# The Effects of Acute Handling Stress on the Secondary Stress Responses of Striped Bass (Morone saxtilis) and its hybrid (Morone chrysops x Morone saxtilis)

by

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# THE EFFECTS OF ACUTE HANDLING STRESS ON THE SECONDARY STRESS RESPONSES OF STRIPED BASS (MORONE SAXTILIS) AND ITS HYBRID (MORONE CHRYSOPS X MORONE SAXTILIS)

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The importance of understanding the stress response can not be underestimated in fisheries research or the aquaculture industry. Three studies were undertaken to quantify the secondary stress responses of striped bass and it's hybrid. These were measured as fluctuations in glucose, glycogen, lactate, and osmolality. Fish were stressed by aerial emersion in a dipnet. The first study was conducted with fingerling inland and anadromous striped bass. The three goals were to: determine if fingerlings responded with the General Adaptation Syndrome, if the two had different responses to the stress, and if feeding state (fed up until the day of the stress vs. starved for three days prior) had an effect on the stress response. The second study was conducted with two year old pure and hybrid striped bass. The two goals were to: determine any differences in the stress response, and to see if feeding state played a role in the response. The third study was conducted with hybrid fingerlings. This study looked at the ability of fed and three day starved fish to moderate their secondary stress responses after a handling stress, when placed in 0, 5, 10, or 15‰ saline recovery water.

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# SECONDARY STRESS RESPONSES TO ACUTE HANDLING BY FINGERLING INLAND AND ANADROMOUS STRIPED BASS, MORONE SAXTILIS

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Both fed and three day starved freshwater (ave. mass: 6.97g) and anadromous (ave. mass: 6.54g) striped bass fingerlings were held in dipnets above water for five minutes in groups of six. This acute stress caused a general adaptation syndrome response in the fish. Severity of the response to the stress was measured by quantitating levels of whole-body glucose, glycogen, and lactic acid in unstressed bass (considered standard level), and then at 30 minutes, 1 hour, 6 hrs, 12 hrs, 24 hrs, and 48 hrs recovery. Blood osmolality was also measured in the freshwater bass. At resting levels, both fed and starved freshwater bass showed significantly higher concentrations of the whole body parameters measured than anadromous bass. All four groups of bass showed a significant increase in lactic acid and glucose immediately after the stress, with a concomitant decrease in glycogen. Peak levels of glucose and lactic acid were similiar in the four groups. Nutritional state did not have an effect on the glucose and lactic acid responses, but did affect the glycogen response. The two starved groups did not return to control glycogen concentrations during the 48 hr recovery period. By 48 hours, both glucose and lactic acid levels had returned to pre-stress levels or lower. It is concluded that freshwater and

anadromous strains of fingerling striped bass do not differ in their sensitivity to an acute handling stress.

# INTRODUCTION

The handling of fish is a common, unavoidable procedure in fisheries management and the aquaculture industry. This handling is often a significant stress and handling has been known to result in mortality of fish (Yeager, 1990). Much work has been done on quantifying the physiological responses to stress (Adams, 1990; Barton & Iwama, 1991; Mazeaud et. al., 1977; Pickering, ed., 1981) in adult fish, but little work has been done on young, immature fish.

It is generally accepted that adult fish respond to stress with a phenomenon called the General Adaptation Syndrome (GAS) which was first described in mammals (Selye, 1950). The reactions of the fish are usually divided into 2 categories: primary and secondary responses (Mazeaud et. al., 1977). During the primary response, epinephrine concentrations spike rapidly from sympathetic nervous system activation, followed by a slightly delayed response of cortisol (Barton & Iwama, 1991; Carmichael et. al., 1984 a,b). Adrenocorticotrophic hormone is released from the adrenohypophysis, which in turn stimulates the hypersecretion of corticosteroids from the interrenal tissue (Mazeaud et. al., 1977). Measurements of these hormonal changes can give a generalized, quantitative portrayal of the stress a fish is under. The primary responses are generally advantageous, mobilizing energy for short term emergencies, and do not necessarily mean that the fish has been injured by the stress. Much of the research on stress has been focused on the changes in cortisol (Barton & Iwama, 1991; Gingerich

& Drottar, 1989; Strange et. al., 1978). Because they are difficult to measure, catecholamine level changes have rarely been assessed.

There are several metabolic and osmoregulatory assessments which can determine if a fish is experiencing actual physiological harm from the stress. These are measurements of the secondary responses, which may be consequences of the primary responses or may represent homeostatic dysfunction (Carmichael et. al., 1984a; Mazeaud et. al., 1977, Robertson et. al., 1987). It is these metabolic and osmoregulatory disturbances that are often the direct causes of mortality or general weakening of the animal. Analysis of these changes also provides physiological information at a higher level of biological organization, than measurements of the primary responses (Wedemeyer et. al., 1990). For these reasons, the focus of the present study was to quantify the secondary responses caused by handling stress, and to determine the length of time needed to regain homeostasis.

Measurements of the secondary changes include whole-body glucose, glycogen, and lactic acid, as well as blood osmolality. Increased concentrations of glucose (hyperglycemia), which reflect lowered concentrations of glycogen, give an indication of the ability of the fish to convert and mobilize energy (Gratzek & Reinart, 1984; Carmichael et. al., 1984a). Hyperlactemia gives an indication of whether or not anaerobic metabolism has been used during the stress (Driedzic & Kiceniuk, 1976; Goolish, 1991; Miles et al., 1974; Swift, 1983). Blood osmolality can reflect the ability of the fish to regulate plasma electrolytes (Eddy, 1981).

Striped bass are one of the most popular sport fish on the near shore East Coast, including the Chesapeake Bay and it's tributaries (Stevens, 1984). Striped bass are primarily an anadromous fish, but there are populations of inland striped bass along the East Coast. Stock from both are used in the aquaculture industry, and by federal and state hatcheries for artificial propagation (Davis & Parker, 1990: Whitehurst & Stevens, 1990). Multiple thousands of fingerlings are produced each year for stocking operations. It is unknown how many fingerlings survive the stress of handling required for stocking, as well as if there is any difference in sensitivity to stress between the anadromous and inland striped bass. If a significant difference can be elucidated, artificial propagation could shift to primarily using the type with lower sensitivity, thereby alleviating some of the post-stress mortality.

It is customary in hatcheries to take fish off feed for a few days before handling or transport. Whether this affects the secondary stress response of striped bass appears to have not been investigated.

The principal aims of this study were to determine: (1) if small (2-15 g) striped bass reacted to stress by responding with the GAS as seen in adults, (2) if there were any significant differences in the stress response between young anadromous and inland striped bass, and (3) if the feeding state(fed vs. 3 day starved) affected the stress response.

## MATERIALS AND METHODS

*Fish* Anadromous striped bass of both sexes were raised at the National Fisheries Research Center in Leetown, West Virginia. Inland striped bass of both sexes were hatched at Brookneal Hatchery, Virginia State Division of Game and Inland Fisheries. Inland fish were transported to the Virginia Tech Aquaculture Center as fry and raised at the Center until time for experimentation. While being raised, both types of bass were kept indoors in a flow-thru well water system. Bass were fed to satiation two times each day with a commercial fish chow. Before transport to our lab at Virginia Tech, Blacksburg, VA for experimentation, both types of bass were starved for three days.

Transportation took place in well-aerated water for both types of bass. Slightly saline (~ 10‰) water was used to transport the anadromous bass to reduce osmotic stress. All of the bass were transferred into 390L holding tanks in the lab. Water in these tanks was matched to the water in the transport tanks. Salinity was slowly decreased over a number of days to 0‰, and the temperature was adjusted to 21° C. Water is dechlorinated, and calcium is added in the form of calcium chloride, to raise hardness concentrations above 150 ppm, before reaching the holding tanks. Flow rate was kept high enough to ensure that concentrations of ammonia remained below 1.0 ppm, pH 6.5-7.0, nitrite concentrations below 0.2 ppm, and dissolved oxygen concentrations with aeration remained at close to saturation. Fish were allowed at least a week and a half for acclimation to laboratory conditions.

At the time of experimentation, the anadromous bass had a mean length of 6.9 cm (range, 3.1-9.5 cm) and a mean mass of 6.53 grams (range, 2.37-11.12 g). Inland bass had a mean length of 7.3 cm(range, 2.9-11.4) and a mean mass of 6.97 grams (range, 2.87-17.80).

*Stress* The same procedure for the handling stress was used for both the anadromous and inland bass. Bass in groups of six were dipnetted, and held out of the water for 5 minutes. After the stress, each group was returned to a static, aerated 40 L Nalgene™ container for a recovery period. One group of bass was not held out of water, but immediately sacrificed and used for controls. Transfer of these controls took less than thirty seconds and did not appear to trigger a stress response. In a preliminary test, these were compared with another group in which the fish were first sedated with benzocaine dissolved in the water. No significant differences were found between the fish sedated and those not sedated before transfer

. Another group was stressed, and then immediately sacrificed and used for $T_0$ . Groups of fish were then sacrificed at .5 hrs, 1 hr, 6 hrs, 12 hrs, 24 hrs, and 48 hrs of recovery.

The anadromous bass were sacrificed by placement in liquid nitrogen with transfer taking less than thirty seconds. A slightly different procedure was used with the inland bass, since blood osmolality was being measured. The inland bass were placed in an acute anesthetizing dose of benzocaine, 50 mg/ L (Soivio et. al.,

1977; Summerfelt & Smith, 1990; Wedemeyer, 1970). After immobilization, the fish were partially decapitated and a drop of blood was taken for osmolality analysis. They were then frozen in the same manner as the anadromous bass. All bass were left in the liquid nitrogen for 2 - 3 minutes to ensure complete freezing. Bass were placed in double-tagged plastic bags for storage at -80° C.

*Analysis* Frozen bass were weighed individually, decapitated, weighed again and heads were discarded. Both anadromous and inland bass were then cut into small pieces and homogenized in 15 ml of 8% perchloric acid for glucose, glycogen, and lactic acid analysis. Homogenization was done in a Waring™ Commercial blender. Approximately 4 mls of homogenate were saved in a labelled centrifuge tube for analyses. These samples were kept refrigerated at 0-5° C for no more than one week.

The homogenate in the tubes was centrifuged for ten minutes and 0.5 mls of the supernatant was removed, placed in another centrifuge tube, and spun again. The supernatant from this tube was used in the glucose and lactic acid analyses. Excess supernatant was returned to the original tubes. The pellet was resuspended in the remaining supernatant, and this was used in the glycogen analyses. It was assumed that the majority of the glycogen remained in the pellet, and was not suspended in the supernatant.

Glucose and glycogen analyses were accomplished by using the enzymatic Sigma® Glucose Kit #510 for blood. It was modified only to the extent that a whole-body sample was used

instead of blood. Glycogen was indirectly measured by conversion into glucose by a procedure (Roehrig & Allred, 1974) using amyloglucosidase. The resulting supernatant was then treated as a glucose sample and analyzed using the Sigma® Glucose Kit.

Lactic acid concentrations were analyzed using an enzymatic tissue procedure modified from Sigma® Kit #826-UV. Results were taken spectrophotometrically as absorbance using appropriate standards to set a standard curve.

Osmolality measurements on the inland bass were completed by taking a drop of blood from the heart after anesthetization for immediate measurement in a Wescor Model 5130A vapor pressure osmometer. Blood-letting was accomplished in less than half a minute, then the fish were then immediately frozen in liquid nitrogen.

*Statistical Analyses* Statistical analyses included analyses of variance, F test for variance, Student's t test and correlation coefficients. The criterion for statistical difference was $p=.05$.

## RESULTS

Handling of all the striped bass, both fed and three day starved caused a significant stress response, as expressed by elevated glucose and lactic acid, and depressed glycogen concentrations. The pattern of events over the time course demonstrated that the small bass were demonstrating a typical

general adaptation syndrome response as described earlier in this paper

*Inland vs. Anadromous*

Whole-body glucose concentrations in all four groups showed a response to the handling stress in the form of a significant increase, and then gradual decline back to initial concentrations. Control concentrations in inland bass were approximately 4.5x those in anadromous bass (Fig. 1). All four groups had similar peak concentrations at six hours post-stress. All groups except the anadromous starved group returned to control glucose concentrations during the 48 hour recovery period. The anadromous starved group remained at slightly higher than resting concentrations, but was stable. Overall, inland bass had a more moderate response.

The control values for whole-body lactate in inland bass were significantly greater, by approximately 1.4x, than in anadromous bass (Fig. 2). Immediately post-stress, whole body lactate peaked at similar concentrations in both fed and starved anadromous, and fed freshwater bass. Inland starved group concentrations peaked at 30 minutes post-stress. Concentrations stabilized in both inland and anadromous bass at six hours post-stress at an average lower than the control values. Inland concentrations were significantly higher than anadromous at this time. After forty-eight hours of recovery, the inland starved group was nonsignificant from controls because of a high variability.

Fig. 1 Changes in the concentration of whole body glucose in inland and anadromous striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are signifcantly different (P = .05).

# Average Whole Body Glucose Concentrations After Stress and Recovery

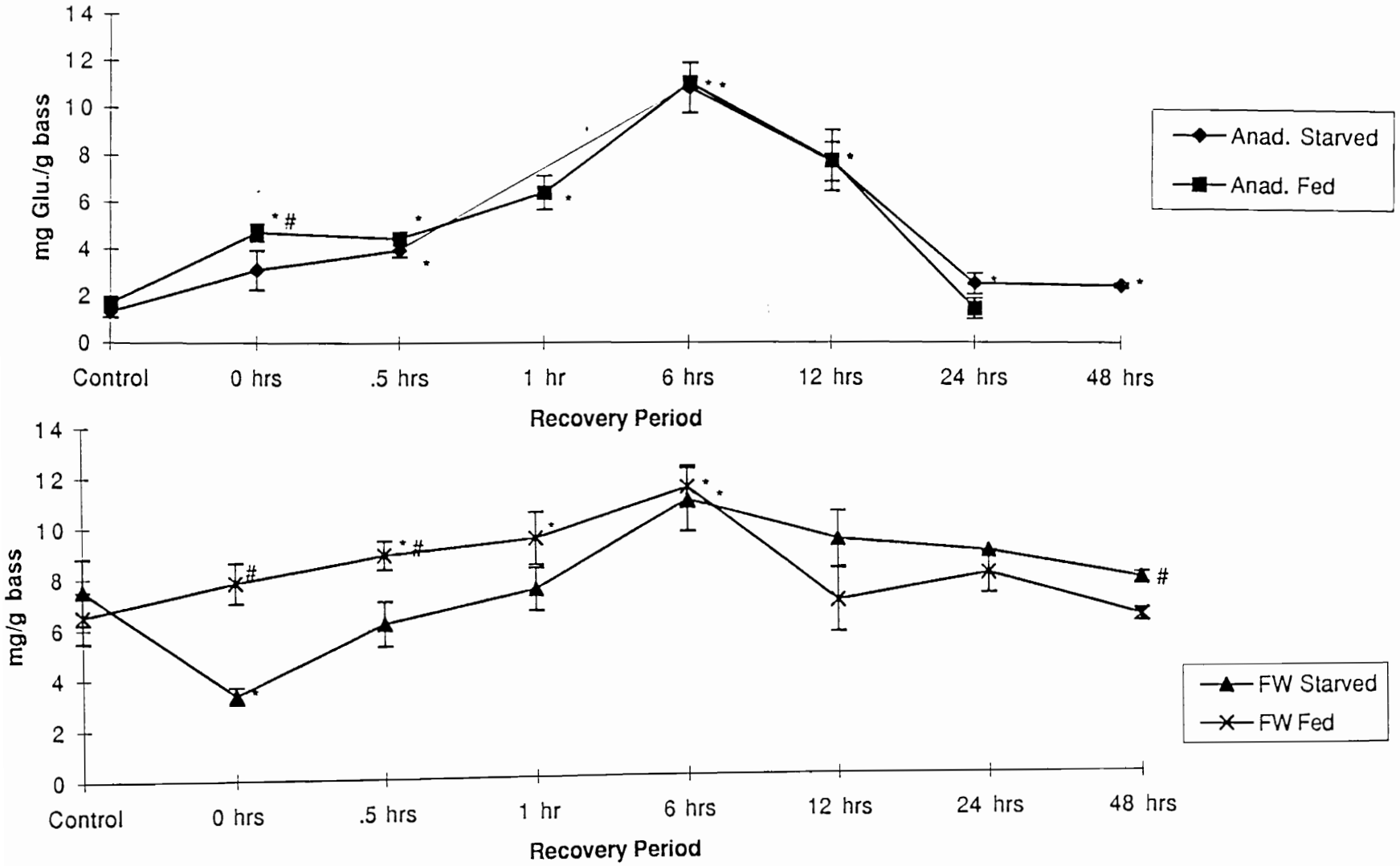
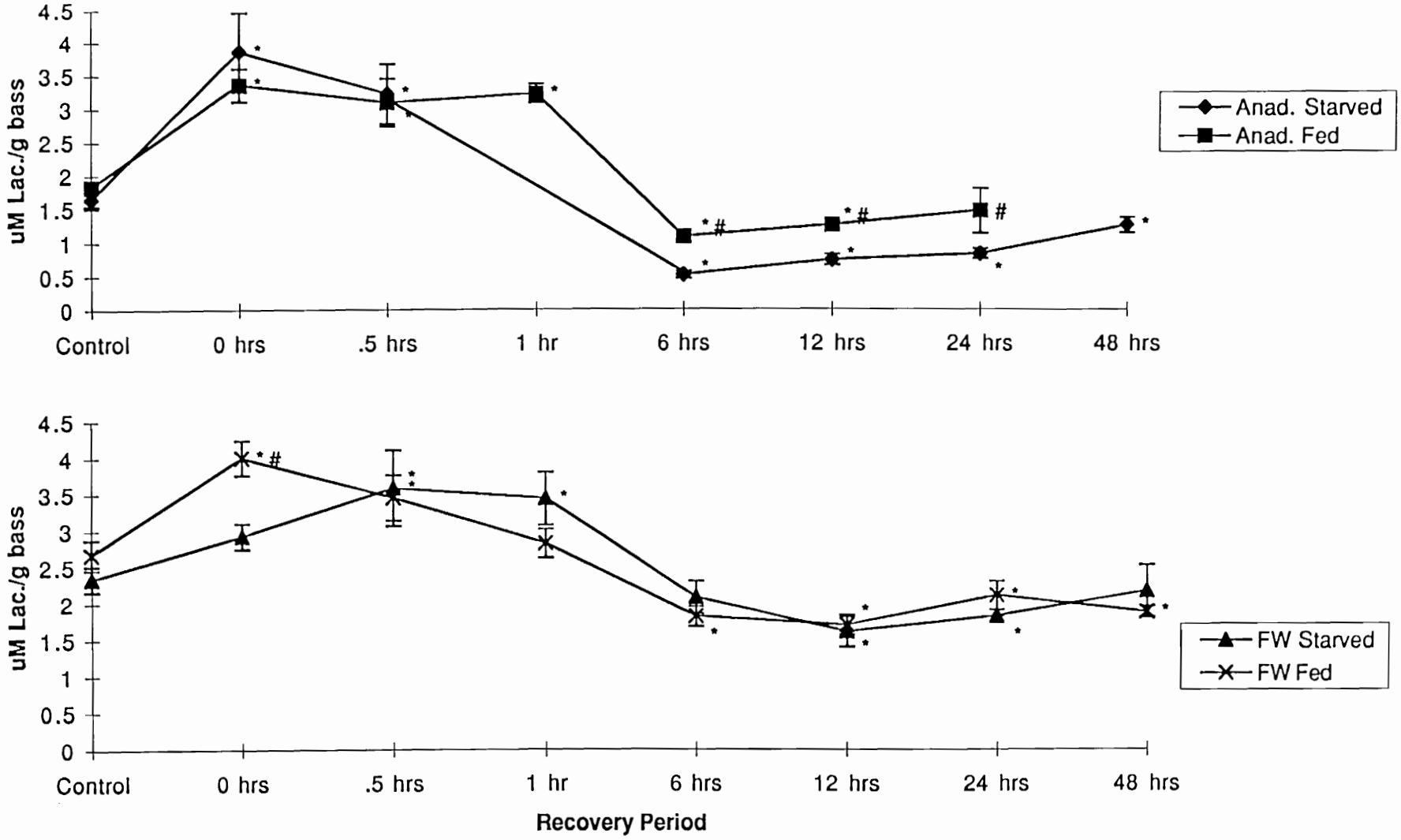


Fig. 2 Changes in the concentration of whole body lactate in inland and anadromous striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

# Average Whole Body Lactate Concentrations in Striped Bass After Stress And Recovery



As with the other variables, inland glycogen control values were significantly higher than anadromous control values (Fig. 3). Glycogen concentrations in all groups decreased immediately post-stress, but inland bass remained significantly higher than anadromous bass. By six hours post-stress, the four groups separated into two separate patterns ruled by diet. The fed groups returned to control values or higher by the end of the time course, while the starved groups remained significantly lower than control concentrations.

