



Effects of whirling disease on recruitment of brown trout in the Ruby River and Poindexter Slough,  
Montana  
by Scott Travis Opitz

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Fish  
and Wildlife Management  
Montana State University  
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**Abstract:**

Brown trout numbers began to decline in the mid 1990s in the Ruby River and Poindexter Slough. Possible causes for the decline were investigated and no likely cause was found. Infection by *Myxobolus cerebralis* was diagnosed in brown trout from both waters in 1995 and was considered the possible cause of the declines. To determine if this was the cause of the declines, abundances of age-0 brown trout were monitored with the use of electrofishing to see if any sudden drastic declines occurred during this susceptible life stage. Exposure experiments were also conducted in both waters with age-0 brown and rainbow trout to determine experimentally if whirling disease was present in these systems. Fish were collected during electrofishing and from the exposure experiments for histological confirmation of whirling disease. Age-0 abundances declined gradually as would normally be expected. Brown trout in the exposure experiments were minimally infected, if at all, and the rainbow trout were moderately to severely infected as confirmed by histology. Histology also confirmed the presence of whirling disease in some of the fish collected during electrofishing. Data collected by Montana Department of Fish, Wildlife & Parks suggest that the populations are increasing. Whirling disease did not affect the recruitment of brown trout in both waters during the time the study was conducted.

**EFFECTS OF WHIRLING DISEASE ON RECRUITMENT OF BROWN TROUT  
IN THE RUBY RIVER AND POINDEXTER SLOUGH, MONTANA**

by

**Scott Travis Opitz**

**A thesis submitted in partial fulfillment  
of the requirements for the degree**

of

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in

**Fish and Wildlife Management**

**MONTANA STATE UNIVERSITY-BOZEMAN  
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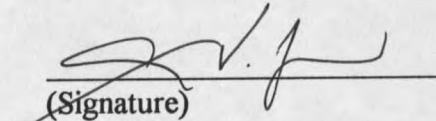
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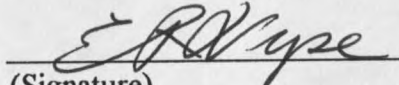
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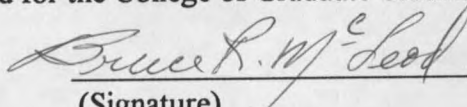
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## ABSTRACT

Brown trout numbers began to decline in the mid 1990s in the Ruby River and Poindexter Slough. Possible causes for the decline were investigated and no likely cause was found. Infection by *Myxobolus cerebralis* was diagnosed in brown trout from both waters in 1995 and was considered the possible cause of the declines. To determine if this was the cause of the declines, abundances of age-0 brown trout were monitored with the use of electrofishing to see if any sudden drastic declines occurred during this susceptible life stage. Exposure experiments were also conducted in both waters with age-0 brown and rainbow trout to determine experimentally if whirling disease was present in these systems. Fish were collected during electrofishing and from the exposure experiments for histological confirmation of whirling disease. Age-0 abundances declined gradually as would normally be expected. Brown trout in the exposure experiments were minimally infected, if at all, and the rainbow trout were moderately to severely infected as confirmed by histology. Histology also confirmed the presence of whirling disease in some of the fish collected during electrofishing. Data collected by Montana Department of Fish, Wildlife & Parks suggest that the populations are increasing. Whirling disease did not affect the recruitment of brown trout in both waters during the time the study was conducted.

## INTRODUCTION

Whirling disease is a parasitic infection of trout and salmon caused by the myxosporean *Myxobolus cerebralis* (Markiw 1992a). *M. cerebralis* developed as a nonpathogenic parasite in brown trout *Salmo trutta* in northern Asia and central Europe (Hoffman et al. 1962; Hoffman 1970, in Halliday 1976; Hoffman 1990). It was discovered in rainbow trout *Oncorhynchus mykiss* in Germany in 1893 (Hofer 1893, in Hoffman 1990). It is believed to have reached the United States in frozen trout from Europe and was diagnosed in 1958 in Pennsylvania (Hoffman 1990). The disease was first diagnosed in the western United States in hatcheries in Nevada and California in 1966 (Yasutake and Wolf 1970). Whirling disease was diagnosed in rainbow trout in the Madison River, Montana, in December 1994 (M. Lere, Montana Department of Fish, Wildlife & Parks (FWP), Bozeman, Montana, personal communication).

### Hosts

*M. cerebralis* has a two-host life cycle. The hosts are salmonids and the aquatic oligochaete *Tubifex tubifex* (Markiw and Wolf 1983; Wolf and Markiw 1984; Wolf et al. 1986). Not all salmonids are equally susceptible to the disease. Susceptibility varies among species and strains and may also vary among individual fish (Markiw 1992b). The species in the following list are ranked according to susceptibility from greatest to least: rainbow trout, sockeye salmon *O. nerka*, golden trout *O. aguabonita*, cutthroat trout *O. clarki*, brook trout *Salvelinus fontinalis*, steelhead *O. mykiss*, chinook salmon *O.*



*tshawytscha*, Atlantic salmon *Salmo salar*, brown trout, coho salmon *O. kisutch*, lake trout *Salvelinus namaycush*, and splake (hybrid between brook and lake trout) (O'Grodnick 1979; Hoffman 1990; Markiw 1992a). Eurasian grayling *Thymallus thymallus* are reported to be infected by *M. cerebralis* (Volf 1957, in Halliday 1976; Havelka and Volf 1970; Bagdanova 1971, in Halliday 1976), but Arctic grayling *T. arcticus* are resistant to infection (Matthew Clow, Montana State University, Bozeman, Montana, personal communication).

Hoffman et al. (1962) stated that brown trout become infected but not diseased. In contrast, Havelka and Volf (1970) reported 80-90% mortality of brown trout infected with whirling disease. The differences in the results from these two studies may have been caused by the age and size of fish exposed, the number of triactinomyxons they were exposed to, the length of the exposures, and environmental conditions associated with the exposure of the fish. Brown trout, which are native to Europe, were introduced to Montana in 1889 by the Bureau of Fisheries (Brown 1971; Luton 1985). Brown trout tend to be found in valley portions of large rivers, but also do well in reservoirs. They must use streams to spawn successfully. Most brown trout become sexually mature by age 3, but may be mature as early as age 2 or as late as age 4 or 5. They spawn from October to December and may move long distances upstream to do so. Females dig the redd and both males and females guard it. The eggs hatch the following February to April. Hatching usually takes 50 days at 10 °C. Brown trout feed on aquatic insects and adults may prey on sculpins, suckers, and trout fingerlings (Brown 1971).

Brown trout exhibiting clinical signs of whirling disease were collected in 1994 in the Colorado River, Colorado (Walker and Nehring 1995). Blacktail was found on 21 of 256 wild brown trout held in sentinel cages on July 14, 1994. On August 4, 1994, 17 of 234 brown trout had blacktail and two had cranial deformities such as protruding eyes, shortened sloping snouts, or both. In wild, free-ranging brown trout, blacktail was easily observed from the banks in mid-July 1994 (Walker and Nehring 1995). The appearance of it at this time is in concordance with evidence showing that blacktail develops 35-45 days after exposure to the parasite (Markiw 1992a; Walker and Nehring 1995). Blacktail was highest in these populations from July to September and in upstream reaches. Sixty percent of the brown trout in the upper reaches had blacktail and some had lordosis or scoliosis.

Nonsalmonids have been reported to be infected by *M. cerebralis* as well. These fish are tench *Tinca tinca*, gudgeon *Gobio gobio*, pike *Esox lucius*, European perch *Perca fluviatilis* (Ramirez-Medina 1962, in Halliday 1976), and young Atlantic herring *Clupea harengus* raised in aquaria (Dannevig and Hansen 1952, in Halliday 1976). However, these reports may be misidentification of other myxosporeans as *M. cerebralis* (Halliday 1976; Markiw 1992a).

#### Life Cycle

*M. cerebralis* myxosporean spores are released from infected fish by death and decay. The spores are biconvex or lenticular with a width of 8 to 10  $\mu\text{m}$  at their widest point. Coiled filaments are contained in two ovate capsules at the anterior or polar end of

the spore (Wolf and Markiw 1985; Markiw 1992a). Spores ingested by *Tubifex* worms transform to actinosporean triactinomyxons (the stage that infects fish) in 3.5 months at 12.5 °C (Markiw 1992a). This transformation takes place in the gut epithelium of the *Tubifex* worm (El-Matbouli and Hoffmann 1989).

Triactinomyxons are anchor shaped and topped with three polar capsules. The anterior end or episore is 36  $\mu\text{m}$  long and the style extends 90  $\mu\text{m}$  below this. The arms taper and extend 170  $\mu\text{m}$  (Wolf and Markiw 1984). After release from the worm host, triactinomyxons enter susceptible fish through the skin, buccal cavity, upper esophagus, fins, or the lining of the digestive tract. The triactinomyxons transform back to *M. cerebralis* spores in the cartilage of the fish (El-Matbouli and Hoffmann 1989). This process takes about 2.6 months at 12.5 °C (Markiw 1992a).

Clinical signs may appear in fish from 35-80 days after infection. Head and axial skeleton deformations, cranial depressions, and shortening of the snout can last throughout the life of the fish (Markiw 1992a). Cranial deformities are caused by interference with osteogenesis (Hoffman et al. 1962; Christensen 1966, in Halliday 1976; Hoffman 1970, in Halliday 1976). Blacktail is caused when the spinal column is infected posterior to the 26th vertebra and pressure is exerted on caudal nerves that control pigment cells (Phlen 1904, in Halliday 1976; Schaperclaus 1954, in Halliday 1976; Hoffman et al. 1962; Hoffman 1966; Hoffman 1970, in Halliday 1976). This pressure can also cause spinal deformities (Hoffman 1966; Havelka et al. 1971, in Halliday 1976; Hoffmann 1970; Hoffman 1970, in Halliday 1976). Blacktail appears 35-45 days after infection at 12.5 °C. Whirling behavior appears around the same time or later (Markiw

1992a). Whirling may be caused by neural damage from lesions and disintegration of cartilaginous tissue around the equilibrium organs and can cause exhaustion, malnutrition, and death (Halliday 1976; Hoffman 1970, in Halliday 1976).

Research conducted in Willow Creek, the Madison River, the Missouri River, and Little Prickly Pear Creek, Montana, in 1997 showed that severe infections in rainbow trout occurred in a temperature range from 9 °C to 17 °C. A peak of infection occurred around 14 °C. This data suggests that there are certain times when young fish are more likely to become severely infected based on water temperatures (E. R. Vincent, FWP, Bozeman, Montana, personal communication). *Triactinomyxon* release from infected *T. tubifex* peaks at 13 °C to 14°C (Thomas Waldrop, National Fish Health Research Laboratory, Kearneysville, West Virginia, personal communication).

#### Whirling Disease in Montana

Concurrent with the discovery of whirling disease in the Madison River, Montana, the rainbow trout population there declined in abundance. In the upper river, a 90% decline in abundance of yearling rainbow trout was observed (Vincent 1996). All possible causes for the decline were investigated, but age-0 monitoring of rainbow trout and histological examination of fish confirmed that whirling disease was responsible for the decline. Whirling disease had no apparent effect on brown trout in this river (Vincent 1996).

Abundances of brown trout in the Ruby River and Poindexter Slough in southwestern Montana declined in the mid-1990s, causing concern among anglers and

managers about the future of these valuable fisheries (R. A. Oswald, FWP, Dillon, Montana, personal communication). Estimated abundances of brown trout >15.2 cm (total length) in the Silver Spring section of the Ruby River exceeded 1,200 fish per 1.6 km in 1989-1992, but were less than 700 fish per 1.6 km in 1993-1995 (Figure 1).

Abundance estimates of brown trout in Poindexter Slough did not decline as sharply, but were generally lower in 1993-1995 than in 1989-1992 (Figure 1). Brown trout from both rivers tested positive for whirling disease in 1995. Six of the 11 fish collected in Poindexter Slough and 5 of the 15 fish collected in the Ruby River were positive for the disease (E. MacConnell, Bozeman Fish Technology Center, Bozeman, Montana, personal communication). Although brown trout are considered resistant to whirling disease, no other factors were identified that could have caused the observed declines in abundances (R. A. Oswald, personal communication).

The objective of this study was to determine if whirling disease was responsible for the declines in abundances in brown trout observed in the Ruby River and Poindexter Slough. Abundances of age-0 brown trout were periodically monitored in both systems to assess survival rates, and individuals were examined for clinical signs of whirling disease and disease severity. Sentinel-fish exposures were conducted to determine experimentally if whirling disease was present in these systems.

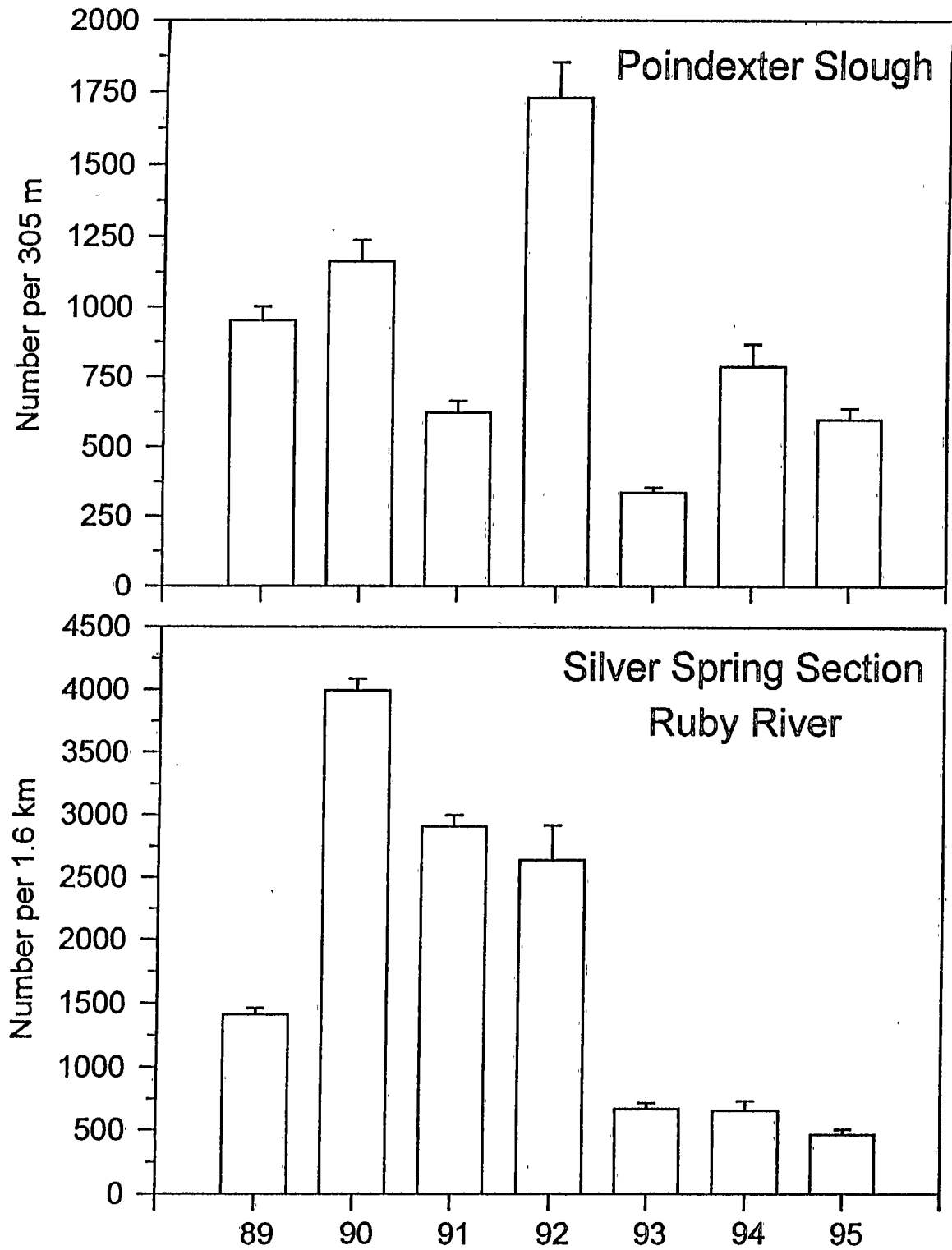


Figure 1: Brown trout population abundance estimates (+1 SD) at Poindexter Slough and the Silver Spring section of the Ruby River made by FWP from 1989-1995.

## STUDY AREAS

The Ruby River and Poindexter Slough are both located in the Jefferson River drainage in southwestern Montana. The Ruby River sites are in Madison County and Poindexter Slough is in Beaverhead County. The Ruby River was originally called Passamari, which means "pleasant valley," by the Shoshone Indians. It was later named Philanthropy in August 1805 by Meriwether Lewis during the Lewis and Clark Expedition. Later, it was named Stinking Water and in 1877 the legislature changed the name to the Ruby because of the large number of red garnets found in the river by miners. The Ruby River has a drainage area of 1,801 square km. It is fed by tributaries draining the Greenhorn, Snowcrest, Gravelly, Tobacco Root, and Ruby Mountains. It flows north for 105 km through Ruby Reservoir to its confluence with the Beaverhead River.

Work was conducted at three sites on the Ruby River, designated the Woodson site, the Barnosky site, and the Snowcrest site. The Woodson site was on the Woodson Ranch, northwest of Laurin, Montana. This site was located in Sections 30, 31, and 32 of Township 5 S, Range 4 W. The study section was 3,000 m long and began 1,000 m downstream from the confluence with Alder Gulch Creek. The section ended at the bridge on the ranch road 1,300 m downstream from the confluence of California Creek. It began at an elevation of 1,540 m and ended at 1,525 m. The gradient for the study section was 5.0 m/km. The section of the river used in the study was sinuous. A section of the river that had recently undergone rehabilitation work was omitted from the study site. The sentinel-fish exposure cages were located about 400 m downstream from the end of

the section used for abundance monitoring (Figure 2). The site for the cages was chosen because it was an area that was not likely to be disturbed by people or animals. It was also shallow enough that water fluctuations would not submerge the cages. Mean daily water temperatures at the Woodson site from June 1996 through March 1998 ranged from 16.8 °C to -1.2 °C (Figure 3). The primary types of vegetation that were found at the site were willow, cottonwood, and various grasses and forbs. Cattle and hay production took place on the ranch. The cattle were excluded from the riparian area by electric fencing.

The Barnosky site was located southwest of Sheridan, Montana, on the Silver Springs Ranch. The section of river used was located in Sections 10, 14, and 15 of Township 5 S, Range 5 W. The study section was 3,900 m long and began about 1,150 m upstream from the ranch-road bridge that crosses the river and ended where the Silver Spring Road crosses the Ruby River. The reach was sinuous. The section began at an elevation of 1,504 m and ended at 1,491 m. The gradient of the study section was 3.3 m/km. Exposure cages were placed about 125 m downstream from the bridge on the ranch road. This was an area that was shallow and unlikely to be disturbed by people or animals (Figure 4). Mean daily water temperatures ranged from 17.0°C to -4.1 °C (Figure 5) from June 1996 to March 1998. The primary vegetation in the area was willow, and various grasses and forbs. The ranch maintained a cattle operation and hay production.

The Snowcrest site, on the Ruby River, was on the Snowcrest Ranch, south of and upstream of Ruby Reservoir. The study section was in Sections 33 and 34 of Township 8 S, Range 4 W. The section was 4,800 m long and began 550 m downstream of the



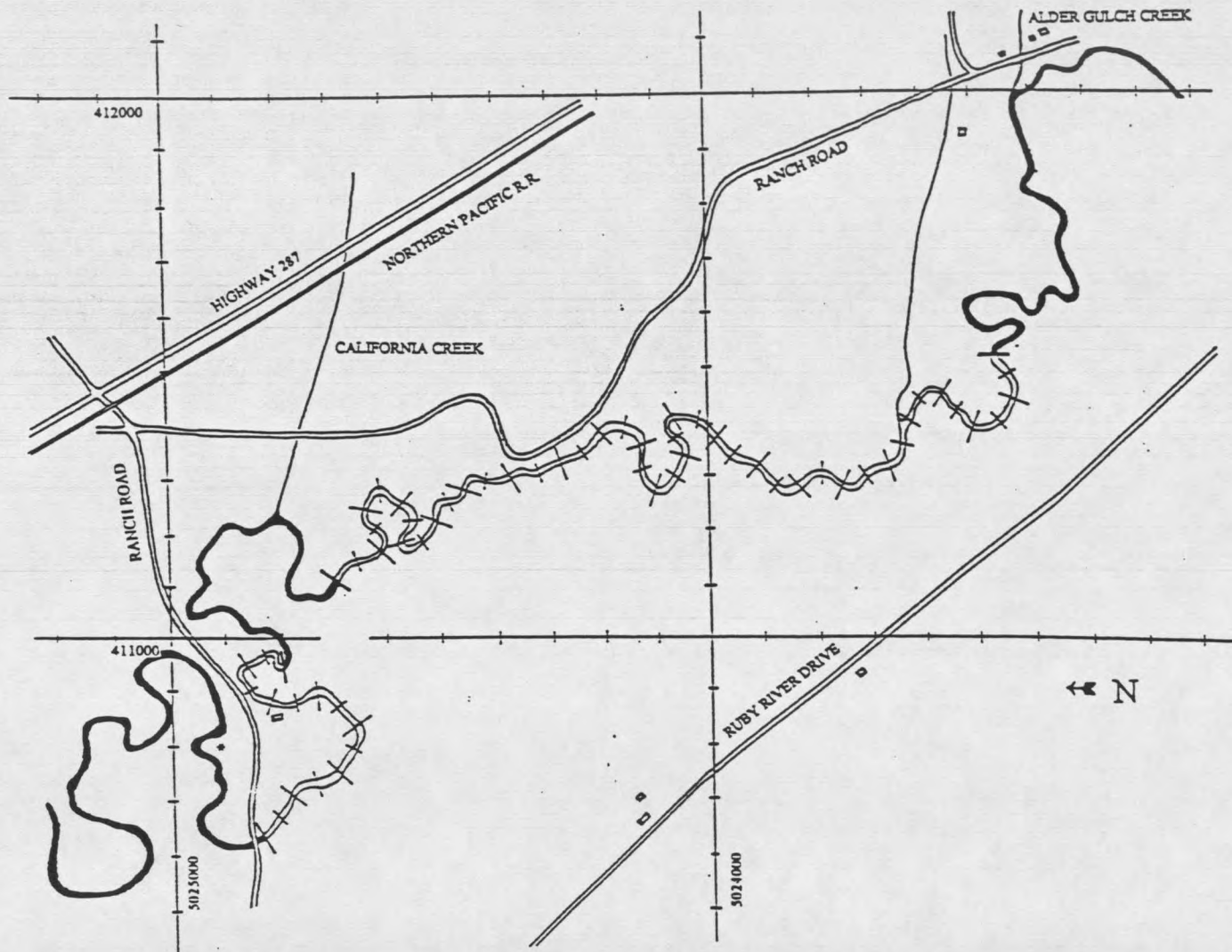


Figure 2. Map of the Woodson site. The vertical and horizontal grid lines represent the UTM coordinates. The tick marks on the river represent the 50 m transects. The exposure experiment cage location is represented by \*.

