THE EFFECTS OF STOCKING INVASIVE RAINBOW TROUT (ONCORHYNCHUS MYKISS) ON ASSEMBLAGE STRUCTURE AND MICROHABITAT USE OF NATIVE FISHES IN A SOUTHERN APPALACHIAN STREAM

by

Duncan C. Elkins

(Under the Direction of Gary D. Grossman)

ABSTRACT

We examined the impacts of stocking with rainbow trout on the native fish community of Betty Creek, Georgia, USA, at the level of the assemblage (population, biomass, species richness, diversity) and the individual (microhabitat use). Our study encompassed the years from 2003-2008 and used a replicated BACI design with two pairs of control and introduction sites. We performed 7 introductions and described 4 microhabitat shifts that were significant at the 90% level and 11 more that were consistent with these but not significant. The persistence of these effects appeared to be related to the number of trout that remained in the site after the introductions, but this relationship was non-significant. We supplemented these results with experiments in a model stream using warpaint shiners and rainbow trout. Trout affected some aspects of the social behavior and space use of the warpaint shiners, including depressing their feeding rate, and caused them to use larger homeranges and experience higher current velocities.

INDEX WORDS: rainbow trout, introduced species, native fish, warpaint shiner, competition

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August 2010

DEDICATION

I dedicate this work to my wife, Leigh, to my parents, and to the native fishes of the southeast.

ACKNOWLEDGEMENTS

This project would not have been possible without the assistance of many, many individuals. To all the student workers, especially Allie, who trained me, and Abby, who gamely endured rain and illness, no graduate student could have asked for better help. To the other students of the Grossman lab, thanks for schlepping, shocking, and running trials. To Amy, who was around for the project's inception, and Megan, who gave it its real momentum, I'm glad I could be here to polish it off. To Gary Sundin and Shannon Albeke, you arrived with observational and analytical skills at the perfect time. To the landowners along Betty Creek, the Wilsons, the Darnells, and the Hambidge Center, thanks for giving us access to your lovely stream. To Dr. Grossman and the members of my committee, thanks for all your help and advice- I look forward to serving you an order of fries someday soon. This project was funded with the help of the Georgia Department of Natural Resources, the Warnell School of Forestry and Natural Resources, and a Coldwater Fisheries Scholarship from the Oconee River and Coosa River Chapters of Trout Unlimited.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), although native only to coastal drainages of the Pacific Rim (MacCrimmon 1971, Behnke 2002), are widely stocked worldwide (Fausch 1988, Welcomme 1988, Crawford and Muir 2008). In 2004, Halverson (2008) calculates State and Federal agencies released approximately 9,960,000 kg of diploid and triploid rainbow trout into US waters, with stocking programs active in 45 of 50 states, the only exceptions being Alabama, Florida, Louisiana, Mississippi, and South Carolina. Despite this widespread introduction little is known about the effects of rainbow trout stocking on native non-game fishes, even though substantial declines have been reported in many of these species in the last 20 years (Jelks et al. 2008). Consequently, an assessment of the effects of trout stocking on non-game fishes is warranted, because these fishes inhabit many of North America's stocked waters, especially because research with both native trout and New Zealand non-game species document negative interactions with stocked trout (e.g., McIntosh et al. 1992, McIntosh et al. 1994, Kaeding and Carty 1996, Clark and Rose 1997, Kruse 1999).

The adverse effects of invasive fishes on native species typically are produced via either interspecific competition for space or food (Grossman and Freeman 1987, Flecker and Townsend 1994) or predation (Garman and Nielsen 1982). In the presence of introduced salmonids, native fishes may display behavioral changes that reduce foraging success (McIntosh et al. 1992, McIntosh et al. 1994, Townsend 1996, McDowall 2003, Townsend 2003, McDowall 2006, Penaluna et al. 2009). The proximate negative effects produced by these interactions include shifts to less profitable micro- and mesohabitats, shift to alternate prey resources, and ultimately, reductions in the density that ultimately increase the probability of extinction. This may be a substantial problem in southern Appalachian streams because they display high amounts of environmental variability, which may increase the probability of extinction for sensitive species (Grossman et al. 1995). Nonetheless, studies in this region have suggested that stocked rainbow trout may not strongly affect habitat use by native cyprinids of the southeastern US (Grossman and Sostoa 1994), in the way that brown trout (Salmo trutta) have affected the native galaxiids of New Zealand. Consequently, we conducted experiments in a representative southern Appalachian stream to test the hypothesis that stocked rainbow trout did not affect both assemblage structure and microhabitat use by native non-game fishes.

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CHAPTER 2

THE EFFECTS OF RAINBOW TROUT STOCKING ON THE NATIVE FISH ASSEMBLAGE OF A SOUTHERN APPALACHIAN STREAM*

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*Elkins, D. C., and G. D. Grossman. To be submitted to Ecological Monographs

ABSTRACT

We examined the impacts of stocking with rainbow trout on the native fish community of Betty Creek, Georgia, USA, at the level of the assemblage (population, biomass, species richness, diversity) and the individual (microhabitat use). Our study encompassed the years from 2003-2008 and used a replicated BACI design with two pairs of control and introduction sites. Beginning in fall of 2005, we performed seven introductions, during which hatchery rainbow trout were stocked into the introduction sites and all sites were enclosed with block-nets. The study period was one of remarkable environmental variability, including high flows in 2004-2005 and drought beginning in 2006. Although the assemblage-level data were variable, we did not detect any impacts of rainbow trout stocking. We used principal components analysis (PCA) to assess whether the microhabitat use of native fishes changed, relative to random habitat availability, between the periods before, during, and after the 48-hour introductions. We observed 8 species present for some or all of seven introductions, resulting in 39 analyses.

Results were highly variable, perhaps because of changing abundances driven by environmental conditions, but native fishes' microhabitat use was significantly less selective during the trout introductions. Four analyses showed microhabitat shifts by native species away from rainbow trout that were significant at the 90% level, and eleven more showed shifts that were consistent with trout avoidance but not significant. Of these fifteen responses, seven disappeared after the removal of the block-nets, while four analyses showed some decline in the effect and four effects persisted unchanged even at greatly reduced densities of rainbow trout. We conclude that these sublethal effects are strongest among drift-feeding minnows in the middle water-column guild but suggest that repeated stocking with invasive salmonids could lower the fitness of many native fish species.

KEYWORDS: rainbow trout, trout stocking, native fish, Cyprinidae

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), although native only to coastal drainages of the Pacific Rim (MacCrimmon 1971, Behnke 2002), are widely stocked worldwide (Fausch 1988, Welcomme 1988, Crawford and Muir 2008). In 2004, Halverson (2008) calculates State and Federal agencies released approximately 9,960,000 kg of diploid and triploid rainbow trout into US waters, with stocking programs active in 45 of 50 states, the only exceptions being Alabama, Florida, Louisiana, Mississippi, and South Carolina. Despite this widespread introduction little is known about the effects of rainbow trout stocking on native non-game fishes, even though substantial declines have been reported in many of these species in the last 20 years (Jelks et al. 2008). Consequently, an assessment of the effects of trout stocking on non-game fishes is warranted, because these fishes inhabit many of North America's stocked waters, especially because of document negative interactions between stocked trout and native salmonids (Fausch 1988) and between stocked trout and New Zealand non-game species (e.g., McIntosh et al. 1992, McIntosh et al. 1994, Kaeding et al. 1996, Clark and Rose 1997, Kruse 1999).

The adverse effects of invasive fishes on native species typically are produced via either interspecific competition for space or food (Grossman and Freeman 1987, Flecker and Townsend 1994) or predation (Garman and Nielsen 1982). In the presence of introduced salmonids, native fishes may display behavioral changes that reduce foraging success (McIntosh et al. 1992, McIntosh et al. 1994, Townsend 1996, McDowall 2003, Townsend 2003, McDowall 2006, Penaluna et al. 2009). The proximate negative effects produced by these interactions include shifts to less profitable micro- and mesohabitats, shifts to alternate prey resources, and ultimately, reductions in density and increased probability of extinction. This may be a substantial problem in southern Appalachian streams where high environmental variability may also increase the probability of extinction for sensitive species (Grossman et al. 1995). Nonetheless, studies in this region have suggested that stocked rainbow trout may not strongly affect habitat use by native cyprinids of the southeastern US (Grossman and Sostoa 1994), in the way that brown trout (Salmo trutta) have affected the native galaxiids of New Zealand (McDowall 2006). Consequently, we conducted experiments in a representative southern Appalachian stream to test whether stocked rainbow trout affect assemblage structure or microhabitat use by native non-game fishes.

METHODS

The Study Site

Our study area consisted of four sites, each 50m long, spread out along approximately 2.5 km of Betty Creek, a third order stream located in northeastern Georgia that is a tributary of the Little Tennessee River. Betty Creek has a watershed area of 33.4 km^2 which is 99.4% forested (Jones et al. 1999). The watershed lies entirely within the boundaries of the Chattahoochee National Forest, with some low-density residential development and pasture land on the valley bottom. All four of our sites had intact riparian vegetation, though this appeared to be second-growth. The stream has an active channel that contains a mixture of riffle, run, and pool habitat that is visually similar to many other streams in the southern Appalachians. Betty Creek receives one annual stocking of rainbow trout, near where it crosses Georgia Highway 441, over 4 km below our first study site. This stocking is timed to coincide with the Georgia chapter of Trout

Unlimited’s annual Trout Camp, and the lower section receives intense angling pressure in the week after stocking (Duncan Elkins, pers. obs.)

We divided our four sites into two sets of paired control (C) and introduction (I) sites [Pair 1 - Upper Patterson Gap (C), Lower Patterson Gap (I), Pair 2 - Darnell (C) and Wilson (I), (Figure 2.1).] Although we chose site boundaries in an attempt to balance the amount of riffle, run, and pool habitat within each pair, several smaller streams join the main channel in the approximately 1 km between the Lower Patterson Gap and Darnell sites and the stream is therefore larger in the lower pair, with a straighter channel and smaller pools, relative to the volume of riffle and run habitat.

We used a BACI (Before-After, Control-Intervention) design (Stewart-Oaten et al. 1986) with paired sites, which is the most powerful design for this type of study (Smith 2002). In our case the impact was the introduction of stocked rainbow trout in the study site so we will refer to the intervention sites as “introduction” sites. Each site was 50m long and was visually representative of stocking sites in mountain streams (G. Grossman & Chris Skelton, pers. obs.)

The Darnell site was added when we lost access to our original site, located downstream of the Wilson site approximately 100m upstream of the bridge at Neville Road, after a year of sampling. Due to the distance between these locations, we discarded the data from the first year of data collection at Neville Road and we delayed the first rainbow trout introduction at the lower site pair by one field season to gather sufficient baseline data at Darnell. Thus, our samples are unbalanced between the lower site pair, Wilson and Darnell, and upper site pair, Lower and Upper Patterson Gap.

Field Sampling

We quantified fish species richness and abundance using three-pass electrofishing and used snorkeling observations for microhabitat-use measurements (see *Fish Microhabitat Use*, below.) We sampled only in spring and fall months because of the additional stress that higher summer temperatures place on shocked fishes and because most stream fishes in the southern Appalachians are quiescent (Grossman and Freeman 1987, Hill and Grossman 1993) and there is little stocking of similar streams during the winter months.

After completing the spring and fall microhabitat observations in all sites, we performed a three pass electrofishing survey to quantify assemblage structure, species richness, evenness, and abundance each species. Sampling effort was kept constant. Before electrofishing, both the top and bottom boundaries of the study section were blocked off with 1/4" mesh seines. A six to nine person team systematically electrofished the entire site (Smith Root Model 12 and LR-24 back pack electrofishers) beginning at the lower block-net and proceeding upstream to the upper net. Fish were collected using a two to three meter long seine (1/4" mesh) held just downstream from the electrodes. We placed captured fish in aerated holding tanks on the stream bank until they could be measured (± 1 mm, standard length, SL) and weighed with an electronic balance (± 0.1 g). On a few occasions when individual species were very abundant (i.e., several hundred captured) we subsampled ($n > 100$) for length and weight measurements and constructed length-weight regressions for these species and used these regressions to estimate total biomass. Upon completion of the third pass, fishes were redistributed throughout the entire reach. Due to high flows during the fall 2003 sample period, only two passes were performed for this sample.

Fish Assemblage Structure

We calculated population estimates using data from the three sequential passes and Program CAPTURE (Rexstad and Burnham 1991). We generally used the M(bh) estimator from Otis, Burnham, et al. (1978), however, on the single occasion when only two passes were made (Fall 2003) we used the Zippin two-pass estimator (Zippin 1958). On occasion, the assumptions of the 3-pass depletion estimator were violated for several rare species (e.g., *Moxostoma* sp. or *Cyprinella galactura* neither of which was observed in $n > 4$ within a single sample). When this occurred, we used the total number of individuals captured as the population estimate. We tested the hypothesis that the variation in flow was significantly correlated to species richness, diversity, and abundance using flow data from USGS gage # 02178400 on the Tallulah River at Clayton, Georgia. We selected this gage because it is the closest USGS gage to our sample site. However, the Tallulah River is much larger than Betty Creek, so we compared these data to flow data from Weir 8 at Coweeta Creek, a nearby stream of similar size (Grossman and Ratajczak 1998) for which flow data were unavailable during the last year of our samples. For the 1982-2007 water years, the 30-day means on these two gauges were highly correlated with an $r^2 = 0.94$ (regression: Tallulah Flow = 598*Coweeta Flow + 8.7742, $p << 0.05$). We calculated species richness as the number of species present in electrofishing samples. To calculate diversity

(Shannon's H) we used the VEGAN (Oksanen et al. 2008) package in R (R Development Core Team 2008).

Fish Microhabitat Use

We made fish microhabitat measurements in Betty Creek seasonally from spring 2003 to spring 2008 using the underwater observation methods of Grossman & Freeman (1987) and Grossman et al. (1998). These methods have been successful in quantifying both microhabitat selection and the effects of potential competitors and predators (including rainbow trout) on stream fishes in the southeastern United States (Grossman et al. 1995, Grossman et al. 1998, Grossman and Ratajczak 1998) and Europe (Grossman and Sostoa 1994). Microhabitat measurements were made on all species observed in the site. Rainbow trout introductions were staggered between paired sites, typically by two weeks, due to logistical constraints.

We made all snorkeling observations under base flow conditions which we operationally defined to preclude observations during a rising hydrograph on the nearby USGS gage at Clayton, GA, (# 02178400) or if more than 0.75 cm of rain had fallen in the previous 24 hours. We made fish observations during daylight hours (from 0900 to 1700 hours) by entering at the downstream end of the site and snorkeling slowly upstream. We covered the entire channel width by slowly moving laterally until the far bank was reached and then moving slowly upstream and repeating the traverse. Upon sighting an undisturbed fish we recorded the species identification, and estimated its standard length (± 1 cm), its vertical distance from the substratum (± 1 cm), its distance from shelter (± 1 cm, defined as any object capable of concealing at least 50% of the fish), and placed a numbered lead weight at its location (Petty and Grossman 2004). After snorkeling 10-15 m of the site, we returned and made the following habitat measurements at the location of each numbered weight: 1) depth (± 1 cm, meter stick), 2) mean water-column velocity (± 0.1 cm/s, electronic velocity meter), 3) focal point velocity (velocity at the fish location, ± 0.1 cm/s, electronic velocity meter), and 4) visual estimates of the percentage composition of seven substratum categories in a 20 X 20 cm square directly underneath the fish location. These categories were as follows: bedrock, particles > 30 cm embedded to the surface; boulders, unembedded particles >30 cm; cobble, ≤ 30 and > 2.5 cm; gravel, ≤ 2.5 and > 0.2 cm; sand, ≤ 0.2 cm; silt, material that was capable of suspension in the water-column, and debris, leaf or woody material.

Consistent with the report of Grossman and Freeman (1987) we observed that stream fishes were often undisturbed by a slow-moving floating observer. We frequently had fishes feed from the drift within 50 cm of a snorkeler and saw Gilt darters (*Percina evides* Jordan & Copeland) exhibit courtship and spawning behavior on at least three occasions. We considered a fish undisturbed if it continued to feed and maintained a stable position within the water column (for drift feeding fishes) or did not attempt to flee or withdraw into a crevice (for benthic fishes). Conversely, we considered a fish to be disturbed if it exhibited a characteristic escape response and we attempted to avoid this disturbance by taking measurements on a maximum of three individuals from the same school.

Habitat Availability

During each set of microhabitat observations we collected habitat availability data from 50 stratified random locations (10 samples from each 10 m) within the 50 m study section. Sample locations were determined using a random number generator to determine X (length) and Y (width) coordinates in the stream. Because each set of fish microhabitat observations consisted of multiple snorkel passes over a period of days, we typically took 25 availability samples in a single pass, one pass occurring early in the sample period, after the conclusion of behavioral observations for that day, and one pass occurring near the end. We used the methods described above to quantify habitat availability in terms of: depth, mean water-column velocity, and the percentage composition of seven substratum categories (bedrock, boulder, cobble, gravel, sand, silt and debris, as above).

Rainbow Trout Introductions

Starting in summer 2005 through spring 2008, we conducted nine rainbow trout introductions (settable). Each rainbow trout introduction trial consisted of three segments, with two observation periods bracketing a 48-hour period during which rainbow trout were stocked into the introduction sites and confined with block-nets to maintain the stocking density. During each segment, we made microhabitat observations for fish and random points to quantify habitat availability. In the pre-stocking segment (hereafter, "before"), we snorkeled each site from two to six times, over 2 to14 days, in order to obtain a minimum of eight measurements for each species common at the site, but we typically recorded 15-20 observations per species. In the rainbow trout introduction phase (hereafter, "during"), we installed block-nets (1/4 inch mesh size) at the upstream and downstream boundaries of the control and introduction sites in a pair in late afternoon. Twenty five rainbow trout provided by the Lake Burton Fish Hatchery (Georgia Dept. Nat. Resources), were then stocked in the introduction sites. This approximated the stocking density used by the Georgia DNR for a typical stream of this size. We observed fish microhabitat use by snorkeling for another two days with the block-nets in place to confine the introduced rainbow trout to the site. The third phase began after this period (hereafter, “after”), when the block-nets were removed and we snorkeled and collected fish microhabitat use data at each site an additional three - five times, beginning approximately 36 hours after the removal of the nets. Every attempt was made to balance the number of observations, per species, during each period. At the conclusion of the “after” observation segment, we performed a three-pass electrofishing survey in both sites, as described in Field Sampling, above, for comparison with samples taken prior to initiation of the introduction experiments.

Although we performed nine introductions, two were excluded from our microhabitat analyses due to logistical or weather problems that prevented us from collecting adequate microhabitat data in the 48-hour segment during the introduction. All observations in the “during” phase were made with an observer in both the introduction and control sites snorkeling simultaneously. The same two observers collected the data from the “before” and “after” phases, although these snorkel passes were not simultaneous. All observers were trained by one of the authors (DE) and each observer’s observations from their training period were compared to his using a graphical PCA procedure (see below) and shown to be indistinguishable at the 95% level of significance.

Statistical Analysis

To test for rainbow trout introduction effects on species richness, diversity, abundance, and biomass estimates, we performed an ANOVA on pooled samples from control and introduction sites and coded the samples as either before (pre-Fall 2005 at the upper site pair, pre-spring 2006 at the lower pair) or after the initiation of rainbow trout stocking. In each case, we modeled the response as a function of time (before or after stocking began), treatment (stocked or not stocked), and included an interaction term for stocking X time. Our null hypothesis was that no differences were present among mean values for each group at $\alpha = 0.1$. Because of the BACI design, a significant introduction X time interaction term would indicate a significant effect of stocking rainbow trout on one of these assemblage-level metrics. To assess statistical significance, we used an experimental α of 0.10 due to the small sample sizes of some species and the short – term nature of the introductions, which limited the number of observations possible.

We tested for differences in microhabitat habitat availability both within and among sites and between fish microhabitat use and availability using the Principle Component Analysis (PCA) technique (correlation matrix solution) of Grossman & Freeman (1987) and Grossman & de Sostoa (1994). We used PCA because microhabitat data represent a constellation of correlated factors that fish most likely perceive as interacting rather than independent variables and PCA is the least biased multivariate technique for use on data of this type (Grossman and Boulé 1991). As applied here, PCA reduces the dimensionality of a data set by applying a linear transformation to the variables, rotating the points in multivariate space and creating new composite axes that maximize the differences between the points in two dimensions. Linear and per cent data were transformed using ln and arcsine-square root transforms, respectively. We only report ecologically interpretable components with eigenvalues greater than one (Grossman and Freeman 1987).

To visualize any microhabitat shifts due to the presence of rainbow trout we conducted a PCA on the habitat availability and fish microhabitat observations before and during each rainbow trout introduction and then plotted the mean values for each on a Cartesian plane (Figure 2.2). On these plots, the distance between a pair of habitat and fish use points represents the level of microhabitat selection displayed by the fish, i.e., the farther the fish use point is from the random habitat availability point, the more selective the fish appeared to be during that time period. For the introduction sites, we also plotted the mean value for rainbow trout microhabitat use during the introduction period. We created a separate paired plot (introduction vs. control) for each species for which we made more than n=8 observations in both observation periods of an introduction.

Effects of Trout Stocking on Microhabitat Use

If our sites were suitable replicates then plots of the available microhabitat in our control and introduction sites should be similar. To test this hypothesis, we performed a PCA on the random habitat availability points for each pair for all years, and plotted these, as above, with 90% confidence intervals around the means for PC1 and PC2. If the 90% confidence ellipses around two microhabitat means did not overlap, we consider the two samples to be significantly different (equivalent to a t-test at alpha = 0.10, Johnson 1999).

We tested the null hypothesis that the presence of trout does not cause native fishes to shift from their pre-introduction microhabitat use by performing a two-way ANOVA on the distances between microhabitat use and availability in the control and introduction sites before and during the introductions (Figure 2.3). A significant ($\alpha = 0.10$) interaction between the time (before vs. during) and treatment (control vs. introduction) factors would indicate that the control and introduction sites responded differently to the experimental manipulation. We blocked this analysis by introduction to account for the substantial environmental variability in our system.

Do rainbow trout produce microhabitat displacement in native fishes?

If the introduction of rainbow trout produced a microhabitat shift in native fishes' microhabitat use, we would expect the distance between trout use and fish microhabitat use during the introduction to be greater than that between trout use and the fish use before the introduction (Figure 2.3). We will refer to this shift as "displacement." To test the hypothesis that native fishes were displaced by trout, we performed a paired t-test on the distances before and during each introduction ($\alpha = 0.10$). We used a one-tailed test, because our alternative hypothesis is that native fishes shift away from trout. Because some of the species (yellowfin shiner, Tennessee shiner, central stoneroller) were only present in sufficient numbers for analysis in 2 or 3 introductions, we first classified species with respect to their microhabitat guild (Grossman et al. 1998), e.g., upper water-column, lower water-column, and benthic, and analyzed displacement with respect to these guilds.

Do rainbow trout produce shifts in microhabitat use by native fishes?

We tested for shifts in microhabitat use by native species by plotting 90% confidence ellipses around the mean scores for the samples of interest (i.e. microhabitat availability, microhabitat use, etc.) for each pair of components (Grossman and Sostoa 1994).

We analyzed these ellipses to test for displacement in more detail. If native fishes were avoiding rainbow trout, their microhabitat use ellipses would shift away from those for the rainbow trout during the introduction segment of experiments. If there were no differences in microhabitat use by native fishes, then the use ellipses for both periods would overlap in both the control and treatment sites. This "no effect" condition is shown in Figure 2.5.

If, however, a native species displayed a strong avoidance response to rainbow trout, then microhabitat use in the introduction site would differ significantly between before and introduction periods in the treatment site but not in the control site and the use ellipses in the treatment site would not overlap. We termed this response a "Type I" effect (Figure 2.6).

Some species exhibited variable responses to rainbow trout, perhaps due to variability in the response of individual fish or because responses were short-term or variable in time. In these cases, we expected microhabitat use to remain constant in the control site but observed a shift away from trout in the treatment site that was not always significant. We termed this a “Type II” response (Figure 2.7). This test is more sophisticated than the ANOVA and t-tests employed, above, on the selectivity and displacement because those tests consider only the distances between the PCA means for availability and use (selectivity) or between means at different times (displacement.) By analyzing each species individually, by introduction, this test includes both the direction of any absolute shift and also any increase in the variance around the fish use means.

In some instances, the relationship of fish microhabitat use to habitat availability was different in the treatment site than the control, throughout, or shifted in a manner inconsistent with avoidance, and we evaluated these plots on a case by case basis and generally scored them as “uninterpretable.”

Persistence of Trout Avoidance Effects

We tested the hypothesis that native fish returned to their pre-introduction microhabitat use pattern after the removal of the block-nets by performing two additional sets of PCA analyses. The first set compared the “during” and “after” segments and the second compared “before” and “after” samples. The first comparison tested whether native fishes altered their microhabitat use after trout dispersed when the block–nets were taken down, and the second tested whether this response differed from microhabitat use displayed in the “before” period. If a native fish species avoided the stocked rainbow trout during the introduction and completely returned to their pre-introduction microhabitat use after the removal of the block-nets, we would expect the plots of the before-after data to show a near-total overlap of the native fish microhabitat use ellipses in the introduction site for species receiving a Type I or Type II designation, above. In these cases, we assigned a score of “fully recovered” (Figure 2.8.)

If a native species had been avoiding the stocked trout during the introduction and these effects declined but did not disappear after the removal of the block-nets, we would expect microhabitat use by native fishes in the “after” period to be intermediate between the “during” and “before” patterns and, when this occurred, we assigned a score of “partially recovered.” If a native species avoided stocked rainbow trout during the introduction and these effects persisted after the removal of the block-nets, we would expect no shift in fish microhabitat use ellipse between the “during” and “after” data. In these cases, we assigned a score of “no recovery.”

Whether as a result of dispersal or removal, we observed far fewer stocked rainbow trout in the period after the removal of the block-nets, so we did not include the use ellipses for trout on the PCA plots used to assess recovery. However, we did record microhabitat use data for the rainbow trout we observed during the snorkel passes in the “after” segments. To test the hypothesis that the persistence of microhabitat shifts was proportional to the number of remaining rainbow trout, we calculated a rough index of trout presence by dividing the number of rainbow trout we observed by the number of snorkel passes we made in the “after” segment. We categorized these as “low” (fewer than one rainbow trout observed, per pass), “medium” (1-3 observed, per pass) and “high” (greater than 3 observed, per pass.) We used a chi-squared analysis to test whether the distribution of cases in the matrix of effect persistence versus trout density was different from random.

RESULTS

Microhabitat Availability and Site comparability

Available habitat varied during our study period, but the control and introduction sites were indistinguishable at the 90% level of significance in 10 of the 15 samples (Figure 2.9). These data indicate that comparisons between the control and introduction sites are generally valid and the two pairs replicate each other sufficiently that we will not distinguish between observations made at the upper and lower site pair in the following discussions.

The variability in the habitat availability data may be explained, in large part, by the drought that began in 2006. Flows between summer 2004 and winter 2006 were relatively higher than the long-term seasonal mean, whereas flows after winter 2007 were lower than the long term means (Figure 2.10.)

Species richness, diversity, population variation, and biomass

Species richness and species diversity (Shannon's H) were variable during the study, although similar levels of variability were observed among sites (Figure 2.11, Figure 2.12). Species richness ranged from five, at the upper Patterson Gap site in Spring 2006, to 19 at the lower Patterson Gap site in Spring 2008, with a mean species count of 12.3 (SD = 3.6). Diversity varied with species richness and ranged from 0.62 to 2.00 with a mean value of 1.30 (SD = 0.42).

Both variables show parallel levels of variation within the site pairs, indicating that our site pairings are valid.

Total fish abundance (Figure 2.13) and total fish biomass (Figure 2.14) also were highly variable, and reflect the relative size difference of the two site pairs. The downstream sites (Wilson and Darnell) probably supported higher fish abundance and biomass because they were physically larger, averaging 8.66m (SD 0.44) and 8.81m (SD 1.23) in width, compared to 7.64m (SD 0.79) at Upper Patterson Gap and 7.97m (SD 0.80) at Lower Patterson Gap during our study.

The last data value (spring 2008) for the Darnell site reflects an additional estimated 4,750 grams of fish biomass from six large rainbow trout which we had not observed in the site on the previous observation period three days earlier. These fish were stocked by a nearby landowner approximately two days before our sample, after the conclusion of our microhabitat observations, and had moved upstream into our site (Stanley Darnell, pers. comm.)

Long-term assemblage level effects

The introduction of rainbow trout did not produce long – term shifts in assemblage structure because none of the Time X Treatment interaction terms were significant for species richness, diversity, abundance, or biomass in the sites

Microhabitat use

Although there were variations reflecting seasonal or episodic changes in habitat availability (see the PCA plots for individual introductions, below), the broad patterns of microhabitat use by native fishes in our sites were consistent. Minnows of the mid-water column guild occupied deep microhabitats relatively far from shelter over depositional substrata and their 90% confidence ellipses often overlapped (Figure 2.15). By contrast, benthic guild members were closer to both the bottom and shelter, in microhabitats that were faster, shallower, and with greater amounts of erosional substrata than either mid-or lower water column guild members. We observed the two members of the lower water-column guild in microhabitats with intermediate characteristics. We observed warpaint shiners in microhabitats most similar to those occupied by rainbow trout during the introduction.

Principal Components for single-species analyses

Although there was some variation among the individual species and introductions, ecological interpretation of the first two PCs generally was consistent. The first PC typically reflected a gradient between negative loadings for percent silt, sand, and debris and positive loadings for mean velocity and percent gravel and cobble. We interpret this axis as describing the range of velocities and substratum from low-velocity microhabitats dominated by depositional substrata and high-velocity habitats dominated by erosional substrata. The second PC consistently had positive loadings for depth and percent boulder. This gradient describes the pool-like microhabitats versus shallow microhabitats with smaller substrata.

Effects of Rainbow Trout on Microhabitat Selection

Rainbow trout produced a shift in the microhabitat selectivity (distance between availability and use means on PC1 and PC2, across all species in all introductions) displayed by fishes in the control and introduction sites (Figure 2.16). The ANOVA yielded significant terms for introduction (blocking) (6 df, $F = 12.92$, $p < 0.01$), treatment (1 df, $F = 12.52$, $p < 0.01$) and the time X treatment interaction term (1 df, $F = 3.35$, $p = 0.07$).

This interaction appears as a significant reduction in microhabitat selectivity by native fish during the introduction in treatment sites. Based on observations, this likely is due to avoidance of the trout by native fishes that resulted in a scattering pattern of reduced selectivity. The paired t-tests on this displacement, by guild (Table 2.2), show that both the upper water-column (17 df, $T = -2.14$, $p = 0.024$) and lower water-column guilds shifted away from rainbow trout (8 df, $T = -2.08$, $p = 0.035$) during the introductions.

Both pooled and guild-based microhabitat selection results confirm that native fishes shifted microhabitat use in the presence of rainbow trout. Nonetheless, these patterns simply indicate a general response and specific responses of individual species follow.

Responses of Individual Species

We quantified the dynamics of these shifts by analyzing PCA data for each of the 39 instances where a native species was sufficiently common for us to make more than 8 observations at both the control and introduction sites in the before and during time segments. We observed several types of responses, including shifts from non-random to random use, shifts away from the trout into faster or slower microhabitats, and frequent increases in the variability of the microhabitats occupied by native fishes when the rainbow trout were present. In total, we assign 4 Type I responses, which are characterized by significant (at the 90% level) changes in microhabitat use by native fishes consistent with trout avoidance. We observed another 11 Type II responses that were consistent in their interpretations with the Type I responses, but less pronounced.

Type I Avoidance Responses

We observed four Type I responses that were broadly consistent with the results of the t-tests described above. Three of four Type I responses represented a decrease in microhabitat selection by minnows in the mid-water column guild (species), either as a result of a positional shift of the ellipse or an increase in its variance (i.e., a change in ellipse shape). In introduction #7, the ellipse for rosyside dace in the "before" period overlaps the location of the rainbow trout ellipse in the "during" period (i.e. microhabitat use for these two species are statistically indistinguishable in these periods). However, during this period, rosyside dace shifted microhabitat use down to significantly shallower microhabitats with greater amounts of erosional substrata (Figure 2.17).

Warpaint shiners displayed similar responses in introduction #6, i.e. warpaint shiners occupied the same microhabitat in the before period as trout in the "during" period then responded to the rainbow trout stocking by dispersing into a wider variety of microhabitats (indicated by the width of the use oval during the introduction) that were slightly shallower and characterized by more depositional substrata, particularly silt and debris (Figure 2.18).

In introduction #8, warpaint shiners initially occupied microhabitats that were deeper, slower, and characterized by substrata that were more depositional (more sand and less cobble) than random but shifted significantly back to random use in the “during” period (Figure 2.19).

Central stonerollers responded to rainbow trout in experiment 6 by shifting from selection for deep microhabitats with high boulder cover to random use in shallower microhabitats with more variable substrata, a usage pattern that became indistinguishable from random (Figure 2.20).

Mottled sculpin in the introduction displayed two significant shifts in the “during” period (introductions #5 and #7). However, microhabitat use by the rainbow trout did not overlap with the sculpins’ pre-introduction use, and the observed shifts were not away from the trout, so we do not classify these as Type I responses.

Type II Avoidance Responses

The most common response observed was a Type II response, in which the species were displaced from microhabitats occupied by rainbow trout but these shifts were not significant. The most pronounced Type II effects occurred when the microhabitat use ellipse for the rainbow trout overlapped the “before” use ellipse for the native fish.

For example, in introduction #5, warpaint shiner shifted away from pool microhabitats which were the microhabitats occupied by rainbow trout, and toward shallower microhabitats characterized by more depositional substrata. In addition, warpaint shiners occurred over more variable substrata when rainbow trout were present (Figure 2.21).

Yellowfin shiners in introduction #6 displayed similar responses to warpaint shiners. In the presence of rainbow trout, yellowfin shiners shifted out of deeper areas and these microhabitats were occupied by rainbow trout during the trout segment. Yellowfin shiners showed greater variability in microhabitat use when trout were present, and these sites had higher current velocities (Figure 2.22).

Although there are only two members of the lower water column guild (river chub and central stoneroller), they displayed six of ten Type II responses. These responses were similar to those displayed in Type I responses but increased variability on the substrate axis (PC1) causes any shift in the mean use during the introduction to appear non-significant. The general pattern was a shift away from microhabitats occupied by rainbow trout, with the new microhabitats being shallower and sometimes having a more variable substratum (e.g., some w/more depositional substrata and others with more erosional substrata.) All four of the responses by river chub (introductions #6, 7, 8, and 9) and one of the two responses by central stoneroller (introduction #8) are shown in Appendix 2.1: Single Species PCA Biplots.

Benthic guild members (mottled sculpin and gilt darters) did not display any Type 1 responses and only exhibited two Type II responses. Mottled sculpins in introduction #8 shifted into habitats that were less pool-like with more erosional substrates when the trout were present in the introduction site. This shift is consistent with the interpretation that the sculpins were minimizing their microhabitat overlap with the trout, although this is the by far the strongest displacement toward erosional microhabitat of any of the benthic species we observed.

Gilt darters in introduction #8 showed a response similar to those described for the water-column fishes, above; when rainbow trout were present, we observed gilt darters in less pool-like microhabitats that were slower, shallower, sandier, and more variable. Their selection of these microhabitats is consistent with minimizing overlap with the rainbow trout.

No Avoidance Response or Uninterpretable

The majority of responses by native fishes (23 of 39) were uninterpretable or showed no response to rainbow trout. Sixteen cases (species) showed similar patterns of microhabitat use in “before” and “during” segments of experiments in both control and treatment sites. In two of the remaining seven plots (rosyside dace in introduction #2, yellowfin shiner in introduction #8), there is a difference between control and treatment sites, but the shift occurs in the control site alone and cannot be attributed to the presence of rainbow trout. There were three cases (yellowfin shiner, warpaint shiner, and rosyside dace, all in introduction #9) in which microhabitat use by the native fish always appeared random in the treatment site, and hence the results are uninterpretable. Finally, there are two cases (warpaint shiner, introductions #2 and

#3) in which we observe a Type II response in the introduction site, but the pre-introduction use, relative to availability, is different between the control and introduction sites, so we do not feel that the experimental control is adequate to assign a response to these plots. These responses are summarized in Table 2.3.

It is not surprising that the mid water-column guild, containing the species whose microhabitat most closely matches that of rainbow trout (Figure 2.15), also showed the strongest responses to trout introductions, displaying 3 of the 4 Type I avoidance responses and 4 of the 11 Type II responses (with two other responses suggesting similar effects in the introduction sites that are confounded by changes in the control sites.) However, when viewed as guilds (Table 2.4) the fishes of the lower water-column guild appear to be the most commonly affected by the introduced rainbow trout, displaying 6 Type I or Type II responses out of 9 analyses.

Persistence of Type I and Type II effects

Comparisons of microhabitat use between the “during” and “after” segments of the experiment enabled us to test for the persistence of both Type I and Type II responses. We observed two full recoveries for Type I responses, rosyside dace in introduction #7 (Figure 2.23) and central stoneroller in introduction #6, however the remaining two Type I responses showed no recovery (warpaint shiners, introductions #6 (Figure 2.24) and #8. Among the type II responses, we observed six full recoveries (Warpaint shiners in introductions #5 and #7, Tennessee shiners in introduction #7, river chub in introduction #7, and gilt darter in introduction #8.

We observed partial recoveries, in which either the position of the “after” use ellipse returned to overlap the “before” use ellipse and increased variance in the “during” use ellipse declined in the “after” ellipse, but not both. These partial recoveries were yellowfin shiner and river chub in introduction #6, mottled sculpin in introduction #8, and river chub in introduction #9.

Finally, there were two instances in which an altered pattern of microhabitat use observed in the “during” segment persisted unchanged into the “after” segment, central stoneroller and river chub in introduction #8.

There were two instances in which we observed less than 1 trout, per snorkel pass, in the “after” segment, three instances in which we observed between 1 and 3 rainbow trout, per pass, and two instances in which we observed more than 3 rainbow trout, per pass (Table 2.5). Breaking down the persistence of rainbow trout effects by the index of trout density, three of the four instances in which the trout effects remained after the introduction period occurred when rainbow trout density was high (>3 stocked trout observed, per snorkel pass), while five of the eight instances of a full recovery occurred when trout persistence was low (<1 rainbow trout observed, per pass) (**Figure** 2.25). A chi-square analysis of these proportions shows that these proportions are not significantly different from the random expectation in a 3x3 matrix of the trout persistence and recovery categories (Chi-Square = 6.0000, df 4, p = 0.200) and Fisher’s Exact Test, due to the small sample size, also indicates no significant relationship between effect persistence and trout density (p = 0.334).

DISCUSSION

Our experiments were conducted during a period in which flows and associated environmental parameters varied due to a severe drought that began in 2006. Nonetheless, the introduction of trout did not produce any identifiable effects on assemblage structure. Viewed in the context of the variability presumably introduced by flow, the diversity and biomass measures indicate that overall fish abundances in our sites were stable, or declined slightly, over the course of our study, while the assemblage was variable in both the control and introduction sites, which suggests that the driver was probably environmental variability and not trout stocking. This may not be surprising given that the introduction and retention of trout necessitated the use of block-nets, which also would have restricted the emigration of affected native fishes. In addition, the maintenance of these block-nets required that our experiments be short-term.

In contrast to assemblage-level parameters, the introduction of rainbow trout did produce microhabitat shifts by a number of native fishes, including warpaint shiners, rosyside dace, Tennessee shiners, yellowfin shiners, central stonerollers, river chubs, gilt darters, and mottled sculpin. In general, members of the middle water-column guild scattered out of the deep, fast sections of pools and into more variable microhabitats that tended to be shallower, slower, and with more depositional substrata, although these responses were variable. Fishes of the lower water-column guild were similarly displaced and occupied more variable microhabitats when rainbow trout were present in high densities, whereas benthic species occasionally moved into habitats with lower mean flows during the introductions.

Some of the variability in these responses may be attributable to the effects of the block-nets. For instance, in introduction #8 (Figure 2.19), our field notes from these dates indicate that we observed few warpaint shiners in the deep pool near the downstream end of the treatment site, where they had frequently been observed before the introduction and where many of the trout quickly collected after the stocking. During this introduction, we observed more warpaint shiners in the shallower glide habitat just upstream of the pool, consistent with avoiding the trout. However, a brief rainfall just after the imposition of the block-nets, coupled with low antecedent flow and the accumulation of leaf litter on the stream banks resulted in some restriction of flow through the lower block-net. This created an area of relatively homogeneous habitat between a riffle at the top end and the pool at the bottom, which may explain the seemingly random distribution of the shiners during the introduction.

More noticeably, the responses of mottled sculpin may be attributable to some combination of trout and net effects. While the Type II use displacement in introduction #8 is clearly away from the trout, and almost sufficient to earn a Type I designation, the introduction site displacements in introductions #5 and #7 are toward random use and not away from the trout (Figure 2.26). While this could be a scattering response, it was not uncommon during fall samples for some small sculpin to disperse into flooded rock bars toward the bottom of the sites that were created as our block-nets began to fill with leaf litter. A similar shift occurs in the control site during introduction #2, which supports our interpretation that this is an effect of the block-nets and not of the trout.

It is unlikely that fishes in the other two feeding guilds were similarly affected by the slight changes in the available microhabitat immediately upstream of our block-nets. We positioned our sites between natural barriers, either bedrock outcrops or riffles, and the block-nets were typically positioned 2-3 meters downstream of the site boundary and above such a barrier. Most of the habitat affected by the nets was riffle or bare bedrock and outside of our survey area. However, the effect of the nets may have propagated upstream into the lowest riffles in our survey areas during the fall samples, when heavy leaf fall occasionally made it difficult for us to keep the block-nets clear. Sculpins were the only species we consistently observed in these shallow riffle areas, so we feel they are likely to be the only species affected by the net effects.

In calculating the persistence of rainbow trout during the “after” segment, the total number of observations probably overestimates the number of trout present in the site because we almost certainly observed the same individuals on different days. Although we could not establish the fate of all the trout we introduced, Ms. Sara Smiley completed a Senior Thesis project in our lab by tagging the 25 trout used for one introduction and attempting to recapture them at the conclusion of the “after” snorkeling observations by electro-shocking the site and two sections spanning 25 meters above and below. Of the 25 tagged rainbow trout released, she recaptured 10 over the course of three days of shocking, only two of which were still within the site boundaries (Smiley 2006).

It was not a goal of our experiment to assess the decay of these rainbow trout effects, so we did not continue snorkeling until the stocked trout or their effects on the native fishes disappeared.

However, we believe these results indicate that the effects we observed during our 48-hour introduction are likely to persist at trout densities well below those present during our experimental manipulation (25 trout / 50 m segment.) Our sample size was insufficient to demonstrate this statistically but our analysis suggests that the density of remaining trout may be a factor in the persistence of these effects, particularly among the fishes in the mid-water feeding guild, whose microhabitat preferences apparently make them more likely to be displaced by rainbow trout. In what amounts to a reciprocal experiment to ours, Penaluna et al. (2009) use a BACI design and selective removal of introduced rainbow and brown trout from a river in Chilean Patagonia. They observed an expansion in the mesohabitat usage of three native species (two Galaxiids and a Trichomyterid) in the reaches with reduced trout density, indicating that trout reduce the habitats used by the native fishes through competition or interactive segregation, even though trout stocking in these rivers was initiated in the early 1900s and the trout populations are naturalized.

These results contrast with several studies of rainbow trout and rosyside dace, one of the species in our assemblage, which demonstrated little evidence of competition in experimental streams (Grossman and Boulé 1991, Rincón and Grossman 1998) or under field conditions near Betty Creek (Grossman and Freeman 1987, Grossman and Ratajczak 1998). However, these differences may be a result of differential responses to small and large trout (McIntosh et al. 1994). In the previous work on rainbow trout and dace, the rainbow trout were naturalized and typically much smaller than the hatchery fish we stocked, and the dace were collected from streams containing trout. We did see some evidence of trout recruitment in Betty Creek, namely parr and rare juveniles ranging from 10-15cm, and in most cases these small fish occupied either small pockets along the edges of riffle habitat (parr, presumably YOY) or in mixed-species schools with minnows in pool habitat, much as described by Grossman and Freeman (1987) and Grossman and Ratajczak (1998). However, we never saw these naturalized rainbow trout engage in any agonistic behaviors, either intra- or inter-specific, which we observed nearly continuously among the larger stocked rainbow trout. The stocked fish were very active, particularly in pool habitats, and we observed them chasing each other and aggressively feeding on large bits of leaves and detritus in the drift. This behavior is not inconsistent with reports of naïve feeding behavior and enhanced aggression in rainbow trout (Berejikian et al. 1996, Marchetti and Nevitt 2003) or other cultured salmonids (e.g., Mesa 1991).

It should be noted that we did not observe direct predation in the field and we found no fish in the gut contents of three of the large recently-stocked rainbow trout we collected in the spring 2008 electrofishing sample at the Darnell site. (We investigated this stocking and were told by a neighboring landowner that no other private stocking had occurred adjacent to that site during our experiment (Stanley Darnell, pers. comm.).

Combined, these results indicate that rainbow trout affect habitat use of native fishes of all three microhabitat guilds, although responses of the upper-water-column guild members showed both the greatest overlap and response to rainbow trout. These effects may be ameliorated in the field by the greater variety of habitat available, though forcing fish into lower velocities may reduce feeding opportunities further by reducing exposure to drift (Hill and Grossman 1993).

The effects we observed were immediate and short-term (48 hours post rainbow trout introduction). These effects seemed to persist even as the number of trout declined following our introductions, though displaced native fishes frequently did return to their pre-introduction pattern of microhabitat usage at the lowest rainbow trout densities. When there are repeated stocking events, native fish may adapt to the presence of rainbow trout in the longer term through microhabitat segregation (Kruse 1999, Penaluna et al. 2009), albeit possibly at the cost of reduced fitness (Flecker and Townsend 1994, McDowall 2003, Simon and Townsend 2003, Townsend 2003, McDowall 2006, Zimmerman and Vondracek 2006). Under conditions of naturalization and invasion, the food web effects of introduced salmonids may also be severe (Townsend 1996, McDowall 2003, Simon and Townsend 2003, Baxter et al. 2004, McDowall 2006).

Nevertheless, we urge caution when considering whether to stock rainbow trout into a stream containing vulnerable native species. Our results for warpaint shiners may reasonably be extended to threatened drift-feeding minnows with similar ecologies which occupy streams that could also support rainbow trout. Furthermore, our results indicate that species such as river chub and stonerollers altered their habitat use when rainbow trout were present, which suggests that these results may be extensible to threatened epibenthic species, as well

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Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management **22**:82-90

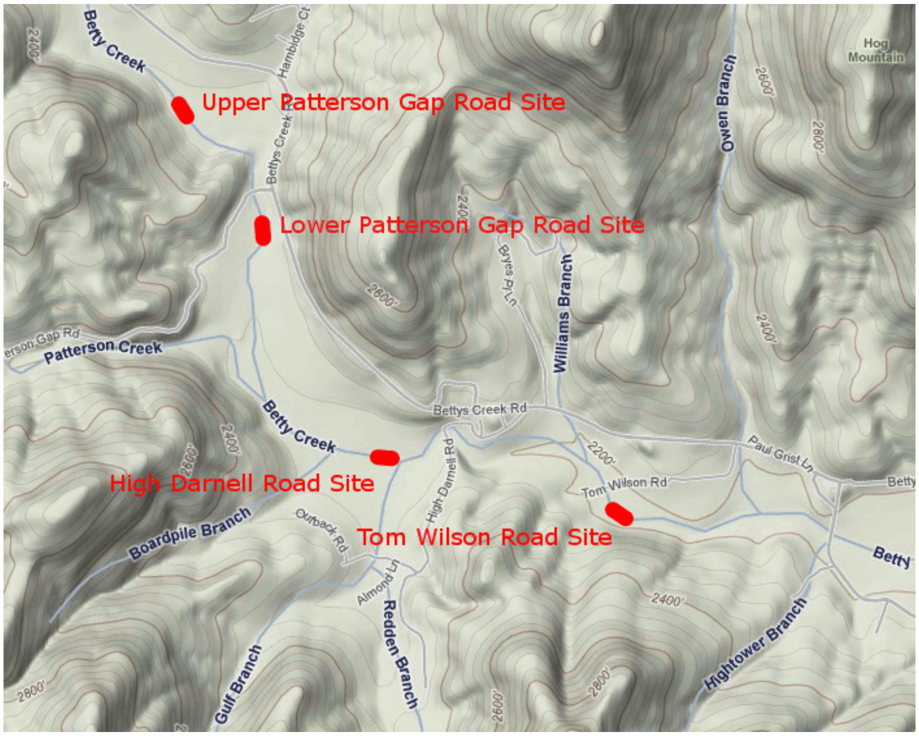


Figure 2.1: Map of the Study sites, near Dillard, GA.

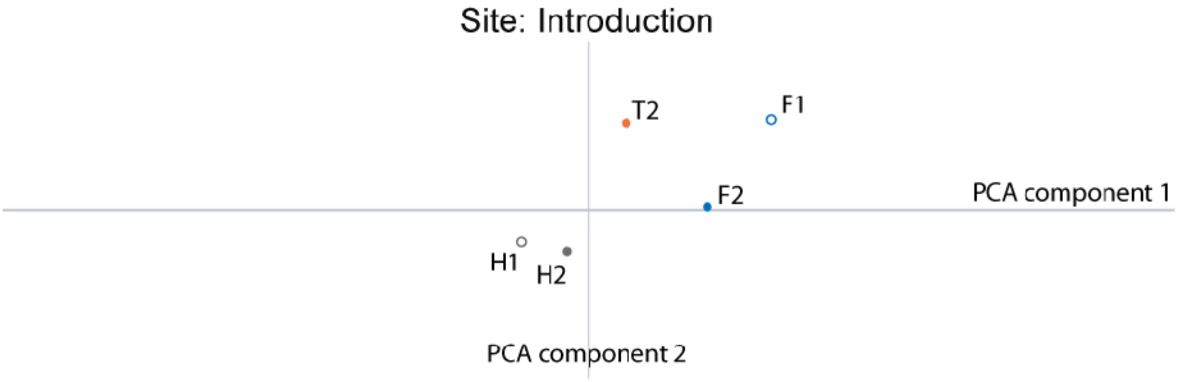


Figure 2.2: Example two-dimensional PCA plot. Points represent means for microhabitat availability within the stream before (H1) and during (H2) the introduction and native fish microhabitat use during the same two periods (F1, F2), and rainbow trout use during the introduction (T2).

