Possible Red Imported Fire Ant (*Solenopsis Invicta* Buren) Ingestion-induced Mortality to Stocked Trout in the Lower Mountain Fork River, Oklahoma

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Abstract: The introduction of hatchery-reared trout into Oklahoma's Lower Mountain Fork River (LMFR) has enhanced angling opportunities. However, both Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) have recently behaved erratically, with subsequent mortality, thought to be the result of Red Imported Fire Ant (*Solenopsis Invicta* Buren) flotilla consumption during flood events. The objectives of this paper are to: (1) enumerate diets, (2) compare physical characteristics and diets between trout species, and (3) determine if there is a relationship between diet and physical characteristics of dead trout collected after two flooding events (February 2023, June 2024) at the LMFR. Analysis of nine dead trout revealed that Fire Ants comprised 99% of their diet by frequency of occurrence, percent composition by number, and percent composition by weight. Statistical analysis showed no significant difference between Brown Trout and Rainbow Trout physical characteristics (length, weight) or diet characteristics (diet weight, number of Fire Ants consumed). Significant positive correlations were observed between fish size (length, weight) and diet weight, though no relationship was found between fish size and number of fire ants consumed. We found larger trout generally consumed a greater mass of Fire Ants, suggesting a potential threshold effect where high ingestion of Fire Ants may lead to mortality. However, more work needs to be done to determine the mechanistic cause and mortality threshold for Red Imported Fire Ant consumption by trout.

Introduction

For more than a century, the introduction of hatchery-reared salmonid fishes into streams and reservoirs has been used to provide angling opportunities where native fish have been extirpated, or to enhance angling opportunities where natural trout populations were reduced or extirpated due to anthropogenic impacts (Hanisch et al. 2012). Trout are typically found in cold-water streams, reservoir tailwaters, or seasonally in small impoundments where water temperatures

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remain suitable for their survival (Snow et al. 2019). Brown Trout (*Salmo trutta*) and Rainbow trout (*Oncorhynchus mykiss*) are not native to Oklahoma (Miller and Robison 2004), but the Oklahoma Department of Wildlife Conservation (ODWC) stocks them in certain waters to provide angling opportunities (ODWC 2023). In 1965 ODWC started a put-and-take trout stocking program on the lower Illinois River below Lake Tenkiller, followed by trout stockings on the Blue River Public Fishing area in southern Oklahoma in 1968 (Gilliland 1989). Since the 1960s ODWC has expanded the Lower Mountain Fork River (LMFR) to a year-round trout fishery and created several other seasonal trout put-and-take fisheries (ODWC 2023). Trout stockings are primarily conducted from November through the end of February when water temperatures are cooler. Both Brown Trout and Rainbow Trout are considered cold-water species with a critical thermal maximum ranging from 23 to 30°C (Beitinger et al. 2000; Carline and Machung 2001; Chen et al. 2015). However, critical thermal maximum for these species can vary based on factors such as acclimation, size, and genetic adaptation to local environmental conditions (Beitinger et al. 2000).

Hatchery-raised trout are opportunistic feeders that quickly adapt to a natural diet, predominantly consuming invertebrates (Tay et al. 2007, Odenkirk and Estes 1991, O'Rouke 2014). Although typically small, the magnitude and severity of impacts on other native fish and invertebrate populations vary considerably from system to system (Rodger et al. 2021, Rodger and Stark 2022). For example, Fenner et al. (2004) found that stocked Rainbow Trout rarely compete with other top predators in these systems, though they might compete with insectivorous fish such as darters, sculpins, and cyprinids. Likewise, Metcalf et al. (1997) suggested stocked trout can potentially influence the recruitment of Smallmouth Bass (*Micropterus dolomieu*) through competition and Snow et al. (2019) illustrated that stocking predatory fish like trout can negatively influence age-0 Gizzard Shad (*Dorosoma cepedianum*) biomass. Given their broad diet and adaptability, trout are able to consume a broad range of diet items. However, consumption of invasive species such as Red Imported Fire Ants (*Solenopsis Invicta* Buren; Figure 1) may have negative consequences on their survivability (*sensu* Hutchins 1960, Prather 1960, Crance 1965).