Coefficients of correlation were run comparing each groups' glucose and lactate concentrations over time with the glycogen concentrations (Table 1). Unexpectedly, the Anadromous Starved and Inland Fed groups had weakly positive correlation coefficients when lactate and glycogen were compared. All other correlations showed the expected negative correlation.

*Fed vs. Three Day Starved*

There were no distinct differences in glucose concentrations between the starved and fed groups of stressed fish for either anadromous or inland bass, nor were the controls different. At six hours post-stress all groups of bass had similar concentrations of glucose. At twenty-four hours post-stress, anadromous starved group concentrations were significantly higher than anadromous fed concentrations.

Fed and three day starved lactate control values in both inland and anadromous bass were similar. Three day starvation did

Fig. 3 Changes in the concentration of whole body glycogen in freshwater and anadromous striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

## Average Whole Body Glycogen Concentrations in Striped Bass After Stress and Recovery

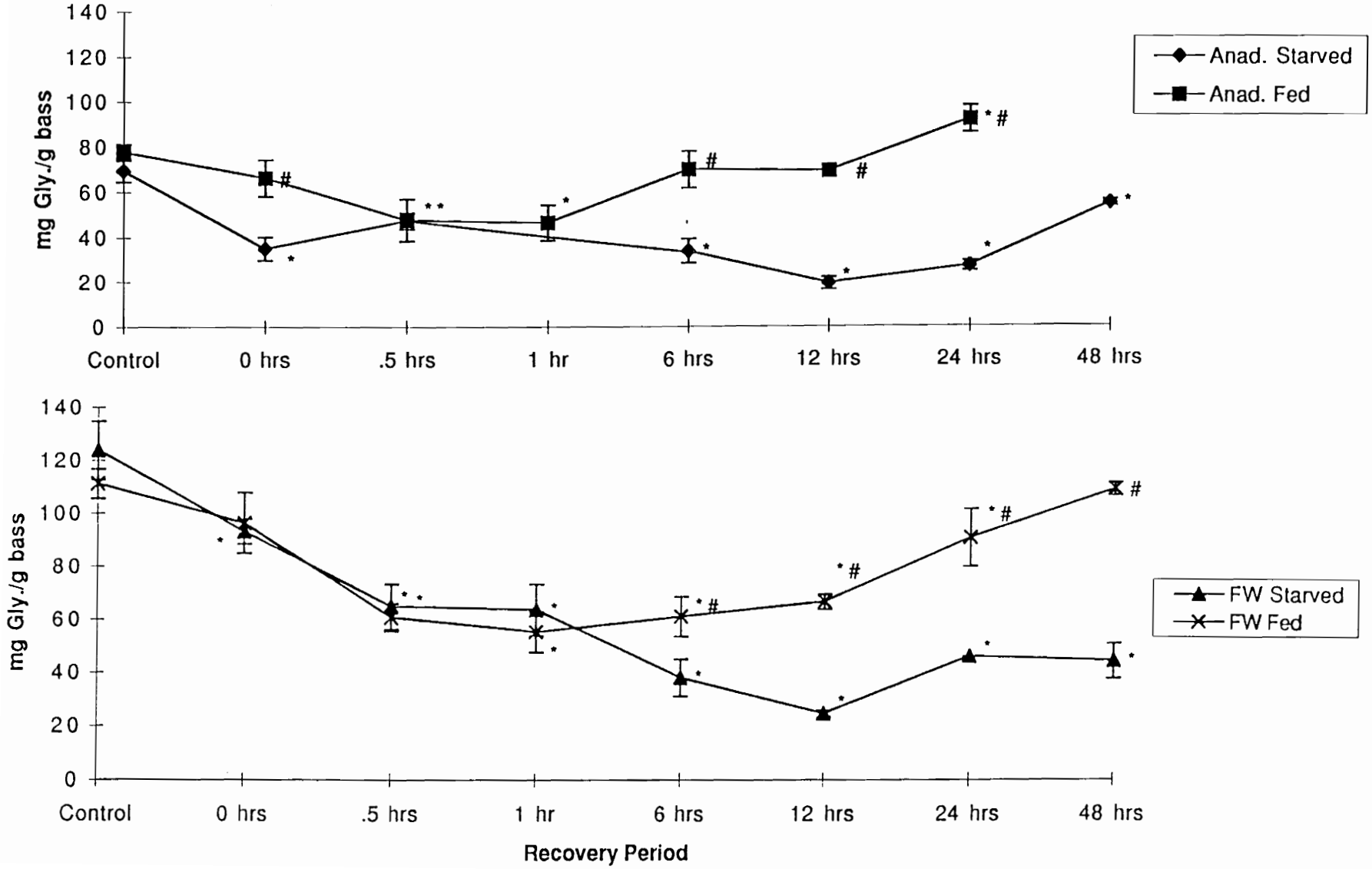


Table 1.

| Comparison | Coefficient of Correlation |
|---|---|
| | |
| Anadromous Fed | |
| - Glucose/Glycogen | -0.36042 |
| - Lactate/Glycogen | -0.69419 |
| Anadromous Starved | |
| - Glucose/Glycogen | -0.56382 |
| - Lactate/Glycogen | +0.22618 |
| Freshwater Fed | |
| - Glucose/Glycogen | -0.73364 |
| - Lactate/Glycogen | +0.0266 |
| Freshwater Starved | |
| - Glucose/Glycogen | -0.29961 |
| - Lactate/Glycogen | -0.33561 |

not affect the lactate response in the inland group. The same pattern of recovery was seen in both anadromous groups but the fed group remained consistently higher.

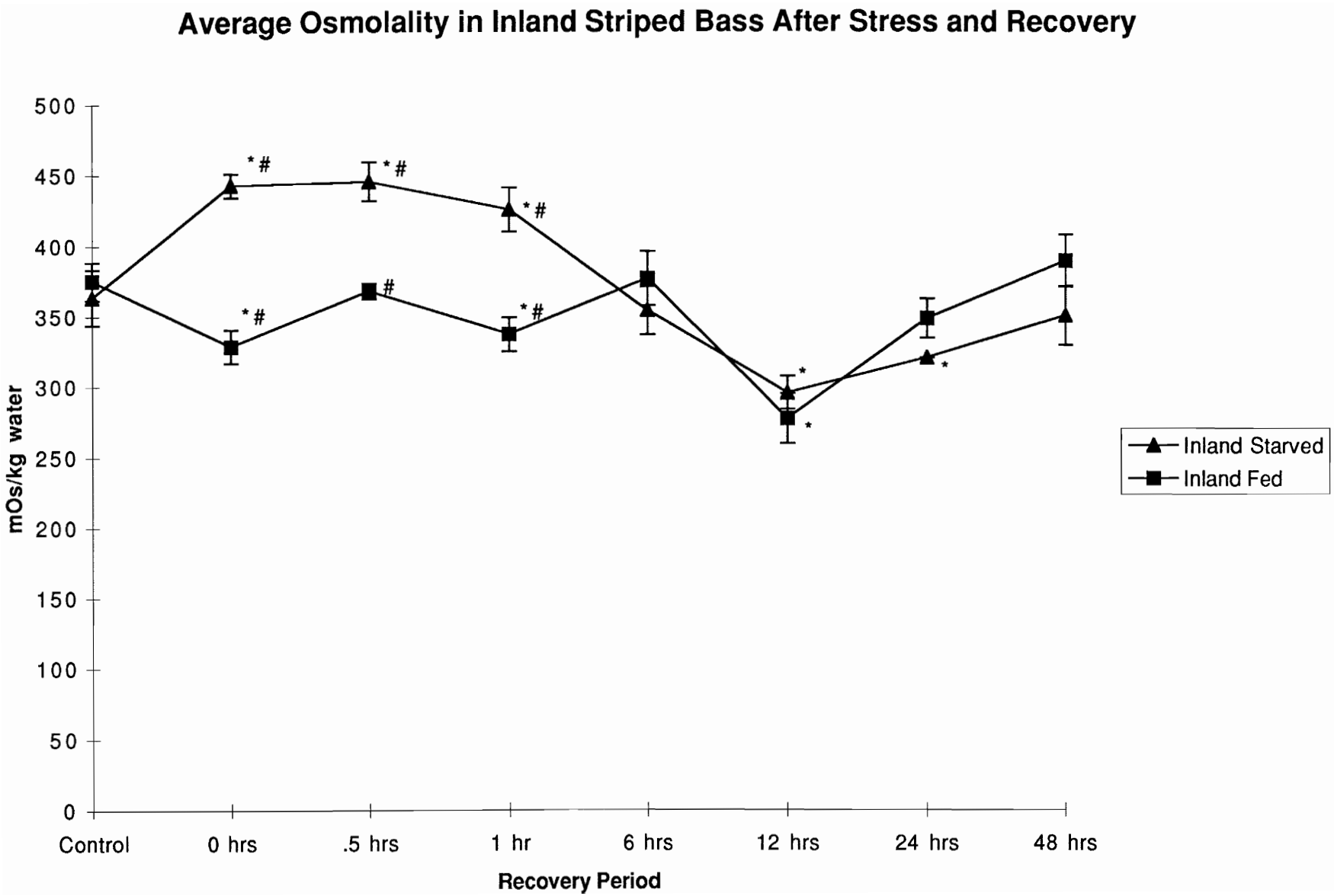
As with the whole-body glucose, there were no significant difference between the fed and starved groups' control glycogen values in either the inland or anadromous sets. At six hours post-stress, the four groups had split into two distinct patterns based on diet, with the two fed groups significantly higher than the starved groups throughout the rest of the time course. Both fed groups returned to control concentrations or higher by the end of the 48 hr recovery period, whereas neither of the starved groups did.

Data on blood osmolality was only taken for inland bass (Fig. 4). Starvation did not affect blood osmolality in unstressed fish. Immediately post-stress, the starved group had a significant increase in blood osmolality, while the fed group decreased significantly. At six hours post-stress, fed and starved values became similar to each other and to control values. Both groups significantly declined at 12 hours post-stress. After forty-eight hours of recovery, blood osmolality concentrations had returned to control values in both groups.

## DISCUSSION

The pattern of physiological changes following the stress indicate that the fingerling striped bass show a typical general adaptation response as described earlier in this paper. Whole-body measurements, except for glycogen, have not been

Fig. 4 Changes in the concentration of plasma osmolality in freshwater and anadromous striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).


Average Osmolality in Inland Striped Bass After Stress and Recovery
mOs/kg water
500
450
400
350
300
250
200
150
100
50
0
Control
0 hrs
.5 hrs
1 hr
6 hrs
12 hrs
24 hrs
48 hrs
Recovery Period
Inland Starved
Inland Fed

not affect the lactate response in the inland group. The same pattern of recovery was seen in both anadromous groups but widely used as indicators of stress. While they are not directly comparable to plasma measurements, whole-body measurements can be more comprehensive, and therefore more useful. To our knowledge, no other studies have investigated differences in inland and anadromous striped bass, even though both are used extensively in the hatchery and aquaculture industries.

The hyperglycemic response in teleosts stressed by exposure to air has been widely noted (Fletcher, 1984; McCormick & McLeod, 1925; Menten, 1927; White & Fletcher, 1986). The severity and length of the fluctuations varies greatly with species and feeding state (Barton et. al., 1988; Wydoski et. al., 1976). Concentrations of plasma glucose generally return to resting values one to four days after an acute stress (Hattingh, 1976; Leach & Taylor, 1980; Mazeaud et. al., 1977). The increase in glucose concentrations seen at the beginning of the stress response is due to the elevation of circulating epinephrine, and functions to provide quick, short-term energy for the "fight or flight" response (Barton & Iwama, 1991; Wedemeyer et. al., 1990). The primary source of the elevated glucose is glycogenolysis, the conversion of glycogen from the liver into glucose (Braley & Anderson, 1992; Mazeaud et. al., 1977; Mazeaud & Mazeaud, 1981; Wedemeyer et. al., 1990). However, increases in glycogenolysis are short lived and then reversed (Braley & Anderson, 1992).

Continued elevated glucose concentrations are thought to be controlled by cortisol, though solid evidence has yet to be found on the exact nature of the effect in fish. In mammals, cortisol reduces glucose utilization by the muscle and other tissues, and stimulates liver gluconeogenesis. It has long been assumed that cortisol has the same effect in teleosts (Leach & Taylor, 1980, Van der Boon, 1991). Work by Anderson et. al. (1991) with rainbow trout, however, casts some doubt on the role of cortisol in hyperglycemia. These workers did not find a glucose response when cortisol was added to the bloodstream by an implanted osmotic pump. There may be wide species differences, so much work still needs to be done.

The inland starved group had a significant decrease in glucose concentrations immediately after the stress, a surprising result because stress almost universally causes an increase in glucose. However, decreases in whole-body glucose have been found in rainbow trout alevins exposed to exhaustive exercise (Krumschnabel & Lackner, 1993). Because glucose metabolism is not limited to muscle, many reactions may be responsible, making interpretation difficult. Krumschnabel & Lackner (1993) found a corresponding rise in glucose-6-phosphate, which indicates the use of free glucose as a glycolytic substrate. The peak response of all the groups to the handling stress was at six hours post-stress. Plasma glucose concentrations usually peak one to three hours post-stress (Fletcher, 1984; Pickering et. al., 1982; Wedemeyer, 1972). However, in our study the delay to six hours can be attributed to using whole-body measurements.

All of the groups' peak responses were similar. From this, we concluded that feeding state does not play a significant role when the starvation period is so short. This has been reported for fish plasma glucose by Fletcher (1984) and Lewis & Epple (1984).

The magnitude of the response was greater in anadromous bass than in inland bass. This may not be significant, because at twenty-four hours post-stress, concentrations of glucose in all of the groups had declined back to resting concentrations, and in the groups monitored at forty-eight hours these concentrations remained stable. Therefore, even though the anadromous bass had a greater response range, they were able to recover in the same time period.

Lactic acid concentrations have been noted to markedly increase under the hypoxic conditions that aerial emersion creates (Hopkins & Cech, 1992; Pickering et. al., 1982; Vijayan & Moon, 1992; Wedemeyer et. al., 1990). Lactic acid is produced in the white muscle of teleost fishes as a result of anaerobic metabolism (Lackner et. al., 1988; Pickering et. al., 1982). Lactic acid must diffuse from the poorly vascularized white muscle into the blood stream, which may cause peak plasma concentrations to be a significant time period after the lactic acid was actually produced (Groman, 1982; Love, 1980). Whole-body measurements, therefore, are of more use than plasma measurements.

Immediately after the stress, peak lactate concentrations were seen in all groups except the inland starved group which peaked at thirty minutes post-stress. This is in

contrast to plasma lactate peak concentrations, which are usually delayed to one or two hours after the stress (Pickering et. al., 1982; Vijayan & Moon, 1992; Wood & Perry, 1985). Feeding state did not significantly affect the lactate responses. This was supported by data from Vijayan & Moon (1992) which did not show any differences in lactate response between fed and thirty day food deprived rainbow trout after an acute handling stress. Vijayan et. al. (1991) also did not find a significant difference between fed and thirty day food deprived brook trout after even a chronic stress.

By six hours post-stress, lactate concentrations in all groups had declined significantly and stabilized at resting values or below. The rapid removal of lactate may be the result of complete oxidation by the tissues, and gluconeogenic conversion of lactate to glucose and glycogen in white muscle (Girard & Milligan, 1992). The former may be shown by the peak concentrations of glucose shown at 6 hours post-stress. Again, even though inland bass had a more moderate response, anadromous bass were able to return to resting values in the same time period.

Glycogen stored in the liver represents an important energy source that is converted to glucose after a stress has occurred. Studies have shown that hepatic glycogen concentrations decrease rapidly in stressed teleosts (Paxton et. al., 1984; Vijayan et. al., 1990). Elevated catecholamine concentrations are known to induce this glycogenolysis (Mazeaud et. al., 1977). Cortisol may also play a role in stress-induced glycogen mobilization (Vijayan et. al., 1991). Evidence shows that the recovery of glycogen is probably

dominated by an as yet undefined intramuscular glyconeogenic pathway from lactic acid, and not a direct incorporation of glucose into glycogen (Arthur et. al., 1992; Moyes et. al., 1992; Schulte et. al., 1992). West et. al. (1994) found that in rainbow trout, glucose, regardless of plasma concentration, accounted for less than 10% of glycogen repletion after exercise. Food deprived fish may have a more difficult time recovering glycogen concentrations (Vijayan & Moon, 1992).

Inland bass glycogen control values were significantly higher than those of anadromous fish. There is no obvious reason for this. Differential rates of feeding are a possibility. Concentrations in all of the groups decreased significantly through one hour post-stress. After that, feeding state played a major role in recovery. Both of the fed groups began recovering, and glycogen concentrations increased through the rest of the recovery period back to resting concentrations or higher. The starved groups, on the other hand, declined through twelve hours post-stress, before increasing at twenty-four and forty-eight hours post-stress. Glycogen concentrations in the starved groups remained significantly lower than control values at termination of the recovery period. Feeding state has been shown to modify the glycogen response in teleosts (Sheridan & Mommsen, 1991; Vijayan & Moon, 1992), however these studies were done with fish starved for a much longer time period than was done here. To our knowledge, differences have not been seen in fish with such a short starvation period.

The correlation coefficients between the glucose and lactate values and glycogen were not completely expected in some areas. Since lactate concentrations increase and then decrease over the recovery time course while glycogen does the opposite, the weakly positive correlations found in the anadromous starved and inland fed groups was unexpected. This positive correlation is possibly due to the fact that both groups during recovery had periods of hypolactemia due perhaps to a temporary cessation of spontaneous activity. All of the other comparisons had the expected negative correlation, but the differences in degree are interesting.

Fish stressed in freshwater tend to gain water and lose electrolytes. Elevated catecholamine concentrations after a stress cause the dilation of gill filamental arteries, and increase branchial blood flow, which in turn cause the increase in uptake of water and ionic loss (Gratzek & Reinert, 1984; Harrel & Moline, 1992; Wedemeyer et. al., 1990). These ionic disturbances, if not corrected, are thought to be the major cause of stress-related death. Unfortunately, only the inland groups were tested for plasma osmolality. The inland fed group followed this pattern, but by twenty-four hours post-stress, blood osmolality was similar to control concentrations and remained that way at forty-eight hours. The significant increase seen in the starved group immediately after the stress was unexpected. The reason for the increase is unclear. Mazeaud et. al. (1977) reported a hemoconcentration which was attributed to an internal redistribution of electrolytes to buffer hyperlactemia. But, since peak lactic acid concentrations were

similar in both the fed and starved group, it seems unlikely this is the cause.