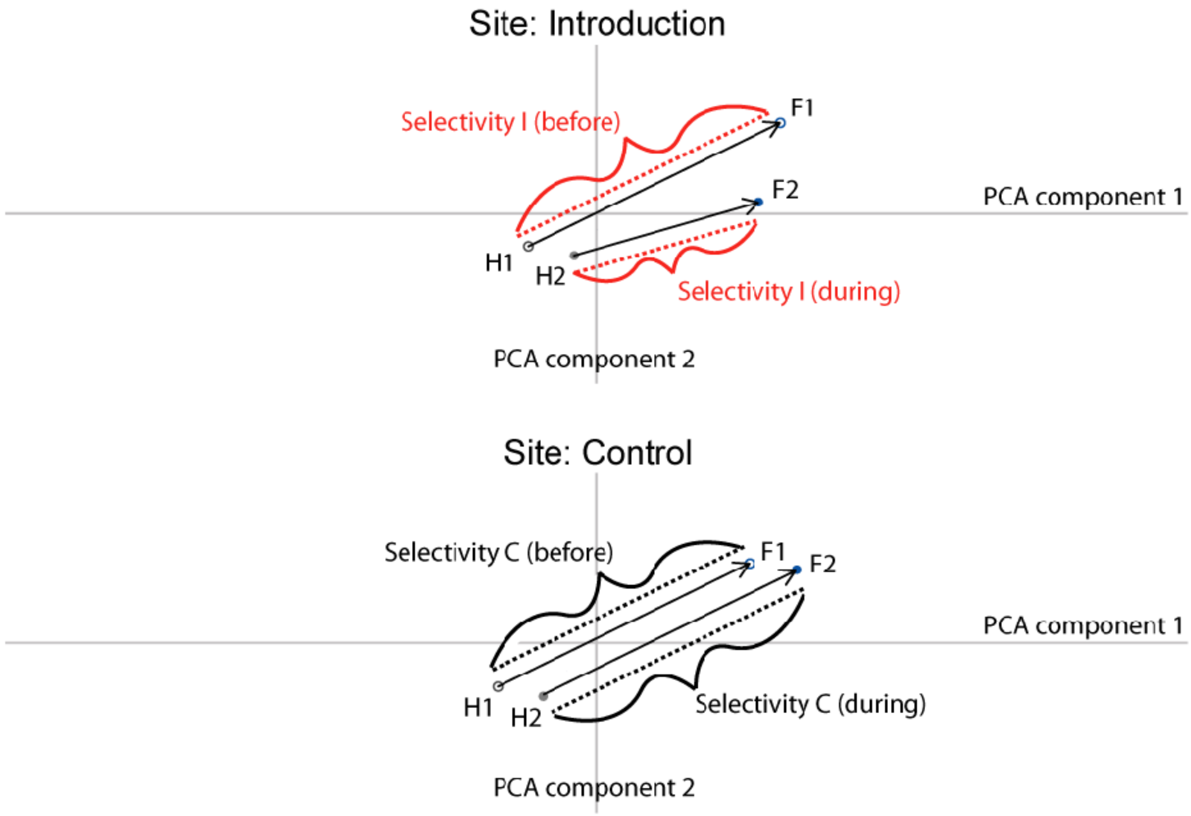


Figure 2.3: Vectors describing the selectivity of native fish microhabitat use. In this idealized presentation, the native fishes' microhabitat selectivity is reduced when trout are present in the introduction site, while selectivity at the control site is unchanged. Point labels are as described for Figure 2.

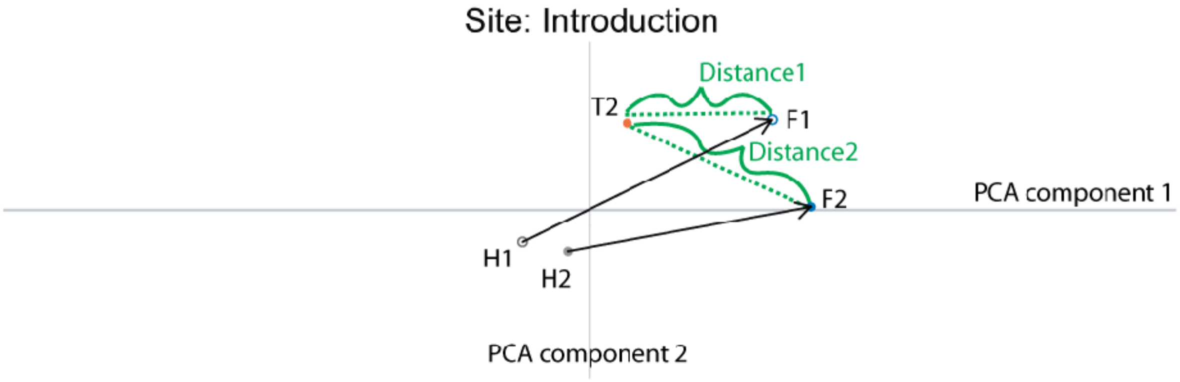


Figure 2.4: Expected avoidance response in native fishes. Avoidance of the rainbow trout would be reflected in an increase in the distance between the native fish use and trout use between the observation periods, or Distance 1 < Distance 2. Point labels are as described for Figure 2.2.

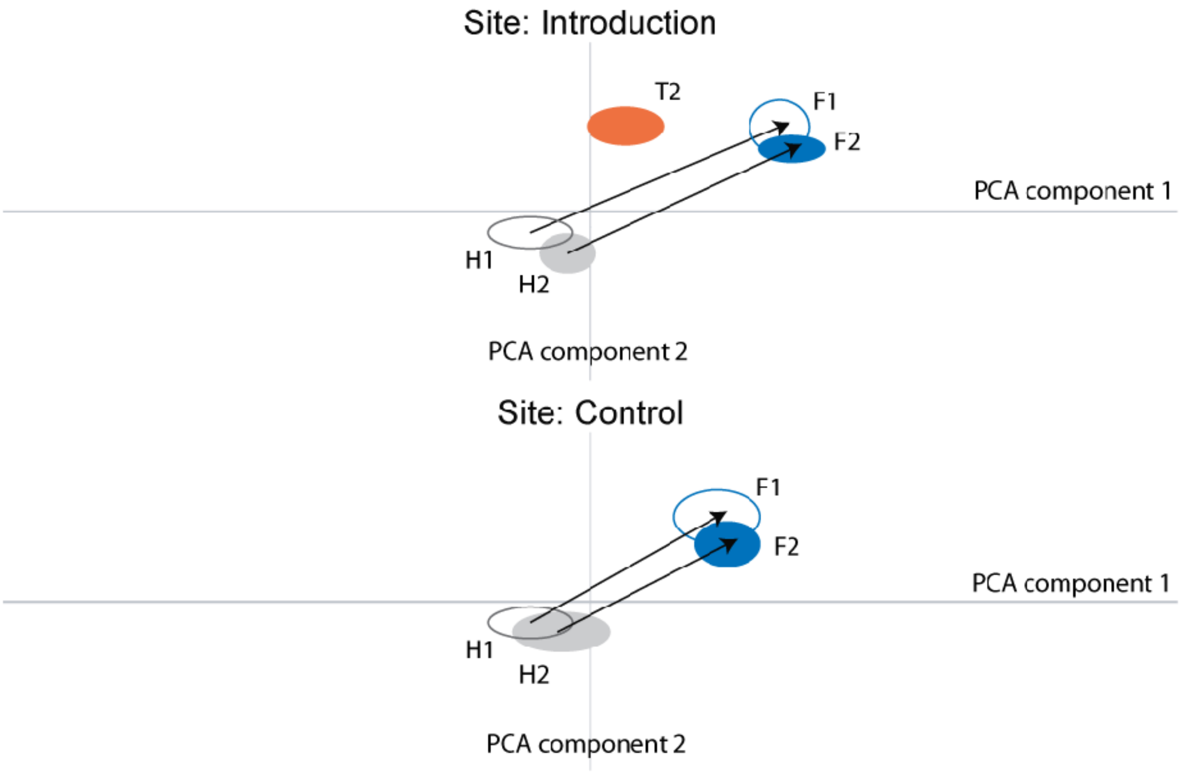


Figure 2.5: Idealized PCA plot showing no response the introduction of rainbow trout. Ellipses represent 90% confidence intervals around the mean values for an observation set and overlapping ovals represent means what are not statistically different. Labels are as described for Figure 1.

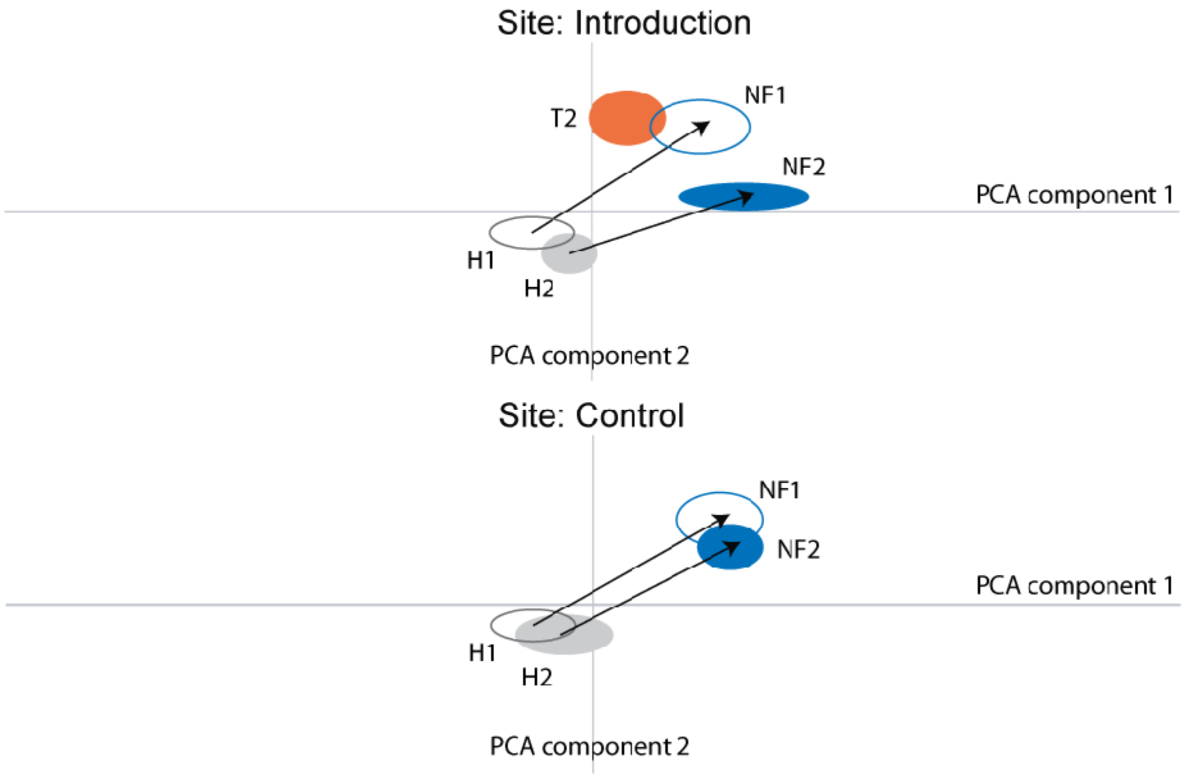
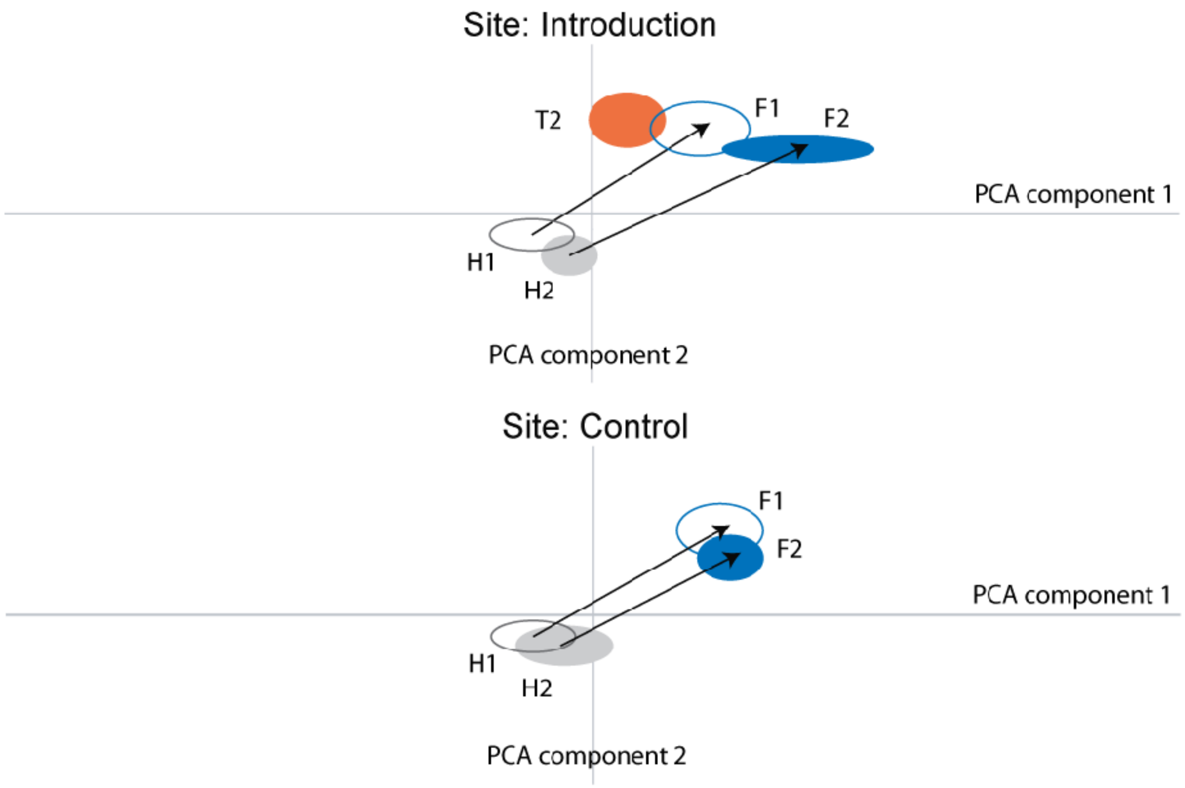


Figure 2.6: Idealized PCA plot of a Type I effect. The two microhabitat use ellipses for the native fish in the impact site do not overlap, the position of the habitat availability ellipses and use ellipses in the control site are consistent, and the displacement of the introduction ellipses is consistent with avoiding overlap with the rainbow trout use ellipses. Labels as described for Figure 2.2.



Site: Introduction

Site: Control

Figure 2.7: Idealized PCA plot showing a Type II effect. Native fish microhabitat use in the introduction site is displaced during the introduction, consistent with native fishes' avoidance of introduced rainbow trout, but overlapping ellipses indicate that this shift is non-significant at the 90% level. Labels as described in Figure 2.2.

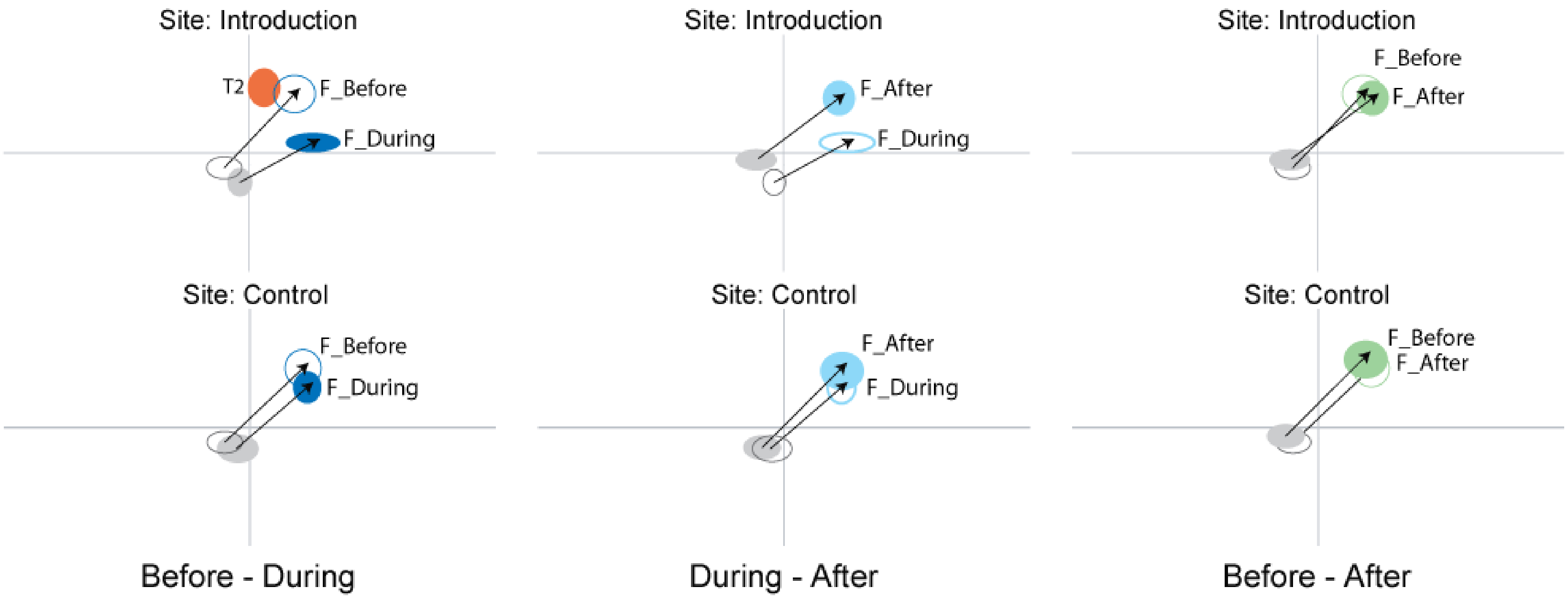


Figure 2.8: Idealized PCA plots showing Type I displacement effect and full recovery. Labels as described in Figure 2.2

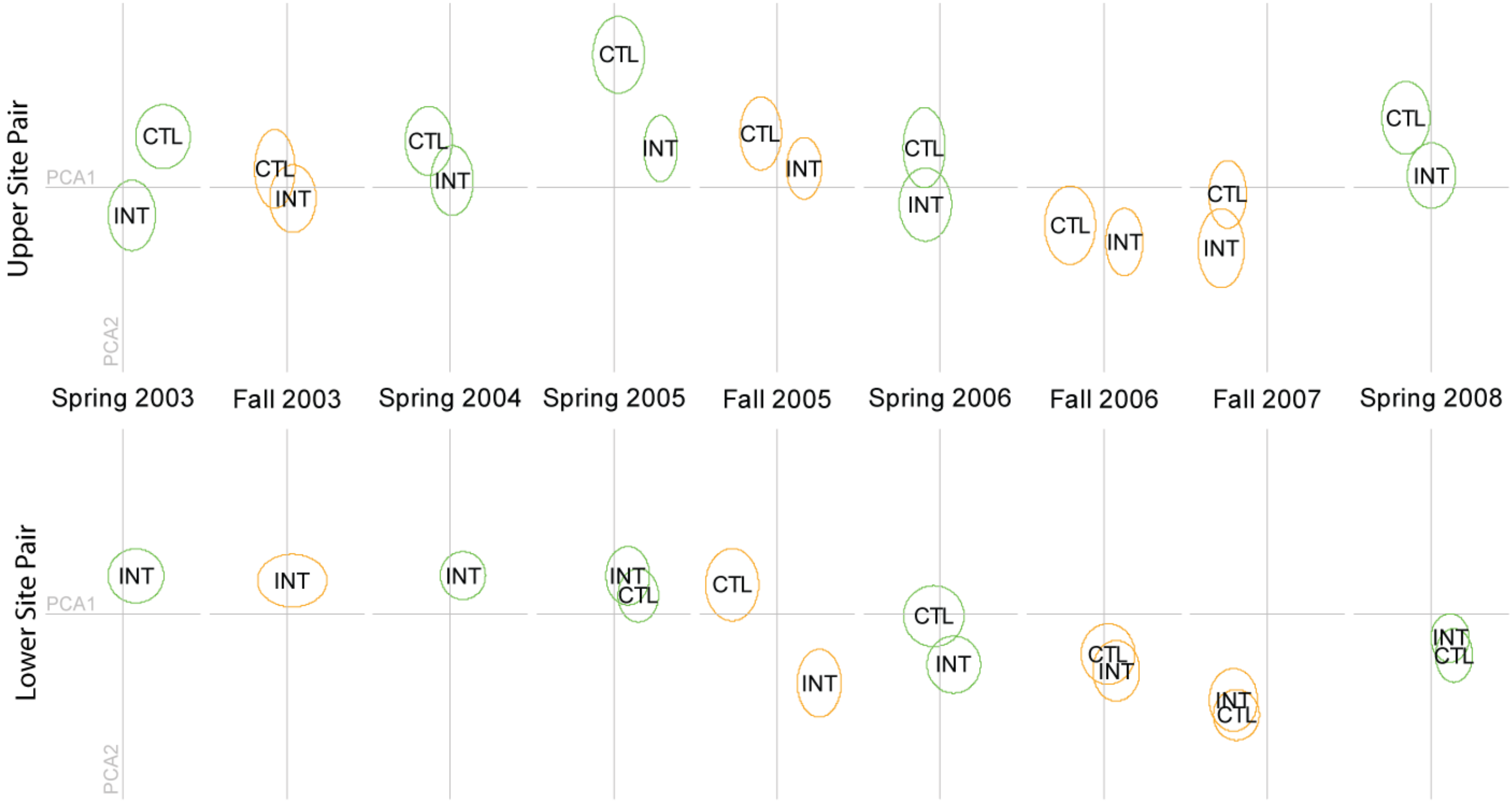


Figure 2.9: PCA plot of random microhabitat availability through time at both site pairs. Ellipses represent 90% confidence intervals around the means for PC1 and PC2. For all plots, the loadings for the first PCA component were (mean flow 0.78, %cobble 0.75, %gravel 0.53, %silt -0.65, %sand -0.58, with an eigenvalue of 2.44, 27.2% variance explained) and the loadings for the second PCA component were (depth 0.68, %boulder 0.44, %cobble -0.48, with an eigenvalue of 1.42, 15.8% variance explained). Ellipses labeled INT represent observations in the Introduction sites and those labeled CTL represent observations from the control sites.

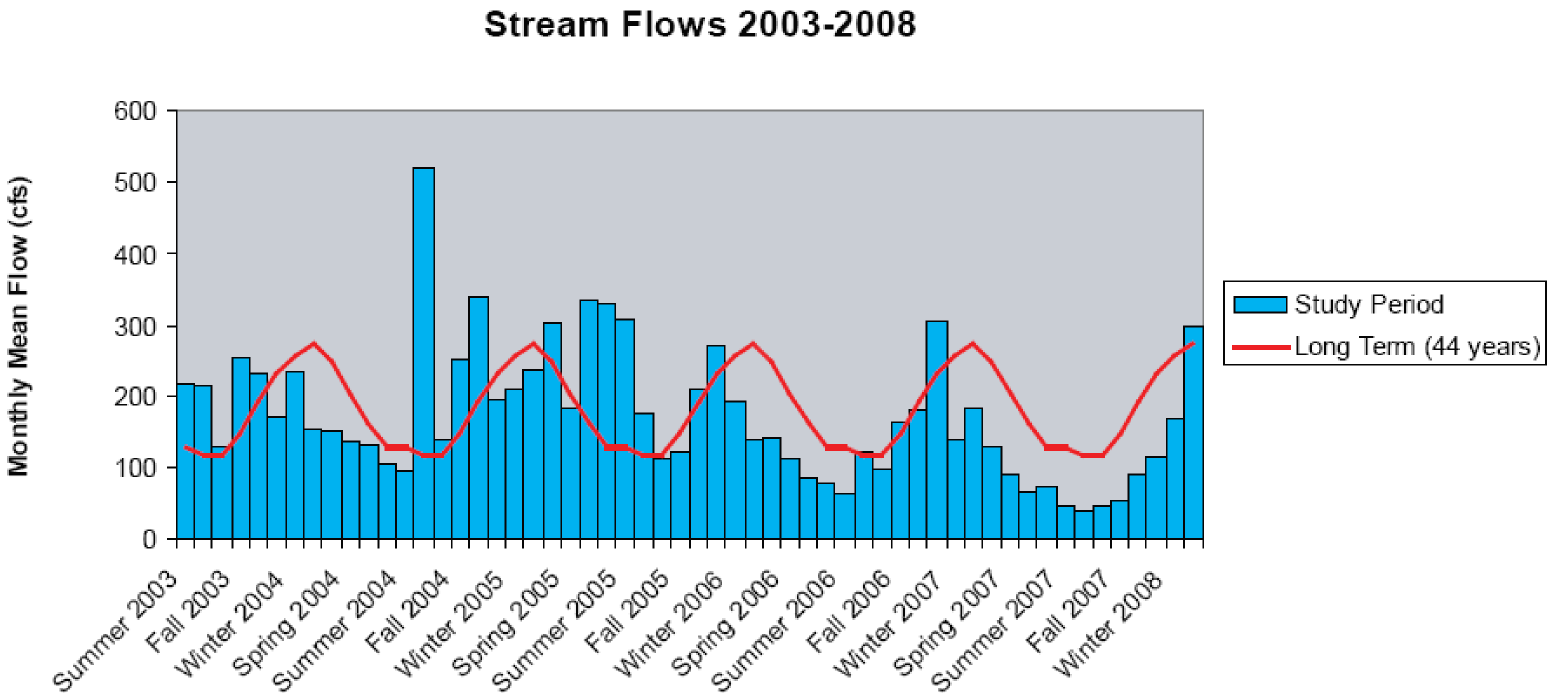


Figure 2.10: Seasonal Stream Flows. 90-day mean and long-term seasonal mean flows at USGS gage #02178400 for the Tallulah River at Clayton for the study period.

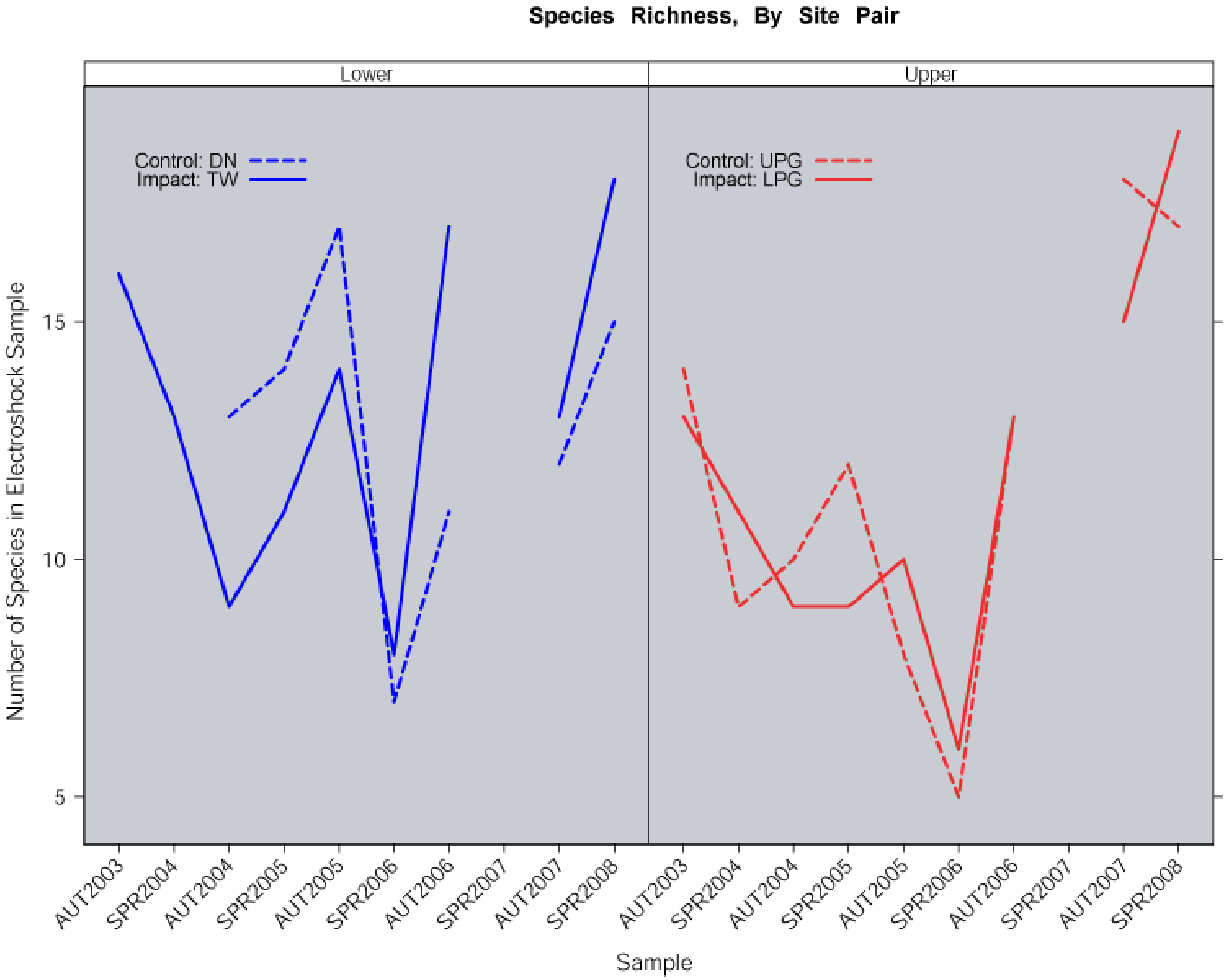


Figure 2.11: Species Richness for each electrofishing sample.

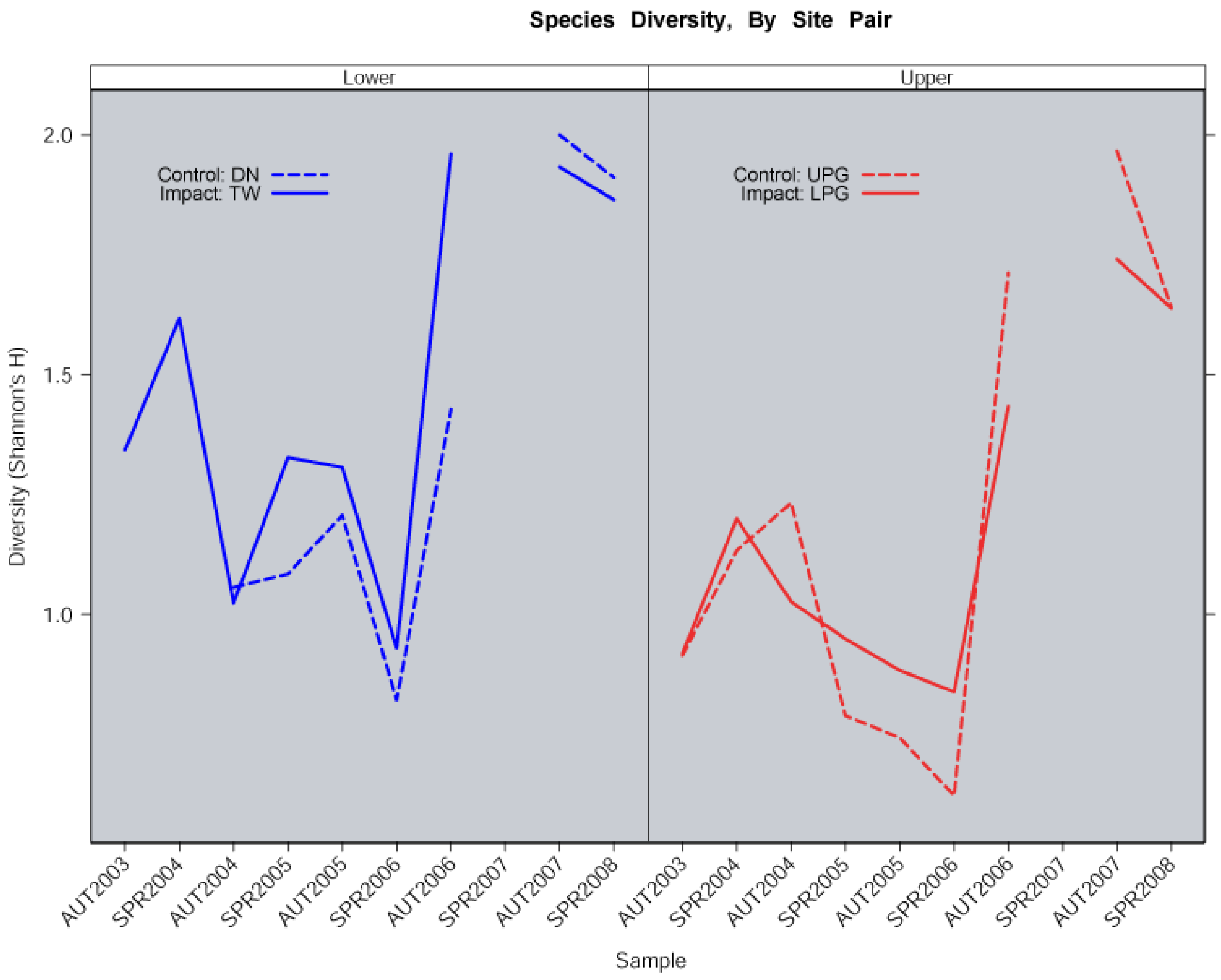


Figure 2.12: Species diversity (Shannon's H) for each electrofishing sample

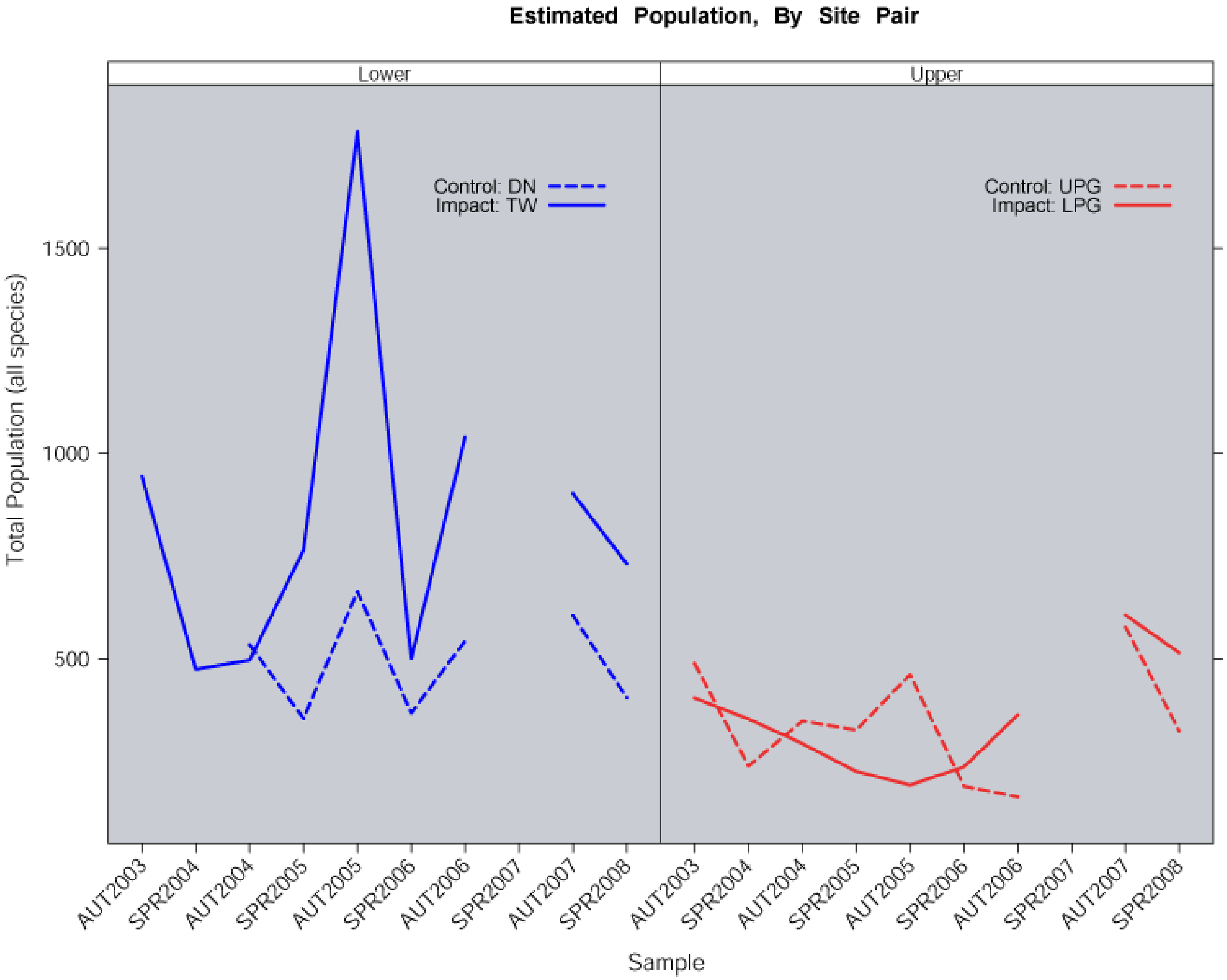


Figure 2.13: Total fish abundance estimates for each electrofishing sample, derived from multi-pass depletion calculations.

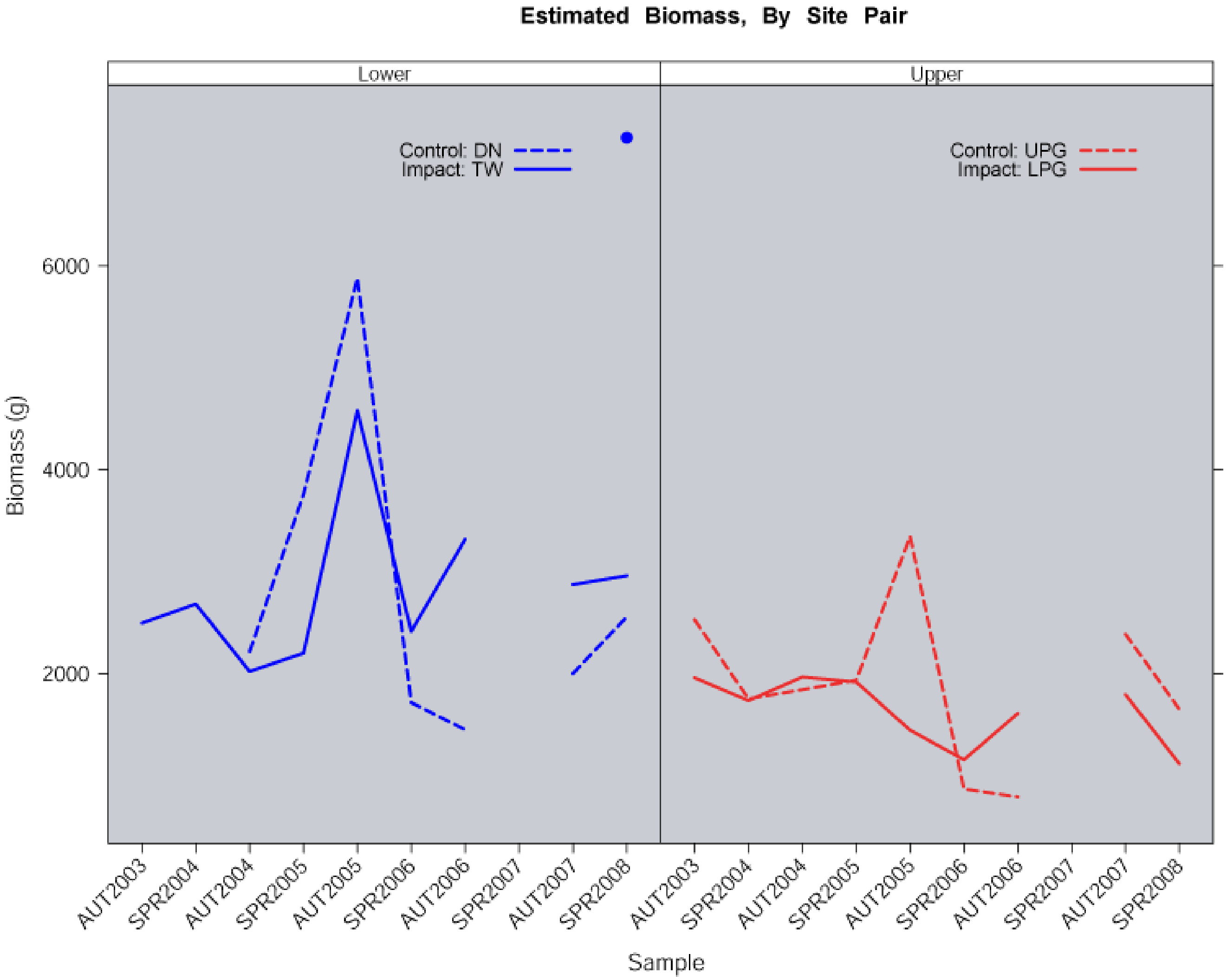


Figure 2.14: Total fish biomass estimates for each electrofishing sample. The point above the values for Spring 2008 in the Lower site pair reflects the measured biomass at the DN site including 6 large rainbow trout that immigrated from downstream.

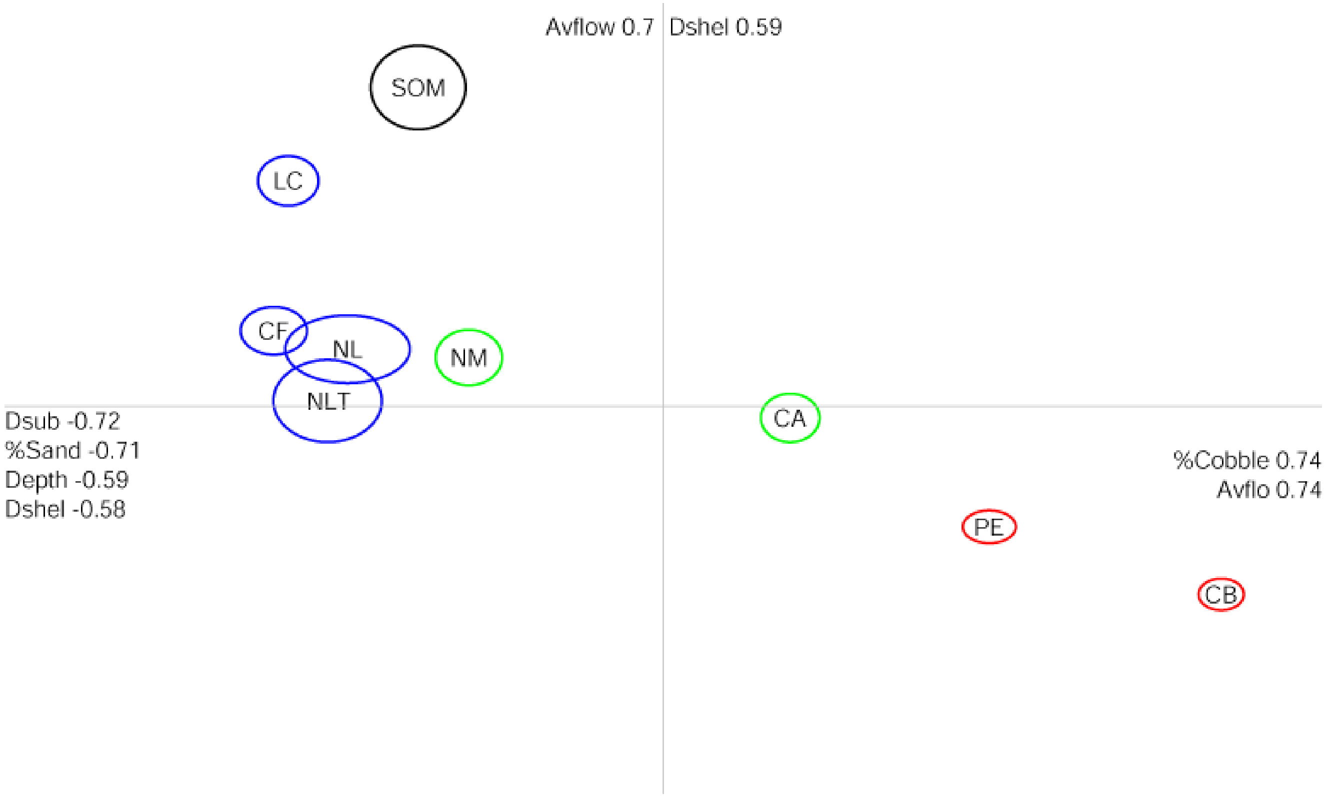


Figure 2.15: PCA Plot of microhabitat use by the 8 native fish species, before rainbow trout introduction. Ellipse centers represent the mean value for all observations on the axes of PCA components 1 (horizontal) and 2 (vertical). The ellipses represent 90% confidence intervals around these means. Species codes: SOM = stocked rainbow trout, LC = warpaint shiner, CF = rosyside dace, NL = Tennessee shiner, NLT = yellowfin shiner, NM = river chub, CA = central stoneroller, PE = gilt darter, CB = mottled sculpin. Members of the upper water-column guild are shown in blue, whereas lower water-column and benthic species are shown in green and red, respectively. All native fish points reflect observations taken in the period before the trout introductions; observations on rainbow trout have been projected into the before-introduction habitat space. The eigenvalues for PC1 and PC2 were 3.28 (27.3% variance explained) and 1.6 (13.3% variance explained).

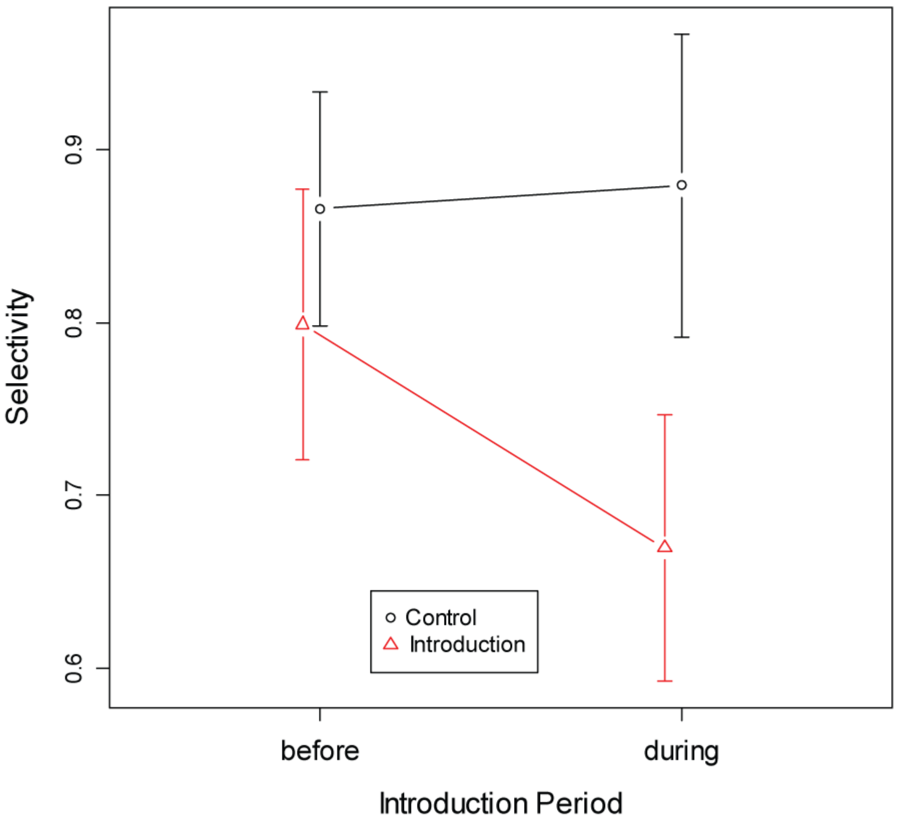


Figure 2.16: Interaction plot of ANOVA on native fish selectivity vectors. Error bars represent 90% confidence intervals around the individual means. Selectivity describes the distance between native fish microhabitat use and availability, in PCA space, and has no meaningful units.

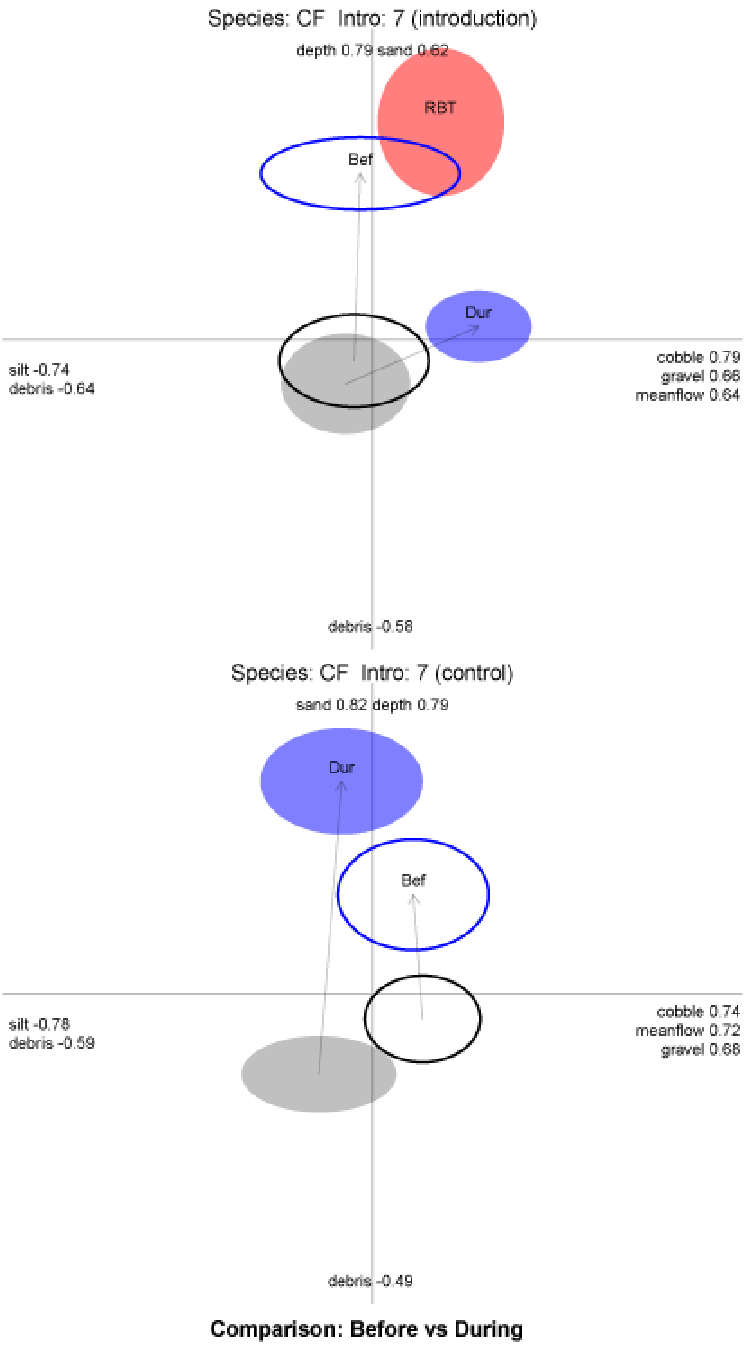


Figure 2.17: PCA plots for rosyside dace in introduction #7. These plots show a Type I rainbow trout avoidance response in which the native fish moved to shallower habitats with more erosional substrates while trout were present. Axes show variables with loadings > 0.4 for PC1 (horizontal) and PC2 (vertical).

