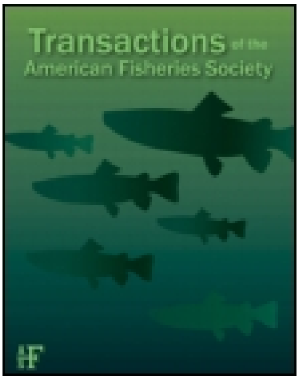

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Changes in Avoidance Response Time of Juvenile Chinook Salmon Exposed to Multiple Acute Handling Stresses

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Abstract.—A simple behavioral test was devised to determine the effects of multiple acute stresses on the avoidance response time of fish. Groups of freshwater-adapted juvenile chinook salmon *Oncorhynchus tshawytscha* were exposed to a sudden overhead light and the time it took each fish to reach cover was noted. Tests were done on fish stressed one, two, or three times with 3 h between stresses, and on fish 1, 3, 6, and 24 h after each level of stress; unstressed fish served as controls. All unstressed fish reached cover within 15 s. Stressed fish took longer to reach cover (often several minutes), with the greatest delay in response occurring immediately after the stress. There was a gradual decrease in response time with recovery from the stresses. Exposure to two or three consecutive stresses increased the delay in response time over that of fish exposed to a single stress, indicating that the effects of the stresses were cumulative.

Juvenile cultured salmonids are subjected to handling stresses while being raised in hatcheries, during transportation to release sites, and in certain by-pass operations designed to transport them around hydroelectric dams. Physiological studies have shown that exposure to a single handling stress causes changes in plasma hormone levels, elevations of plasma glucose and lactic acid, changes in blood pH and electrolyte levels, histological changes, depletion of liver glycogen, and suppression of the immune system (Pickering 1981). Barton et al. (1986) examined some of the above responses in freshwater-adapted chinook salmon *Oncorhynchus tshawytscha* after two or three consecutive handling stresses and found that the physiological responses were greater after two and three stresses than after a single stress.

Because stress affects a number of physiological conditions, it is important to know if stress also affects the performance of the fish. Behavioral tests are one way of determining sublethal effects of stress on the whole organism and have been used for many years in toxicity testing (Sprague 1971). One simple type of behavior test is the stimulus-response experiment in which some type of stimulus is applied to the fish and the response is measured quantitatively or qualitatively, or both. For example, Webb (1975) applied an electrical shock to fish and measured their reaction time and acceleration with high-speed photography.

The first objective of our study was to determine if handling stress reduces the tendency of juvenile chinook salmon to respond to a stimulus. The ability of fish to go to cover after sudden exposure to an overhead light was chosen for the stimulus-response behavior because of simplicity of experimental design and relevance to research on transport stress. When fish are transported by truck for stocking or for bypassing hydroelectric dams, they are held in the dark. At the release site, they are suddenly exposed to daylight as they are transferred from the trucks to the receiving waters. In order to avoid aerial or aquatic predators, the fish must quickly find cover. Previous physiologial studies have shown that, upon release, transported fish are stressed (Barton et al. 1980; Specker and Schreck 1980). If stress reduces the ability of fish to seek cover, it could decrease their chance for survival.

A second objective of this study was to determine if two or more acute stresses in succession cause additional changes in the tendency of juvenile chinook salmon to seek cover. In transport, fish are handled in the loading and unloading procedures, both of which are stressful (Barton et al. 1980). If subsequent stresses further reduce a fish's ability to respond to stimuli, they could affect the fish's chances of survival.

Methods

Fish.—Juvenile spring chinook salmon (15.0 cm mean fork length; 41.4 g mean wet weight) were obtained from Eagle Creek National Fish Hatchery and transported to the Oregon State University Smith Farm research facility in Corvallis. Before experimentation, the fish were acclimated for at least 2 weeks in 0.6-m in diameter, circular,

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flow-through tanks containing approximately 200 L and receiving 4 L/min of 12 ± 1°C aerated well water. There were 70–80 fish in each tank. The fish were kept on a natural photoperiod (12-h-light: 12-h-dark) and fed daily with Oregon moist pellets at a rate of about 1.5% body weight/d.

Apparatus.—Behavior experiments were carried out in three wooden Y-troughs each 0.7 m wide, 2.5 m long, and 0.25 m deep (Figure 1). The flow-through troughs received 4 L/min of 12 ± 1°C aerated water and were equipped with three gates to divide the troughs into compartments. These gates could be pulled upward with a pulley system to allow the fish undisturbed access to other compartments. A permanent 44-cm-long black plastic cover was fixed to the center compartment of each trough. Two 20-W fluorescent lights were positioned 38 cm above the Y-troughs, one over the arm area and the other over the leg area. The arm areas of each trough were fitted with removable black plastic sheets that completely covered the arms. The leg portion of the apparatus below the leg gate was not used in these experiments.

