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A Before-After Control-Impact Comparison of the Effects of Whirling Disease Epizootics on Trout Population Dynamics in Montana Rivers

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Project Summary: Whirling disease (WD) has spread rapidly throughout the United States the past 20 years, but predicting its impacts to trout populations has been problematic. Using a database that contained capture-recapture information for 384,938 trout during the years 1980-2007, a before-after control-impact (BACI) study design was used to analyze data from infected river sections and non-infected reference sections on six different Montana rivers infected with severe whirling disease (>50% of sentinel fish with grade ≥3 infection). The BACI comparison allowed us to estimate how much change in rainbow and brown trout populations was due to whirling disease versus other factors such as variation in flow. Effects of WD on trout recruitment, growth, condition, biomass, and age structure were also examined. A Bayesian mark-recapture model indicated that disease had a strong negative effect on abundance of small rainbow trout, with abundance declining to an average of 50% (range 30-69%) of pre-disease levels. This marked decline was consistent across all study rivers. In contrast, a parallel decline in larger fish was not observed; instead, the numbers of rainbow trout >300 mm either remained the same or increased sharply after WD, with the magnitude of the changes varying by river. Rainbow trout of all size classes did not show reduced growth or condition after a WD outbreak, suggesting that those fish that survive do not suffer continued survival or performance deficits even in highly infected systems. In the Missouri River, rainbow trout age 4 and older show marked increase in survival and growth after WD. Changes in rainbow trout density were generally compensated for by opposite changes in brown trout density. This pattern of replacement was particularly evident in Rock Creek, where decline in rainbow trout from 90% of total trout density to 20-30% after WD was met by a similar magnitude increase in brown trout. High infectivity levels coincided with low stream flows since 2000, indicating drought may have exacerbated whirling disease impacts on rainbow trout.

3.1. **Introduction**

Whirling disease (WD), an infection of salmonids caused by the nonindigenous metazoan parasite *Myxobolus cerebralis*, has been rapidly expanding throughout North America over the past 20 years (Hedrick et al. 1998; Bartholomew and Reno 2002). The parasite has now been detected in 22 states, and continues to spread, threatening wild salmonid populations (Bartholomew and Reno 2002; Arsan et al. 2007). WD has led to major declines in high value recreational trout fisheries throughout the western United States (Nehring and Walker 1966; Vincent 1996). In Colorado, at least 560 km of premier trout streams have experienced long-term declines in rainbow trout populations; in some locales, declines of 90% of rainbow trout density and biomass have persisted for over 10 years (Nehring and Thompson 2003). For example, in the Gunnison River, Colorado, numbers of 150-mm and larger rainbow trout during pre-WD years of the 1980s averaged about 3,400 per km but subsequent population estimates yielded 531 per km in 1998 and 86 in 2003 (Nehring 2006).

Whirling disease was first confirmed in Montana in 1994 following sharp declines in Madison River rainbow trout (Vincent 1996). Since the 1970s, Montana trout rivers have been managed as wild fisheries, without supplemental stocking (Vincent 1987). In other states, stocking of *Myxobolus cerebralis*-infected rainbow trout led to rapid spread and magnification of infection in wild rainbow trout (Nehring 2006). The discovery of *M. cerebralis* in the Madison River precipitated a statewide program to monitor the spread and disease risk of the parasite utilizing caged sentinel fish (Baldwin et al. 1999).

One of the perplexing problems of WD, given the wide spread of the parasite into many waters in the U.S., has been the high variation reported in population responses to infection. Some infected trout populations have been reported to have severely declined (Nehring and Walker 1996; Vincent 1996; Nehring 2006), whereas others reportedly showed no detectable effects (Modin 1998; Kaeser et al. 2006). In a review of WD impacts in Colorado, such a wide range of responses led Nehring (2006:40) to conclude that "it is very difficult to predict with any degree of certainty where, when and under what circumstances the impact of *M. cerebralis* might be devastating and where it would be benign." Although the difficulty in forecasting population impacts from the disease is not unexpected given the dynamic and complex nature of the host-parasite-environment relationship (Hedrick et al. 1999; Kerans and Zale 2002), how the parasite affects salmonids at the population and assemblage level is a key metric of interest for assessing disease impacts; however, there have been few in-depth studies of trout population dynamics following epizootic outbreaks of WD (Karr et al. 2005).

Vincent (pers, comm.) noted that rainbow trout population declines in some Montana rivers occurred when 50% or more of sentinel fish had disease severity scores of 3 or more on the MacConnell-Baldwin scale (0 = uninfected, 5 = severe infection). Fish with this level of infection exhibit clinical symptoms of disease including whirling behavior, blacktail, cranial and spinal deformities, exophthalmia, and poor survival and performance (Thompson et al. 2002; Ryce et al. 2004; DuBey et al. 2007). However, the linkage between disease severity observed in sentinel fish and disease severity and population effects in wild fish is uncertain. If population-level effects could be tied to a disease-severity threshold measured from sentinel fish, trout population response could be more reliably predicted based on measured infectivity levels in the field, which would thereby result in improved risk-assessment tools (Bartholomew et al. 2005).

In this study, we used a before-after control-impact (BACI) study design to assess if trout populations in six different river drainages in Montana exhibit similar responses to severe WD epizootics. These drainages have a unique combination of long-term fish population, disease severity, and environmental data that allow a detailed analysis of population response to a WD epizootic under varying biotic and abiotic factors. We also assessed possible compensatory growth and survival responses to WD outbreaks by examining other metrics in addition to changes in abundance including recruitment, growth, condition, size structure, and trout species composition before and after the onset in WD.

3.2 Study design

A BACI study design was used to assess population responses to whirling disease across multiple drainages in Montana. The BACI experimental design was developed to answer the difficult question of quantifying environmental impacts that vary over space and time (Underwood 1992). The invasion of *M. cerebralis* in a trout river can be likened to an 'unplanned impact' that precludes random selection of treatment and control sites that characterize ideal experimental designs used in statistical analysis (Wiens and Parker 1995). However, statistical comparison of the amount of change among multiple reference and impact sites before and after an impact allows for quantification of effects of the impact with a greater degree of certainty than traditional impact studies that employ just a before-after comparison without controls, or else only one control and treatment site (Wiens and Parker 1995). The BACI design has been employed to quantify seabird declines after the *EXXON VALDEZ* oil spill (Murphy et al. 1997); the impact of the 2000 Bitterroot, Montana, wildfire on fish populations by comparing data from burned and reference streams before and after the fire (Sestrich 2005); and the effects of logging on cutthroat trout populations (DeGroot et al. 2007). Inclusion of reference sections allows partitioning of changes from the impact versus other environmental factors that may have changed independently of whirling disease such as water temperature and flow. Thus, comparison of before and after differences between reference and WD positive sites can be used to account for the effect of natural variation that could mask or artificially amplify the estimated impact (Wiens and Parker 1995).

Within-drainage reference sections, described below, were identified for 5 of the 6 study rivers. In our study 'reference section' refers to a river reach that has had no or low infection (0-2 disease severity ranking) relative to 'impacted' river sections where there has been a sustained infection risk of 50% or more of sentinel cage fish showing moderate to severe lesions (>3 disease severity ranking) indicative of severe WD infection. Reference sections were located from 16 to 55 km from impacted sections, a sufficient distance such that the sections can be reasonably considered independent of each other.

A methodological limitation of many environmental impact studies is the lack of consistency in data collection among sites or a limited time scale of available data (Wiens and Parker 1995). However, trout population data have been collected in a consistent fashion on Montana trout rivers since the early 1980s using electrofishing mark-recapture techniques over the same, long sampling sections (1.5-9.0 km) at multiple sites within each drainage (Vincent 1982).

3.3 Methods

Data Sources and Acquisition

Trout Population Data and Timing of WD Infection

We obtained trout population data from two sources. We first queried Montana Fish, Wildlife, and Parks' (FWP) fishery database compiled by the Fisheries Information Services (FIS) office in Bozeman. The database has been developed as a repository for all fish population data collected by FWP fish biologists. However, not all available data has been compiled in the database, so we filled in missing data by contacting fish biologists who conduct population surveys for each river used in our analysis. Substantial time was required to acquire the data and format it into a common database. The final database was comprised of 384,938 trout collected during the years of 1980 to 2007.

We initially identified 7 Montana rivers that met the requirements for a WD outbreak outlined in the Introduction: the Missouri, Madison, Big Hole, Ruby, Bitterroot, Blackfoot rivers, and Rock Creek. All rivers had a long time series of data for trout populations, WD infection, and environmental conditions that allowed an in-depth analysis of long-term whirling disease impacts to wild trout populations. We subsequently omitted the Madison River from the analysis as Dick Vincent, FWP Bozeman, was concurrently analyzing trout population response to WD for this river in detail in a study separate from our own. In addition, we omitted the Big Hole River from our analysis to avoid lengthy delays in our analyses that would have been necessary because of the difficulties in obtaining data in electronic format for this river. There is a long time series of data on the Big Hole, but little of it is has been digitized to date. We expended considerable time and effort trying to compile the complete dataset for this river, but due to time constraints for digitizing the data, we omitted this site from inclusion in the analysis. However, in its stead, we substituted the Gallatin River because it met our data requirements for having a long time series of population data available in electronic format and presence of both reference and WD-infected reaches in the system. The final dataset included 6 study rivers, although the analysis of Bitterroot River data was restricted by limited post-WD data for some sites. Descriptions of trout population data available for each river as well as how the timing of WD infection for the BACI analysis was determined (pre- vs post-WD years and reference vs. infected sites) are detailed below. Kerans et al. (2009) provides maps of sentinel fish cage sites, as well as more detailed information on temporal and spatial patterns of infection severity within for each drainage.

1) Bitterrroot River: We incorporated five population sampling sections into our analysis. On the East Fork of the Bitterroot River, the Trinity and Maynard sampling sections are located 4.2 and 20 km, respectively, upstream of the confluence (see Kerans et al. 2009); the lengths of the respective reaches are 1.3 and 0.8 km[1]. The 4.6-km-long Connor sampling section on the West Fork of the Bitterroot River is located 2 km from the confluence. The distance between the Connor and Trinity sections is about 20 km. Two sampling sections were incorporated from the mainstem Bitterroot River: Darby, an 8.2 km-long reach located at river kilometer 120, and Bell Crossing, a 5.8-km-long reach located about 55 km downstream at river kilometer 65. Population sampling has occurred every 1-3 years since the 1980s in most sections, and since the 1990s in all sections. The East Fork sections are free-flowing and not influenced by dams; the West Fork Connor section is located in a tailwater below Painted Rocks Reservoir. Minimum summer flows in the Darby section, and to a

[1] Although section lengths sampled were typically the same from year to year, there was some variation in sampling distance, especially during the early years of sampling in the 1980s. The most common sampling distance is cited in the text; in the analysis, all population estimates were standardized to number per km.

lesser extent in the Bell Crossing section, are partially moderated by Painted Rocks Reservoir upstream.

No *M. cerebralis*-infected fish were found during examination of free-ranging trout from 1996 to 2001 in the mainstem Bitterroot River from Missoula to the West Fork confluence (Vincent 2003). WD monitoring with sentinel fish cages first began in 2000 and has continued sporadically throughout the East and West Forks (see Kerans et al. 2009). We compared tributary and mainstem reaches separately due to differences in river size. The West Fork Connor section served as the reference reach to the infected East Fork Trinity and Maynard sections. Mean WD severity in the Connor section has tested 0 to <1.0 in all sampling done from 2000-2007, whereas sentinel cages on the East Fork increased from 0.3 in 2000, to 1.0 in 2002, to peak infections >2.75, including mean severity rankings of >4.5 from 2004 to 2007, although many of these samples were taken in the fall, and the infection severity during rainbow trout spawning in the spring is unknown (Kerans et al. 2009) but presumably as high or higher than fall samples. Bell Crossing section served as the reference reach and Darby was classified as the infected reach on the mainstem. Sentinel fish cages located in or near the Bell Crossing section have been well below 1.0 mean infection severity from 2001 to 2007. Infection severity on the Darby section was about 0.5 when first measured in 2001, increasing to >3.0 beginning in 2005, and measuring >3.3 in 2006 and 2007. Mean infection severity of >2.75 was first observed in fall 2005. Based on these data, our best estimate for when a population level severity first appeared in both infected reaches was 2004.

2) Blackfoot River: Two population-sampling sections, Johnsrud and Scotty Brown, were used in the analysis. The Johnsrud section is a 5.8 km-long reach located in the lower Blackfoot River. The Scotty Brown section is a 6.1 km-long reach located in the middle Blackfoot River drainage near Ovando, Montana, about 50 km upstream of the Johnsrud section. Biannual population sampling for both sections began in 1989.

WD was detected in a few free-ranging trout during initial sampling of the study area in 1995. Sentinel cages were first deployed in 1998. Johnsrud was designated as the reference reach as infection levels from the nearby sentinel cage site at Gold Creek ranged from mean severity of 0.2 in 1998 to a peak of 2.4 in 2000, and has fluctuated between 0.5 to 2.2 since that time. Scotty Brown was designated as the infected reach. Sentinel cages in this area (Cottonwood Creek and Monture Creek), had low infection (<1) in the mainstem and nearby tributaries in 1998, but infection severity rose to levels exceeding 2.75 in 2002. Radiotagging revealed that most spawners in this section of the river appear to spawn in Monture Creek (Pierce et al. 2009). The infection in this creek was low from 1998 to 2000 (0.2 to 1.72; no data in 2001) and reached >2.75 levels in 2002 and rose to levels >4 thereafter. The estimated time when infection first exceeded a severity of 2.75 was 2002.

3) Gallatin River: The two sections are the 3.1 km-long Hoffman section on the East Gallatin near Bozeman, Montana, and the 3.5 km-long Jack Smith section on the mainstem Gallatin River near Big Sky (Kerans et al. 2009). The sections are separated by about 50 river kilometers. The Hoffman section is a smaller, warmer, lower gradient reach in contrast to the wider, colder, high gradient Jack Smith section of the

Gallatin Canyon. Population data were available for both sections beginning in 1982 and continuing through 2006-07.

Hoffman was the designated infected reach. Vincent (FWP Bozeman, pers. comm.) noted that the East Gallatin has one of the more severe WD infections in Montana. Infection severity measured in sentinel cages near the Hoffman section were 1.3 when first deployed in 1999, 0.6 in 2000, 1.47 in 2001 and 2.9 in 2002. In 2003-2005, cages in this area increased markedly to very high levels, well above 4.0. The estimated time when infection exceeded a severity of 2.8 was 2002.

Jack Smith was designated the reference reach. Although sentinel cage data is somewhat limited in this river section, infection levels measured in 2006 in three separate cages within or directly upstream or downstream of this section ranged from 0 to 0.5. Infection levels in nearby cages downstream at Gallatin Gateway were 0 to 0.5 in 2001 and 2005, lending further support of the designation as a low infection reference reach. It could be argued that this is not a true reference site because upstream infections in the West Fork of the Gallatin River have been quite high, ranging from 4.8 in 2004, 3.7 to 4.9 in 2005 and 2.9 in 2006. However, it is important to note that sentinel cages in the mainstem within a kilometer downstream of the confluence with the West Fork have always recorded infection levels of <0.5. Thus, the designation of the Jack Smith section as a reference reach seems reasonable.

4) Missouri River: The Missouri River below Holter dam contains some of the most extensive time series of trout population data in Montana. The Craig and Cascade sections have been sampled almost annually in the fall and spring since 1980. The 9.0

km-long Craig section near Wolf Creek, Montana, is located just downstream of Holter Dam (Kerans et al. 2009). The 6.6 km-long Cascade section near Cascade, Montana, is located 27 km downstream of the Craig section.

Craig was designated as the infected reach. Little Prickly Pear (LPP) Creek, a major spawning and rearing tributary, supports juvenile and adult recruitment to the Craig section. WD testing of free-ranging fish in LPP in 1996 confirmed the presence of WD but at a low severity. Sentinel cages were first deployed in 1997. WD infection severity rose from a low of <1 in 1997 to >2.75 in 1998. Infection severity has remained >3 through 2005. The sharp drop in number of yearling rainbow trout migrating to the mainstem from LPP in spring 1999, a level much reduced from the previous year of outmigrant trapping, provides corroborating evidence that high WD infection began in 1998. These 1998 year class fish would likely have recruited to the population of rainbow trout >200 mm as two-year-olds in the fall of 2000. Thus, we designated 1980 to 1999 as the pre-WD time period (before impact), and 2000 to 2005 as the post-WD (after impact) time period.

Cascade served as the reference reach. Similar to LPP, the Dearborn River serves as the primary spawning and rearing tributary supporting trout populations in the Cascade section. Previous coded wire tagging work with juvenile fish indicated that most rainbow trout that spawned and reared in LPP or Dearborn River recruited to the Craig and Cascade sections, respectively, with relatively little movement between the two sections (Munro 2004). Sentinel cages were first deployed on the Dearborn in 1996. Mean WD severity was 0 from 1996 to 2000, and was first detected at a low level (<1.0) in 2001. Infection severity rose to >2.75 levels (>3.5) in 2003 and has remained very high (>4.5) thereafter. Peak infection severity >2.75 first occurred in 2003, about 5 years after severe infection was first observed in LPP. With a two year lag prior to recruiting to the trout population in the Cascade section, it was therefore estimated that population effects from severe infection (post WD) would first occur in 2005. Based on these data, we designated the Cascade section as a reference reach to Craig prior to 2005.

5) Rock Creek: Berg (2004) provided a detailed review of trout population dynamics in Rock Creek over the last 20 years, including some initial assessment of the effects of whirling disease. Population monitoring began in this drainage in the 1970s. For our analysis, following the recommendations of Berg (2004), we omitted inclusion of electrofishing data from the 1970s due to differences in electrofishing gear and questions about the comparability with sampling methods used from the 1980s to the present. Two sections of Rock Creek were selected for analysis: the 2.0 km-long Fish and Game section, located in lower Rock Creek at river kilometer 23, and the 2.2 km-long Hogback section, located in middle Rock Creek at river kilometer 51, roughly 28 km upstream from the Fish and Game section.

There are no uninfected 'reference' reaches in Rock Creek. In 1997, nearly all free-ranging trout tested positive for WD indicating *M. cerebralis* had been present in the system for some time. Sentinel cages near Hogback and Fish and Game sections measured infection levels greater that 3.75 in 1999 to 2002, suggesting that the population threshold of 2.75 had occurred some years earlier. Vincent (2006) and Granath et al. (2007) hypothesized that high infections probably began present in the early to mid 1990s. Thus, the best estimate for when infection levels >2.75 probably first occurred is 1995**.** We considered the years 1980 to 1994 as the pre WD time period, and the years 1995 to 2006 as the post WD time period. We considered using a reference reach from other nearby rivers, but long term data were not available or the rivers were impacted by other confounding factors (trout populations are depressed in the nearby lower Clark Fork River from heavy metal pollution not present in Rock Creek)(Pat Saffel, FWP Missoula).

6) Ruby River: We included four population-sampling sections on the upper Ruby River upstream of Ruby Reservoir in our analysis (Kerans et al. 2009). The lower two sections, Greenhorn (rkm 105, 3.4 km long) and Canyon (rkm 121, 1.3 km long) were designated infected reaches, and the upper two sections, Vigilante (rkm 131, 3.4 km long) and Three Forks (rkm 147, 1.9 km long), were designated reference reaches.

Several WD infected fish were detected among free-ranging trout in 1995. Sentinel cage sampling began in 2002; infection severity measured 3.0 in the Greenhorn section and 1.9 in the Canyon section. In 2003, the sections measured 3.7 and 3.2, respectively, indicating a sharp increase in numbers of highly infected fish. In 2005, these same sites were a 4.8 in May 2005 in Greenhorn and 2.9 in Canyon. Biologists noted a high percentage of deformed heads in recent years, also indicative of a high level of WD infection (Jim Magee, FWP Dillon). The lack of cage data prior to 2002 makes it difficult to determine when the >2.75 infection severity first began. However, given that infection exceeded this threshold in Greenhorn in 2002 and in

Canyon the following year, we believe using the year 2002 as the divider between pre- and post-WD time periods is a reasonable estimate.

In contrast, infection severity in the Vigilante and Three Forks sections were 0 in 2002, 2003, and 2004. Vigilante showed a moderate increase to 1.3 in 2005. These data indicate that both these sites would be suitable as reference reaches to the infected Canyon and Greenhorn reaches.

Flow

Flow level could influence trout abundance and thereby response to WD by influencing WD infection risk (MacConnell and Vincent 2002; Hallett and Bartholomew 2008), trout recruitment and survival (Lobon-Cervia and Mortenson 2005), and capture efficiency of electrofishing. We included flow as a covariate in the population estimation model (see below) and to account for potential synergistic effects of drought and WD infection risk. Flow data for each river were partitioned into 4 biologically meaningful time periods:

1) Spring, prior to main runoff (March 15-May 15);
2) Late spring/early summer: time of peak runoff and peak WD infection (May 15-July 15);
3) Summer low flow/maximum fish growth period (July 15-Sept 15);
4) Fall/winter period of low growth and stable flows (Sept 15-Mar 15).

Continuous flow records are available for the Bitterroot River near Darby (USGS site number 12344000), Blackfoot River near Bonner (site 12340000), Rock Creek at Clinton (site 12334510), and Ruby River above the reservoir near Alder (site 06019500). For the Missouri River, flow data for Little Prickly Pear Creek (LPP) and the Dearborn River were analyzed separately as these are the main spawning tributaries supporting the mainstem Craig and Cascade sections, respectively. However, flow records are incomplete (missing pre-1991 for LPP and pre-1993 data for Dearborn River). To fill in missing values, regression equations were developed using flow data from Prickly Pear Creek near Helena, Montana (USGS site 06061500), which has a much longer period of record than LPP or the Dearborn River, is in the same county, and has a similar drainage size. Regression coefficients were sufficiently high to indicate the predictive relationship was adequate for filling in missing values; the regression coefficient was $r = 0.85$ ($p < 0.001$) for the Dearborn River (USGS site 06073500)(-7.5% average deviation between predicted and observed flows for years 1993-2005), and $r = 0.76$ ($p < 0.001$) for LPP (USGS site 06071300)(average deviation, +0.7% for years 1991-2005).

For the Gallatin River, flow data were available on the mainstem of the Gallatin at Gallatin Gateway (site 06043500) back to 1980, except for missing years 1982-1984. Flow data for the East Gallatin (near Bridger Creek, site 06048700) was only available for the period of 2001 to 2007. We attempted to fill in missing values on the East Gallatin by regressing the two Gallatin flow datasets, but the regression was moderate ($r = 0.62$), and inspection of the scatter plot revealed a poor relationship at higher discharges. This was not surprising given the much different size and geographic distance between the two sites. In lieu of the lack of flow data on the East Gallatin, we therefore used the longer time series of flow data for the mainstem Gallatin River to represent flows in both study sections.

Plots of mean flows for each river and for each flow period used in the analyses are given in Appendix 6.

<u>Temperature</u>

Water temperature has an important influence on WD infection rates. Optimum temperature for infection is about 12 to 15ºC (Vincent 1999; MacConnell and Vincent 2002). Temperature can influence disease severity by altering trout fry emergence times (Vincent 1999), the timing and magnitude of release of free-swimming, infective triactinomyxon spore (Blazer et al. 2003, Kerans et al. 2005), and trout growth rates (Bear 2005), which in turn affect the window of vulnerability of young trout to whirling disease (Ryce et al. 2005). We originally planned to include water temperature as a covariate in our BACI analysis, but a review of USGS station data and polling of FWP biologists revealed a lack of extensive time series of water temperature data paralleling that available for the fish population and flow data. We did find USGS water temperature station data at many sites after about 2000, but data were generally scarce for prior years. Therefore, we were not able to incorporate water temperature into our analysis. However, because water temperature is generally inversely correlated with flow (low flow, drought years are warmer and vice versa), we likely captured much of the effects of temperature variation on WD infection and on trout population dynamics via the inclusion of a flow covariate in our analysis.

Population Estimation:

Fish Sampling

A standardized fish sampling methodology developed in the 1970s for acquiring trout population estimates on Montana rivers (Vincent 1982) was used on all sites. Mark-recapture population estimates were conducted using electrofishing estimates spaced about a week apart to allow for redistribution of marked fish. Sampling was conducted annually on most rivers either during the spring (Blackfoot River and Rock Creek) or the fall (Bitterroot, Gallatin, and Ruby rivers), except for the Missouri River where semiannual estimates were conducted on the mainstem river during both the spring (for brown trout during tributary spawning by rainbow trout) and the fall (for rainbow trout during tributary spawning by brown trout). Electrofishing sampling was consistently applied over the course of the study using three different sampling methods, depending on river size (Vincent 1982): 1) *large rivers*: drift boat with fixed boom anode, both banks electroshocked during mark and recapture runs (mainstem and West Fork Bitterroot rivers, Blackfoot River, Missouri River, Rock Creek); 2) *moderate-sized rivers*: drift boat with mobile anode (Canyon and Greenhorn sections on Lower Ruby River, Gallatin River); 3) *small rivers*: towed raft with mobile anode (East Fork Bitterroot River, Three Forks and Vigilante sections on Ruby River). During marking runs, fish were marked with a fin clip, measured for length, and weighed. During recapture runs, marks were noted and marked fish were measured and unmarked fish were measured and weighed. In our analysis, fish were separated into 25-mm length classes, starting at 100 mm and progressing in 25 mm increments to the maximum lengths of 500 to 600 mm. For population estimation, the population was assumed closed with no in- or out-migration and minimal mortality between the electrofishing runs. Previous research on this method in Montana rivers using marked fish confirmed little movement or mortality between marking and recapture runs, supporting these assumptions (Vincent 1982).

Population Estimation Model Development

Mark-recapture population estimation based on electrofishing data for fish populations must be done with consideration of the fact that capture probabilities likely vary with fish size. First, electrofishing is strongly size biased against small fish, with smaller fish less vulnerable to stunning by electrical currents (Reynolds 1996). Further, in large rivers, capture probability also may decline among the largest fish in the population, presumably because they have an improved ability to detect and avoid the electrical current due to their larger body size (FA+ 2004). As a result, a key assumption of some mark-recapture models, that capture probability is constant and equal for all individuals, is frequently not met, which can be problematic when analyzing data from 2-pass electrofishing. Further difficulties arise in estimating population sizes for these data because electrofishing catches may be low in number due to a naturally small population present, or low capture probability (even if N is large). To counteract this problem, catch efficiency curves have been incorporated into mark-recapture programs to stabilize estimation by combining information about capture probability over different length classes. To date, Montana trout population abundance estimates have been made using a partial logn-likelihood estimator, developed by Gould et al. (1991), and incorporated into a population analysis program for Montana FWP, Fisheries Analysis (2004) or FA+. This model adjusts capture probabilities according to fish length in recognition that not all lengths of fish are equally susceptible to capture by electrofishing. In this approach to estimating population size, capture probability is modeled as a quadratic function of fish length, and the modeling assumes a fixed intercept value (-5) and estimates a unique pair of coefficients relating fish length and fish-length-squared to the capture rate on each river section in each year.

Because estimated capture probabilities strongly influence estimates of population abundance in mark-recapture estimation, it is important to model capture probability in a realistic manner. Further, it is desirable if reasonable models of capture rate are precise as this allows precise estimation of abundance. Therefore, prior to applying the traditional maximum-likelihood estimator to our WD datasets, we first sought to test its robustness. The estimator had not been published, so we were uncertain as to its acceptability within the scientific community. Additionally, the estimator was developed in the early 1990s prior to the advent of the now widespread model selection analysis used to evaluate and select among competing mark-recapture models (Hayes et al. 2007). We used a variety of test datasets to evaluate whether it seemed advisable to (1) use a fixed intercept and (2) to always assume that a quadratic form of the relationship between capture rate and fish length. We used generalized linear models (McCullagh & Nelder 1989) to evaluate competing models that relaxed the assumption of a fixed intercept and a quadratic functional form of fish length. We found evidence that these generalizations would be useful because different intercepts were sometimes needed and the quadratic form could sometimes (but not always) be simplified to a linear form for some river-year combinations. Further, it was noted that capture probability did seem to follow similar enough patterns among years on the same rivers that it might be reasonable to consider methods that would share information about capture probability across years within a given river section. This was of interest as it relates to seeking precise estimates of capture probability that are also reasonable estimates of the actual underlying rates.

We also sought an estimation method that would allow us to account for possible yearly differences in capture probabilities due to environmental variation from weather, flow conditions, and other factors. Based on our initial testing of the FA+ methodology and our desire to account for potential year to year differences on capture probability in our estimation model, the statistician on our team, Jim Robison-Cox, developed a Bayesian mark-recapture population estimator for our analysis. Though there has been some recent description of the use of Bayesian mark-recapture models for fisheries applications (Grabowski et al. 2009), the approach has not been widely used to date, so we describe the approach in detail below.

Bayesian inference techniques are useful when the number of parameters is large, as it is for these data. The first step in Bayesian analysis is to select an appropriate likelihood for the data and prior distributions for all parameters. The data in our dataset consists of counts of the number of fish of a given length class caught in the first pass (n_1), the number caught in the second pass (n_2), and the number of marked fish caught in the second pass (m). The Bayesian mark-recapture population estimate model was developed with the following components.

1) The likelihood of observed counts based on the capture probability, p, and the population size, N, uses the following labels for the various sample sizes:

	No. caught in 1st pass	No. missed in 1st pass	Total
No. caught in 2nd pass	M	$n_2 – m$	n_2
No. missed in 2nd pass	$n_i – m$	$N – n_1 - n_2 + m$	$N – n_2$
Total	n_1	$N – n_1$	N

2) Considering a fish of a given length with capture probability, p, of being caught on either pass, the assumption of independent captures (vulnerability to capture is equivalent for each pass) gives the following probabilities for the four possible outcomes: caught twice, caught only in pass 1, caught only in pass 2, or not ever caught.

	Caught in 1st pass	Missed in 1st pass

Caught in 2nd pass	p^2	$(1-p)p$
Missed in 2nd pass	$p(1-p)$	$(1-p)^2$

Non-informative (vague) priors were used for population size N_{tl} (where t denotes the year and l the length class of trout), and for capture probabilities p_{tl}, to avoid the possibility of prior choice determining results. To model capture probabilities, we used a “hockey stick” model in which p is small for smaller lengths (based on the physics of electroshock), and increasing to a mean value which holds for moderate and large fish. Within each site and species combination, every year was given a random effect across all length classes, and each length class was given a random effect across all years (both were added at the logit(p) scale). The model is flexible in that probabilities may go up or down for any length class and for any year. At the same time, the model allows data sharing across length classes, years, or both in keeping with whatever sharing is supported by the data. For further model development details, see Appendix 1.

Model Application

For each site and species, six Markov chain Monte Carlo chains were begun in WinBugs (Spiegelhalter et al. 2002) each starting in a different extreme of the parameter space. The Gelman-Rubin diagnostic was used to determine when the chains had converged ($R < 1.05$). When numbers of captured fish were large, as on the Missouri, convergence was obtained more quickly than for smaller datasets. After convergence, another 2000 runs (times six chains) were generated and every fifth realization was stored for further processing and for estimation of percentiles of the posterior distribution of the parameters of interest. Each estimate required several hours of computation time.

Population size estimates and accompanying 95% posterior probability intervals were generated for each year, species, and site across all length classes. Figure 3.1 shows the posterior distribution of capture probabilities for the Craig and Cascade sampling sections on the Missouri river. Note that the dots (posterior medians) do follow a hockey stick pattern that increases from a very low value for small fish to a moderate probability for medium fish, flattening out to stay constant for large fish. The modeling process does allow flexibility so that if any one length (across all years) departs from this pattern, it is able to do so. Capture probability curves for all species and river sections are shown in Appendix 2.

Total population estimates were standardized to number per kilometer of river to allow comparisons across all sites. As stated above, no statistical technique gives reliable population estimates when counts are small and when capture probabilities are low. Therefore, because of a noticeable decline in capture probabilities to low levels in fish less 200 mm ($p_{tl} < 0.1$), we truncated the data in our population estimates to consider only fish >200 mm in length.

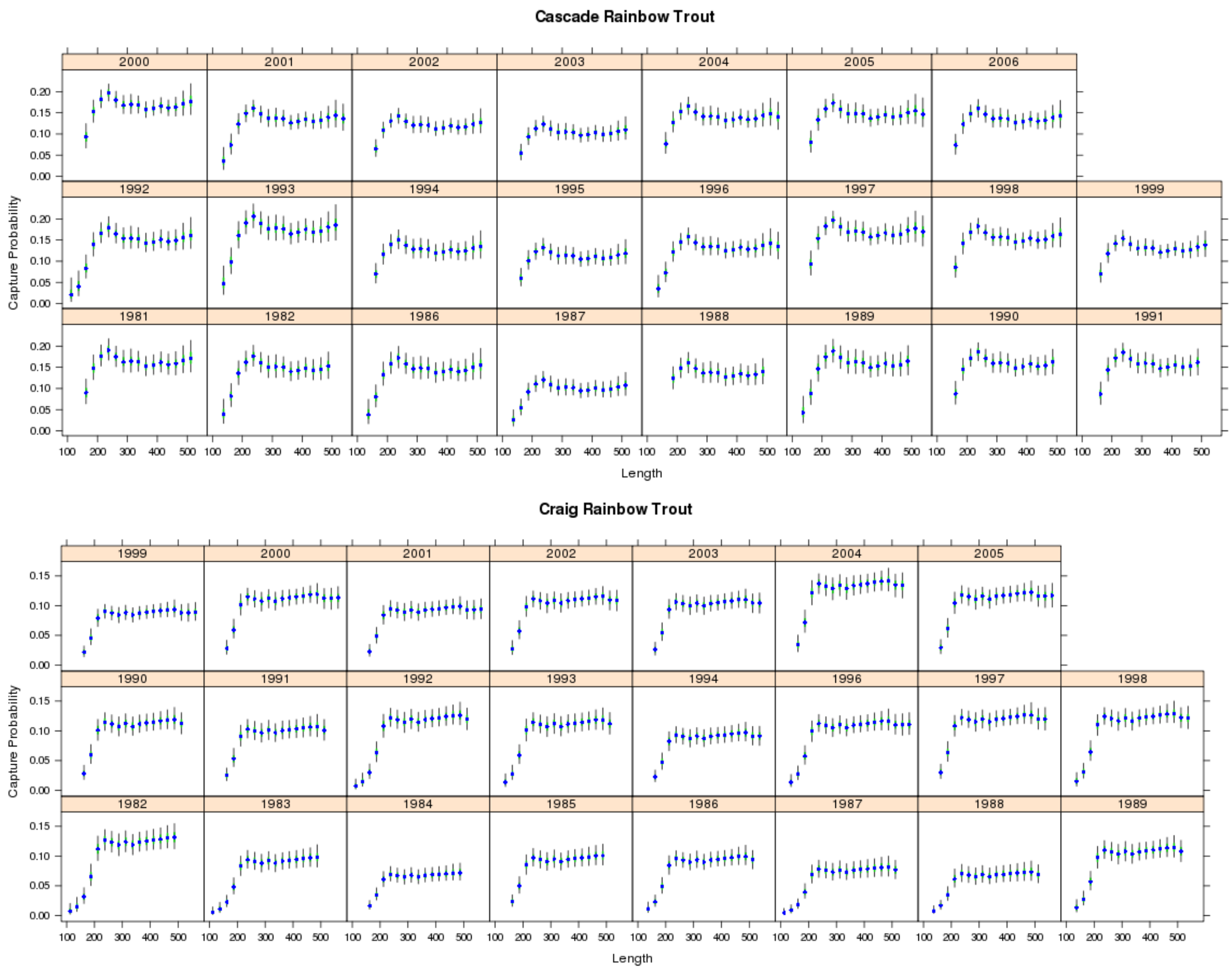


Figure 3.1. Capture probabilities of rainbow trout by size class in the Cascade and Craig sampling sections of the Missouri River.

Age Class Population Estimates

As part of the sampling protocol on the Missouri River, biologists took a scale from a subsample of fish and aged them by counting annuli. Fish were aged as age 1, 2, 3, or 4+. Fish in the latter category could not be aged accurately beyond age 4, although trout in the Missouri River live to be much older (T. Horton, FWP, Helena, unpub data). From these data, we obtained the distribution of ages for each length class, and we randomly generated ages for each fish represented in the sampled posterior of *N*. To include variability from the fact that the empirical age distribution is an estimate, not known, proportions were sampled from a Dirichlet distribution with means equal to the empirical proportions.

WD Effect Estimates using the BACI Model

Obtaining the population estimates was an initial step toward assessing the impact of whirling disease. The next stage in the process was to compare population sizes before and after WD appeared in each river in order to assess the possible effects on trout population abundance. Models were developed similar to Before-After-Control-Impact (BACI) models in the literature (Wiens and Parker 1995). Delineation of before and after WD time periods for each river are given in section 3.2.

