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Rainbow Trout (Oncorhynchus mykiss) Movement and Mortality in the Flat Brook-Roy Catch and Release Section of New Jersey

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Abstract

Rainbow trout are a widely distributed species for recreational angling. The state of New Jersey has 14 streams in which the Bureau of Freshwater Fisheries stocks hatchery raised Rainbow Trout. This paper focuses on the Flatbrook-Roy catch and release section of the Big Flat Brook in northwest NJ. From 2012 to 2015, electrofishing surveys conducted by the NJDEP reported significantly less fish within the Flatbrook-Roy catch and release area, compared to the state's other catch and release area in the South Branch of the Raritan River. This study was designed to investigate and understand the fate of the trout introduced into the Flat Brook-Roy section by looking at their survivorship and movement in and/or out of the catch and release section of the stream. 79 fish were surgically implanted with radio transmitters and introduced into three separate stocking locations from April-September of 2017 and 2018. The data collected across the two years showed that the trout were not traveling outside of the catch and release designated waters. Across the summer months of each year, there was a 100% mortality rate within the sample, all credited to specific mammalian or avian predation events. Between the stocking sites, the trout both stayed longer and survived better at the site with the largest pool dimensions and most in-stream cover. The findings help fisheries management decisions by confirming that the introduced trout do in fact remain within the designated catch and release waters. Future decisions related to fish allocation and fishery regulation should consider stocking sites of adequate size that provide enough cover to increase survivorship and mitigate predation in order to maximize the recreational angling experience.

MONTCLAIR STATE UNIVERSITY

Rainbow Trout (*Oncorhynchus mykiss*) Movement and Mortality in the Flat Brook-Roy Catch and Release Section of New Jersey

By

Christopher J. Shea

A Master's Thesis Submitted to the Faculty of

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RAINBOW TROUT (*Oncorhynchus mykiss)* MOVEMENT AND MORTALITY IN THE FLAT BROOK-ROY CATCH AND RELEASE SECTION OF NEW JERSEY

A THESIS

Submitted in partial fulfillment of the requirements

For the Degree of Master of Science

By

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Introduction

In freshwater ecosystems across the world, Salmonids are important species for maintaining ecological function, acting as both effective predators and sources of food, and impacting the flow of nutrients and energy within a stream or lake ecosystem. Salmonids are a family of bony fish consisting of the subfamilies Salmoninae (salmon and trout) and Coregoninae (whitefish). Phenotypically these sub families can be distinguished by their differences in mouth size. Whereas Salmoninae have a large mouth with a jaw that extends past the eye, Coregoninae have a characteristically small mouth (Kraft et al 2006). Among the Salmonids the Salmoninae are popularly sought after as a sport fish by recreational anglers. Within New Jersey freshwaters, rainbow trout (*Oncorhynchus mykiss)* are the most widely distributed recreational Salmonid, with 618,480 fish stocked in public rivers and lakes in 2019 alone.

Rainbow trout are native to the waters in the watersheds west of Colorado in North America, ranging from Alaska and British Columbia south through Mexico (Staley and Mueller 2000). Within the native range the species includes subspecies of resident rainbow trout and an anadromous migratory trout known as steelhead trout. The anadromous steelhead trout migrates towards the Pacific Ocean as juveniles and returns to freshwaters as mature adults to spawn (Staley and Mueller 2000). Beyond the native range, rainbow trout have been introduced to waters on every continent (with the exception of Antarctica) due to popularity as a sport fish and ability to thrive in aquaculture rearing facilities (Kopack et al 2016). The ubiquitous distribution of the species as either native, introduced or an invasive fish calls for the need to understand their form, function and behavior to comprehend their role within the freshwater systems in which they are found or introduced.

Rainbow Trout Life History

The rainbow trout is a streamlined salmonid characterized by a single adipose fin and soft rayed fins, lacking stiff spines found on other bony-fish, and 8 to 12 rays on the anal fin (Kraft et al 2006). Phenotypic coloration includes a reddish pink line at the midline of the fish, black spots along the lateral line and on the upper fins and tail, and scales below the midline appearing silver or white (Delany 2008). Size and shape of the rainbow trout may vary between habitat, sex, and maturity of the fish. Rainbow trout are considered to reach adulthood when they are over 20 cm (approximately 8 inches) in length and sexually mature at the age of 2 to 3 years (Raleigh et al 1984). The size and weight of a rainbow trout can vary greatly, but they can grow to be as large as 114 cm (45 inches) in length.