Response time.—Response times were measured by exposing fish to a sudden continuous overhead light and measuring the time it took for each fish to swim under cover. Preliminary experiments were done to determine the number of fish to use per trial, the size of the cover, the distance to cover, the type of cover, and the position of the light source.

A standard handling stress consisted of holding the fish in a dip net in the air directly above the trough for 30 s; unstressed fish served as controls. Fish were stressed either one, two, or three times, with a 3-h delay between each stress as in the multiple-stress protocol of Barton et al. (1986). The behavioral response was then measured either immediately or after a 1, 3, 6, or 24-h recovery period.

Each behavioral test was performed as follows: Six fish were introduced to each arm of the Y-trough with the gates between the arms and the permanent cover closed. The holding tanks were close to the experimental apparatus to minimize transfer time (it took about 5 s between the time that the fish were netted and the time that they were placed in the apparatus). The arms of the trough were then covered with black plastic and the fish were allowed to adjust to the apparatus overnight. The next morning, fish, with the exception of the control groups, were captured within 5 s by a net the same width as the trough and then subjected to the standard handling stress. After each stress, the fish were returned to the apparatus and covered until the next stress or until the appropriate recovery time had elapsed. After the recovery period, an avoidance response trial was performed that consisted of turning on the fluorescent light above the arms of the Y-trough while simultaneously removing the cover and opening the gate between the arm and the covered center compartment. The time taken for each fish to swim under the permanent cover was recorded. A trial ended when the last fish reached cover or after 30 min, whichever came first. Some aspects of the behavior of the fish during the trials were also noted, such as whether the fish came out of cover again, whether the fish would explore the tank or just remain still, and the position of the fish at the top or bottom of the water column.

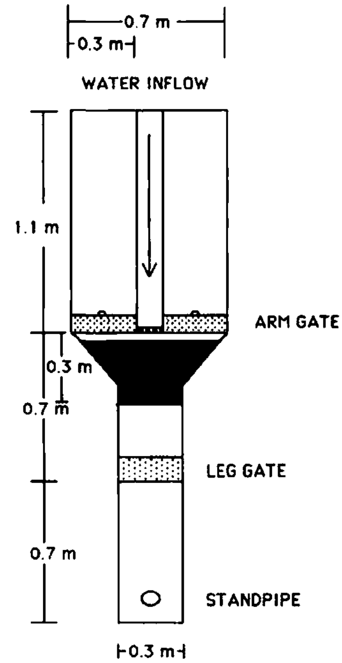

FIGURE 1.—Diagram of Y-trough apparatus used to test response times of juvenile chinook salmon to light. Black section represents permanent 44-cm-long black plastic cover.

Initially, two replicates of six fish each were used for each experimental group and the data were pooled. Because a maximum of six trials (three Y-troughs each with two compartments) could be run per day and the experimental design required 32 trials (16 groups of two replicates), groups were assigned randomly to troughs and days. On each day, the initial stress was applied at about 0900 hours. For some experimental groups, a third replicate was run to verify results. Because fish occasionally escaped from the apparatus and because three replicates were used for some groups, total sample size per group varied from 10 to 17 fish.

The median response time was calculated for each group of fish. Median response time was used because data did not follow a normal distribution (more fish responded early than late) and because some fish did not respond in the allotted time period so that calculation of the mean and standard error could not be exact (Sokal and Rohlf 1969). The nonparametric Kruskal-Wallis test was used to examine the effect of stress over time and to determine whether repeated stresses had a cumulative effect. A nonparametric multiple-comparison test (Daniel 1978) was used to compare medians when median response times were significantly different.

A second experiment was conducted in which fish not subjected to a handling stress were tested in the apparatus individually instead of in groups of six. Response times of the single fish were compared to those of fish tested in groups by the nonparametric Mann–Whitney *U*-test.

Results

The median response times of stressed fish were significantly greater than those of fish that were not subjected to the 30 s handling stress (Figure 2). The fish that did not receive the stress darted to cover almost immediately (0.02–0.23 min). Stressed fish generally took longer to reach cover (0.02 to more than 30 min); the longest median response times occurred immediately after stress and a gradual decrease in median response times was evident with recovery from stress. Response times of stressed fish were highly variable, as indicated by the range of response times in each group (Table 1). In all groups, more fish responded at the beginning of the range than at the end. In general, the range of response times decreased in size with recovery from stress as fish responded in shorter time intervals.

