 1, 3, 6, 12, and 24 h after the stress. Samples for liver glycogen were obtained before the stress and at 6, 12, and 24 h.

Basal plasma cortisol levels were similar for all three temperatures (7.5, 12.5, and 21.0 C) and were relatively low (Fig. 73). Peak cortisol levels at 1 h after the 30-s handling stress were also similar to each other, being 190, 209, and 224 $ng \cdot ml^{-1}$ for the 7.5, 12.5, and 21.0 C groups, respectively (Fig. 73). The only significant differences in plasma cortisol were at 6 and 12 h after stress, where plasma cortisol was slightly higher in the low temperature group. Plasma glucose levels in response to the handling stress were substantially higher in the 21.0 C group than at lower temperatures (Fig. 74). The peak concentration of 113 $mg \cdot dl^{-1}$ at 6 h is more than double those found at the two lower temperatures. Plasma glucose returned to pre-stress levels within 12 h at 7.5 and 12.5 C, but not until 24 h at 21.0 C (Fig. 74). Hepatosomatic indices (HSI, i.e., % liver weight$\cdot$body $weight^{-1}$) were highest in the low temperature group, being 1.76, 1.28, and 1.01 % for the low, ambient, and high temperature groups,

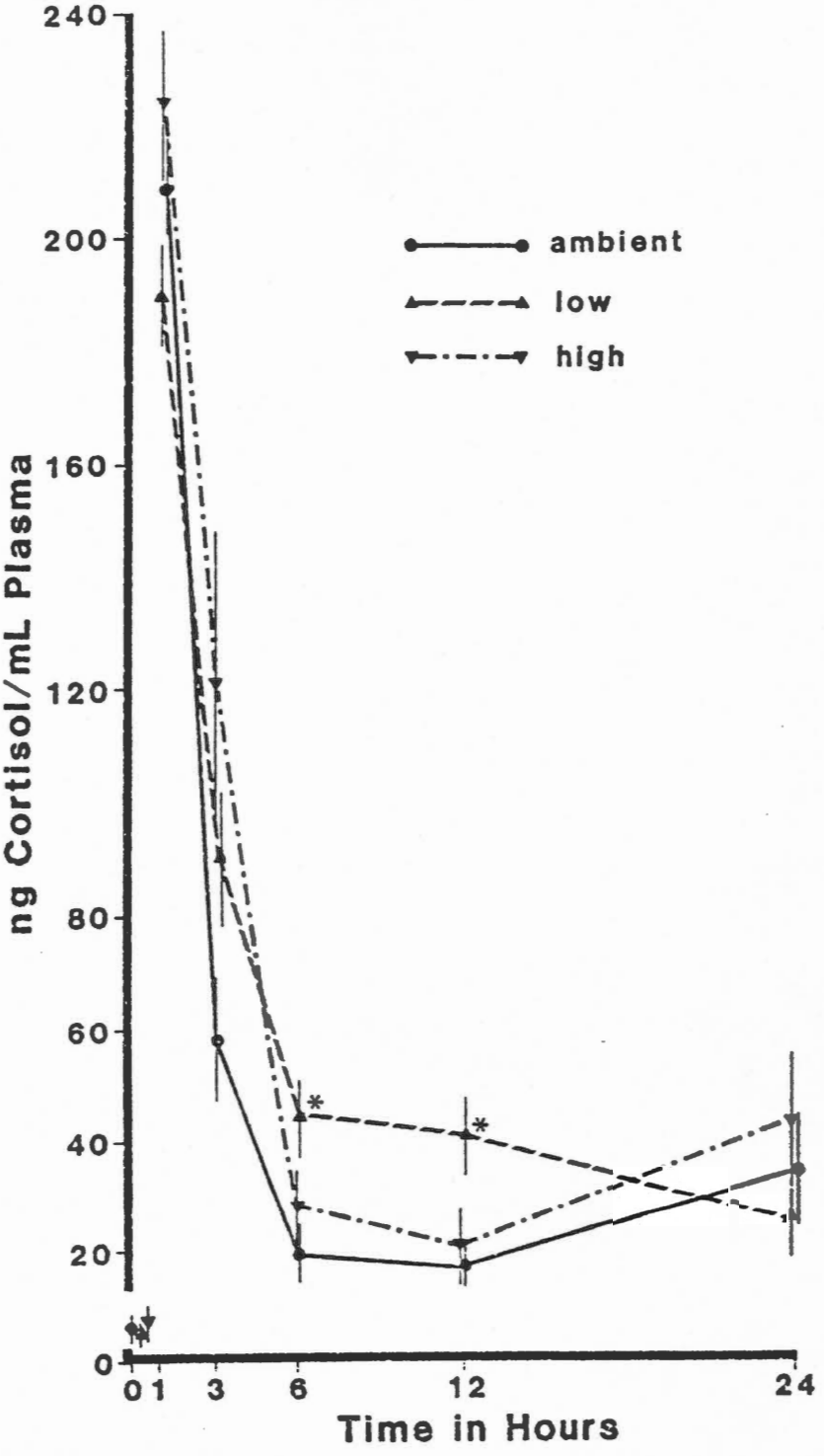

Figure 73. Plasma cortisol (ng/ml +SE) in juvenile fall chinook salmon acclimated to ambient (12.5 C), low (7.5 C), and high (21.0 C) temperatures and subjected to a 30-s handling stress. Sample sizes of n = 12 represent pooled data from duplicate treatments. Values marked with an asterisk (*) indicate a significant difference from the ambient temperature at that time point (Duncan's new multiple-range test at 5%).

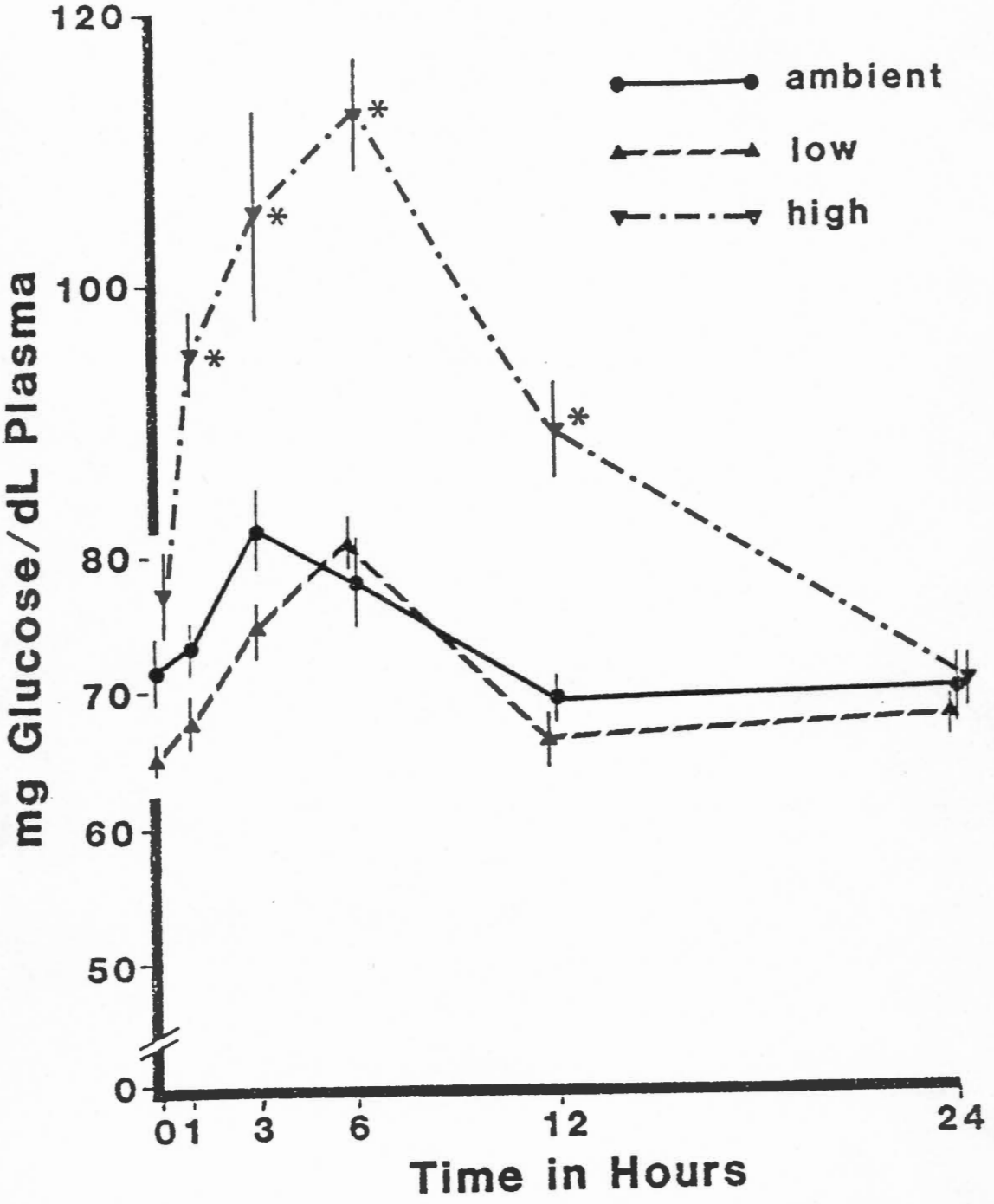

Figure 74. Plasma glucose (mg/dL ±SE) in juvenile fall chinook salmon acclimated to ambient (12.5 C), low (7.5 C), or high (21.0) temperatures and subjected to a 30-s handling stress. Sample sizes of n = 12 represent pooled data from duplicate treatments. Values marked with an asterisk (*) indicate a significant difference from the ambient temperature at that time point (Duncan's new multiple-range test at 5%).

respectively. There was a decline in HSI in all groups in response to the stress.

Acclimation temperature did not appear to affect the peak plasma cortisol level after an acute handling stress. The higher levels of cortisol observed at 6 and 12 h probably resulted from a reduction in the rate at which cortisol was cleared from the body at the lower temperature. The significantly increased plasma glucose levels in response to handling at 21.0 C illustrate the combined effect of the handling stress plus the high temperature and are a reflection of the general level of metabolism at this temperature, as compared to the lower temperatures. Thus, we conclude that at higher temperatures there is a greater metabolic cost associated with stress and that performance capacity may be reduced.

Nutrition and Stress Response

Experimental design, results, and discussion. During our first year of study at McNary Dam, we found that many smolts had empty stomachs and, subsequently, that liver glycogen levels were relatively low (see: System Evaluation). Stress can cause metabolic disturbance in fish (Wendt and Saunders 1973, Leach and Taylor 1980), and the nutritional state of a fish might seriously influence the fish's ability to respond to stress encountered in the collection system. We conducted a controlled diet experiment to determine if the type of diet or fasting alters the physiological response to an acute stress.

Groups of fall chinook salmon were reared on three different diets (i.e., low [7%], normal [13%], and high [19%] fat diets) for a period of

approximately 3 months (April-June 1983) at Abernathy Salmon Cultural Development Center, Longview, Washington. The diets were based on the Abernathy fish food with either 1, 7, or 13% fish oil added, which resulted in total fat contents of 7, 13, or 19%, respectively. Fish were acclimated at a density of ca. 5 $g \cdot L^{-1}$ in 700-L circular tanks, each receiving a 12-$L \cdot min^{-1}$ inflow of 12 C aerated well water. Prior to stress experiments, duplicate groups from each diet regime were fasted for 20 d. At day 20, all groups were subjected to a standard 30-s handling stress and then allowed to recover in their home tank. Samples for assay of plasma cortisol and glucose were taken prior to the stress and at 1, 3, 6, 12, 24, and 48 h; liver samples for glycogen determination for the fish fed normal and high fat diets were obtained before the stress and at 6, 12, and 24 h.

At the time of the experiment, fasted fish were smaller than their fed counterparts for all three of the diet regimes. Also, condition factor ($K = g \times 100 \cdot cm^{-3}$) was lower in the fasted fish, being 1.06, 1.08, and 1.10 compared to 1.20, 1.23, and 1.27 in the fed fish for the low, medium, and high fat diets, respectively. Similarly, the hepatosomatic index (HSI) in the fasted fish was lower than that for the fed fish except for those fish on the high fat diet, where HSI was the same for both fed and fasted fish. After the stress, HSI varied considerably across the different treatment groups. Liver glycogen levels in the fed fish were 35, 20, and 23 $mg \cdot g^{-1}$ for the low, medium, and high fat diet groups, respectively (Table 7). Conversely, in the fasted fish, levels were 6, 5, and 4 $mg \cdot g^{-1}$ for the low, medium, and high fat diet groups, respectively, at the start of the experiment (Table 7). This represents a drop in liver glycogen of approximately 80% over the 20-d fast. At 6 h after handling, both medium

Table 7. Plasma cortisol ($ng \cdot mL^{-1}$), plasma glucose $mg \cdot dL^{-1}$) and liver glycogen ($mg \cdot g^{-1}$) ±SE in fed and 20-d fasted juvenile chinook salmon after receiving low (7%), normal (13%), or high (19%) fat diets and then subjected to a 30-s handling stress. Sample sizes of n = 9-11 represent pooled data from duplicate treatments.

