Experimental Assessment of Mortality and Hyperglycemia in Tiger Muskellunge Due to Stocking Stressors

MARTHA E. MATHER AND ROY A. STEIN

Department of Zoology, The Ohio State University
Columbus, Ohio 43210, USA

ROBERT F. CARLINE[1]

Ohio Cooperative Fishery Research Unit, 1735 Neil Avenue
Columbus, Ohio 43210, USA

Abstract. — Tiger muskellunge (the F_1 hybrid of female muskellunge *Esox masquinongy* and male northern pike *E. lucius*) have survived poorly when stocked in reservoirs. To understand why, we quantified, in the laboratory, both mortality and plasma glucose responses to three common stocking stressors: dipnet handling, confinement, and temperature increase. No young-of-year hybrids died within 48 h when the temperature was abruptly increased 10°C and only 5% died when the temperature was increased 12°C, but 98% died within 4 h when the temperature was increased 15°C. Thus, we concluded that thermal stress is an important determinant of poststocking mortality. Mortalities in response to three multiple-stressor treatments—(1) handling and temperature increase, (2) handling, confinement at a fish density of 83 g/L, and temperature increase, and (3) handling, confinement at 135 g/L, and temperature increase—did not differ from each other or from mortality associated with a temperature increase alone. Thus, handling and moderate-density confinement during transport do not necessarily increase poststocking mortality of tiger muskellunge. Abrupt temperature increases of 12 and 15°C increased peak plasma glucose concentrations significantly. Handling and confinement together caused a significant hyperglycemia both with and without a temperature increase. However, the relative magnitude of the hyperglycemia caused by individual handling and confinement stressors depended on the presence of a thermal stressor. Finally, we found that plasma glucose concentrations and mortality were not correlated. Although glucose is easily measured and sensitive to small changes in stress, it is not a good indicator of reduced survival and should not be used as such in studies intended to quantify stress-induced mortality.

The tiger muskellunge (the F_1 hybrid of female muskellunge *Esox masquinongy* and male northern pike *E. lucius*) is a popular sportfish that can be economically reared (Buss et al. 1978; Graff 1978). Like other stocked fish, tiger muskellunge may suffer stress-related mortality at stocking as high as 30% (Stein et al. 1981; Carline et al. 1986). Stress during rearing and stocking cannot be eliminated but it can be reduced (e.g., Wedemeyer 1972; Mazeaud et al. 1977; Pickering et al. 1982). Effects on survival of stocking fish in isolation coves, stocking them at night, and adding salts to their transport water have been widely studied (e.g., Belusz 1978; Stein et al. 1981; Johnson and Metcalf 1982; Nikinmaa et al. 1983; Carmichael et al. 1984b). In this study, we examined ways to reduce mortality caused by stocking stressors. Because mortality during field stocking is variable, we chose a more controlled approach. We first identified the stressors associated with stocking, then tested these stressors individually in the laboratory. In this way, we could isolate each stressor, assess its relative severity, and then propose mortality-reducing modifications of the stocking process.

In most studies of stress, either fish performance or physiological responses to stressors have been monitored, but not simultaneously (Pickering 1981). Effects of stocking stressors on performance indices such as resistance to disease, reproduction, feeding, growth, and survival have been examined (e.g., Specker and Schreck 1980; Billard et al. 1981; Elliot 1981; Johnson and Metcalf 1982; Pickering et al. 1982). As an alternative method of quantifying the stress response, physiological responses have been measured. When a fish perceives a stressor, the activation of the hypothalamic–pituitary–interrenal axis causes a rapid increase in plasma catecholamines and corticosteroids which, in turn, cause a suite of metabolic and osmoregulatory changes (Mazeaud et al. 1977; Wedemeyer and McLeay 1981). Increased concentrations of plas-

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[1] Present address: Pennsylvania Cooperative Fish and Wildlife Research Unit, The Pennsylvania State University, University Park, Pennsylvania 16802, USA.

ma glucose, a secondary stress response, have been measured in response to numerous disturbances including temperature increase (e.g., Wedemeyer 1973; Schneider et al. 1981), handling (e.g., Chavin and Young 1970; Wedemeyer 1972; Pickering et al. 1982), and confinement during transport (e.g., Miles et al. 1974; Specker and Schreck 1980; Carmichael et al. 1983, 1984b). Elevation of glucose concentration is an adaptive response, not in itself harmful to the fish (Pickering 1981; Schreck 1981). Nevertheless, plasma glucose has been used as a measure of the relative severity of the stress response (e.g., Silbergeld 1974; McLeay 1977; Strange 1980; Carmichael et al. 1984a, 1984b) and has been suggested as a diagnostic test for the severity of stress (Wedemeyer and McLeay 1981).

