POPULATION VIABILITY OF ARCTIC GRAYLING (*THYMALLUS ARCTICUS*) IN THE GIBBON RIVER, YELLOWSTONE NATIONAL PARK

by

Amber Christine Steed

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

of

Master of Science

in

Fish and Wildlife Management

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2007

APPROVAL

of a thesis submitted by

Amber Christine Steed

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the Division of Graduate Education.

Dr. Alexander V. Zale

Approved for the Department of Ecology

Dr. David W. Roberts

Approved for the Division of Graduate Education

Dr. Carl A. Fox

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master’s degree at Montana State University, I agree that the Library shall make it available to borrowers under rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice page, copying is allowable only for scholarly purposes, consistent with “fair use” as prescribed in the U.S. Copyright Law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Amber Christine Steed

August 2007

ACKNOWLEDGMENTS

Many individuals deserve thanks for contributing to the success of this project from its incipience to its completion. Al Zale provided invaluable guidance and support throughout its duration. Thank you to my committee members Todd Koel and Steven Kalinowski for their direction and assistance. I am indebted to Ninh Vu, John Borkowski, Steve Cherry, and Michael Meeuwig for their guidance. Thanks to Yellowstone National Park (YNP) for funding support.

Deepest thanks to Colleen Blaine and Bonnie Griffis for contributing with selfless and patient dedication for little more than the experience. I am grateful for Dan Mahony's continual support, advice, and loyal commitment throughout the years I have known him. Chris Romankiewicz, Nicole Legere, Michelle Hall, the YNP Fisheries volunteers and Student Conservation Association interns, Tim Bywater, William Voigt, and the Yellowstone Park Foundation's Fly-Fishing Volunteer Program made invaluable contributions to the project. Additional thanks to Christie Hendrix, Wes Miles, and Dennis Young.

I especially thank my fellow graduate students for their empathy, support, advice, and assistance in countless ways large and small. I have gained immeasurable benefit by them both personally and professionally. And of course, I thank my parents and family for their unwavering and unquestioning support and love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES – CONTINUED

Table Page

LIST OF FIGURES

LIST OF FIGURES – CONTINUED

ABSTRACT

The fluvial Arctic grayling *Thymallus arcticus* is restricted to less than 5% of its native range in the contiguous United States and was listed as Category 3 under the Endangered Species Act (ESA) until a 2007 ruling removed its protection. Fluvial grayling were thought to be restricted to the Big Hole River, Montana, where abundances were declining. Although fluvial grayling of the lower Gibbon River, Yellowstone National Park (YNP) were considered extirpated by 1935, anglers frequently reported catching grayling throughout the river since 1980. My goal was to determine if a viable population of fluvial grayling persisted in the Gibbon River, or if fish caught in the river were downstream emigrants from lacustrine populations in headwater lakes. I developed three objectives to address this goal: 1) determine grayling abundances in the Gibbon River, 2) determine the source of grayling in the Gibbon River detected downstream of headwater lakes (occupied by lacustrine populations), and 3) determine if grayling are successfully spawning in the Gibbon River. In 2005 and 2006, estimated abundances ranged from 0 to 95 and 0 to 109, respectively. Larger estimated abundances resulted when the Chapman modification was incorporated into electrofishing estimates, increasing ranges to vary from 0 to 421 and 0 to 506 in 2005 and 2006, respectively. However, both methods of estimation were accompanied by large standard errors reflecting the few grayling detected in the river. Relatively few grayling were caught by anglers in comparison to other systems. Genetic analyses indicated that grayling from throughout the Gibbon system likely belonged to the same population, most notably supported by very low genetic differentiation ($F_{ST} = 0.0021 \pm 0.002$) between headwater lake and river fish. Lengths at most ages were similar among all Gibbon system fish and successful river spawning was not documented below Little Gibbon Falls (the first barrier to upstream movement downstream of headwater lakes). Few grayling adults and no fry were detected in the Gibbon River, implying that a reproducing fluvial population does not likely exist. These findings may affect future ESA considerations of fluvial grayling while providing data for management within and outside of YNP.

INTRODUCTION

The fluvial Arctic grayling was previously designated as Category 1 and declining in Montana under the Endangered Species Act (ESA)—the final stage before listing as Threatened or Endangered (USFWS 1991). A petition was presented to the U.S. Fish and Wildlife Service (USFWS) in 1991 to elevate its status to Endangered (USFWS 1993). The USFWS concluded that Endangered listing was “warranted but precluded by other higher priority listing actions” (USFWS 1994). The fluvial grayling was elevated to Candidate standing in 1996 (USFWS 1996). It was listed as Category 3 under the ESA until a ruling in April 2007 revoked its federal protection (USFWS 2004; USFWS 2007). However, an appeal to reconsider the listing of fluvial grayling reflects the lack of consensus on the issue (D. Peterson, USFWS, personal communication). The fluvial form is presently restricted to less than 5% of its historic range and continues to decline (Magee et al. 2006). The fluvial grayling in Montana was designated a fish of “special concern” by the Montana Chapter of the American Fisheries Society, the Endangered Species Committee of the American Fisheries Society, Montana Fish, Wildlife & Parks (MFWP), and the Montana Natural Heritage Program of the Nature Conservancy in 2006.

In the contiguous United States, fluvial Arctic grayling are thought to be limited to a segment of the Big Hole River, Montana, in which abundances have been declining since the mid-1980s (Magee et al. 2006). Whereas lacustrine populations of grayling exist throughout Montana and elsewhere, fluvial grayling are behaviorally adapted to riverine environments and are genetically and behaviorally distinguishable from adfluvial fish (Kaya 1991; Kaya and Jeanes 1995). Consequently, all attempts to stock river and stream systems with lacustrine grayling have failed in Montana and in Yellowstone National Park (YNP) (Varley 1981; Kaya and Jeanes 1995). Moreover, the Big Hole population is genetically distinguishable from other lacustrine grayling populations (Everett and Allendorf 1985; Kaya 1991, 1992). However, efforts initiated by MFWP through the Arctic Grayling Restoration Program, aimed to establish and maintain five distinct populations of fluvial Arctic grayling within the historic range of the species by 2020, have resulted in limited but encouraging success through the use of fluvial grayling broodstocks and remote site incubators (Montana Fluvial Arctic Grayling Workgroup 1995; Magee et al. 2006).

Within and near to YNP, fluvial Arctic grayling were present until about 1900 in the Gallatin River and until 1935 in the Madison River, the Firehole River below Firehole Cascade, and the Gibbon River below Gibbon Falls (Vincent 1962; Kaya 2000). Nonnative brown trout (*Salmo trutta*) introductions and the creation of Hebgen Reservoir were followed by the near-complete loss of fluvial grayling within YNP by the mid-1900s (Kaya 2000). Prior to their sharp decline in the Madison River, grayling taken from two of its tributaries near Ennis Lake were planted in Georgetown Lake near Anaconda, Montana (Everett and Allendorf 1985). This brood-stock was used to supply 1,000,000 grayling fry to the historically-fishless Grebe Lake at the headwaters of the Gibbon River in 1921

(Varley 1981). The Grebe Lake stock is genetically distinct from Red Rock Lakes and Big Hole River populations, despite its fluvial lineage (Everett and Allendorf 1985).

A hatchery at Grebe Lake was established in 1921, resulting in the continued stocking of over 12.5 million grayling back into the lake from 1933 to 1955 (Varley 1981). Grayling population abundance estimates (fish greater than 109 mm in total length) for Grebe Lake obtained from trap nets and mark and recapture methods were about 27,000 fish in 1953 and 26,000 fish in 1954 (Kruse 1959). Wolf Lake (downstream of Grebe Lake) also supports grayling (Varley et al. 1976), but a population estimate has not been made there. At least two grayling have been caught in the Gibbon River by anglers annually since 1980 (except for 1987) and periodically by YNP survey crews (Koel et al. 2005). It is unknown if grayling detected in the Gibbon River below Little Gibbon Falls since about 1935 are fluvial (including successful reproduction within the Gibbon River), or if they are downstream emigrants.

Research Need

An assessment of the population viability of Arctic grayling in the Gibbon River, YNP, was necessary based on the declining status of the Big Hole River fluvial grayling population, the ongoing reassessment of the fluvial grayling's status under the ESA, the paucity of data on grayling in the Gibbon River system, YNP, and the prospective benefits to MFWP restoration efforts. The confirmation of a viable population of fluvial grayling in the Gibbon River would affect future stocking and subsequent genetic adulteration of grayling in the Gibbon River. A fluvial population in the Gibbon River would also be considered one of five populations to be established and maintained by the Arctic Grayling Restoration Program. By deeming the Gibbon River grayling as one of these five populations, resources would be freed for additional grayling research and conservation. If a viable population of fluvial grayling does not exist in YNP, this knowledge may bolster support for conservation of the Big Hole River population, allow efforts to re-establish fluvial grayling within the Gibbon River, and influence future listing determinations under the ESA.

My goal was to determine if a viable population of fluvial Arctic grayling exists in the Gibbon River. I developed three objectives to address this goal: 1) determine grayling abundances in the Gibbon River, 2) determine the source of grayling in the Gibbon River detected downstream of headwater lakes (occupied by lacustrine populations), and 3) determine if grayling are successfully spawning in the Gibbon River. Viable abundances are necessary to minimize the effects of inbreeding over time and maintain adaptive genetic variation (Rieman and Allendorf 2001). However, putatively viable abundances of grayling could exist in the Gibbon River without representing a self-sustaining population if fish are lacustrine emigrants (Kaya 1991; Kaya and Jeanes 1995) or if they do not successfully reproduce in the river. I determined if a viable population of fluvial grayling exists in the Gibbon River by considering the evidence produced from all objectives.

STUDY AREA

The Gibbon River originates at Grebe Lake, located at 2,445 m elevation, about 2.9 km northwest of Canyon Village, YNP. From its headwaters, the Gibbon River flows 54.3 km to its confluence with the Firehole River to form the Madison River (Figure 1) (2,072 m elevation).

Fish assemblages vary longitudinally along the Gibbon River, reflecting the three successive barriers to upstream movement. The farthest downstream barrier (Gibbon Falls, 25.6 m) exists in Gibbon Canyon, about 11.3 km upstream from the Firehole River confluence. Historically, Arctic grayling, mottled sculpin (*Cottus bairdi*), mountain whitefish (*Prosopium williamsoni*), longnose dace (*Rhinichthys cataractae*), and westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) existed in the Gibbon River below Gibbon Falls (Varley and Shullery 1998). Only brown trout, dace, rainbow trout (*Oncorhynchus mykiss)*, sculpin, and whitefish were known to exist in this stretch of river in 2005 (Varley and Shullery 1998; Koel et al. 2004). The next barrier to upstream movement (Virginia Cascade, 19.4 m) is located about 28.4 km upstream of Gibbon Falls, less than 1 km downstream of Virginia Meadows. Before nonnative fish introductions, sculpin were the only inhabitants above Gibbon Falls (Varley and Shullery 1998). The Gibbon River harbored brown trout, dace, eastern brook trout (*Salvelinus fontinalis*)*,* rainbow trout, and sculpin between Gibbon Falls and Virginia Cascade in 2005 (Koel et al. 2004). The final barrier to upstream movement (Little Gibbon Falls, 7.6 m) lies about 3.9 km above Virginia Cascade.

A dense population of eastern brook trout resides between Virginia Cascade and Little Gibbon Falls, accompanied by rainbow trout and sculpin (D.L. Mahony, YNP, personal communication). Only grayling and rainbow trout are found above Little Gibbon Falls (Varley and Shullery 1998).

METHODS

Arctic Grayling Abundances in the Gibbon River, YNP

I electrofished and snorkeled during 2005 and 2006 to determine Arctic grayling abundances in the Gibbon River, YNP. Electrofishing was used from June through mid-July during 2005 and from late-May through mid-July during 2006. Snorkeling was used from mid-July through September during both years because low discharge and personnel and equipment limitations precluded electrofishing during those months. Grayling abundances were estimated from electrofishing data by dividing grayling counts by electrofishing efficiency for all salmonids caught (Pearsons and Temple 2007). Abundance estimates were calculated from snorkeling data by determining the relationship between snorkeling counts and estimated abundances from electrofishing data to establish snorkeling efficiency ($\hat{R}$) for subsequent application to snorkeling counts (Dolloff et al. 1993). Estimates of grayling per km in each stretch of river between barriers to upstream movement were produced for each sample period. I estimated the minimum number of grayling present by these criteria using data collected by the Yellowstone Park Foundation’s Fly-Fishing Volunteer Program. Grayling and non-grayling (i.e., other salmonids) catch rates (i.e., number of grayling caught per angler-hour) were also produced.

Although total abundance is more easily estimable for fish populations, the effective population size (N_e) is necessary to determine the rate of inbreeding and loss of genetic variation (i.e., population viability) (Frankham 1995). Direct estimates of N_e were not available for grayling in the Gibbon River system because they required extensive demographic or genetic data spanning multiple generations (Frankham et al. 2002). However, ratios have been developed to estimate N_e from abundance estimates for various taxa (Frankham 1995). I used the ratio of about 0.3 to 1 to infer N_e from estimated grayling abundances per stretch of river between barriers to upstream movement (Frankham 1995).

The Gibbon River was divided into four strata for sampling in 2005 based on the presence of multiple barriers to upstream fish movement (Gibbon Falls, Virginia Cascade, Little Gibbon Falls) and the disparate fish assemblages among strata (D.L. Mahony, YNP, personal communication; Brown and Austen 1996; Van Den Avyle and Hayward 1999; Devore and Peck 2005). After the river was visually surveyed for areas that were impossible to sample by electrofishing and snorkeling (e.g., waterfalls, rapids, thermal features), the remaining portions of each stratum were partitioned into potential 500-m sample reaches (Figure 1). I used proportional allocation and random selection to select sample reaches comprising about one-quarter of each stratum (Thompson et al. 1998; Peterson et al. 2002) (Table 1).

Figure 1.—Randomly-selected sample reaches and strata along the Gibbon River, YNP, 2005.

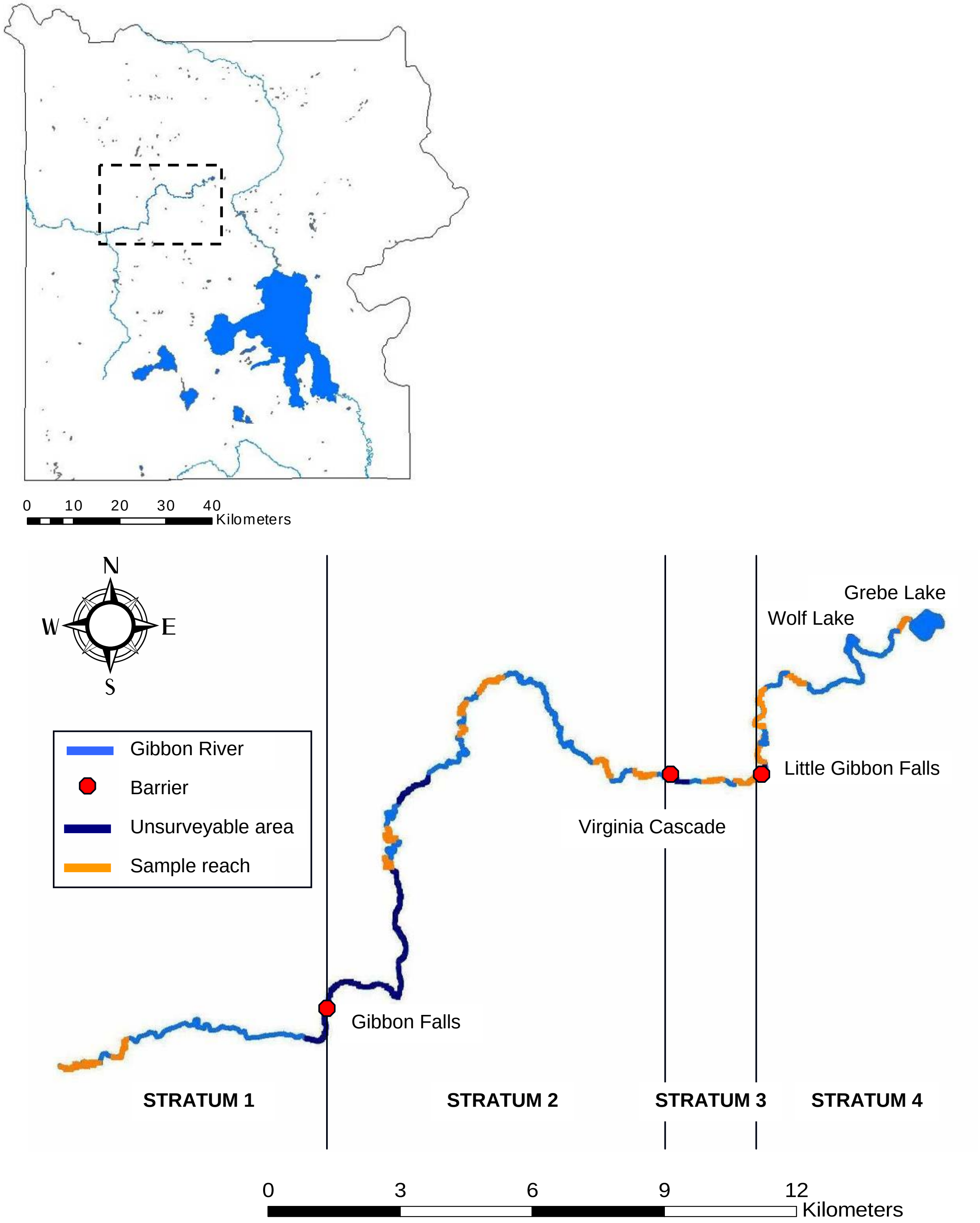


Table 1.—Proportional allocation of sample reaches within strata along the Gibbon River, YNP, 2005.