The spawning season for rainbow trout can vary based on locality, resident vs anadromous, and wild vs hatchery raised trout. As a characteristic of Salmonids, movement is required for spawning. The anadromous Steelhead trout will migrate out to the Pacific returning to inland freshwaters to spawn. The resident, river dwelling rainbows will move up-stream as an obligate requirement for successful spawning. In natural environments, the rainbow trout breeding season occurs in the spring, however, over the course of decades of aquaculture manipulations, many hatchery-raised rainbow trout have been conditioned to spawn in the fall, including the rainbow trout that are stocked into New Jersey waters. This alteration of the spawning season has been achieved through the hatchery methods including generations of selective breeding, altered light cycles and hormone injections (Leitriz and Lewis 1980). Hatchery-raised rainbow trout are typically poorly adapted to their introduced stocking locations and as a result reproduction of these populations is rare, occurring in only ideal conditions, with occasional hybridization occurring with naturalized or wild populations, as seen in previous research within the Lake Superior fishery (Close 1999).

Rainbow trout have been known to live for as long as 11 years, however their typical life expectancy is approximately 6 years. The fish reach reproductive maturity at the beginning of their 3rd year and spawning typically takes place in the 3rd or 4th year of life (Raleigh et al 1984). The fecundity of river-dwelling females is directly related to length of the individual but may range from 200 to 8,000 eggs (Delaney 2008). Eggs are deposited in nests, called redds, created by the female's caudal fin approximately 4 to 12 inches deep and 10 to 15 inches in diameter, and are fertilized by males (Delaney 2008). After fertilization the female or hen covers the eggs beneath the river substrate she had previously removed, and the eggs remain relatively well protected at the heads of stream riffles or down-stream edge of pools (Mills 1989).

The ideal habitat for rainbow trout contains clear, cold waters, deep pools and riffles, as well as in-stream cover and substantial vegetation on stream banks (Raleigh et al 1984). Like most aquatic dwelling organisms, rainbow trout are ectothermic, with the water conditions determining their body temperature and in turn their metabolism and respiration. Rainbow trout also require a high concentration of dissolved oxygen in the water, for this reason cold water streams are the ideal habitat for this species. Temperatures from 12oC to 18oC are considered to be the optimal range for suitable rainbow trout waters (Huang et al 2018). As a response to heat stress, the pathways of energy metabolism, protein metabolism, immune system, as well organ tissue maintenance can be disrupted (Huang et al 2018). In waters with temperatures of 21oC, studies have shown some evidence of adaptive responses in heat-stressed rainbow trout, however, temperatures exceeding the critical temperature of 24oC moves the heat response beyond adaptive regulation to injury (Huang et al 2018).

The diet of both young and mature Rainbow trout primarily consists of aquatic insects and "drift" organisms such as mayflies (Heydarnejad and Purser 2015). Eventually as a trout matures it may feed on small fish or larger benthic invertebrates such as crayfish. Foraging patterns can be driven by food availability, trout metabolism, and available prey sizes. Foraging ecology and spatial memory suggest that an efficient forager should be able to identify food locations and visit them based on their perceived rates of renewal (Heydarnejad 2015). Rainbow trout are visual feeders relying on visual cues to indicate the presence of prey items while foraging. One study (Heydarnejad and Purser 2015) demonstrated that rainbow trout may prefer a "win-stay" strategy of spatial memory during foraging. In this strategy, trout sit and wait at the location of their last successful food forage, anticipating additional drift organisms arriving at that site by the stream current (Heydarnejad and Purser 2015). However, other studies have shown that foraging behavior may be driven by a critical rate of prey capture, dependent on prey density and substrate composition of the stream, more so than other influences (Ware 1972).

Rainbow trout Hatchery and Stocking Programs

Rainbow trout are characterized as portion feeders that will readily feed on a wide variety of aquatic insect organisms. They are also a fast-growing fish species that displays rapid weight gain (Aitkaliyeva et al 2019). The USDA estimates that rainbow trout grow one inch per month throughout the spring and summer months in natural waters (Oregon USDA 1971). These characteristics make rainbow trout an easy species to manage in modern hatchery breeding conditions (Aitkaliyeva et al 2019). Fish stocking programs are used to both bolster and maintain fisheries for recreational sport fishing as well as for conservation and/or reintroduction (Kopack

et al 2016). Some stocking programs have introduced efforts to increase anti-predator behavior in stocked fish by introducing chemical alarm cues consistent with a specific predator in order to help the fish avoid predation through associative learning within the hatchery environment (Kopack et al 2016). This practice aligns with evidence that predation is the number one source of mortality for stocked rainbow trout (Kopack 2016).