BACI models allowed estimation of the effects of WD independent of the many other potential factors affecting year-to-year variation in trout abundance, including such factors as flow variation. The BACI model used here includes two indicator variables as fixed effects. The ‘control-impact’ indicator variable is labeled as ‘1’ for the infected site and ‘0’ for the reference site, with the variable coefficient estimating the mean difference between the two sites. The ‘before-after’ indicator variable is ‘1’ for post-WD (after) and ‘0’ for pre-WD (before), with the variable coefficient estimating the mean temporal effect common to both sites. Additionally, an interaction term was added between the two indicator variables, which allow an estimate of the difference in temporal effect between the two sites. Assuming the ‘impact’ (WD infection) is the only factor that has changed; the coefficient estimate for the interaction is therefore the effect of WD.

Population sizes vary dramatically from one river to another and a sensible common metric is needed for any combined analysis. We chose the year of first severe WD infection for the ‘infected’ reach as a dividing line, and for each length class at each site, averaged population size before that time point to create a 'baseline' abundance estimate, the 'before WD'. To quantify population changes after WD, all abundance estimates were divided by their respective baselines, and these 'population change' values were transformed using log base 2. The $\log_2$ scale was used because on this scale a doubling of population will be conveniently indicated as a +1 and a halving by -1. Interval estimates in output tables are shown in $\log_2$ scale and are also back-transformed to the proportion change scale.

Three models were fitted for each set of data.

1) No effect of WD. Uses only the indicators for BA and CI as follows:

$$y_{tls} = \mu_{river,l} + \beta_{1l} I_{infected} + \beta_{2l} I_{postWD} + \epsilon_{tls}$$

2) WD effect on population abundance. The interaction between BA and CI is included as an effect of WD common to all rivers:

$$y_{tls} = \mu_{river,l} + \beta_{1l} I_{infected} + \beta_{2l} I_{postWD} + \beta_{3l} I_{infected} I_{postWD} + \epsilon_{tls}$$

3) WD effect by river. The models adds an additional variable to account for variation in response to WD among individual rivers:

$$y_{tls} = \mu_{river,l} + \beta_{1l} I_{infected} + \beta_{2l} I_{postWD} + \beta_{river,l} I_{infected} I_{postWD} I_{river} + \epsilon_{tls}$$

Additionally, we modeled the error term, ε_{tls}, as including random year-to-year variation (common random year effects across all rivers) and a river-within-year random effect, as well as *iid* residual variance.

Comparisons of competing models are complicated by the fact that there is not a single sample of data to fit with these models. Instead, we have the model output from WinBUGS runs, i.e., samples of size 2,400 from each posterior distribution of population estimates. Rather than extracting a mean or median from each posterior distribution of population estimates and assuming to have a representative sample of data points, we fit the models to each of the 2,400 samples. By storing the output of each fitted model, we obtained a sample from the posterior distribution of the parameters of interest, namely β_{3l} or $\beta_{river,l}$. The technique is analogous to obtaining a bootstrap estimate of regression coefficients. Instead of resampling the data in each iteration, a different draw from the posterior is used, thus converting a sample from the posterior of $\log_2$ population change to a sample from the posterior for the coefficient estimates of the model. As in bootstrapping, no assumption of normality is needed; inference comes from the range of observed values, not from *t* and *F* tests. Model comparison is complicated by the fact that in our analysis there are not just three fitted models to compare, but 2,400 replicates of output for each of three models. Therefore, we followed the recommendation of Spiegelhalter et al. (2002) and used the 'deviance information criterion' or DIC, a generalization of Akaike's AIC model selection procedure, to select among the three competing models. In this report, models which differ by less than 5 DIC units are considered equally plausible, whereas models with the combination of the lowest DIC values and with greater than 5 DIC unit difference indicate the most plausible or best fit among competing models.

BACI analyses were run for the four rivers with adequate BACI data: Blackfoot, Gallatin, Missouri, and Ruby rivers; all four rivers had both reference and WD+ sections and multiple years of post-WD data. The two other study rivers, Rock Creek and the Bitterroot River, were treated separately; Rock Creek had before-after data but no reference section, and the Bitterroot River had no post-WD data for the West Fork reference section and only one year post WD data for the mainstem Bell reference section.

Total Density and Biomass

The above BACI analysis compared population responses by individual length classes. We also wished to analyze response of total density and of biomass to WD. For each species, year *t*, length *l*, and site *s*, the WinBUGs output provides a sample from the posterior made up of y_{tls}^{j}, where *j*= 1, …2,400 random draws. To convert the sample of *y*'s to a sample of total counts, *t*, from the posterior of total count, we simply summed over lengths: $t_{ls}^{j} = \sum_{l} y_{tls}^{j}$. For biomass, similar to what was done for density, posterior counts for length class *l*, year *t*, and site *s*, were incorporated into the WinBUGS model and 2,400 samples of weights were drawn (with replacement) from the weights of captured fish for each site, species, year, and length class. Weights were first summed to obtain a total biomass for each length class, and then weights for all length classes summed to obtain a total biomass. By repeating the process for 2,400 iterations, we obtained a sample from the posterior distribution of biomass for each site/species/year combination. Typically, biomass is derived by multiplying the number of fish in a length class by the mean weight (Hayes et al. 2007), but the calculation ignores the variability of sampling from the population and results in overly small standard errors. Finally, biomass response to WD was estimated by fitting the three BACI model equations described above for each species and river.

Relative Species Composition and Age Structure

In addition to changes in absolute numbers, we also examined how the proportion of rainbow trout and brown trout varied over time in the presence and absence of WD. The proportion of rainbow trout comprising the total density of both species was compared over years for each river in WD+ and WD- sections. We also examined how the abundance of various age classes for Missouri River trout changed in the WD+ Craig section after WD relative to pre-WD conditions and compared to the WD- Cascade reference section.

Relative Weight

Relative weight, W_r, the weight of an individual fish of a given length compared to a national average, can be a sensitive indicator of fish condition and their response to environmental stressors and food availability. We computed W_r using the equations from Anderson and Neumann (1996) for lotic rainbow trout and brown trout:

$$\text{Rainbow trout, } W_r = W * 10^{[2 + 5.023 - 3.024 \text{ Log}_{10}(L)]}$$

$$\text{Brown trout, } W_r = W * 10^{[2 + 4.867 - 2.960 \text{ Log}_{10}(L)]},$$

where W is the weight in grams and L the total length in mm for individual fish. Fish with W_r of 100 are considered to be in good condition relative to other fish in that size range.

We compared W_r before and after WD for all fish combined and within three different length classes using ANOVA with year as a nested factor. Length classes corresponded to small (200-300mm or 8-12”), medium (300-400 mm or 12 to 16”) and large trout (>400 mm or 16”). We hypothesized that rainbow trout in highly infected river sections would be in poorer condition relative to WD-reference sections and compared to fish in infected sections prior to WD.

Growth Rate

We compared changes in growth rates before and after WD for rainbow trout and brown trout from the Missouri River, the only population with available age data. Mean lengths at ages 1, 2, 3, and 4+ were compared before and after WD using GLM ANOVA, with year as a nested factor. We hypothesized that rainbow trout (and potentially brown trout) would show reduced growth after heavy infection from WD. Alternatively, we postulated that growth could substantially increase among surviving fish after a WD epizootic, especially among smaller size classes most affected by the disease, due to much lower densities causing a compensatory growth response.

Flow Effects

Although the inclusion of reference sites accounts for ‘control’ of environmental variables that also may be affecting population abundance in addition to the presence of the main ‘treatment’ (presence of WD), the BACI models do not utilize flow, water temperature, or other covariates as explicit fixed effects. In particular, flow could play a role in population declines because Montana has had drought conditions coincident with WD outbreaks, resulting in lower summer and winter flows and a reduction in duration and magnitude of spring peak flow.

We explored relationships between flow and abundance of smaller rainbow trout and brown trout (200-225, 225-250, and 250-275 mm) because the smaller length classes are likely most sensitive to changing flow conditions (Lobon-Cervia and Mortensen 2005). We examined associations among the four seasonal flow periods (section 3.2) across all four BACI rivers combined (Blackfoot, Gallatin, Missouri, and Ruby). For each flow variable and length class, we ran WinBUGS to provide 2400 population estimates for each year and river. To account for differences among rivers, we standardized flow by computing an average flow across all years for each river and used the standard deviation from the average flow as a standardized flow variable. Trout abundance was standardized in a similar way, yielding a median $\log_2$ proportion change in abundance as the response variable. We then averaged together the $\log_2$ proportion changes for a given length class and computed correlation of the mean abundance to flow. Because the length classes we examined included fish of 1-2 years of age, we also examined possible lag effects between flows and abundance for lags of 1 to 3 years.

3.4 Results

WD Effects on Rainbow Trout Abundance

BACI Analysis: Based on analyses that used deviance information criterion (DIC) values to assess the relative plausibility of three competing models (Model 1: no WD effect; Model 2: same WD effect across all rivers; Model 3: interaction of WD effect and river (site), for the four rivers with adequate BACI data: Blackfoot, Gallatin, Missouri, and Ruby rivers), we found evidence of negative effects of WD for shorter fish (with effects varying by river for some length classes but not others). Results for longer fish were strikingly different from those of shorter fish, and abundance estimates for some length classes on some rivers were higher post-WD than for other situations.

Table 1 gives DIC values for each of the three competing models fit to rainbow trout abundance for each length class from 200 mm to 500 mm. Model 1, the “no WD" model, did not have the smallest DIC in any row, and thus was never favored as the best model. Model 2, which incorporates a global WD effect across all rivers, had the lowest DIC value for rainbow trout length classes 200-300 mm and 450-475 mm (Table 1, boxed values) and was greater than 5 DIC units from the Model 1 values, indicating a significant effect of WD on abundance of these size classes. Adding an interaction term to account for differences among rivers in response to WD (model 3) did not improve DIC values for these size classes (difference in DIC values <5).

In contrast, DIC values for size class 300-450 mm in Model 3 were >5 units from Model 2 and had lower overall values. This difference indicated that the WD-by-river interaction model was the more plausible model and that WD had significant effect on fish 300-450 mm but that variation in response varied by river. For the largest rainbow trout size class (475-500 mm), all three models had nearly equal DIC values, indicating all three were equally plausible, suggesting the lack of a strong effect of WD.

The magnitude of the effects of WD on abundance of each rainbow trout size class is shown graphically in Figure 3.2. Individual values of proportional change for small rainbow trout 200-300 mm long is shown for all rivers combined in Table 3.2, and for each individual river for all size class in Table 3.3. Plots of population density by size class for each river across all sampling years are shown in Appendix 3.

For all rivers combined, the median proportional decline in small rainbow trout 200-300 mm in length after WD was greater than two-fold (0.55 or -1.15 on the $\log_2$ scale). For individual rivers, the decline was highest for the Gallatin (0.69) and Missouri rivers (0.59) and less so for the Blackfoot (0.46) and Ruby (0.30) rivers.

Table 1. DIC values for RBT models by length class.

Length	no WD	WD	WD by river
200 – 225	364.18	$\boxed{353.30}$	354.99
225 – 250	330.67	$\boxed{311.11}$	314.27
250 – 275	312.59	$\boxed{300.42}$	302.00
275 – 300	312.22	$\boxed{299.86}$	299.69
300 – 325	345.80	339.04	$\boxed{308.65}$
325 – 350	335.55	325.38	$\boxed{299.60}$
350 – 375	331.24	330.13	$\boxed{309.93}$
375 – 400	340.25	340.91	$\boxed{309.28}$
425 – 450	286.28	286.54	$\boxed{255.27}$
450 – 475	230.58	$\boxed{169.40}$	167.37
475 – 500	208.08	205.17	205.12

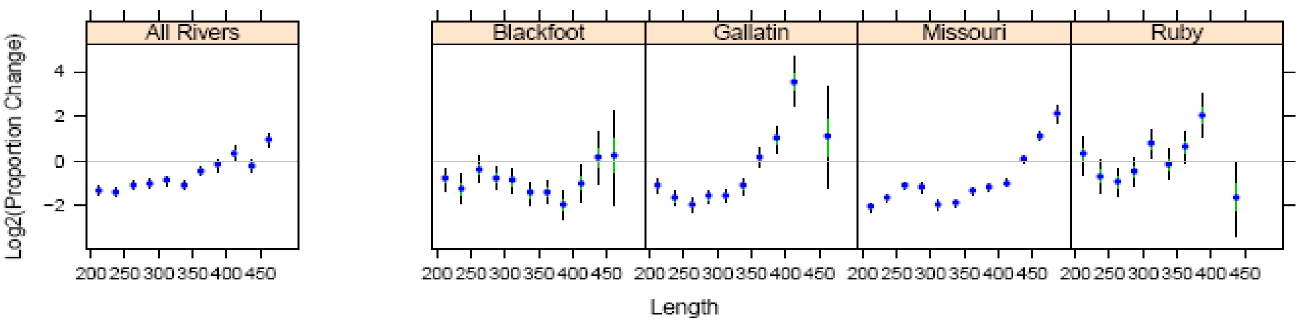


Figure 3.2. WD effects on rainbow trout density for individual length classes for all rivers combined and for each individual study river used in the BACI analysis. The “0” line indicates no difference between reference and WD-infected sites. Values below the line indicate a decrease in density, and values above the line indicate an increase in density compared to the baseline ‘pre-WD’ population density.

for four rivers used in the BACI model and for Rock Creek (before-after only, no reference section). Intervals are shown for $\log_2$ scale and by proportion change.

In contrast to the negative effect on smaller fish, there was a significant increase in large rainbow trout after WD. Rainbow trout in the 450-475 mm length class showed a nearly two-fold increase in abundance after WD compared to reference reaches without WD, and this effect was evident across all study rivers. Rainbow trout in the 300-450 mm size range also showed an increase in abundance in response to WD, though the response was much more variable among rivers, with the strongest increase (median 2-fold increase) shown in the Gallatin and Missouri Rivers, and little to no response shown rainbow trout of this size in the Ruby and Blackfoot rivers, respectively (Figure 3.2; Table 3.3).

River	length	Rainbow Log$_2$	Rainbow Proportion	Brown Log$_2$	Brown Proportion
Blackfoot	225–250	(-1.91 , -0.58)	(0.27 , 0.67)	(-1.08, 0.33)	(0.47 , 1.26)
	250–275	(-0.95 , 0.21)	(0.52 , 1.15)	(-1.27, 0.23)	(0.42 , 1.17)
	275–300	(-1.28 , -0.28)	(0.41 , 0.82)	(-0.51, 1.38)	(0.70 , 2.61)
	300–325	(-1.41 , -0.35)	(0.38 , 0.78)	(-1.73, 0.24)	(0.30 , 1.18)
	325–350	(-1.93 , -0.94)	(0.26 , 0.52)	(-2.11, -0.61)	(0.23 , 0.66)
	350–375	(-1.91 , -0.87)	(0.27 , 0.55)	(-0.34, 1.17)	(0.79 , 2.25)
	375–400	(-2.57 , -1.31)	(0.17 , 0.40)	(-1.52, 0.07)	(0.35 , 1.05)
	400–425	(-1.81 , -0.19)	(0.28 , 0.88)	(-3.53, -1.89)	(0.09 , 0.27)
	425–450	(-1.00 , 1.35)	(0.50 , 2.54)	(-1.69, -0.28)	(0.31 , 0.82)
	450–475	(-1.97 , 2.29)	(0.26 , 4.88)	(-2.33, -0.70)	(0.20 , 0.62)
	475–500			(-1.16, 0.78)	(0.45, 1.72)
Gallatin	225–250	(-1.96 , -1.37)	(0.26 , 0.39)	(-1.53, 1.55)	(0.35 , 2.93)
	250–275	(-2.23 , -1.64)	(0.21 , 0.32)	(-0.82, 1.52)	(0.57 , 2.88)
	275–300	(-1.90 , -1.32)	(0.27 , 0.40)	(-0.27, 1.66)	(0.83 , 3.15)
	300–325	(-1.84 , -1.25)	(0.28 , 0.42)	(-0.33, 1.62)	(0.80 , 3.07)
	325–350	(-1.46 , -0.81)	(0.36 , 0.57)	(-0.33, 1.66)	(0.79 , 3.16)
	350–375	(-0.22 , 0.58)	(0.86 , 1.49)	(-0.63, 1.10)	(0.65 , 2.14)
	375–400	(0.40 , 1.54)	(1.32 , 2.91)	(-1.44, 0.15)	(0.37 , 1.11)
	400–425	(2.47 , 4.63)	(5.53 , 24.69)	(-2.03, -0.43)	(0.25 , 0.74)
	450–475	(-1.19 , 3.38)	(0.44 , 10.38)	(-3.12, -1.36)	(0.12 , 0.39)
	450–475			(-3.11, -0.88)	(0.12, 0.54)
	475–500			(-3.52, -0.32)	(0.09 ,0.80)
Missouri	225–250	(-1.84 , -1.50)	(0.28 , 0.35)	(-1.27, 0.53)	(0.41 , 1.44)
	250–275	(-1.29 , -0.93)	(0.41 , 0.52)	(-1.04, 0.35)	(0.48 , 1.27)
	275–300	(-1.39 , -0.93)	(0.38 , 0.53)	(-1.34, -0.49)	(0.40 , 0.71)
	300–325	(-2.20 , -1.71)	(0.22 , 0.31)	(-1.73, -1.12)	(0.30 , 0.46)
	325–350	(-2.06 , -1.70)	(0.24 , 0.31)	(-1.58, -1.01)	(0.33 , 0.50)
	350–375	(-1.53 , -1.20)	(0.35 , 0.44)	(-0.95, -0.31)	(0.52 , 0.81)
	375–400	(-1.34 , -1.04)	(0.39 , 0.49)	(-1.04, -0.48)	(0.49 , 0.71)
	400–425	(-1.14 , -0.82)	(0.45 , 0.56)	(-0.87, -0.35)	(0.55 , 0.79)
	425–450	(-0.11 , 0.22)	(0.92 , 1.17)	(-0.35, 0.16)	(0.79 , 1.12)
	450–475	(0.89 , 1.30)	(1.86 , 2.47)	(-0.02, 0.56)	(0.98 , 1.47)
	475–500	(1.74 , 2.52)	(3.33 , 5.73)	(0.32, 0.91)	(1.25 , 1.88)
Ruby	225–250	(-1.42, 0.10)	(0.37, 1.07)		
	250–275	(-1.60, -0.31)	(0.33, 0.80)		
	275–300	(-1.10, 0.12)	(0.47, 1.09)		
	300–325	(0.16, 1.37)	(1.11, 2.58)		
	325–350	(-0.81, 0.50)	(0.57, 1.41)		
	350–375	(-0.12, 1.34)	(0.92, 2.53)		
	375–400	(1.05, 3.03)	(2.07, 8.14)		
	425–450	(-3.34, -0.01)	(0.10, 1.00)		

Table 3.3. 95% credible intervals for change in population due to WD for rainbow trout and brown trout by size class and by river. Brown trout numbers were too low to perform the analysis on Ruby River.

<u>Rock Creek Before-After Analysis</u>: Although both sites on Rock Creek were infected and a full BACI analysis was not possible, a before-after (B-A) analysis is possible if one assumes

that WD is the only change in the environment. We fit a model with an effect for the post-WD indicator and a site (section) effect. Additionally, a model with those terms and site by infection interaction was fit. DIC comparison between the models (Table 3.4) indicated that model 1 (no WD) is adequate for lengths of 325 to 425 mm, model 2 is preferred for lengths below 325 mm, and model 3 is preferred for fish over 425 mm. Interestingly, the before-after coefficients for Rock Creek are similar to those of the four BACI rivers combined. However, in Rock Creek, the WD effects on smaller trout seem to be stronger than in other rivers (Table 3.2). For rainbow trout 200-300 mm long, there was nearly a four-fold (-1.99 on log2 scale) decline in abundance after WD. As in other rivers, there was a significant increase in the largest rainbow trout (425-450 mm) after WD, although this response varied by section, being present in the Fish and Game section, but absent in the Hogback section (Figure 3.4; Table 3.5).

Bitterroot River

There was only one year of post-WD population data for the mainstem Bitterroot River. However, it is noteworthy that the abundance of small rainbow trout 200-300 mm in the WD+ Darby section was only 30 fish per km in the post-WD year of 2005, 90% below the long-term average density of 286 per km from 1989-2002 (Appendix 3). In contrast, the abundance of small rainbow trout in the WD- Bell section was similar across all pre- and post-WD years. For East and West Fork Bitterroot tributaries, there was no post-WD data on the WD- Connor section to serve as a reference. However, for the WD+ Trinity and Maynard sections of the East Fork, density of small rainbow trout (200-300 mm) in the post-WD years of 2004-2006 was 62 to 179 fish per km, respectively, 50-67% lower than pre-WD density of

157 to 363 per km (Appendix 3), suggesting a possible WD effect on recruitment of young fish into the population.

Length	no WD	WD	WD by site
200–225	64.80	60.00	59.77
225–250	71.29	61.70	60.55
250–275	57.62	49.77	49.94
275–300	60.71	54.80	54.87
300–325	64.44	54.93	53.29
325–350	65.82	61.73	57.02
350–375	62.31	62.80	61.36
375–400	74.30	75.22	72.62
400–425	61.80	62.46	62.83
425–450	24.34	19.17	-26.64

Table 3.4. DIC comparisons of rainbow trout models without a WD effect, with a common WD effect, and with a WD x site interaction effect for Rock Creek.

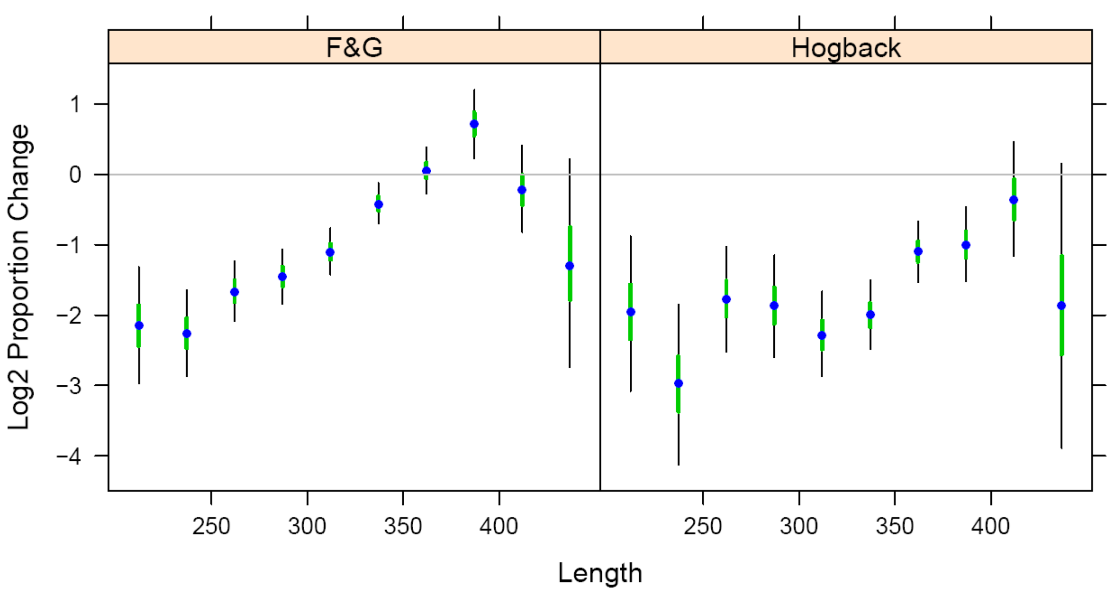


Figure 3.4. Proportional change (log2 scale) in rainbow trout density after WD in Rock Creek by river section and by length class.

		Rainbow		Brown	
Site	length	Log_2	Proportion	Log_2	Proportion
F&G	200–225	(-2.97, -1.31)	(0.13, 0.40)	(-3.80, 0.86)	(0.07, 1.82)
	225–250	(-2.87, -1.64)	(0.14, 0.32)	(-0.43, 2.25)	(0.74, 4.76)
	250–275	(-2.08, -1.23)	(0.24, 0.42)	(0.60, 3.01)	(1.52, 8.04)
	275–300	(-1.83, -1.07)	(0.28, 0.48)	(-0.19, 1.50)	(0.88, 2.83)
	300–325	(-1.42, -0.76)	(0.37, 0.59)	(0.28, 2.15)	(1.21, 4.42)
	325–350	(-0.69, -0.12)	(0.62, 0.92)	(-0.08, 1.86)	(0.95, 3.64)
	350–375	(-0.27, 0.38)	(0.83, 1.31)	(-0.39, 1.64)	(0.76, 3.13)
	375–400	(0.23, 1.20)	(1.17, 2.30)	(0.61, 3.05)	(1.53, 8.28)
	400–425	(-0.82, 0.41)	(0.57, 1.33)	(1.36, 3.91)	(2.57, 15.02)
	425–450	(-2.74, 0.22)	(0.15, 1.17)		
Hogback	200–225	(-3.08, -0.88)	(0.12, 0.55)		
	225–250	(-4.13, -1.85)	(0.06, 0.28)		
	250–275	(-2.52, -1.03)	(0.17, 0.49)		
	275–300	(-2.59, -1.15)	(0.17, 0.45)		
	300–325	(-2.87, -1.66)	(0.14, 0.32)	(0.47, 5.70)	(1.39, 51.90)
	325–350	(-2.48, -1.50)	(0.18, 0.35)	(1.29, 5.94)	(2.44, 61.45)
	350–375	(-1.53, -0.66)	(0.35, 0.63)	(1.44, 7.21)	(2.72, 147.57)
	375–400	(-1.51, -0.46)	(0.35, 0.73)	(0.63, 7.47)	(1.55, 177.19)
	400–425	(-1.16, 0.47)	(0.45, 1.38)	(-2.85, 2.70)	(0.14, 6.50)
	425–450	(-3.89, 0.16)	(0.07, 1.12)		

Table 3.5. Rock Creek 95% credible intervals for change in rainbow trout and brown trout population due to WD.

WD Effects on Brown Trout Abundance

BACI analyses were performed for brown trout on the Blackfoot, Gallatin, and Missouri rivers, and a B-A analysis for brown trout on Rock Creek. There were too few brown trout in the Ruby River to perform an analysis in that study river. Table 3.6 lists DIC values for each of the three competing models fit to brown trout abundance. The magnitude of the effects of WD on abundance of each brown trout size class is shown graphically in Figure 3.5. Individual values of proportional change for small brown trout 200-300 mm long are shown in Tables 3.3 for the Blackfoot, Gallatin, and Missouri rivers, and Table 3.5 for

Rock Creek. Plots of population density by size class for each river across all sampling years are shown in Appendix 3.

DIC values showed no consistent pattern in response of brown trout to WD. WD did not appear to have an effect on abundance of brown trout 250-300 mm and 350-400 mm in the three BACI rivers as Model 1 (no WD) was the most plausible model. For other size classs, Model 3 (WD x river interaction) is preferred over the common WD effect model (Model 2), indicating that post-WD effects varied by river. On these rivers, the response to WD showed no identifiable pattern (Figure 3.5).

For Rock Creek, Model 2 or Model 3 was favored, indicating a significant effect of WD, except for the smallest and largest size classes, where all models had similar DIC values. The strongest response was shown for brown trout 325-400 mm, where abundance increased about 2-4-fold after WD.

Brown trout abundance for all size classes on the mainstem WD+ Darby section and WD- Bell section was similar pre- vs. post-WD, though there was only one year of post-WD data (Appendix 3). For East and West Fork Bitterroot tributaries, there were too few brown trout to allow comparisons on the WD- Connor section. Abundance patterns were similar across all size classes pre- and post-WD on the WD+ Maynard and Trinity sections of the East Fork.

Blackfoot, Gallatin, and Missouri Rivers			
Length	no WD	WD	WD by river
225 – 250	256.1	260.0	249.2
250 – 275	236.8	242.6	235.7
275 – 300	271.9	277.6	269.7
300 – 325	273.5	277.3	260.9
325 – 350	280.2	281.3	266.0
350 – 375	259.0	265.3	258.2
375 – 400	252.6	256.3	249.6
400 – 425	271.6	269.4	261.0
425 – 450	264.0	265.5	254.5
450 – 475	248.7	250.2	230.3
475 – 500	221.7	227.5	207.4

Rock Creek			
Length	no WD	WD	WD by site
300–325	41.88	42.65	41.68
325–350	61.02	55.39	53.13
350–375	58.61	50.82	45.32
375–400	57.69	47.88	45.80
400–425	39.16	38.86	36.08

Table 3.6. DIC values for brown trout models by length class for the Blackfoot, Gallatin, and Missouri rivers (left table) and Rock Creek (right table).

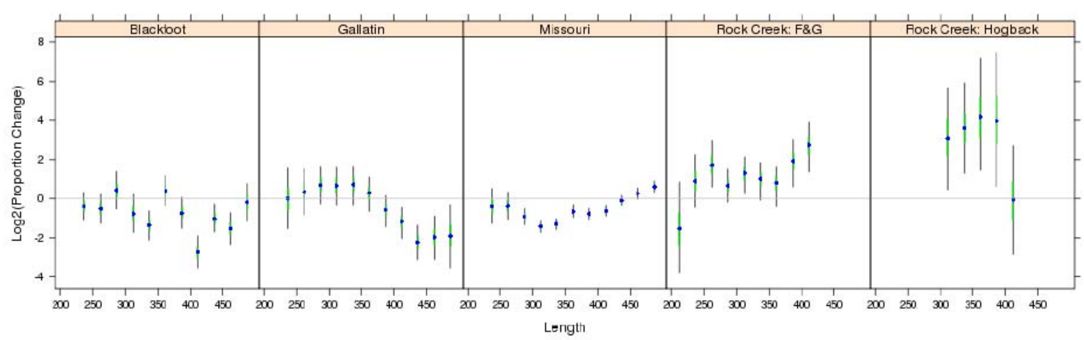


Figure 3.5. Proportional change in brown trout density after WD for individual study rivers.

Total Density and Biomass

Table 3.7 lists DIC values for each of the three competing models fit to rainbow trout and brown trout total density and biomass. Median values for proportional change in density and biomass for all rivers combined are shown in Figure 3.6 and in Figure 3.7 for individual rivers. Table 3.8 lists the 95% credible intervals around each median for the different study rivers.

There was little difference in DIC values between the "no WD" model 1 and the global WD effect model 2 for either species. Model 3, the WD x river interaction model was the most plausible model, as DIC values were substantially lower, indicating a significant effect of WD on total density and biomass of both rainbow trout and brown trout, with the effect varying by river. After WD, rainbow trout total density and biomass in WD infected sections declined 4-fold in the Blackfoot River relative to WD- reference sections, and 2-fold in the Gallatin and Missouri rivers; in the Ruby River, there was little change in total density but a slight decline in biomass (Figure 3.6; Table 3.8). Brown trout showed a nearly opposite pattern to rainbow trout. After WD, brown trout total density and biomass increased about two-fold in the Blackfoot, 1-fold in the Gallatin River, and declined by about 25% in the Missouri River. In the Ruby River, there were too few brown trout in the WD- reference sections for an accurate estimate of biomass or total density.

		Model		
Species	Response	No WD	WD	WD by River
Brown	Biomass	209.34	210.22	190.29
Brown	Total	208.12	208.98	189.04
RBT	Biomass	222.93	222.90	212.82
RBT	Total	219.92	214.99	204.37

Table 3.7. DIC comparisons for three competing models fit to total density and biomass of rainbow trout and brown trout.

A. Total Population Density B. Biomass

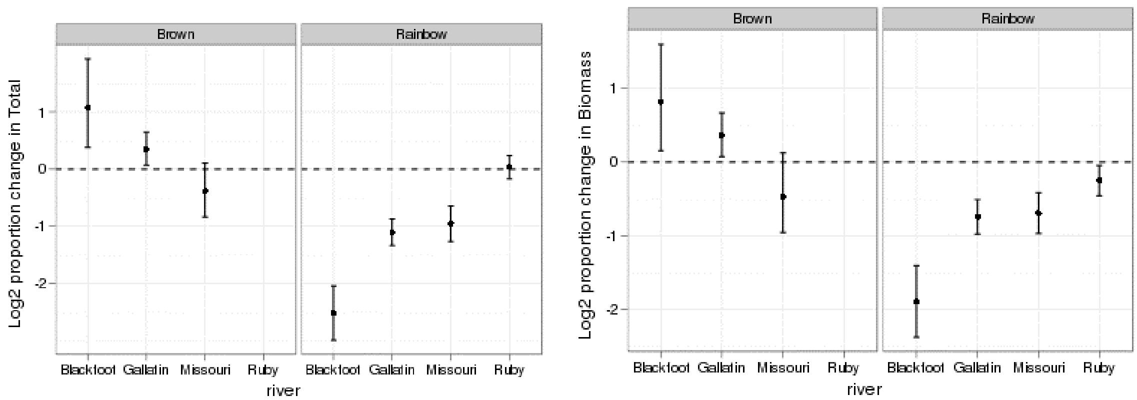
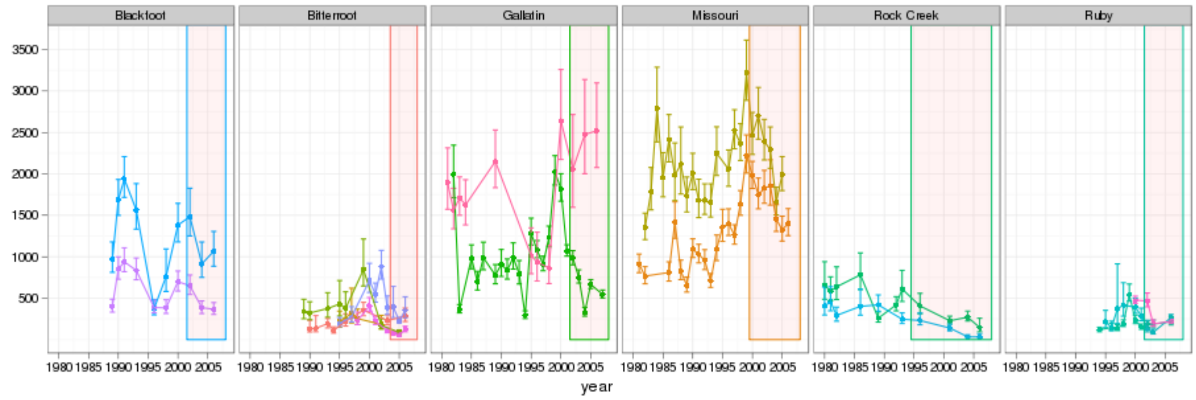


Figure 3.6. Proportional change ($\log_2$ scale) in total population density (A) and biomass (B) of brown trout and rainbow trout after WD in the Blackfoot, Gallatin, Missouri, and Ruby rivers.

		Log_2 scale		Proportion	
Species	River	Biomass	Total	Biomass	Total
Brown	Blackfoot	(0.15, 1.60)	(0.38, 1.93)	(1.11, 3.03)	(1.30, 3.81)
	Gallatin	(0.073, 0.66)	(0.067 0.65)	(1.05 1.58)	(1.05 1.56)
	Missouri	(-0.96 0.12)	(-0.83 0.11)	(0.51 1.09)	(0.56 1.08)
Rainbow	Blackfoot	(-2.37, -1.41)	(-2.99, -2.04)	(0.19, 0.38)	(0.13, 0.24)
	Gallatin	(-0.98, -0.51)	(-1.34, -0.87)	(0.51, 0.70)	(0.40, 0.55)
	Missouri	(-0.97, -0.41)	(-1.26, -0.64)	(0.51, 0.75)	(0.42, 0.64)
	Ruby	(-0.46, -0.045)	(-0.17, 0.23)	(0.73, 0.97)	(0.89, 1.18)

Table 3.8. 95% credible intervals for effects of WD on biomass and total population density or brown trout and rainbow trout using the BACI model.