Stratum	Location	Total surveyable distance (m)	Sample reaches
1	Below Gibbon Falls	9,000	4
2	Gibbon Falls to Virginia Cascade	18,500	9
3	Virginia Cascade to Little Gibbon Falls	3,500	2[a]
4	Little Gibbon Falls to Wolf Lake	10,000	5
Total		41,000	20

[a] Data collected from one of the two reaches was not used because an insufficient portion of the reach was sampled.

Sampling effort was reallocated among reaches in 2006 to target areas of Arctic grayling detection and pool-dominated habitat, preferred by grayling during summer (Liknes 1981; Hubert et al. 1986; Liknes and Gould 1987; Reynolds 1989). This adjustment reflected 2005 results indicating grayling detection was exclusive to pool-dominated reaches (Steed and Zale 2006). This modification was an attempt to minimize the variance in abundance estimates within strata while maximizing sampling efficiency. Habitat delineation was performed prior to sampling in 2006. All surveyable sample reaches along the Gibbon River were classified according to pool-habitat frequency. Pools were defined as areas with maximum depths greater than or equal to 0.5 m, reduced water velocities, and undisturbed water surfaces (Liknes 1981). Reaches containing greater than or equal to 50% pool habitat were assigned a rating of type-1 (H1) whereas reaches containing less than 50% pool habitat (i.e., dominated by riffles) were assigned a rating of type-2 (H2)(Table 2). After all reaches were rated, standard errors were calculated for counts obtained during 2005 by electrofishing and snorkeling, respectively, according to habitat type to calculate the number of reaches to sample in 2006 (Table 2). Because no grayling were observed in H2 reaches using these techniques in 2005, conservative hypothetical standard errors were calculated to produce values greater than zero (Table 2)(J. Borkowski, Montana State University, personal communication).

Table 2.—Standard errors (*s*) associated with electrofishing and snorkeling counts of Arctic grayling, by habitat type, within each stratum along the Gibbon River, YNP, 2005. Substrata correspond to habitat-type reaches within strata.

				Electrofishing		Snorkeling	
Stratum	Habitat rating	Substratum	Sample reaches	Count[a]	*s*	Count[a]	*s*
1	1	1	10	2/8	0.463	11/16	0.946
	2	2	8	0/0	0.186[b]	0/0	0.186[b]
2	1	3	16	3/10	0.483	6/20	0.801
	2	4	21	0/8	0.186[b]	0/12	0.186[b]
3	2	5	7	0/4	0.213[c]	0/6	0.213[c]
4[d]	2	6	15	0/10	0.213[c]	0/20	0.213[c]

[a] Total number of grayling observed/total number of sampling events.

[b] Standard error calculated from hypothetical count of (1/29). All H2 reaches in strata 1 and 2 were combined (29) to estimated hypothetical standard error because strata 1 and 2 are more similar to each other than they are to strata 3 and 4.

[c] Standard error calculated from hypothetical count of (1/22).

[d] Reaches in stratum 4 located between Grebe and Wolf lakes were eliminated in 2006 because of their proximity to spawning adfluvial Arctic grayling (i.e., reduced from 20 to 15).

After standard errors were calculated, the following formula was used to determine the number of reaches to sample per substratum by electrofishing and snorkeling during 2006 (Table 3) (Thompson 2002):

$$n_h = \frac{n N_h \sigma_h}{\sum_{h=1}^{6} N_h \sigma_h},$$

where

n = minimum number of reaches to sample,

h = substratum,

N_h = total number of reaches per substratum, and

σ_h = standard deviation (estimated as s_h) associated with each sampling technique per substratum.

Assuming a 20-reach minimum sampling effort based on 2005 efforts, an average number of reaches to sample per substratum was determined based on the numbers of reaches calculated for electrofishing and snorkeling, respectively (Table 3). Twenty-two reaches were ultimately required based on a minimum of two sample reaches per substratum. Reaches to sample in 2006 were then randomly selected from all surveyable reaches (Figure 2). All reaches selected except for one reach in stratum 4 were electrofished once within about a two-week period once per month from late-May through mid-July and snorkeled within about a one-week period once per month from mid-July through September. One reach in stratum 4 was not sampled because of accessibility constraints.

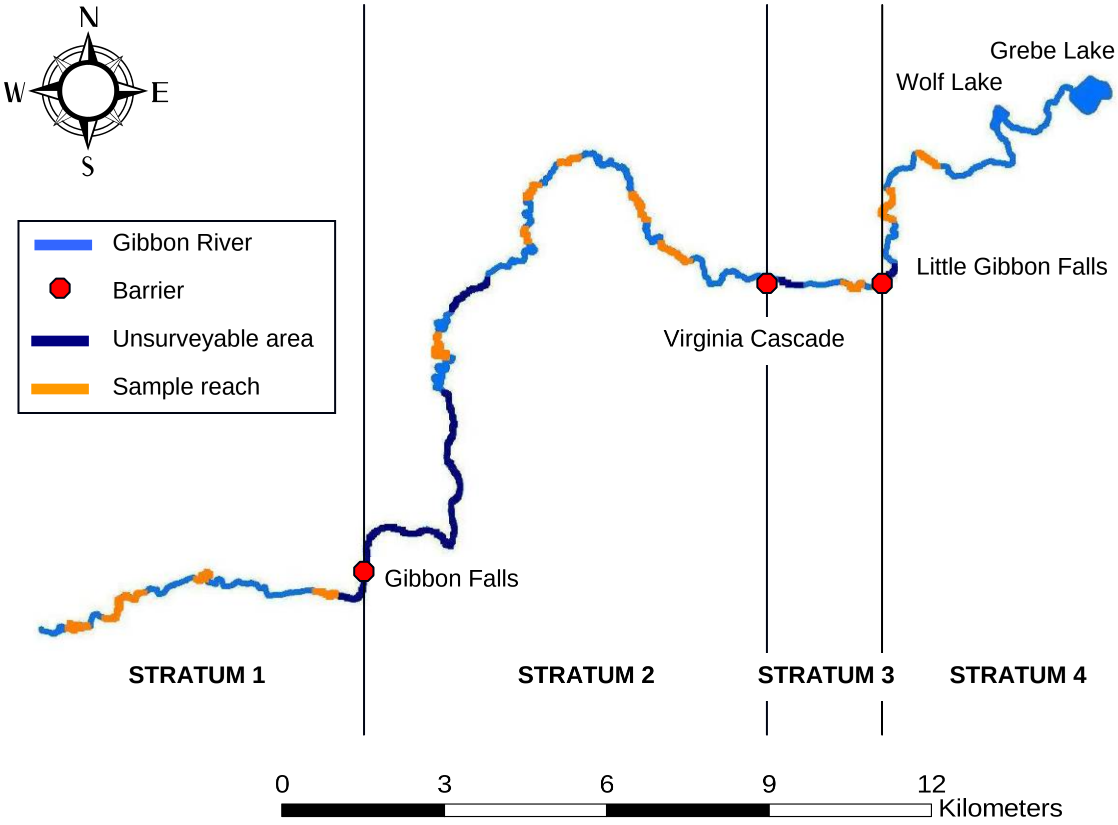
Table 3.— Calculated number of reaches (n_h) to sample per substratum (h) out of all possible reaches (N_h) by electrofishing and snorkeling in the Gibbon River, YNP, 2006. Estimates are based on standard errors (s_h) derived from data collected during 2005 and a 20-reach minimum sampling effort. Averaged number of reaches ($\bar{n}$) to sample using both techniques are also shown.

	Electrofishing					Snorkeling						
h	N_h	s_h	$N_h s_h$	$nN_h s_h$	n_h	N_h	s_h	$N_h s_h$	$nN_h s_h$	n_h	$\bar{n}$	Sample reaches[a]
1	10	0.463	4.63	92.60	4.49	10	0.946	9.46	189.20	5.65	5.07	5
2	8	0.186	1.49	29.76	1.44	8	0.186	1.49	29.76	0.89	1.17	2
3	15	0.483	7.25	144.90	7.03	15	0.801	12.02	240.39	7.18	7.11	7
4	22	0.186	4.09	81.84	3.97	22	0.186	4.09	81.84	2.44	3.21	3
5	7	0.213	1.49	29.82	1.45	7	0.213	1.49	29.82	0.89	1.17	2
6	15	0.213	3.20	63.90	3.10	15	0.213	3.20	63.90	1.91	2.50	3[b]
Total	77	- -	22.14	- -	21.5	77	- -	31.75	- -	18.97	- -	22

[a] At least two reaches were selected per substratum.

[b] Only two of the three selected reaches were sampled because of logistical constraints.

Figure 2.—Randomly-selected sample reaches and strata along the Gibbon River, YNP, 2006.



Electrofishing methods were consistent with those used by Dan Mahony and the YNP Streams Crew on the Gibbon River since 1999 and followed MFWP guidelines with different gears used in different sections because of variation in river depth and width (Kennedy and Strange 1981). Fish were collected using a Smith-Root, Inc., battery-powered backpack electrofisher in all selected reaches of strata 4, 3, and two reaches in stratum 2. These reaches were sampled by moving upstream whereas the remaining reaches were sampled by moving downstream using a boat-based or shore-based Coffelt 2C electrofishing unit powered by a 3.5 kW generator because of greater depths and discharge. Two reaches in stratum 2 were sampled during the first sample period in 2006 using the Coffelt unit described above based on shore and subsequently sampled based from a boat about 1.8 m in length. Remaining reaches in stratum 2 and all of stratum 1 were sampled using the Coffelt unit while floating downstream in a raft about 4.3 m in length. All reaches were electrofished at least twice from late-May through July using the single-pass method (Peterson and Cederholm 1984; Kruse et al. 1998; Magee et al. 2005) and by mark and recapture in July 2006 (Ricker 1975).

Total length of each fish caught was measured to the nearest millimeter. Sex identification and gonadal ripeness of Arctic grayling caught were determined when possible, with induced discharge of gametes indicating spawning condition. Visible implant alphanumeric (VI Alpha) tags were administered to each grayling greater than or equal to 130 mm (total length) after examination for previous tags or clips; this length corresponds to the minimum grayling size for hard VI Alpha tag retention (McMahon et al. 1996).

The number of Arctic grayling in each sample reach in each stratum in each sample period was estimated by dividing the number of grayling caught by the estimated electrofishing efficiency ($\hat{q}$) (Pearsons and Temple 2007). Values of $\hat{q}$ were calculated from mark and recapture data collected during 2005 and 2006 (Ricker 1975). Estimated efficiency was calculated in each reach as the number of salmonids recaptured (R_s) divided by the number captured (C_s)

during the second sampling event (Ricker 1975; Pearsons and Temple 2007). Grayling and all other salmonids caught were assumed to have similar catchability because too few grayling were sampled to accurately estimate species-specific efficiency (J. Magee, MFWP, personal communication). Estimates of *q* were not made by habitat-type within strata because of low recapture rates. However, estimates of *q* were similar in habitat types (H1 and H2) across strata (chi-square test, $p > 0.10$). Therefore, $\hat{q}$ values were pooled by habitat type. Pooling produced a weighted-average $\hat{q}$ for each habitat type (Table 4). Confidence intervals were calculated using the relationship between the *F* and the binomial distributions (Zar 1984).

Table 4.—Mean electrofishing efficiency estimates ($\bar{\hat{q}}$) calculated by habitat type using pooled salmonid mark and recapture data from the Gibbon River, YNP, 2005 and 2006.

Habitat type	N_{captured}	$N_{\text{recaptured}}$	$\bar{\hat{q}}$	95% CI
H1	367	39	10.6%	7.7 – 14.2%
H2	655	48	7.3%	5.5 – 9.6%

Estimates of grayling abundance using a variation of the Petersen method (Ricker 1975; S. Cherry, Montana State University, personal communication) incorporating the Chapman modification (Chapman 1951) were reported alongside those calculated without the modification to produce the range of potential abundances and because one assumption associated with each estimate could not be validated (S. Cherry, Montana State University, personal communication). Thus, grayling counts were divided by Chapman $\hat{q}$ values to produce comparative estimates. Chapman $\hat{q}$ values were calculated in the same manner as non-Chapman $\hat{q}$ values except 1 was added to each of the pooled R_s and C_s values (Table 5).

Table 5.—Mean electrofishing efficiency estimates ($\bar{\hat{q}}$) incorporating the Chapman modification and calculated by habitat type using pooled salmonid mark and recapture data from the Gibbon River, YNP, 2005 and 2006.

Habitat type	N_{captured}	$N_{\text{recaptured}}$	$\bar{\hat{q}}$	95% CI
H1	368	40	10.9%	7.9 – 14.5%
H2	656	49	7.5%	5.6 – 9.8%

Although the Chapman modification is useful when estimating abundances from small sample sizes (Chapman 1951; Ricker 1975), applying it to zero counts can inflate estimates considerably. Omitting the Chapman modification assumes grayling are absent when no fish are encountered whereas incorporating the modification assumes fish are present when none are observed (S. Cherry, Montana State University, personal communication). The following equations illustrate the application of estimated efficiencies to grayling counts to produce estimates of abundance:

Petersen variation: $$\hat{\tau}_{gt} = \frac{C_s C_{gt}}{R_s},$$

Petersen variation with Chapman modification: $$\hat{\tau}_{gt} = \frac{(C_s + 1)(C_{gt} + 1)}{R_s + 1} - 1,$$

where

$C_s =$ number of salmonids caught,

$C_{gt} =$ number of grayling caught at time *t*,

$R_s =$ number of marked salmonids recaptured, and

$\hat{\tau}_{gt} =$ estimated grayling abundance at time *t*.

The mean number of grayling in each H1 and H2 reach sampled, respectively, in each stratum in each sample period was calculated by dividing the estimated total number of grayling in each habitat-type reach by the number of H1 and H2 reaches sampled, respectively. These values were then multiplied by the respective number of habitat-type reaches in a stratum. Abundances in all H1 and H2 reaches in a stratum were combined to produce estimates for each stratum. Variability in estimated grayling abundances in stratum 1 was underestimated in 2005 because no H2 reaches were sampled that year. Standard errors (*s*) in abundance estimates were calculated using the following formula (Thompson 2002):

$$s(\hat{\tau}_j) = \sqrt{\hat{\mathrm{var}}(\hat{\tau}_j)} = \sqrt{\hat{\mathrm{var}}(\hat{\tau}_{j,H1})} + \sqrt{\hat{\mathrm{var}}(\hat{\tau}_{j,H2})} \quad ,$$

where

$\hat{\tau}_j$ = estimated total grayling abundance in stratum *j*,

$s(\hat{\tau}_j)$ = standard error in $\hat{\tau}_j$,

$$\hat{\text{var}}(\hat{\tau}_{j,H1}) = N_{j,H1}(N_{j,H1} - n_{j,H1})\frac{s^2_{j,H1}}{n_{j,H1}} \; ,$$

$$\hat{\text{var}}(\hat{\tau}_{j,H2}) = N_{j,H2}(N_{j,H2} - n_{j,H2})\frac{s^2_{j,H2}}{n_{j,H2}} \, ,$$

$$s^2_{j,H1} = \frac{\sum_{i=1}^{n}(x_{j,i} - \bar{x}_j)^2}{n_{j,H1} - 1} \; ,$$

$$s^2_{j,H2} = \frac{\sum_{i=1}^{n}(x_{j,i} - \bar{x}_j)^2}{n_{j,H2} - 1} \; ,$$

$N_{j,H1}$ = total number of H1 reaches in stratum *j*,

$N_{j,H2}$ = total number of H2 reaches in stratum *j*,

$n_{j,H1}$ = number of H1 reaches sampled in stratum *j*, and

$n_{j,H2}$ = number of H2 reaches sampled in stratum *j*.

