Stocking and Handling-induced Stress in Red Drum Fingerlings

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Abstract: Stress induced by handling, hauling, and net confinement was evaluated in 0.2–0.8-g red drum fingerlings (*Sciaenops ocellatus*). Changes in plasma glucose concentrations were used as general indicators of stress, and changes in plasma chloride concentrations were used as indicators of osmoregulatory dysfunction. Hematocrit dynamics were also monitored. Net confinement (for ≤ 9 hours) and transport (for ≤ 10.0 hours) caused elevated plasma chloride concentrations and decreased hematocrit. Changes in plasma glucose concentrations in net confined and hauled fish were not consistent. Fifty percent cumulative mortality was observed after 9 hours of net confinement. Almost no mortality occurred during transport. Fingerling red drum, subjected to short term stressors, such as standard hauling and stocking procedures, generally respond well, but some may die shortly after removal of the stressors.

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The red drum is a popular game and commercial fish along the Texas coast. Increases in fishing pressure and lack of regulatory control resulted in the decline of coastal red drum populations during the 1970s and early 1980s (Matlock 1982). Texas Parks and Wildlife Department, in conjunction with the Gulf Coast Conservation Association, established intensive aquaculture, stocking, and management programs in order to help restore red drum populations. Present culture and stocking operations are centered at the John Wilson fish hatchery, Corpus Christi, Texas.

Fish are often stressed during culture and stocking procedures. Examples of stressors identified for fish include crowding, handling, transport, low dissolved oxygen, extreme temperatures, and osmotic shock. Nonspecific physiological responses occur when an animal is exposed to a stressor (Selye 1950). These responses in fish include release of corticosteroid hormones from interrenal tissue and

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release of catecholamines from chromaffin cells (Mazeaud and Mazeaud 1981). Releases of corticosteroids and catecholamines result in secondary increases in plasma glucose concentrations (Wedemeyer 1972, 1976; Specker and Schreck 1980; Strange 1980; Mazeaud and Mazeaud 1981; Schreck 1981; Carmichael et al. 1984).

Plasma chloride fluctuations have been used as an indicator of osmoregulatory dysfunction during handling, hauling, and stocking procedures (Wedemeyer 1972, Barton et al. 1980, Pickering et al. 1982, Carmichael et al. 1984). Stress induced changes at the gills precipitate a gain or loss of water and an increase in ion permeability (Pic et al. 1974, 1975).

The objectives of this study were to document mortality and characterize specific physiological changes during and after the handling and hauling of red drum. Changes in plasma glucose concentrations were used as indicators of stress, and changes in plasma chloride concentrations were used as indicators of osmoregulatory dysfunction. Hematocrit was also monitored to evaluate its effectiveness as an indicator of stress.

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Methods

Red drum fingerling 20–35 mm, SL, and 0.2–0.8 g were used in all experiments. The 30- to 35-day-old fingerlings were the product of indoor spawning by the control of photoperiod and temperature and extensive rearing in fertilized 0.81-ha ponds. At harvest, ponds were drained for 2 days. Fish were collected at the kettle with a dip net, weighed, and then transferred into waiting hauling tanks containing nitrofurazone (10 mg/liter) in water similar in salinity to the pond water. Four experiments were conducted: a 3-hour net confinement; a 9-hour net confinement; transporting fish to the stocking site and following their recovery for 1 day, *in situ;* and transport of fingerlings for 6.5 to 10 hours. Each experiment was conducted in triplicate and data within the replicates of each experiment were pooled.

Net confinement was conducted in cement holding troughs receiving a constant supply of aerated seawater to maintain satisfactory water quality. Fish were crowded in a net and forced into constant contact with one another. As fish were sampled, net size was adjusted to maintain a similar density throughout each trial. The initial 3-hour net confinement was conducted in 46 g/liter seawater (796 milliequivalents/liter chloride). Samples were taken at 15-minute intervals. Dissolved oxygen was 5.0–6.0 mg/liter and temperature was 29° C. Fish were collected from the kettles of ponds prior to lowering water levels for harvest and values from the initial fish sampled in this trial were designated "baseline."

