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Matthew G. Mesa for the degree of Master of Science

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Title: Electrofishing Mark-Recapture and Depletion Methodologies Evoke Behavioral and Physiological Changes in Cutthroat Trout (Oncorhynchus clarki)

Redacted for privacy

Abstract approved: _____
Carl B. Schreck

We examined the behavioral and physiological responses of wild and hatchery-reared cutthroat trout Oncorhynchus clarki subjected to a single electroshock, electroshock plus marking, and multiple electroshocking in natural and artificial streams. In a natural stream, trout released after capture by electrofishing and marking showed distinct behavioral changes: fish immediately sought cover, remained relatively inactive, did not feed, and were easily approached by a diver. An average of 3 to 4 h were required for 50% of the fish to return to a seemingly normal mode of behavior, although responses varied widely among collection sites. Using the depletion method, we observed little change in normal behavior of fish remaining in the stream section (i.e. uncaptured fish) after successive passes with electrofishing gear. In an artificial stream, hatchery-reared and wild cutthroat trout immediately decreased their rates of feeding and aggression after electroshocking and marking. Hatchery trout

generally recovered in 2 to 3 h whereas wild trout required at least 24 h. Analysis of feeding and aggression data by hierarchical rank revealed no distinct recovery trends among hatchery trout of different ranks; in wild trout, however, recovery seemed to be faster in social dominants than in intermediates and subordinates. Physiological indicators of stress (plasma cortisol and blood lactate) increased significantly in cutthroat trout subjected to electroshock plus marking, or single or multiple electroshocking. As judged by the magnitude of the greatest change in cortisol and lactate, multiple electroshocking elicited the most severe stress response; however, concentrations had returned to unstressed control levels by 6 h after treatment. It was evident that electrofishing and the procedures involved with estimating fish population size elicited a general stress response that manifested itself not only physiologically but behaviorally as well. We believe these responses could affect the accuracy of estimating population size by violating key assumptions of the methods.

ELECTROFISHING MARK-RECAPTURE AND DEPLETION METHODOLOGIES EVOKE BEHAVIORAL AND PHYSIOLOGICAL CHANGES IN CUTTHROAT TROUT (*Oncorhynchus clarki*)

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INTRODUCTION

Because electrofishing is widely used to collect fish for various purposes, the effects of electricity on fish have received much attention. The physiological effects of electricity on fish include a variety of sub-lethal changes in blood chemistry charachteristics that last for various lengths of time (Caillouet 1967; Schreck et al. 1976; Bouck et al. 1978; van Waarde and Kesbeke 1983). Morphological effects include physical injury or mortality of fish; however, many studies of the lethality or incidence of injury due to electroshock have been conducted in unnatural situations and often under severe electrical conditions (Collins et al. 1954; Spencer 1967; Ellis 1973; Whaley et al. 1978; Hudy 1985). The effects of electricity on fish behavior have mainly concentrated on the galvanotaxic and electronarcotic aspects (Haskell et.al. 1954; Taylor et.al. 1957; Vibert 1963; Ellis 1975; Balayev and Fursa 1980; Balayev 1981).

The importance of investigating the effects of electricity on fish becomes manifest when one considers that electrofishing is commonly used to estimate fish population size. Underlying all mark-recapture or depletion estimators are assumptions that, if violated, can affect the accuracy of estimates. Of particular importance is the assumption of equal catchability, which has been the subject of much

statistical investigation (Eberhardt 1969; Otis et al. 1978; Carothers 1979; Burnham and Overton 1979; Seber 1982) and its failure reported to affect the accuracy of fish population estimates (Beukema and de Vos 1974; Cross and Stott 1975; Bohlin and Sundstrom 1977; Yundt 1983; Peterson and Cederholm 1984). If it is assumed that, to meet the assumption of equal catchability, fish must show normal behavior and physiology, an understanding of how the procedures involved in population estimators (e.g., electroshock plus handling and marking) affect these factors may provide insight that should lead to more accurate population estimates.

Our ultimate goal was directed at evaluating the efficacy of electrofishing for population estimation. We investigated the possible effects of capturing, handling, marking, and multiple electroshocking on the normal behavior and physiology of coastal cutthroat trout Oncorhynchus clarki. Although there are variations in the methodologies for mark-recapture and depletion experiments, we attempted to conduct what we considered to be fairly representative procedures. Specifically, our objectives here are to (1) determine the effects of electroshocking plus marking and multiple electroshocking on trout behavior in a natural stream; (2) determine the effects of electroshocking plus marking on the integrity of social hierarchies, frequency of aggressive behavior, and feeding rate of wild and hatchery trout in an artificial stream; and (3) determine the effects of a single electroshock, multiple electroshock, and electroshock plus marking on physiological indicators of stress.

Methods

NATURAL STREAM OBSERVATIONS

Experiments were conducted at Mill Creek, a third order tributary of the Yamhill River, which in turn flows into the Willamette River in Polk County, Oregon. Twelve sections were selected for experiments, on the basis of their suitability for effective electroshocking and snorkeling and the number of trout present. Conductivity ranged from 59 to 80 micromhos/cm, average water velocity from 3 to 10 cm/s, and water temperatures from 11 to 13 C during the study. Other physical characteristics of the study sections, and electrofishing treatment used in each, are listed in Table 1. Experiments were conducted during summer and fall in 1987 and 1988.

A preliminary dive in each section enabled us to qualitatively observe the behavior of normal, undisturbed cutthroat trout. This information, along with observations on cutthroat trout behavior in other streams, enabled us to describe characteristics of normal fish behavior and was the basis for assessing any changes in behavior caused by the electrofishing treatments.

To evaluate the effects of capture by electrofishing and subsequent marking, we subjected 10 of the 12 experimental sections (site number 8 was done in 1987 and 1988) to the following protocol. Block seines were placed at the upstream and downstream ends of the section to prevent fish emigration or immigration. A single upstream pass followed by a relatively quick downstream pass was made by three or four people; two were fitted with backpack electroshockers (Coffelt

Table 1.-Selected physical characteristics of 12 sites in Mill Creek used for electrofishing experiments during the summer and fall, 1987-1988.

Site No.	Mean length (m)	Mean width (m)	Mean depth (cm)	Mean canopy cover (%)	In stream boulders (No.>50 cm)	treatment[a] received
1	53	7.7	30.6	43	1	mr,dep
2	42	6.3	18.7	28	7	mr,dep
3	34	5.5	32.0	5	3	mr
4	43	8.2	22.4	96	19	mr
5	62	5.3	19.6	27	10	mr,dep
6	63	7.3	27.0	48	16	mr
7	86	6.7	22.0	43	11	mr,dep
8	60	5.4	17.5	90	-	mr
9	55	9.5	27.8	70	15	dep
10	58	4.8	31.5	63	5	dep
11	75	8.1	23.5	50	9	mr
12	50	-	-	40	4	mr

[a]abbreviations: mr = mark-recapture; dep = depletion method

Model II-A used to deliver 600 V DC), and either one or two (depending on the size of the section) were netters. Trout longer than 100 mm (all measurements were in total length) were anaesthetized in unbuffered 3-Aminobenzoic Acid Ethyl Ester (MS-222), measured to the nearest millimeter, and weighed to the nearest gram. Fish were then marked with colored fingerling tags on monocord thread inserted by needle through the dorsal musculature behind the dorsal fin, so that the tag hung about 2.5 cm from the point of insertion. These tags had no apparent short-term deleterious effects and were highly visible under water. Fish were allowed to recover (to the point of swimming upright) in buckets of fresh water and released one or two at a time throughout the length of the section. On average, it required 20 to 30 min to complete the electrofishing runs and less than 1 min to weigh, measure, and tag each fish once the electrofishing was completed.

Upon release and at 1 to 6 h, 24 h, and 168 h after release, we snorkeled in the stream section (average time 15 min) to qualitatively observe the behavior of marked and unmarked fish. The diver followed each marked fish released to record the immediate behavioral response in terms of choice of specific location within the section. At each observation interval, the diver counted the number of marked fish showing seemingly normal behavior (herein after referred to as "normal" fish, as determined by comparisons with the behavior of undisturbed trout) and marked fish behaving abnormally ("abnormal" fish). We plotted the percentage of marked trout behaving normally, abnormally, or not observed over time to determine the length of time

required for half of the marked fish to return to the normal pre-shock behavioral condition.

In 1988, six sections, some re-used from the previous year (Table 1), were subjected to a multiple electroshock protocol. At each section, after block seines were placed, two divers counted the number of cutthroat trout longer than 100 mm. One diver swam upstream and counted the fish in cover and out of cover; the second followed after the first diver had snorkeled approximately 75% of the length of the section. After completion of both fish surveys, we made a single-pass electrofishing run, as previously described. Captured trout were held in buckets along the stream bank. Immediately after the first pass, the divers again snorkeled along the section, reversing the order of entry, to count fish located in and out of cover. This sequence continued for a maximum of three electrofishing passes. The counts of fish after each electrofishing pass were compared to note any changes in the proportion of fish in and out of cover, in relation to those seen in the initial dive.

ARTIFICIAL STREAM OBSERVATIONS

An oval completely recirculating stream aquarium was used for all trials (Reeves et al. 1983). The stream was 4.3 x 4.9 m on the sides, 0.76 m wide, and 0.61 m deep. It was lined with varying depths of gravel and cobble substrates to produce a stream with four pools (50 cm deep) and three riffles (40 cm deep). A perforated feeding tube along the stream bottom evenly distributed food, to simulate insect drift. Each pool contained hollow tiles or stacked bricks to provide additional shelter. Lighting, provided by nine 60-

W incandescent bulbs spaced evenly above the stream channel, was controlled by a timer (Everest and Rodgers 1982) that provided a graded intensity photoperiod of 12-h light:12-h darkness. Water temperature was maintained by a cooling and heating unit set at 12° to 13° C, and water velocity (0.0 - 10.0 cm/s) by a rotating paddle wheel. Water was continually passed through a sand filter and UV sterilizer; make up water was added to the channel at 0.5 L/min. A curtain with screened windows surrounded the inside perimeter of the stream to permit observation of the fish without disturbing them. Fish were fed frozen brine shrimp, which were thawed in collecting tanks and then passed through the feeding tube to simulate drift. We conducted six trials--three with hatchery-reared cutthroat trout (average weight 63 ± 2.7 g SE) and three with wild fish (average weight 46 ± 5.7 g SE) captured by angling in local streams with artificial flies and barbless hooks. The stream was drained, sterilized, refilled and stocked with new fish between trials, which were conducted from May to July 1988.