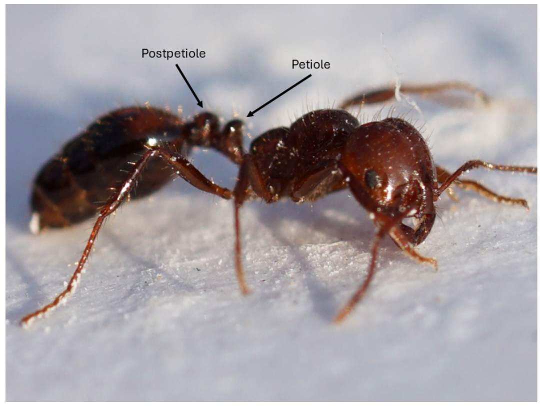

Figure 1. Photograph of a Fire Ant collected in July of 2024. The distinguished feature of a Red Imported Fire Ant is the two nodes on the waist portion of the abdomen called the petiole and postpetiole. Picture courtesy of Brandon Brown.

The Red Imported Fire Ant is a global invader that has been introduced to at least seven countries, including the United States in the mid-1930s, where it has spread across fourteen states (Mooney and Cleland 2001, Robbin et al. 2012). They have successfully invaded the southern United States, specifically Oklahoma, due to the lack of interspecific competition with other ant species and the absence of more diverse aggressive ant fauna found in South America (Allen et al. 2004, Morrison et al. 2004). Anthropogenic soil disturbance increases the density of Red Imported Fire Ants, while they can achieve high densities in both dry and wet undisturbed habitats, their populations are substantially influenced by soil disturbance, with densities decreasing over time as disturbed areas return to conditions similar to undisturbed ones (LeBrun et al. 2012). This pattern of fire ant density related to soil disturbance is a novel finding and highlights the significant impact of anthropogenic activities on their population dynamics as it relates to the highly developed LMFR area.

Anthropogenic influences, such as the development of infrastructure like parking lots, have created the perfect habitat for introduced Red Imported Fire Ants, and the colonization of these areas of the LMFR (LeBrun et al. 2012). The LMFR cuts through an area with shallow rocky soils which allow little rainfall to be absorbed into the ground. During heavy or prolonged rainfall this often results in flooding of low-lying areas. Heavy rain events wash out some Red Imported Fire Ants into sounding tributaries that are within the mound portion of their nest that can range from 30 cm to 90cm tall (Green et al. 1999), into the LMFR. Red Imported Fire Ants survive flooding events by creating a flotilla (Figure 2), a behavioral response in which ants connect to one another to form a structure that allows them to float on the surface of the water (Roeder et al. 2018). While flotillas are present in the river, predation by trout or other fishes in the river is likely occurring. Although anecdotal, angler observational records indicate that when Red Imported Fire Ant flotillas are observed floating in the LMFR, trout are also observed acting erratically or are found dead.

Figure 2. This photograph shows a Red Imported Fire Ant flotilla formed by ants linking together (shown in the close-up photograph of the structure) to float on the water's surface. This behavior enables them to survive flooding events. These flotillas can become prey for fish, including trout, as they drift into aquatic habitats.

Reports of dead trout were received from anglers on February 23, 2023, and June 17, 2024, from the LMFR. Anglers observed Red Imported Fire Ants in a stomach removed from one of the dead trout (Figure 3). Afterward, the angler collected a subsample of dead trout was for diet analysis to verify stomach content. The objectives of this study were to: (1) enumerate diets of dead trout, (2) compare physical (i.e., length, weight) and diet characteristics of expired Rainbow Trout and Brown Trout to determine if there are differences between each species, and (3) determine if there is a relationship between diet (i.e, weight, Red Imported Fire Ants consumed) and physical characteristics of dead trout.