State Trout Stocking and Site Selection

New Jersey's spring trout stocking program is supported exclusively with rainbow trout raised at the Pequest Trout Hatchery in Oxford, New Jersey. The trout season opens on the first Friday in April of each year. Leading up to the opening day of the trout season approximately 184,400 rainbow trout are stocked within the states fourteen trout streams, with a total of approximately 570,000 hatchery raised rainbow trout stocked into all of the trout waters in New Jersey including ponds and lakes. The Flat Brook of Northwest Sussex County, where this study took place, is allocated approximately 34,000 rainbow trout each spring making it the third highest stocked stream in the state.

New Jersey has provisions for protecting open state waters, the Surface Water Quality Standards. These standards use abiotic and biotic parameters for classifying state surface waters, including dissolved oxygen, suspended solids, pH and temperature. There are two general classifications in New Jersey, FW1 and FW2. FW1 waters receive the highest priority on protection and maintenance, ensuring they remain in a natural state (Hamilton and Barno 2005). Trout-specific waters have a set of three further potential classifications determined by the suitability of the system for trout. The classifications are: Trout Production, defined by the waters used by trout for spawning and nursery purposes; Trout Maintenance, defined by waters which support trout all year; and Non-trout waters not suitable for the sensitivity of trout due to physical, chemical or biological characteristics (Hamilton and Barno 2005). The Flat Brook system at the headwaters is classified as non-trout due to the water reaching temperatures not suitable for trout (Shramko 2017). However, downstream in the system there are stretches classified as both Trout production and Trout maintenance. The Flat Brook watershed as a whole remains relatively undisturbed compared with other New Jersey trout fisheries: only 4.5% is considered urban and 2.5% agricultural, leaving 94% of the watershed forested (Shramko 2017). The Trout Production and Trout Management classifications result in protected, undisturbed riparian buffers to support the trout within the river. Coupled with the Stream's picturesque setting and forested surroundings, the Flat Brook is an extremely desirable recreational area for New Jersey anglers.

In 2012 the NJDEP Bureau of Freshwater Fisheries began an electro-fishing stream assessment to document the number of trout found within a specific 6.7 kilometer (4.2 mile) section of the Flat Brook. The purpose of this assessment was to survey the number of three trout species; brook trout, brown trout and rainbow trout found within a proposed catch and release section on that stretch of the Flat Brook. The electro-shocking data collected in 2012-2013 resulted in 39 total trout collected along what will be referred to as the Flat Brook-Roy transect. This study resulted in the implementation of catch and release regulations in 2014. The shocking efforts post regulation in 2014 and 2015 resulted in 72 total trout collected from the electro-shocking including three new locations not previously sampled in 2012-2013 (see Appendix 1) (NJDEP BFF 2015).

Results from the Flat Brook-Roy transect electrofishing study supported the implementation of the catch and release regulation which still remains in 2020. A subsequent stocking assessment was conducted in 2016 and 2017 comparing New Jersey's only two catch and release regulated streams, the Flat Brook-Roy transect and the South Branch of the Raritan River. The results of the electro-shocking assessment suggested that considerably less stocked trout were holding over in the Flat Brook-Roy transect than in the South Branch of the Raritan after being stocked (Shramko 2017). This begs the question, what is the fate of hatchery raised rainbow trout that are stocked into the Flat Brook-Roy transect catch and release section and where do they go?

The current study was designed to address this question through the use of a radio telemetry tracking protocol for 80 rainbow trout raised at the Pequest Trout Hatchery and introduced into the Flat Brook-Roy transect. This study investigated trout activity, movement, and mortality across three different stocking locations along the catch and release Flat Brook-Roy transect, to better understand the fishery and to help optimize the state's resources for the recreational angling experience along the Flat Brook-Roy catch and release transect.

Methods

Study Site

The selected study site, the Flat Brook-Roy transect, is the only catch and release section in the Flat Brook in Sussex County NJ. The transect spans a 6.7 kilometer stretch of stream, the upstream limit of the transect is located at the GPS coordinates 41.20646, -74.80377, while The downstream limit of the transect is located at the GPS coordinates 41.17869, -74.86164 (see Appendix 1). The whole transect is located within a section of the stream which has a 94% forested watershed and undisturbed riparian buffers (Shramko 2017). The 6.7 kilometer stretch of the stream has differences in water flow rates both before and after rainfall events and varies in sinuosity, width, and depth. The stream substrate is consistently rock and gravel from the upstream to the downstream limits of the transect (see Appendix 2).