In conclusion, the results indicate that inland and anadromous striped bass do not differ significantly in their secondary responses to stress. It is interesting to note, however, that the anadromous groups' resting concentrations for all parameters were significantly lower than the inland groups. This may indicate that the inland groups were unknowingly stressed before the beginning of the experiment, though protocols before the experiment were identical for both groups. Peak glucose and lactic acid concentrations in all groups were similar, as were the time to recovery. The lowest depletions of glycogen were also similar, though recovery was more variable. Feeding state did play a role in glycogen recovery, but not in the glucose or lactic acid responses. The starved groups failed to return to resting concentrations of glycogen during the time course of the experiment. While short term food deprivation does not seem to affect glucose concentrations, continued low glycogen reserves could have a serious effect on the glucose response if the fish were to be stressed again before reserves could be restored.

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# SECONDARY STRESS RESPONSES TO ACUTE HANDLING STRESS BY STRIPED BASS (MORONE SAXTILIS) AND HYBRID STRIPED BASS (MORONE CHRYSOPS X MORONE SAXTILIS)

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Both fed and three day starved pure and hybrid striped bass (ave. mass: 487g) were held in dipnets above water for three minutes in groups of six. Severity of the stress response was measured by quantitating levels of plasma glucose and chloride ions, and whole blood lactic acid, sodium ions, and potassium ions. Levels were measured in the bass at rest (considered standard levels), immediately after the stress, and then at 12 hours, 24 hours, and 48 hours of recovery. Both fed and starved hybrid bass had more moderate responses than the pure groups in all of the parameters, except potassium where there was no significant difference. The handling was not severe enough to cause any significant glucose response in the hybrid groups. Both hybrid groups regained sodium and chloride ion homeostasis within twenty four hours of recovery, while ion concentrations in both the fed and starved pure groups remained significantly depressed after 48 hours. Though recovery was in a hypoosmotic environment, elevated plasma potassium was seen in all four groups. Feeding state did not affect the stress

response. It is concluded that hybrid striped bass have a significantly higher tolerance to an acute handling stress than pure striped bass.

# INTRODUCTION

Fish used in fisheries management and the aquaculture industry must often be handled in the process of raising them. This handling leads to a significant stress response by the fish, and can result in mortality if the stress is severe enough (Yeager et. al., 1990). Many studies have been conducted to quantify the physiological responses to stress in fish ( for reviews see: Adams, 1990; Barton & Iwama, 1991; Mazeaud et. al., 1977; Pickering, ed., 1981). Most of these have focused on adult salmonid fishes (Barton et. al., 1988; McDonald et. al., 1993; McGeer et. al., 1991), and there are still considerable gaps in the knowledge of the stress response in other species. Since fish species react differently to changing environmental factors (Carmichael et. al., 1984a), it is important to quantify the stress response in these other species.

Adult fish respond to stress with a non-specific phenomenon commonly called the General Adaptation Syndrome (GAS), which was first described in mammals by Selye (1950). It has evolved as an adaptive mechanism to allow animals to regain homeostasis after a stress (Pickering, 1981a). Reactions in fish are usually divided into two categories for convenience: the primary responses, and the secondary responses (Mazeaud et. al., 1977).

During the primary response, catecholamine concentrations increase because of sympathetic nervous system activation, and adrenocorticotrophic hormone is released from the

adenohypophysis stimulating the hypersecretion of corticosteroids (Selye, 1950). It has also been determined that the neuroendocrine control of these two major hormone groups is interrelated (Axelrod & Reisine, 1984). Epinephrine concentrations spike rapidly followed by a slightly delayed response of cortisol (Barton & Iwama, 1991; Carmichael et. al., 1984a,b). Measurements of these hormonal changes can give a generalized, quantitative portrayal of the stress a fish is under. These hormonal changes are usually advantageous, mobilizing energy for short term emergencies, and do not necessarily mean that the fish has been injured by the stress. Much of the research on stress has been focused on the changes in cortisol (Barton & Iwama, 1991; Gingerich & Drottar, 1989; Strange et. al., 1978).

Changes in the secondary responses are often used to determine if the fish is actually experiencing physiological harm. The secondary responses may be consequences of the primary responses or may represent homeostatic dysfunction (Carmichael et. al., 1984a; Mazeaud et. al., 1977). It is these metabolic and osmoregulatory disturbances which are often the direct causes of handling-related mortality. Analysis of these changes also provides physiological information at a higher level of biological organization than measurements of the primary responses (Wedemeyer et. al., 1990). For these reasons, the main focus of the present study was to quantify the secondary responses caused by handling stress, and to determine the length of time needed to regain homeostasis.

Assessments of the secondary changes included plasma glucose, lactic acid, sodium, chloride, and potassium. Increased concentrations of glucose (hyperglycemia) give an indication of the ability of the fish to convert and mobilize stored energy ( Carmichael et. al., 1984a; Robertson et. al., 1987). Hyperlactemia gives an indication of whether or not the stress has caused anaerobic metabolism (Driedzic & Kiceniuk, 1976; Goolish, 1991; Miles et. al., 1974; Swift, 1983). Fluctuations in plasma sodium, chloride, and potassium are especially important to monitor because osmoregulatory dysfunction is thought by many to be the leading cause of mortality in stressed fish (Carmichael et. al., 1984a,b; Tomasso et. al., 1980; Wedemeyer, 1972; Wedemeyer et. al., 1990).

Both hybrid (*Morone chrysops x Morone saxtilis*) and pure striped bass (*Morone saxtilis*) are important in sport fisheries along the East Coast. Both have been extensively stocked in lakes and reservoirs (Stevens, 1984). Hybrid striped bass have demonstrated high survival rates, and a wide tolerance of temperatures and salinities (Smith & Jenkins, 1985a,b; Smith et. al., 1989), but fairly little is known about their response to handling stress. Quantification of the response is important if hybrid striped bass are to become as significant to the aquaculture industry as are pure striped bass.

The principal aim of this study was to compare the stress response of both hybrid and pure striped bass, in order to

determine if hybrids had a significantly lower stress response than pure striped bass. A secondary aim was to determine if feeding state (fed vs. 3 day starved) affected the response. This is of importance to evaluate, because it is customary practice in hatcheries to not feed fish for a few days before handling or transport. It is unknown if this affects the stress response.

## MATERIALS AND METHODS

*Fish* Both hybrid and pure striped bass (ave. length & mass: 27.9 cm, 487g) were raised at the Aquaculture Center at Virginia Tech for approximately two years. They were kept in 700 liter round tanks with a flow-thru well water system. Hardness was kept above 150 ppm, while ammonia concentrations were kept below 1.0 ppm, and nitrite concentrations remained below 0.2 ppm. The bass were fed to satiation once every day, and this protocol was followed up until the beginning of experimentation, except for one group of each type of bass which was starved for three days prior.

*Stress* Both hybrid and pure striped bass (n=6) were stressed by aerial emersion and crowding in a dipnet for 3 minutes. The bass were returned to 700 L circular tanks for the appropriate recovery regimen. Groups were taken for blood samples at 0 hrs, 6 hrs, 12 hrs, 24 hrs, and 48 hrs after the handling stress. The control groups were not stressed.

After the appropriate recovery period, fish were quickly transferred to an acutely anesthetizing dose of benzocaine, 50 mg/L

(Soivio et. al., 1977; Summerfelt & Smith, 1990; Wedemeyer, 1970). When immobilized, the caudal peduncle was severed, and blood taken by heparinized (Ammonium heparin) 3 ml syringe fitted with 22 gauge needles.

Blood was placed in labelled 1.5 ml centrifuge tubes. 35 µl of whole blood was immediately used in $Na^+$ and $K^+$ analysis. 20 additional µl of whole blood was transferred to a labeled 0.5 ml centrifuge tube containing 40 µl 8% perchloric acid to be used in lactic acid analysis. All centrifuge tubes were kept on ice until placed in a 2-6° C refrigerator or centrifuged.

Blood samples were kept on ice for a maximum of an hour and a half before being centrifuged for 10 minutes. Plasma was removed and placed in a new labeled 1.5 ml centrifuge tube. These tubes were kept in a -80° C freezer until analyzed.

*Analysis* Sodium and potassium blood analyses were run using a Ciba Corning ISE Na/K Analyzer (Model 614). The 614 is a direct potentiometric ISE analyzer that does not require any pretreatment of the samples as does the commonly used flame photometer technique. This allows for a significantly smaller sample size to be used, as well as allowing for the monitoring of the ions in their usual physiological matrix.

For lactic acid analysis, an enzymatic Sigma Kit #826-UV was used. The original procedure called for using 2 mls of supernatant, but to conserve blood, it was scaled down to a microprocedure using only 20 µl. All other steps were followed.

Results were taken spectrophotometrically as absorbance using appropriate standards to establish a standard curve.

The plasma glucose analyses were run using Sigma Kit #510, which utilizes an enzymatic procedure.

Chloride concentrations were measured in plasma samples using Sigma Kit #460 which utilizes thiocyanate.

*Statistical Analysis* The statistical analyses run included analysis of variance, F test for variance, and Student's t test. The criterion for statistical difference was p=.05.

## RESULTS

Resting plasma glucose concentrations in the hybrid and pure groups were significantly (p=.05) different, the pure groups were significantly higher (Fig. 1). Immediately after the stress, the pure groups had a significant increase, while the hybrid groups did not change. Throughout the experiment, the hybrid groups remained similar to control values. The pure starved group's glucose concentrations remained significantly higher than controls through out the recovery period, while the pure fed group exhibited hypoglycemia.

Hybrid bass control concentrations of lactic acid were significantly lower than pure bass. All groups had a statistically significant (p=.05) peak increase immediately after the stress (Fig. 2). Both of the hybrid groups and the pure starved group stabilized at control concentrations during the recovery period. The pure fed fish

Fig. 1 Changes in the concentration of plasma glucose in hybrid and pure striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point. * denotes significant difference from control (P = .05), # denotes hybrid and pure groups are significantly different (P = .05). 1 denotes significant difference between pure groups (P = .05).

## Average Plasma Glucose Concentrations in Pure and Hybrid Striped Bass After Stress and Recovery

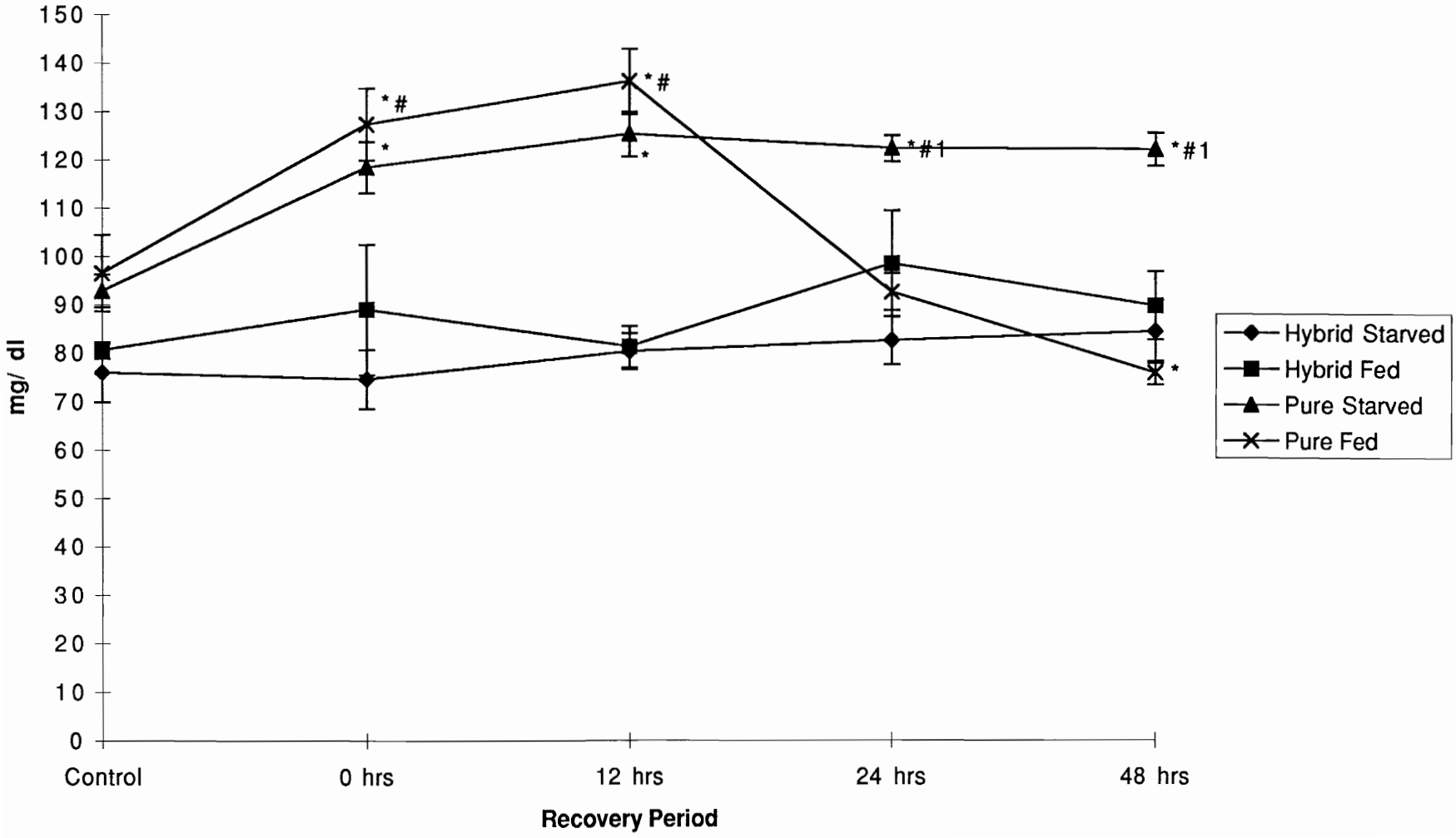
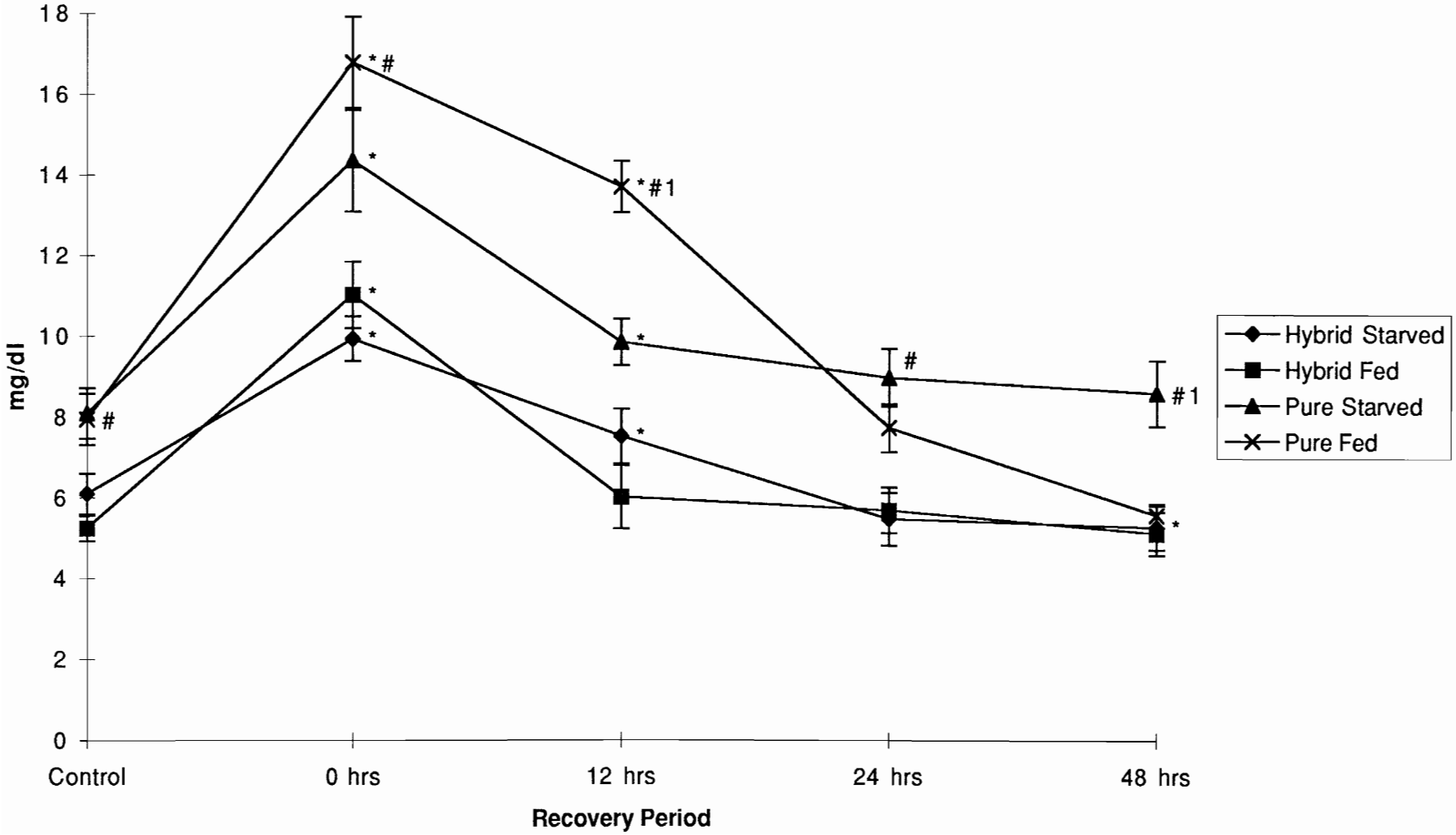


Fig. 2 Changes in the concentration of whole blood lactate in hybrid and pure striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point. * denotes significant difference from control (P = .05), # denotes hybrid and pure groups are significantly different (P = .05). 1 denotes significant difference between pure groups (P = .05).

## Average Plasma Lactate Concentrations in Hybrid and Pure Striped Bass After Stress and Recovery



showed a hypolactemic response at the end of the recovery period. Diet appeared to play a significant role only in the pure fish.

Resting blood potassium concentrations were similar for all four groups of fish. Immediately after the stress, concentrations in all increased significantly to peak concentrations (Fig. 3), 5.75 - 6.25 mM/l, and were similar to each other. By twelve hours post-stress, potassium concentrations in all groups had returned to resting and remained stabilized through the end of the time course. There was no dietary influence on the potassium response in any of the groups.

All of the groups had similar resting concentrations of blood sodium ( approx. 180 mM/L) and chloride (approx. 115 mEq/L). There was no significant response in either of the variables immediately after the stress by any of the groups (Figs. 4, 5). By twenty-four hours post-stress, the hybrid groups' sodium and chloride concentrations regained resting concentrations and stabilized, whereas the pure groups remained significantly below resting even after 48 hours. Feeding state did not have a significant effect on the sodium or chloride response.

## DISCUSSION

The hybrid striped bass had more moderate responses to the handling stress in all but one of the parameters, than the pure striped bass. Feeding state only appeared to affect the pure glucose and lactate response.

Fig.3 Changes in the concentration of plasma potassium in hybrid and pure striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point. * denotes significant difference from control (P = .05), # denotes hybrid and pure groups are significantly different (P = .05).