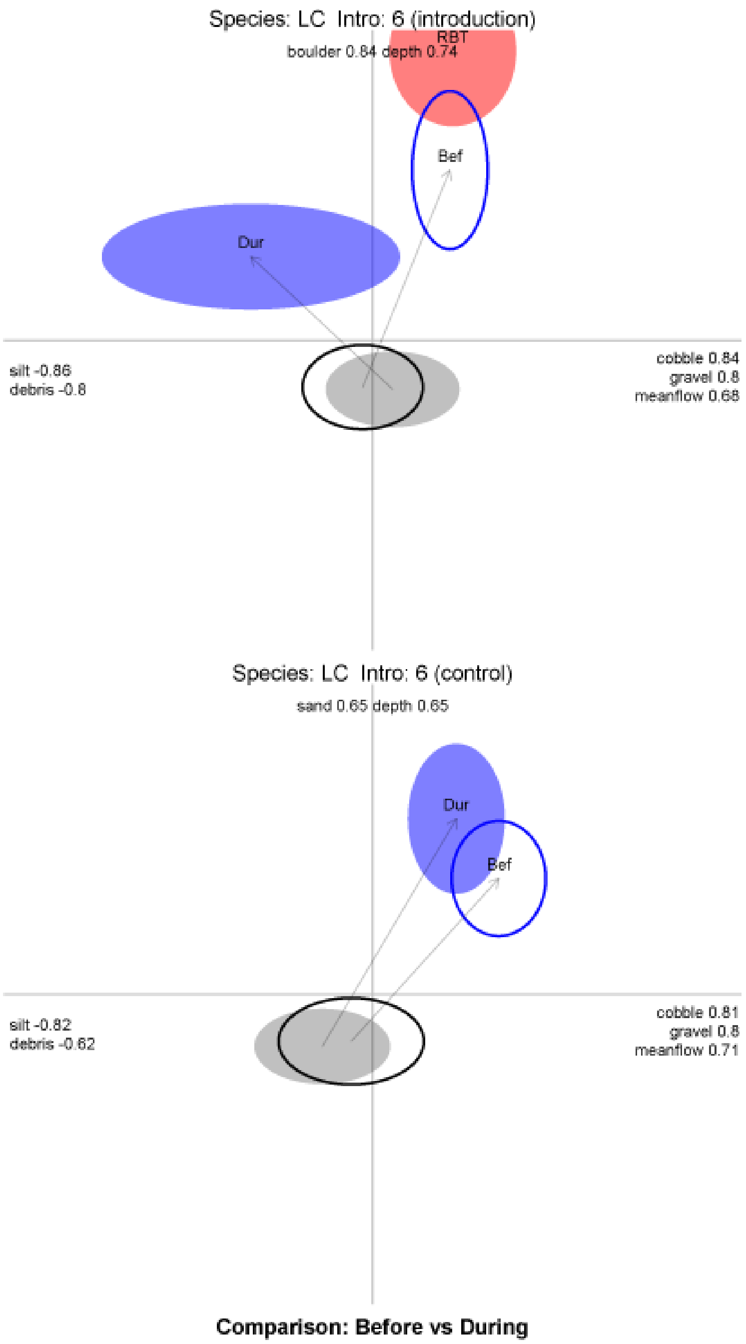


Figure 2.18: PCA plots for warpaint shiner in introduction #6. These plots show a different Type I rainbow trout avoidance response, in which the native fish moved to shallower habitats with a wider variety of more depositional substrates while rainbow trout were present.

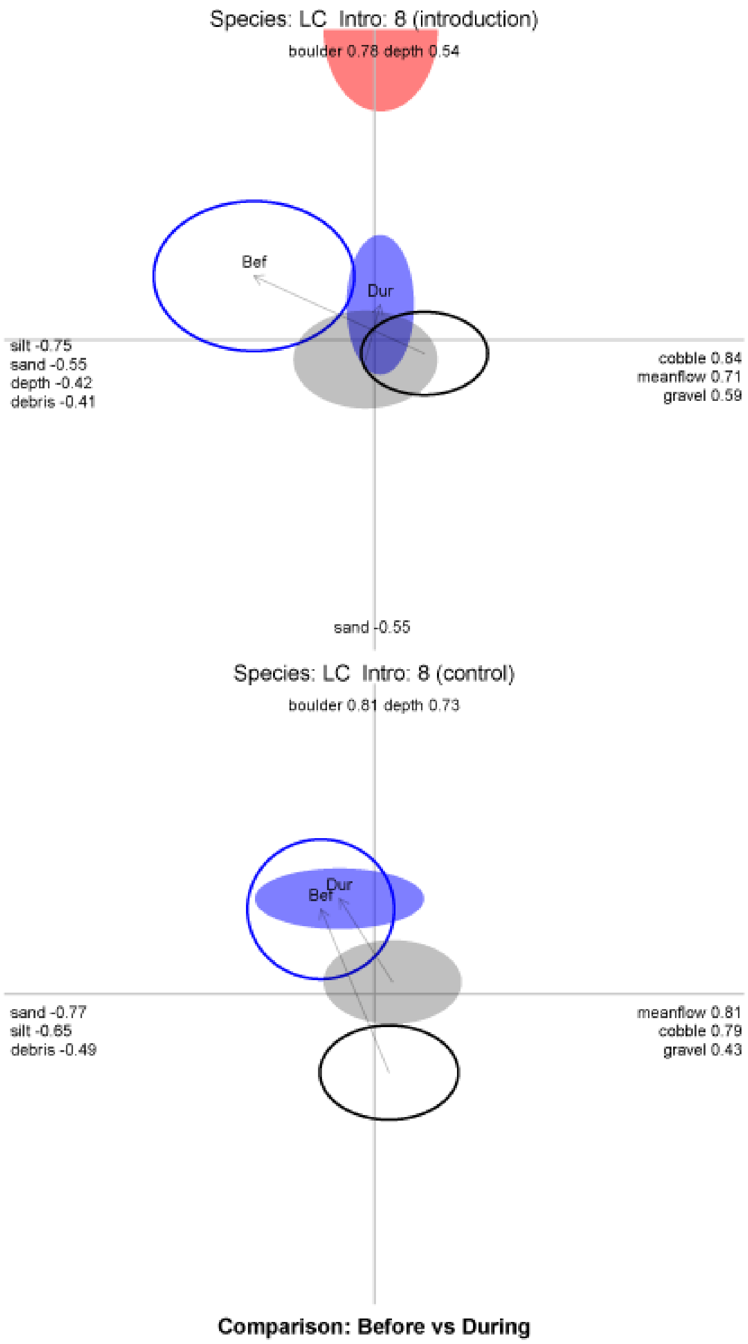


Figure 2.19: PCA plots for warpaint shiner in introduction #8. These plots show a Type I rainbow trout avoidance response, in which previously non-random habitat use becomes indistinguishable random during the rainbow trout introduction.

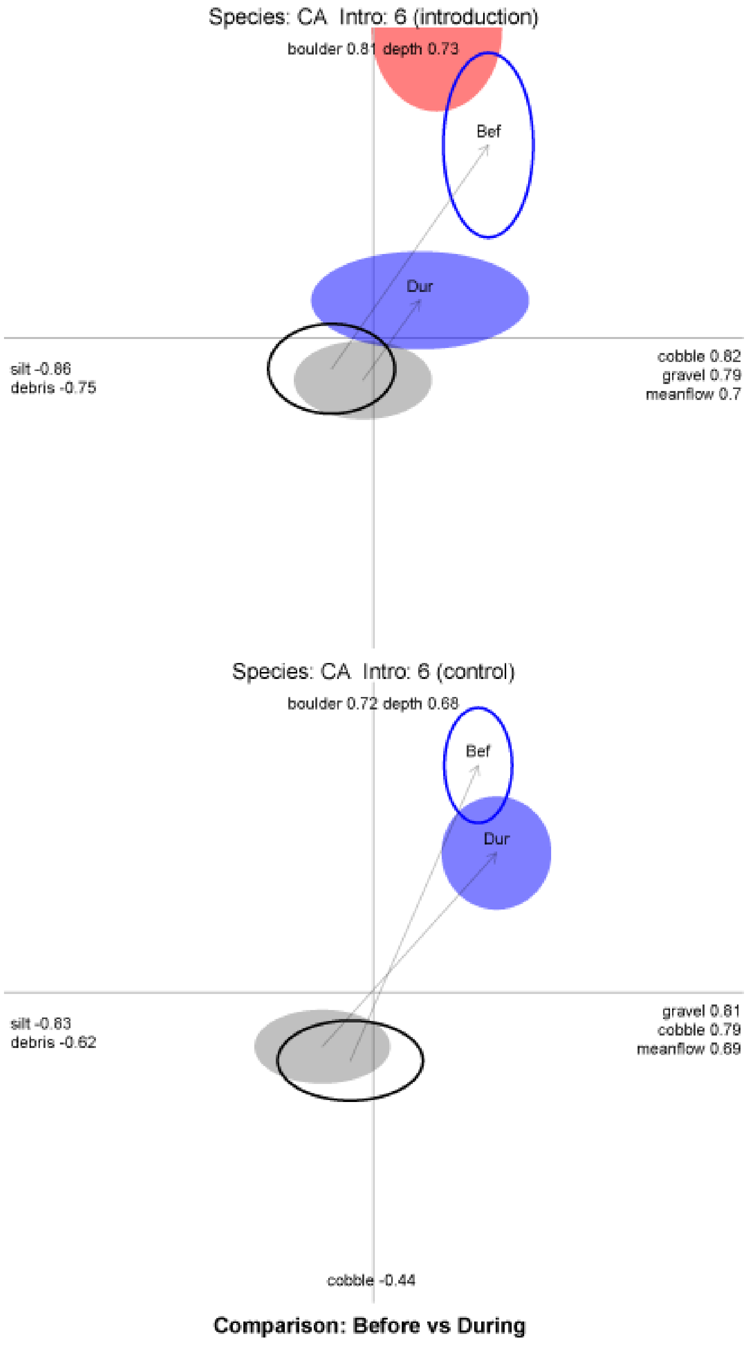


Figure 2.20: PCA plots for central stonereoller in introduction #6. These plots show a Type I response similar to that shown in Figure 2.17.

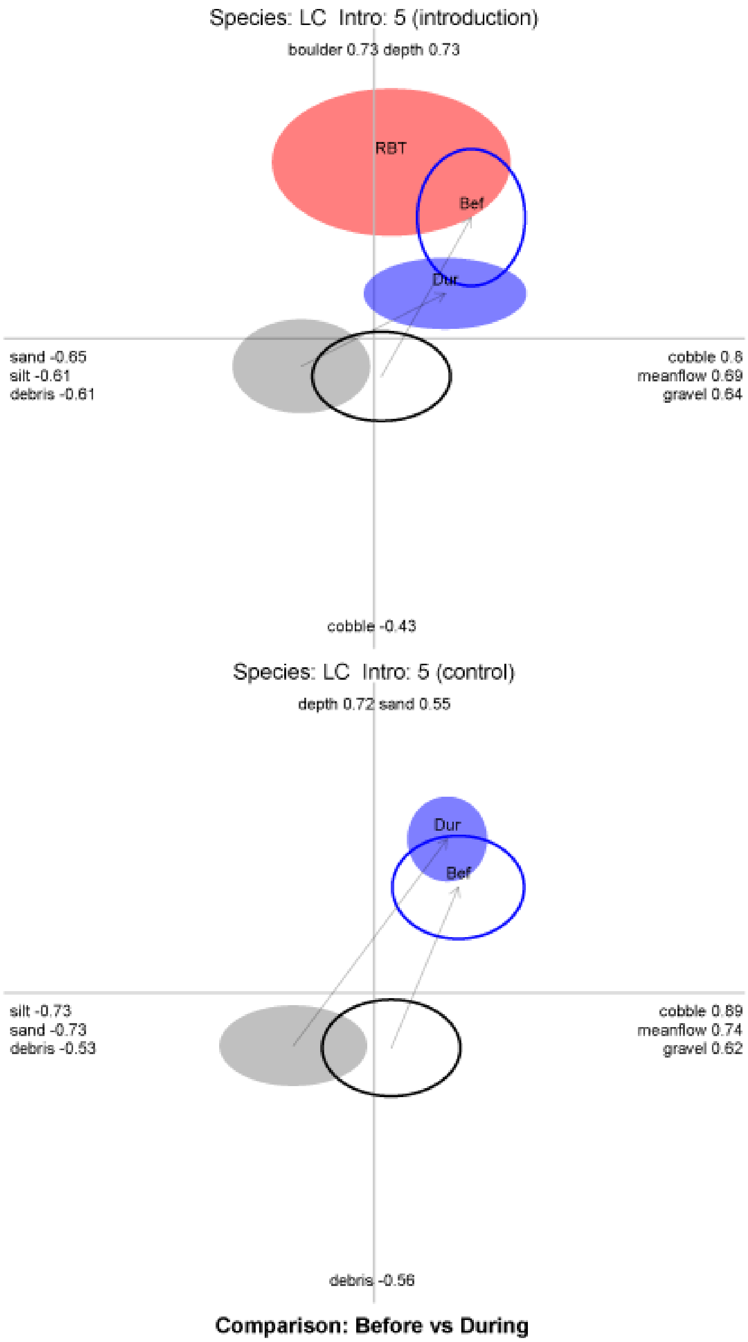


Figure 2.21: Type II response for warpaint shiners in introduction #5.

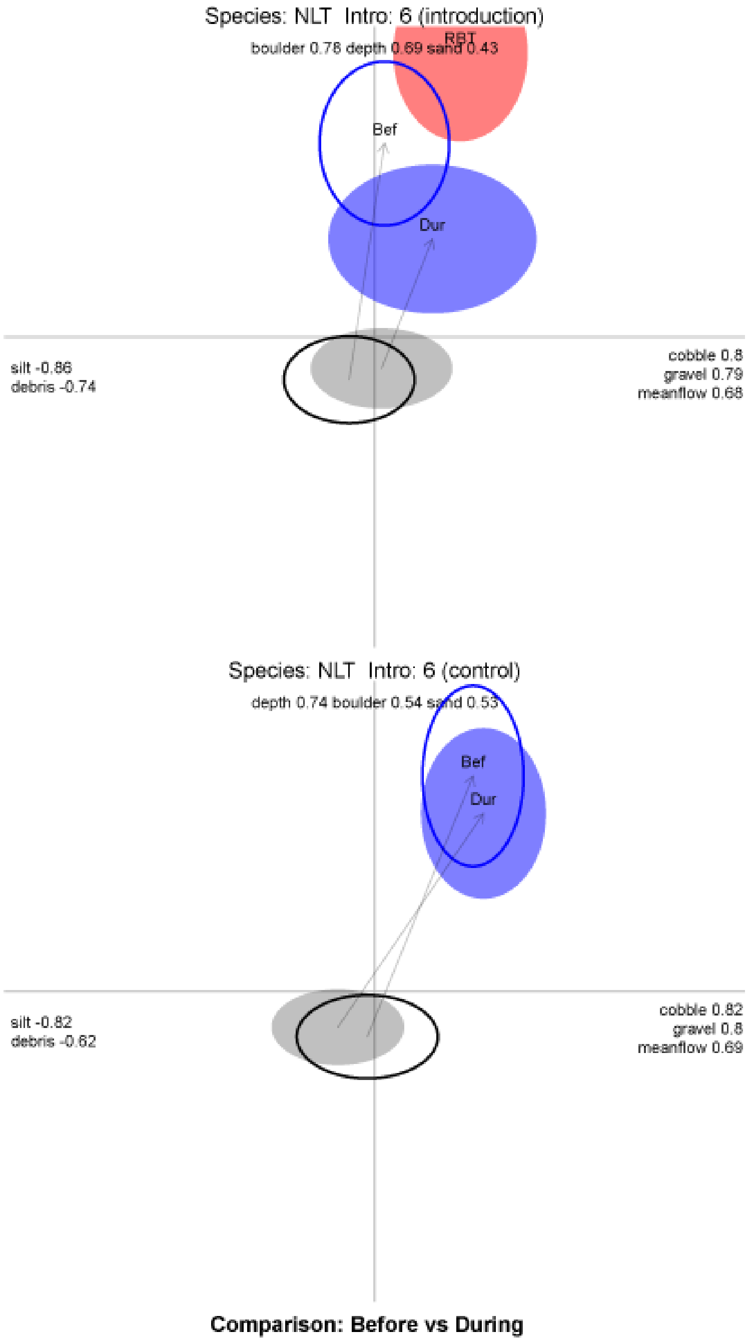


Figure 2.22: Type II response for yellowfin shiners in introduction #6.

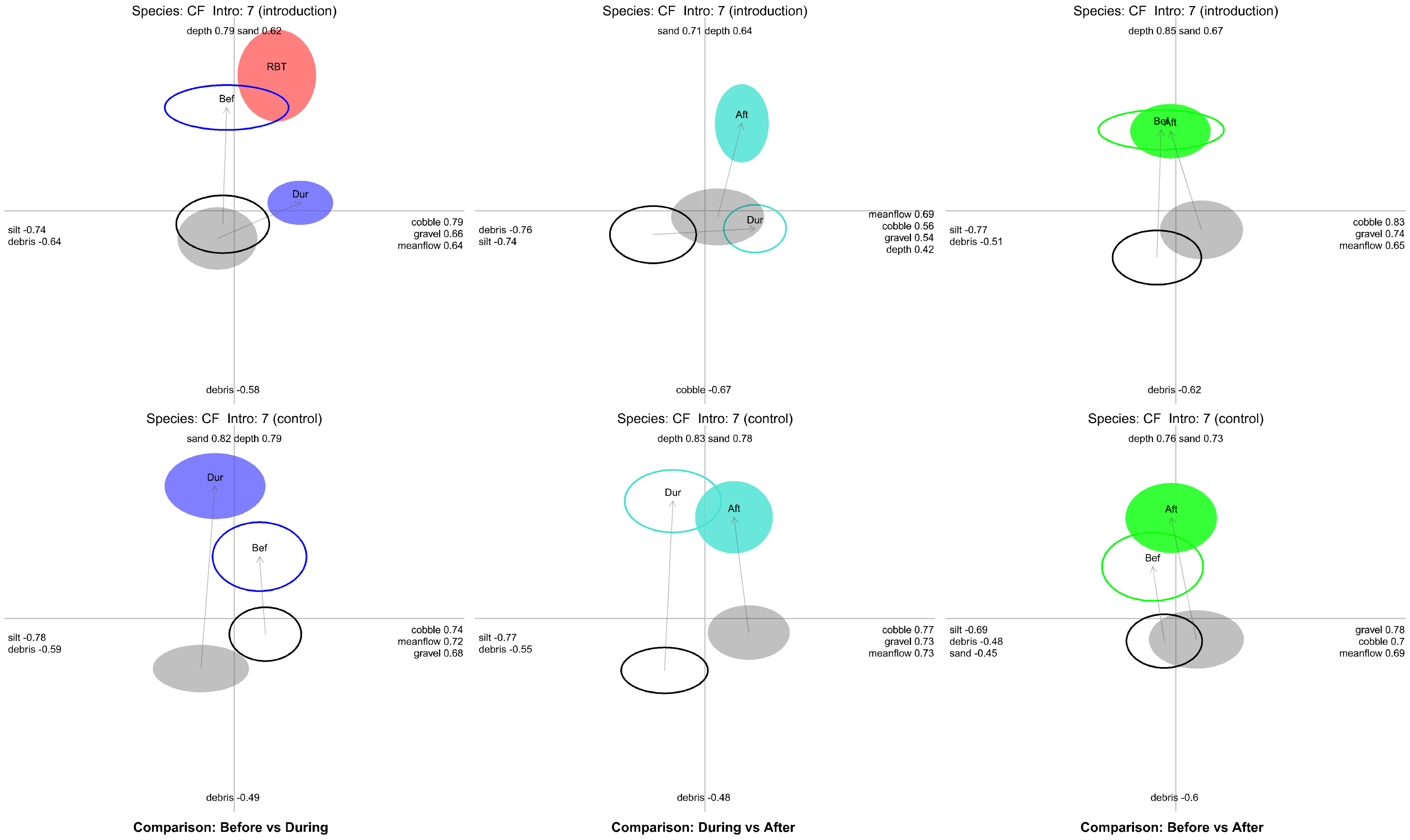


Figure 2.23: Paired PCA plots for rosyside dace in introduction 7. The during-after (middle) and before-after (right) comparisons show a full recovery of the type I trout introduction effect.

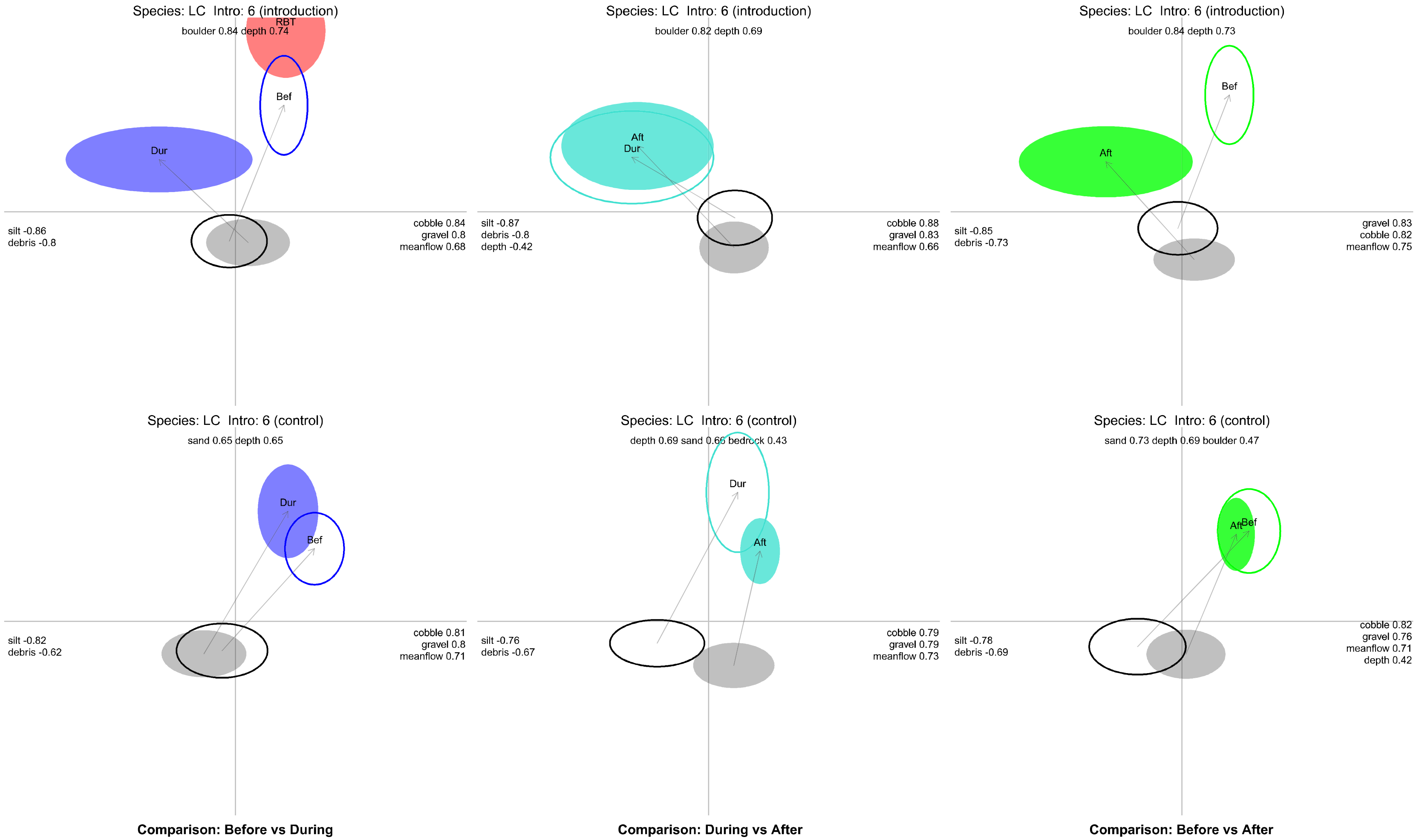


Figure 2.24: Paired PCA plots for warpaint shiner in introduction 6. The during-after (middle) and before-after (right) comparisons show no recovery of the type I introduction effect.

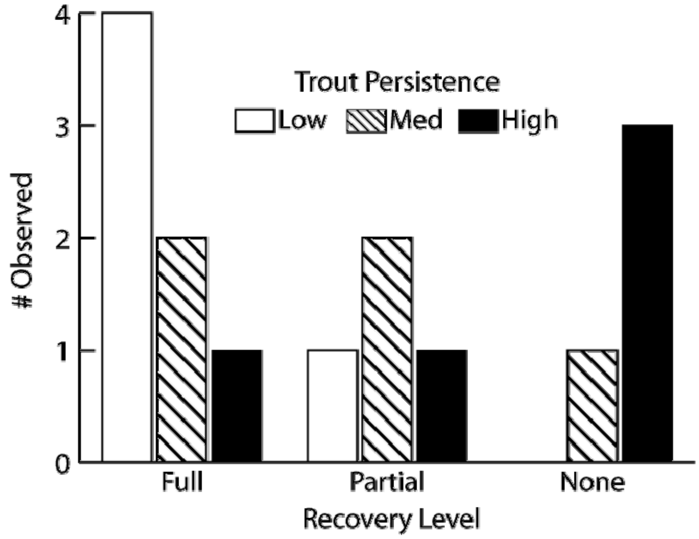


Figure 2.25: Frequency of recovery at three levels of trout persistence following block net removal.

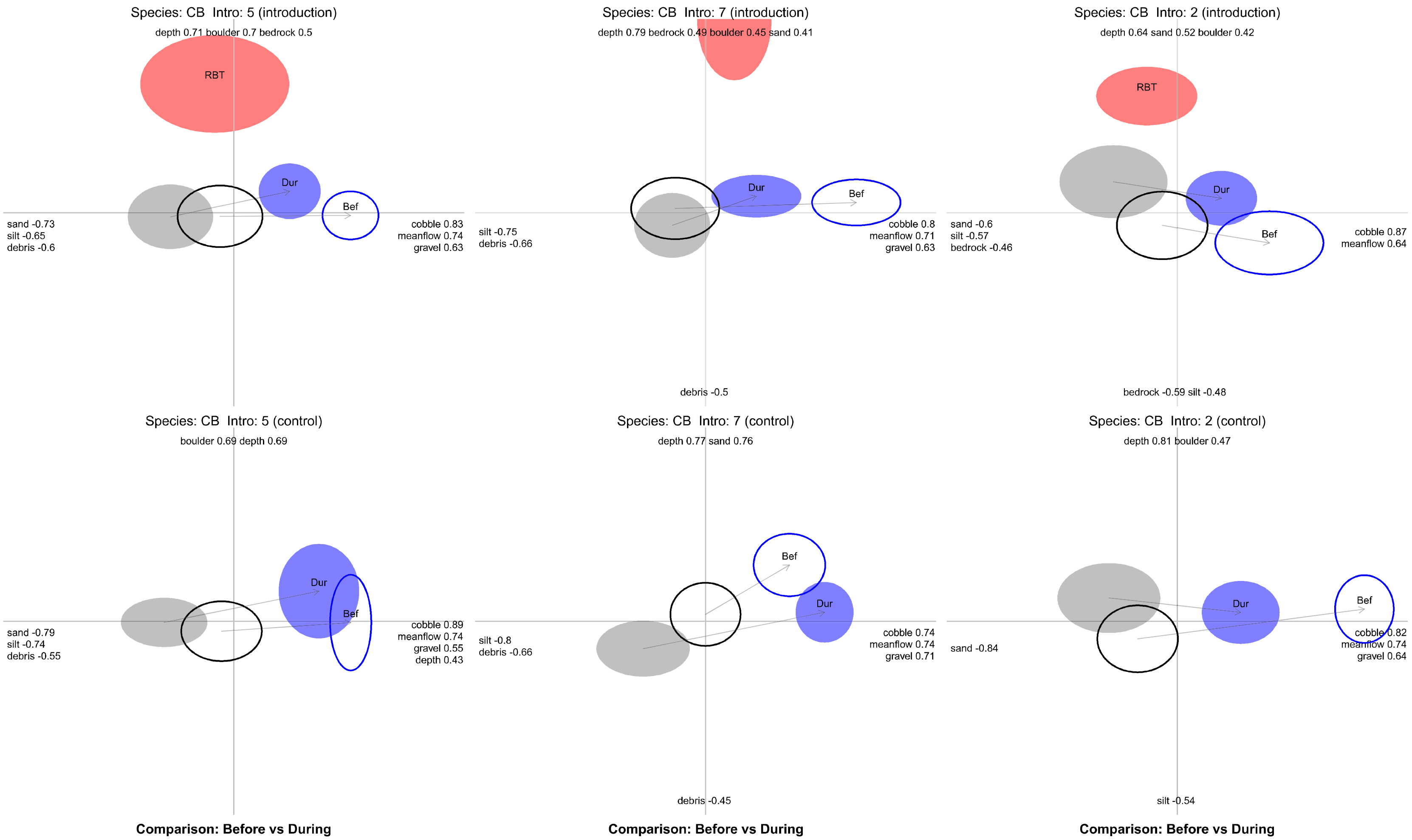


Figure 2.26: Plots of three introductions for mottled sculpin. These plots show a similar response in the introduction sites for introductions #5 and #7 (top row, left and middle) and the control site in introduction #2 (bottom row, right), consistent with a response to the imposition of block nets.

Table 2.1: Summary of field sampling activity. Site codes in the Introduction column indicate which of the site pairs received a stocking of rainbow trout, PG = the upper pair, near Patterson Gap Road, TW = the lower pair, near Tom Wilson Road. Parentheses indicate samples that are not analyzed here, due to unbalanced observations (Fall 2005) or weather conditions that prevented sampling during the introduction period (Spring 2006).

		Activity			
Year	Season	Microhabitat	Electrofishing	Introduction	Introduction Dates
2003	Spring	X			
2003	Fall	X	X		
2004	Spring	X	X		
2004	Fall	X	X		
2005	Spring	X	X		
2005	Summer	X		(1 PG)	(8/4-8/25)
2005	Fall	X	X	2 PG	9/25-10/3
2006	Spring	X	X	3 PG, (4 TW)	4/23-5/11, (5/3-5/28)
2006	Summer	X			
2006	Fall	X	X	5 TW	9/21-10/10
2007	Summer	X			
2007	Fall	X	X	6 PG, 7 TW	9/28-10/8, 10/11-10/31
2008	Spring	X	X	8 PG, 9 TW	4/25-5/12, 5/7-6/2

Table 2.2: Paired t-test statistics for mean displacement of native fish microhabitat use. A negative mean difference indicates that the centroid of the native fish microhabitat use ellipse was farther from the trout centroid during the introduction than it had been, before the introduction. Asterisks indicate $p < 0.05$.

Guild	**t Statistic**	**D.F.**	**Mean Difference**
Upper water-column	-2.14*	17	-0.163
Lower water-column	-2.08*	8	-0.211
Benthic	0.19	11	0.019

Table 2.3: Summary of individual species' responses to rainbow trout introductions.

	Introduction						
Species	#2	#3	#5	#6	#7	#8	#9
Warpaint Shiner	Inc (Type II)	Inc (Type II)	**Type II**	**Type I**	**Type II**	**Type I**	*Uninterp*
Rosyside Dace	None	▨	None	None	**Type I**	*Uninterp*	*Uninterp*
Yellowfin Shiner	▨	▨	▨	**Type II**	▨	None	▨
Tennessee Shiner	▨	▨	▨	▨	**Type II**	None	*Uninterp*
Central Stoneroller	▨	▨	▨	**Type I**	▨	**Type II**	None
River Chub	▨	None	None	**Type II**	**Type II**	**Type II**	**Type II**
Gilt Darter	▨	▨	None	None	None	**Type II**	None
Mottled Sculpin	None	None	None	None	None	**Type II**	None

Table 2.4: Summary of responses to rainbow trout introductions, by guild.

	Response Type			
Guild	**Type I**	**Type II**	**None / Uninterp**	**Total**
Upper water-column	3	4	11	18
Lower water-column	1	5	3	9
Benthic	0	2	10	12

Table 2.5 Trout persistence in introduction sites after block net removal

Intro#	Snorkel Passes	Total Trout Observed	Mean Trout/Pass	Trout Persistence
2	2	9	4.5	High
3	4	11	2.8	Med
5	3	8	2.7	Med
6	3	6	2.0	Med
7	6	1	0.2	Low
8	3	10	3.3	High
9	4	0	0.0	Low

APPENDIX 2.1: SINGLE SPECIES PCA BIPLOTS

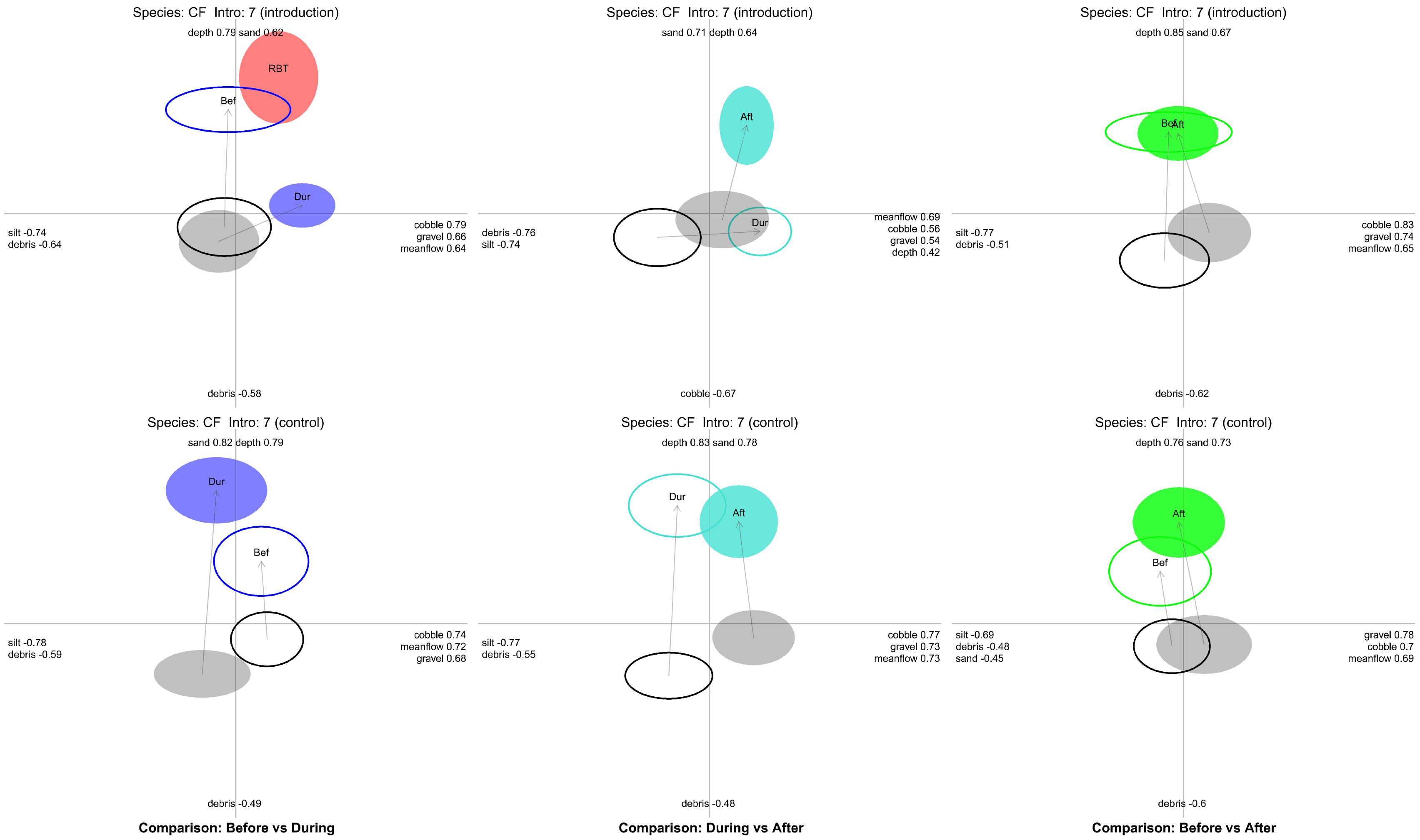
This appendix contains a table of rainbow trout introduction effects, derived from microhabitat PCA analysis, followed by all before-after, during-after, before-after PCA biplots, arranged by response designation (Type I, Type II, Uninterpretable, No Effect).

	Introduction						
Species	#2	#3	#5	#6	#7	#8	#9
Warpaint Shiner	Inc (Type II)	Inc (Type II)	**Type II**	**Type I**	**Type II**	**Type I**	*Uninterp*
Rosyside Dace	None	▨	None	None	**Type I**	*Uninterp*	*Uninterp*
Yellowfin Shiner	▨	▨	▨	**Type II**	▨	None	▨
Tennessee Shiner	▨	▨	▨	▨	**Type II**	None	*Uninterp*
Central Stoneroller	▨	▨	▨	**Type I**	▨	**Type II**	None
River Chub	▨	None	None	**Type II**	**Type II**	**Type II**	**Type II**
Gilt Darter	▨	▨	None	None	None	**Type II**	None
Mottled Sculpin	None	None	None	None	None	**Type II**	None

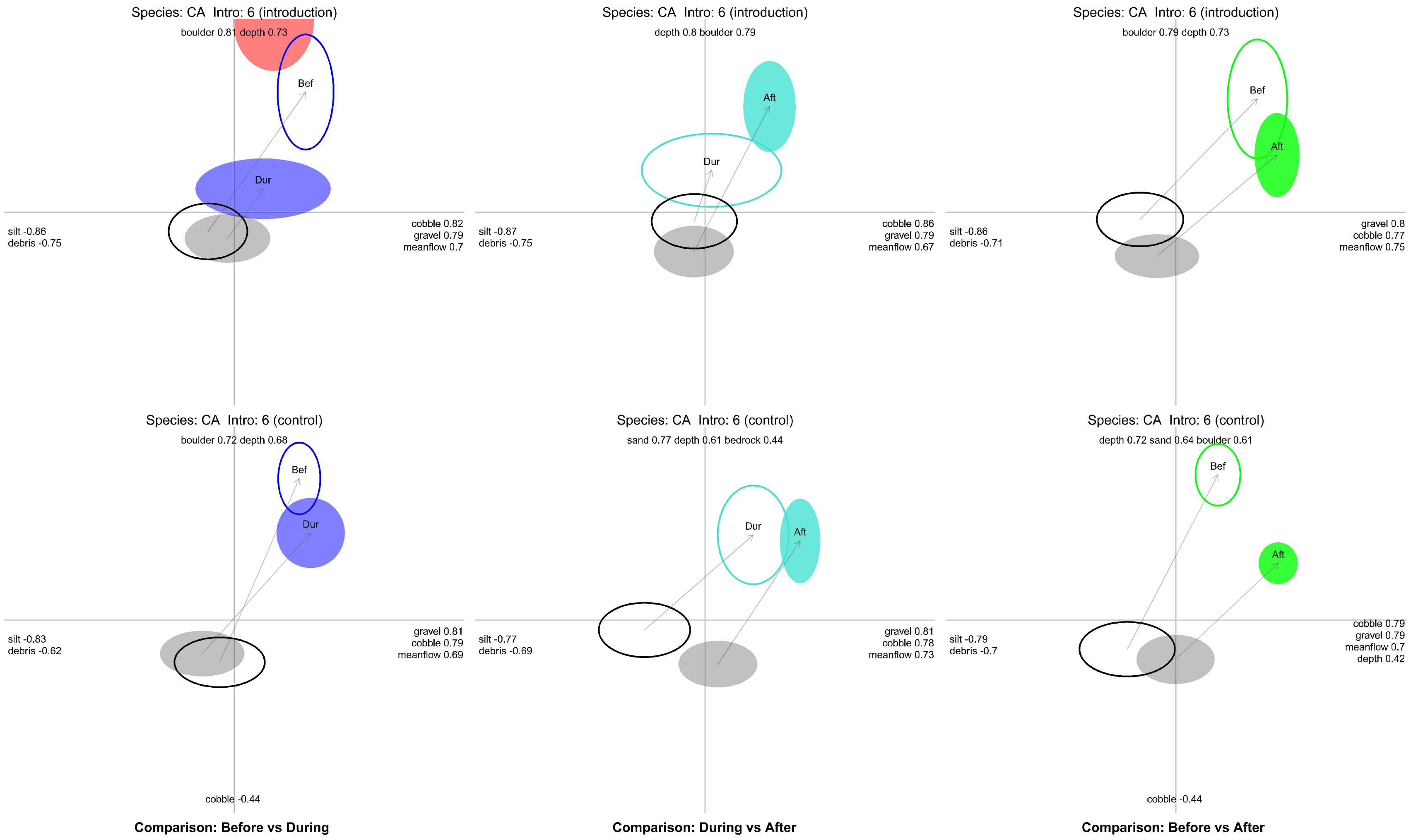
Shaded cells represent instances where a species was not present in sufficient numbers for analysis.

For PCA biplots in this section, the species abbreviation codes are as follows:

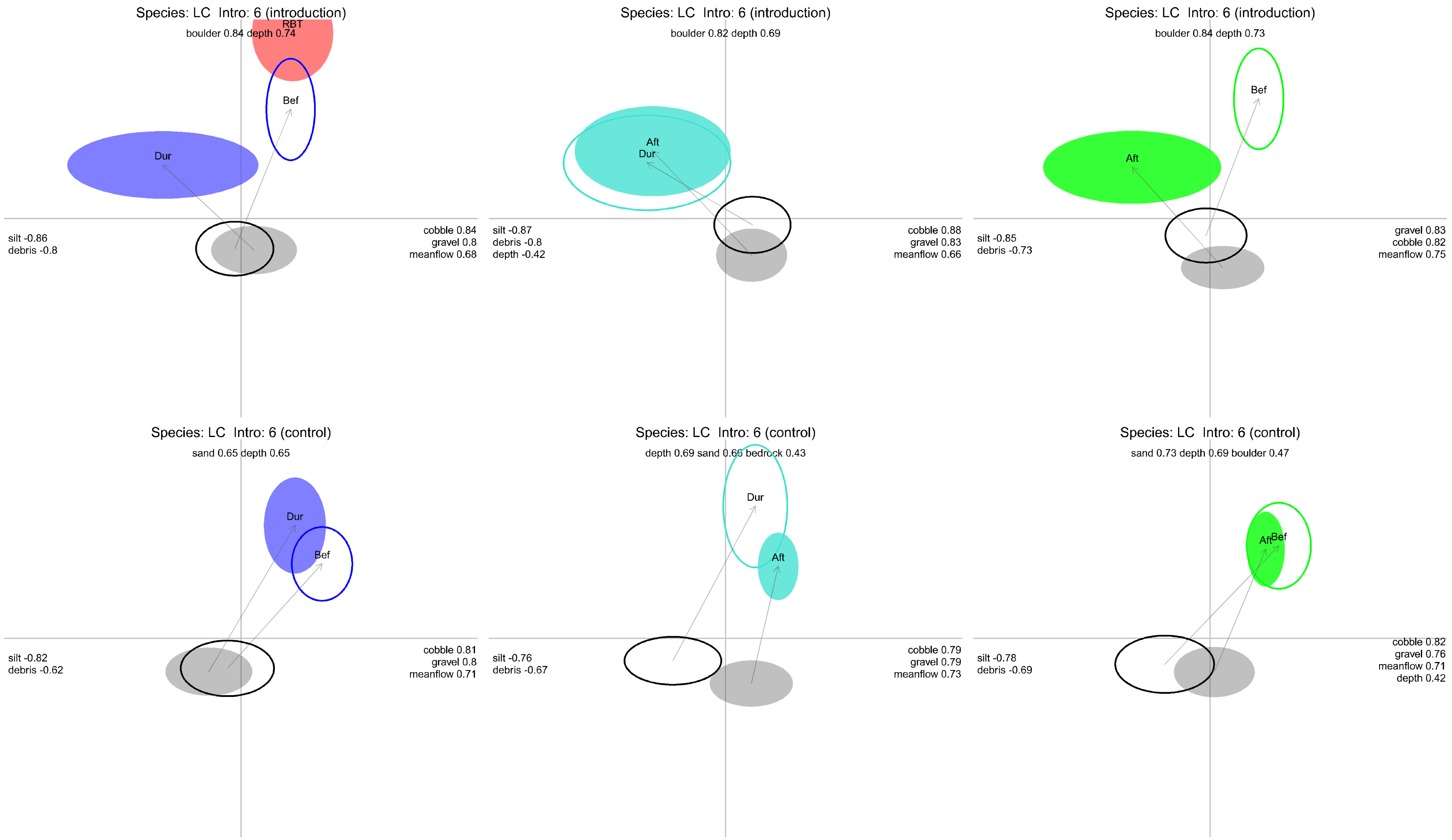
CA = central stoneroller CB = mottled sculpin CF = rosyside dace LC = warpaint shiner
NLT = yellowfin shiner NL = Tennessee shiner NM = river chub PE = gilt darter



