THESIS

REPRODUCTION AND RECRUITMENT DYNAMICS OF FLATHEAD CHUB *PLATYGOBIO GRACILIS* RELATIVE TO FLOW AND TEMPERATURE REGIMES IN FOUNTAIN CREEK, COLORADO

Submitted by

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ABSTRACT

REPRODUCTION AND RECRUITMENT DYNAMICS OF FLATHEAD CHUB *PLATYGOBIO GRACILIS* RELATIVE TO FLOW AND TEMPERATURE REGIMES IN FOUNTAIN CREEK, COLORADO

A paucity of basic ecological information for flathead chub *Platygobio gracilis* has made effective conservation planning difficult for this declining species. The objective of this study was to contribute insight to the poorly understood reproductive ecology of flathead chub, and enable prediction of effects of future hydrologic alterations in Fountain Creek, Colorado, to avoid or mitigate negative impacts from these actions. To accomplish this I investigated the influence of flow and water temperature regimes on reproduction and recruitment dynamics of flathead chub in Fountain Creek from May 2012 to October 2013 through collection of eggs, and analysis of otoliths from larvae and juveniles. Presence of flathead chub eggs and larvae in drift nets and Moore egg collectors indicated a protracted spawning season spanning a four-month period from mid-May to mid-September. Species composition of fish hatched from eggs reared in the laboratory showed the majority of eggs captured in drift nets were flathead chub. This enabled identification of peak reproduction periods based on captures of eggs in preserved samples. Reproduction began in each year when water temperatures exceeded 15°C, and highest egg densities occurred in later May and June in both 2012 and 2013. Unlike literature suggestions of need for flow spikes to induce reproduction, spawning occurred during both steady low flow conditions and to a lesser extent, under fluctuating flows caused by convective storms. Larvae hatching also peaked in May and June but, unlike egg production, was restricted to periods of stable low flows of about 1-2 m^3/s. Recruitment, in this study defined as the addition of an individual to the population by survival to the juvenile stage, occurred only in a subset of the egg production season during periods of low and steady flows, usually in late May and June. In contrast, egg production preceding or during flow spikes that reached approximately 20 m^3/s produced few recruits, presumably because eggs and weak-swimming larvae were transported downstream or destroyed. Recruitment sometimes occurred prior to flow spikes, but the minimum duration of relatively steady flows required was about three weeks. Both episodic and frequent high magnitude flow events had large and negative impacts on recruitment of flathead chub, and potentially population dynamics of the species in Fountain Creek. This is mostly counter to the prevailing paradigm that high flows are required for many plains-adapted minnow species to reproduce, a hypothesis formulated mostly from observations in flow-depleted streams where such patterns may be an artifact of an altered environment. This study was successful in identifying environmental conditions suitable for flathead chub reproduction and recruitment related to temperature and flow regimes in Fountain Creek. Managers should use these insights to predict how future hydrologic alterations may affect the flathead chubs so the population in Fountain Creek can be conserved.

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DEDICATION

For Mom,

who instilled in me the importance of a passionate life

For Dad,

who exemplified the importance of hard work in all you do

For Kelly,

for unwavering love and support in all my endeavors

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INTRODUCTION

Reproduction and recruitment are important life history phases that affect fish population abundance, and each can influence population dynamics of species independently (Roughgarden et al. 1988; Jones 1990; Caley et al. 1996; Garvey et al. 2002). Recruitment, often used in fisheries science to describe the addition of young to the population (Ricker 1975), is of particular importance as the survival rate of a year class or within-year cohort can have significant impact on abundance of a species later in life (Thorson 1950; Shepherd and Cushing 1980; Bestgen et al. 2006, 2007). Understanding the mechanisms controlling recruitment is complicated because life history processes such as timing and success of reproduction, growth, and dispersal all influence recruitment, and are each affected by spatially and temporally variable physical and biological conditions (Underwood and Fairweather 1989; Sponaugle and Cowen 1997; Schlosser 1998; King et al. 2003; Zeug and Winemiller 2008).

Those complicated mechanisms may be particularly germane for fishes endemic to the Great Plains, because physical characteristics of streams and biota distribution and abundance fluctuate widely over periods of days to years (Cross and Moss 1987; Dodds et al. 2004; Hoagstrom et al. 2007). Study of reproduction and recruitment dynamics of plains stream fishes is further hindered by the physical environment, where direct observation of reproduction is difficult because of elevated flows and increased turbidity during the spring and summer (Matthews 1988; Fausch and Bestgen 1997). In particular, cyprinids have received little study relative to other fish taxa so the reproductive ecology of many species is poorly understood (Johnston and Page 1992). However, clarifying the effects of physical factors such as flow and temperature regimes is important because they can regulate recruitment of stream fishes by influencing growth and survival of larvae, (Crecco and Savoy 1985; Harvey 1991; Bestgen 1997, 2008; Durham and Wilde 2009; Falke et al. 2010a), which can in turn direct strength of biological interactions such as predation and competition (Bestgen et al. 2006; Craig et al. 2006).

Understanding effects of various physical and biological factors on reproduction and recruitment is especially important in the face of altered habitat conditions. Construction and operation of dams, diversions, and reservoirs for flood control, irrigation, and generation of electricity has extensively altered habitat connectivity, as well as flow, temperature, and sediment regimes in streams of the North American Great Plains (Poff et al. 1997; Fausch and Bestgen 1997; Dodds et al. 2004; Poff et al. 2007). These alterations have led to widespread decline of plains cyprinids across their range (Cross et al. 1985; Winston et al. 1991; Bonner and Wilde 2000; Dudley and Platania 2007; Hoagstrom et al. 2011).

Flathead chub *Platygobio gracilis* is a North American Great Plains cyprinid that is declining over parts of its range, including Colorado, where it is classified as a Species of Special Concern (Loeffler et al. 1982; Bramblett and Fausch 1991; Nesler et al. 1999; Rahel and Thel 2004; Hayer et al. 2008). Flow and temperature regimes and stream length may directly influence the reproduction, recruitment, and persistence of some plains cyprinids, including flathead chub (Bestgen et al. 1989; Taylor and Miller 1990; Platania and Altenbach 1998; Dudley and Platania 2007; Perkin and Gido 2011). Proposed water development may further alter flow and temperature regimes of Fountain Creek, a stronghold for flathead chub in southeastern Colorado near Colorado Springs, and gaining understanding of how these regimes regulate flathead chub reproduction and recruitment is central to effective conservation planning. Therefore, objectives of this study were to gain information on the reproductive ecology of flathead chub as it relates to flow and water temperature regimes in Fountain Creek, Colorado, including:

1. Identify the reproductive season of adult flathead chubs,
2. Analyze recruitment dynamics and measure growth rates of age-0 flathead chub,
3. Investigate the influence of water temperature and hydrologic regime on these processes.

Results will provide insight into the poorly understood reproductive ecology of this species, and enable prediction of effects of future hydrologic alterations in Fountain Creek, which should assist managers with conservation actions for flathead chub.

BACKGROUND

Spanning the geographic center of North America from Canada to Mexico, the Great Plains is one of the largest ecoregions on the continent (Samson and Knopf 1994). Plains river networks are an integral component of ecosystems in this region, and have been characterized as highly variable environments (Dodds et al. 2004). Variability of these systems stems in part from their water sources. Larger rivers in mountainous regions on the western edge of the plains have high flow from snowmelt in the late spring, whereas smaller streams or tributaries with headwaters in the plains experience high flows from spring and summer rainstorm events (Fausch and Bestgen 1997). Spring runoff and summer storm events bring potential for sudden and drastic changes in water temperature, flow regime, and physicochemical conditions, all of which create a harsh environment for fishes and other aquatic biota (Matthews 1987; Fausch and Bestgen 1997). The challenges for fishes inhabiting streams of this region have been greatly exacerbated by human alterations including water storage, diversion, and urbanization (Poff et al. 1997; Bunn and Arthington 2002; Poff et al. 2007).

Despite those variable and unpredictable conditions, a large amount of biotic diversity occurs in streams of the Great Plains, including fishes in the family *Cyprinidae*, collectively known as minnows (Fausch and Bestgen 1997; Hoagstrom and Berry 2008). Cyprinids are the largest family of North American freshwater fishes, and since the Oligocene have greatly diversified, dispersed (Johnston and Page 1992), and evolved life history strategies to persist in highly variable plains stream environments (Matthews 1987). For example, in a study of the brain morphology of the genus *Hybopsis*, Davis and Miller (1967) observed differences between species in concentrations of taste buds and sizes of optic lobes, and concluded three different feeding strategies were used; sight, skin tasting, and mouth tasting. They also concluded that the variability in size of brain lobes was greatest in fishes that inhabited the most variable environments, allowing for a generalist approach to feeding behavior. This means that species inhabiting plains streams that are subject to elevated turbidity levels from spring runoff and summer storm events are able to feed during times of low visibility by having higher concentrations of cutaneous and internal taste buds, whereas species inhabiting clear water showed decreased numbers of taste buds and enlarged optic lobes signifying reliance on a sight-based feeding approach.