## Average Blood Potassium Concentrations in Pure and Hybrid Striped Bass After Stress and Recovery

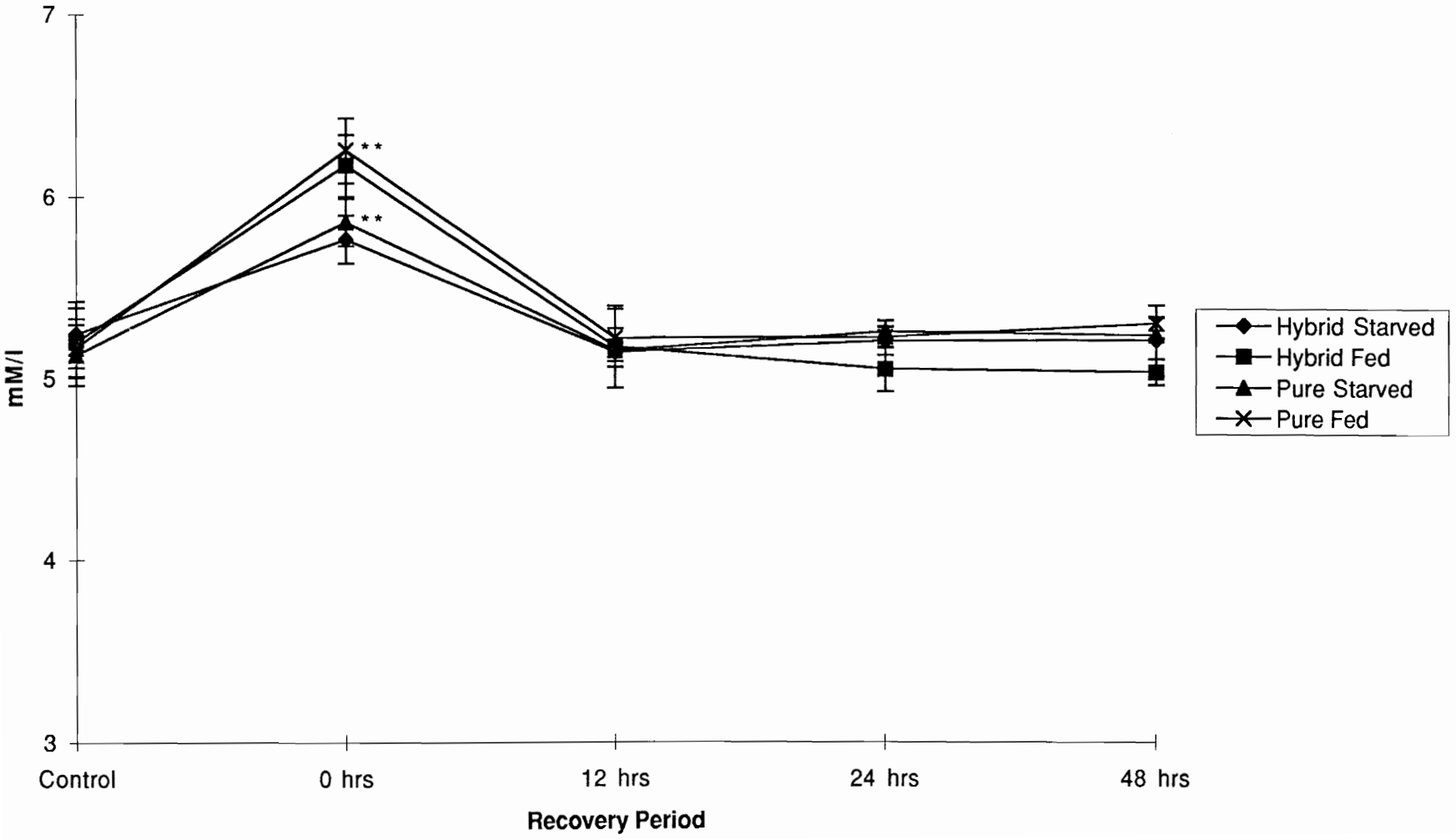


Fig.4 Changes in the concentration of whole blood sodium in hybrid and pure striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point. * denotes significant difference from control (P = .05), # denotes hybrid and pure groups are significantly different (P = .05).

## Average Blood Sodium Concentrations in Pure and Hybrid Striped Bass After Stress and Recovery

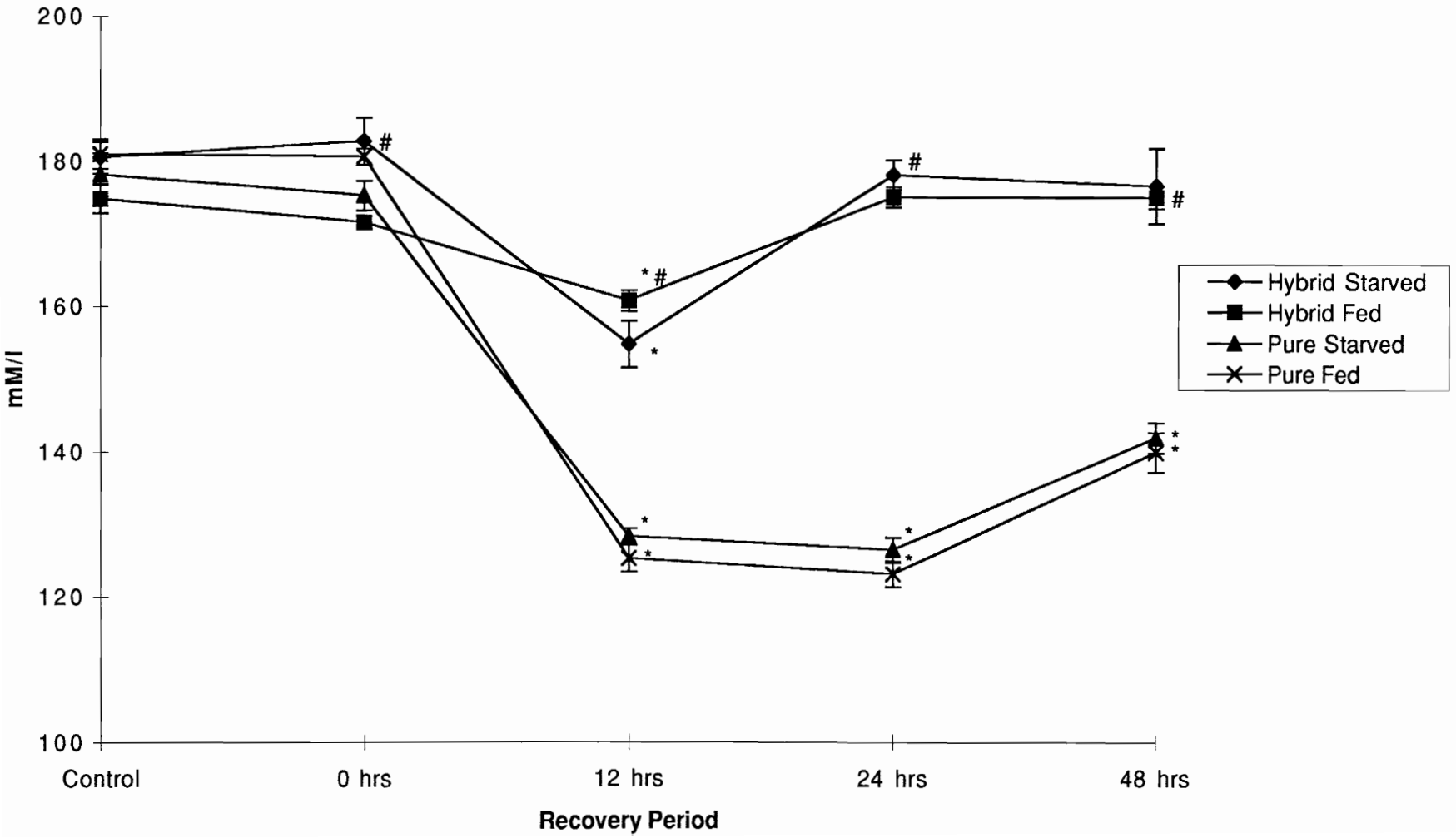
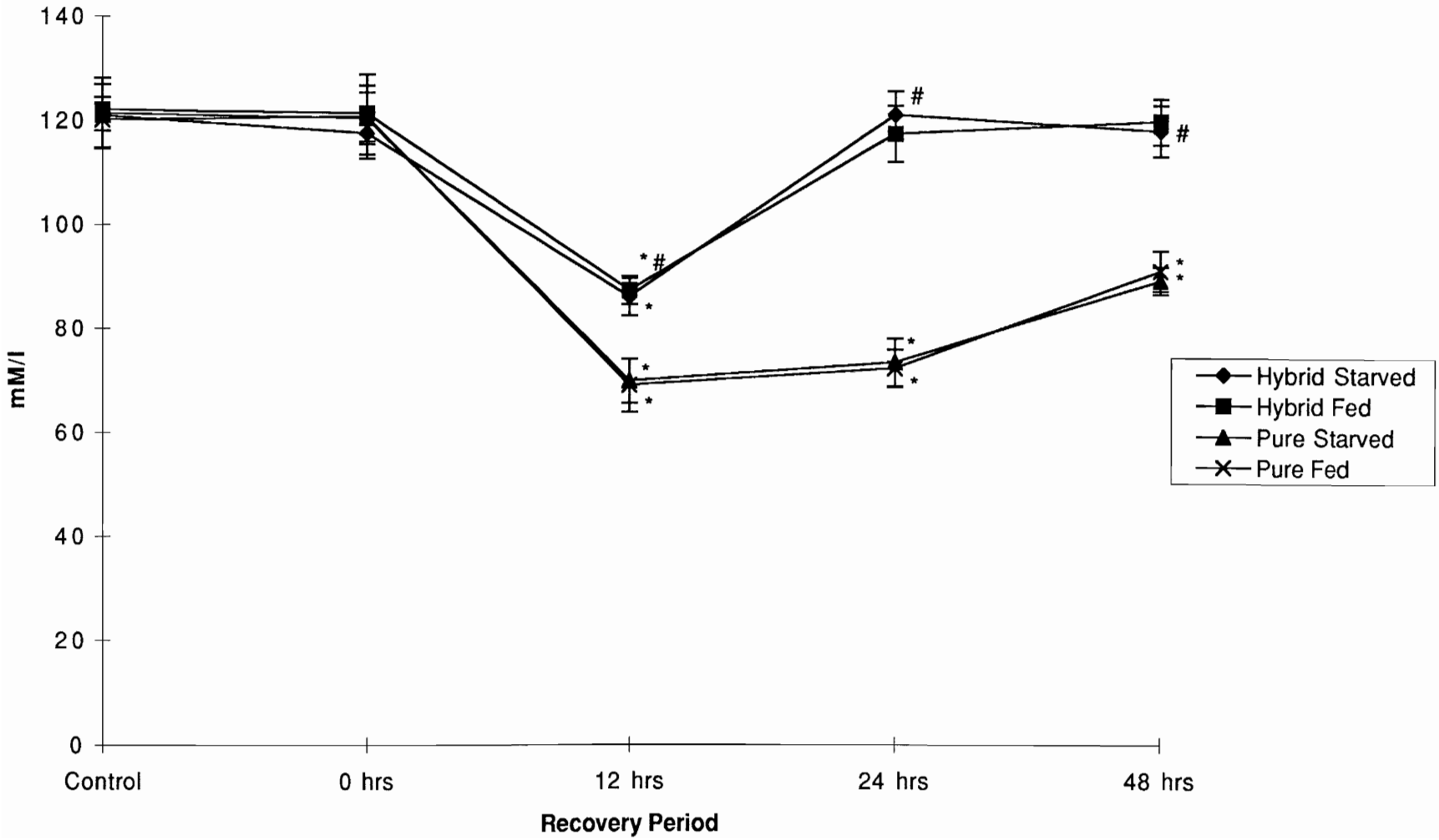


Fig.5 Changes in the concentration of whole blood chloride in hybrid and pure striped bass subsequent to an acute handling stress. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point. * denotes significant difference from control (P = .05), # denotes hybrid and pure groups are significantly different (P = .05).

## Average Blood Concentrations of Chloride in Pure And Hybrid Striped Bass After Stress and Recovery



Resting glucose concentrations of the pure striped bass groups were similar to those found by Strange & Cech (1992) in the same species, but slightly lower than those found in wild fish (Tisa et. al., 1983). The concentrations reported in the wild bass were similar to those found in the hybrid striped bass. The hybrid bass glucose concentrations remained unchanged following handling, indicating that the stress did not affect them greatly. Immediately, after the stress, the plasma glucose in the pure starved bass rose significantly and continued to increase through twelve hours of recovery. This hyperglycemia is caused by gluconeogenesis (formation of glucose from amino and fatty acids) and glycogenolysis (mobilization of glycogen) in the liver (Braley & Anderson, 1992; Mazeaud & Mazeaud, 1981; Nakano & Tomlinson, 1967) stimulated by elevated concentrations of catecholamines (Barton & Iwama, 1991; Mazeaud & Mazeaud, 1981; Wedemeyer et. al., 1990). The sustainment of hyperglycemia has been thought to be caused by cortisol (Barton & Iwama, 1991; Robertson et. al., 1988; Wedemeyer et. al., 1990). More recent work by Anderson et. al. (1991) with rainbow trout, however, casts some doubt on the role of this hormone. They did not find a glucose response when cortisol was added to the bloodstream by an implanted osmotic pump. There may be wide species differences, so much work still needs to be done.

The pure starved group stabilized at elevated glucose concentrations and remained significantly higher than controls throughout the rest of the experiment. At twenty four hours post

stress, the pure fed group began declining and at the end of the experiment, significantly decreased concentrations were seen that were similar to the hybrid groups values. Hypoglycemia has not been seen in many teleosts and an explanation for it is not known. There was a concomitant decrease in lactic acid to below resting concentrations at the same time in the pure fed group.

Lactate is produced by anaerobic metabolism in the white muscle of teleosts under conditions of hypoxia (Hopkins & Cech, 1992; Pickering et. al., 1982). Hypoxic conditions were obtained while the fish were being held in the air during the stress. Resting lactate concentrations in the hybrid groups were similar to those found by Strange & Cech (1992) and Nikinimaa et. al. (1984) in striped bass. The resting concentrations of the pure groups were slightly higher. The peak concentrations found immediately after the stress probably do not represent the maximum that was reached. Lactic acid must diffuse from the poorly vascularized white muscle into the blood stream, causing peak concentrations to be delayed until one or two hours after the stress (Pickering et. al, 1982; Vijayan & Moon, 1992; Wood & Perry, 1985). The next time period that lactate concentrations were measured during recovery was at twelve hours post-stress when it is expected that the concentrations would have decreased. Thus, it is probable that concentrations continued to rise after the stress. Lactate concentrations in all of the groups declined back to resting concentrations in the twenty-four period after the stress. This return is in agreement with data found in other teleosts after an

acute stress (Black, 1957a,b; Soivio & Oikari, 1976). The pure fed group continued to have declining concentrations at forty-eight hours. This made the pure fed group's concentrations similar to the hybrid groups' resting concentrations at that time.

The decline in both lactate and glucose concentrations to concentrations significantly below controls is difficult to explain, especially since the pure starved group did not follow the same pattern. If the pure starved group had followed the same pattern, the logical conclusion would be that the pure striped bass resting concentrations were artificially inflated perhaps due to an unknown stress before the experiment. While feeding state appears to be the significant difference between the two pure groups, it is difficult to use that to explain the decrease. It would be much more likely that if feeding state were the cause that the pure starved group would have been the one affected. Therefore at this time, the only conclusion that can be drawn is that for an unknown reason, the pure fed group was under a mild stress before the handling stress was applied, even though conditions were identical to all of the other groups.

Fish exposed to a hypoosmotic environment tend to lose electrolytes and gain water. This is aggravated after a stress, because elevated catecholamine concentrations dilate the gill filamental arteries, increasing branchial blood flow, which in turn causes the increase in uptake of water and ionic loss (Eddy, 1981; Gratzek & Reinart, 1984; Harrel & Moline, 1992; Mazeaud et. al., 1977; Wedemeyer, 1972; Wedemeyer et. al., 1990). The rise in

plasma cortisol concentrations after a stress may amplify the osmotic imbalance (Umminger & Gist, 1973). However, most research has shown that the rise in cortisol is actually beneficial, and helps to correct electrolyte imbalance ( see review, Baron & Iwama, 1991).

Chloride resting concentrations were similar to those found in striped bass by Davis et. al. (1982). These did not change significantly until twelve hours post-stress, when they declined in all groups, with the pure groups showing by far the greater effect. This delay in hypochloremia has been seen in hybrid striped bass (Tomasso et. al., 1980), but is in contrast to the immediate response seen in salmonids (Wedemeyer, 1972). The recovery water was fairly high in calcium (>150 ppm) in the form of calcium chloride and, this has been shown to reduce the permeability of the gill to sodium and chloride ions (Potts, 1984). By twenty-four hours post-stress, the hybrid groups had returned to resting concentrations and stabilized. The pure groups remained significantly lower than resting concentrations even 48 hours after the handling stress, indicating that the stress was more severe for the pure bass.

Similar results to the chloride ions were seen with the sodium concentrations in all four groups. A positive covariance was found for the two variables in all four groups. Decreasing sodium concentrations after a stress have been reported by Redding & Schreck (1983) and Randall et. al. (1972). Again, the hybrid striped bass were able to return to resting concentrations, while the pure groups remained significantly reduced.

Resting concentrations of potassium in all four groups were similar and were throughout the experiment. An increase in plasma potassium concentrations was seen immediately after the stress in all of the groups. This has also been seen in juvenile coho salmon (Redding & Schreck, 1983), and rainbow trout (Graham et. al., 1982; Turner et. al., 1983). This hyperkalemia is probably caused by intercellular acidosis due to the production of carbon dioxide and lactic acid (Heisler, 1980), which causes an increase in plasma potassium due to a potassium/hydrogen exchange mechanism between intra- and extra- cellular fluid (Turner et. al., 1983). Concentrations decreased back to resting and stabilized in all groups by twenty four hours post-stress. It is unknown why potassium concentrations returned to resting in the pure groups, while sodium and chloride concentrations did not.

In conclusion, the hybrid groups had more moderate responses than the pure groups in all parameters except potassium. The hybrids were not stressed enough to show any significant glucose response. Feeding state did not affect any of the groups' blood electrolyte parameters, and did not appear to affect the hybrid groups' lactate and glucose responses. Hybrid fish are generally considered hardier than either of the pure parental fish. The data from this experiment support that conclusion. Hybrid striped bass may therefore be better adapted to the rigors associated with the hatchery and aquaculture industries.

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# THE EFFECTS OF VARYING SALINITY RECOVERY WATERS ON THE SECONDARY STRESS RESPONSES OF HYBRID STRIPED BASS FINGERLINGS, MORONE CHRYSOPS X MORONE SAXTILIS

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Fed and three-day starved fingerling hybrid striped bass raised in freshwater, were subjected to an acute handling stress in groups of six. Fish were then allowed to recover in 0, 5, 10, or 15 ‰ salinity water. Severity of the stress response was quantitated by measuring whole-body glucose, glycogen, lactic acid, and blood osmolality. Measurements were taken at rest, immediately after the stress, and at 30 minutes, 1 hour, 6 hrs, 12 hrs, 24 hrs, and 48 hrs post-stress. All results followed a typical general adaptation syndrome response. Feeding state and salinity of the recovery water had effects on the stress response. The glucose response was affected by both feeding state and salinity. The response was greater in fed fish, and severity also increased with increasing salinity. The lactate response in fed fish was similar in all of the salinity recovery treatments. Salinity did affect the starved groups' responses, the 0‰ group had a significantly lower stress response than any of the salinity groups. Except for the starved 0‰ group, starved groups had similar stress responses in each of the

salinities. The glycogen response was dominated by feeding state; salinity of the recovery water did not have a significant effect. None of the starved groups regained resting glycogen levels, while all the fed groups did. Blood osmolality was not affected greatly, but fish recovering in 0‰ lost electrolytes, those in saline water gained electrolytes. Resting osmolality levels were higher in starved fish. Overall, fish, either fed or starved, in the 0‰ and 5‰ saline water recovery treatments had the more moderate responses. Higher salinites seemed to add to the stress.

# INTRODUCTION

The handling of fish cannot be avoided in aquaculture and fisheries research. Fish are subjected to aerial emersion, which may last minutes during the processes of tagging, sorting, and transport. This handling causes stress and can lead to mortality (Strange et. al., 1978; Yeager, 1990). The severity of the stress response and the length of time before homeostasis is recovered is directly related to the duration of the stressor, and possibly the characteristics of the recovery water.