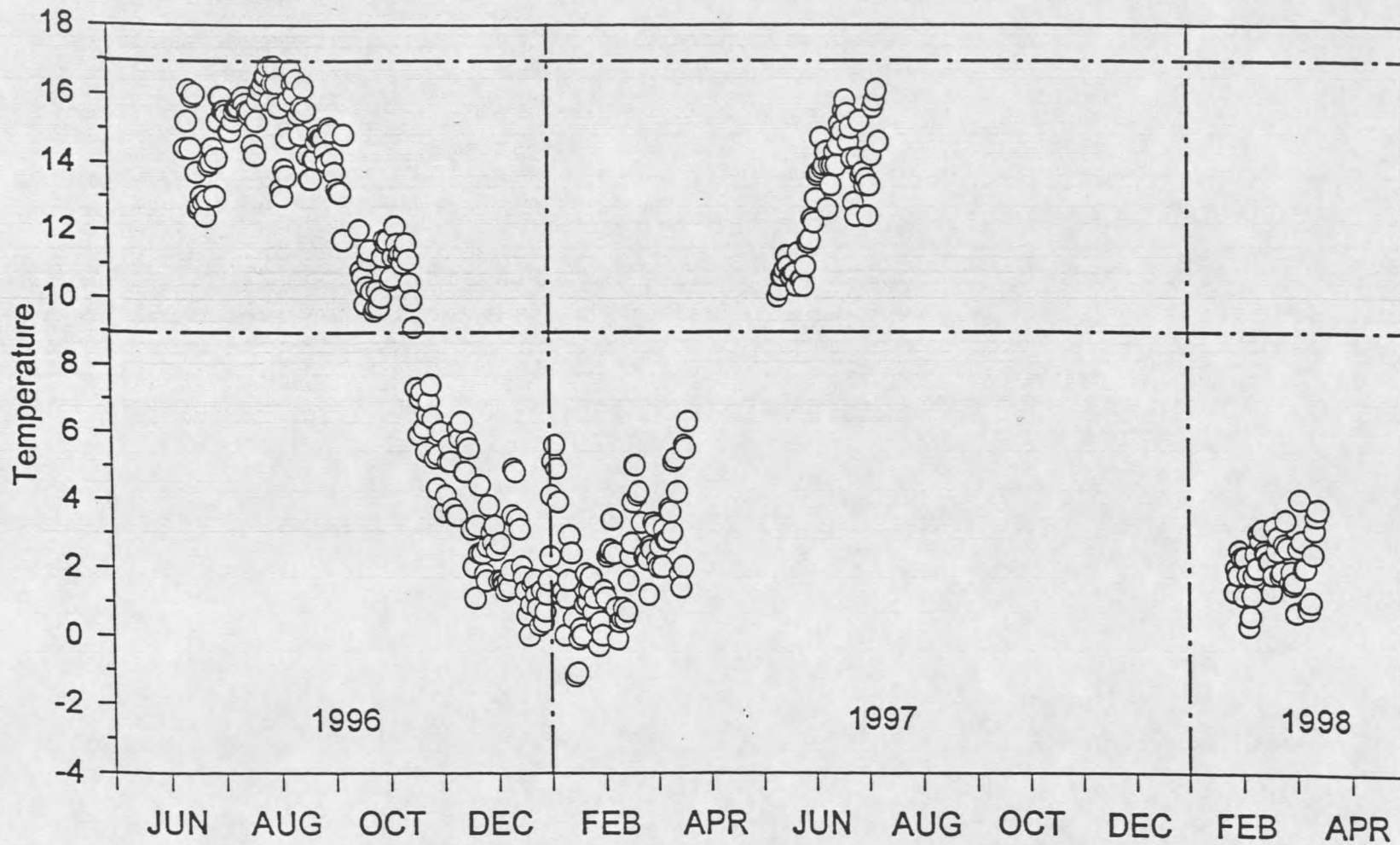
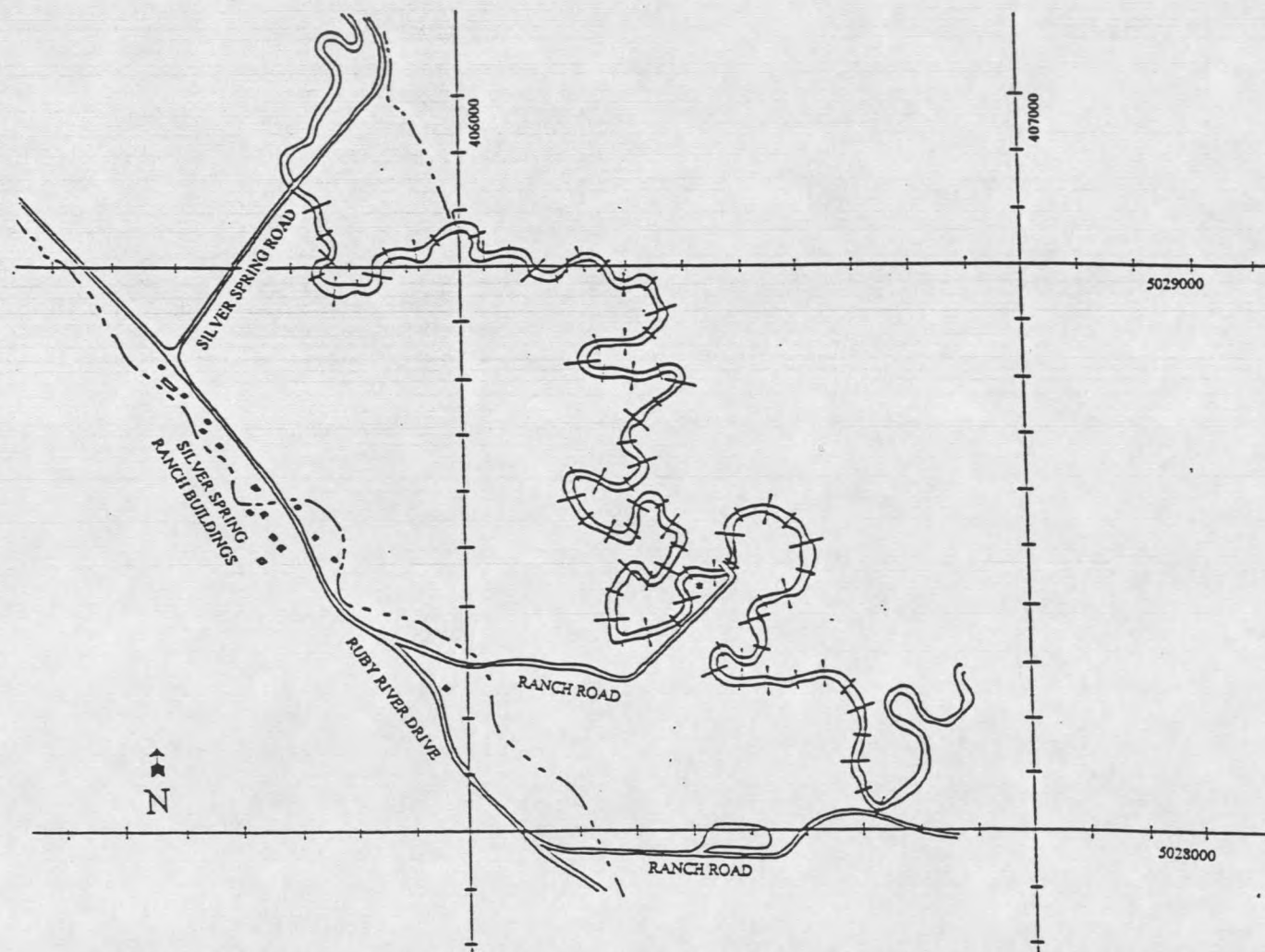


Figure 3. Mean, daily water temperature ( $^{\circ}\text{C}$ ) at the Woodson site from June 1996 to March 1998. The vertical, dashed lines denote years and the horizontal, dashed lines show the 9 to 17  $^{\circ}\text{C}$  range.



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Figure 4. Map of the Barnosky site. The vertical and horizontal grid lines represent the UTM coordinates. The tick marks on the river represent the 50 m transects. The exposure experiment cage location is represented by \*.

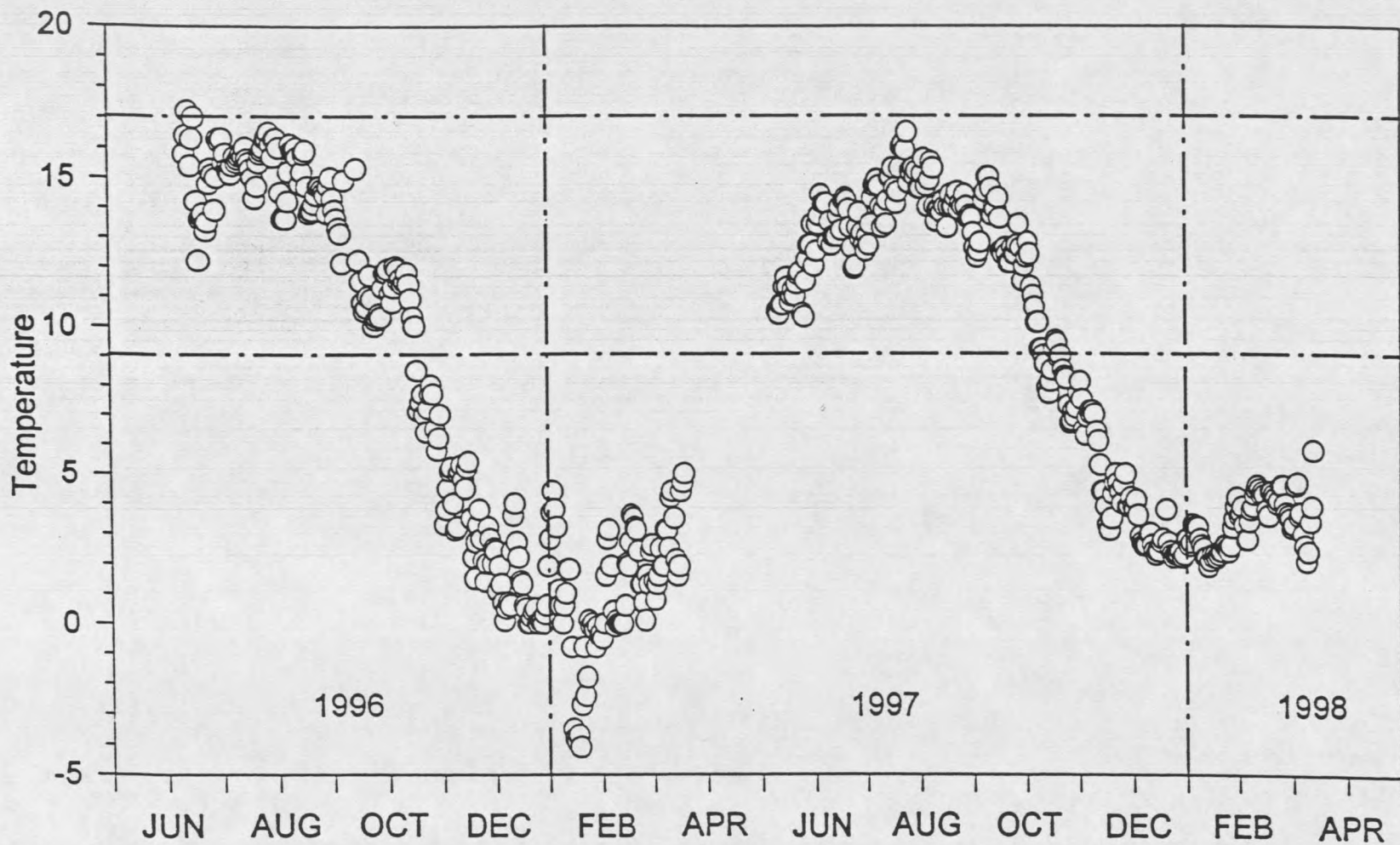


Figure 5. Mean, daily water temperature ( $^{\circ}\text{C}$ ) at the Barnosky site from June 1996 to March 1998. The vertical, dashed lines denote years and the horizontal, dashed lines show the 9 to 17  $^{\circ}\text{C}$  range.

confluence with Ledford Creek. It ended about 50 m upstream from the confluence of Greenhorn Creek with the Ruby River (Figure 6). The reach was sinuous. The study section began at an elevation of 1,739 m and ended at an elevation of 1,726 m. The gradient of the section was 2.7 m/km. Sentinel-fish exposures were not conducted at this site. Various types of vegetation were found on the ranch. They included willow, juniper, cottonwood, and various grasses and forbs. The ranch was used for bison and hay production.

The Poindexter site was on the State-owned Poindexter Slough fishing access site southwest of Dillon, Montana. This study section was located in Sections 26 and 35 of Township 7 S, Range 9 W. The study section was 3,300 m long and sinuous. The section began at an elevation of 1,571 m and ended at 1,566 m. The gradient for the section was 1.5 m/km. It began at the parking lot for the fishing access just off Highway 91 and ended at the confluence of the Beaverhead River. Exposure cages were placed 750 m upstream from the confluence with the Beaverhead River (Figure 7). Mean daily water temperatures ranged from 16.9 °C to 1.2 °C at the Poindexter site from June 1996 through March 1998 (Figure 8). The area was used for agricultural purposes before the purchase of the Poindexter Slough fishing access by the Montana Department of Fish, Wildlife & Parks in July 1979. The area was flood irrigated, hayed, and grazed by livestock. The site has not been grazed since 1979 and it has not been hayed since 1990. Primary types of vegetation that were found on the site were willow, cottonwood, Kentucky bluegrass, reed canarygrass, bearded wheat grass, alkali grass, alkali sacuton, smooth brome, sedges, Garrison foxtail, rose, and snowberry. The site also had large

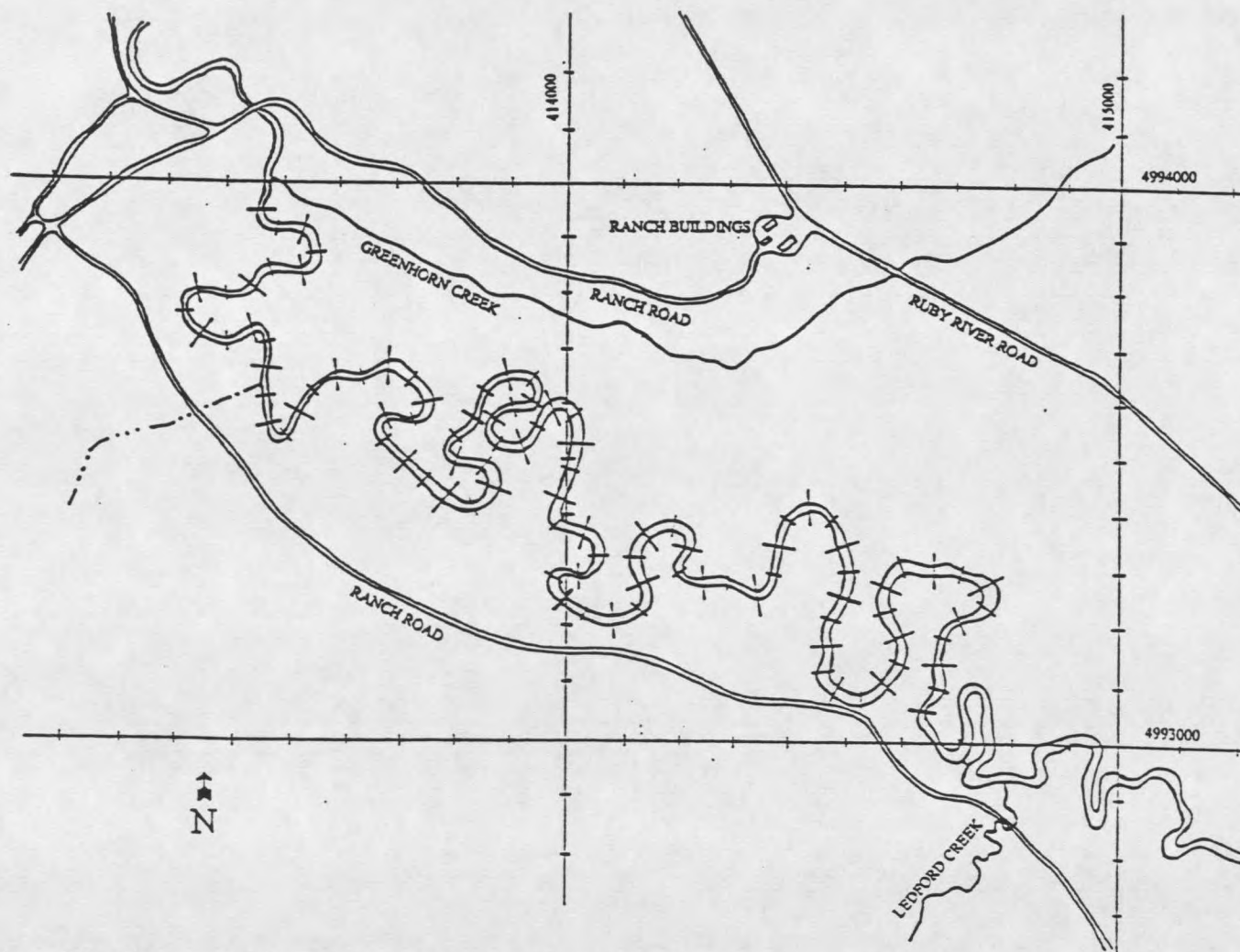


Figure 6. Map of the Snowcrest site. The vertical and horizontal grid lines represent the UTM coordinates. The tick marks on the river represent the 50 m transects.

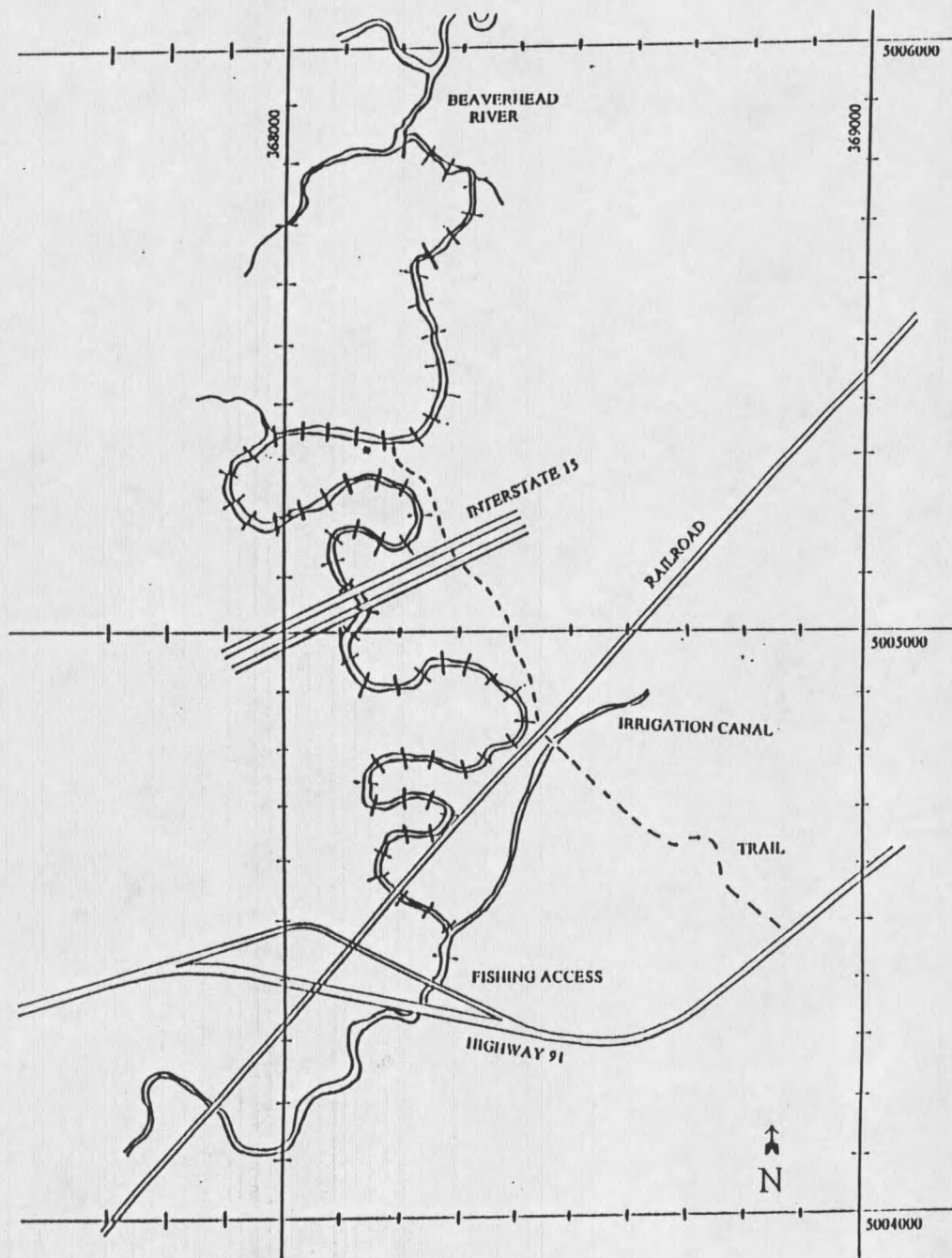


Figure 7. Map of the Poindexter site. The vertical and horizontal grid lines represent the UTM coordinates. The tick marks on the river represent the 50 m transects. The exposure experiment cage location is represented by \*.

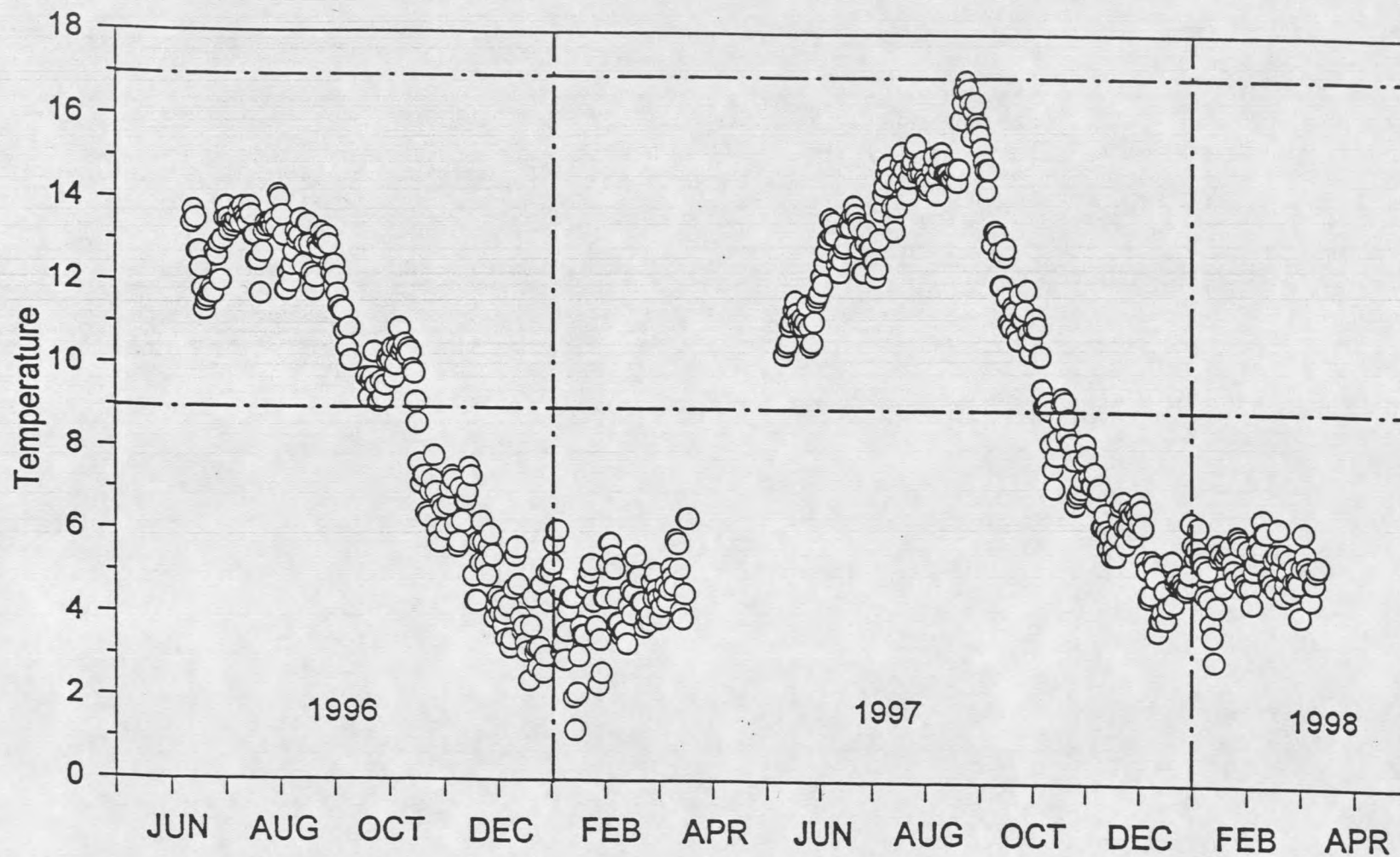


Figure 8. Mean, daily water temperature ( $^{\circ}\text{C}$ ) at the Poindexter site from June 1996 to March 1998. The vertical, dashed lines denote years and the horizontal, dashed lines show the 9 to 17  $^{\circ}\text{C}$  range.



amounts of Canada thistle that is classified as a noxious weed in Montana (Barb Landgraf, Natural Resources Conservation Service, Dillon, Montana, personal communication).

## METHODS

### FWP Abundance Data

Population data collected through electrofishing surveys by Dick Oswald at the Poindexter site and the Silver Spring section of the Ruby River from 1989 through 1998 were analyzed. This was done to look for population trends. Abundances of age-1 brown trout at the Poindexter site during 1989-1992 and 1993-1997 were compared using a one-way t-test (Neter et al. 1993) to determine if recruitment was lower after the suspected introduction of *M. cerebralis* to the system. Abundances at the site during 1989-1992 and 1994-1997 were compared in the same manner. A similar analysis could not be done on age-1 brown trout data from the Silver Spring Section of the Ruby River because age-1 fish were excluded from samples collected there from 1989 through 1995. Abundances of all age classes in aggregate at both sites were plotted by year and examined for trends in abundance.

### Abundance Monitoring

Age-0 brown trout were monitored to determine what effect whirling disease had on recruitment of brown trout. This was done in two sections of the Ruby River in the 1996-97 field season and three sections of the Ruby River in the 1997-98 field season. It was also done in one section of Poindexter Slough during both field seasons. The monitoring was similar to that done by Mark Lere on the Madison River, Montana, in his

study assessing effects of whirling disease on recruitment of rainbow trout where brown trout are believed to be unaffected by whirling disease. The abundance monitoring allowed for the detection of sudden, drastic declines, as seen in the Madison River.