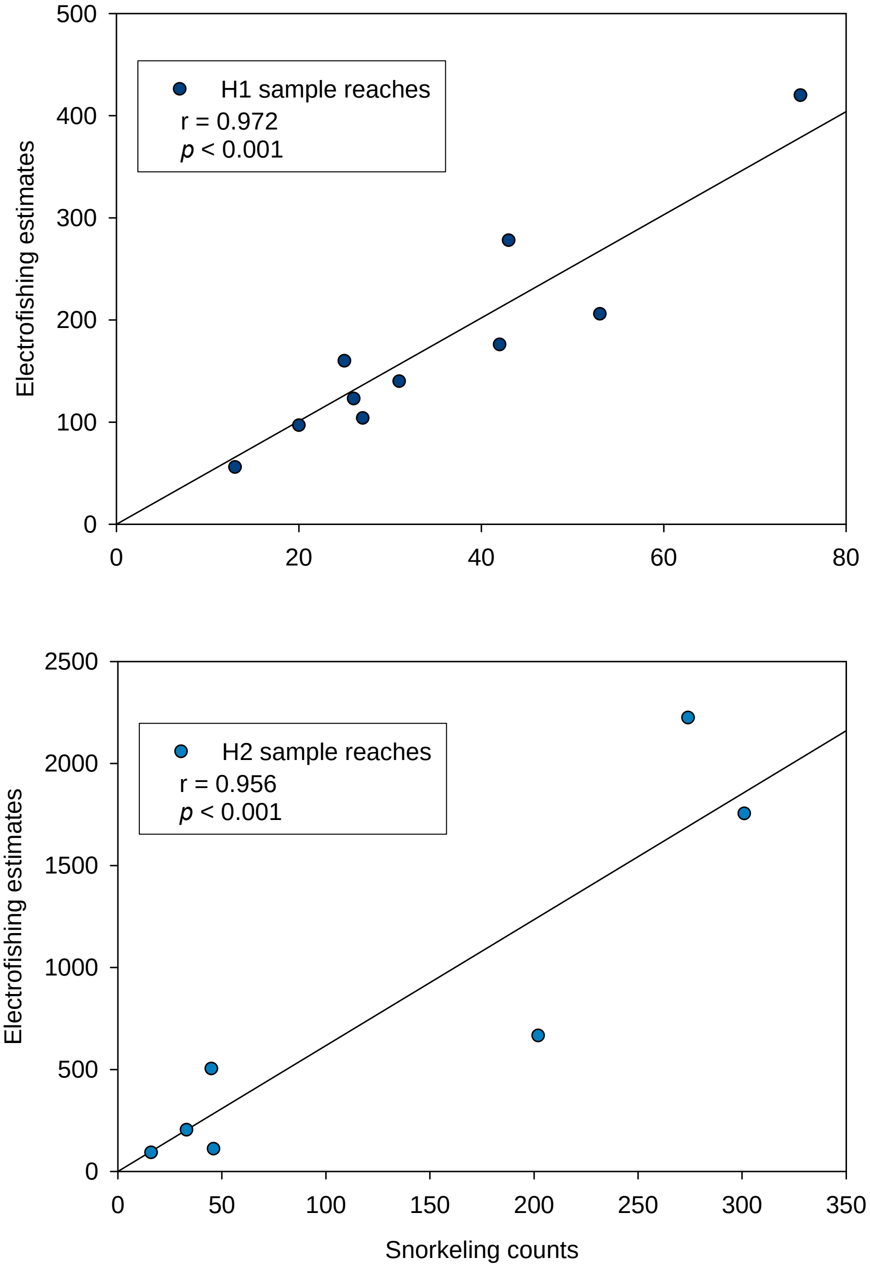
Standard errors in all estimates were reported in lieu of 95% confidence intervals because too few grayling were caught to assume a normal distribution and the variability in $\hat{q}$ values could not be incorporated into expansions to strata (S. Cherry, Montana State University, personal communication). Thus, reported errors were conservative estimates of deviation from mean abundances.

Sample reaches were snorkeled at least once per month from July through September during 2005 and 2006. Surveys involved floating reaches in a dry-suit with snorkel and mask while recording the size category of salmonids observed (Dolloff et al. 1996). Two observers counted fish if the river was too wide for one observer to see both banks (Dolloff et al. 1996). Observers maintained counts for their respective lanes (Schill and Griffith 1984), moving in a regular zigzag pattern parallel to each other thereby maximizing efficiency while minimizing bias (Mullner et al. 1998). Counts were made in a downstream direction if river depth and flow did not permit observers to crawl upstream (Dolloff et al. 1996). Observers counted all salmonids, identified tags or clips, and recorded fish in one of four size categories (< 101, 101-200, 201-300, and > 300 mm total length) on a PVC arm cuff with a pencil. Observers were trained to estimate lengths *a priori* by estimating known lengths of sticks underwater. Counts were made between 1000 and 1600 hours to ensure optimal visibility (Schill and Griffith 1984; Dolloff et al. 1996). Although block nets are useful in minimizing bias, they were not employed because of limited evidence of necessity and logistical constraints (Peterson et al. 2005).

The association between snorkeling counts and the absolute fish abundance estimated in each habitat type by electrofishing was strong (Figure

3), supporting the relationship’s use as an abundance estimator based on snorkeling counts.

Figure 3.—Electrofishing abundance estimates and snorkeling counts for all salmonids caught in habitat-type-1 (H1) and habitat-type-2 (H2) reaches sampled in the Gibbon River, YNP, respectively, in 2005 and 2006.



Snorkeling efficiency ($\hat{R}$) was estimated by substratum in all sample reaches using the following formula to relate salmonid counts (x_i) to estimated "true" abundances (y_i) obtained by electrofishing (Table 8)(Dolloff et al. 1993):

$$\hat{R} = \frac{\sum_{i=1}^{n'} y_i}{\sum_{i=1}^{n'} x_i} = \bar{y}' / \bar{x}' ,$$

where

$$\bar{y}' = \sum_{i=1}^{n'} y_i / n' ,$$

$$\bar{x}' = \sum_{i=1}^{n'} x_i / n' , \text{ and}$$

$n' =$ number of reaches sampled by both snorkeling and electrofishing (i.e., all sample reaches).

Snorkeling efficiency was estimated for each substratum because it produced the best site-specific estimate while minimizing variance. Adjusted Petersen estimates of salmonid abundance were calculated from electrofishing data in nine reaches for $\hat{R}$ estimation (Ricker 1975). Abundances were estimated by dividing electrofishing counts by the appropriate (i.e., H1 or H2) weighted-average $\hat{q}$ (Table 5)(Ricker 1975), incorporating the Chapman modification, for reaches with recapture rates too low to use the adjusted Petersen method (S. Cherry, Montana State University, personal communication). Snorkel counts for each sample reach were performed one to three days prior to electrofishing surveys during July 2006 to minimize potential biases (Thurow and Schill 1996; Mullner et al. 1998). Snorkeling efficiency estimated during July 2006 was applied to counts made during 2005 and to subsequent 2006 surveys, assuming the relationship between abundance and $\hat{R}$ did not change significantly over time. Although snorkel estimates are more variable in winter than in summer months (Rodgers et al. 1992), surveys ceased in late September thereby minimizing temporal bias.

Table 6.—Snorkeling efficiency estimates ($\hat{R}$), calculated by substratum, using snorkeling and electrofishing data collected from 7/24/06 through 8/5/06 on salmonids detected in the Gibbon River, YNP.

Stratum	Substratum	$\bar{x}'$	$\bar{y}'$	$\hat{R}$
1	1	151	1544	10.22
	2	61	864	14.17
2	3	264	2611	9.90
	4	206	2918	14.16
3	5	476	2889	6.10
4	6	324	5472	16.89

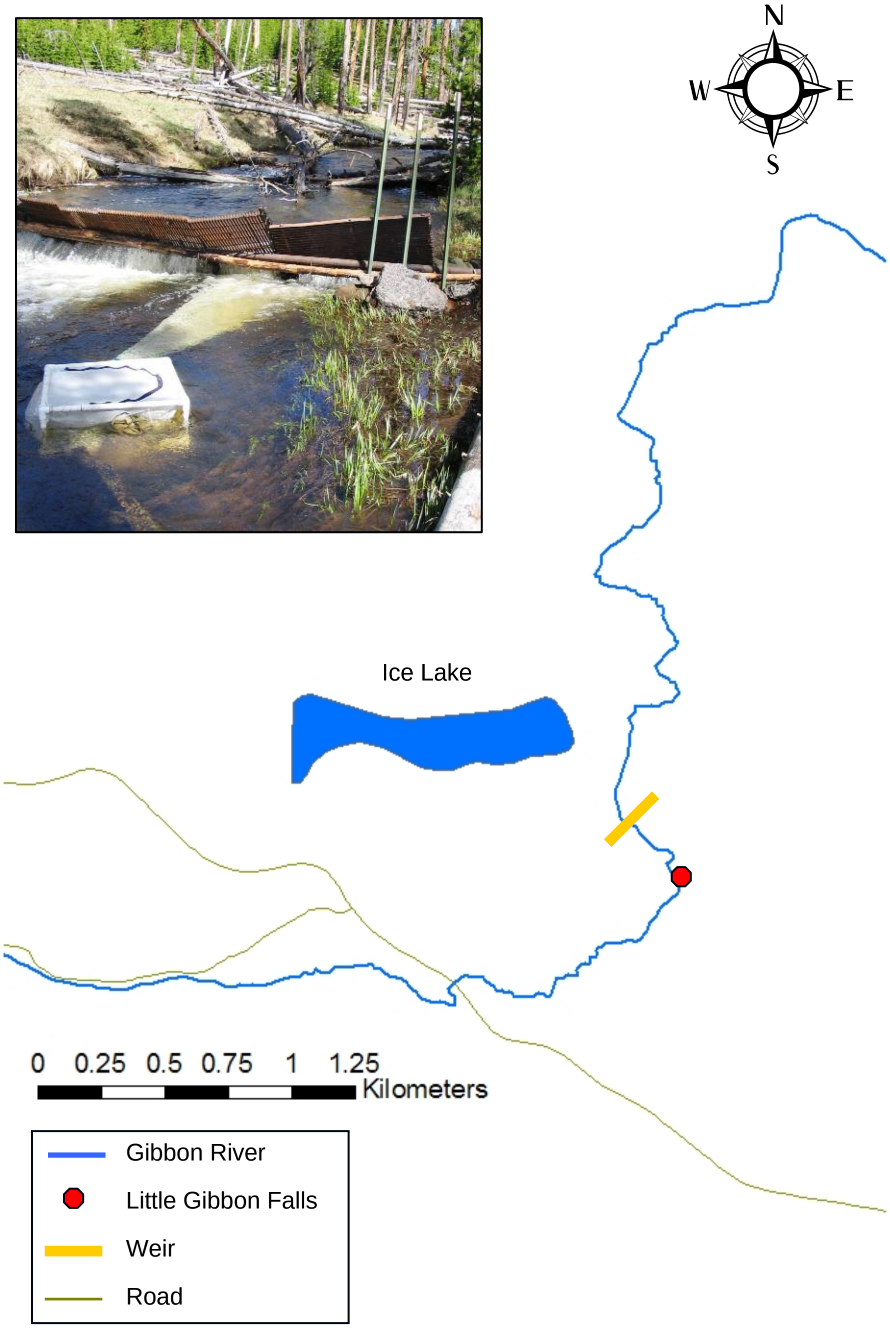
n = number of reaches sampled in each substratum.
$\bar{x}'$ = number of salmonids counted by snorkelers.
$\bar{y}'$ = total number of salmonids estimated from mark and recapture electrofishing data.
$\hat{R}$ = the ratio of the estimated number of fish present to the number observed by snorkelers (e.g., there are about 10 fish present in substratum 1 for every one fish observed).

Anglers with the Yellowstone Park Foundation’s Fly-fishing Volunteer Program were employed periodically from mid June through early September in 2005 and 2006 to capture grayling throughout the Gibbon River system. All angling was performed using fly-fishing tackle. Anglers targeted pool-dominated habitat and areas of prior grayling detection to maximize the likelihood of grayling capture in the river. Grebe and Wolf lakes were sampled only in 2005 and angling was shore-based. Estimates of minimum abundance for each sample period in each stratum were calculated (when dates corresponded) for comparison with estimated total abundances calculated from electrofishing and snorkeling data. Extrapolations to strata could not be made because sampling was temporally irregular and non-random. Estimates of grayling caught per angler-hour (i.e., catch-per-unit-effort (CPUE)) were calculated by dividing the number of grayling caught by the reported number of angler-hours per sampling event (Malvestuto 1996). The number of angler-hours per sampling event was calculated by multiplying the number of anglers present by total fishing time (Malvestuto 1996). Estimates of non-grayling CPUE were calculated as described above, substituting all other salmonids caught for grayling.

A panel weir (Hunter 1954) was installed in 2006 to compare the number of Arctic grayling emigrating from Grebe and Wolf lakes to the estimated abundances of riverine grayling. Equivalent or greater abundances of weir-caught to river-caught grayling would suggest lake-based origins of river-caught grayling. However, support for the existence of one or more populations of fluvial grayling would exist if few or no grayling entered the Gibbon River from headwater lakes.

Weirs have been used successfully to examine fish movement in systems similar to the Gibbon River (Nelson 1999; Nelson et. al 2002; Schmetterling and Adams 2004). I could not install a weir on the Gibbon River in 2005 because of limited personnel availability. However, increased sampling efficiency during 2006 freed resources for the implementation and maintenance of a weir. The weir was installed about 300 m upstream of Little Gibbon Falls and was invisible from nearby trails (Figure 4). This location maximized the distance from headwater lakes while remaining above the first major barrier to upstream movement on the Gibbon River (Little Gibbon Falls).

Figure 4.—Weir location on the Gibbon River, YNP, 2006.



The Source of Arctic Grayling Detected in the Gibbon River

The source of Arctic grayling detected in the Gibbon River, YNP, was determined by analyzing genetic, growth, and length-at-age data collected from grayling caught throughout the Gibbon River system. Genetic comparisons were made based on demonstrated differences between known fluvial and lacustrine grayling (Kaya 1991; Kaya 1992; Everett and Allendorf 1985) using microsatellite loci developed for Arctic grayling in Montana (Diggs and Ardren, in press). Age and growth data were used to detect differences in growth and lengths-at-age of grayling within the Gibbon River system.

Arctic grayling genetic samples were collected in the Gibbon River system, YNP, by electrofishing and fly-fishing from 1999 to 2006 and by weir-trapping in 2006. Grayling caught on the weir were presumed to be of headwater lake origin because this portion of the river was historically fishless (Varley and Shullery 1998) and because of the weir's proximity to lacustrine grayling sources. About one-third of a fin (average clip = 20 mg) was taken from each grayling caught (unless it was a recapture) during 2005 and 2006. Effects of fin-clipping are negligible in goldfish and coho salmon and this procedure has been used by fisheries biologists since the 1800s (Radcliffe 1950; McFarlane et al. 1990). Differential fin clips were taken from grayling caught throughout the Gibbon River system in each of 2005 and 2006 for source determination during recapture events. Left and right pelvic fin clips were taken from grayling caught in headwater lakes and in the Gibbon River, respectively, during 2005. Upper and lower caudal fin clips were taken from grayling caught in headwater lakes and in the Gibbon River, respectively, during 2006. Fin clips were preserved upon collection in glass tubes containing 95% ethanol (Kelsch and Shields 1996; Stamford and Taylor 2004).

I extracted DNA from fin clips using the DNeasy tissue kit (Qiagen, CA). I amplified eleven unique Arctic grayling microsatellite loci (Diggs and Ardren, in press) and one rainbow trout unique locus, *OMM*1037 (Rexroad 2002). All forward primers were fluorescently labeled at the 5' end for visualization purposes. I multiplexed these loci into three polymerase chain reactions (PCR) for efficiency: (1) *Tar*100, *Tar*101, *Tar*104, and *Tar*109, (2) *Tar*103, *Tar*105, and *Tar*106, (3) *Tar*108, *Tar*110, and *Tar*115. The remaining two loci, *Tar*114 and *OMM*1037, were amplified separately. The multiplex PCR mixtures consisted of about 50 to 150 ng of extracted DNA, 5.0 µL of 2 x QIAGEN Multiplex PCR Master Mix (Qiagen, CA), 1 µM of each primer, and enough water for a 10 µL reaction volume. The thermoprofile consisted of an activation step of 15 min at 95°C followed by 30 cycles at 94°C for 30 s, 62°C for 90 s, 72°C for 30 s, and a final one-step extension at 72°C for 10 min. Polymerase chain reaction mixtures for *Tar*114 and *OMM*1037 consisted of 1 x Gold Buffer (Applied Biosystems, CA), 1.5 mM $MgCl_2$ for *Tar*114 or 2.5 mM $MgCl_2$ for *OMM*1037, 200 ng BSA, 1.0 mM dNTPs, 1.0 µM forward and reverse primers each, 0.5 Unit AmpliTaq Gold© DNA Polymerase (Applied Biosystems, CA) and enough water for a 10 µL reaction volume. The thermoprofile consisted of one 10 min activation/denaturing step at

95 °C followed by 45 cycles at 95°C for 30 s, T_m °C for 30 s (*Tar*114 at 58 °C and *OMM*1037 at 55 °C), 72°C for 30s, and a final one-step extension at 72 °C for 30 min. The PCR products were visualized using the 3100-*Avant* Genetic Analyzer and analyzed with GeneMapper v3.7 (Applied Biosystems, CA). Although bins were set up for automatic genotype scoring, each genotype score was manually inspected for accuracy (Hoffman and Amos 2005).