Although it is not unreasonable to assume that physiological changes affect performance, the relation between physiological changes and fish performance has not been explicitly evaluated. If there is a relation between plasma glucose concentration and mortality, fisheries biologists and managers can easily evaluate the severity of specific components of a stocking regimen by measuring glucose. If there is no relation between glucose concentration and mortality, it is important to acknowledge that physiological studies assessing glucose response have limited use for managers. With this in mind, our study had three specific objectives: (1) measurement of hybrid mortality in response to common stocking stressors; (2) measurement of plasma glucose in response to the same stressors; and (3) determination of the relation between mortality and glucose and evaluation of the utility of plasma glucose as an index of mortality during field stockings.

Methods

Mortality.—Young-of-year tiger muskellunge (means ± SD: 43 ± 18 g; 207 ± 29 mm) were obtained from the Ohio Division of Wildlife's Kincaid Fish Farm, Latham, Ohio, and the London Fish Farm, London, Ohio. Fish were held in a 4,000-L tank (volume exchanged every 2–4 d) for at least 14 d under the following conditions: 15 ± 1°C (range); 5.0–8.0 mg/L dissolved oxygen; 0.0–0.5 mg/L ammonia nitrogen; 5–30 mg/L total hardness (as $CaCO_3$); mean pH, 7.4; a fish density of 4–10 g/L; and a photoperiod of 16 h light : 8 h darkness. Ammonia nitrogen and total hardness were monitored with Hach colorimetric tests. Fish were fed dry pellets daily (W7 formulation, Orme 1978) equal to 1% of their wet weight. Based on

TABLE 1.—Summary of stressors used in laboratory treatments quantifying mortality and glucose responses of 15°C-acclimated young-of-year tiger muskellunge to simulated stocking conditions.

Descriptive code for treatments	Stocking stressors		
	Temperature increase T (°C)	Held in dip net D (s)	Confinement density C^a (g/L)
T0			
T10	10		
T12	12		
T15	15		
D + T12	12	30	
D + CL + T12	12	30	83
D + CH + T12	12	30	135
D + T0		30	
D + CL + T0		30	83
D + CH + T0		30	135

^{a} CL = low confinement density; CH = high density.

observation of Ohio Department of Natural Resources stockings, we identified three stressful elements associated with the stocking process: (1) handling in the hatchery, (2) confinement during transport, and (3) temperature increase at stocking. We tested different combinations of these stressors (Table 1). Throughout the text, we use acronyms for treatments: D represents dipnet handling; CL, low-density confinement; CH, high-density confinement; T0, a temperature control; and TX, an X°C temperature increase.

In treatments T10, T12, and T15, we transferred 10–15 fish to experimental tanks containing 15°C water (density = 4–5 g/L) and allowed fish to recover from the handling stress associated with aquarium transfer for 24 h. Then we raised temperatures by pumping 40°C water into the test tank while concurrently removing excess water. Temperature increase required 8–12 min at a rate of 1.4 ± 0.3°C/min. Elevated test temperatures were maintained (within ±1°C) for 48 h, the duration of the experiment. We chose this duration because only one fish of all those tested died between days 2 and 7 in initial experiments. In T0, the control treatment, 15°C water was pumped into the 15°C test tank for 8 min. In all treatments, dissolved oxygen was held between 6.0–8.0 mg/L and ammonia nitrogen ranged from 0.0 to 1.0 mg/L. Thermal tolerance treatments consisted of four to seven replicate experiments.