Figure 3. Photograph taken by an angler of a dead trout that had consumed Red imported Fire Ants at the Lower Mountain Fork River, OK, on June 17, 2024.

Methods

A total of five trout (3 Brown Trout and 2 Rainbow Trout) were collected by anglers on February 23, 2023. An additional four trout (2 Brown Trout and 2 Rainbow Trout) were collected by the ODWC on June 17, 2024. All trout were frozen upon capture or received frozen from anglers and remained frozen until processing. Trout were thawed and processed at the Oklahoma Fisheries Research Lab in Norman, Oklahoma.

Fish were identified using information present in Oats et al. (1993) and Miller and Robison (2004). Fish were measured for total length (mm) and weight (g). Stomachs were extracted, prey items were removed, identified and enumerated, and prey items were weighed (g). All prey items were identified to species when possible, using scientific taxonomic keys to identify aquatic invertebrates (Merrit et al. 2008). Due to the presence of ants, we used a key in Hung et al. (1977) to determine the species of Red Imported Fire Ants. Stomach samples were analyzed by percentage of empty stomachs, frequency of occurrence (O_i), percent composition by number (N_i), and percent composition by weight (W_i; Bowen 1996; Chipps and Garvey 2007).

Several species-specific comparisons were made between Rainbow Trout and Brown Trout. We compared Rainbow Trout versus Brown Trout weights and TLs, and recorded if they exhibited differences in the number of Red Imported Fire Ants consumed present in the stomachs or the weight of their diet samples. Weights for both species were $\log_{10}$ transformed prior to analysis (Zar 1999). If data were normal and variance was equal between species samples a Student's t-test was used (Student 1908), if data were normal and variance was unequal between species samples a Welch's t-test was used (Welch 1951), and if data were nonnormal but variance was equal between species samples a Mann-Whitney U test (Mann and Whitney 1947) was used ($\alpha = 0.05$). Normality was assessed for each species using a Shapiro-Wilks test (Shapiro and Wilks 1965) and equality of variance between each species sampled determined via an F-test ($\alpha = 0.05$).

We then compared diet and fish size metrics. Pearson correlations were used to assess and test the strength of the association between weights and TLs of fish and the number of Red Imported Fire Ants consumed and diet weight (Pearson 1896; $\alpha = 0.05$). Fish weight, TL, number of Red Imported Fire Ants consumed, and diet weight were all $\log_{10}$ transformed for these analyses (Zar 1999). Normality of each variable was determined via a Shapiro-Wilks test and homoscedasticity was determined via a studentized Breusch-Pagan test (Breusch and Pagan 1979, Koenker 1981; $\alpha = 0.05$). Outliers for each variable were assessed by transforming each $\log_{10}$ transformed variable into a z-score and determining if they fell within the range of -3.29 – 3.29. All analyses were conducted in program R version 4.4.1 (R Core Team 2024).

Results

Of the 9 specimens collected, 0% had empty stomachs. Brown Trout and Rainbow Trout ranged in TL from 193 - 246 mm and 176 - 307 mm (Table 1). Brown Trout and Rainbow trout ranged from 60 - 322 g in weight (Table 1). Diets by O_i, W_i, and N_i of these fish contained 99% Red Imported Fire Ants and <1% other, which consisted of a rubber grub lure, crawfish claw, Isopod, Leafhopper, and Odonate. The weight of ants consumed ranges from 1.13 – 10.76 g with a mean of 3.41 g of Red Imported Fire Ants per fish with total weight 31.23 g of total ants consumed by these 9 trout. In total, 1,285 Red Imported Fire Ants were enumerated, ranging from 27 – 426 ants per trout stomach (Figure 4A).

Table 1. Species, total length (TL; mm), and weight (g) of expired trout from the Lower Mountain Fork River, OK. Included are the number of Red Imported Fire Ants (Fire Ants) within each trout stomach and the weight of the diet items from each stomach (Diet Weight).