An exact probability test for departures from Hardy-Weinberg (HW) equilibrium was conducted individually and across all loci using Fisher's method in GENEPOP version 3.4 (Raymond and Rousset 1995). Expected heterozygosity (H_e) was compared between source groups using both sign and paired *t*-tests with significance set at the $\alpha = 0.05$ level (Sokal and Rohlf 1995). Allelic richness was also used to quantify the level of genetic diversity present in each group. Because the number of alleles found in a sample is influenced by sample size, and the sample size varied between study sites (Grebe and Wolf lakes, $N = 95$ individuals; Gibbon River, $N = 66$ individuals) rarefaction was used to standardize sample size. The program HP-RARE was used for this analsyis, and the sample size was standardized to 100 genes (or, equivalently 50 individuals) per population (Kalinowski 2005). The number of private alleles (alleles found in only one population) was also estimated using rarefaction at the sample size (100 genes per population). Differences in private allelic richness between source groups were compared using a sign test with significance set at the $\alpha = 0.05$ level (Sokal and Rohlf 1995; Kalinowski 2005). Genetic differentiation measured by F_{ST} between source groups was estimated using GENEPOP (Raymond and Rousset 1995) and FSTAT version 2.9.3.2 (Weir and Cockerham 1984; Goudet 1995). Bootstrapping was used to calculate associated 95% confidence limits in FSTAT. The statistical significance of differences in allele frequencies between source groups was tested using the genic differentiation test of Fisher as implemented by GENEPOP (Raymond and Rousset 1995). Last, we used STRUCTURE version 2.2 (Pritchard et al. 2000) to group individuals into populations using only the genotypes of the individuals. The number of populations assumed to be present was varied from one to four, and the posterior probability (Ln(D)) associated with each number was used as a measure of support present in the data.

In addition to genetic comparisons, lengths-at-age of Arctic grayling sampled in the Gibbon River system were compared to determine if differences existed between source groups. Data were not compared with fish from other sources (including the Big Hole River, Montana) because productivity in the Gibbon system may differ considerably from other systems because of drainage size, latitude, and thermal regime, potentially confounding length-at-age comparisons. Significantly higher lengths-at-age in lacustrine versus stream-dwelling fish have been documented in multiple systems (Hutchings 1986; O'Connell and Ash 1993; Halvorsen 1996; Dempson et al. 1996; Erkinaro et al. 1998). Thus, significantly higher lengths-at-age in lake-caught versus river-caught fish would provide support for the existence of a fluvial grayling population in the Gibbon River.

Scales were used to age Arctic grayling caught in the Gibbon River system because they can be collected non-lethally (Devries and Frie 1996) and are relatively accurate (Stuart and Chislett 1979). Although otoliths have been deemed more appropriate for ageing grayling in northern latitudes (DeCicco and Brown 2006), discrepancies in age determination between the two methods are minimal within the average lifespan of grayling found in Montana and Wyoming (Shepard and Oswald 1989; Kaya 1990). Scale samples and total length (mm) were collected from each grayling caught by electrofishing, weir-trapping, and fly-fishing in the Gibbon River system during 2005 and 2006. About five to ten scales were removed from a location dorsal to the lateral line on the left side of each grayling (Devries and Frie 1996).

Scales were impressed onto acetate slides and photographed under 25 X magnification using a Pixera Pro 150ES digital camera coupled with an Olympus SZ-CTV microscope. Images were uploaded and prepared for analysis using Viewfinder version 3.0 software. Growth increments were measured from scale radii with SigmaScan Pro image analysis software version 5.0.0 (SPSS Inc., Chicago, Illinois). Ages were assigned based on the number of annuli present (Devries and Frie 1996). I independently measured each image twice to estimate precision using the coefficient of variation (CV) (Chang 1982; Campana 2001):

$$CV_j = 100 \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j},$$

where

X_{ij} = the *i*th age determination for the *j*th fish,

X_j = the mean age estimate for the *j*th fish, and

R = the number of age determinations made for each fish.

The majority of Arctic grayling scale samples collected during 2005 and 2006 in the Gibbon River system originated from Grebe and Wolf lakes (Table 7). Few samples were unsuitable for analysis of age and growth because of poor scale condition (Table 7). Mean CV values and associated standard errors (*s*) of age determinations were similar for lake and river-caught fish (Table 7).

Table 7.—Number of Arctic grayling scale samples obtained from the Gibbon River system, YNP, during 2005 and 2006 with associated average, minimum, and maximum coefficient of variation (CV) values and standard errors (*s*) associated with age determinations.

Source	n_1	n_2	CV	Min CV	Max CV	*s*(CV)
Grebe and Wolf lakes	74[a]	71[a]	3.41	0.00	28.28	7.68
Gibbon River	27	24	3.86	0.00	28.28	8.74
Total	101	95				

n_1 = number of scale samples collected and analyzed.
n_2 = number of scale samples suitable for age estimation.
[a] Sixteen of these samples were weir-caught.

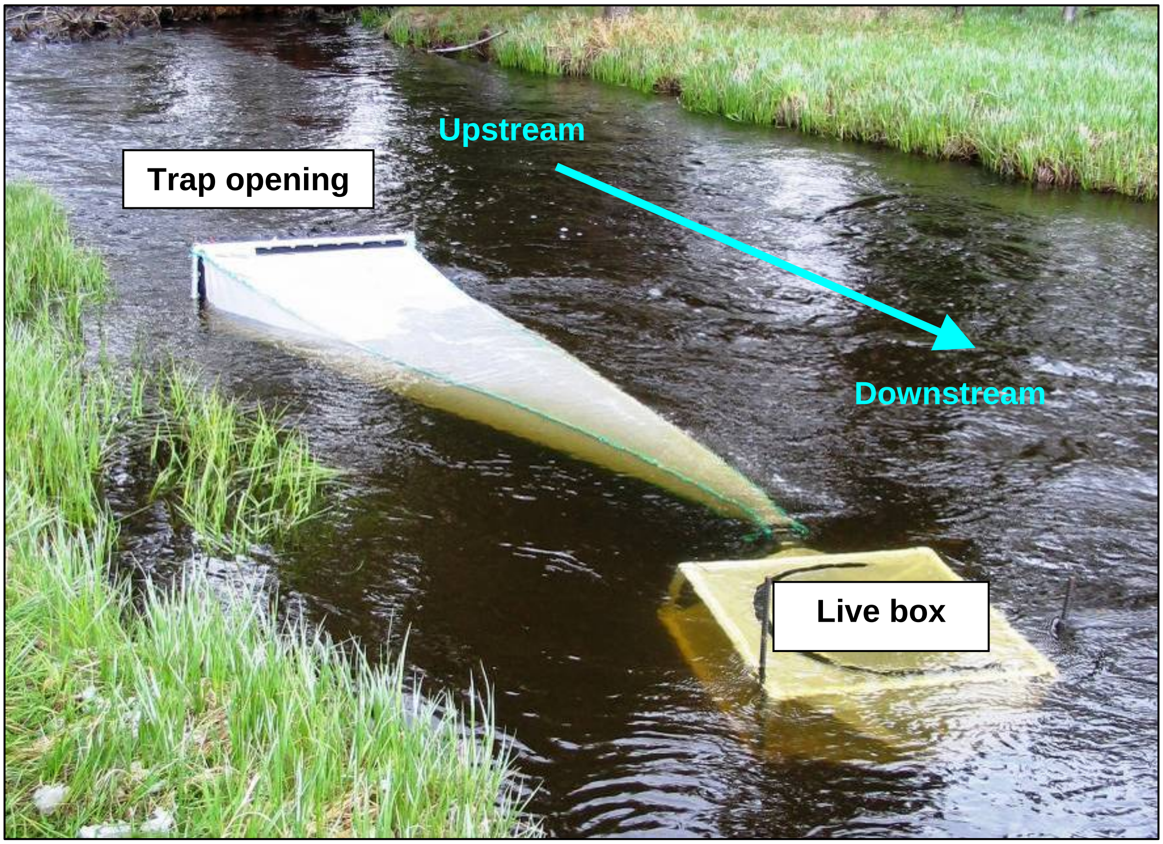
Lengths-at-age were back-calculated using the Fraser-Lee method (Devries and Frie 1996) employing the intercept value (*a*) of 51.5 mm (total length) for Montana grayling (Brown 1943) because too few fish were sampled to develop a system-specific value. Mean lengths and associated 95% confidence intervals were calculated for age-1 through age-4 fish by source (i.e., Gibbon River and Grebe and Wolf lakes fish). Age-5 and age-6 fish from Grebe and Wolf lakes were not included in analyses because none were sampled from the Gibbon River. Mean lengths of grayling at each age were compared between source groups using Welch's two-sample *t*-test ($\alpha = 0.05$) because heteroscedasticity was detected among ages (Zar 1984). Factorial analyses of variance and covariance were not employed to compare lengths of grayling between sources and between age groups because an appropriate transformation was not detected to normalize heteroscedasticity.

Spawning Success of Arctic Grayling in the Gibbon River, YNP

The presence of Arctic grayling fry in the Gibbon River below Little Gibbon Falls would indicate successful spawning in the river. Stationary fry traps were used to detect the presence of grayling fry in the river. This method was used because electrofishing is ineffective for small fish (Reynolds 1996) and identification of fry to species while snorkeling is difficult (Dolloff et al. 1996).

My trap nets were identical to those used by Gale (2005). They were manufactured by Research Nets, Inc., and had a rectangular entrance 61 cm high x 91 cm wide with a 305-cm long net (1.6 mm mesh) attached to a PVC collar (11.4 cm diameter) that led to a 61 x 61 x 61 cm live box (1.6 mm mesh) (Figure 5).

Figure 5.—Fry trap at site 8 in stratum 2 in the Gibbon River, YNP, where it was periodically deployed during 2005 and 2006.

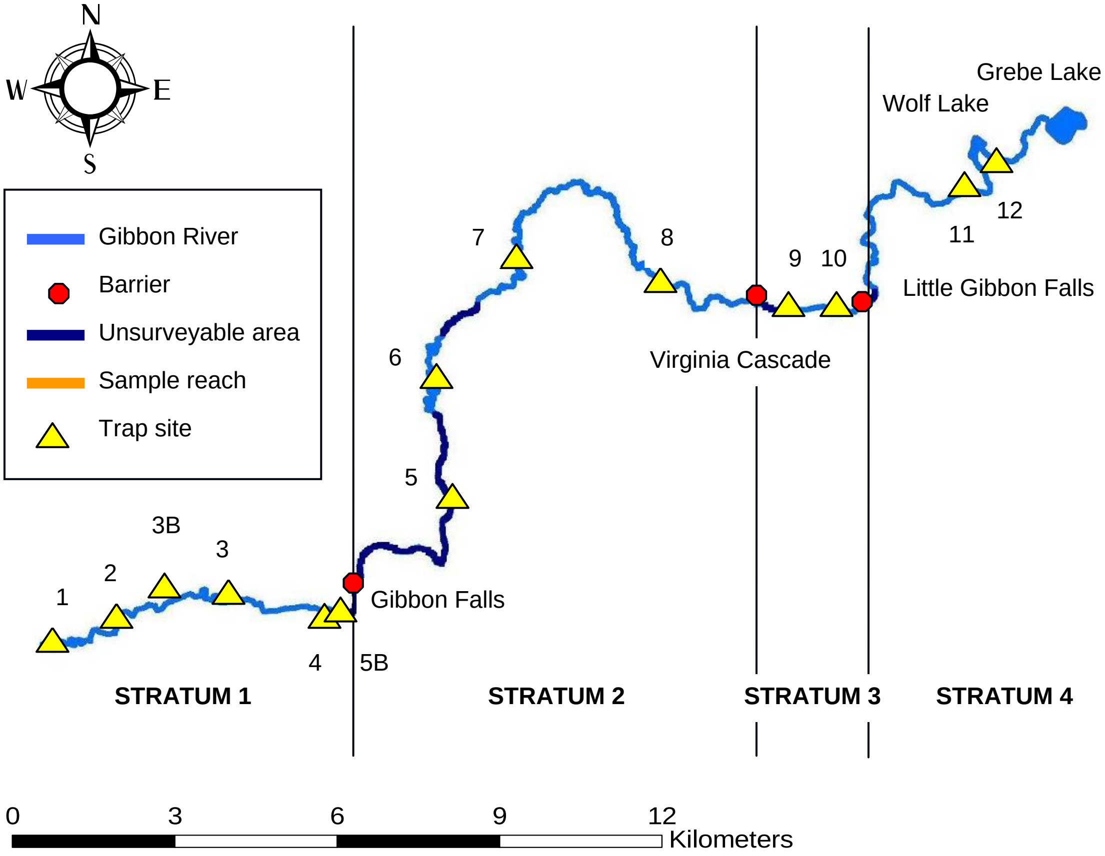


Traps were longitudinally distributed at 12 sites (Figure 6) along the Gibbon River system during 2005, within the confines of access and substrate (e.g., bedrock prevented installation), in preferred age-0 Arctic grayling habitat (Nelson 1954; Armstrong 1986; Deleray 1991). Traps were deployed twice per month for 24 to 48 hours to capture potential daytime and presumed nocturnal movements (Kruse 1959; Lund 1974; Deleray 1991). Forty-eight-h deployment was performed when possible, yielding an average number of fry caught per 24-h period. Traps were deployed from early June through mid-September, relating to previously observed spawning times for grayling near Grebe and Wolf lakes (Kruse 1959; D. Mahony, YNP, personal communication), temperature-based predictions for grayling emergence in Montana (Nelson 1954; Watling and Brown 1955; Northcote 1995), and observed spawning times for Montana grayling populations (Nelson 1954; Shepard and Oswald 1989; Kaya 1990; Deleray 1991; Northcote 1995; Mogen 1996; Kaeding and Boltz 1999). All fry were counted and frozen for subsequent identification in the laboratory if field identification was impossible (Weisel 1966; Barndt 1996). Fish other than grayling were not identified to species.

Fry-trapping effort was reallocated in 2006, targeting areas of prior fry detection to maximize sampling effort (Figure 6). Sites 11 and 12 in stratum 4 were eliminated because of their proximity to adfluvial Arctic grayling in Grebe and Wolf lakes. Sites 10, 7, 5, 3, and 1 were not retained because of their poor fry yields in 2005. Two new sites (3B and 5B) were added in stratum 1 based on its relatively high fry yield in 2005. Traps were deployed on a three-week rotation to avoid lunar-cycle bias. Traps were deployed earlier in 2006 than in 2005 (mid-May versus mid-June) because sites were already established and logistical constraints were reduced. Deployment continued through mid-September in

2006 as in 2005. All other sampling protocols used in 2005 were repeated in 2006.

Figure 6.—Stationary fry trap distribution along the Gibbon River system, YNP, during 2005 and 2006. Sites 1-12 (excluding 3B and 5B) were deployed during 2005 whereas sites 2, 3B, 4, 5B, 6, 8, and 9 were deployed during 2006.



Trap efficiency was directly and indirectly tested during 2005 and 2006 (Hennessey 1998). Indirect efficiency was estimated by calculating the percentage of discharge sampled by each trap. Depth and velocity were measured at five equidistant points upstream (less than 0.5 m) from each trap. Discharge sampled by each trap was estimated as the mean velocity multiplied by the area sampled. The area sampled was estimated as the mean depth multiplied by the width of the trap. The percent discharge sampled by each trap was calculated by dividing the discharge sampled by each trap by the total discharge. Total discharge was estimated for the entire channel as the sum of all discharge estimates. Depth, velocity, and channel width were recorded channel-wide every 0.5 m concurrently at each site. Discharge was estimated every 0.5 m by multiplying the velocity by the area associated with each velocity. The area associated with each velocity was calculated by multiplying depth at each recording by the distance between recordings. The number of fry in the river at a given site was then calculated as a function of the number of fry caught and the sampling efficiency of that site’s trap. Cross-sectional discharge estimates were taken at trap sites using a Marsh-McBirney Flowmate™ digital flow meter, coinciding with trap deployment to account for any trap-induced discharge impediment.

Direct trap efficiency testing involved marking and re-trapping of fry. Fry marking was performed if at least 10 fry were collected (Gale 2005). Fry were marked by immersion in a 1:30,000 solution of Bismarck Brown Y dye for about 1 h with an aerator to minimize stress-induced mortality (Kelsch and Shields 1996). Fry were then released in a suitable pool about 50 m upstream from the trap (Gale 2005). The trap remained deployed for a second 24-h period, with efficiency calculated as the number of marked fry caught divided by the total number dyed and multiplied by 100 (Gale 2005). Direct efficiency was estimated only once per week at any given site, if enough fish were captured, because dyed fish remain discolored for at least 4 days (Hennessey 1998). Efficiency was directly estimated only at sites 12 and 2 during 2005 and 2006, respectively, because of limited catches of live fry at other sites.