A. Total Population Density of Rainbow Trout



B. Biomass of Rainbow Trout

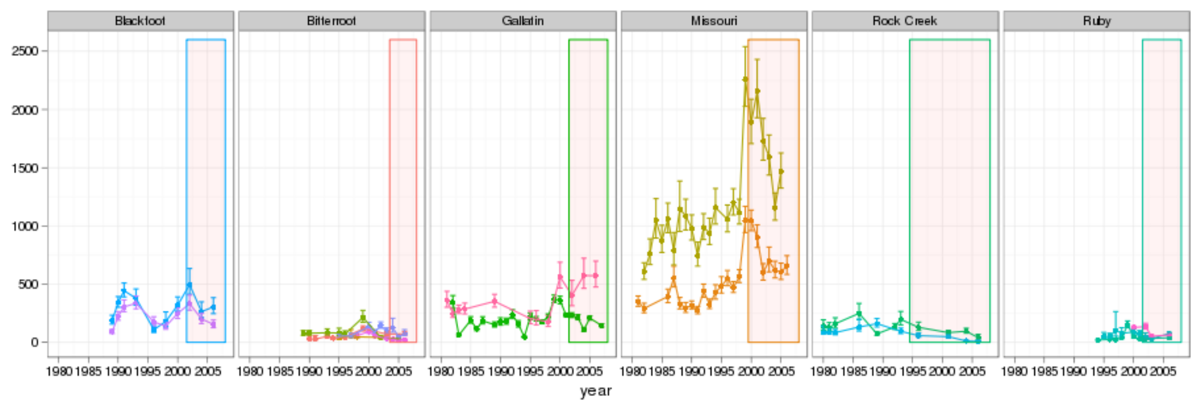
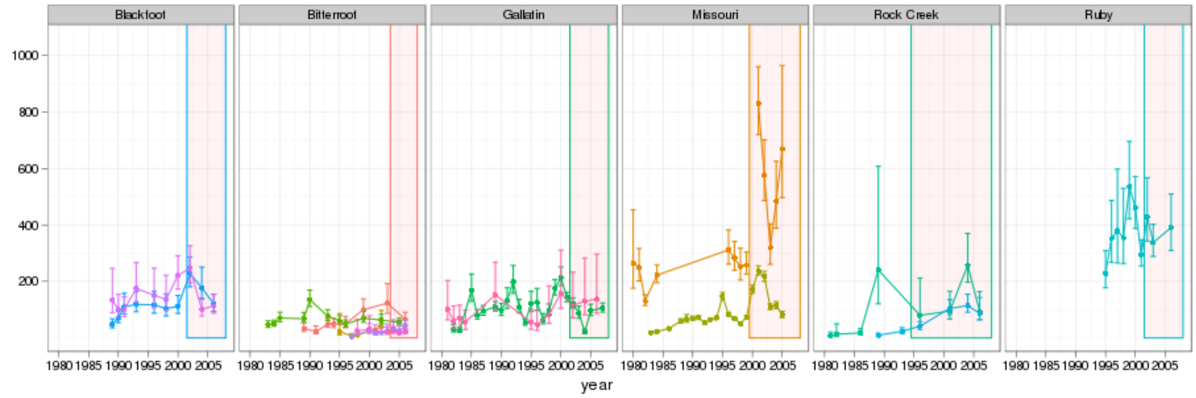


Figure 3.7. Total population density (A) and biomass (B) of rainbow trout per km over time for each individual study river. 95% credible intervals are shown, and medians within each site are connected. Sampling sections (shown in color legend below) for each river are: Blackfoot (Johnsrud and Scotty Brown); Bitterroot (Bell, Connnor, Darby, Maynard, and Trinity); Gallatin (East and West); Missouri (Cascade and Craig); Rock Creek (Fish and Game, Hogback); and Ruby (Greenhorn, Vigilante, 3Forks, Trinity).

A. Total Population Density of Brown Trout



B. Biomass of Brown Trout

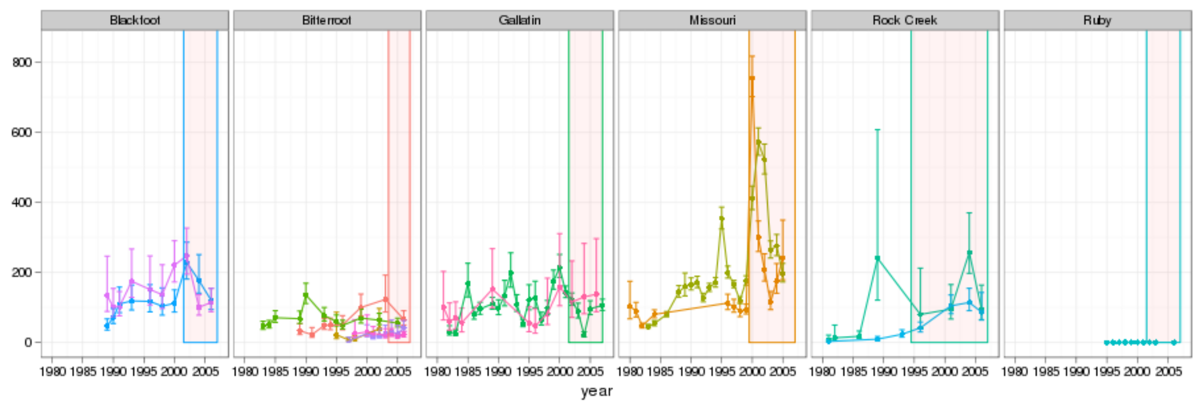


Figure 3.8. Total population density (A) and biomass (B) of brown trout per km over time for each individual study river. 95% credible intervals are shown, and medians within each site are connected.

Relative Abundance and Age Structure

The proportion of rainbow trout for each study river is shown in Figure 3.9 and 3.10. Rainbow trout were the predominant trout in all study rivers, ranging in proportion from 60-90% pre WD. Most rivers showed little change in the overall proportion of rainbow trout and brown trout pre- and post-WD (Figure 3.9) with the exception of Rock Creek (Figure 3.10), which showed a major shift from rainbow trout to brown trout over the study period. Pre-WD, rainbow trout comprised >90% of the total rainbow and brown trout population in Rock

Creek, but this level sharply declined in the mid 1990s and comprised only about 20-30% of total density from 2000-2006. Rainbow trout show a steady decline in proportion in the Blackfoot and Bitterroot rivers, but this pattern is similar between both WD- and WD+ sections.

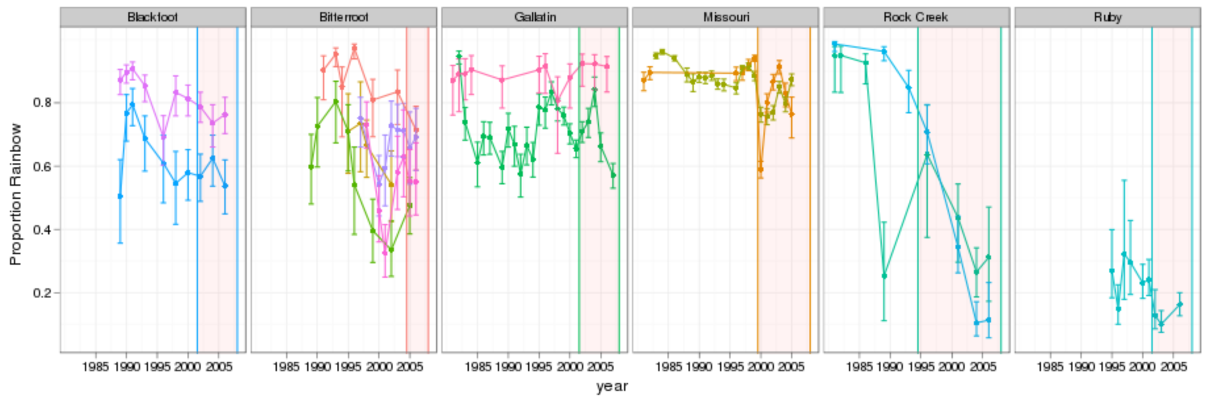


Figure 3.9. Proportion of total trout density comprised of rainbow trout across years for each study river and section. Lines connect posterior medians. Vertical lines represent 95% credible intervals. See Figure 3.7 for site (color) legend.

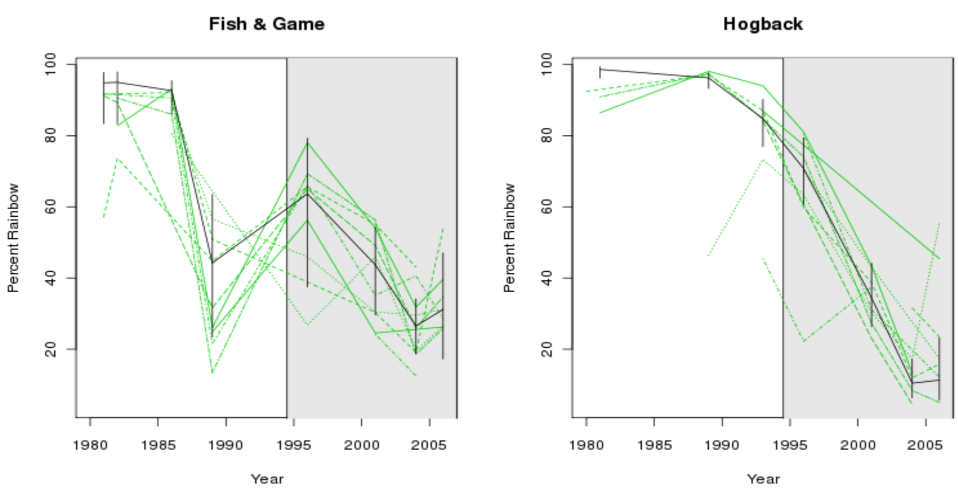


Figure 3.10. Relative abundance of rainbow trout as proportion of total trout density for Rock Creek. Shaded portion indicates years after WD. Solid line is median and lighter lines represent individual size classs.

For the Missouri, there was little change in the relative age structure of brown trout from before to after WD, other than a slight increase in trout age 3 and older (Figure 3.11; Table 3.9). Notably, there was little change in the proportion of age 1 and age 2 brown trout before and after WD for both the WD- Cascade and the WD+ Craig sections, ranging from 37 to 47% pre WD to 43% post WD (Table 3.9). However, young rainbow trout were severely affected by WD. Proportionally, age 1 and 2 rainbow trout in the Craig section declined from 64% of total abundance pre-WD (12,383 per km) to 26% post-WD (6,255 per km) whereas there was little change in proportion of age 1 and 2 fish in the Cascade section (64% pre and post WD). In contrast, older rainbow trout increased markedly after WD (Figure 3.11). Pre WD, age 3 and 4+ rainbow trout averaged 36% of total rainbow trout numbers in the Craig section, but increased to 74% post WD. This represented about a doubling of age 3 rainbow trout from 5,464 to 12,363 per km and about a tripling of age 4+ rainbow trout from 1,631 to 4,821 per km. Older rainbow trout increased during the post-WD period in the WD- Cascade section, but the increase was much more moderate (25% pre WD to 35% post WD).

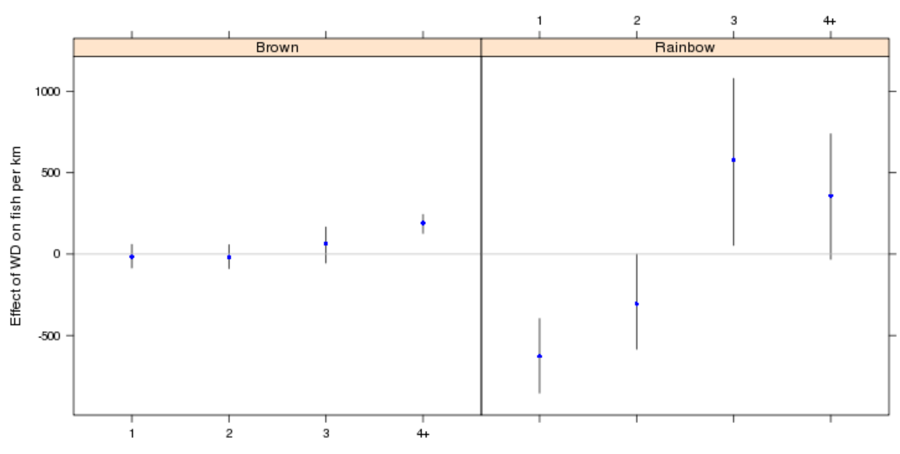


Figure 3.11. Relative change in abundance of brown trout and rainbow trout by age class following the WD epizootic in the Missouri River.

Table 3.9. Proportion of rainbow trout and brown trout by age class before and after WD in the Cascade (WD-) and Craig (WD+) sampling sections on the Missouri River.

Species	Site	Age Class	Pre WD	Post WD
Rainbow	Cascade	1	0.44	0.39
		2	0.30	0.25
		3	0.19	0.29
		4+	0.06	0.06
	Craig	1	0.34	0.13
		2	0.30	0.13
		3	0.28	0.53
		4+	0.08	0.21
Brown	Cascade	1	0.14	0.08
		2	0.23	0.35
		3	0.30	0.32
		4+	0.33	0.26
	Craig	1	0.22	0.09
		2	0.25	0.34
		3	0.19	0.34
		4+	0.35	0.23

Condition

Table 3.10 lists the comparisons between relative weight for rainbow trout and brown trout before and after WD for each study river. Plots of Wr for each species over time are shown in Appendix 4. Wr generally averaged in the 90s for rainbow trout and brown trout across all study rivers (Blackfoot River rainbow trout >300 mm were the exception with Wr's of 75-85), indicating fish were in moderately good condition.

WD did not appear to adversely affect trout condition. There was no difference in Wr of rainbow trout and brown trout pre vs. post-WD for fish in Rock Creek and the Blackfoot and Ruby rivers in both WD+ and WD- sections (Table 3.10). Trout condition increased significantly post-WD in the Bitterroot and Gallatin rivers, but the increase was generally slight (<5%), and was not consistent among species or sections with and without WD. In the highly infected East Gallatin, Wr increased significantly post-WD for both rainbow trout and brown trout; however, brown trout in the WD- West Gallatin also exhibited a significant increase as did brown trout in the WD+ East Fork of the Bitterroot River.

Trout in the Missouri River were the only group among study rivers that showed a significant decline in Wr after WD. However, this decline was observed in rainbow trout in both the WD+ (Craig) and WD- (Cascade) sections and in brown trout in the WD+ Craig section so the increase could not be clearly attributed as a response to WD. Rainbow trout Wr in both sections decreased from a pre-WD level of 102-103 to a post-WD level of 91-92, a 12% decline; this pattern was shown among all three length classes with the greatest decline among small fish in the 200-300 mm length class. Brown trout in the WD+ Craig section showed a similar significant decline in Wr, decreasing from a pre-WD mean of 96 to a post-WD mean of 89, a 7% decline in condition. In contrast, brown trout from the WD- Cascade section showed no change in Wr between the pre- and post-WD periods.

Table 3.10. Relative weights {mean (SE)}of rainbow trout and brown trout in reference (WD-) and WD-positive (WD+) study rivers. Underlined values indicate significant differences (p < 0.05) in relative weight before vs. after WD.

			Rainbow trout,Wr			Brown trout, Wr		
River	section	WD	Before	After	*F*(*P*)	Before	After	*F*(*P*)
Bitterroot	E. Fork	+	97 (0.6)	97 (1.1)	0.1 (0.8)	94 (0.9)	97 (0.7)	6.9 (0.03)
	W. Fork	-	96 (0.6)			96 (0.6)		
	Darby	+	93 (0.6)	89 (2.3)	2.9 (0.1)	95 (0.9)	93 (3.1)	0.2 (0.7)
	Bell	-	89 (1.0)	90 (2.3)	0.1 (0.7)	90 (1.0)	91 (2.4)	0.2 (0.7)
Blackfoot	Johnsrud	+	94 (1.7)	90 (2.3)	0.1 (0.8)	93 (1.2)	92 (1.4)	0.3 (0.6)
	Scotty Brown	-	92 (1.5)	89 (2.8)	0.8 (0.41)	94 (1.7)	90 (2.3)	1.0 (0.3)
Gallatin	East	+	95 (0.7)	102 (1.2)	20.3 (0.00)	94 (0.6)	99 (1.0)	18.5 (0.00)
	West	-	96 (1.1)	97 (2.1)	0.3 (0.6)	96 (0.7)	100 (1.1)	9.4 (0.01)
Missouri	Craig	+	103 (0.9)	91 (1.9)	34.1 (0.00)	96 (0.9)	89 (1.2)	20.9 (0.00)
	Cascade	-	102 (1.1)	92 (1.9)	24.3 (0.00)	91 (1.2)	90 (5.4)	0.1 (0.8)
Rock	F&G,Hogback	+	96 (1.0)	94 (1.5)	1.3 (0.3)	92 (1.2)	94 (2.6)	0.4 (0.5)
Ruby	Greenhorn, Canyon	+	91 (3.7)	96 (2.0)	1.8 (0.2)	99(5.9)	93 (7.7)	0.4 (0.5)
	3Forks,Vigilante	-	98 (1.7)	96 (1.9)	0.7 (0.4)	94 (4.5)	92 (5.4)	0.1 (0.8)

Growth rate

Table 3.11 lists the comparisons between growth in rainbow trout and brown trout before and after WD for the WD- Cascade section and the WD+ Craig section of the Missouri River. Plots of length at age for each species over time are shown in Appendix 5.

Both rainbow trout and brown trout exhibited high growth rates in both river sections. Rainbow trout at age 1 averaged about 240 mm. The majority of rainbow trout that recruit to the mainstem population migrate into the Missouri River from Little Prickly Pear Creek and Dearborn River spawning tributaries as 100-120 mm-long yearlings in the spring (Munro 2004). Thus, about 140 mm of this growth occurs just in the 3-4 month growing period after their entry into the Missouri River and the electrofishing sampling for rainbow trout in October. Growth in subsequent year classes is about 100 mm from age 1 to 2, 80 mm for age 2 to 3, and 60 mm for age 3 to 4+. Brown trout were smaller at age 1 than rainbow trout, but showed similar growth rates and lengths at age in subsequent year classes. For both species, growth rates were similar in the Craig and Cascade sections (Table 3.11).

WD appeared to result in increased growth of larger rainbow trout and brown trout (Table 3.11). Mean length at age 4+ rainbow trout in the WD+ Craig section was 499 mm after WD, an increase of 31 mm from pre-WD years. Mean length of age 3 rainbow trout was also substantially higher after WD (+22 mm; $P = 0.07$). In contrast, there were no differences in length at age for any age class among rainbow trout from the Cascade reference section, although 3 and 4+ age class fish did show higher growth (+16 mm) in the post-WD period. There was no difference in length between pre- and post-WD periods for age 1 and for age 2 rainbow trout from either section.

Table 3.11. Mean length at age (SE) of rainbow trout and brown trout in reference (WD-) and WD-positive (WD+) sections of the Missouri River before and after WD. Underlined values indicate significant differences ($p < 0.05$) in growth before vs. after WD.

			Rainbow trout			Brown trout		
River section	WD		Before	After	*F*(*P*)	Before	After	*F*(*P*)
Cascade	-	Age 1	242 (3.3)	234 (7.5)	1.0 (0.3)	167 (7.5)	162 (5.7)	0.3 (0.6)
		Age 2	337 (4.8)	338 (11.1)	0.1 (0.9)	300 (6.1)	304 (5.6)	0.4 (0.6)
		Age 3	413 (4.1)	419 (8.5)	3.2 (0.09)	376 (11.8)	400 (11.3)	2.1 (0.19)
		Age 4+	465 (4.3)	481 (10.8)	1.9 (0.19)	479 (5.7)	500 (6.2)	6.7 (0.04)
Craig	+	Age 1	240 (3.0)	234 (6.4)	0.7 (0.4)	179 (2.8)	160 (4.8)	11.3 (0.00)
		Age 2	334 (3.6)	341 (7.6)	0.6 (0.4)	290 (4.4)	287 (6.5)	0.1 (0.8)
		Age 3	411 (4.9)	433 (10.2)	3.7 (0.07)	388 (5.5)	399 (9.0)	1.0 (0.3)
		Age 4+	468 (6.1)	499 (11.8)	5.4 (0.03)	480 (4.0)	499 (6.2)	7.0 (0.02)

Length at age 4+ for brown trout increased significantly after WD, but this was observed in both the WD+ Craig (+19 mm) and the WD- Cascade (+ 21 mm) sections. Age 1 brown trout decreased significantly in length in the WD+ Craig section after WD (-19 mm), whereas age 2 and 3 brown trout had similar growth rates between pre- and post-WD years.

Flow effects

Graphs of seasonal flow by year for each of the six study rivers are shown in Appendix 3. For all rivers, flows during late spring (May 15-July 15, peak discharge period) and summer (July 15-Sept 15) generally have been below the long-term average flows since the year 2000.

Table 3.12 shows the correlations between the three length classes and four seasonal flow periods across all four BACI rivers combined (Blackfoot, Gallatin, Missouri, and Ruby). Correlations are shown for trout abundance and flows in the current year (lag = 0) and one (lag =1), two (lag =2), and three years (lag = 3) previous.

		Brown				Rainbow			
size	lag	Mar-May	May-July	Jul-Sep	Sep-Mar	Mar-May	May-Jul	Jul-Sep	Sep-Mar
200-225	0	0.11	-0.14	-0.12	-0.07	-0.02	0.07	0.21	0.12
	1	0.04	-0.02	-0.02	0.00	-0.06	-0.03	-0.03	-0.01
	2	0.07	0.05	-0.01	0.00	-0.04	0.02	0.02	-0.03
	3	0.07	0.01	0.01	0.01	0.02	-0.03	0.05	0.04
225-250	0	0.01	-0.14	-0.15	-0.06	-0.09	-0.06	-0.04	-0.07
	1	-0.07	-0.16	-0.15	-0.13	-0.13	-0.10	-0.08	-0.06
	2	-0.04	-0.11	-0.15	-0.10	-0.12	-0.04	-0.01	-0.09
	3	-0.01	-0.10	-0.13	-0.10	0.01	-0.07	0.02	0.02
250-275	0	-0.05	-0.19	-0.14	-0.08	-0.03	-0.01	0.02	-0.01
	1	-0.13	-0.16	-0.17	-0.13	-0.05	-0.07	-0.04	-0.01
	2	-0.04	-0.16	-0.18	-0.10	-0.06	0.00	0.04	0.01
	3	-0.08	-0.10	-0.12	-0.14	0.09	0.04	0.09	0.08

Table 3.12. Correlations between seasonal flows and the abundance of brown trout and rainbow trout.

We found little evidence that flows influenced abundance of small trout (200-275 mm). Most of the correlations were close to 0, indicating little association between abundance of small trout and flow. The strongest correlations were negative associations between brown trout and flow for the May-July and July-Sept flow periods (-0.15 to –0.19), and a positive association between rainbow trout and Jul-Sept flow (0.21), although no values indicated a strong association.

The possibility of nonlinear and river-specific flow effects was explored further by plotting the median proportion change in abundance of 200-225 mm long rainbow trout with June 15-Sept 15 (summer) flow for each river (Figure 3.12). The strongest association was a positive relation between rainbow trout abundance and flow for the Missouri River. The Ruby and Gallatin show a weak positive relationship between abundance and flow, and there appears to be no relation between flow and abundance on the Blackfoot River.

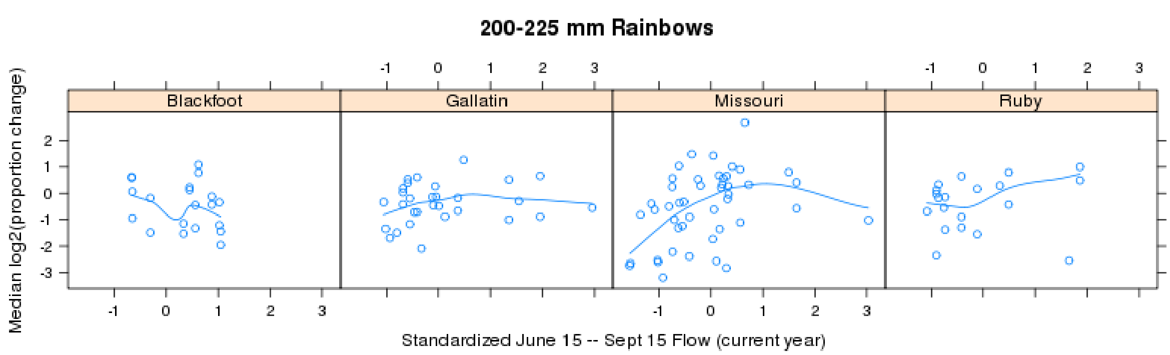


Figure 3.12. Relationship between flow and abundance of small rainbow trout (200-225 mm) by river. Zero on the graphs represents the standardized average abundance or average flow, whereas –1" or +1 represent a 2-fold decrease or increase in abundance (±2 would be a 4-fold difference); for flow (x-axis), values represent the number of standard deviation decrease or increase from the average.

Finally, inclusion of flow into the BACI model for rainbow trout was also investigated to assess BACI model fit with and without flow as a covariate. Summer low flow (lag = 0) from

Figure 3.12 was added to BACI model 3 with and without an interaction with river to assess improvement in model fit as measured by DIC values. For rainbow trout of length 200-225 mm, DIC values from Table 1 were 364.18, 353.30, and 354.99 for BACI models 1, 2, and 3, respectively, indicating a significant effect of WD on abundance. Addition of summer flow as a covariate yielded a DIC value of 335.1 with, and a value of 327.6 without, a river interaction. Thus, inclusion of flow as a covariate substantially improved model fit even further (>5 DIC change), suggesting an interaction of WD and flow on abundance of small rainbow trout, with lower flows and high WD both negatively affecting young rainbow trout in the years since 2000. We caution that the relationships estimated here between flow and abundance may be spurious because the particular flow variable used was selected as the one with the strongest correlation to abundance from 48 possibilities. However, the correlation was in the direction that we predicted *a priori* even though the exact flow variable was chosen through data inspection.

3.5 Discussion

Our results demonstrated a clear linkage between a disease-severity threshold in sentinel fish (50% ≥ grade 3) and marked declines in abundance of small rainbow trout. These results therefore support a key assumption in WD monitoring– that infection risk as measured by controlled exposures of age 0 hatchery rainbow trout is correlated with disease severity and population decline in wild salmonids (Pierce et al. 2009). Though previous studies also demonstrated substantial recruitment declines in wild rainbow trout populations following epizootic outbreaks of whirling disease (Nehring and Thompson 2003; Nehring 2006; Vincent in press), in our study, the inclusion of extensive pre-WD data and reference sections not impacted by the disease allowed us to more definitively measure the magnitude of the effect of WD separate from other potential factors that may have influenced trout population abundance, and to compare population responses to a WD outbreak among multiple rivers.

Overall, we found that whirling disease outbreaks led, on average, to a 50% decline (range 30 to 69%) in rainbow trout in the 200-300 mm size class. This pattern was consistent among the four BACI study rivers (Blackfoot, Gallatin, Missouri, and Ruby rivers) as well as in Rock Creek and the Bitterroot River, although post-WD and reference section data were more limited for the latter two sites. However, the decline of this size class, corresponding to fish 1 to 3 years old, is unlikely due to direct mortality from whirling disease. In the laboratory, rainbow trout of this size and age do not develop clinical whirling disease and show no diminished performance after infection even with high parasite exposure (Ryce et al. 2004; DuBey et al. 2007). Moreover, rainbow trout in this size class in our study did not show reduced growth or condition after a WD outbreak, suggesting that those fish that do survive to this size do not suffer continued survival or performance deficits even in highly infected systems. Rather, the decline is likely due to poor survival of age 0 cohorts, though we were unable to accurately measure abundance of this age class due to the low capture probabilities for such short fish (Figure 3.1; Appendix 2). Rainbow trout < 9 weeks of age and <40 mm in length are the most highly vulnerable life stage to whirling disease (Ryce et al. 2005); those with infection scores of 3 and greater exhibit a high incidence of clinical signs of disease and likely suffer high mortality rates in the wild due to poor growth and diminished predator avoidance (MacConnell and Vincent 2002; Ryce et al. 2004; DuBey et al. 2007).

Contrary to our expectations, the marked decline in smaller rainbow trout after WD outbreak did not lead to major declines in larger fish, as observed in Colorado rivers (Nehring and Thompson 2003). Instead, the numbers of rainbow trout >300 mm either remained the same or increased after WD, with the effects varying by river. Though the lack of decline could be partially attributed to a lag effect wherein low age-0 recruitment has not yet had time to negatively affect subsequent numbers of larger fish, rivers with 5 or more years since the inception of high infectivity (Blackfoot, Gallatin, and Missouri rivers, Rock Creek) showed a similar pattern of stable or increasing numbers of larger trout. Alternatively, the rapidity of the response in abundance, particularly among very large rainbows >400 mm, coupled with increased growth shown by large Missouri River rainbow trout following WD, suggests a compensatory response in survival and growth. Small and large fluvial rainbow trout share a similar (insectivorous) diet and display strong intercohort competition; marked reductions in the density of small trout, as observed in our study, has been shown to increase growth and survival of large trout (Nordwall et al. 2001; Kaspersson and Hojesjo 2009). In the Missouri River, adult declines have been anticipated for many years once older rainbow trout died out (Leathe et al. 2002a), but the adult population remains robust and the average size of large trout continues to increase (FWP 2008), suggesting that survival and growth of large, old rainbow trout in river systems may be much more flexible than previously thought. More detailed examination of age, growth, and survival of these older fish would be a fruitful area for further research.

As expected, WD did not appreciably influence brown trout recruitment. Brown trout have low susceptibility to WD (Hedrick et al. 1999; Baldwin et al. 2000), presumably as a result of having evolved resistance during coevolution with the parasite in Eurasia. Brown trout populations remain largely unchanged in multiple Colorado rivers experiencing major declines in rainbow trout after WD outbreaks (Nehring 2006). In our study, reductions in rainbow trout biomass or density after WD were generally compensated to a similar degree by an increase in biomass and density of brown trout. Though the two species generally occupy different fluvial habitats as adults, the two species may still compete for preferred habitats (Gatz et al. 1987). The major shift in dominance from rainbow trout to brown trout observed in Rock Creek has not been reported in any other rivers following a WD outbreak. Berg (2004) hypothesized that a combination of high infection risk, low flows, and warmer temperatures over the past 10 years has likely promoted this shift to brown trout dominance in that system.

Why the recruitment declines we observed were not as severe as those in previous reports of trout response to WD epizootics is uncertain. Declines of juvenile trout recruitment by 90% or more were reported for multiple Colorado rivers (Nehring and Thompson 2003; Nehring 2006) and for the Madison River in the first 8 years following WD outbreak (Vincent in press) compared to the 50% decline observed in our study. High infectivity of young trout by the parasite requires high spatial overlap between infective spore release and fry emergence within a relatively narrow time window (Downing et al. 2002; MacConnell and Vincent 2002). Nehring (2006) hypothesized that severe population declines in Colorado rivers have persisted from a combination of 1) initial stocking of parasite-infected rainbow trout which infused large numbers of parasite spores into the system; and 2) a high density brown trout population which have served as natural reservoirs of sustained high levels of parasite proliferation that have kept age 0 rainbow trout recruitment negligible over the past 10 years in many rivers. In the Madison River, a combination of high spatial overlap between rainbow trout spawning areas and sites of infection hotspots in the river, coupled with a low number of spawning sites, contributed to high infectivity levels and poor juvenile recruitment (Downing et al. 2002; Vincent in press). In our study rivers, spore production may be dampened compared to that in Colorado rivers because brown trout densities were generally low in our study areas (<100-400 per km; Appendix 3) and remained about 20-30% of total trout densities, and no large stockings of infected hatchery fish were involved in parasite establishment. Finally, we cannot rule out the possibility that the lessened severity of WD in our study rivers is due to rainbow trout resistance to WD as Vincent (2006) and Miller and Vincent (2009) reported for the Madison River and Willow Creek, Montana, respectively. In these cases, resistance appeared related to genetic input in the past from stocking of DeSmet rainbow trout, which have a much greater resistance to WD than other wild rainbow trout stocks (Wagner et al. 2006), followed by strong selection for more resistant fish over about a 10 year period. However, limited sentinel cage testing of Missouri River rainbows showed no such resistance among age 0 fish (Leathe et al. 2002b).

We suspect that the lack of severe recruitment decline of juvenile rainbow trout was due to the continued presence of uninfected spawning areas even in highly infected rivers (Kerans et al. 2009; see also Granath et al. 2007 [Rock Creek]; Pierce et al. 2009 [Blackfoot]). Recruitment from these sites likely serves to maintain rainbow trout populations in these systems, although the relation between recruitment sources and infection risk has not been investigated in detail (but see Pierce et al. 2009). Increased egg deposition in these areas as a result of the increased size and number of very large rainbow trout may also help offset high losses of young trout (Zorn and Nuhfer 2007). Although some uninfected spawning sites in our study rivers have environmental characteristics that may offer some protection from parasite proliferation (e.g., high elevation, high gradient streams with low fine sediment; de la Hoz Franco and Budy 2004; Pierce et al. 2009), the recent high infectivity observed in some areas previously uninfected for long time intervals (e.g., Dearborn River; Kerans et al. 2009), suggests that recruitment declines could become more severe in the future if the parasite continues to spread in these drainages, as documented in Kerans et al. (2009).

High infectivity and recruitment declines in juvenile rainbow trout in our study occurred concurrently with significant drought during 2000-2007. Summer flows during this period were 25% or more below the long-term average flow in at least 6 of the last 7 years on most rivers (except for the Bitterroot River where an upstream reservoir moderates summer flow; Appendix 6). Flow level during the period of incubation, emergence, and early rearing has been shown to strongly affect recruitment in fluvial trout populations (Lobon-Cervia and Rincon 2004; Lobon-Cervia and Mortenson 2005; Zorn and Nuhfer 2007), and the relatively low strength of flow and trout abundance relationship in our study, which included pre- and post-WD periods, suggests that factors other than flow were more strongly affecting recruitment. The association between flow and rainbow trout population response to WD lends support to the hypothesis that lower flows contribute to higher infectivity of salmonid hosts by *M. cerebralis* (MacConnell and Vincent 2002; Hallett and Bartholomew 2008). The reduced velocities at lower flows are thought to promote retention and accumulation of infective stages, settlement of salmonid carcasses, and the deposition of fine sediments that create habitat and a food source for the tubificid worm host (Kerans and Zale 2002; Krueger et al. 2006; Hallett and Bartholomew 2008). Warmer temperatures associated with lower flow also appear to promote higher infectivity (Kerans et al. 2005; Hallett and Bartholomew 2008). In turn, at high flows, dilution of spore concentration may account for decreases in infection severity (Vincent 2002; Hallett and Bartholomew 2008). If this flow-infectivity relationship is true, it is predicted that improved summer flows as a result of wetter climate or of greater dam release during periods of peak infectivity in tailwater rivers should significantly reduce infectivity and improve juvenile survival and recruitment (Hallett and Bartholomew 2008).

Conclusions and Management Considerations

- Outbreaks of whirling disease epizootics in our Montana study rivers led to an average 50% decline in juvenile rainbow trout in the 200-300 mm size class in comparison to rainbow trout populations prior to WD and to reference river sections with no or low levels of infection risk. Although the degree of recruitment decline in relation to infection grade level was not evaluated in this study, the results suggest that marked recruitment declines will occur in wild rainbow trout populations when sentinel cage infection levels exceed 50% or more with grade $\geq$3. Sentinel cage infectivity level may therefore be the best measure available to evaluate risk of population decline from WD in contrast to other methods that measure the presence or absence of *M. cerebralis* or abundance of spores in the water column or within infected fish.
- Given that some cutthroat trout subspecies (DuBey et al. 2007) and mountain whitefish (MacConnell et al. 2000) show even more susceptibility to whirling disease than rainbow trout, population declines of a similar or greater magnitude would also be expected to occur in native cutthroat trout and mountain whitefish populations if this infectivity threshold is exceeded.
- The marked decline in small rainbow trout after WD outbreaks may not always lead to reduction in abundance of medium to large rainbow trout, and in some of the rivers in the this study, the number of large fish increased dramatically after WD. Why severe recruitment declines in larger trout were not observed is uncertain. It is hypothesized that the continued presence of uninfected spawning and early rearing areas, even in highly infected rivers, may be the primary mechanism that buffers recruitment, although increased resistance to WD is another possible alternative.

- Increased growth and survival of adult rainbow trout in the face of a WD epizootic may be the result of a compensatory response to dramatic reductions in density of small rainbow trout. Continued long term monitoring of survival and growth of various size and age classes is needed to determine the stability of this pattern or if maintenance of adult recruitment is only a transitory response.
- The lack of decline in growth or condition of rainbow trout after a WD outbreak suggests that young fish that do survive the infection window of high susceptibility do not suffer later survival or performance deficits even in highly infected systems. Recruitment from WD-free spawning and early rearing areas appears crucial for preventing collapse of rainbow trout populations as observed in many Colorado rivers. Protection and enhancement of a diversity of spawning areas and spawning and rearing life histories appears to allow resilience in the face of high infectivity (Pierce et al. 2009) Identification and protection of these 'clean' sites will be vital to maintain recruitment in WD infected rivers. More research is needed to test the proposal that habitat improvement of key infected spawning areas can reduce infectivity and result in population rebound (Thompson and Nehring 2004).
- The lower flows and increased temperatures associated with drought will likely increase WD severity. Continued summer low flows in the face of climate change will likely lead to further declines of WD-susceptible trout species such as rainbow trout and cutthroat trout and replacement by more resistant species such as brown trout. Research is needed to test ideas to reduce increases in anticipated WD severity in the face of climate change, including 1) habitat restoration measures that control temperature increases (e.g., riparian

shading) and reduce tubificid worm fine sediment habitat (Pierce et al. 2009); and 2) flow manipulation (Hallett and Bartholomew 2008).