To simulate the handling stress that occurs when fish are transferred from hatchery raceways to a transport truck, fish were held out of water for 30 s in a dip net then released into an experimental

tank containing 15°C water. In treatment D + T12, 12°C, the highest temperature increment unhandled fish could survive, followed this handling stress. In treatments D + CL + T0 and D + CL + T12, 120 min of confinement at a density typically encountered during transport followed the handling. In treatments D + CH + T0 and D + CH + T12, a higher confinement density was tested (Table 1). During confinement, water temperature was held at 15 ± 1°C and dissolved oxygen exceeded 6.0 mg/L. In these multiple-stressor treatments, 15 fish were used per replicate, resulting in densities of 10–14 g/L except during confinement. Because these treatments had more variable results, each treatment consisted of 10–16 replicate experiments.

Plasma glucose. — In a second independent set of experiments, groups of 42–106 fish were transferred to a 400-L experimental tank initially at 15°C. Before any stressors were applied, we took a within-experiment control blood sample (time 0). Then we applied the stressor treatments described above. At 1, 4, 24, and 96 h after the final temperature increase or other stressor was applied, we removed fish for blood samples. Treatments, including a control water exchange T0, involved six fish per sample. When results were more variable, we increased the number of fish per sample. For example, treatments including a 12°C temperature increase had eight to 12 fish per sample and treatment T15 involved 15 fish per sample. For sampling, fish were netted and anesthetized with quinaldine. Caudal peduncles were cleaned with alcohol and cut. Whole blood was collected in heparinized capillary tubes within 11 min of the initial disturbance and centrifuged within 20 min of collection. We found no glucose elevation in unstressed fish sampled within 15 min of capture and thus concluded that the observed glucose response was caused by the treatments and not by the sampling procedure. Plasma was isolated, frozen (-5°C), and analyzed within 2 weeks for glucose by a Sigma hexakinase bioassay (Sigma Chemical Company 1982). We did not test glucose response to treatments T10, D + CH + T0, or D + CH + T12. Treatment D + CL + T12 was replicated four times and all other treatments were replicated twice.

Plasma glucose was measured from fish after two typical stockings (Acton Lake, Preble County, and North Lake, Summit County, Ohio) to determine if laboratory results were consistent with field results. A control (time-0) glucose sample was taken at the hatchery before fish were disturbed. At each stocking, fish were isolated in two cages. In one cage, 12 fish were sampled at 1, 4, 24, and 96 h after stocking. Cumulative mortality was determined from the undisturbed cage. Because we had little control over the field stocking operation, stressors encountered in these field stockings were qualitatively but not always quantitatively similar to those used in laboratory experiments. At Acton Lake, fish were transported at densities of 61 g/L for 3 h, then subjected to a 3°C temperature increase. At North Lake, fish were transported at densities of 80 g/L for 4.5 h, then subjected to a 7°C temperature increase. In the field stockings, fish were netted once when moved from the hatchery to truck and again when transferred from the truck to the lake.

Results and Discussion

Mortality

We used an arcsin transformation of mortality data to stabilize the variance, as Steel and Torrie (1980) recommended for proportions. Differences among treatments were examined by analysis of variance and linear-contrast multiple comparisons (SAS 1982). Comparisons were considered significant at $P \leq 0.05$. Neither treatment T10 nor T12 caused mortality significantly greater than zero (one-sample t-tests) and mortalities in these two treatments did not differ from each other (Figure 1). However, when temperature was increased 15°C, 98% of all fish died within 4 h and mortalities in T15 were significantly higher than those in either treatment T10 or T12 (Figure 1). Differences among treatments that included a 12°C temperature increase (T12, D + T12, D + CL + T12, and D + CH + T12) were not significant (Figure 1). Mortalities in multiple-stressor treatments with a 12°C temperature increase were significantly greater than zero (means: D + T12, 20%; D + CL + T12, 8%; D + CH + T12, 11%; one-sample t-tests) although mortality in T12 was not. No mortality occurred in any of the treatments without a temperature change (T0) or in Acton or North lakes (data not shown).