Direct and indirect efficiency estimates were similar at site 2 (4.3% and 4.7%, respectively) in 2006. Efficiency estimated directly at site 12 was lower than efficiency estimated indirectly (18.2% and 52.6%, respectively). However, indirect efficiency was used to approximate fry abundances because of the limited number of direct efficiency estimates. Indirect efficiency estimates for closest dates in 2006 were used in lieu of missing 2005 estimates because values were similar for comparable dates between years. Indirect efficiencies ranged across all trapping periods from 1.1% at site 10 to 52.6% site 12 in 2005 whereas they ranged from 1.1% at site 5B to 36.6% at site 9 in 2006. The greatest variability between years occurred at site 9 in mid June, ranging from 16.7% in 2005 to 35.7% in 2006. No variability in efficiency (1.2%) between years was detected at site 6 in mid September in both years. The area-under-the-curve method (AUC) (Sigma Plot 9.0, English et al. 1992) was used to estimate the annual number of fry that moved downstream at each trap site during each of 2005 and 2006.

RESULTS

Arctic Grayling Abundances in the Gibbon River, YNP

Few Arctic grayling were detected in the Gibbon River, YNP, during 2005 and 2006. Four grayling were caught electrofishing in both 2005 and 2006 whereas no to four fish were observed by snorkelers in each sample period in 2005 and 2006. Fewer grayling were detected by snorkelers in 2006 than in 2005. Nearly all grayling were detected in strata 1 and 2 and similar numbers of grayling were detected in those strata in both years (Tables 8 and 9). No and one grayling were detected in strata 3 and 4, respectively, in both years. Most grayling were detected during mid June through late August in both years. Anglers caught 12 and 9 grayling in 2005 and 2006, respectively, with the majority of these fish caught in stratum 2 during June in both years.

Estimated Arctic grayling abundances varied considerably in both 2005 and 2006 (Tables 8 and 9). Abundances estimated using the Chapman modification were consistently higher, reflecting the low numbers of grayling caught (Tables 8 and 9). However, estimated *s* values (i.e., “representative” deviations from the mean) associated with abundances were generally high. Estimated mean numbers of grayling per km ranged from 0 to 8 and 0 to 19 without and with the Chapman modification, respectively, in 2005 (Figure 7). Estimated mean numbers of grayling per km ranged from 0 to 9 and 0 to 27 without and with the Chapman modification, respectively, in 2006 (Figure 8).

Table 8.—Estimated abundances ($\hat{\tau}$) with associated standard errors (*s*) of Arctic grayling in the Gibbon River, YNP, in each stratum in each sample period, 2005. Estimates incorporating the Chapman modification are shown in italics. Estimates were not made for stratum 3 because only one reach there was adequately sampled in 2005.

	Sample period									
	6/17-7/1[b]		6/29-7/28[b]		7/28-8/9[c]		8/23-8/25[c]		9/22-9/25[c]	
Stratum	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*
1	0	0.00	62	22.59	102	107.43	0	107.43	26	107.43
	82	*0.00*	*128*	*16.80*	- -	- -	- -	- -	- -	- -
2	0	0.00	25	4.95	95	3538.17	95	3538.17	32	3538.17
	260	*16.69*	*421*	*16.69*	- -	- -	- -	- -	- -	- -
4[a]	0	0.00	0	0.00	0	12450.29	0	12450.29	0	12450.29
	186	*0.00*	*186*	*0.00*	- -	- -	- -	- -	- -	- -

[a] Arctic grayling in stratum 4 were considered adfluvial fish based on proximity to headwater lakes.
[b] Estimates based on electrofishing counts.
[c] Estimates based on snorkeling counts.

Table 9.—Estimated abundances ($\hat{\tau}$) with associated standard error (*s*) of Arctic grayling in the Gibbon River, YNP, in each stratum in each sample period, 2006. Estimates incorporating the Chapman modification are shown in italics.

	Sample period									
	5/30-6/9[b]		6/19-7/4[b]		7/24-8/5[b]		8/22-8/25[c]		9/23-9/26[c]	
Stratum	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*	$\hat{\tau}$	*s*
1	0	0.00	74	46.30	50[d]	42.87	57	1457.95	0	1457.95
	181	*0.00*	*253*	*48.17*	*235*[d]	*46.38*	- -	- -	- -	- -
2	0	0.00	109	30.67	0	0.00	23	1492.31	0	1492.31
	391	*0.00*	*506*	*36.39*	*391*	*0.00*	- -	- -	- -	- -
3	0	0.00	0	0.00	0	0.00	0	4611.58	0	4611.58
	87	*0.00*	*87*	*0.00*	*87*	*0.00*	- -	- -	- -	- -
4[a]	93	76.57	0	0.00	0	0.00	0	12450.29	0	12450.29
	286	*89.62*	*186*	*0.00*	*186*	*0.00*	- -	- -	- -	- -

[a] Arctic grayling in stratum 4 were considered adfluvial fish based on proximity to headwater lakes.

[b] Estimates based on electrofishing counts.

[c] Estimates based on snorkeling counts.

[d] Estimates based on recapture of one grayling caught in prior sample period.

Figure 7.—Estimated mean numbers of Arctic grayling per km (without and with the Chapman modification, respectively) in the Gibbon River, YNP, 2005. Estimates were not made for stratum 3 because only one reach there was adequately sampled in 2005. Estimated mean abundances are zero where no bars are shown. The Chapman modification was only applicable to estimates produced from mark and recapture electrofishing data (i.e., the first two sample periods).

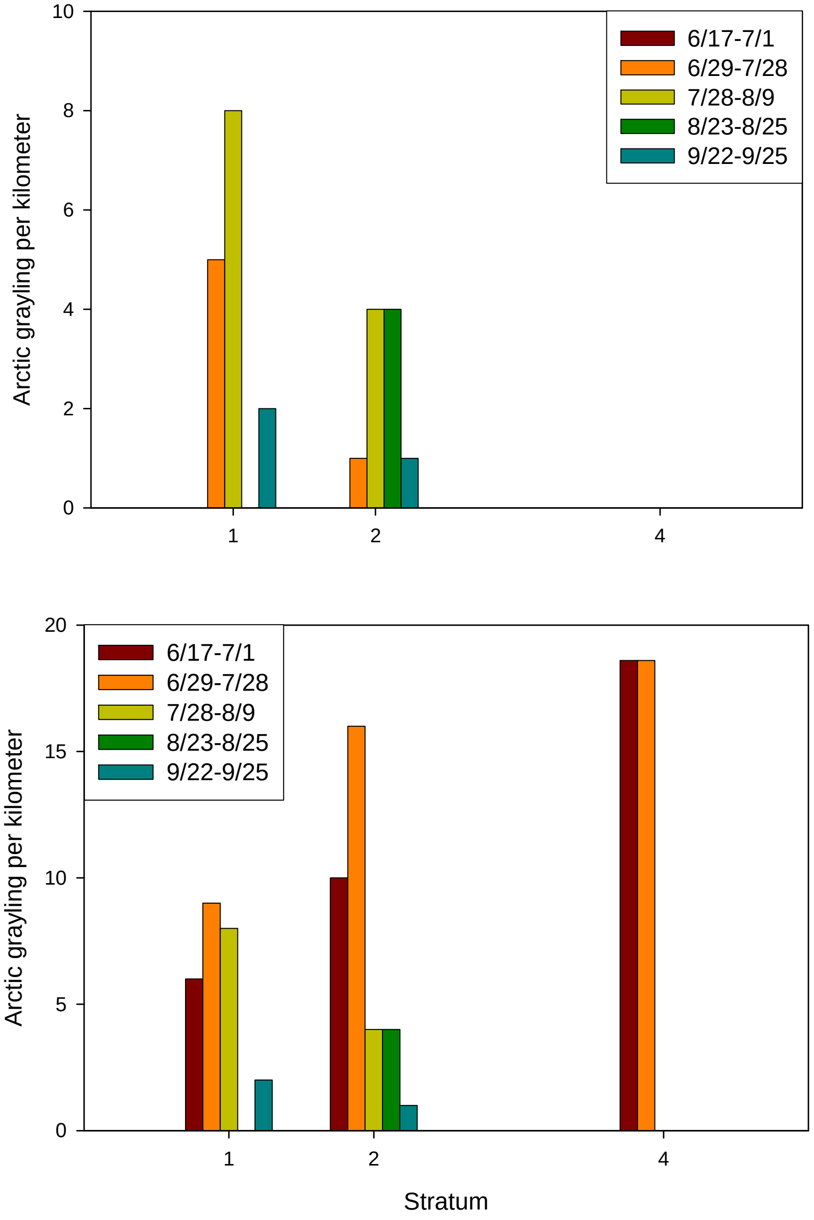
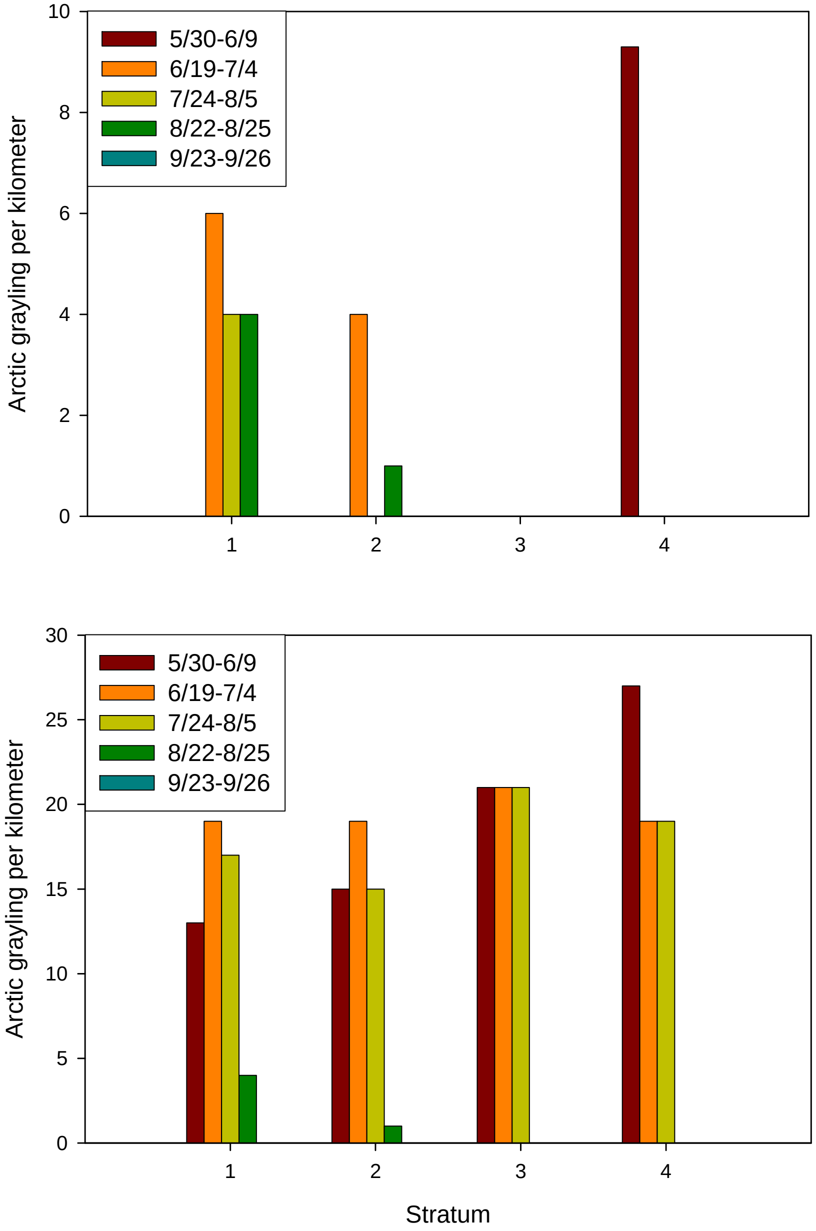


Figure 8.—Estimated mean numbers of Arctic grayling per km (without and with the Chapman modification, respectively) in the Gibbon River, YNP, 2006. Estimated mean abundances are zero where no bars are shown. The Chapman modification was only applicable to estimates produced from mark and recapture electrofishing data (i.e., the first three sample periods).



Estimated minimum grayling abundances produced from angler data ranged from no to three and no to five fish per sample period per stratum in 2005 and 2006, respectively. Estimates of angler CPUE (i.e., number of grayling caught per angler-hour) for grayling in the Gibbon River ranged from 0.17 to 0.37 (n = 4) and 0.03 to 0.29 (n = 4) in 2005 and 2006, respectively. Estimated grayling CPUE in headwater lakes ranged from 0.42 to 0.80 (n = 2) in 2005. Non-grayling CPUE estimates ranged from 0.50 to 1.89 (n = 4) and 0.09 to 0.65 (n = 6) in 2005 and 2006, respectively.

Seventeen adfluvial Arctic grayling were caught on the weir installed upstream of Little Gibbon Falls during 2006 (Figure 9). All of these fish were caught during June and the majority were caught during the first half of the month (Figure 9). All grayling caught were dead upon detection except for one fish. Mortality likely occurred because of fish impingement and subsequent suffocation on weir surface. Grayling caught ranged from 60 to 273 mm (total length) though all fish but one were greater than 200 mm (Figure 10). Sex was determined for 12 of the 17 fish caught with 6 males and 6 females.

Figure 9.—Numbers of Arctic grayling caught on a weir installed on the Gibbon River, YNP, from June through September 2006. No grayling were caught after 6/20.

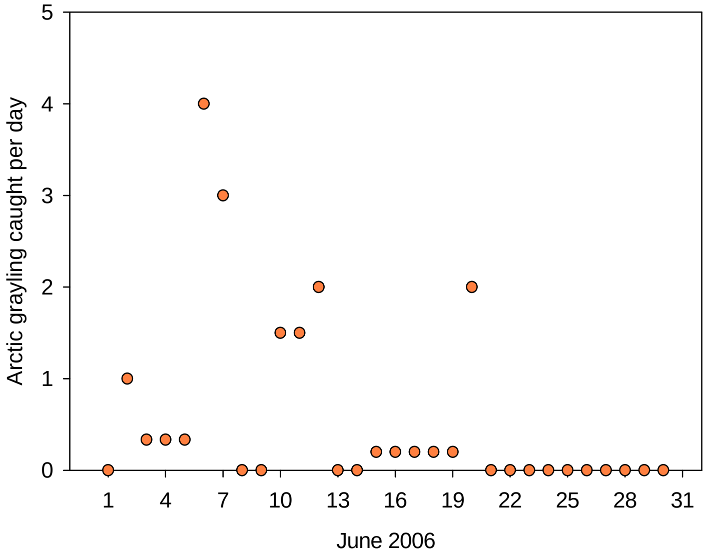
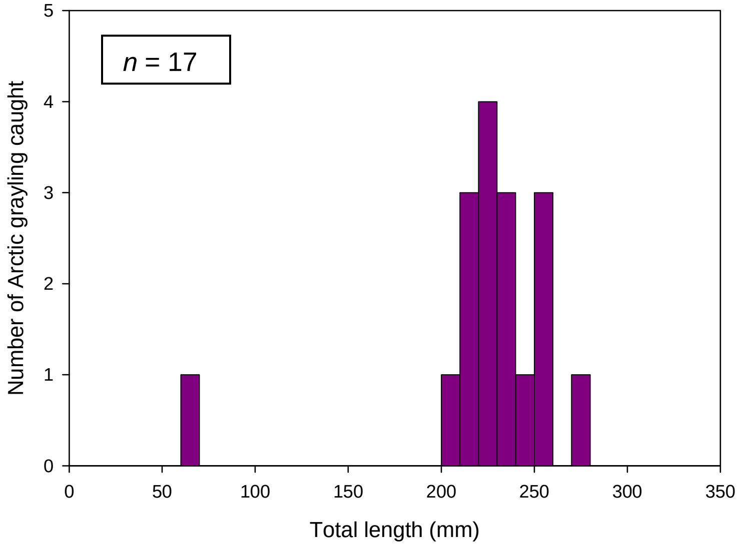


Figure 10.—Length frequencies of Arctic grayling caught on a weir installed on the Gibbon River, YNP, from June through September 2006.



The Source of Arctic Grayling Detected in the Gibbon River

Hardy-Weinberg tests showed a few statistically significant departures from expectations, but no noteworthy trends. Differences between H_e and observed heterozygosity (H_o) within both source groups were not significant except at the *Tar*100 locus for river-origin fish ($p = 0.05$). Lake-source fish deviated significantly from HW equilibrium at two of the twelve loci examined (*Tar*109, $p = 0.01$ and *Tar*114, $p = 0.01$), leading to significant deviation across all loci ($p = 0.02$).