For each trial, seven cutthroat trout were allowed to acclimate to the stream for at least 2 weeks (wild trout often required more time). We judged acclimation by the sustained presence of active feeding and aggressive behavior, leading toward the formation of a dominance hierarchy. During this period, we fed the fish twice daily (at random times from 0800 to 1000 h and 1500 to 1700 h) and observed their behavior.

After the period of acclimation, we conducted focal animal sampling (Altmann 1974) during feeding sessions for 3 consecutive days. Each fish was observed for 5 min while the number of feeding

bites taken and number of aggressive acts elicited and received were recorded. The number of aggressive acts consisted of a sum of the individual agonistic elements of salmonids, including nips, charges, chases, and lateral and frontal displays, as described by Kalleberg (1958), Keenleyside and Yamamoto (1962), and Hartman (1965). The sequence in which fish were observed was randomized for each feeding session. At 0800 hours on the fourth day, fish were captured by electrofishing (Coffelt Model II-A or Smith-Root Model VII backpack units set at 300 V DC) and then marked as described for the natural stream observations. At release, the location and behavioral state of each fish was recorded. Feeding and aggression data were recorded using focal animal sampling for 2 min each hour during hours 1 to 7, and once 24 h after release. Because the observations were made each hour, we reduced observation time to maximize the time interval between observations and minimize any effect of satiation later in the day. All data were converted to fish per minute for comparison.

We calculated mean feeding and aggression rates for each fish from the pre-treatment data. We then calculated a grand mean for each trial, based on the individual fish means. After testing for homogeneity of variance, we found no difference among hatchery trial grand means by one-way analysis of variance (ANOVA; $p < 0.05$); therefore all data were pooled to calculate a single, pre-treatment grand mean. Because of problems encountered with using two disproportionately large fish in one trial, only two of three wild fish trials were considered experimentally sound. When a two-sample t-test revealed no significant difference between the grand means, the data were combined. The pre-treatment grand means and post-

treatment hourly rates were then compared: We subtracted the hourly post-treatment rates of feeding and aggression for each fish from the corresponding pre-treatment grand mean. Within each time interval, we summed the individual fish differences and used a one-sample t-test (for use with equal or unequal variances) to determine whether the average difference for the group at that hour differed significantly from zero (i.e., the null hypothesis being no difference between the pre-treatment grand mean and hourly post-treatment means). For any hour, if the null hypothesis was rejected ($p < 0.05$), we concluded that the pre-treatment grand mean rates of feeding and aggression and post-treatment rates were different. Data were compared within hatchery and wild groups, between groups, and within a hierarchical ranking based on dominance matrices of aggression received and elicited, comparisons of food intake between individuals, and general behavioral observations of each fish.

PHYSIOLOGICAL EXPERIMENTS

Cutthroat trout (average weight 56 $\pm$ 1.1 g SE) used to evaluate the effects of electroshock and marking were obtained from Alsea (Oregon) State Fish Hatchery; cutthroat trout (average weight 18 $\pm$ 0.32 g SE) used to evaluate the effects of multiple electroshocks were obtained from Cedar Creek (Oregon) State Fish Hatchery. All fish were transferred to the Oregon State University Smith Farm research facility and held in flow through, circular tanks (0.9 m in diameter) receiving aerated well water at $12^{\circ} \pm 1^{\circ}$ C and exposed to a natural photoperiod. Fish were fed Oregon Moist Pellets daily and acclimated for at least 2 weeks before each experiment.

To assess the effects of the procedures involved with mark-recapture protocols, we used a completely randomized design with three treatments. A group of 35 fish distributed in three tanks received a single 4-s electroshock (300 V DC from a Coffelt Model II-A backpack unit). We exposed 35 fish distributed in three tanks to a single 4-s electroshock; the fish were then captured, anaesthetized with MS-222, weighed, measured, marked as in the field study, allowed to recover in buckets, and released back into their original tank. As a control, 25 fish in two tanks were left undisturbed. We collected five fish from each group at times 0, 1, 3, 6, 12, 24, and 168 h post treatment (control fish sampling times were alternated between trials). The fish were rapidly removed from the tanks with dip nets and placed in a lethal dose (200 mg/L) of MS-222. The fish were then removed from the anaesthetic and bled from the caudal vasculature (after severance of the caudal peduncle) into an ammonium heparinized capillary tube. Plasma was obtained by centrifugation and stored at -15°C for future assay. Processing time for a sample of five fish generally required less than 5 min. We conducted two trials of this experiment during February-March 1988.

To assess the effects of multiple electroshocking, we distributed 45 fish into two tanks, and exposed them to three consecutive 8-s, 500 V DC electroshocks separated by 0.5 h. Forty fish in each of two other tanks received only a single, 8-s 500 V DC electroshock. Because fish in this experiment were smaller than those used in the first physiology experiment, we increased the voltage and shocking time to elicit a similar electronarcotic response in the fish in the two experiments. Controls were as previously described; in

addition, we assigned 30 fish to a handling stress that consisted of netting the fish from the tank, holding them in the air for 30-s, then returning them to the tank for recovery. Our objective was to compare stress responses between electroshocking and acute handling. We obtained plasma samples as previously described, using the same sampling intervals, and also collected samples 30 min after shocking and handling and immediately after each successive electroshock. We conducted two trials of this experiment in October-November 1988.

Plasma cortisol was determined by ^{3}H - radioimmunoassay (Foster and Dunn 1974), as modified by Redding et al. (1984) for use with salmonid plasma. Plasma lactate was assayed by fluorimetry (Passonneau 1974). All data were tested for homogeneity of variance (Bartlett's Test; Sokal and Rholf 1981). Those found to be homogenous were analyzed by either a t-test or analysis of variance, followed by Fisher's Least Significant Difference test at the 5% probability level (Ott 1977). Data with heterogeneity among variances were tested by a t-test for means with unequal variances, or a Kruskal-Wallis One-Way Analysis by Ranks (Sokal and Rholf 1981).

Results

NATURAL STREAM OBSERVATIONS

The general behavioral responses of cutthroat trout released back to the stream after they were shocked and marked included the immediate seeking of cover, lying motionless on the stream bottom, not feeding, and a general lethargy evidenced by their becoming easily approachable by a diver. These behaviors were observed at all sections and persisted for various periods. Normal cutthroat trout behavior consisted of swimming actively in the water, feeding, behaving skittishly in the presence of a diver, and interacting socially with conspecifics. Immediately after release, the percentage of abnormal fish at several sections was high; increasing numbers of fish regained normal activity as time progressed (Figure 1). The response of marked fish returning to normal behavior was highly variable among sections and we were rarely able to account for 100% of the marked fish after release throughout the course of the observations. The percentage of normal, abnormal, and marked fish not observed for all sections combined is shown in Figure 2a. We also combined data from sections with a catch greater than nine and combined data from sections with a catch less than or equal to nine, which was the overall mean catch of all sections (Figures 2b,c). Recovery was faster in fish from sections where many fish were captured than in those where fewer fish were caught. There was generally a higher percentage of marked trout unaccounted for in sections where few fish were captured at the outset. The behavioral

Figure 1. Percentages of the total number of marked cutthroat trout showing normal or abnormal behavior patterns at various times after release, after capture by electrofishing in 11 sections of Mill Creek. Numbers in parentheses are the number of marked fish released at each section.

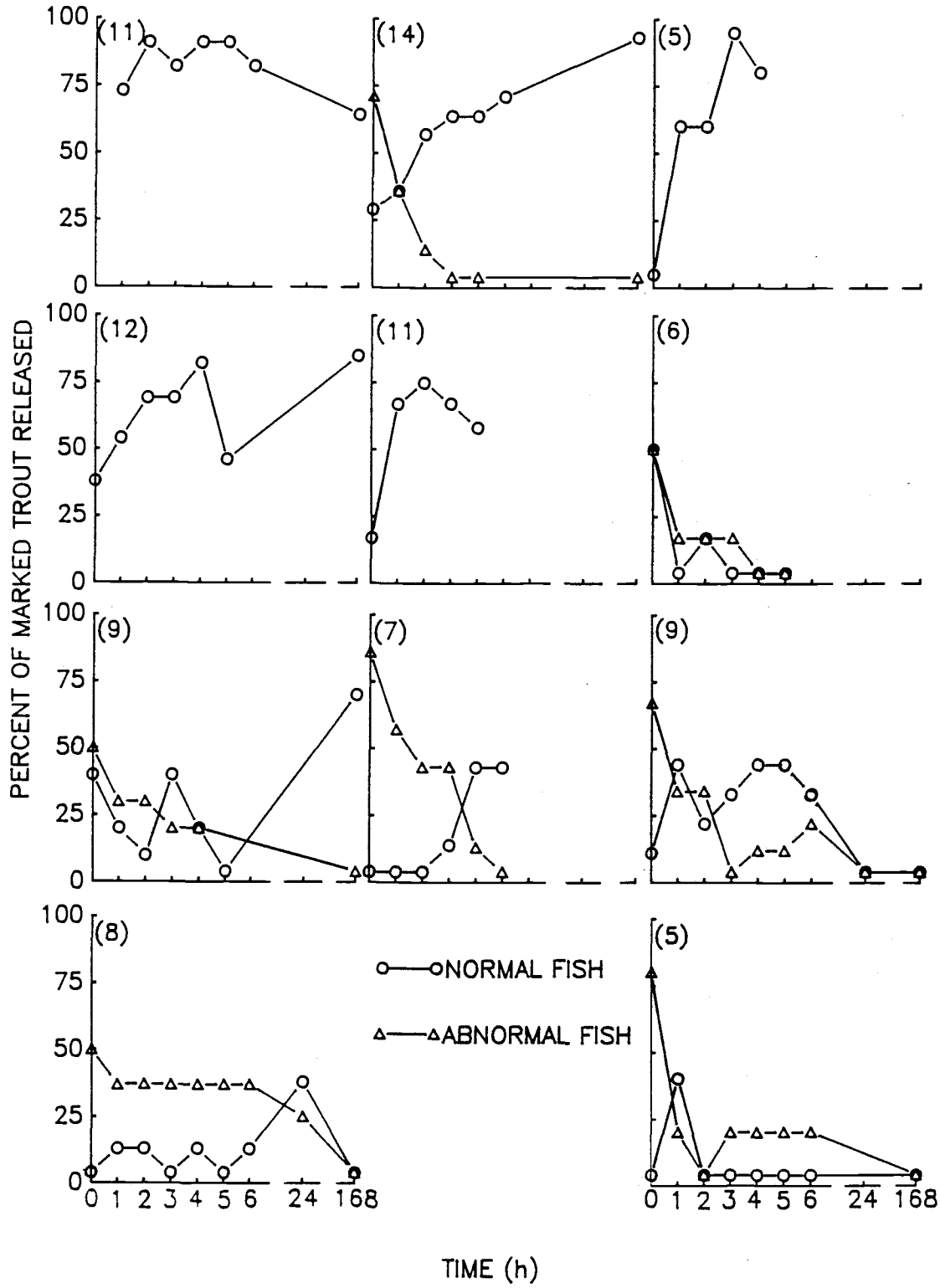

PERCENT OF MARKED TROUT RELEASED
100
75
50
25
0
(11)
(14)
(5)
(12)
(11)
(6)
(9)
(7)
(9)
(8)
(5)
NORMAL FISH
ABNORMAL FISH
0 1 2 3 4 5 6 24 168
TIME (h)