Four, randomly chosen transects at each of the study sites were electrofished using a Coleman Crawdad boat and a mobile shocking unit every two weeks from June through November 1996 and 1997. The Poindexter site was electrofished in March 1996 and the Poindexter, Barnosky, and Woodson sites in March 1997 so that overwinter survival could be examined as well. Transects were 50 m long and extended 1.0-1.5 m from the bank out into the river. The transects were shortened if the water became too deep or spawning redds were found. Locations of each transect were determined using site maps on which the streams were divided into 50-m transects (Figures 2, 4, 6, and 7). They were measured off using a 50-m rope marked at 1-m intervals. A three-pass removal was made at each transect to obtain an estimate of age-0 brown trout abundance (Zippin 1958; Raleigh and Short 1981). If no fish were captured in the first two passes, the third pass was omitted. The same transect was not sampled more than once per year to avoid bias caused by fish movement or mortality resulting from electrofishing. Fish were collected and placed into separate 20-liter buckets for each pass. Estimates of fish numbers for each transect sampled were calculated using MICROFISH Version 3.0 (Van Deventer and Platts 1983). Overwinter survival was calculated by dividing the mean abundance estimate of fish in the March sample by the mean abundance estimate for the last sampling date from the preceding autumn.

### Clinical Signs

Fish collected were identified, counted, measured, and weighed. The lengths and weights were used to calculate Fulton condition factors (Anderson and Willis 1996). Fish were examined for clinical signs of whirling disease. These were (1) cranial deformity, (2) exophthalmia, (3) blacktail, (4) bent caudal, (5) shortened operculum, and (6) whirling behavior (Markiw 1992a).

### Histology

Both clinical signs and demonstration of the presence of *M. cerebralis* spores are needed for definitive diagnosis of whirling disease (Post 1987) because clinical signs typical of whirling disease can be caused by other diseases or electrofishing injury (Halliday 1973).

At least twenty fish from each study site were collected and preserved during each sampling period for histological examination for presence of *M. cerebralis*, if enough fish were present. Each sample consisted of any fish that showed clinical signs, if present, and the remainder of the twenty was made up of fish that did not show clinical signs. If no fish with clinical signs were present, twenty fish that did not show clinical signs were collected. The collected fish were euthanized with tricaine methanesulfonate (MS-222) and were slit down the belly from the isthmus to the vent (Yasutake 1987). Internal organs were moved outside the body cavity to allow for better preservation. The samples were placed in a Nalgene bottle labeled with site, date, and transect data and fixed in Davidson's

solution for 72 hours. They were then preserved in 70% ethyl alcohol until they were processed for histology (Humason 1979).

As an oversight, age-0 brown trout that showed clinical signs of whirling disease were not kept separate from fish that did not show clinical signs in the 1996-97 field season. This made determining whether fish that showed clinical signs were also infected with *M. cerebralis* impossible. The fish examined histologically were only from transects that contained fish with clinical signs. During the 1997-98 field season, age-0 brown trout collected for histological examination that showed clinical signs of whirling disease were kept separate from those that did not. All brown trout collected that showed clinical signs of whirling disease were examined histologically. Some fish that did not show clinical signs of whirling disease (nonclinical) were also collected for histological examination during the 1997-98 field season. Fish from the Snowcrest site collected only in September and October were examined because no abundance monitoring was done in November or March. The number of nonclinical fish that were collected and examined varied. In some transects, few or no nonclinical fish were collected because of low abundances of fish or high numbers of fish showing clinical signs. Samples that were ruined during histological preparation because of processor problems also reduced the number of nonclinical fish examined.

Histological tissue preparation and reading of slides were done at the Bozeman Fish Technology Center, in Bozeman, Montana, under the direction of Elizabeth MacConnell. Preserved fish were processed using standard histological techniques. First, the tail section of the fish behind the dorsal fin was removed. The fish was then cut in half

longitudinally. One half was processed through graded alcohols, clearant, and paraffin wax. The sample was then embedded in paraffin wax and was cut into five- $\mu$ m thick sections. A gill section and sagittal section were placed on 2.5 cm by 7.6 cm glass slides. The sections were stained with hematoxylin and eosin (H&E) and Giemsa (Luna 1968). Slides were examined using light microscopy.

The severity of the infection was graded using the MacConnell-Baldwin scale. The scale of grades ranging from zero to four, were defined as follows: 0. No Infection. 1. Minimal - No host inflammatory cells; few areas of cartilage (1-2 areas of cartilage in a section) infected. Often difficult to detect because of little to no cartilage degeneration, especially when infection is in prespore stages. 2. Mild - Several areas of cartilage but minimal or no host response (few inflammatory cells). Inflammatory response has not caused any bone distortion or involvement of other tissues (very localized response). Often seen early in the infection when the parasite arrives at the cartilaginous tissue and phagocytoses chondrocytes. 3. Moderate - Several areas of cartilage infected in each section, cartilage lysis (degeneration and necrosis) and associated inflammatory response. More diffuse with minimal or mild impact on surrounding tissue. 4. Severe - Several to numerous areas of cartilage are infected and associated inflammatory response is extensive. There is also moderate to severe involvement of surrounding tissues (bone displacement and distortion). Granulomatous lesions found in cartilage or surrounding connective tissues.

Histological examination was used to determine if clinical signs were caused by whirling disease or another factor. It was also used to determine if fish that did not show

clinical signs of whirling disease were infected with *M. cerebralis*. This is important because fish may have subclinical infections, carry spores, and transmit the infection (Kozel et al. 1980; Markiw 1992b).

### Exposure Experiments

Exposure experiments were conducted at the Poindexter, Woodson, and Barnosky study sites in 1996 and 1997. Sentinel cages with rainbow trout are a reliable method of determining the presence or absence of *M. cerebralis* (Hnath 1970; Horsch 1987). These experiments allowed for daily observation of exposed fish to detect the time of appearance of clinical signs and death. One location at each of the three study areas was selected where the sentinel cages would be unlikely to be disturbed by people or animals and would not be affected by high water from spring runoff. Four sentinel cages were placed in this location at each site each year. Fifty post-hatch fish were placed in each of the 12 sentinel cages in early June. The fish were about four months old and averaged 2.5 to 3.8 cm in length when they were placed in the cages in 1996. They were about three and a half months old and averaged 2.5 cm in length when they were placed in the cages in 1997. At each site, three of the cages contained brown trout and one contained rainbow trout. In 1996, all of the trout came from Big Springs Trout Hatchery in Lewistown, Montana. The brown trout were Soda Lake, Wyoming, strain and the rainbow were Eagle Lake strain. In 1997, the rainbow were Desmet strain from Big Springs Trout Hatchery and the brown trout were Bitterroot strain from the Harriman Trout Company in St. Ignatius, Montana. Water temperature data were collected during the exposures using HOB-

TEMP temperature loggers placed at the exposure cage locations. The water temperature data were collected to see if fish in the exposure experiments were exposed within the 9 °C to 17 °C range when infection is most likely (E. R. Vincent, personal communication). Each cage was equipped with a 12-hour belt feeder filled with Nelson's Sterling Silver Cup Fish Food #3 five times a week. This was done to rule out lack of food as a cause of disease or mortality. The amount of feed used was 4% of the average body weight of fish in each group. This was determined by weighing 10 fish from each group to obtain an average weight. The sentinel cages were checked daily Monday through Friday to make sure that they did not suffer any damage, that the fish were not able to escape, and to remove mortalities. Mortalities were recorded and preserved for histological examination using standard techniques (Humason 1979) as described earlier. If necessary, repairs to the cages were made.

One sentinel cage of brown trout was removed after two, four, and six weeks of exposure from each site. The rainbow trout were removed after six weeks of exposure. The fish were transferred to two Living Streams in Pony, Montana, in 1996 and to three Living Streams in the Wild Trout Research Lab (WTRL) in Bozeman, Montana, in 1997. After the fish were transported to the laboratories, they were maintained until October 31, in 1996 and November 21, in 1997. Negative-control groups of 50 unexposed brown trout and 50 unexposed rainbow trout were also maintained in these Living Streams both years. Lots of fish were maintained separately in the Living Streams at 12 °C. The fish were provided 4% of their average body weight in feed using 12-hour belt feeders in 1996



and by hand in 1997. Clinical signs and deaths were monitored daily Monday through Friday. Mortalities were collected and fixed in Davidson's solution (Humason 1979) for histology.

### Histology

Fish from the exposure experiment in 1996 were not examined histologically because of large losses of fish caused by mechanical failures and brown blood disease. Subsamples of fish from the 2, 4, and 6-week exposure groups and the positive-control rainbow trout groups were collected in July, August, October, and November 1997 for examination. Fish from the negative-control brown and rainbow trout groups were collected in July, August, and November 1997. In 1997, a collection of five fish from each group each month was planned because of the inability to maintain fish until November in 1996. The number of fish that were collected varied according to the number of surviving fish in each group. Collections from groups that had very few surviving fish were not made during some months. This helped to insure that at the end of the trial in November there would be fish in these groups for comparison if needed.

## RESULTS

FWP Abundance DataPoindexter Site

No obvious declines in recruitment were observed except for 1993 when population data were graphed by year (Figures 9 and 10). Numbers of large fish did not decline after 1993. Abundance was statistically higher in 1989-1992 than 1993-1997 at the 95% confidence level. When a one-way t-test was done on abundances of age-1 brown trout from 1989-1992 and 1994-1997 to see how much 1993 was affecting the results, no significant difference was found at the 95% confidence level. When overall population abundances of brown trout at the Poindexter site were graphed, it was observed that abundances stopped decreasing in 1996 and continued to increase through 1998. No obvious decline for an extended period of time occurred at this site (Figure 11).

Silver Springs Section

An obvious decline in recruitment in the Silver Springs Section was observed that began in 1993 and continued through 1996 as noted by decreased numbers of large fish. Recruitment then increased in 1997 and 1998 as noted by increasing numbers of large fish and the return of age-1 fish (Figures 12 and 13). No one-way t-test on age-1 abundances was done because no data on age-1 fish was collected in 1989-1995. In 1996, almost no age-1 fish were found during sampling. An obvious decline in overall population

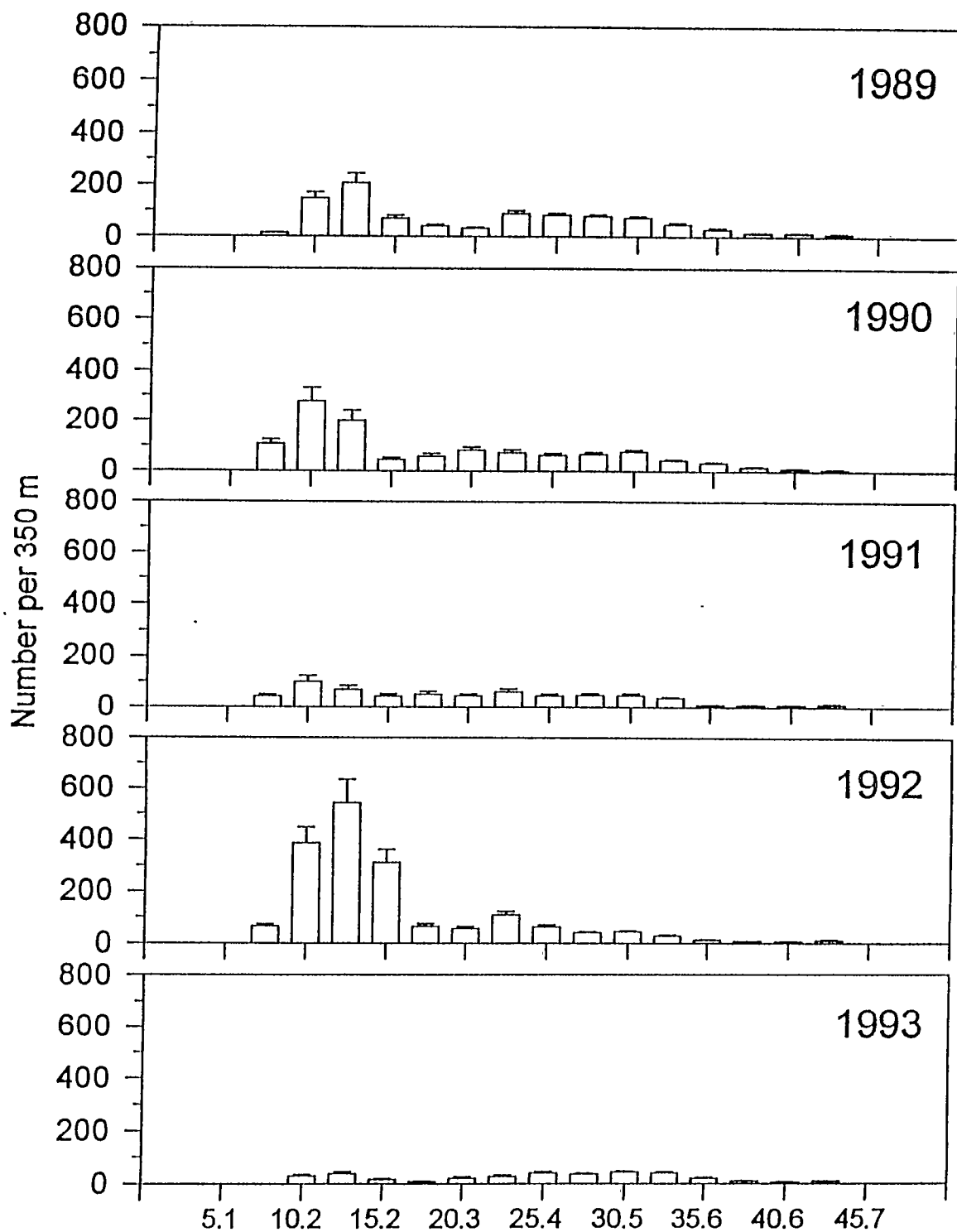


Figure 9. Number of brown trout (+ 1 SD) for each length group (cm) at the Poindexter site from 1989 through 1993.

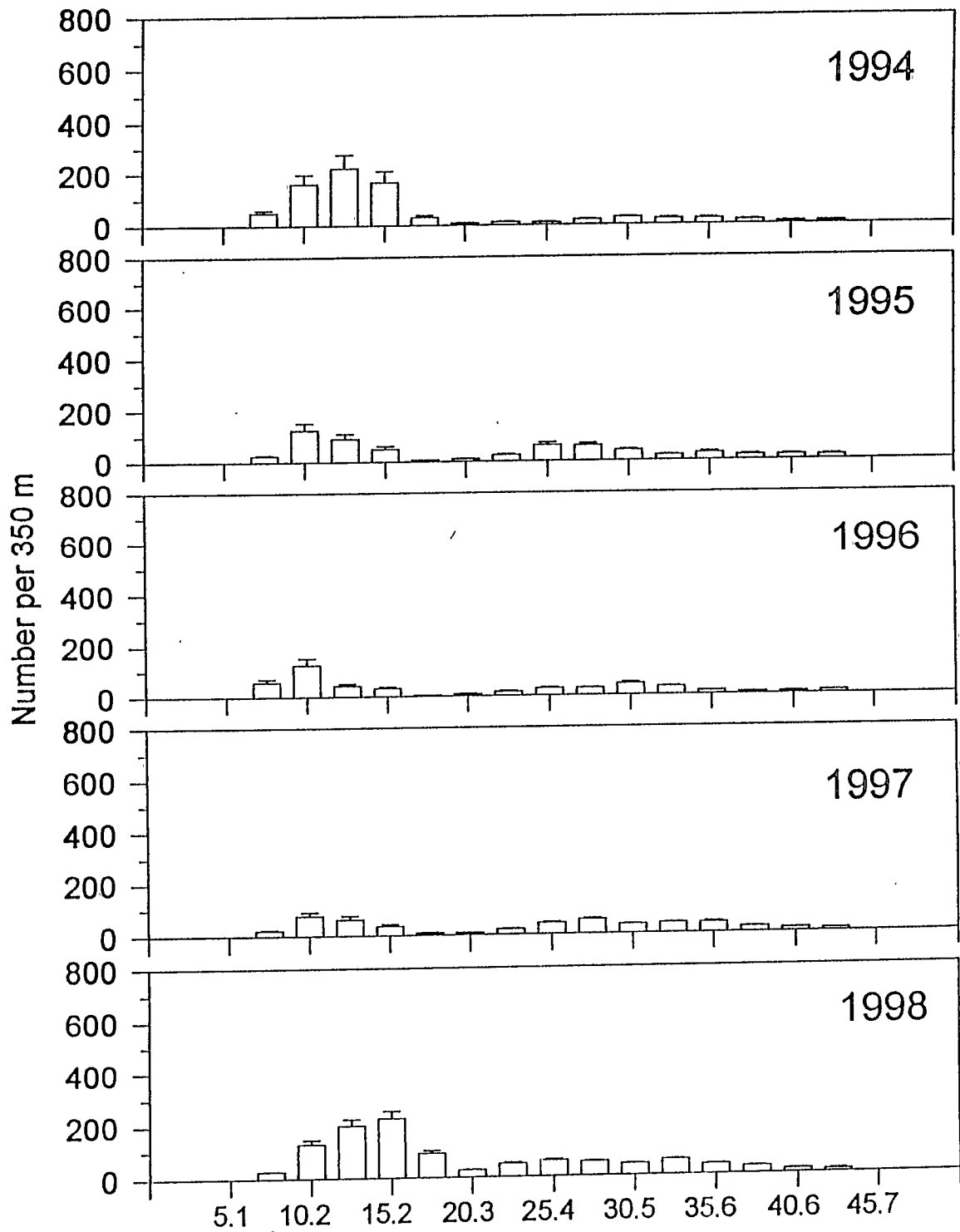


Figure 10. Number of brown trout (+ 1 SD) for each length group (cm) at the Poindexter site from 1994 through 1998.

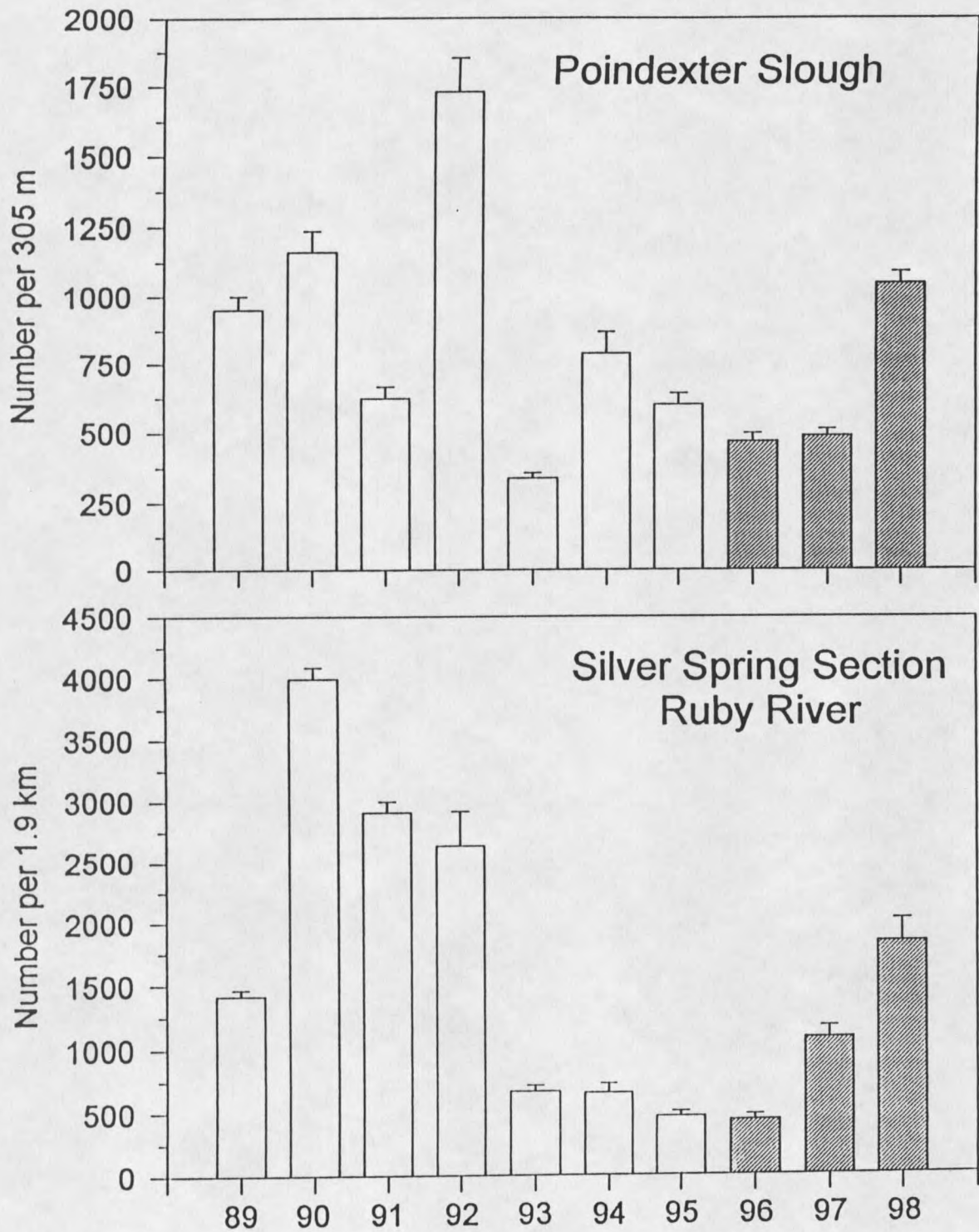


Figure 11. Brown trout population abundance estimates (+1 SD) at Poindexter Slough and the Silver Spring section of the Ruby River made by FWP from 1989 through 1998. The shaded bars represent the years during and after the study.

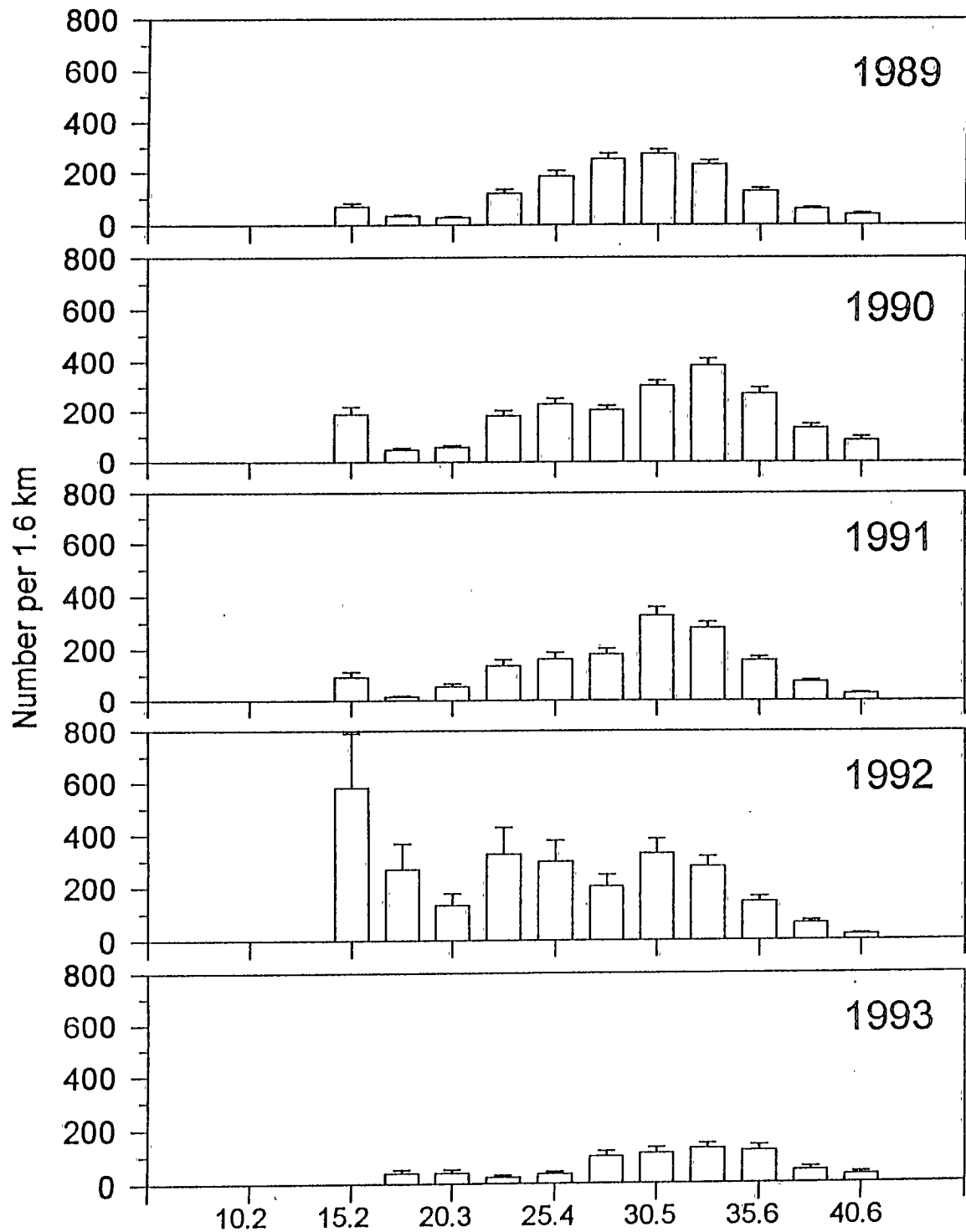


Figure 12. Number of brown trout (+ 1 SD) for each length group (cm) at the Silver Springs section of the Ruby River from 1989 through 1993.