The level of H_e for individual loci within source groups ranged from 0.67 (river-origin, *Tar*103) to 0.92 (lake-origin, *Tar*115; river-origin, *Tar*110). The H_e of river-origin fish was significantly lower than that of lake-origin fish according to sign-test ($p = 0.02$) and paired *t*-test (p = 0.005) results (Table 10).

Table 10.—Genetic variability at 12 microsatellite loci in Arctic grayling caught in the Gibbon River system, YNP.

	Population	
	Grebe and Wolf lakes (n = 95)	Gibbon River (n = 66)
Locus	H_e	H_e
*OMM*1037	0.76	0.69
*Tar*100	0.89	0.89
*Tar*101	0.86	0.85
*Tar*103	0.70	0.67
*Tar*104	0.86	0.81
*Tar*105	0.87	0.84
*Tar*106	0.91	0.83
*Tar*108	0.83	0.83
*Tar*109	0.83	0.80
*Tar*110	0.91	0.92
*Tar*114	0.88	0.87
*Tar*115	0.92	0.91
Multilocus average	0.85	0.83

H_e = Expected heterozygosity

Allelic richness test results demonstrated little difference between Arctic grayling source groups, unlike H_e trends. Rarefaction results indicated a total of 163 and 154 alleles in lake-origin and river-origin fish, respectively. The total number of alleles per locus among all grayling caught varied from 7 at *Tar*103 in both source groups to 21 at *Tar*114 in lake-origin grayling. Twenty-four and 15 private alleles were found in lake-origin and river-origin groups, respectively, after rarefaction. Further, the number of private alleles were not significantly different between source populations according to sign test results (p = 0.30). Eleven of 24 private alleles in the lake-origin fish were found at *Tar*108, *Tar*109, and

*Tar*114 whereas 7 of 15 were found at *Tar*100, *Tar*105, and *Tar*114 in river-origin fish.

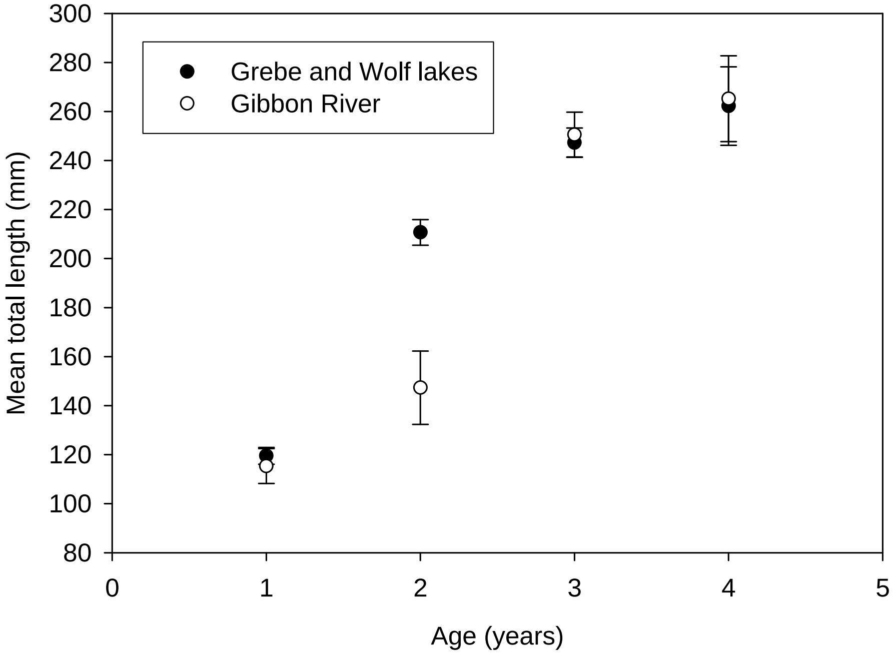
Multilocus genetic differentiation between lake-origin and river-origin Arctic grayling was low (F_{ST} = 0.0021 ± 0.002) (Frankham et al. 2002). Although genic differentiation was significant between source groups (p = 0.01), only two of the twelve loci analyzed were significant (p = 0.04 at *Tar*100 and p = 0.04 at *OMM*1037). Output from STRUCTURE corroborated genetic differentiation results, indicating that all samples most likely belonged to one population rather than two or three separate groups. The estimated posterior probability of K, the hypothetical number of populations, was greatest as K approached 1 (K = 1, -8763.2; K = 2, -8831.7; K = 3, -8956.4, K = 4, -9284.0).

Age-1, age-3, and age-4 fish from Grebe and Wolf lakes and the Gibbon River did not differ in size (Figure 11 and Table 11). However, age-2 fish from the Gibbon River were significantly smaller than fish of the same age from Grebe and Wolf lakes (Figure 11 and Table 11). Fish from Grebe and Wolf lakes grew more than Gibbon River fish between ages 1 and 2 whereas fish from the Gibbon River grew more between ages 2 and 3 than Grebe and Wolf lakes fish (Figure 11).

Table 11.—Sample sizes (*n*) and *p*-values from Welch's two-sample *t*-tests ($\alpha = 0.05$) comparing mean lengths-at-age (mm) of Arctic grayling sampled in the Gibbon River system, YNP. Age-5 and age-6 fish from Grebe and Wolf lakes were not included in analyses because none were sampled from the Gibbon River.

	Age					
n	1	2	3	4	5	6
Grebe and Wolf lakes	71	71	71	17	4	1
Gibbon River	24	23	22	5	- -	- -
p-value	0.287	< 0.001	0.540	0.767	- -	- -

Figure 11.—Mean total lengths (mm) with associated 95% confidence limits for age-1 through age-4 Arctic grayling from the Gibbon River system, YNP.



Spawning Success of Arctic Grayling in the Gibbon River, YNP

No Arctic grayling fry were detected in the Gibbon River below Little Gibbon Falls in both 2005 and 2006. Although an estimated 1,299 ± 1,265 grayling fry were present at site 12 (near Wolf Lake) in 2005, these fish are distinct from any river population because of the site's proximity to lacustrine grayling populations (Tables 12 and 13). However, these data confirm that the nets used were capable of catching grayling fry when present. An estimated 54,151 ± 31,220 salmonid fry were present at twelve trap sites from June through September 2005 whereas an estimated 160,757 ± 35,860 salmonid fry were present at seven trap sites during May through September 2006 (Table 12). The increase in the estimated number of fry present from 2005 to 2006 likely reflects the reallocation of effort to areas of prior fry detection and the extended trapping season. The relatively large estimated numbers of fry present from mid-June through mid-July during both 2005 and 2006 correspond to emergence periods for salmonids residing throughout the Gibbon River (Figure 12) (Varley and Schullery 1998).

Table 12.—Estimated number of salmonid fry present (based on the AUC method) in the Gibbon River, YNP, 2005 and 2006.

		Number present	
Stratum	Site	2005	2006
1	1	9,491 ± 6,958	- -
	2	31,005 ± 42,164	40,828 ± 27,011
	3	0	- -
	3B	- -	46,242 ± 19,320
	4	10,493 ± 2,909	63,593 ± 32,289
	5B	- -	2,999 ± 1,860
2	5	0	- -
	6	426 ± 258	0
	7	0	- -
	8	308 ± 59	6,723 ± 12,274
3	9	37 ± 8	372 ± 87
	10	0	- -
4	11	716 ± 141	- -
	12	1,299 ± 1,265[a]	- -
		376 ± 376[b]	- -
		1,675 ± 1,775[c]	- -
Total		54,151 ± 31,220	160,757 ± 35,860

[a] Total estimated number of Arctic grayling fry present at site 12.

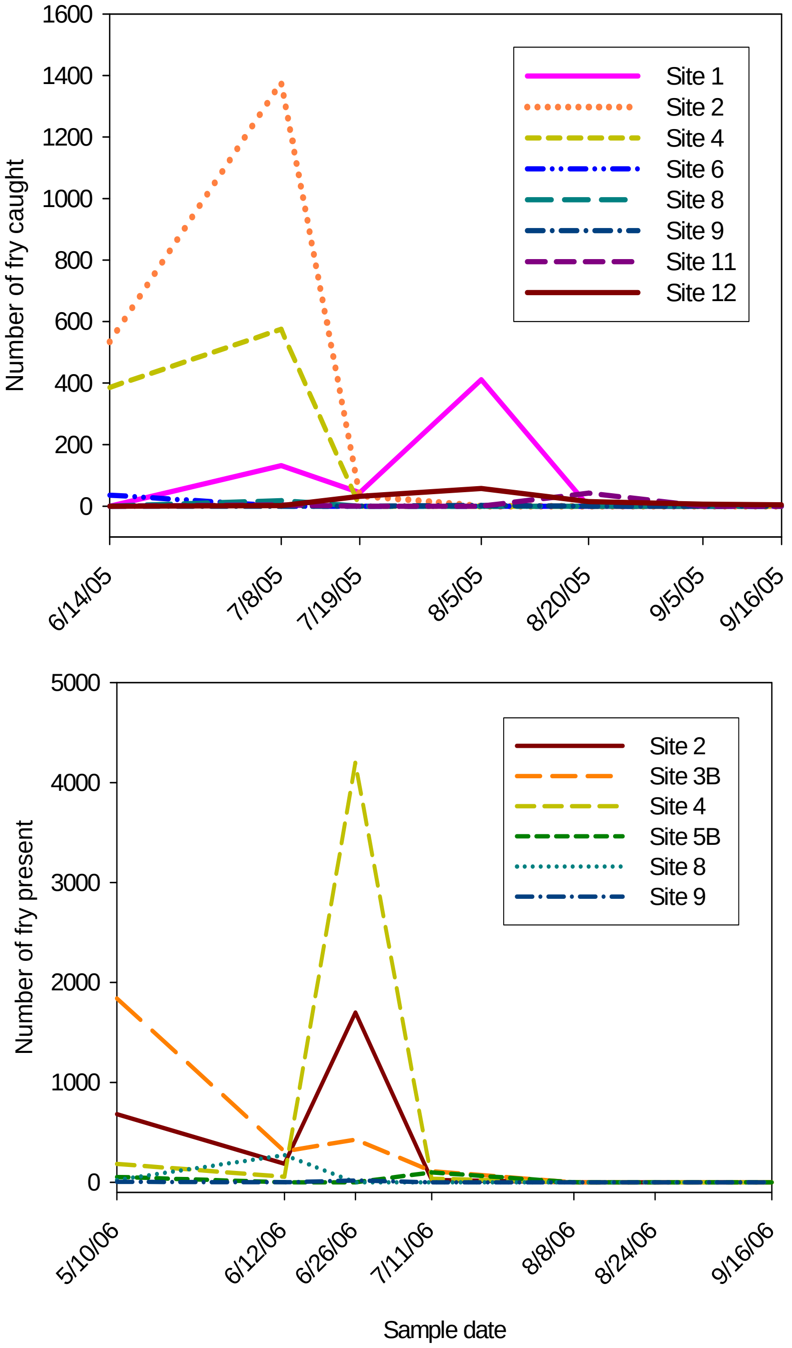
[b] Total estimated number of rainbow trout fry present at site 12.

[c] Total estimated number of salmonid (i.e., rainbow and grayling) fry present at site 12.

Table 13.—Fry caught per 24 hours during each trapping period at site 12 in the Gibbon River, YNP, 2005. Arctic grayling caught were adfluvial fish originating in Grebe or Wolf lakes.

	Catch by trapping period							
Species	6/13-6/15	7/6-7/8	7/18-7/20	8/3-8/5	8/18-8/20	9/3-9/5	9/16-9/19	Total
Arctic grayling	0	1	15	27	0	0	0	43
Rainbow trout	0	0	0	0	7	3	2	12

Figure 12.—Estimated mean number of salmonid fry present at each trap site in the Gibbon River, YNP, during each trapping period in 2005 and 2006, respectively. Sites not shown include 3, 5, 7, and 10 during 2005 and site 6 during 2006 because no fry were caught at these locations during respective years.



DISCUSSION

Abundances of Arctic Grayling in the Gibbon River, YNP

It is unlikely that estimated abundances of Arctic grayling represent a viable population in the Gibbon River. The largest estimated abundances would be expected in stratum 1 if a viable population of fluvial grayling existed in the river because it is the only stratum grayling historically inhabited (Varley and Schullery 1998). However, the estimated abundances were comparable between stratum 1 and stratum 2 in both years (Tables 8 and 9), potentially representing lacustrine emigrants temporarily occupying those strata. Further, estimated mean numbers of grayling per km incorporating the Chapman modification decrease with increasing distance from headwater lakes, suggesting downstream movement of lacustrine fish. Estimated grayling densities in the Gibbon River are comparable to those of the Big Hole River, Montana (Kaya 1990; Magee et al. 2006) and far below densities in Canada and Alaska (Northcote 1995), indicating that abundances do not represent a viable population. Additionally, if effective population sizes per stratum are about one-third estimated abundances (Frankham 1995), no to 152 grayling (equivalently zero to six fish per km) contribute to population persistence in the river (Tables 8 and 9; Figures 7 and 8). Impaired reproductive success (i.e., Allee effects) may result as a function of the difficulty in finding a mate (Myers et al. 1995), particularly because the majority of estimated effective population densities in the Gibbon were well below densities found in most other systems (Northcote 1995). Effects may also include the breakdown of social and migratory patterns, reduced predator or competitor evasion, and the failure to secure adequate food resources (Frank and Brickman 2000).

Although angler data is often prone to bias (Gabelhouse and Willis 1986) and few estimates of minimum abundance and CPUE were made in the Gibbon River, data represent targeted efforts and demonstrate the scarcity of Arctic grayling in the river. Estimates of angling CPUE for grayling were well below those of the Kakisa River, Northwest Territories (Moshenko and Low 1983) and the Ruby River, Montana (Lamothe and Magee 2004), where grayling have been introduced by MFWP since 1997 (Magee et al. 2006). Estimated CPUE for grayling was lower in the Gibbon River than it was in headwater lakes. Additionally, estimated CPUE was lower for grayling than it was for other salmonids. These data further suggest that very few grayling reside in the Gibbon River.

Estimating abundance of rarely encountered species is difficult (Bayley and Peterson 2001) and often plagued by high variability (S. Cherry, Montana State University, personal communication). Variable abundance estimates based on sparse detection data illustrate the scarcity of grayling in the river. Reported estimates of representative deviation (i.e., estimated standard errors) underscore the variability in estimated abundances (Tables 8 and 9). Although there were various sources of potential bias and large errors associated with estimates of

Arctic grayling abundance, it should be emphasized that very few fish were detected in the Gibbon River in both 2005 and 2006 despite intensive sampling efforts employing multiple techniques. Further, the most appropriate approach to abundance estimation was used and the associated variability should not overshadow the demonstrated scarcity of grayling in the Gibbon River (S. Cherry, Montana State University, personal communication).

Estimating accurate and precise parameters for rarely detected species is difficult (Bayley and Peterson 2001) and prone to bias (Gu and Swihart 2004). Myriad methods of abundance estimation are employed by fishery biologists to minimize bias, from maximum-likelihood estimators (Seber 1973; Otis et al. 1978; Kruse et al. 1998; Mitro and Zale 2000; Pearsons and Temple 2007) to the Petersen method (Ricker 1975). I did not employ one of the many maximum-likelihood models because each required a minimum capture probability of 0.10 with five or more recapture events to produce unbiased estimates (Otis et al. 1978). My electrofishing capture probabilities were generally less than 0.10 (Tables 4 and 5) and only three electrofishing estimates were logistically feasible in each of 2005 and 2006. Additionally, the Petersen method used, with and without the Chapman modification, is a well-established and unbiased estimator of abundance (Ricker 1975).

Several approaches can be employed to estimate capture probability (*q*) for single-pass electrofishing using mark and recapture techniques (Ricker 1975; Kruse et al. 1998; Pearsons and Temple 2007). Though some calculate estimated *q* ($\hat{q}$) as the number of marked fish caught in the second sample (*r*) divided by the total number of fish marked (*m*) (Ricker 1975), this ignores the total number of fish caught in the second sample (*c*) (S. Cherry, Montana State University, personal communication). However, I used the formula (*r*/*c*) because it addresses this discrepancy (S. Cherry, Montana State University, personal communication) and is preferred when many fish are recaptured (Ricker 1975). Further, this application of $\hat{q}$ for similar species to counts of rarely encountered fish produces valid estimates of abundance (Pearsons and Temple 2007).

Applying $\hat{q}$ values pooled by habitat-type to Arctic grayling counts was not the only possible approach to estimating abundances. Estimated efficiencies could have been pooled by stratum, altogether, or averaged by any one of these criteria. Estimates could not be pooled by habitat-type within strata because of low recapture rates. However, $\hat{q}$ values were not pooled by stratum or altogether because this would have ignored potential variability between habitat-type reaches. Averaging $\hat{q}$ values by any criterion would not have accounted for differences in numbers of fish caught among estimates and resulting 95% confidence intervals would have assumed a normal distribution.