Two major results emerged from our thermal tolerance experiments with tiger muskellunge. First, hybrids were very tolerant of rapid temperature increase. Second, for these 15°C-acclimated hybrids, a critical increment existed (between 12 and 15°C); little mortality occurred below this increment but most fish died above it. Scott (1964), using abrupt temperature increases, found an upper temperature limit of 32.5°C that could be re-
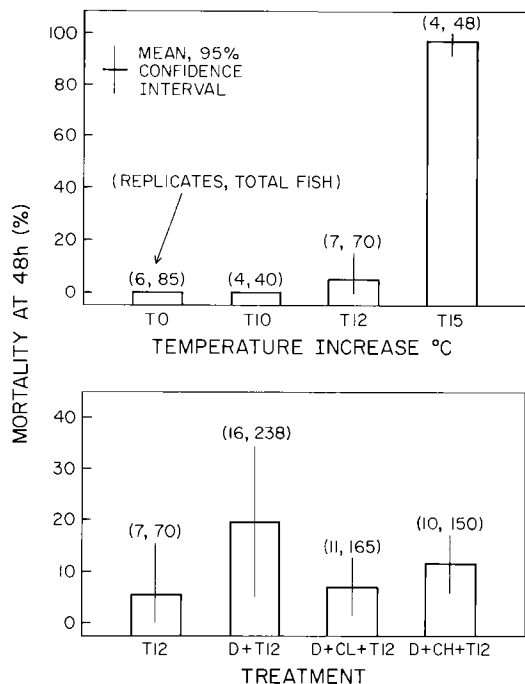
FIGURE 1.—Mortality response of 15°C-acclimated young-of-year tiger muskellunge to stocking stressors. The upper panel shows mortality in response to abrupt temperature increases of 0°C, 10°C, 12°C, and 15°C. The lower panel shows mortality in response to multiple-stressor treatments; D denotes handling in a dip net; CL and CH denote confinement at low and high fish densities, respectively.

sisted 1,000 min (17 h) by 50-mm tiger muskellunge acclimated to 25°C. Fry (1947) reported that a 3°C increase in acclimation temperature increases thermal tolerance by 1°C for several fish species. If this extrapolation holds for esocids, then 30°C, the lowest temperature that killed 15°C-acclimated hybrids in our experiments is consistent with Scott's lethal temperature of 32.5°C for 25°C-acclimated hybrids.

Because dipnet handling, confinement at low density (83 g/L), or confinement at high density (135 g/L) did not increase mortality in our experiments, we concluded that, for tiger muskellunge, neither handling nor moderate-density confinement during transport contributed to stress-related mortality. Tiger muskellunge may acclimate to handling and crowding in the hatchery and thus be less susceptible to these stressors than other species. However, freshwater drum Aplodinotus grunniens, not a hatchery-reared species, also had lower delayed mortality after being transported

(1–6 h; 60–120 g/L, 5 mg NaCl/L) than when released immediately after capture (Johnson and Metcalf 1982). Salt contributes to this decrease in mortality, of course, but the isolation experienced during hauling may enhance recovery.

In our study, we did not observe high mortality in response to the handling and confinement associated with stocking either in the laboratory or the field. Both Stein et al. (1981) and Carline et al. (1986) reported variable poststocking mortality among tiger muskellunge. Because this field mortality approached 30% only when the temperature increase at stocking exceeded 9.5°C, they concluded that thermal shock is an important cause of poststocking mortality of tiger muskellunge. In our laboratory study, we also found that large temperature change was a severe stressor; if tiger muskellunge survived the temperature increase, handling and confinement caused little additional mortality. The critical temperature increment fish can survive probably varies not only across but within species in relation to the nutritional status and disease history of the fish. Differences in fish health may explain why Stein et al. (1981) and Carline et al. (1986) found 30% mortality in response to a 9.5°C thermal stressor and, in this study, we found only 8% mean mortality in response to simulated stocking incorporating a 12°C thermal stressor. The two field stockings reported in this study provide further support for the importance of thermal stress in poststocking mortality. In both lakes, no tiger muskellunge died when the temperature increase was less than 8°C. In contrast, 92% of largemouth bass Micropterus salmoides, handled and confined in untreated transport water at 180 g/L for 30 h, died even when no thermal stress occurred (Carmichael et al. 1984b). The largemouth bass were confined at densities 1.5–3.0 times higher for 15 times longer than our tiger muskellunge, which may explain the difference between posttransport mortalities in the two studies. Salts in the transport water reduce mortality. Average largemouth bass mortality in response to these same transport stressors was reduced to 9% when stress-reducing additives were used during transport and recovery (Carmichael et al. 1984b). Belusz (1978) also found that use of various combinations of chemicals (salt and furacin) dissolved in the transport water, nighttime stocking, and stocking into isolation coves reduced poststocking mortality of muskellunge to an insignificant level in 5 of 6 years. Thus, poststocking mortalities in our experiments and in those discussed above are influenced by thermal stress, fish

health and loading density, duration of confinement, and additives used during transport.