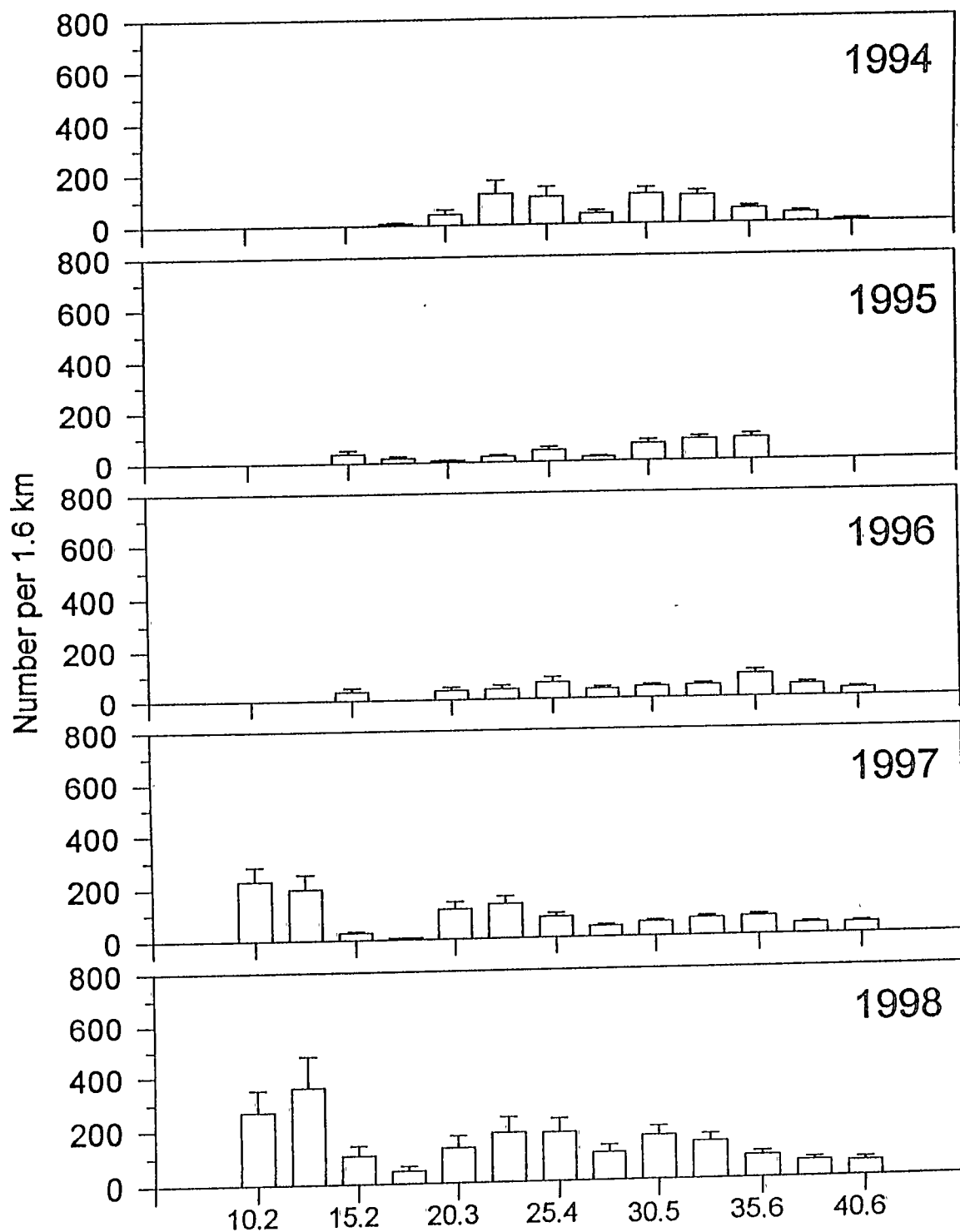


Figure 13. Number of brown trout (+ 1 SD) for each length group (cm) at the Silver Springs section of the Ruby River from 1994 through 1998.

abundances was observed from 1993-1996. Overall abundance began to increase in 1997 and continued in 1998 (Figure 11).

### Abundance Monitoring

#### Woodson Study Site

Mean abundances at the Woodson site in the 1996-97 field season gradually declined from July to October with some fluctuation in August and October. Mean abundance was highest in August at 24 (SD 10) brown trout per 50 m and was lowest in October at 13 (SD 5) brown trout per 50 m (Figure 14). Overwinter survival was not estimated in the 1996-97 field season because of late snowfalls followed by early runoff in the spring that prevented sampling. A gradual decline with some fluctuation was also seen in the 1997-98 field season. Mean abundances had high variability on July 18 and September 12. Mean abundance was highest in July at 71 (SD 40) age-0 brown trout per 50 m and lowest in March at 1 (SD 1) age-0 brown trout per 50 m (Figure 14). Overwinter survival was 6% from October 1997 to March 1998. Mean abundances, at the Woodson site, tended to be higher in the 1997-98 field season than during the 1996-97 field season.

Mean lengths of age-0 brown trout at the Woodson site gradually increased from July to October in the 1996-97 field season. The mean length was 65.5 mm (SD 9.6) in July and reached 92.9 mm (SD 13.4) in October (Figure 15). Mean lengths of brown trout in the 1997-98 field season gradually increased from July through March. The mean length of age-0 brown trout was 64.6 mm (SD 9.6) in July and reached 108.3 mm (SD



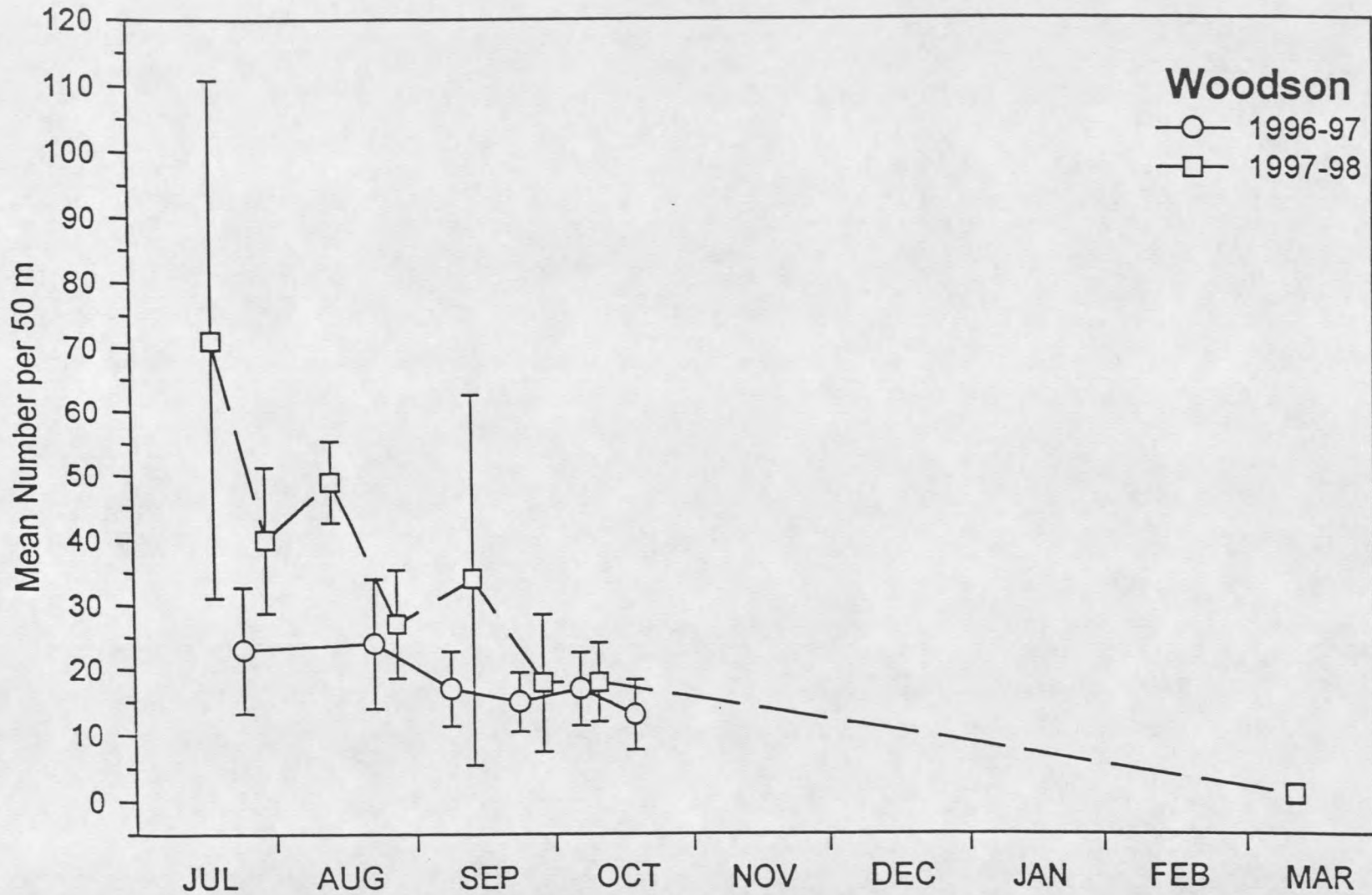


Figure 14. Mean abundances of age-0 brown trout ( $\pm 1$  SD) for the 1996-97 and 1997-98 field seasons at the Woodson site.

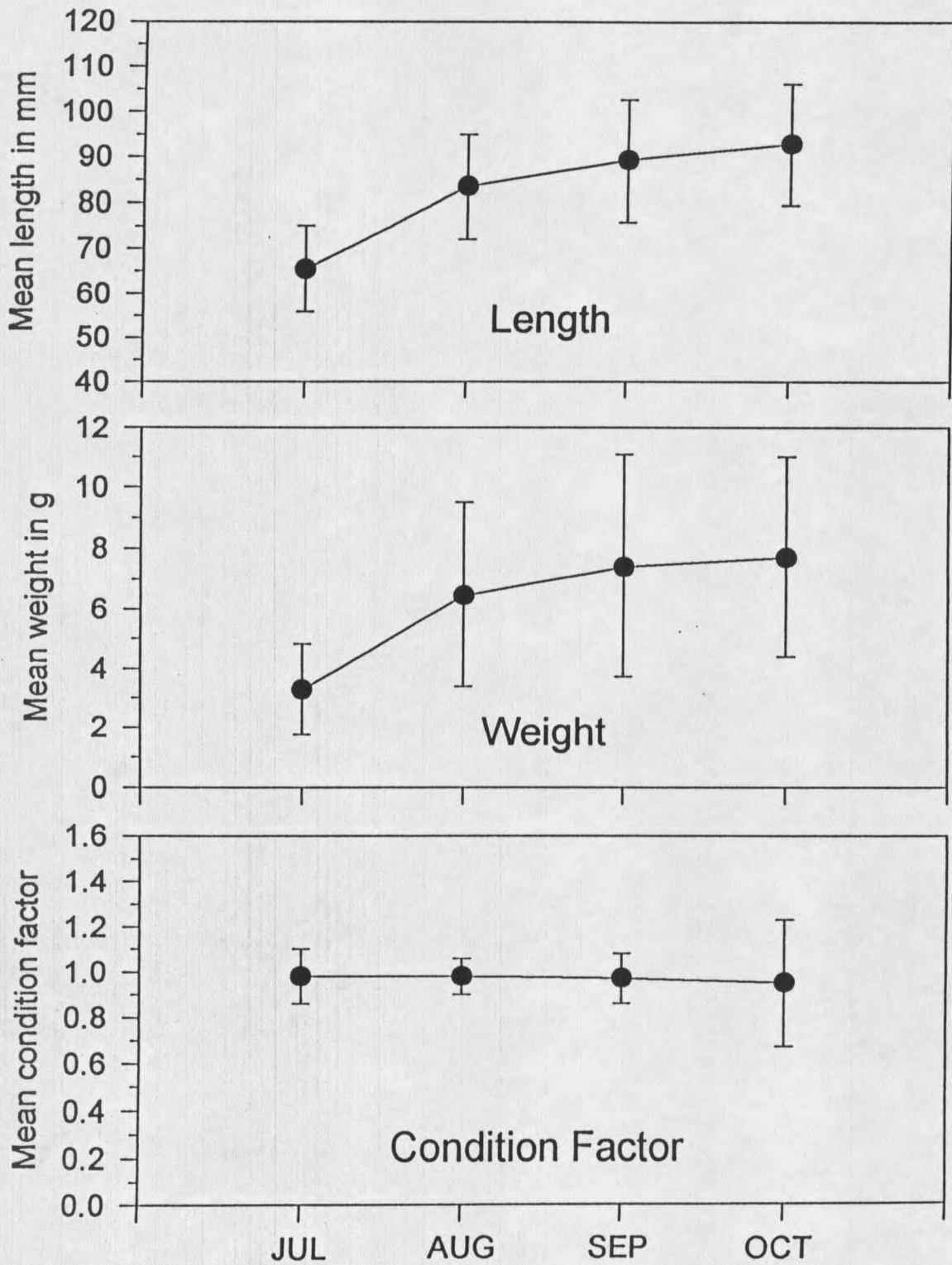


Figure 15. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Woodson site during the 1996-97 field season.

20.6) in March (Figure 16). Mean lengths were higher in the 1997-98 field season than the 1996-97 field season. Mean weights of age-0 brown trout gradually increased from 3.3 g (SD 1.5) in July to 7.7 g (SD 3.3) in October during the 1996-97 field season (Figure 15). The mean weight of age-0 brown trout, in the 1997-98 field season, increased from 3.8 g (SD 1.4) in July to 11.2 g (SD 5.3) in March (Figure 16). Mean weights in the 1997-98 field season were also higher than those in the 1996-97 field season. The mean condition factor was constant from July through September within a range of 0.98 (SD 0.12) to 0.96 (SD 0.11) and decreased slightly to 0.95 (SD 0.28) in October (Figure 15). The mean condition factor gradually decreased during the 1997-98 field season from 1.03 (SD 0.10) in July to 0.84 (SD 0.04) in March and was higher than the 1996-97 field season (Figure 16).

During the 1996-97 field season, 422 age-0 brown trout were examined for clinical signs at the Woodson site. Of those, 6.9% showed some form of clinical sign. Clinical signs were only seen in 5.8% of the 806 brown trout examined during the 1997-98 field season (Table 1). Blacktail made up the majority of the clinical signs observed in the 1996-97 field season and more than 75% of those seen in the 1997-98 field season. Jaw deformities made up the next highest percentage of clinical signs observed during both field seasons. Combinations of clinical signs were also observed in a few fish collected during both field seasons (Table 2). A gradual increase in the percentage of age-0 brown trout showing clinical signs each month was seen from July to October during the 1996-97 field season at the Woodson site. No obvious trend was seen during the 1997-98 season. No clinical signs were observed in March of 1998 (Figure 17).

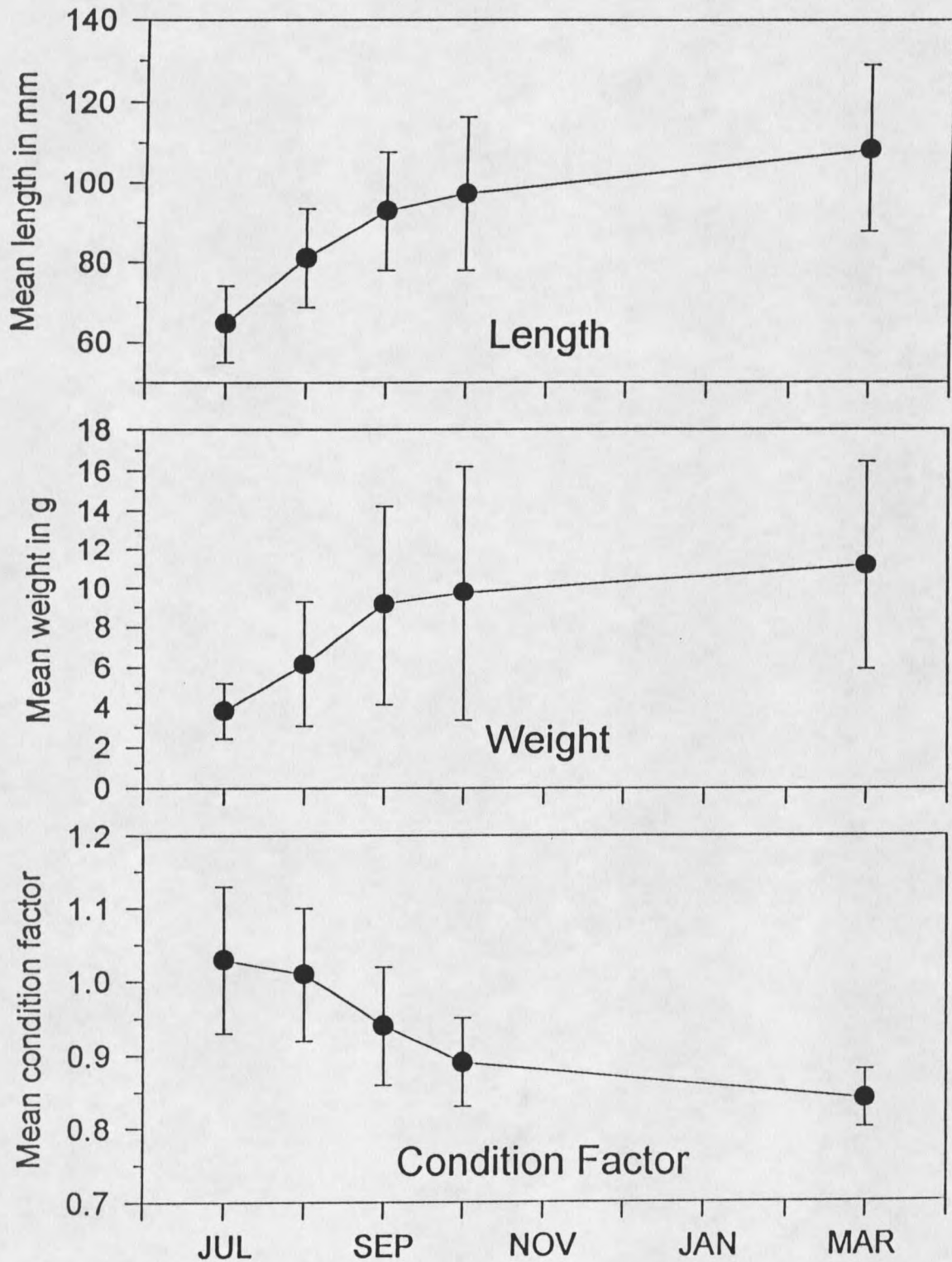


Figure 16. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Woodson site during the 1997-98 field season.

## WOODSON

Field Season	Number of Fish Examined	Number of Fish With Clinical Signs	Percentage of Fish With Clinical Signs
1996-97*	422	29	6.9%
1997-98	806	47	5.8%

## BARNOSKY

Field Season	Number of Fish Examined	Number of Fish With Clinical Signs	Percentage of Fish With Clinical Signs
1996-97*	360	15	4.9%
1997-98	467	16	3.4%

## POINDEXTER

Field Season	Number of Fish Examined	Number of Fish With Clinical Signs	Percentage of Fish With Clinical Signs
1996-97*	1423	32	2.2%
1997-98	2953	21	0.7%

Table 1. Number of age-0 brown trout that were examined during the fall and spring for clinical signs of whirling disease as well as the number of and percentage that showed clinical signs at each study site. \* No examinations were conducted in the spring.

Field Season	Number of Fish With Clinical Signs	Blacktail (%)	Jaw Deformities (%)	Short Operculum (%)	Bent Caudal (%)	Blacktail and Black Head (%)	Blacktail and Bent Caudal (%)
1996-97	29	48.3	44.8	13.4	0	3.4	0
1997-98	47	76.6	14.9	0	2.1	4.3	2.1

Table 2. Number of age-0 brown trout showing clinical signs of whirling disease as well as the percentage of each clinical sign observed during the 1996-97 and 1997-98 field seasons at the Woodson site.

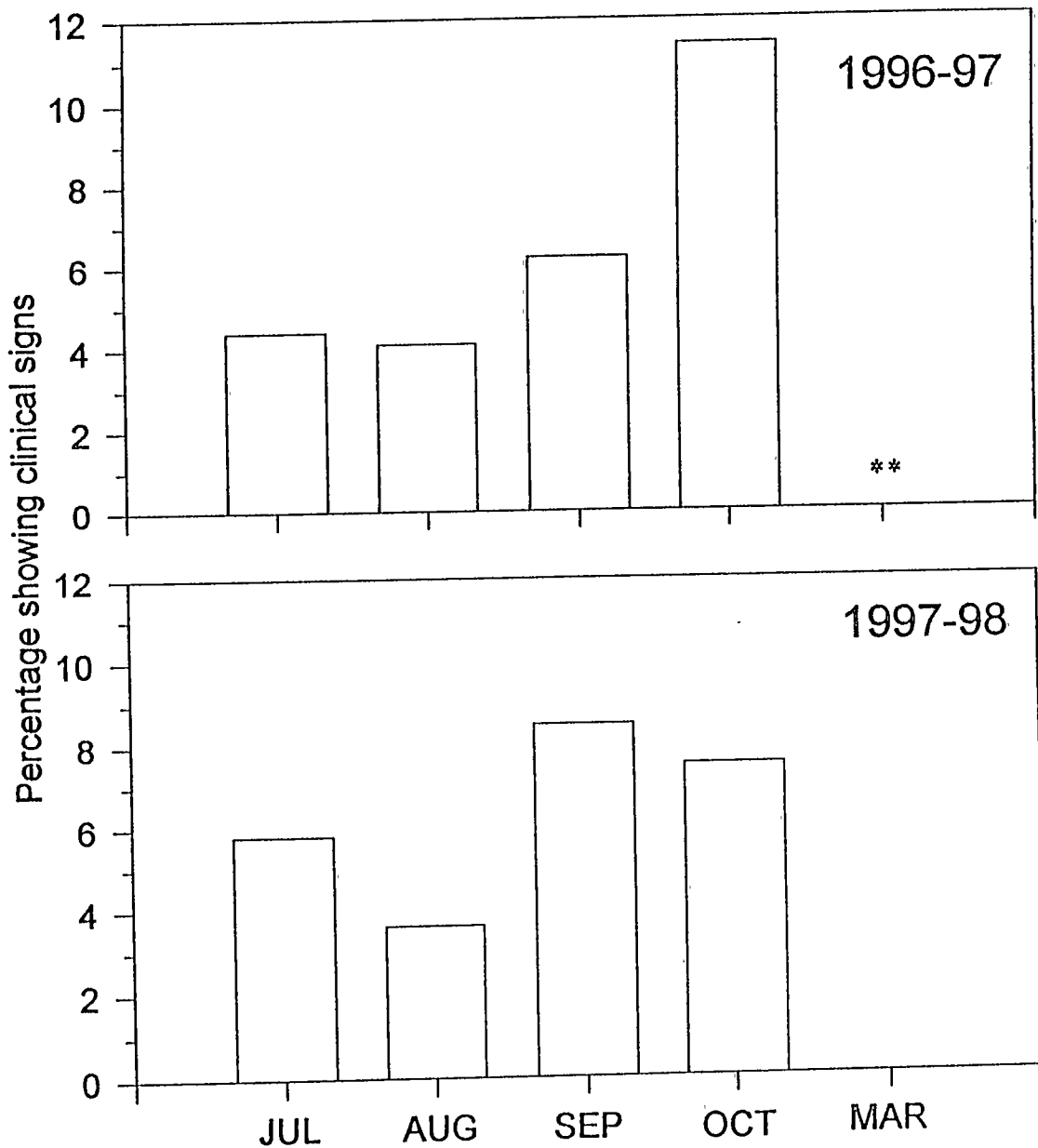


Figure 17. Percentage of age-0 brown trout that were examined each month at the Woodson site, during the 1996-97 and 1997-98 field seasons, that showed clinical signs of whirling disease. \*\* No monitoring was done.

Histological examination of brown trout that included both clinical and nonclinical fish collected during the 1996-97 field season revealed that all of the fish had either a Grade 0 or Grade 1 infection of *M. cerebralis* (Table 3). Brown trout that showed clinical signs of whirling disease that were collected during the 1997-98 field season showed a range of infection from Grade 0 to Grade 4. No Grade 1 infections were seen in any of the fish and the majority of infections were Grade 2 and higher (Table 4). Grade 0 infections made up 23.4% of the clinical fish examined. Nonclinical fish collected and examined during the 1997-98 showed only Grade 0 and Grade 1 infections of *M. cerebralis* with one exception. A nonclinical fish collected in September had a Grade 2 infection. The majority of infections were Grade 0 and Grade 1 infections only made up 32% of the nonclinical fish examined (Table 5).

#### Barnosky Study Site

Mean abundances of age-0 brown trout in the 1996-97 field season at the Barnosky site declined gradually from July through October with some variability. Abundance was highest at 16 (SD 12) per 50 m in August and declined to 6 (SD 6) per 50 m in October (Figure 18). Overwinter survival was not estimated for the 1996-1997 field season due to late snowfalls and early runoff in the spring that did not allow for sampling. Mean abundances of age-0 brown trout, in the 1997-98 field season, fluctuated and showed occasional high variability from July to March. Abundance was highest at 25 (SD 7) brown trout per 50 m in August and 25 (SD 11) per 50 m on October 31 and lowest in March at 3 (SD 4) per 50 m (Figure 18). In the 1997-98 field season, overwinter survival



## WOODSON

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	4	1	3	0	0	0
September	2	1	1	0	0	0
October	7	4	3	0	0	0

## BARNOSKY

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	5	2	1	2	0	0
September	3	1	1	1	0	0
October	1	1	0	0	0	0

## POINDEXTER

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	4	4	0	0	0	0
September	11	7	4	0	0	0
October	3	3	0	0	0	0

Table 3. Number of age-0 brown trout that were examined and exhibited each histological grade of infection, by month, from the Woodson, Barnosky, and Poindexter sites in 1996.