Ignoring basic assumptions associated with sampling techniques is a common “sin” amongst biologists (Sutherland 2006) and a large potential source of bias in abundance estimation (Koper and Brooks 1998). Bias in abundance estimates of Arctic grayling in the Gibbon River may have stemmed from the violation of one or more assumptions associated with the sampling techniques employed. Potential violations associated with mark and recapture electrofishing and the Petersen estimator of abundance include non-random sampling, loss of marks, recruitment, mortality, emigration, immigration, and unequal catchability of all targeted individuals (including marked and non-marked fish) (Ricker 1975; Bohlin et al. 1989). Random sampling implies that fish are independently and randomly distributed throughout the sample reach and are not avoiding or seeking capture. Although violating this assumption may have lead to abundance underestimation, fish avoidance of electrofishing equipment is generally brief (Paller 1995; Young and Schmetterling 2004), negligibly affecting resulting estimates (Young and Schmetterling 2004). Loss of marks can also be ignored because fin clips were used to identify fish. Violating the assumption of a closed target population may have lead to negative bias in abundance estimates (Peterson et al. 2005). Although block nets are useful for minimizing this bias (Peterson et al. 2005), they were not employed because they do not significantly affect mark and recapture estimates of salmonid abundance in montane streams (Young and Schmetterling 2004).

Erroneously assuming equal q amongst all fish may have contributed most to bias in Arctic grayling abundance estimates. Although estimating grayling-specific q was preferable, low catches precluded this approach. However, the grayling’s general territoriality (Northcote 1995, 1997), preference for pool habitat (Shepard and Oswald 1989; Kaya 1990), and large scales imply it may have been more susceptible to capture by electrofishing than other salmonids. This discrepancy would have lead to an overestimation of abundance when salmonid $\hat{q}$ was applied to grayling counts. Inflated values for each stratum would have resulted when biased estimates of abundance for each reach were expanded. This bias was minimized by estimating $\hat{q}$ for all salmonids caught, reflecting a range of habitat uses and behaviors (Varley and Schullery 1998).

Snorkeling may have also produced bias in estimates of Arctic grayling abundance (Thurow 1994). Although precise estimates of fish abundance can be obtained by underwater observation (Schill and Griffith 1984; Dolloff et al. 1996), bias can result from diver-induced fish response (Peterson et al. 2005) and inadequate detection resulting from poor visibility (Hagen and Baxter 2005), species misidentification, and variation in depth, temperature, and habitat use (Dolloff et al. 1996). The calibration ratio ($\hat{R}$) was used to account for these biases, correcting snorkel counts using electrofishing estimates (Dolloff et al. 1993). Sampling occurred when turbidity was low during optimal daylight (1000 to 1600) using multiple divers when necessary (Dolloff et al. 1996), further diminishing bias. Although grayling resemble mountain whitefish, the species were morphologically distinguishable by divers. Temperature-related bias was minimized by performing surveys during summer (Rodgers et al. 1992). Further, the grayling’s habitat use and territoriality (Northcote 1995, 1997) likely increased its detectability by divers (Hillman et al. 1992).

Spurious conclusions can result when an inadequate area is sampled on the reach and system scale (Paller 1995). The distribution of Arctic grayling may have been insufficiently represented by the relatively small proportion of the Gibbon River sampled. However, portions of the river I was unable to sample by electrofishing or snorkeling included little preferred grayling habitat (e.g., waterfalls, rapids, thermal features) (Kaya 2000) and were therefore unlikely to harbor many fish. Further, stationary fry traps were employed in these areas to capture grayling fry if present. Reach-scale bias may have resulted by sampling fewer large reaches rather than many small ones (Sutherland 2006). However, longer reach lengths are required when attempting to sample sporadically occurring species (Paller 1995). Thus, I sampled 500-m reaches to maximize sampling effort and the chance of encountering grayling. Sampling by habitat-type using longer reaches further minimized bias associated with rare species detection and low electrofishing efficiency (Paller 1995).

The detection of 17 Arctic grayling on the weir installed above Little Gibbon Falls in 2006 demonstrates movement of grayling from headwater lakes into the river. This movement is likely associated with spawning activity because all but one grayling caught were adults and no grayling were detected after mid June, corresponding to the end of spawning by Grebe Lake grayling (Kruse 1959). Although more grayling may have been captured if the weir had been installed before June, the movement observed suggests that grayling likely enter the Gibbon annually.

The Source of Arctic Grayling in the Gibbon River, YNP

It is unlikely that Arctic grayling caught in the Gibbon River represent a native, self-sustaining fluvial population. Arctic grayling detected in the Gibbon River can be explained by one of two hypotheses: 1) river fish represent a self-sustaining native population of fluvial grayling, or 2) fish detected in the river originated from stocked headwater lakes. Although sign and paired *t*-test results indicating significantly lower H_e in river grayling may reflect a river population suffering from founder effects (Frankham et al. 2002), analyses of genetic differentiation, private allelic richness, and genetic structure do not support this theory. The degree of genetic differentiation between lake-source and river-source fish ($F_{\mathrm{ST}} = 0.0021 \pm 0.002$) indicates that over 99% of genetic variation in all Arctic grayling sampled resided within groups and less than 1% between them. European grayling populations separated for less than 60 years demonstrate higher levels of differentiation than was found in the Gibbon River system (Melgaard et al. 2003), rendering the persistence of a glacially relic fluvial population unlikely. Even grayling separated by 55 km without barriers to movement exhibit higher F_{ST} values than detected in the Gibbon system (Koskinen et al. 2001). The failure to detect a significant difference in private allelic richness after rarefaction between source groups supports the hypothesis that river-caught fish originated in headwater lakes. Further, STRUCTURE assigned all samples to a single population.

Although genetic analyses generally support the hypothesis that Arctic grayling in the Gibbon River represent emigrants from headwater lakes, lower H_e in river fish suggests a more complex scenario. The very low F_{ST} value indicates that grayling from throughout the Gibbon system unlikely originated from different sources, supported by the association of higher values with geographically connected (Koskinen et al. 2001) and recently separated (Melgaard et al. 2003) European grayling populations. Comparable allelic and private allelic richness between groups indicates common origins, further supported by STRUCTURE's assignment of all fish to a single cluster. However, lower H_e in river fish indicates that successful reproduction occurred recently in the river or that population structure exists among headwater lake fish. Successful river reproduction may have resulted between emigrants from headwater lakes or between headwater lake fish and native fluvial grayling. A strong bottleneck signal would have likely been detected as low allelic richness, low H_e, and no private alleles (Kinnison et al. 2002; Ramsted et al. 2004) if fish from Grebe and Wolf lakes colonized the Gibbon River since original lake stocking in 1921 (Varley 1981). However, allelic richness of river grayling was notably higher than in some European grayling populations (Koskinen et al. 2001) and freshwater fishes in general (DeWoody and Avise 2000). Also, no difference in the number of private alleles was detected. Further, lacustrine fish do not successfully adapt to the fluvial life history despite the success of fluvial fish in lacustrine habitat (Kaya 1991; Kaya and Jeanes 1995). Fluvial grayling demonstrated loss of rheotaxis about two generations after Meadow Creek, a tributary to the Madison River, was converted to Meadow Lake (i.e., Ennis Reservoir) in 1900 (Kaya 1992). However, no empirical studies have examined this phenomenon (Kaya 1992).

Alternatively, if an extant Gibbon River population was founded by both headwater lakes and native fluvial fish, allelic richness and H_e may be high and few to no private alleles would be observed (Small et al. 2006). However, I failed to detect a significant difference in numbers of private alleles between source groups. Further, very little genetic differentiation was detected between groups and STRUCTURE assigned all fish to a single population. Although overall high allelic richness and H_e were observed in river fish, life history differences between lacustrine and fluvial fish undermine the mixed-source hypothesis. Spawning times of fluvial and lacustrine Arctic grayling in Montana generally differ as a function of temperature gradient between river and lake systems (Kaya 2000), a phenomenon potentially amplified by the geothermal influences in the Gibbon River. As indicated previously, lacustrine fish do fully adapt to the fluvial life history (Kaya 1991; Kaya and Jeanes 1995).

Based on available evidence, the lower H_e in river fish most likely reflects low levels of population structure among headwater lake fish. In this scenario, Arctic grayling caught in the Gibbon River represent emigrants from a subset of a Grebe or Wolf Lake population that spawn in the Wolf Lake outlet (i.e., the Gibbon River), suggesting structure in these lakes. Strays of this subset may have passed over barriers to upstream movement (i.e., Little Gibbon Falls, Virginia Cascade, Gibbon Falls) seeking suitable spawning habitat, supported by the territoriality (Northcote 1995, 1997) and considerable movements of grayling during spawning season (Shepard and Olswald 1989; Northcote 1995). Headwater lake population structure is further supported by the HW disequilibrium detected in lake-source rather than river-source fish (Kalinowski 2006). However, disequilibrium may also reflect genotyping error undetected during double-scoring (Kalinowski 2006). Although some lacustrine grayling exhibit low intrapopulation genetic diversity, considerable structure (Koskinen et al. 2001) and local adaptation (Haugen & Vøllestad 2000) can exist over small geographic scales. Lake structure may have developed because spawning is restricted to tributaries and outlets, implying assortative mating among grayling exhibiting fidelity to natal waters (Northcote 1995, 1997).

Corroborating genetic analyses, the overall similarity in lengths-at-age between Arctic grayling caught in the Gibbon River and those from headwater lakes imply a common origin for all Gibbon fish. However, the significantly different lengths of age-2 fish between source groups may reflect error in age determination, a fluvial population in the Gibbon River, or lacustrine emigrants detected in the Gibbon River. Although ageing error may have occurred, it unlikely manifested only in age-2 Gibbon River fish. The significantly lower lengths at age-2 fish more likely reflects differences in productivity between headwater lakes and the Gibbon River because lacustrine fish have demonstrated significantly higher lengths-at-age than their fluvial counterparts in multiple systems (Hutchings 1986; O’Connell and Ash 1993; Halvorsen 1996; Dempson et al. 1996; Erkinaro et al. 1998). Less growth was observed between age-1 and age-2 grayling from the Big Hole River, Montana (Shepard and Oswald 1989) compared to Red Rock Lake, Montana (Nelson 1954) fish, potentially reflecting reduced food availability for age-1 fish in the river system. This difference may also represent metabolic costs associated with transition to a fluvial environment (Jonsson et al. 1997). Further, gape size increases with length allowing prey-switching and increased growth (Kahilainen and Lehtonen 2003). The greater growth observed between age-2 and age-3 grayling in the Gibbon River compared to headwater lakes fish may reflect prey-switching to a food source more abundant in the river.

Although the observed differences in length at age-2 between Gibbon River and Grebe and Wolf lakes fish supports the existence of a fluvial population of Arctic grayling in the Gibbon River, very similar lengths at age-1, age-3, and age-4 fish detected throughout the system suggests fish are emigrants from headwater lakes. Grayling from Red Rock Lake were larger than Big Hole River grayling after age-1 (Nelson 1954; Shepard and Oswald 1989), though Liknes (1981) determined Big Hole fish were larger than Red Rock Lake fish at age-1 and smaller at subsequent ages. Arctic grayling from numerous systems across Norway, Sweden, Slovakia, and Siberia also varied in length at multiple age classes (Northcote 1995), likely reflecting differences in system productivity. Although adfluvial grayling do not successfully adapt to the fluvial life history (including production of viable offspring) (Kaya 1991; Kaya and Jeanes 1995), offspring of Wolf Lake outlet spawners may have survived in the Gibbon River until detection.

Spawning Success of Arctic Grayling in the Gibbon River, YNP

The failure to detect Arctic grayling fry in the Gibbon River below Little Gibbon Falls in both of 2005 and 2006 indicates that successful river spawning did not likely occur during those years. Although stationary trap nets sampled a relatively small portion of the river, nets captured non-grayling fry during both years (Table 11; Figure 9). Though grayling in Montana are very small at emergence (7-11 mm) (Watling and Brown 1955), adfluvial grayling fry were caught near headwater lakes in 2005 (Table 13). Further, grayling fecundity is high in comparison to other salmonids (Northcote 1995), increasing the likelihood of detection.

Conclusion

The preponderance of evidence indicates that a viable population of fluvial Arctic grayling does not exist in the Gibbon River, YNP. Variability in low estimated abundances is an artifact of the few grayling caught, illustrating the species' scarcity in the river despite intensive sampling employing multiple techniques. Anglers targeting grayling habitat caught relatively few fish and grayling likely enter the river annually from headwater lakes. River and headwater lakes grayling were genetically similar relative to differences within other grayling populations (Koskinen et al. 2001; Melgaard et al. 2003). Lengths at most ages were similar among all Gibbon system fish and successful river spawning was not documented. Though many have assumed that fluvial grayling were extirpated from the Gibbon River by the mid-1930s (Vincent 1962; Kaya 2000), this study constitutes the first substantial evidence that a fluvial population does not currently exist. This information will help direct fisheries management within YNP, providing the opportunity to re-establish fluvial grayling without genetically adulterating a native population. These data also support the continued conservation and restoration of the last remaining population of fluvial grayling in the lower 48 United States, residing in the Big Hole River, Montana. Although fluvial grayling were recently removed from federal protection under the ESA (USFWS 2007), opinions on appropriate conservation measures will likely remain controversial for years to come (D. Peterson, USFWS, personal communication). Nevertheless, the scarcity of fluvial grayling within its local native range supports careful and objective consideration of its future.

LITERATURE CITED

Armstrong, R.H. 1986. A review of Arctic grayling studies in Alaska, 1952-1982. Biological Papers of the University of Alaska 23:3-17.

Barndt, S. 1996. The biology and status of the Arctic grayling in Sunnyslope Canal, Montana. Master's thesis. Montana State University, Bozeman.

Bayley, P.B., and J. T. Peterson. 2001. An approach to estimate probability of presence and richness of fish species. Transactions of the American Fisheries Society 130:620-633.

Bohlin,T. S. Hamrin, T.G. Heggberget, G. Rasmussen, and S.J. Saltveit. 1989. Electrofishing—theory and practice with special emphasis on salmonids. Hydrobiologia 173:9-43.

Brown, C.J.D. 1943. Age age growth of Montana grayling. The Journal of Wildlife Management 7:353-364.

Brown, M.L., and D.J. Austen. 1996. Data management and statistical techniques. Pages 17-61 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

DeCicco, A.L., and R.J. Brown. 2006. Direct validation of annual growth increments on sectioned otoliths from adult Arctic grayling and a comparison of otolith and scale ages. North American Journal of Fisheries Management 26:580-586.

Deleray, M.A. 1991. Movement and utilization of fluvial habitat by age-0 Arctic grayling, and characteristics of spawning adults, in the outlet of Deer Lake, Gallatin County, Montana. Master's thesis. Montana State University, Bozeman.

Dempson, J.B., M.F. O'Connell, and M. Shears. 1996. Relative production of Atlantic salmon from fluvial and lacustrine habitats estimated from analyses of scale characteristics. Journal of Fish Biology 48:329-341.

Devore, J., and R. Peck. 2005. Statistics: the exploration and analysis of data, 5th edition. Brooks/Cole—Thomson Learning, Belmont, California.

Devries, D.R., and R.V. Frie. 1996. Determination of age and growth. Pages 483-512 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

DeWoody, J.A., and J.C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology 56:461-473.

Diggs, M.D., and W.R. Ardren. In press. Characterization of 12 highly variable tetranucleotide microsatellite loci for Arctic grayling (*Thymallus arcticus*) and cross amplification in other *Thymallus* species. Molecular Ecology Notes.

Dolloff, A., J. Kershner, and R. Thurow. 1996. Underwater observations. Pages 533-554 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Dolloff, C.A., D.G. Hankin, and G.H. Reeves. 1993. Basinwide estimation of habitat and fish populations in streams. U.S. Forest Service Technical Report SE-83, Asheville, North Carolina.

Erkinaro, J., and E. Niemelä. 1995. Growth differences between the Atlantic salmon parr, *Salmo salar*, of nursery brooks and natal rivers in the River Teno watercourse in northern Finland. Environmental Biology of Fishes 42:277-287.

Erkinaro, J., E. Niemelä, A. Saari, Y. Shustov, and L. Jørgensen. 1998. Timing of habitat shift by Atlantic salmon parr from fluvial to lacustrine habitat: analysis of age distribution, growth, and scale characteristics. Canadian Journal of Fisheries and Aquatic Sciences 55:2266-2273.

English, K. K., R. C. Bocking, and J. R. Irvine. 1992. Robust procedure for estimating salmon escapement based on the area-under-the-curve method. Canadian Journal of Fisheries and Aquatic Sciences 49: 1982-1989.

Everett, R.J., and F.W. Allendorf. 1985. Population genetics of Arctic grayling: Grebe Lake, Yellowstone National Park. University of Montana, Missoula. Genetics Laboratory Report 85(1).

Frank, K.T., and D. Brickman. 2000. Allee effects and compensatory population dynamics within a stock complex. Canadian Journal of Fisheries and Aquatic Sciences 57:513-517.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: A review. Genetical Research 66:95-107.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2002. Introduction to conservation genetics. Cambridge University Press, Cambridge.