Results of the multiple-comparison tests revealed that exposure to two or three consecutive stresses increased both the median response times and the recovery times, though not significantly except for the 24-h recovery groups ($P < 0.05$). Ranges of response times remained high through-

TABLE 1.—Range of time (min) for juvenile chinook salmon to swim to cover after exposure to a sudden overhead light for fish stressed one, two, or three times (3 h between stresses) and tested immediately (0 h), and for fish allowed to recover 1, 3, 6, or 24 h after stress. The range represents the minimum and maximum response times for each group. Trials were stopped after 30 min. Controls were unstressed, and responded to light in 0.02–0.2 min.

Time after stress (h)	Response time (min) after		
	One stress	Two stresses	Three stresses
0	0.13–30.2	0.02–30+	2.8–30+
1	0.12–5.8	0.15–30+	0.1–30+
3	0.02–12.1	0.03–30+	0.02–23.1
6	0.05–2.2	0.03–23.8	0.02–30+
24	0.02–2.3	0.6–7.3	0.02–30+

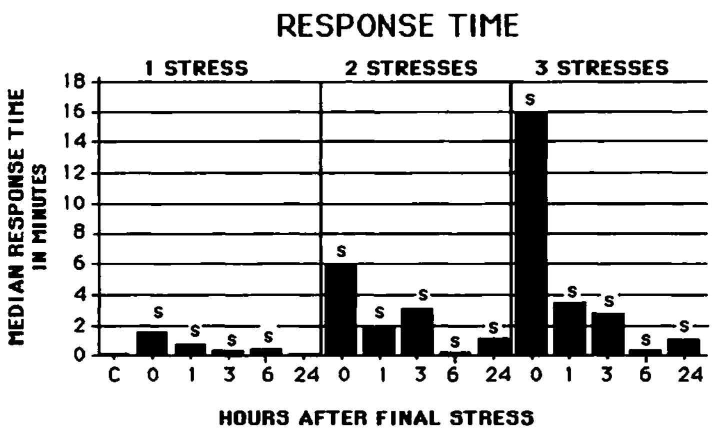

FIGURE 2.—Median response times in minutes (i.e., time to swim to cover after exposure to a sudden overhead light) of fish stressed one, two, or three times (3 h between stresses) and of fish allowed to recover 0, 1, 3, 6, or 24 h after stress. C indicates control fish (i.e., unstressed fish subjected to the behavioral test). Bars with an s over them indicate that they are significantly different from controls ($P < 0.05$).

out the recovery period of the three-stress group while variability tended to decrease with recovery time in the two-stress and one-stress groups.

Before seeking cover, stressed fish tended to remain motionless either on the bottom or the top of the water column, and usually against a side. In a few cases, fish remained inverted for several minutes after the stress. Stressed fish tended to move around slowly or not at all. Some appeared oblivious to movement of other fish around them even if bumped by the swimming fish, and some swam at the surface with their snout out of water. Preliminary experiments also showed that stressed fish exposed to the light were less likely than unstressed fish to respond to an additional stimulus such as a shadow passing over the water or a hand splashing the water (personal observations).

The unstressed fish that were tested individually had a median response time of 1.99 min (range, 0.04–6.22 min; N = 10). This was significantly different from the control fish tested in groups; they had a median response time of 0.05 min (range, 0.02–0.23 min).

Discussion

The results of our study indicated that acute handling stresses not only increased the time that it took for fish to respond to a stimulus but also elicited general lethargic behavior. Other investigators have noted similar changes in the behavior of fish after a handling stress. Bouck and Ball (1966) observed that rainbow trout *Salmo gairdneri* stressed by hooking were lethargic and stopped feeding. Herting and Witt (1967) found that seined bluegill *Lepomis macrochirus* behaved sluggishly and did not exhibit darting avoidance reactions when confronted with a predator; this caused increased vulnerability to predation. Other stresses, including thermal stress (Sylvester 1972; Coutant 1973) and various pollutants (Hatfield and Anderson 1972; Bull and McInerney 1974; Kleerekoper 1976; Woltering et al. 1978; Henry and Atchison 1979; Hedtke and Norris 1980), also cause decreased activity, stuporous behavior, and increased susceptibility to predation.

We also demonstrated that fish subjected to two or more stresses had less tendency to respond to a stimulus and required longer recovery times than fish stressed only once. These results indicated that the degree of response to a given stress increased when it occurred shortly after a previous stress; however, the differences in response times between various levels of stress were not statistically significant for most groups. This was probably due to the high variability between individuals, not uncommon in behavioral studies (Martin and Bateson 1986), and to the reduced sensitivity of nonparametric statistics relative to analysis of variance in detecting departures from the null hypothesis (Sokal and Rohlf 1969).