Fish react to stress with a series of physiological changes called the General Adaptation Syndrome which was first described in mammals (Selye, 1950). Most of the work previously done on the stress response in fish has been done with juveniles and adults, while this study used fingerlings. For convenience, in fish these changes have been grouped into two categories: the primary responses and the secondary responses (Mazeaud et. al., 1977). During the primary response, activation of the sympathetic nervous system causes catecholamines (epinephrine and norepinephrine) to increase and release of adrenocorticotrophic hormone from the pituitary causes hypersecretion of corticosteroids such as cortisol (Barton & Iwama, 1991; Mazeaud et. al. 1977). Measurements of these two hormonal changes, especially of cortisol, can give a general, quantitative portrayal of the extent of stress a fish is experiencing. The physiological response is generally advantageous,

and does not necessarily mean that the fish has been injured by the stress.

The release of these two classes of hormones induces a large variety of secondary responses. These include elevated blood glucose (McCormick & Macleod, 1925; Mazeaud et. al., 1977), elevated blood lactic acid (Love, 1980; Swift, 1983), increased glycogen metabolism (Gratzek & Reinart, 1984; Mazeaud et. al., 1977, Schwalme & MacKay,1991), and osmoregulatory disturbances (Harrel & Moline, 1992; Weirich et. al., 1992). If these biochemical disturbances are too great, and the fish is unable to recover homeostasis, then mortality may result. Measurement of the changes in these parameters has been used to elucidate the severity of the stressor, including responses brought on by handling (McGeer et. al., 1991; Robertson et. al., 1987).

A number of mitigation techniques have been used to lessen the severity of the stress response as a result of handling. These techniques have involved a pre-stress three day starvation period, anesthetic treatment, raising the water hardness, and addition of salts to the recovery water (Grizzle et. al., 1993; Thomas & Arnold, 1988; Tomasso et. al., 1980).

The addition of salts is perhaps the most common technique used with freshwater fish to alleviate the severity of the stress response. By raising the salinity, the inside and outside environments are closer to being balanced, and it is easier for the fish to recover and maintain osmotic homeostasis. Several researchers (Maetz, 1974; Randall et. al., 1972; Wedemeyer et. al.,

1990) have shown that recovering and maintaining osmotic homeostasis may be the most important key in recovering from a stress. Adult pure striped bass have an especially hard time maintaining electrolyte balance when stressed (J.J. Cech, pers. comm.).

Unfortunately, in most of the literature a precise salinity is not specified. Thus, an aim of this study was to determine the most effective salinity for reducing the stress response when handled hybrid striped bass were placed in the water for a recovery period. A second objective of this study was to evaluate the aquaculture industry practice of starving fish for three days before handling and any other subsequent procedure, so that the gastrointestinal tract is clear. For this purpose, the hybrids were separated into two sets: Fed and Three Day Starved before the beginning of the experiment. Most published literature has only shown significant difference in the stress response between fed fish and those starved for a long period of time (30 days).

## MATERIALS AND METHODS

*Fish* Hybrid striped bass of both sexes for the 0‰ experiment were raised until fingerling size at Pintail Point Farm in Queenstown, Maryland. They were transported to the lab at Virginia Tech in slightly saline (5‰), well aerated water.

Upon arrival at Virginia Tech, the hybrids were placed in 390L holding tanks with similar temperature and salinity to travel tank water (i.e. 21°C, 5‰). Salinity was slowly decreased over a number of days to 0‰, and the temperature was maintained at 21° C. The water used is dechlorinated, and calcium is added in the form of calcium chloride,to raise concentrations of hardness above 150 ppm, before reaching the holding tanks. Flow rate was kept high enough to ensure that concentrations of ammonia remained below 1.0 ppm, nitrite concentrations below 0.2 ppm, and dissolved oxygen concentrations with aeration remained at close to saturation. The bass were fed a commercially available fish chow two times daily to satiation. Fish were allowed almost four weeks to acclimate, because there was a large die-off attributable to a parasite three days after arrival at the lab. Only enough fish were left after the die-off to use in the 0‰ experiments. The mean mass at the time of experimentation was 6.6g and length 6.85 cm.

Hybrids for the 5, 10, and 15‰ trials were obtained as fingerlings from Southland Fisheries Corporation in Hopkins, South Carolina. The fish were transported by the company to the Virginia Tech Aquaculture Center, where they were kept in 700 L four ft circular tanks. A flow-thru well water system was employed. Hardness was kept above 150 ppm, oxygen concentrations were close to saturation, and ammonia and nitrite concentrations were low enough to be undetectable. All experimentation on these fish took place at the Aquaculture Center to avoid stressing the fish by moving them again. An acclimation period of three weeks was

allowed. At the time of experimentation, the mean weight and length were 4.17 g and 4.4 cm. This is too small for much in the way of blood analyses, so whole-body parameters were used for most of the measurements.

After discussion with the head of the VA Tech Aquaculture Center, it was determined that there is little or no difference in stocks of striped bass on the East Coast, because fingerlings are often transfered between states. Therefore, there should not be any significant differences between the hybrid striped bass' stress responses even though they are from different facilities.

*Stress* The same procedure for the handling stress was used for all four salinity sets. Bass in groups of six were dipnetted, and aerially emersed for 5 minutes. After the stress, each group was returned to a static, aerated 40 L. Nalgene™ container for a recovery period. One group of bass was not held out of water, but immediately sacrificed and used for controls. Transfer took less than thirty seconds and did not appear to trigger a stress response. In a preliminary test, this group was compared with another group in which the fish were first sedated with benzocaine dissolved in the water before transfer. No significant differences were found. Another group was stressed, and then immediately killed and frozen and used for $T_0$ . Sets were taken at .5 hrs, 1 hr, 6 hrs, 12 hrs, 24 hrs, and 48 hrs of recovery.

The bass after stress and recovery were placed in an acute anesthetizing dose of benzocaine, 50 mg/ L (Soivio et. al., 1977; Summerfelt & Smith, 1990; Wedemeyer, 1970). After immobilization, the fish were partially decapitated and a drop of blood was taken for osmolality analysis. Blood letting was accomplished in less than thirty seconds. The bass were then placed and left in liquid nitrogen for 2 - 3 minutes to ensure complete freezing. Bass were placed in double-tagged plastic bags for storage at -80° C.

*Analysis* Frozen bass were weighed individually, decapitated, weighed again, and heads were discarded. The bass were then cut into small pieces and homogenized in 15 ml of 8% perchloric acid in preparation for glucose, glycogen, and lactic acid analysis. Homogenization was done in a Waring™ Commercial blender. Approximately 4 ml of homogenate were saved in a labelled centrifuge tube for analyses. These samples were kept refrigerated at 0-5° C for no more than one week.

The homogenate in the tubes was centrifuged for ten minutes. 0.5 mls of the supernatant was removed, placed in another centrifuge tube, and spun again. The supernatant from this tube was used in the glucose and lactic acid analyses. Excess supernatant was returned to the original tubes. The pellet was resuspended in the remaining supernatant, and this was used in the glycogen analyses. It was assumed that the majority of the glycogen remained in the pellet, and was not suspended in the supernatant.

Glucose and glycogen analyses were accomplished by using Sigma® Glucose Kit #510 for blood. It was modified only to the extent that a whole-body sample was used instead of blood. Glycogen was indirectly measured by conversion into glucose by a procedure (Roehrig & Allred, 1974) using amyloglucosidase. The resulting supernatant wasthen analyzed using the Sigma® Glucose Kit.

Lactic acid concentrations were analyzed using an enzymatic tissue procedure modified from Sigma® Kit #826-UV. Results were taken spectrophotometrically as absorbance using appropriate standards to prepare a standard curve.

Osmolality measurements were done by taking a drop of blood from the heart after anesthetization for immediate placement in a Wescor Model 5130A vapor pressure osmometer.

*Statistical Analyses* statistical analyses included analyses of variance, F test for variance, Student's t test and coefficient of correlation. The criterion for statistical difference was p=.05.

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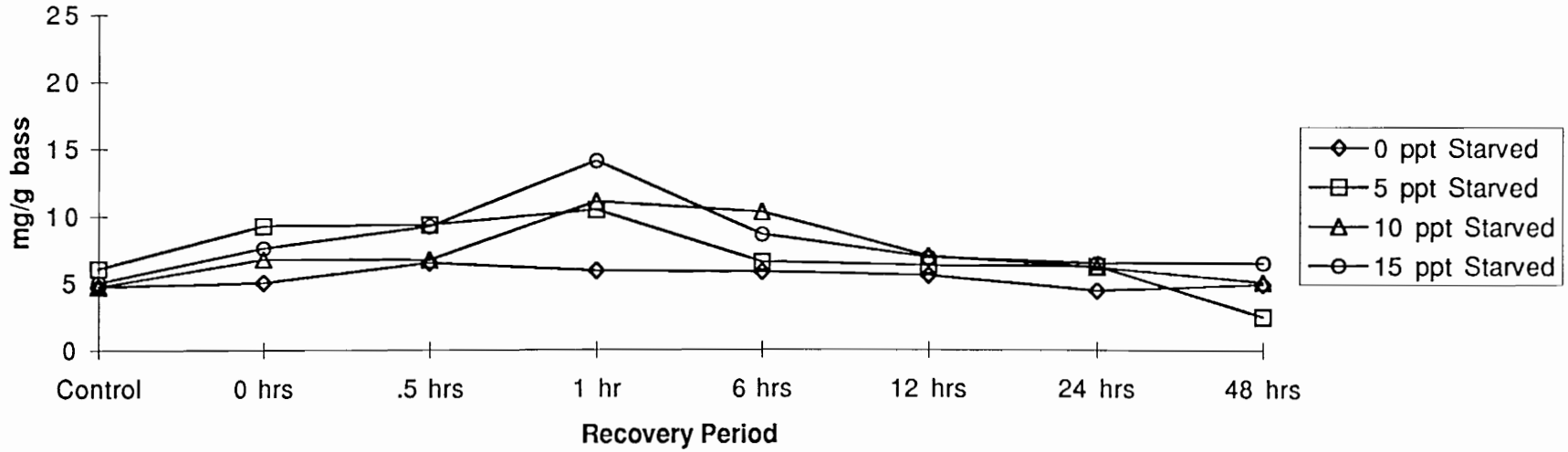
## Results

Resting glucose concentrations of the starved and fed groups overlapped, but the starved groups tended to be slightly lower than the fed ones. Immediately after the stress, all of the groups recovering in saline water had a significant increase in glucose concentrations (Figs.1, 2a&b), but those recovering in

Fig. 1 Changes in the concentration of whole body glucose in hybrid striped bass after stress and recovery in various salinity waters. Fish were handled at 0 hrs. Standard error not included for clarity. 0‰ group from different stock facility.

## Average Whole Body Glucose Concentrations in Hybrid Striped Bass After Stress and Recovery in Various Salinity Waters



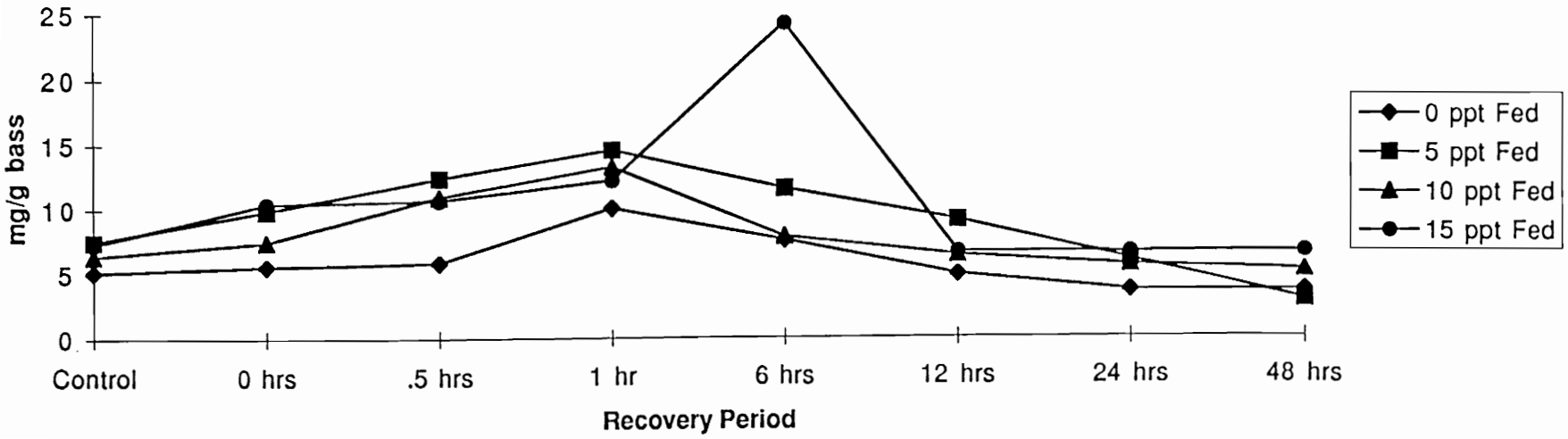



Fig. 2a Changes in the concentration of whole body glucose in hybrid striped bass after stress and recovery in 0 & 5 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05). 0‰ group from different stock facility.

## Average Whole Body Glucose Concentrations in Hybrid Striped Bass After Stress and Recovery in 0 & 5 ppt Saltwater

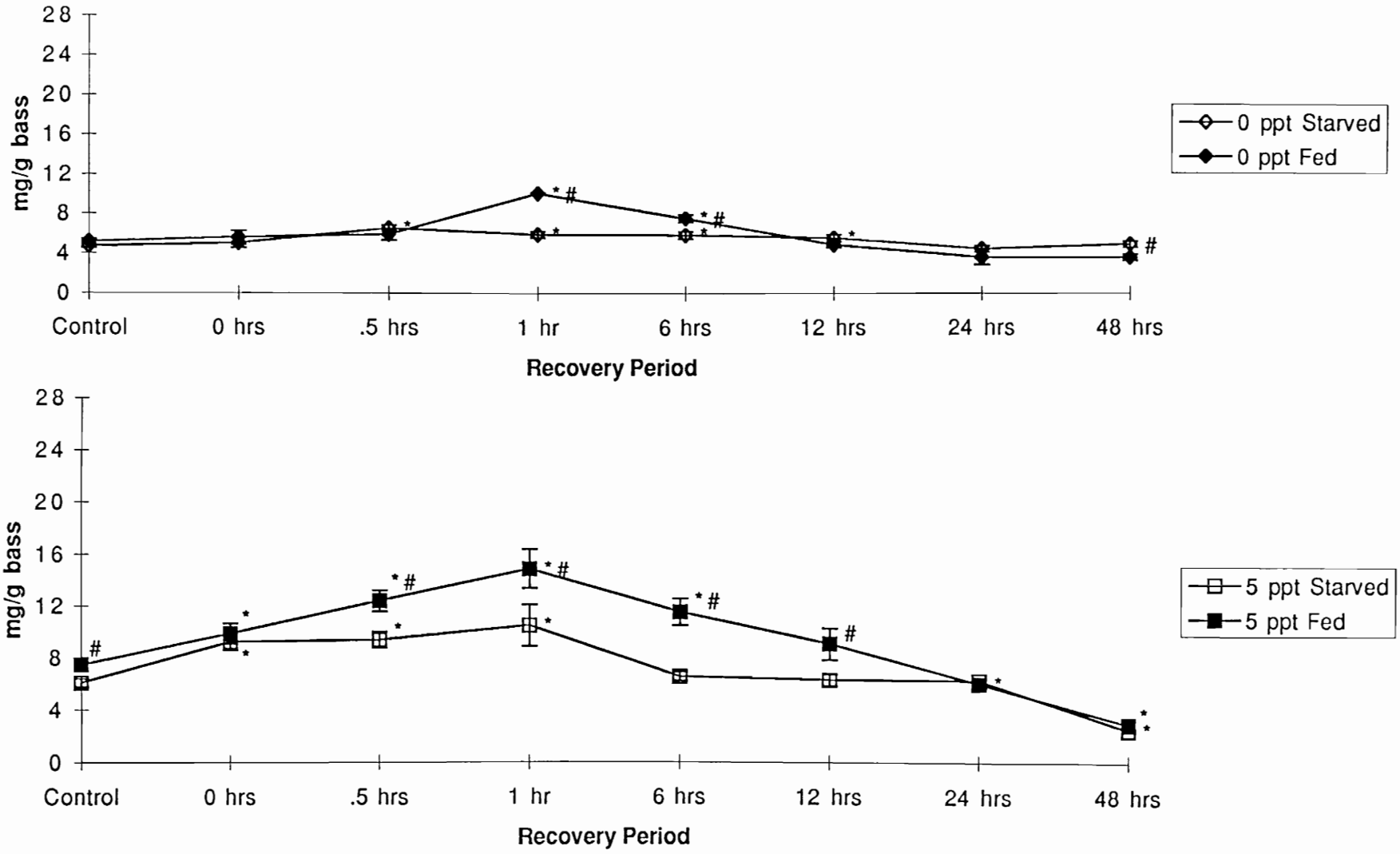
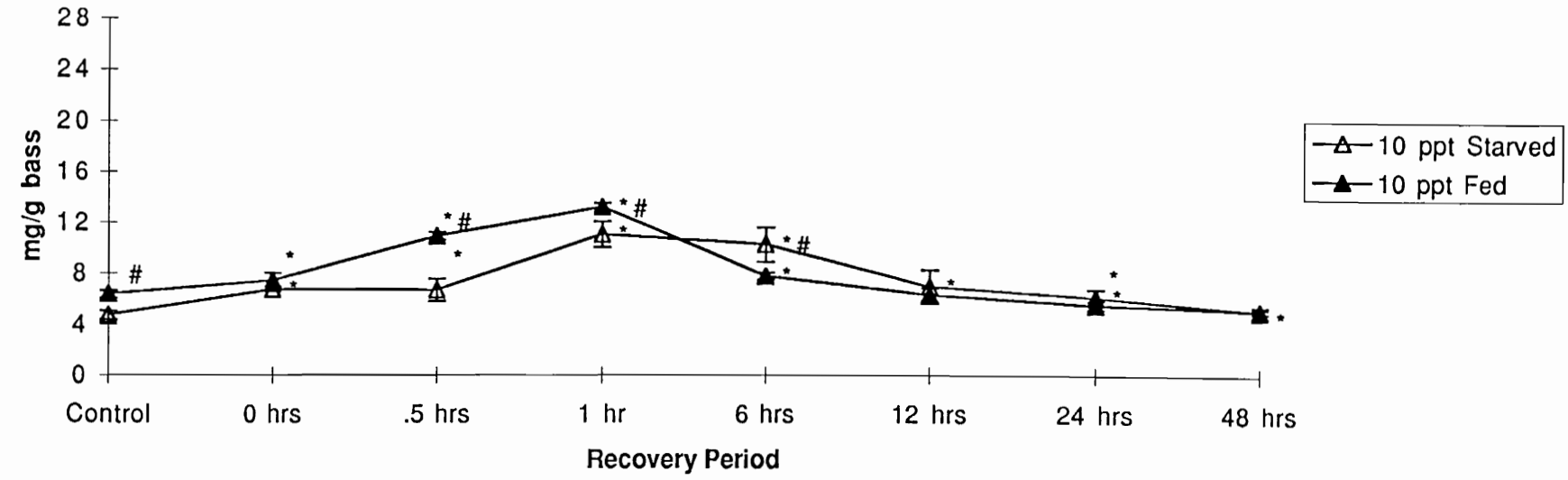
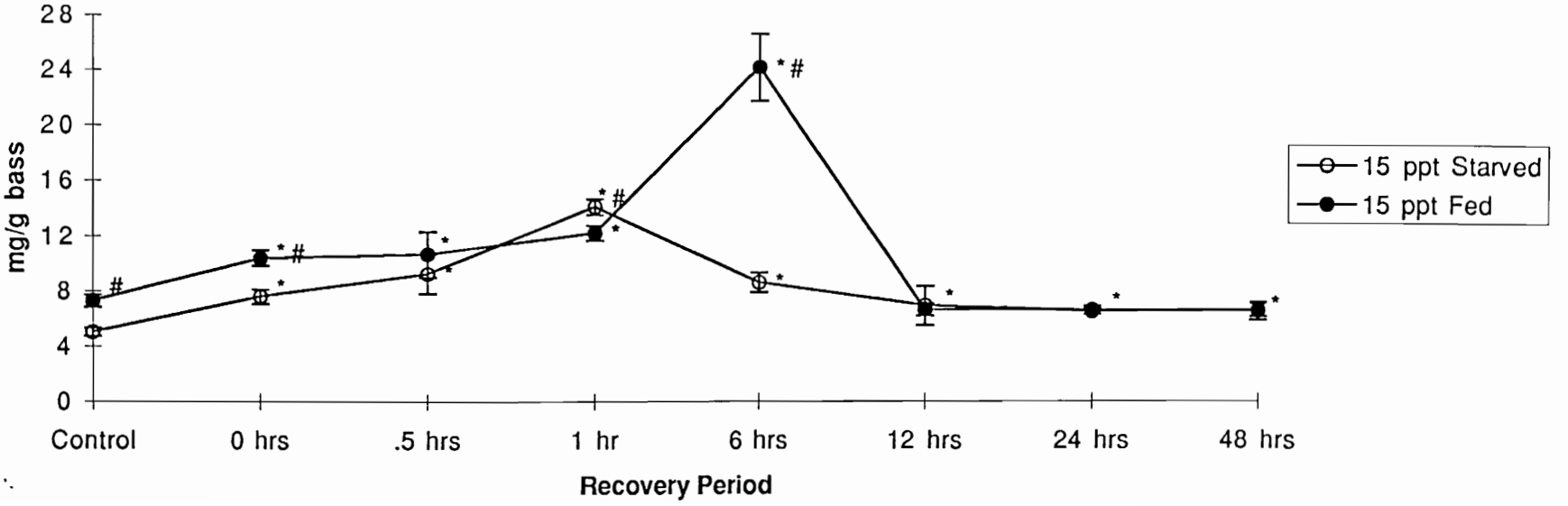


Fig. 2b Changes in the concentration of whole body glucose in hybrid striped bass after stress and recovery in 10 & 15 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

## Average Whole Body Glucose Concentrations in Hybrid Striped Bass After Stress Allowed to Recover in 10 & 15 ppt Saltwater



freshwater (0‰ ) did not. Severity of the starved groups'glucose response increased with salinity. At the end of the 48 hr recovery period, the 0‰ and 10‰ starved groups were stabilized at control concentrations, while the 5‰ group was significantly below, and the 15‰ group significantly higher than control concentrations.