		Fed Fish			Fasted Fish		
Time (h)		Low Fat	Normal Fat	High Fat	Low Fat	Normal Fat	High Fat
0	Cortisol	66 ±11	41 ±9	49 ±7	9 ±3	20 ±5	19 ±8
	Glucose	82 ±3	78 ±3	83 ±2	68 ±3	67 ±3	61 ±2
	Glycogen	35 ±4	20 ±3	20 ±3	6 ±1	5 ±1	4 ±1
1	Cortisol	229 ±11	204 ±17	185 ±23	217 ±17	216 ±13	186 ±16
	Glucose	109 ±5	100 ±5	107 ±5	79 ±3	80 ±3	77 ±3
	Glycogen	-	-	-	-	-	-
3	Cortisol	67 ±8	56 ±12	78 ±12	67 ±10	73 ±8	59 ±11
	Glucose	112 ±5	106 ±8	132 ±9	83 ±3	98 ±3	85 ±4
	Glycogen	-	-	-	-	-	-
6	Cortisol	38 ±7	58 ±13	89 ±14	80 ±17	63 ±24	36 ±9
	Glucose	89 ±4	105 ±7	144 ±3	102 ±10	103 ±9	97 ±8
	Glycogen	-	13 ±2	16 ±3	-	4 ±1	5 ±2
12	Cortisol	111 ±16	96 ±15	37 ±7	45 ±5	44 ±13	32 ±8
	Glucose	88 ±3	101 ±5	115 ±6	77 ±4	93 ±11	74 ±4
	Glycogen	-	15 ±4	16 ±2	-	6 ±1	5 ±1
24	Cortisol	25 ±6	64 ±9	42 ±11	25 ±7	10 ±5	18 ±10
	Glucose	88 ±3	99 ±5	116 ±9	70 ±3	75 ±4	81 ±7
	Glycogen	-	11 ±3	19 ±2	-	6 ±1	5 ±1
48	Cortisol	33 ±10	36 ±13	32 ±8	23 ±3	29 ±13	29 ±10
	Glucose	78 ±3	80 ±4	84 ±4	58 ±4	65 ±3	69 ±3
	Glycogen	-	-	-	-	-	-

and high fat diet groups showed decreased glycogen levels of 13 and 16 $mg \cdot g^{-1}$, respectively, and remained below initial levels for the following 24-h period (Table 7). Liver glycogen levels in the fasted fish remained low and did not change as a result of the handling stress. Basal levels of both plasma cortisol and glucose were lower in the fasted fish for all three diet groups (Table 7). However, plasma cortisol levels subsequent to the handling stress were similar in both the fasted and fed fish (Table 7), except for an unexplained increase in the low and normal fat diet groups at 12 h. Conversely, plasma glucose levels were noticeably higher in the fed fish than in the fasted fish, particularly in the high fat diet group where the peak glucose level of 144 $mg \cdot dl^{-1}$ occurred at 6 h (Table 7).

As expected, the handling stress caused a decline in liver glycogen in fed groups but not in the fasted groups in which glycogen levels were already low. The unusually high basal liver glycogen content in the low fat diet group was probably due to the higher proportion of carbohydrate added to the diet formulation in place of the fat. Although basal levels of plasma cortisol appeared to be reduced in fasted fish, neither fasting nor type of diet affected levels of plasma cortisol in response to an acute handling stress. However, plasma glucose levels after handling were greater in fed fish as compared to fasted fish. Moreover, the greatest glucose level in response to the stress exhibited by the high fat group suggests that stored lipids may be more important than glycogen as a source of energy during stress. Thus, speculatively, fish fed a high fat diet prior to release from a hatchery may be better suited for coping with stresses in a new environment, such as when released into the Columbia River for emigration, by being able to more effectively mobilize their energy reserves.

Cortisol and Disease Resistance

Experimental design, results, and discussion. Stress encountered at the dam elevated plasma levels of cortisol for significant periods of time (see: System Evaluation). It is known that corticosteroids can have an adverse effect on the immune system and disease resistance (see reviews: Baxter 1976, Ellis 1981). Hence, we conducted the following experiment at the Oregon Department of Fish and Wildlife Fish Disease Laboratory, Corvallis, Oregon, to examine the effects of elevated plasma cortisol titers on juvenile salmons' disease resistance. Unfortunately, coho salmon were the only fish available at the time of this experiment; however, we believe that the results are illustrative of mechanisms found in salmonids in general. Reagent hydrocortisone (i.e. cortisol) was dissolved in molten (45 C) cocoa butter at a concentration of 40 $mg \cdot ml^{-1}$ and 0.1 ml was injected intraperitoneally into each fish after the procedure of Pickering and Duston (1983). The molten cocoa butter solidifies immediately upon injection and forms a bolus which slowly leaches cortisol into the fish's system, resulting in elevated plasma cortisol levels for at least 7 weeks (Maule et al., unpublished data). The concentration of cortisol used was selected based on a dose response study comparing 20, 40, and 80 $mg \cdot ml^{-1}$ cortisol in cocoa butter. The 40 $mg \cdot ml^{-1}$ dose resulted in plasma cortisol levels close to the lowest levels seen in fish at McNary Dam.

After anesthetization in 50 $mg \cdot ml^{-1}$ MS-222, 5 groups of 25 fish each were injected with cortisol-cocoa butter and put in 64.5 L cuboidal tanks with a continuous supply of aerated, fish-pathogen-free well water, preheated to 15 C. Additionally, 3 groups of 25 fish were injected with

molten cocoa butter and placed in similar tanks. Eleven days after injection, two groups of cortisol-injected fish and two groups of cocoa butter-injected fish were inoculated with Vibrio anguillarum (see: Disease challenge). The remaining groups of fish were used as controls and to monitor plasma cortisol levels. All groups were checked daily for mortalities, and V. anguillarum was isolated from the kidneys of all mortalities. The disease challenge was terminated 14 d after inoculation and all survivors were bled for plasma cortisol determination and were examined for the presence of a cocoa butter bolus in the peritoneal cavity. Percent mortality and mean of the time to death were calculated for the replicated groups.

The intraperitoneal cortisol injections resulted in plasma cortisol levels equivalent to those in fish sampled from the collection and transportation system and resulted in significantly higher mortalities than controls when exposed to V. anguillarum (Fig. 75). There was no significant difference in the mean of the times to death for the two groups, 3.6 ± 0.1 and 3.4 ± 0.1 days for the cortisol-treated and control groups, respectively. Cortisol is known to cause the lysis of white blood cells in the mammalian system (Ellis 1981), and we have seen reduction in the numbers of white blood cells which may be the result of the stress-induced plasma cortisol elevation in fish entering the collection system at McNary Dam (Figs. 22-24). We believe that the connections between stress, cortisol, immunocompetence, and disease resistance may be affecting the long-term survival of salmonids in the Columbia River.

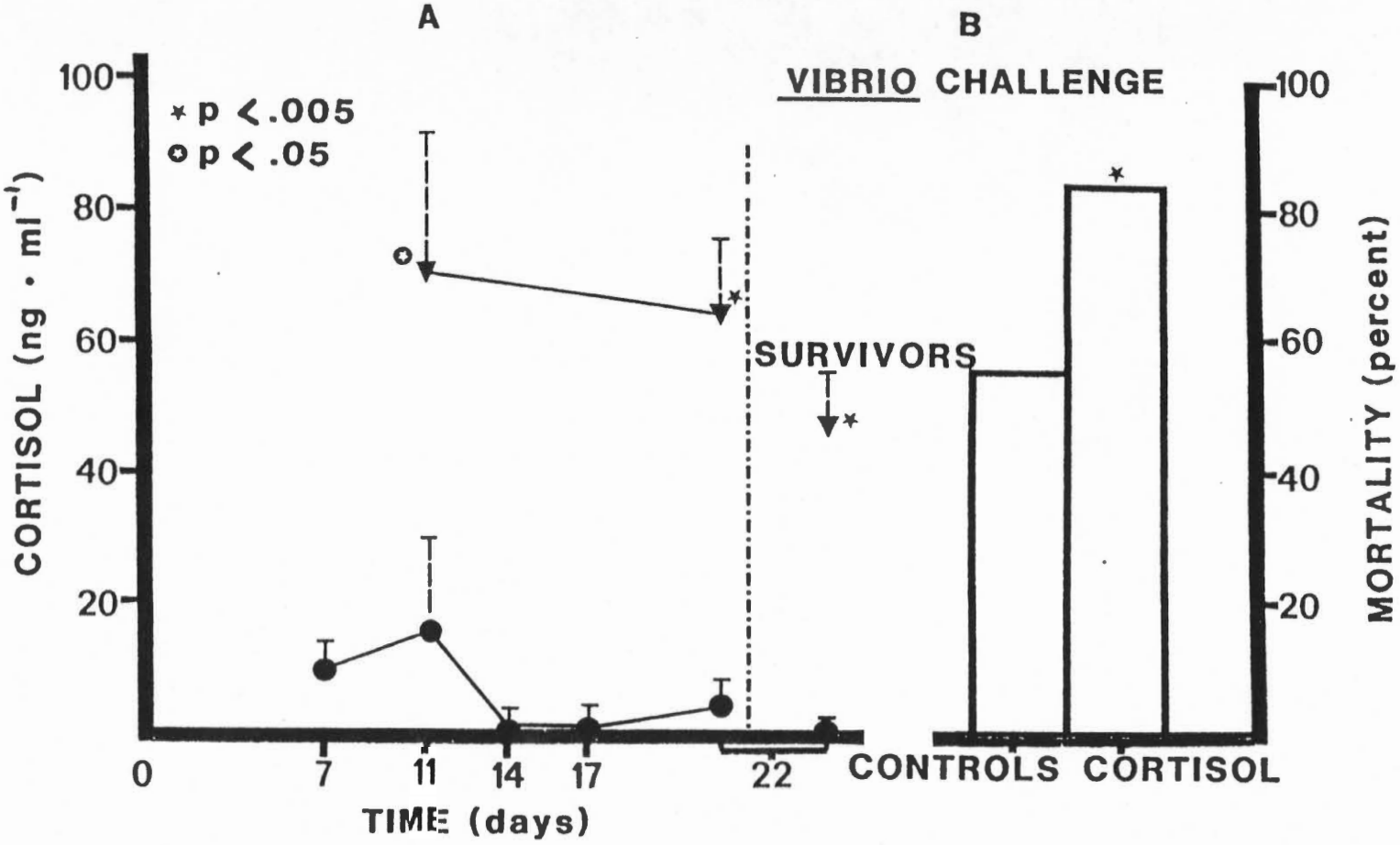

Figure 75. A. Plasma cortisol levels of juvenile coho salmon which were intraperitoneally injected with cortisol in molten cocoa butter (▼) and then injected with just cocoa butter (●). B. Mortality and plasma cortisol levels of coho injected as in A and subsequently exposed to Vibrio anguillarum.

Stress and Swimming Performance

Experimental design, results, and discussion. A smolt's capacity to swim quickly or for a sustained time may be impaired if the fish is in a stressed condition. This may compound the already reduced swimming efficiency of fish consequent to smoltification (Flagg et al. 1983). This impaired performance capacity may then result in reduced survival when the fish encounters predators or physical obstructions in the water. Experiments were conducted at the Marine Science Center to examine the effects of stress on critical swimming speed, the maximum speed that a fish can maintain for a given length of time, and fatigue time, the length of time that a fish can maintain a given swimming speed. These variables are defined by the structure and use of the swimming tube in which they are measured. The portion of our tubes in which fish swim was .076 m diameter by 1.5 m in length (.25 x 3.0 ft) with screening at both ends to contain the fish. The inflow end of the tube also had a baffling screen to eliminate turbulence. The flow meter was positioned on the inflow end of the tube behind the barrier screen, so as not to interfere with fish in the tube, but it was calibrated to the flow in the swim portion of the tube. Water inflow was through a 36 cm (11/4 inch) PVC pipe, and velocities in excess of 105 $cm \cdot s^{-1}$ could be achieved.

In these experiments, critical swimming speed was measured by increasing the water velocity in the tube and noting the velocity at which the fish stopped swimming. The swim tubes were calibrated to the fall chinook prior to the experiments; that is, we determined the length of time required to acclimate fish to the tube (18-24 h), optimum number of fish to

be used in a trial (3 fish), magnitude of increments (10 $cm \cdot s^{-1}$) and length of time at each water velocity increment (5 min), and the minimum water velocity at which the fish must actively swim (25 $cm \cdot s^{-1}$).

In the critical swimming speed experiments, 3 fish were allowed to acclimate in each tube overnight with the water velocity at 5 $cm \cdot s^{-1}$. Stress was applied by draining the water from the tube for 30 s (similar to 30 s in a dipnet, out of water). Fish which were not stressed served as controls, and other fish were stressed 1, 2, or 3 times with 1 h delay between stresses. After each stress, the water velocity was maintained at 5 $cm \cdot s^{-1}$ until the next stress or until the swimming trials which were conducted immediately after the last stress or at 1, 3, 6, 12 or 24 h after the last stress. A swimming trial consisted of increasing water velocity immediately to 25 $cm \cdot s^{-1}$ and maintaining it there for 30 min. This 30 min is apparently necessary for fish to fully shift into a swimming mode and results in less variability in the data (Brett 1965). Water velocity was then increased 10 $cm \cdot s^{-1}$ every 5 min, and the time and water velocity at which each fish stopped swimming was noted. Critical swimming speed (CSS) was calculated as:

$$CSS = V_{max-I} + \frac{(I)\ (t)}{T_{max}}$$

where: T_{max} = maximum length of time at any water velocity, here equal to 5 min

V_{max-I} = maximum water velocity achieved and maintained for T_{max}

t = the length of time the fish swam at the maximum water velocity achieved (V_{max})

I = increment at which water velocity was increased, here equal to 10 $cm \cdot s^{-1}$.

For example, if a fish was able to swim for 2 min at 50 $cm \cdot s^{-1}$, then

$V_{max-I} = 40\ cm \cdot s^{-1}$, $t = 2$ min

and $CSS = 40\ cm \cdot s^{-1} + \frac{(10\ cm \cdot s^{-1})\ (2\ min)}{5\ min} = 40\ cm \cdot s^{-1}$

Experiments to determine fatigue time were conducted in much the same way as the critical swimming speed experiments. Three juvenile fall chinook were acclimated overnight and stressed either 0, 1, 2, or 3 times as above. Fatigue trials were run at 0, 1, 3, 6, and 24 h after the last stress and consisted of immediately increasing the water velocity to 25 $cm \cdot s^{-1}$ for 30 min. Water velocity was then increased to 60 $cm \cdot s^{-1}$ and the time at which each fish stopped swimming was noted for a maximum of 60 nin. After each fish quit swimming or at 60 min, the fish was removed from the tube and a blood sample was collected. Blood samples were also taken from unstressed fish not used in the swim tubes. Blood samples were analyzed for hematocrit, cortisol, glucose, lactic acid, osmolarity, sodium and potassium. The time to 50% fatigue was calculated for each group by plotting cumulative percent fatigued on a probit scale versus time to fatigue on a logarithmic scale and extrapolating the 50% fatigue time from the graph.