Glucose Response

To assess the relative severity of stocking stressors on plasma glucose concentrations, we compared peak values across treatments. For every 96-h replicate, we chose the sample time (1 or 4 h) with the maximum glucose mean. Within each treatment, we combined the values from this interval for the two to four replicates and compared these pooled peak samples across treatments by analysis of variance and linear-contrast multiple comparisons.

A 12°C temperature increase significantly elevated plasma glucose in young-of-year tiger muskellunge when no other stressor was used (T0 versus T12: Figure 2). A 15°C increase also caused significant hyperglycemia compared to the control (T15 versus T0) but not compared to the 12°C increase. A 12°C temperature increase caused a significant response when a handling stressor was included in the stressor combination (D + T0 versus D + T12) and when both handling and confinement stressors were used (D + CL + T0 versus D + CL + T12). Glucose concentrations returned to pretreatment levels by 96 h for D + T12 and D + CL + T12 and by 24 h for all other treatments (Figure 2). Handling and confinement together increased plasma glucose levels significantly both with (T12 versus D + CL + T12) and without a temperature increase (T0 versus D + CL + T0). In both field stockings, temporal patterns of glucose response were similar to laboratory results (Figure 3). To assess whether handling or confinement was responsible for this glucose elevation, we examined the effect of each separately. Initial handling alone caused a significant temporary hyperglycemia when a temperature increase occurred (T12 versus D + T12: Figure 2), but not in the absence of a temperature increase (T0 versus D + T0). Confinement alone did not affect glucose concentrations when temperature was increased (D + T12 versus D + CL + T12: Figure 2) but confinement alone did cause a significant hyperglycemia when temperature was unaltered (D + T0 versus D + CL + T0). Hence, the relative impact of handling and confinement stressors depended on the presence of a thermal stressor.

For young-of-year tiger muskellunge, abrupt temperature increases of 12 and 15°C are major stressors. Abrupt temperature increases of 10, 12, and 15°C also caused significant temporary hyperglycemia in juvenile salmonids (Wedemeyer
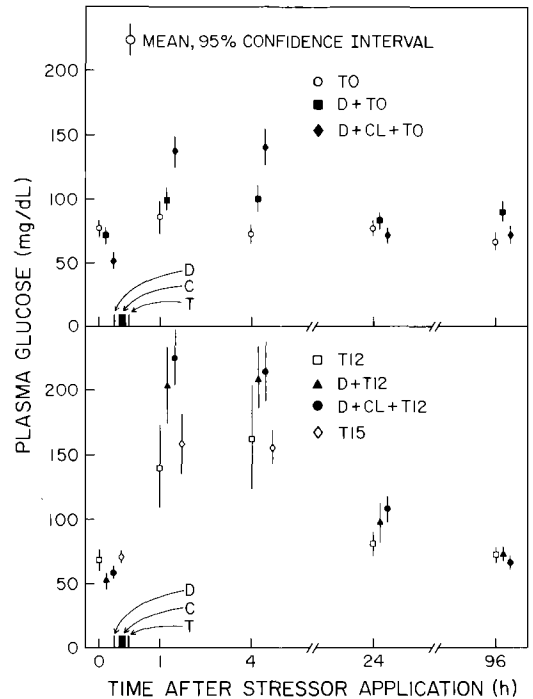

FIGURE 2.—Plasma glucose response to handling (in a dip net, D) confinement (at low, CL, and high, CH, fish density), and abrupt temperature increase (T°C) in 15°C-acclimated young-of-year tiger muskellunge. The upper panel shows the plasma glucose response to treatments T0, D + T0, and D + CL + T0. Each point represents two pooled replicates or a total of 12 fish. The lower panel shows plasma glucose response to treatments T12, D + T12, D + CL + T12, and T15. In T12 and D + T12, each point represents two pooled replicates or a total of 16–24 fish. In D + CL + T12, each point represents four pooled replicates or a total of 32–48 fish. In T15, each point represents two replicates or a total of 30 fish. In all treatments, fish were sampled at five times: 0 (before the stressor was applied) and 1, 4, 24, and 96 h after the stressor was applied. Points are offset slightly for clarity.