Stocking Locations

Along the 6.7 km Flat Brook-Roy transect, three independent stocking locations were chosen to distribute the radio-tagged rainbow trout in order to track differences in movement and survivorship/mortality at each of the sites, as well as within the entirety of the transect. The stocking locations will be referred to as Site A through C for the duration of this paper. Site A, located at the GPS coordinates 41.20581, -74.807, was the furthest up-stream stocking location. The pool in which the fish were stocked at Site A measured 1.55 meters at its deepest point, 20.67 meters in length and 18.69 meters wide. Site A was characterized by slow moving water, a rock and gravel bottom and several Eastern hemlock trees partially submerged across the pool (See Appendix 3). The Site B stocking pool was located down-stream at the GPS coordinates 41.20176, -74.81168. This pool measured 0.63 meters at its deepest depth, 16.27 meters in length and 11.25 meters in width. Site B was characterized by shallow depth and contiguous rock substrate, relatively fast-moving water and one singular partially uprooted Eastern hemlock minimally submerged (See Appendix 4). Site C was located the furthest downstream at the GPS coordinates 41.18544, -74.85067. Site C measured 1.19 meters at its deepest depth, 14.66 meters in length and 12.53 meters wide. Site C had slow moving water, no cover provided by fallen trees or partially submerged trees, and a silted bottom. This site had a foot bridge located at the upstream end of the stocking pool (See Appendix 5).

Pre-Stocking Radio-tagging Procedure

In the Spring of 2017 and 2018, 40 rainbow trout rainbow trout were surgically implanted with individually tuned radio transmitters resulting in a total of 80 implanted fish to be stocked and tracked over the spring and summer of 2017 and 2018. The battery life of the Advanced Telemetry Systems F-1580 transmitters (ATS) was projected to last 230 days (see appendix 6). For this reason, the study was designed for 40 fish to be tracked in 2017 and 40 in 2018 for a 230-day period, or until the naked transmitter was found in or around the stream or a signal was no longer detectable in the area surrounding the transect. Additionally, for each year, two surgery dates implanted 20 fish at a time. The first surgery date in April, April 12th, 2017, a week after the opening of the rainbow trout season and the second surgery date a month later on May 17th, 2017. Following the same protocol in 2018 the implantation surgeries were conducted on April 11th, 2018 and on May 14th, 2018. The reason for stocking 20 fish a month apart was to allow for

potential effects of differing river conditions to influence fish behavior upon their introduction to the stream.

The surgeries were conducted in the lab of the Pequest trout hatchery where individual 1½ year old fish were selected from the outdoor trout bays averaging 10.5 inches and 0.5lbs. In the lab a surgical board was set-up with a recycling diluted maintenance dose of MS-222 tricaine-S,tricaine methanesulfonate**.** Before the surgery the trout were put in an oxygenated bucket of water with an induction dose of MS-222 tricaine-S, tricaine methanesulfonate until fully anesthetized. The trout were then placed on the surgical board, ventral side up, with a tube containing a maintenance dose of anesthetic to keep the fish immobile and their gills well oxygenated (See Appendix 7). The incision to insert the transmitter was first sterilized with iodine then the cut was made on the ventral side just above the cloaca roughly half an inch in size. The transmitter, weighing 3.6 grams, was inserted into the body cavity of the fish using a plastic tube to hold the incision open, and a curved needle in order to place the transmitter into the trout and create an exit to pull the transmitter's antenna through the side of the fish without impeding their ability to swim (see Appendix 8). To finalize the surgery, the incision was closed using 2 non-dissolvable sutures (see Appendix 9). The trout was then moved to a designated empty raceway at the hatchery in order to monitor health of the trout, assess the success of the surgery and to ensure transmitter function in the specimen. All implanted fish had the same surgical procedure and were held for a 24-hour period before the introduction into the study site.

Stock and Post Stocking Protocol

The three sites (A, B, and C) along the transect received 6-7 of the radio-transmitter implanted fish respectively during each of the two stocking events, 12 to 14 at each site per year and 24 to 28 across the duration of the two year study. The stocking protocol included a total sample size of 80 fish over the 2017-2018 monitoring period. For the purpose of data analysis, the intended sample size of 80 was reduced to 79 due to complications during the transport of one trout from the hatchery to the stream.

At the downstream end of the study transect, an Advanced Telemetry Systems R4500C unmanned datalogger unit was positioned with an attached standing antenna in order to monitor any implanted fish leaving the downstream demarcation of the transect area. In order to appropriately monitor the implanted fish, the R4500C unit was pre-programmed with each individual transmitter frequency and set to constantly scan and record any frequency encountered. To ensure constant surveillance, the R4500C was powered by a fully charged external marine battery which was replaced every 2 weeks.