Reproductive strategies of fishes have also evolved for life in variable plains river conditions. Upstream movements and spawning in response to high flow events in spring and summer is common in some Great Plains cyprinids (Moore 1944; Bottrell et al. 1964; Bestgen et al. 1989; Taylor and Miller 1990; Fausch and Bestgen 1997; Platania and Altenbach 1998; Bonner and Wilde 2000). Movements may be substantial, as some cyprinids were able to swim 50 or more km in less than 72 hours in a swim chamber (Bestgen et al. 2010) and also used designed fishways to bypass in-stream barriers in field settings (Archdeacon and Remshardt 2012). The purpose of these movements was presumably both for re-colonization of suitable upstream habitat by adults (Cross et al. 1985; Bonner 2000) and to repopulate downstream reaches via transport of eggs and larvae (Cross et al. 1985; Bestgen et al. 1989; Platania and Altenbach 1998).

Means by which some plains fishes are able to repopulate a downstream area lies with anatomy and development of eggs and newly hatched larvae, another reproductive adaptation for life in fluctuating plains streams. Moore (1944) and Bottrell et al. (1964) noted that Arkansas River shiner *Notropis girardi* and speckled chub *Macrhybopsis aestivalis* each produce non-adhesive, semi-buoyant eggs. This egg type was also observed in six Rio Grande basin cyprinids, which released gametes in the water column, a behavior known as pelagic broadcast spawning (Platania and Altenbach 1998). Following a spawning event, eggs absorbed water by osmosis, expanding the perivitelline space to achieve a semi-buoyant state, where slight vertical currents keep them suspended as they are carried downstream during development (Moore 1944; Bottrell et al. 1964; Platania and Altenbach 1998). After hatching, larvae immediately enter a swim-up stage which enables them to remain suspended while they develop a gas bladder, absorb yolk, and transition to exogenous feeding. Upon development of the gas bladder, they are able to move into areas of lower velocity and higher temperature, light penetration, and primary productivity, which aids growth (Bottrell et al. 1964; Platania and Altenbach 1998). Eggs of Arkansas River shiner and speckled chub hatched within 24-48 h depending on water temperature (Moore 1944; Bottrell et al. 1964), and the entire development process can take anywhere from 3-7 d depending on temperature and discharge volume following spawning (Platania and Altenbach 1998). This rapid early life development is a survival strategy for species in systems with rapidly fluctuating flow regimes. For example, rapid development to a larger size may enable recently hatched fish to seek and use refuges during flood events that may pose threats to less developed fishes (Matthews 1986; Harvey 1987).

In addition to upstream spawning migrations and rapid development, many plains fishes have developed a reproductive strategy to avoid population crashes due to catastrophic events. Most small-bodied cyprinids have a short life span of two or three years, meaning that poor or non-existent recruitment in a year would significantly reduce abundance the following year (Bestgen et al. 1989; Bonner 2000). To reduce this potential, multiple clutches of eggs are produced during an extended spawning season usually lasting throughout the late spring and summer (Bestgen et al. 1989; Taylor and Miller 1990; Bonner 2000; Durham and Wilde 2006; 2009). Doing so presumably ensures that some cohorts of young will be produced during times when conditions are favorable for survival and subsequent recruitment to the population. Early accounts of plains cyprinid reproductive ecology suggested spawning coincided with large increases in flow (Moore 1944; Bestgen et al. 1989), an idea that was extended to other species without sufficient data. Alternatively, other more recent studies have found reproduction in several plains cyprinids occurs continuously during periods of low flow without flow spikes (Bonner 2000; Durham and Wilde 2006). Both spawning approaches were documented for Arkansas River shiner among these studies, suggesting this species, and perhaps other plains cyprinids, are capable of adopting a reproductive strategy to fit localized conditions. Collectively these adaptations are characteristic of a reproductive guild of fishes known as pelagic broadcast spawners, which were originally common in stream fish communities of the Great Plains (Gido et al. 2010; Perkin and Gido 2011, Hoagstrom and Turner 2013).

Flathead chub inhabit Great Plains river systems as far north as the Northwest Territories of Canada and south to New Mexico, Texas, and Louisiana (Olund and Cross 1961; Martyn and Schmulbach 1978; Kucas 1980; Rahel and Thel 2004). Historic distribution of flathead chub in Colorado includes the Arkansas River basin, with early specimens captured far upstream in the Arkansas River near the city of Salida at an elevation of 2,160 m (Ellis 1914). Population declines in Colorado led to listing of flathead chub as a Species of Special Concern, a designation defined as vulnerable and where further declines may lead to threatened or endangered status, and strong populations remain only in portions of the Arkansas River, Purgatoire River, and Fountain Creek (Loeffler et al. 1982; Bramblett and Fausch 1991; Nesler et al. 1999). To date, flathead chub life history investigations have focused on adult morphology and meristics, habitat use, diet, growth rate, and length of reproductive season (Olund and Cross 1961; Bishop 1975; Martyn and Schmulbach 1978; Cross et al. 1985; Scarnecchia et al. 2000; Fisher et al. 2002), but reproductive ecology remains poorly studied. Similarities in distribution and morphology to other plains cyprinids has prompted suggestion that flathead chub may belong to the pelagic broadcast spawning guild (Durham and Wilde 2005, 2006; Perkin and Gido 2011). However, documentation of spawning behavior, egg characteristics, and recruitment dynamics of flathead chub are insufficient to support this hypothesis.

STUDY AREA

Fountain Creek is a tributary to the Arkansas River in southeastern Colorado (Figure 1). The headwaters of Fountain Creek form near the town of Green Mountain Falls, Colorado, at an elevation of approximately 2,400 m. This upstream section flows southeast approximately 25 river kilometers (rkm), dropping almost 600 m in elevation, before the confluence with Monument Creek in Colorado Springs, Colorado. The upstream section of Fountain Creek is a cold-water stream with a trout-dominated fish assemblage, but downstream of Monument Creek it becomes a warm-water stream with a largely native fish community dominated by cypriniform species (Nesler et al. 1999). From Colorado Springs, Fountain Creek flows south along the base of the Front Range Mountains for approximately 90 rkm until it joins the main stem of the Arkansas River at Pueblo, Colorado. The total land area drained in the Fountain Creek watershed is approximately 2,300 km^2 upstream of the U.S. Geological Survey (USGS) gage near Pueblo (#07106500).

Fountain Creek exhibits a highly variable spring and summer flow regime due to surface runoff from snowmelt and summer convective storms, both historically (pre-1950) as well as recently (Figure 2), but flows have changed over time. Variability of summer flows has increased further from urbanization in the Colorado Springs area that has resulted in a higher proportion of impervious land cover in the watershed. This increases runoff directly into streams, particularly through roadways and storm drains with resulting increased frequency of flood events, increased bankfull flooding, and reduced groundwater recharge. In contrast, urbanization has also eliminated historical low-flow conditions, and steadily increased base flow levels in Fountain Creek over time. Trans-basin diversions to meet increased demand of a growing population are the driver of these increases, and have resulted in sewage effluent comprising a large proportion of the water in Fountain Creek.

Flow regimes during 2012-2013 sampling seasons were typical of historical patterns, but varied between years in timing and magnitude of flow spikes (Figure 3). For example, in 2012 a single large and relatively early flow spike occurred whereas in 2013, more frequent flow spikes occurred later in summer. In addition to a seasonally variable flow regime, Fountain Creek also now has a variable daily flow regime in some areas (Figure 4). This typical daily pattern was presumably caused by periodic effluent releases from a wastewater treatment facility in Colorado Springs. Releases from the Owens-Hall diversion dam, located 60 rkm upstream of the Arkansas River, also created occasional discharge spikes that also sluiced sediment from behind the dam. The gate was opened periodically to flush accumulations of fine sediment, algae, and detritus built up behind the diversion dam that interfered with the water diversion intake upstream. Sluicing events released a high magnitude pulse of extremely turbid water for approximately 30 min during base flow conditions.