Stress initially reduced the time to 50% fatigue from 3.8 to 2.0 min as shown by differences between fish that were not stressed and those tested for fatigue immediately after the first stress (Fig. 76). Immediately after the first stress, the fish were experiencing an oxygen debt as evidenced by elevated plasma lactate (Fig. 77) and the stress response was just beginning to occur. Many of the fish may not have been able to recover from this oxygen debt and thus fatigue time was reduced. By one hour after the first stress, plasma glucose levels were elevated (see: Multiple Stresses of

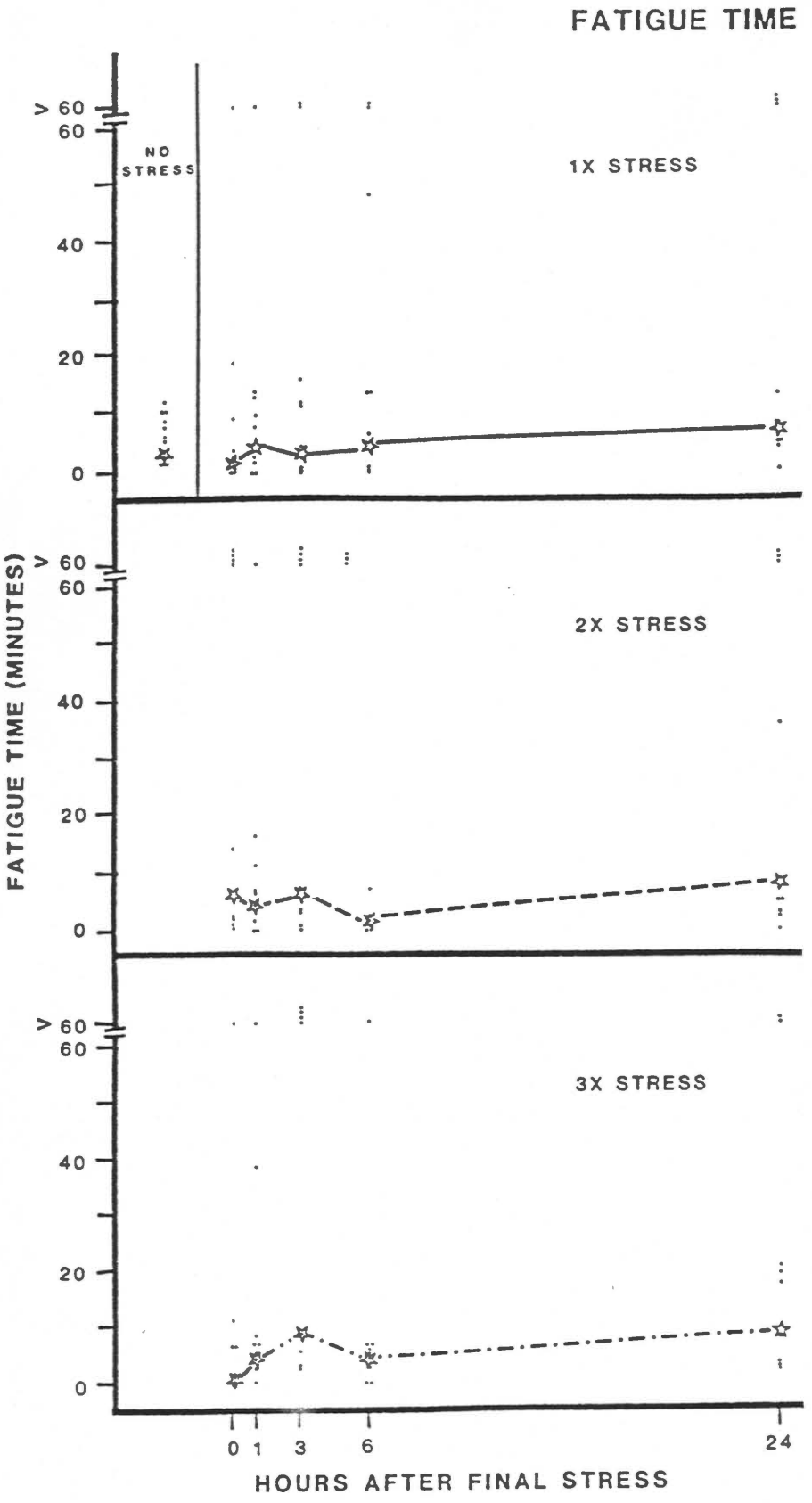

Figure 76. Fatigue times, i.e. time that fish can maintain position in a flow of water (60 cm/sec), of juvenile fall chinook either not stressed or stressed 1, 2 or 3 times with one hour between stresses. Each point represents an individual fish. Stars indicate the time to 50% fatigue. The > 60 represents fish that were still swimming at 60 minutes when the test was terminated.

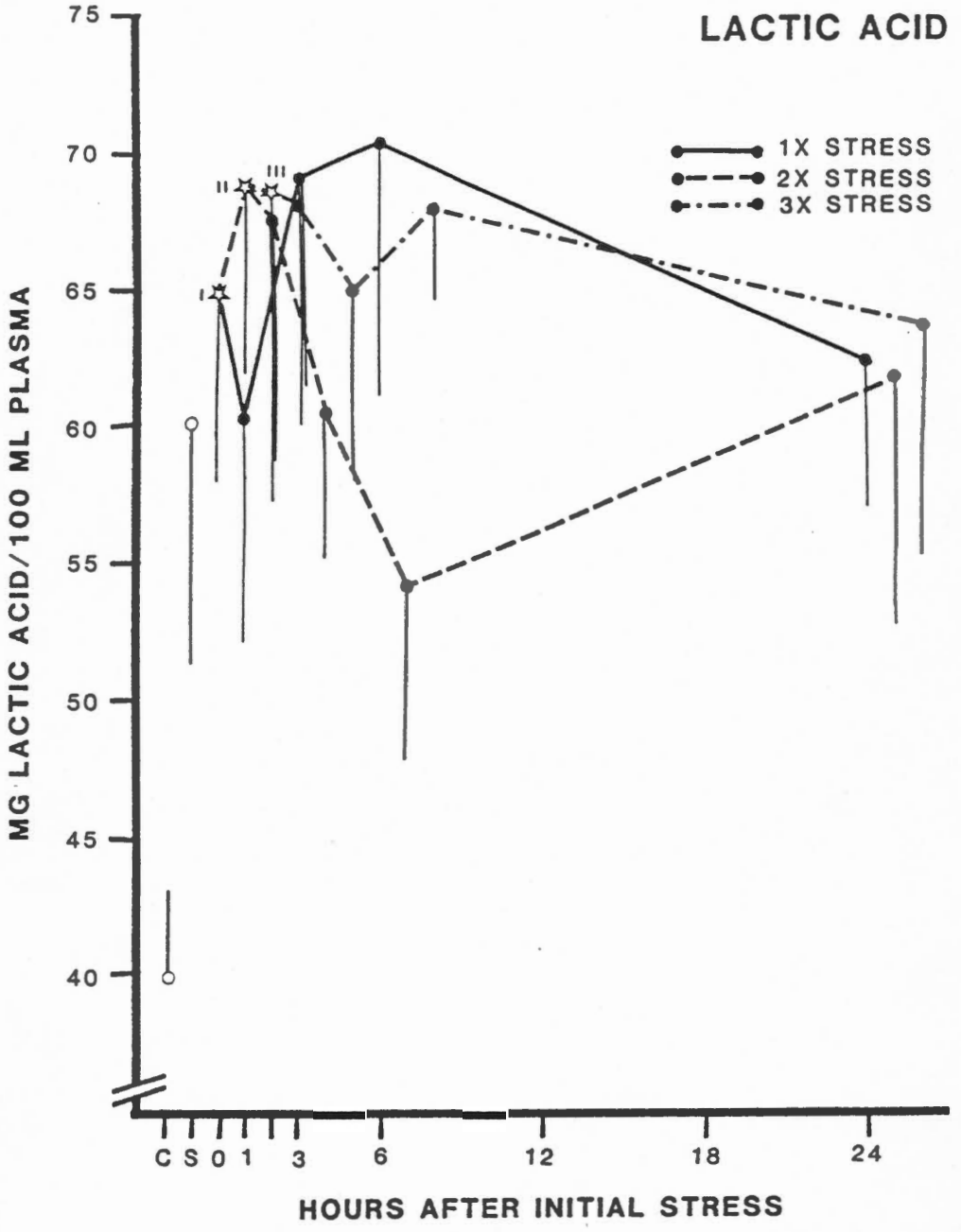

Figure 77. Mean plasma lactic acid (mg/100 ml ± SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Stars indicate the time fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time.

Saltwater-acclimated Fish). A swimming fatigue test at this time yielded a time to 50% fatigue of 4.8 min which was slightly higher than that for non-stressed fish. Perhaps the energy mobilization caused by the stress response provided a benefit of increased swimming endurance for some of the fish. The continued elevation in plasma glucose 24 h after stress (see: Multiple Stresses of Saltwater-Acclimated Fish) correlates with the increase in fatigue time 24 h after one stress when fish were subjected to two stresses. The time to 50% fatigue immediately after the second stress was 6.4 min, and 44% of the fish swam longer than 60 min. Fish subjected to three stresses had a reduction in time to 50% fatigue immediately after the third stress to 1.1 min.

It should be noted that in nearly all of the fatigue tests after stress, some fish would not swim and some swam longer than 60 min. Thus it appears that there are some individuals better adapted to cope with stress. However, when plasma cortisol and glucose, hematocrit, osmolarity, lactic acid, sodium and potassium blood levels (Figs. 77-82) were compared between the fish that fatigued early (< 20 min) and those that swam longer (> 40 min), there were no significant differences. It is likely that there was some other parameter not measured, perhaps hemoglobin concentration, glycogen stores or muscle lactic acid, that caused the differences in the two groups of individuals.

Plasma cortisol and glucose were higher in non-stressed, fatigued fish than in non-stressed, non-fatigued controls, indicating that swimming itself causes a stress response. No significant differences in plasma cortisol, glucose, hematocrit or lactic acid were found between non-stressed, fatigued fish and fish fatigued after one, two, or three stresses at any of the time

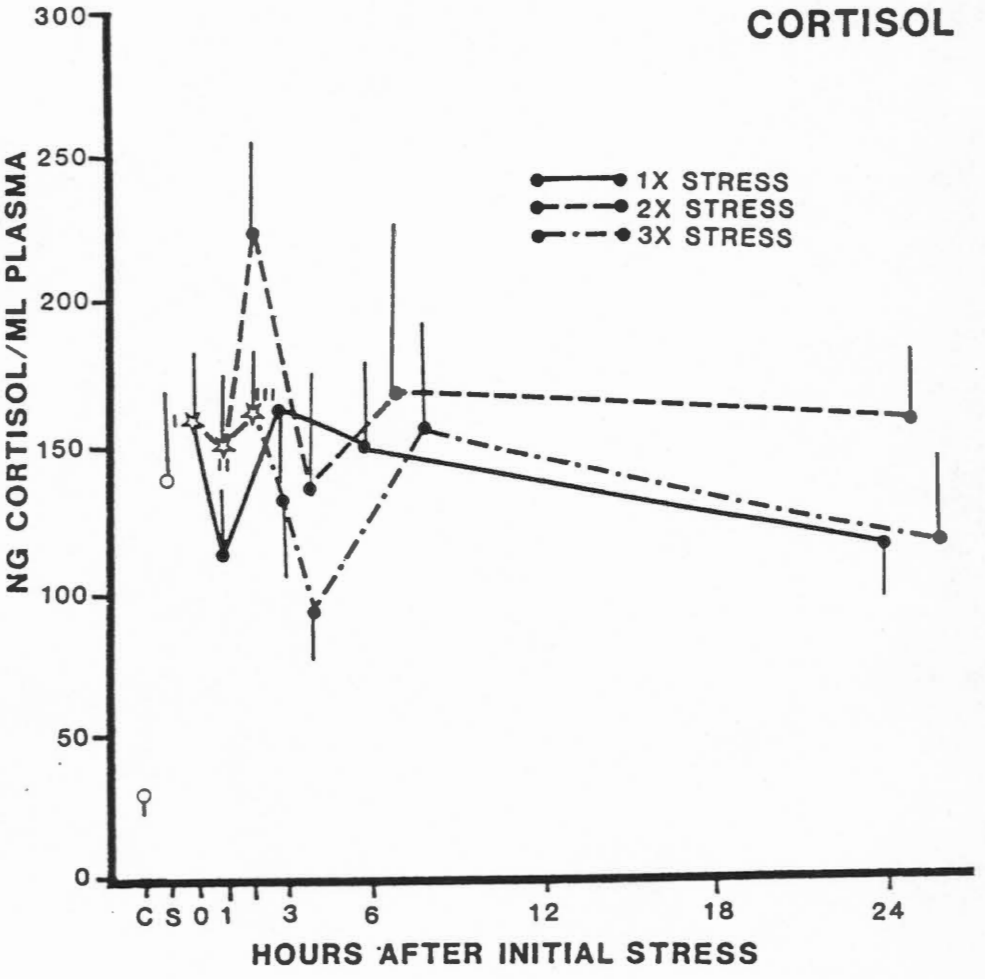

Figure 78. Mean plasma cortisol (mg/ml ± SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Stars indicate the times when fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time.