On the first day of each morning stocking event, each site was revisited in the afternoon and each individual fish was located using Advanced Telemetry Systems R410 handheld radio receiver and a yagi directional antenna. The afternoon telemetry was executed in order to record initial movement of the stocked fish. The research protocol called for recording the location of all stocked fish every day for the first 2 weeks after introduction. After the initial two weeks all fish were located every other day until a fish was determined dead, by finding a dropped transmitter or by a missing signal from the study site. In the event a specific fish was tracked to the same location for 5 consecutive days, protocol was to enter the water to encourage subtle fish movement to determine if the fish was still alive. In the event no movement was detected the

transmitter was retrieved from the stream bottom using an underwater sighting device and a net on a long pole.

For the purpose of logging data recorded by field technicians, movement was defined as travel from one specific riffle-run or pool to another riffle-run or pool. Fish found at the same location as the most recent survey, were recorded as having no movement. No subtle movement within the pool or riffle was recorded as movement in the movement data. In order to record data on fish activity, movement of each individual fish, as defined above, was recorded as "a move" and the distance it traveled was recorded in meters.

For the purpose of this study, the following assumptions were made regarding predation events. Mammalian predation events were assumed to have taken place when a naked transmitter was retrieved from the stream or the stream bank. Avian predation events were assumed when a signal was completely lost from the study site. In some cases when signals were lost from the study site, a survey of local heron rookeries turned up some transmitters. Regardless of whether transmitters were located near heron rookeries, any instance where signals were lost at the study site were considered avian predation events.

Wildlife field cameras were placed at each stocking location and at random locations throughout the transect. The cameras were set to record 30 second videos in the event their sensors were activated by motion. These cameras were effective in capturing the presence of predators both mammalian and avian. However, footage captured by the cameras was not used for any further data analysis due to the discrete nature of the transmitters and the uncertainty if the predation events captured by the cameras were fish specific to the study sample.

To analyze the recorded data on movement and mortality/predation the JMP Pro statistical analysis software from SAS was used. With this software a series of t-tests was used to compare differences in data by year. Additionally, a series of t-tests were used to make pairwise comparisons on data recorded from the fish at each stocking site location. JMP and Microsoft Excel we used to develop descriptive figures and charts for the data collected on the sample.

Results

Movement Results by Year

An analysis of the farthest movement data from the initial stocking site by year for fish with recorded movements, 29 trout from 2017 and the 25 trout in 2018, was analyzed using a two-tailed t-test. The results show that the farthest distance travel from initial stocking site in 2017 and 2018 were not significantly different from each other (t=.78614; df=47.96068; p=.4356) (Figure 1, Table 1)

Figure 1. Analysis of Furthest Distance (m) By Year

An analysis of trout activity measured in meters traveled by year for each sample population (2017 and 2018) was analyzed using a two-tailed t-test. The results showed that the total activity measured in meters between the years was not significantly different from each other (t= -0.77415; df= 76.46179; p=.4412) (Figure 2, Table 2).

Figure 2. Distance Moved Distribution by Year

A two-tailed t-test showed that there was no statistically significant difference between the average distance per movement in 2017 and 2018 (t= 1.15046 ; df= 54.76135 ; p= $.2550$) (Figure 3, Table 3).

Figure 3. Distance per Move Distribution by Year

For each year of the study, activity was monitored by tracking the number of movements, defined as the tracking of a fish to a new riffle run or pool from the most recent location tracked during previous telemetry survey. In 2017 the 40 fish averaged 3 movements per fish across the sample and in 2018 the 39 trout averaged 1.28 movements per fish across the sample (see Table 4).

Between the 2017 and 2018 data for number of days spent at initial stocking site, a twotailed t-test showed no significant difference between the annual groups ($t= 0.447702$; df= 74.52588; p=.6557) (Figure 4, Table 5).

Table 5. Average Days Spent at Stocking Location by Year

Figure 4. Distribution of Number of Days at Stocking Location by year

Thus, none of the trout movement data collected in 2017 and 2018 was significantly different. The lack of significant difference between the two years allows for the full sample to be treated as one population and therefore the remaining analyses on movement will be reported for the movement data of all 79 trout.

Total Sample Movement

Across both years only one trout was found via radio telemetry tracking to have moved outside of the confines of the transect (see Appendix 1). Using this fish along with 53 other fish that dispersed from their original stocking site, the *average farthest distance* moved from the original stocking site was 615.42 meters (see Table 6).

The average distance traveled measured in meters of the entire 79 trout sample was 549.23 meters; this number includes the cumulative distance of all moves both away from and back to their initial stocking location for all fish, even those that did not move from their stocking site (Table 6).