Figure 1

Figure 2. (a) Percentages of marked cutthroat trout showing normal or abnormal behavior patterns or not observed at various times after release, after electroshocking plus marking experiments at Mill Creek. Bars show the percentages based on data from all sections combined; (b) Mean percentages of cutthroat trout showing normal or abnormal behavior patterns or not observed for sections in which greater than nine marked fish were released. (c) Mean percentages of cutthroat trout showing normal or abnormal behavior patterns or not observed for sections in which nine or less marked fish were released.

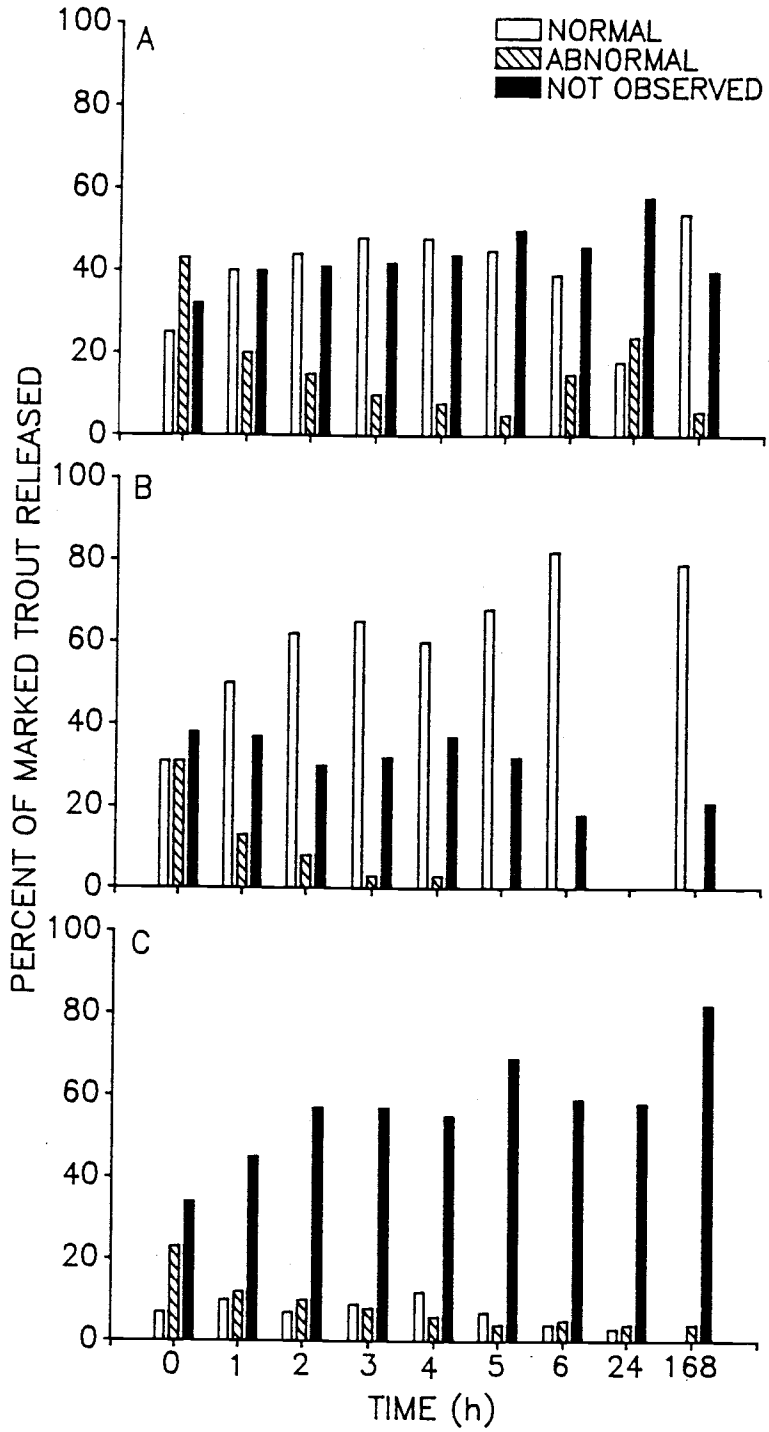

NORMAL
ABNORMAL
NOT OBSERVED
A
B
C
100
80
60
40
20
0
PERCENT OF MARKED TROUT RELEASED
0
1
2
3
4
5
6
24
168
TIME (h)

Figure 2

responses of marked fish upon release included rapidly swimming to large rocks for cover (48% of the marked fish released), lying motionless on the stream bottom (17%), swimming to a large group of conspecifics (16%), remaining in the water column (15%), or swimming to undercut banks, rootwads, or other debris for cover (4%).

Using a multiple electroshock protocol, we observed little change in normal behavior of fish remaining in the stream section after successive electrofishing passes. During most dives, we found the fish out of cover and behaving normally, indicating that the disturbance to the stream section did not elicit a significant fright or hiding response (Table 2). In only one instance (2 fish at section 1) were uncaptured fish found in heavy cover and behaving lethargically after the first electrofishing pass.

ARTIFICIAL STREAM OBSERVATIONS

There were no differences between hatchery and wild fish in grand mean rates of feeding (hatchery: $\bar{X}$ = 9.9 bites·fish^{-2}·min^{-1}, N = 19; wild: $\bar{X}$ = 11.0, N = 12) and aggression (hatchery: $\bar{X}$ = 0.76 aggressive acts elicited·fish^{-2}·min^{-1}; wild: $\bar{X}$ = 0.48) before electroshocking and marking. Relative to the grand mean rate, hatchery fish fed at a significantly lower rate 1 h after release but fed normally at all other times (Figure 3). The aggression rate of hatchery fish after release was variable, being significantly lower than the preshock mean rate at 1, 2, 5, 6, and 30 h post shock (Figure 3). In wild fish, mean rates of feeding and aggression decreased significantly after shocking and marking; feeding rates returned to normal 24 h post treatment; however, aggression rates did not return to normal during

Table 2.-Summary of fish counts made by divers and catch by electrofishing when the depletion method was used at Mill Creek, 1988. Fish counts were made before each electrofishing pass and are shown as the mean of counts by two divers.

Site No.	Fish count		Electrofishing	
	In cover	Out of cover	Pass No.	Catch[a]
1	1	13	1	8
	2	0	2	5
	0	0	3	NA
2	5	43	1	10
	4	33	2	12
	2	24	3	5
3	2	25	1	18
	2	19	2	13
	2	12	3	9
4	2	7	1	13
	0	1	2	3
	NA	NA	3	NA
5	5	12	1	13
	1	4	2	8
	1	3	3	0

[a]NA = not available

Figure 3. Mean ± SE feeding and aggression rates for hatchery cutthroat trout (upper panel) and wild cutthroat trout (lower panel) before and several hours after electroshocking and marking in an artificial stream. All trials within a group are combined.