Response: Type I
Recovery: Full



Response: Type I
Recovery: Full



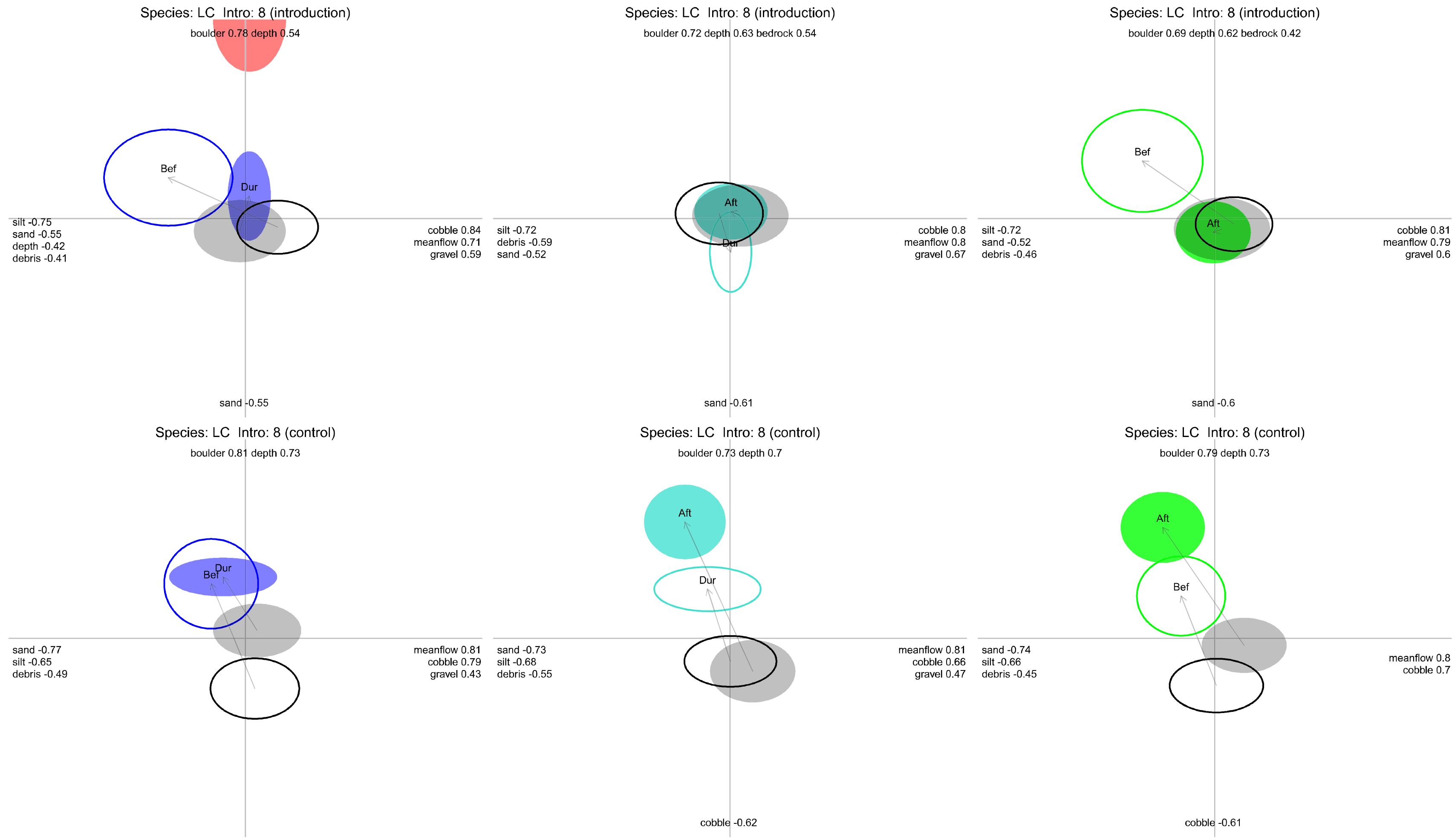
Comparison: Before vs During

Comparison: During vs After

Comparison: Before vs After

Response: Type I

Recovery: None



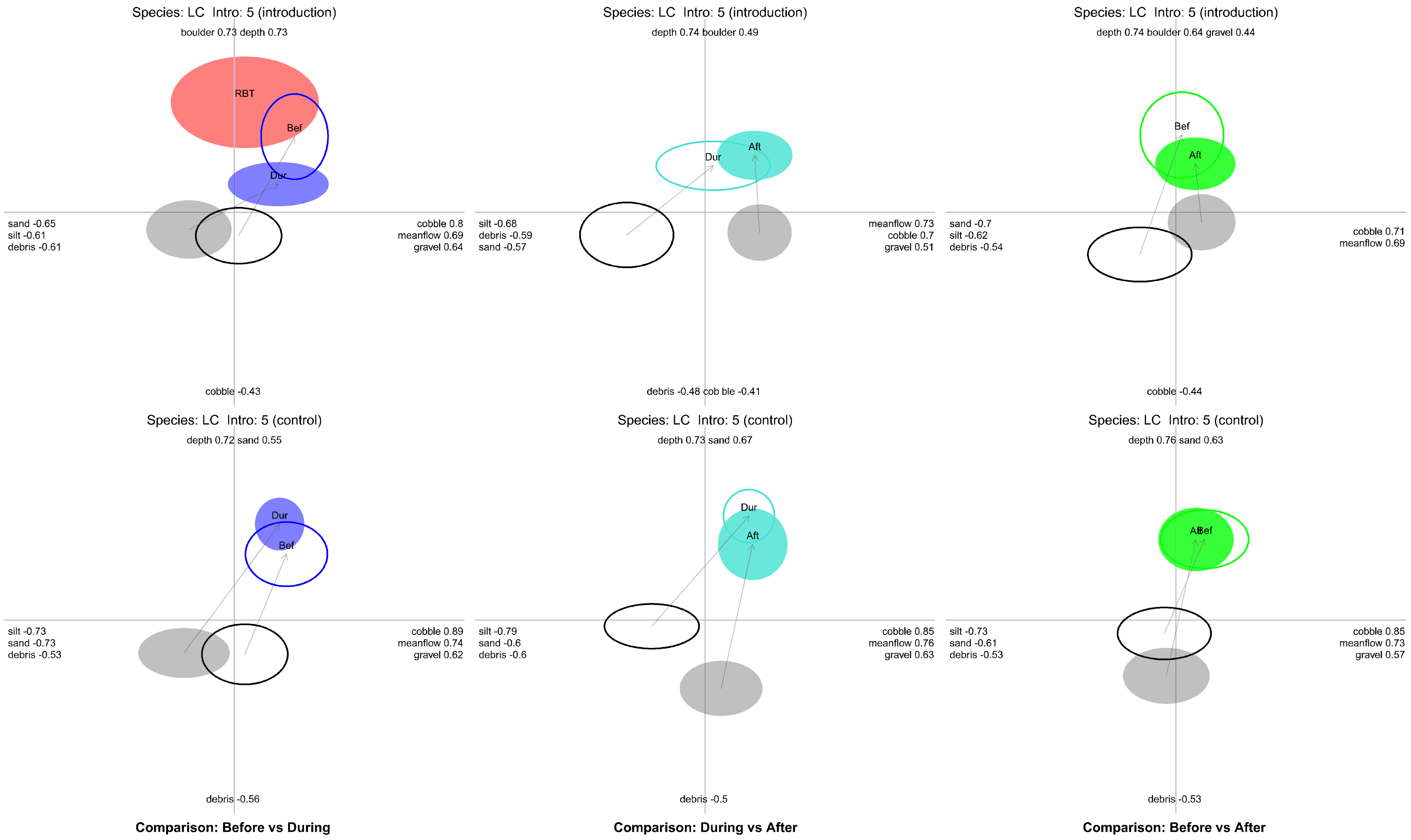
Comparison: Before vs During

Comparison: During vs After

Comparison: Before vs After

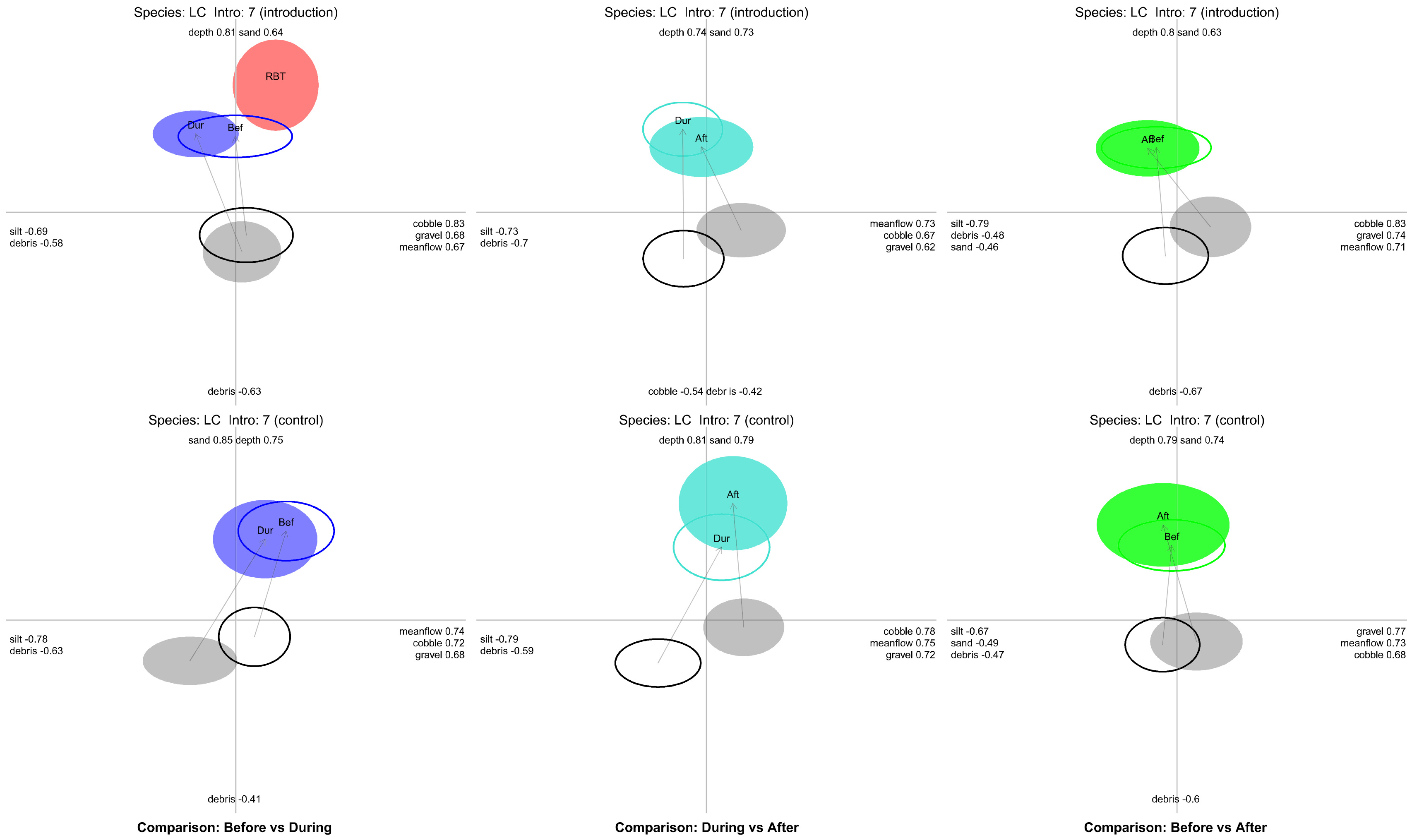
Response: Type I

Recovery: None



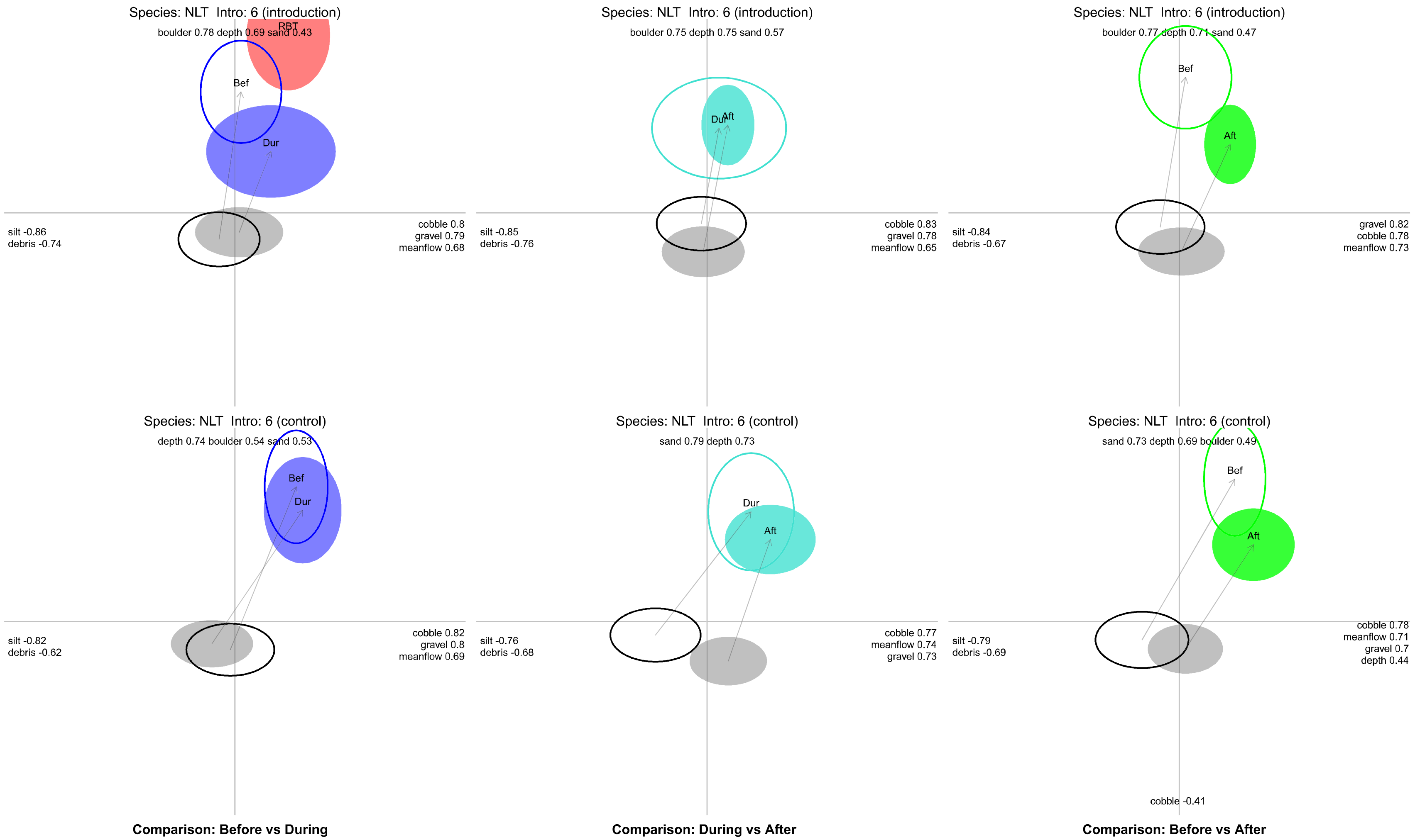
Response: Type II

Recovery: Full



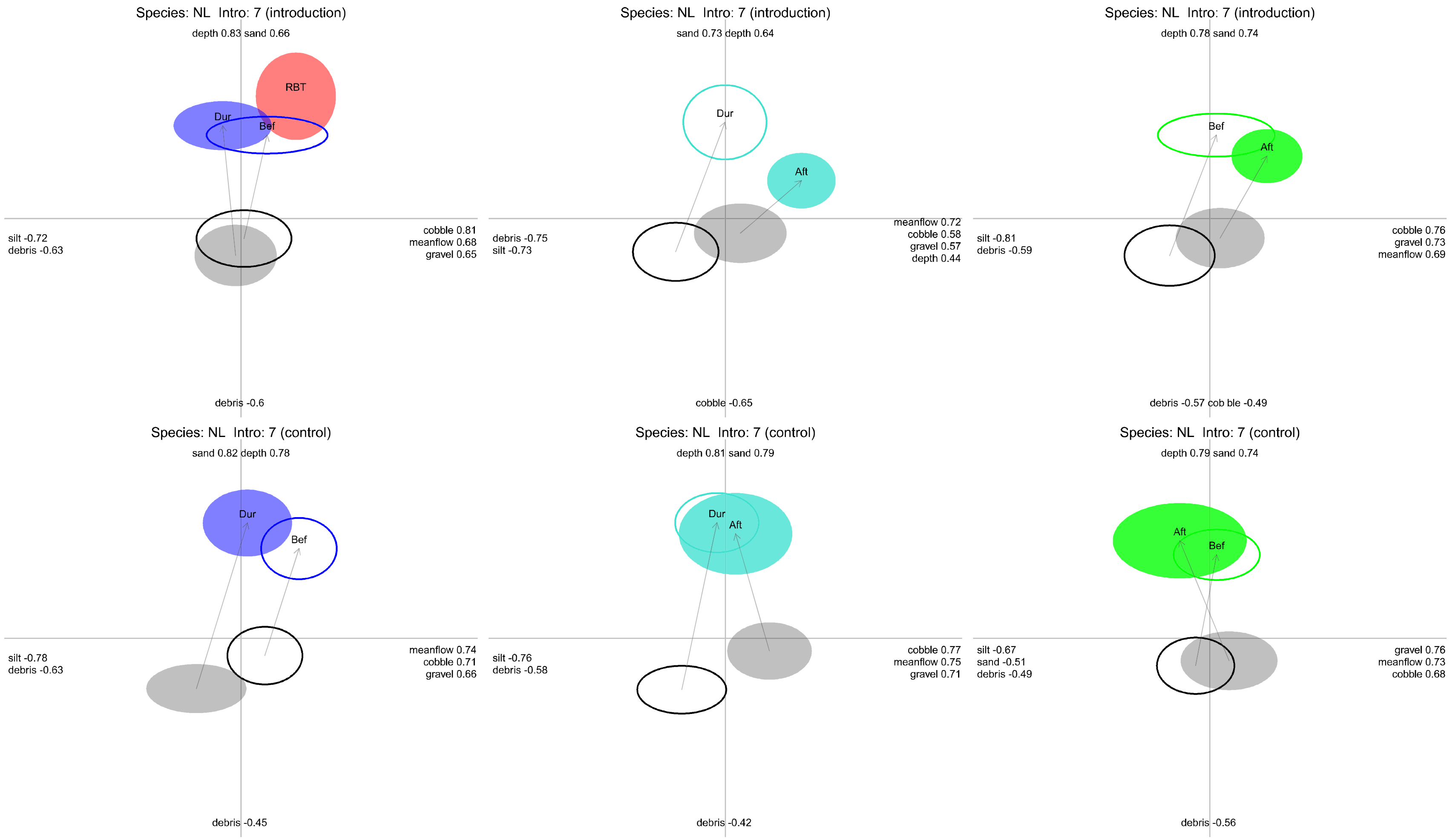
Response: Type II

Recovery: Full



Response: Type II

Recovery: Partial



Species: NL Intro: 7 (introduction)

Species: NL Intro: 7 (introduction)

Species: NL Intro: 7 (introduction)

Species: NL Intro: 7 (control)

Species: NL Intro: 7 (control)

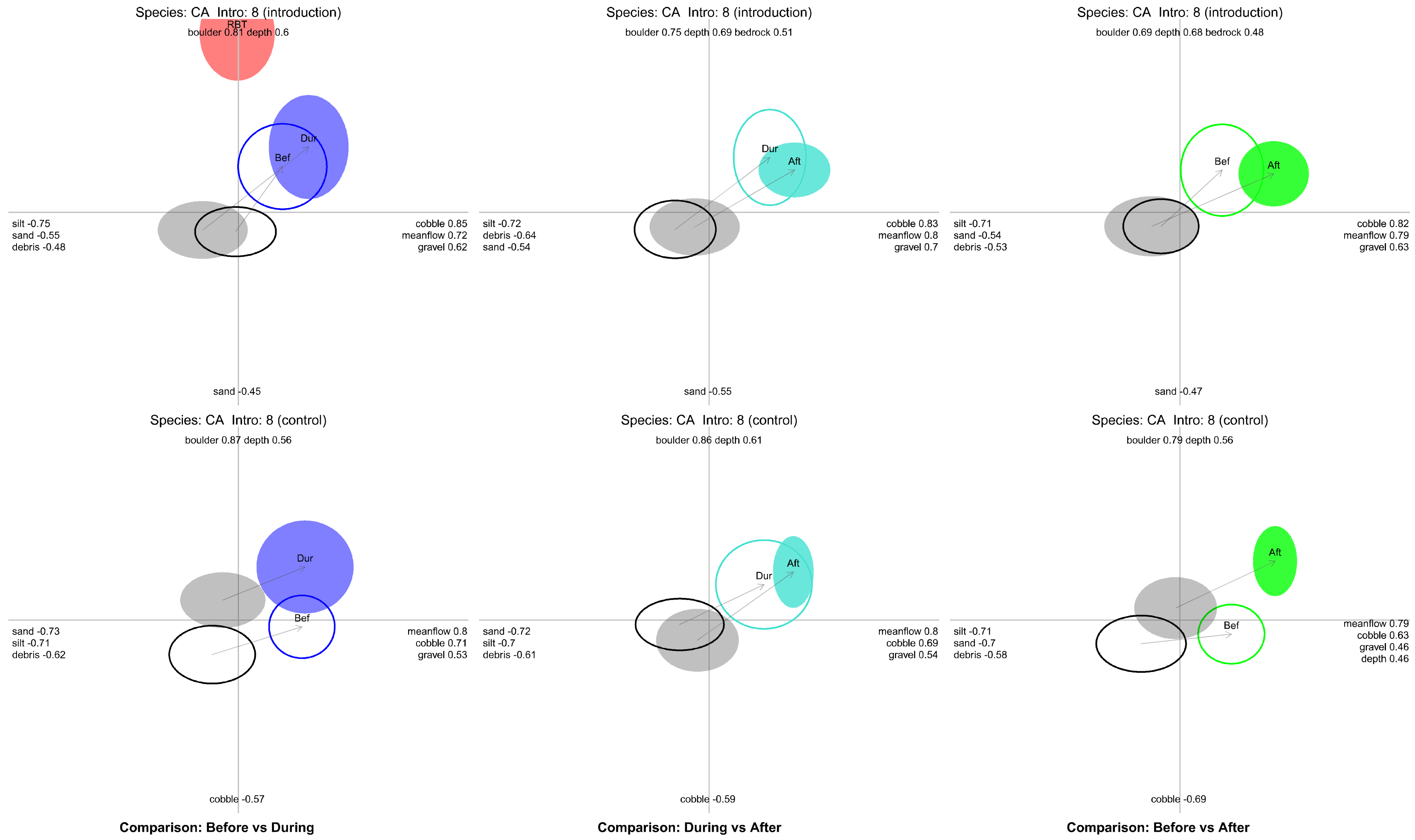
Species: NL Intro: 7 (control)

Comparison: Before vs During

Comparison: During vs After

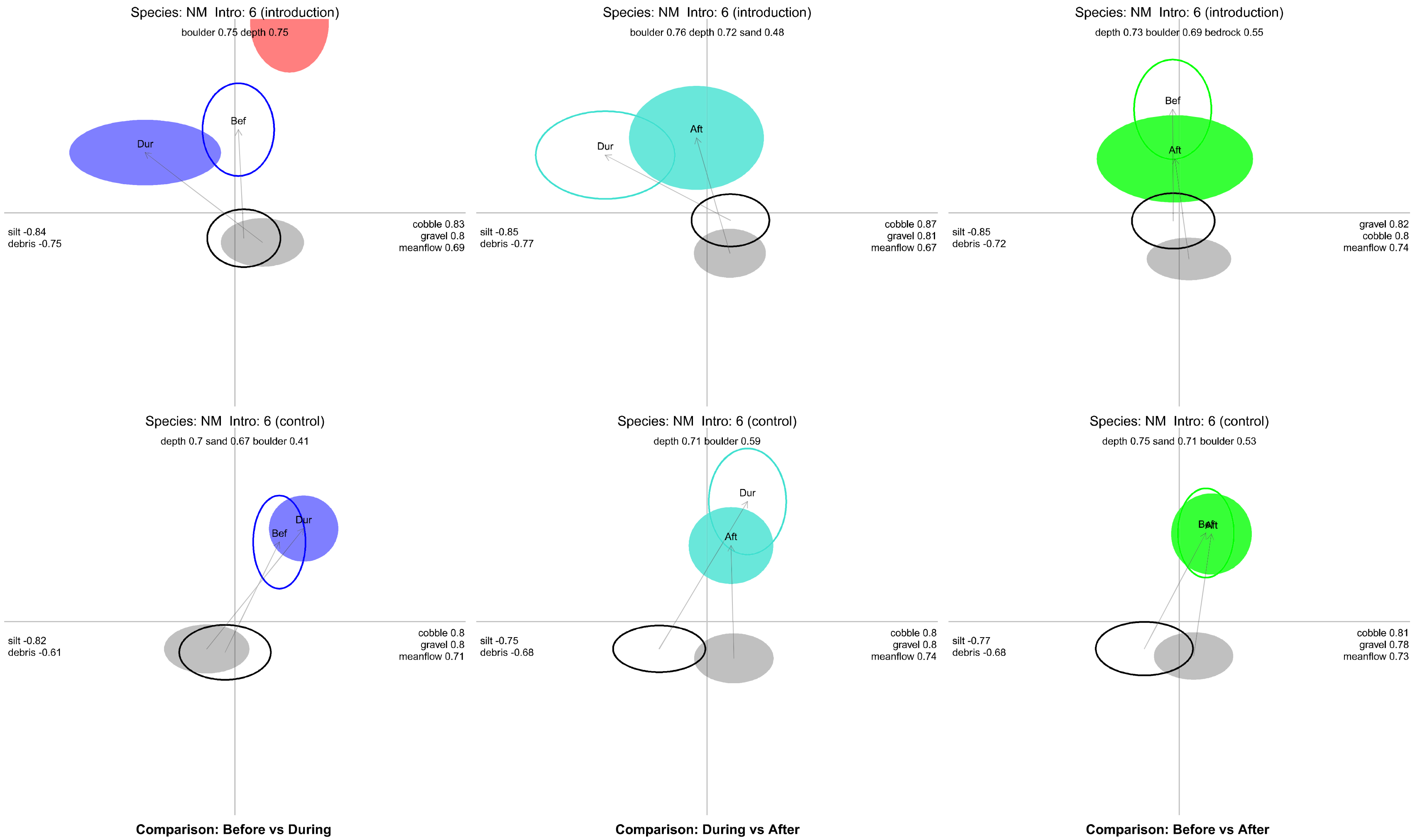
Comparison: Before vs After

Response: Type II
Recovery: Full



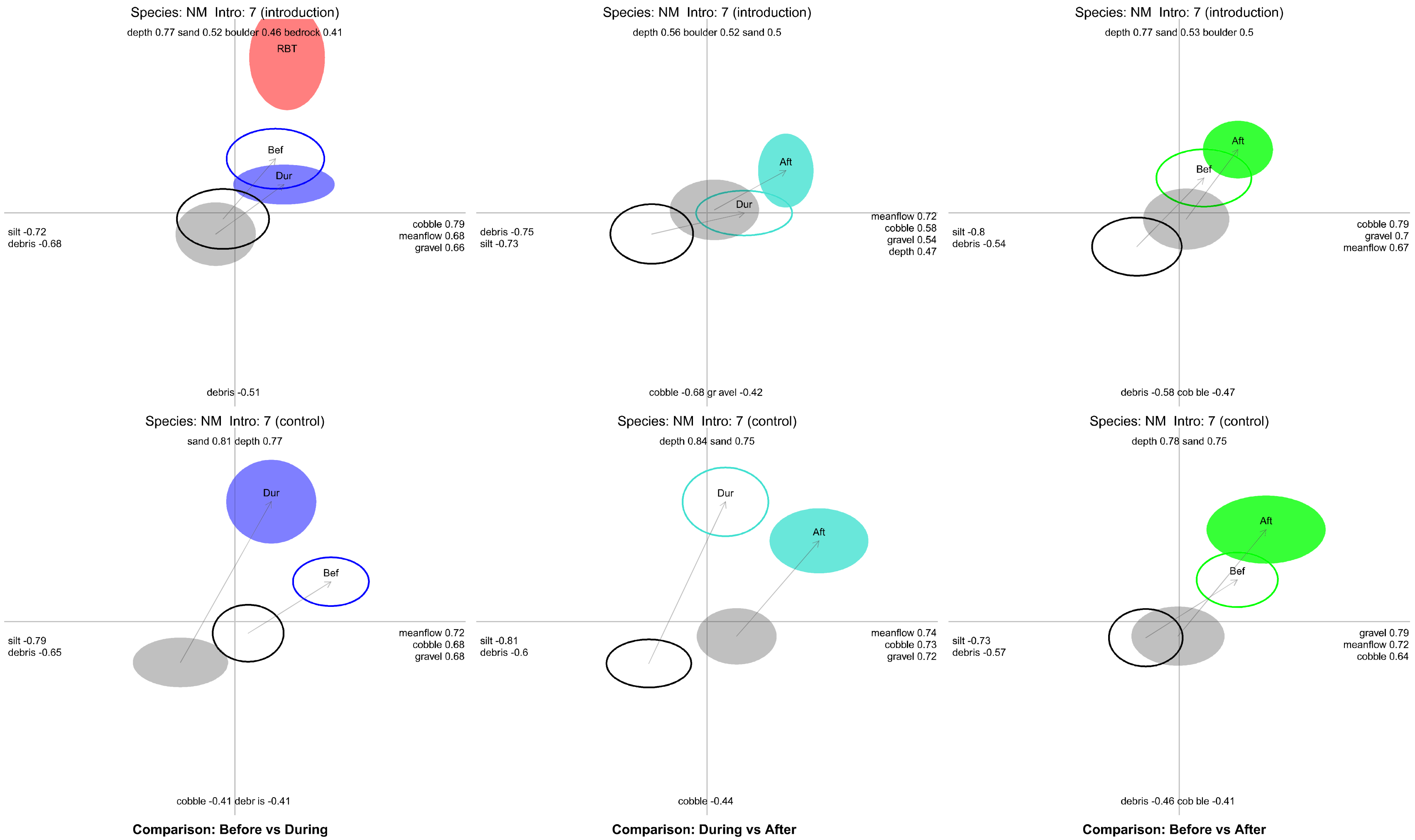
Response: Type II

Recovery: None

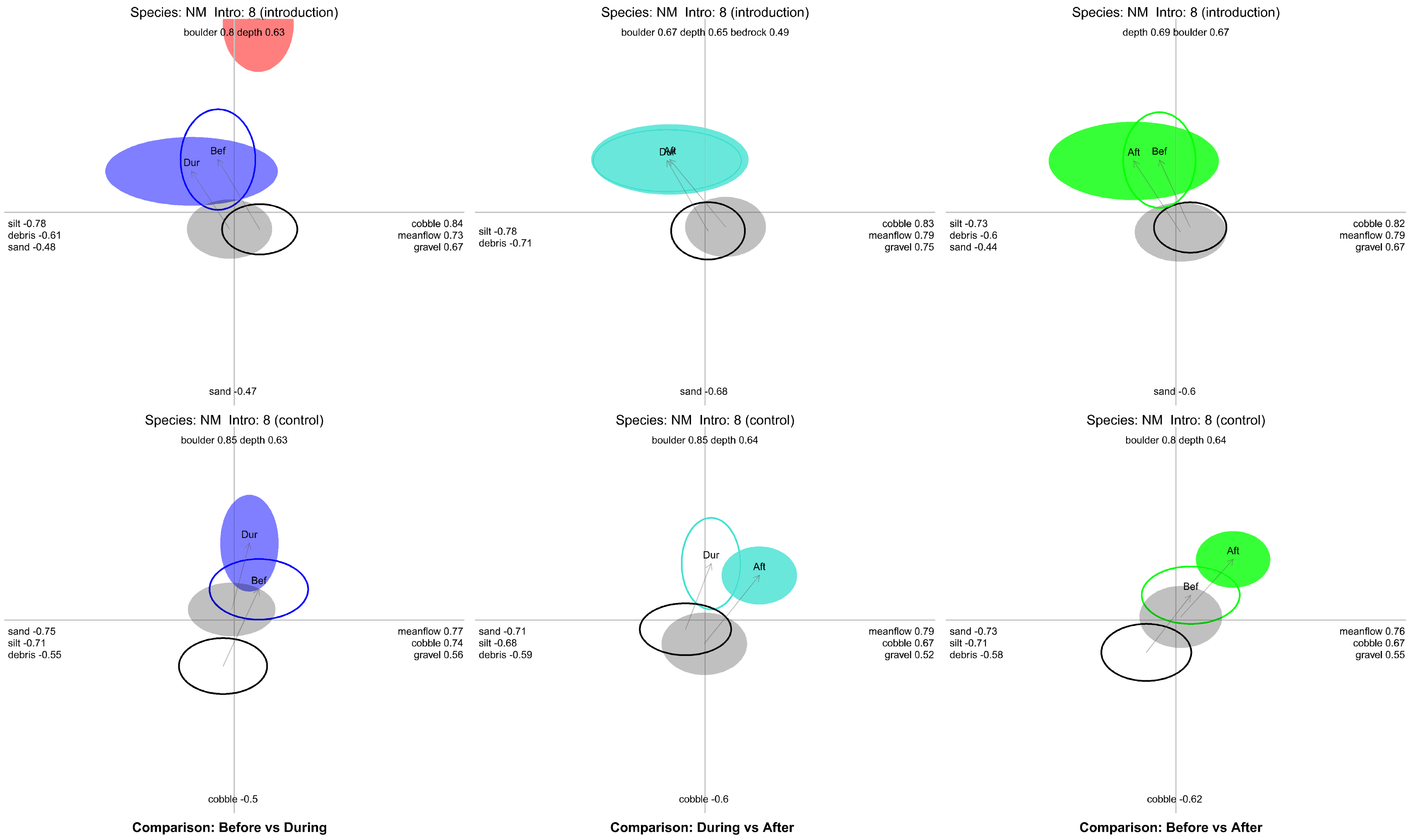


Response: Type II

Recovery: Partial

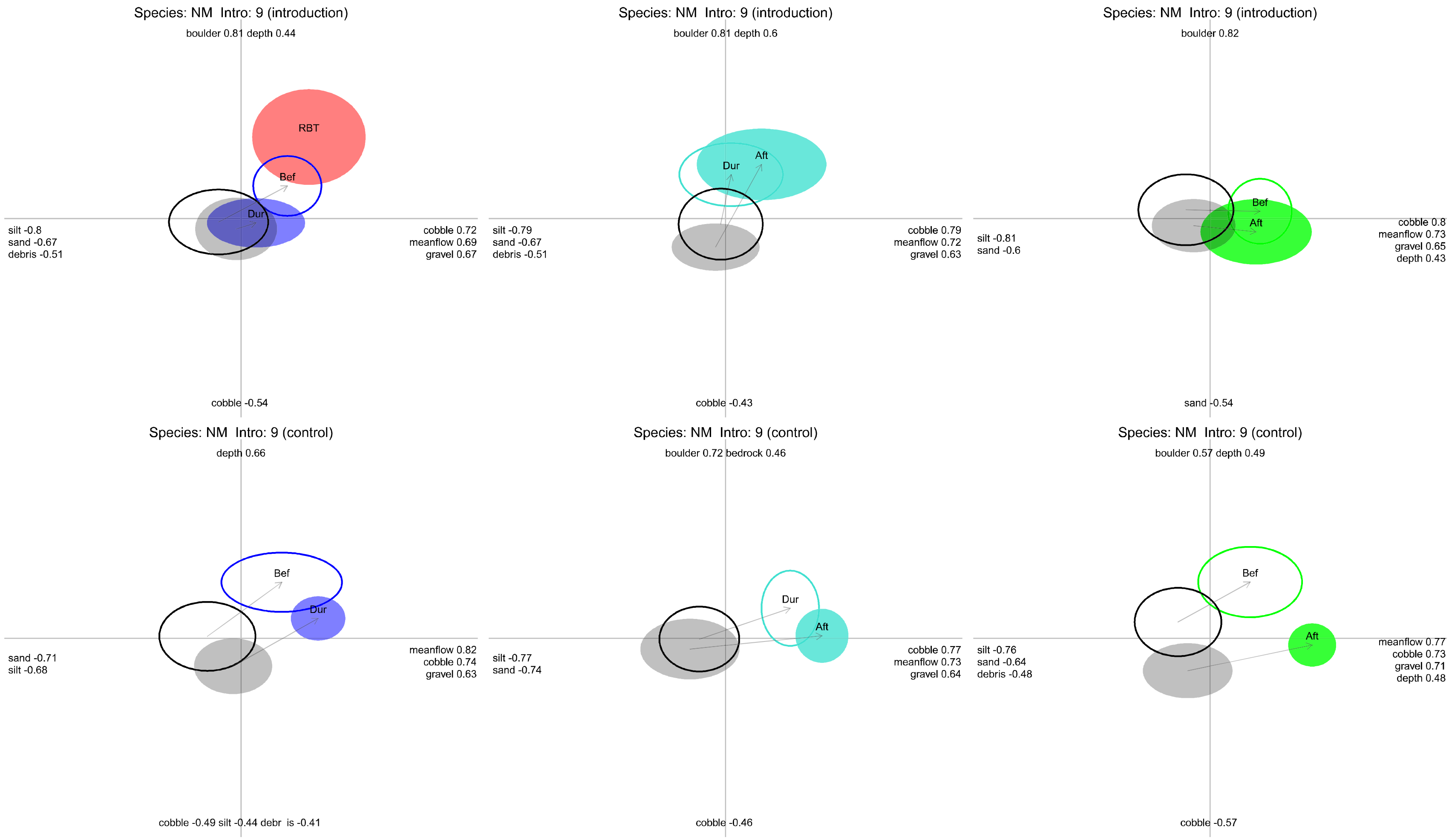


Response: Type II
Recovery: Full



Response: Type II

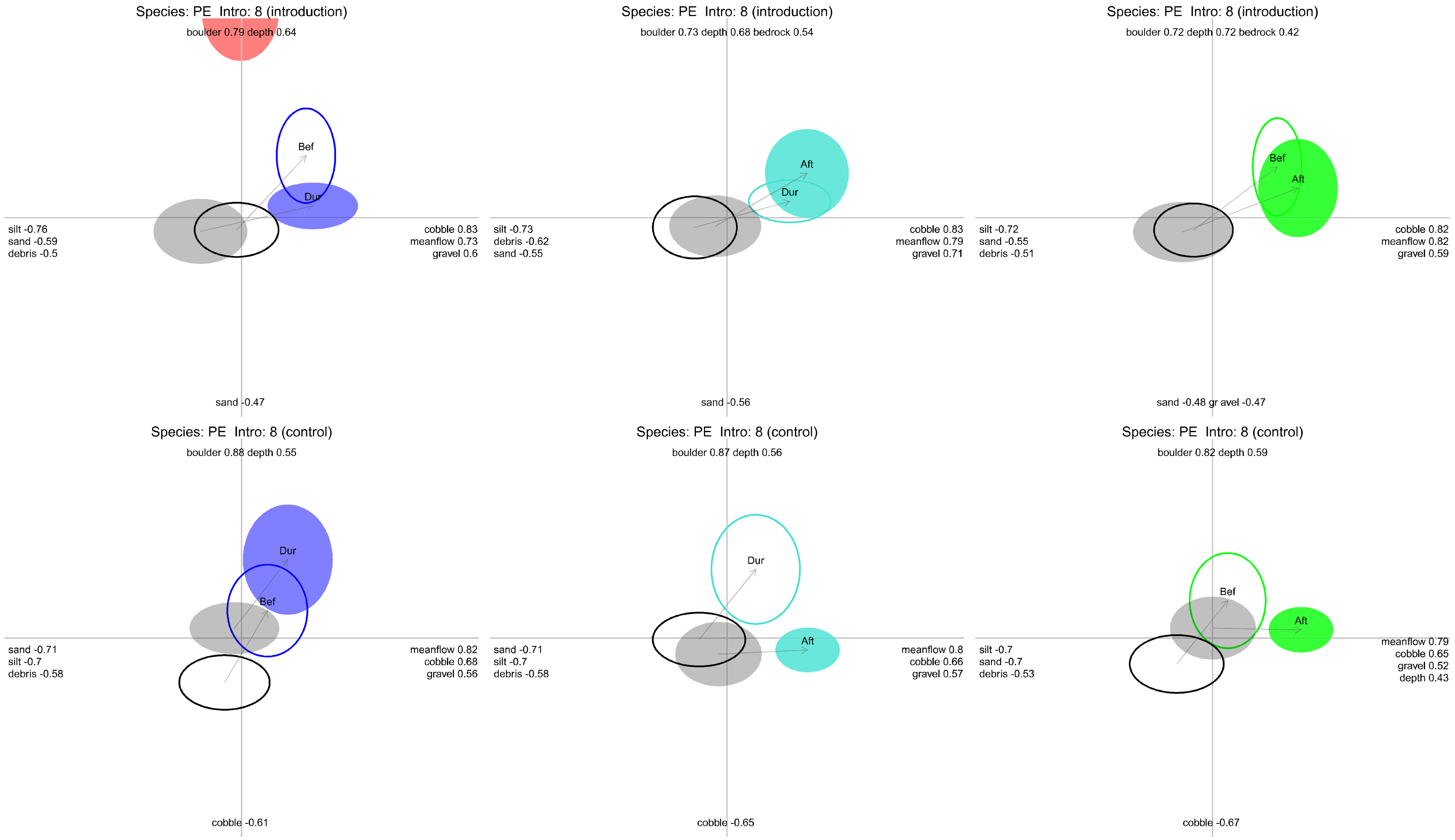
Recovery: None



Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

Response: Type II

Recovery: Partial



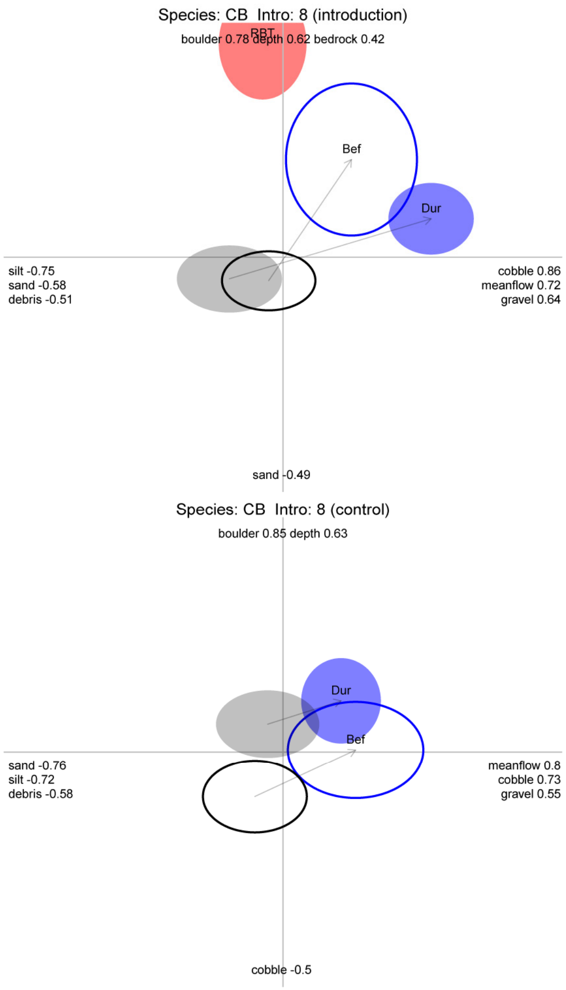
Comparison: Before vs During

Comparison: During vs After

Comparison: Before vs After

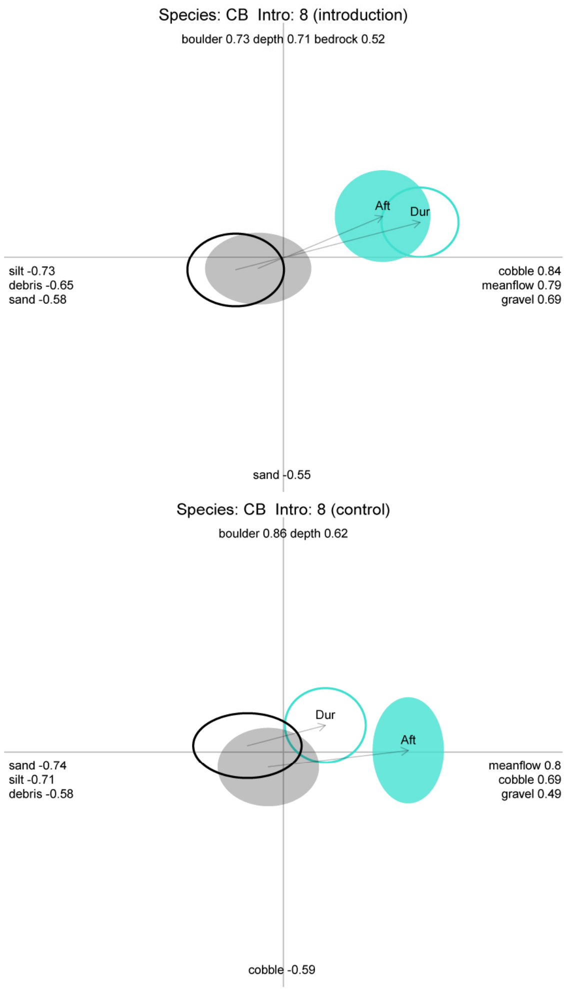
Response: Type II

Recovery: Full


Species: CB Intro: 8 (introduction)

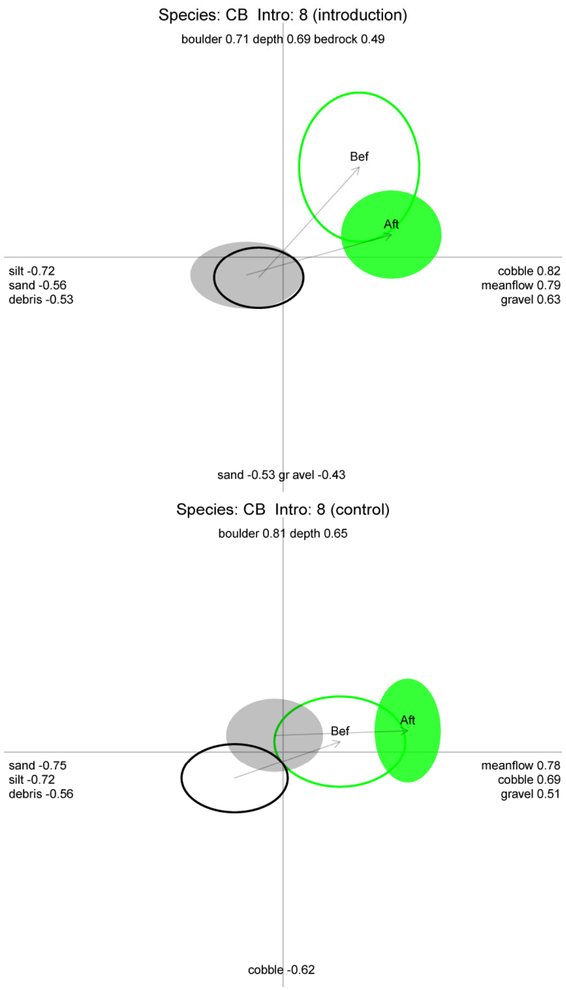
Species: CB Intro: 8 (control)

Comparison: Before vs During


Species: CB Intro: 8 (introduction)

Species: CB Intro: 8 (control)

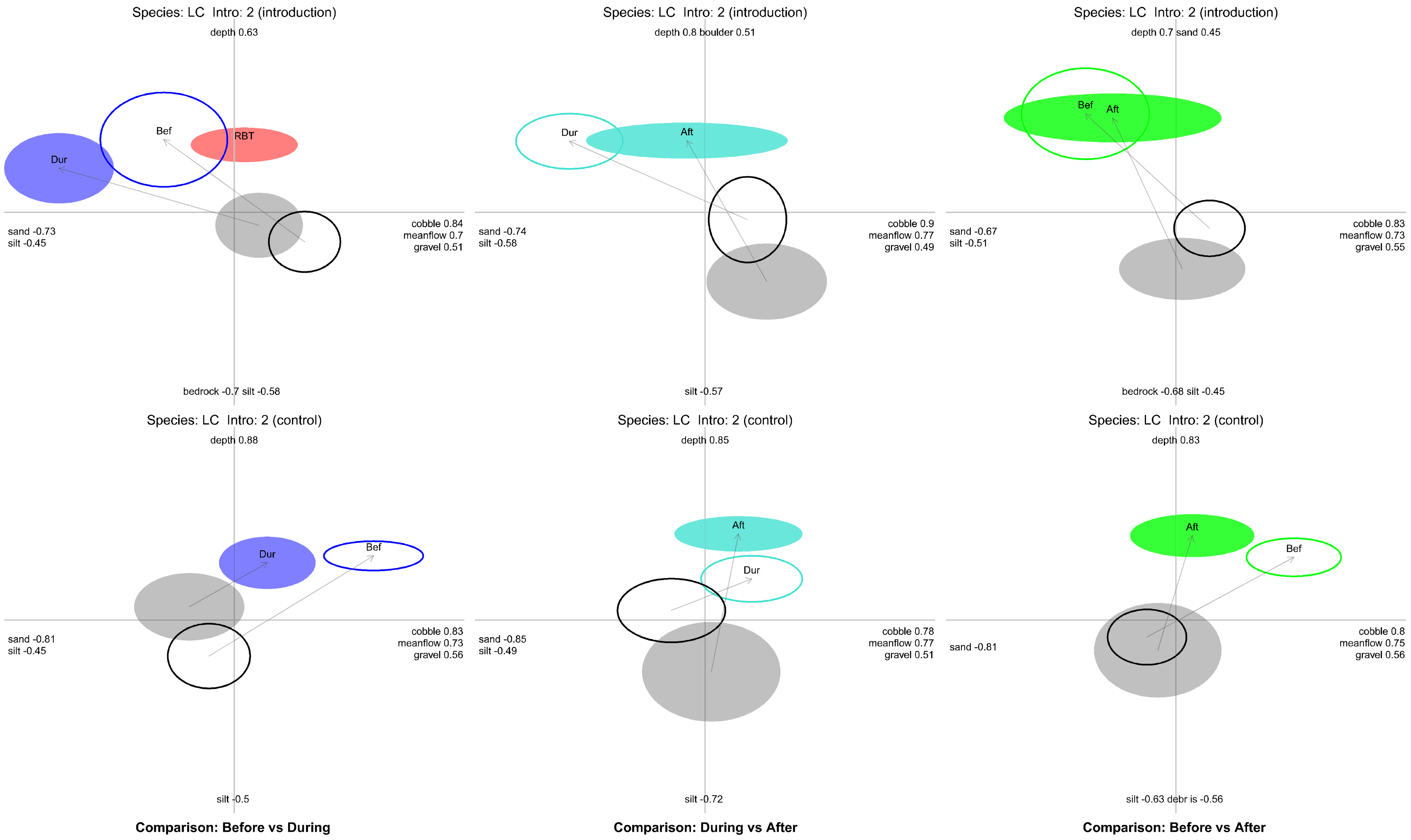
Comparison: During vs After


Species: CB Intro: 8 (introduction)

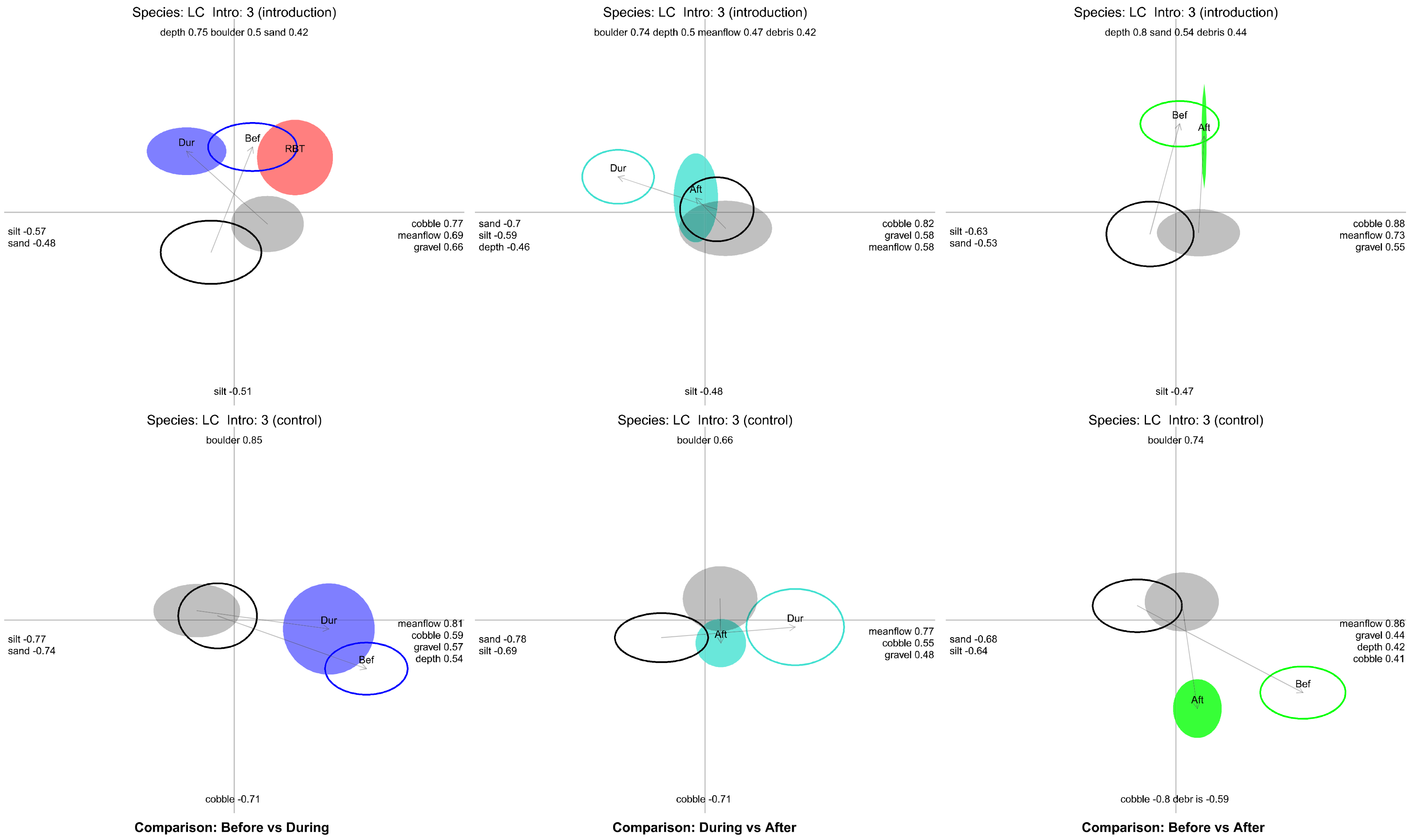
Species: CB Intro: 8 (control)