## WOODSON

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	20	3	0	7	4	6
August	8	3	0	0	1	4
September	16	5	0	2	4	5
October	3	0	0	1	0	2

## BARNOSKY

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	5	1	0	2	2	0
September	5	2	1	0	1	1
October	5	2	0	0	2	1
March	1	1	0	0	0	0

## POINDEXTER

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
June	2	1	0	1	0	0
July	8	2	0	3	2	1
August	6	0	1	4	0	0
September	3	2	1	0	0	0
October	1	1	0	0	0	0
November	1	0	1	0	0	0

Table 4. Number of age-0 brown trout showing clinical signs of whirling disease that were examined and exhibited each histological grade of infection, by month, from the Woodson, Barnosky, and Poindexter sites in the 1997-98 field season.

## WOODSON

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	25	19	6	0	0	0
August	23	16	7	0	0	0
September	18	8	9	1	0	0
October	8	6	2	0	0	0
March	4	3	1	0	0	0

## BARNOSKY

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	17	16	1	0	0	0
August	33	27	6	0	0	0
September	12	7	5	0	0	0
October	26	17	9	0	0	0
March	4	3	0	0	1	0

Table 5. Number of nonclinical, age-0 brown trout that were examined and exhibited each histological grade of infection, by month, from the Woodson and Barnosky sites in the 1997-98 field season.

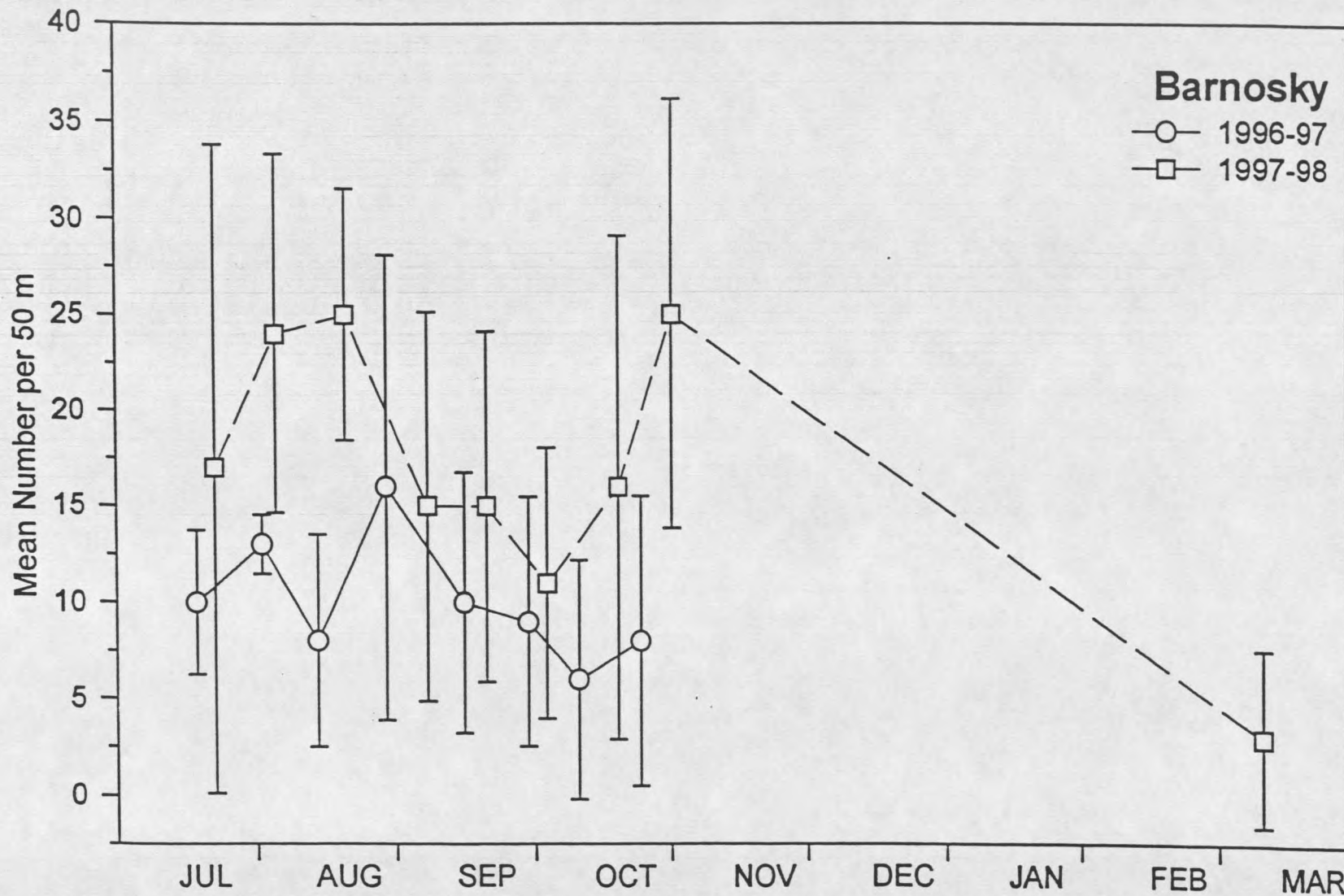


Figure 18. Mean abundances of age-0 brown trout ( $\pm 1$  SD) for the 1996-97 and 1997-98 field seasons at the Barnosky site.

from October 1997 to March 1998 was 12%. Abundances in the 1997-98 field season were higher than the abundances in the 1996-97 field season.

Mean length of age-0 brown trout increased gradually from July through October in the 1996-97 field season. The mean length was 62.7 mm (SD 7.0) in July and increased to 102.4 mm (SD 13.5) in October (Figure 19). Mean lengths of brown trout in the 1997-98 field season increased gradually from 71.5 mm (SD 10.1) in July to 125.9 mm (SD 24.1) in March (Figure 20). The mean weight of brown trout during the 1996-97 field season gradually increased from 2.7 g (SD 0.9) in July to a high of 10.3 g (SD 4.5) in October (Figure 19). The mean monthly weight of age-0 brown trout during the 1997-98 field season increased from 3.7 (SD 1.7) in July to 17.6 (SD 9.6) in March (Figure 20). The mean monthly condition factor of age-0 brown trout declined slightly from 1.02 (SD 0.18) in July to 0.92 (SD 0.07) in October during the 1996-97 field season (Figure 19). The mean condition factor of brown trout during the 1997-98 field season was fairly constant from July through October and declined into March. The mean condition factor was highest in August at 0.97 (SD 0.14) and lowest in March at 0.81 (SD 0.07) (Figure 20). The mean condition factor was lower in the 1997-98 field season than in the 1996-97 field season.

At the Barnosky site, 306 age-0 brown trout from the 1996-97 field season were examined for possible clinical signs of whirling disease. Only 4.9% showed some form of clinical signs (Table 1). Of the 467 age-0 brown trout that were examined in the 1997-98 field season, clinical signs were observed in 3.3% (Table 1). Blacktail made up more than half the clinical signs in the 1996-97 field season and 75% of those in the 1997-98 field

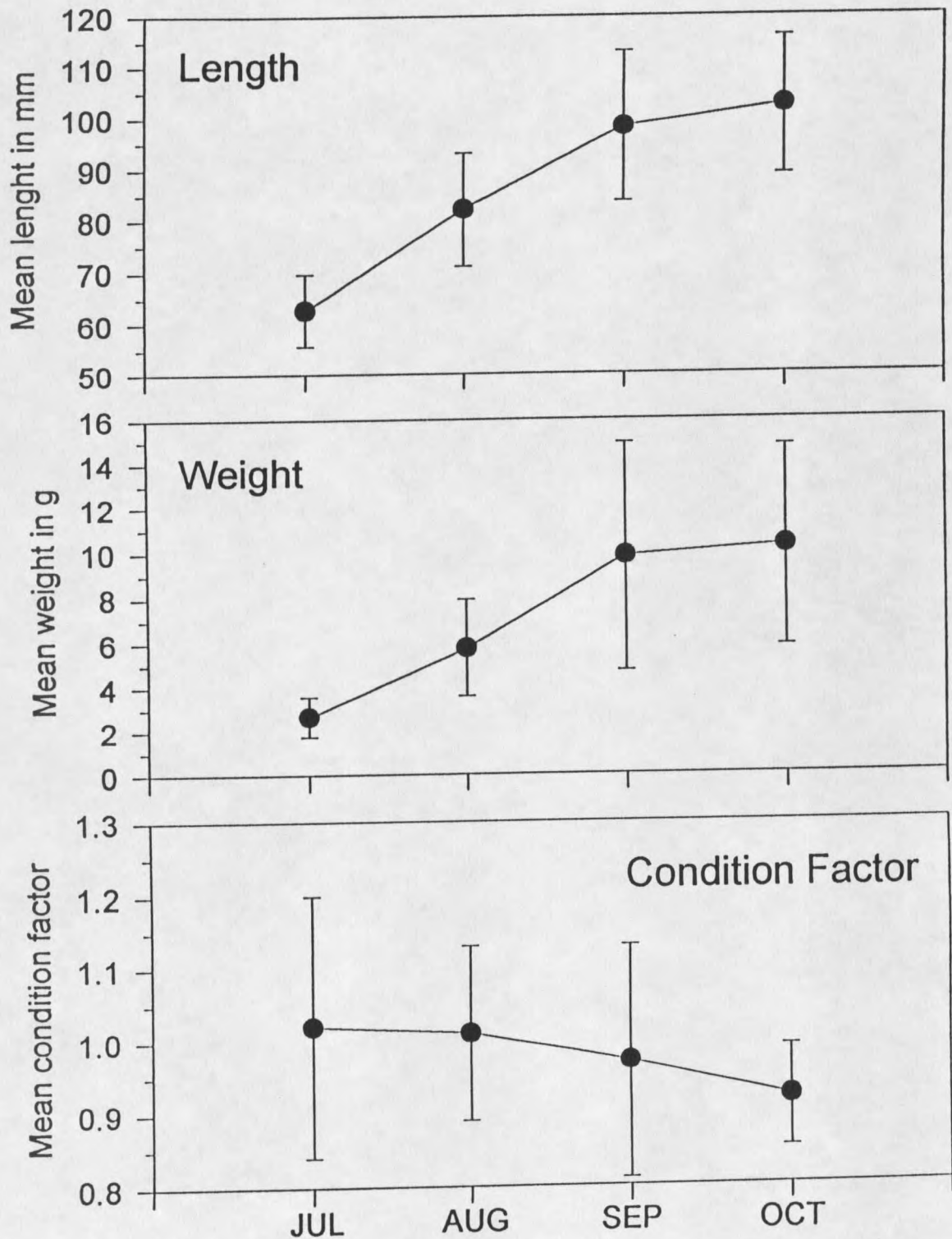


Figure 19. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Barnosky site during the 1996-97 field season.

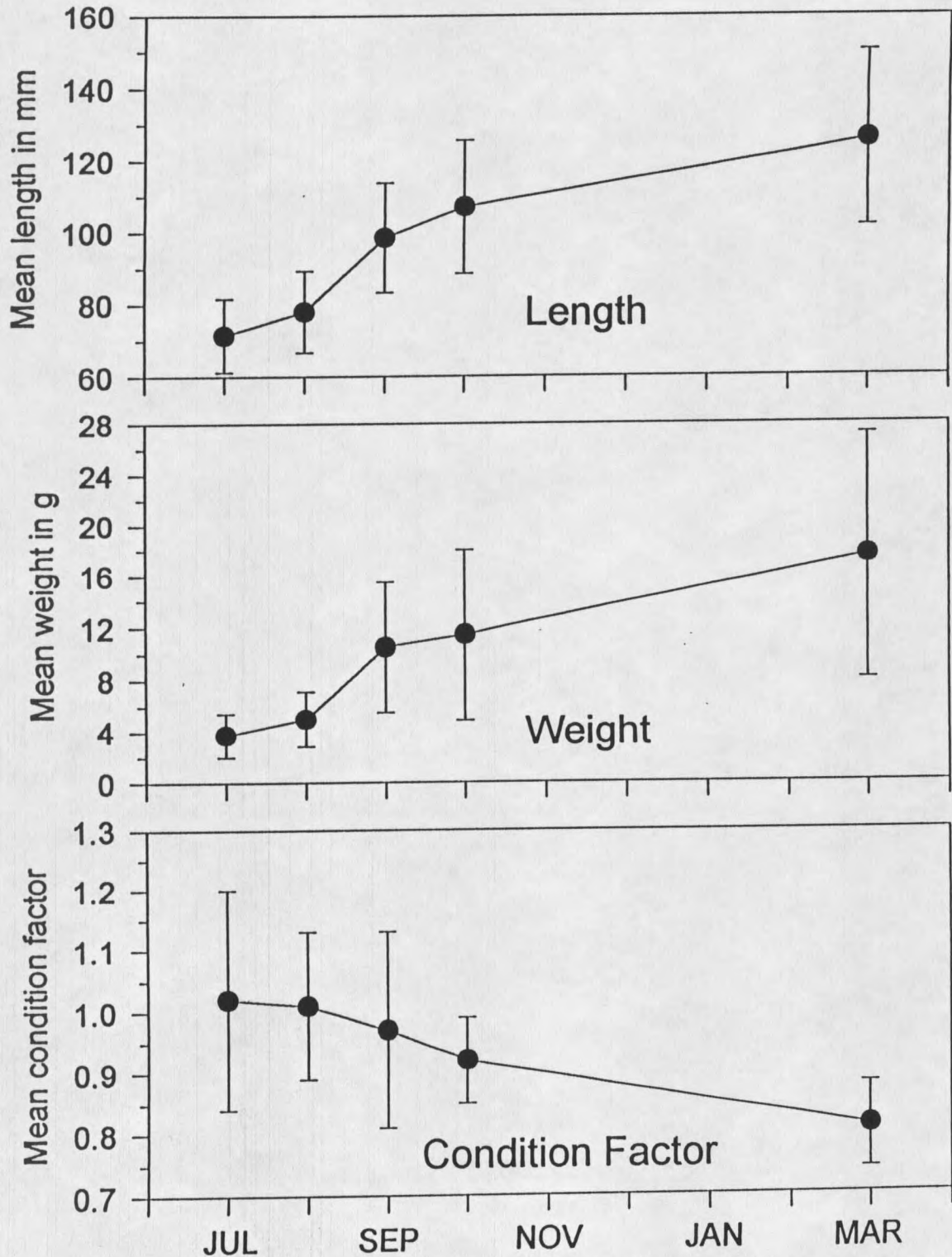


Figure 20. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Barnosky site during the 1997-98 field season.

season (Table 6). Jaw deformities made up the next highest percentage of clinical signs. Brown trout were observed with a combination of clinical signs during both field seasons and were among the smallest percentages of clinical signs observed (Table 6). No obvious trends in the percentage of age-0 brown trout showing clinical signs each month were seen during either field season (Figure 21).

Brown trout that were collected during the 1996-97 field season at the Barnosky site and were examined histologically showed infections ranging from Grade 0 to Grade 2 (Table 3). Brown trout that showed clinical signs of whirling disease that were collected during the 1997-98 field season had infections ranging from Grade 0 to Grade 4 (Table 4). Grade 0 infections made up 37.5% of these fish. The nonclinical fish that were examined had Grade 0, 1, and 3 infections. The Grade 1 infections made up 23% of the examined fish. There was only one Grade 3 infected fish (Table 5).

#### Poindexter Study Site

Mean abundances during the 1996-97 field season, at the Poindexter site, declined from July 1996 through March 1997. Mean abundance was highest at 70 (SD 32) age-0 brown trout per 50 m in September and lowest at 11 (SD 11) age-0 brown trout per 50 m in March (Figure 22). Overwinter survival from November 1996 to March 1997 was 55%. Mean abundance during the 1997-98 field season was the highest at 195 (SD 197.0) age-0 brown trout per 50 m in June. The lowest abundance was 15 (SD 10.4) per 50 m in



Field Season	Number of Fish With Clinical Signs	Blacktail (%)	Jaw Deformities (%)	Bent Caudal (%)	Cranial Deformities (%)	Blacktail and Short Operculum (%)	Blacktail and Bent Caudal (%)
1996-97	15	53.3	26.7	6.7	6.7	6.7	0
1997-98	16	75.0	12.5	0	6.3	0	6.3

Table 6. Number of age-0 brown trout showing clinical signs of whirling disease as well as the percentage of each clinical sign observed during the 1996-97 and 1997-98 field seasons at the Barnosky site.

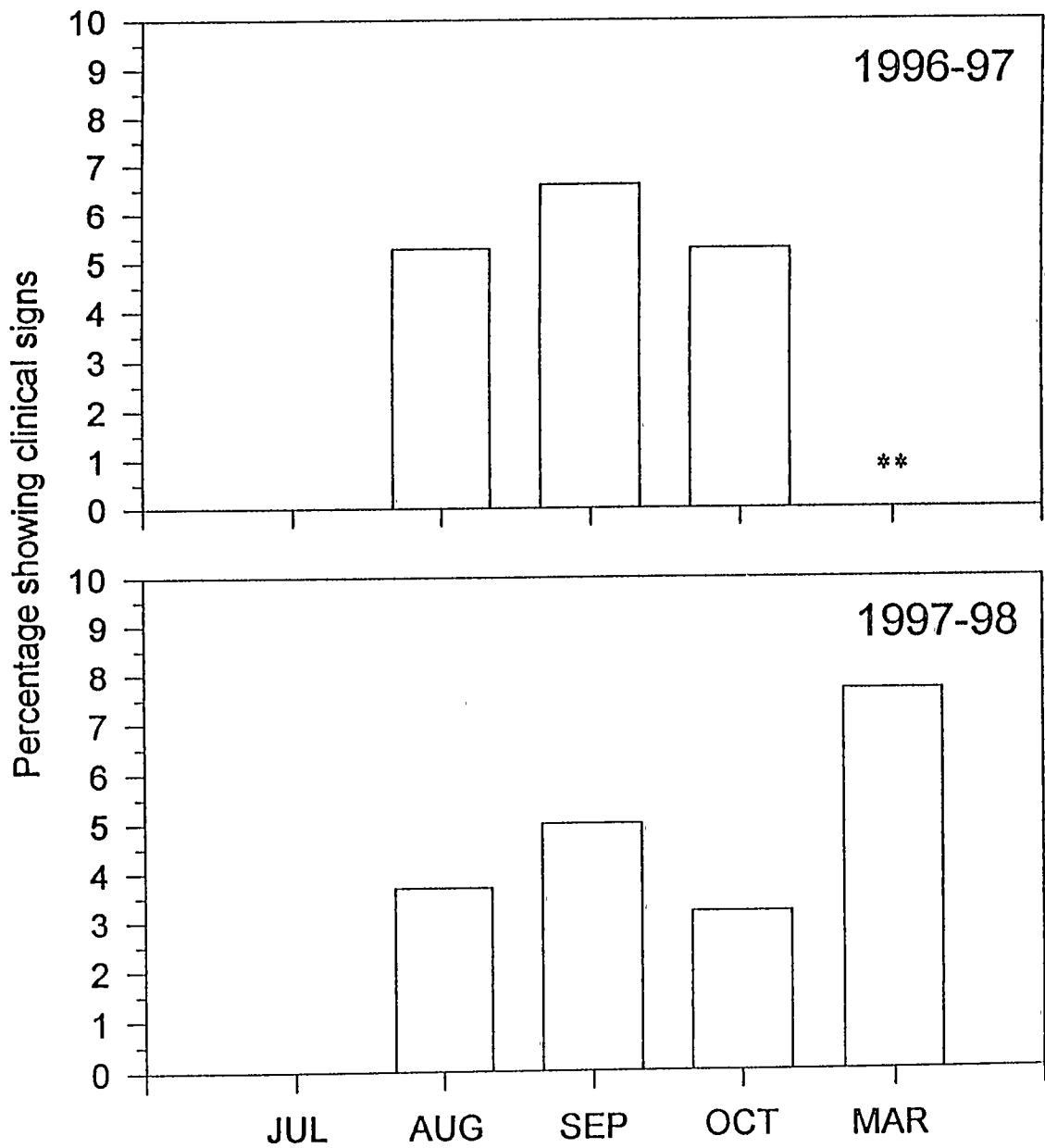


Figure 21. Percentage of age-0 brown trout that were examined each month at the Barnosky site, during the 1996-97 and 1997-98 field seasons, that showed clinical signs of whirling disease. \*\* No monitoring was done.

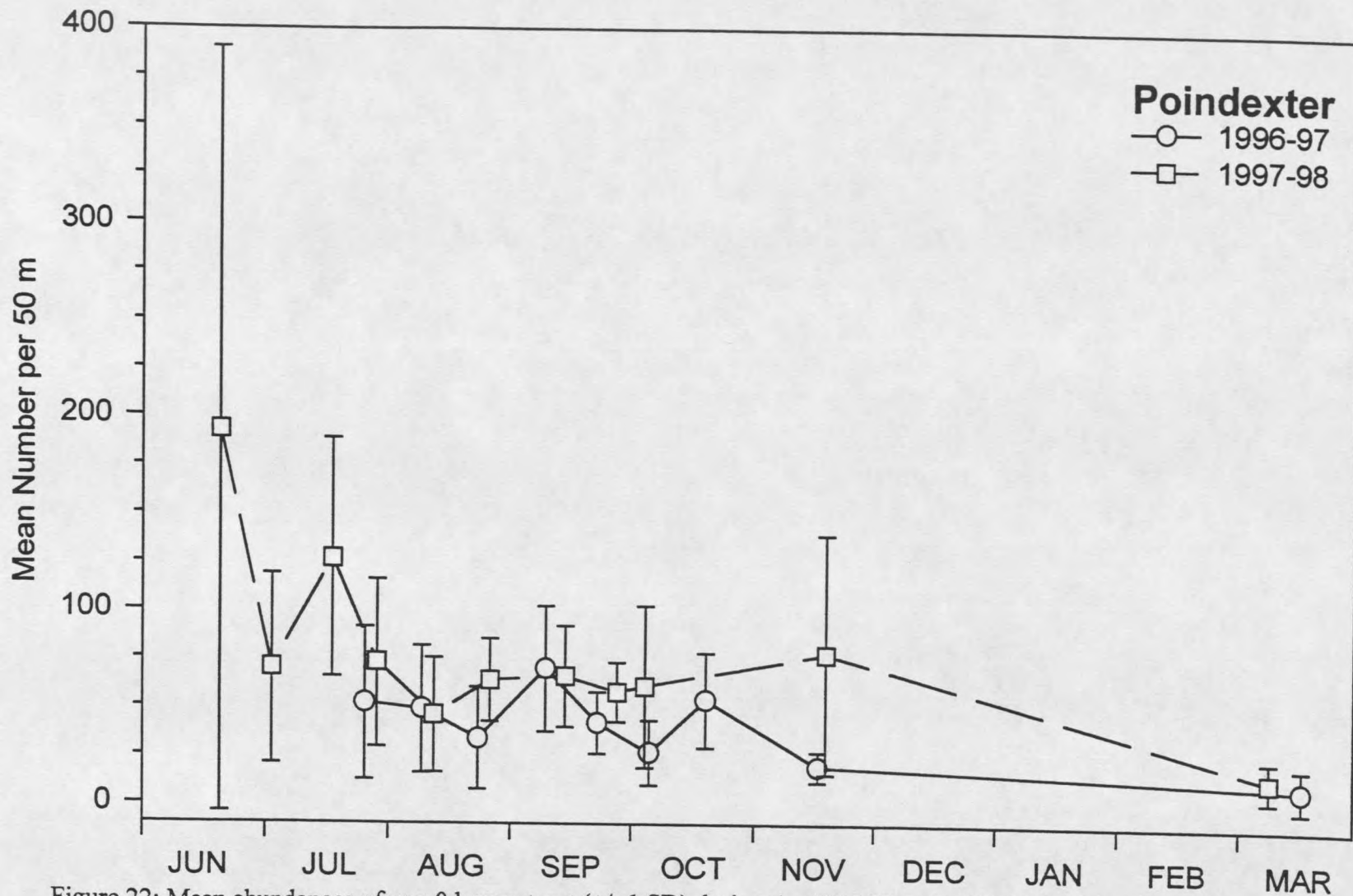


Figure 22: Mean abundances of age-0 brown trout ( $\pm 1$  SD) during the 1996-97 and 1997-98 field seasons at the Poindexter site.

March (Figure 22). Abundances in the 1997-98 field season were higher than those in the 1996-97 field season. Overwinter survival of age-0 brown trout was 19% in the 1997-98 field season.