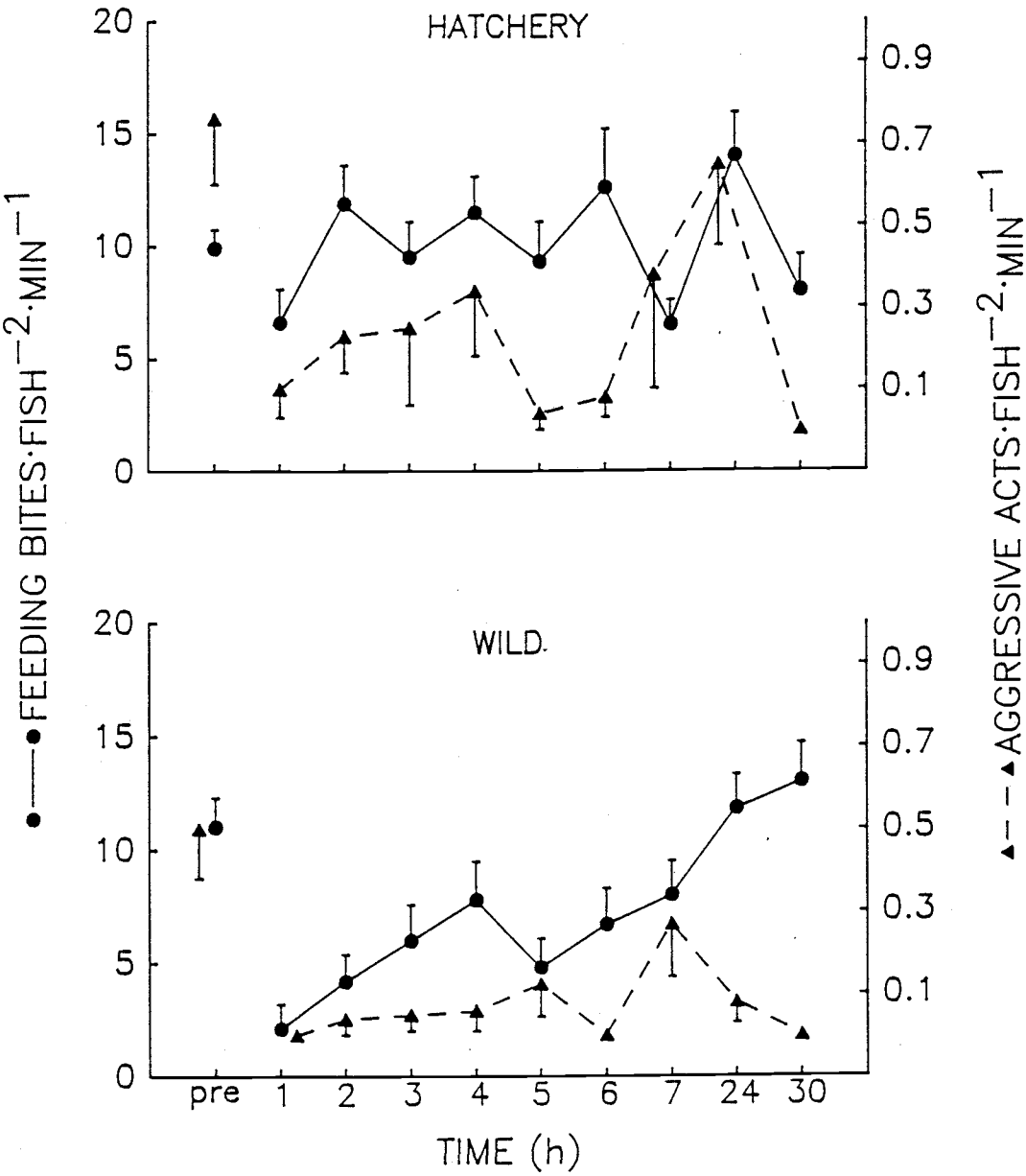

HATCHERY
WILD
20
15
10
5
0
0.9
0.7
0.5
0.3
0.1
pre
1
2
3
4
5
6
7
24
30
TIME (h)
●—●FEEDING BITES·FISH^{-2}·MIN^{-1}
▲- -▲AGGRESSIVE ACTS·FISH^{-2}·MIN^{-1}

Figure 3

the observations (Figure 3).

Analysis of feeding and aggression data by hierarchical rank of fish revealed that hatchery dominants were generally feeding normally by 2 h post-treatment (Figure 4). Aggression was low for the first three hours post-treatment and highly erratic thereafter. Feeding rate in hatchery intermediates showed no significant changes, although aggression rates were generally lower than the pre-treatment rate during much of the observation period. Subordinant hatchery trout showed no real changes in either feeding or aggression. Dominant wild fish returned to normal feeding rates by 2 h post-treatment and maintained a slightly lower rate of feeding for the first day (Figure 4). Intermediate wild trout did not return to normal feeding rates until the second day, while subordinates showed no appreciable changes in feeding rate aside from the first hour following treatment. After electroshocking and marking, rates of aggression generally remained low for wild dominants and intermediates throughout the observation period.

PHYSIOLOGICAL EXPERIMENTS

Electroshock plus marking

Because there were no differences in overall mean levels of cortisol between experimental trials, we combined all data. Mean concentrations of plasma cortisol in fish subjected to electroshock plus marking increased immediately and peaked at 1 h (Figure 5). They returned to control concentrations at 3 h, were elevated at 6 h, and again returned to control concentrations for the rest of the experiment. Plasma cortisol concentration in fish receiving only a

Figure 4. Mean ± SE feeding and aggression rates for socially ranked hatchery (panels at left) and wild cutthroat trout (panels at right) before and several hours after electroshocking and marking in an artificial stream.

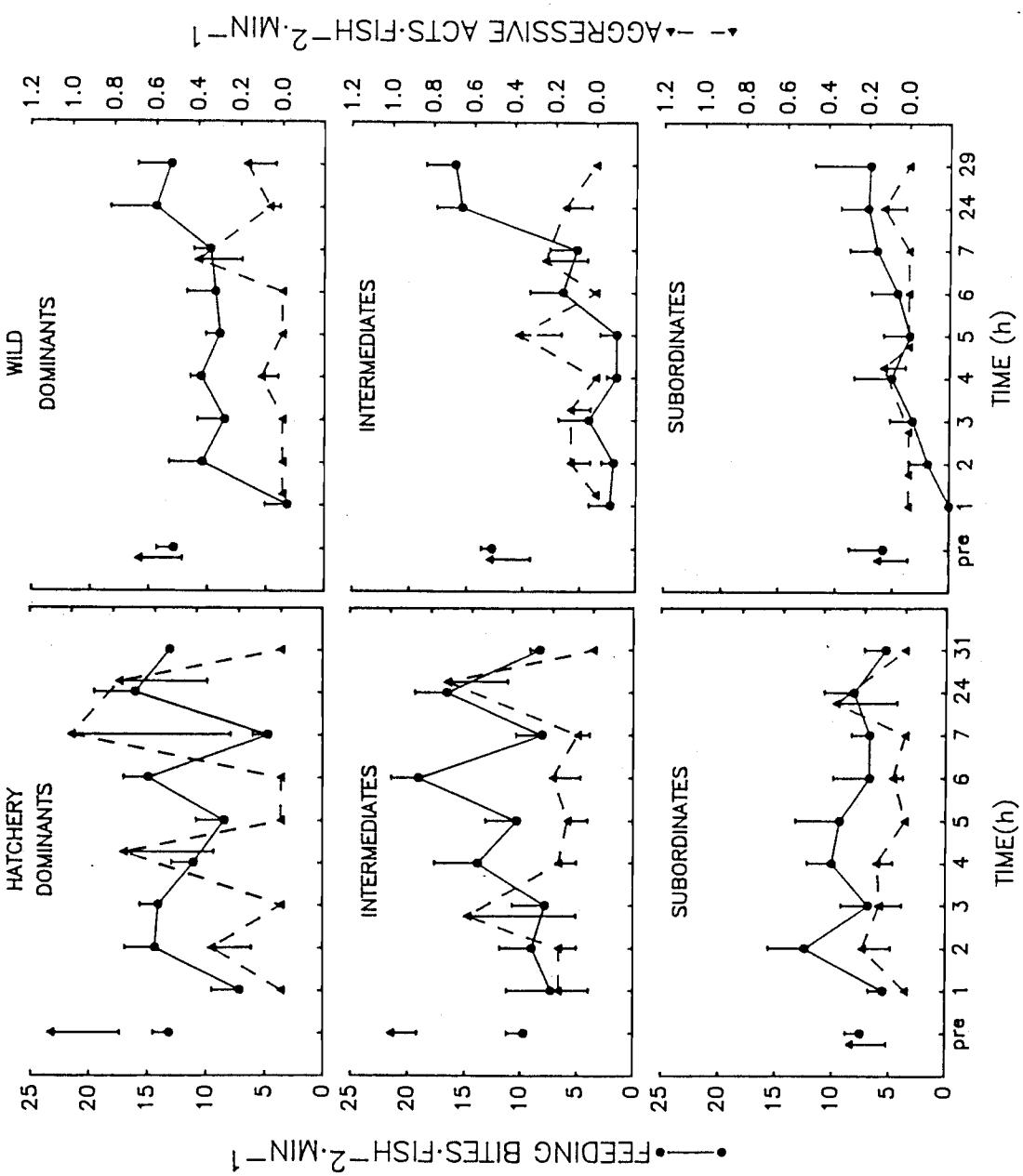
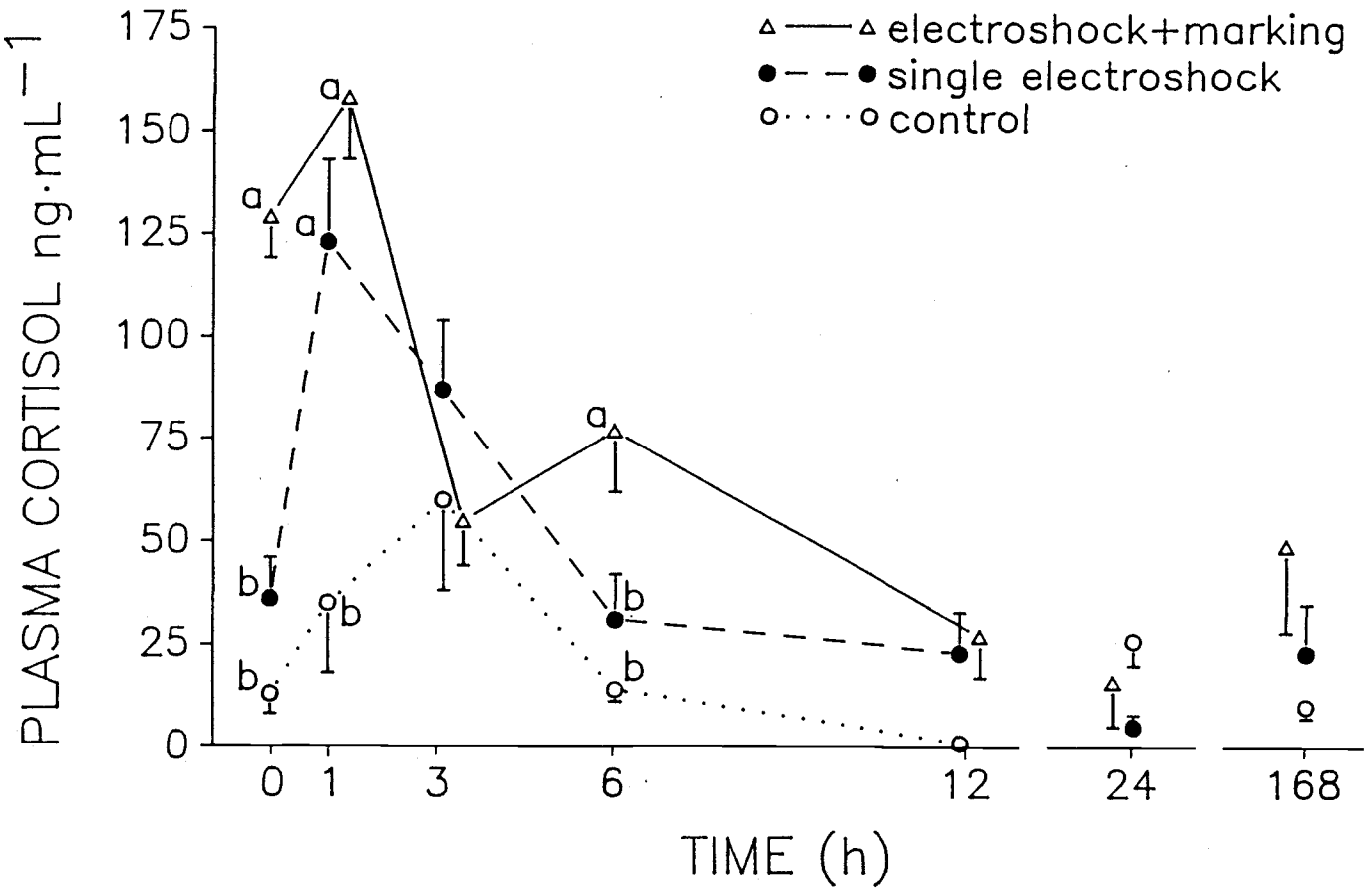