Comparison: Before vs After

Response: Type II
Recovery: Partial



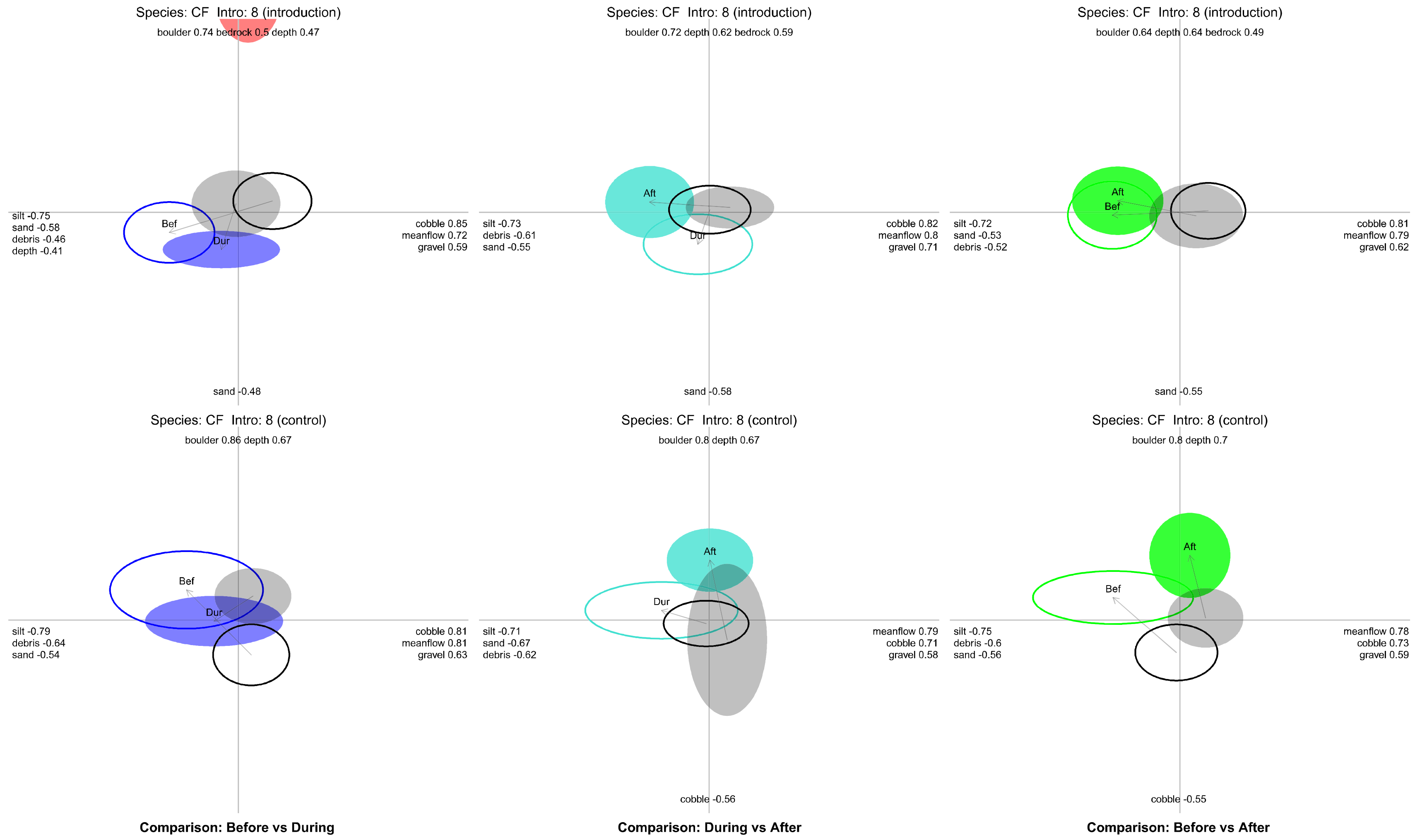
Response: Uninterpretable
Recovery: N/A



Response: Uninterpretable

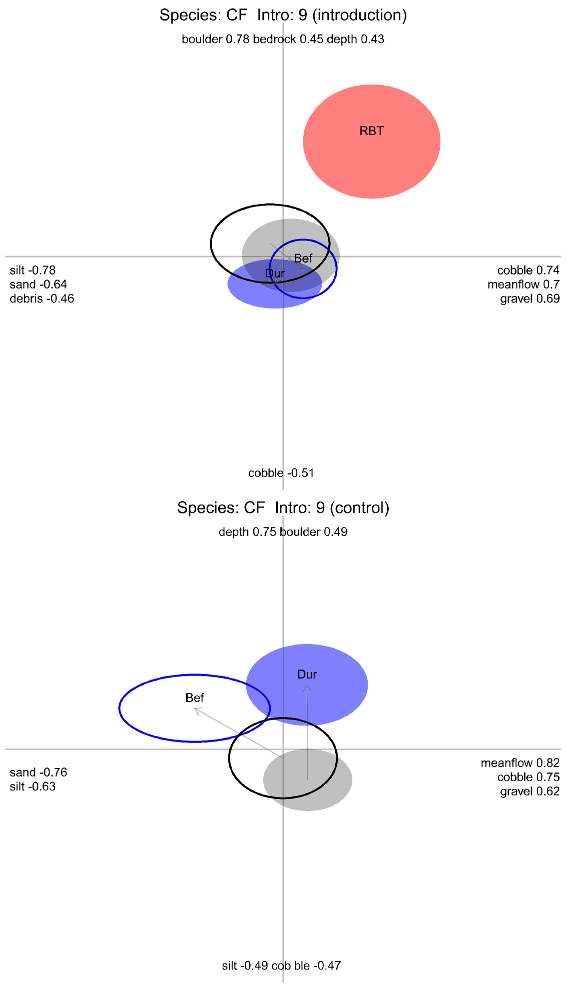
Recovery: N/A

Response: Uninterpretable
Recovery: N/A

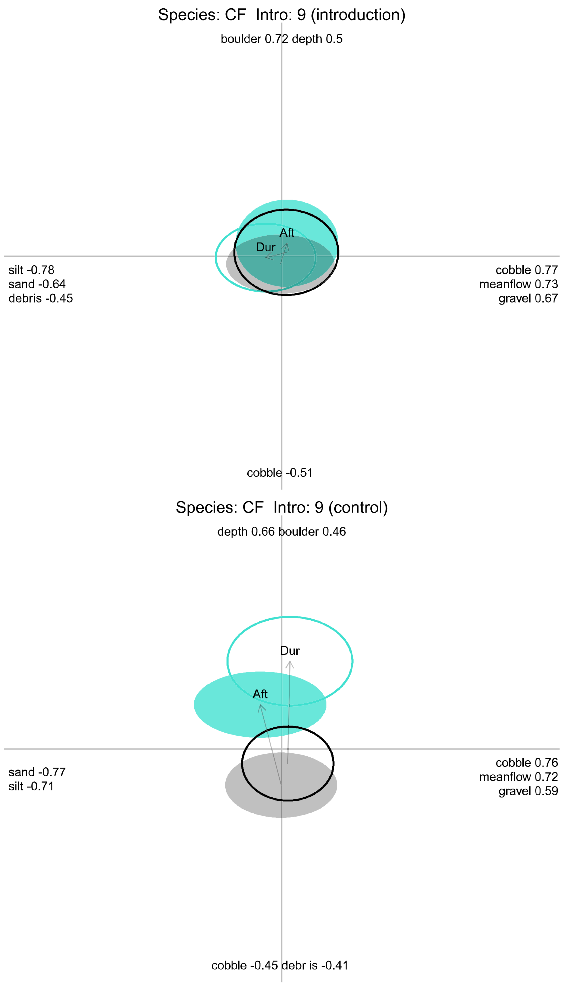


Response: Uninterpretable

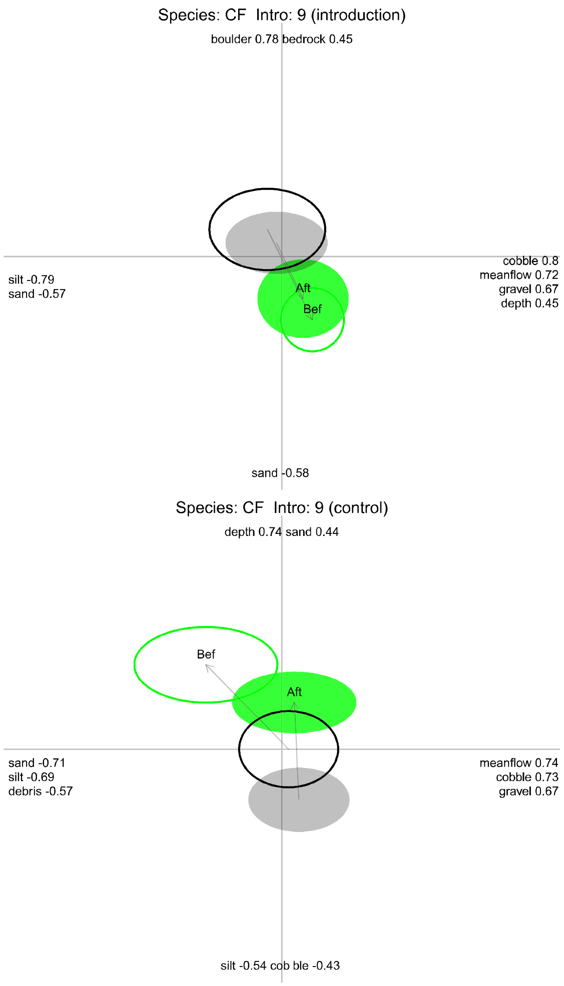
Recovery: N/A



Comparison: Before vs During

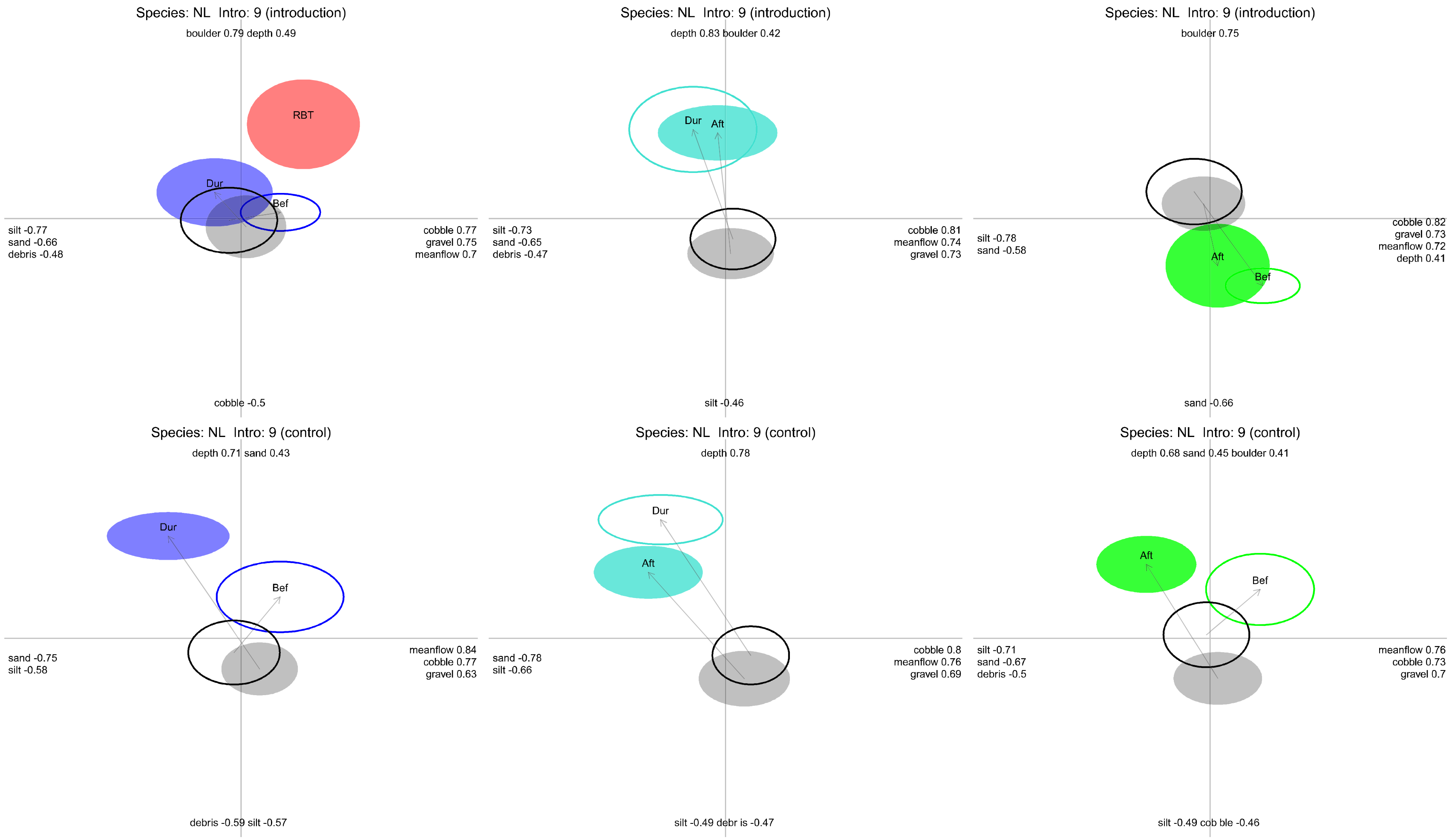


Comparison: During vs After



Comparison: Before vs After

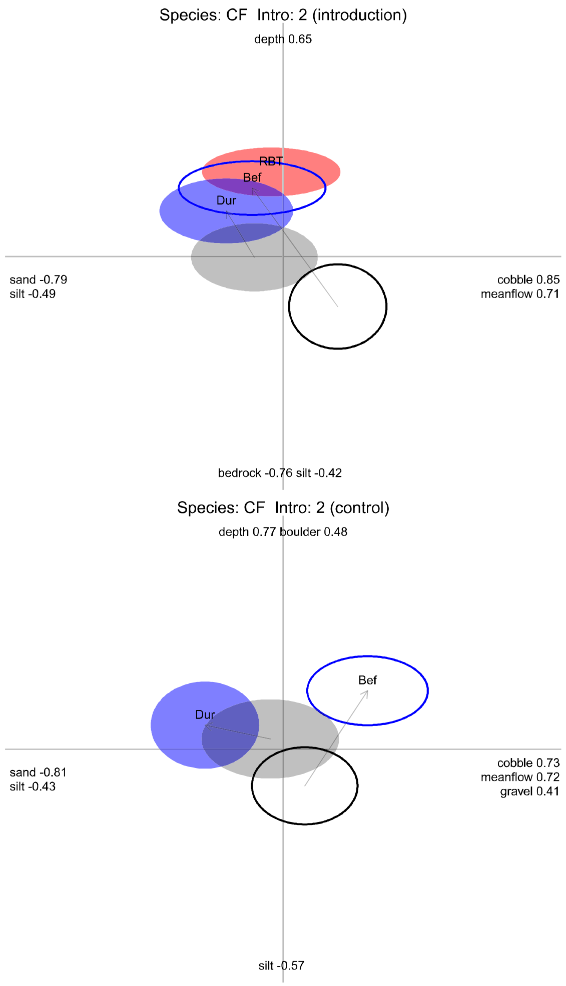
Response: Uninterpretable
Recovery: N/A



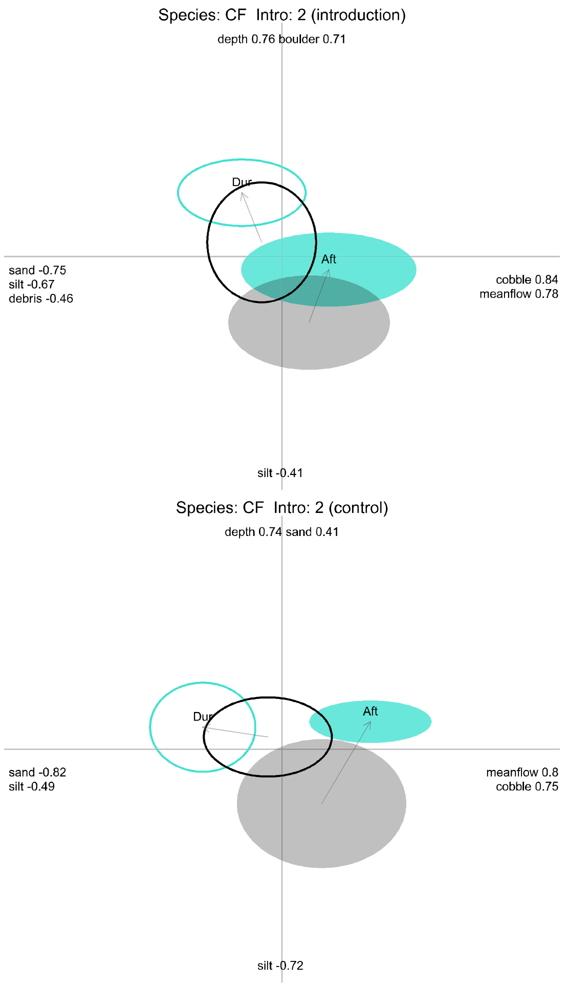
Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

Response: Uninterpretable

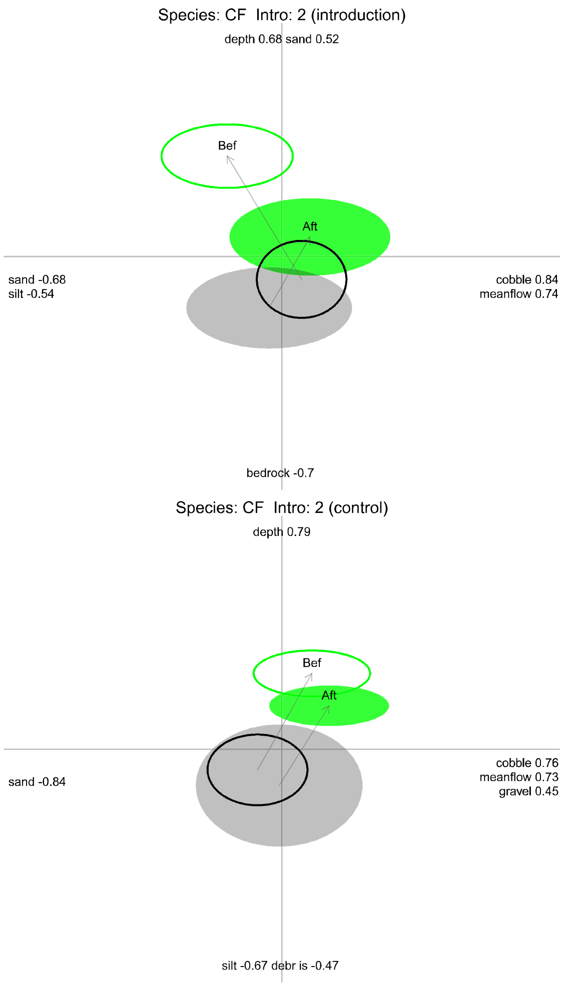
Recovery: N/A



Comparison: Before vs During



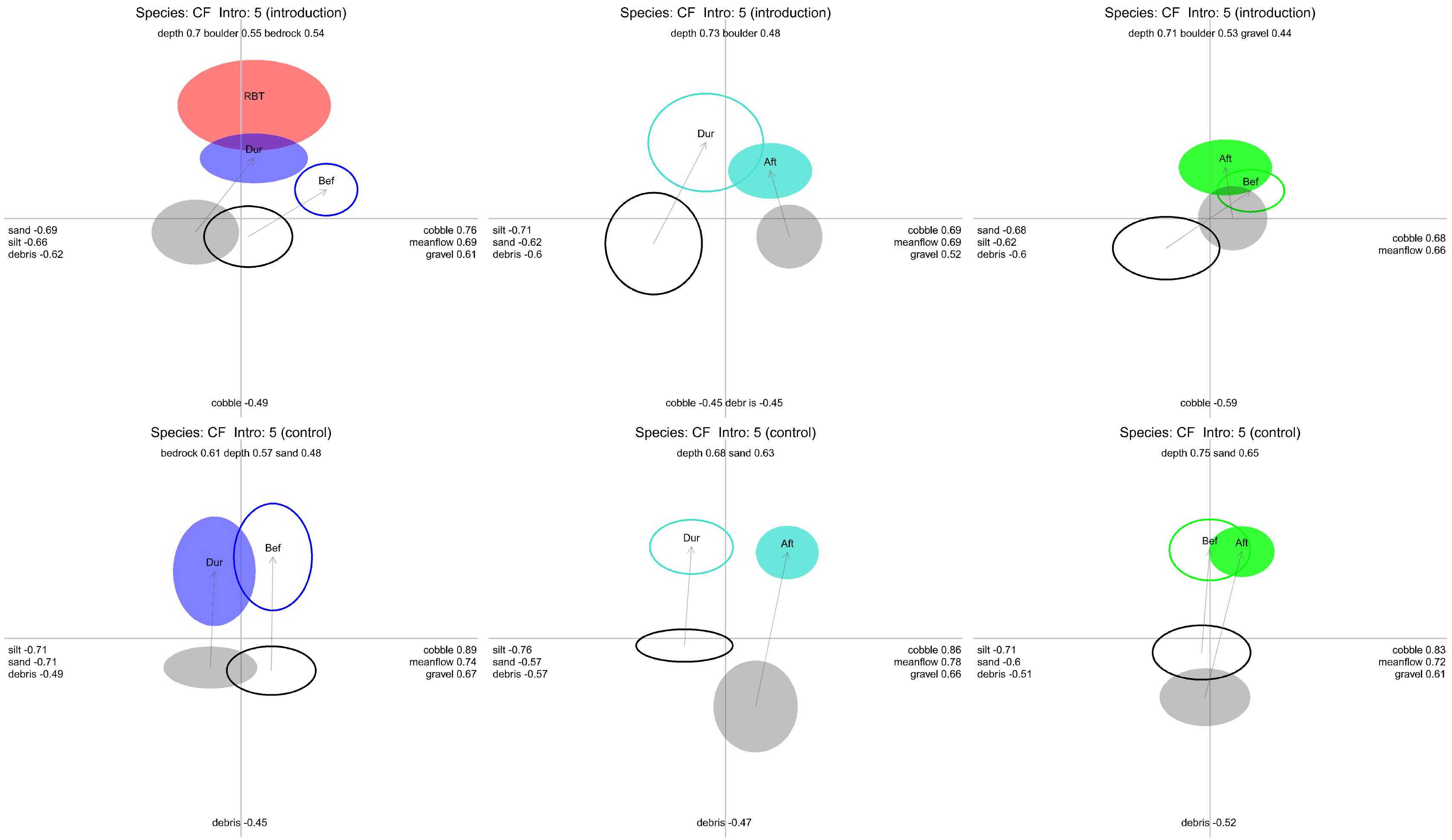
Comparison: During vs After



Comparison: Before vs After

Response: None

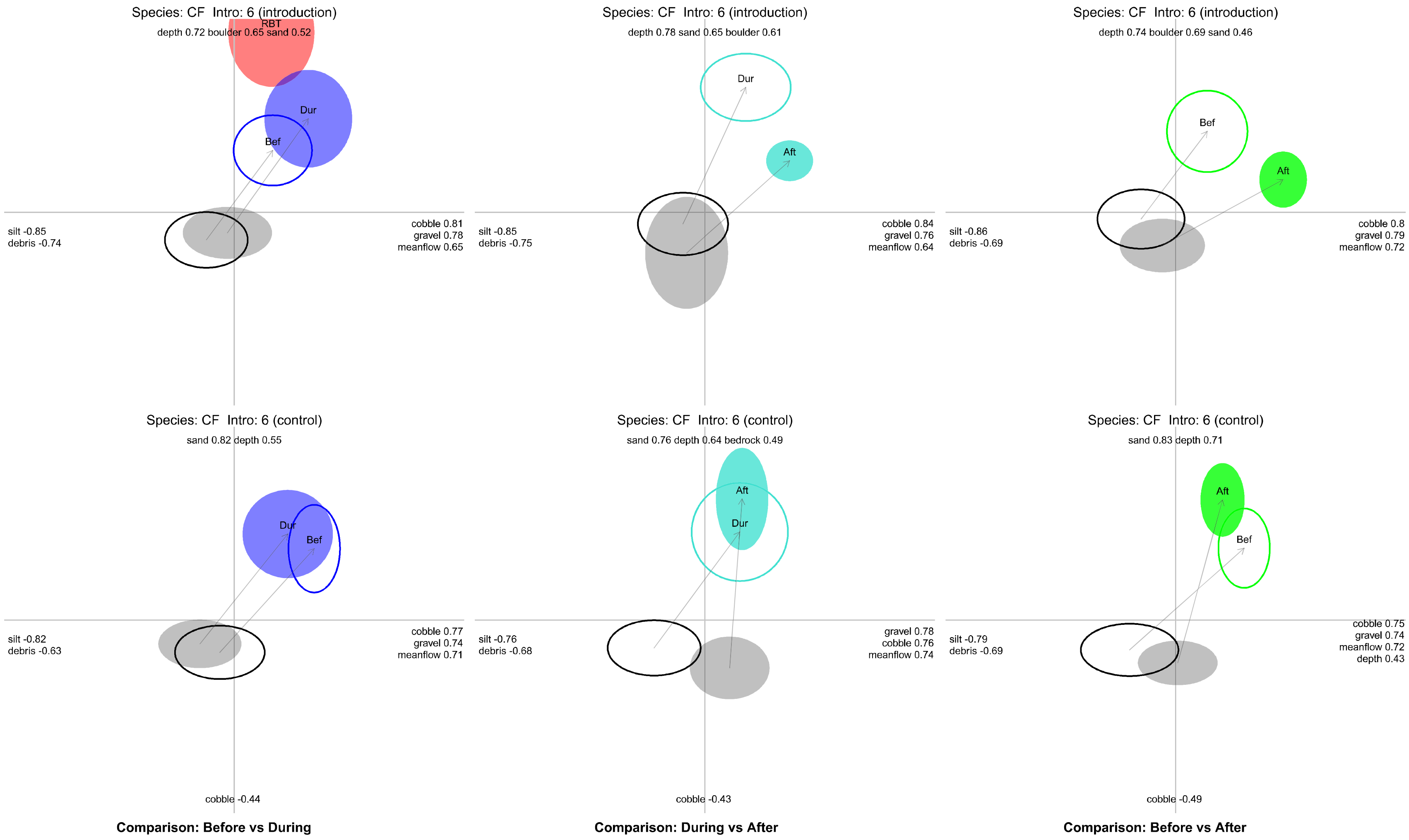
Recovery: N/A



Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

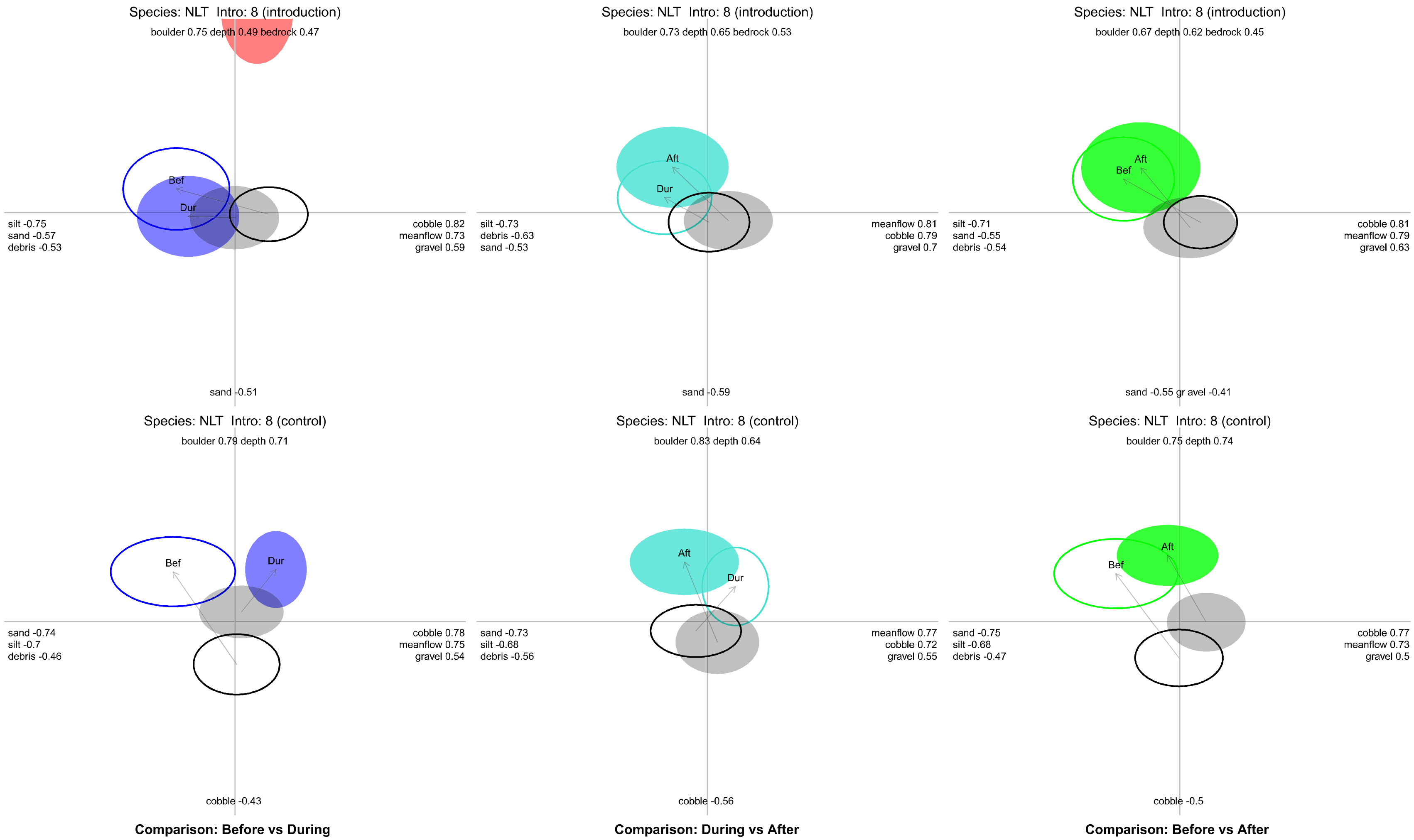
Response: None

Recovery: N/A



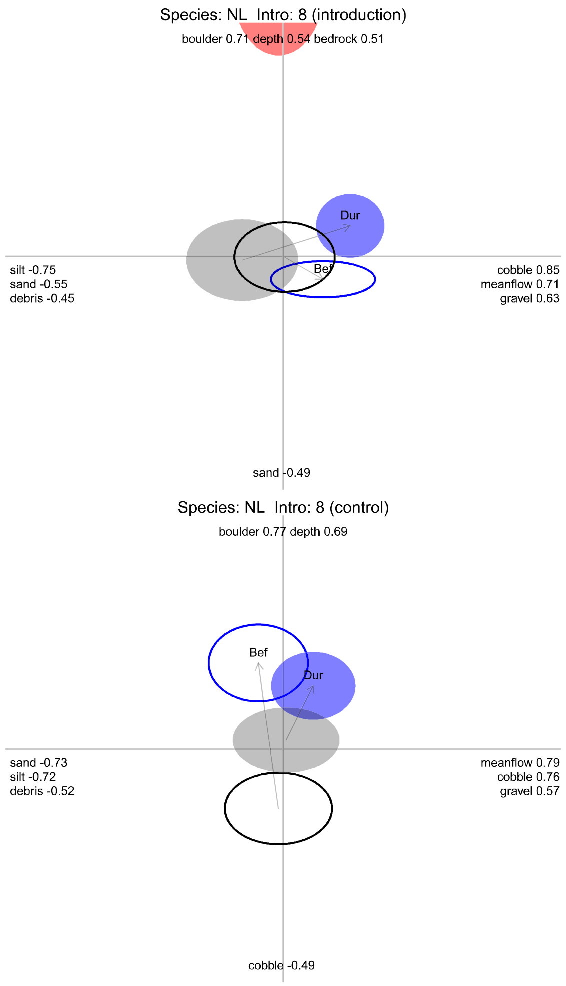
Response: None

Recovery: N/A

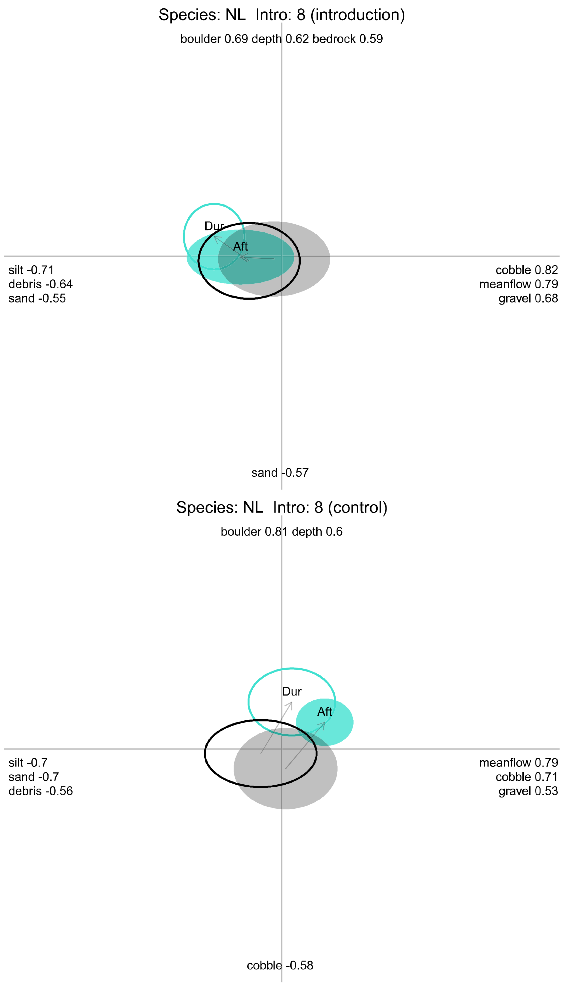


Response: None

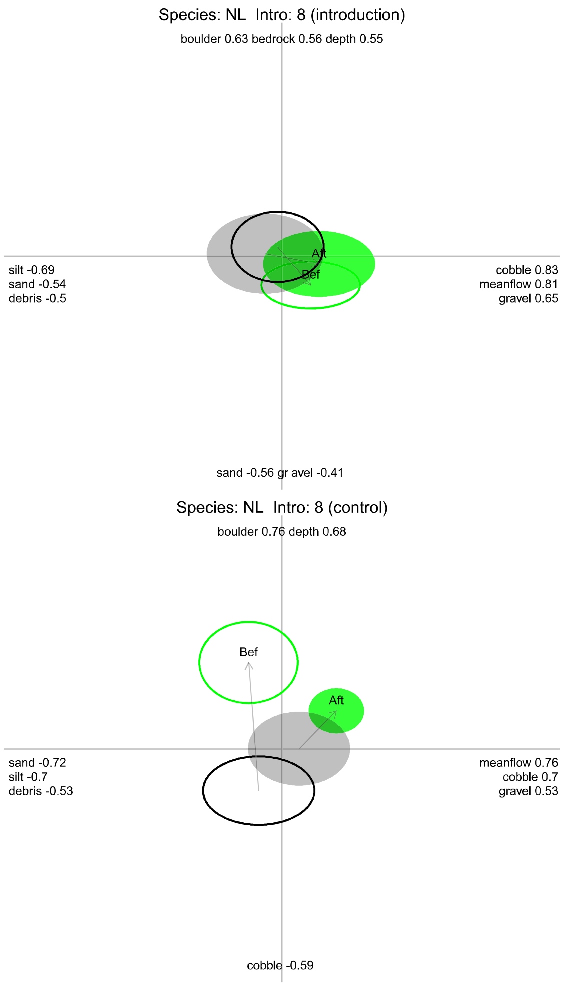
Recovery: N/A



Comparison: Before vs During



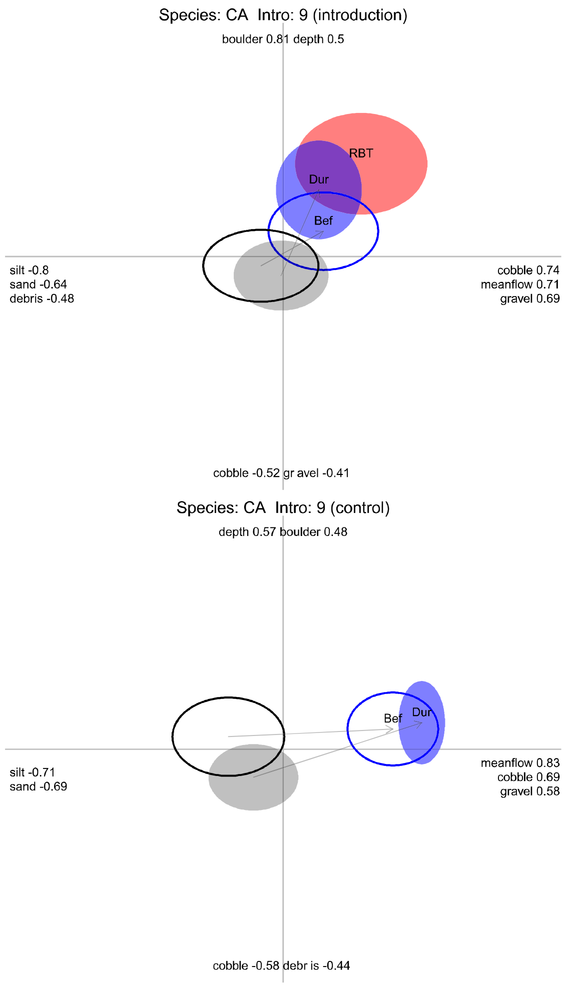
Comparison: During vs After



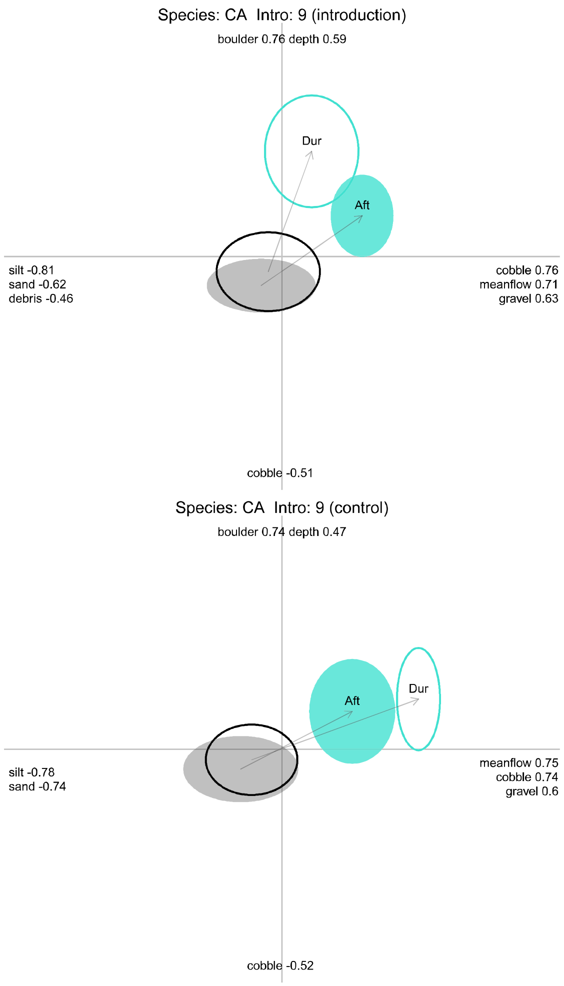
Comparison: Before vs After

Response: None

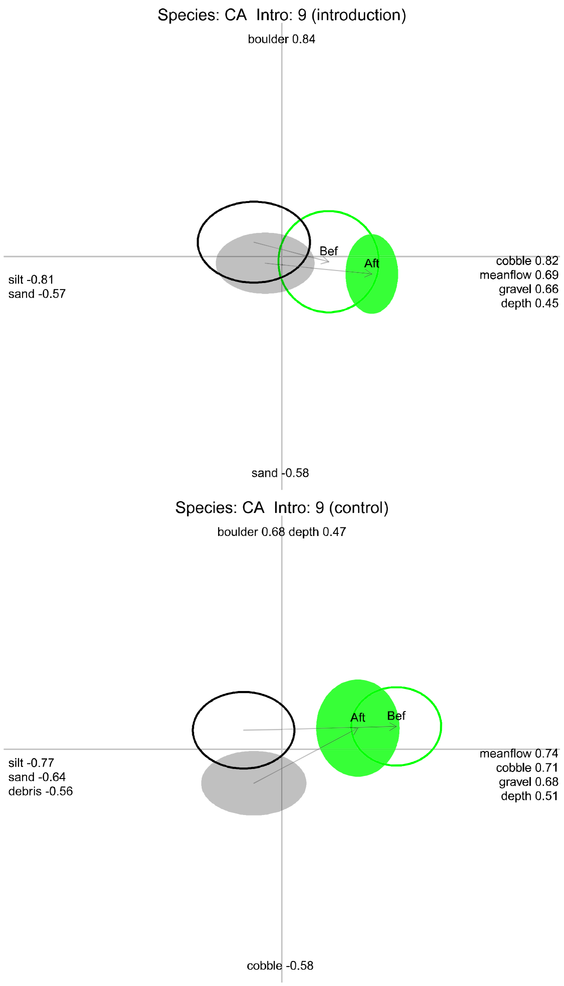
Recovery: N/A



Comparison: Before vs During



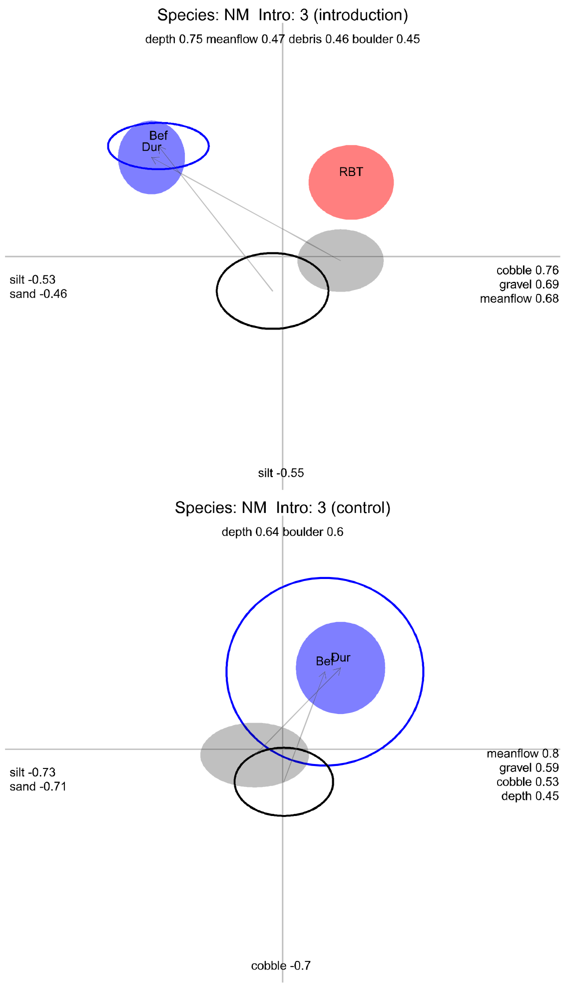
Comparison: During vs After



Comparison: Before vs After

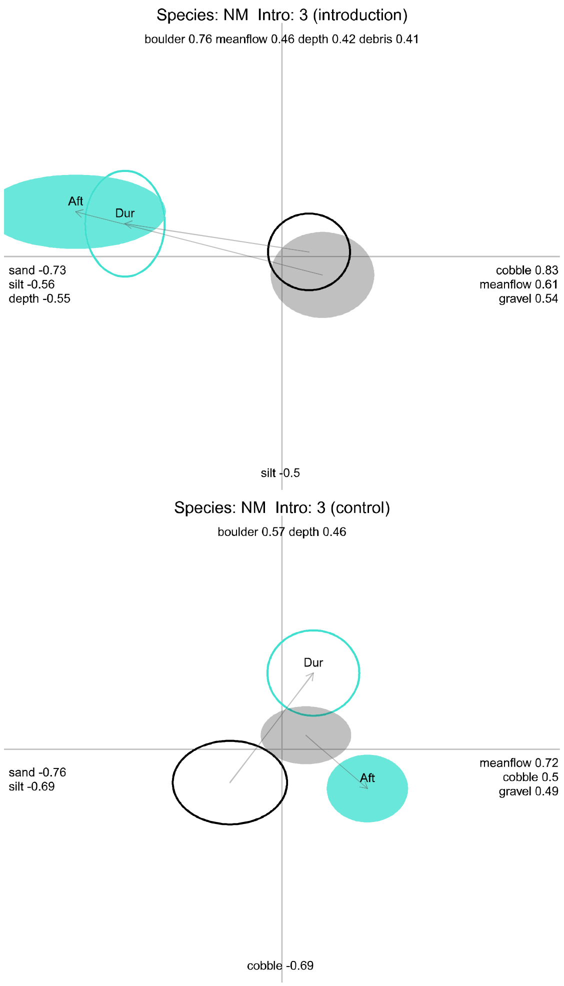
Response: None

Recovery: N/A


Species: NM Intro: 3 (introduction)

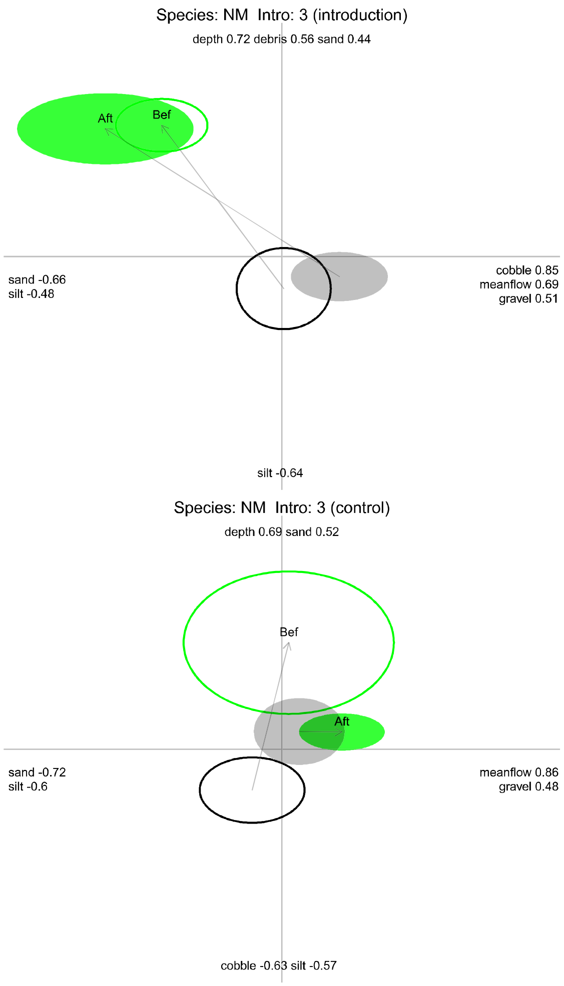
Species: NM Intro: 3 (control)

Comparison: Before vs During


Species: NM Intro: 3 (introduction)

Species: NM Intro: 3 (control)

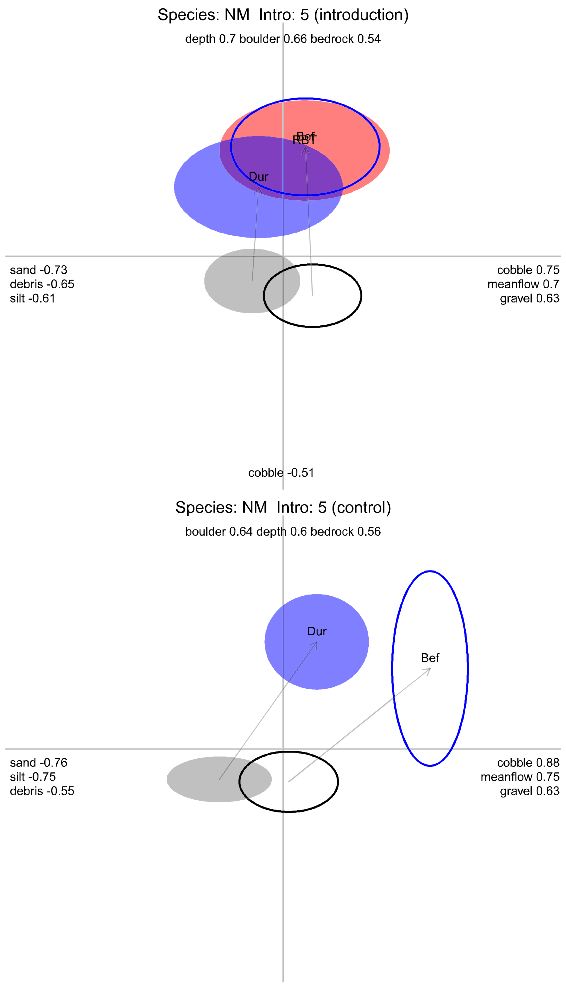
Comparison: During vs After


Species: NM Intro: 3 (introduction)

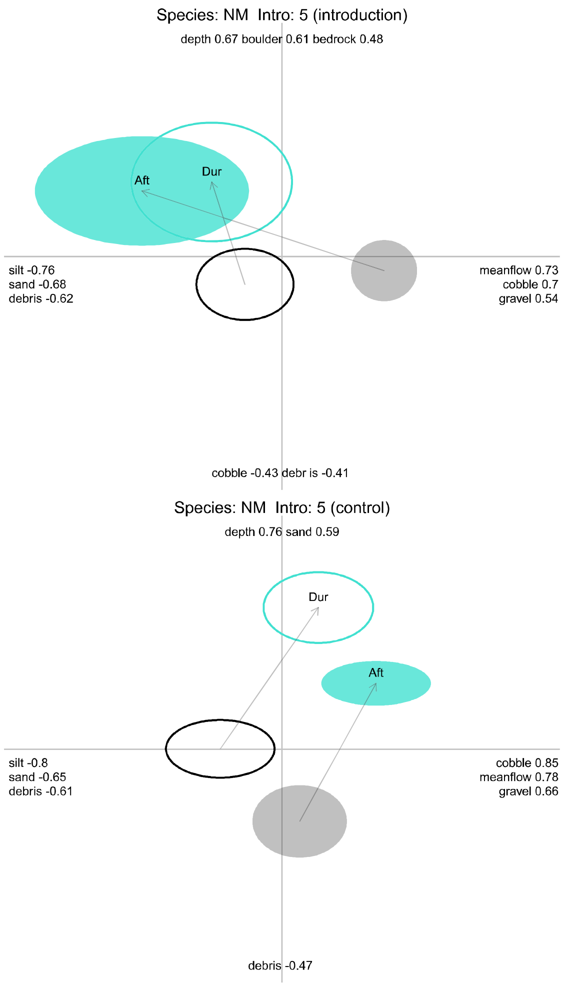
Species: NM Intro: 3 (control)