Species	TL	Weight	Fire Ants	Diet Weight
Brown	193	80	86	1.77
Brown	205	84	83	2.14
Brown	216	88	35	1.19
Brown	241	148	51	1.47
Brown	246	150	48	2.29
Rainbow	176	60	211	2.48
Rainbow	191	70	27	1.13
Rainbow	301	278	318	8.00
Rainbow	307	322	426	10.76

$\log_{10}$ transformed weight, TL, number of Red Imported Fire Ants consumed, and diet sample weight were all normal for samples from Rainbow Trout (W range = 0.80 – 0.98, all $p > 0.05$).

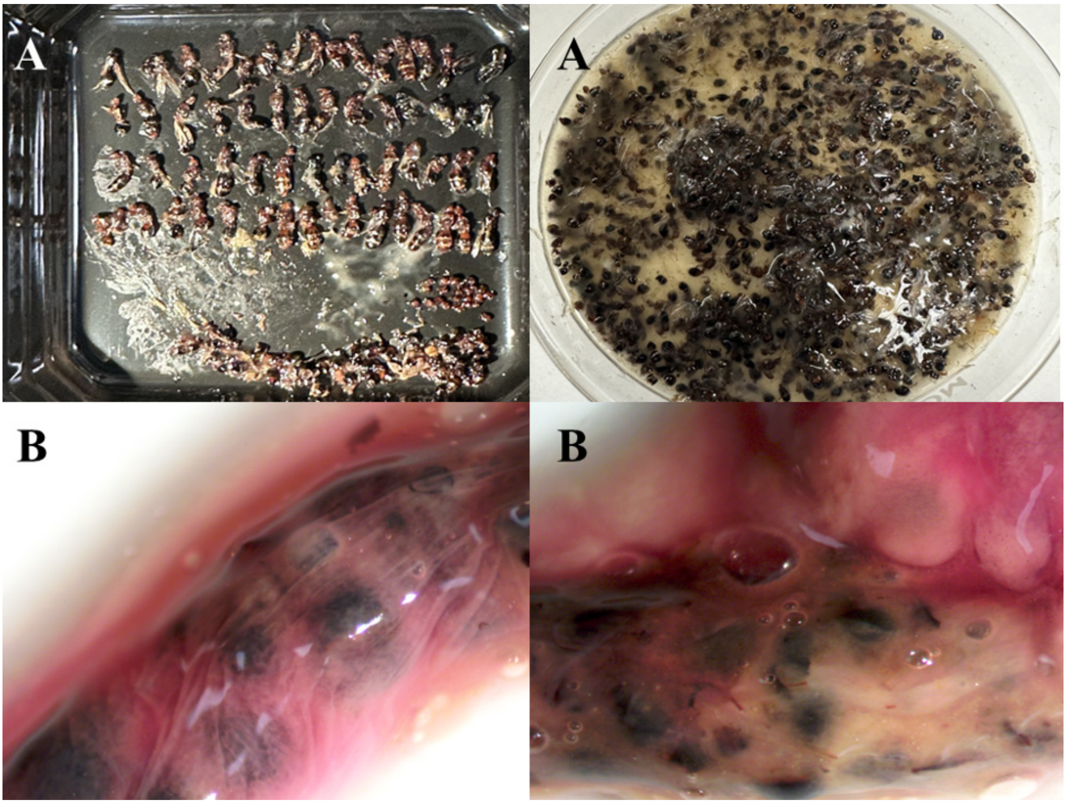

Figure 4. Images show the stomach and intestinal contents of trout sampled from the Lower Mountain Fork River. The upper panel (A) depicts a trout stomach filled primarily with Red Imported Fire Ants, while the lower panel (B) shows a section of the trout's intestines containing Red Imported Fire Ants and other minor dietary items.