The average distance traveled measured in meters for all trout in the total sample is 241 meters (Table 6). All 79 trout in the sample spent an average of 23.9 day at their original stocking site before their first recorded movement (Table 6). The average number of moves per trout within the whole sample was 2.24 moves (Table 6). Hand-held radio telemetry tracking data showed the earliest movement from the stocking site to be on the first day of introduction into the stream and the longest time spent at original stocking location to be 125 days (Figure 5).

Table 6. Total Sample movement data averages.

Figure 5. Total Sample: Number of Fish at Initial Stocking Location by Days after introduction

Mortality and Predation by Year

In 2017 the average number of days alive for the 40 trout in the sample was 52.4 days. In 2018 the average number of days alive for the 39 trout in the sample was 46.2 days (Table 7). A two-tailed t-test was used to test for significance and determined no difference between the two years (t= -0.62866; df=76.70946; p=.5314) (Table 7 Figure 6).

Figure 6. Distribution of Number of Days Alive by Year

Mortality events in relation to calendar dates followed a similar curve between both 2017 and 2018 with mortality events being most significant in weeks 6 through 10 (Figure 7). In 2017, there were 21 mammalian predation events and 19 avian predation events. In 2018, there were 25 mammalian predation events and 14 avian predation events (see Figure 8). In total, there were 46 mammalian predation events and 33 avian predation events (see Figure 8). The lack of significant difference between the mortality data from each year allows for the total sample size to be reported as one population.

Figure 7 Mortality in Population by Year

i.

Predation by Year

Figure 8 Predation Events by Year

Total Sample Mortality and Predation Results

Using the entire sample of 79 trout, the average number of days alive was 52.42 days (sd=35.57; n=79). The longest-lived trout survived for 125 days whereas the shortest-lived trout was alive in the stream for 0 days. There was no trout alive in the sample past 125 days which was September 21st. Between weeks 6 and 10, respective to the calendar dates May 22 to June 30, mortality was highest with 33 predation events during that span of time (Figure 9).

Figure 9. Total Population Mortality by Week of Study

Results by Stocking Site Location

Movement Results by Site

Movement data was recorded for each stocking site in Tables 8 & 9. Table 8 shows the average distance traveled over the course of the study while table 9 shows the average number of days spent at each stocking site.

Table 8. Average Distance Traveled by Site

Table 9. Average Number of Days spent at Stocking Site by Site

The average distance traveled at each site was analyzed using a t-test and ordered difference report and showed that the average distance traveled was not significantly different between any of the sites (See Table 10, Figure 10).

Table 10. Student's t-test results for Site Comparison of Total Distance Traveled

Figure 10. Distribution of Total Distance Traveled by Site.

The average number of days spent at the initial stocking site did show statistical significance between sites. A Student's t-test and ordered differences report showed that trout at Site A remained at their initial stocking location significantly longer than trout at Site B and Site C (Table 11, Figure 11 & 12).

Table 11. Student's t-test results for Site Comparison of Number of Days Spent at initial Site

Figure 11. Number of Fish at Site by Days at Stocking Site

Figure 12. Distribution of # Days Spent at Stocking Location

The activity of trout at each site was tracked by logging the number of movements each fish made. A Student's t-test was used to analyze the number of movements and the results showed that Site C had statistically significant difference in activity (in terms of number of moves made) than Sites A and B (Table 12)

Table 12. Student's Test results for Number of Moves at each Site

Mortality and Predation by Site

The average number of days alive at each site was analyzed using a Student's t-test and the ordered differences report showed significant difference in number of days alive between site A and B, but not between A and C or B and C. (See Table 13, Figure 13 & 14)

Table 13. Students t-test results for Site Comparison of Number of Days Alive

Figure 13. # of Fish by Days Alive across Stocking Sites

Figure 14. Distribution of Days Alive by Site, ranging from 0 to 125 days.

The length of time fish survived at Site A was significantly longer than at Site B and there was a slight interaction between survivorship at Site A and Site C.

Discussion

Across two years of congruent data collection, this study confirmed the initial findings reported by Shramko (2017) that the fish introduced into the Flat brook through the NJDEP stocking program are not leaving the Flat Brook and moving into the Delaware River (see figure 2). The location where the Flat Brook empties into the Delaware River is 13.25 km or 13250 m downstream from the downstream boundary of the Flat Brook-Roy transect. That distance far exceeds the furthest distance traveled by any individual fish within the sample, from any one of the three stocking locations. The movement results from 2018 align in with the movement results of 2017 as initially reported in Shramko (2017; See Fig. 2,3,4 & Table. 6). These congruent results confirm the expected movement behavior for the trout stocked into the Flatbrook-Roy catch and release transect and will help with future planning and implementation of the NJDEP's trout stocking efforts within this section of stream. This investigation indicates that stocked rainbow trout will not only remain within the Flat Brook but will also remain within the catch and release section in close proximity to their initial stocking locations. These findings are important when developing a stocking program that supports a recreational fishery that is designed to maximize angler success rate.