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<u>Appendices</u>

Appendix 1. Additional Bayesian mark-recapture model development information.

Appendix 2. Capture probability plots for rainbow trout and brown trout by river and river sampling section.

Appendix 3. Population density of rainbow trout and brown trout by size class by river and river sampling section.

Appendix 4. Relative weights of rainbow trout and brown trout by river and river sampling section.

Appendix 5. Length at ages 1, 2, 3, and 4+ of rainbow trout and brown trout in the Missouri River for the Craig (WD+) and Cascade (WD-) sections.

Appendix 6. Seasonal flow data for each study river. Horizontal line represents long-term average flow.

Appendix 1. Additional Bayesian mark-recapture model development information.

- Prior on Population sizes, N_{tl}, where l indicates length class and t the year:
  A vague prior proportional to inverse population size was used as in Raftery (1988).

- The prior distribution for p_{tl} is hierarchical prior based on historical data and the physics of electroshock which suggests that smaller fish are less susceptible to electroshock. Random effects were used to model year and length effects in the logit scale.

  $$\text{logit}(p_{tl}) = b_l + \alpha_t$$

  Each component is described below.

- Length classes effects, b_l:
  For each length class, site, and species we first used a maximum likelihood estimate of capture probability as in Royle and Dorazio (2008). The plots in Figure 1 show the estimates and variability in these quantities across lengths and years for Brown and for Rainbow trout in two sections of the Missouri River.

  The black line in each plot is a smoothed average of the capture probabilities. All four plots show a similar pattern for smaller lengths with $E(p)$ increasing with size up to a "cut point" which appears to be near 250–275 mm. After the cut point, there seems to be a plateau. We model capture probability by making a parameter out of cut-point, and allowing it to occur at the length-class boundaries. A second parameter is the mean of (logit) capture probability to the right of the cut-point. The third parameter is the slope of the mean (logit) probability to the left of the cut-point. We experimented with an additional cut-point and slope to allow the right-hand tail to have some other linear trend, but found that the additional two parameters were very poorly estimated and did not improve the model.

  $$b_l = \epsilon_b + \begin{cases} \mu_l & \text{if } l \geq c \\ \mu_l + \beta(c - l) & \text{if } l < c \end{cases} \quad \text{where } \epsilon_b \sim N(0, \sigma_l^2)$$

  - Prior on cut-point: c must equal one of the length class boundaries below the median, all of which are equally likely a priori.
  - Random length effect for lengths greater than c: $b_l \sim N(\mu_l, \sigma_l^2)$ with empirical prior on μ_l: $\mu_l \sim N(-1.0, 4^2)$, and non-informative prior on σ_l^2: $\sigma_l^2 \propto \sigma_l^{-2} I(\sigma_l^2 < 4)$.
  - Random length effect for lengths less than the cut point: $b_l \sim N(\mu_l + \beta(c - l), \sigma_l^2)$, where l is the midpoint of the length class. Vague prior on initial slope: $\beta \sim N(0, 4^2)$.

- Year effects, a_t:
  The colored lines in the plots of Figure 1 connect estimates for each year. No strong year effects are obvious across all length classes, but it seems sensible to include a potential random year effect: to account for differences in temperature, flow, work crew, etc.

  $$a_t \sim N(0, \sigma_t^2)$$

  Vague prior for $\sigma_t^2 \propto \sigma_t^{-2} I(\sigma_t^2 < 25)$.

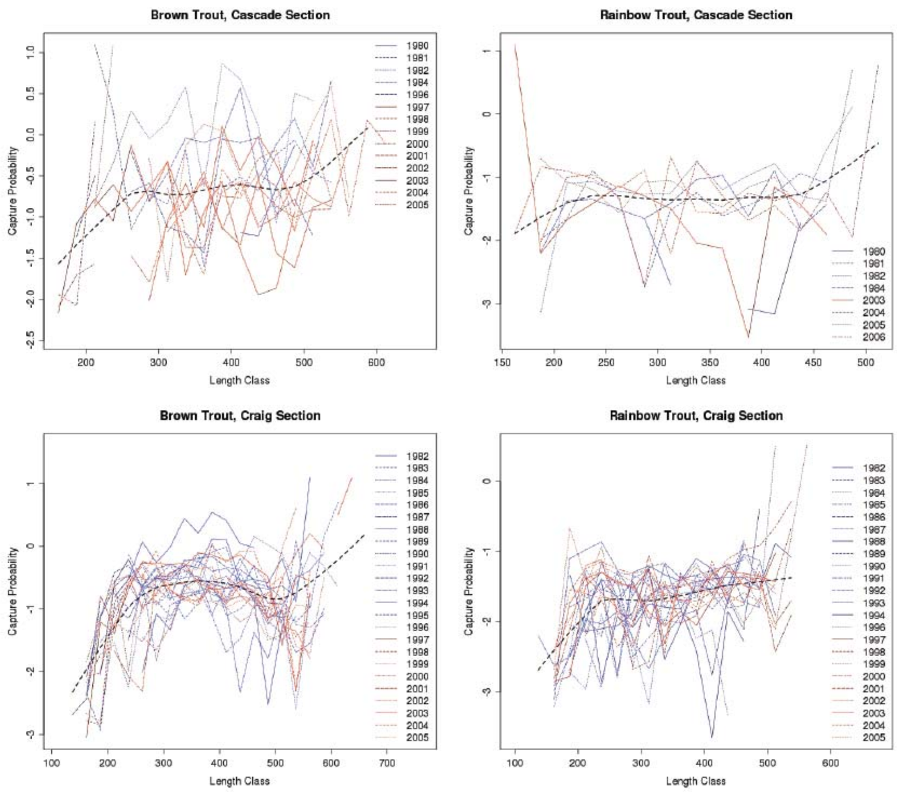


Figure 1: Maximum likelihood estimates of capture probabilities for two species and two sections of the Missouri River. Vertical axis is logit(capture probability) and x axis is length class. Each blue or red line connects estimates for a given year. The black dashed line is a smoothed average across all years.

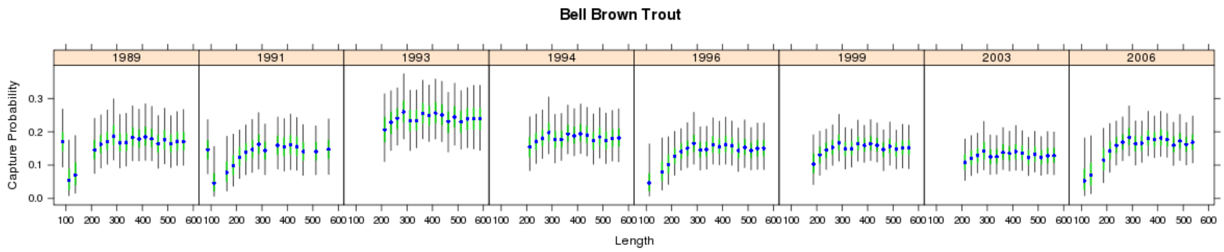
References

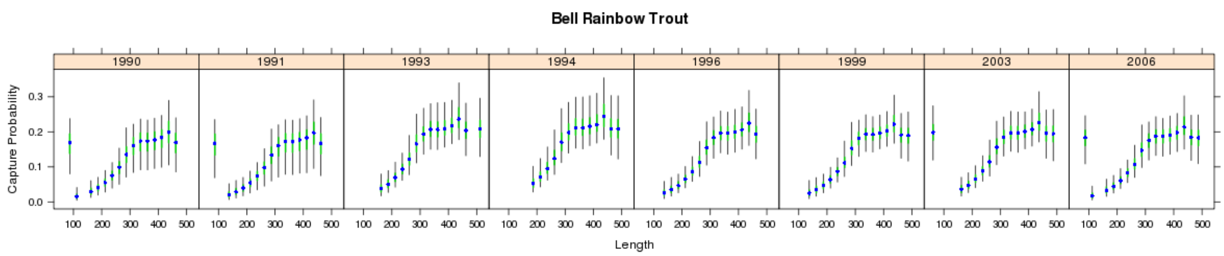
A.E. Raftery. Inference for the binomial N parameter: A hierarchical Bayes approach. *Biometrika*, 75:223–228, 1988.

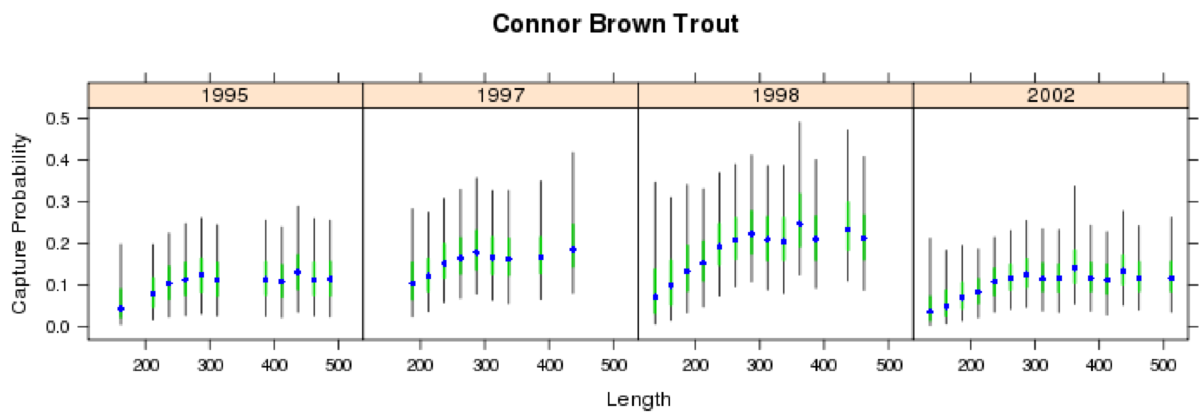
J.A. Royle and R.M. Dorazio. *Hierarchical Modeling and Inference in Ecology.* Elsevier, Amsterdam, 2008.

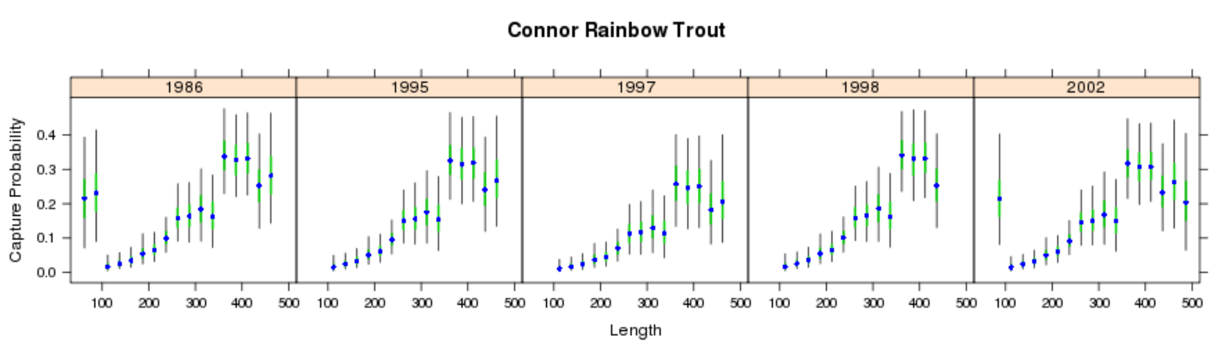
Appendix 2. Capture probability plots for rainbow trout and brown trout by river and river sampling section.

1. **Bitterroot River**

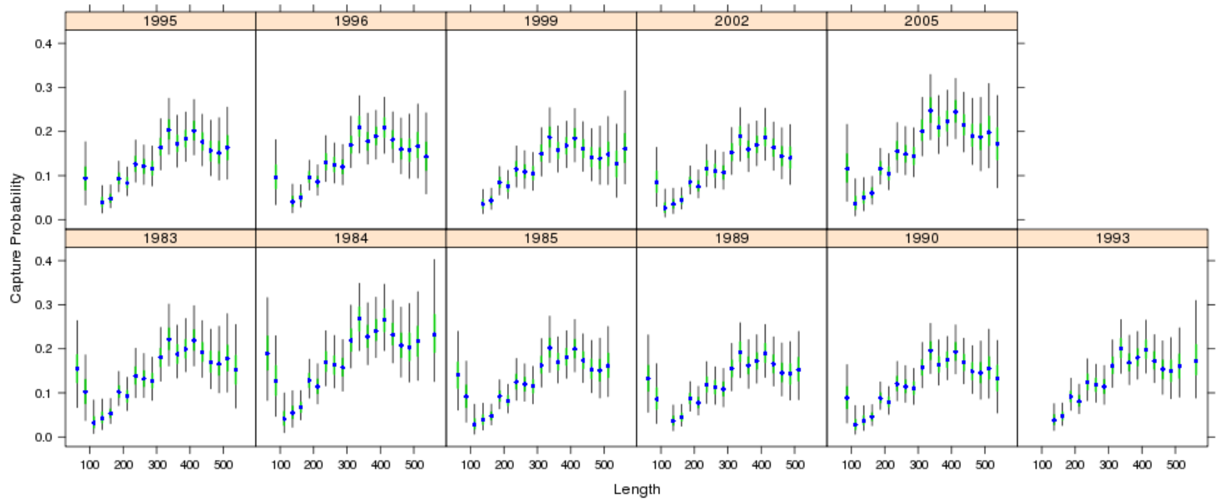




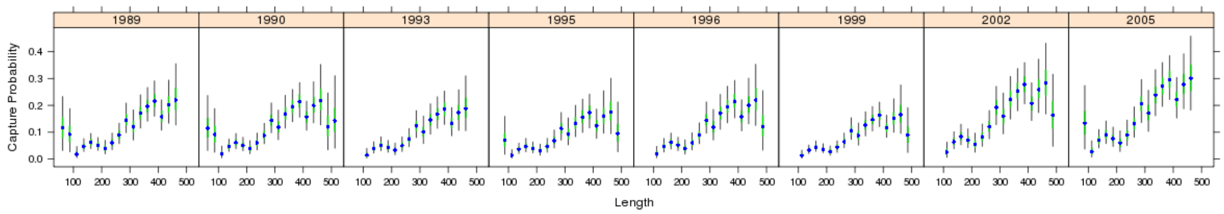




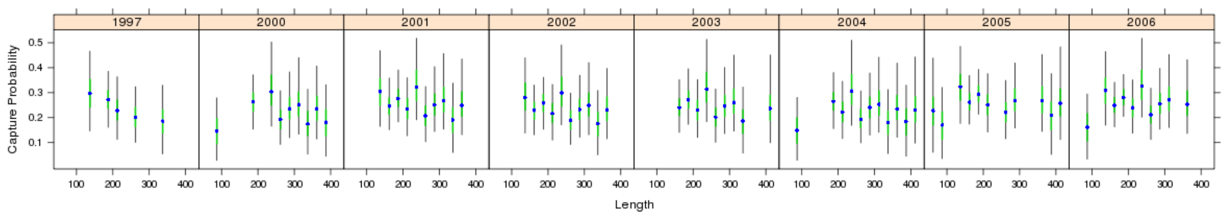
Darby Brown Trout



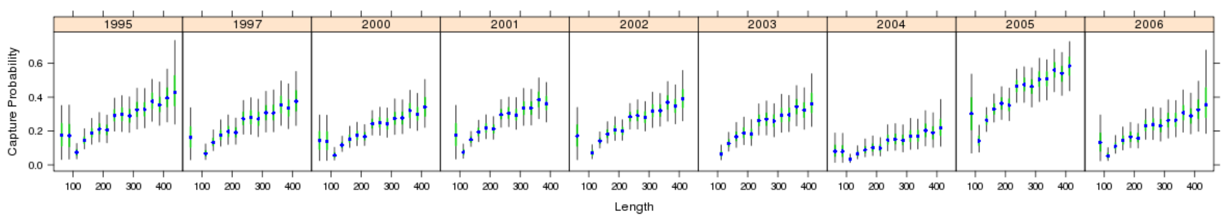
Darby Rainbow Trout



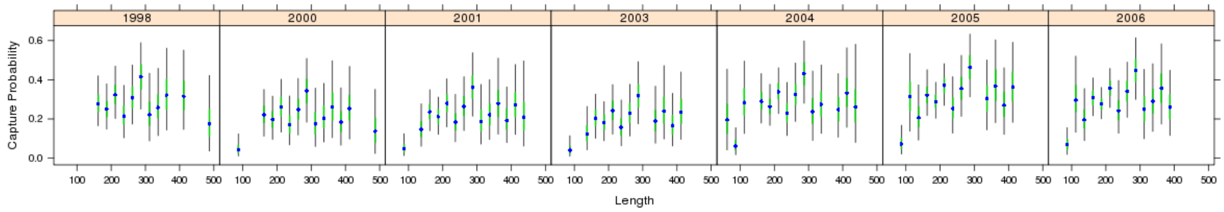
Maynard Brown Trout



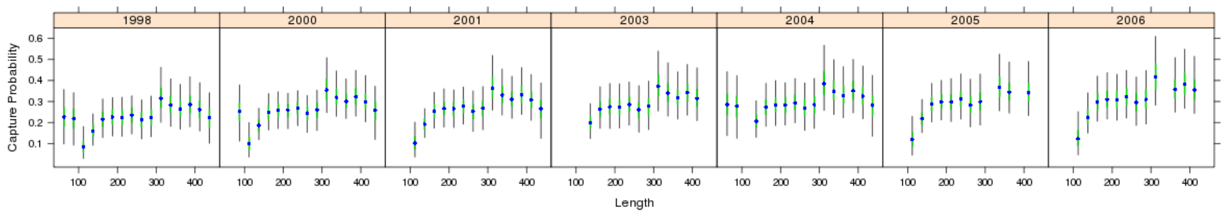
Maynard Rainbow Trout



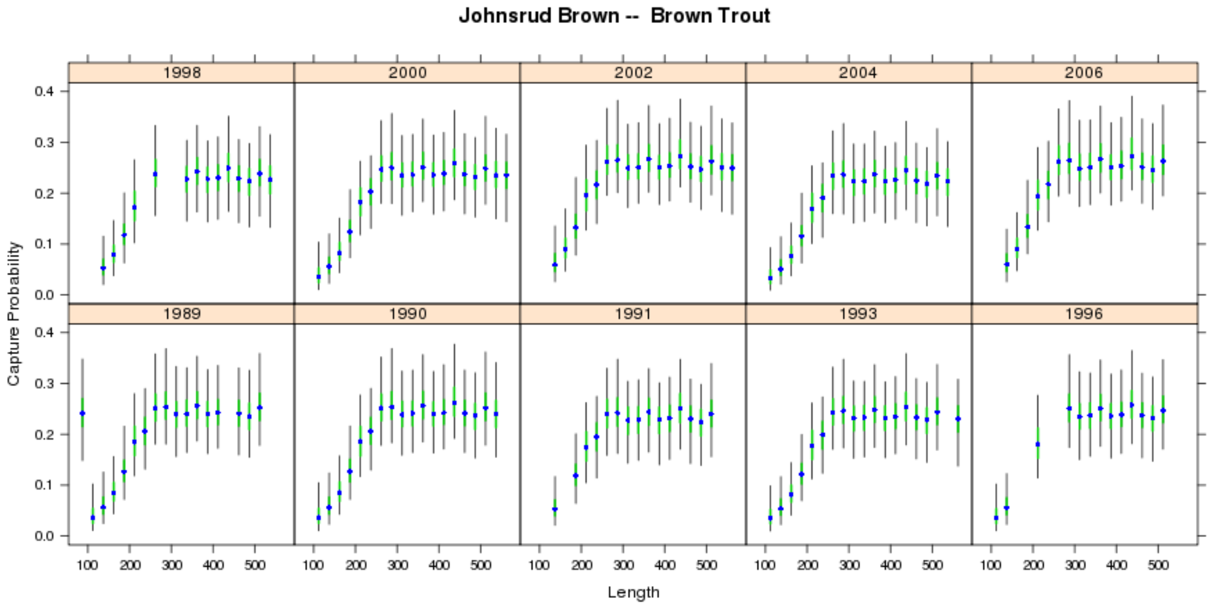
Trinity Brown Trout

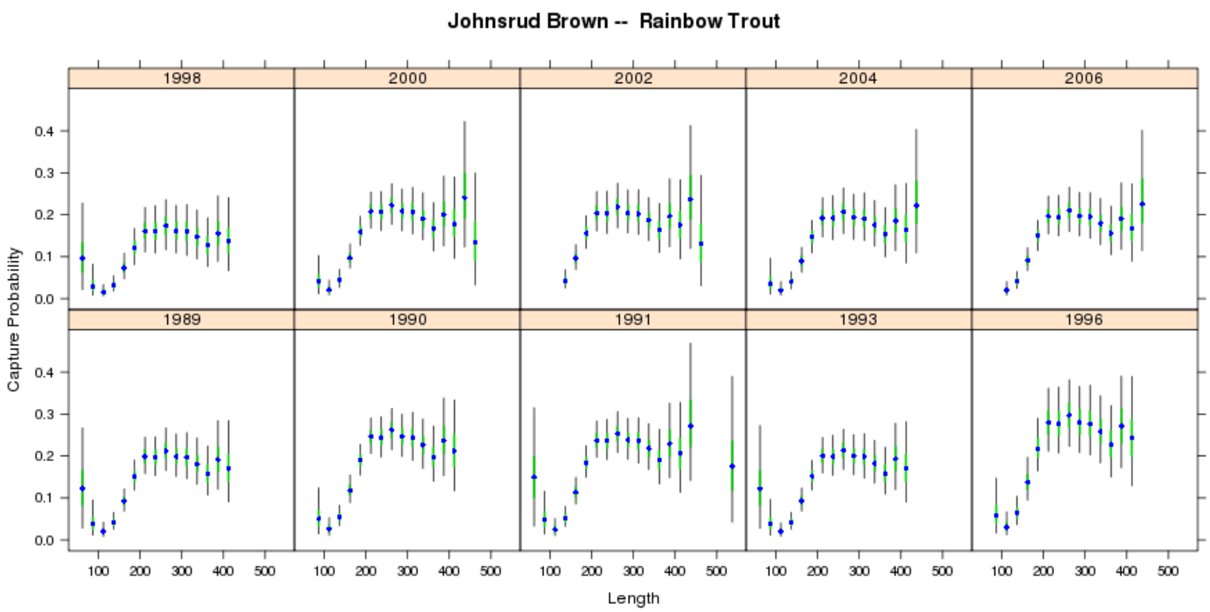


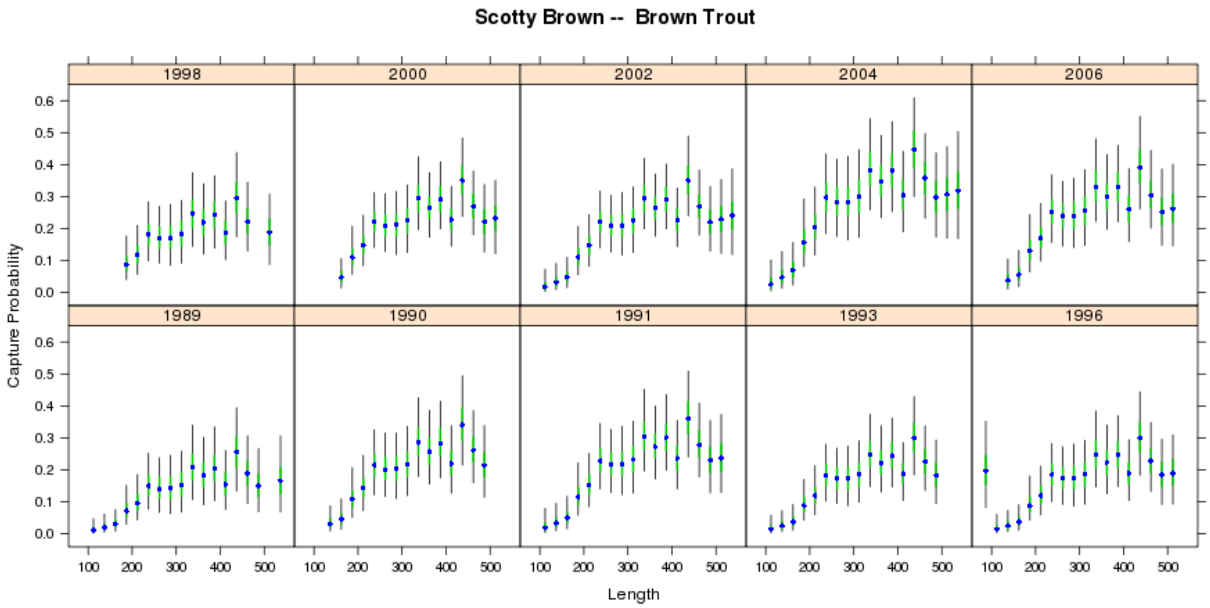
Trinity Rainbow Trout

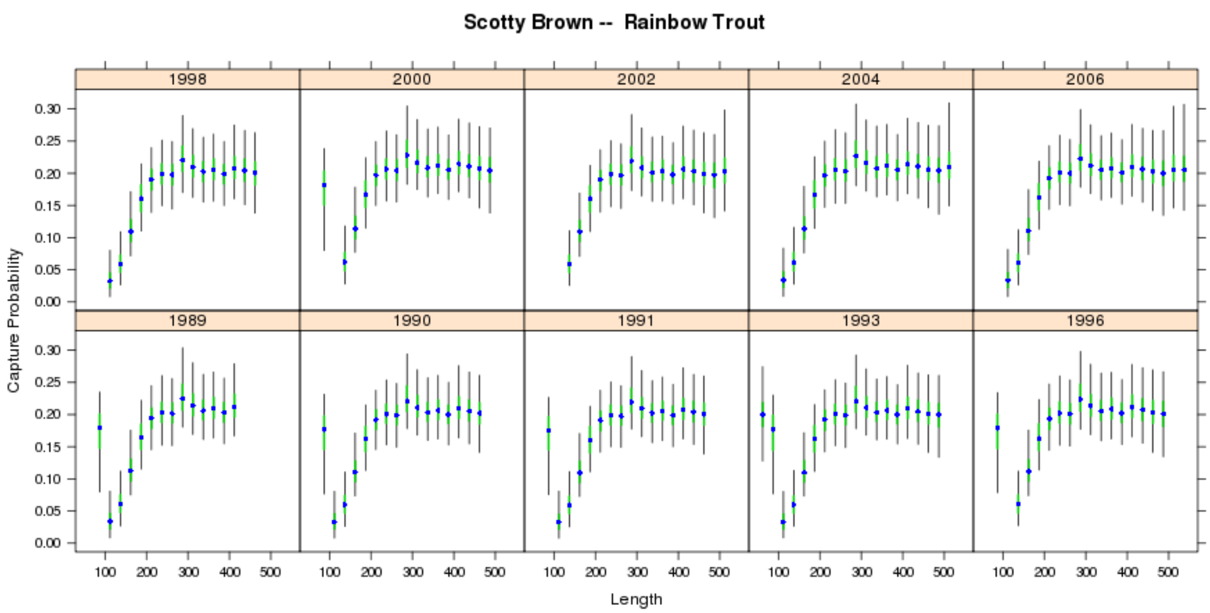


1) Blackfoot River

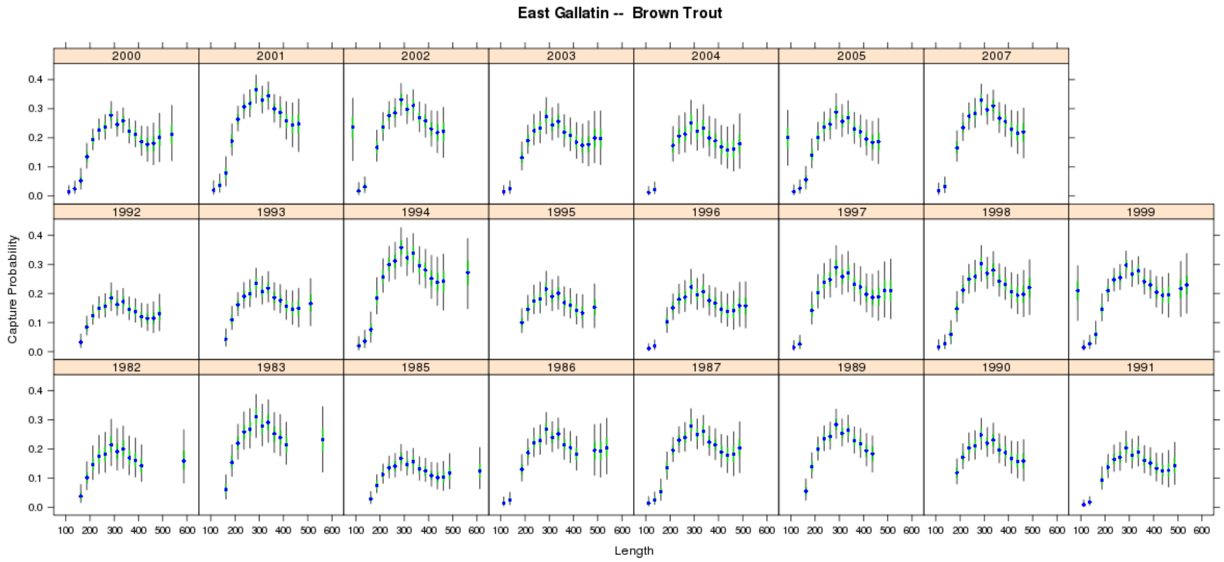


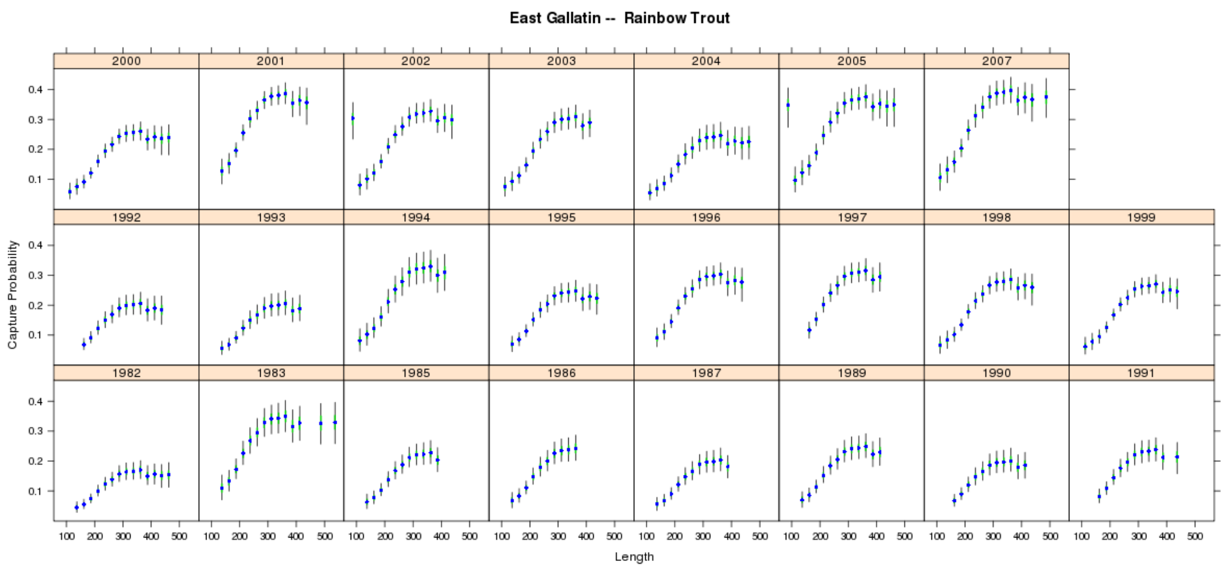


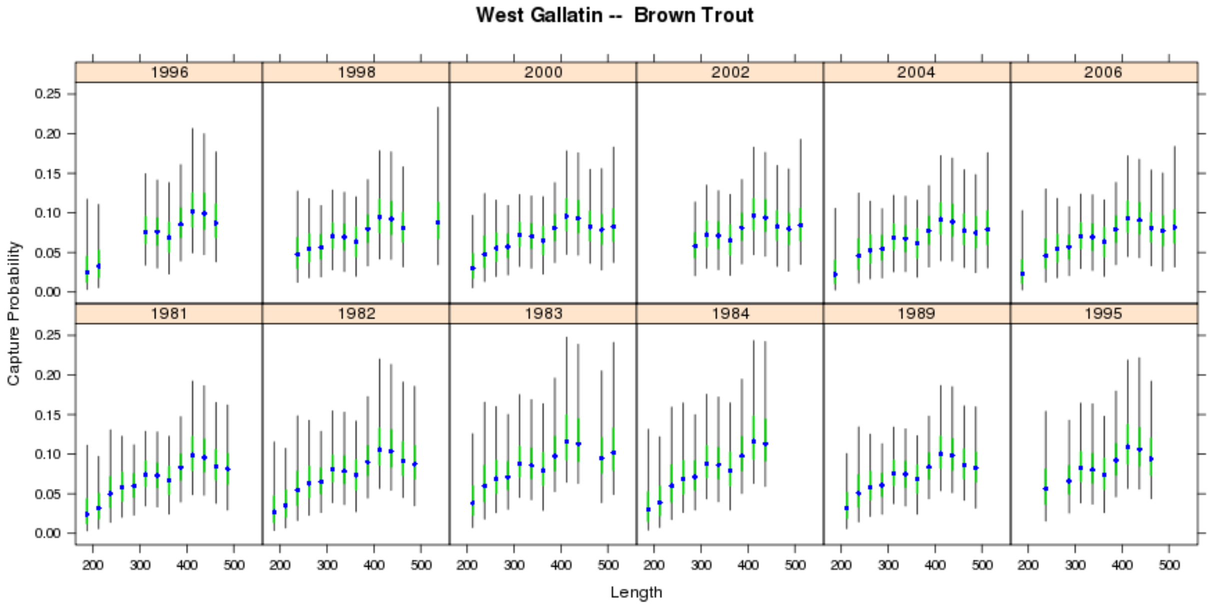

Scotty Brown -- Brown Trout
1998
2000
2002
2004
2006
1989
1990
1991
1993
1996
Capture Probability
Length

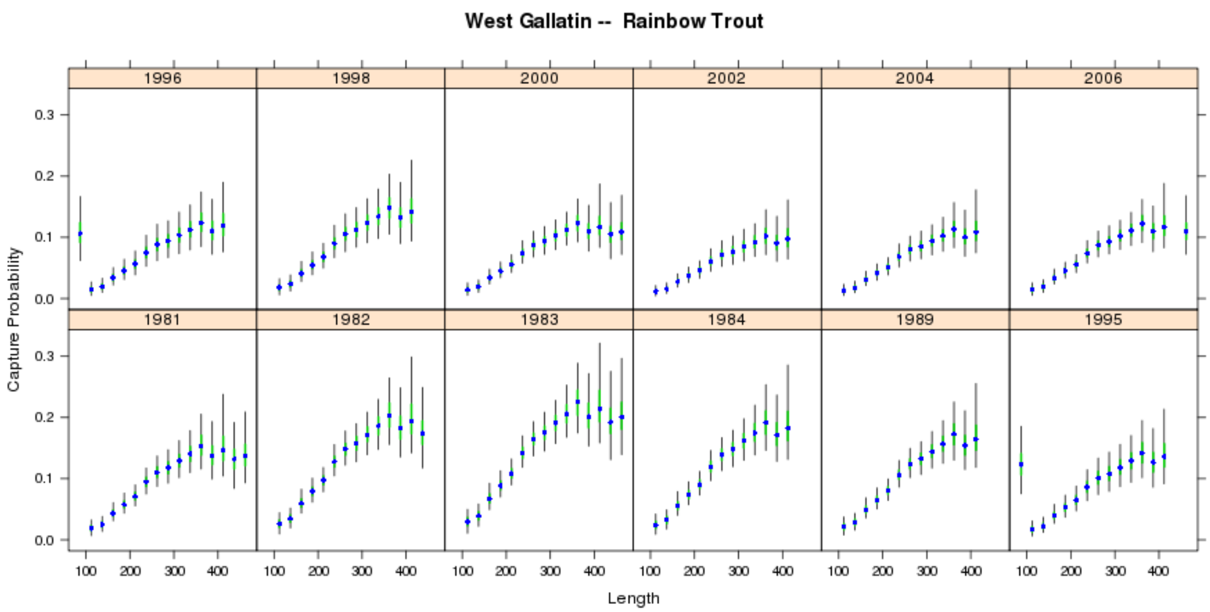

Scotty Brown -- Rainbow Trout
1998
2000
2002
2004
2006
1989
1990
1991
1993
1996
Capture Probability
Length