Flow regimes in Fountain Creek will be further altered by construction of the Southern Delivery System Project (SDSP) (U.S. Dept. of Interior 2008). This water project will pump water via pipeline from the Arkansas River in Pueblo uphill approximately 70 km to Colorado Springs for municipal use. Upper Williams Creek Reservoir, southeast of Colorado Springs, will store untreated water, which will then be sent to water treatment facilities prior to municipal use. Following municipal use, treated water will be stored in Lower Williams Creek Reservoir and released periodically down Fountain Creek to satisfy downstream water needs. Water releases will be via pipeline from the reservoir and enter Fountain Creek approximately 15 rkm downstream of the Owens-Hall diversion dam, which is approximately 45 rkm upstream of the

Arkansas River confluence. Operation of the SDSP under the most probable scenario (alternative 2) is projected to have “minor adverse effects” on the streamflow conditions and aquatic life in Fountain Creek from Security, Colorado, downstream to the confluence of the Arkansas River (U.S. Dept. of Interior 2008). Magnitudes of Fountain Creek flows are projected to increase at varying levels both up and downstream of the SDSP water exchange point. Downstream of the exchange point average increases from nearly 20% to greater than 60% are projected throughout the year, with highest increases (54-61%) expected from April–June, the presumptive reproductive period for many warmwater fishes in Fountain Creek (Figure 5). Monthly streamflow projections are averaged across wet and dry years, meaning observed increases from the SDSP will vary between years in timing, magnitude, and duration but could be substantially higher than averages.

METHODS

Timing and Duration of Reproduction - Drift Netting

To document the timing and duration of flathead chub reproduction in Fountain Creek, eggs and fish larvae were collected throughout the late spring and summer via daily drift net sampling. This approach provided *de facto* evidence of spawning, compared to presence of ripe fish, which may be inadequate if some fishes have a fractional spawning strategy. Drift nets were used to estimate egg abundance in Fountain Creek because they capture the non-adhesive eggs as they are transported downstream during development (pers. comm., K.R. Bestgen, Colorado State University). Because drift nets were intended to capture non-adhesive eggs like those of flathead chub, and egg types of other fishes found in Fountain Creek differ or are unknown, the species composition of egg catches does not necessarily reflect the composition of the entire fish community. Three drift net sampling stations were established to monitor egg and larval fish presence and abundance: FC1, FC2, and FC3. Sampling stations FC1 and FC2 were located 7.25 and 0.9 rkm upstream from USGS gaging station 07106000, respectively, and FC3 was located 7.5 rkm downstream, so this gage was used to monitor Fountain Creek flow and water temperature during this study. Sampling stations were all located downstream of the first potential barrier to upstream movement that fish encounter, the channel-spanning Owens-Hall diversion dam located 60 rkm upstream of the Fountain Creek-Arkansas River confluence. This section of Fountain Creek has a large population of flathead chubs, and sampling by personnel from Colorado Parks and Wildlife from 2010 to 2013 documented large concentrations of reproductively-ready adult chubs directly downstream of the diversion structure and farther downstream throughout the summer months (pers. obs., M.R. Haworth, Colorado State

University). Thus, the FC1 sampling station was placed approximately 0.2 km below the Owens-Hall diversion dam and sampled daily throughout the study, whereas sampling at the FC2 and FC3 stations occurred on alternating days.

A 500 µm mesh drift net (0.76 m wide x 0.38 m high x 2.0 m long, tapered to a cod end measuring 11 cm diameter) was used to sample fish eggs and larvae. Samples were collected between 0600 and 1200 hrs nearly daily throughout the study. The drift net was set in the channel thalweg to sample the greatest volume of water possible, although sampling did not occur during extreme high flow events due to safety issues and reduced gear efficiency. The drift net opening was fitted with a General Oceanics Inc. Model 2030R mechanical flow meter, and time and flow meter readings were recorded at the beginning and end of each net set, which made calculation of average water velocity (m/s) possible. When drift nets were fully submerged, the effective net sampling area was 0.29 m^2. By multiplying the average water velocity by the area of the submerged net mouth, the total volume of water (m^3) filtered during each sampling event was obtained. During periods of low flow when nets were not fully submerged, the depth of the portion of the net submerged was recorded and used to adjust the total volume of water sampled. Water temperature was measured (°C) at the beginning and end of each net set with a handheld thermometer, as was water clarity (cm) with a Fieldmaster ® model 78-070 transparency tube. Stream conditions recorded during sampling included dominant habitat and substrate types. Nets were deployed until detritus and fine particulate matter restricted flow of water through the mesh and the net mouth began to form a backwater, after which debris and fish were strained using a 500 µm sieve and immediately preserved in 100% ethanol. All eggs and fish larvae were removed from preserved samples within 4 days, counted, and stored in 100% ethanol to allow for otolith analysis. Number of eggs or larval fish captured was divided by total volume of water filtered during each net set to estimate catch density (n/m³), which enabled comparisons between samples under various flow conditions.

Additionally, paired samples were occasionally taken at FC1 immediately before and during sediment sluicing events at the Owens-Hall diversion dam. This was done to compare drift rates of eggs and larval fish during lower base flows prior to sluicing versus higher flows when sediment was released from behind the diversion. Eggs and larvae captured during sediment sluicing events were included in final drift densities. This is because sluicing events were unscheduled and could not all be sampled, therefore the effects of undocumented sluicing events on drift densities of eggs and larvae were unknown.

Timing and Duration of Reproduction - Live Egg Collection and Rearing

Concurrent with drift net sampling at each site, Moore egg collectors (0.3 mm fiberglass window screen material; Altenbach et al. 2000) were used to collect live eggs transported downstream in Fountain Creek. However, Moore egg collector samples were for a relatively short duration during sluicing events due to high debris loads and high water velocities. Thus, egg densities in Moore egg collectors may be biased low compared to drift nets, especially at site FC1. Live eggs were transferred to the laboratory and hatched and reared to a taxonomically-identifiable size or life stage. This information allowed me to determine the likely species composition of eggs captured concurrently in drift net samples, which were otherwise unidentifiable to species due to preservation. Collection of live eggs also provided an estimate of flathead chub egg density transported downstream based on captures per m³ of water sampled. Daily egg collections were grouped as weekly cohorts in the lab due to space constraints, where they were placed into glass trays with aeration, and treated with an anti-fungal agent. Eggs were monitored approximately every 6 h, during which dead eggs were removed and newly hatched fish were transferred into a separate, non-aerated tray. Monitoring continued until all eggs from the weekly collection had hatched or were determined dead. Fish larvae were fed live *Artemia* spp. (brine shrimp) *ad libitum* until they grew to a size when species identification was possible, at which time they were preserved in 100% ethanol. Using reared specimens of known identity and unpublished illustrations, species identity of reared specimens was verified by two investigators (myself and D.E. Snyder, Larval Fish Laboratory, Colorado State University). Our species identifications had 100% agreement.

Recruitment Patterns - Seining

Age-0 flathead chub were collected from mid-July to early October to determine patterns of recruitment, here defined as survival of larvae to a juvenile developmental state, which occurred beginning at about 20 mm total length (TL). The maximum size of recruited age-0 juveniles observed was approximately 60 mm TL. Seine sampling was conducted fortnightly with various-sized fine mesh seines (0.76 m long x 0.61 m high, mesh sizes 0.8 and 1.5 mm, or 6.1 m long x 1.2 m high, 3.1 mm mesh) to ensure all age-0 cohorts that survived the sensitive early life history stages in summer were represented in samples. Seining occurred at the FC2 and FC3 drift net sampling stations, as well as an additional downstream station, FC4, located approximately 19 rkm downstream of FC3. Seining stations were located downstream of drift net sampling locations to account for the transport of eggs and larvae during early life stages to downstream reaches. Thus, recruitment observed at seine sample sites were thought to reflect patterns of reproduction that occurred upstream, and therefore the recruitment patterns throughout Fountain Creek. Seven samples from each of the three seining locations were made in each of 2012 and 2013.

Seining was conducted in all habitat types present within 200 m upstream or downstream of drift netting locations. At FC4, the Fountain Creek access point served as the center point of this sampling. Seine hauls with the 3.1 mm mesh seine were performed in a downstream direction in higher velocity main channel habitats, whereas 0.8 and 1.5 mm-mesh seine samples were performed in the upstream direction in lower velocity and shallow channel margins, braids, riffles, and backwater habitats. In riffle habitats, the net was often held stationary while one person disturbed substrate upstream, which dislodged fish downstream into the net. For each sampling occasion, the sampling duration was recorded and effort was generally sufficient to capture approximately 40 or more individuals. Specimens were stored in 100% ethanol for later identification, and measured in the laboratory to the nearest 0.01 mm TL with digital calipers.