HATCHERY DOMINANTS
WILD DOMINANTS
INTERMEDIATES
SUBORDINATES
TIME(h)
TIME (h)
pre
•—• FEEDING BITES·FISH⁻²·MIN⁻¹
•– –• AGGRESSIVE ACTS·FISH⁻²·MIN⁻¹

Figure 4

Figure 5. Mean ± SE plasma cortisol concentrations in Alsea hatchery cutthroat trout subjected to a single 4-s, 300 V DC electroshock or electroshock plus marking relative to unstressed controls. Means represent pooled data from two trials (N=10). Means within a time interval with no letters in common are significantly different ($P<0.05$); time intervals with no letters shown indicate no significant difference among the means.

Figure 5


electroshock+marking
single electroshock
control
PLASMA CORTISOL ng·mL⁻¹
175
150
125
100
75
50
25
0
0
1
3
6
12
24
168
TIME (h)
a
b

single electroshock did not increase immediately (Figure 5), but increased significantly 15 min after shocking, peaked at 1 h, and then returned to control concentrations by 3 h. Plasma cortisol concentrations in shocked and marked fish and those only shocked differed significantly immediately after application of the treatment and 6 h later.

Because average lactate levels differed significantly between trials, we analyzed the data independently. In both trials, lactic acid increased significantly after electroshocking plus marking and remained elevated for 1 h (Figure 6). Concentrations returned to control levels by 3 h post treatment. Lactic acid dynamics in fish receiving only a single electroshock were similar to those in fish that were both shocked and marked.

Multiple electroshock

Because there were no differences in average cortisol levels between trials, we pooled all data for analysis. In fish receiving multiple electroshocks, cortisol concentrations were significantly elevated after the second and third shocks (Figure 7). Cortisol peaked after the third shock, did not return to control levels until 6 h after application of the first shock, and was elevated again 24 h later. Cortisol of fish receiving only a single shock peaked at 0.5 h post-treatment and remained elevated for 6 h. Cortisol concentrations for fish receiving the 30-s handling stress peaked at 0.5 h post treatment and returned to control levels by 3 h.

Figure 6. Mean ± SE lactic acid concentrations in Alsea hatchery cutthroat trout subjected to a single 4-s, 300 V DC electroshock or electroshock plus marking relative to unstressed controls. Panels represent two replicate trials. Means (N=5) within a time interval with no letters in common are significantly different ($P<0.05$); time intervals with no letters shown indicate no significant difference among the means.

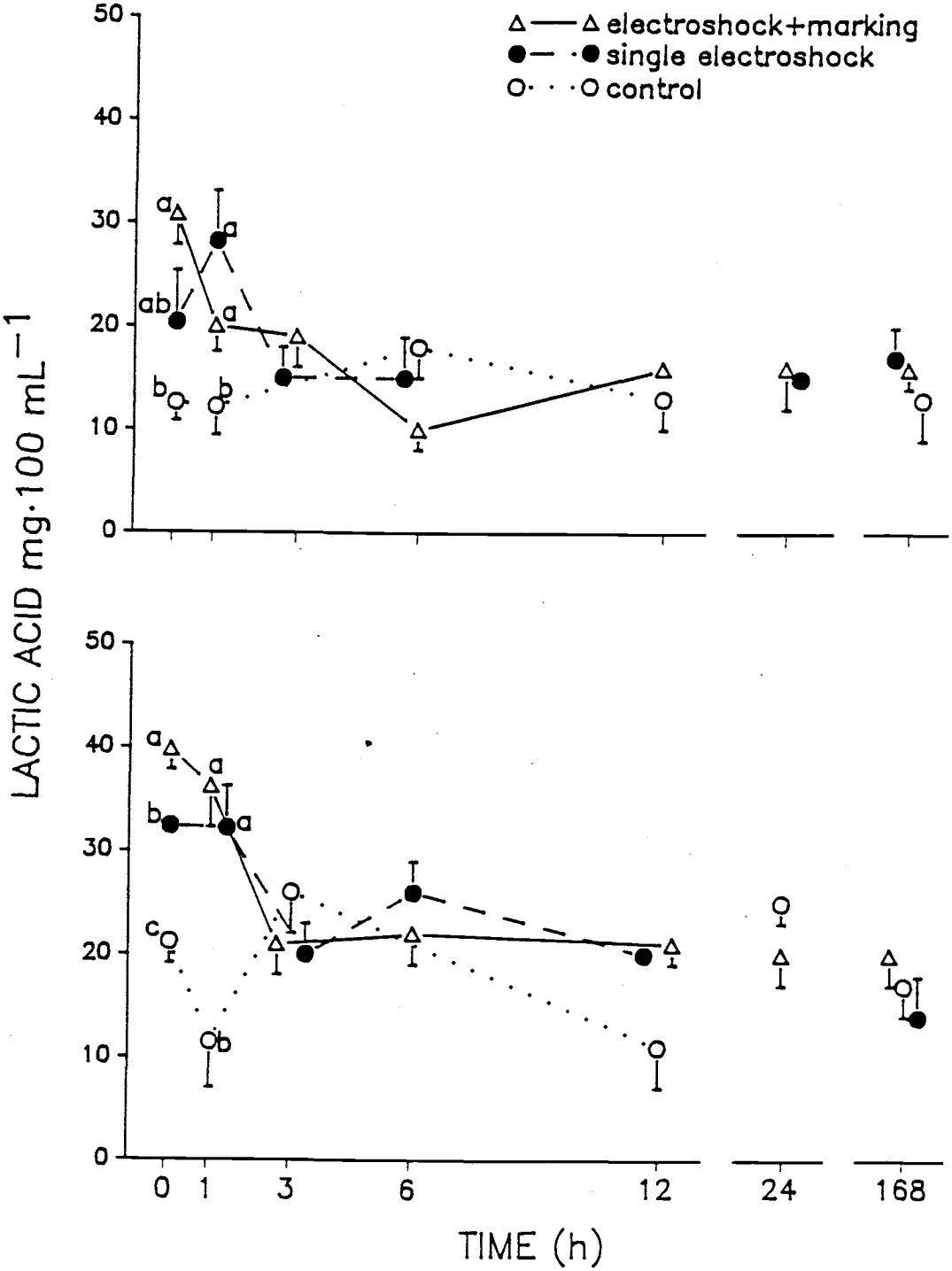
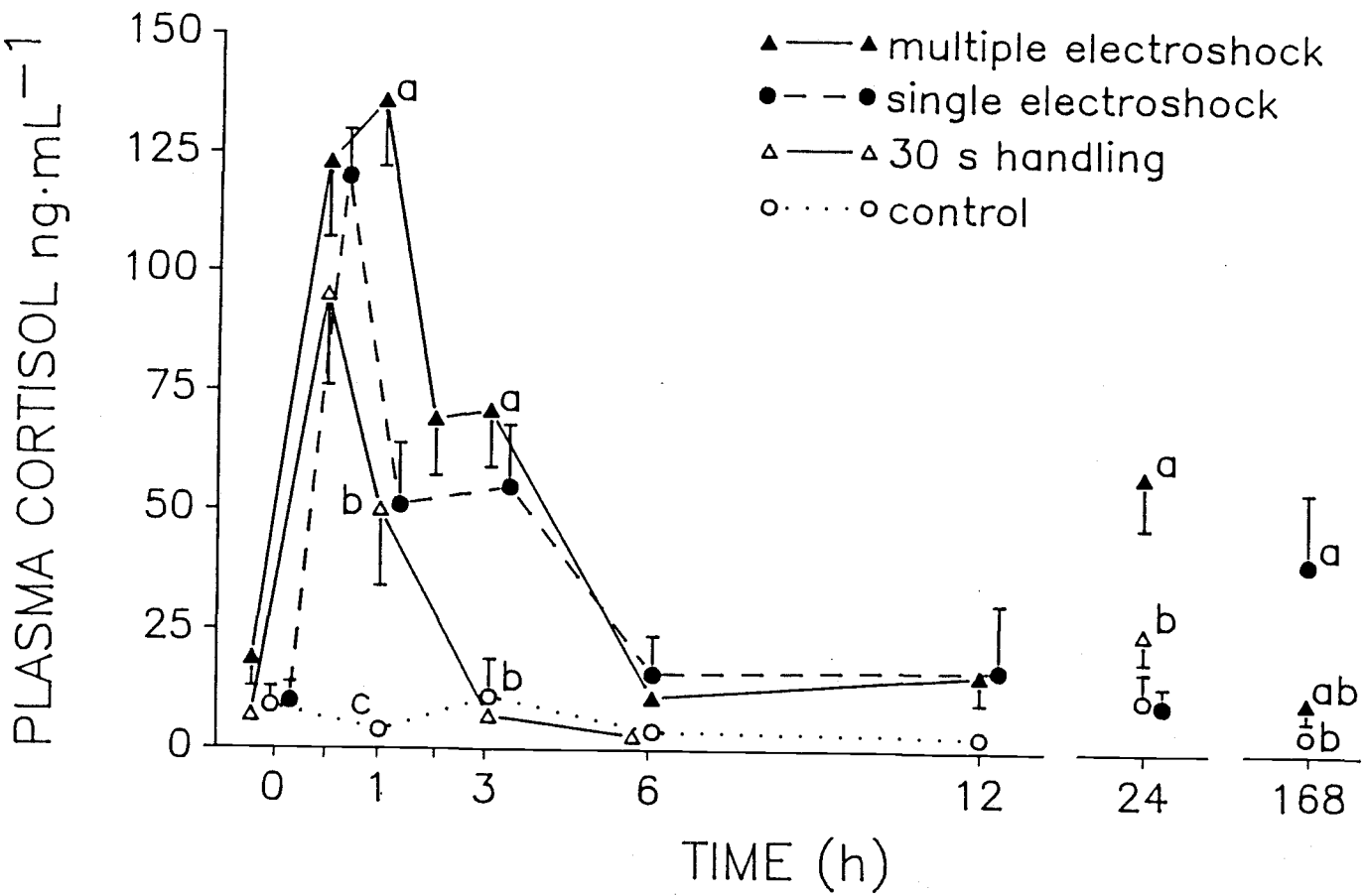

electroshock+marking
single electroshock
control
LACTIC ACID mg·100 mL⁻¹
TIME (h)
0
10
20
30
40
50
0 1 3 6 12 24 168
a
ab
b
c