The results from this study suggest that the quality of the aquatic habitat coupled with the behavioral characteristic of the hatchery raised fish, limited their dispersal out of the Flat Brook-Roy transect. Perhaps the age of the fish in this study is the reason for low emigration outside of the catch and release transect. A previous radio-telemetry study conducted in 2010 on resident rainbow trout in South Dakota acknowledges the fact that juvenile rainbow trout will often spend two years in their natal streams before dispersing into larger waters (Jones 2010). Jones found that most pre-spawn trout did not disperse from their initial location. All of the fish used in this study were $1\frac{1}{2}$ year old trout and not in spawning condition. According to NJDEP Fisheries Biologist Ross Shramko, there are very few locations in NJ with wild spawning trout populations, the Flatbrook-Roy Transect is not one of those locations (Shramko per comm). In addition, the NJ hatchery raised fish introduced to the stream, over years of manipulation, have become fall spawning fish and would probably revert back to wild spring spawning tendencies, however they do not persist in the streams of NJ long enough to establish breeding populations in most cases (R. Shramko pers comm).

These results are consistent with a study on non-native stocked rainbow trout in Arizona (Sweetser et al 2002). Sweetser reported that none of the 54 Rainbow trout from his study location left the study area (Sweetser et al 2002). These results are consistent with the Flatbrook-Roy transect where only 1 out of 79 left the confines of the transect. These consistent results between stocked trout studies and this study suggest that the rearing of hatchery trout in confined raceways leads to a sedentary lifestyle, exhibiting primarily short distance movements after stocking into a natural habitat.

The low numbers of movement events made within the sample may also be indicative of trout foraging behavior. Wild Rainbow trout are drift feeders, feeding as the stream current brings food from upstream to them. For this reason, trout can use their spatial memory to learn locations where they successfully forage, and the perceived rates of renewal of those locations (Heydarnejad and Purser 2015). This leads to rainbow trout exercising a "sit and wait" win-stay foraging strategy (Heydarnejad 2015). In win-stay foraging behavior, an organism will return or remain to a previous reward location instead of avoiding a location where it has already had success (Heydarnejad and Purser 2015). Perhaps the hatchery raised fish in this investigation

have had that "sit and wait" strategy reinforced by the hatchery's method of food delivery, where they are fed directly at a perceived consistent time and location along the hatchery raceway. The Heydarnejad study chose to look at hatchery raised trout and their spatial decisions on foraging. The results of that study indicate that hatchery raised trout operated under a "win-stay" foraging strategy and hatchery feeding processes may in fact reinforce the spatial behavior that the wild variation of rainbow trout exhibit (Heydarnejad and Purser 2015).

This study has established the philopatric nature of trout introduced into the catch and release section of the Flat Brook-Roy transect, however the fate of these fish goes beyond their movement behavior. Mortality data collected over the duration of this study, sheds light on the fate of the trout introduced into the Flat Brook-Roy catch and release section. In 2018 the mortality data collected were similar to the data collected in 2017. All 79 fish considered in the data analysis were lost from the stream as a result of direct predation from either an avian or mammalian predator. The predation events across both years follow a similar curve and correlate to predator reproductive periods, where energetic demands increase for both mammalian and avian predators. Predators of rainbow trout with confirmed sightings using wildlife cameras strategically positioned at stocking locations along the stream included Great Blue Heron, Common Merganser, Osprey, River Otter, American Mink, Black Bear and Raccoon.

The results of this study agree with a study conducted in Southern Idaho fisheries (Chairamonte et al 2019). The Idaho investigation included stocked rainbow trout and determined that avian predation reduced the amount of catchable fish within the study area, mirroring the findings of the current study conducted in the Flat Brook-Roy transect.