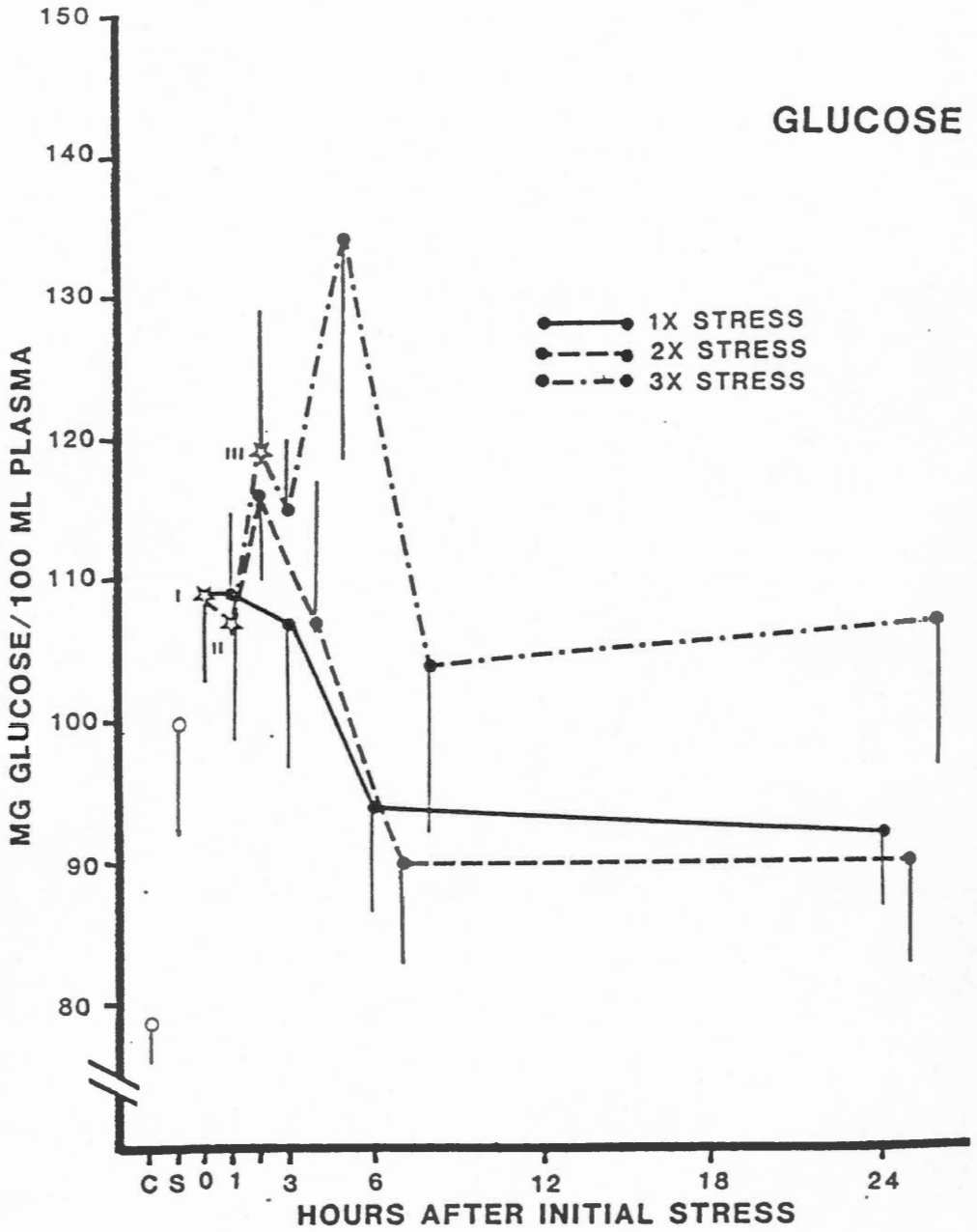

Figure 79. Mean plasma glucose (mg/100 ml ± SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Stars indicate the times when fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time.

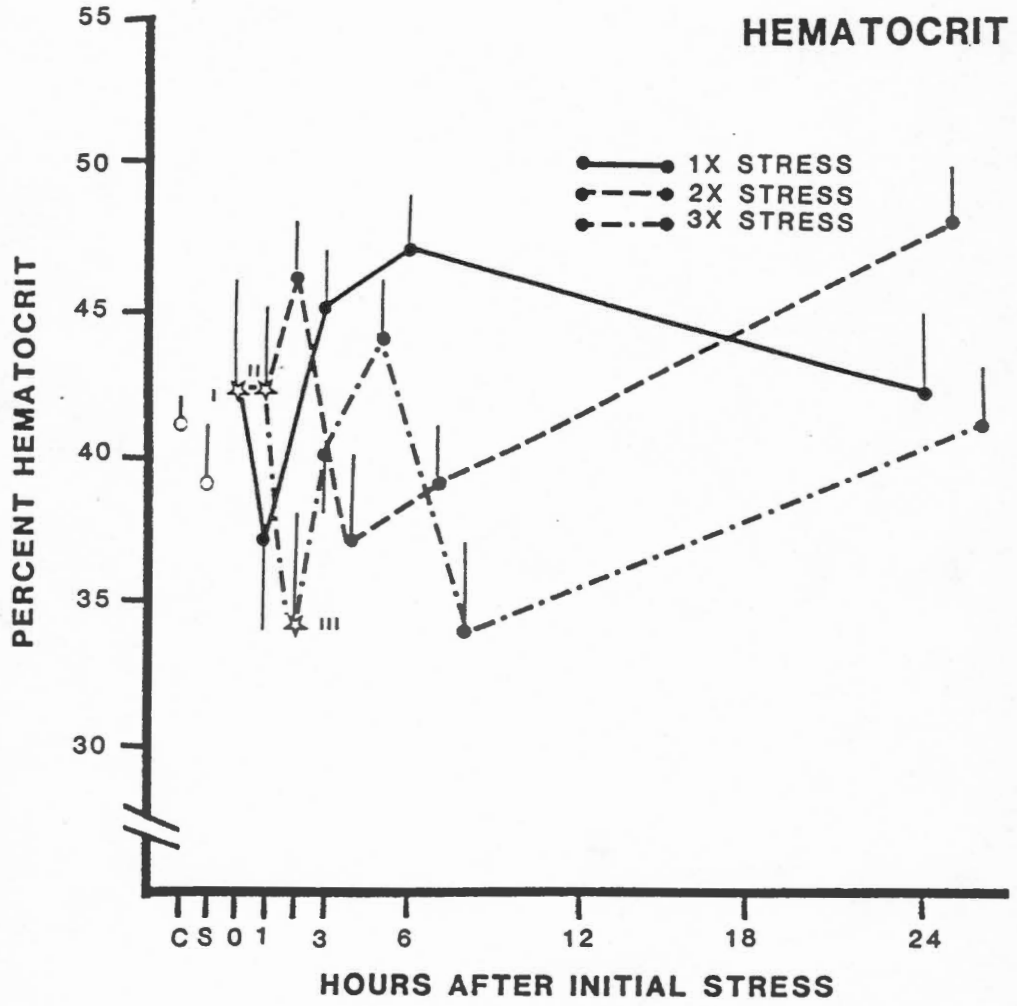

Figure 80. Mean hematocrit (percent ± SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Stars indicate the times when fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time.

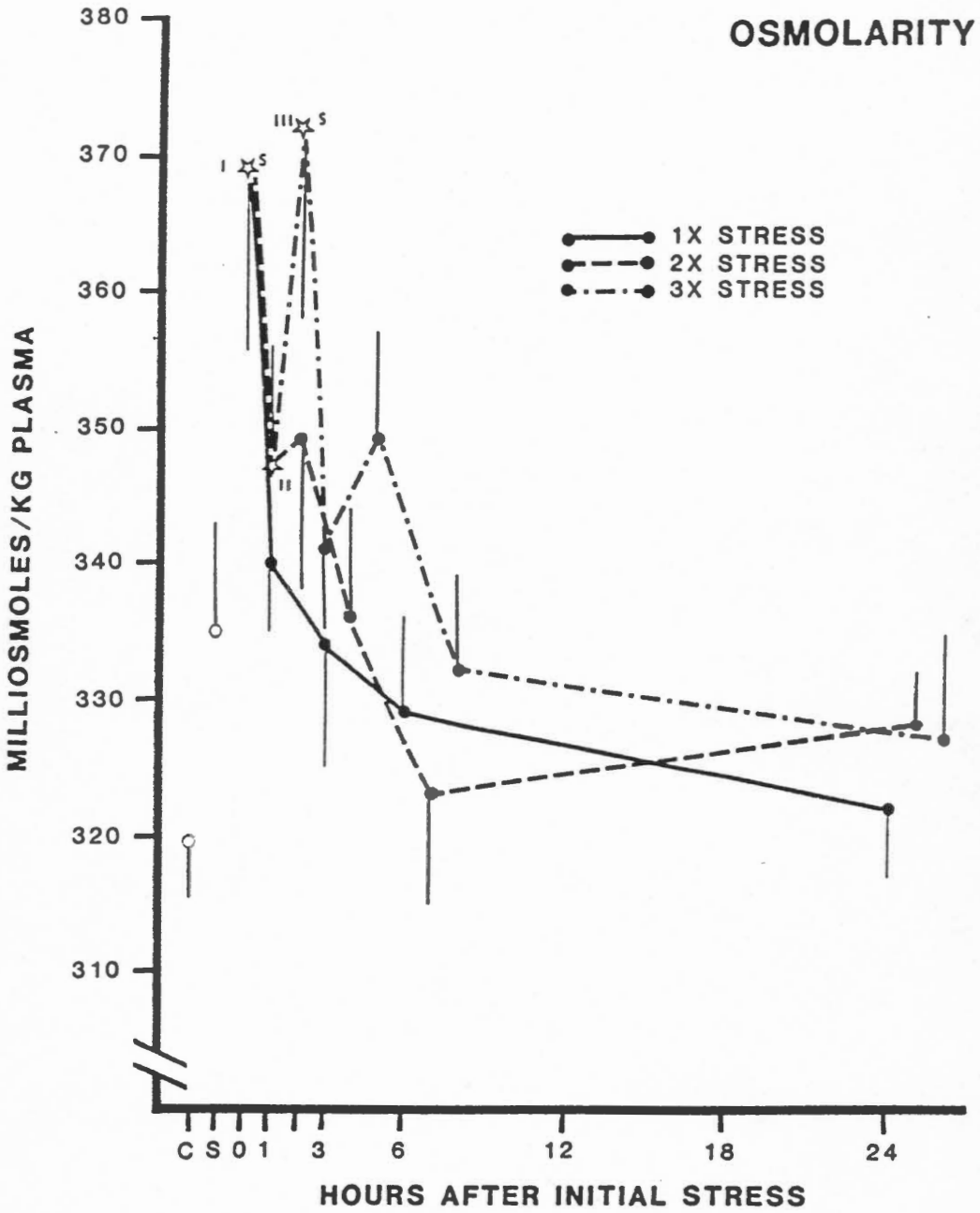

Figure 81. Mean plasma osmolarity (mosmoles/kg ± · SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Stars indicate the times when fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time. Points with an adjacent s indicate that they were significantly different from controls (Sum of squares simultaneous test procedure).

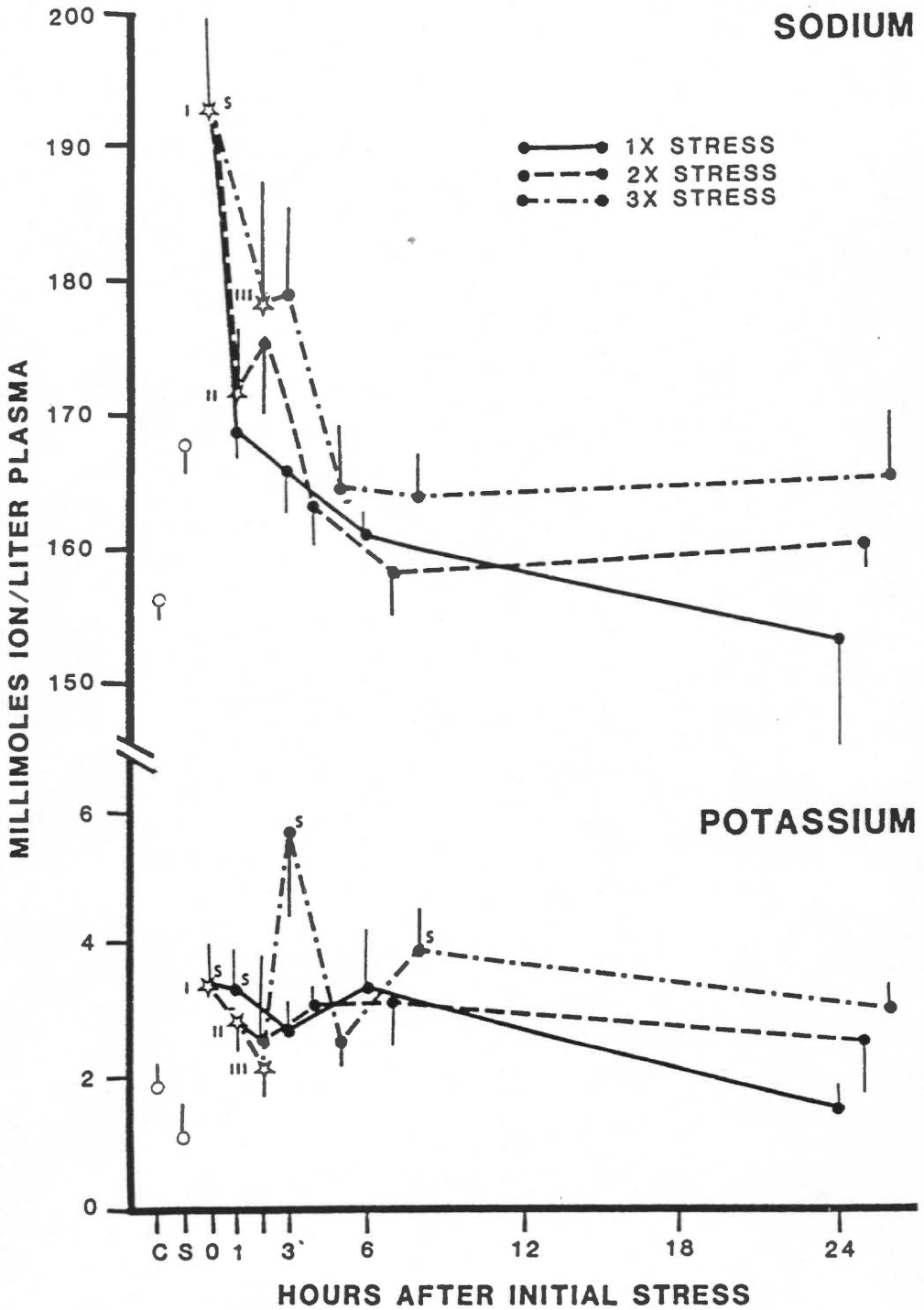

Figure 82. Mean plasma sodium and potassium (mmoles/l ± SE for n=9) in juvenile fall chinook following a fatigue time test (the amount of time the fish could maintain position in a 60 cm/sec flow of water) on fish stressed 1, 2 or 3 times with one hour between stresses. C represents control fish which were not stressed and not subjected to a fatigue test. S represents control fish that were unstressed and subjected to a fatigue test. Starts indicate the times when fish were stressed and adjacent Roman numerals indicate the number of stresses applied up to that time. Points with an adjacent s indicate that they were significantly different from controls (Sum of squares simultaneous test procedure).

periods after stress. These results indicate that swimming places a physiological demand on fish that is further compounded by handling stresses. The effect of swimming tends to mask the cumulative stress response seen in handling stress tests without swimming (see: Multiple Stresses of Saltwater-Acclimated Fish).