3. **Gallatin River**

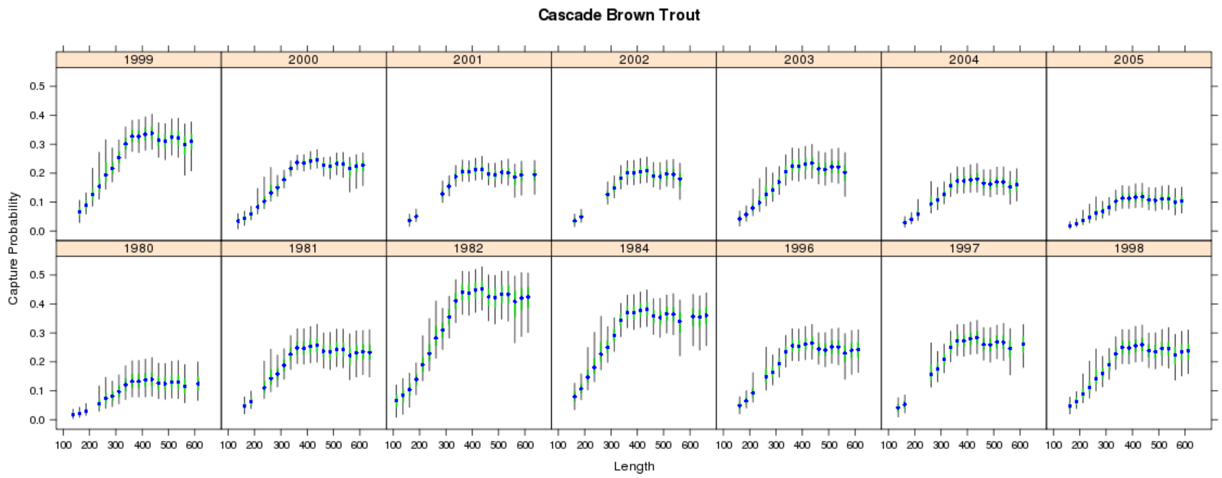


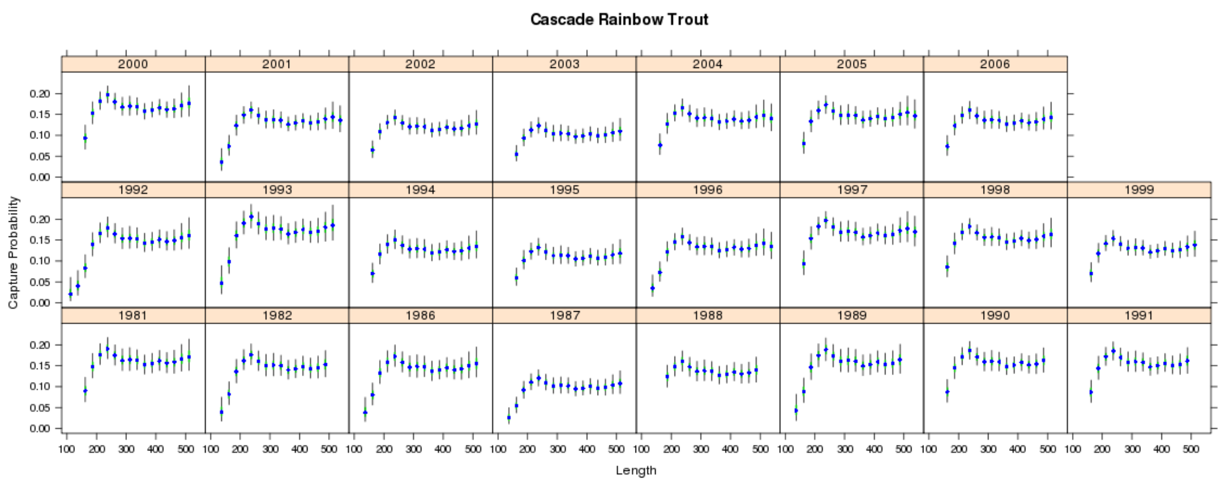



West Gallatin -- Brown Trout
1996
1998
2000
2002
2004
2006
1981
1982
1983
1984
1989
1995
Capture Probability
Length

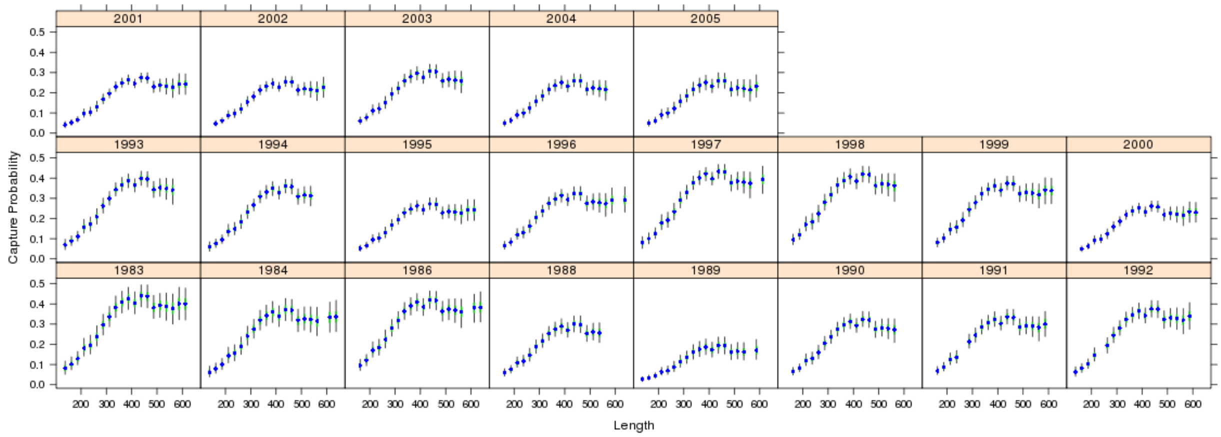

West Gallatin -- Rainbow Trout
1996
1998
2000
2002
2004
2006
1981
1982
1983
1984
1989
1995
Capture Probability
Length

4. **Missouri River**

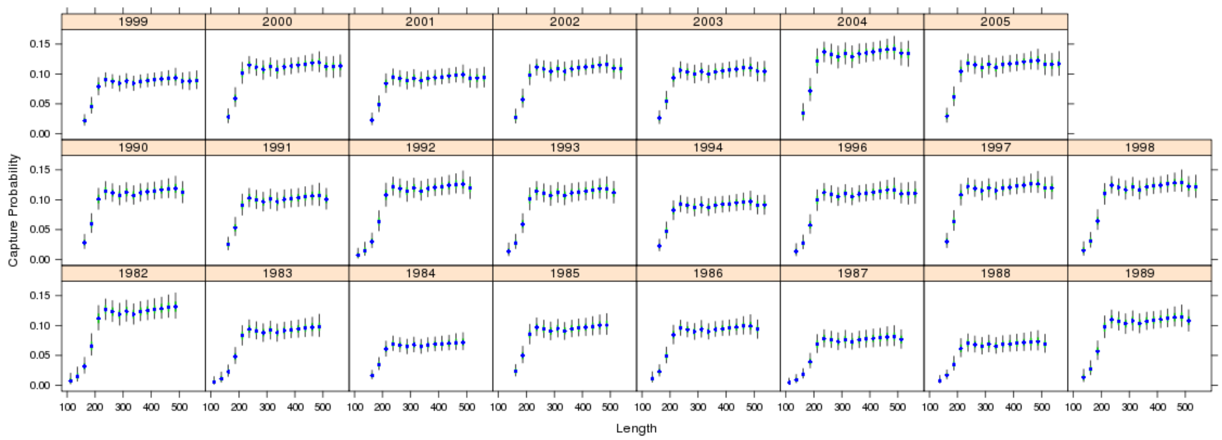




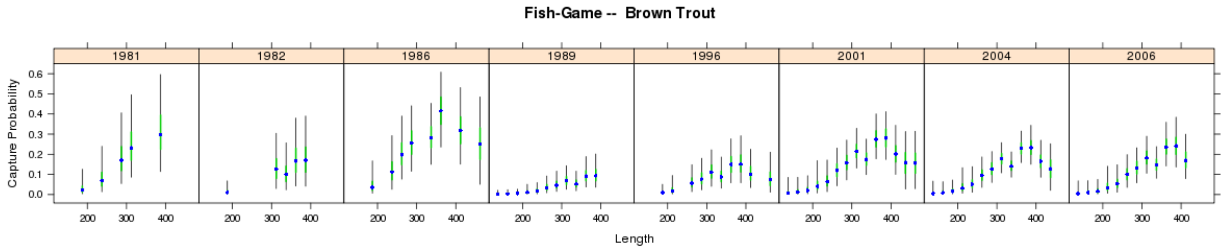
Craig Brown Trout

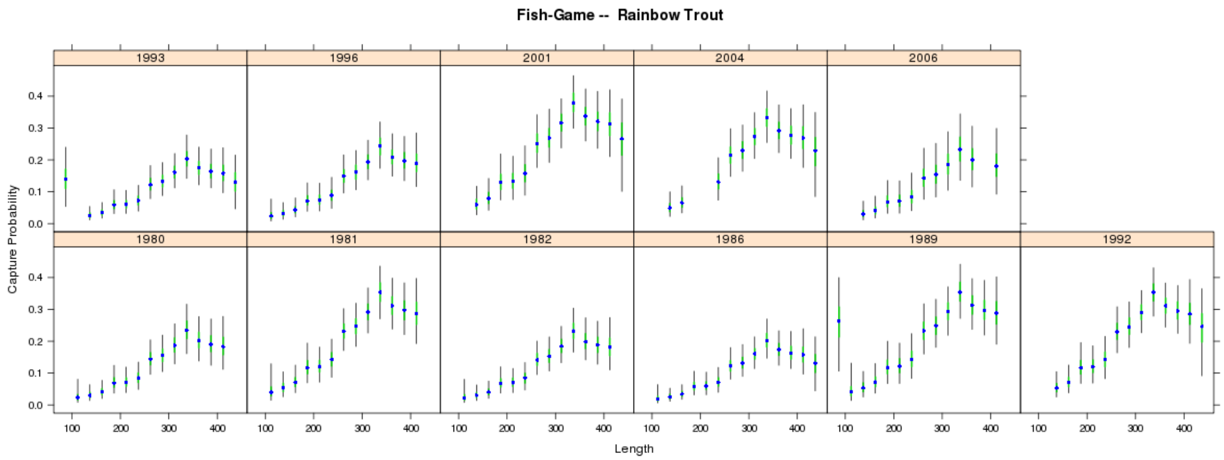


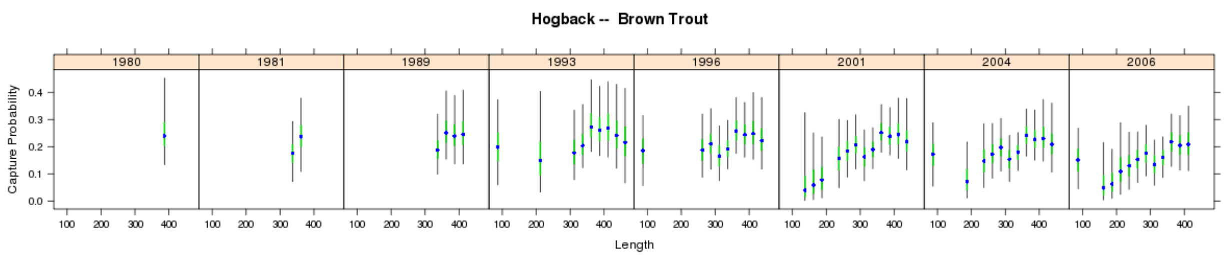
Craig Rainbow Trout

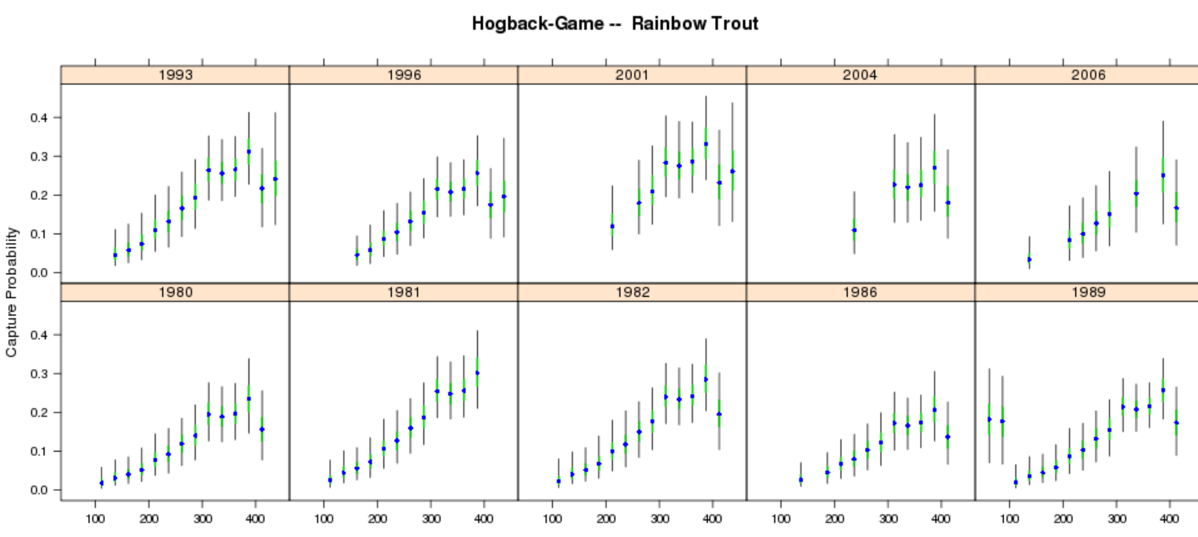


5. Rock Creek

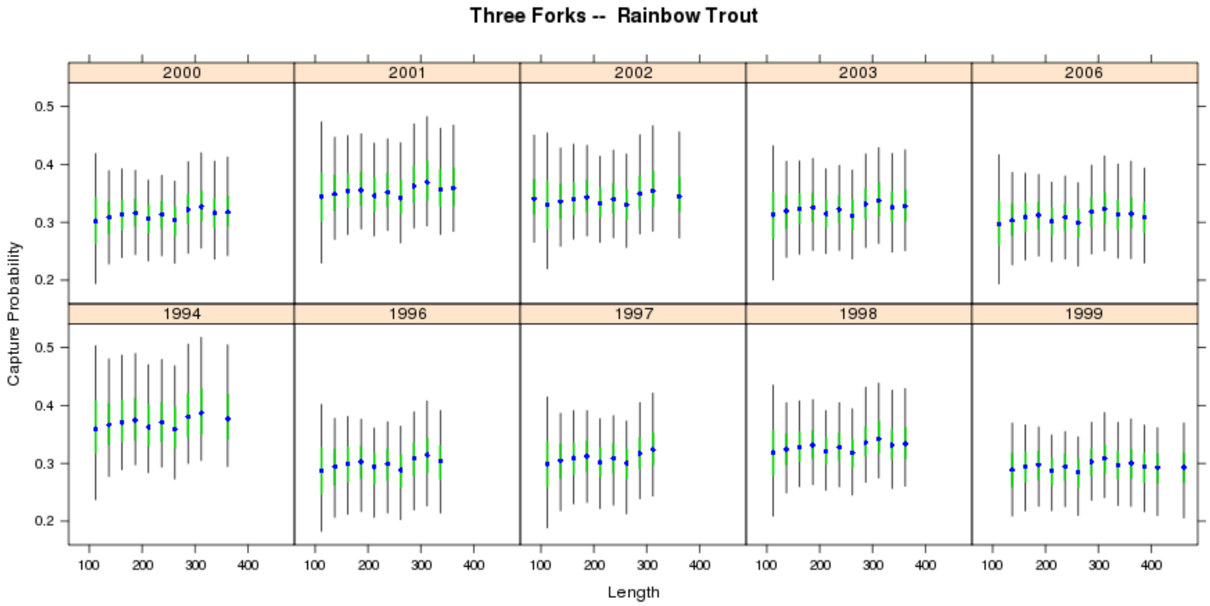

Fish-Game -- Brown Trout
1981
1982
1986
1989
1996
2001
2004
2006
Capture Probability
Length


Fish-Game -- Rainbow Trout
1993
1996
2001
2004
2006
1980
1981
1982
1986
1989
1992
Capture Probability
Length

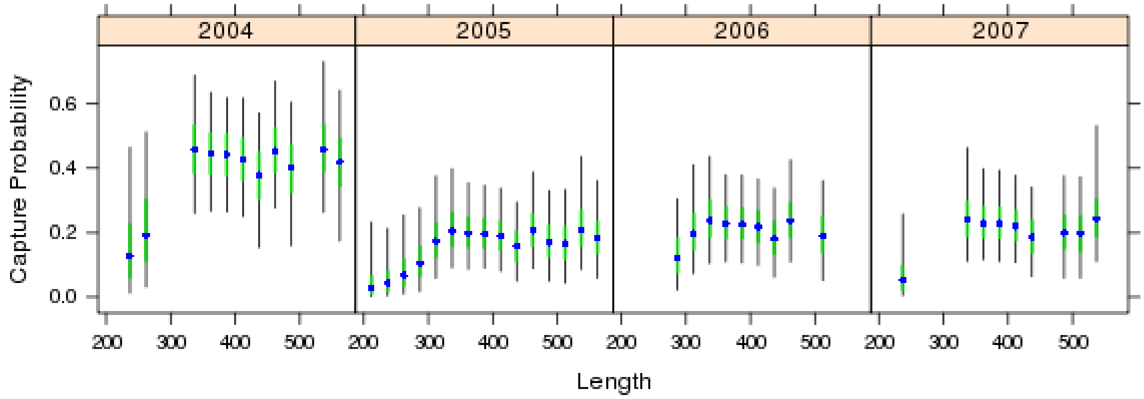

Hogback -- Brown Trout
1980
1981
1989
1993
1996
2001
2004
2006
Capture Probability
Length


Hogback-Game -- Rainbow Trout
1993
1996
2001
2004
2006
1980
1981
1982
1986
1989
Capture Probability
Length

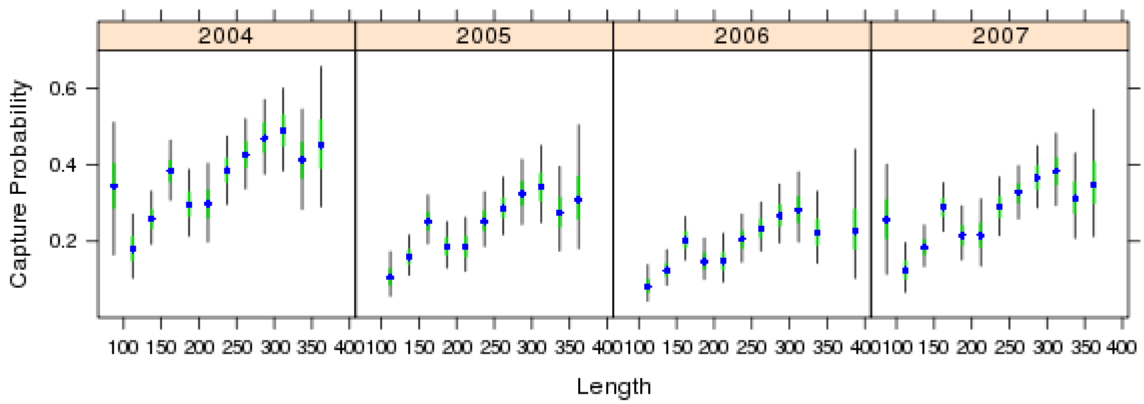
6. Ruby River


Three Forks -- Rainbow Trout
2000
2001
2002
2003
2006
1994
1996
1997
1998
1999
Capture Probability
0.2
0.3
0.4
0.5
Length
100
200
300
400

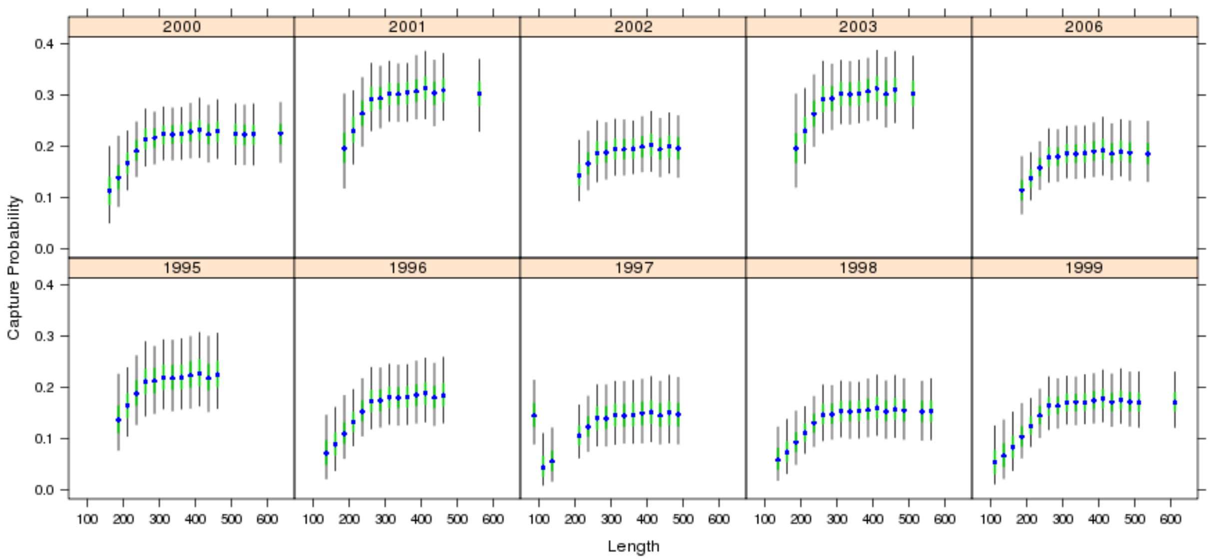
Canyon -- Brown Trout

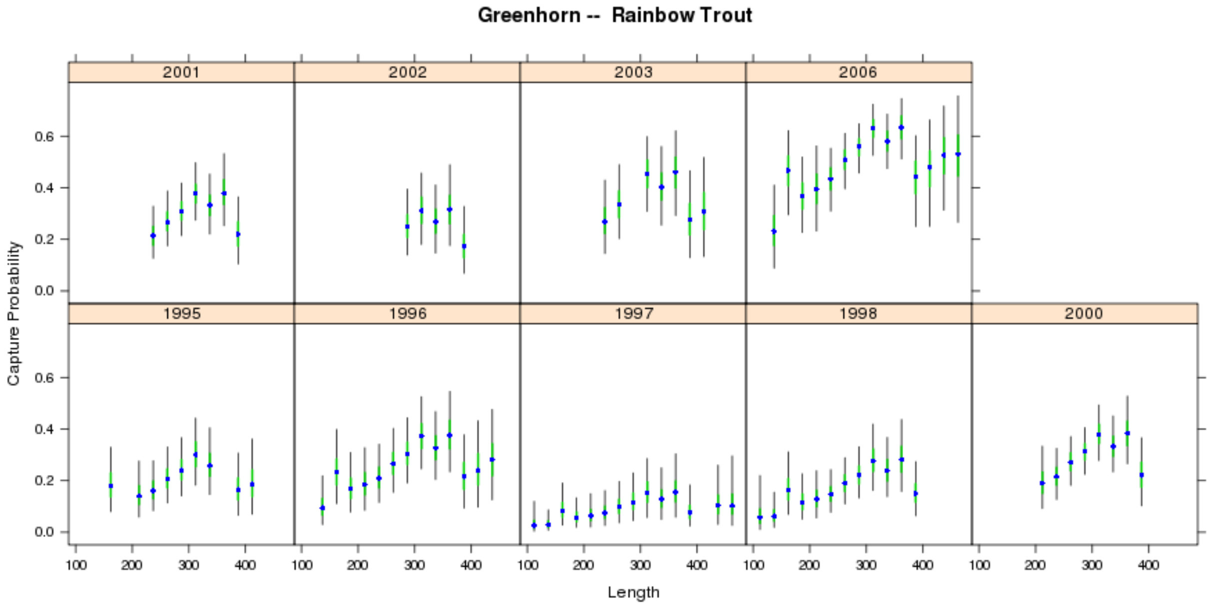


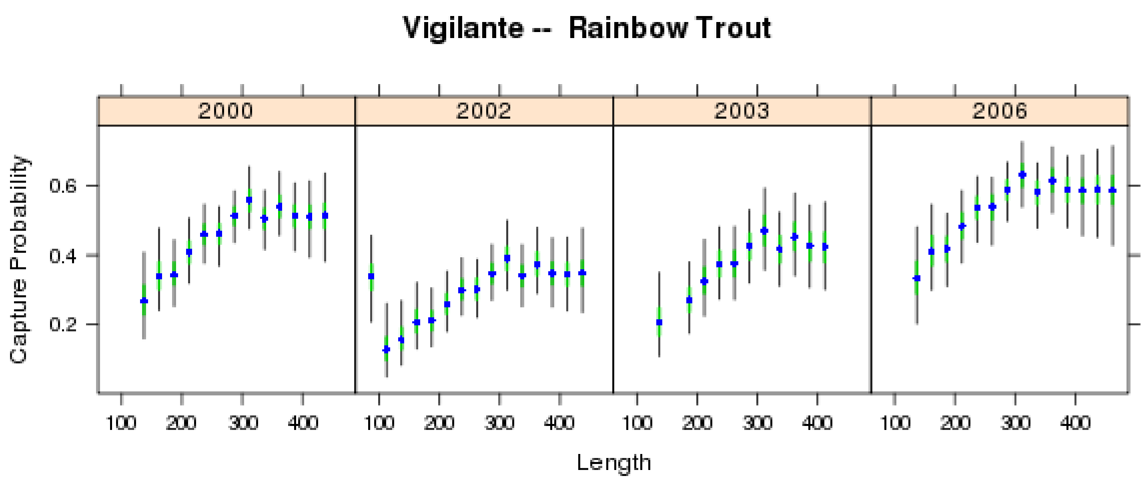
Canyon -- Rainbow Trout



Greenhorn -- Brown Trout

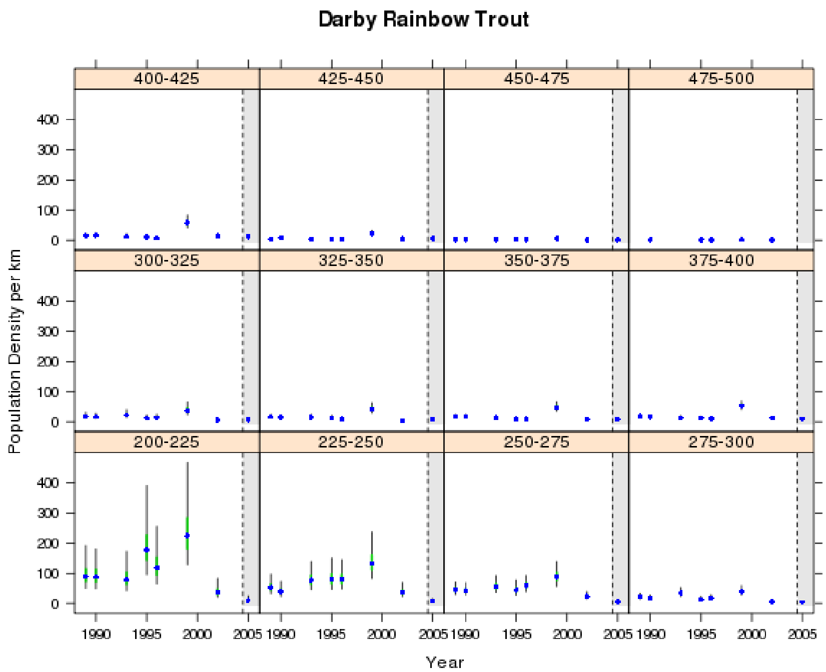


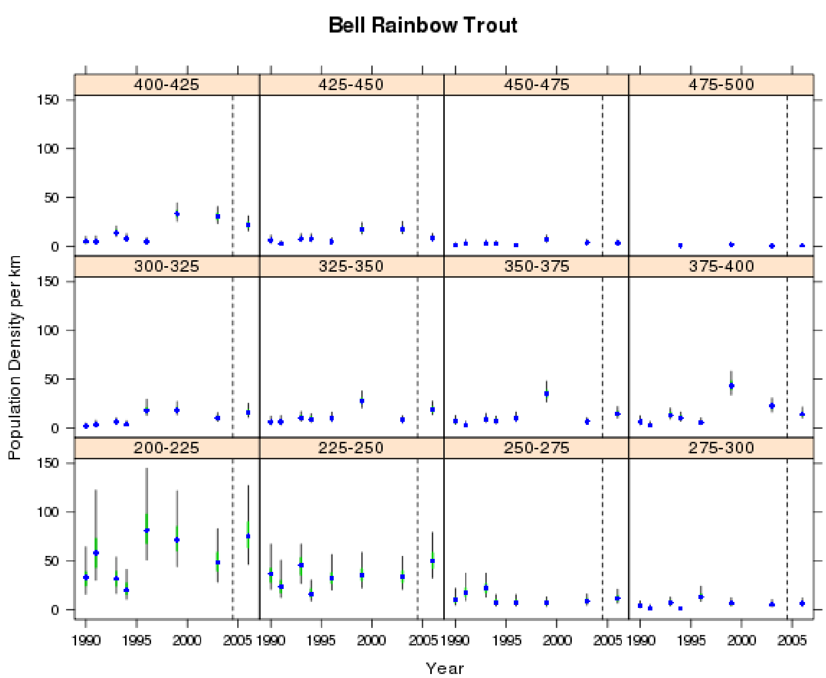

Greenhorn -- Rainbow Trout
2001
2002
2003
2006
1995
1996
1997
1998
2000
Capture Probability
Length

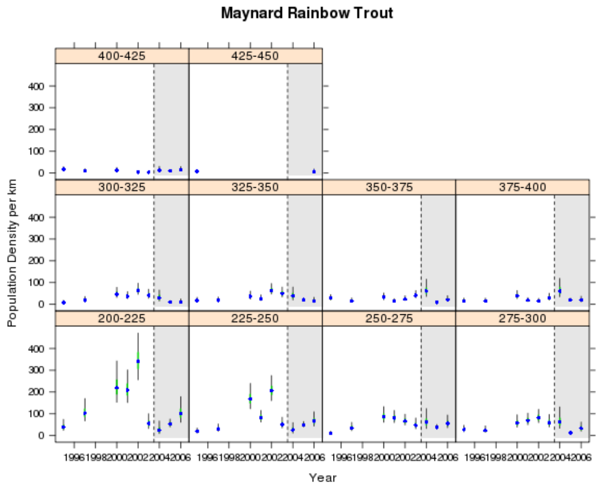

Vigilante -- Rainbow Trout
2000
2002
2003
2006
Capture Probability
Length

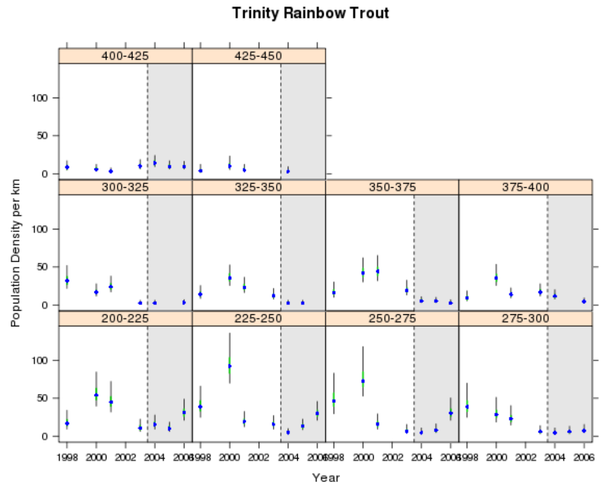
Appendix 3. Plots of population density by size class for rainbow trout and brown trout by river and river sampling section. Shaded section indicates post-WD years for WD+ river sections, and dotted vertical line indicates start of post-WD period for WD- reference sections.