In a second net confinement, fish were confined in a net for up to 9 hours in 36 g/liter seawater (623 meq/liter chloride). Dissolved oxygen was 5.5–6.5 mg/liter and temperature was 27° C. Fish were collected from kettles of ponds already lowered

for harvesting, and initial plasma values were designated "time zero." Samples of blood were taken after 0.5, 3, 6, and 9 hours of confinement in the net.

During normal hauling and stocking procedures, fish were transported in trailer-mounted hauling tanks (15–32 g fish/liter) 113 km (approximately 2.5 hours) by truck to a barge loading site. They were then transferred to similar tanks mounted on a barge and further transported to a predetermined stocking site in the Espiritu Santo Bay estuarine grass flats. As fish were in transit (from 0.5 to 1.5 hours) the barge tank was tempered to match ambient salinity and temperature. Samples were collected from fish removed from kettles of ponds lowered for harvesting (time zero); 30 minutes later from fish in the hauling tanks before leaving the hatchery; in transit; at the barge site before being loaded into tanks on the barge; and before release at the stocking site. Some fish were placed into holding cages at the stocking site and sampled 24 hours later. Dissolved oxygen was 8.0 mg/liter, temperature was 25° C, and salinity was 28 g/liter (484 meg/liter chloride) at the stocking site at the time of stocking.

A second transport experiment consisted of hauling the fingerlings for up to 10.0 hours. Salinity in the ponds at time of transport was 36 g/liter (623 meg/liter). Dissolved oxygen was 5.5–6.5 mg/liter and temperature was 27° C. Samples were collected from fish immediately after removal from the ponds, 30 minutes after being placed in the hauling tanks, and 6.5 to 10.0 hours in transit.

Blood was collected for analysis of hematocrit, glucose, and chloride by severing the tail at the caudal peduncle and touching the tip of a heparinized capillary tube to the hemal vessels. Dead or moribund fish were not sampled. During each sampling, blood from 10 to 25 fingerlings was collected in 1 capillary tube because of the small size of the fingerlings. Prior to collection of blood, in order to reduce seawater chloride contamination, fish were briefly washed in fresh water and then dried using a paper towel. Blood collection took 4–6 minutes from the initial disturbance to completion of bleeding of fingerlings into the tube. Capillary tubes were centrifuged within 1 hour of sampling and hematocrit determined. The plasma samples were frozen using dry ice, then were stored at −20° C before analysis.

Glucose concentrations were determined on 10-microliter samples of plasma using a kit (Sigma Chemical Co., Saint Louis, Mo.) based on glucose oxidase and peroxidase; 0-dianisidine was the chromagenic oxygen receptor. Correlation coefficients for glucose standards exceeded 0.99. Chloride concentrations were determined using 10-microliter samples of plasma with a Buchler, Cotlove chloridometer (amperometric-coulometric titration). Dissolved oxygen and temperature were measured using a Yellow Springs Instrument Company, oxygen and temperature meter. Salinites were measured using a American Optical refractometer. Statistical analyses were not applied to the data due to pooling of the samples.

Results

Experiment One—Plasma glucose concentrations fluctuated between 35 mg/100 ml and 55 mg/100 ml throughout the first 3 hours of net confinement (Fig. 1).

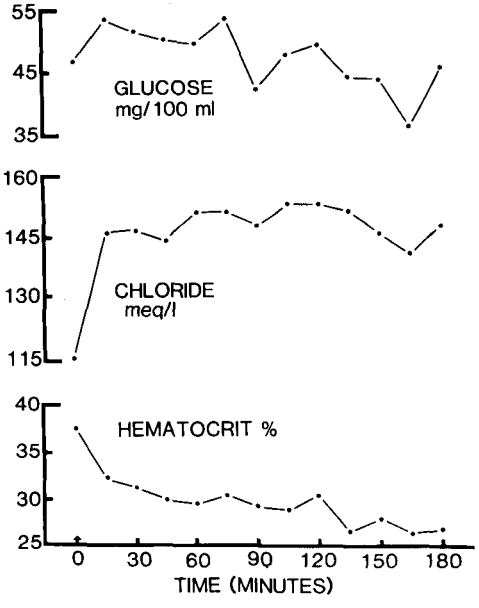

Figure 1. Blood characteristics of fingerling red drum harvested from a pond and then confined in a dipnet for 3 hours (time of initial disturbance, referred to as "baseline" and marked with an arrow). Each data point represents pooled samples of approximately 60 fish.