Log_{10} transformed TL, number of Red Imported Fire Ants consumed, and diet sample weight were normal for samples all Brown Trout (W range = 0.87 – 0.96, all p > 0.05); however, log_{10} weight was not normal (W = 0.77, p = 0.04). Variances were unequal between species for number of Red Imported Fire Ants consumed (F = 0.02, p < 0.01) and diet weight (F = 0.01, p < 0.01). Welch's t-tests suggested that the two species consumed a similar number of Red Imported Fire Ants (t = -2.16, p = 0.12) and that diet weights were similar between species (t = -1.67, p = 0.19). Variance was similar between species TLs (F = 0.11, p = 0.06) and student's t-tests suggested TLs were similar between species (t = -1.90, p = 0.10). Variance was similar between log_{10} transformed weights (F = 0.13, p = 0.08) and Mann-Whitney U tests suggested log_{10} transformed weights were similar between species (W = 10, p = 1.00).

Statistically similar sizes of expired Rainbow Trout and Brown Trout were sampled from the Lower Mountain Fork River, OK. Both species appeared to consume a statistically similar number of Red Imported Fire Ants, and their stomach contents exhibited statistically similar weights. Though a small number of other organisms were present in diets, most of the matter within each stomach consisted of Red Imported Fire Ants. These tests suggest that there was not a species-specific bias in weight or TL of observed expired trout. It also suggests that no species-specific preference for consuming Red Imported Fire Ants was exhibited in the Lower Mountain Fork River. However, these findings are likely driven by low sample size.

All data were normally distributed after $\log_{10}$ transformations based on our Shapiro-Wilks test (W range = 0.84 – 0.92, all $p > 0.05$). Likewise, the relationships between $\log_{10}$ transformed weight and TL was homoscedastic for both $\log_{10}$ transformed number of Red Imported Fire Ants consumed and diet weight (BP range = 0.09 – 1.20, all $p > 0.05$). No outliers were detected in any $\log_{10}$ transformed variable (z-score range = -1.27 – 1.83). The Pearson correlation between $\log_{10}$ transformed weight and number of Red Imported Fire Ants consumed was 0.57 and was determined to be insignificant ($t = 1.85$, $df = 7$, $p = 0.11$). The Pearson correlation between $\log_{10}$ transformed TL and number of Red Imported Fire Ants consumed was 0.51 and was determined to be insignificant ($t = 1.56$, $df = 7$, $p = 0.16$). The Pearson correlation between $\log_{10}$ transformed weight and diet weight was 0.81 and was significant ($t = 3.70$, $df = 7$, $p < 0.01$). The Pearson correlation between $\log_{10}$ transformed TL and number of diet weight was 0.77 and was significant ($t = 3.22$, $df = 7$, $p = 0.02$).

Number of ants consumed did not appear to be significantly correlated with fish weight or TL (Figure 5). However, the weight of diet items was significantly positively correlated with fish weight and TL (Figure 5). Given Red Imported