Critical swimming speeds of these stressed fish tended to be slightly below that of unstressed acclimated fish (Fig. 83), indicating that stress can reduce fall chinooks' swimming performance. However, the results of these experiments were highly variable and no significant differences (Duncan's multiple range test) were found between stressed and non-stressed fish at any level of stress or time after stress.

Stress and Behavior

Experimental design, results, and discussion. A smolt's capacity to respond to a stimulus may be impaired if the fish is in a stressed condition. An experiment was performed in fresh water at the Smith Farm facility to examine the effect of stress on the ability of fish to swim to cover when exposed to a bright light. Tests were conducted in three Y-troughs measuring 0.3 m in arm and leg width and 2.5 m in total length (Fig. 84). The troughs were equipped with several gates to compartmentalize the fish. A permanent 44-cm-long black plastic cover was placed on the center portion of each trough. Water was supplied to the center of the trough at a flow rate of 1 L/min. Six fish were placed in the arms of the troughs the evening before the experiment, and removable black plastic covers were placed over each arm. The next morning, fish were tested for response time or stressed by holding them in a dip net for 30 sec. Fish were stressed

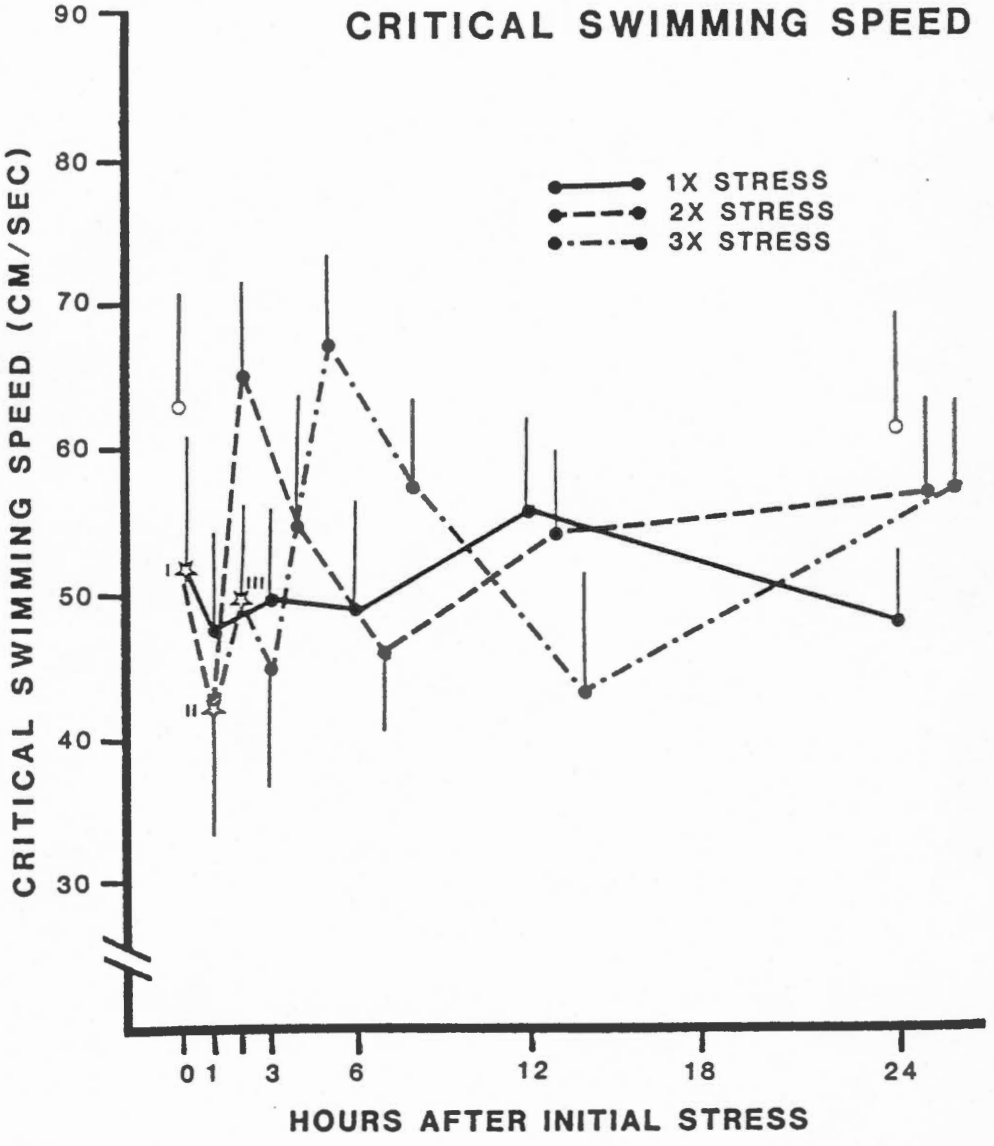

Figure 83. Critical swimming speed, i.e. maximum speed at which fish can maintain position in a flow of water, of juvenile fall chinook either not stressed (controls) or stressed 1, 2 or 3 times with one hour between stresses. Points are the means ± standard error for 12 fish. Stars indicate times where stress was applied and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish.

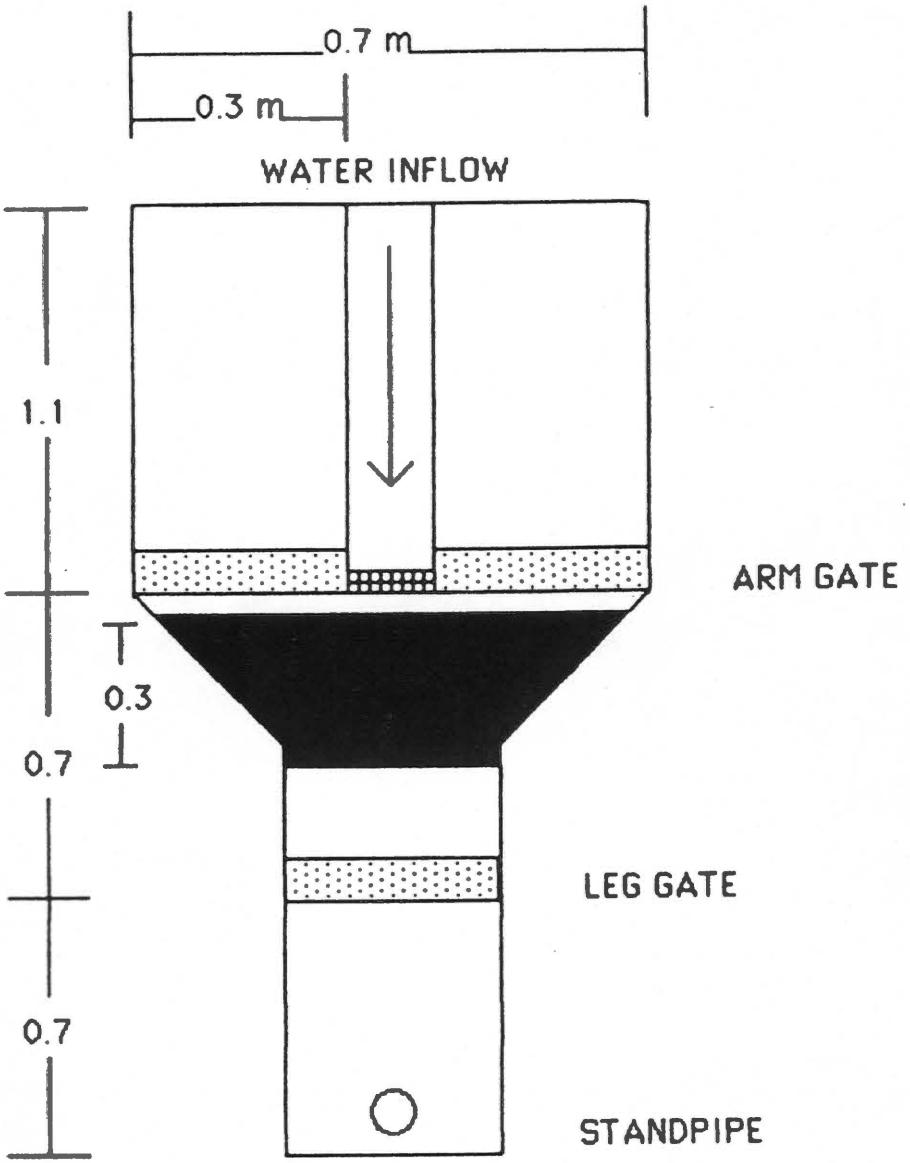

Figure 84. Diagram of apparatus used in the response time experiment.

1, 2, or 3 times with three hours between stresses. After each stress, the fish were returned to the apparatus and covered until the next stress or until the behavior trial was begun at 0, 1, 3, 6, or 24 h after the last stress. An experimental trial consisted of turning on a bright fluorescent light above the arms of the Y, simultaneously removing the cover and opening the gate and noting the time it took for each fish to swim under the permanent cover at the center of the trough. Tests were discontinued after 30 min, at which time most of the fish had reached cover. The median response time was calculated for each group of fish, and the non-parametric Kruskal-Wallis test was used to examine the effect of stress over time and to determine whether repeated stresses had a cumulative effect.

Unstressed fish went to cover within 0.23 min. Stressed groups tended to have many fish with response times longer than the controls and some fish did not respond in 30 min (Fig. 85). The stresses tended to be cumulative, with fish stressed three times having longer response times and slower recovery than fish stressed twice, which in turn had longer response times and a slower recovery than fish stressed once. Thus stress may have a detrimental effect on behavior, which may affect the fish's survival in the wild.

The response times of stressed fish peaked immediately after stress and then gradually recovered with time. In contrast, plasma cortisol in fish stressed in freshwater (see: Multiple Stresses) peaked at 1 h after stress, and plasma glucose peaked 3-5 h after stress. The initial high response times may be due to the oxygen debt and osmoregulatory problems experienced by fish immediately after stress.

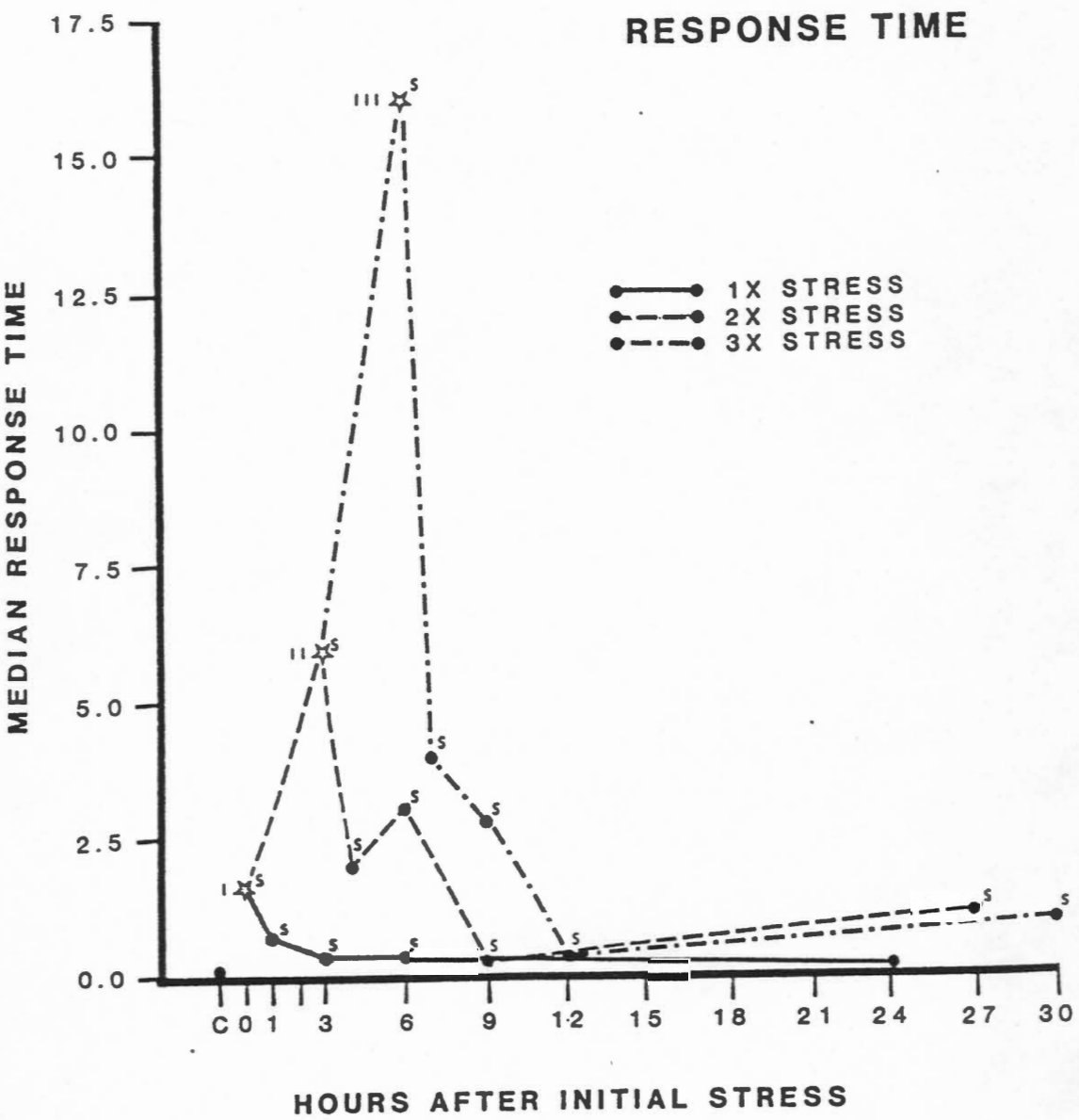

Figure 85. Response time, i.e. the time required for fish to reach cover when exposed to a sudden bright light, of juvenile fall chinook either unstressed (controls) or stressed 1, 2, or 3 times with three hours between stresses. Points are the medians for 10-17 fish. Stars indicate the times when stress was applied and adjacent Roman numerals indicate the number of stresses applied up to that time. Points with adjacent (S) indicate points that are significantly greater than controls.