Plasma chloride increased 26% (from 115 meq/liter to 145 meq/liter) in 15 minutes following initial disturbance and remained elevated for the duration of the confinement. Hematocrit decreased (from 37% to 26%) during the 3 hours of net confinement (Fig. 1). Thirty-five percent of the confined fish died during the 3-hour study.

Experiment Two—Plasma glucose concentrations decreased 42% (from 95 mg/100 ml to 55 mg/100 ml) throughout 9 hours of net confinement (Fig. 2). Chloride concentrations increased within 30 minutes of the initial disturbance at time zero (from 113 meq/liter to 125 meq/liter) and remained elevated for the duration of the confinement. Hematocrit decreased from 34% to 24% during 6 hours in the net. Fifty percent mortality occurred after 9 hours of net confinement (Fig. 3).

Experiment Three—Glucose concentrations increased 100% within 30 minutes of time zero in fish harvested from ponds and placed in hauling tanks (Fig. 4). Plasma glucose levels then decreased from 140 mg/100 ml to 40 mg/100 ml from the time the fish left the hatchery until they reached the stocking site. Glucose remained low after 24 hours in fish maintained in the holding cages at the stocking site. Plasma chloride concentrations decreased 24% between the time the fingerlings left the hatchery until they reached the stocking site by barge and remained low after 24 hours in the holding cages. Hematocrit did not show any changes during this transport series. No mortality was observed during haulage.

Experiment Four—Plasma glucose concentrations decreased 21% (from 95 mg/100 ml to 75 mg/100 ml) between 30 minutes after initial disturbance and the time the hauling tanks returned to the hatchery (6.5–10 hours) (Fig. 5). Plasma chloride concentrations increased 32% (from 95 meq/liter to 125 meq/liter) after the

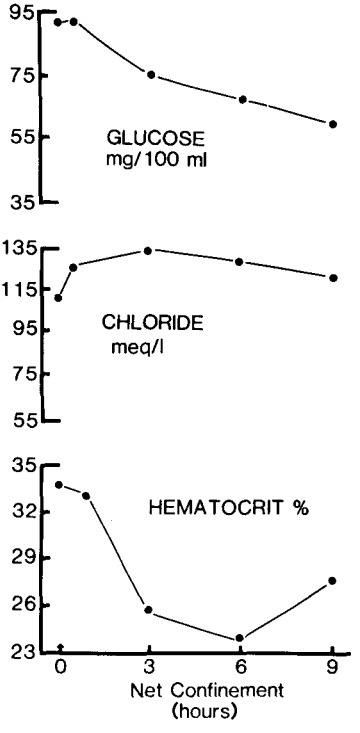

Figure 2. Blood characteristics of fingerling red drum sampled from a lowered pond and then confined in a dipnet for up to 9 h (time of initial disturbance referred to as "time zero" and marked with an arrow). Each data point represents pooled samples of approximately 60 fish.

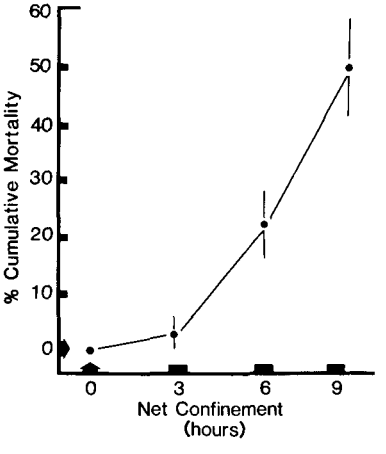

Figure 3. Cumulative mortality (% means + S.E.) of approximately 2000 fingerling red drum during 9 h of net confinement.