Recruitment Patterns - Otolith Daily Increment Validation

A main tenet of any aging study is to validate the accuracy and precision of the technique used (Beamish and McFarlane 1983; Bestgen and Bundy 1998; Hill and Bestgen 2014). Therefore, a study was performed to determine the timing of first daily increment deposition in otoliths of flathead chub and evaluate if increment deposition rate was one per day in the post-hatch period (Campana and Nielson 1985; Campana 2001). Validation of daily increment patterns is needed to ensure that otolith increment counts yield accurate and precise estimates of individual fish age, and the subsequent growth rates and hatch dates that are derived from fish ages. In a previous study (2010; pers. comm. K.R. Bestgen, Colorado State University), flathead chub embryos were obtained by introducing twelve ripe adult flathead chub (4 female, 8 male) collected from Fountain Creek, Colorado, into a 302 L aquarium with sand, gravel, and cobble substrate with 20°C water. Spawning was noted when fertilized eggs were discovered on the tank bottom, and these were subsequently collected and removed for incubation in separate aquaria at 20°C fluctuating $\pm$ 2°C daily. At hatching, flathead chub larvae were reared following methods previously discussed, and larval fish were preserved in 100% ethanol on 12 occasions at intervals ranging from 0-47 d post-hatch to generate a series of known-age flathead chub. For age validation, 20 individuals were randomly chosen from each of the 12 lots and measured to the nearest 0.01 mm TL. Right and left sagittal otoliths were removed from specimens and mounted on microscope slides in immersion oil for interpretation. Preservation date and TL of each individual was recorded separately to allow the reader to perform a blind count of increments. I examined otoliths under a compound microscope at 320X magnification and recorded diameter and core radius measurements using an ocular micrometer. A single reader (the author) counted increments in otoliths from each fish twice, each on separate occasions. The two readings for each individual were averaged to obtain a final age. I report those findings here to streamline the Results section.

Estimated age of flathead chub early life stages corresponded closely with known age in a nearly 1:1 fashion, and indicated that one otolith increment was deposited for each day of life. This was evidenced by the linear regression relationship of estimated age as a function of known age, which was significant (Figure 6: $r^2 = 0.99$, $p < 0.001$), and had a slope near 1 (0.97 $\pm$ 0.05 95% CI). The intercept of the relationship was not significantly different from 0 (0.89 $\pm$ 0.95 95% CI, $p = 0.07$) which indicated increment deposition began at hatching, a result confirmed with observation of one increment in 1-day-old fish. Because flathead chub deposited daily growth increments in otoliths beginning the day of hatching and at a rate of one per day, increment counts were simply the age of the fish in days after hatching.

Recruitment Patterns - Hatch Date Estimation

Hatching dates of larval flathead chub captured in drift nets, and hatching dates for larger age-0 flathead chub juveniles collected by seining, were estimated to construct distributions of hatching dates and provide insight to recruitment patterns during 2012-2013. Hatching dates for larvae provided insight to suitable hatching conditions over the reproductive season, whereas juvenile hatching dates revealed periods when conditions promoted subsequent survival and recruitment of larvae to older life stages. Sub-samples of individuals were chosen to represent the complete range of flathead chub sizes present in each collection, presumably representing the range of hatching dates and the relative abundance of various life stages present in recruited age-0 fish. Right and left sagittal otoliths were dissected from fish and mounted on a standard microscope slide in a drop of cyanoacrylate glue and allowed to harden for at least 48 hours. Otoliths were then ground and polished, covered with a drop of immersion oil, and read as for the increment validation. Juvenile otoliths were read on three non-successive occasions rather than two, as they were not as easy to read as otoliths of larvae. Age estimates within a fish were compared, and differences exceeding 10% resulted in discarding that fish in subsequent analyses. Acceptable otolith age estimates were averaged to yield a final estimate of age in days for the individual. Hatch date was estimated as the date of collection minus the estimate of age in days. This was completed for 54 (13% of total) larval flathead chub collected in drift nets, and 594 (24% of total) juvenile flathead chub collected by seining.

Following aging, I obtained individual growth rates for fish as follows:

$$\text{i.} \quad growth\ rate\left(\frac{mm}{d}\right) = \frac{TL(mm) - 5.5mm}{age(d)}$$

where 5.5 mm was the mean TL at hatching of flathead chub, obtained from specimens of larval flathead chub reared in the laboratory. Subtracting length at hatch ensures growth rate estimates were restricted to the post-hatch time period when daily increments are formed.

Rearranging the equation above, growth rates of otolith-aged fish were used to solve for the age of remaining larval (n = 376) and juvenile (n = 1,892) flathead chub not aged by otoliths as follows:

$$\text{ii.} \quad age(d) = \frac{TL(mm) - 5.5mm}{growth\ rate\left(\frac{mm}{d}\right)}$$

where growth rates of otolith aged fish were inserted to solve for age in days of non-otolith aged fish from the same collection. Fish from the same collection were used because differences were found in growth rates across seasons (see Results). Consistent with fish aged by otoliths, hatching date for fish estimated from growth rates was derived from collection date minus the estimate of age in days.

Recruitment Patterns – Age-0 Growth Rates

Growth rate information obtained from analysis of age-0 flathead chub otoliths was used to model predictions of age as a function of TL, and to describe the influence of hatching date on flathead chub summer growth patterns in 2012 and 2013. A total of 594 individuals from 2012 (n = 300) and 2013 (n = 294) were analyzed, representing 21 and 27% of total catches, respectively. Linear regression of $\log_{10}$-age as a function of $\log_{10}$-length was used to model age at length predictions, and to assess the effect of hatch date on growth. Multiple linear regression was used to adjust for the effects of age on length.   This was done to discern whether a seasonal effect on growth rate variation was present.

Flow and Temperature Regime Influence

Timing and duration of spawning, and distribution of larval and juvenile flathead chub hatching dates were compared to annual flow and temperature regimes in Fountain Creek during 2012 and 2013 to describe the relationship between these events and stream conditions. To do so, the distribution of juvenile hatching dates were grouped into 10-day intervals after first hatching and tallied to represent a percentage of the total juvenile catch in each of 2012 and 2013. This number was then divided by the percentage of drift net captured eggs during the same 10-day interval, which produced a ratio of juveniles that survived to egg production. This yielded an estimate of relative survival. The hypothesis of no differential mortality throughout the reproductive season assumed that the proportion of juvenile fish in each interval would be consistent with egg production and represented by a relative survival value of 1.0. Alternatively, relative survival values < 1 indicated higher than expected mortality of fish occurred in that period while values > 1 indicated survival of juveniles was higher than would be expected based on egg captures. Relative survival values were compared to flow and temperature conditions during each 10-day interval, which enabled identification of temperature and flow conditions that promoted or hindered survival at each life stage. Analysis of these patterns added to the understanding of spawning, hatching, and survival of flathead chub in Fountain Creek, and provided the basis to inform recommendations for conservation that are discussed later.

RESULTS

Timing and Duration of Reproduction - Live Egg Collection and Rearing

Eggs captured in Moore collectors in Fountain Creek from May through August in 2012, and April through August in 2013 (Table 1), were reared to determine species composition of drifting eggs. Flathead chub were the most abundant species reared in both 2012 and 2013 (Table 2). Laboratory hatching success of eggs was low in 2012 (8%) with 98 larvae hatched from 1,278 eggs, but increased substantially in 2013 (30%) with 276 larvae hatched from 931 eggs. Time required for eggs to hatch was 1-7 d, with variation due to differences in developmental stage at the time of capture.

Sampling effort was greatest at station FC1, but those samples yielded lower egg densities compared to FC2 and FC3. Flathead chub hatched from eggs were first captured in early May, were present into August, and were the most abundant species in samples in each of 2012 and 2013. Following first egg captures in early to mid-May, densities increased rapidly and peaked in late May and early June (Figure 7). First flathead chub reproduction, based on presence of eggs in samples, coincided with mean daily water temperatures of approximately 15°C. Egg densities generally declined in the months of July and August, but a second mode was observed in August 2013. Counter to expectations for some plains cyprinids, neither initiation of spawning nor peak egg density was perceptibly linked to a change in discharge in 2012 or 2013 because flows were relatively stable at those times.