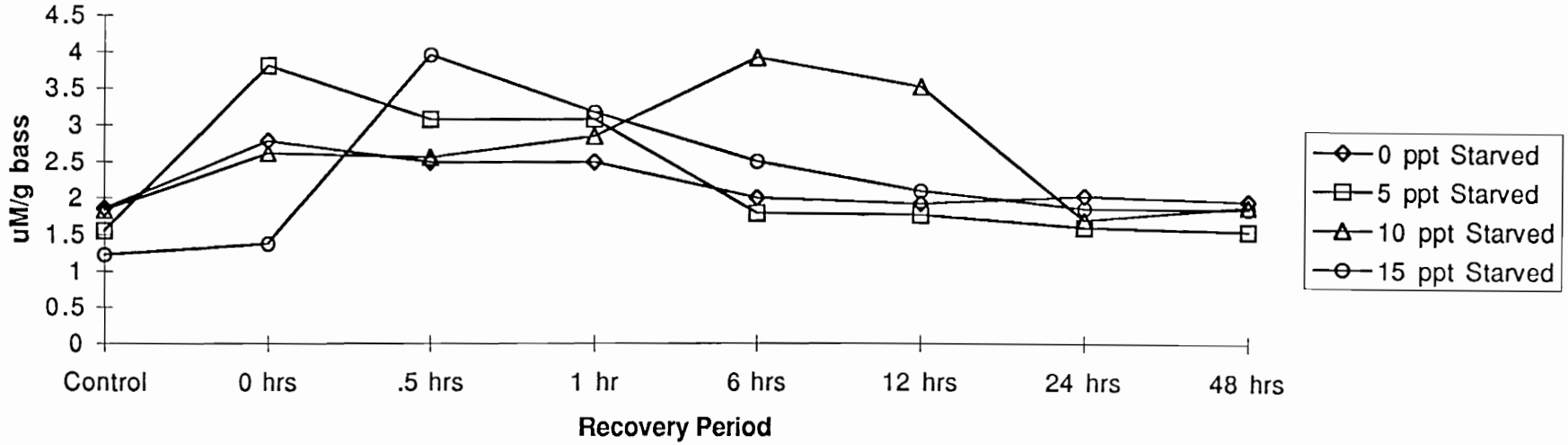
Severity also tended to increase with salinity in the fed groups. The peak level of glucose in the 15‰ group, was especially severe at 330% resting concentrations. Glucose concentrations in all of the fed groups returned to resting by twelve hours post-stress, and in all but the 15‰ group, stabilized at slightly lower than resting concentrations at twenty-four hours. Feeding state did play a role in the glucose stress response.

Control values of lactate in the fed and starved groups were similar, except the 15‰ starved group which was significantly below all of the other groups (Figs. 3, 4a&b). All of the groups had significant increases immediately after the stress. Severity of the peak response in the starved groups differed only in the absolute, with the 0‰ group having a much lower peak response than the saline recovery groups. The 0, 5, and 10‰ starved groups stabilized during the 48 hr recovery period, while the 15‰ group remained elevated at the end of the recovery period.

Peak lactate values in the fed fish occurred at thirty minutes post-stress in all of the groups, and were insignificant from each other. All of the groups regained resting concentrations at six hours post-stress, and except for the 5‰ group stabilized. The

Fig. 3 Changes in the concentration of whole body lactate in hybrid striped bass after stress and recovery in various salinity waters. Fish were handled at 0 hrs. Standard errors not included for clarity. 0‰ group from different stock facility.

## Average Whole Body Lactate Concentrations in Hybrid Striped Bass After Stress and Recovery in Various Salinity Waters



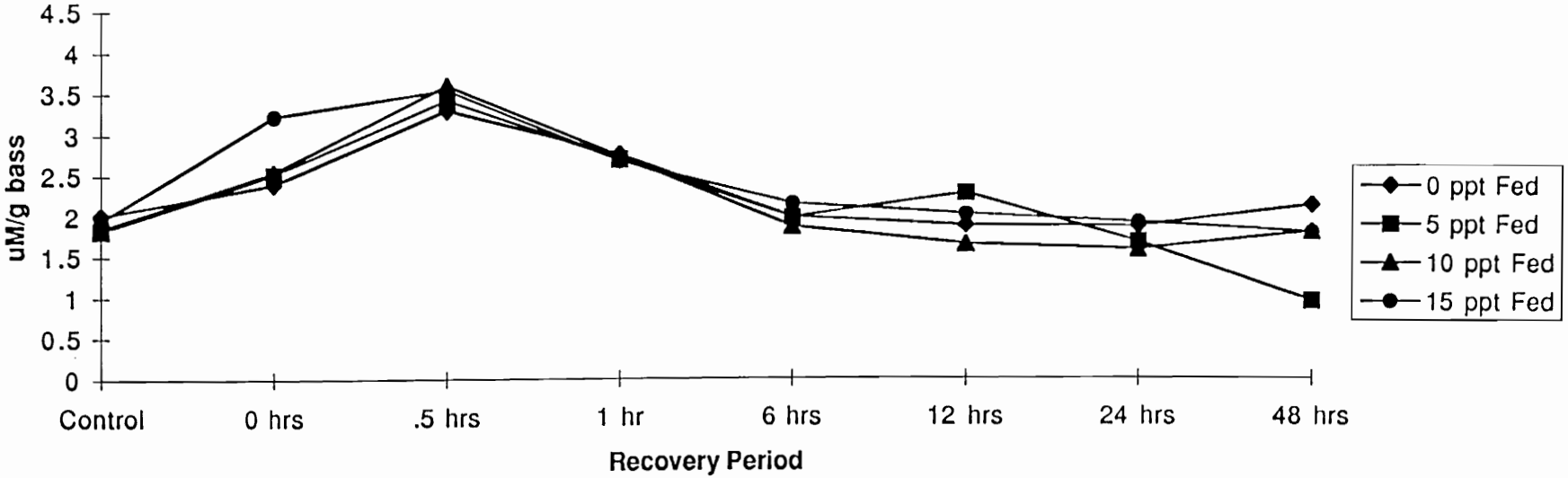
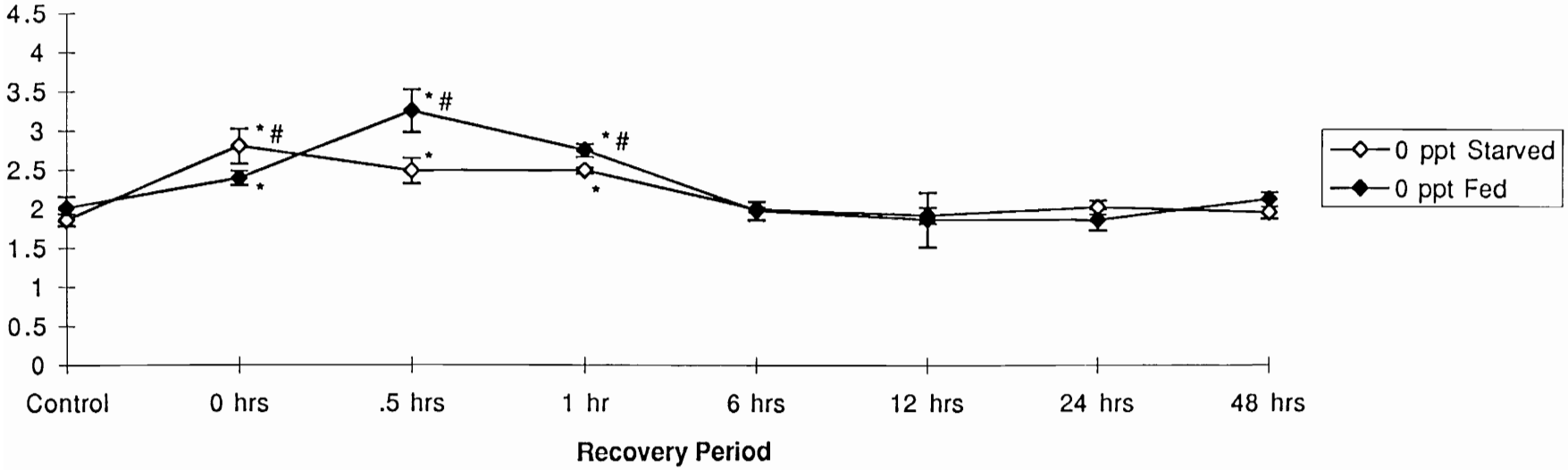



Fig. 4a Changes in the concentration of whole body lactate in hybrid striped bass after stress and recovery in 0 & 5 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes siginficant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05). 0‰ group from different stock facility.

## Average Whole Body Lactate Concentrations in Hybrid Striped Bass Allowed to Recover in 0 & 5 ppt Saltwater



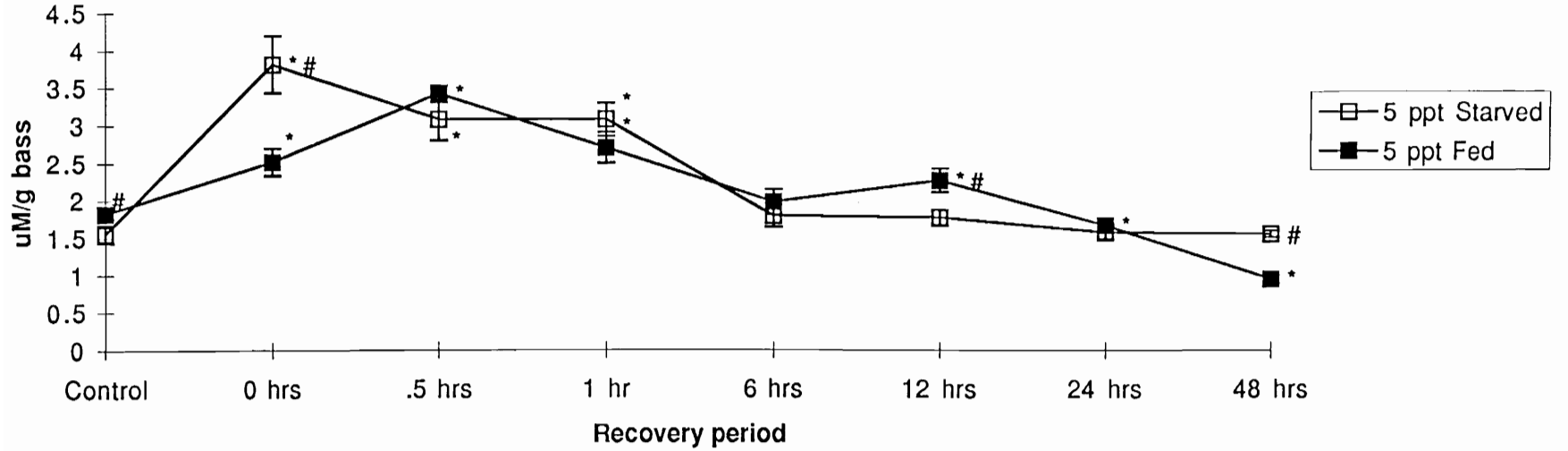
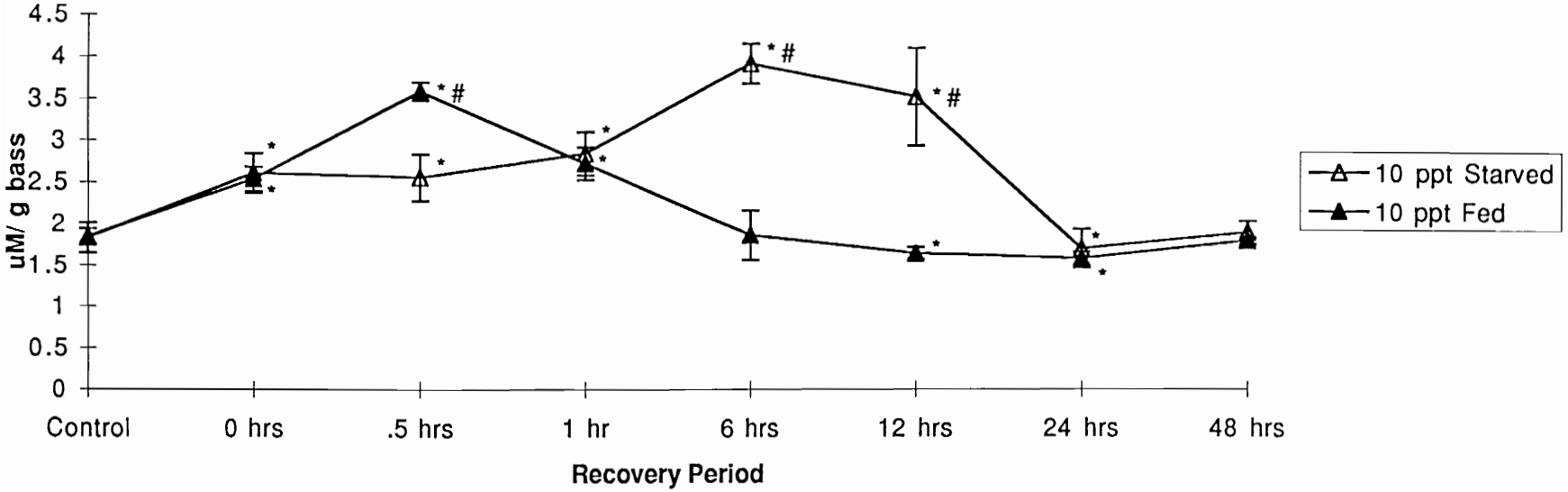
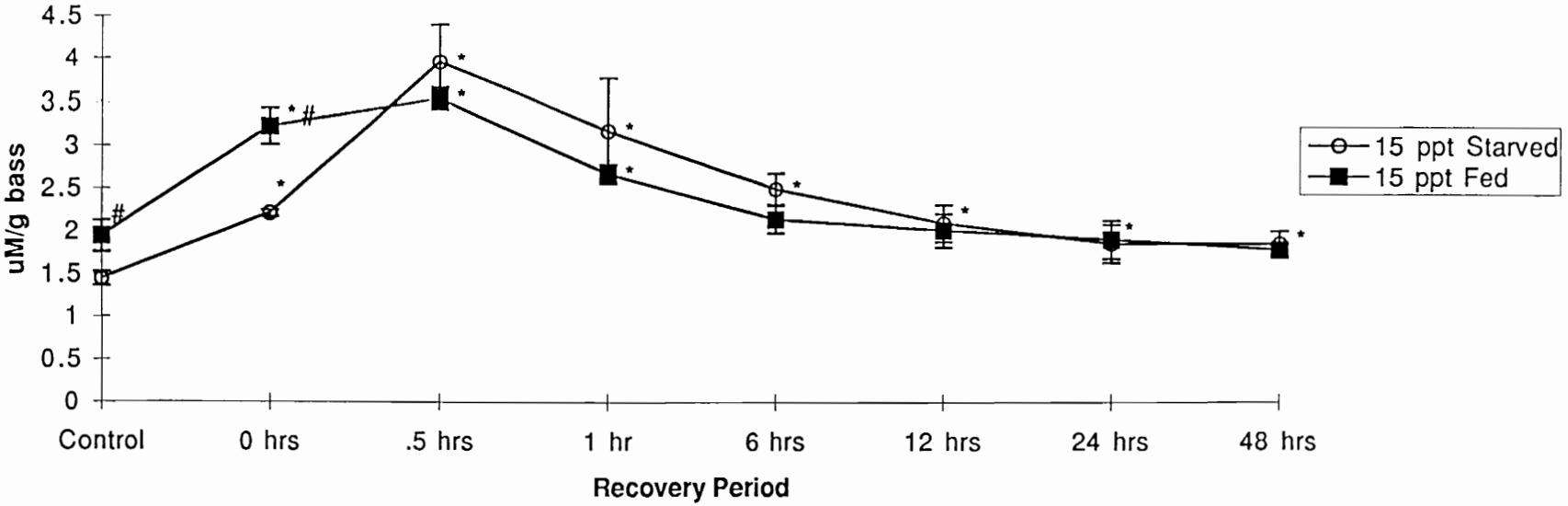



Fig. 4b Changes in the concentration of whole body lactate in hybrid striped bass after stress and recovery in 10 & 15 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

## Average Whole Body Lactate Concentrations in Hybrid Striped Bass After Stress and Recovery in 10 & 15 ppt Saltwater



5‰ group continued to decline through the rest of the recovery period.

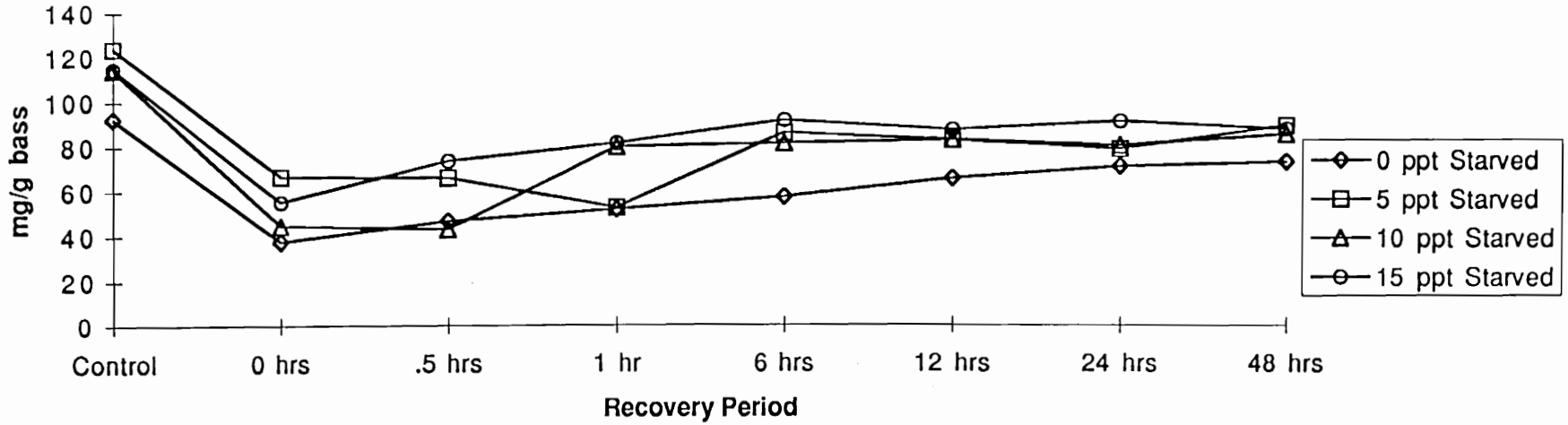
The starved groups' control glycogen concentrations varied widely, while the fed groups' were similar (Figs. 5, 6a&b). The starved groups' decline after the stress averaged approximately half (55%) of their glycogen reserves. None of the starved groups returned to resting glycogen concentrations by forty-eight hours post-stress. The fed groups' initial declines were not as severe, and all the groups remained similar. On average, fed groups lost approximately one third (37%) of their glycogen reserves. All of the fed groups regained control concentrations or higher during the 48 hr recovery period. Feeding state played a large role in the glycogen response.