Multiple Stresses of Saltwater-acclimated Fish

Experimental design, results, and discussion. Laboratory experiments were conducted in salt water at the Marine Science Center to determine the physiological responses of fish acclimated to salt water to standardized multiple stresses. These permitted a better understanding of the swimming experiments that were likewise conducted in salt water. Juvenile fall chinook were obtained from Fall Creek Hatchery, transported to the Marine Science Center, placed in fresh water and gradually acclimated to full-strength sea water. In the experiment, acclimated fish were exposed to the standardized handling stress consisting of dip-netting the fish from the tank and suspending them in the air for 30 s before returning them to a tank. Plasma samples were collected from fish stressed 1, 2, and 3 times with 1 h between stresses, and from fish allowed to recover 1, 3, 6, 12, and 24 h after the last stress.

Plasma cortisol in juvenile chinook reached a maximum of 222 $ng \cdot ml^{-1}$ in 1 h and returned to control levels within 6 h following a single acute 30-s handling stress (Fig. 86). In fish subjected to a second and third identical handling stress 1 h apart, plasma cortisol followed the same pattern but with peaks at 288 and 329 $ng \cdot ml^{-1}$, respectively. In all cases, plasma cortisol declined to control levels in 24 h after the last stress. Plasma glucose levels peaked at 3 h (139 $mg \cdot 100\ ml^{-1}$) in fish stressed once (Fig. 87). Fish stressed 2 or 3 times had peak plasma glucose levels within 6 h after stress of 133 and 144 $mg \cdot 100\ ml^{-1}$, respectively. Unlike cortisol levels, the glucose response was not cumulative and glucose did not decline to control levels within 24 h. We also assayed plasma samples for lactic

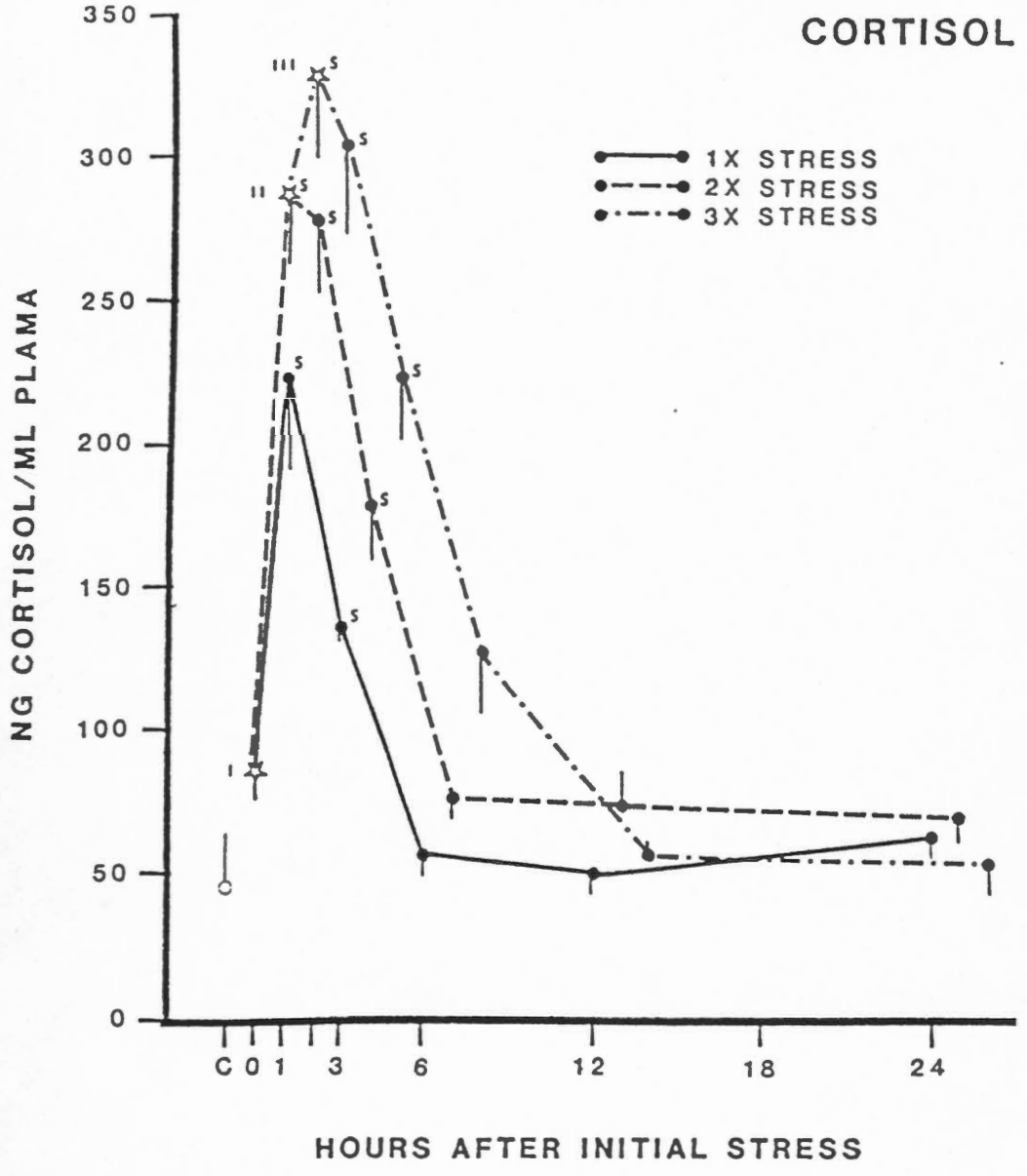

Figure 86. Mean plasma cortisol (ng/ml ± SE for n = 10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12, and 24 h after the final stress. Stars indicate the samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates unstressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison tests ($P < .05$) are summarized below in the form of x+y where x is the number of stresses and y is the number of hours post-stress (underlined values are not significantly different).

0 h:	1+0 <	2+0	3+0	6 h:	1+6	2+6	3+6
1 h:	1+1	2+1	3+1	12 h:	1+12	2+12	3+12
3 h:	1+3	2+3	3+3	24 h:	1+24	2+24	3+24

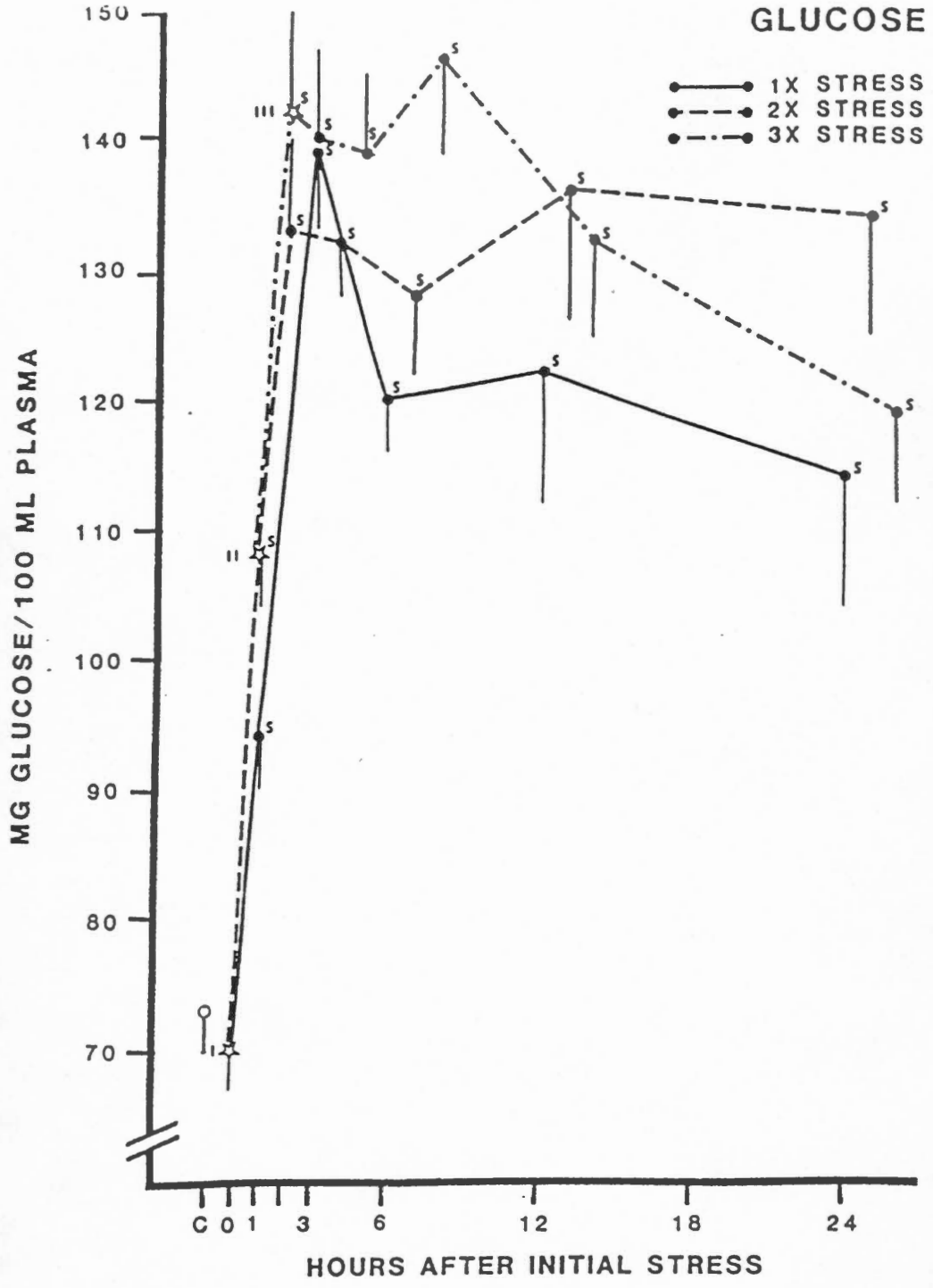

Figure 87. Mean plasma glucose (mg/100 ml ± SE for n = 10) of fish exposed to 1, 2 or 3 handling stresses (30 sec in a dip net) with 1 h between stresses and of fish allowed to recover 1, 3, 6, 12, and 24 h after the final stress. Stars indicate samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates non-stressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison tests ($P < .05$) are summarized below in the form of x + y where x is the number of stresses and y is the hours post-stress (underlined values are not significantly different).

0 h:	1+0 <	2+0 <	3+0		
1 h:	1+1 <	2+1	3+1		
3 h:	1+3	2+3	3+3		
6 h:	1+6	2+6 <	3+6		
12 h:	1+12	2+12	3+12		
24 h:	1+24	2+24	3+24		

acid, osmolarity, and sodium (Figs. 88-90) which showed cumulative increases with increased number of stresses. Plasma potassium and hematocrits decreased when fish were stressed, but did not show clear differences with increased number of stresses (Fig. 90-91).