Timing and Duration of Reproduction - Drift Netting

Eggs captured in drift nets were assumed to be flathead chub based on the high proportion of that species in reared egg samples. Flathead chub began spawning (indicated by egg captures and hatching dates) in mid to late May and peaked in early to mid-June in each of 2012 and 2013 (Figures 8 and 9). More samples were taken during 2013 creating a more continuous distribution of eggs, but the proportion of samples with eggs was similar between years (Table 3), which allowed for equitable comparisons.

Flathead chub spawning was extended in each year, continuing into early September, but varied in magnitude by year. After peaking in June 2012, egg catches were modest through July and August. In contrast, in July and August of 2013, egg catches were as much as two to four times higher than in 2012. Temperature regimes were similar in each year, wherein both the initiation and conclusion of spawning coincided with mean daily water temperatures of approximately 15°C. Water clarity of Fountain Creek fluctuated throughout sampling, and ranged from < 1 cm to > 70 cm. Each year, the FC1 station samples had markedly higher egg densities than FC2 and FC3, which were similar to one another (Table 3).

The five paired egg samples collected just before and during sediment sluicing downstream of the Owens-Hall diversion dam in 2012 indicated that many eggs were transported from upstream of the diversion dam. This was true because background levels of egg transport were relatively low before sluicing but increased to higher levels during sluicing events (Figure 10). Higher egg densities measured during sluicing events contributed to higher egg densities at FC1 compared to other downstream sites, but did not account exclusively for very high single-day densities. This was true because elevated egg densities were recorded on 16 May and 31 May 2012 when paired samples were collected before and during sediment sluicing, whereas similarly high densities recorded 24 May and 3 July 2012 were in the absence of sediment sluicing.

Larval flathead chub were captured in drift nets between early June and mid-August in 2012, and mid-May to early September in 2013 (Table 4). Average densities of larvae were similar between sampling stations and years, but were low overall, and occurred in a smaller proportion of samples compared to eggs. Eleven species of fish representing four families were collected in drift net samples (Table 5). Unidentified specimens were those damaged prior to or during preservation, or had poorly defined morphological characteristics and thus, could not be identified. Flathead chub and longnose dace *Rhinichthys cataractae* were the dominant species in both years comprising over 65% of all fish captured, but varied by station. Flathead chub were more abundant in downstream Fountain Creek samples, where habitat was mainly braided sand channels. Longnose dace were more numerous at stations FC1 and FC2, where more riffle habitat composed of gravel and cobble was present. Flathead chub larvae were predominantly 6.5 mm TL or less, with fewer larger individuals exceeding 10 mm TL and those were captured primarily at the FC3 station (Figure 11). The small size of flathead chub larvae in drift nets confirmed that capture took place shortly after hatching occurred and that larger individuals were less susceptible to capture.

Recruitment Patterns – Seining

Seine sampling began in mid-July and continued at two-week intervals into early October in each of 2012 and 2013 (Table 6). Mean TL of flathead chub captured was nearly identical between years, although was slightly lower in September and October samples from 2013. The maximum length of flathead chub captured increased on each sampling occasion (33-66 mm

TL), while the minimum length fluctuated (12-29 mm TL) between samples in 2012 and 2013. The widest TL range of flathead chub occurred during samples from September and October, indicating presence of small fish late in each year. Total and number per hour of age-0 flathead chub collected varied by sample in both years, and was affected by flow conditions during sampling. Mean daily discharge ranged from 1-191 m^3/s in 2012 and 2013, and at the upper end of this range, sampling was difficult. Mean daily water temperatures ranged from 20-23°C during July sampling, 18-23°C during August sampling, 14-23°C during September sampling, and 10-16°C during October sampling.

Recruitment Patterns – Hatch Date Estimation

Hatching date estimates were derived for 430 larval flathead chub collected in drift nets by both otolith analysis (n = 54, 13% of total) and estimation from growth rates (n = 376, 83% of total, Figures 8 and 9). Estimated hatching dates for larvae ranged from mid-May to early September, 2012 and 2013, and similar to egg captures, the greatest proportion occurred from mid-May through June each year.

No larvae apparently hatched in May 2012 despite presence of highest drift net egg densities of the year (Figure 8). The earliest larval flathead chub hatch date estimate was 2 June, but no flathead chub larvae were captured again until 11 June after which a large mode of larvae were collected over a two week period. Modest numbers of larvae hatched in July and August, and were partitioned into two separate modes also lasting approximately two weeks in duration.

Similar to 2012, observed larval hatching dates were not uniformly distributed during the 2013 reproductive season. Larvae first hatched on 17 May in 2013, and hatching peaked through late May into mid-June (Figure 9). No larvae hatched after 25 June except for a few individuals in late summer, a smaller mode between 20 August and 8 September. Hatched larvae were absent from late June through early August 2013 despite high densities of eggs captured in drift nets in the same period.

In addition to larvae, hatching date estimates were also derived for 2,486 juvenile flathead chub collected by seining, some via counting otolith daily increments (n = 594, 24% of total) and the remainder from growth rate analysis (n = 1,892, 76% of total, Figures 8 and 9). The range of hatching dates of those recruited juveniles among samples collected through summer and autumn typically increased, but the oldest fish captured (the earliest date) remained about the same in all samples (Figure 12). For example, seine samples from early July as well as late September each had fish with early hatching dates (15 May) as well as a mix of fish of other ages. This indicated samples represented the spectrum of fish of different ages that were available in Fountain Creek.

Juvenile flathead chub recruited over an extended period from May through August in each year, but recruitment was concentrated in a shorter interval of approximately six weeks (Figures 8 and 9). More than 90% of flathead chub that recruited to the juvenile stage hatched from mid-May through the end of June in 2012 and 2013, although the full range for hatching date estimates was over a 15-week period from 9 May to 25 August. Earliest hatching date for a juvenile recruit in 2012 was 21 May. The majority of 2012 juvenile recruitment occurred in June, and was consistent with high abundance of larvae. However, similar to larvae, juvenile recruitment was greatly reduced in May and early June despite abundant egg catches during this period. Modest recruitment continued through July and August, but was reduced relative to larval hatching during this period.

Earliest recruitment for flathead chub juveniles in Fountain Creek in 2013 was on 9 May and continued through the end of June (Figure 9). That period accounted for nearly all 2013 recruitment. Recruitment during this period occurred over a duration similar to that of larval hatching. Hatching date estimates showed a near complete absence of juvenile recruitment in July, August, and September in 2013, with the exception of a few individuals in early July and late August. This recruitment pattern was nearly the same as that of larval hatching dates, but few juveniles were evident from the mode of larvae hatched in late August.

Recruitment Patterns – Age-0 Growth Rates

Flathead chub length and age were positively correlated (Figure 13), and a linear regression of $\log_{10}$-age as a function of $\log_{10}$-length was significant and fit the data reasonably well for fish captured in both 2012 ($r^2 = 0.78$, $p < 0.001$) and 2013 ($r^2 = 0.83$, $p < 0.001$). When hatching date was included in a multiple linear regression, models remained significant in both 2012 ($r^2 = 0.88$, $p < 0.001$) and 2013 ($r^2 = 0.95$, $p < 0.001$) where hatching date explained an additional 10-12% of the variation in length-age relationship, respectively.

Mean daily growth rates in age-0 flathead chub were greater in 2012 than 2013 (Table 7). Range of daily growth rates was greatest in early samples, but narrowed later in the year. Because the possible range of individual fish ages grew wider with each sample (Figure 12), a more useful way to describe growth patterns was through the relationship with hatching date (Figure 14). Individual growth rates varied widely, from 0.79 mm/d for a fish hatched in June 2012, to 0.28 mm/d in August 2013. Growth rate was negatively correlated with hatching date in both 2012 and 2013, meaning individuals hatched later in the year grew slower than those hatched earlier. The greater negative slope coefficient in the 2013 regression equation reflected a faster decline in growth rate through the reproductive season than in 2012.

Flow and Temperature Regime Influence

Relative survival values for 10-day intervals throughout the 2012 and 2013 reproductive seasons revealed effects of flow and temperature regimes on flathead chub reproduction and recruitment. Peak daily flows in Fountain Creek were considered in relative survival comparisons because short term changes in discharge were not consistently evident in a mean daily discharge calculated over a 24-hour period. Therefore, instantaneous flow data was examined for days when mean daily discharges exceeded 4 m^3/s to better describe flow conditions during each interval (Figures 15 and 16).