The considerable variability in response times among the fish exposed to the handling stresses indicated that some fish were better able than others to respond after stress. In a natural environment with predators or other hazards, a short response time could be a selective advantage.

We also observed that unstressed fish tested individually had a longer median response time than did fish tested in groups. Fish often use visual and chemical signals as cues for social behavior (Bond 1979). In groups, there may be some social interactions in which some fish follow others to cover, thus decreasing their median response time over fish tested individually. Such group behavior may be disrupted by stress. In some of our stressed groups, particularly those tested immediately after a stress, many fish remained motionless even if bumped by other fish. It is possible that the stressed fish did not either perceive or respond to cues that trigger social behavior.

A problem in performing this type of behavioral test is that fish must be transferred to the study apparatus and this involves brief handling, which is stressful. In our study, handling was kept to a minimum by locating the holding tanks near the apparatus. The fish were also given 18–24 h to recover from the transfer before starting the behavior test. Thus, although the control fish may not have been totally unstressed, they were at least less stressed than fish subjected to the 30 s handling stresses. Also, because all groups of fish were handled in the same manner in the transfer, the differences in response times should be due to differences in experimental treatment.

The possibility that the changes in response time were due to a diurnal rhythm and not to the applied stresses was considered. However, preliminary trials on control fish taken at various times in the morning and early afternoon had median response times ranging from 0.04 to 0.11 min, similar to the controls in our experiment (personal observations).

Behavioral differences between individuals are sometimes correlated with physiological changes. For example, Ejike and Schreck (1980) found that dominant fish have lower plasma cortisol levels than subordinate fish. For comparative purposes, the timing of stresses and the recovery times used in our study were chosen to coincide with the timing of physiological measurements made by Barton et al. (1986). The longest response times to a sudden overhead light occurred immediately after stress, followed by an improvement in response time with increasing recovery time. However, for selected plasma constituents, Barton et al. (1986), using the same test protocol, found that the peak responses occurred (depending on the number of stresses) at 0.5–1 h after stress for cortisol and lactic acid, 3–6 h after stress for glucose, 1–12 h after stress for sodium, and 0.5–3 h after stress for potassium. Thus, the longest behavioral response times occurred before these peak physiological stress responses would be expected. However, there are other physiological responses that are more immediate, for example, stimulation of the sympathetic nervous system and catecholamine release (Mazeaud and Mazeaud 1981), that may contribute to the delay in response time. The high response times evident immediately after the stress could also be a result of lack of oxygen to the brain causing a stunning and disorientation similar to that documented for mammals (Selkurt 1982; Ganong 1983). Thus, as the physiological stress response reached a maximum and the fish recovered from the oxygen lack, its response time to light shortened.

This view is consistent with that of Redding and Schreck (1983) who concluded that the increase in plasma cortisol and the secondary responses it causes are adaptive responses to compensate for the energetic costs of stress. However, the response times did not completely return to prestress levels, although Barton et al. (1986) found that the selected plasma constituents recovered in 6–12 h, an indication that the stressed fish may have had an increased sensitivity to environmental factors after 24 h even though some of the physiological factors probably would have recovered (Barton et al. 1986). Other physiological factors such as plasma lactate and number of circulating lymphocytes can take several days to recover from a single stress (Pickering et al. 1982) and may be involved in this increased sensitivity. Other studies indicate increased sensitivity of fish to a second stress. Specker and Schreck (1980) observed that transportation reduced the ability of coho salmon *Oncorhynchus kisutch* to withstand a second stress of crowding confinement as indicated by increased mortality and higher cortisol levels. Barton et al. (1985) noted that elevations in plasma cortisol in response to handling were higher in rainbow trout first exposed to low environmental pH than in handled control fish exposed to a normal pH. Conversely, Pickering and Pottinger (1987) observed that poor water quality suppressed cortisol responses during confinement stress.

In conclusion, acute handling stresses increased the response time of juvenile chinook salmon to light. Likely consequences of both this delay and the general lethargic behavior in newly stocked fish are increased exposure and vulnerability to predation or other environmental hazards. Because these changes in behavior can adversely affect fish survival, they should be considered in conjunction with physiological changes when management decisions are made. For example, in transporting fish for stocking, efforts could be made to reduce the severity of the handling and to provide optimum conditions for recovery. Releasing the fish at night might also be beneficial because the need to find cover would not be as urgent.

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