Correlation coefficients were run on each group comparing the glucose and lactate concentrations over time with the glycogen concentrations (Table 1). All of the groups had strong negative correlations, except the 10‰ and 15‰ groups starved groups in both comparisons, and the 15‰ group glucose-glycogen comparison.

Resting blood osmolality values were significantly different between the fed and starved groups (Fig. 7, 8a&b). There was also a significant difference within the groups. Both freshwater recovery control groups had significantly higher concentrations than the saline recovery groups of the same feeding state. After the stress, the osmolality of both fed and starved fish recovering in saltwater increased, while those in freshwater decreased. Bass, fed

Fig. 5 Changes in the concentration of whole body glycogen in hybrid striped bass after stress and recovery in various salinity waters. Fish were handled at 0 hrs. Standard errors left not included for clarity. 0‰ group from different stock facility.

## Average Whole Body Glycogen Concentrations in Hybrid Striped Bass After Stress and Recovery in Various Salinity Waters



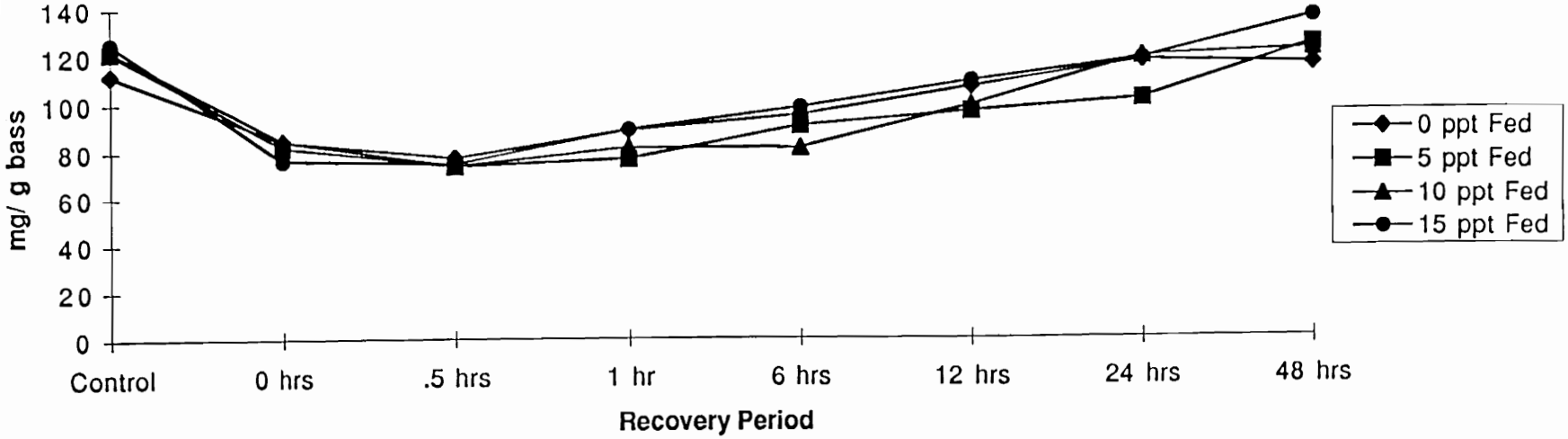
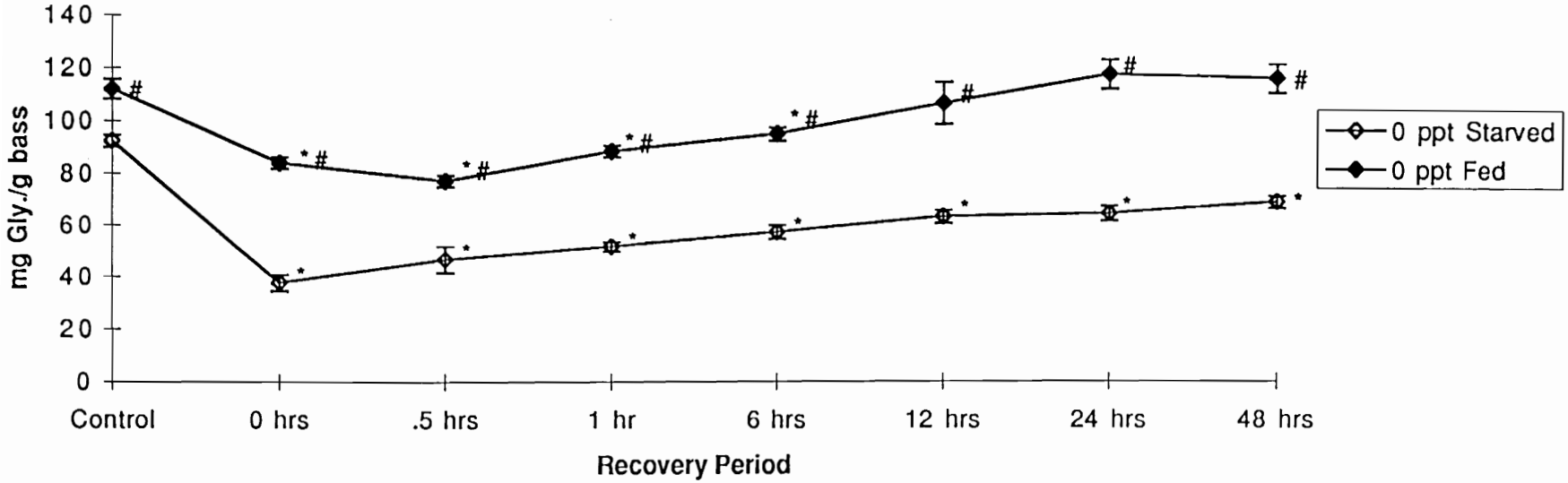



Fig. 6a Changes in the concentration of whole body glycogen in hybrid striped bass after stress and recovery in 0 & 5 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes siginficant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05). 0‰ group from different stock facility.

## Average Whole Body Glycogen Concentrations in Hybrid Striped Bass After Stress and Recovery in 0 & 5ppt Saltwater



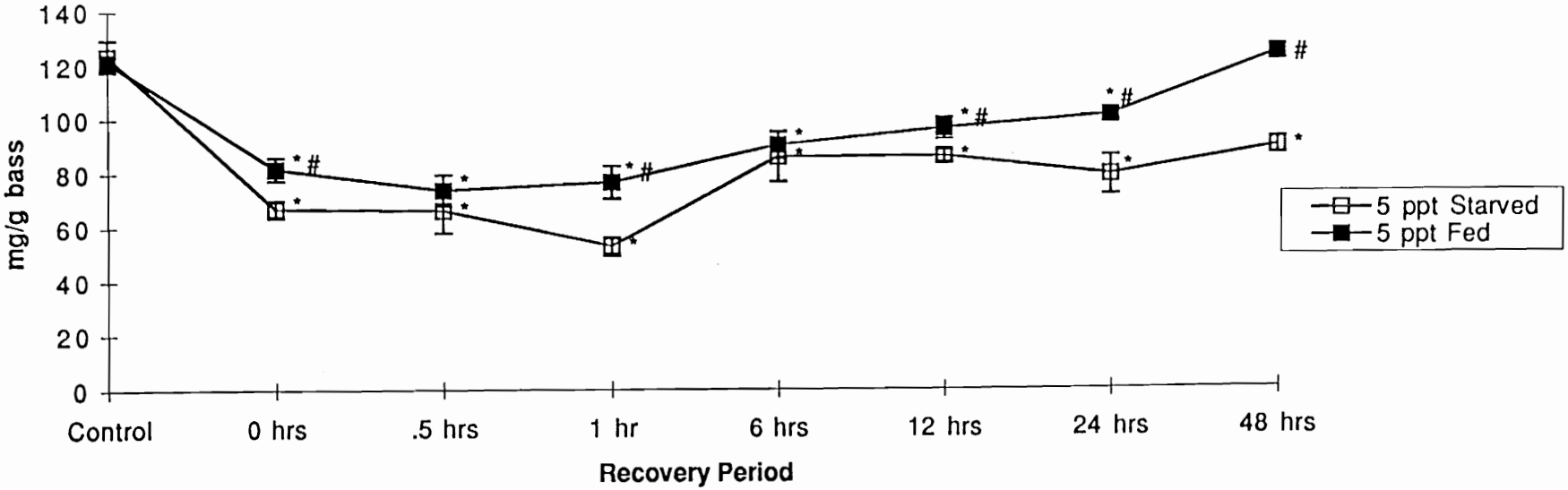
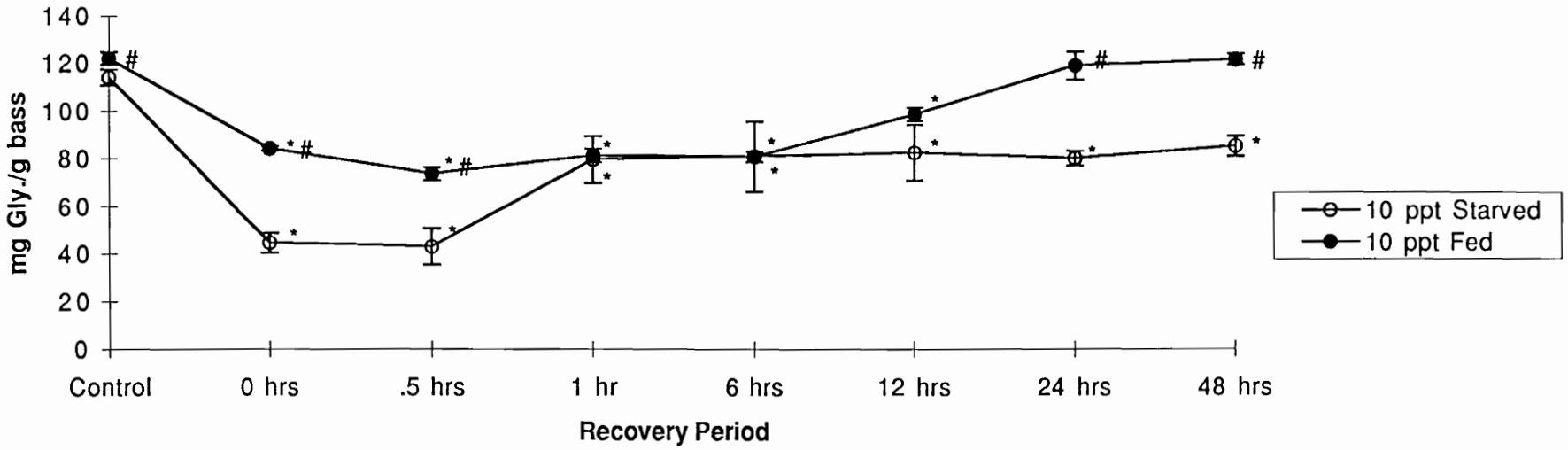



Fig. 6b Changes in the concentration of whole body glycogen in hybrid striped bass after stress and recovery in 10 & 15 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point,
* denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

## Average Whole Body Glycogen Levels in Hybrid Striped Bass After Stress And Recovery in 10 & 15 ppt Saltwater



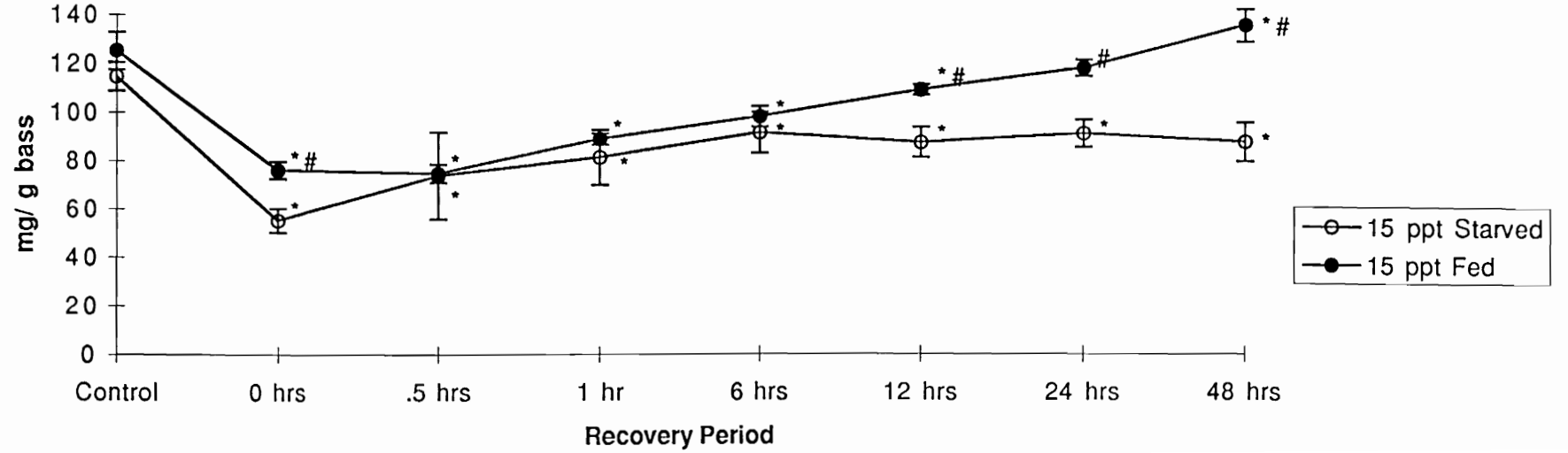
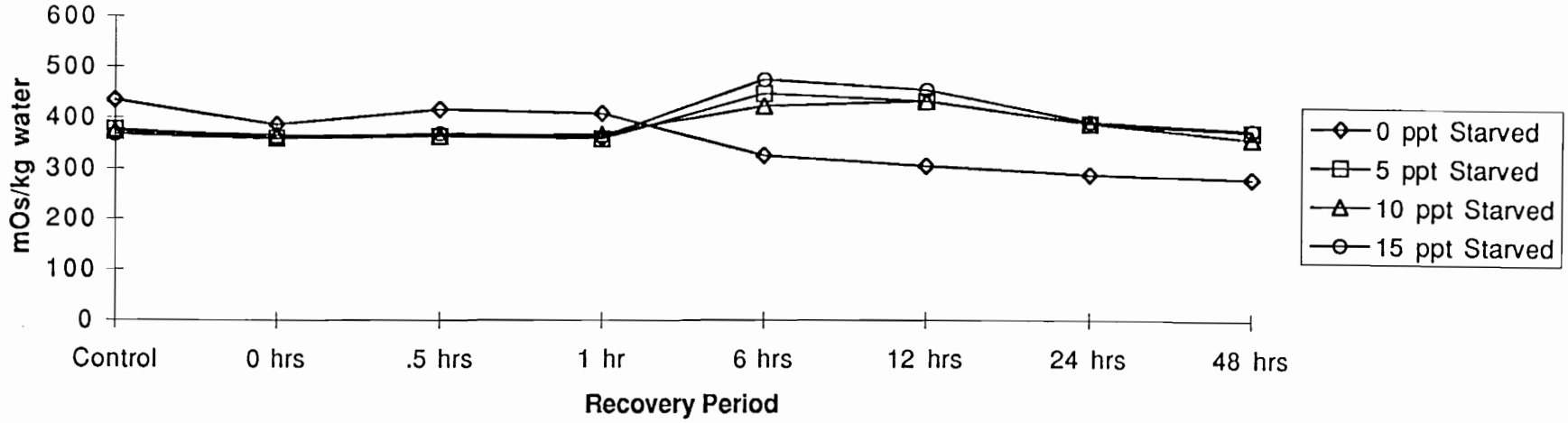



TABLE 1

| Comparison | Correlation Coefficient |
|---|---|
| | |
| 0 ppt Fed | |
| - Glucose/Glycogen | -0.6136 |
| - Lactate/Glycogen | -0.8115 |
| 0 ppt Starved | |
| - Glucose/Glycogen | -0.5816 |
| - Lactate/Glycogen | -0.8717 |
| 5 ppt Fed | |
| - Glucose/Glycogen | -0.8643 |
| - Lactate/Glycogen | -0.8767 |
| 5 ppt Starved | |
| - Glucose/Glycogen | -0.6442 |
| - Lactate/Glycogen | -0.7124 |
| 10 ppt Fed | |
| - Glucose/Glycogen | -0.7602 |
| - Lactate/Glycogen | -0.7301 |
| 10 ppt Starved | |
| - Glucose/Glycogen | -0.1902 |
| - Lactate/Glycogen | -0.2656 |
| 15 ppt Fed | |
| - Glucose/Glycogen | -0.3997 |
| - Lactate/Glycogen | -0.9215 |
| 15 ppt Starved | |
| - Glucose/Glycogen | -0.3789 |
| - Lactate/Glycogen | -0.2696 |

Fig. 7 Changes in the concentration of whole blood osmolality in hybrid striped bass after stress and recovery in various salinity waters. Fish were handled at 0 hrs. Standard errors not included for clarity. 0‰ group from different stock facility.