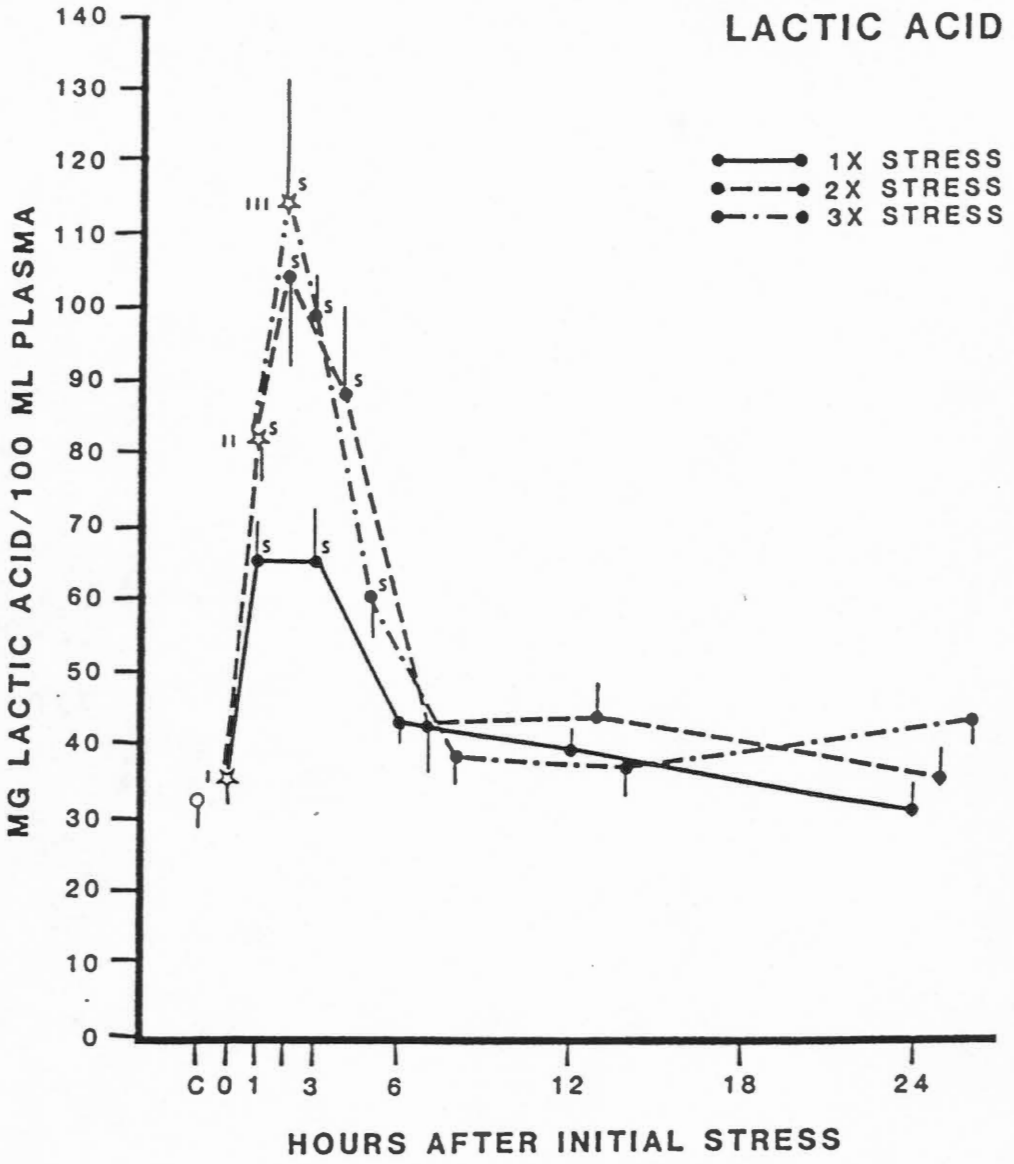

Figure 88. Mean plasma lactic acid (ng/100 ml ± SE for n = 10) of fish exposed to 1, 2, or 3 handling stresses (30 sec in a dip net) with 1 h between stresses, and of fish allowed to recover 1, 3, 6, 12, and 24 h after final stress. Stars indicate the samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates non-stressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison test ($P < .05$) are summarized below in the form of x + y where x is the number of stresses and y is the hours post-stress (underlined values are not significantly different).

0 h:	1+0 <	2+0	3+0	6 h:	1+6	2+6	3+6
1 h:	1+1 <	2+1	3+1	12 h:	1+12	2+12	3+12
3 h:	1+3	2+3	3+3	24 h:	1+24	2+24	3+24

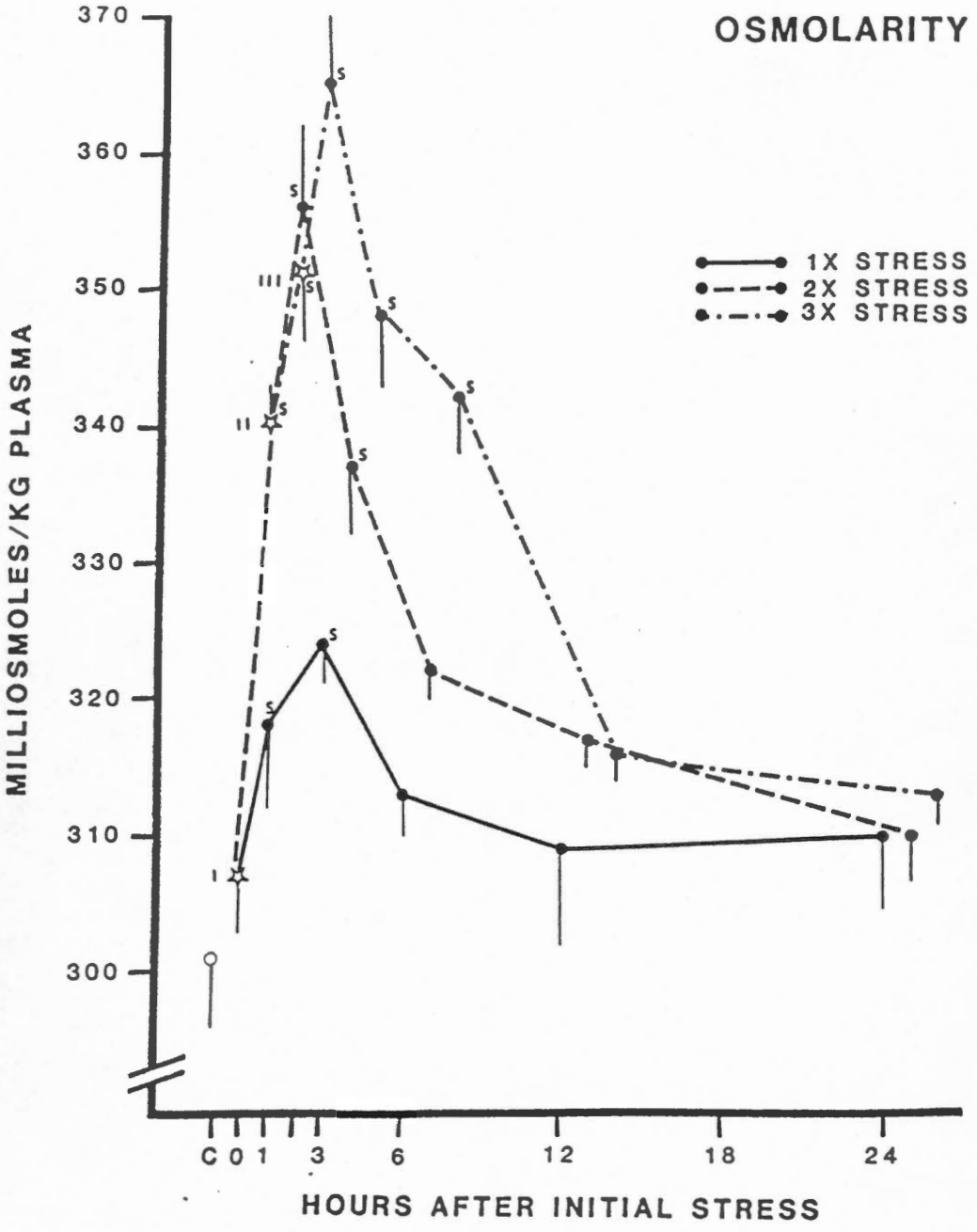

Figure 89. Mean plasma osmolarity (mosmoles/kg ± SE for n = 10) of fish exposed to 1, 2 or 3 handling stresses (30 sec in a dip net) with 1 h between stresses, and of fish allowed to recover 1, 3, 6, 12 and 24 h after final stress. Stars indicate the samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates non-stressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison tests ($P < .05$) are summarized below in the form of x + y where x is the number of stresses and y is the hours post-stress (underlined values are not significantly different).

0 h:	1+0 <	2+0	3+0	6 h:	1+6	2+6	< 3+6
1 h:	1+1 <	2+1	3+1	12 h:	1+12	2+12	3+12
3 h:	1+3	2+3	3+3	24 h:	1+24	2+24	3+24

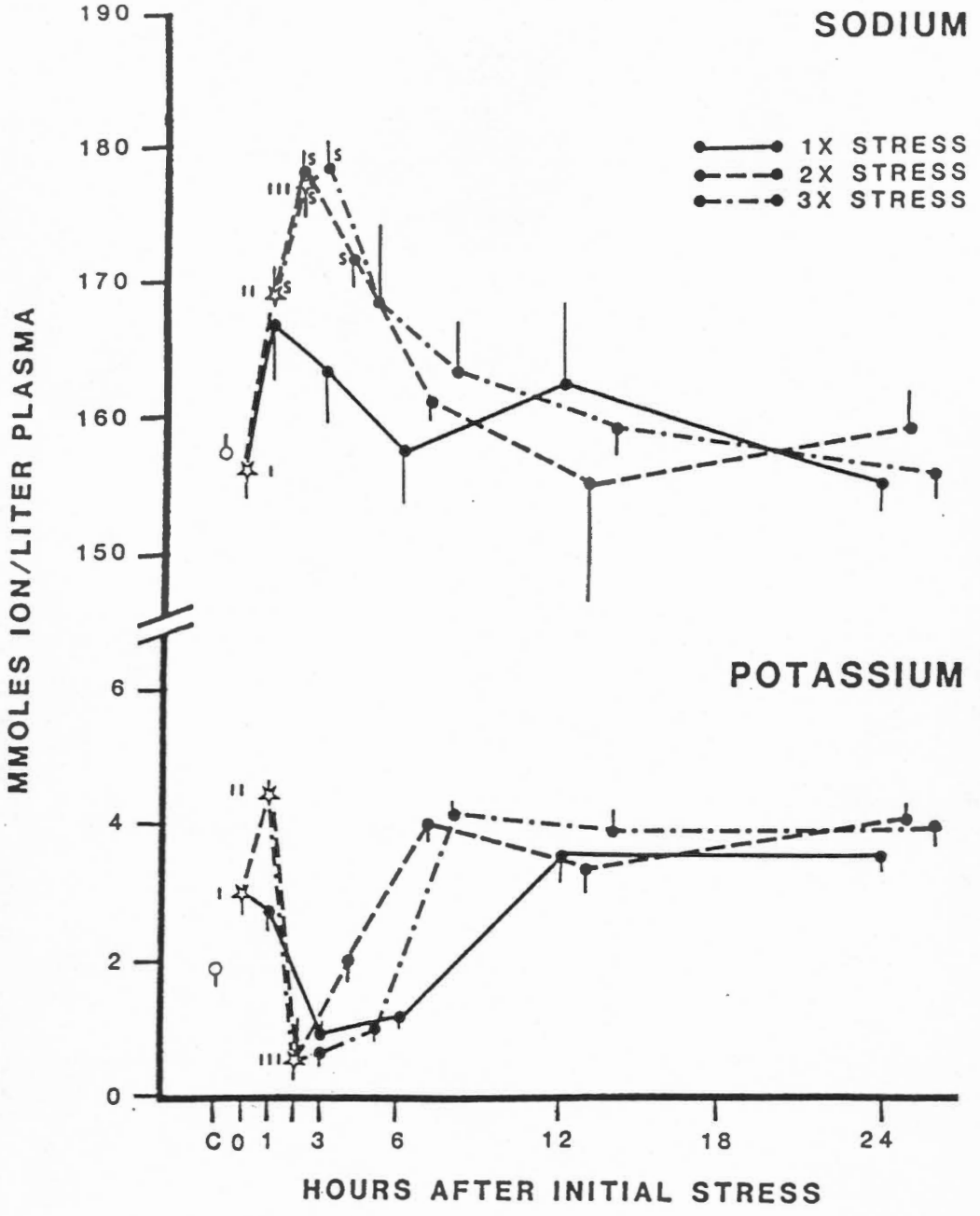

Figure 90. Mean plasma sodium and potassium (mmole/l ± SE for n = 10) of fish exposed to 1, 2 or 3 handling stresses (30 sec in a dip net) with 1 h between stresses, and of fish allowed to recover 1, 3, 6, 12, and 24 h after the final stress. Stars indicate the samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates non-stressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison tests ($P < .05$) are summarized below in the form of x + y where x is the number of stresses and y is the hours post-stress (underlined values are not significantly different).

0 h:	1+0	<	2+0	<	3+0	0 h:	3+0	< 1+0 < 2+0
1 h:	1+1	<	2+1		3+1	1 h:	2+1	3+1 < 1+1
3 h:	1+3		2+3		3+3	3 h:	1+3	3+3 < 2+3
6 h:	1+6		2+6		3+6	6 h:	1+6	< 2+6 3+6
12 h:	1+12		2+12		3+12	12 h:	1+12	2+12 3+12
24 h:	1+24		2+24		3+24	24 h:	1+24	2+24 3+24

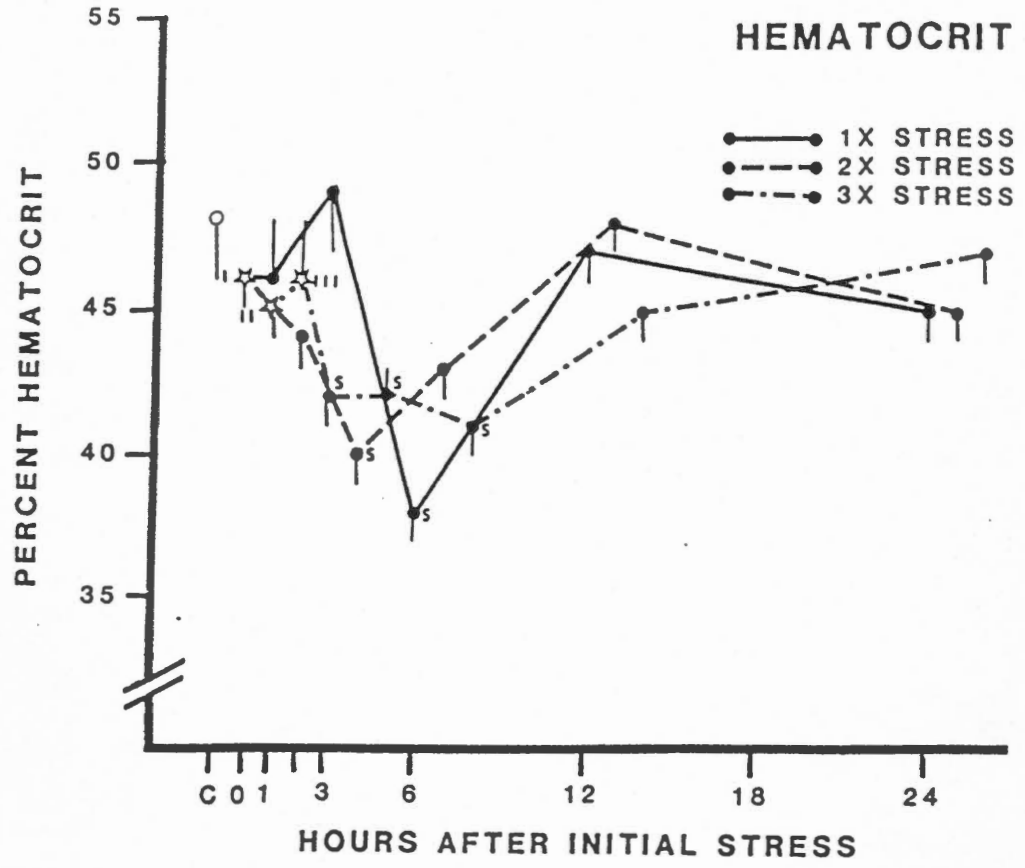

Figure 91. Mean hematocrit (percent ± SE for n = 10) of fish exposed 1, 2 or 3 handling stresses (30 sec in a dip net) with 1 h between stresses, and of fish allowed to recover 1, 3, 6, 12, and 24 h after the final stress. Stars indicate the samples taken immediately after stress, and adjacent Roman numerals indicate the number of stresses applied up to that time. Open circles indicate non-stressed fish. C indicates non-stressed control fish. Points with an adjacent (s) indicate that they are significantly different from controls. Results of multiple comparison tests ($P < .05$) are summarized below in the form of x + y where x is the number of stresses and y is the hours post-stress (underlined values are not significantly different).