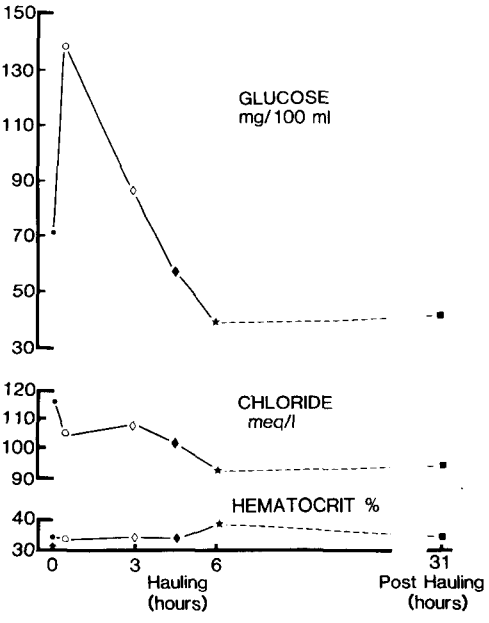

Figure 4. Blood characteristics of hauled fingerling red drum sampled first from a lowered pond and then periodically until release at the stocking site. Each data point represents pooled samples of approximately 60 fish. (●–taken at initial disturbance ("time zero"); ○–taken 30 minutes after loading into hauling tanks; ◇–3 h in transit; ◆–at barge site; ★–at stocking site; ■–24 h later in holding cages at stocking site).

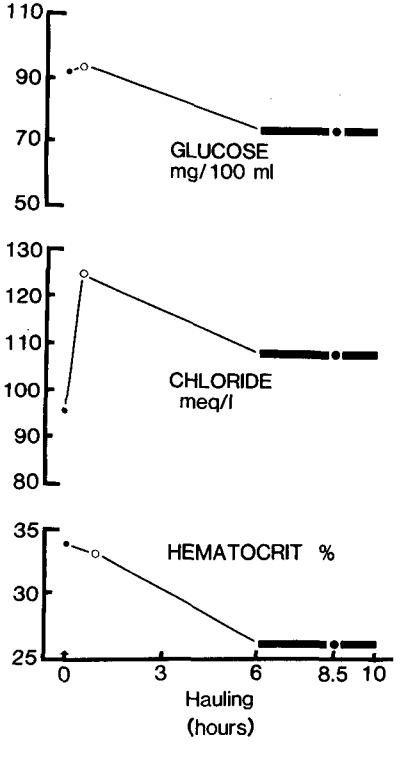

Figure 5. Blood characteristics of fingerling red drum hauled from 6.5 to 10.0 h. Fish were sampled from a lowered pond (time zero). Each data point represents pooled samples of approximately 60 fish. (●–initial sample marked with an arrow; ○–taken 30 minutes after loading into hauling tanks; ▬●▬–mean 8.5 h of transit.

initial disturbance and before leaving the hatchery. Chloride concentrations remained elevated throughout hauling, and during the first day after transport. Hematocrit decreased 24% (from 33% to 25%) throughout the haul.

Discussion

Fingerlings collected from the kettles of ponds prior to lowering water for harvest have been shown to have exhibited baseline plasma glucose concentrations (46.5 mg/100 ml) similar to undisturbed red drum (45.6 ± 8.3 mg/100 ml, mean ± S.D. N = 86) reported by Robertson (1984). In contrast, fingerlings collected from kettles of ponds lowered for harvest exhibited elevated glucose concentrations (83.3 mg/100 ml), suggesting that lowering ponds for harvest is stressful although the fingerlings are not handled directly. Handling procedures such as capture, crowding, and dipnetting, have been observed to be the most stressful in coho salmon *Oncorhynchus kisutch* (Specker and Schreck 1980), in muskellunge *Esox masquinongy* (Miles et al. 1974), and in rainbow trout *Salmo gairdneri* (Pickering et al. 1982).

In this study, glucose concentrations of fingerling red drum observed during the net confinement studies (Figs. 1, 2) and the final transport study did not show the expected increases observed in larger red drum (Robertson 1984) and other fish species (Wedemeyer 1972, 1976; Fletcher 1975; Strange 1980; Pickering et al. 1982). Robertson (1984) found no relationship between red drum size (130–260 mm SL) and glucose values within the range tested. During hauling and stocking (Fig. 4) a sharp increase did occur 30 minutes after the initial disturbance. Variable plasma glucose concentrations observed in this study may have occurred in part due to the differences in nutritional status of the fish, size of the fish, environmental pH, environmental temperature, and salinity (Nakano and Tomlinson 1967, Wedemeyer 1972, Barton et al. 1980, Specker and Schreck 1980, Pickering et al. 1982, Nikinmaa et al. 1983, Brown et al. 1984).