Figure 6

Figure 7. Mean ± SE plasma cortisol concentrations in Cedar Creek hatchery cutthroat trout subjected to a single 8-s, 500 V DC electroshock, three 8-s, 500 V DC electroshocks separated by 0.5 h, or a 30-s handling stress relative to unstressed controls. Means represent data from two trials combined (except for handling stress fish). Means (N=10) within a time interval with no letters in common are significantly different ($P<0.05$); time intervals with no letters shown indicate no significant difference among the means.

Figure 7


PLASMA CORTISOL ng·mL⁻¹
150
125
100
75
50
25
0
multiple electroshock
single electroshock
30 s handling
control
a
a
b
b
c
a
b
a
ab
b
0
1
3
6
12
24
168
TIME (h)

Because average lactate concentrations differed between trials, we analyzed the data separately. In one trial, lactic acid increased in fish receiving multiple electroshocks, peaked immediately after the third shock, and gradually decreased to normal by 6 h (Figure 8). Lactate increased only slightly at 0.5 h in fish receiving only a single shock before it returned to normal by 1 h post treatment. In the second trial, lactate increased rapidly and peaked immediately after the third shock in fish that received multiple electroshocks. Lactate concentrations remained elevated for 3 h after the first shock before returning to control levels by 6 h. Although lactic acid in fish receiving a single electroshock peaked at 1 h post treatment, this concentration was not different from controls; however, lactate was significantly elevated at 3 h and returned to control titers for the remaining sample periods. In fish receiving the 30-s handling stress, lactate peaked at 0.5 h and returned to control levels by 6 h.

Figure 8. Mean ± SE lactic acid concentrations in Cedar Creek hatchery cutthroat trout subjected to a single 8-s, 500 V DC electroshock, three 8-s, 500 V DC electroshocks separated by 0.5 h, or a 30-s handling stress relative to unstressed controls. Panels represent two replicate trials. Means (N=5) within a time interval with no letters in common are significantly different ($P<0.05$); time intervals with no letters shown indicate no significant difference among the means.

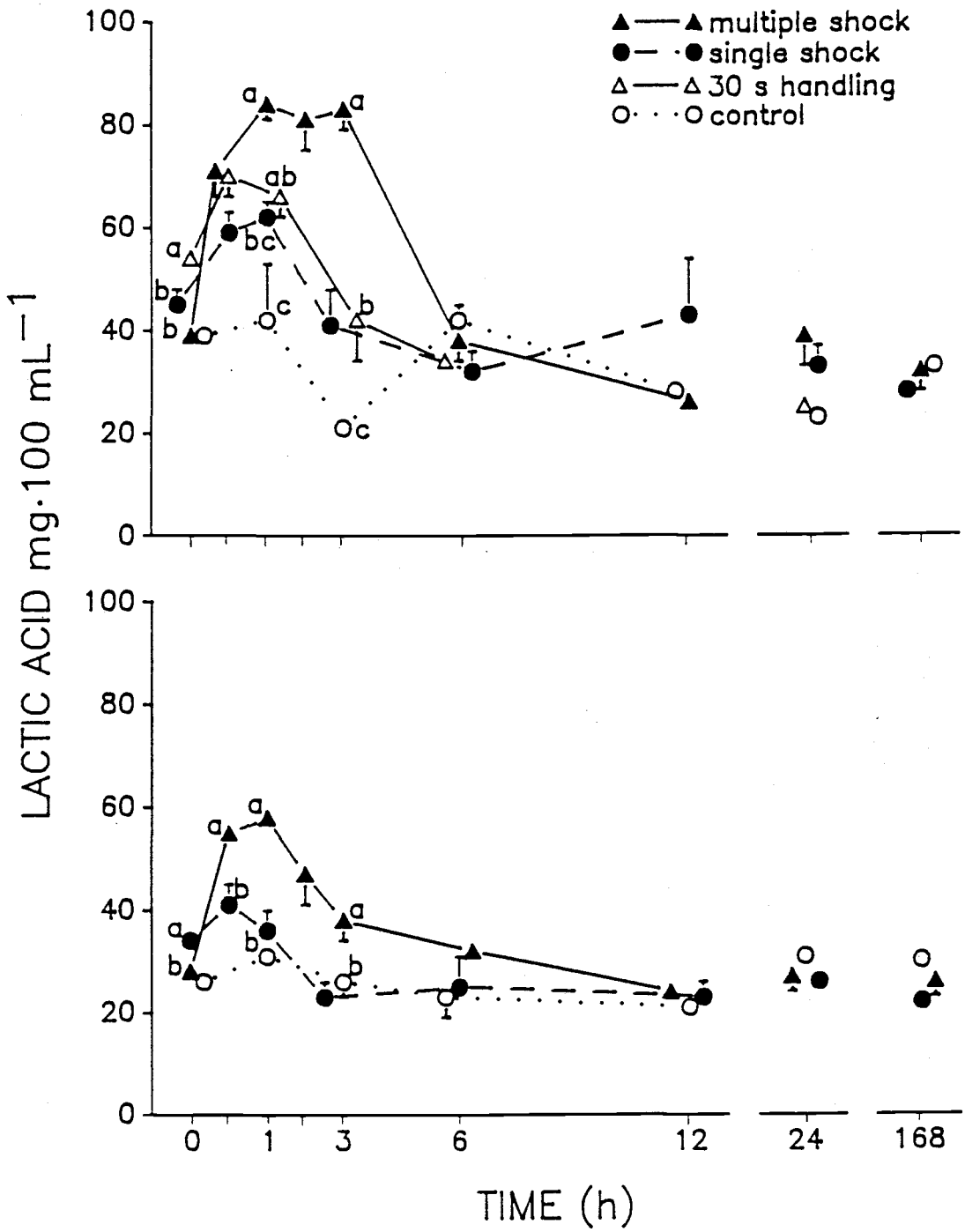

multiple shock
single shock
30 s handling
control
LACTIC ACID mg·100 mL⁻¹
TIME (h)
0
20
40
60
80
100
0
1
3
6
12
24
168

Figure 8

Discussion

It is evident that electroshock and the procedures involved with estimating fish population size elicit a general stress response that manifests itself not only physiologically but behaviorally as well. Our results showed that this response lasted for several hours and suggested that wild trout may be affected more severely than hatchery trout.

Our findings at Mill Creek clearly reveal a marked behavioral change in fish that were captured by electrofishing and marked. Although variability among sections was high, on average we were typically unable to account for more than 50% of the marked fish behaving normally. Perhaps the most striking response was the general lethargy and cryptic behavior of the trout, which lasted for several hours. Other investigators have noted general lethargic behavior in fish after various stresses (Bouck and Ball 1966; Hertig and Witt 1967; Coutant 1970; Curry and Kynard 1978; Sigismondi and Weber 1988). Most of our fish sought some type of cover immediately after release and several were observed trying to dig into the substrate or wedge themselves between rocks. This cover-seeking behavior was in direct contrast to normal fish behavior in Mill Creek where fish were seldom found in cover, behaved skittishly, and often fleed in the presence of a diver.

These behavioral changes might alter the catchability of marked fish during subsequent electrofishing attempts. Halsband (1967) reported that sick or fatigued fish do not react well to electric

current. Fish may become so affected by electricity that they become physically unable to be drawn towards the anode (Cross and Stott 1974). Saul (1980) noted that cobble and boulder substrates provided refuge for fish that resulted in lower percentages entering the catch. Our inability to locate a high percentage of marked fish in several sections of Mill Creek suggests that had subsequent electrofishings taken place, the efforts might have occured over a reduced population. This may have been due to fish emigration or mortality; however marked fish were rarely seen outside the study section. We believe that fish remained in the sections but were not located by divers because they were in uncharacteristically heavy cover. This cover-seeking response is one factor that might alter catchability (e.g. see Saul 1980) and suggests that habitat quality is an important variable to consider when conducting population estimates. Areas with a lot of instream cover, such as woody debris, log jams, deep undercut banks, and large boulders, may be areas where electrofishing would be inefficient and some other technique (e.g. estimates by snorkeling) may have increased efficacy. The question of whether marked fish become more catchable or less catchable during subsequent electrofishing attempts has produced equivocal answers (Cross and Stott 1975; Bohlin and Sundstrom 1977; Yundt 1983; Peterson and Cederholm 1984), and, given our findings, indicates a need for further investigation.

We observed that fish seemed to recover faster at sections with a relatively large catch and also that these sections had a relatively low percentage of marked fish unaccounted for. This may be because sections with a large catch were also areas where cutthroat trout were

found in large, close knit groups in deep water. Marked fish often returned to such groups soon after release and were classified as normal fish, even though they might still be affected by the stress and were actually seeking refuge. Another possible explanation is section differences in the amount and type of instream cover available for refuge. We noted that in areas with deep water and cobble and boulder substrates it was difficult to maintain position while trying to observe any fish that may be hiding. In addition, the low light levels within large boulder complexes and undercut banks added to the difficulty of observing fish potentially using such areas for shelter.