Mean lengths of age-0 brown trout increased from July to November and then declined slightly in March during the 1996-97 field season. Mean length was lowest at 68.5 mm (SD 12.6) in July and highest at 114.1 mm (SD 21.8) in November (Figure 23). Mean lengths in the 1997-98 field season increased gradually from a low of 49.6 mm (SD 8.1) in June to a high of 127.2 mm (SD 25.6) in March (Figure 24). Mean lengths were higher than those in the 1996-97 field season. The mean weights of brown trout during the 1996-97 field season increased from a low of 3.7 g (SD 2.4) in July to a high of 15.7 g (SD 9.1) in November (Figure 23). Mean weights of age-0 brown trout during the 1997-98 field season gradually increased from June to October and then declined in November. They increased again in March. Mean weights were lowest at 1.9 g (SD 0.9) in June and highest at 16.8 g (SD 11.9) in October (Figure 24). Mean weights were higher in the 1997-98 field season than the 1996-97 field season. The mean condition factor increased slightly from July to August and then declined gradually through March during the 1996-97 field season. Mean condition factor was highest at 1.0 (SD 0.11) in August and lowest at 0.84 (SD 0.07) in March (Figure 23). During the 1997-98 field season, the mean condition factor was constant from June to August and then fluctuated from September to March. The mean condition factor of brown trout was highest at 1.05 (SD 0.18) in July and lowest at 0.79 (SD 0.09) in March (Figure 24). There was little difference in mean condition factor during both field seasons.

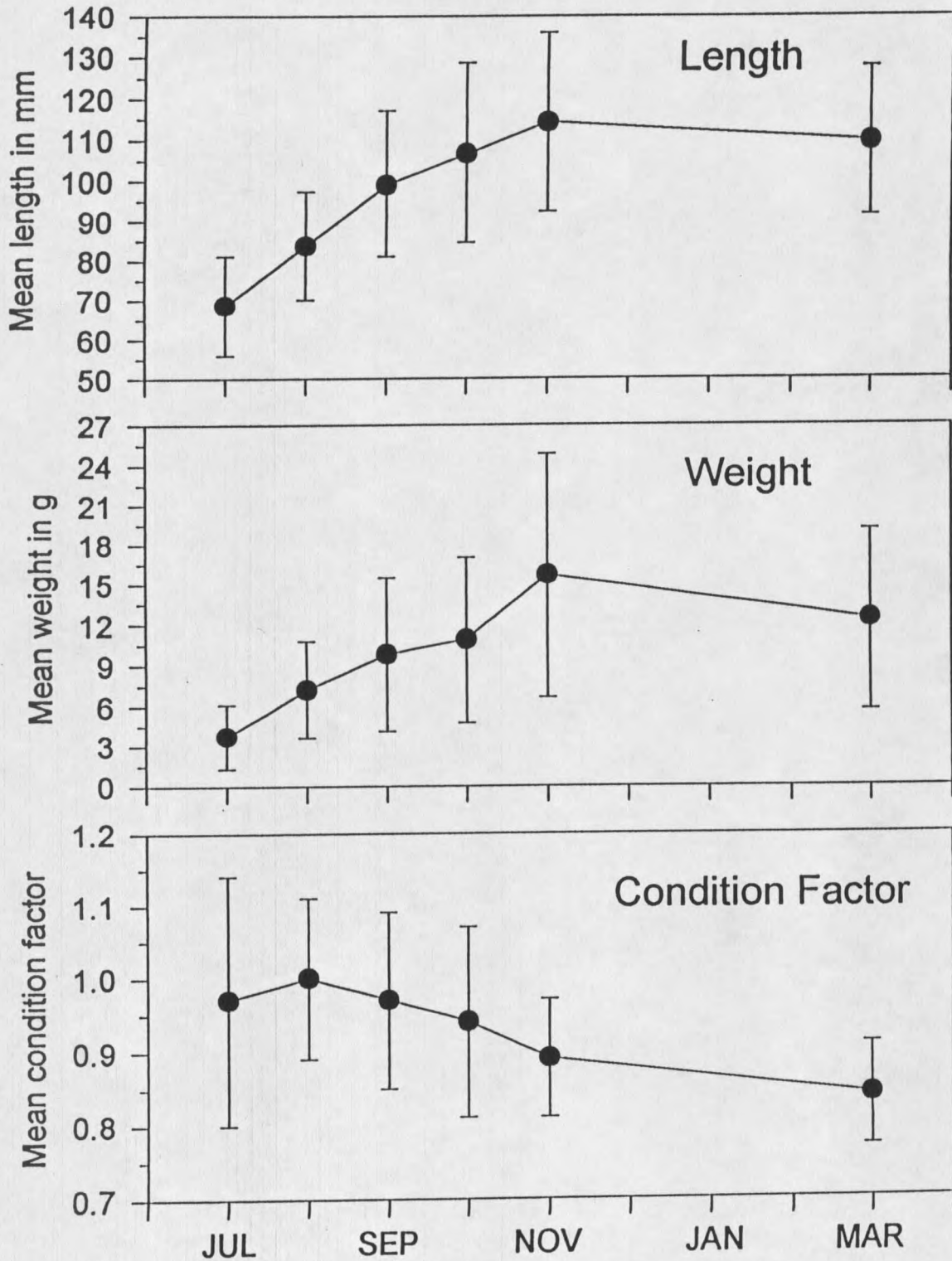


Figure 23. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Poindexter site during the 1996-97 field season.

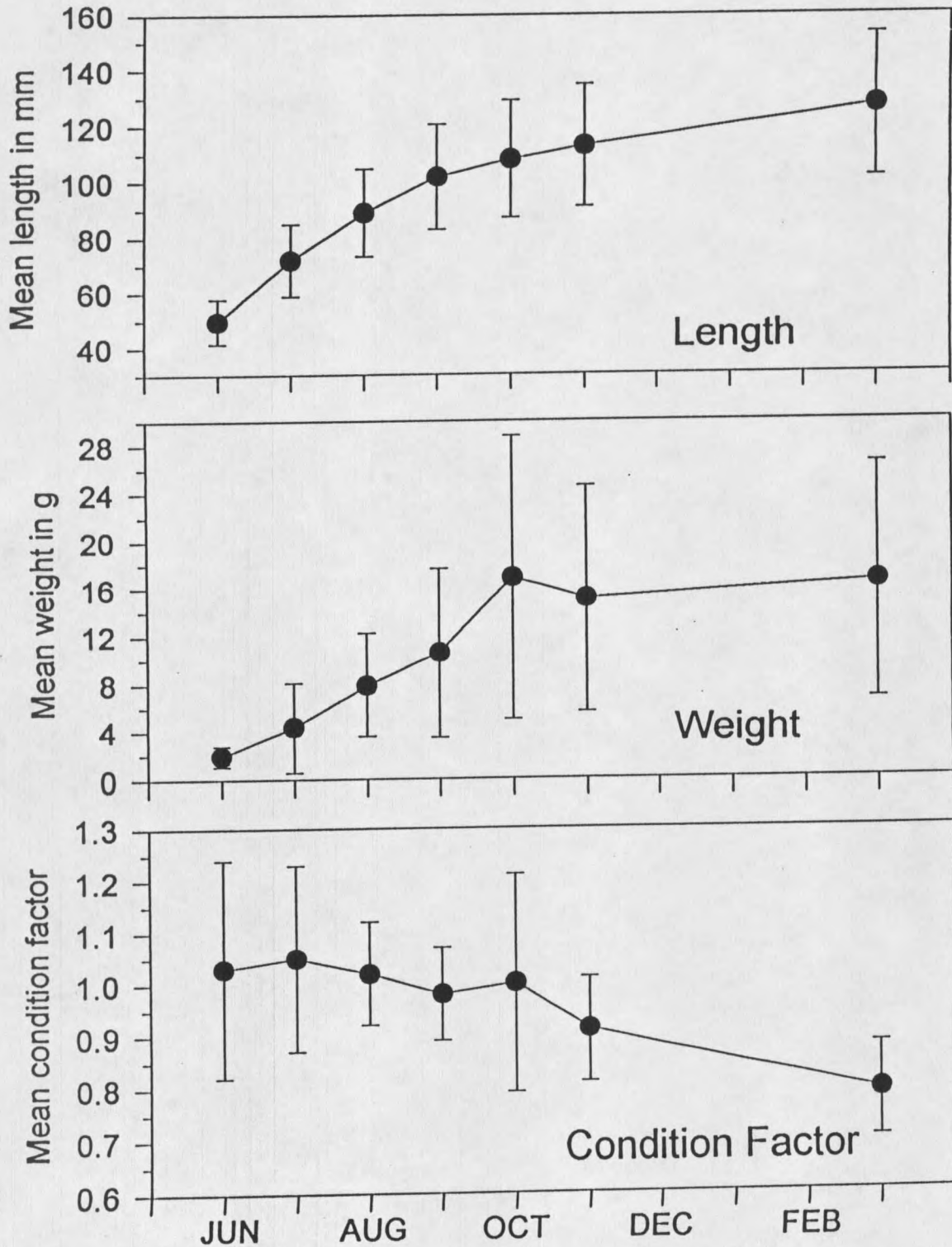


Figure 24. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Poindexter site during the 1997-98 field season.

At the Poindexter site, 1,423 age-0 brown trout were examined for clinical signs during the 1996-97 field season. Clinical signs were observed in 2.2% of them. Of the 2,953 age-0 brown trout examined during the 1997-98 field season, clinical signs were observed in 0.7% (Table 1). Blacktail made up the largest percentage of clinical signs observed in both field seasons. The next highest percentage of clinical signs was made up by jaw deformities in the 1996-97 field season and shortened opercula in the 1997-98 field season (Table 7). Some brown trout examined during both field seasons exhibited a combination of more than one clinical sign. These combinations made up the smallest percentage of clinical signs seen in both field seasons (Table 7). A gradual increase in the percentage of age-0 brown trout showing clinical signs each month was seen from August to March during the 1996-97 field season. No clinical signs were observed in July. No obvious trend was seen in the 1997-98 field season and no clinical signs were observed in March (Figure 25).

Age-0 brown trout collected during the 1996-97 field season and examined histologically had infections that ranged from Grade 0 to Grade 4. Of the 18 fish examined, 4 showed a Grade 1 infection of *M. cerebralis* (Table 3). Brown trout that showed clinical signs of whirling disease and were collected during the 1997-98 field season had infections ranging from Grade 0 to Grade 4. The majority of fish examined showed Grade 1 or higher infections (Table 4). Grade 0 infections made up 28.6% of the clinical fish examined. Most nonclinical fish collected and examined had Grade 0 infections. A small number of fish collected in July through November did have Grade 1

Field Season	Number of Fish With Clinical Signs	Blacktail (%)	Jaw Deformity (%)	Short Operculum (%)	Cranial Deformity (%)	Bent Caudal (%)	Blacktail and Bent Caudal (%)	Blacktail and Jaw Deformity (%)	Jaw Deformity and Short Operculum (%)	Blacktail and Cranial Deformity (%)
1996-97	28	39.3	32.1	3.6	7.1	7.1	3.6	3.6	0	3.6
1997-98	26	42.3	11.5	19.2	11.5	11.5	0	0	7.8	0

Table 7. Number of age-0 brown trout showing clinical signs of whirling disease as well as the percentage of each clinical sign observed during the 1996-97 and 1997-98 field seasons at the Poindexter site.



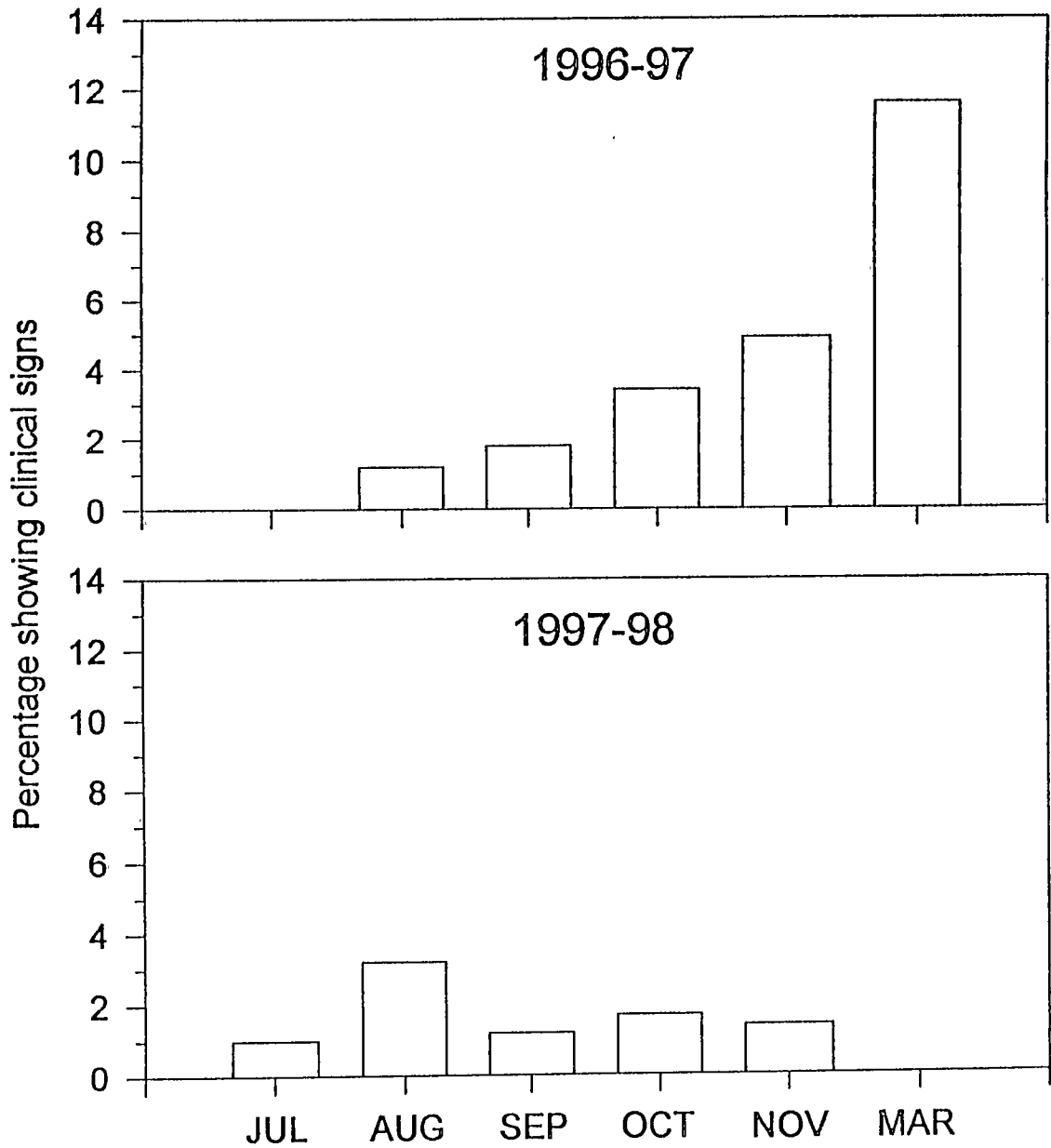


Figure 25. Percentage of age-0 brown trout that were examined each month at the Poindexter site, during the 1996-97 and 1997-98 field seasons, that showed clinical signs of whirling disease.

infections. These made up 9% of the nonclinical fish examined (Table 8). One nonclinical rainbow trout was collected and examined in October and was found to have a no infection.

### Snowcrest Study Site

Mean abundances of age-0 brown trout at the Snowcrest site decreased gradually with some fluctuation from July to November. High variability was seen in July and September. Overall abundances were low compared to the other study sites. Mean abundance was highest at 6 (SD 7) age-0 brown trout per 50 m in July and lowest at 1 (SD 1) age-0 per 50 m in November (Figure 26). No overwinter survival estimate was made at the Snowcrest site in the 1997-98 field season because of an extremely wet spring and inability to access the study site.

Mean length and weight of age-0 brown trout at the Snowcrest site increased from July to August. They then remained stable from August to October and increased again in November. Mean length of age-0 brown trout was lowest at 73.5 mm (SD 6.1) in July and highest at 125.8 mm (SD 15.8) in November (Figure 27). Mean monthly weight of age-0 brown trout was lowest at 4.4 g (SD 1.1) in July and highest at 17.7 g (SD 5.6) in November (Figure 27). The mean condition factor declined from July to November with some fluctuation. The mean monthly condition factor was highest at 1.09 (SD 0.16) in July and lowest at 0.84 (SD 0.05) in October (Figure 27).

No clinical signs were observed in the 109 age-0 brown trout and 10 age-0 rainbow trout collected at the Snowcrest site in the 1997-98 field season.

## POINDEXTER

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
June	9	9	0	0	0	0
July	50	48	2	0	0	0
August	28	25	3	0	0	0
September	26	23	3	0	0	0
October	25	21	4	0	0	0
November	1	0	1	0	0	0
March	12	12	0	0	0	0

Table 8. Number of nonclinical, age-0 brown trout that were examined and exhibited each histological grade of infection, by month, from the Poindexter site in the 1997-98 field season.

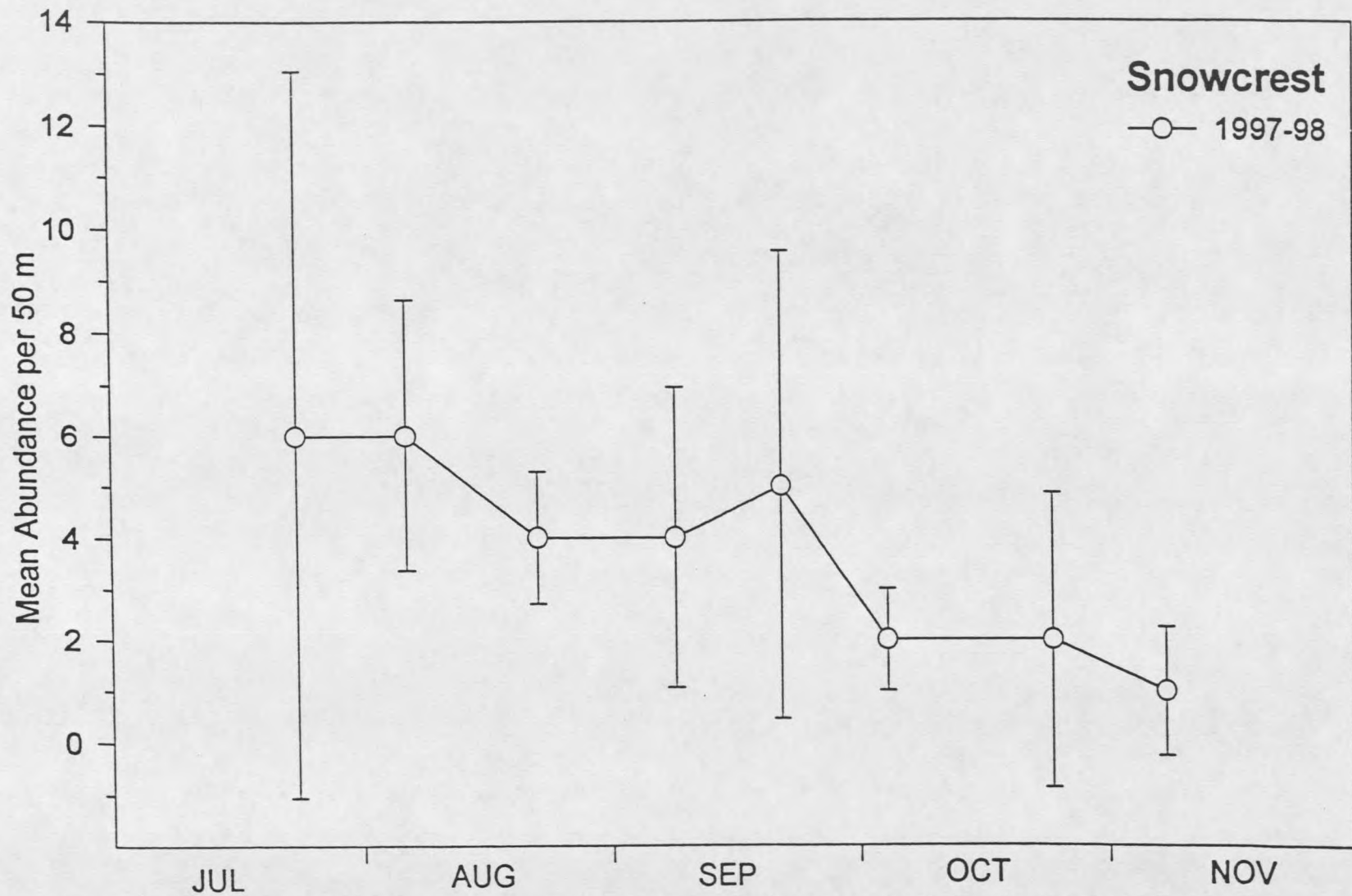


Figure 26. Mean abundances of age-0 brown trout (+/- 1 SD) at the Snowcrest site during the 1997-98 field season.

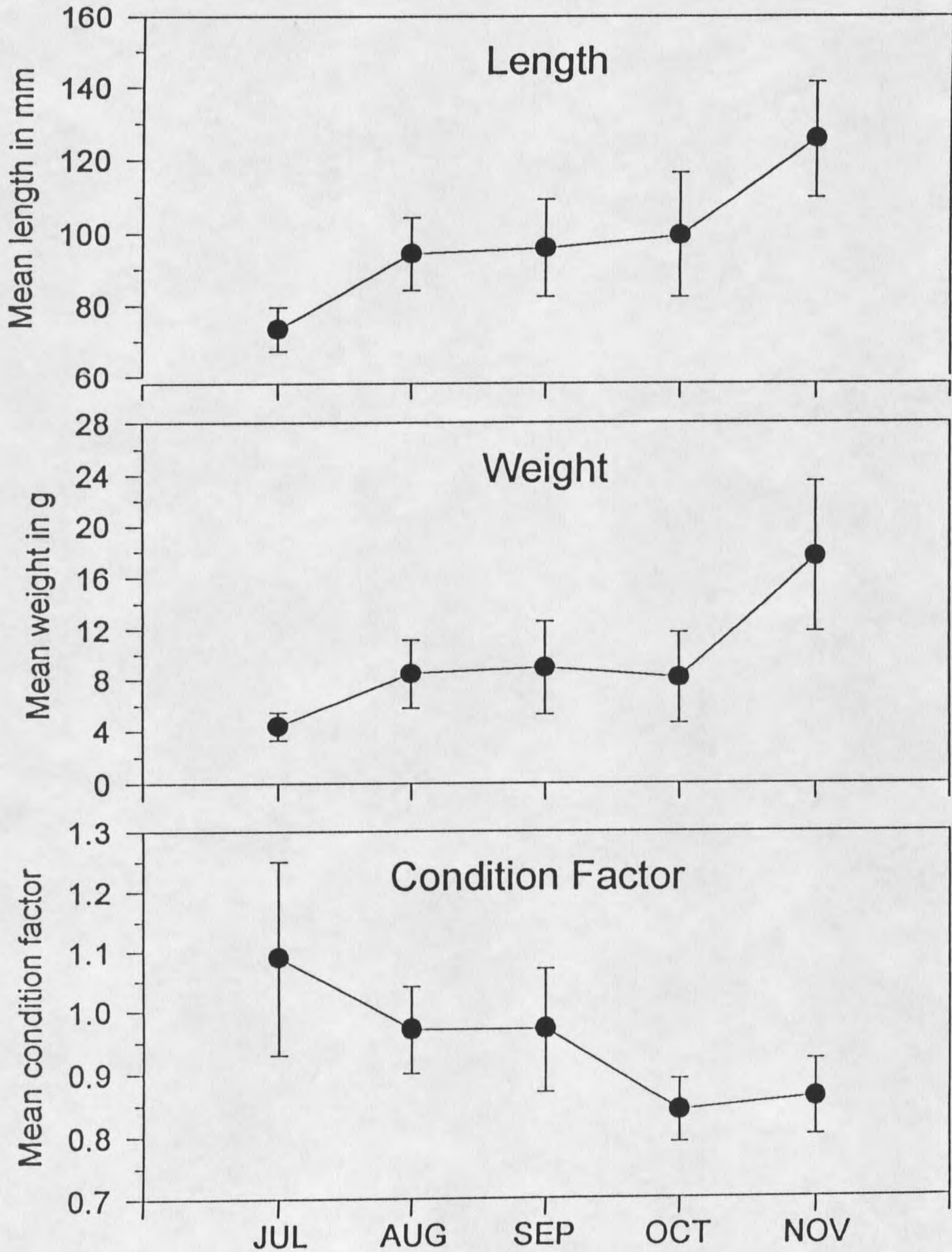


Figure 27. Mean length, weight, and condition factor ( $\pm 1$  SD) of age-0 brown trout at the Snowcrest site during the 1997-98 field season.

Age-0 brown trout collected at the Snowcrest site that were examined histologically showed no infection by *M. cerebralis* (Table 9). Two rainbow trout that were collected and examined also showed no infection by *M. cerebralis*.

### Abundance Summary

Mean abundance estimates at the Poindexter site, in the 1996-97 field season, were the highest and the Barnosky site was the lowest of the three sites used. In the 1997-98 field season, the mean abundance estimates at the Poindexter site were again the highest of all of the sites used. The Woodson site was the second highest followed by the Barnosky site. The Snowcrest site had the lowest mean abundance estimates of the four sites used. Gradually declining abundance estimates of age-0 brown trout were seen in both the 1996-97 and 1997-98 field seasons at all of the study sites. No obvious, sudden declines that would suggest that whirling disease was affecting survival were observed at any of the sites during either field season.