Comparison: Before vs After

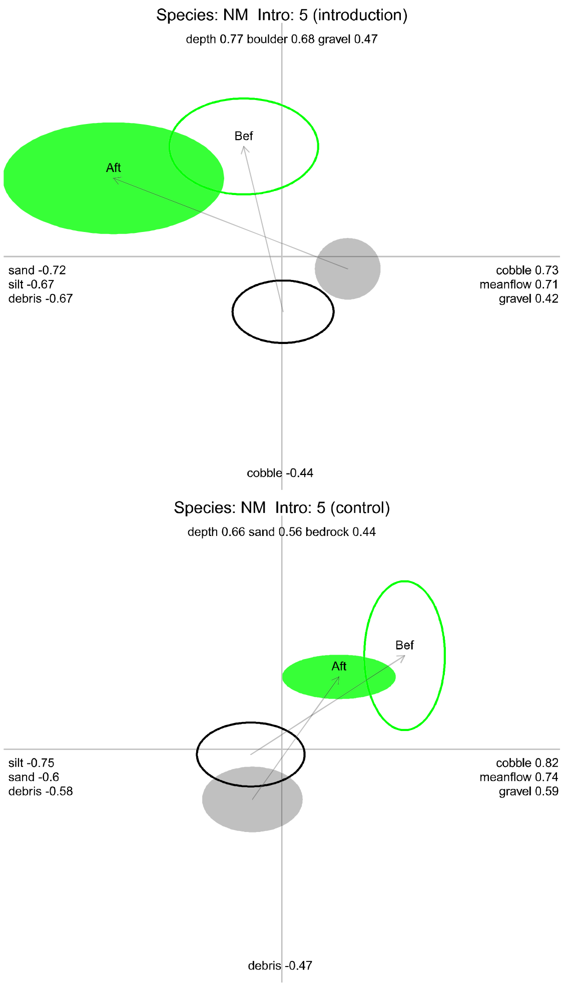
Response: None
Recovery: N/A



Comparison: Before vs During



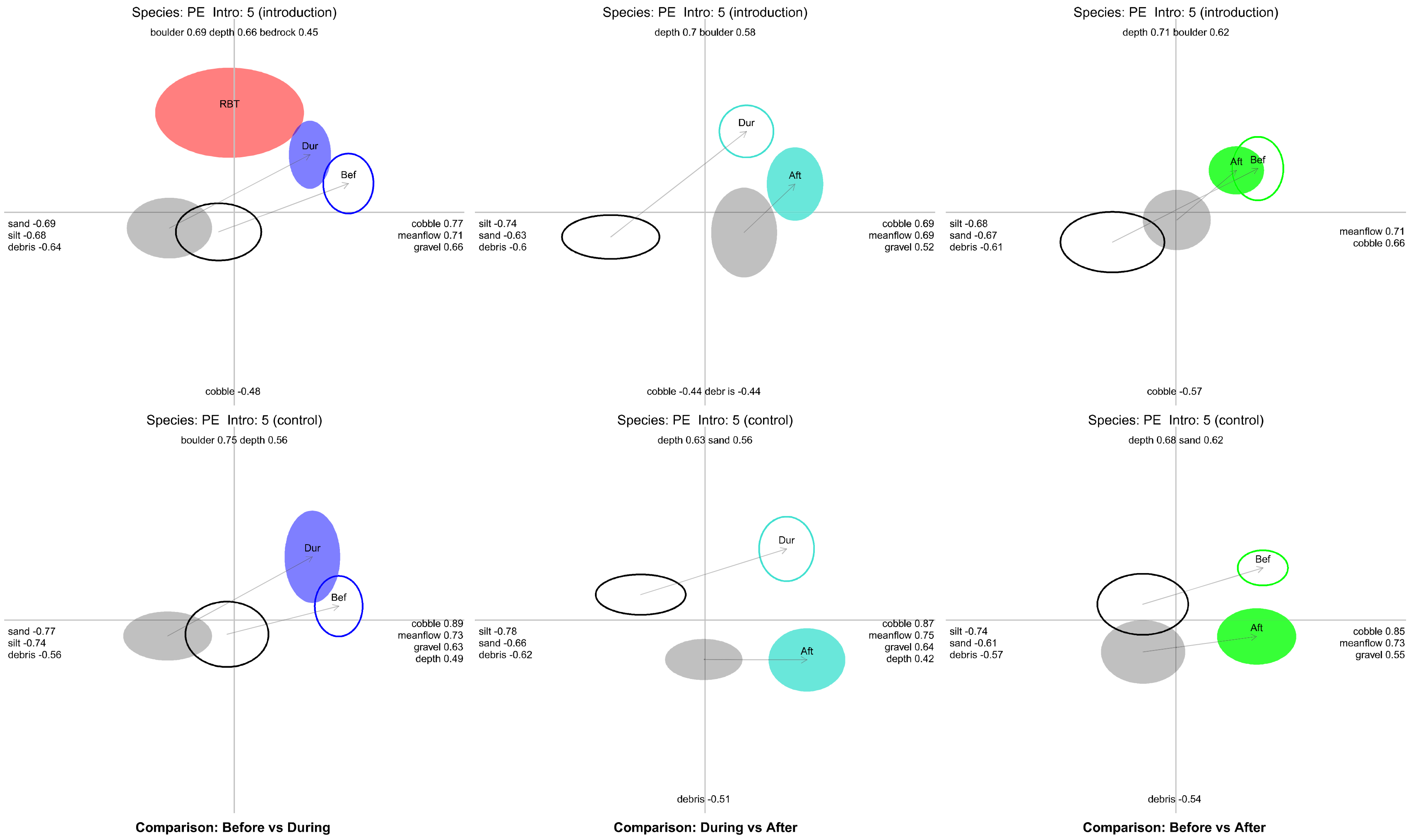
Comparison: During vs After



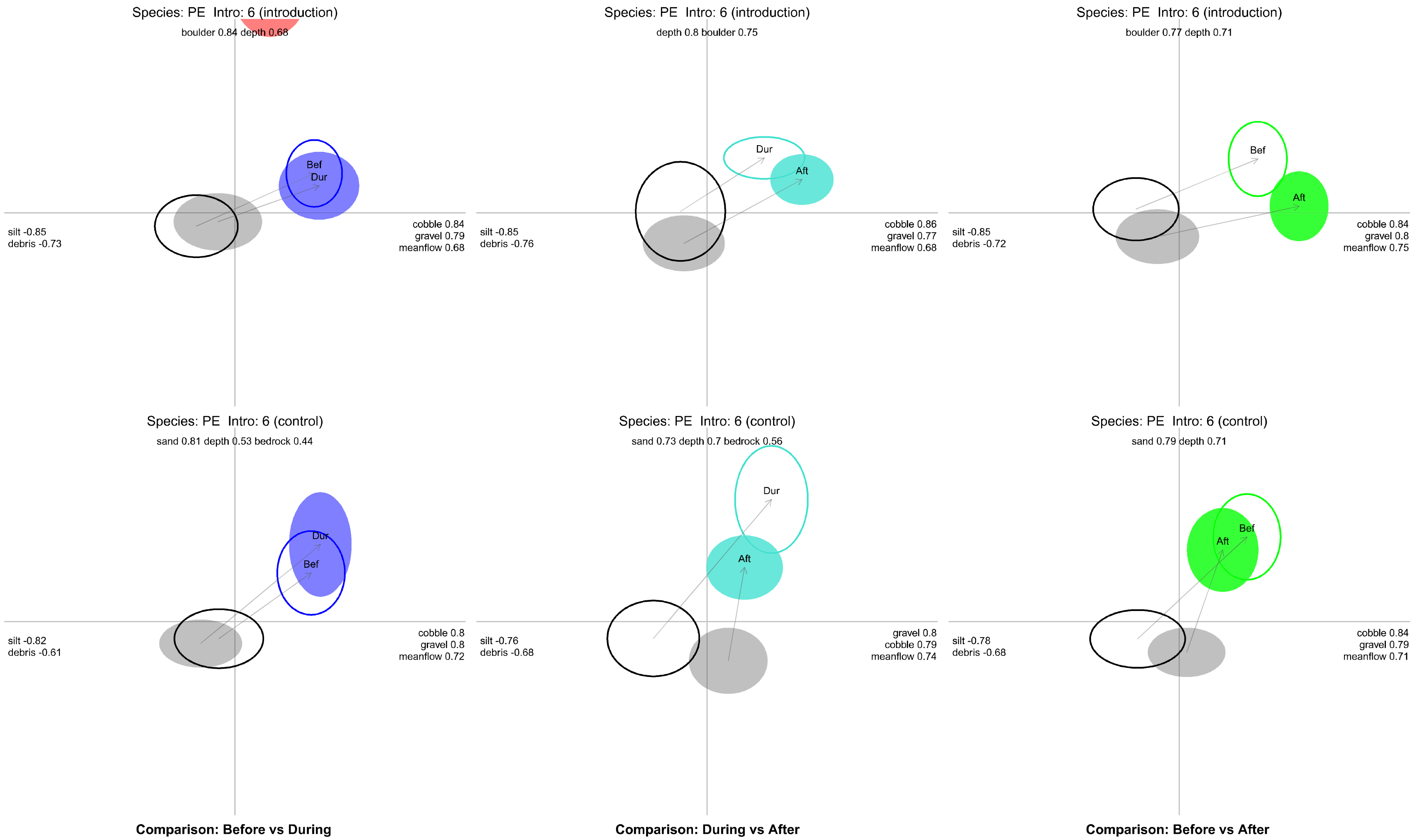
Comparison: Before vs After

Response: None

Recovery: N/A

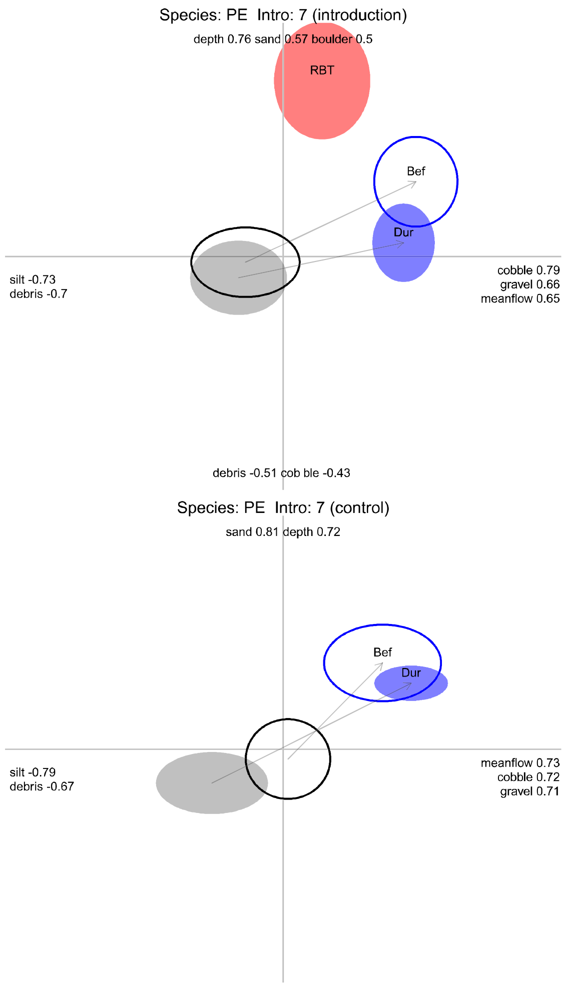


Response: None
Recovery: N/A

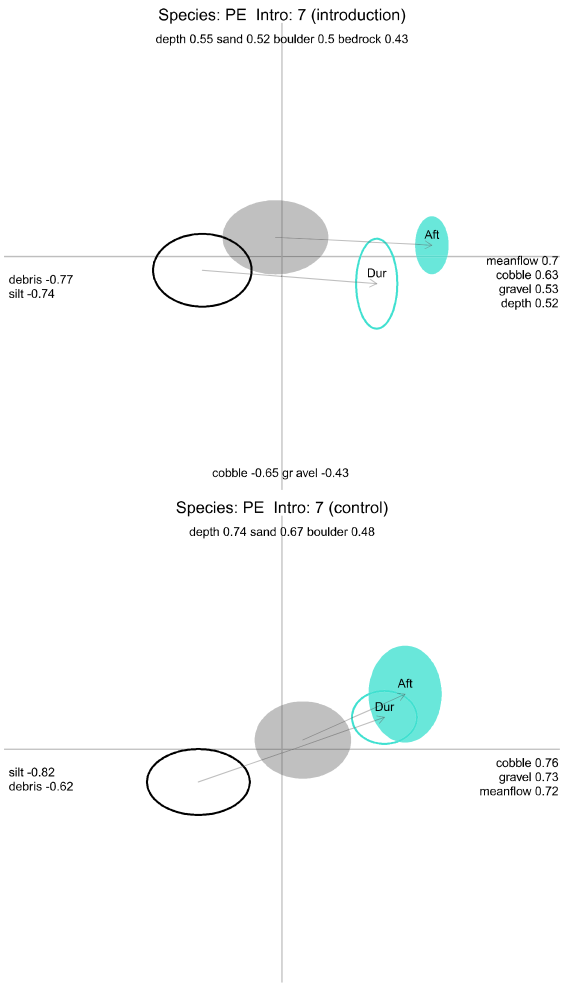


Response: None

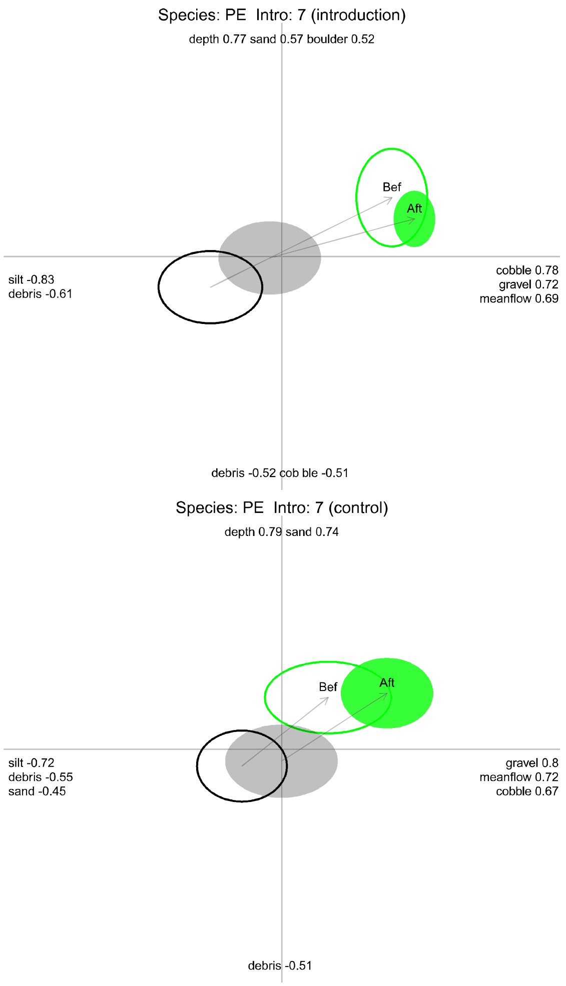
Recovery: N/A



Comparison: Before vs During

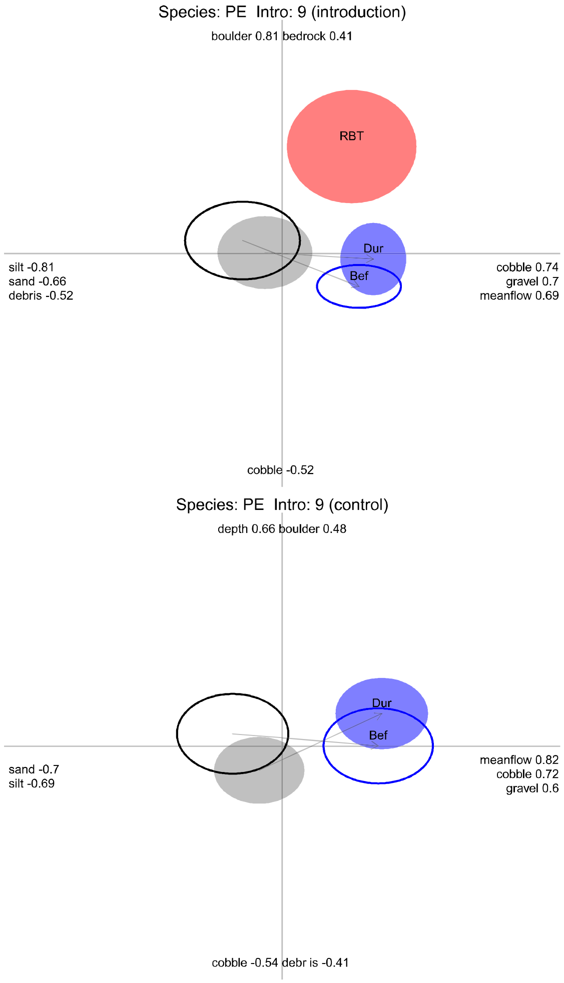


Comparison: During vs After

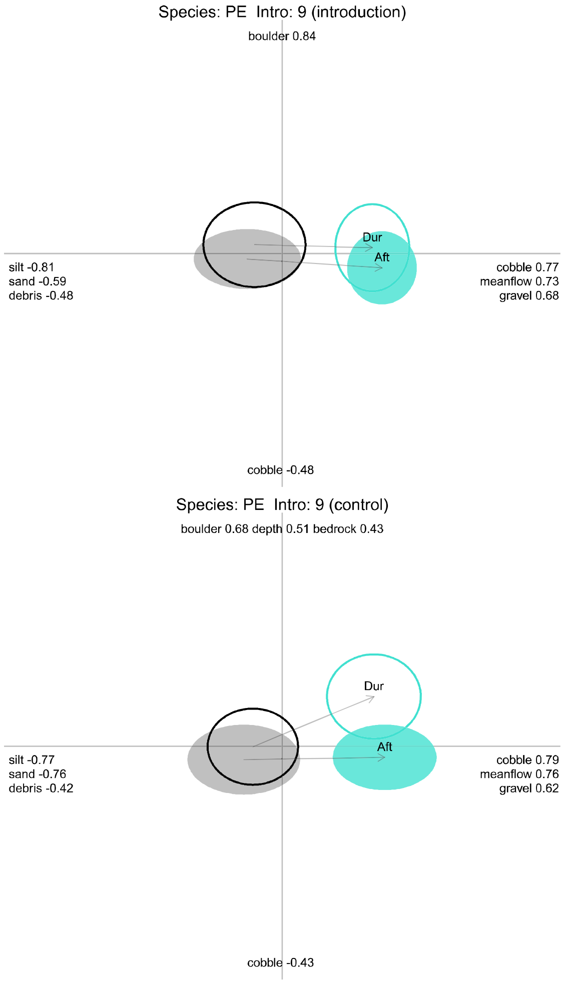


Comparison: Before vs After

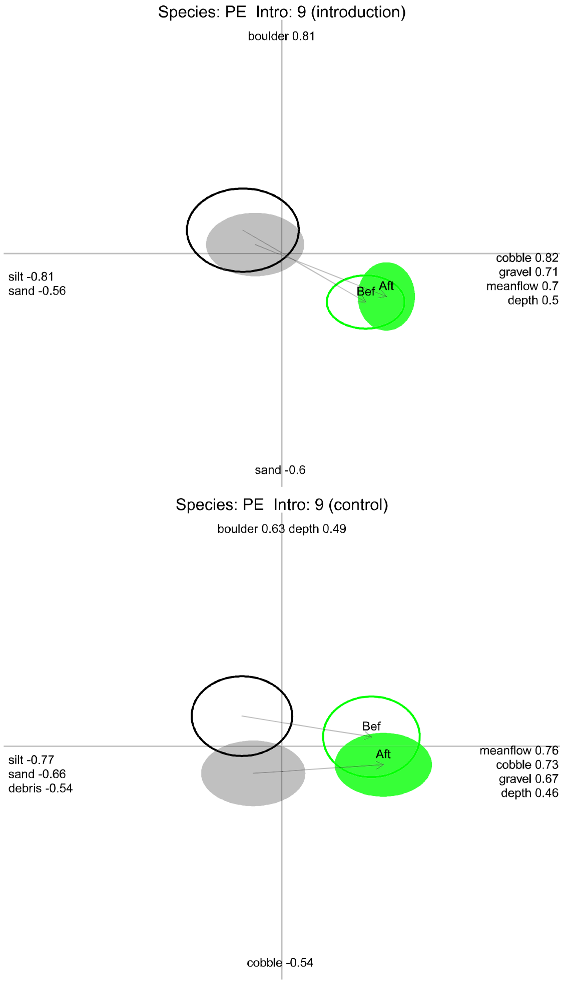
Response: None
Recovery: N/A



Comparison: Before vs During

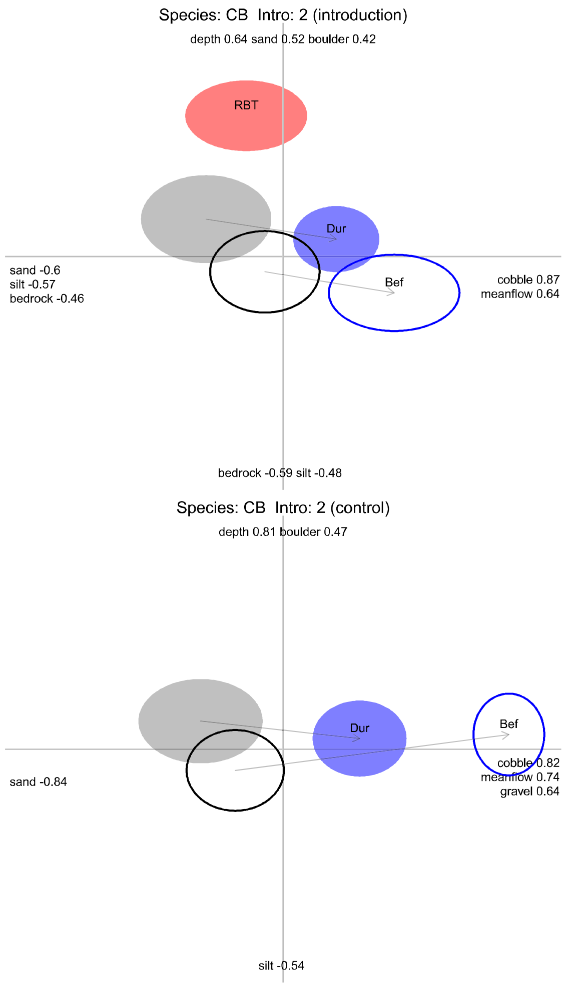


Comparison: During vs After

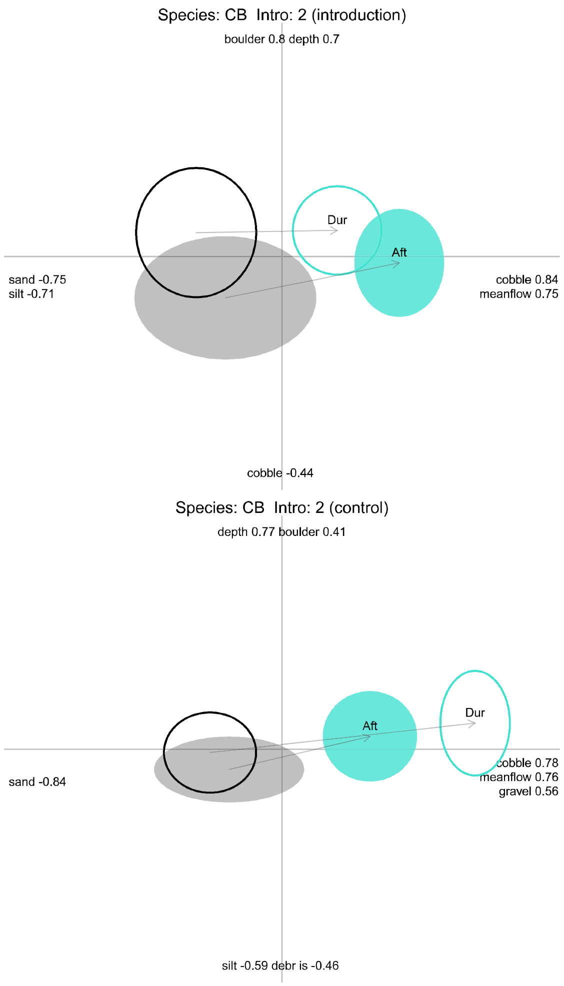


Comparison: Before vs After

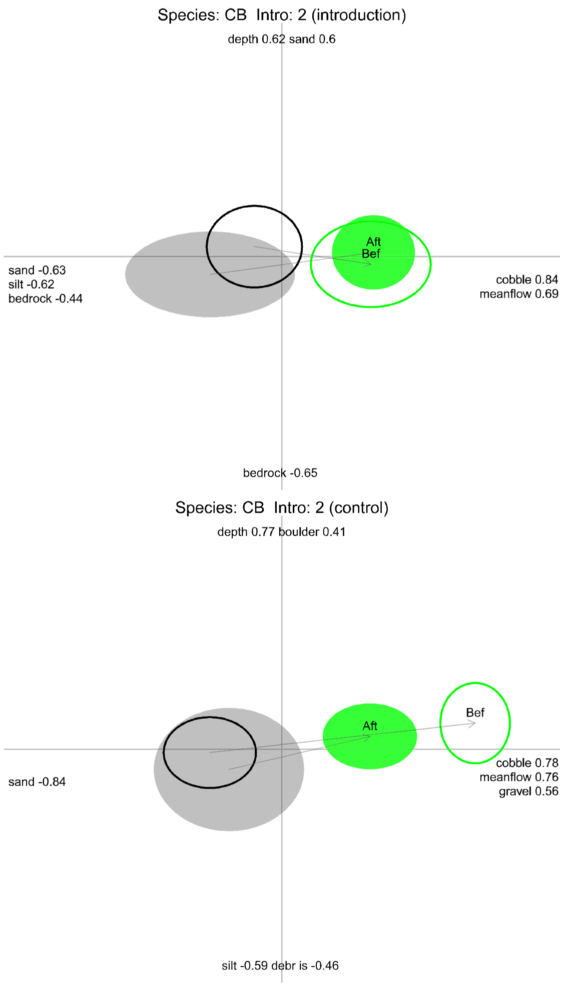
Response: None
Recovery: N/A



Comparison: Before vs During



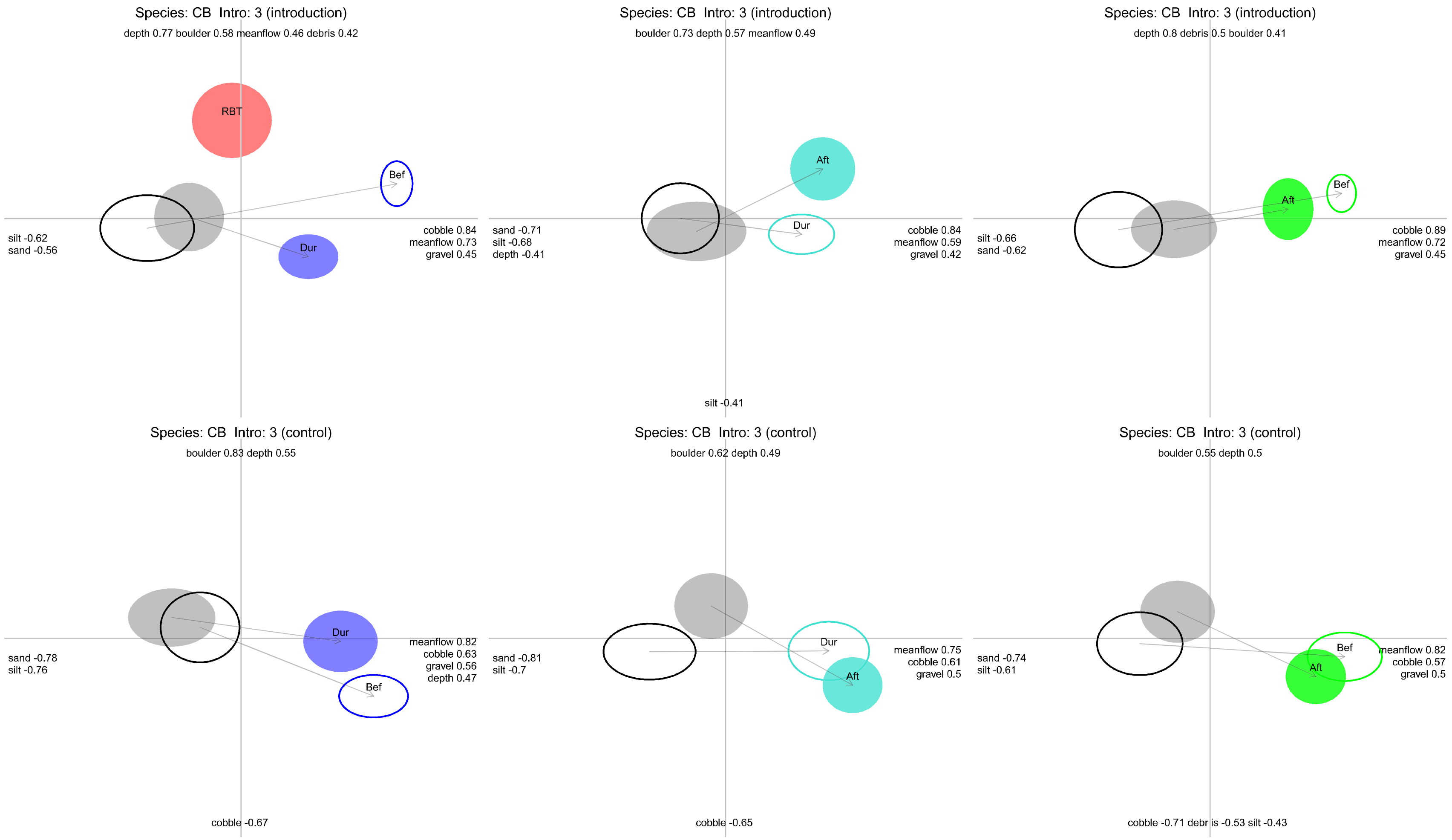
Comparison: During vs After



Comparison: Before vs After

Response: None

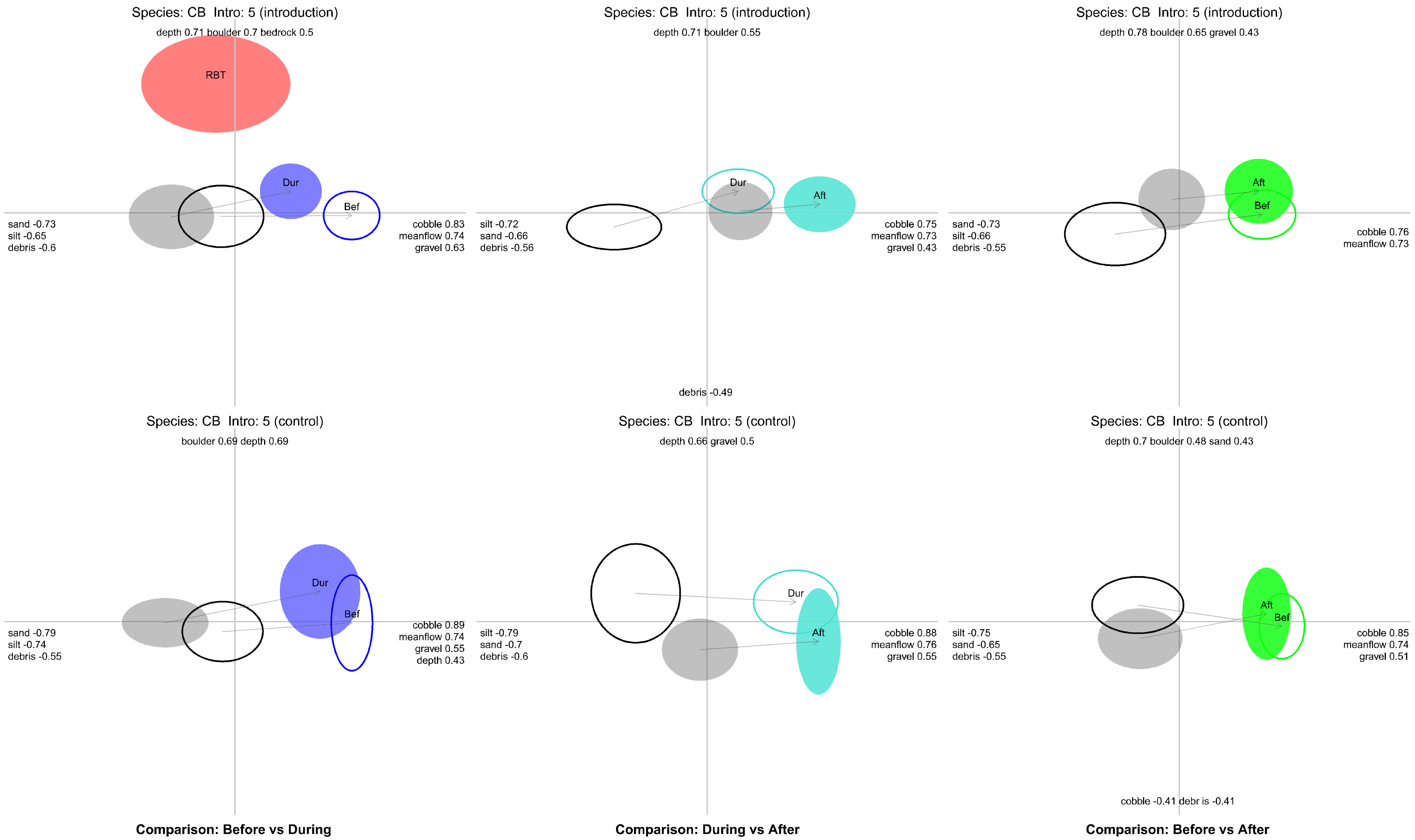
Recovery: N/A



Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

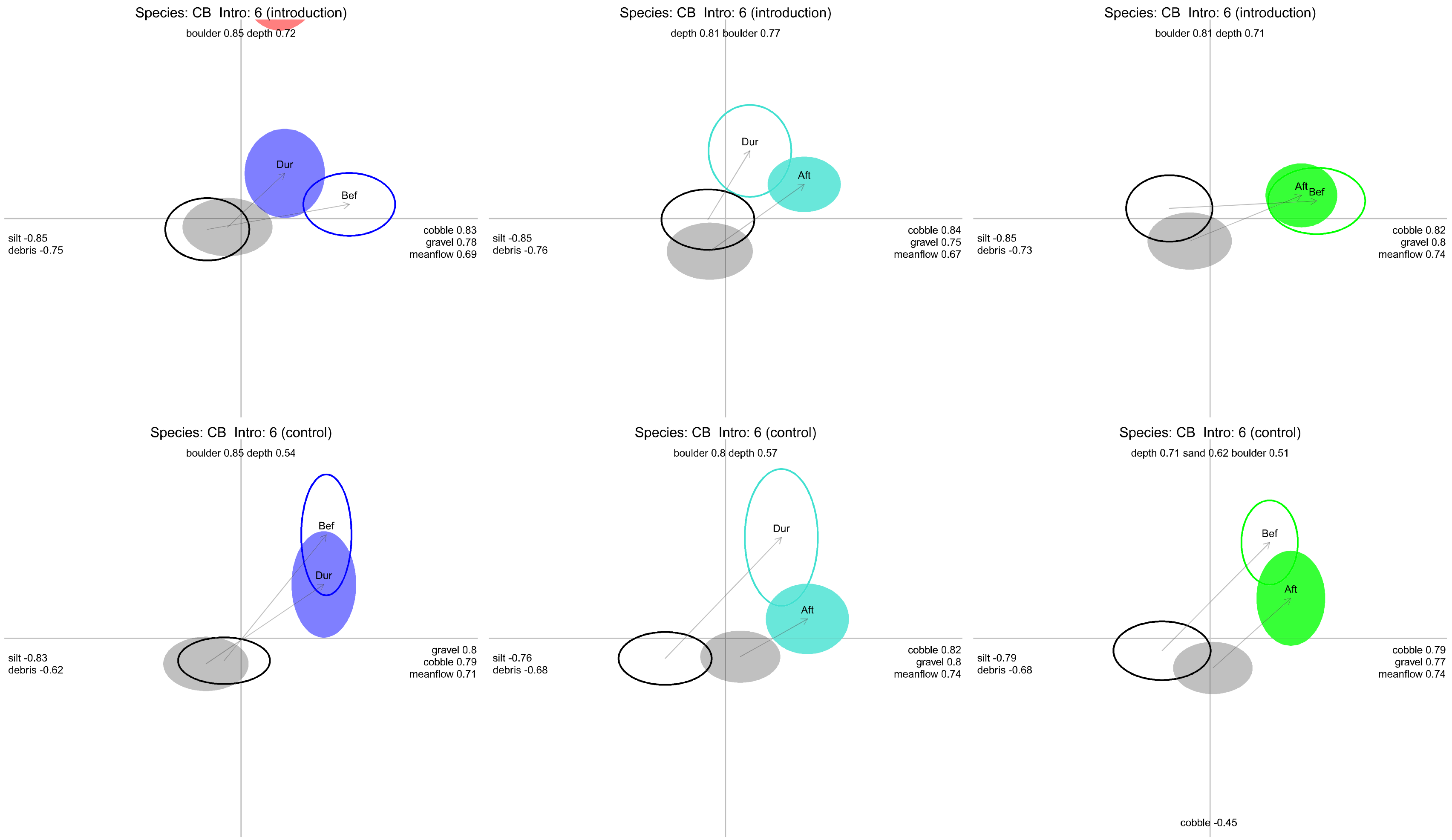
Response: None

Recovery: N/A



Response: None

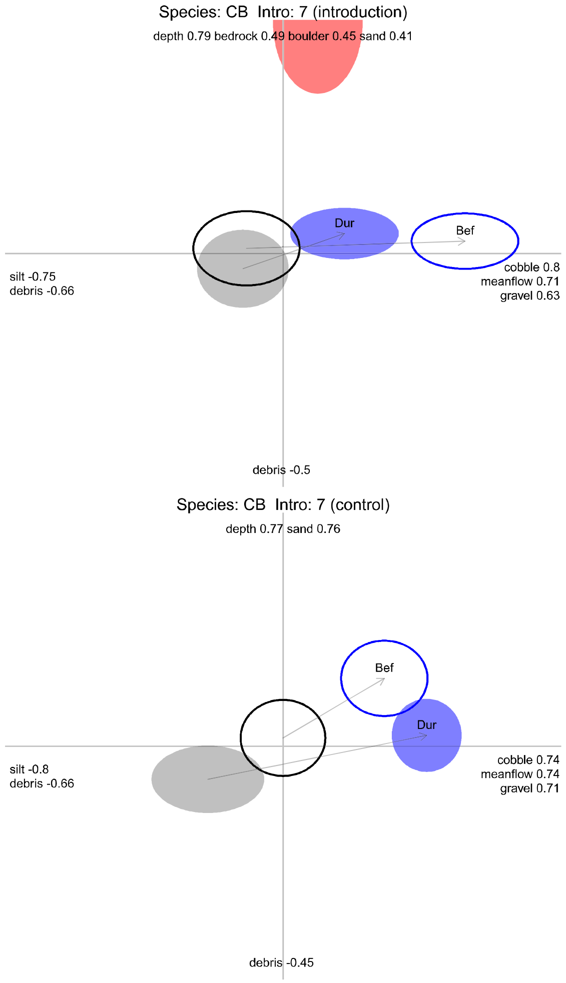
Recovery: N/A



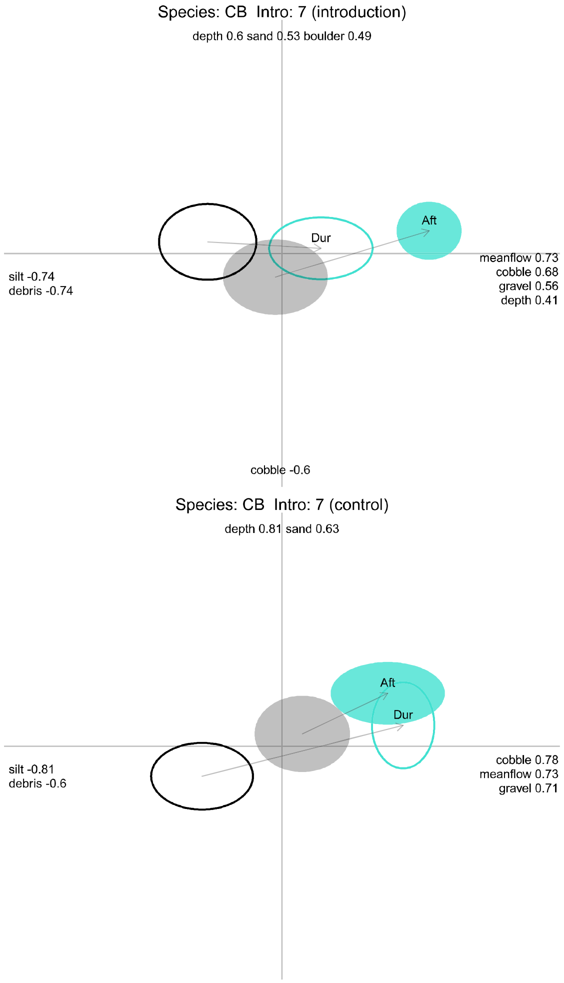
Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

Response: None

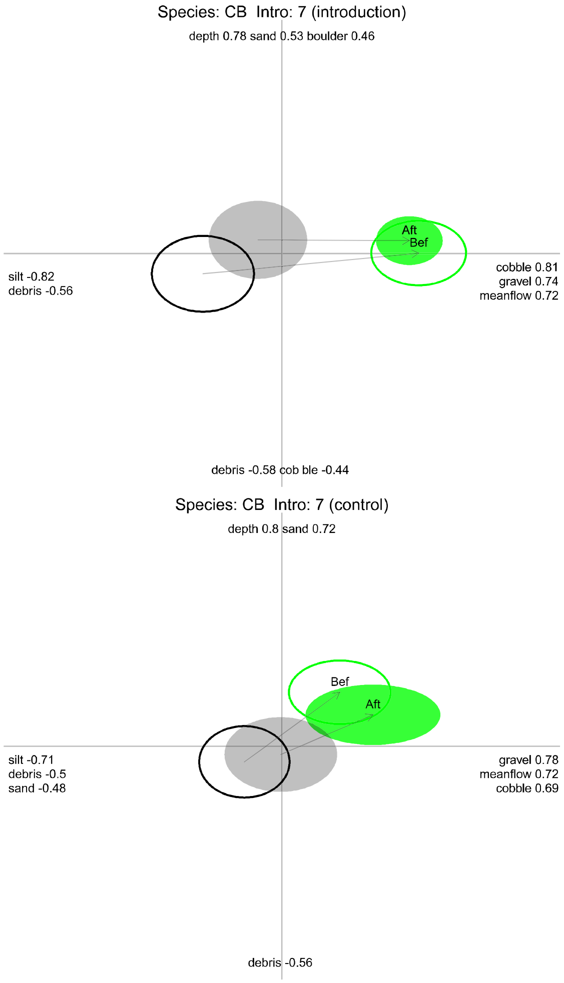
Recovery: N/A



Comparison: Before vs During

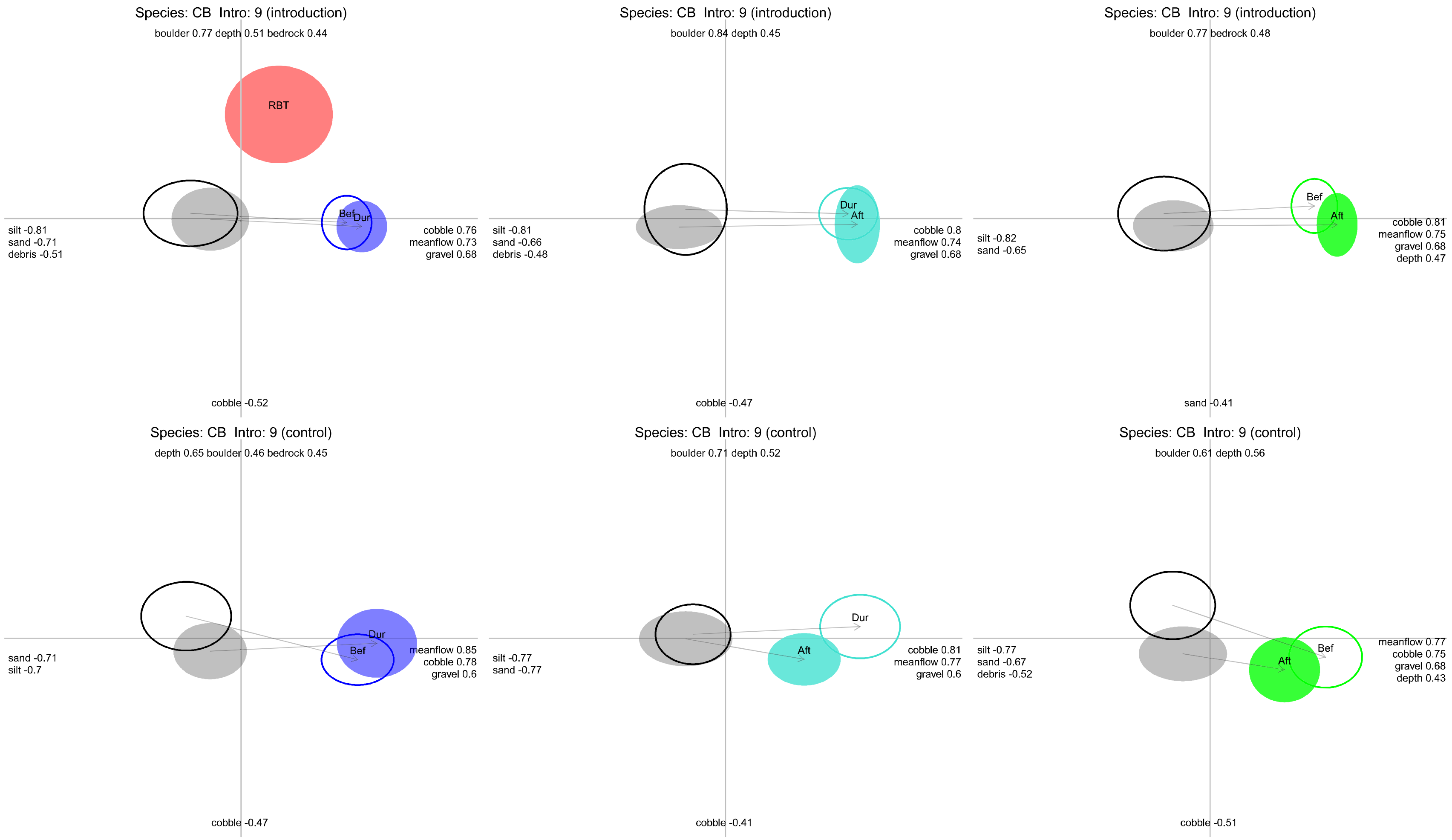


Comparison: During vs After



Comparison: Before vs After

Response: None
Recovery: N/A



Comparison: Before vs During **Comparison: During vs After** **Comparison: Before vs After**

Response: None

Recovery: N/A

APPENDIX 2.2: PCA LOADINGS

Mean values for the variable loadings on the first two factors in the PCA analyses (Standard Deviation). Values greater than 0.4 are shown in bold.

Segments	Factor	Depth	Mean Flow	Silt	Sand	Gravel	Cobble	Boulder	Bedrock	Debris
Before-After	F1	0.17	**0.74**	**-0.71**	**-0.50**	**0.62**	**0.76**	0.06	-0.09	**-0.46**
		(0.23)	**(0.04)**	**(0.12)**	**(0.27)**	**(0.14)**	**(0.10)**	(0.10)	(0.16)	**(0.21)**
	F2	**0.63**	0.11	-0.20	0.25	-0.08	-0.34	**0.52**	0.10	-0.17
		(0.26)	(0.14)	(0.21)	(0.44)	(0.23)	(0.22)	**(0.23)**	(0.27)	(0.29)
Before-During	F1	0.09	**0.72**	**-0.68**	**-0.53**	**0.62**	**0.78**	0.05	-0.08	**-0.45**
		(0.23)	**(0.06)**	**(0.15)**	**(0.27)**	**(0.13)**	**(0.07)**	(0.14)	(0.15)	**(0.24)**
	F2	**0.66**	0.12	-0.22	0.20	-0.10	-0.34	**0.54**	0.15	-0.18
		(0.11)	(0.17)	(0.18)	(0.37)	(0.17)	(0.19)	**(0.28)**	(0.31)	(0.27)
During-After	F1	0.05	**0.74**	**-0.73**	**-0.48**	**0.62**	**0.77**	0.07	-0.07	**-0.53**
		(0.26)	**(0.05)**	**(0.09)**	**(0.32)**	**(0.13)**	**(0.08)**	(0.15)	(0.09)	**(0.19)**
	F2	**0.66**	0.10	-0.20	0.25	-0.09	-0.33	**0.55**	0.20	-0.21
		(0.12)	(0.14)	(0.19)	(0.38)	(0.21)	(0.22)	**(0.27)**	(0.23)	(0.21)

CHAPTER 3

THE EFECTS OF RAINBOW TROUT STOCKING ON THE MESOHABITAT USE, FEEDING EFFICIENCY, AND SOCIAL BEHAVIOR OF WARPAINT SHINERS IN AN ARTIFICIAL STREAM*

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*Elkins, D. C., and G. D. Grossman. To be submitted to Freshwater Biology

ABSTRACT

Rainbow trout are widely stocked, worldwide, to supplement naturalized populations and support put-and-take fisheries, despite the evidence that salmonid introductions may negatively impact native fishes. We conducted experiments in a model stream to assess the effects of hatchery rainbow trout on the mesohabitat use, foraging success, and social behavior of warpaint shiners. When rainbow trout were absent, warpaint shiners occupied pool mesohabitats; however, when rainbow trout were present, warpaint shiners moved away from the trout, and into shallower habitats with higher current velocities and more variable substrata. In addition, the presence of rainbow trout significantly reduced capture success, feeding efficiency, and increased crowding among warpaint shiners. Feeding rate effects were exacerbated during spring/fall conditions. These effects were consistent across two realistic densities of warpaint shiners and were not observed during controls.

KEYWORDS: rainbow trout, warpaint shiner, trout stocking, competition, artificial stream

INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), although native only to coastal drainages of the Pacific Rim (MacCrimmon 1971, Behnke 2002), are widely stocked worldwide (Fausch 1988, Welcomme 1988, Crawford and Muir 2008). In 2004, Halverson (2008) calculates State and Federal agencies released approximately 9,960,000 kg of diploid and triploid rainbow trout into US waters, with stocking programs active in 45 of 50 states, the only exceptions being Alabama, Florida, Louisiana, Mississippi, and South Carolina. Despite this widespread introduction little is known about the effects of rainbow trout stocking on native non-game fishes, even though substantial declines have been reported in many of these species in the last 20 years (Jelks et al. 2008) and stocking with salmonids has been implicated in many such declines (Crawford and Muir 2008, Gozlan 2008). Consequently, an assessment of the effects of trout stocking on non-game fishes is warranted, because these fishes inhabit many of North America's stocked waters, especially because research with both native trout and New Zealand non-game species document negative interactions with stocked trout (e.g., McIntosh et al. 1992, McIntosh et al. 1994, Kaeding et al. 1996, Clark and Rose 1997, Kruse 1999).