## Average Blood Osmolality in Hybrid Striped Bass After Stress and Recovery in Various Salinity Waters



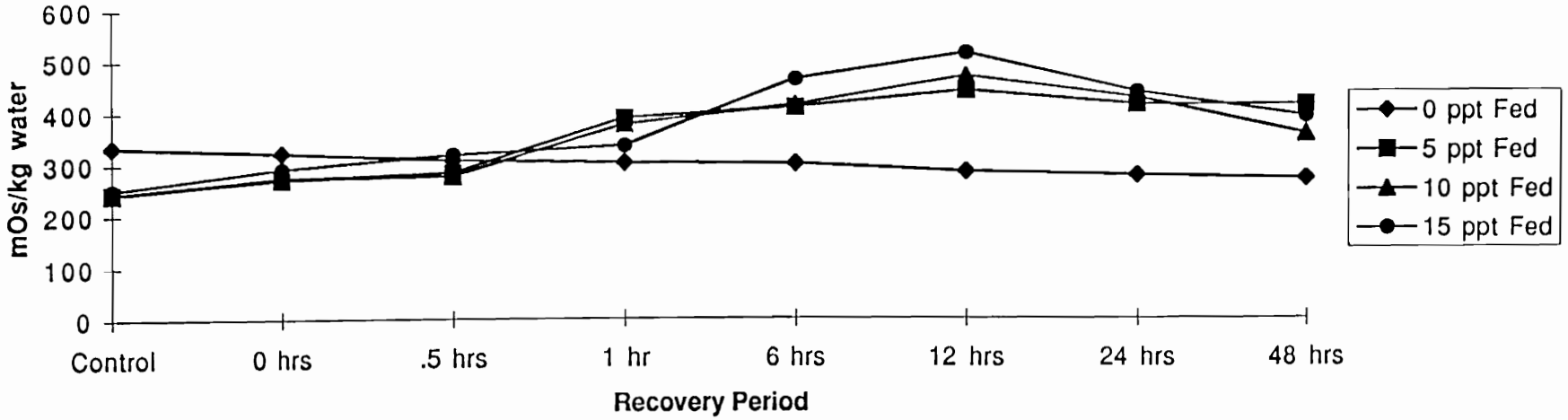
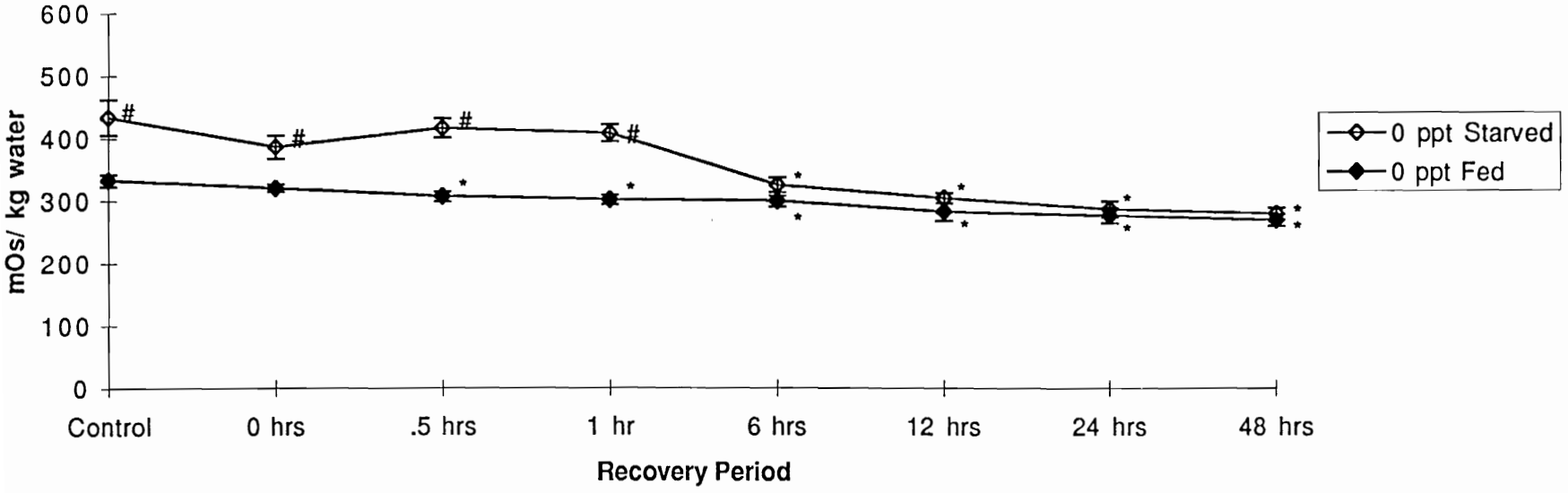



Fig. 8a Changes in the concentration of whole blood osmolality in hybrid striped bass after stress and recovery in 0 & 5 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point, * denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05). 0‰ group from different stock facility.

## Average Blood Osmolality in Hybrid Striped Bass After Stress and Recovery in 0 & 5 ppt Saltwater



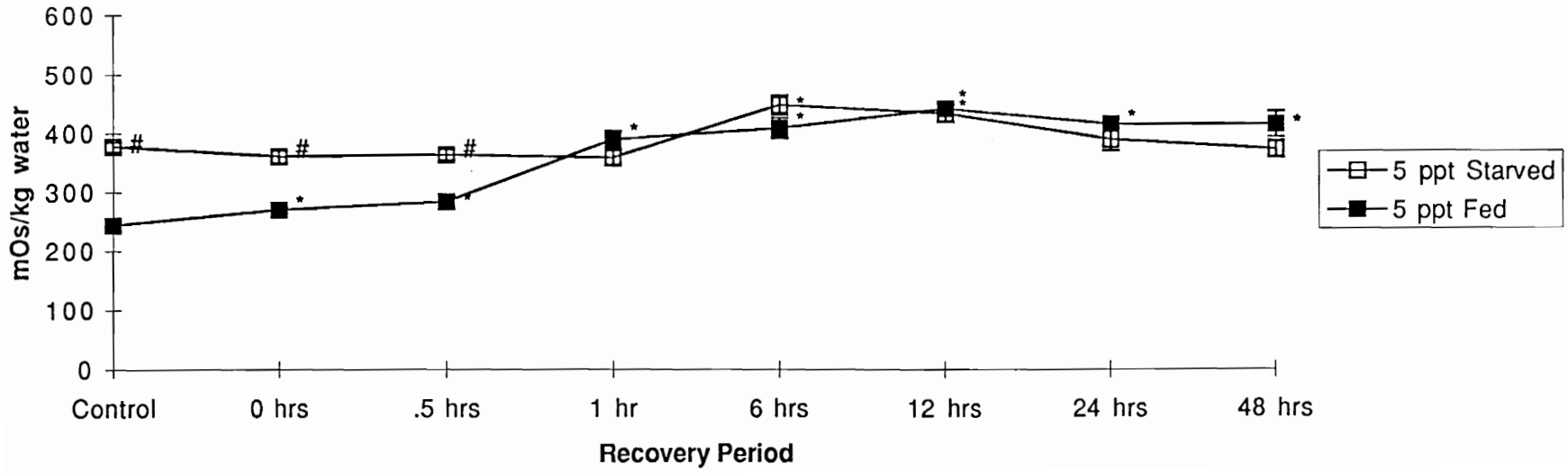
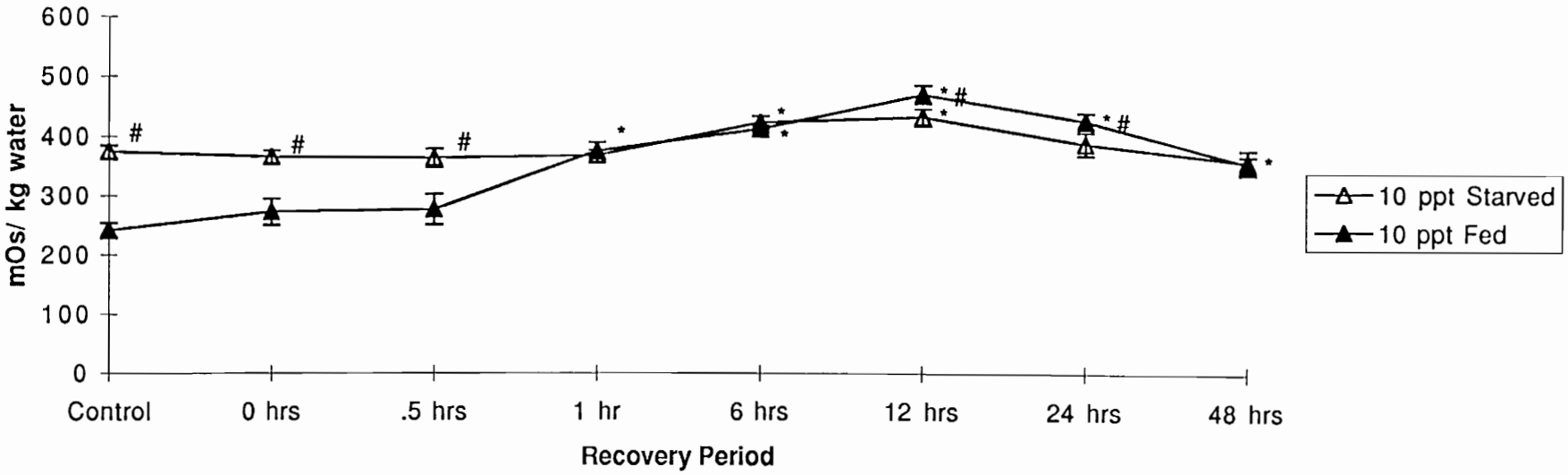
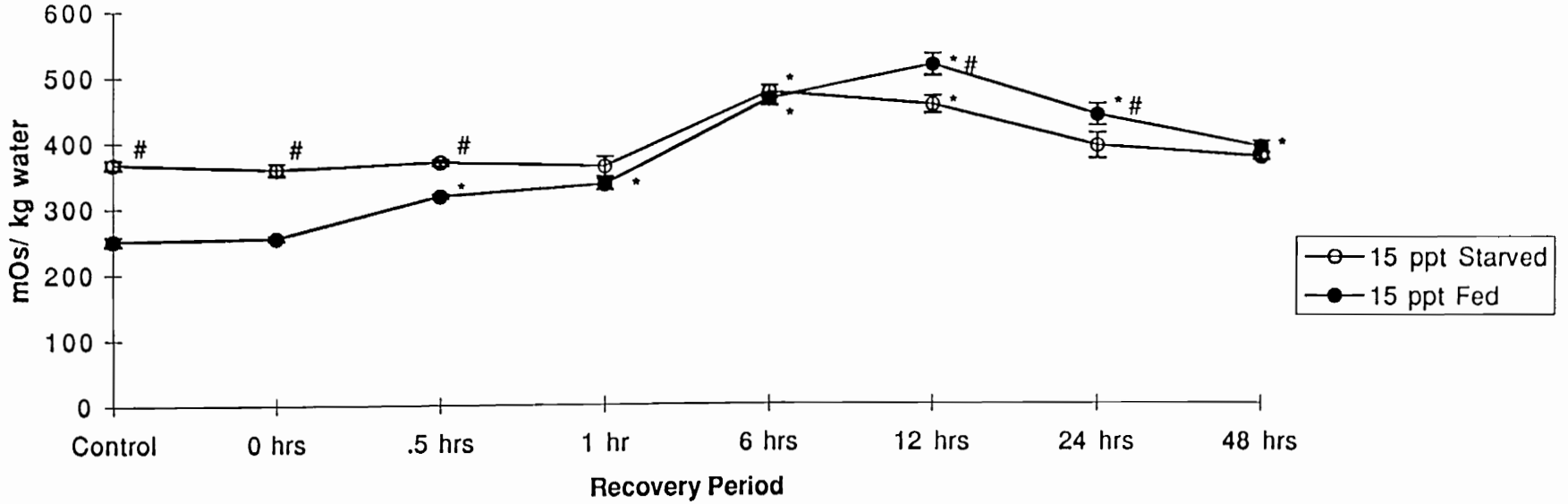



Fig. 8b Changes in the concentration of whole blood osmolality in hybrid striped bass after stress and recovery in 10 & 15 ppt saltwater. Fish were handled at 0 hrs. Vertical lines indicate ± S.E.M., n = 6 for each point,
* denotes significant difference from control (P = .05), # denotes fed and starved groups are significantly different (P = .05).

## Average Blood Osmolality in Hybrid Striped Bass After Stress and Recovery in 10 & 15 ppt Saltwater



or starved, recovering in freshwater had a slow steady decline of blood osmolality throughout the experiment. All of the saline starved groups returned to control concentrations during the 48 hr recovery period, while the saline fed groups remained elevated at the end of the recovery period.

## Discussion

When hybrid striped bass were subjected to an acute handling stress, and then allowed to recover in various salinity waters, several whole-body parameters and blood osmolality imbalances occurred. These imbalances persisted in some cases for at least 48 hours. Both salinity of the recovery water and feeding state (fed vs. three day starved) of the test fish impacted the stress response.

Elevated glucose concentrations are one of the most commonly used indicators of stress in fish. These increased concentrations are caused by rising concentrations of catecholamines, which in turn cause an increase in glycogenolysis, the conversion of glycogen to glucose, and gluconeogenesis (Mazeaud et. al., 1977; Nakano & Tomlinson, 1967; Schwalme & McKay, 1991; Wedemeyer et. al., 1990). Elevated concentrations of glucose are thought to be sustained by cortisol, which stimulates liver gluconeogenesis, and may suppress peripheral sugar uptake (Barton & Iwama, 1991; Robertson et. al., 1988; Vijayan et. al., 1991; Wedemeyer et. al., 1990). A study by Anderson et. al. (1991) with rainbow trout however, failed to show a glucose response to cortisol

when the cortisol was administered by an osmotic pump surgically implanted in the fish. There may be wide variations among species so more research needs to be conducted.

Resting concentrations of glucose in the fed and starved groups, though there was some overlap, were for the most part significantly different. The lower concentrations in the starved groups appears to indicate that even a short term starvation has some consequences. In each of the salinity recovery sets, the fed group had significantly higher peak concentrations of whole-body glucose than the starved group (Fig. 1,2 a-b). Feeding has been known to affect the glucose response, but it has only been shown in fish starved for twenty days (Barton et. al., 1988) or longer (Foster & Moon, 1991; Vijayan & Moon, 1992), and not for fish starved for only a short time (Fletcher, 1984; Lewis & Epple, 1984). Since the glucose response is at least partially dependent on liver glycogen stores (Mazeaud et. al., 1977), it follows that fish with lower liver glycogen reserves would not be capable of exhibiting as strong a glucose response (Aldrin et. al., 1979; Barton et. al., 1988). Although the starved groups had a lesser response, concentrations remained elevated for a longer period of time than the fed groups. This may indicate that the stress had a greater effect, as well as indicating that another energy pathway is being used. Stored body lipids have been shown to be an important energy source during fasting in fish (Jezierska et. al., 1982).

The severity of the glucose stress response tended to increase with increasing salinity of the recovery water. The fed and

starved groups recovering in freshwater had the most moderate responses.

Hypoxic conditions, such as were obtained during the air exposure, have been shown to cause increases in lactic acid production (Hopkins & Cech, 1992; Pickering et. al., 1982; Vijayan & Moon, 1992; Wedemeyer et. al., 1990). Lactic acid is produced in the white muscle, as a result of anaerobic metabolism (Lackner et. al., 1988; Pickering et. al., 1982). Whole-body lactic acid measurements may present a more accurate picture of the stress than blood measurements. Lactic acid must diffuse from the poorly vascularized white muscle into the blood stream (Groman, 1982), and this may cause peak plasma concentrations to occur a significant time period after the lactic acid was produced (Love, 1980). With fish of fingerling size, as these were, limited blood volume prevents many blood analyses.

Resting concentrations of lactic acid were similar to each other, except the 15‰ treatment, which was significantly lower for an unknown reason. Resting concentrations were similar to those found by Goolish (1991), but significantly lower than those reported by Krumschnabel & Lackner (1992). Peak whole-body concentrations occurred immediately after the stress, or thirty minutes post-stress in all groups except the 10‰ starved group. This is in contrast to peak plasma concentrations, which usually occur at one to four hours post-stress (Love, 1980; Pickering et. al., 1982; Vijayan & Moon, 1992; Wood & Perry, 1985). There was no significant difference in peak concentrations between the fed and

starved groups in each salinity set. Vijayan & Moon, (1992) also did not find a significant difference in the lactate responses of fed and trout starved thirty days.

Salinity of the recovery water did not affect the fed groups' lactate responses significantly. All of the fed groups recovered at six hours post-stress. Salinity of the recovery water did affect the starved groups in two ways. The freshwater group had a more moderate response than any of the saline groups, and the 0‰ and 5‰ groups were able to recover in twenty five percent or less of the time that it took the fish in the 10‰ or 15‰ recovery environments. This appears to indicate that salinity of recovery water does not affect fed fish, but in starved fish, increasing salinity aggravates the stress response.

Glycogen stored in the liver represents an important energy source. Studies have shown that hepatic glycogen concentrations decrease in stressed teleosts (Paxton et. al., 1984, Vijayan et. al., 1990). This glycogenolysis is stimulated by catecholamine elevation (Mazeaud et. al., 1977; Wedemeyer et. al., 1990). Evidence shows that the recovery of glycogen is probably dominated by an as yet undefined intramuscular glyconeogenic pathway from lactic acid, and not a direct incorporation of glucose into muscle glycogen (Arthur et. al., 1992; Moyes et. al., 1992; Schulte et. al., 1992). West et. al. (1994) found that in rainbow trout, glucose, regardless of plasma concentration, accounted for less than 10% of glycogen repletion after exercise. Feeding state has been known to modify glycogen metabolism (Vijayan & Moon, 1992), and

changes in glycogen reserves may have a direct effect on the glucose response (Aldrin et. al., 1979).

Resting concentrations of glycogen in the fed groups were similar, while the starved groups' concentrations ranged widely, suggesting that utilization of glycogen during fasting is variable among fish. All of the groups showed significant declines in glycogen immediately after the handling stress. The starved groups lost a significantly greater percentage of their reserves than the fed group. This may indicate that the starvation had compound effects. Not only did it lower resting concentrations of glycogen, it also caused the stress to have a greater effect. None of the starved regained resting concentrations during the recovery period. All of the fed groups began recovering at one hour post-stress, and by forty-eight hours had regained resting glycogen concentrations. Salinity of the recovery water did not appear to affect the fed or starved groups' glycogen response.

The correlation coefficients between the glucose and lacate values and glycogen were as expected. The high negative correlations were recorded because the glucose and lactate responses over the recovery period were the inverse of the glycogen response. This is indicative of a general adaptation syndrome response.

Fish stressed in freshwater, a hypoosmotic environment, tend to gain water and lose electrolytes (Mazeaud et. al., 1977; Redding & Schreck, 1983). The opposite occurs when fish are stressed and allowed to recover in saline environments that are

hyperosmotic (Eddy, 1981; Mazeaud et. al., 1977). Elevated catecholamine concentrations after a stress cause the dilation of gill filamental arteries, and increase branchial blood flow (Gratzek & Reinart, 1984; Maetz, 1974; Wedemeyer et. al., 1990), and this increases the rate of exchange between the internal and external environments (Waring et. al., 1992; Wedemeyer, 1972). These ionic disturbances if not corrected are thought to be a major cause of stress related death.

The resting blood osmolality concentrations of the fed and starved groups of bass fingerlings were significantly different. Osmolality of the fed fish were much lower than the starved groups. At this time, there does not appear to be a clear explanation for this effect of feeding. To our knowledge, these differences have not been seen by other researchers. The starved groups' concentrations were slightly higher than those reported by Weirich et. al. (1992), while the fed groups were significantly lower. The freshwater groups both declined throughout the recovery period, though significant change did not occur until thirty minutes post-stress for the fed group, and six hours post-stress in the starved group. Concentrations in both were similar after six hours post-stress.In the saline recovery groups, there was a longer delay in the osmolality change for fed groups. The delay of at least a half hour in significant change in all of the groups may have been caused by the concentration of calcium (>150 ppm) in the water. Calcium ions have been shown to reduce gill permeability (Potts, 1984), thereby decreasing ion flux and diffusion of water. Interestingly, the increase in all of the fed groups in

saline recovery environments at one hour post-stress, made them nonsignificant from the starved groups. All of the starved groups recovering in saline environments regained resting blood osmolality at twenty-four hours of recovery, while the fed groups remained significantly higher than controls. There was no significant difference in the responses among the fed or starved groups in the different salinities. Salinity was expected to play a role in the response, not only in the difference between freshwater (0‰ ) and saltwater, but also among the salinities. There was a difference in the response between the two types of recovery water. The lack of a large difference in the response among the salinities is interesting.

In conclusion, feeding state and salinity of the recovery water both affected the physiological response to handling. The importance of each varied from parameter to parameter. The glucose response was affected by both the salinity of the recovery water, and feeding state. The fed group recovering in 15‰ salt water had the most severe response. Salinity of the recovery water did not affect the fed groups' lactate responses, but did affect the starved groups. The glycogen response was dominated by feeding state, in that the best recovery occured in fed fish. Blood osmolality was not affected much by either nutrition or salinity of recovery water, except in the difference between freshwater and any of the salinity treatments. Overall, the fish recovering in freshwater (0‰) and 5‰ saltwater had the most moderate response indicating that a mildly hypoosmotic recovery environment is most effective. The

higher salinity recovery treatments may actually have been a slight stress in themselves for the hybrid striped bass.

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Relevant Courses

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Skills

- Specialized in the area of stress physiology of fish, and techniques to mitigate stress.
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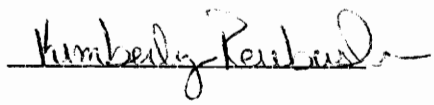

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