Mean lengths and weights of age-0 brown trout increased from the beginning to the end of each field season at all of the study sites. They were also higher in the 1997-98 field season than the 1996-97 field season. Mean condition factors of age-0 brown trout generally decreased for the beginning to the end of the field season. The mean condition factors were higher in the 1997-98 field season than the 1996-97 field season at the

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
September	4	4	0	0	0	0
October	1	1	0	0	0	0

Table 9. Number of nonclinical, age-0 brown trout that were examined and exhibited each histological grade of infection, by month, from the Snowcrest site in the 1997-98 field season.

Woodson and Barnosky sites. There was little difference in mean condition factor between field seasons at the Poindexter site.

Clinical signs at the Woodson, Barnosky, and Poindexter sites declined from the 1996-97 field season to the 1997-98 field season. The Woodson site had the highest percentage of clinical signs during both field seasons. The Barnosky site was the second highest during both field seasons. The Poindexter site was the lowest during the 1996-97 field season and the Snowcrest site was the lowest in the 1997-98 field season. Blacktail and jaw deformities made up the majority of the clinical signs observed at the Woodson, Barnosky, and Poindexter sites during the 1997-98 field season.

Histology showed that the majority, but not all, of age-0 brown trout that showed clinical signs in the 1997-98 field season were infected with *M. cerebralis*. The majority of the nonclinical fish that were examined had Grade 0 infections. Grade 1 infections made up 32% or less of the fish examined at each site. There was only one fish that had a Grade 2 infection and one that had a Grade 3 infection. Monthly infection rates were calculated for the Woodson, Barnosky, and Poindexter sites in light of the histology data. The infections rates were calculated by first calculating the percentage of nonclinical fish that were actually infected. The total number of nonclinical fish was then multiplied by that percentage. This generated the number of nonclinical fish that were actually infected. The number of clinical fish that were infected was added to the number of nonclinical fish that were infected. This generated the total number of fish that were infected at the site during a specific month. Dividing the total number of fish that were actually infected by the number of fish examined during electrofishing during a specific month gives the



percent infection rate for that month. These infection rates allowed comparison among sites and months.

The infection rates at the Woodson and Barnosky sites gradually increased from July through September (Table 10). Infection rates at both sites decreased in October, possibly indicating selective mortality of infected individuals. The Woodson site had the highest infection rate in comparable months of the three sites except for October when the Barnosky infection rate was highest. The lowest infection rates in comparable months were seen at the Poindexter site.

### Exposure Experiments

#### 1996 Experiments

Age-0 brown trout in the 2, 4, and 6-week exposure groups and the positive-control rainbow groups at the Woodson, Barnosky, and Poindexter sites all had a greater than 96% survival rate from the time the exposures began until they were transported to Pony. All of the exposure groups were exposed at mean daily water temperatures between 9°C and 17 °C, which is considered the optimal range for infection of whirling disease (Figure 28).

Mechanical failures and poor water quality caused the 1996 exposure experiments to fail. A drain valve on a Living Stream at Pony failed and the tank was drained on July 6. The negative-control rainbows and the 4-week exposure groups from the Woodson, Barnosky, and Poindexter sites were almost completely lost. Moreover, dark fish began appearing in all of the lots of fish on July 29. About half the fish being held at Pony were

Month	Woodson	Barnosky	Poindexter
June	--	--	0.003%
July	27.2%	2.24%	4.5%
August	31.7%	20.4%	12.0%
September	56.8%	42.6%	11.6%
October	29.2%	35.4%	15.9%
March	33.3%	30.8%	0%

Table 10. Monthly infection rate of *M. cerebralis* at the Woodson, Barnosky, and Poindexter sites during the 1997-98 field season.

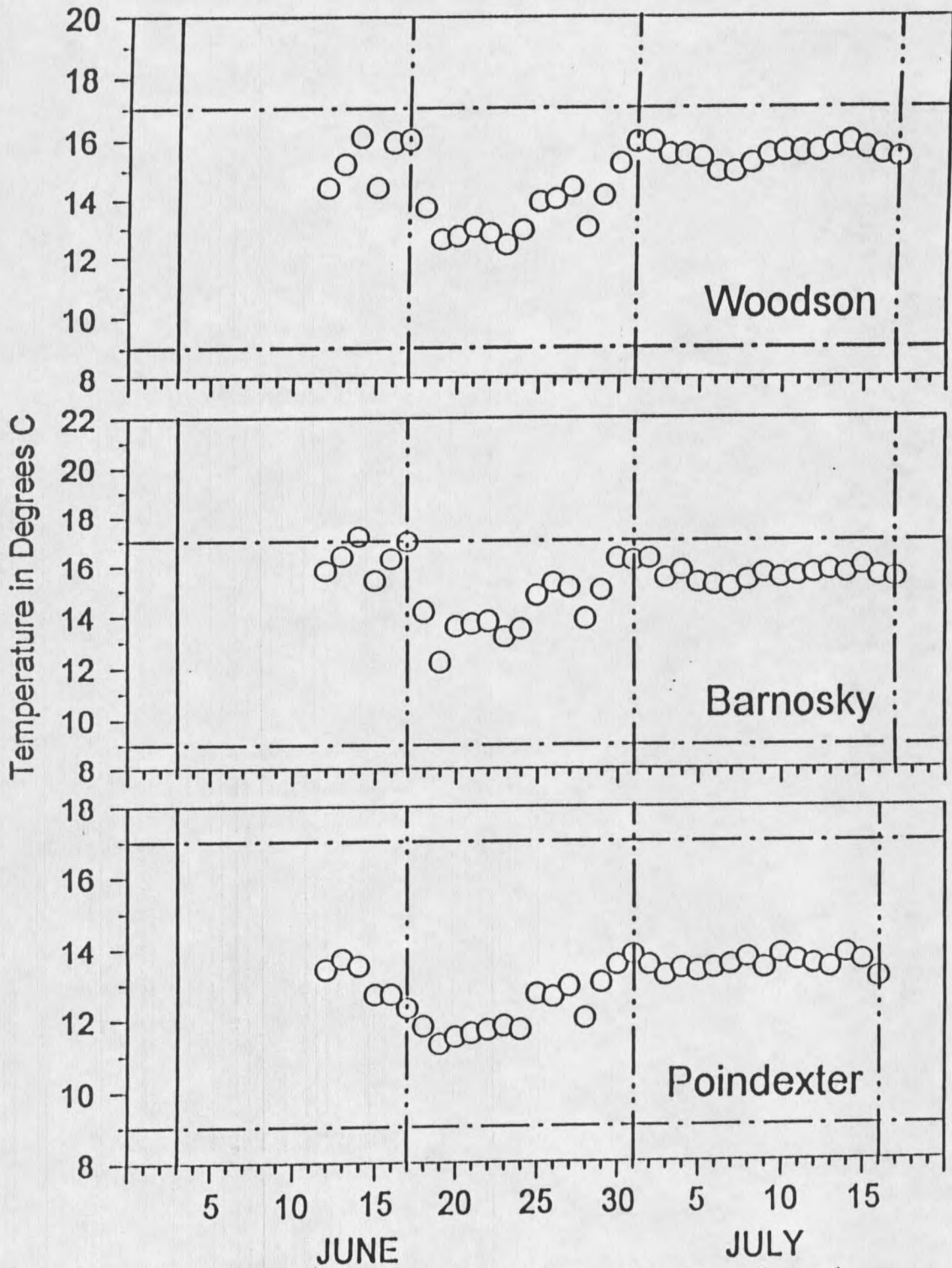


Figure 28. Mean daily water temperatures ( $^{\circ}\text{C}$ ) at the Woodson, Barnosky, and Poindexter sites during the 1996 field season. The horizontal, dashed lines show the 9 to 17  $^{\circ}\text{C}$  temperature range and the vertical, dashed lines show when the different exposure groups were removed from each site. The solid, vertical lines show when the exposures began.

dark in color and mortalities were beginning to occur on July 30. Four dark-colored rainbow trout exposed at the Poindexter site were collected and taken to the U.S. Fish and Wildlife Service Fish Technology Center in Bozeman, Montana, for examination. The fish were diagnosed as having brown blood disease (E. MacConnell, personal communication). Brown blood disease is caused by methemoglobin formation in the blood and decreases the ability of the blood to carry oxygen. This is caused by an increase in nitrites in the water. The use of salt (sodium chloride) can reduce the level of methemoglobin in fish (Post 1987). All fish at Pony were placed in a 1% salt bath for up to 15 minutes to lower the methemoglobin levels on August 10. They were placed into a 20-liter bucket of fresh water to recover and then back into the Living Streams. The Poindexter positive-control rainbow, the Barnosky 2 and 6-week brown trout, and the negative-control brown trout groups were placed in a salt bath again on August 20 because of continuing large numbers of dark-colored fish. To control ammonia and nitrite levels Bacta-Pur, a commercial bacterial preparation used to seed biological filtration, was added to the water in the Living Streams at Pony. Three hundred ml were added on August 27 and one hundred and fifty ml were added every three days after that. No fish were left in the 4-week group from Poindexter on September 4. The Poindexter 2 and 6-week groups and the negative-control brown trout only had one fish remaining then. Only two fish remained in the Barnosky 4 and 6-week groups and the Woodson 4-week group had three fish. The negative-control rainbow, Poindexter positive-control rainbow, and Woodson positive-control rainbow had 7, 13, and 19 fish, respectively. The Woodson and Barnosky 2-week groups had 15 and 5 brown trout remaining, respectively. Twenty-one fish were left in the

Woodson 6-week group and the Barnosky positive-control rainbow. The trial was ended on October 31, 1996 because of continued loss of fish.

Only a single specimen, a brown trout exposed for two weeks at the Woodson site, exhibited a possible clinical sign (whirling behavior) of whirling disease, but this may have been a symptom of brown blood disease.

Histology was not done on these fish because such large numbers were lost to mechanical failure and poor water quality, as well as brown blood disease. This left very few fish from each group to examine and the added stresses may have made it impossible to detect what effect if any whirling disease had on the fish.

#### 1997 Experiments

Woodson Site The age-0 brown trout that were in the 2-week exposure group at the Woodson site had a 97% survival rate from the beginning of exposure until they were transported to the WTRL on June 25, 1997. These fish were exposed to *M. cerebralis* during mean daily water temperatures within the range of 9 °C to 17 °C (Figure 29). The brown trout that were in the 4 and 6-week exposure groups and the positive-control rainbows were lost in the field during the first part of July when the cages were overturned, possibly by animals or large debris. The loss of these groups occurred before any survival data could be collected. At the laboratory, an obvious decline in survival of the 2-week group was seen from June to August. When the study was ended, on November 21, survival of this group was 38% (Figure 30). Two brown trout in this group exhibited whirling behavior on June 28.

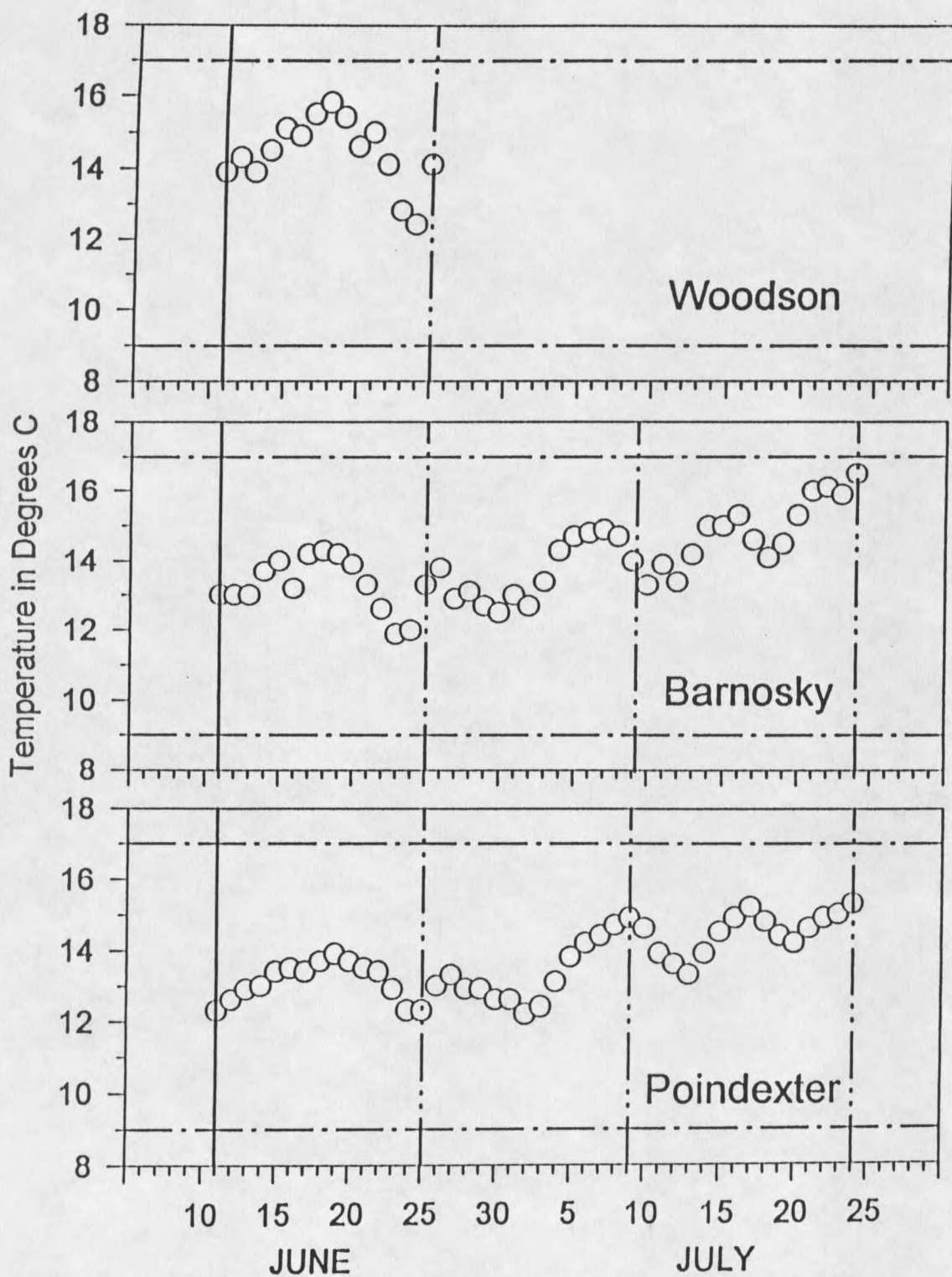


Figure 29. Mean daily water temperatures ( $^{\circ}\text{C}$ ) at the Woodson, Barnosky, and Poindexter sites during the 1997 field season. The horizontal, dashed lines show the 9 to 17  $^{\circ}\text{C}$  temperature range and the vertical, dashed lines show when the different exposure groups were removed from each site. The solid, vertical lines show when the exposures began.

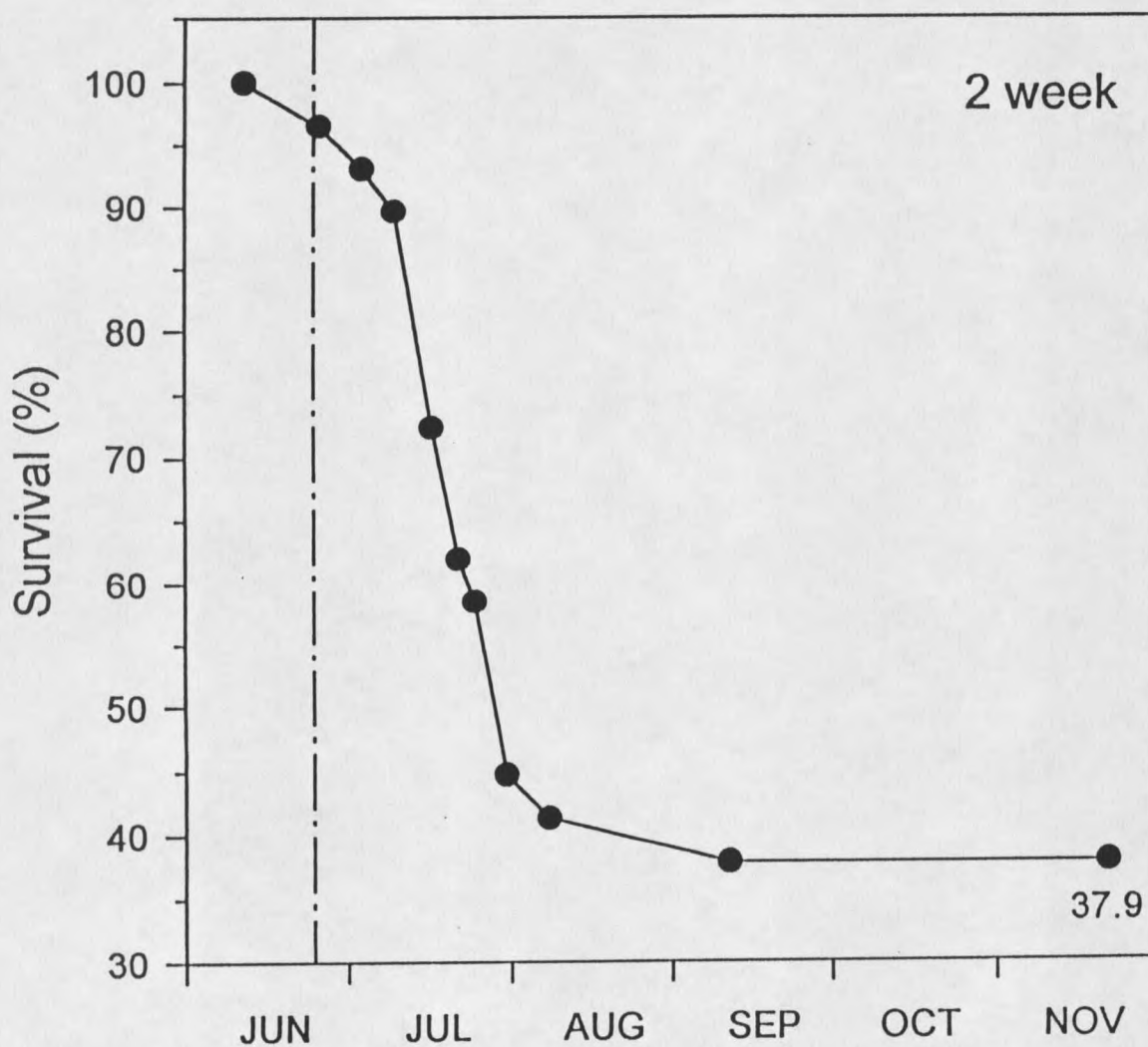


Figure 30. Survival rates of age-0 brown trout exposed for two weeks at the Woodson site during the 1997 field season. The vertical, dashed line denotes when the fish were moved to the Wild Trout Research Laboratory.

Histology done on brown trout from the Woodson 2-week exposure group showed that only two fish collected in July had Grade 2 infections. The remaining fish that were examined showed no infection by *M. cerebralis* (Table 11).

Barnosky Site The age-0 brown trout that were in the 2 and 4-week exposure groups at the Barnosky site had a 100% survival rate from the beginning of exposure to the time they were transported to the WTRL. The 6-week exposure group had a 97% survival rate during this time, and the survival rate of the positive-control rainbows was 95%. Mean daily water temperatures at the site during the time of exposure were within the 9 °C to 17 °C range (Figure 29). The 2-week exposure group survival rate decreased to 67% by August 1 and remained at that level until the trial was terminated in November. The 4 and 6-week groups had very similar survival rates at the end of the study with 95% and 97%, respectively (Figure 31). The survival rate of the positive-control rainbows decreased to 53% in November when the project was ended (Figure 32). Most of the decline in survival of this group occurred after August. The positive-control rainbows began exhibiting whirling behavior in September and continued through November when the trial was ended.

Histology done on brown trout from the 2-week exposure group revealed no infection by *M. cerebralis*. The same was true for brown trout from the 4 and 6-week exposure groups (Table 12). No infection was seen in the positive-control rainbow trout until October when four fish exhibited Grade 2 infections. In November, Grade 3 and 4 infections of *M. cerebralis* were seen in the positive control rainbows (Table 13).



Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	8	6	0	2	0	0
August	2	2	0	0	0	0
October	5	5	0	0	0	0

Table 11. Number of age-0 brown trout from the 2-week exposure group at the Woodson site that were examined and exhibited each histological grade of infection, by month, in 1997.

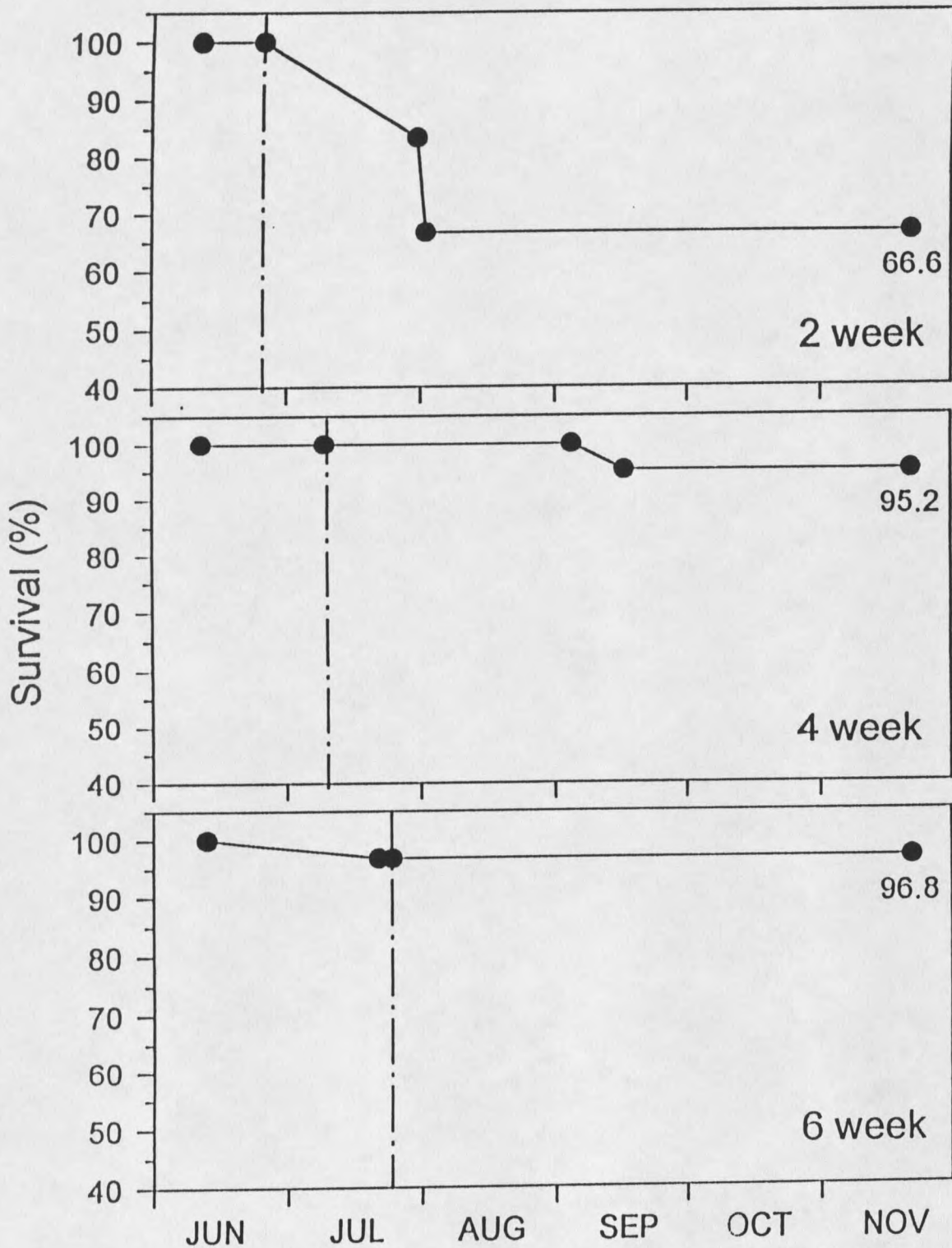


Figure 31. Survival rates of age-0 brown trout exposed for two, four, and six weeks at the Barnosky site during the 1997 field season. The vertical, dashed lines denote when the fish were moved to the Wild Trout Research Laboratory.