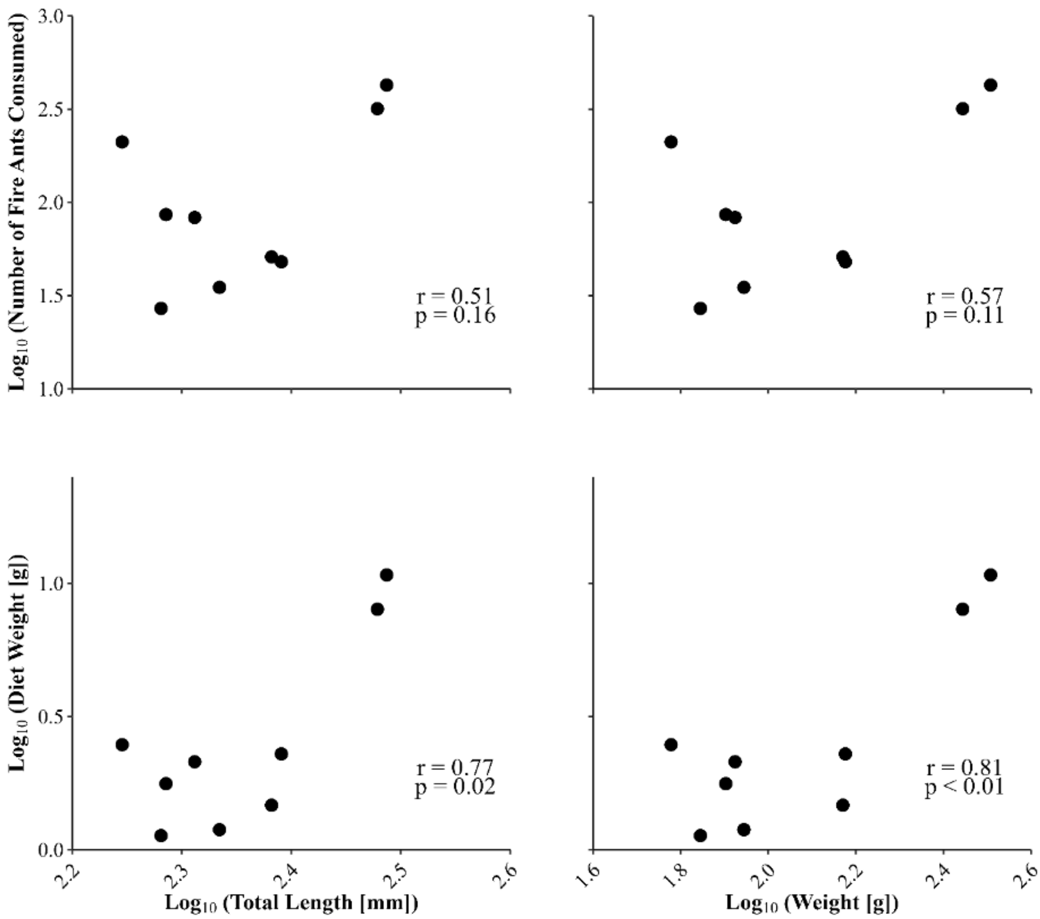

Figure 5. $\log_{10}$ transformed number of Red Imported Fire Ants consumed and diet weights plotted against $\log_{10}$ transformed total lengths and weights from dead trout collected from the Lower Mountain Fork River, OK (black circles). Included are Peterson correlation coefficients (r) and significance of each correlation (p).

Fire Ants constituted most of the diet weight in each stomach, it is likely larger fish consumed a greater mass of Red Imported Fire Ants prior to expiring. Given the phenotypic variation in Fire Ant colonies this is likely the reason that the number of Red Imported Fire Ants did not exhibit a relationship with either weight or TL of expired fish.

Discussion

This is the first study in Oklahoma to demonstrate a connection between the consumption of Red Imported Fire Ants by trout in the LMFR and the observed deaths of these fish. Red Imported Fire Ants were found in the highest abundance of all prey items in the diets of both Brown and Rainbow Trout found dead within the LMFR. Although the sample size is limited, there is little variation between Brown and Rainbow Trout in terms of the number and weight of ants consumed. Despite this, the number of Red Imported Fire Ants consumed did not exhibit a significant correlation with trout weight or total length. Instead, the weight of diet items, including Red Imported Fire Ants, was significantly correlated with trout size. This suggests that while the proportion of Red Imported Fire Ants in the diet was high across all sampled fish, larger trout may be consuming a greater total mass of these ants. The finding of similar diet composition between Brown and Rainbow Trout indicates that both species are equally affected by the presence of Red Imported Fire Ants, but it also highlights the need for further investigation into how different factors, such as prey availability and trout size, influence the effects of consuming Red Imported Fire Ants.