Relative survival in 2012 was highest between 10 and 19 June, when 53% of all recruited juveniles hatched (Table 8, Figure 8). That high recruitment episode occurred when only a small percent (4.3%) of 2012 eggs were produced indicating substantial recruitment can occur from few eggs. Immediately following this interval, an additional 27% of juvenile recruitment occurred between 20 and 29 June with a relative survival value slightly greater than 1 (1.2). Average daily water temperature during these peak recruitment intervals was 19.1-21.1°C. Similar water temperatures and egg production levels occurred prior to and following these intervals from 31 May to 9 June and 30 June to 9 July, but relative survival was less than 1.0 (0.3, 0.5) during those intervals. During those intervals with reduced relative survival, peak instantaneous discharges of 255 (7 June) and 55 m^3/s (9 July) occurred. Less than 10% of egg production and juvenile recruitment occurred from 10 July to 30 September, and although relative survival was low through these intervals, these values represent a very small proportion of all reproduction and recruitment. These reductions in July and early August were coincident with water temperatures exceeding 22°C, while temperatures through the remaining later intervals were similar to those during peak recruitment intervals in June.

Highest relative survival in 2013 occurred during late May and June, and was observed several weeks earlier than in 2012 (Table 8, Figure 9). Approximately 95% of all recruited juveniles hatched over four intervals between 21 May and 29 June 2013, or about six weeks. Average daily water temperature was 17.2-20.6°C throughout these intervals. Similar to 2012, the interval with the highest relative survival was between 10 and 19 June, 2013. This period accounted for 33% of juvenile recruitment but similar to 2012 eggs were relatively uncommon at 7.7% of the 2013 production. Relative survival was greater than 1.0 in each interval during this six week period except 21 to 30 May, where a lower relative survival of 0.4 accounted for 15.8% of all recruitment and the largest proportion of the total egg production for any interval at 43.4%. Relative survival was less than 1.0 during all intervals in which a flow event exceeding 20 m^3/s occurred, and recruitment was zero in all but two intervals with such flows. Egg production during intervals with high flow events were 18% of the 2013 total catch.

DISCUSSION

Flathead chub in Fountain Creek reproduced over a protracted season during 2012 and 2013 as evidenced by presence of eggs and larvae in drift net collections, spanning the approximately four month period mid-May through mid-September. This is the first documentation of the duration of the reproduction season for flathead chub and confirms scattered life history details reported from other portions of its range that placed spawning between June and September (Olund and Cross 1961; Bishop 1975; Martyn and Schmulbach 1978; Gould 1985). Species composition of fish hatched from eggs reared in the laboratory showed the majority of eggs captured in drift nets were flathead chub, enabling identification of peak reproduction periods. Consistent presence of eggs showed that spawning began in May and continued into September, and highest egg catch densities occurred in late May through the end of June in 2012 and 2013. Reproduction was influenced more by water temperature than streamflow, because spawning occurred under both steady low flow conditions and during fluctuating higher flows. Larval hatching also peaked in May and June, but unlike eggs was restricted to periods of stable low flow conditions. Recruitment of juveniles occurred only in a subset of the reproductive season, and was associated with periods of low and steady flows, usually in late May and June. Periods with flow spikes produced few juvenile recruits in spite of ample egg production. Growth was variable between and within years, which has implications for recruitment of young. These findings are discussed in detail below.

Timing and Duration of Reproduction-Egg Catch

Flow and thermal regimes affect structure and persistence of stream communities (Schlosser 1985; Poff et al. 1997, Olden and Naiman 2010) and understanding how these abiotic processes affect flathead chub in Fountain Creek is fundamental to conservation planning. Fountain Creek is an unusual environment to understand effects of exogenous factors on cyprinid reproduction because base flow steadily increased over the past century from increased urbanization, eliminating occasional periods of discharge intermittency typical of streams in the southern and western Great Plains (Fausch and Bestgen 1997). Many of these streams and their associated fish assemblages have recently experienced more frequent and longer periods of intermittency owing to surface and groundwater depletions caused by water impoundment and extraction (Cross et al. 1985; Falke et al. 2010b). As such, plains stream cyprinid reproduction is often studied when discharge is a limiting factor (Moore 1944; Bestgen et al. 1989; Taylor and Miller 1990), and a common conclusion is that plains cyprinids reproduce in response to rising hydrographs or spates. Consistent egg catches throughout a protracted reproductive season showed that flathead chub in Fountain Creek spawned continuously even in steady flows and do not require increased discharge to do so. This was particularly evident in late May and early June 2013 when the peak of reproduction coincided with an extended period of stable low flow conditions of 1-2 m^3/s. These findings are similar to those for several Great Plains cyprinids in the Canadian River, Texas, where presence of discharge in the flow-depleted system was more important for successful spawning than the magnitude of flows (Bonner 2000; Durham and Wilde 2006).

Photoperiod and temperature are also important environmental factors regulating reproduction by temperate zone fishes (DeVlaming 1972), and may serve as the controlling mechanism(s) for spawning by flathead chub in Fountain Creek given presence of ample stream discharge throughout the year. Onset of spawning each year, based on presence of eggs in samples, consistently occurred in mid-May of 2012 and 2013, meaning photoperiod may cue initiation of spawning. Onset also coincided with increasing water temperatures, when mean daily temperature exceeded about 15°C. Reproduction continued at temperatures as high as 23°C, indicating this range may be optimal. Endogenous and exogenous factors interact to regulate reproduction in fishes (Munro et al. 1990) but it is unclear if environmental thresholds exist that would preclude reproductive success of flathead chub. It is possible that high mid-summer temperatures exceeded thermal limits for flathead chub reproduction or survival. However, this was unlikely as mean daily water temperatures in Fountain Creek did not exceed 23°C in 2012 or 2013, values well below the range of mean hyperthermia tolerances (34.9-38.8°C) observed in 35 Great Plains stream fish species (Smale and Rabeni 1995). The timing and duration of egg catches during 2012-2013 sampling indicated that spawning took place in late spring or summer during stable low flow conditions coupled with mean daily temperatures of 15-23°C, and thus, should be considered optimal conditions to maintain flathead chub reproduction in Fountain Creek.

Timing and Duration of Reproduction-Larval Catch

Drift of flathead chub larvae occurred over an extended period, May through August, but timing varied between study years. Similar to egg catches, earliest larvae were in mid-May and peak densities were observed in May and June of 2012 and 2013. Hatching and drift patterns of flathead chub larvae are poorly known, but timing in Fountain Creek was similar to that observed by Durham and Wilde (2008), who captured low densities of drifting larvae in late-May to mid-June in the Canadian River, Texas.

Distribution of larval flathead chub hatching dates showed flow conditions affected larval presence in both 2012 and 2013. Hatching of chub larvae in 2012 was concentrated after isolated high-magnitude flow events, and occurred mainly during lower and more stable flow conditions. Similarly, nearly all 2013 larvae hatched in May and June when flows were stable and high magnitude flow events were absent. These patterns showed relatively stable low flow conditions were required for successful hatching to occur. High magnitude flow spikes were apparently not required to promote hatching.

The duration of the interval between high magnitude flow events may also regulate successful hatching of larval flathead chub. The shortest interval between high flow events when successful hatching occurred in 2012 was three weeks, 10 to 30 July (21 days); successful hatching also occurred in a similar duration window from 8 June to 7 July (30 days). The same was true in 2013, when hatching occurred during a 19 day window of low flows from 24 August to 11 September. However, a similar 18 day stretch of low flow conditions occurred between 16 July and 2 August 2013, and despite documented spawning (presence of eggs) during this period, no larvae were captured.

Apparent absence of larvae from late May and early June collections in 2012 was likely due to sample processing error from the onset of sampling until that was corrected on 13 June, after which larvae were detected in samples. An extreme flow event occurred prior to this correction on 7 June and may have also have influenced low abundance of larvae from samples if conditions were capable of causing mortality of newly hatched fish or downstream displacement outside of sampling areas (Harvey 1987). Larval drift was very low or absent during flow events of similar magnitude later in 2012, suggesting a combination of these two factors likely influenced larval absence in May and June 2012.

Density of larvae captured was several orders of magnitude lower than that of eggs at all drift net sampling stations. The broadcast spawning technique employed by flathead chub may in part explain low larval densities. A large amount of gametes are released during spawning, of which a proportionally small amount will hatch resulting in a lower number of individuals available for capture relative to eggs (Johnston and Page 1992; Johnston 1999). Additionally, drift net sampling is a passive capture technique, and different capture rates would be expected amongst egg and fish larvae, given the ability of larvae to swim shortly after hatching and find low velocity areas so they are not transported downstream (Platania and Altenbach 1998, Hoagstrom and Turner 2013). Flathead chub are mobile at hatching, and become capable swimmers 3-4 d post-hatch and as small as 7 mm TL. Mobility at a relatively small size was supported by the large proportion of larvae < 7 mm TL in samples captured in drift nets and the few longer fish captured (Fig. 11), suggesting that larger fish with higher swimming capacity were not susceptible to drift or the sampling gear. Drift nets were set in the channel thalweg, and movement of precocial larvae into channel margins and backwater habitat shortly after hatching may explain low larval densities in samples.