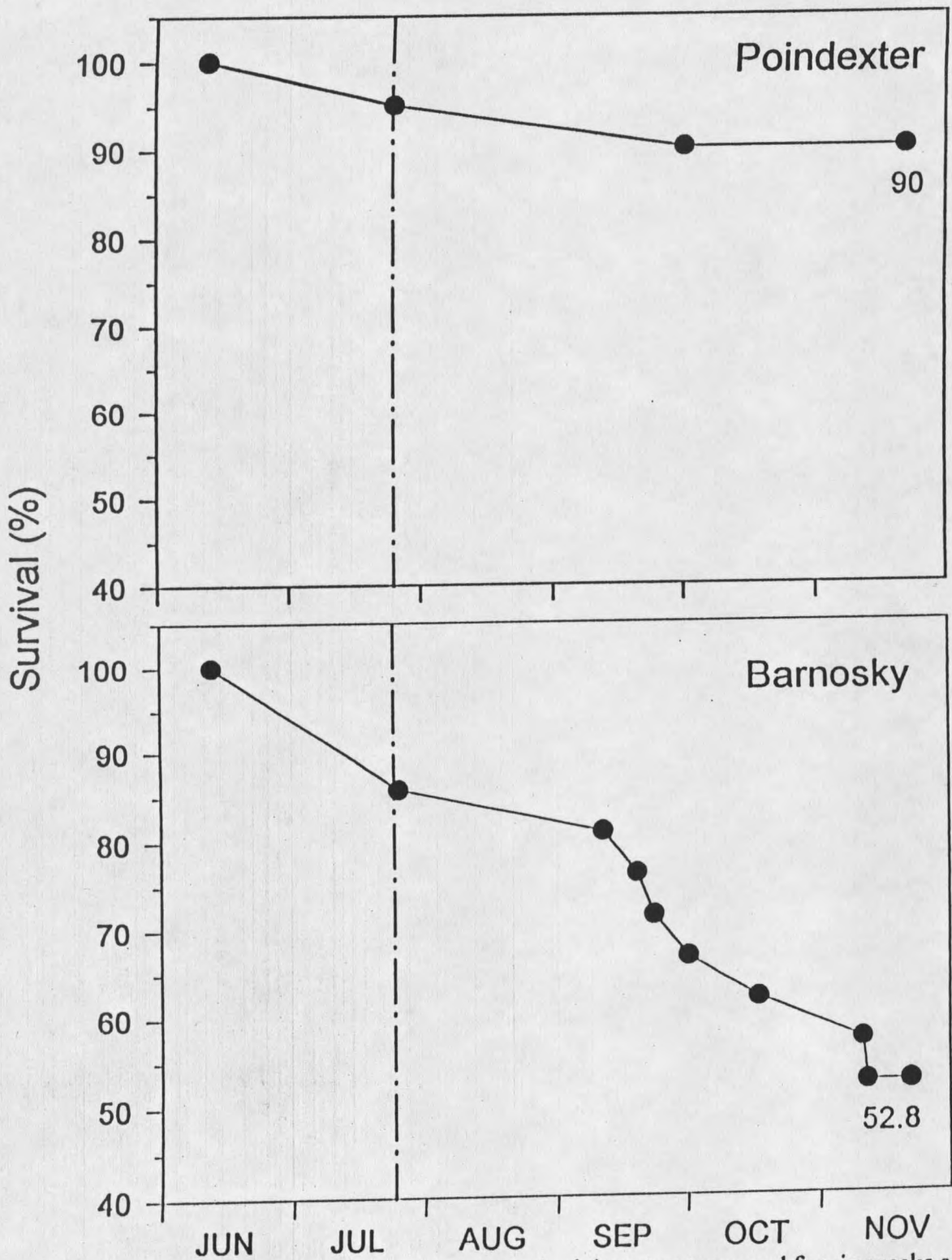


Figure 32. Survival rates of age-0, positive-control rainbow trout exposed for six weeks at the Poindexter and Barnosky sites during the 1997 field season. The vertical, dashed lines denote when the fish were moved to the Wild Trout Research Laboratory.

2-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	2	2	0	0	0	0
November	9	9	0	0	0	0

4-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	1	1	0	0	0	0
August	3	3	0	0	0	0
October	5	5	0	0	0	0
November	3	3	0	0	0	0

6-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
August	2	2	0	0	0	0
October	5	5	0	0	0	0
November	5	5	0	0	0	0

Table 12. Number of age-0 brown trout from the 2,4, and 6-week exposure groups at the Barnosky site that were examined and exhibited each histological grade of infection, by month, in 1997.

## BARNOSKY

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	2	2	0	0	0	0
August	3	3	0	0	0	0
October	5	1	0	4	0	0
November	5	1	0	0	1	3

## POINDEXTER

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	1	1	0	0	0	0
August	2	2	0	0	0	0
October	5	2	0	0	3	0
November	10	3	0	0	2	5

Table 13. Number of age-0, positive-control rainbow trout that were examined and exhibited each histological grade of infection, by month, from the Barnosky and Poindexter sites in 1997.

Poindexter Site The survival rate of age-0 brown trout that were in the 2 and 4-week exposure groups at the Poindexter site in the 1997-98 field season was 100% from the beginning of exposure until they were transported to the WTRL. The survival rate of the 6-week group was 96% from the beginning of the exposure until they were transported to the laboratory. The positive-control rainbows had a survival rate of 86% during this time. During the exposure time, the mean daily water temperatures were between 9°C and 17 °C (Figure 29). The survival rate of the 2 and 4-week groups at the end of the study were identical at 62%. The 6-week exposure group survival rate declined gradually to 88% on November 21 when the trial was ended. Most of the declines in these groups occurred in July and August (Figure 33). After the positive-control rainbows were transported to the lab, the survival rate decreased to 90% at the end of the trial (Figure 32). The positive-control rainbows began exhibiting whirling behavior in September and continued through November when the trial was ended.

Histology that was done on brown trout collected from the 2, 4, and 6-week exposure groups revealed no infection by *M. cerebralis* with one exception. One brown trout from the 2-week exposure group that was collected in October had a Grade 2 infection (Table 14). The positive-control rainbow trout showed no infection until October when Grade 3 infections were observed. Grade 3 and 4 infections of *M. cerebralis* were seen in November (Table 13).

The age-0, negative-control rainbow trout that remained unexposed had a 100% survival rate from June through November when the trial was ended. The negative-

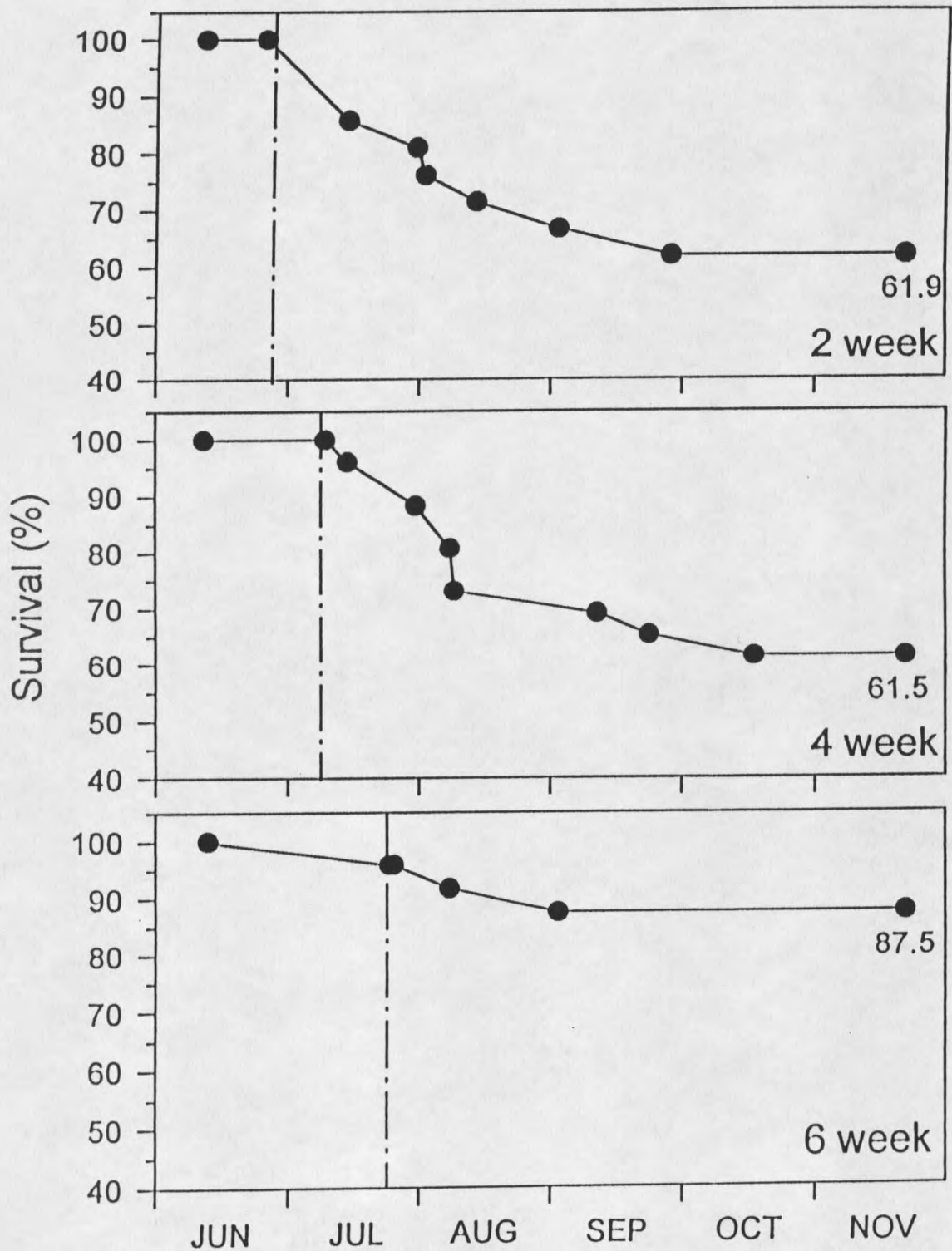


Figure 33. Survival rates of age-0 brown trout exposed for two, four, and six weeks at the Poindexter site during the 1997 field season. The vertical, dashed lines denote when the fish were moved to the Wild Trout Research Laboratory.

2-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	2	2	0	0	0	0
August	2	2	0	0	0	0
October	5	4	0	1	0	0
November	8	8	0	0	0	0

4-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	1	1	0	0	0	0
August	3	3	0	0	0	0
October	5	5	0	0	0	0
November	3	3	0	0	0	0

6-week						
Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	1	1	0	0	0	0
August	2	2	0	0	0	0
October	5	5	0	0	0	0
November	2	2	0	0	0	0

Table 14. Number of age-0 brown trout from the 2,4, and 6-week exposure groups at the Poindexter site that were examined and exhibited each histological grade of infection, by month, in 1997.



control, age-0 brown trout survival rate declined to 76% from June to November. Most of this decline occurred during July. This was during the same time that the other groups of exposed brown trout declined (Figure 34). Both the negative-control rainbow and brown trout showed no infection of *M. cerebralis* when examined histologically (Table 15).

#### Exposure Experiment Summary

Only three of the brown trout exposed at the study sites showed an infection by *M. cerebralis*. Two brown trout in the 2-week exposure group from the Woodson site had Grade 2 infections. One brown trout from the 2-week exposure group at the Poindexter site had a Grade 2 infection as well. The positive-control rainbows exposed at the Barnosky and Poindexter sites had Grade 3 and 4 infections in October and November. The negative-control brown and rainbow trout all showed no infection by *M. cerebralis*.

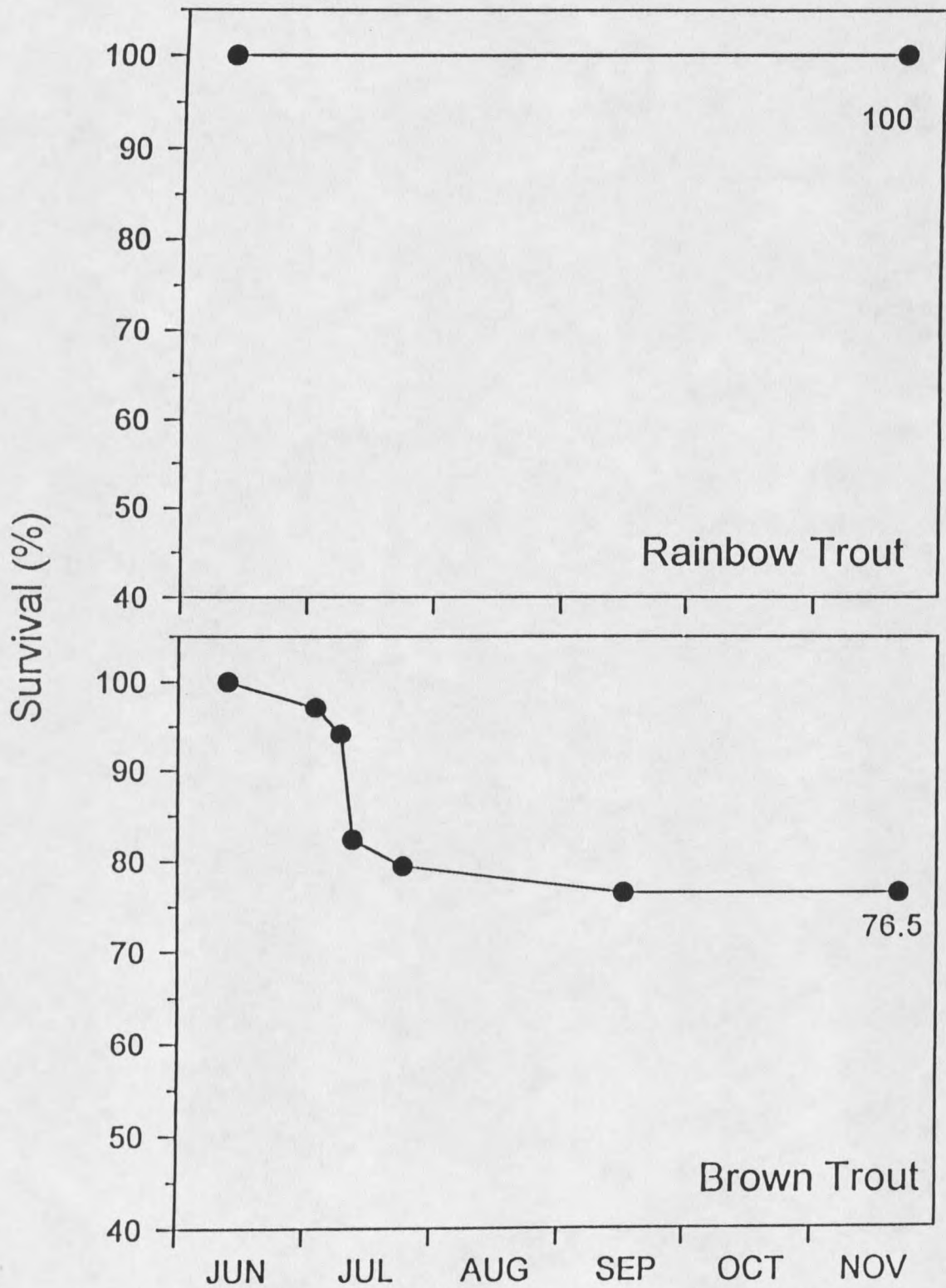


Figure 34. Survival rates of age-0, negative-control rainbow and brown trout that were unexposed and remained at the Wild Trout Research Laboratory.

## Rainbow Trout

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	2	2	0	0	0	0
August	2	2	0	0	0	0
November	5	5	0	0	0	0

## Brown Trout

Month	Number of fish examined	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
July	1	1	0	0	0	0
August	5	5	0	0	0	0
November	5	5	0	0	0	0

Table 15. Number of age-0, negative-control rainbow and brown trout that were examined and exhibited each histological grade of infection, by month, in 1997.

## DISCUSSION

### FWP Abundance Data

Analysis of brown trout population data collected by FWP at Poindexter Slough suggests that there was no obvious decline at the Poindexter site except in 1993. It appears that Poindexter Slough went through a natural fluctuation in abundance. Natural fluctuations are caused by many factors. These factors can include year class strength, reproductive success, and environmental factors. Review and analysis of the data did show that there was an obvious and extended decline at the Silver Spring section of the Ruby River. Recent data also suggests that the populations of brown trout in Poindexter Slough and the Silver Spring section of the Ruby River are increasing toward previous levels. This suggests that whirling disease was not a cause in the decline at Poindexter Slough but may have been the cause of decline in the Ruby River.

### Abundance Monitoring

The gradual declines in mean abundance of age-0 brown trout seen in the 1996-97 and the 1997-98 field seasons were as expected of normal unaffected populations. No obvious, sudden declines in abundance, as seen in the Madison River, were observed. Age-0 brown trout abundances were similar to those in the Madison River in that they did not appear to be affected by the presence of whirling disease in these systems (Vincent 1996). The results are also similar to those found in the Colorado River and Gunnison River in Colorado. Rainbow trout were severely affected and brown trout showed no

precipitous declines in abundance (Nehring et al. 1998). The increases in mean abundance seen in the 1997-98 field season suggest that the populations of brown trout in the Ruby River and Poindexter Slough are beginning to increase rather than continuing to decrease.

The variability seen in the abundance estimates of the age-0 brown trout can be explained by the variability in the habitat of the transects that were sampled. Some transects had abundant cover and habitat for age-0 brown trout whereas others had none. Transects that provided more habitat and cover generally had more age-0 brown trout. This caused variability in the abundance estimate for those transects. Mid-channel aquatic vegetation at the Poindexter site increased during the summer, providing habitat for age-0 brown trout out of the areas along the banks that were monitored. This may have reduced abundance estimates for transects where this additional habitat was present. Available habitat should be considered when selecting areas to sample in order to reduce bias in abundance estimates.

Few age-0 rainbow or brown trout were found at the Snowcrest site compared with the other study sites. Woody debris such as fallen willows that would be used by age-0 fish was absent from this site. Few areas contained gravels suitable for spawning. Actual spawning and rearing of fish may be taking place either up or downstream from the study site. They may also be taking place in lower reaches of tributaries such as Greenhorn Creek and Ledford Creek. These factors may have contributed to the low

number of age-0 fish that were found at this study site. Further investigation may determine the usefulness of this site for spawning and rearing of both brown and rainbow trout.

Overwinter survival rates for salmonid populations are not well documented (Handy 1997), but studies that have been done indicate a wide range of values. Smith and Griffith (1994) reviewed studies incorporating 24 salmonid populations and reported that average first-winter survival rates for salmonids was 49.8% with a standard deviation of 18.0%. The overwinter survival rate of age-0 wild brown trout in Convict Creek, California, had a maximum of 84%, a minimum of 15%, and an average of 38% over a four-year period (Needham et al. 1945). Brook trout in Lawrence Creek, Wisconsin, had a maximum overwinter survival rate of 73%, a minimum of 35%, and an average of 54% over an 11-year period (Hunt 1969). A study on survival rates of rainbow trout in the Henrys Fork of the Snake River, Idaho, by Smith and Griffith (1994) used cages with and without cover. Overwinter survival rates for fish in cages with cover ranged from 100% to 68% with an average of 86%. Survival rates of fish in cages without cover ranged from 100% to 57% with an average of 76%. Overall survival rates of fish in both types of cages ranged from 100% to 63% with an average of 80%.

The overwinter survival rate of age-0 brown trout for the Poindexter site, 55%, was within the ranges suggested in other studies at in the 1996-97 field season but was below those ranges in the 1997-98 field season at 19%. Survival rates at the Barnosky and Woodson sites were below the ranges reported in the literature at 12% and 6%, respectively in the 1997-98 field season. These low overwinter survival rates may be due

to limited carrying capacity for age-1 brown trout at the Barnosky and Woodson sites. The survival rates may have also been an artifact of low sampling effort. Only one abundance estimate was done at each site in the spring. If a second estimate had been done at each site, more fish may have been captured as water temperatures rose and fish began to leave interstitial spaces in the substrate used for overwinter survival. Sampling more than once and later in the spring as water temperatures begin to rise may give a more accurate overwinter survival rate.

Percentages of age-0 brown trout that showed clinical signs of whirling disease in this study were lower than would be expected to be lost to natural mortality. The percentage of clinical signs decreased from the 1996-97 to the 1997-98 field season. This suggests that whirling disease is not affecting population levels and its impacts on age-0 brown trout declined during the time of the study. The percentages of age-0 brown trout that showed blacktail were much lower than brown trout in the upper Colorado River. Up to 60% of the brown trout in some reaches showed blacktail (Walker and Nehring 1995). Percentages of other clinical signs such as cranial deformities and shortened operculum were similar to those found in the Colorado river.

Rainbow trout that were observed in the tailwater below Ruby Reservoir had obvious cranial and caudal deformities. This suggests that the tailwater may be a point source for whirling disease in this system. It may provide good habitat for *T. tubifex*, and rainbow trout are abundant in this area. The percentage of clinical signs of whirling disease was higher at the Woodson site than the Barnosky site located farther downstream. Both study sites are below Ruby Reservoir. This may indicate that the

potential for infection by *M. cerebralis* is decreased as distance from the reservoir increases. A well designed study may show this to be true.

Histology was a useful tool in studying the potential impacts of whirling disease. Most of the age-0 brown trout that showed clinical signs were positive for *M. cerebralis* with up to Grade 4 infections. This confirmed that the clinical signs were caused by whirling disease. Less than 38% of the fish that showed clinical signs from the Barnosky site had Grade 0 infections. At the Poindexter site less than 28.6% of the fish were not infected. The Woodson site only had 23.4% of the brown trout that showed clinical signs that were not infected. The clinical signs that were seen in these fish may have been caused by other factors such as electrofishing or other diseases. Some nonclinical brown trout that were from the Woodson, Barnosky and Poindexter sites were positive with Grade 1 and 2 infections. These levels of infection are considered to be nonlethal. There was also a fish with a Grade 3 infection at the Barnosky site. Less than 33% of the nonclinical brown trout from Woodson site were infected with *M. cerebralis*. The Barnosky and Poindexter sites had 23.7% and 8.6% of the nonclinical fish that were infected with *M. cerebralis*. This provides further evidence that nonclinical fish can be infected by *M. cerebralis* and may be carriers. These results also show that presence or absence of clinical signs are not definitive for the presence or absence of whirling disease. The variation in infection rates that was seen in the age-0 brown trout has some possible explanations. Poindexter Slough and the Ruby River have springs and upwellings located throughout the reaches that were studied. These may have provided more suitable habitat for tubifex worms, in turn increasing the possibility of infecting fish more severely. These



may also be areas that age-0 brown trout are being hatched and reared in. This relates to the idea of "hot spots" in the upper Colorado River suggested by Walker and Nehring (1995). They found that in some areas of the upper Colorado River age-0 brown trout were more severely affected by whirling disease than in other areas. It may be that conditions in certain areas of these waters are ideal for supporting an increased level of whirling disease. The level of whirling disease in some of these areas may be high enough to overwhelm brown trout. These areas may support high numbers of tubifex worms and salmonid fish, the two hosts needed for the persistence of whirling disease. These areas would have high production of triactinomyxons as well as high levels of contact between triactinomyxons and fish.

#### Exposure Study

Age-0 brown trout exposed during the 1997-98 field season did not become infected except for two at the Woodson site and one at the Poindexter site. These fish were exposed for two weeks and had Grade 2 infections that are considered nonlethal (E. R. Vincent, personal communication). Eight age-0 rainbow trout exposed at the Barnosky site were infected with *M. cerebralis* and had up to Grade 4 infections. Ten age-0 rainbow trout exposed at the Poindexter site were also infected with *M. cerebralis* with up to Grade 4 infections. This suggests that whirling disease was present in these areas but not at levels that would infect brown trout severely or in high numbers. By not beginning the exposures until the first part of June, a period of higher presence of *M. cerebralis* may

have been missed. The brown trout would be younger and possibly more susceptible earlier in the spring. Mean daily water temperatures are in the 9 °C to 17 °C range before June (Figures 3, 5, and 8).

Obvious declines in survival rates were seen in the negative-control brown trout, the Poindexter 2 and 4-week exposure groups, the Barnosky 2-week exposure group, and the Woodson 2-week exposure group once the fish were at the WTRL in 1997. These declines may have been caused by poor water quality in the Living Streams at the WTRL. Location and crowding of these groups in the Living Streams may have also contributed to the declines. The declines in survival rate may have been caused in part by a bacterial gill infection detected in some fish from these groups.

The results of rainbow trout being more severely infected than brown trout is similar to that seen in rainbow and brown trout from the Madison River.

### Conclusions

The effects of whirling disease on recruitment of brown trout in both the Ruby River and Poindexter Slough were negligible during the time this study was conducted. It does not appear that whirling disease was the cause of the decline in Poindexter Slough, but it may have been the cause of the decline seen in the Ruby River.

Brown trout were introduced into Montana more than 100 years ago (Brown 1971; Luton 1985). During this time, resistance to whirling disease may have been lost. *M. cerebralis* was introduced and conditions in these water may have become suitable for whirling disease to be expressed. Brown trout then became susceptible. Fish lacking

resistance may have been removed from the populations. Fish that retained some resistance may have been able pass the resistance on to their offspring. Brown trout that have resistance may be the fish that are reproducing and causing the increases that are occurring in these waters. Lack of samples that could be tested for whirling disease and age-0 abundance data prior to 1996 makes this theory unprovable. Research done on bacterial kidney disease (Withler and Evelyn 1990) and infectious hematopoietic necrosis (McIntyre and Amend 1978) showed that genetic variation in mortality occurs and can be used in selective breeding programs to produce resistant strains of fish. Resistance to vibriosis, furunculosis, and bacterial kidney disease in chum *Oncorhynchus keta*, chinook and coho salmon can also be improved through genetic strain selection in breeding programs (Beacham and Evelyn 1992). Resistance to *Ceratomyxa shasta* in different strains of Columbia River summer steelhead is believed to be due to natural selection (Buchanan et al. 1983). Parasites have also been associated with increased resistance in salmonids by being causative agents of natural selection (Withler and Evelyn 1990). This information shows that natural selection for resistance to disease in a fish species is possible. It however, does not provide information on how much time is required for this selection to take place and become obvious at the population level.

Continued population monitoring and whirling disease sampling will help to assess the impact, if any, whirling disease is having on brown trout in these waters. The continuation of age-0 abundance monitoring could provide insight for possible future recruitment and whirling disease problems.

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