The adverse effects of invasive fishes on native species typically are produced via either interspecific competition for space or food (Grossman and Freeman 1987) or predation (Garman and Nielsen 1982). In the presence of introduced salmonids, native fishes may display behavioral changes that reduce foraging success (McIntosh et al. 1992, McIntosh et al. 1994, Townsend 1996, McDowall 2003, Townsend 2003, McDowall 2006, Penaluna et al. 2009). The proximate negative effects produced by these interactions include shifts to less profitable micro- and mesohabitats, shift to alternate prey resources, and ultimately, reductions in the density that ultimately increase the probability of extinction. This may be a substantial problem in southern Appalachian streams because they display high amounts of environmental variability, which may increase the probability of extinction for sensitive species (Grossman et al. 1995). Nonetheless, studies in this region have suggested that stocked rainbow trout may not strongly affect habitat use by native cyprinids of the southeastern US (Grossman and Sostoa 1994), in the way that brown trout (Salmo trutta) have affected the native galaxiids of New Zealand (e.g., McDowall 2006). In this study we conducted experiments in an artificial stream to assess the impacts of invasive rainbow trout on foraging and social behavior of warpaint shiners, *Luxilus coccogenis* (Cope), a common native minnow that frequently occupies streams stocked with trout in the southern Appalachian mountains.

METHODS

The System

Warpaint shiners are a large (max T.L. 120mm), active minnow (Cyprinidae) found in the Blue Ridge, Ridge and Valley, Cumberland Plateau, and southern Highland Rim portions of the Tennessee River Drainage, the Savannah and Santee drainages of Georgia and the Carolinas, and the upper New River system of Tennessee, the latter probably as the result of an introduction (Etnier and Starnes 1993). Outten (1957) cites an 1892 report by Woolman of warpaint shiners in the Big Sandy River system of Kentucky, a tributary of the Ohio River system. Warpaint shiners typically inhabit swift riffles and pools and feed on a variety of aquatic insects, including ephemeroptera, hymenoptera, coleoptera, and diptera, which are taken both in the drift and from the surface (Outten 1957).

Although originally native to coastal drainages of the Pacific Rim, the most likely origin for the hatchery strain of rainbow trout is the McCloud River of California (Needham and Behnke 1962, MacCrimmon 1971). Since the late nineteenth century, rainbow trout have been widely introduced to promote sport fisheries, and are now regularly stocked into tailwaters, rivers, lakes, and streams throughout the Southeastern United States, including those where warpaint shiners are found.

We selected warpaint shiners as the test minnow species because they occupy microhabitats that overlap most closely with rainbow trout in the field (Elkins and Grossman, 2010) and show significant behavioral overlap; Etnier and Starnes (1993) note that warpaint shiners are occasionally taken by anglers in waters supporting trout. This similarity should maximize the interpretability of any observed effects. Furthermore, warpaint shiners are a reasonable surrogate for other medium-sized drift-feeding minnows, including species of conservation concern, such as the spotfin chub (*Erimonax monacha* Cope), blue shiner (*Cyprinella caerulea* Jordan) or bluestripe shiner (*Cyprinella callitaenia* Bailey & Gibbs) that may be present in waters where rainbow trout are introduced in the southeast, because their foraging behavior is typical of fishes in this guild.

Experimental Design

We conducted a series of laboratory experiments as an adjunct to a field study of the effects of trout stocking on non-game fishes (Elkins & Grossman 2010), because it was possible to quantify behavioral responses under controlled conditions. In these experiments, we observed small groups of warpaint shiners in an artificial stream and tested for the effects of the introduction of single rainbow trout on the 1) per capita aggression rate, 2) feeding rate, 3) feeding efficiency, 4) distance to the nearest conspecific, 5) distance to a newly introduced fish, and 6) position within the stream, relative to the pool. Our experimental design consisted of three controls and one treatment, nested in two temperature and two density treatments to ensure our results were representative of the range of natural conditions present in the wild.

We began with a focal group of either two or five warpaint shiners (density treatments) and observed them over a series of two-day periods: alone (control), or after the addition of a rainbow trout (trout treatment, hereafter abbreviated "TR"), a river chub, *Nocomis micropogon* (Cope), (large fish control, hereafter LF), or another warpaint shiner (density control, hereafter "DC"). We used the extra warpaint shiner to control for simple density effects and the chub served as a competitor/non-predator "large fish" control, since these are the largest drift-feeding species we consistently collected in pools with warpaint shiners. We varied temperature, conducting trials at either 12ºC or 17ºC, and photoperiod to test for seasonal effects (spring/fall or summer). We used two densities of warpaint shiners (2 fish, hereafter "LD", or 5 fish, "HD") because stream fishes tend to be patchily distributed and these densities are commonly observed in the southern Appalachian streams (Elkins & Grossman pers. obs.). Given the tank dimensions, described below, these groups created initial densities of 2.1 and 0.7 warpaint shiners per m^2 of tank area. In all, we completed nine trials at each warpaint shiner density: five LD trials at 12ºC and four at 17ºC; and four HD at 12ºC and five at 17ºC (see Table 3.1). (In order to minimize the acclimation stress on the shiners, the choice of experimental conditions was largely dictated by the conditions under which they were collected.)

We conducted three observation sessions per day, with the first occurring before noon, and the last after noon. To avoid satiation effects, we suspended feeding for at least 30 minutes between one pair of sessions and at least 90 minutes between the other pair. We began each trial with two days of observation on the focal warpaint shiners in the experimental group. We then began the series of randomly assigned two day treatments. Treatments were assigned randomly to minimize interactions between tank acclimation effects and treatment order (see Table 3.1).

Treatment 1- Focal Fish Control (WP)

During the first two days of each trial, we observed the focal group of two or five warpaint shiners, as described above.

Treatment 2- Density Control (DC)

In late afternoon, after the completion of the day's third observation session, one new warpaint shiner was introduced to the tank, followed by an overnight acclimation period. We performed an additional two days of observations of the fish in the experimental group, as described above. After the third observation session of the second day, the extra warpaint shiner was removed from the tank.

Treatment 3 -Rainbow Trout Treatment (TR)

We added one rainbow trout in late afternoon, followed by an overnight acclimation period, and performed two days of observations. In later trials, the absolute position of the rainbow trout was recorded, from which the Euclidean distance to the focal warpaint was calculated. After the third observation session of the second day of the rainbow trout treatment, the rainbow trout was removed.

Treatment 4 - Large Fish Control (LF)

We added one river chub which was larger than the warpaint shiners and as comparable in size to the rainbow trout as was practical, in late afternoon. Observation of the experimental group continued for an additional two days, as described in treatment above.

Tank design and physical parameters

We performed trials in an experimental stream located at the Whitehall Fisheries Laboratory at the University of Georgia. The experimental stream was constructed of 1.9 cm thick acrylic [3.05 m (L) X 1.52 m (W) X .76 m (H)]. The stream had a false bottom approximately 30 cm above the base of the tank that allowed for flow to be generated by two electric trolling motors (@24.95 kg thrust). Water reemerged at the opposite end of the platform and flowed across the top of this platform in a nearly laminar fashion (Figure 3.1). An experimental arena was created on the platform with a pair of block nets across the width of the tank, and this area was subdivided with two platforms constructed of acrylic and PVC to create three discrete depth zones of equal area: a “riffle” zone with a depth of 15 cm, a “run” zone with a depth of 25 cm, and a “pool” zone with a depth of 35 cm. Riffles were covered with a 5cm layer of small cobbles (maximum diameter 15 cm) and gravel, whereas the run was covered with a 4 cm layer of gravel (max diameter 2cm) and the pools with a 2 cm layer of gravel and sand, with a small transition zone between each section consisting of sloping piles of substrate. The tank was shrouded with sheets of opaque fiber board which ensured visual isolation of observers. All observations (see below) were made from within a black plastic blind constructed around a small window in the tank screen to minimize any disturbance to the fishes.

We set the water temperature and photoperiod to approximate both spring/fall (water temperature 12 C ± 1 C photoperiod 10.5 hours including 15 minute periods at "dawn" and "dusk" during which the light intensity was gradually stepped up or down) and summer conditions (water temperature 17 C ± 1.5 C, photoperiod 12 hours, including dawn and dusk) conditions (Duncan Elkins, unpublished data.)

We measured flows in the tank with an electronic flow meter (± 0.01 cm/s) before and after each trial to ensure consistency within and among trials. Mean velocities in the three microhabitats were maintained at 22 cm/s, 16 cm/s, and 11 cm/s in the riffle, run and pool sections of the tank respectively. Velocities ranged from zero in the pool to 32 cm/s near the upstream end of the riffle. We maintained pH at 7.3 and added aquarium salt (NaCl) at the rate of 7.5g per 10 gallons of dechlorinated tap water to ease osmotic stress. Water velocity, temperature, and pH ranges were consistent with those repeatedly recorded in the field at the collection site (Grossman and Ratajczak pers. obs.).

We installed 8 prey release outlets spaced evenly across the front of the tank (see Figure 3.2) and even with the substratum. *Chironomidae* larvae (bloodworms) were dispensed at the head of the riffle and run sections of the tank, entrained in the current, and carried the length of the tank, simulating the natural drift of prey items. For each trial, we added a total of between 10.8 and 11g of frozen bloodworms to two water reservoirs above the tank, which were aerated to melt the frozen blocks and to keep food items in suspension. These reservoirs were connected to the eight release points by four nylon tubes, each of which passed through a computer-controlled solenoid valve and a Y connection. These release valves were programmed on staggered 90-second cycles such that one of the four valves was open for one second out every 22.5. Although the prey delivery rate was constant, in terms of the volume of water released, per minute, stochastic variation in the suspension of prey within the reservoir and the path through the Y-connectors simulated natural variability in prey abundance.

Collection of Experimental Subjects

We obtained a subsample of rainbow trout to be stocked that week by Georgia Department of Natural Resources (DNR) personnel from the trout hatchery at Lake Burton, Habersham County, Georgia. Trout were held in a closed tank (480 l) and fed a diet of commercial trout chow (2.4 mm pellets) to simulate hatchery conditions. We collected warpaint shiners and river chubs from Coweeta Creek, Macon County, North Carolina, using seine nets as described by Wagner (2004). After acclimation of at least 12 hours in our species-specific holding system, all warpaint shiners were anesthetized using a buffered solution of MS222 (Tricaine Methane Sulphonate) at a concentration of 1.07 g to two liters of holding tank water, weighed (± 0.01 g), measured (standard length, ± 1mm).

While anesthetized, each fish was marked with a colored acetate tag (approximately 2mm^2) just below the dorsal fin as described in Wagner (2004), where it was further established that these tags do not affect general behavior or feeding behavior of small minnows. We then returned warpaint shiners to the holding tanks to recover for two days prior to the beginning of each set of experiments, during which we fed them bloodworms and administered a prophylactic dose (3mg/L) of the antibiotic Kanamycin Sulfate.

The typical period between collection and initiation of an experiment was four days (collect Thursday, mark Friday, recover Saturday and Sunday) although this period was occasionally extended to six days due to weather or other scheduling constraints. We did not collect any fish that were visibly diseased or deformed in the field (although many fish did exhibit trematode *metacercaria*, which we enumerated but did not attempt to treat.) We attempted to collect shiners of similar size (range less than 10 mm) in the field and marked 3-4 more fish than were required for a trial so we could select the individuals that recovered fastest from the anesthesia for use in the trial. The remaining fish were kept in our holding system and fed bloodworms for the duration of the trial. Although several fish in our pre-experiment feasibility trials succumbed to an external secondary infection around the marking site (probably *Flavobacterium columnare*), we experienced no mortality from infection after the installation of an ultraviolet sterilizer in the holding system and initiation of the Kanamycin prophylaxis; the most common source of mortality during our trials was fish jumping out of the tank through gaps in the screen cover around the water return plumbing. All fish were euthanized after our trials in a supersaturated solution of CO_2.

Fish Observations

We based experimental procedures on those of Rincón and Grossman (1998). Warpaint shiners were introduced into the experimental stream in late afternoon and left to acclimate overnight. We made our experimental observations during three sessions each day. All observation sessions began 30 minutes after the initiation of the feeding system to allow the fishes to acclimate to the prey releases. We observed individual fish for periods of two minutes each, during which we recorded the X, Y, and Z coordinates and identity and distance of the nearest con- and heterospecific every 30 seconds.

Within the 30-second observation periods, we recorded feeding activity (number of prey items captured and missed) and aggressive encounters involving the focal individual (both initiator and receiver and intensity of encounters, plus fish identity). Aggressive behaviors were qualified as a flash, charge, or nip, as follows: We recorded a flash when a fish changed its posture or orientation relative to another fish, provided this change did not include a movement of more than one body length. Typically, a flash involved angling the body upward, turning sideways relative to the target fish, and briefly flaring the fins. A chase involved the initiator moving more than one body length towards another fish, including active pursuit. We recorded a nip if a charge resulted in contact between the head of the initiator and the target fish. We also recorded aggressive interactions with focal individuals and any treatment fish, as well as their position in the tank in the last seven trials.

After two minutes of observations, we proceeded to a new focal fish, in a random order chosen before the start of the session, until all of the individuals had been observed. In all, we made three circuits resulting in 30 minutes of observations (5 fish * 2 minutes * 3 rounds) in each session of the high-density trials or 12 minutes of observations (2 fish * 2 minutes * 3 rounds) per session of the low-density trials. All observations were recorded on microcassette and transcribed after the completion of the trial.

Warpaint Behavior Statistical Analysis

We tested whether treatments (temperature, focal fish only, rainbow trout, large fish control, and density) significantly affected mesohabitat use, aggression, feeding attempts, prey captured, strike success, the distance to the front of the tank, distance to the nearest conspecific, and distance to the treatment fish (rainbow trout, river chub, or unfamiliar warpaint shiner) in the experimental group of warpaint shiners. To test for mesohabitat effects we classified each observation by the mesohabitat (riffle/ run/ pool) occupied. We then used partitioned chi-square tests ($\alpha = 0.05$) to test for differences in mesohabitat occupancy among treatments. The partitioned chi-square (or "subdivided" chi-square, Zar 1999) assigns both an overall p-value to the combined distribution for the null hypothesis that observations are randomly distributed among the 12 cells of the treatment x habitat matrix and a sub-score for each of the cells that may be compared to a chi-square table with two degrees of freedom to assess the deviance of that particular cell from the random expectation. This approach allows us to identify which mesohabitats were over- or under-used by the shiners, relative to the expectation of random habitat use.

We used linear mixed models (McLean et al. 1991) to estimate the effects of the various treatments on aggression rates, feeding rates, and microhabitat measures. The mixed modeling approach estimates the mean response under baseline conditions (five warpaint shiners at 17ºC) as well as the change from the baseline value produced by the treatments. All models included a random trial effect to account for individual variation in the fishes or other experimental parameters between trials. We used observation session as an experimental unit, during which each fish was observed for six minutes. To account for the differences in fish density between the low and high density trials, all rates were converted to per capita rates. All models use a null hypothesis of no effect of density, season, or treatment with $\alpha = 0.05$. We created separate models for total aggression (total aggressive acts, per warpaint shiner, per six minutes of observation in a session) and for each of the three levels of aggression (flashes, chases, or nips • fish^{-1} • session^{-1}) to investigate how the experimental parameters affected both the amount and intensity of intra-specific aggression among the warpaint shiners. Similar models were created for per capita prey capture rate (total prey items caught • fish^{-1} • session^{-1}), prey miss rate (misses • fish^{-1} • session^{-1}) and feeding efficiency (proportion of successful strikes • fish^{-1} • session^{-1}). Finally, we modeled the space use by the warpaint shiners using the mean distance (to another fish or the front of the enclosure), per session, as the response and assumed a null hypothesis that the mean distance would not vary between treatments, seasons, or densities.

RESULTS

Mesohabitat use

Mesohabitat use by warpaint shiners in the experimental stream was significantly different than random during the control, the density control, and the rainbow trout treatment (Figure 3.3). During the initial two day control period, when the tank contained only two or five warpaint shiners, these fish preferentially occupied the pool mesohabitat instead of the riffle and run (all p values <0.001). The same result was obtained during the DC control. However, during the BF control, mesohabitat use did not differ from random. Conversely, during the RT treatment, warpaint shiners were under-represented in the pool habitat ($p< 0.001$) and over-represented in the riffle and run mesohabitats ($p < .001$). We have included the full partitioned chi-square table for this analysis as Table 3.2.

Effects of rainbow trout on behavior and spatial organization of warpaint shiners

The baseline (five warpaint shiners at 17C) mean per capita aggression rate was 4.9 aggressive acts • per fish^{-1} • six-minutes^{-1} ($p<0.001$). The estimated effect of the DC treatment was the only one that differed significantly from 0; we estimate that the effect of an additional warpaint produced an additional 1.7 ($p=0.004$) acts of aggression • fish^{-1} • session^{-1}(Figure 3.4). Our observations indicate that warpaint shiners would establish relatively stable positions in feeding lanes during the first two days of the experiment and the addition of a conspecific disrupted this feeding hierarchy. The new fish would frequently attempt to establish itself in or near the holding position of another warpaint shiner, which produced additional aggression as new positions were established.

The relative influence of temperature, treatment, and density were similar when we analyzed aggressive acts flash, charge, nip separately. However, warpaint shiners significantly increased the frequency of specific aggressive acts during the large fish (river chub) control and rainbow trout treatments (baseline estimate: 1.42 charges • fish^{-1} • session^{-1}, $p= 0.002$; chub effect: 0.42, $p = 0.021$; rainbow trout effect: 0.37, $p= 0.044$) . Parameter estimates for all linear models, including these, are presented in Appendix 2.

The baseline strike rate (i.e., all strikes, successful and unsuccessful) was 3.5 strikes fish^{-1} ($p <.0001$), per session, and only rainbow trout significantly affected this value. During the TR treatment, strike rates significantly decreased by 0.8 strikes • fish^{-1} • session^{-1} ($p = 0.01$) (Figure 3.5).

We observed two significant effects of the treatments on prey capture by the focal warpaints. The baseline per-capita prey capture rate was 2.4 prey items per warpaint shiner per six minutes of observation ($p < 0.001$). Both BF and RT significantly altered prey capture rates, with an 18% increase ($p = 0.034$) during the BF treatment and a 26% decrease ($p = 0.003$) during the TR treatment (Figure 3.6).

To further elucidate the difference in the response between the warpaint shiners' responses to the RT and LF introductions, we modeled the proportion of total strikes that resulted in a capture. The baseline feeding efficiency was 0.74 captures/strike ($p <.0001$). Warpaint density did not affect feeding efficiency, however the LT seasonal temperature treatment reduced feeding efficiency by 0.14 captures/strike ($p = 0.033$), as did the RT treatment, which reduced the feeding efficiency by 0.12 captures/strike ($p < 0.001$, Figure 3.7).

The presence of a rainbow trout also affected intraspecific spatial relationships of warpaint shiners. Under baseline conditions the mean distance from a warpaint shiner to its nearest neighbor was 56.3 cm (Figure 3.8). This distance nearly doubled in the LD treatment, increasing by 45.1 cm ($p<0.0001$). By contrast, under LT conditions, mean nearest neighbor distances decreased by 19.2 cm ($p = 0.039$). The presence of a river chub increased nearest-neighbor distances by 4.4 cm ($p <.0001$), while the mean nearest-neighbor distance decreased by 4.5 cm during the DC control ($p<.0001$) and 10.1 cm during TR ($p<0.001$).

We observed multiple significant treatment effects on the distance of warpaint shiner to the introduced fish (DC, CH, TR). In this analysis, DC was the baseline (five warpaint shiners, 17C) and the mean distance of the focal warpaint shiners to the introduced warpaint was 87.0 cm (p<0.001, Figure 3.9). The only significant treatment was RT, during which the focal warpaints were, on average, 116.9 cm from the rainbow trout (effect size 29.9 cm, p<0.001).

We observed three significant effects in the analysis of the focal warpaints' forward position. During the baseline period the mean distance of warpaint shiners to the front of the tank was 79.6 cm (p <.0001, Figure 3.10). The spring/autumn temperature treatment significantly increased this distance by almost 19cm (p= 0.0243,). When rainbow trout were present, this distance decreased by 33.5 cm (p <.0001). During the CH control, distance to the front of the tank was reduced by 8.8 cm (p=0.022). None of the remaining estimates, (LD, DC) were significant.

DISCUSSION

Drift feeding fish compete for profitable positions within a stream and maintain them through agonistic interactions with both conspecific and interspecific competitors (Noakes 1980, Fausch and White 1986, Freeman and Grossman 1992b, a, Fausch 1998, Rincón and Grossman 2001, Ward et al. 2006). However, previous research in the stream where we collected our warpaint shiners has demonstrated little evidence of competition between rainbow trout and another small cyprinid, rosyside dace (*Clinostomus funduloides* Girard) (Grossman and Freeman 1987, Grossman and Ratajczak 1998), nor have these two species exhibited competition in another model stream (Grossman and Boulé 1991, Rincón and Grossman 1998). These studies largely used rainbow trout that were equivalent in size to the rosyside dace or slightly larger. As in a similar experiment using juvenile brown trout and a galaxiid (Bonnett and McIntosh 2004), introduced salmonids may not compete, at the mesohabitat level, with native fishes of similar size and ecology.

In our experiments, large rainbow trout significantly affected habitat use, social behavior and foraging success of warpaint shiners. When rainbow trout were absent, warpaint shiners occupied pool mesohabitats; however, when rainbow trout were present, warpaint shiners moved away from the trout, out of pool mesohabitats into shallower habitats with more variable substrata. In addition, the presence of rainbow trout resulted in reduced capture success, reduced feeding efficiency, and greater crowding among warpaint shiners than during other treatments. Feeding rate effects were exacerbated during spring/fall conditions. These effects were consistent across the two densities of focal fish and were not observed during either the DC or LF control. Relative to the baseline the average warpaint shiner would be predicted to feed almost 19% (-0.14/0.74 = -18.9%) less successfully at 12 C and 17% less effectively when a rainbow trout is present. These effects are additive, so the feeding efficiency of an average warpaint at spring or fall temperatures when rainbow trout were present would be reduced by 35%, relative to the baseline rate.

The increase we observed in the prey capture rate and the non-significant effect of the BF treatment on the total number of strikes are likely a consequence of river chub foraging behavior. Unlike warpaint shiners and rainbow trout, river chubs frequently nosed among the gravel and cobble for prey items that had settled out of the drift, primarily in the run mesohabitat. This activity frequently stirred up additional particles that were consumed by the warpaint shiners.

Indeed, in several trials, one warpaint shiner followed and hovered over the river chub while it foraged in the gravel, perhaps anticipating resuspended prey. Similarly, although the forward shift in warpaint position during BF is statistically significant, it is small, relative to the rainbow trout effect, and may be explained by the alteration in foraging behavior displayed by some warpaint shiners during the river chub treatment.

The warpaint shiners' shift forward in the presence of a rainbow trout, however, could have significant energetic implications for the warpaint shiners, because previous work has shown that minnows in the front of a group have a higher prey capture rates (Wagner 2004; Hazelton 2008). Nonetheless, in our experimental tank forward positions also had higher current velocities because of the positions of the riffle and run and, while the mean position at the baseline is 96 cm from the front of the tank (near the boundary between the pool and riffle/run mesohabitats), the effect of a rainbow trout is to move the average position forward 33 cm, well into the higher velocity regime of the riffle or run. Maintaining a feeding position in the riffle and run mesohabitats would therefore require more energy, on average, and fishes very near the front of the experimental area often could not react to a released prey item until it had drifted past them. Pursuing these items would be expected to incur a marginal energy cost over simply anticipating and catching them in the oncoming drift (Hazelton 2008).

Although we did observe direct predation in two of our experimental trials, the rainbow trout were only able to catch and consume the warpaint shiners after a protracted chase that ultimately resulted in the shiner being pinned to the net barrier at one end of the tank, an outcome we suspect is unlikely in nature. This study suggests that the effects of rainbow trout on warpaint shiner may come not through direct predation but, rather, through reduction in fitness, by precipitating mesohabitat shifts that increase energetic costs of maintaining swimming position and reduce food intake. These effects may be ameliorated in the field by the greater variety of habitat available, though forcing fish into lower velocities may reduce feeding opportunities further by reducing exposure to drift (Hill and Grossman 1993, Grossman et al. 2002). Since warpaint shiners are ecologically similar to a number of drift-feeding cyprinids that frequently inhabit streams where rainbow trout are stocked, these results may extend to other systems and justify caution in the assessment of stocking programs that might affect imperiled native fishes.

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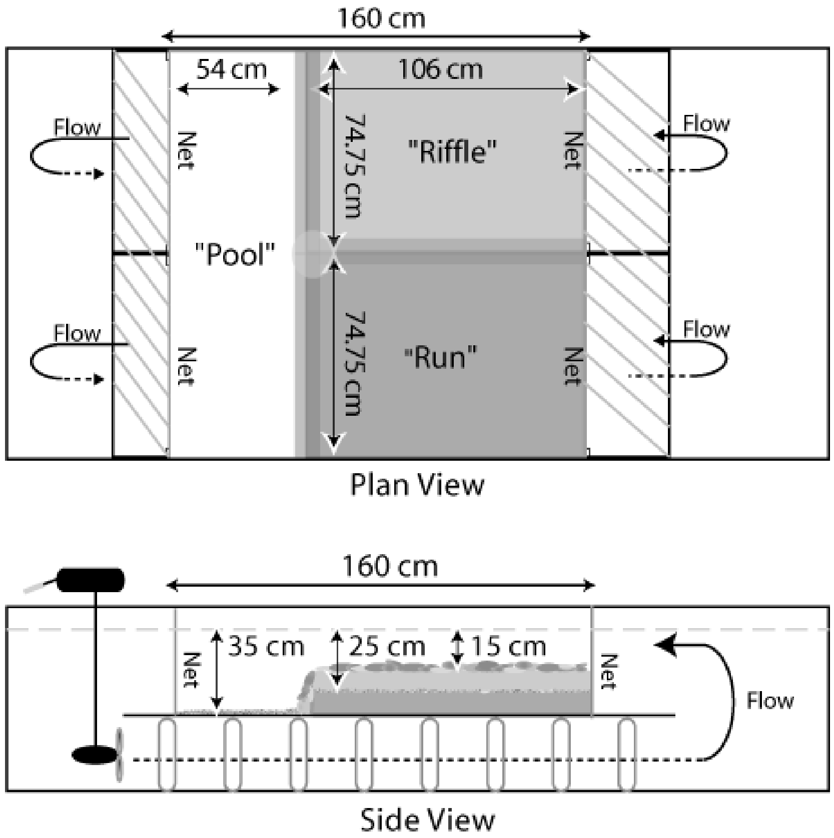
Plan View

Side View

Figure 3.1: Design of Experimental Stream. Not shown are intake and output plumbing for the filter and chiller systems, which were located in the bottom corner at the head of the tank and angled so as to minimize any potential disruption of laminar water flow.

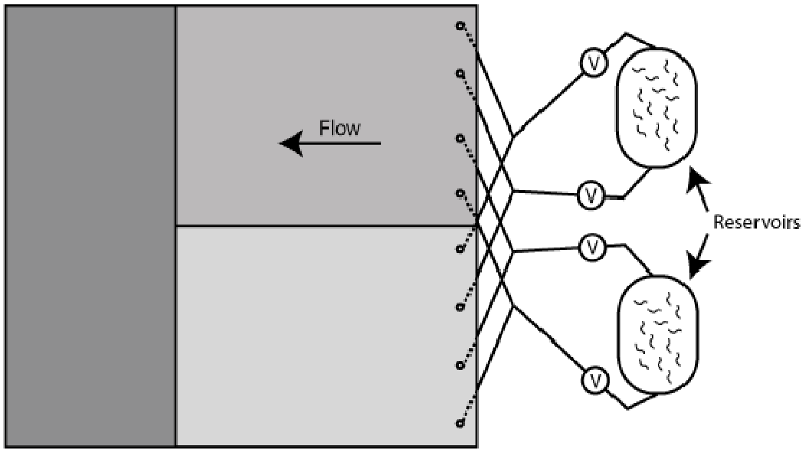

Figure 3.2: Feeder design in experimental stream. Two reservoirs were located above the tank and released suspended prey items into the stream via gravity flow while the computer-controlled valves (V) were in the open position.

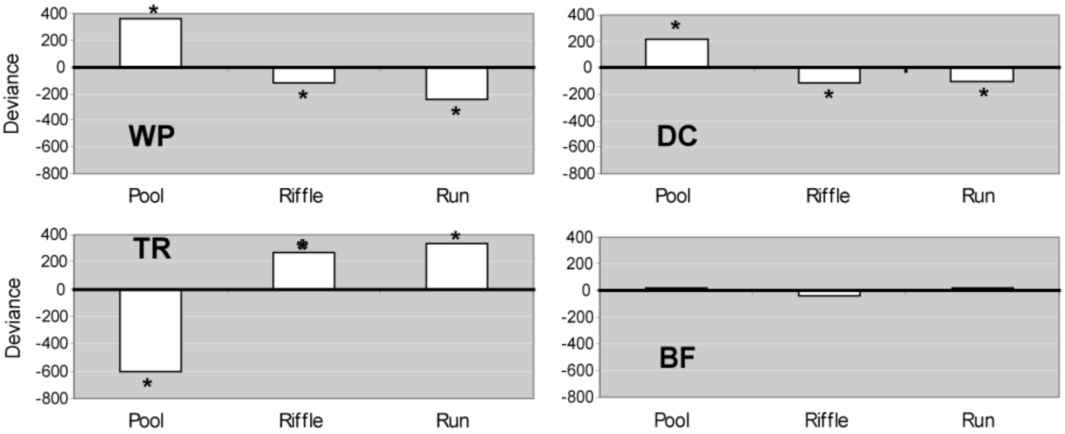

Figure 3.3: Relative deviance of observed habitat use frequencies from expected values. Asterisks indicate which habitats were significantly ($\alpha = 0.05$) over or underutilized by warpaint shiners, relative to the assumption of random use.

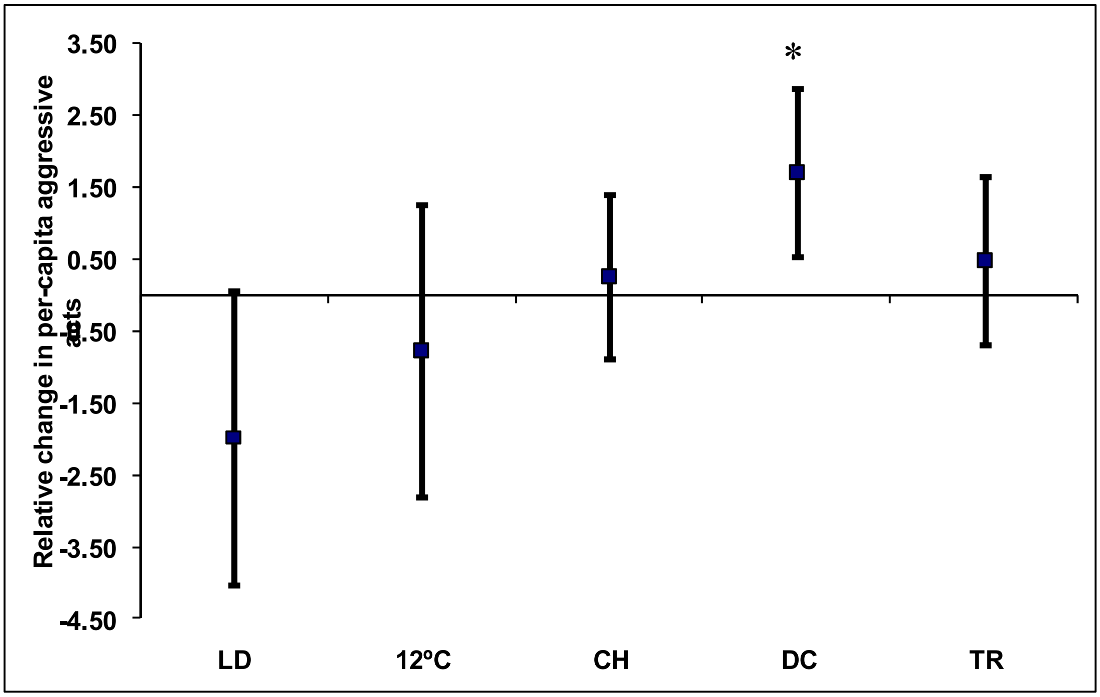

Figure 3.4: The effect of density, temperature, and treatment on per capita aggressive acts. The zero line represents baseline conditions (five warpaint shiners at 17ºC). Mean aggression rate for baseline conditions was 4.94 aggressive acts, per warpaint shiner, per six minutes. Significant effects (α = 0.05) are indicated with an asterisk, and mean and 95% confidence intervals are presented.

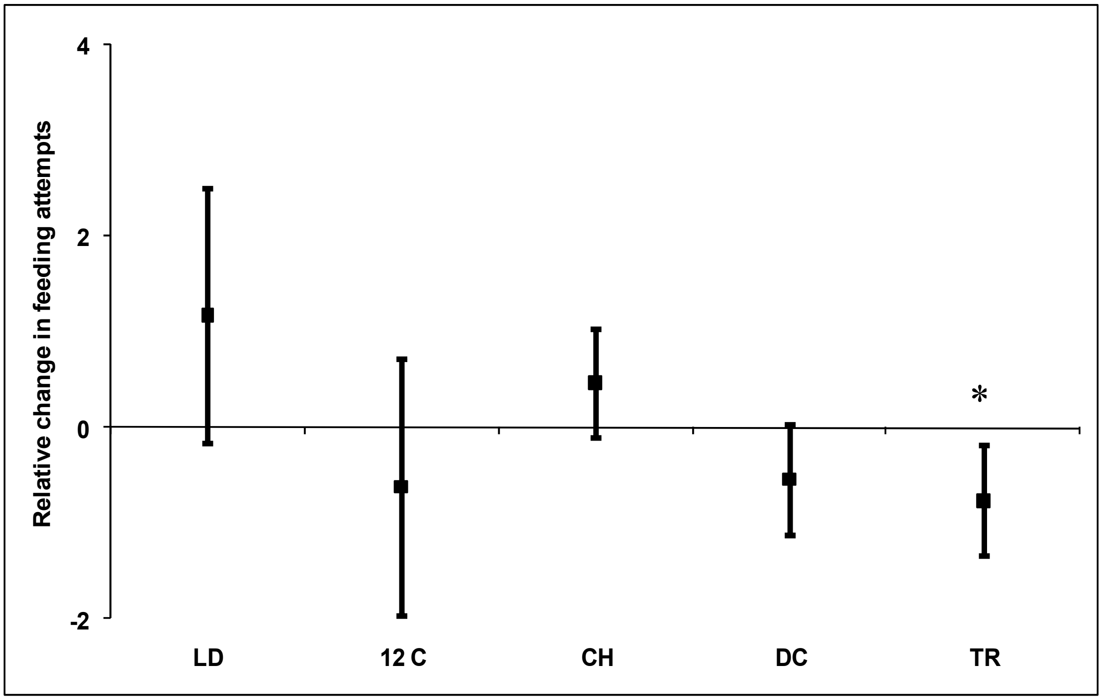

Figure 3.5: The effect of density, temperature, and treatment on per capita strike rate (successful strikes + misses). The zero line represents the baseline conditions (five warpaint shiners at 17ºC). At this baseline, an average fish made 3.5 strikes, per session. Significant effects (α = 0.05) are indicated with an asterisk, and the illustrated ranges indicate predicted 95% confidence intervals.

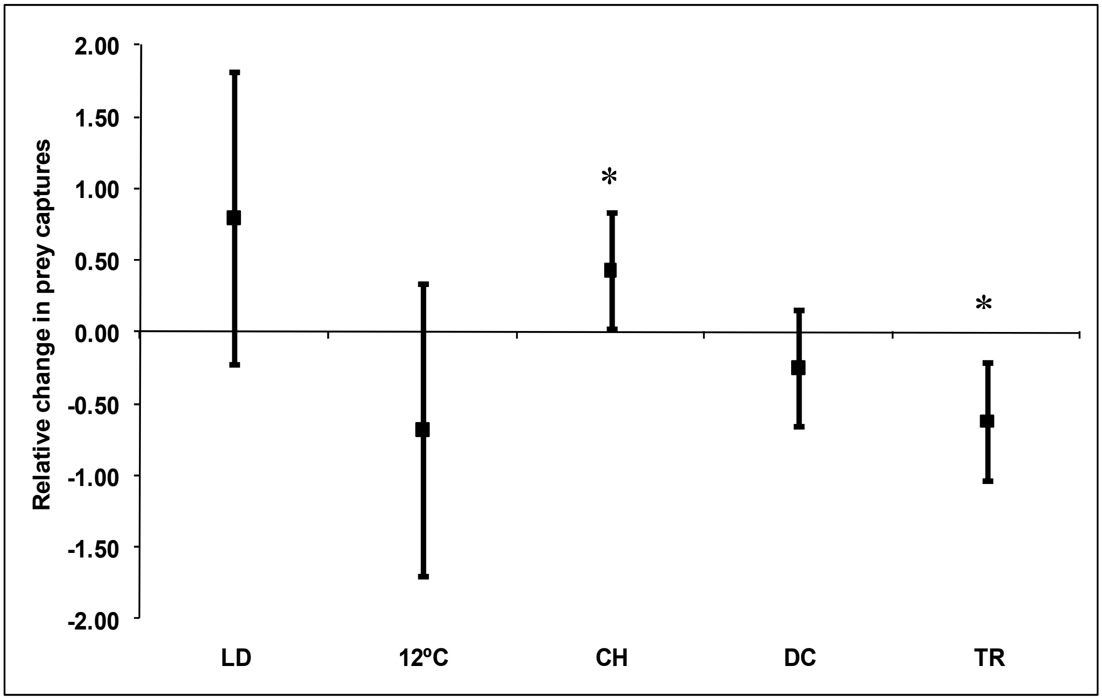

Figure 3.6: The effect of density, temperature, and treatment on per capita prey capture (i.e., successful strikes.) The zero line indicates the baseline conditions of five warpaint shiners at 17ºC. At this baseline, each warpaint shiner captured 2.39 prey items, per six minutes of observation, on average. Significant effects (α = 0.05) are denoted with an asterisk, and the illustrated ranges indicate predicted 95% confidence intervals.

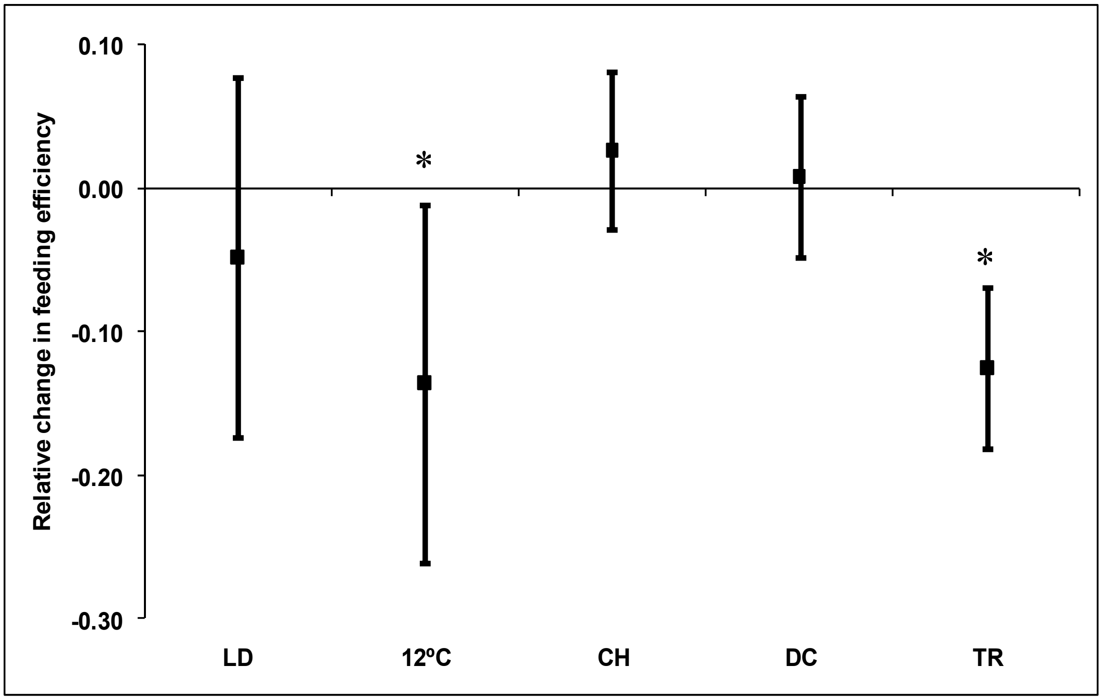

Figure 3.7: The effect of density, temperature, and treatment on feeding efficiency (captures/captures + misses). The zero line represents baseline conditions (five warpaint shiners at 17ºC). At this baseline, 0.74 of prey capture attempts were successful, on average. Significant effects (α = 0.05) are indicated with an asterisk, and mean and 95% confidence intervals are presented

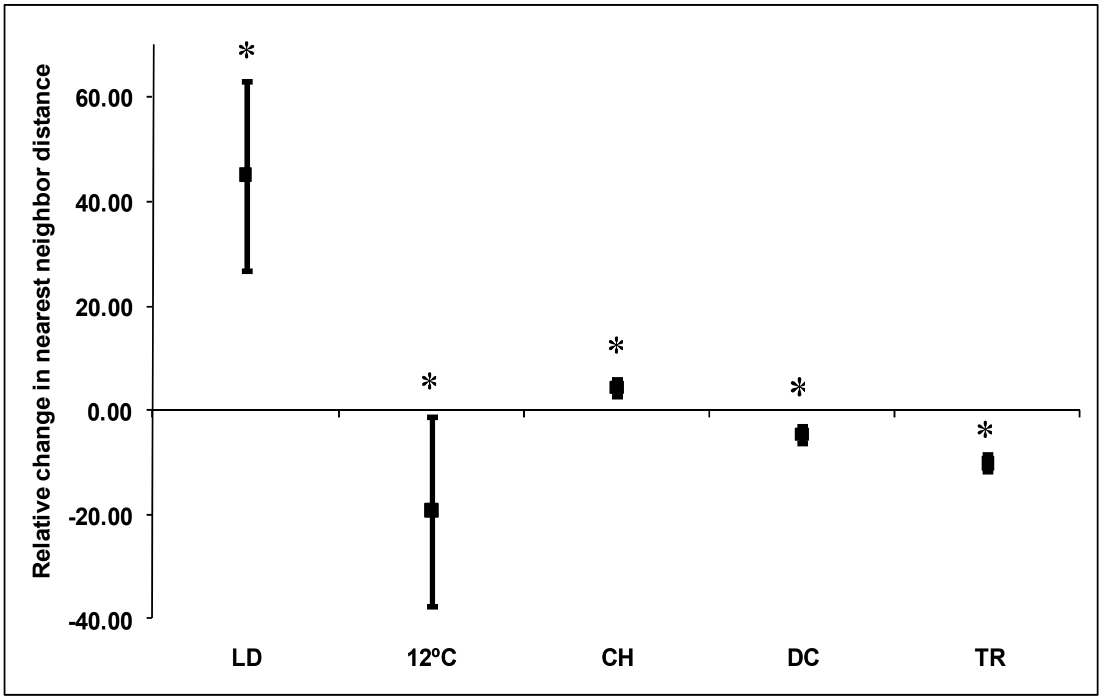

Figure 3.8: The effect of density, temperature, and treatment on nearest neighbor distance between focal warpaints (cm). The nearest neighbor was the closest warpaint (excluding the extra warpaint during the DC treatment) to the focal fish. The zero line represents baseline conditions (five warpaints at 17ºC), when focal warpaints were 56.32 cm from their nearest neighbor, on average. Significant effects ($\alpha = 0.05$) are indicated with an asterisk, with means and 95% confidence intervals indicated.

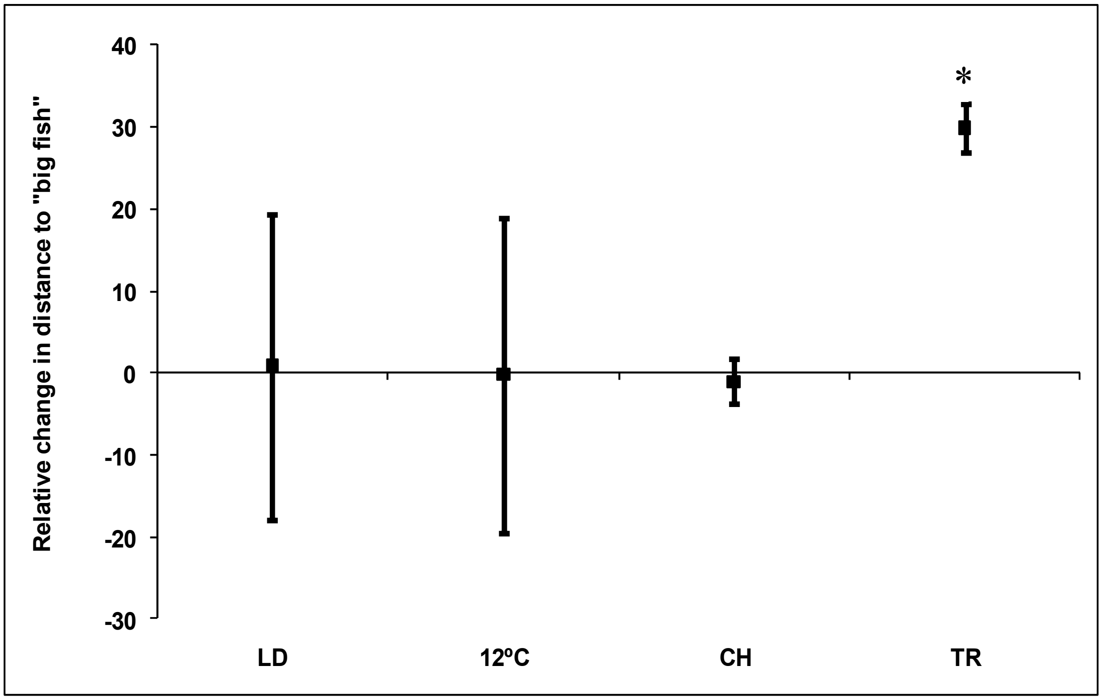

Figure 3.9: The effect of density, temperature, and treatment on distance from focal warpaint to the "big fish" (cm). The big fish referred to either the river chub (CH) or the rainbow trout (TR). Effects were estimated relative to the distance between the focal warpaint and the additional warpaint (DC) at 17ºC, which represents the zero line in the figure. At this baseline, focal warpaints were 87.0 cm from the new warpaint, on average. Significant effects ($\alpha = 0.05$) are indicated with an asterisk, and the illustrated ranges indicate predicted 95% confidence intervals.

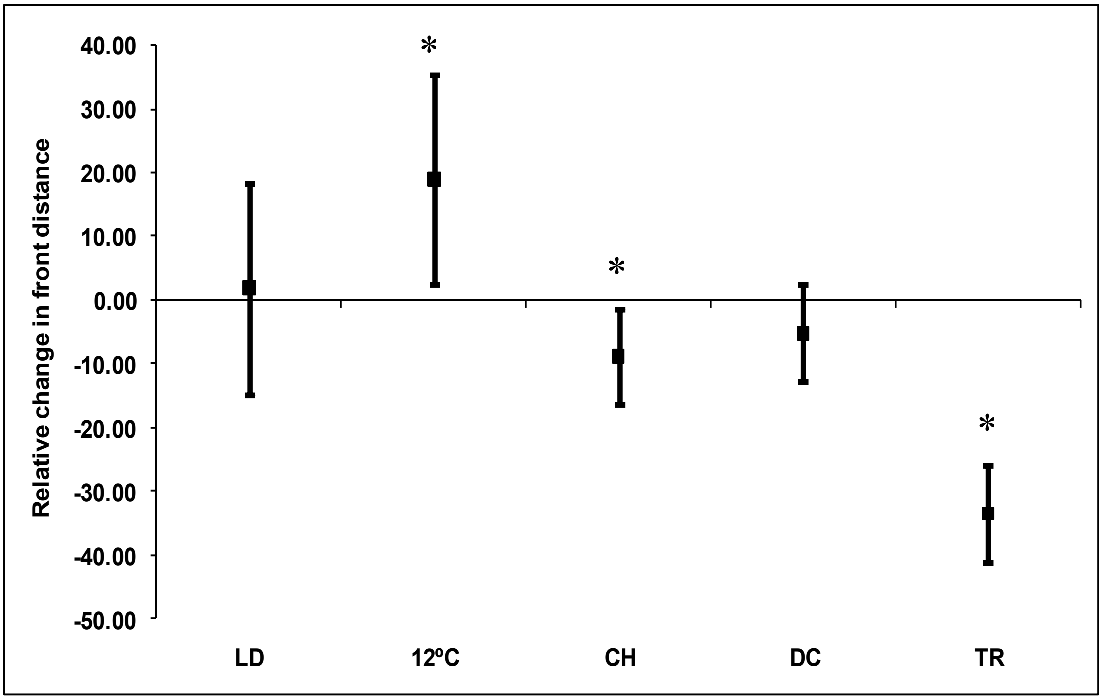

Figure 3.10: The effect of density, temperature, and treatment on distance to front of experimental tank (cm). Warpaint shiners were 96.4 cm from the front of the tank, on average, during the baseline conditions of five fish at 17ºC, which is denoted by the zero line. Significant effects ($\alpha = 0.05$) are indicated with an asterisk, and mean and 95% confidence intervals are shown.

Table 3.1: Summary of parameters for experimental trials.