Stochastic disturbance events may have also influenced hatching of flathead chub larvae in July and August 2013. Two large wildfires burned in the Fountain Creek drainage in each of the study years; the Waldo Canyon Fire (23 June – 10 July, 2012) that burned 74 km^2, and the Black Forest Fire (11 June – 20 June 2013) that burned 58 km^2. Wildfires are common to the Western United States and the Front Range Mountains of Colorado, and can alter stream temperature, chemistry, and sediment dynamics (Rieman and Clayton 1997; Moody and Martin

2001; Rhoades et al. 2011). Effects of wildfire, particularly increased sediment loads, has been extensively studied for adult fish, but little information is available regarding the response of fish eggs and larvae. Furthermore, most fire-related research has focused on salmonid species inhabiting fluvial systems with lower and less fluctuating sediment levels than those typical of Great Plains streams (Chapman 1988; O'Connor and Andrew 1998; Argent and Flebbe 1999).

Fires occurred in the Fountain Creek drainage in each of 2012 and 2013, but convective storms centered over burn scars that mobilized large amounts of fine sediments were restricted to 2 July and 10 July, 2013. Water clarity was less than 1 cm deep immediately following these events, but returned to typical levels in 2 and 6 d after each storm, respectively. It is possible that changes in sediment load and water chemistry had negative impacts on flathead chub larvae as none were captured during these run-off events between 2 and 16 July. However, it is difficult to attribute this absence solely to effects of fire given the elevated flow event that occurred on 15 July which may also have destroyed larvae. Stream conditions returned to a typical state following the 15 July storm event, and spawning continued uninterrupted through July and August of that year. High flow events of a magnitude similar to the event on 15 July also occurred at a high frequency during this period, and no larvae were captured. Thus, it is difficult to separate the effects of altered stream conditions due to fire from a flow event that was of a magnitude capable of displacing or destroying eggs and larvae.

Collectively these findings showed that flathead chub larvae successfully hatched during low flow conditions in spring and summer in Fountain Creek, and high magnitude flow events were not required to promote hatching. Additionally, it appears that run-off from rain over burn scar areas in July 2013 may have had negative effects on flathead chub larvae, but were difficult to separate from flow regime effects.

Recruitment Patterns - Hatch Date Estimation

Distributions of hatching dates revealed several key factors controlling of juvenile flathead chub recruitment patterns. Flathead chubs recruited over an extended period from May to August, nearly all recruited fish hatched in May and June, and recruitment occurred differentially relative to both spawning and flow regime (Figures 8 and 9). Durham and Wilde (2006) also documented peak flathead chub recruitment in May and June, and recruitment of multiple cohorts over two years in the Canadian River, Texas. Similar to patterns in Fountain Creek, the consistent peak of recruitment occurred under different flow conditions between study years. Recruitment of multiple cohorts is a strategy employed by several plains cyprinids as an adaptation to ensure population persistence despite highly fluctuating environmental conditions (Bestgen et al. 1989, Taylor and Miller 1990; Platania and Altenbach 1998; Bonner 2000; Durham and Wilde 2006, 2009).

Recruitment patterns of juvenile flathead chub, similar to larval fish distributions, were not similar to patterns of egg production in 2012 or 2013. Instead, distributions of hatching dates for recruiting juveniles in Fountain Creek were from a relatively small subset of eggs produced in each year. In 2012 and 2013, the majority of flathead chub juvenile recruitment in Fountain Creek was represented as a single mode with peaks in mid-June, but peaks were distributed differently in each year owing to variable flow regime. Prior to the first high flow event of the summer on 7 June 2012, low recruitment occurred in spite of high egg densities. It was possible a lag associated with time from spawning to hatching explained some of this pattern, but was unlikely to be the sole driver of that pattern given the time period between peak egg catches to the peak in recruitment exceeded the 1-7 d needed for eggs to hatch in laboratory rearing. Recruitment patterns later in the summer provided evidence that high flow events likely accounted for the lack of recruitment in May despite high levels of reproduction. This was because some of the highest larval flathead chub densities were observed in mid-July, between two high flow events on 9 and 31 July 2012, and similar to late May, did not result in proportional amounts of recruitment. This suggested that favorable conditions for both spawning and hatching must have been followed by conditions that were unsuitable for recruitment, and was likely explained by the storm event on 31 July approximately 10 days following peak July larval catches. Alternatively, immediately following the storm on 31 July 2012, ambient levels of reproduction and low density larval catches accounted for a second mode of recruitment which coincided with low flow conditions lasting approximately six weeks. These patterns suggested that high magnitude flow events and the frequency at which they occurred regulated recruitment dynamics of flathead chub in Fountain Creek.

In contrast to 2012, Fountain Creek remained at low flow conditions during peak reproduction in May and June 2013, resulting in uniform recruitment patterns of juvenile flathead chub when compared to capture patterns for eggs and larvae. High magnitude flows in 2013 were restricted to later summer and occurred at a much higher frequency than 2012, beginning in mid-July and continuing into October almost uninterrupted. Spawning continued throughout this time as relatively high egg densities were observed, but only a small mode of larvae were captured in late August and early September, and juvenile recruitment was nearly absent. Those low-recruitment events showed, similar to single high magnitude flows, that high frequency of lower magnitude flow events also greatly reduced recruitment of flathead chub despite spawning during that time period. Taken together, observations of reproduction and recruitment from 2012 and 2013 revealed that suitable spawning and hatching conditions did not ensure successful recruitment, and that flow disturbances of high magnitude and or frequency had a large and negative effect on survival of early life stages and population dynamics of flathead chub in Fountain Creek.

Under estimation of age is a potential source of error in estimating hatching dates because increment width is reduced as fish growth slows (Campana and Nielson 1985). Narrow increments increases the likelihood of not counting all daily increments present in the otolith during age estimation. However, several factors indicate this was not the case when I aged flathead chubs. First, daily increment validation showed high levels of accuracy and precision when estimating increment counts from known age fish ranging from 1-50 d old. Furthermore, the timing of initial recruitment estimates makes sense biologically as they coincided with the appearance of eggs and larvae in drift net samples in mid-May. Lastly, if under-estimation of age was occurring, the expected effect would be that earliest observed hatching dates would shift later throughout collections. This was not observed as the ranges of hatching date estimates by sample remained consistent across all collections, and estimates of earliest hatch between the first and last collections did not differ by more than four days in either year.

Potential displacement of eggs and larvae from drift netting stations into lower Fountain Creek and the downstream Arkansas River may also have impacted observed recruitment patterns. The most downstream juvenile sampling station FC4 was located 24.5 rkm upstream of the confluence with the Arkansas River. Because juvenile sampling did not occur in this lower portion of Fountain Creek or in the Arkansas River, it is unknown if the lack of juvenile recruitment observed at sampling stations may have occurred at downstream locations. Densities of adult flathead chub are reduced in lower Fountain Creek and the Arkansas River relative to reaches where sampling occurred (pers. obs., M.R. Haworth, Colorado State University), and suggested that recruitment was not likely to have occurred in downstream areas where sampling did not occur, or that fish recruited in downstream reaches and subsequently moved upstream. Ongoing research by Colorado Parks and Wildlife investigating adult flathead chub movement in Fountain Creek and the Arkansas River will provide insight into this scenario.

Recruitment Patterns - Age-0 Growth Rates

Growth patterns of flathead chub sampled in both years indicated individuals hatched earlier in the reproductive season experienced higher growth rates than later-hatched fish. This was consistent with the results reported by Durham and Wilde (2005), who found growth of five plains cyprinids, including flathead chub, declined for fish hatched later in the year. In Fountain Creek this pattern may be explained by the differences in exposure to varying environmental conditions in early versus later portions of the growing season. Fish hatching in late spring and early summer were exposed to the warmest temperatures of the year in June, July and August, while fish hatched in late summer or early fall would have been exposed to these warm temperatures for a shorter period of time.