The general behavioral observations of fish (i.e., general lethargy and cover-seeking response) in the artificial stream were consistent with observations at Mill Creek, especially for wild trout. However, the dynamics of recovery from the stress were different. In the artificial stream, normal behavior patterns--aside from a decrease in aggression--returned by 24 h after treatment. At Mill Creek, it was often difficult to even locate marked fish 24 h after shocking and marking. This discrepancy in recovery dynamics between fish in the two systems is most likely due to the much more complex environment of the natural stream, making it more difficult to locate and observe marked fish.

Wild cutthroat trout appeared more severely affected by the stresses involved with the mark-recapture protocol than did hatchery fish in our artificial stream. Various investigators have compared the ability of hatchery and wild rainbow trout Oncorhynchus mykiss to deal with various stresses (Wydoski et al. 1976; Casillas and Smith 1977; Woodward and Strange 1987), but results have been equivocal.

Although we found that both groups decreased their activity upon release, hatchery fish generally recovered in about 1 to 2 h, whereas wild fish required at least 24 h. Wild fish showed a general lethargy that was evidenced primarily by a marked decrease in aggressive behavior. Although there was ample opportunity for aggression to occur throughout the day after treatment, it never consistently returned to pre-treatment rates; when aggression did occur, it was less intense. The relative absence of overt, agonistic behaviors throughout the post-treatment observation period suggests that normal behavior patterns were altered, even though the fish were feeding during this time. However, for at least the first 4 h after the fish were shocked and marked, most fed sluggishly and did not swim far to acquire food items. Feeding intensity increased later in the day and at 24 h, presumably after the effects of the stress had decreased.

The effects of electroshocking and marking on the integrity of a dominance hierarchy may affect the accuracy of population size estimates. Our results suggest that, at least for wild fish, intermediate and subordinate fish may require more time for recovery from stress than social dominants. It appears that hatchery fish, regardless of rank, are more resilient to the stresses involved in this experiment. The hypothesis that there is an inverse relation between dominance status and a low level, chronic state of stress in fish, in which dominants are under the least stress, has been inferred by several investigators (Erickson 1967; Noakes and Leatherland 1977; Ejike and Schreck 1980), and is consistent with our results for wild fish. Whether this differential ability to handle stress would have

any effect on estimating population size is speculative but might be related to the proportions of differently ranked individuals residing in the stream section.

Our inability to observe much behavior change in fish during multiple electroshock experiments at Mill Creek does not lead to the conclusion that this method has no effect. Although we observed distinctly abnormal behavior in one section, most fish remaining in the other sections after successive electrofishing passes were showing the characteristic normal behavior of cutthroat trout at Mill Creek. However, because the depletion method is popular in small streams and multiple electroshock affects fish physiology, its effects on fish behavior may warrant further investigation.

Exposure of fish to electricity plus handling, marking, etc., subjects them to a significant amount of stress which becomes apparent as changes in blood constituents (Caillouet 1967; Madden and Houston 1976; Schreck et al. 1976; Burns and Lantz 1978; Bouck et al. 1978; van Waarde and Kesbeke 1983). Schreck et al. (1976) attributed these changes to the combined effects of trauma, paying off an oxygen debt, and the general adaptation syndrome of stress. Cutthroat trout subjected to a single electroshock did not show an immediate (i.e. less than 10-s) increase in plasma cortisol and lactic acid; this reaction is consistent with results obtained by Woodward and Strange (1987) for rainbow trout and contrasts with results of Schreck et al. (1976), although a relatively longer time period elapsed before Schreck et al. (1976) collected their first samples. Lactic acid typically returned to control levels after 3 h, whereas cortisol required 6 h. The addition of stressors involved with the mark-

recapture procedure immediately elevated concentrations of cortisol and lactate beyond levels observed in fish experiencing only electroshock. Perhaps fish that were electroshocked and marked simply had more time to liberate cortisol from the interrenal tissue; their stressful experience averaged about 10 min in contrast to about 15-s in those that were only shocked. Indeed, cortisol concentrations in fish 15 min after a single electroshock did not differ significantly from time 0 fish receiving electroshock and marking.

Studies investigating the effects of repetitive electroshocking on fish have reported that growth rates may be reduced (Gatz et al. 1986; Gatz and Adams 1987) and mortality may be induced in a predictable manner on fish populations (Saul 1980). However, the time intervals between successive electroshocking treatments in these studies were large relative to what might be considered standard protocol in a depletion based population estimate (Libosvarsky 1966; Bohlin and Sundstrom 1977; Peterson and Cederholm 1984; Hankin and Reeves 1988). Our information provides insight into stress responses of fish that are subjected to multiple electroshocks in a stream but not captured, which may be more common than might be expected, especially in areas with a lot of instream cover.

The cortisol dynamics of trout receiving multiple electroshocks indicated that cortisol was not liberated cumulatively, at least not in the manner reported by Barton et al. (1986) for healthy chinook salmon Oncorhynchus tshawytscha subjected to acute, multiple handling experiences. However, a cumulative effect may become manifest in the secondary stress response, as evidenced by the relatively long

recovery periods of lactate concentration in fish receiving multiple electroshocks. We surmise that the severity of the shocking stress was sufficient to produce a maximal response, and that no capacity was left in the interrenal tissue to further elevate cortisol after subsequent shockings. However, Strange et al. (1977) noted that plasma concentration of cortisol is a function of secretion and clearance; therefore, the lack of a further increase in cortisol after the third shock may have been due to an increased clearance rate. As judged by the magnitude of the maximum change, the response of lactate to multiple electroshocks was greater than that to a single shock, shocking plus marking, or a single handling. Although lactate concentrations for cutthroat trout receiving a single electroshock were higher than values reported by Schreck et al. (1976) for shocked rainbow trout, the recovery of lactic acid levels in the blood was similar to that noted by Black et al. (1959) and Schreck et al. (1976) for rainbow trout and Burns and Lantz (1978) for largemouth bass Micropterus salmoides. Fish receiving multiple electroshocks maintained elevated lactate concentrations for 6 h. This period of elevated lactate concentrations may be critical to the health of the fish because, as stated by Schreck et al. (1976), it apparently reflects the period of anaerobic muscular activity (i.e., severe exercise). Although the proximate cause of death in fish after severe exercise is speculative (Wood et al. 1983), high levels of lactate in the blood may be a factor contributing to mortality of fish (Black 1958; Parker and Black 1959; Parker et al. 1959; Caillouet 1971). Whether high levels of lactate in fish released back into a stream contribute to mortality may depend on environmental factors such as

temperature (Dean and Goodnight 1964) or the individual fish, as it has been suggested there is considerable variation in susceptibility to increased blood lactic acid (Caillouet 1967). Indeed, individual fish variability, coupled with disturbance to the tanks caused by periodic sampling, may explain differences between trials in both experiments and variable lactate concentrations in control fish.

Recovery from the stresses of shocking and marking, as judged from behavioral observations at both Mill Creek and the artificial stream, are generally supported by the recovery characteristics of the physiological systems. Based on our findings, a recovery period of 3 to 6 h after release of marked fish might appear to be a reasonable assumption. To provide some insight into the relation between behavior and physiology, we correlated feeding and aggression rates of wild and hatchery fish from our artificial stream experiments with cortisol concentrations from our physiological experiments. There was an inverse relation between both feeding ($r = -.77$) and aggression ($r = -.57$) rates and cortisol concentrations, which makes intuitive sense; when a fish "feels" well physiologically, it will be evidenced externally in the form of normal behavior. However, because of the behavioral changes observed in fish at Mill Creek and the longer recovery period required for wild trout in the artificial stream, the 3- to 6-h period of recovery may be an underestimate for applied field situations.

In conclusion, the procedures involved with common fish population size estimators subjected fish to considerable physiological stress and altered normal behaviors of stream-dwelling cutthroat trout. Because these responses were highly variable and generally lasted for

several hours, the likely consequences are that some assumptions of population size estimators may be invalid. In particular, we refer back to a question posed by Schreck et al. (1976) based on their findings that rainbow trout failed to recover from electroshock within the span of a working day: Would the assumption of equal vulnerability be met in mark-recapture estimates where fish are marked, released, and recaptured on the same day? Our findings suggested that this assumption would appear to be invalid; we therefore recommend that the minimum time between mark and recapture runs be at least 24 h. Rapid observations by snorkeling may be used to count marked fish and observe behavior in a section before a recapture run is started to ensure the validity of the assumptions. Habitat quality, i.e., the amount and type of instream cover, seems to be an important variable to consider in stream electrofishing population estimates. Electrofishing may fail to produce adequate electrotaxis if fish are in low conductivity water and under heavy cover. We acknowledge that the efficacy of electrofishing depends on many factors and our results are not applicable to all situations. However, an understanding of the fundamentals of electrofishing can only help maintain the effectiveness of a technique seemingly all too often taken for granted.