1973), largemouth bass (Carmichael et al. 1984a), and rainbow trout *Salmo gairdneri* (Schneider et al. 1981). Other investigators have found, as we did, that handling and confinement during stocking (or stocking simulation) cause significant elevations in plasma glucose concentration. For tiger muskellunge, handling and confinement caused a significant physiological response both with and without a thermal stressor. Handling and moderate-density confinement during transport in untreated water (2.5–12 h at 12–250 g/L) caused significant temporary hyperglycemia in *Labeo capensis* (Hattingh 1976), coho salmon *Oncorhyn-*
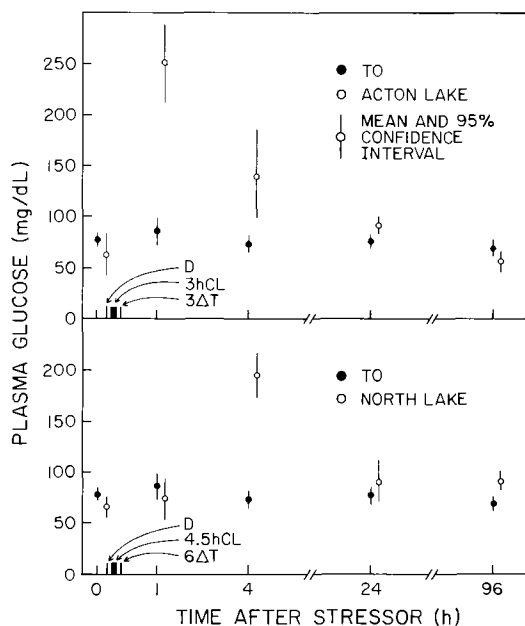
FIGURE 3.—Plasma glucose concentrations in young-of-year tiger muskellunge through 96 h following stocking in two Ohio reservoirs. Field data are compared with the laboratory control treatment T0 (zero temperature increase for 15°C-acclimated fish). Each point represents 12 fish. In both field stockings, a second dipnet handling (D) occurred between the confinement (CL) and the temperature increase (°CΔT).

chus kisutch (Specker and Schreck 1980), and smallmouth bass Micropterus dolomieui (Carmichael et al. 1983). Adding salts during transport reduces the physiological response. Both brown trout Salmo trutta and largemouth bass exhibited a significant hyperglycemia after handling and confinement in untreated water (100–180 g/L for 14–30 h) and a markedly reduced response when salts were added (Nikinmaa et al. 1983; Carmichael et al. 1984b). Conversely, Aldrin et al. (1979) found that handling and 3-h transport has little effect on mean salmonid glucose concentrations. They attributed this lack of response to the unusually low nutritional condition of the fish.

Whether handling or confinement is more stressful depends on fish species, transport conditions, and presence of a thermal stressor. Temporary hyperglycemia in response to dipnet handling alone (30–180 s) has been demonstrated for salmonids (Wedemeyer 1972; Pickering et al. 1982), esocids (Soivio and Oikari 1976), goldfish Carassius auratus (Chavin and Young 1970), smallmouth bass (Carmichael et al. 1983), and

sand dab Limanda limanda (Fletcher 1984). Unlike our results, handling in these studies produced a significant physiological response by itself. For tiger muskellunge, initial handling has little impact when no thermal stress occurs and the overall stress level is low. Perhaps being reared in a hatchery where handling and disturbance are routine makes tiger muskellunge less susceptible to handling stress alone.

When tiger muskellunge are subjected to thermal stress and a high overall stress level, however, initial handling is more severe than subsequent confinement. Other investigators have had similar results. Miles et al. (1974) concluded that confinement during transport does not increase muskellunge plasma glucose concentrations more than the initial handling alone. Wendt and Saunders (1973) found that transport did not increase glucose concentrations in Atlantic salmon Salmo salar. Specker and Schreck (1980) found that increasing the density of transport confinement from 12 to 120 g/L and the duration from 4 to 12 h caused elevations of salmonid glucose and cortisol concentrations, but they concluded that initial handling was the most severe element of the stocking process. Barton et al. (1980) also concluded that initial handling was more severe than confinement for young rainbow trout. The psychological aspect of stress influences the magnitude of the physiological response (Schreck 1981); perhaps this explains the more severe response of several fish species to the initial handling stressor. Conversely, smallmouth bass hauled at 250 g/L for 2.5 h had significantly higher glucose after transport than after loading (Carmichael et al. 1983). High density during transport, the temporary hypoxia and thermal stress that inadvertently occurred during the experiment, or species-specific differences may explain the discrepancy between responses of smallmouth bass and tiger muskellunge. For tiger muskellunge, some degree of thermal stress usually accompanies stocking. Hence, we believe that, in the presence of a thermal stressor, the less severe nature of confinement during transport relative to initial handling is a fair representation of relative stocking stresses on tiger muskellunge.