A study of river otters, conducted within Yellowstone National Park, Wyoming, reported on the diets of river otter by investigating latrine sites and analyzing scat (Crait 2005). The results of their investigation found river otters to be highly dependent on riverine trout, with trout occurring in 72% of all river otter scat (Crait and Ben-David 2006). These findings are consistent with the Flat Brook-Roy findings on mammalian predation and supports the assumption that predators will return to feed at a specific location until that location has been depleted of available food. The location of the Flat Brook-Roy catch and release section falls within one of the state's ecological strongholds, with several state forests and wildlife management areas being adjacent to the Flat Brook's waters. In addition to the camera trap data showing otters living in the area, the investigator found several otter slides and tracks within the study area. Perhaps the amount of predator presence along the transect can explain why mortality was so high compared to the stocked fish at the Ken Lockwood Gorge catch and release site where survivorship is much higher (NJDEP BFF 2015). The mortality of trout documented by this study should direct future management decisions made for the Flatbrook-Roy fishery. If these stocked trout are being completely removed by predation and not holding over through the summer and fall, perhaps reconsidering the number of fish allocated to this area is appropriate. Another alternative would be to remove the catch and release mandate along the Flat Brook-Roy transect in order to allow anglers access and use of the fish before they are inevitably removed by predation.

An analysis of the differences in fish longevity across stocking sites in this investigation indicate that fish preferred stocking sites with a large pool size and greatest amount of in-stream cover. This study showed that fish introduced to smaller pool sizes with reduced cover will more readily disperse and/or be preyed upon. These results are supported by a study that examined territory value of cover and predation risk in trout (Johnsson et al 2004). The results showed a strong preference for cover, both when threatened and when not threatened (Johnsson et al 2004). The Johnsson study supports the site-specific movement results from the Flat Brook-Roy

transect and should be assimilated into the management considerations for the Flatbrook-Roy Catch and Release section. In order to maximize the survivorship of stocked trout and thus accentuate the angling experience, stocked trout should be allocated to stocking locations that exhibit large pool size and adequate stream cover.

Study Limitations

One of the major assumptions of this study concerned predation events. This study assumed that transmitters whose signals disappeared from the transect were cases of avian predation. In some cases, the missing signals were discovered at a considerable distance from the immediate stream area, at local heron rookies. In other cases, the signals were never again found. However, regardless of whether the signals were found at the heron rookeries or never found again, all of these events were treated as avian predation events. Wildlife camera data provided evidence of avian predator presence along the study transect.

This study also assumed that mammalian predation events occurred when naked transmitters were retrieved from the stream bottom or along stream banks. This assumption was supported with additional evidence like scat, dens sites, runs, physical remains of the trout, tracks and wildlife camera footage of mammalian predator presence.

The time constraints of this investigation limited the number of quantitative habitat measurements taken. These included fluctuations in water depth during precipitation events and water temperature changes over the course of the study, which could have provided important information regarding the movements of the trout.

Unfortunately, due to the potentially harmful process of identifying the gonads in the $1\frac{1}{2}$ year old trout, the sex of the introduced fish was not determined, so any sex differences in movement data and mortality events could not be analyzed.

Suggestions for Further Research

The sample in this study consisted of 79 1¹/₂ year old trout, weighing approximately a half a pound and ranging in size from ten and a half to eleven inches. Future studies into the movement patterns of introduced rainbow trout should include fish of different ages and sizes: older and larger fish may have different movement patterns and survival rates.

Since predation plays such a major role in the fate of fish introduced into the Flat Brook-Roy catch and release section of the Flat Brook, further research is needed to better understand the nature of predation events. Monitoring predator pressure along the Flat Brook-Roy transect, using a full array of camera traps, should give a clearer understanding of predation events and could lead to management strategies that reduce the mortality of stocked rainbow trout.

Currently, the Pequest Trout hatchery does not have a protocol during rearing that exposes the rainbow trout to predatory cues to trigger anti-predatory behavior (Shramko, Per. Comm.). There is research that suggests chemical cues introduced during hatchery rearing may increase anti-predatory behavior of the stocked fish, however further research is needed to confirm its effectiveness in the natural environment.

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Appendices

Apdx. 1. Electro-shocking results, NJDEP study investigating fish presence in the Flat Brook

Electrofishing locations along the Big Flat Brook and Flat Brook from $2012 - 2015$.

Apdx. 2. Map of Entire Flat Brook-Roy Transect

Apdx. 3. Underwater Go-Pro Stream Substrate showing the typical benthic environment for the entire stream.

Apdx 4. Photo of stocking site A with downed trees creating the pool where the fish were released

Apdx. 5. Stocking Site B Photo. Shallow pool where the fish were released, narrow in width, slight instream cover.

Apdx. 6. Stocking Site C photo, No in-stream coverage at all in the pool where the fish were Released

Apdx. 7. Transmitter measuring 2.5 cm and weighing 3.6 grams

Andx. 8. Surgery Table with fish in operation cradle

Apdx. 9. Transmitter Implantation

Apdx. 10. Suturing the incision. On average the entire surgery took 5 minutes from opening to closing the incision.