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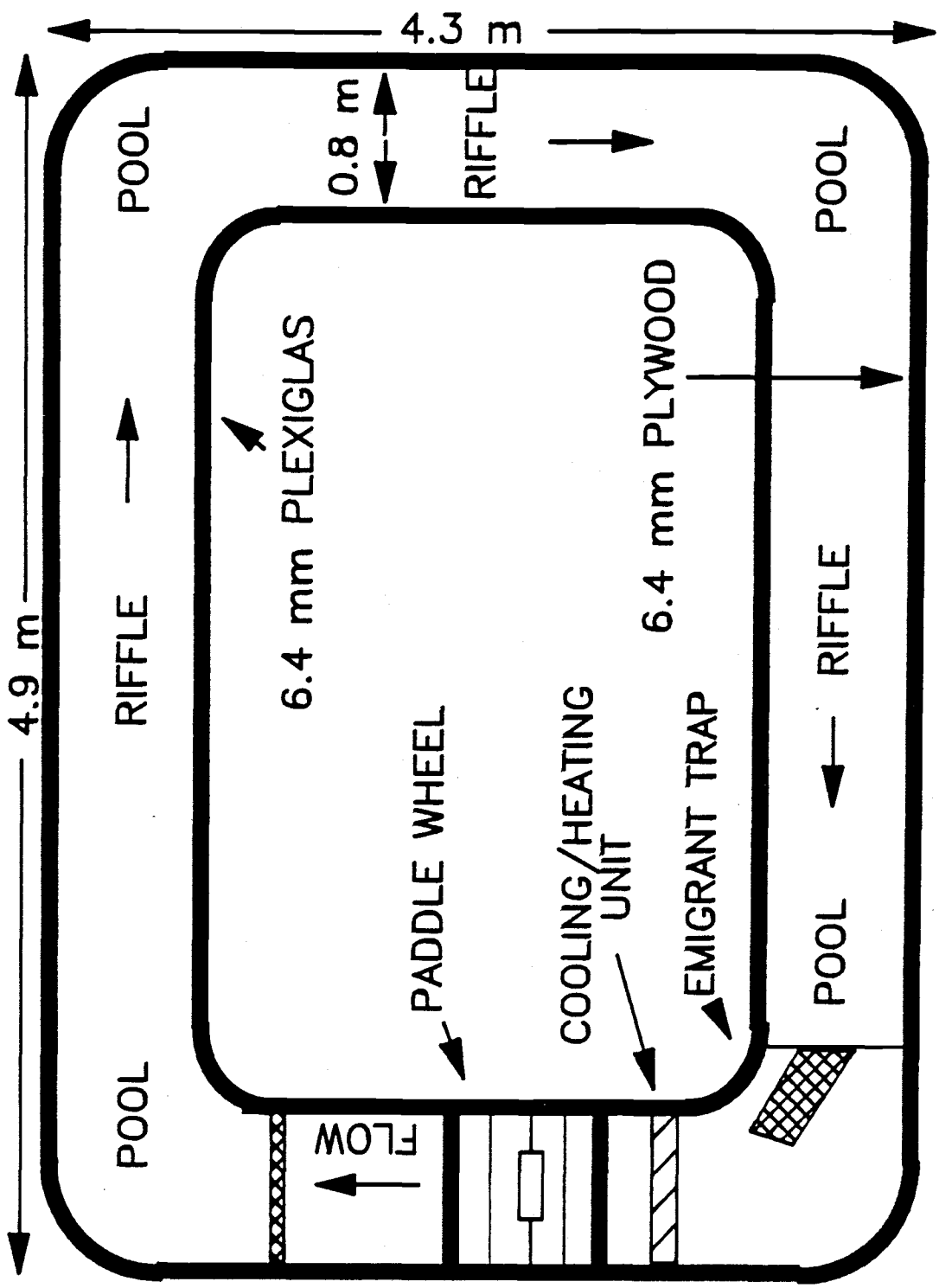
APPENDIX

Figure 9. Diagram of the artificial stream used in investigating the effects of electrofishing plus marking on the behavior of hatchery and wild cutthroat trout.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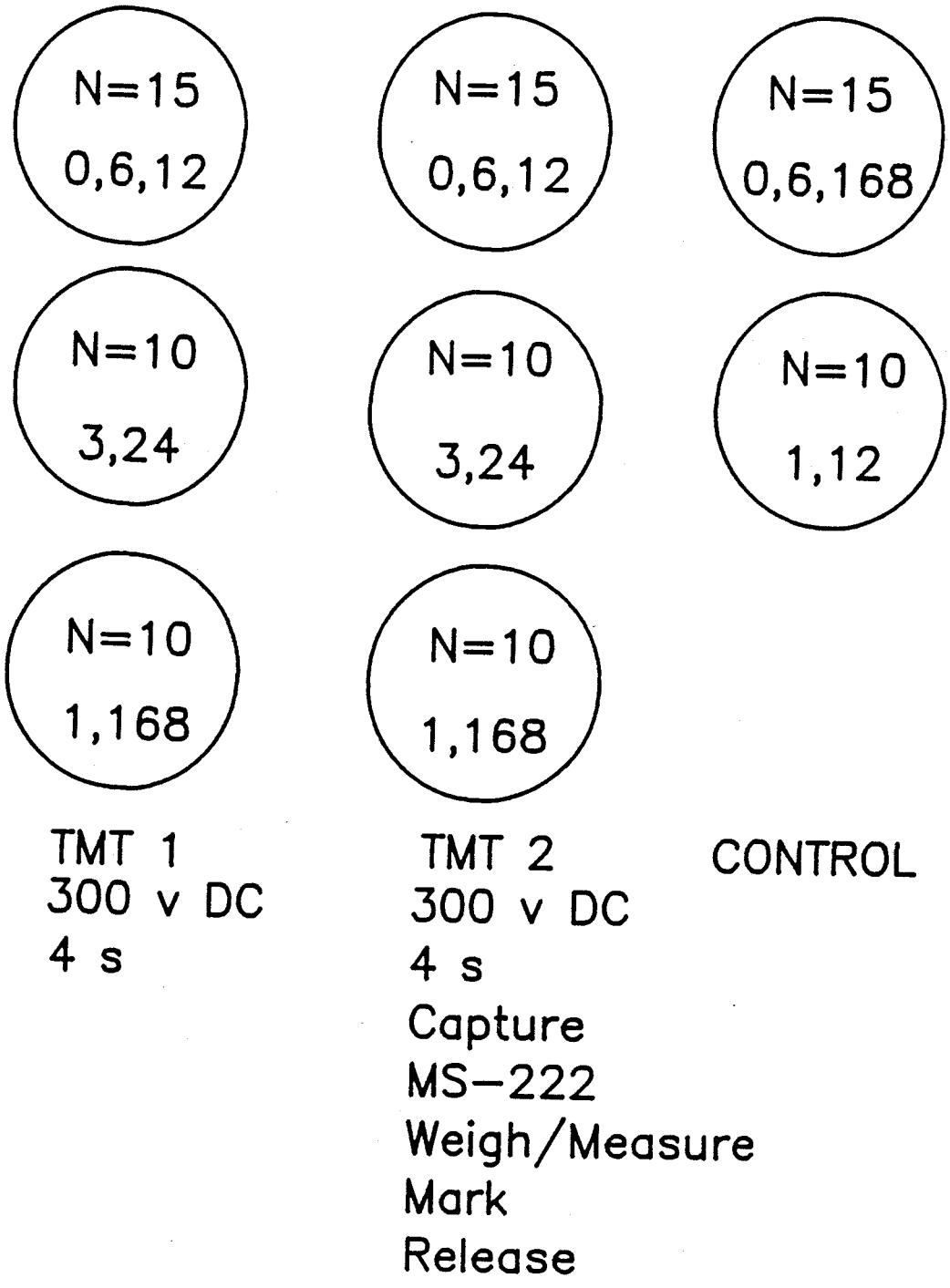
4.9 m
POOL
0.8 m
RIFFLE
POOL
RIFFLE
6.4 mm PLEXIGLAS
6.4 mm PLYWOOD
RIFFLE
PADDLE WHEEL
COOLING/HEATING UNIT
EMIGRANT TRAP
POOL
POOL
FLOW

Figure 9

Figure 10. Experimental design used in physiological experiments investigating the effects of a single electroshock (TMT 1) and electroshock plus marking (TMT 2) on cutthroat trout. Circles represent replicate tanks within a treatment; N is the number of fish in the tank; numbers within circles are the sampling intervals (h) post-treatment. Two complete trials of the experiment were conducted.


N=15
0,6,12
N=15
0,6,12
N=15
0,6,168
N=10
3,24
N=10
3,24
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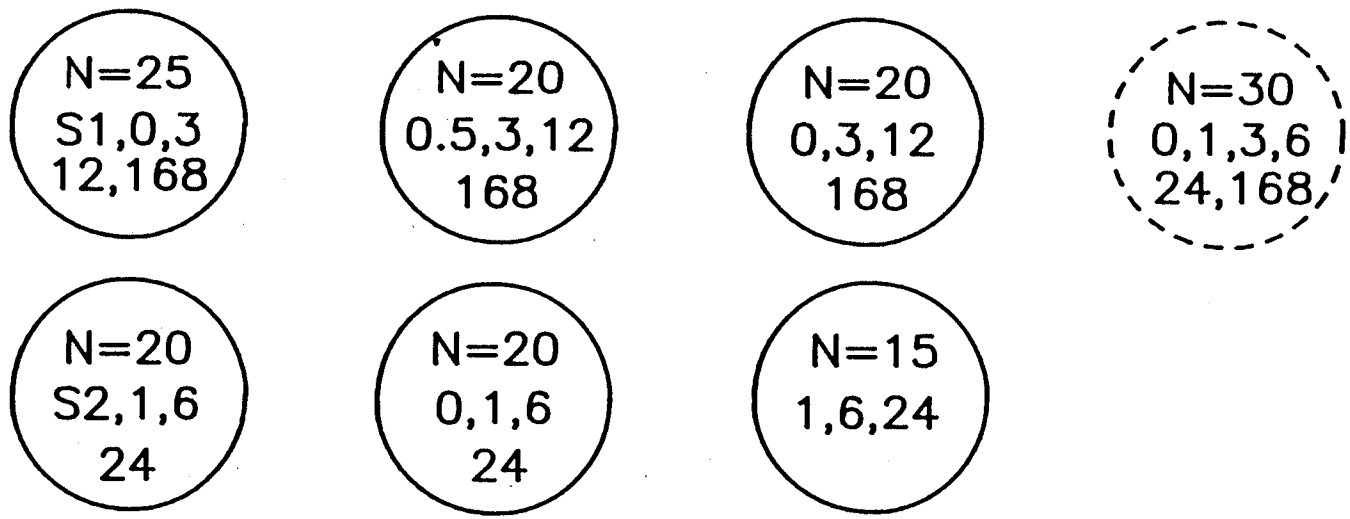
1,12
N=10
1,168
N=10
1,168
TMT 1
300 v DC
4 s
TMT 2
300 v DC
4 s
Capture
MS-222
Weigh/Measure
Mark
Release
CONTROL

Figure 10

Figure 11. Experimental design used in physiological experiments investigating the effects of a multiple electroshock (TMT 1), single electroshock (TMT 2), and a 30-s handling stress (TMT 3) on cutthroat trout. Circles are replicate tanks within a treatment. N is the number of fish in the tank; numbers within the circles are the sampling intervals (h) post-treatment. Two complete trials of the experiment were conducted.

Figure 11



TMT 1
500 v DC
8 s
0.5 h
shock
0.5 h
shock

TMT 2
500 v DC
8 s

CONTROL

TMT 3
dipnet
confine
30 s