Plasma Glucose as an Indicator of Survival

We did not find a convincing relation between plasma glucose concentrations and mortality ($r = 0.35$; $P > 0.05$: Figure 4), nor did high glucose values indicate mortality in the two field stockings (Figure 4). For the laboratory portion of this anal-
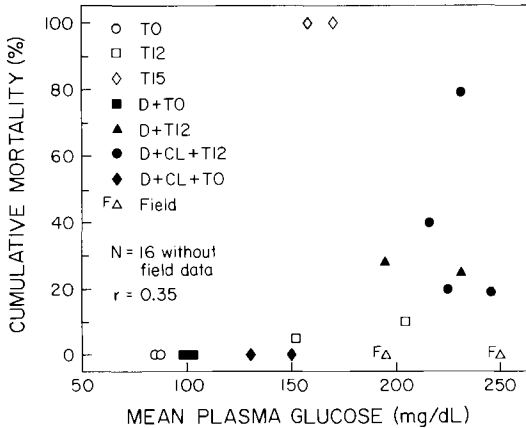
FIGURE 4.—Correlation between cumulative mortality at 96 h and mean maximum glucose concentration for young-of-year tiger muskellunge subjected to stocking stressors in the laboratory. Data from field tests are included for comparison. Temperature increases above 15°C are denoted by T, dipnet handling by D, and confinement at low fish density by CL.

ysis, we removed fish from experimental tanks for blood samples and corrected mortality estimates for a decreasing sample size by actuarial estimation with fixed removal intervals (Cox and Oakes 1984). These data demonstrate that, although plasma glucose concentration is easily measured and reflects small changes in fish response to different stressors, it is not a good indicator of the probability of mortality and should not be used as such in studies intended to quantify stress-induced mortality.

Whether or not a performance index is related to a physiological index depends upon the specific variable being examined. Carmichael et al. (1984a) noted that largemouth bass fed only when plasma constituents were within normal concentration ranges. Schreck (1981) has demonstrated a direct relation between clinical indicators of stress and dominance rank of coho salmon. Specker and Schreck (1980) concluded that increased cortisol responses in transported salmon were correlated with increased mortality in response to severe confinement stress. Conversely, Specker and Schreck (1980) did not find a relation between clinical indices and other indicators of reduced performance, namely, increased expression of latent kidney disease or reduced ability to migrate. Changes in cortisol concentration (Schreck 1981) or changes in cortisol and glucose concentrations (Pickering et al. 1982) have not corresponded to long-term changes in growth or survival. Thus, physiological

changes cannot be assumed to cause changes in other aspects of fish condition. Perhaps another physiological index would provide more information about stress-induced mortality, and a combination of several indices is likely to be more informative than any single index (Carmichael et al. 1984a, 1984b; Barton et al. 1985). However, before we accept that any physiological measure might be correlated with the severity of stress, as expressed by subsequent mortality, a functional relationship between the two must be demonstrated.

Management Considerations

The magnitude of temperature stress appears to be the most important factor determining the extent of mortality when fish are stocked. Stocking procedures, therefore, should minimize temperature increases. Because mortality is such a variable response, managers should keep thermal stress well below the critical thermal increment for their species. Unnecessary handling of fish should be minimized, of course, but our results suggest that handling need not increase mortality if thermal shock is minimized. Our results also suggest that, for tiger muskellunge, moderate-density confinement need not be a concern if water quality remains high. Managers are ultimately interested in mortality at stocking but mortality is an extreme measure of stress response. Sublethal stress may reduce realized performance capacity (Schreck 1981) and should be considered when optimal stocking procedures are designed and poststocking survival is assessed.

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