Fingerling red drum exhibited elevated plasma chloride concentrations in both net confinements and the final hauling experiment in this study. These changes in plasma chloride concentrations probably reflect the impaired ability of the animal to effectively ionoregulate in a hypertonic environment (concentrated seawater) while also responding to a stressor. Handling and hauling of freshwater fish in a medium hypoosmotic to plasma has been shown to have depressed plasma chloride concentrations in largemouth bass (Carmichael et al. 1984), smallmouth bass *Micropterus dolomieui* (Carmichael et al. 1983), coho salmon, steelhead trout *Salmo gairdneri* (Wedemeyer 1972, 1976), brown trout *Salmo trutta* (Nikinmaa et al. 1983), and muskellunge (Miles et al. 1974). Plasma chloride concentrations were elevated in hybrid stripped bass (*Morone saxatalis* × *Morone crysops*), hauled in a hyperosmotic medium (Tomasso et al. 1980). The winter flounder, *Pseudopleuronectes americanus*, exhibited a similar increase in plasma chloride concentration during stress in hyperosmotic conditions (Fletcher 1975). Release of catecholamines also affects plasma electrolyte flux during stress (Mazeaud and Mazeaud 1981). Endo-

genous catecholamines may have an effect on the water balance of the fish (Maetz 1974). In seawater-adapted grey mullet *Mugil capito,* catecholamine injections caused parallel changes in diffusion permeability (disruption of electrolyte balance by inhibition of salt extrusion at the gills) and osmotic permeability (increased osmotic water loss at the gills) (Pic et al. 1974, 1975).

In addition to metabolic disturbances, disruption of the ionoregulatory processes through physiological stess causes abrupt and persistent changes in the hemodynamics of teleosts (Kirk 1974; Carmichael et al. 1983, 1984). Electrolyte shifts which induce changes in osmotic permeability can result in an increase in hematocrit (hemoconcentration) in salt water fish (Mazeaud et al. 1977). Decreases in hematocrit in the 3- and 9-hour net confinements (Fig. 1 and 2) and in the second set of hauling experiments indicated hemodilution rather than hemoconcentration occurred in the fingerling red drum. These results were opposite to those seen in the winter flounder (Fletcher 1975), in which significant increases in hematocrit were observed when the fish was stressed in salt water (32–33 ppt). Rainbow trout stressed in salt water (28–30 ppt) displayed decreased hematocrit levels (Redding and Schreck 1983). However, the authors could not conclude the lowered hematocrit was the result of blood dilution because osmolality and shifts in electrolyte concentrations suggested hemoconcentration. Rainbow trout stressed in freshwater had increased hematocrit due to the osmotic shift of water from the blood into the muscle, rather than the addition of the cells to the blood by splenic contractions (Stevens 1968). At times of stress, red blood cells can be released by splenic contractions, causing increased hematocrit (Stevens 1968); or removed from circulation, as observed in lymphatic diseases, thus causing a decrease in hematocrit as observed in lower vertebrates (Guyton 1981).

During this study, an attempt was made to determine the degree of mortality in post-confined and post-hauled fingerlings. Seventy percent of the fish which survived the 9-hour net confinement of experiment 2 died by the second day after confinement. At the same time, lower mortality rates were observed in fish confined for 3 and 6 hours. Fish from the second hauling experiment exhibited 36% mortality 4 days after hauling. Most of the dead fish exhibited evidence of bacterial infection (*Vibrio sp.* as determined by J. E. Marks, College of Veterinary Medicine, Texas A&M University). Our experimental design was not adequate to distinguish among mortalities due strictly to handling and confinement, stress-induced epizootics, or incidental bacterial infections. However, high post-stress mortalities such as these should not be dismissed and warrant further research to accurately characterize delayed mortality which follows handling and transport.

In summary, the magnitude and duration of plasma glucose response in red drum fingerlings to handling and net confinement appear inconsistent. Hematocrit exhibited an uncharacteristic decrease rather than the expected increase in a hyperosmotic environment. Expected responses of plasma chloride were apparent during the stocking procedures and net confinement. In the related net confinements, a large differential in salinity (46 g/liter in the 3-hour net confinement and 36 g/liter in

the 9-hour net confinement) may have influenced the stress response. Also, the small size of the fingerlings may have been a factor in their varied physiological response to stressors.

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