0 h:	1+0	2+0	3+0	6 h:	1+6	<	2+6 3+6
1 h:	1+1	2+1	3+1	12 h:	1+12		2+12 3+12
3 h:	1+3	<	2+3 3+3	24 h:	1+24		2+24 3+24

SUMMARY

Collection System

The results indicate that fall and spring chinook smolts are stressed as they are manipulated by the elements of the collection and transportation system at McNary Dam. These stressses are physiologically characterized by transient increases in plasma cortisol without increases in interrenal cell nuclear diameters, indicating that the overall stress in acute rather than chronic. Stress-induced metabolic disturbances are evidenced by increases in plasma glucose and decreases in hepatic glygogen; however, the variable nutritional status of emigrating fish could greatly affect the changes in these indices. Although there is a wide variability in hematocrit and leucocrit responses, there appears to be a latent depression in WBC count within 24 to 48 h after the stress of collection at McNary Dam or transport to Bonneville Dam. The results of the secondary stress challenges, saltwater challenges, and swimming performance tests suggest that the smolts' performance is impaired by the stresses of the collection and transportation for as long as 48 h.

The patterns of plasma cortisol levels in fall chinook smolts taken from various elements in the system (Figs. 3-9, 35, 36)) and of fish exposed to a secondary stress (Figs. 29-32 and 43) indicate that the elements of the collection system have cumulative effects on the fishes' physiological responses to stress. Therefore, any modification in the collection system that reduces stress at one point in the system should lessen the total physiological impact on the fish.

Prior to the 1983 smolt emigrations, several modifications were made in the collection system, which resulted in increased water flow from the collection flume through the vertical pipe and to the upwelling box on the downstream side of the dam (Delarm et al. 1984). The primary objective of these modifications was to prohibit the build-up of adult American shad at the end of the flume, as their presence there late in the summer appeared to inhibit the movement of fall chinook smolts into the vertical pipe. The modifications succeeded in flushing the shad through the system (Brad Eby, U.S. Army Corps of Engineers, personal communication) and this may have reduced the stress experienced by fall chinook late in the run. Plasma cortisol levels in fish sampled during the late run, 1982, were considerably higher than those of fish sampled earlier in the run (Fig. 3). However, in 1983 and 1984 after the modifications increased flow, plasma cortisol levels were not elevated later in the run. We speculate that the increased water velocity through the system may have decreased the stress experienced by smolts at all times of the run, as plasma cortisol levels were lower in all aspects of the collection system in 1983 and 1984 as compared to 1982 except in fish from the gatewell (i.e. prior to the modified collecton channel) (Fig. 4-9). We reason that the increased flow through the vertical pipe may have flushed smolts to the upwelling box before they were more severely stressed by swimming against the flow. Additional support for this interpretation is that there were no between-year differences in plasma cortisol levels in fish transported and held at Bonneville Dam (Figs. 48, 49, and 61). If the differences in plasma cortisol levels of fall chinook at McNary Dam between years were the result of differences in the river environment or variability in the fish, responses of fish transported to

Bonneville Dam should have paralleled those in fish at McNary Dam. However, this was not the case, indicating that the differences seen at McNary Dam may have been the result of modifications in the collection system.

Osmoregulatory ability was reduced late in the run in 1982, 1983 and 1984, probably the result of the increased water temperatures as the runs progressed. We found that fall chinooks' plasma cortisol response to stress was independent of acclimation temperature (Fig. 73) but that plasma glucose response was elevated at the highest temperature (Fig. 74). This suggests that at higher temperatures, a greater metabolic cost accompanies the response to stress. Osmoregulatory stress placed energetic demands on fish, and the increased temperatures may have interfered with smolts' ability to efficiently use the energy required to perform a variety of tasks. The physiological indices which normally show changes in energetic demands placed on fish (hepatic glycogen and plasma glucose) were highly variable in all years (Figs. 14, 15, 37-39, and 51), perhaps the result of variable nutritional states of the fish.

Comparison of responses of fall chinook and spring chinook to the collection system seem to indicate that spring chinook are more sensitive to the stresses of the system than are fall chinook, or that factors in the system such as the presence of juvenile steelhead trout make the system more stressful during the spring chinook emigration than during the fall chinook emigration. Peak plasma cortisol levels in spring chinook held in the raceway were quite high (350-400 $ng \cdot ml^{-1}$; Figs. 35 and 36) and did not increase above these levels in response to a secondary stress challenge (Fig. 43) . Fall chinook had relatively low peak plasma cortisol levels (125-135 $ng \cdot ml^{-1}$; Figs. 4-6) which increased significantly in response to

the secondary stress (Fig. 30). In other words, spring chinook appeared to be maximally stressed by the collection system while fall chinook were not, as evidenced by plasma cortisol levels. Morover, our laboratory study demonstrated that in response to stress, plasma glucose levels were significantly greater in stressed juvenile fall chinook acclimated to high temperature (21° C) than low temperature (7-12° C; Fig. 74). However, fall chinook which were migrating in warmer water (15-20° C) showed no significant increases in plasma glucose after passing through the collection system (Figs. 16-18), while plasma glucose in spring chinook in cooler water (11-13° C) increased by as much as 100% after collection (Figs. 38 and 39). We caution that interracial comparisons of absolute values of plasma cortisol and glucose may not be biologically sound. However, the failure of spring chinook to respond to secondary stress may be indicative of the relative sensitivity of stress of fall and spring chinook salmon. Furthermore, the failure of transported spring chinook to survive to adulthood at the same rate as transported fall chinook (relative to non-transported fish; Park et al. 1983) leads us to conclude that spring chinook experience more stress than fall chinook during collection and transportation.

Juvenile salmon usually avoid bright light by staying in shadows or deep water during the day (Hoar 1985, Ali 1959). The sudden exposure to bright sunlight when fish reach the upwelling box and continued bright light, both natural and artificial, throughout the fishes' stay in the holding facility is stressful to fall chinook as evidenced by plasma cortisol levels (Fig. 45). We believe that darkening the collection facility is a low-cost management option which can result in a significant reduction in the stress of collection on outmigrants.

We do not believe that the raceways themselves were particularly stressful independent of bright sunlight. The changes in physiological indices of stress in fish after a short time in the raceway were latent responses to stresses of the collection procedures. It appears that the post-collection recovery time of 12 to 48 h is optimal, given the operational necessities at the dam, the rates at which the clinical indices of stress return to baseline, and fishes' responses to various challenges. However, emigrating smolts can be held too long as evidenced by reduced osmoregulatory capacity (Figs. 26-28) and disease resistance (Table 5) in fish held for 5 to 8 d. Aggressive behavior is apparently maintained in migrants (B. Olla, National Marine Fisheries Service, personal communication); social interactions encountered while in the raceways could acocunt for the delayed stress of being held in the raceways, and the reduction in stress in covered raceways where fishes' vision was impaired.

The maximum density at which fish were held in raceways (0.5 lbs•gal^{-1} was not excessive. In all but one of our tests, plasma cortisol levels of fall and spring chinook were reduced within 24 h of entry into the raceway, independent of fish density, at or below the maximum (Figs. 3-9, 35, 36, and 44).

The anesthetization, handling, and marking of smolts at McNary Dam apparently did not cause stress in addition to that experienced by fish going directly into the raceways (Fig. 46) as evidenced by plasma cortisol changes. This does not imply, however, that the marking procedure does not impair the performance abilities of the fish. Care must be taken to ensure that marked fish have a reasonable time to recover from the procedure

before transport or release into the river (ca. 24 to 48 h). Moreover, anesthetizing fish prior to moving them from the holding tank to the marking shed appears to be beneficial in reducing the stress of handling (Fig. 47).

Transportation System

The most stressful event in the transportation system appears to be loading the fish into the truck or barge (Figs. 48, 49, 57-59, 61). Immediately after loading into a truck, fish appeared to be stressed, independent of the density of fish in the tank. However, higher densities enroute did appear to affect the fishes' ability to recover from the stress of loading. The maximum allowable density of fish in a transport truck (0.5 $lbs \cdot gal^{-1}$) was not excessive, but exceeding this level might interfere with the enroute recovery. Moreover, transporting fish at less than the maximum density allowed greater enroute recovery from the stress of loading (Fig. 57).

Fall chinook smolts removed from the transport vehicles at Bonneville Dam recovered from the stress of the transport and sampling procedures within 24 h, as evidenced by plasma cortisol levels (Figs. 48, 49, 58, 61). Moreover, these procedures elicited uniform responses in fish, with limited variation occurring between years within the runs or with transport by truck or barge. This lack of variation in plasma cortisol response appears to indicate that before the fish were transported, they had recovered from the stresses of collection at McNary Dam, which do show within- and between-years variation (Figs. 3-9).

Fall chinook smolts transported to Bonneville Dam did show reduced osmoregulatory ability as the runs progressed (Figs. 28, 29, 42, and 55), again suggesting that environmental temperature is a critical factor in performances such as osmoregulation. This could mean that the ability of fish to perform any energy-demanding task was more impaired by the combination of stress and high temperature than by either factor alone.

The results of the disease challenge (Table 5), saltwater challenges (Figs. 28, 29, and 55), secondary stress challenge (Figs. 65 and 66), and swimming performance (Table 4) suggest that a 1-d recovery period after transport may be optimal. However, out of necessity, we handled the fish used for these challenges prior to the tests. We do know that upon arrival at Bonneville Dam, fish were in the process of recovery (i.e., have lower plasma cortisol levels) from the stress of being loaded into the truck or barge. We do not know the nature of fishes' response to the release procedures; however, we speculate that fish released from the barge by draining the tanks directly into the river would not be stressed as seriously as fish released from a truck through a 100-m flexible tube as was done in 1982. Moreover, we speculate that the disorientation of being returned to the river after 1-2 d in the collection and transportation system may compound the stress of the physical disturbance of release.

Smolts primarily emigrate at night (Hoar 1958, Ali 1959) and, consequently, it seems reasonable that transporting and releasing fish at night might be less stressful and cause less disorientation upon release. Our comparison of night versus day transport appears to indicate that day transport allows more rapid recovery than night transport (Fig. 58). However, there were several uncontrolled variables in the test (e.g. length

of time in the raceway prior to transport) and we cannot conclude that day transport is superior to night transport.

We have presented data on the effects of collection and transportation on short-term dynamics of physiological indices of stress and performance capacities in juvenile chinook salmon in the Columbia River. The combination of laboratory experiments and field data show that the stresses of collection and transportation can cause short-term perturbations in the physiology of emigrating salmon. Furthermore, these perturbations can potentially affect the short-term survival of emigrating fall chinook salmon by altering disease resistance, swimming ability, and osmoregulatory function. However, it has been shown that changes in the collection system can reduce the total stress of the system to juvenile fall chinook and we have recommended additional changes which should result in further stress reduction. We believe that, given the operational necessities of the system, the stresses of the system are within acceptable limits for juvenile fall chinook.

CONCLUSIONS

Juvenile fall chinook were stressed by the collection system at McNary Dam.

The elements of the collection system had cumulative effects on the fishes' response to the system.

Changes in the collection system between 1982 and 1983 decreased the total stress experienced by fall chinook collected.

The collection system is more stressful to spring chinook salmon than to fall chinook salmon during their respective emigrations.

There were seasonal variations in some physiological responses to stress, probably the result of changes in the environment.

The maximum raceway density of 0.5 $lbs \cdot gal^{-1}$ was not excessive.

Darkening the upwelling box, bar-sorter, and raceways reduced the stress of the collection system on fall chinook.

Fall chinook which were anesthetized, handled, and marked were no more stressed than fish which just went through the collection system, but required a day's recovery time before transport or liberation.

Anesthetizing fish prior to transferring them from the subsample holding tank to the marking shed reduced the stress of handling.

Optimum length of time for fall chinook to recover from the stresses of collection is 12 to 48 h.

Loading fish into the transport vehicle was the most stressful event in the transportation procedure.

The transport vehicles were not stressful and the fish showed some recovery from the stress of loading while enroute.

There was no significant difference in the stress of transport by truck or barge.

The maximum truck transport loading density of 0.5 $lb \cdot gal^{-1}$ was not excessive.

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