Despite the high percentage of Red Imported Fire Ants found in the trout stomachs, the study did not observe a direct statistical correlation between the number of Red Imported Fire Ants consumed and fish size, suggesting that other factors might also play a role in the mortality of the trout. However, the significant correlation between diet weight and fish size implies that larger fish are more likely to have ingested a greater mass of Red Imported Fire Ants, which could exacerbate the impact of consuming these invasive ants. This underscores the potential for a threshold effect, where exceeding a certain amount of ingested Red Imported Fire Ants might result in acute health issues leading to the trout's death. For example, based on the weight of the diet sample divided by the number of ants counted, each ant weighed ~.024 g. Extrapolating out the percentage of total Fire Ant weight to trout weight results in an estimated value of 2.3% (Table 1). This suggests that an individual trout consuming ≥ 2.3% of it is weight in Red Imported Fire Ants may die. However, further experimentation would be needed to confirm this value. This is especially true as trout examined in this study consumed 66.2 to 304.7 g of Red Imported Fire Ants and that value on average is 1.5 times higher than Red Imported Fire Ants enumerated in diets. Further investigation into the digestive tract of the fish showed that Red Imported Fire Ants were prevalent throughout the entire digestive tract of all dead fish (Figure 4B). This suggests that investigation of the total digestive tract may be more representative of the total number of Red Imported Fire Ants consumed by the fish resulting in a better estimate of the number of Red Imported Fire Ants that would potentially cause trout mortality.

This is the first documented event of Red Imported Fire Ants causing death to fish in Oklahoma waters; however, it is not the first documented fish death due to Red Imported Fire Ants in the United States. Green and Hutchins (1960) fed Red Imported Fire Ants to a variety of sunfish in ponds after reports of Red Imported Fire Ants causing fish kills and did not record any mortality; however, when sunfish were force-fed 1-2 millimeters of macerated Red Imported Fire Ants in a lab setting, fish died ≤1 hr. Furthermore, Prather (1960) fed gelatin pills to sunfish containing 100 Red Imported Fire Ants twice daily for 4 weeks with no fish mortalities being observed. Crance (1965) examined 153 sick or dead bluegills, with 151 of those individuals containing Red Imported Fire Ants in diet samples. In 1963 the Alabama Department of Conservation received reports of several fish kill events. During the ensuing investigation, 183 sick or dead fish were taken from

26 ponds and examined, with 96.7% of the fish stomach content containing whole or pieces of winged Red Imported Fire Ants (Crance 1965).

Anglers and ODWC staff have observed interesting behaviors in trout after ingesting Red Imported Fire Ants, such as loss of equilibrium and swimming ability. During these observations, some fish have been seen recovering from this state after some time, and others that are dying or dead are collected. This behavior is likely due to the consumption of a small amount of Red Imported Fire Ants, although this antidotal observation it is supported by examining the stomachs of expired trout in the same area where trout were observed with loss of equilibrium and swimming ability. Although this is extrapolated from our data with a low sample size, it does provide a plausible reference on where to start to understand what the threshold is for what amount of Red Imported Fire Ants a trout can consume until death may occur. For example, Crance (1965) documented fish becoming sick or distressed after being exposed to swarms of Red Imported Fire Ants. In one observation, hundreds of fish in ponds were affected, but most recovered within 12 to 18 hours (Crance 1965). Further experiments documented seven sick or distressed bluegills from ponds that were brought to the lab; all seven recovered within 12 hours and remnants of Red Imported Fire Ants were found at the bottom of the holding tanks (Crance 1965).

Data from this study indicate that mortality due to the consumption of Red Imported Fire Ants is likely occurring on the LMFR. However, the mechanistic cause remains unknown. The physical damage caused by consuming many Red Imported Fire Ants might interfere with the trout's digestive system, leading to complications or even death. Likewise, the potential toxicity of consuming many Red Imported Fire Ants may cause complications or mortality. The mechanistic cause of these symptoms should be an area of future study. Regardless, Red Imported Fire Ant consumption could become a critical concern for the management of trout fisheries in the LMFR. For example, given Red Imported Fire Ants appear to be consumed when flooding occurs on the LMFR and flooding is becoming more common, mortalities due to Red Imported Fire Ant consumption may increase in frequency. Investigation into the mechanism behind mortality due to Red Imported Fire Ants consumption may become important to fisheries managers in other areas where Red Imported Fire Ants are common or increasing as they expand their geographic range. Understanding the precise mechanisms behind this mortality is crucial for mitigating the impacts of invasive species on aquatic ecosystems and developing strategies to protect both the trout populations in the LMFR and the overall health of the environment.

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