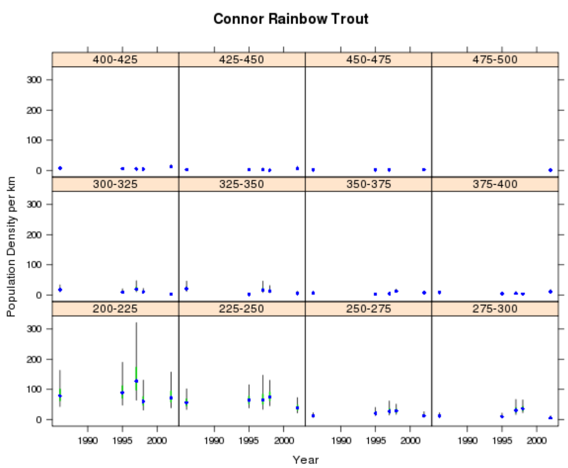
1. **Bitterroot River**:

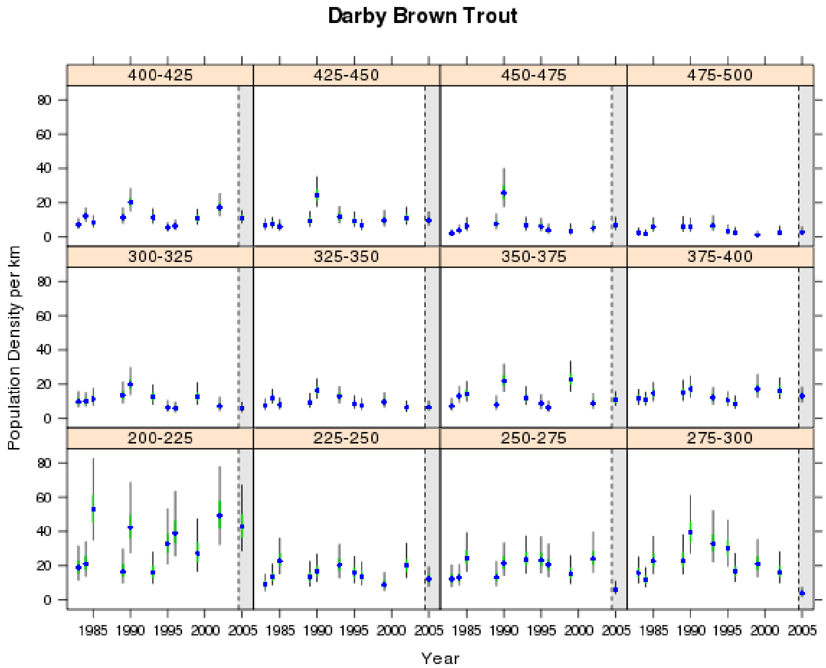


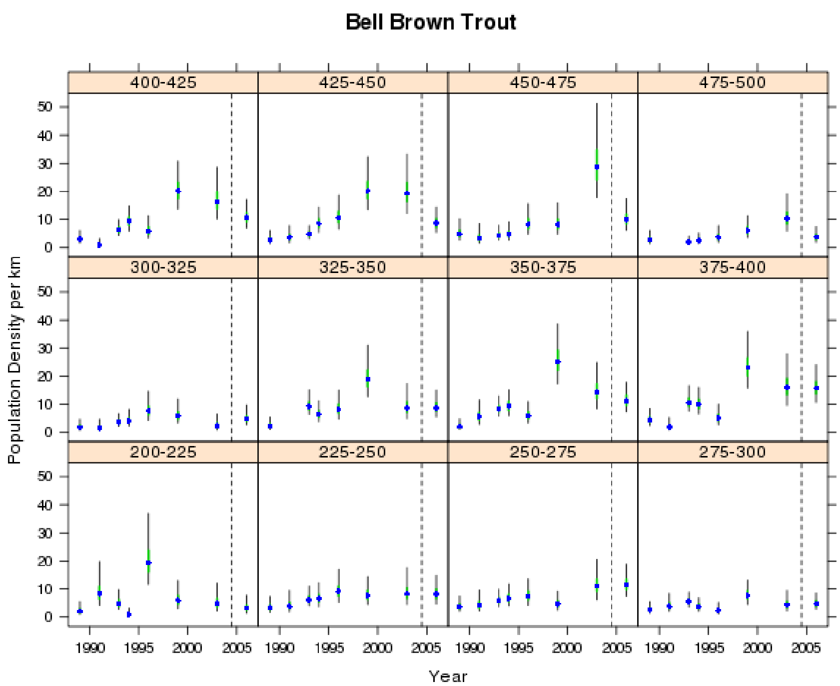



Maynard Rainbow Trout
400-425
425-450
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

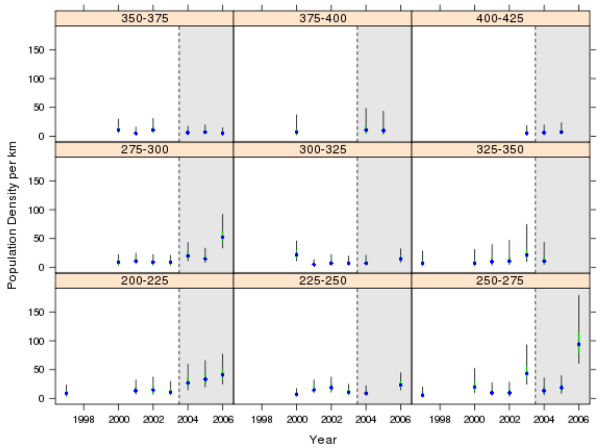

Trinity Rainbow Trout
400-425
425-450
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year


Connor Rainbow Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

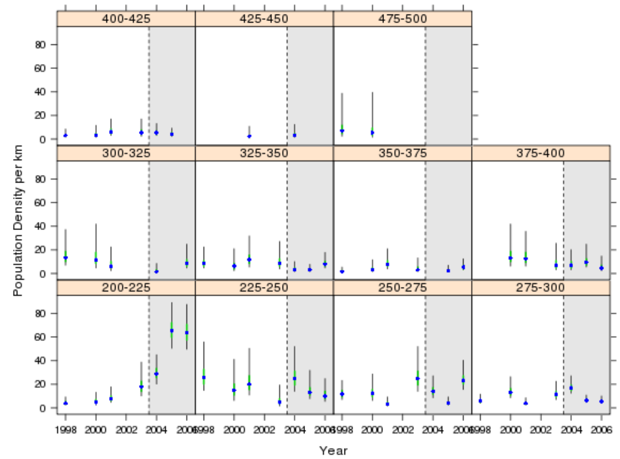

Darby Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year


Bell Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

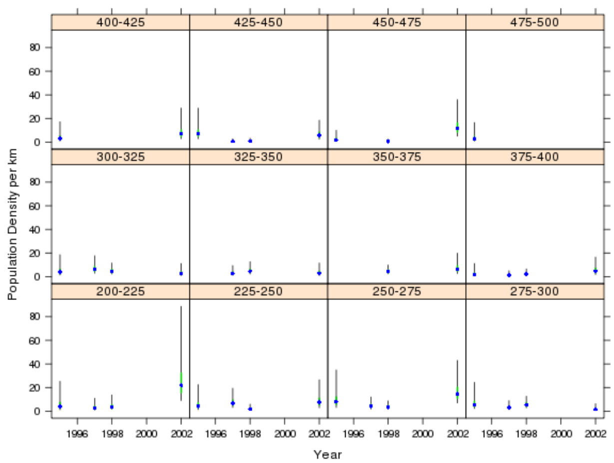
Maynard Brown Trout



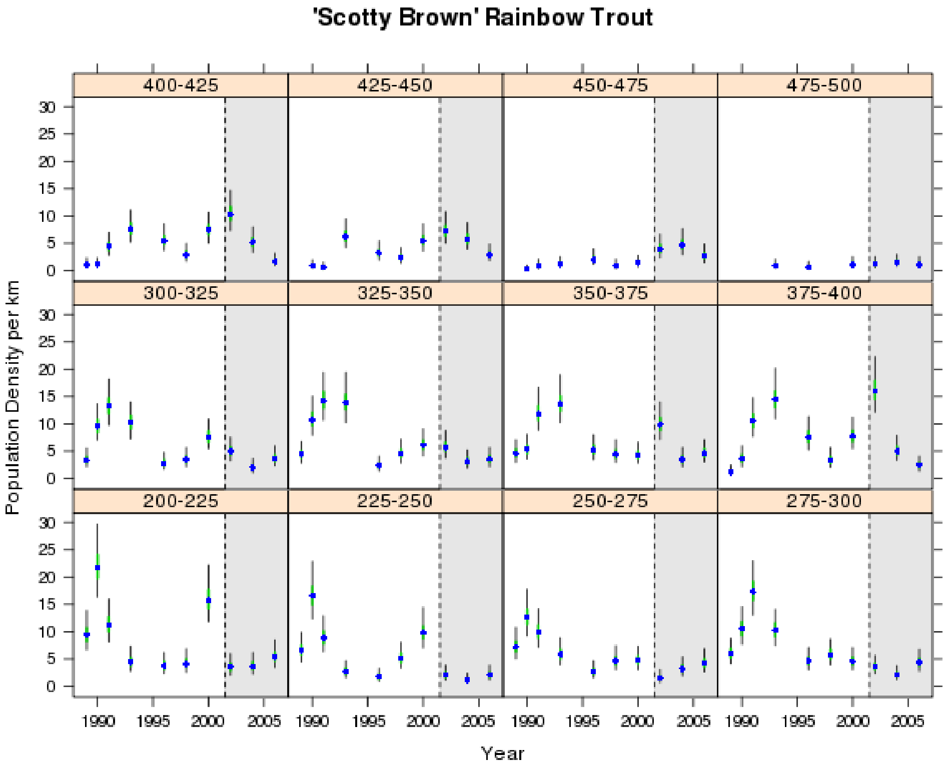
Trinity Brown Trout

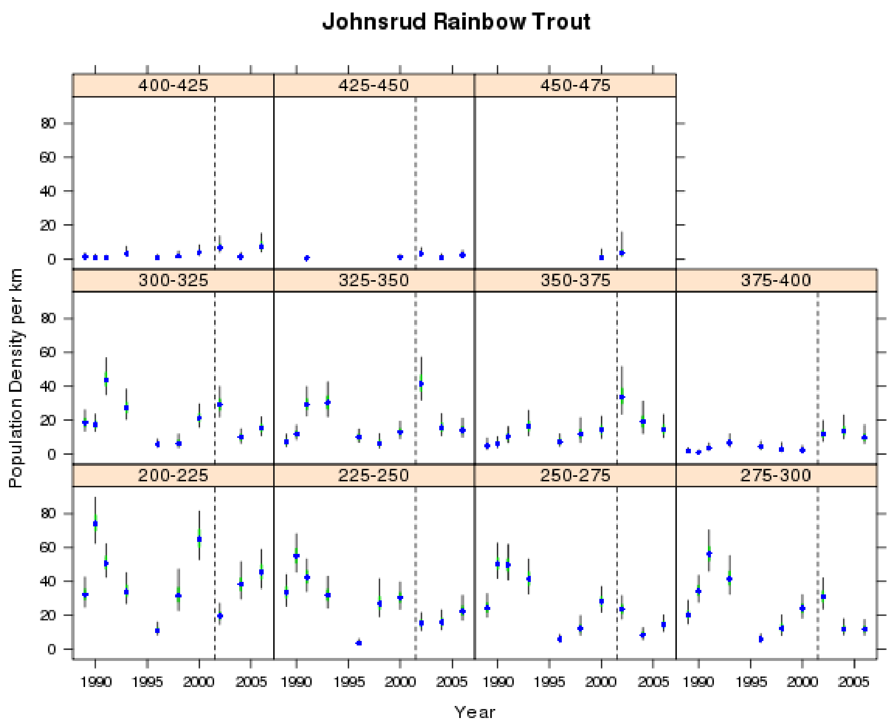


Connor Brown Trout

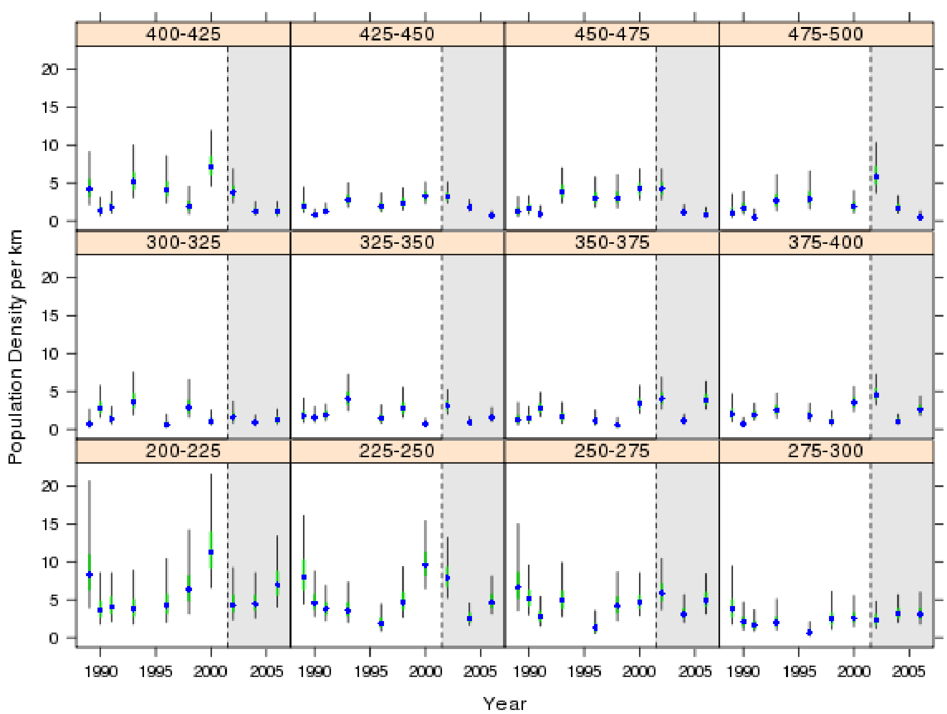


2. Blackfoot:

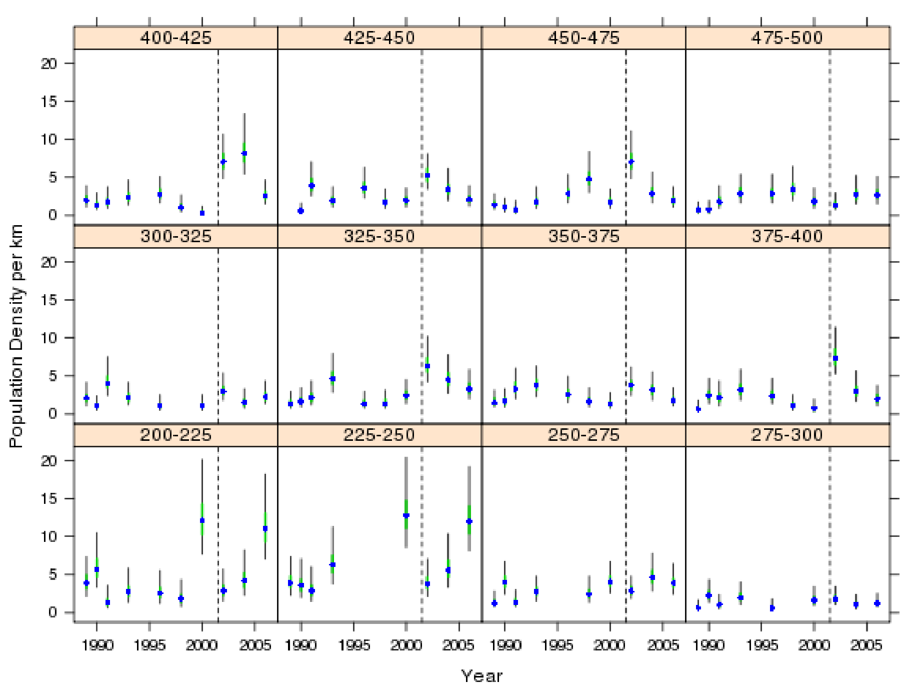

'Scotty Brown' Rainbow Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year


Johnsrud Rainbow Trout
400-425
425-450
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

'Scotty Brown' Brown Trout

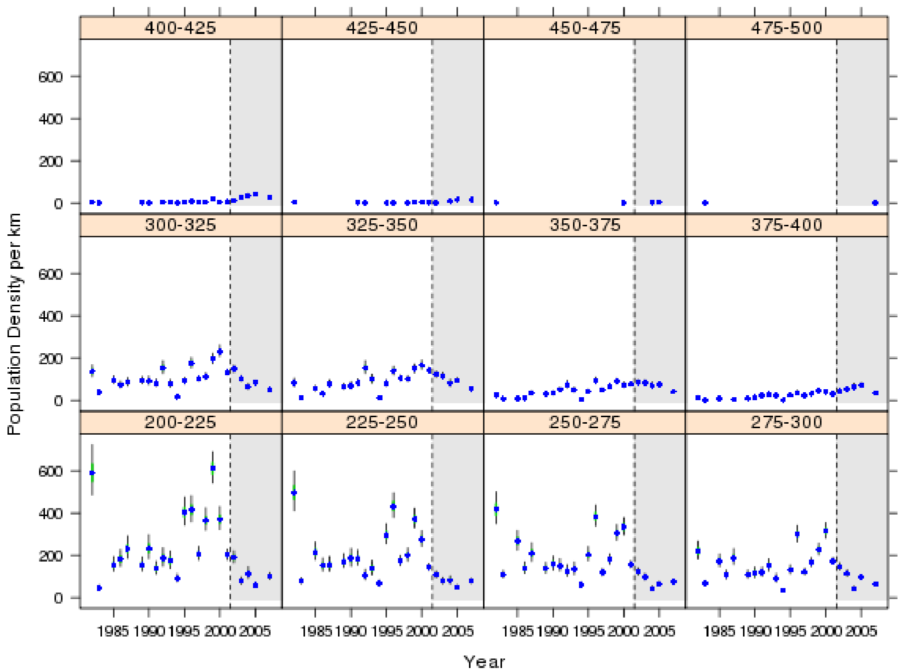


Johnsrud Brown Trout

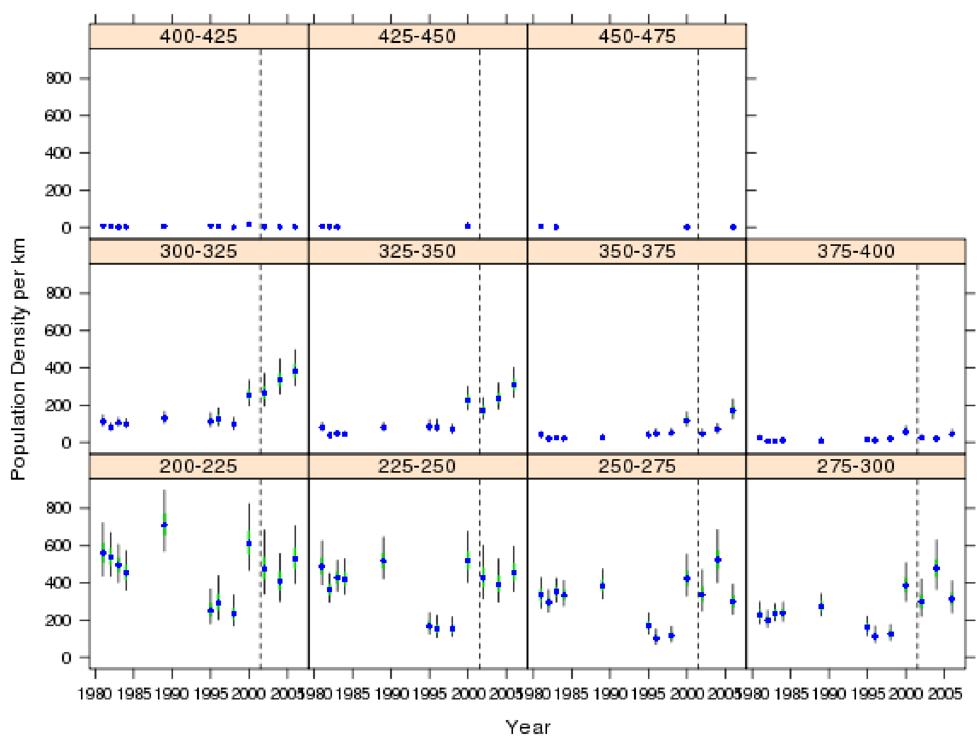


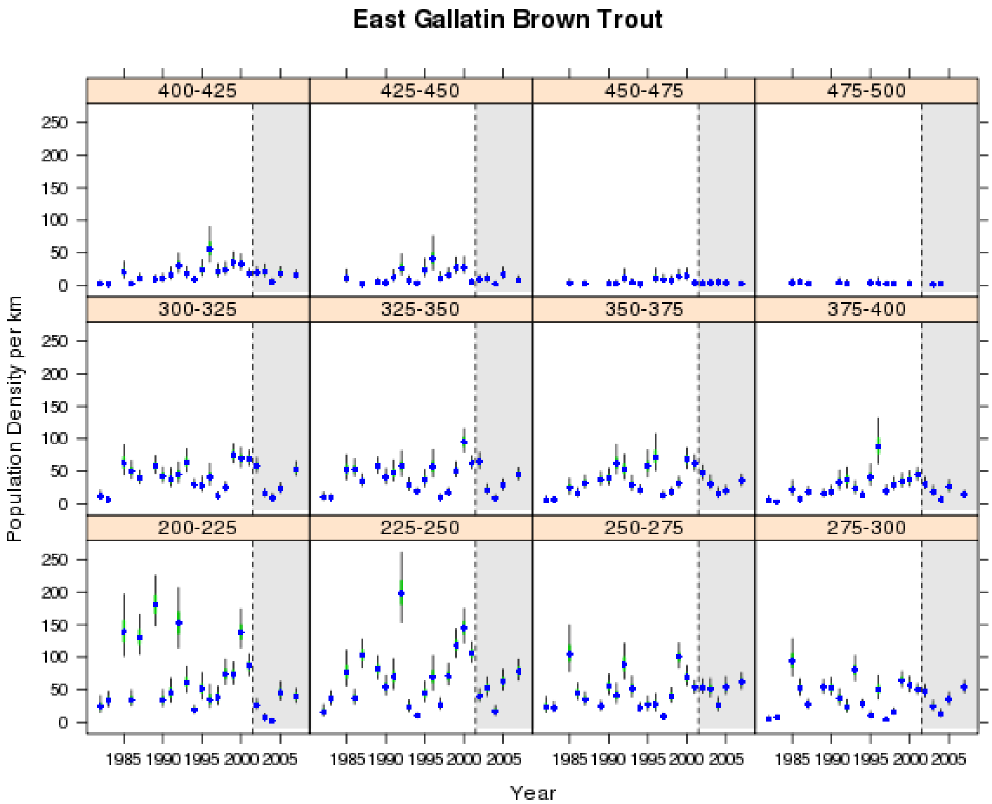
3. Gallatin:

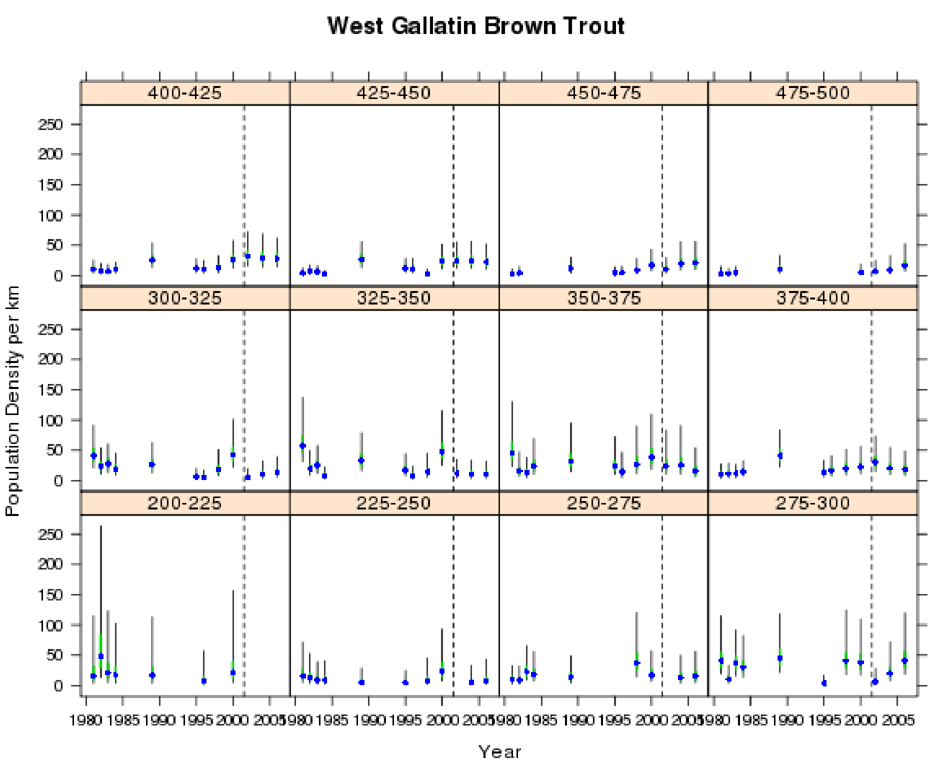
East Gallatin Rainbow Trout



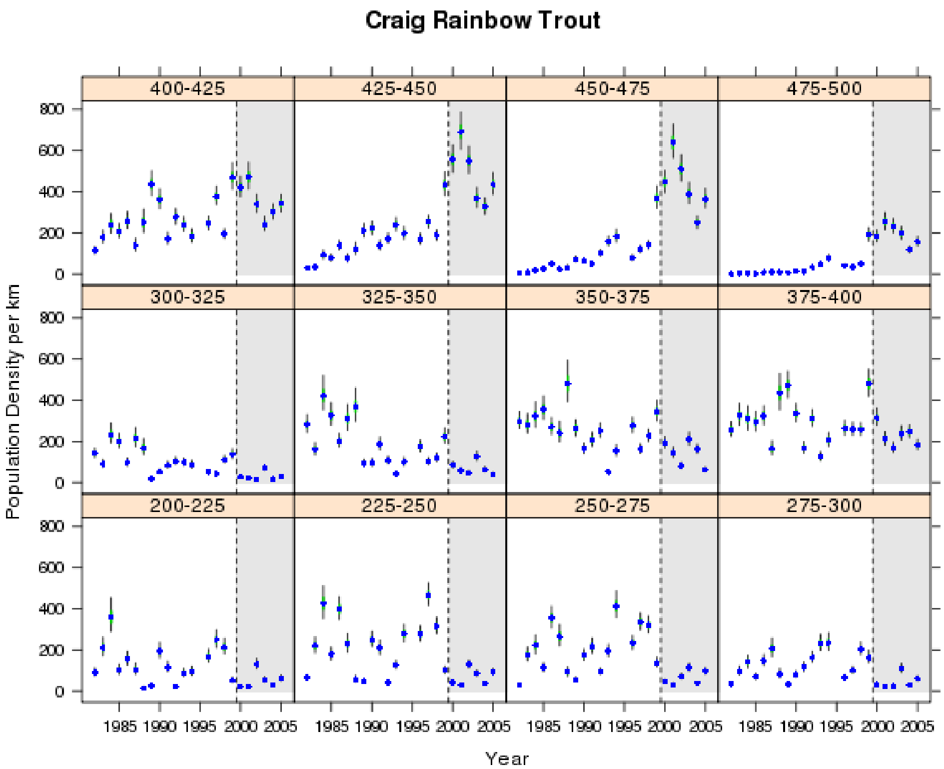
West Gallatin Rainbow Trout

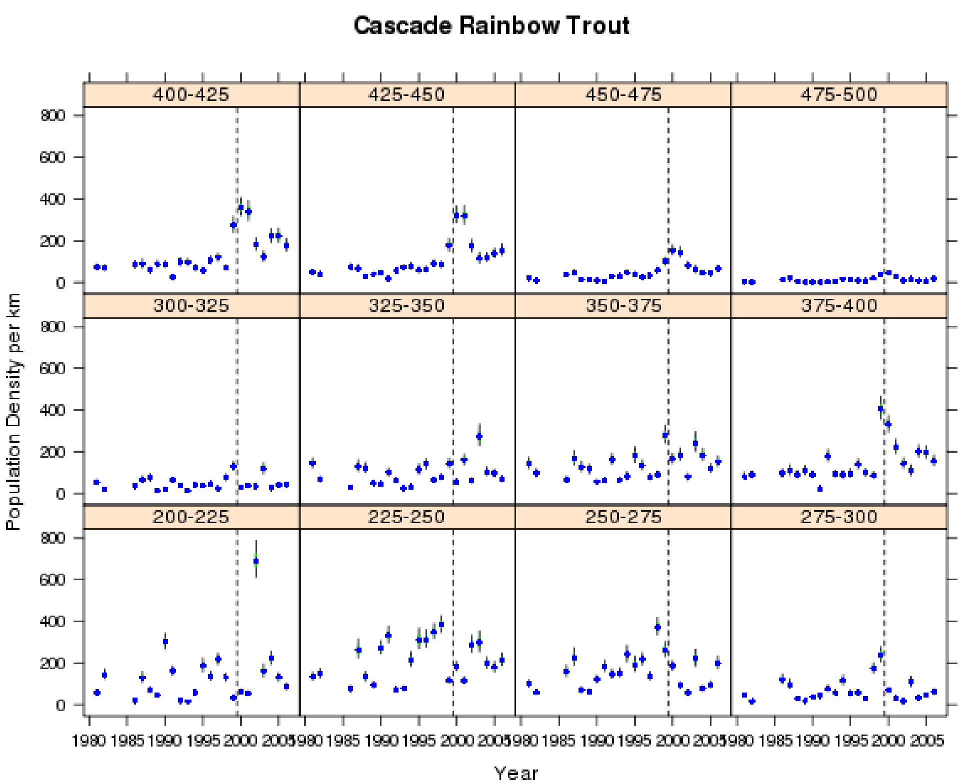


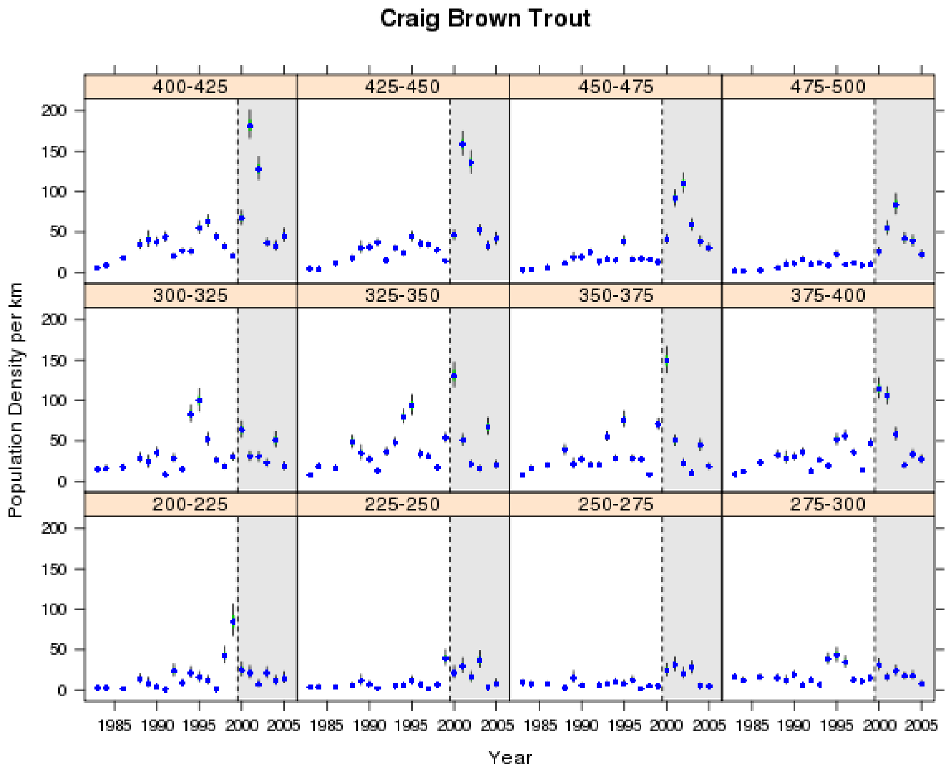

East Gallatin Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

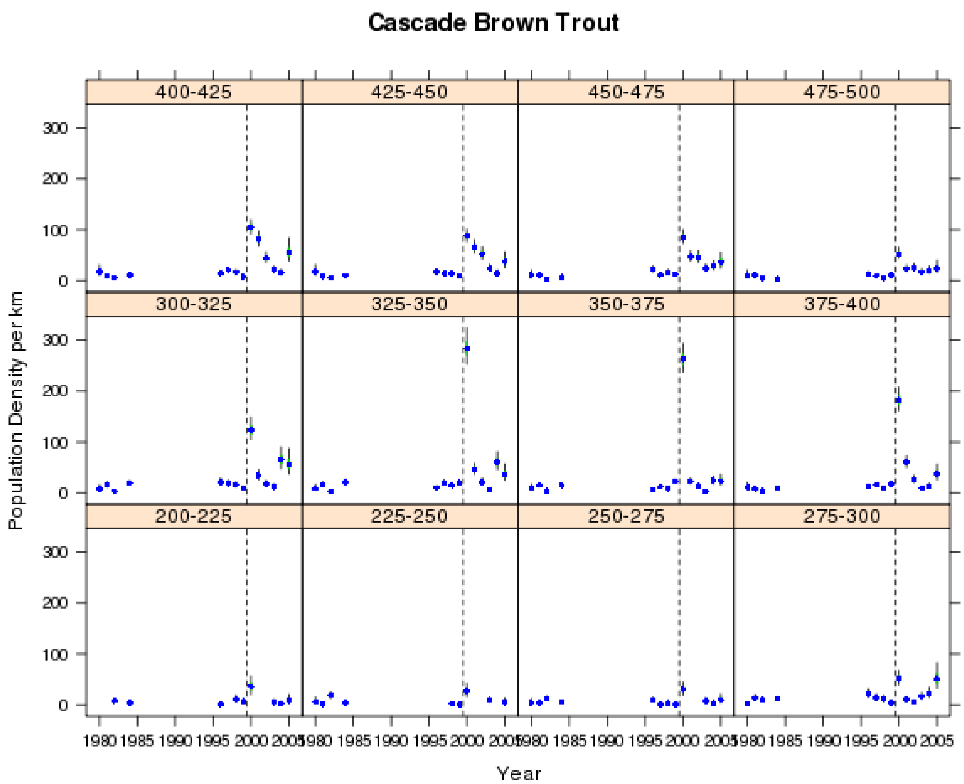

West Gallatin Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

4. Missouri:


Craig Rainbow Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

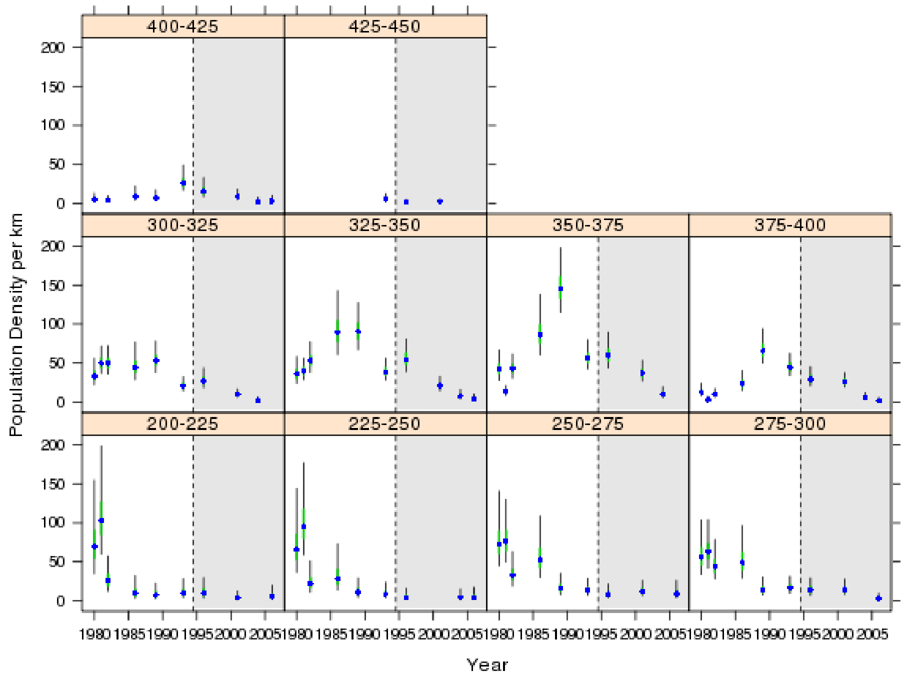

Cascade Rainbow Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year


Craig Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year
0
50
100
150
200
1985
1990
1995
2000
2005

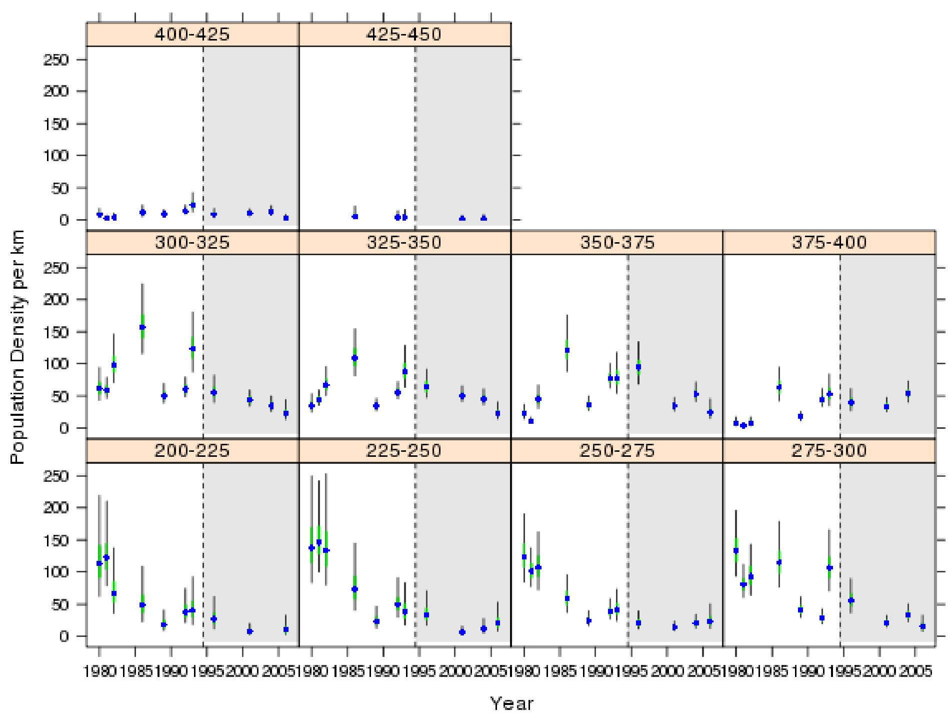

Cascade Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year
0
100
200
300
1980
1985
1990
1995
2000
2005

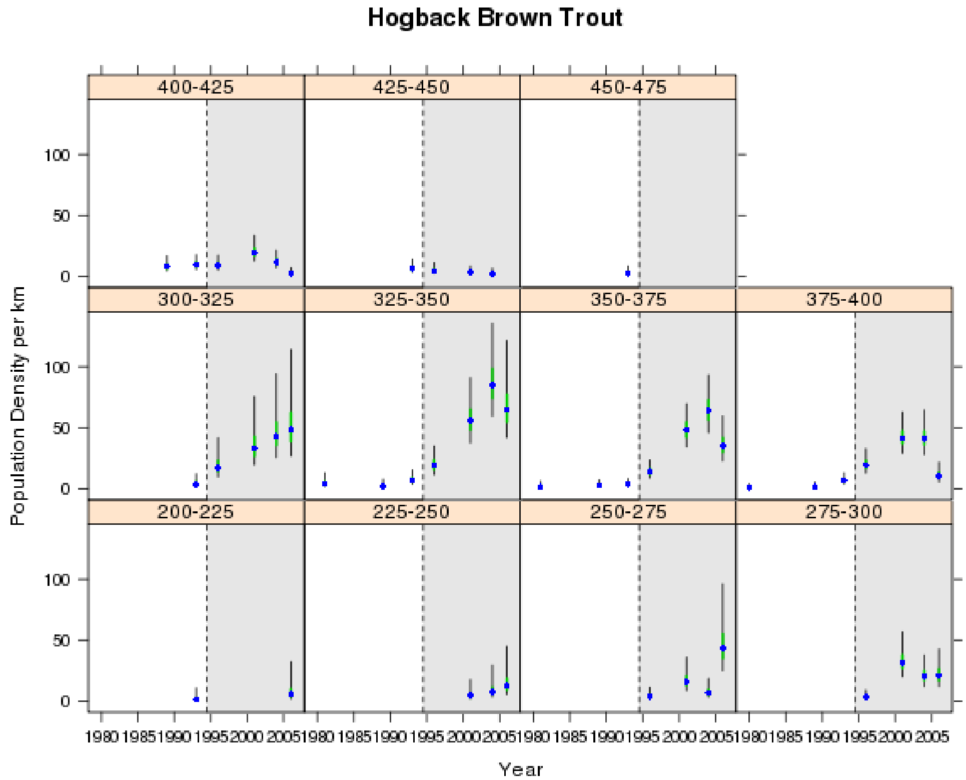
5. Rock Creek:

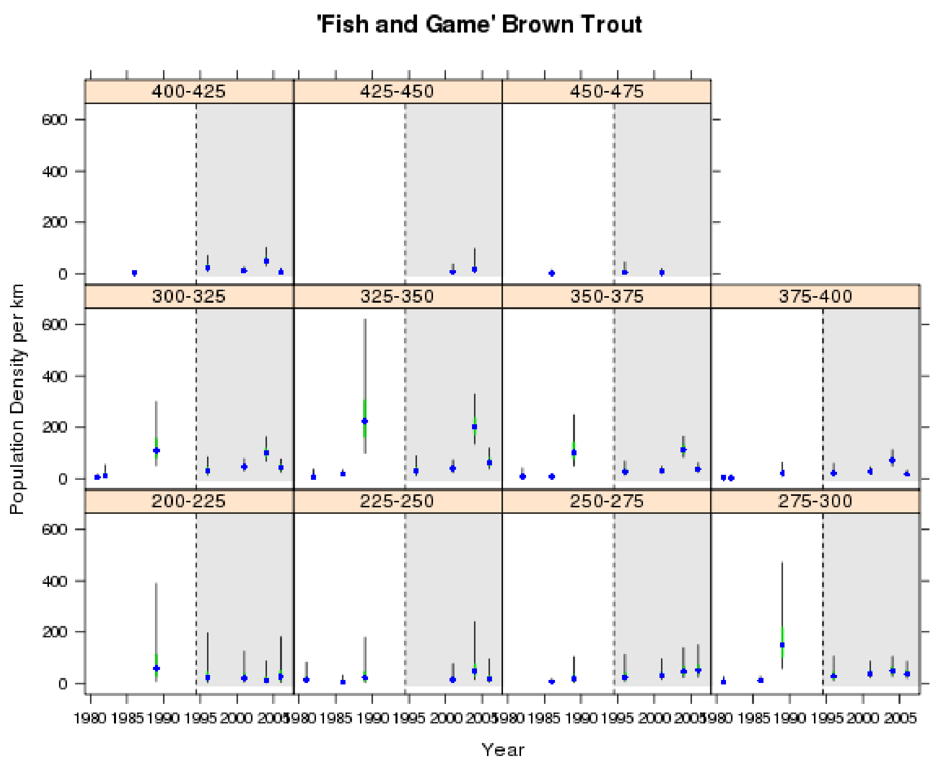
Hogback Rainbow Trout



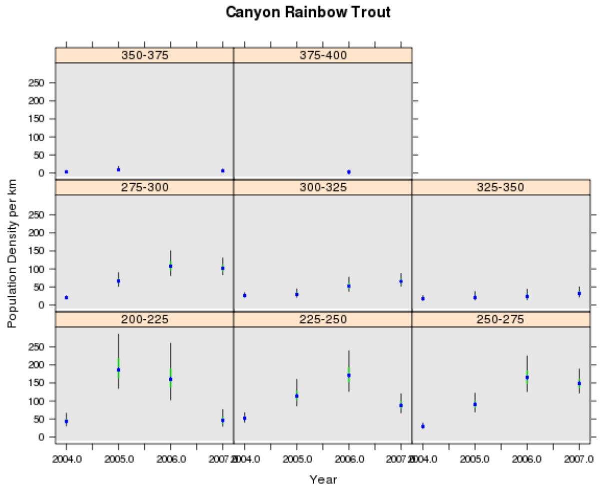
'Fish and Game' Rainbow Trout

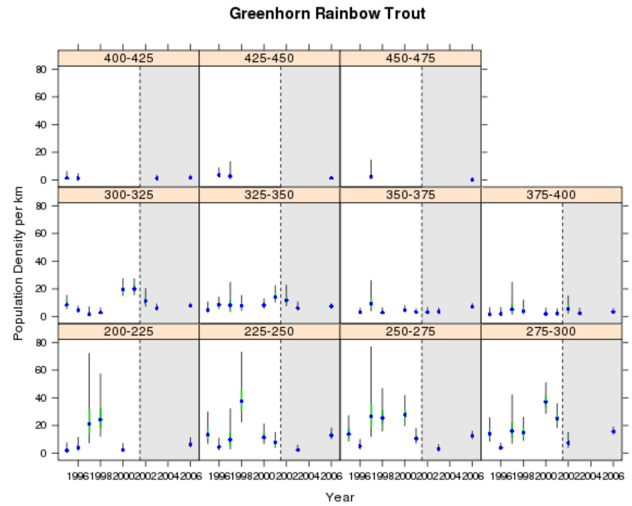


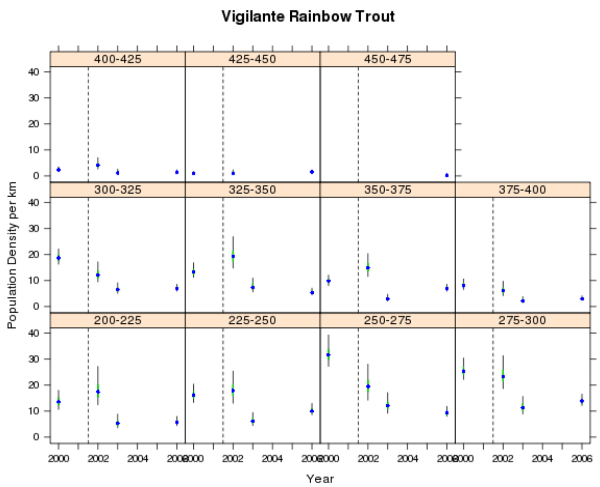

Hogback Brown Trout
400-425
425-450
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

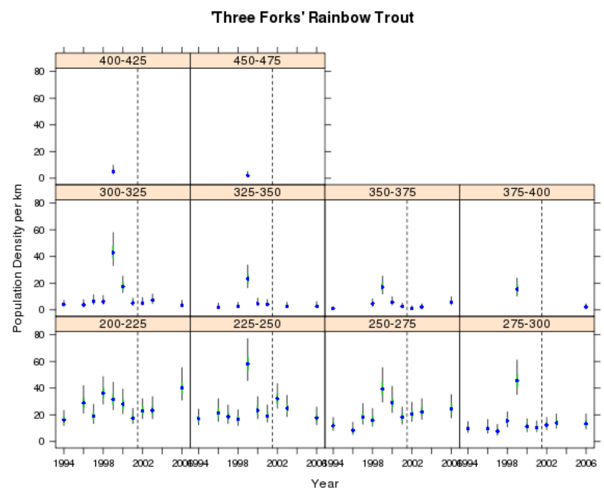

'Fish and Game' Brown Trout
400-425
425-450
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

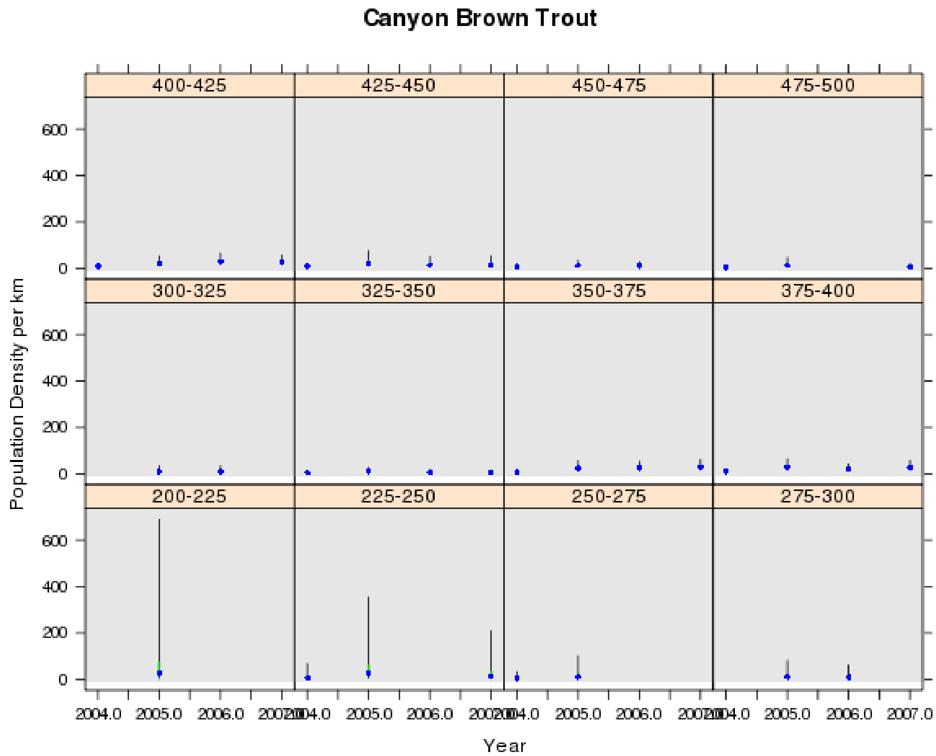
6. Ruby:

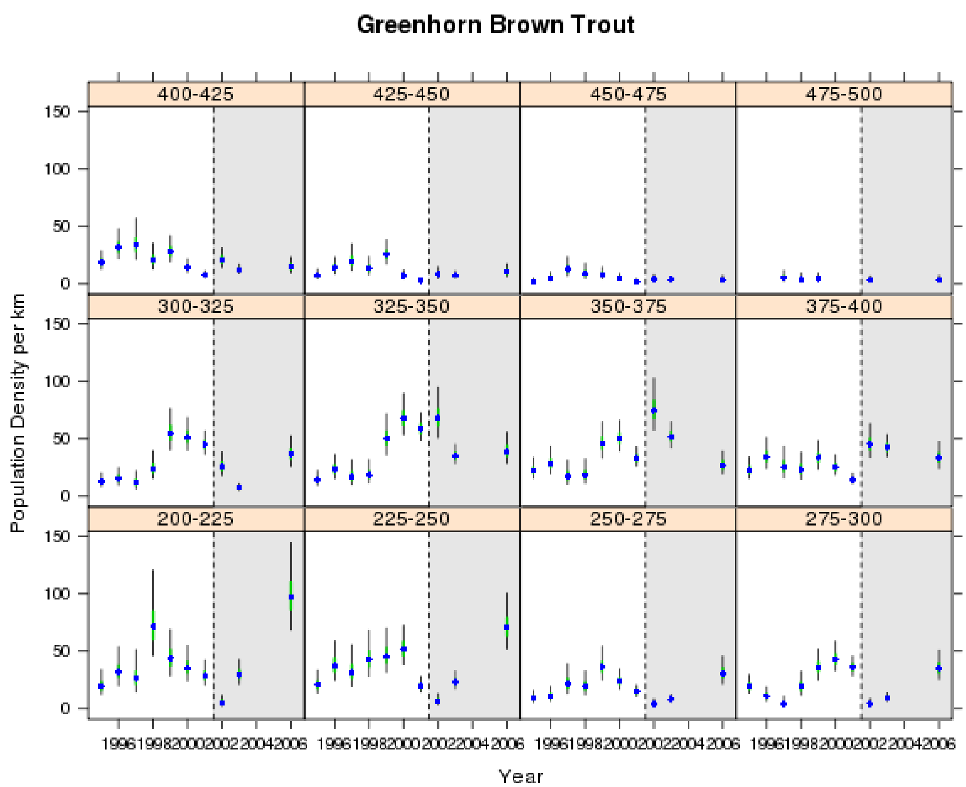

Canyon Rainbow Trout
350-375
375-400
275-300
300-325
325-350
200-225
225-250
250-275
Population Density per km
Year


Greenhorn Rainbow Trout
400-425
425-450
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year


Vigilante Rainbow Trout
400-425
425-450
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

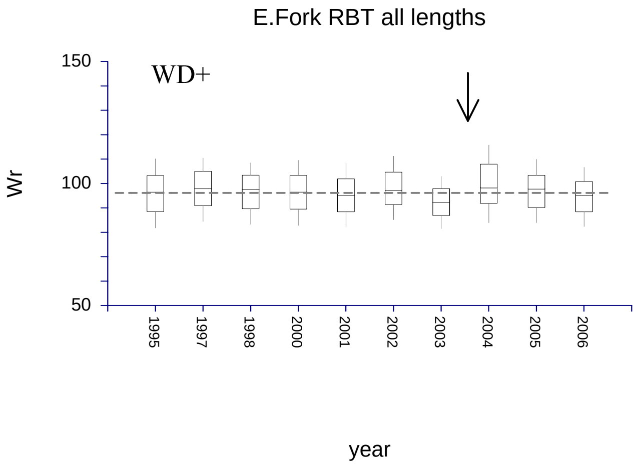

'Three Forks' Rainbow Trout
400-425
450-475
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

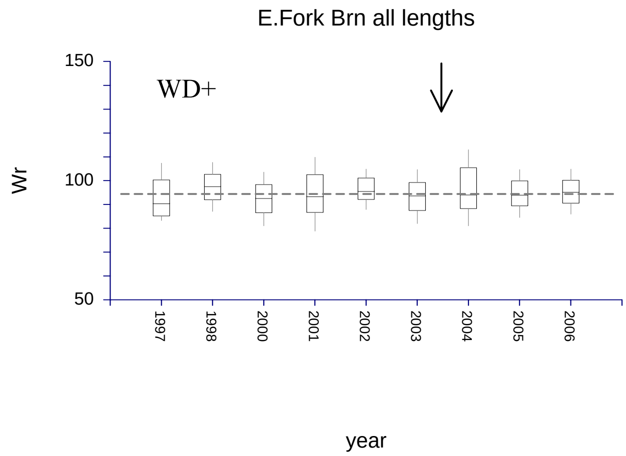

Canyon Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

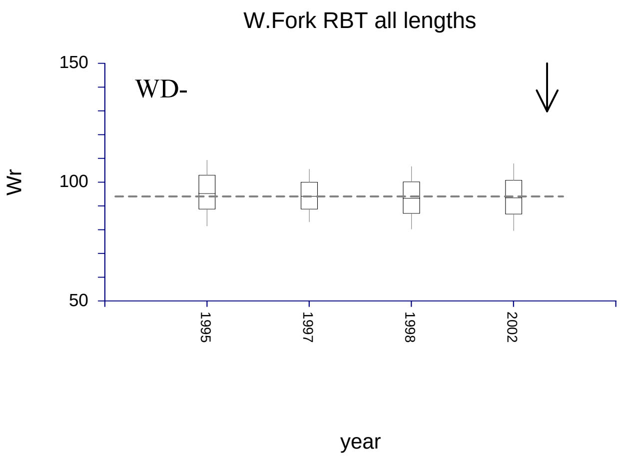

Greenhorn Brown Trout
400-425
425-450
450-475
475-500
300-325
325-350
350-375
375-400
200-225
225-250
250-275
275-300
Population Density per km
Year

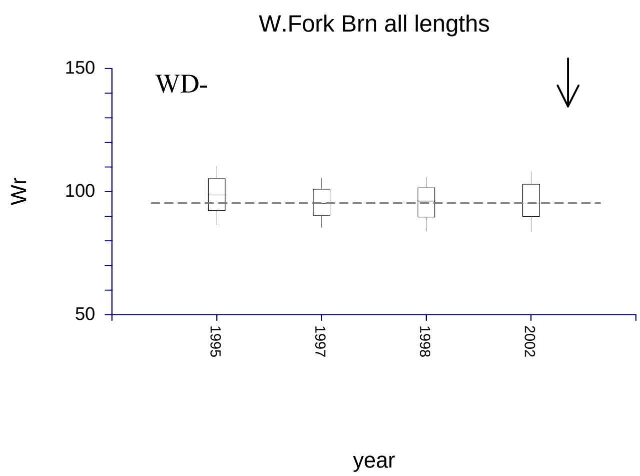
Appendix 4. Relative weight (Wr) plots for rainbow trout and brown trout by river and river sampling section. Horizontal lines indicate long-term average Wr, and arrow indicates timing of WD. WD+ indicates WD-infected section and WD- indicates reference section.

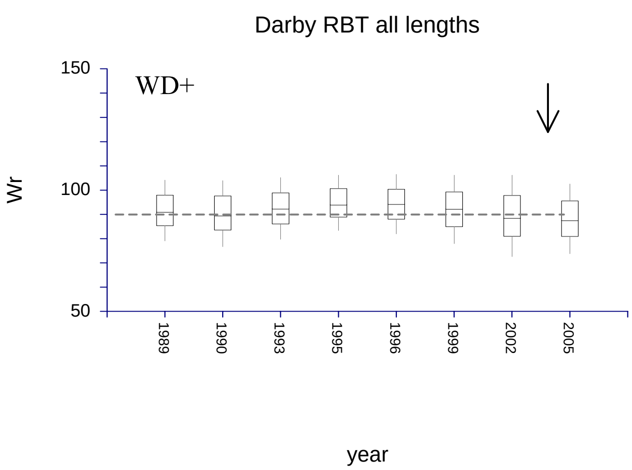
1. **Bitterroot River**

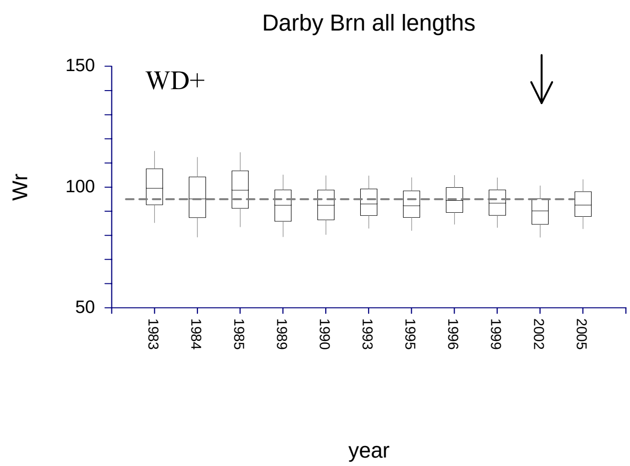


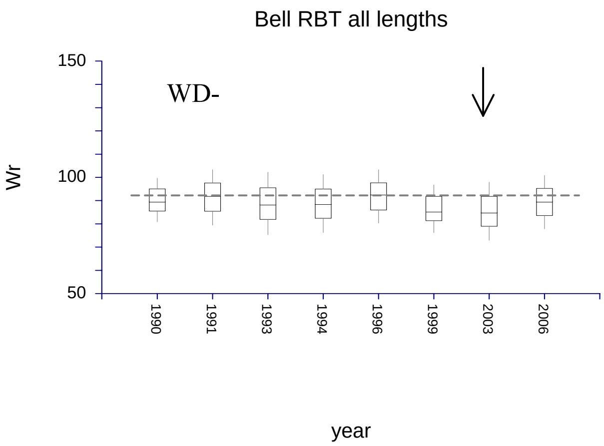


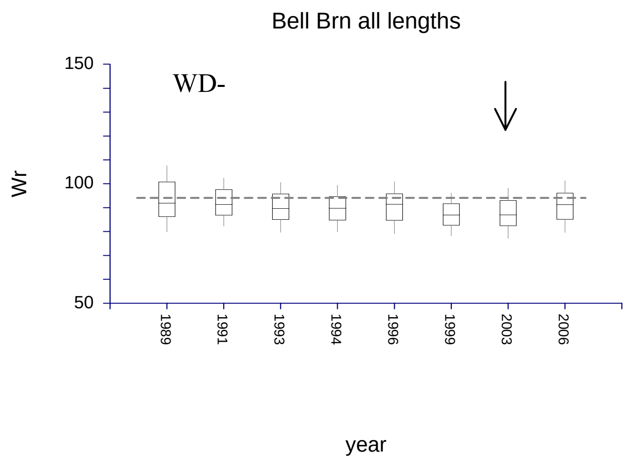




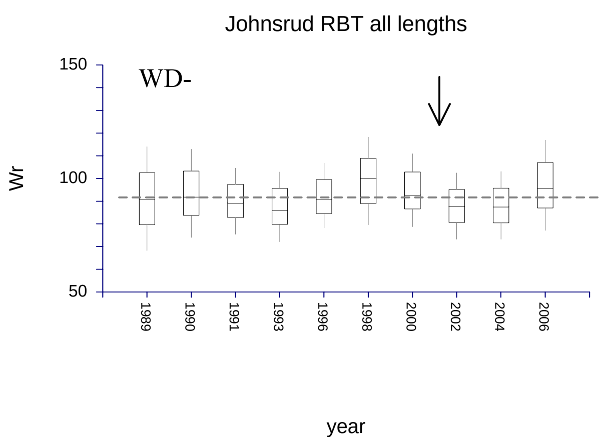


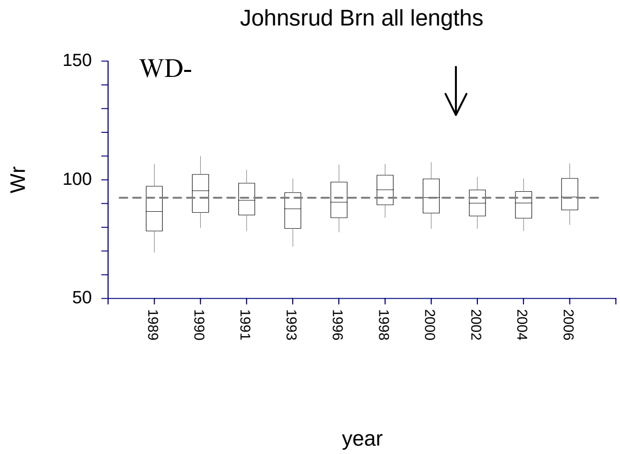


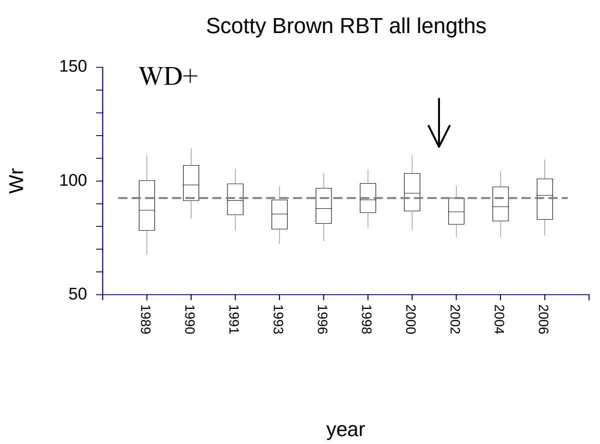

Bell RBT all lengths
WD-
Wr
150
100
50
1990
1991
1993
1994
1996
1999
2003
2006
year

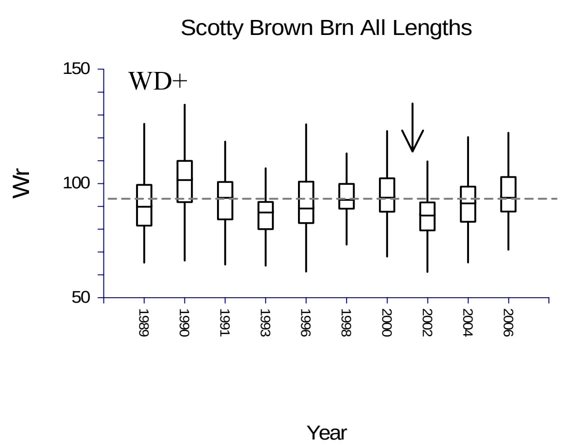

Bell Brn all lengths
WD-
Wr
150
100
50
1989
1991
1993
1994
1996
1999
2003
2006
year

2. **Blackfoot**

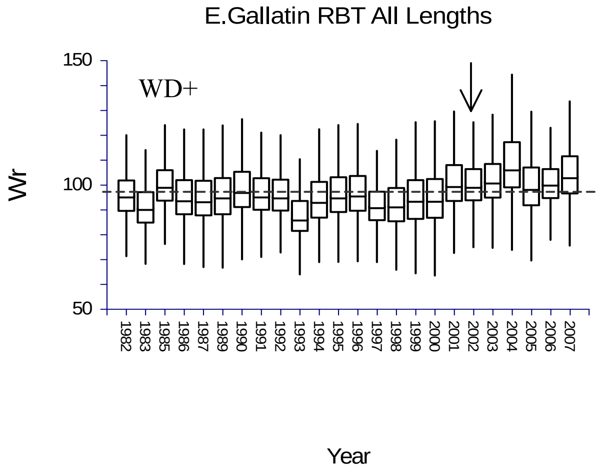

Johnsrud RBT all lengths
WD-
Wr
150
100
50
1989
1990
1991
1993
1996
1998
2000
2002
2004
2006
year

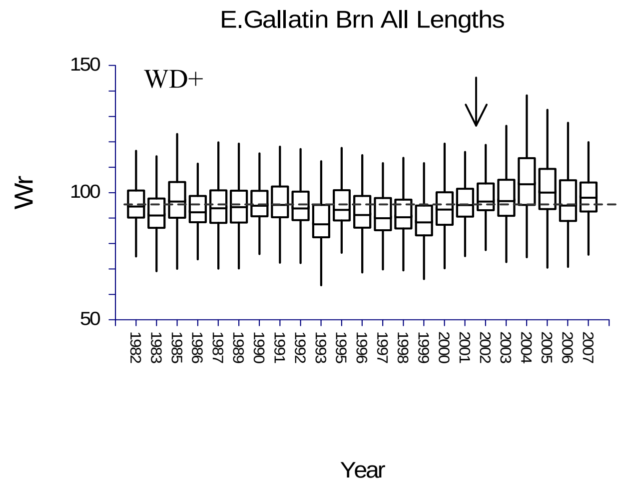

Johnsrud Brn all lengths
WD-
Wr
150
100
50
1989
1990
1991
1993
1996
1998
2000
2002
2004
2006
year

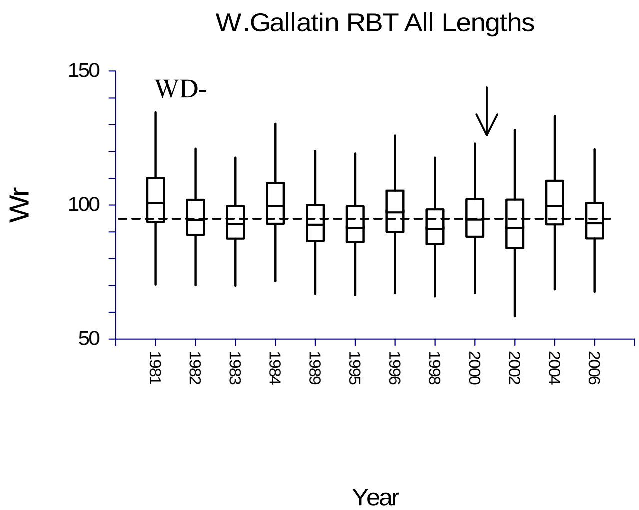

Scotty Brown RBT all lengths
WD+
Wr
150
100
50
1989
1990
1991
1993
1996
1998
2000
2002
2004
2006
year

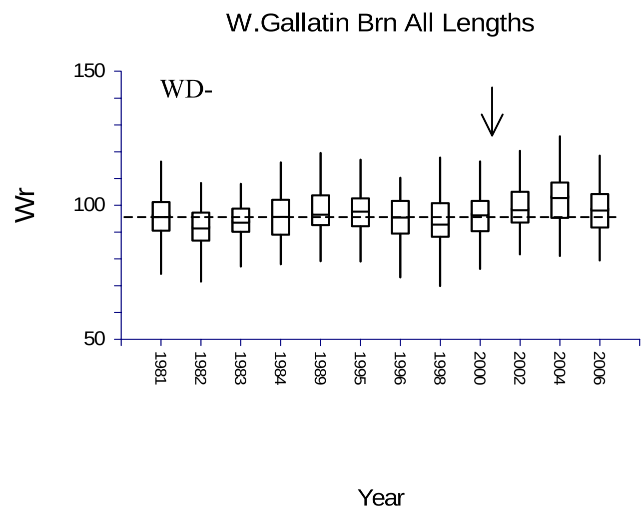

Scotty Brown Brn All Lengths
WD+
Wr
150
100
50
1989
1990
1991
1993
1996
1998
2000
2002
2004
2006
Year

3. **Gallatin**

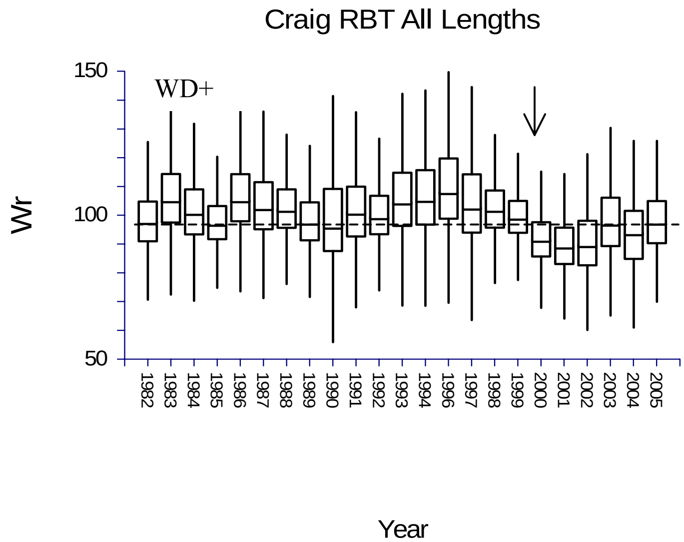

E.Gallatin RBT All Lengths
WD+
Wr
150
100
50
1982 1983 1985 1986 1987 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007
Year

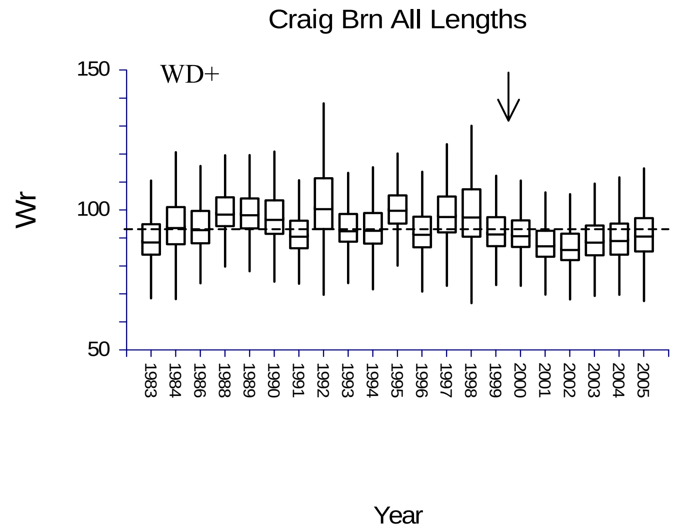

E.Gallatin Brn All Lengths
WD+
Wr
150
100
50
1982 1983 1985 1986 1987 1989 1990 1991 1992 1993 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007
Year

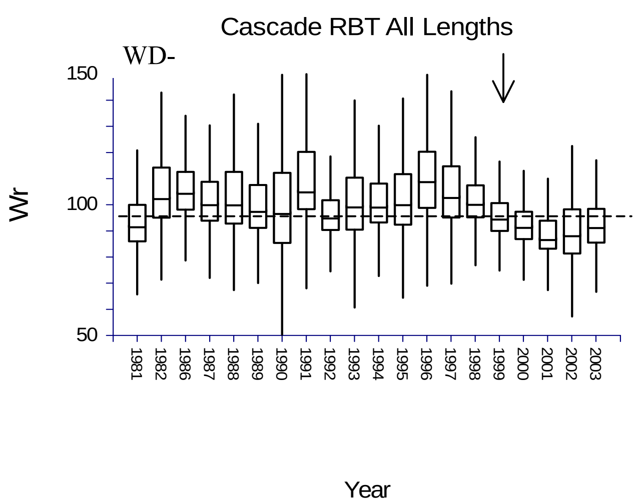

W.Gallatin RBT All Lengths
WD-
Wr
150
100
50
1981 1982 1983 1984 1989 1995 1996 1998 2000 2002 2004 2006
Year

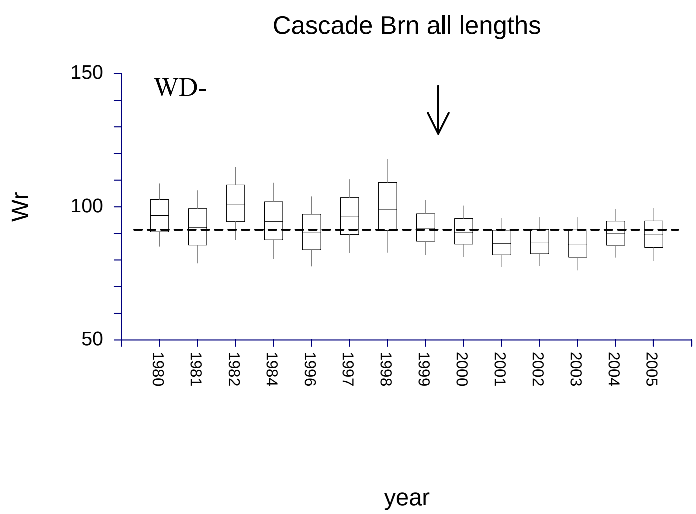

W.Gallatin Brn All Lengths
WD-
Wr
150
100
50
1981 1982 1983 1984 1989 1995 1996 1998 2000 2002 2004 2006
Year

4. **Missouri**

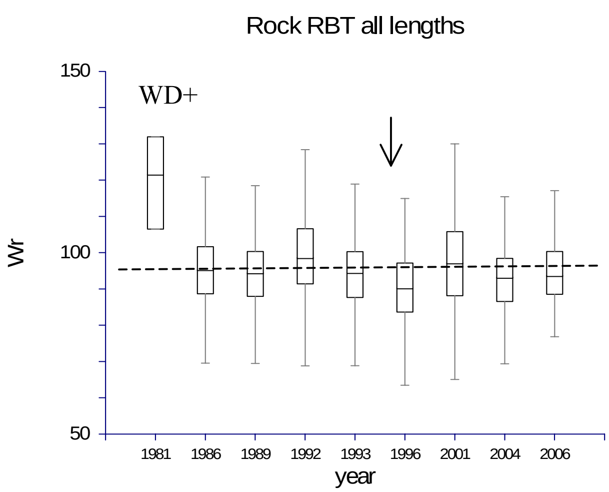


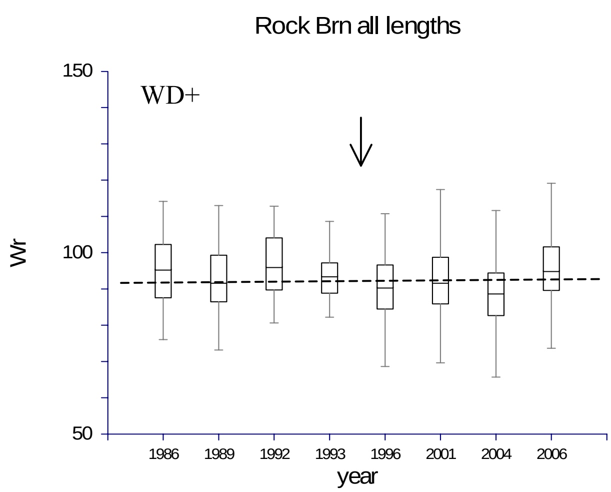




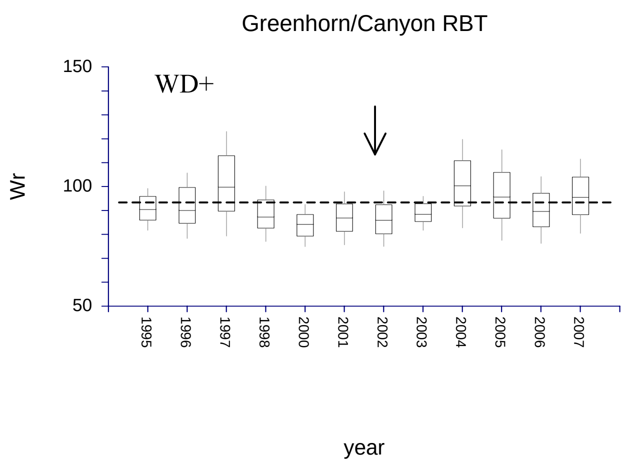


5. **Rock Creek**

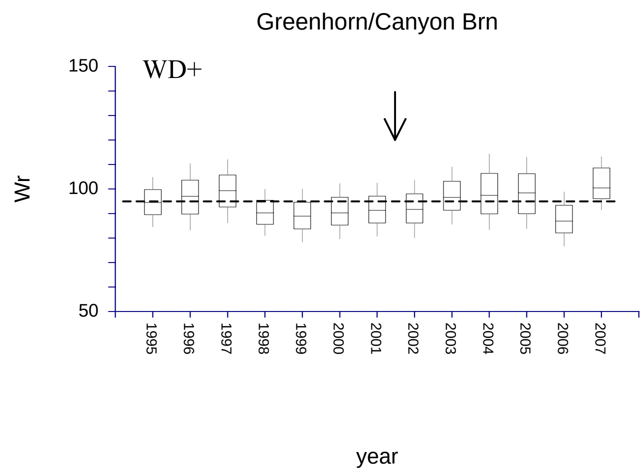




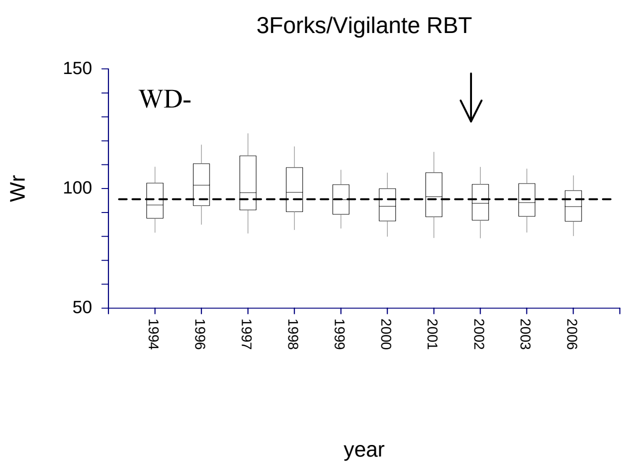
6. **Ruby**



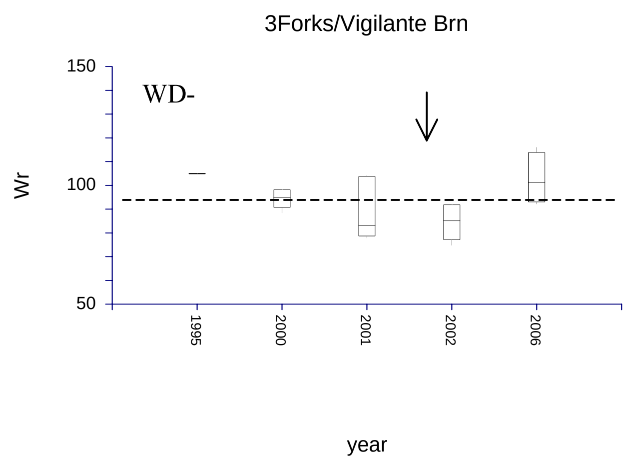
Greenhorn/Canyon RBT
WD+
Wr
150
100
50
1995
1996
1997
1998
2000
2001
2002
2003
2004
2005
2006
2007
year