Gabelhouse, D. W., Jr., and D. W. Willis. 1986. Biases and utility of angler catch data for assessing size structure and density of largemouth bass. North American Journal of Fisheries Management 6:481–489.

Gale, S.B. 2005. Entrainment losses of westslope cutthroat trout into screened and unscreened irrigation canals on Skalkaho Creek, Montana. Master's thesis. Montana State University, Bozeman.

Goudet, J. 2001. FSTAT: a program to estimate and test gene diversities and fixation indices, version 2.9.3. Available: http://www.unilch/izea/softwares/fstat.html. (June 2007).

Hagen, J., and J.S. Baxter. 2005. Accuracy of diver counts of fluvial rainbow trout relative to horizontal underwater visibility. North American Journal of Fisheries Management 25:1367-1377.

Halvorsen, M., and L. Jørgensen. 1996. Lake-use by juvenile Atlantic salmon (*Salmo salar* L.) and other salmonids in northern Norway. Ecology of Freshwater Fish 5:28-36.

Haugen, T.O., and L.A. Vøllestad. 2000. Population differences in early life-history traits in grayling. Journal of Evolutionary Biology 13:897-905.

Hennessey, L. E. 1998. An evaluation of Yellowstone cutthroat trout fry recruitment related to water leases on four tributaries of the Yellowstone River. Master's thesis. Montana State University, Bozeman.

Hoffman, J.I., and W. Amos. 2005. Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. Molecular Ecology 14:599-612.

Hubert, W.A., R.S. Helzner, L.A. Lee, and P.C. Nelson. 1985. Habitat suitability index models and instream suitability curves: Arctic grayling riverine populations. U.S. Fish and Wildlife Service Biological Report 82(10.110).

Hunter, J.G. 1954. A weir for adult and fry salmon effective under conditions of extremely variable run-off. The Canadian Fish Culturist 16:27-33.

Hutchings, J.A. 1986. Lakeward migrations by juvenile Atlantic salmon, *Salmo salar*. Canadian Journal of Fisheries and Aquatic Sciences 43:732-741.

Jonsson, N., B. Jonsson, and P. Hansen. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo salar*. Journal of Animal Ecology 66:425-436.

Kaeding, L.R., and G.D. Boltz. 1999. A study of Arctic grayling and their stream habitat in support of reserved water right applications, Red Rock Lakes National Wildlife Refuge, Montana. U.S. Fish and Wildlife Service, Bozeman, Montana.

Kahileinen, K., and H. Lehtonen. 2003. Piscivory and prey selection of four predator species in a whitefish dominated subarctic lake. Journal of Fish Biology 63:659-672.

Kalinowski, S. 2005. HP-RARE: a computer program for performing rarefaction on measures of allelic diversity. Molecular Ecology Notes 5:187-189.

Kalinowski, S. 2006. HW-QUICKCHECK: an easy-to-use computer program for checking genotypes for agreement with Hardy-Weinberg expectations. Molecular Ecology Notes 6:974-979.

Kaya, C.M. 1990. Status report on fluvial Arctic grayling (*Thymallus arcticus*) in Montana. Montana Department of Fish, Wildlife and Parks, Helena.

Kaya, C.M. 1991. Rheotactic differentiation between fluvial and lacustrine populations of Arctic grayling (*Thymallus arcticus*), and implications for the only remaining indigenous population of fluvial Montana grayling. Canadian Journal of Fisheries and Aquatic Science 48:53–59.

Kaya, C.M. 1992. Review of the decline and status of fluvial Arctic grayling in Montana. Proceedings of the Montana Academy of Sciences 52:43-70.

Kaya, C.M. 2000. Arctic grayling in Yellowstone: status, management, and recent restoration efforts. Yellowstone Science 8(3):12-17.

Kaya, C.M., and E. D. Jeanes. 1995. Retention of adaptive rheotactic behavior by F1 fluvial Arctic grayling. Transactions of the American Fisheries Society 124:453-457.

Kelsch, S.W., and B. Shields. 1996. Care and handling of sampled organisms. Pages 121-145 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Kennedy, G.J.A., and C.D. Strange. 1981. Efficiency of electric fishing for salmonids in relation to river width. Fisheries Management 12:55-60.

Kinnison, M.T., P. Bentzen, M.J. Unwin, and T.P. Quinn. 2002. Reconstructing recent divergence: evaluation nonequilibrium population structure in New Zealand chinook salmon. Molecular Ecology 11:739-754.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2005. Yellowstone Fisheries & Aquatic Sciences: Annual Report, 2004. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Koel, T.M., J.L. Arnold, P.E. Bigelow, P.D. Doepke, B.D. Ertel, and D.L. Mahony. 2004. Yellowstone Fisheries & Aquatic Sciences: Annual Report, 2003. National Park Service, Yellowstone Center for Resources, Yellowstone National Park, Wyoming.

Koskinen, M.T., J. Piironen, and C.R. Primmer. 2001. Interpopulation genetic divergence in European grayling (*Thymallus arcticus*, Salmonidae) at a microgeographic scale: implications for conservation. Conservation Genetics 2:133-143.

Kruse, T.E. 1959. Grayling of Grebe Lake, Yellowstone National Park, Wyoming. United States Department of the Interior Fish and Wildlife Service, Fishery Bulletin 149, Washington.

Kruse, C.G., W.A. Hubert, and F.J. Rahel. 1998. Single-pass electrofishing predicts trout abundance in mountain streams with sparse habitat. North American Journal of Fisheries Management 18:940-946.

Kuehl, R. O. 1994. Statistical principles of research design and analysis. Duxbury Press, Belmont, CA.

Lamothe, P., and J. Magee. 2004. A summary of angler surveys from the Upper Ruby River (1999-2003). Montana Department of Fish, Wildlife & Parks, Dillon.

Liknes, G.A. 1981. The fluvial Arctic grayling (*Thymallus arcticus*) of the upper Big Hole River drainage, Montana. Master's Thesis. Montana State University, Bozeman.

Liknes, G.A., and W.R. Gould. 1987. The distribution, habitat and population characteristics of fluvial Arctic grayling (*Thymallus arcticus*) in Montana. Northwest Science 61:122-129.

Lund, J.A. 1974. The reproduction of salmonids in the inlets of Elk Lake, Montana. Master's thesis. Montana State University, Bozeman.

Magee, J., E. Rens, and P. Lamothe. 2005. Fluvial Arctic grayling monitoring report 2004. Montana Department of Fish, Wildlife & Parks, Dillon.

Magee, J., E. Rens, and P. Lamothe. 2006. Fluvial Arctic grayling monitoring report 2005. Montana Department of Fish, Wildlife & Parks, Dillon.

Malvestuto, S.P. 1996. Sampling the recreational creel. Pages 591-623 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

McFarlane, G.A., R.S. Wydoski, and E.D. Prince. 1990. Historical review of the development of external tags and marks. American Fisheries Society Symposium 7:9-29.

McMahon, T.E., S.R. Dalbey, S.C. Ireland, J.P. Magee, and P.A. Byorth. 1996. Field evaluation of visible implant tag retention by brook trout, cutthroat trout, rainbow trout, and Arctic grayling. North American Journal of Fisheries Management 16:921-925.

Melgaard, T., E.E. Nielsen, and V. Loeschcke. 2003. Fragmentation by weirs in a riverine system: A study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. Conservation Genetics 4:735-747.

Mogen, J.T. 1996. Status and biology of the spawning population of Red Rock Lakes Arctic grayling. Master's thesis. Montana State University, Bozeman.

Montana Fluvial Arctic Grayling Workgroup. 1995. Montana fluvial Arctic grayling restoration plan. Montana Fish, Wildlife & Parks. Helena, MT.

Moshenko, R.W., and G. Low. 1983. Data from the Arctic grayling sport fishery on the Kakisa River, Northwest Territories, 1971-78. Department of Fisheries and Oceans, Report 388, Winnipeg, Manitoba.

Mullner, S.A., W.A. Hubert, and T.A. Wesche. 1998. Snorkeling as an alternative to depletion electrofishing for estimating abundance and length-class frequencies of trout in small streams. North American Journal of Fisheries Management 18:947-953.

Myers, R.A., N.J. Barrowman, J.A. Hutchings, and A.A. Rosenberg. 1995. Population dynamics of exploited fish stocks at low population levels. Science 269:1106-1108.

Nelson, M.L. 1999. Evaluation of the potential for ‘resident’ bull trout to re-establish the migratory life-form. Master’s thesis, Montana State University, Bozeman.

Nelson, M.L., T.E. McMahon, and R.F. Thurow. 2002. Decline in the migratory form of bull char, *Salvelinus confluentus*, and implications for conservation. Environmental Biology of Fishes 64:321-332.

Nelson, P.H. 1954. Life history and management of the American grayling (*Thymallus signifier tricolor*) in Montana. Journal of Wildlife Management 18:324-342.

Northcote, T. C. 1995. Comparative biology and management of Arctic and European grayling (Salmonidae, *Thymallus*). Reviews in Fish Biology and Fisheries 5:141-194.

Northcote, T.C. 1997. Potamodromy in Salmonidae—living and moving in the fast lane. North American Journal of Fisheries Management 17:1029-1045.

O’Connell, M.F., and E.G.M. Ash. 1993. Smolt size in relation to age at first maturity of Altantic salmon (*Salmo salar*): the role of lacustrine habitat. Journal of Fish Biology 42:551-569.

Otis, D.L., K.P. Burnham, G.C. White, and D.R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:3-135.

Pearsons, T.N., and G.M. Temple. 2007. Impacts of early stages of salmon supplementation and reintroduction programs on three trout species. North American Journal of Fisheries Management 27:1-20.

Peterson, N.P., and C.J. Cederholm. 1984. A comparison of the removal and mark-recapture methods of population estimation for juvenile coho salmon in a small stream. North American Journal of Fisheries Management 4:99-102.

Peterson, J.T., J. Dunham, P. Howell, R. Thurow, and S. Bonar. 2002. Protocol for determining bull trout presence. Bull Trout Committee, Western Division, American Fisheries Society, Bethesda, Maryland.

Peterson, J.T., N.P. Banish, and R.F. Thurow. 2005. Are block nets necessary?: Movement of stream-dwelling salmonids in reponse to three common survey methods. North American Journal of Fisheries Management 25:732-743.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotypic data. Genetics 155:945-959.

Purkett, Jr., C.A. 1951. Growth rate of trout in relation to elevation and temperature. Transactions of the American Fisheries Society 80: 251-259.

Radcliffe, R. W. 1950. The effect of fin-clipping on the cruising speed of goldfish and coho salmon fry. Journal of the Fisheries Research Board of Canada 8:67-73.

Ramsted, K.M., C.A. Woody, G.K. Sage, and F.W. Allendorf. 2004. Founding events influence genetic population structure of sockeye salmon (*Oncorhynchus nerka*) in Lake Clark, Alaska. Molecular Ecology 13:277-290.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248-249.

Reynolds, J.B. 1989. Evaluation of the HIS model for riverine grayling in relation to Alaksan project impacts. Unit Contribution Number 32, Alaska Cooperative Fishery Research Unit. University of Alaska, Fairbanks.

Reynolds, J.B. 1996. Electrofishing. Pages 221-253 *in* B.R. Murphy and D.W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Ricker, W.E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada, Number 191.

Rieman, B.E., and F.W. Allendorf. 2001. Effective population size and genetic conservation criteria for bull trout. North American Journal of Fisheries Management 21:756-764.

Rodgers, J.D., M.F. Solazzi, S.L. Johnson, and M.A. Buckman. 1992. Comparison of three techniques to estimate juvenile coho salmon populations in small streams. North American Journal of Fisheries Management 12:79-86.

Schill, D.J., and J.S. Griffith. 1984. Use of underwater observations to estimate cutthroat trout abundance in the Yellowstone River. North American Journal of Fisheries Management 4:479-487.

Schmetterling, D.A., and S.B. Adams. 2004. Summer movements within the fish community of a small montane stream. North American Journal of Fisheries Management 24:1163-1172.

Seber, G.A.F. 1973. The estimation of animal abundance and related parameters. Hafner Press, New York.

Shepard, B.B., and R.A. Oswald. 1989. Timing, location, and population characteristics of spawning Montana Arctic grayling (*Thymallus arcticus montanus* [Milner]) in the Big Hole River drainage, 1988. Montana Department of Fish, Wildlife and Parks, Bozeman.

Small, M.P., A.E. Frye, J.F. Von Bargen, and S.F. Young. 2006. Genetic structure of chum salmon (*Oncorhynchus keta*) populations in the lower Columbia River: are chum salmon in Cascade tributaries remnant populations? Conservation Genetics 7:65-78.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry, 3rd edition. W.H. Freeman, New York.

Stamford, M.D., and E.B. Taylor. 2004. Phylogeographical lineages of Arctic grayling (*Thymallus arcticus*) in North America: divergence, origins, and affinities with Eurasian *Thymallus*. Molecular Ecology 13:1533-1549.

Stuart, K.M., and G.R. Chislett. 1979. Aspects of the life history of Arctic grayling in the Sukunka drainage. Internal report of the British Columbia Fish and Wildlife Branch, Prince George.

Sutherland, W.J. 2006. The twenty commonest surveying sins. Pages 408-410 *in* W.J. Sutherland, editor. Ecological census techniques, 2nd edition. Cambridge University Press, Cambridge.

Thompson, S.K. 2002. Sampling. John Wiley and Sons, New York.

Thompson, W. L., G. C. White, and C. Gowan. 1998. Monitoring vertebrate populations. Academic Press, New York.

Thurow, R.F. 1994. Underwater methods for study of salmonids in the Intermountain West. U.S. Forest Service General Technical Report INT-GTR-307, Ogden, Utah.

Thurow, R.F., and D.J. Schill. 1996. Comparison of day snorkeling, night snorkeling, and electrofishing to estimate bull trout abundance and size structure in a second-order Idaho stream. North American Journal of Fisheries Management 16:314-323.

USFWS (U.S. Fish and Wildlife Service). 1991. Endangered and threatened wildlife and plants; animal candidate review for listing as endangered or threatened species, notice of review. Federal Register 56(225):58804-58836.

USFWS. 1993. Endangered and threatened wildlife and plants: 90-day finding and commencement of status review for a petition to list the fluvial population of the Arctic grayling as endangered. Federal Register 58(11):4975-4976.

USFWS. 1994. Endangered and threatened wildlife and plants; finding on a petition to list the fluvial population of the Arctic grayling as endangered. Federal Register 59(141):37738.

USFWS. 1996. Endangered and threatened wildlife and plants; review of plant and animal taxa that are candidates for listing as endangered or threatened species. Federal Register 61:7596.

USFWS. 2004. Endangered and threatened wildlife and plants; review of species that are candidates or proposed for listing as endangered or threatened; annual notice of findings on resubmitted petitions; annual description of progress on listing actions. Federal Register 69(86):24881.

UWFWS. 2007. Endangered and threatened wildlife and plants: revised 12-month finding for upper Missouri River distinct population segment of fluvial Arctic grayling. Federal Register 72(78):20305-20314.

Van Den Avyle, M.J., and R.S. Hayward. 1999 Dynamics of exploited fish populations. Pages 127-166 *in* C.C. Kohler and W.A. Hubert, editors. Inland fisheries management in North America, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Varley, J.D., R.E. Gresswell, S.M. Hoskins, R.B. Mathias, D.E. Jennings, M.D. Carpenter, and J. Dolan. 1976. Fishery and Aquatic Management program in Yellowstone National Park. Annual project report for calendar year 1975. U.S. Department of the Interior, Fish and Wildlife Service, Fisheries and Federal Aid, Wyoming.

Varley, J.D. 1981. A history of fish stocking activities in Yellowstone National Park between 1881 and 1980. National Park Service and U.S. Fish and Wildlife Service, Report 35:1-94, Yellowstone National Park, Wyoming.

Varley, J.D., and P.D. Schullery. 1998. Yellowstone fishes: ecology, history, and angling in the park. Stackpole Books, Mechanicsburg, Pennsylvania.

Vincent, R.E. 1962. Biogeographical and ecological factors contributing to the decline of Arctic grayling, *Thymallus arcticus* (Pallas), in Michigan and Montana. Doctoral dissertation. University of Michigan, Ann Arbor.

Watling, H., and C.J.D. Brown. 1955. The embryological development of American grayling *(Thymallus signifer)* from fertilization to hatching. Transactions of the American Microscopical Society 74:85-93.

Weir, B.S., and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Weisel, G.F. 1966. Young salmonid fishes of western Montana. Proceedings of the Montana Academy of Sciences 26:1-21.

Young, M.K., and D.A. Schmetterling. 2004. Electrofishing and salmonid movement: reciprocal effects in two small montane streams. Journal of Fish Biology 64:750-761.

Zar, J. H. 1984. Biostatistical analysis, second edition. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.