Trial	Focal Fish Dens.	Season	Warpaint SL (mm)	Order	Trout SL (mm)	Trout Mass (g)	Chub SL (mm)	Chub Mass (g)
1	HD	SUM	69-75	DC, TR, BF	189	78.3	145	58.74
2	HD	SUM	55-91	DC, BF, TR	216	144.6	142	50
3	HD	SUM	80-118	BF, DC, TR	232	180	148	51.73
4	HD	SUM	67-82	DC, TR, BF	192	130.3	180	82.48
5	LD	SUM	68-74	BF, DC, TR	189	128.3	180	83.02
6	LD	SPR	105-112	DC, BF, TR	133	35.0	116	25
7	LD	SPR	87-88	DC, TR, BF	201	109.5	152	61.5
8	HD	SPR	81-108	TR, BF, DC	173	77.3	152	61.5
9	LD	SPR	50-51	BF, TR, DC	127	28.5	107	22.5
10	LD	SUM	83-90	TR, BF, DC	174	69.4	173	77.9
11	HD	SPR	60-63	BF, DC, TR	212	128.0	140	38.12
12	HD	SUM	62-66	TR, BF, DC	250	232.5	144	36.94
13	HD	SPR	71-79	TR, BF, DC	210	127.1	141	35.75
14	LD	SPR	62-64	TR, BF, DC	145	54.4	100	16.15
15	LD	SPR	77-78	TR, DC, BF	171	57.3	110	19.24
16	HD	SPR	68-73	BF, DC, TR	255	229.7	110	18.15
17	LD	SUM	69-69	TR, BF, DC	223	92.8	130	33.03
18	LD	SUM	76-77	TR, DC, BF	217	184.8	116	27.2

Table 3.2: Partitioned chi-square for mesohabitat usage. Overall Chi-Square Test for Equal Proportions: 2 DoF, Chi-square = 3454.2, Pr > ChiSq <.0001, n=15117

Value Key:
Observed
Expected
Deviation
Cell Chi-Square

		Habitat		
Treatment	pool	riffle	run	Total
WP	2368	412	1011	3791
	2006.2	526.63	1258.1	
	361.78	*-114.6*	*-247.1*	
	65.24	**24.952**	**48.55**	
BF	2112	506	1332	3950
	2090.4	548.72	1310.9	
	21.638	*-42.72*	*21.082*	
	0.224	**3.3259**	**0.339**	
DC	2206	406	1142	3754
	1986.6	521.49	1245.9	
	219.36	*-115.5*	*-103.9*	
	24.222	**25.578**	**8.6598**	
TR	1314	776	1532	3622
	1916.8	503.16	1202.1	
	-602.8	*272.84*	*329.94*	
	189.56	**147.95**	**90.56**	

APPENDIX 3.1: LAB TRIALS- LINEAR MODELING PARAMETER ESTIMATES

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	Baseline	2.8	4.9	7.1	0.000
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	2 fish	-4.0	-2.0	0.1	0.057
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	12 C	-2.8	-0.8	1.3	0.460
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	CH	-0.9	0.3	1.4	0.657
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	DC	0.5	1.7	2.9	0.004
Total per-capita aggression	Agg. acts • fish^{-1} • session^{-1}	TR	-0.7	0.5	1.7	0.409

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	Baseline	1.14	2.03	2.92	0.413
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	2 fish	-1.7	-0.8	0.0	0.055
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	12 C	-1.3	-0.5	0.3	0.249
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	CH	-0.3	0.2	0.7	0.389
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	DC	0.2	0.7	1.3	0.005
type1 per-cap aggression	Flashes • fish^{-1} • session^{-1}	TR	0.3	0.5	1.0	0.078

Response	Units	Effect	low 95% CI	Effect Estimate	high 95% CI	p-value
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	Baseline	0.64	1.42	2.19	0.002
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	2 fish	-1.2	-0.4	0.3	0.265
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	12 C	-1.3	-0.6	0.2	0.145
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	CH	0.1	0.4	0.8	0.022
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	DC	0.0	0.4	0.7	0.058
type2 per-cap aggression	Charges • fish^{-1} • session^{-1}	TR	0.0	0.4	0.7	0.044

Response	Units	Effect	low 95% CI	Effect Estimate	high 95% CI	p-value
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	Baseline	0.65	1.46	2.27	0.002
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	2 fish	-1.5	-0.8	0.0	0.043
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	12 C	-0.3	0.4	1.1	0.286
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	CH	-1.0	-0.4	0.1	0.132
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	DC	0.1	0.6	1.2	0.031
type3 per-cap aggression	Nips • fish^{-1} • session^{-1}	TR	-1.0	-0.5	0.1	0.092

Response	Units	Effect	low 95% CI	Effect Estimate	high 95% CI	p-value
Feeding attempts	Strikes • fish^{-1} • session^{-1}	Baseline	2.1	3.5	4.8	<.0001
Feeding attempts	Strikes • fish^{-1} • session^{-1}	2 fish	-0.2	1.2	2.5	0.086
Feeding attempts	Strikes • fish^{-1} • session^{-1}	12 C	-2.0	-0.6	0.7	0.366
Feeding attempts	Strikes • fish^{-1} • session^{-1}	CH	-0.1	0.5	1.0	0.102
Feeding attempts	Strikes • fish^{-1} • session^{-1}	DC	-1.1	-0.5	0.4	0.066
Feeding attempts	Strikes •fish^{-1} • session^{-1}	TR	-1.3	-0.8	-0.2	0.010

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	Baseline	1.37	2.39	3.42	0.000
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	2 fish	-0.2	0.8	1.8	0.128
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	12 C	-1.7	-0.7	0.3	0.192
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	CH	0.0	0.4	0.8	0.034
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	DC	-0.7	-0.2	0.2	0.232
per-capita prey captures	Prey captures • fish^{-1} • session^{-1}	TR	-1.0	-0.6	-0.2	0.003

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	Baseline	0.60	1.09	1.59	<0.0001
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	2 fish	-0.1	0.4	0.9	0.119
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	12 C	-0.4	0.1	0.5	0.798
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	CH	-0.2	0.0	0.3	0.768
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	DC	-0.6	-0.3	0.0	0.035
per-capita misses	Missed strikes • fish^{-1} • session^{-1}	TR	-0.4	-0.1	0.1	0.314

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	Baseline	0.62	0.74	0.87	<.0001
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	2 fish	-0.2	0.0	0.1	0.453
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	12 C	-0.3	-0.1	0.0	0.034
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	CH	0.0	0.0	0.1	0.337
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	DC	0.0	0.0	0.1	0.766
feeding efficiency	Captures/Strikes • fish^{-1} • session^{-1}	TR	-0.2	-0.1	-0.1	<.0001

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
distance from front of tank	cm	Baseline	79.64	96.37	113.09	<.0001
distance from front of tank	cm	2 fish	-14.7	1.8	18.3	0.829
distance from front of tank	cm	12 C	2.5	19.0	35.5	0.024
distance from front of tank	cm	CH	-16.3	-8.8	-1.3	0.022
distance from front of tank	cm	DC	-12.8	-5.2	2.4	0.180
distance from front of tank	cm	TR	-41.2	-33.5	-25.9	<.0001

Response (trials>11)	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
distance to big fish	cm	Baseline	72.3	87.0	101.7	<.0001
distance to big fish	cm	2 fish	-17.8	0.9	19.5	0.911
distance to big fish	cm	12 C	-19.4	-0.2	19.1	0.981
distance to big fish	cm	CH	-3.8	-1.0	1.7	0.455
distance to big fish	cm	TR	27.0	29.9	32.8	<.0001

Response	**Units**	**Effect**	**low 95% CI**	**Effect Estimate**	**high 95% CI**	**p-value**
Nearest warpaint dist.	cm	Baseline	38.78	56.32	73.86	<.0001
Nearest warpaint dist.	cm	2 fish	26.9	45.1	63.3	<.0001
Nearest warpaint dist.	cm	12 C	-37.3	-19.2	-1.0	0.039
Nearest warpaint dist.	cm	CH	2.9	4.4	6.0	<.0001
Nearest warpaint dist.	cm	DC	-6.0	-4.5	-3.0	<.0001
Nearest warpaint dist.	cm	TR	-11.7	-10.1	-8.5	<.0001

CHAPTER 4

THE EFECTS OF RAINBOW TROUT STOCKING ON THE SPACE USE OF WARPAINT SHINER IN AN EXPERIMENTAL STREAM[*]

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[*] Elkins, D. C., and G. D. Grossman. To be submitted to Freshwater Biology

ABSTRACT

Rainbow trout are widely stocked, worldwide, to supplement naturalized populations and support put-and-take fisheries, despite the evidence that salmonid introductions may negatively impact native fishes. We conducted experiments in a model stream to assess the effects of hatchery rainbow trout on the space use of warpaint shiners, a common cyprinid in Southern Appalachian streams. We used the LoCoH algorithm to generate polygons describing the home ranges used by warpaint shiners. Rainbow trout did not affect the edge/area ratio of the shiners' homeranges, but significantly increased their size by 57%. Rainbow trout and control treatments reduced the average overlap between individual shiners' home ranges. When rainbow trout were present, warpaint shiners were displaced and experienced significantly higher current velocities. These effects were consistent across two realistic densities of warpaint shiners and were not observed during controls.

KEYWORDS: rainbow trout, warpaint shiner, space use, LoCoH

INTRODUCTION

Rainbow trout are the most widely stocked fish in the United States; Halverson (2008) calculates State and Federal agencies released approximately 9.96 x 10^6 kg of rainbow trout into US waters in 2004. Although trout stocking is a common management technique in Georgia, little is known about the effects of this practice on the fishes inhabiting stocked waters. An assessment of the effects of trout stocking on non-game fishes is overdue, because many of the non-game fishes of the United States (including some threatened and endangered species) inhabit stocked waters, and previous research demonstrates that stocked or introduced trout may negatively affect native fishes (e.g., McIntosh et al. 1992, McIntosh et al. 1994, Kaeding and Carty 1996, Clark and Rose 1997, Kruse 1999). Jelks, et al (2008) describe substantial declines in the North American fish fauna since 1989 and cite a variety of factors producing these declines, including introduced species. However, they do not address the effects of deliberate and repeated introductions of invasive species through stocking programs.

The risks invasive salmonids pose to native salmonids are well explored, from both a genetic (e.g.,Waples 1991, Reisenbichler and Rubin 1999) and behavioral or competitive perspective (e.g., Fausch and White 1986, Fausch 1988, Mesa 1991, Fausch 1998, Harwood et al. 2002). Where they have been explored, the effects of trout introductions on native fishes that are not salmonids have focused primarily on the galaxiids in New Zealand (McIntosh et al. 1992, McIntosh et al. 1994, McDowall 2003, Bonnett and McIntosh 2004, McDowall 2006) or Patagonian Chile (Penaluna et al. 2009). In most of these cases, the introduced salmonid is brown trout (*Salmo trutta* L.) or a combination of brown trout and rainbow trout.

The adverse effects of introduced fishes on native species typically are produced via either interspecific competition for space or food (Grossman and Freeman 1987) or predation (Garman and Nielsen 1982). In fact, the threat of predation, alone, may be sufficient to induce behavioral changes in prey fishes that reduce their foraging ability (Fraser and Gilliam 1992). The proximate negative effects produced by these interactions include shifts to less profitable micro- and mesohabitats and reductions in the density of native fishes, which ultimately may lead to extinction. The probability of extinction may be higher in systems that display high amounts of environmental variability such as southern Appalachian streams (Grossman et al. 1995). Nonetheless, several studies have suggested that introduced trout may not strongly affect habitat use by native fishes (Hill and Grossman 1993, Grossman and Sostoa 1994, Grossman et al. 1998, Rincón and Grossman 1998). However, these studies typically used or observed naturalized trout which were comparable in size to the native cyprinids with which they were not shown to compete, whereas stocked trout are typically larger and may display behavioral differences as a result of their hatchery conditioning (Berejikian et al. 1996, Marchetti and Nevitt 2003).

In this study we introduced large rainbow trout to an artificial stream to document the effects of stocking with hatchery-raised rainbow trout on the spatial organization of groups of warpaint shiners, *Luxilus coccogenis*, a native non-game fish that commonly inhabits streams where rainbow trout are stocked.

METHODS

The System

Warpaint shiners are a large (max T.L. 120mm), active shiner found in the Blue Ridge, Ridge and Valley, Cumberland Plateau, and southern Highland Rim portions of the Tennessee River Drainage, the Savannah and Santee drainages of Georgia and the Carolinas, and the upper New River system of Tennessee, the latter probably as the result of an introduction (Etnier and Starnes 1993). Outten (1957) cites an 1892 report by Woolman of warpaint shiners in the Big Sandy River system of Kentucky, a tributary of the Ohio River system. Warpaint shiners typically inhabit swift riffles and pools and feed on a variety of aquatic insects, including ephemeroptera, hymenoptera, coleoptera, and diptera, which are taken both in the drift and from the surface (Outten 1957).

Although originally native to coastal drainages of the Pacific Rim, the most likely origin for the hatchery strain of rainbow trout is the McCloud river of California (Needham and Behnke 1962, MacCrimmon 1971). Since the late nineteenth century, rainbow trout have been widely introduced to promote sport fisheries, and are now regularly stocked into tailwaters, rivers, lakes, and streams throughout the Southeastern United States, including those where warpaint shiners are found.

We selected warpaint shiners as the test minnow species because they occupy microhabitats that overlap most closely with rainbow trout in the field (Elkins and Grossman, 2010) and show significant behavioral overlap; Etnier and Starnes (1993) note that warpaint shiners are occasionally taken by anglers in waters supporting trout. This similarity should maximize the interpretability of any observed effects. Furthermore, warpaint shiners are a reasonable surrogate for other medium-sized drift-feeding minnows, including species of conservation concern, such as the spotfin chub (*Erimonax monacha* Cope), blue shiner (*Cyprinella caerulea* Jordan) or bluestripe shiner (*Cyprinella callitaenia* Bailey & Gibbs*)* that may be present in waters where rainbow trout are introduced in the southeast, because their foraging behavior is typical of fishes in this guild.

Experimental Design

We conducted a series of laboratory experiments as an adjunct to a field study of the effects of trout stocking on non-game fishes (Elkins & Grossman 2010), because it was possible to quantify behavioral responses under controlled conditions. In these experiments, we observed small groups of warpaint shiners in an artificial stream and tested for the effects of the introduction of single rainbow trout on the space use of individual warpaint shiners. Our experimental design consisted of three controls and one treatment, nested in two temperature and two density treatments to ensure our results were representative of the range of natural conditions present in the wild.

We began with a focal group of either two or five warpaint shiners (density treatments, hereafter abbreviated) and observed them over a series of two-day periods: alone (control, hereafter "WP"), or after the addition of a rainbow trout (trout treatment, hereafter "TR"), a river chub, *Nocomis micropogon* (Cope), (large fish control, hereafter "LF"), or another warpaint shiner (density control, hereafter "DC"). We used the extra warpaint shiner to control for simple density effects and the chub served as a non-competitor/predator "large fish" control, since these are the largest drift-feeding species we consistently collected in pools with warpaint shiners. We varied temperature, conducting trials at either 12ºC or 17ºC, and photoperiod to test for seasonal effects (spring/fall or summer, hereafter “SPR” and “SUM”). We used two densities of warpaint shiners (2 fish, hereafter “LD”, or 5 fish, “HD”) because stream fishes tend to be patchily distributed and these densities are commonly observed in the southern Appalachian streams (Elkins & Grossman pers. obs.) Given the tank dimensions, described below (Figure 4.1), these groups created initial densities of 2.1 and 0.7 warpaint shiners per m^2 of tank area. In all, we completed nine trials at each warpaint shiner density: five LD trials at 12ºC and four at 17ºC; and four HD at 12ºC and five at 17ºC (see Table 4.1). (In order to minimize the acclimation stress on the shiners, the choice of experimental conditions for a particular trial was largely dictated by the conditions under which they were collected.)

We conducted three observation sessions per day, with the first occurring before noon, and the last after noon. To avoid satiation effects, we suspended feeding for at least 30 minutes between one pair of sessions and at least 90 minutes between the other pair. We began each trial with two days of observation on the focal warpaint shiners in the experimental group. We then began the series of randomly assigned two day treatments. Treatments were assigned randomly to minimize interactions between tank acclimation effects and treatment order.

Treatment 1- Focal Fish Control (WP)

During the first two days of each trial, we observed the focal group of two or five warpaint shiners, as described above.

Treatment 2- Density Control (DC)

In late afternoon, after the completion of the day's third observation session, one new warpaint shiner was introduced to the tank, followed by an overnight acclimation period. We performed an additional two days of observations of the fish in the experimental group, as described above. After the third observation session of the second day, the extra warpaint shiner was removed from the tank.

Treatment 3 -Rainbow Trout Treatment (TR)

We added one rainbow trout in late afternoon, followed by an overnight acclimation period, and performed two days of observations. In later trials, the absolute position of the rainbow trout was recorded, from which the Euclidean distance to the focal warpaint was calculated. After the third observation session of the second day of the rainbow trout treatment, the rainbow trout was removed.

Treatment 4 - Large Fish Control (LF)

We added one river chub which was larger than the warpaint shiners and as comparable in size to the rainbow trout as was practical, in late afternoon. Observation of the experimental group continued for an additional two days, as described in treatment above.

Tank design and physical parameters

We performed trials in an experimental stream located at the Whitehall Fisheries Laboratory at the University of Georgia. The experimental stream was constructed of 1.9 cm thick acrylic

[3.05 m (L) X 1.52 m (W) X .76 m (H)]. The stream had a false bottom approximately 30 cm above the base of the tank that allowed for flow to be generated by two electric trolling motors (@24.95 kg thrust). Water reemerged at the opposite end of the platform and flowed across the top of this platform in a nearly laminar fashion. An experimental arena was created on the platform with a pair of block nets across the width of the tank, and this area was subdivided with two platforms constructed of acrylic and PVC to create three discrete depth zones of equal area: a “riffle” zone with a depth of 15 cm, a “run” zone with a depth of 25 cm, and a “pool” zone with a depth of 35 cm (Figure 4.1) Riffles were covered with a 5cm layer of small cobbles (maximum diameter 15 cm) and gravel, whereas the run was covered with a 4 cm layer of gravel (max. diameter 2cm) and the pools with a 2 cm layer of gravel and sand, with a small transition zone between each section consisting of sloping piles of substrate. The tank was shrouded with sheets of opaque fiber board which ensured visual isolation of observers. All observations (see below) were made from within a black plastic blind constructed around a small window in the tank screen to minimize any disturbance to the fishes.

We set the water temperature and photoperiod to approximate both spring/fall (water temperature 12 C ± 1 C photoperiod 10.5 hours including 15 minute periods at “dawn” and “dusk” during which the light intensity was gradually stepped up or down) and summer conditions (water temperature 17C ± 1.5C, photoperiod 12 hours, including dawn and dusk) conditions (Duncan Elkins, unpublished data.)

We measured flows in the tank with an electronic flow meter ($\underline{+}$ 0.01 cm/s) before and after each trial to ensure consistency within and among trials. Mean velocities in the three microhabitats were maintained at 22 cm/s, 16 cm/s, and 11 cm/s in the riffle, run and pool sections of the tank respectively. Velocities ranged from zero in the pool to 32 cm/s near the upstream end of the riffle. We maintained pH at 7.3 and added aquarium salt (NaCl) at the rate of 7.5g per 10 gallons of dechlorinated tap water to ease osmotic stress. Water velocity, temperature, and pH ranges were consistent with those repeatedly recorded in the field at the collection site (Grossman and Ratajczak pers. obs.).

We installed 8 prey release outlets spaced evenly across the front of the tank (see Figure 4.2) and even with the substratum. *Chironomidae* larvae (bloodworms) were dispensed at the head of the riffle and run sections of the tank, entrained in the current, and carried the length of the tank, simulating the natural drift of prey items. For each trial, we added a total of between 10.8 and 11g of frozen bloodworms to two water reservoirs above the tank, which were aerated to melt the frozen blocks and to keep food items in suspension. These reservoirs were connected to the eight release points by four nylon tubes, each of which passed through a computer-controlled solenoid valve and a Y connection. These release valves were programmed on staggered 90-second cycles such that one of the four valves was open for one second out every 22.5. Although the prey delivery rate was constant, in terms of the volume of water released, per minute, stochastic variation in the suspension of prey within the reservoir and the path through the Y-connectors simulated natural variability in prey abundance.

Collection of Experimental Subjects

We obtained a subsample of rainbow trout to be stocked that week by Georgia Department of Natural Resources (DNR) personnel from the trout hatchery at Lake Burton, Habersham County, Georgia. Trout were held in a closed tank (480 l) and fed a diet of commercial trout chow (2.4 mm pellets) to simulate hatchery conditions. We collected warpaint shiners and river chubs from Coweeta Creek, Macon County, North Carolina, using seine nets as described by Wagner (2004). After acclimation of at least 12 hours in our species-specific holding system, all warpaint shiners were anesthetized using a buffered solution of MS222 (Tricaine Methane Sulphonate) at a concentration of 1.07 g to two liters of holding tank water, weighed (± 0.01 g), measured (standard length, ± 1mm).

While anesthetized, each fish was marked with a colored acetate tag (approximately 2mm^2) just below the dorsal fin as described in Wagner (2004), where it was further established that these tags do not affect general or feeding behavior of small minnows. We then returned warpaint shiners to the holding tanks to recover for two days prior to the beginning of each set of experiments, during which we fed them bloodworms and administered a prophylactic dose (3mg/L) of the antibiotic Kanamycin Sulfate.

The typical period between collection and initiation of an experiment was four days (collect Thursday, mark Friday, recover Saturday and Sunday) although this period was occasionally extended to six days due to weather or other scheduling constraints. We did not collect any fish that were visibly diseased or deformed in the field (although many fish did exhibit trematode *metacercaria,* which we enumerated but did not attempt to treat.) We attempted to collect shiners of similar size (range less than 10mm) in the field and marked 3-4 more fish than were required for a trial so we could select the individuals that recovered fastest from the anesthesia for use in the trial. The remaining fish were kept in our holding system and fed bloodworms for the duration of the trial. Although several fish in our pre-experiment feasibility trials succumbed to an external secondary infection around the marking site (probably *Flavobacterium columnare*), we experienced no mortality from infection after the installation of an ultraviolet sterilizer in the holding system and initiation of the Kanamycin prophylaxis; the most common source of mortality during our trials was fish jumping out of the tank through gaps in the screen cover around the water return plumbing. All fish were euthanized after our trials in a supersaturated solution of CO_2.

Fish Observations

We based experimental procedures on those of Rincón and Grossman (1998). Warpaint shiners were introduced into the experimental stream in late afternoon and left to acclimate overnight. We made our experimental observations during three sessions each day. All observation sessions began 30 minutes after the initiation of the feeding system to allow the fishes to acclimate to the prey releases. We observed individual fish for periods of two minutes each, during which we recorded the X,Y, and Z coordinates and identity and distance of the nearest con- and heterospecific every 30 seconds.

Within the 30 second observation periods, we recorded feeding activity (number of prey items captured and missed) and aggressive encounters involving the focal individual (both initiator and receiver and intensity of encounters, plus fish identity). Aggressive behaviors were qualified as a flash, charge, or nip, as follows: We recorded a flash when a fish changed its posture or orientation relative to another fish, provided this change did not include a movement of more than one body length. Typically, a flash involved angling the body upward, turning sideways relative to the target fish, and briefly flaring the fins. A chase involved the initiator moving more than one body length towards another fish, including active pursuit. We recorded a nip if a charge resulted in contact between the head of the initiator and the target fish. We also recorded aggressive interactions with focal individuals and any treatment fish, as well as their position in the tank in the last seven trials.

After two minutes of observations, we proceeded to a new focal fish, in a random order chosen before the start of the session, until all of the individuals had been observed. In all, we made three circuits resulting in 30 minutes of observations (5 fish * 2 minutes * 3 rounds) in each session of the high-density trials or 12 minutes of observations (2 fish * 2 minutes * 3 rounds) per session of the low-density trials. All observations were recorded on microcassette and transcribed after the completion of the trial.

Current Mapping

To ensure that velocities were consistent from trial to trial, we measured the current before each trial at four locations (the centroids of the run and riffle sections and two points directly behind these centroids at the midpoint of the pool) at 10cm above the substrate and adjusted the trolling motors, as necessary. At the conclusion of our trials, we mapped the current in the tank at a finer scale for use in modeling the current regime experienced by each fish. We gridded the tank off in 10x10cm squares and measured the current at 2 cm from the substrate in every square. We completed similar measurements using a 20 x 20 cm grid at 10 cm from the substrate and a 30 x 30 cm grid at the surface. Substrate-associated measurements were made using a smaller grid to capture turbulence caused by cobbles and the sloping tank bottom between habitat sections and because the majority of the fish activity we observed was near the bottom.

We used a 2-dimentional spline procedure in Arc/Info (ESRI 2006) to interpolate between the measured velocities and create a continuous current map for the experimental area with a cell size of 1x1 cm. This raster layer then served as a reference, containing the predicted velocity for every location within the tank.

Warpaint Space Use

We estimated warpaint shiner home ranges for each treatment from the 30-second positional data using the LoCoH tool in R (R Development Core Team 2008). LoCoH is a non-parametric, kernel-based method of home range estimation citation. In contrast to other algorithms that describe space use by aggregating points, such as minimum convex polygons, LoCoH can generate home ranges containing non-contiguous areas and is particularly well suited for describing habitat use in patchy environments containing distinct habitat types e.g., pools within a stream reach (Getz and Wilmers 2004).

We used the ‘adaptive sphere-of-influence’ α -LoCoH method to calculate home range, as recommended by Getz, Fortmann-Roe et al. (2007). This method assembles polygons that enclose home ranges by first considering each observed location and identifying all the neighboring points within a cumulative radius such that the distances of all points within the radius to the reference point sum to a value less than or equal to α. It then produces minimum convex polygons (local hulls) enclosing this group of points. After iteratively creating a local hull for each observed location, the hulls are sorted in increasing order of size, then merged until a given size home range is produced. Hence, for a 25th percentile isopleth, hulls are merged until 25 per cent of points are included. We chose a value for α by following the 'minimum spurious hole covering' rule (as described in Getz et al. 2007). We allowed the algorithm to selectively add up to 2cm of random 'jitter' to our observations, since overlapping points are discarded, otherwise, and 2cm is probably well within the range of our observation error.

Although it is possible to ensure that the 100% isopleth contains all the recorded points by selecting a value of α greater than the maximum distance between any two observations (for our tank, approximately 440cm), the isopleths generated using values of α greater than ~150cm consistently resulted in home ranges that were unrealistically large and covered areas rarely occupied by warpaint shiners (the equivalent of spurious hole-covering in our habitat). Getz, Fortmann-Roe et al. (2007) showed that the adaptive method was fairly insensitive to small changes in alpha. Hence, we used an α value of 100 for our data, which produced home ranges consistent in shape and scale with our observations, and did not exhaustively tune the value for each individual trial. We also specified that each hull must contain at least 2 points to ensure that 100% isopleths always would be generated, ensuring that the 95% isopleths would be available for our analysis.

We generated four home ranges for each individual focal warpaint to describe the space used by that fish during each experimental treatment (WP, DC, LF, and TR). For these individual fish home ranges, we selected only the 95% isopleths, or the polygons that enclosed 95% of the fish observations, in order to describe the overall area used by an individual fish during the two days of a treatment.

We also generated a series of core home ranges (Wray et al. 1992), using the 50% isopleths, for which we pooled all fish observations for all trials of a given density and season, and created maps of the areas that were most commonly used by warpaint shiners in each treatment, across trials. We used core areas for this analysis because 95% polygons were unrealistically large, probably due to individual variation among the warpaint shiners in the different trials.

Space Use Analysis

To test whether rainbow trout altered the spatial attributes of the warpaint shiners' homeranges, we used ArcMap (ESRI 2006) to calculate the area, perimeter, and edge/area ratio of each set of 95% isopleths. To test the hypothesis that trout increased the overlap between warpaint shiners, we overlaid the isopleths within each trial and treatment and calculated the amount of overlap between the focal warpaints in a treatment both as an absolute area and as a percentage of each fish's home range. We used mixed linear modeling to test for significant differences in mean home range size, % overlap, or edge/area ratio of the warpaint shiners, using density, season, and treatment as fixed effects (including an interaction between density and season) and a random effect of individual fish (alpha = 0.05). All models estimate the additive effects of the other parameters relative to a baseline of LD and SPR conditions

We calculated the mean velocity and standard deviation within each core polygon using the Iterative Zonal Statistics ArcScript (Albeke 2009), which used the core layer as an analysis mask for the velocity map to extract just the pixels describing the current profile within each area of fish use. Using the standard deviation reported by the script and number of pixels in each polygon, we calculated 95% confidence intervals around each mean.

RESULTS

Modeled velocities ranged from slightly negative (-2 cm/sec) in the pool mesohabitat to approximately 35 cm/sec at the top of the riffle, with intermediate velocities in the run and some turbulence at the boundaries between the mesohabitats (Figure 4.3). The LoCoH algorithm frequently generated multiple polygons for each fish, which is consistent with our observations, and these polygons were fairly stable for the WP, DC, and CH treatments (Figure 4.4 and Figure 4.5).

Rainbow trout did not affect the edge/area ratio for the warpaint shiners' homeranges. Under the baseline conditions (LD, SPR, WP) the mean edge/area ratio as 0.37, and none of the other effects were significant. (All parameter estimates for linear models are listed in Appendix 4.1.)

Rainbow trout significantly increased the average homerange of a warpaint shiner, from 641 cm^2 under baseline conditions (LD, SPR, WP) to 1009 cm^2 (df 566, $t = 5.18$, $p < 0.01$), an increase of 57%. The only other significant effect was the BF control, which reduced the mean home range to 440 cm^2 (df 566, $t = 4.09$, $p < 0.01$).

All of the fish addition treatments decreased the mean homerange overlap significantly from 18% at the baseline (LD, SPR, WP). When a river chub was present (BF), the mean homerange overlap dropped to 5% (df 566,$t=-6.95$, $p < 0.01$) and when an additional warpaint shiner was present the overlap dropped to 9%(df 566, t=-4.73, p<0.01). The presence of a rainbow trout (RT) reduced the home range overlap the least, to 11% (df 566, t=-3.84, p<0.01).

Velocity Profiles

Under both densities and in both seasons, the mean velocity in the core areas used by warpaint shiners in the focal fish control (WP) was either the lowest of the treatments or statistically indistinguishable from the lowest (Figure 4.6). In most cases (3 of 4), the mean velocity experienced by the focal warpaints was indistinguishable from that in the DC treatment, i.e., when the introduced fish was a conspecific. In all cases, the mean velocity experienced by a focal warpaint shiner during TR was significantly higher than under WP.

DISCUSSION

The polygons we calculated and refer to as "home ranges" describe only the locations used by the fish while they were feeding during the day, so they almost certainly do not accurately describe the entirety of the habitat used by these fish in a 24-hour period.   Nevertheless, this study suggests that the presence of rainbow trout may substantially reduce the minnows' fitness, by increasing energetic costs of maintaining swimming position and reducing food intake. For drift-feeding fishes in streams, microhabitat selection is strongly a function of velocity. Using a foraging model based on velocity, as it affects capture success, Hill and Grossman (1993) showed that both minnows and trout occupy microhabitats that maximize their energy gain. In an extension of this work, an optimal-foraging model based on energy gain successfully predicted the velocities where minnows were observed in the field in 79% of cases (Grossman et al. 2002).

Among drift-feeding salmonids, there is a well-established relationship between fitness and feeding position, with the most-dominant fishes getting the best feeding lanes. As shown by Fausch (1984), a model based on potential profit, in terms of net energy gain, almost perfectly predicts the linear rank for drift-feeding Coho salmon (*Oncorhynchus kisutch*) in an aquarium, with dominant fish occupying the more profitable locations. A similar pattern holds for rainbow trout (Metcalfe 1986): dominant fish occupy positions that allowed them to obtain a greater energy intake for a given expenditure, while subordinates that adopt a "high-return/high-cost foraging strategy" experience a net energy loss. Furthermore, in a natural system containing multiple salmonid species, subordinate species shift to use more profitable positions when the dominant competitor is removed (Fausch and White 1986). Evidence suggests that similar effects may occur when other families are subordinate to introduced trout: three native species (2 galaxiids, 1 Trichomycterid) have been shown to expand their mesohabitat use when non-native trout are selectively removed from a stream in Patagonian Chile (Penaluna et al. 2009).

Particularly in the presence of a predator, there may be other aspects of microhabitat selection that override net energy gain, such as position relative to other prey fishes. When confronted with an artificial predator in a lake, schools of spottail shiner (*Notropis hudsonius*) have lower reaction distance (i.e., spend more time on activities such as feeding) than do solitary individuals (Seghers 1981). However, in tests within a stream system Freeman and Grossman (1992) show that foraging groups of rosyside dace are not shoaling but aggregating, attracted not to each other but to patchy food resources and only incidentally polarized by flow, with the only evidence of shoaling occurring in response to a seasonally-occurring predator.

If the spatial responses the warpaint shiners displayed in the present of trout were anti-predator schooling, these effects were slight. In another analysis of these experiments Elkins and Grossman (2010- Chapter 2) showed that the mean distance to the nearest conspecific decreased significantly, from 56 to 46cm, when trout were present. However, the current analysis shows that mean homerange size increased and overlap decreased when trout were present, suggesting that the warpaints were not clumping tightly. Perhaps this is a result of "conflicting pressures" hypothesis (Magurran 1990), which suggests that advantage of schooling as an anti-predator defense may be outweighed by other selection pressures, such as the need to feed, at certain times. We did observe that the warpaint shiners were typically tightly clumped before the initiation of feeding during the morning session, which lends support to this idea.

When a rainbow trout was present, the positions of the warpaint shiners were less stable (the larger home ranges during this treatment suggesting more movement between observations) and the shiners experienced significantly higher velocities than they experienced when alone (WP) or when adjusting to a new conspecific (DC, 3 cases out of 4). The mean velocity experienced by the shiners increased during the BF control, as well, though less than when trout were present (3 cases out of 4). This result may have more to do with collaborative foraging behind the river chub. Unlike the trout, the river chubs in our trials would occasionally forage between the cobbles in the riffle and run sections of the tank for prey items lodged there. When this occurred, one warpaint shiner would trail the chub as into these higher velocity microhabitats, presumably to feed on the detritus dislodged by the chub. Whether or not this foraging behavior was behind the change in mesohabitat usage, we frequently observed overlap between the warpaint shiners' home range polygons and the points where the river chubs occurred, while this was almost never the case with the rainbow trout.

Because of the competitive dominance conferred by the size advantage of the rainbow trout, the warpaint shiners appear to be adopting the "high-return/high-cost" strategy for subordinate fish described by Metcalfe (1986) by moving more and occupying the higher-velocity mesohabitats. However, Elkins and Grossman (2010 – Chapter 2) showed that warpaint shiners actually fed less effectively and caught fewer prey when a trout was present, making this strategy one of higher cost by diminished returns.

Although direct predation is the clearest mechanism by which salmonids might affect native fishes, our results suggest a valid alternative pathway. Although our trials were short-term (48 hours or trout exposure), if these effects occur and persist in the field, we would expect them to significantly decrease the fitness of the warpaint shiners. Since many streams are stocked repeatedly throughout the trout-fishing season, rainbow trout have shown a propensity to naturalize and become invasive (Crawford and Muir 2008), and the ecology of warpaint shiners is broadly similar to that of many other minnows, there is a potential for substantial and ongoing negative impacts to native fish communities. Clearly, managers should use caution in making decisions to stock streams that currently support threatened or vulnerable native fish populations with rainbow trout.

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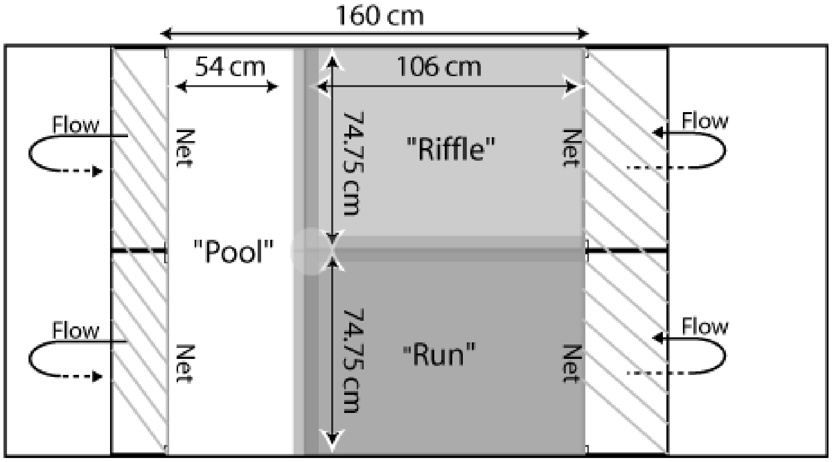
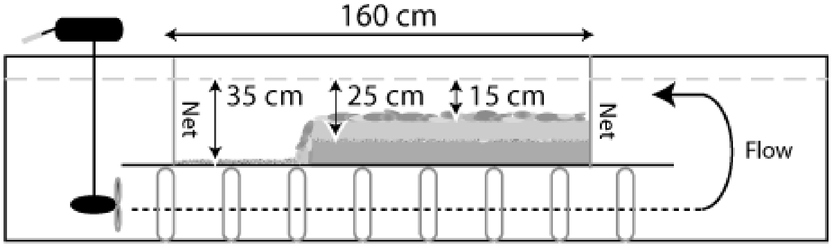


Plan View



Side View

Figure 4.1: Design of Experimental Stream. Not shown are intake and output plumbing for the filter and chiller systems, which were located in the bottom corner at the head of the tank and angled so as to minimize any potential disruption of laminar water flow.

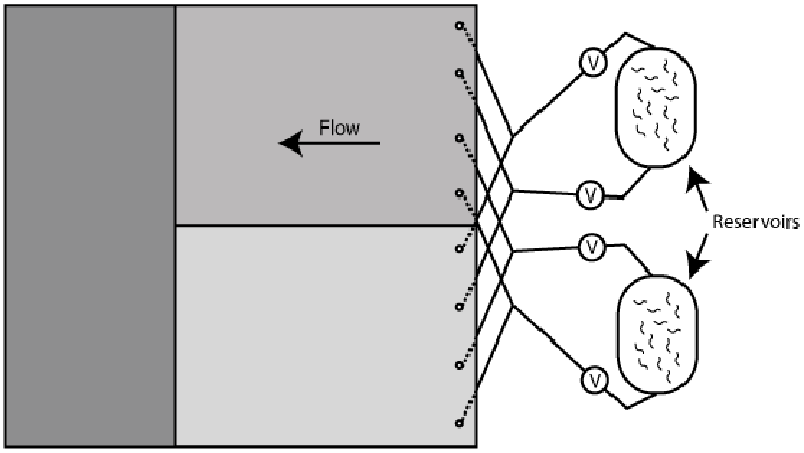


Figure 4.2: Feeder design in experimental stream. The two reservoirs were located above the tank and released suspended prey items into the stream via gravity flow while the computer-controlled valves (V) were in the open position.

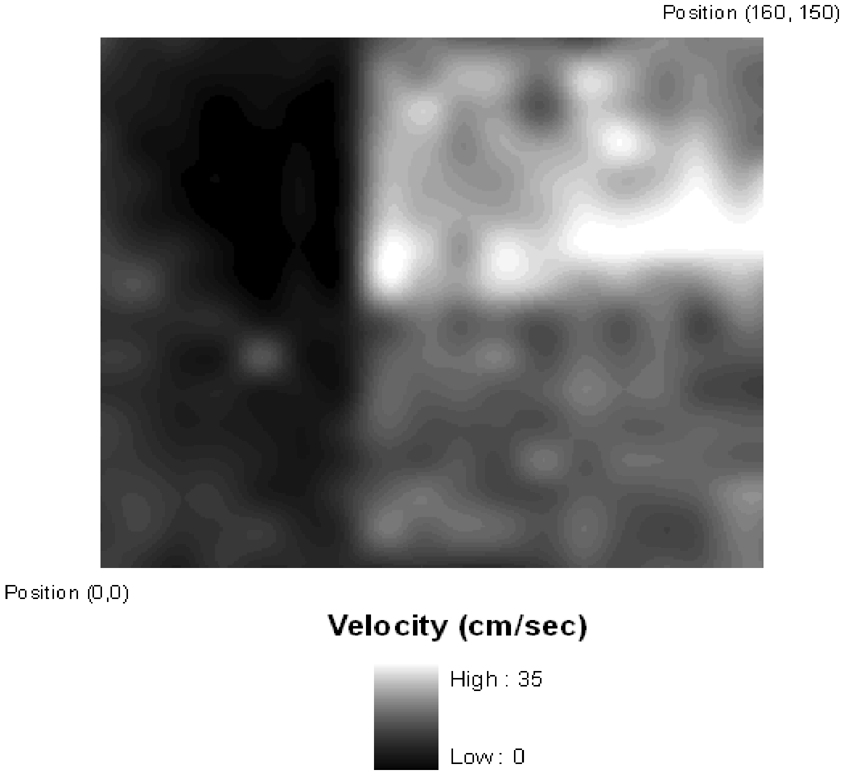


Figure 4.3: Current Map at z=2 cm interpolated from point measurements.

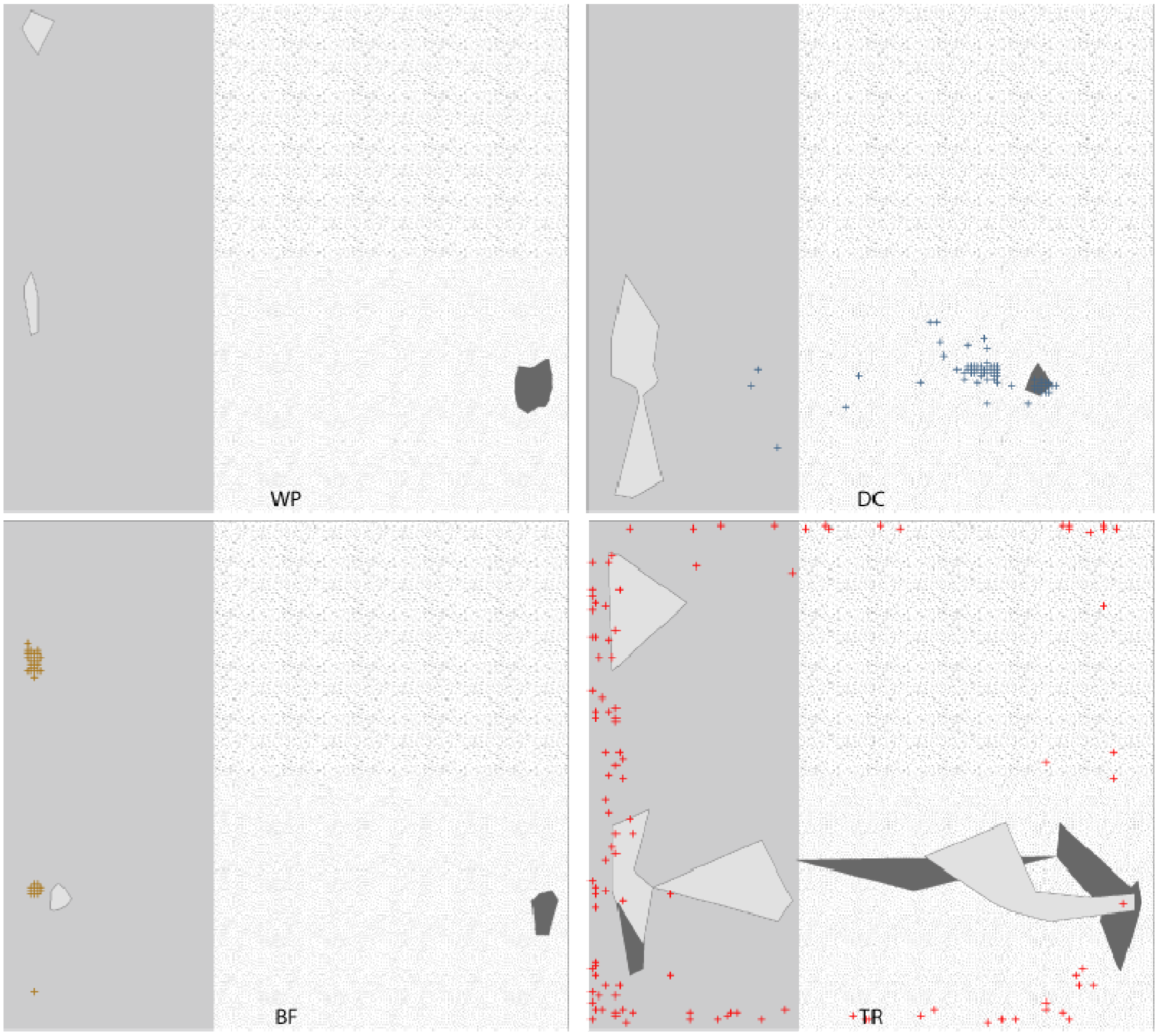


Figure 4.4: Homeranges derived for Trial 20. The colors of the polygons reflect the colors of the tags applied to the focal warpaint shiners in this LD trial under SUM conditions. Colored crosses reflect the measured positions of the additional fish in each treatment.

Figure 4.5: Homeranges derived for Trial 19. The colors of the polygons reflect the colors of the tags applied to the focal warpaint shiners in this LD trial under SUM conditions. Colored crosses reflect the measured positions of the additional fish in each treatment.

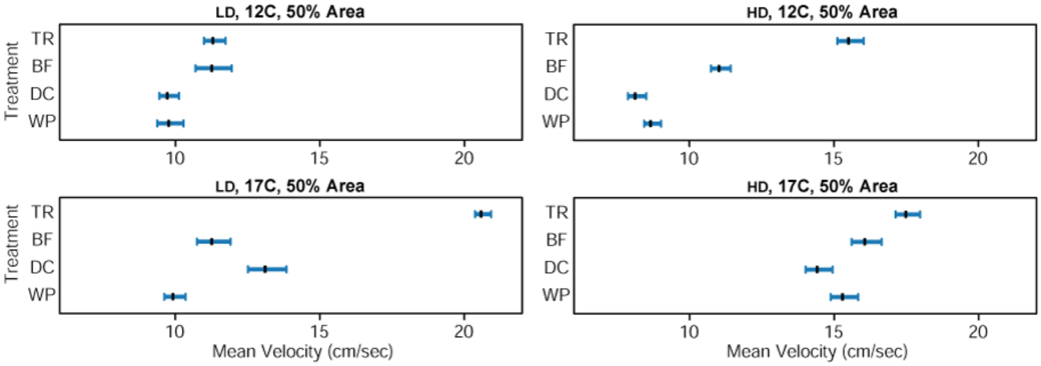


Figure 4.6: Mean (95% CI) velocity for the core areas used by warpaint shiners in all trials.

Table 4.1: Summary of parameters for experimental trials.

Trial	Focal Fish Dens.	Season	Warpaint SL (mm)	Order	Trout SL (mm)	Trout Mass (g)	Chub SL (mm)	Chub Mass (g)
1	HD	SUM	69-75	DC, TR, BF	189	78.3	145	58.7
2	HD	SUM	55-91	DC, BF, TR	216	144.6	142	50.0
3	HD	SUM	80-118	BF, DC, TR	232	180	148	51.7
4	HD	SUM	67-82	DC, TR, BF	192	130.3	180	82.5
5	LD	SUM	68-74	BF, DC, TR	189	128.3	180	83.0
6	LD	SPR	105-112	DC, BF, TR	133	35.0	116	25.0
7	LD	SPR	87-88	DC, TR, BF	201	109.5	152	61.5
8	HD	SPR	81-108	TR, BF, DC	173	77.3	152	61.5
9	LD	SPR	50-51	BF, TR, DC	127	28.5	107	22.5
10	LD	SUM	83-90	TR, BF, DC	174	69.4	173	77.9
11	HD	SPR	60-63	BF, DC, TR	212	128.0	140	38.1
12	HD	SUM	62-66	TR, BF, DC	250	232.5	144	36.9
13	HD	SPR	71-79	TR, BF, DC	210	127.1	141	35.8
14	LD	SPR	62-64	TR, BF, DC	145	54.4	100	16.2
15	LD	SPR	77-78	TR, DC, BF	171	57.3	110	19.2
16	HD	SPR	68-73	BF, DC, TR	255	229.7	110	18.2
17	LD	SUM	69-69	TR, BF, DC	223	92.8	130	33.0
18	LD	SUM	76-77	TR, DC, BF	217	184.8	116	27.2

APPENDIX 4.1: LINEAR MODEL ESTIMATES

Edge/Area Ratio

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.37	0.03	147	10.98	<0.001
BF	0.00	0.03	147	-0.03	0.975
DC	0.04	0.03	147	1.14	0.255
TR	-0.04	0.03	147	-1.42	0.158
SUM	-0.05	0.04	46	-1.13	0.265
HD	-0.03	0.03	46	-0.96	0.341
SUMxHD	0.01	0.05	46	0.12	0.904

Homerange Size

	Value	Std.Error	DF	t-value	p-value
(Intercept)	640.65	156.45	566	4.09	<0.001
BF	-136.45	69.33	566	-1.97	0.0495
DC	-73.19	69.33	566	-1.06	0.292
TR	368.76	71.22	566	5.18	<0.001
SUM	240.57	225.78	51	1.07	0.292
HD	-101.59	173.83	51	-0.58	0.562
SUMxHD	281.84	261.68	51	1.08	0.287

Homerange Overlap

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.18	0.03	566	5.40	<0.001
BF	-0.13	0.02	566	-6.95	<0.001
DC	-0.09	0.02	566	-4.73	<0.001
TR	-0.08	0.02	566	-3.84	<0.001
SUM	-0.03	0.05	51	-0.56	0.579
HD	0.06	0.03	51	1.59	0.119
SUMxHD	-0.08	0.05	51	-1.63	0.111

CHAPTER 5

SUMMARY: RAINBOW TROUT STOCKING AND NATIVE FISHES

SUMMARY

Although rainbow trout are commonly stocked into coldwater streams in Georgia, little is known about the effect that this practice has on the rich fish biota of these streams. In this project, we quantified the short-term effects of rainbow trout stocking on native fishes, using both small scale field introductions in two sets of paired control and treatment sites, and laboratory experiments in an experimental stream. Specifically, we assessed the effect of stocking on non-game fish species richness and abundance, patterns of habitat selection by non-game fishes, and habitat use and foraging success of warpaint shiner, *Luxilus coccogenis*, a native non-game fish that may serve as a surrogate for T/E minnows.

Our field experiments demonstrated that environmental variability is manifest as changes in habitat availability for native fishes and at the assemblage level in fluctuating species richness, diversity, fish populations, and fish biomass. Short term field stocking experiments did not produce any significant changes in species richness, diversity, population size or biomass of non-game fishes in our paired sites. The field stocking experiments did produce significant short-term effects on microhabitat use of several native fishes. When responses were present, the general response was movement out of the deep, fast sections of pools into more variable microhabitats that tended to be shallower, slower, and more depositional. In addition, some species occupied more variable microhabitats with lower flows.

Our field results are consistent with those of the laboratory experiment: in the absence of rainbow trout, the warpaint shiners occupied the pool mesohabitat in the tank. During rainbow trout treatments, warpaint shiners were significantly closer to one another, caught significantly fewer prey and had significantly reduced feeding efficiency (items captured per strike), than during the other treatments, and the effects on feeding efficiency were exacerbated during spring/fall conditions

Our spatial analysis gives additional insights into the effects of the microhabitat shifts we observed in the field and in the lab. Using a methodology developed for tracking mammals, we described the “homeranges” used by warpaint shiners and used the changes in these homeranges to quantify both the additional movement (increased homerange size when trout were present) and swimming velocity (increased mean velocities when trout were present) that, together, describe significantly increased energy expenditure by the warpaint shiners when trout were present.

Combined, these studies show that rainbow trout affect habitat use of native fishes and that these effects were observed over a range of water – column and benthic species. Nonetheless the strongest and most consistent responses were observed in upper – water column guild members that overlap rainbow trout the most in microhabitat use. The lab studies suggest that rainbow trout may significantly reduce the fitness of non-game species by increasing energetic costs of maintaining swimming position and reducing food intake. Although, results from field experiments are short-term and not always consistently manifest by all species, combined with lab results they suggest that the presence of stocked rainbow trout may result in displacement from preferred microhabitats and increased energetic costs for non-game species.

Because the field sites are visually similar to many stocked streams in Georgia and the warpaint shiner is a “typical” southeastern medium–sized minnow, our results should yield insights into the potential effects of stocking rainbow trout on other species and sites. Consequently, we urge caution when considering the stocking of rainbow trout in streams containing vulnerable native species.