Greenhorn/Canyon Brn
WD+
Wr
150
100
50
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
year

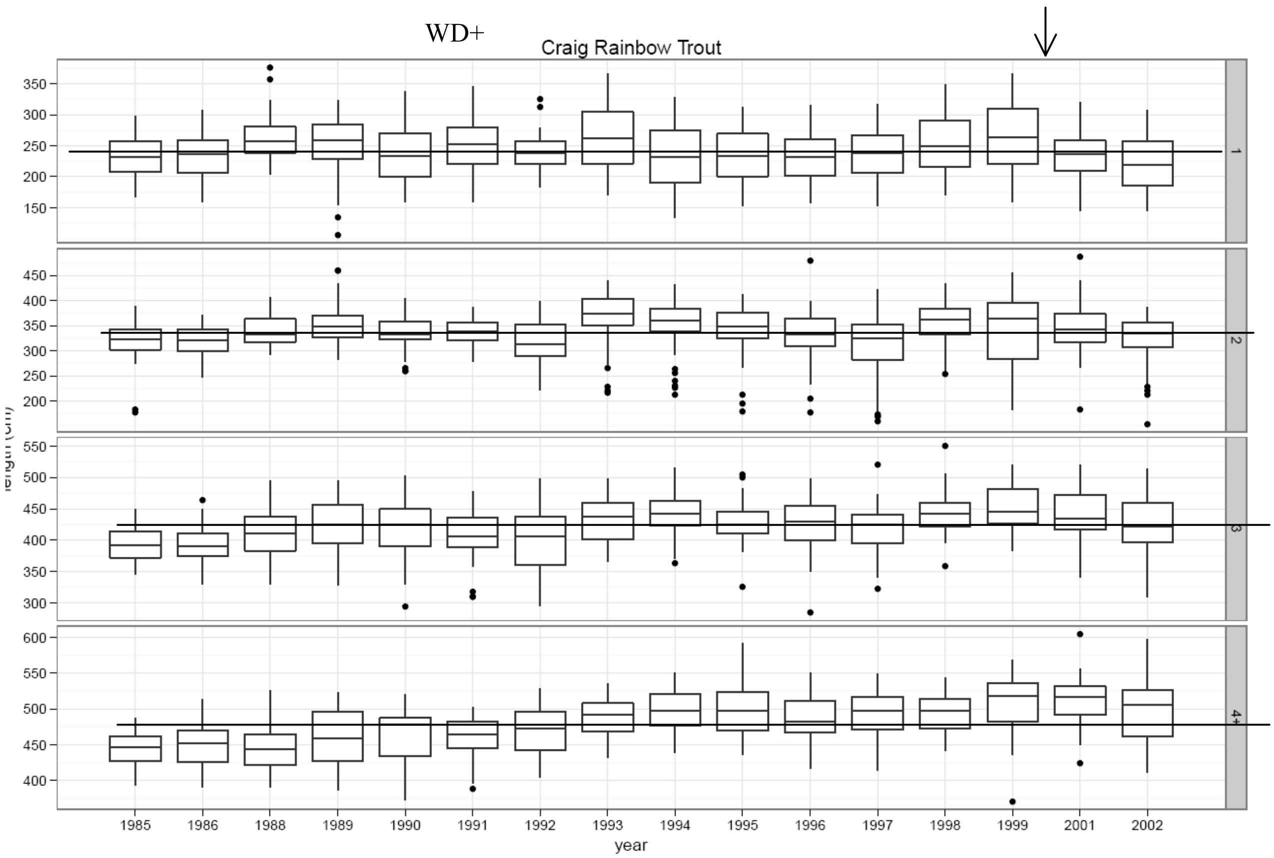


3Forks/Vigilante RBT
WD-
Wr
150
100
50
1994
1996
1997
1998
1999
2000
2001
2002
2003
2006
year

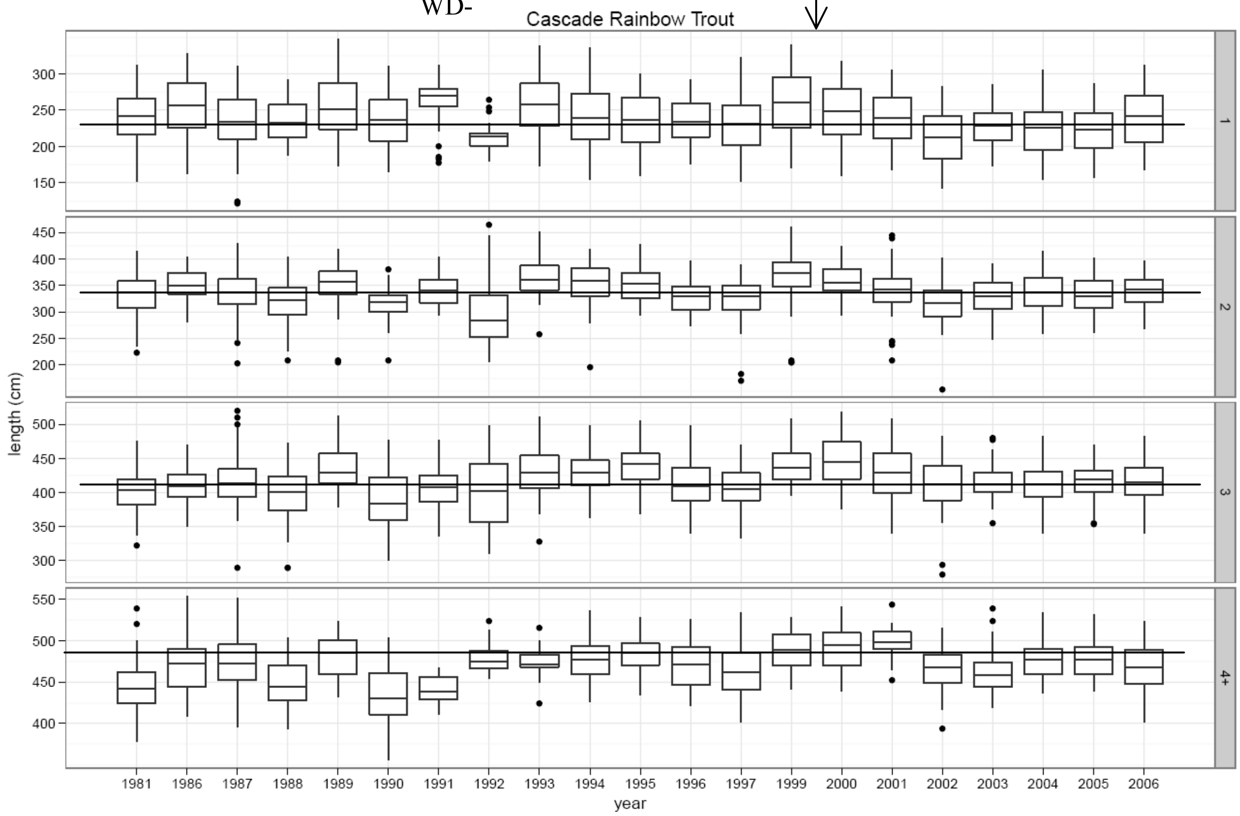


3Forks/Vigilante Brn
WD-
Wr
150
100
50
1995
2000
2001
2002
2006
year

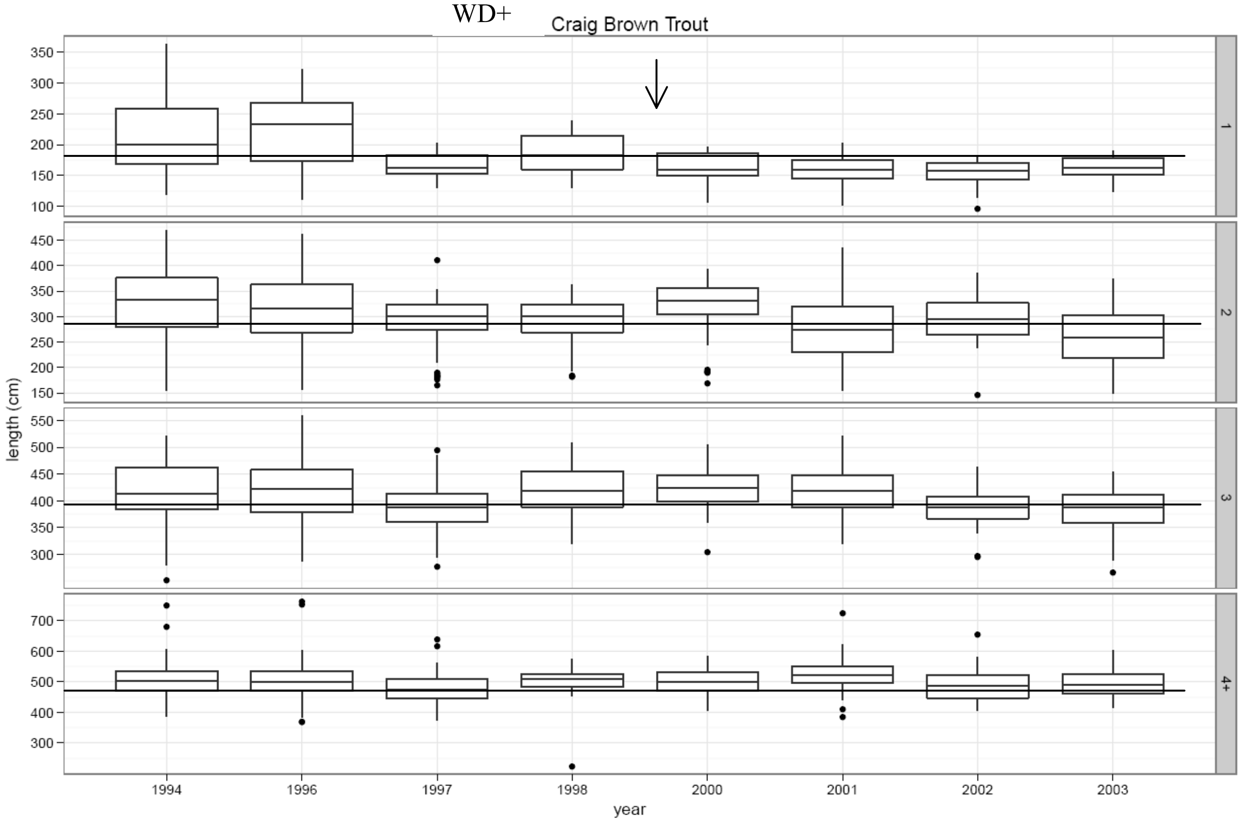
Appendix 5. Length at ages 1, 2, 3, and 4+ of rainbow trout and brown trout in the Missouri River for the Craig (WD+) and Cascade (WD-) sections. Horizontal lines indicate long-term average length for each age, and arrow indicates timing of WD.



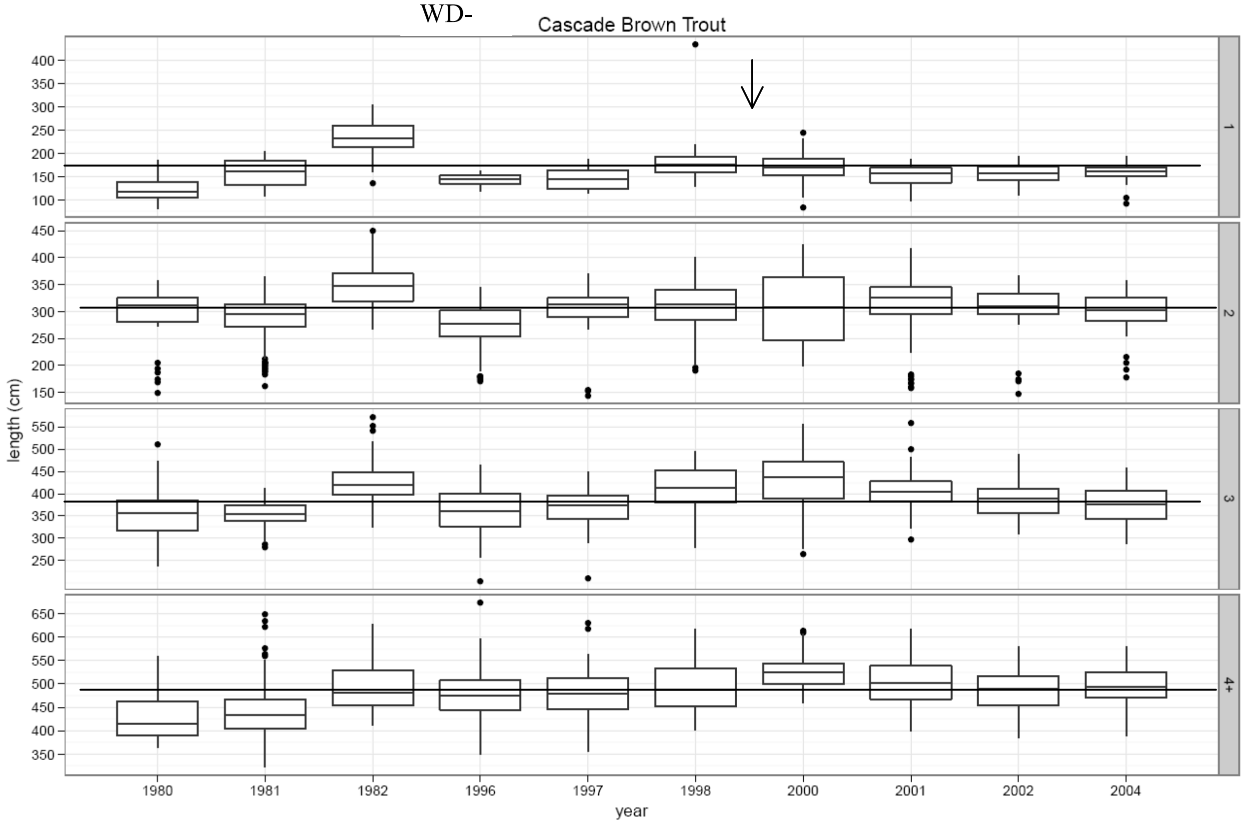
WD-


Cascade Rainbow Trout
length (cm)
year
1
2
3
4+
1981
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1999
2000
2001
2002
2003
2004
2005
2006

WD+

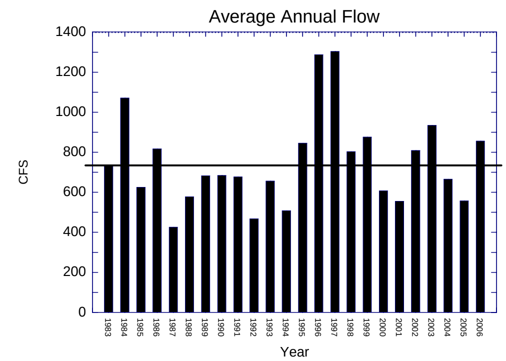

Craig Brown Trout
length (cm)
year
1994
1996
1997
1998
2000
2001
2002
2003
1
2
3
4+

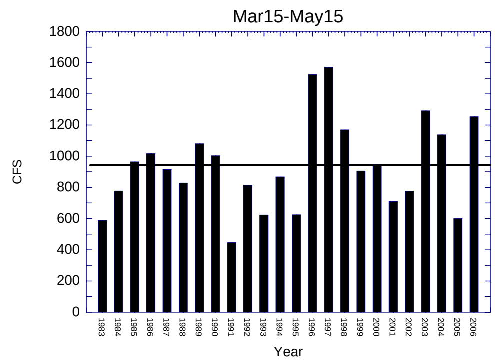
WD-

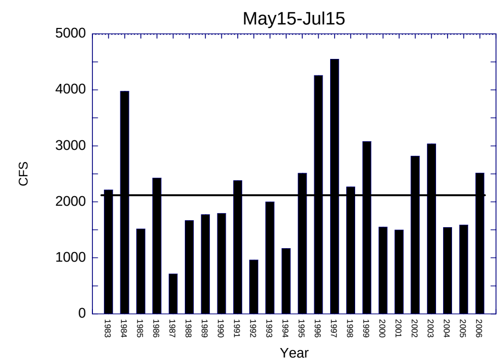

Cascade Brown Trout
length (cm)
year
1980
1981
1982
1996
1997
1998
2000
2001
2002
2004
1
2
3
4+

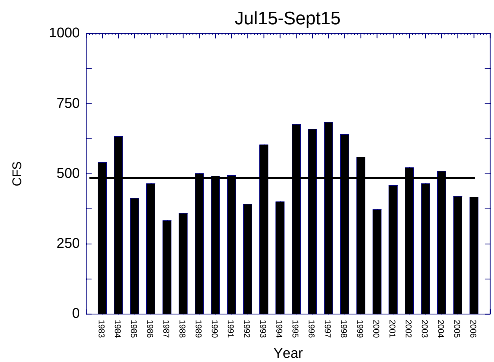
Appendix 6. Seasonal flow data for each study river (Bitterroot, Blackfoot, Gallatin, Missouri, and Ruby rivers, and Rock Creek). Horizontal line represents long-term average flow.

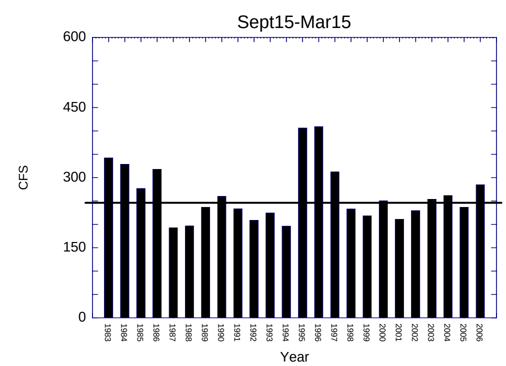
Bitterroot River Flow at Darby, 1983-2006



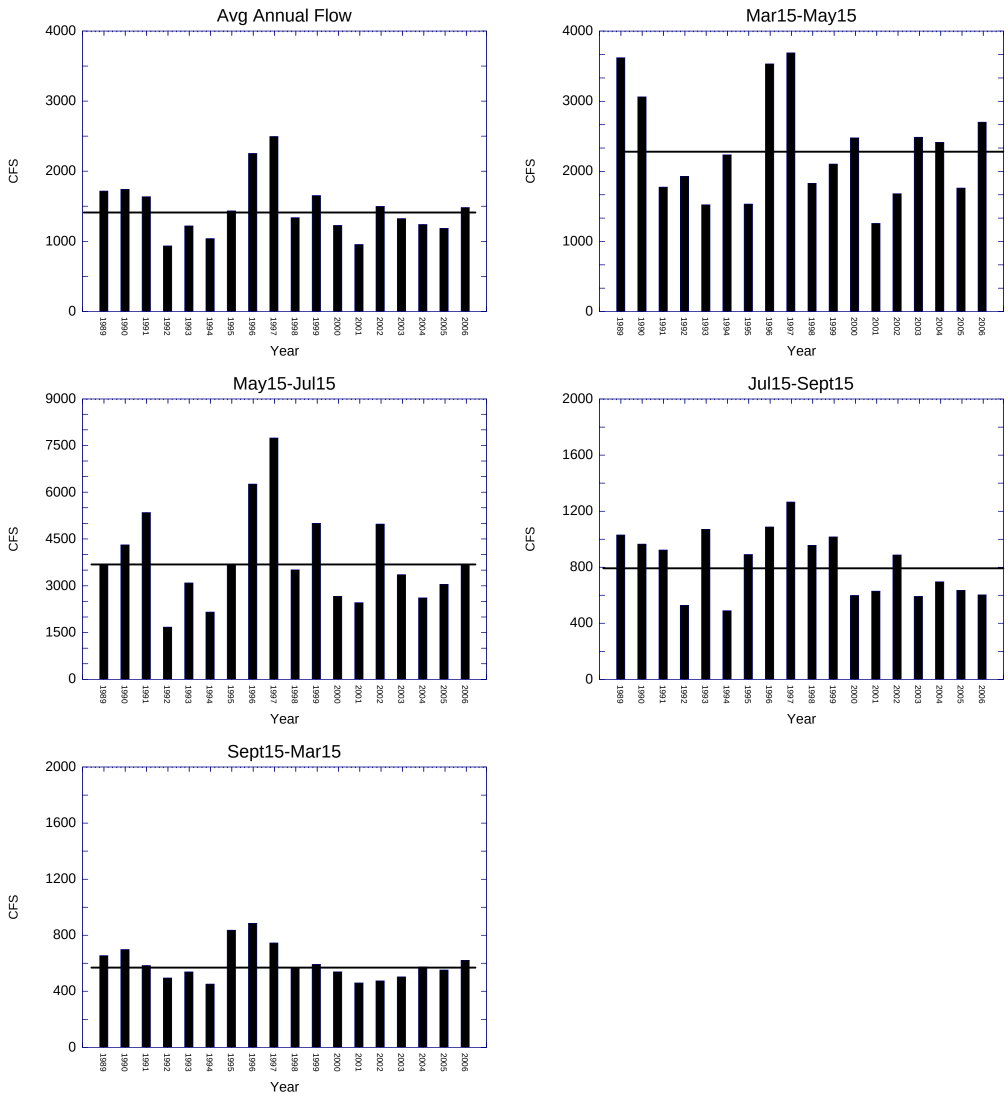




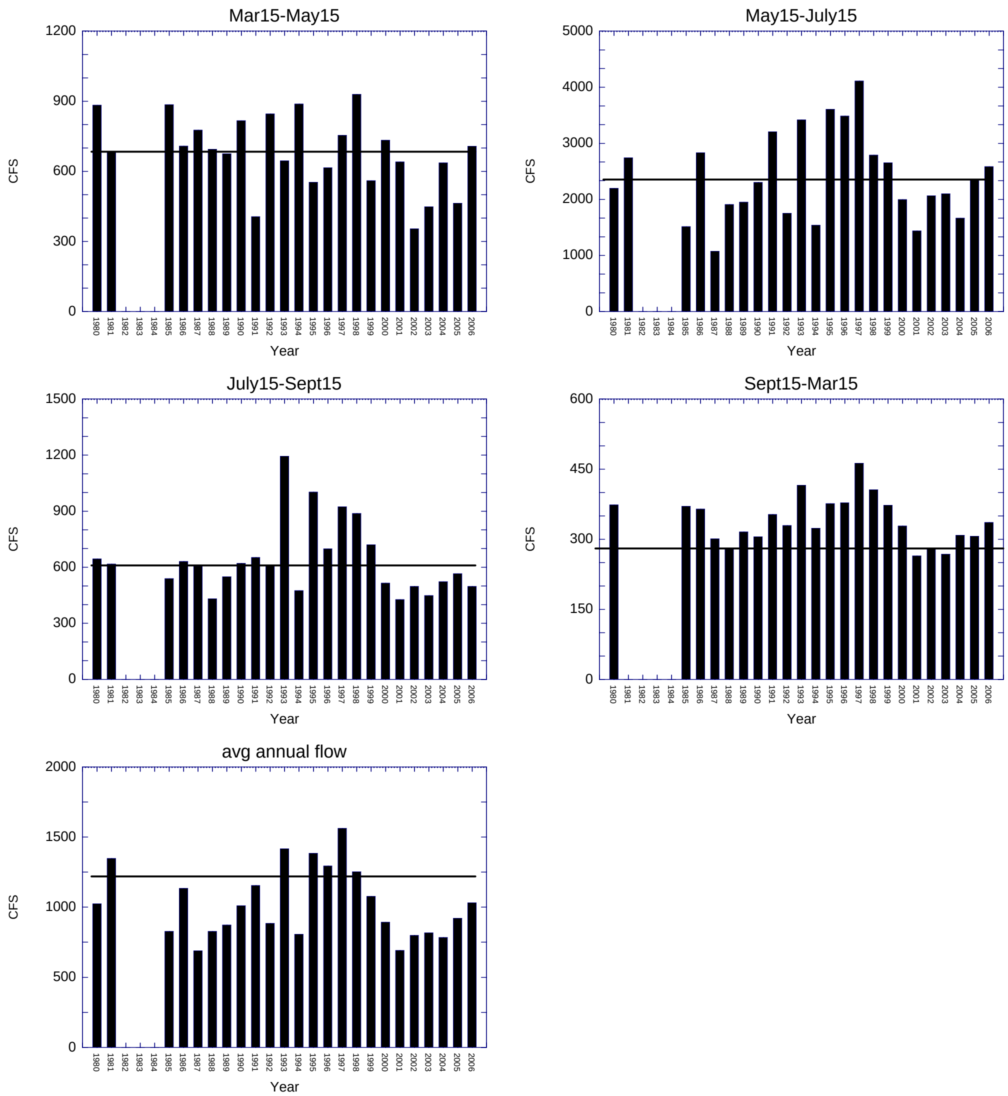




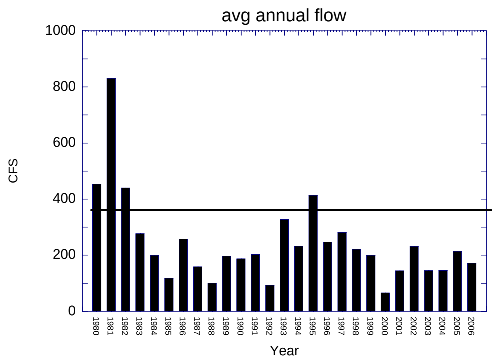
Blackfoot River Flow 1989-2006

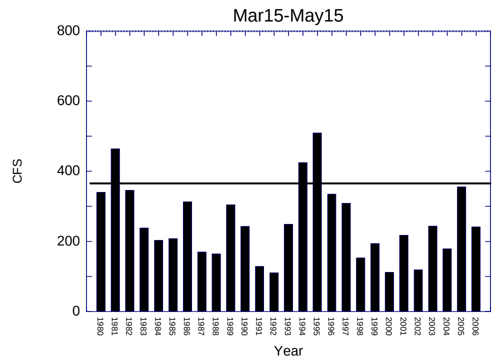


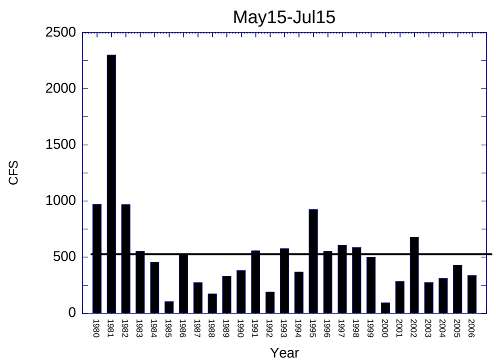
West Gallatin River Flow, 1980-2006

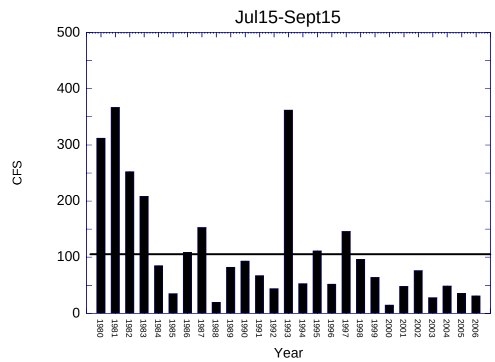


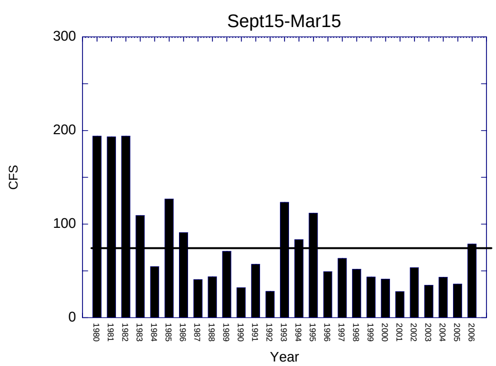
Missouri River: Dearborn River flow, 1980-2006



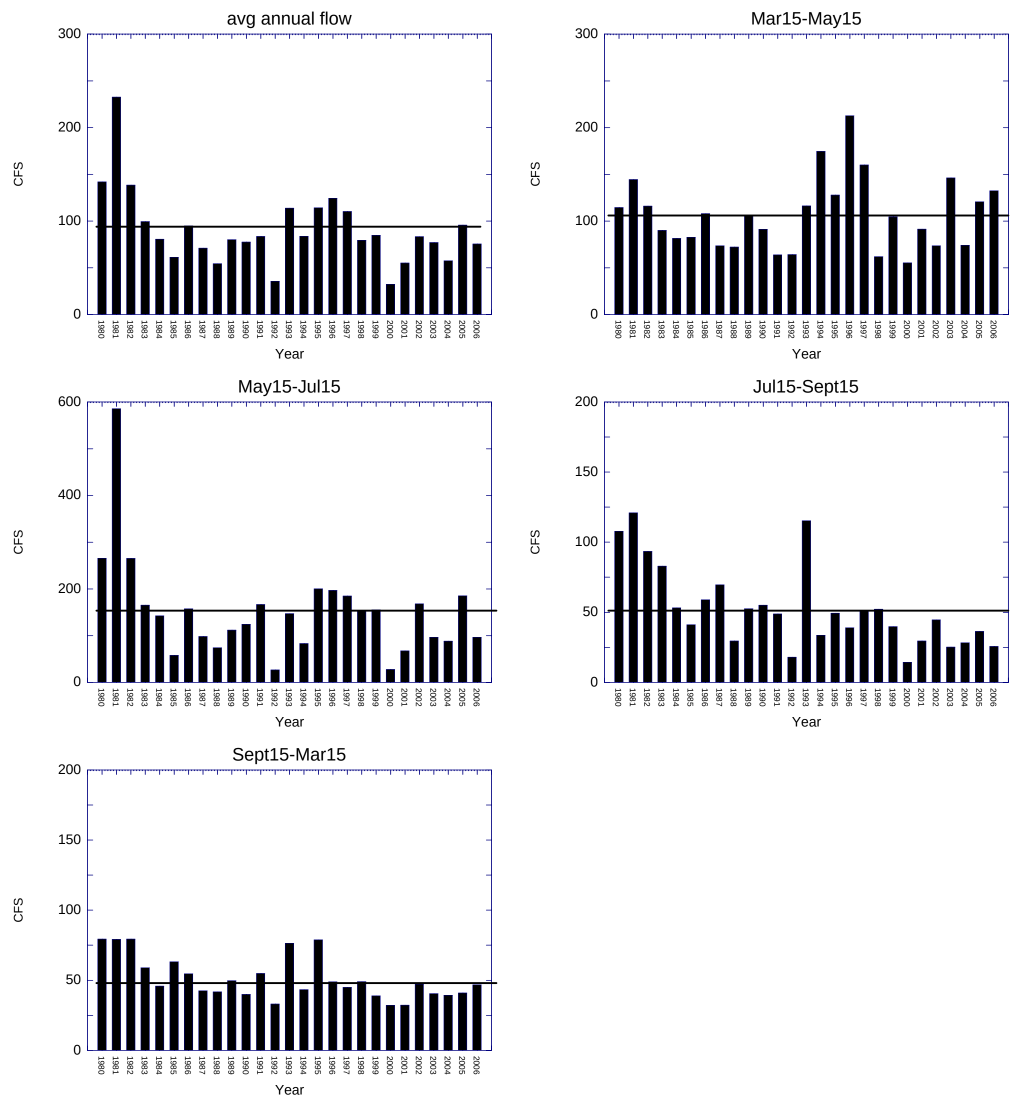




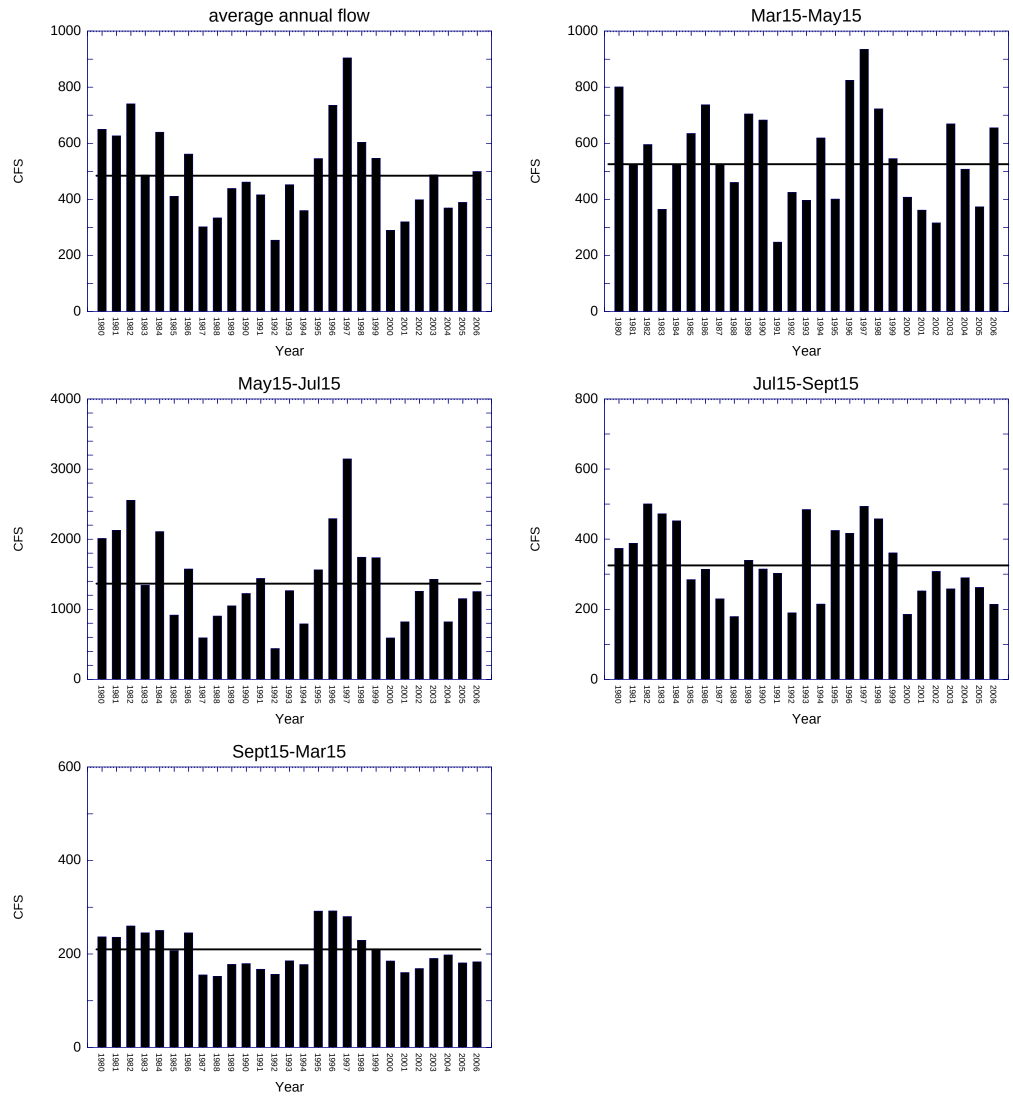




Missouri River: Little Prickly Pear Creek Flows, 1980-2006



Rock Creek Flow at Clinton, 1980-2006



Ruby River at Alder Flow, 1994-2006