Other studies have also shown seasonal temperature differences regulate growth of fishes. For example, Crecco and Savoy (1985) found that growth of American shad *Alosa sapidissima* larvae slowed when exposed to cooler water temperatures in the Connecticut River, resulting in lower survival rates. Larval bloater *Coregonus hoyi* from Lake Michigan hatched earlier in the season grew half as fast during the first three weeks of life than individuals hatched later (Rice et al. 1987). Bestgen et al. (2006) also found differential growth among cohorts of Colorado pikeminnow in the upper Colorado River basin, with higher mean growth rates for the few individuals that survived from earlier produced cohorts. Consistently faster growth in earlier hatched flathead chub in both 2012 and 2013, despite differing flow regimes between years, showed thermal regimes earlier in the year promoted faster growth of fish in Fountain Creek. Faster early growth rates of flathead chubs may provide additional explanation of higher levels of recruitment for fish hatched earlier in the season. This was because survival may be length-dependent, and by maximizing the length of growing season, larger body size may confer a survival advantage over smaller individuals (Conover 1990; Kirjasniemi and Valtonen 1997; Bestgen et al. 2006).

In addition to length of growing season and water temperatures, larval growth may be driven by prey availability (Houde 1978; Werner and Blaxter 1980; Bestgen 1996; 2008). For example, earlier hatched flathead chub may have higher food resources, which may be depleted later in the reproductive season (Welker et al. 1994). Biotic interactions may also contribute to faster growth of early hatching individuals through advantages in resource competition (Persson 1983; Ward et al. 2006) or predation (Polis 1981; Takasuka et al. 2003). Enhanced competitive abilities may be realized in earlier hatched fish that undergo ontogenetic niche shifts to maximize growth and survival by utilizing food resources unavailable to smaller, later hatched individuals, and attaining sizes that reduce predation pressure (Werner and Gilliam 1984, Cowan and Houde 1992; Gagliano et al. 2007). In this way direct individual metabolic influences combined with indirect biotic interactions may explain the seasonal effect in growth rate variation of flathead chub in Fountain Creek.

Flow and Temperature Regime Influence

Relative survival analysis illustrated how flow and temperature regimes influenced flathead chub reproduction and recruitment in each of 2012 and 2013. Reduction of relative survival values nearly always coincided with discharge events that met or exceeded 20 m^3/s

(Table 8). The interval from 30 July to 8 August, 2012, was the only one to have a relative survival greater than 1.0 despite a high magnitude flow that occurred. Timing of elevated flow in that interval likely caused relative survival value greater than 1.0. For instance, high discharge events on 7 June and 9 July, 2012 that lowered relative survival values of their respective intervals below 1.0 occurred at or near the end of the 10-day interval. Alternatively, the high flow on 31 July occurred early in that 10-day interval, and a high relative survival of 1.4 occurred. However, relative survival was reduced in the interval that preceded (20-29 July) which otherwise experienced low, stable flow conditions that were commonly associated with high relative survival. These patterns show that eggs and larvae produced immediately prior to high flow events are most vulnerable to negative effects of high flow disturbance, and refuted the hypothesis of no differential mortality.

The effect of high flow frequency on juvenile recruitment was also elucidated through relative survival analyses. Only two consecutive intervals with high flows occurred in 2012, and were later in the reproductive season when egg production was nearly absent. In contrast, many high flow events occurred from July to September of 2013 (Figure 16), where only one of the last eight intervals of the year did not experience a flow spike. Five intervals from 10 July to 28 August produced 17.4% of all eggs captured in 2013, but produced nearly no juvenile recruitment (0.2%). This showed that low, stable flow conditions over periods ample to promote survival of eggs and larvae were not realized due to frequent increases in discharge.

Stream conditions favorable for reproduction and recruitment of flathead chub were also identified through patterns of relative survival. Onset of reproduction was in early May in both 2012 and 2013 evidenced by modest egg catches. However, the relationship of reproduction relative to flow is not entirely clear. Spawning does appear to increase with some flow events as in August, 2013 (Figure 9), but most egg production occurred during low, stable conditions of 1-2 m^3/s, and showed that flow spikes are not required for reproduction to occur. Initiation of spawning coincided with mean temperatures of approximately 15°C in 2012 and at 11.5°C in 2013, but large egg catches began in both years when temperatures reached approximately 17°C, and continued at high levels up to water temperatures of 21°C in each year. This temperature range may also promote juvenile recruitment, as the majority occurred during the same interval (10-19 June) in both study years from proportionally small amounts of egg production. Mean water temperature was nearly identical during these intervals in 2012 and 2013 at 19.7 and 19.4°C, respectively, as were flows, which were relatively constant at 1-2 m^3/s. Collectively these patterns showed that a relatively modest amount of reproduction can account for a majority of recruitment in a single year under favorable temperature and flow conditions, approximately 17-21°C and 1-2 m^3/s.

Relative survival values depend largely on the amount of measured egg production in an interval, which can be greatly affected by singular capture events. The interval of 21-30 May, 2013, contained three of the highest egg captures of the year which elevated the total percentage of eggs to 43.4%, and a corresponding relative survival value of 0.36. The reduced relative survival is likely a product of the arbitrary selection of ten day intervals, as 15.8% of the annual recruitment took place during this interval. If the 21-30 May interval were combined with the intervals preceding and following, they would account for 62.6% of egg production and 77.2% of recruitment, with a relative survival of 1.23. Therefore, relative survival may be less useful to describe survival patterns over consecutive intervals that experienced similar flow and temperature conditions and lacked high flow disturbances. Additionally, relative survival in intervals that experienced low levels of egg production may be less useful to describe broad patterns. For example, the earliest 2013 interval from 1-10 May had a low relative survival of 0.19, but less than 1% of egg production occurred during this interval, meaning this interval had potential for only a very small contribution to total annual reproduction and recruitment. Therefore, relative survival values, while useful to describe the effects of localized high flow events or annual temperature regimes, may require consideration of additional factors to best assess broad patterns of flathead chub reproduction and recruitment in Fountain Creek.

Operation of the SDSP has the potential to create additional high flow spikes in Fountain Creek, which negatively impact recruitment of flathead chub in late spring and summer. Such hydrograph spikes should be avoided to allow for successful recruitment of juveniles, with a minimum of three week intervals of steady low flow conditions of approximately 1-2 m^3/s, preferably in the months of May and June when reproduction is high. Alternatively, reduction in stream discharge may also negatively affect flathead chub recruitment. Base flows in Fountain Creek have become increasingly stabilized and continuous through time, conditions that favor increased recruitment. As such future unforeseen changes to water management, for example the recapture and storage of treated wastewater rather than its release, would reduce Fountain Creek flows and may reduce recruitment success, although it is unclear what may constitute a minimum flow threshold.

Effect of Sluice Gate Operation

Peak flathead chub reproduction in 2012 and 2013 occurred during the months of May and June, but extremely high egg catch densities in drift nets may be an artifact of egg captures during sediment sluicing at the Owens-Hall diversion dam directly above station FC1 (rkm 58.0). Samples collected during sluicing exhibited densities several orders of magnitude higher than typical catches, elevating the average drift net egg density at the FC1 station relative to FC2 (rkm 50.9) and FC3 (rkm 43.4) (Figure 17). Eggs in sluice release drift net samples at FC1 were mostly opaque, and appeared dead upon capture. This was confirmed by unsuccessful laboratory rearing of eggs concurrently captured with Moore egg collectors.

It was not possible to know if and when sluicing occurred during sampling at FC2 and FC3 due to the short duration and punctuated nature of such events, but lack of opaque eggs from those downstream samples suggested little or no transport from upstream. Presence of opaque eggs only at station FC1, elevated drift net egg densities, and the low proportion of live eggs captured with Moore egg collectors collectively indicated some eggs deposited in the pool upstream of the Owens-Hall diversion dam were released in high densities during sluicing. Prairie stream impoundments are capable of entraining propagules of obligate drifting species, in some instances leading to population reductions in upstream reaches (Winston et al. 1991; Platania and Altenbach 1998; Dudley and Platania 2007). Depending on the interval at which sluicing occurs at the Owens-Hall diversion dam, duration of egg deposition may be long enough to cause mortality. Further research would elucidate this possible effect, but until such information is available, opening the sluice gate frequently during the reproductive season may reduce effects of egg deposition and mortality upstream of the diversion. Because laboratory rearing indicated that hatching of eggs can take up to seven days, and because the time required to kill deposited eggs is not clear, frequent, perhaps daily, sluicing operation would be ideal.

SUMMARY

Effective planning for the conservation and management of imperiled North American cyprinids is made more difficult by a lack of information regarding reproductive ecology of the species of interest (Johnston 1999). This study contributed to the ecological knowledge of flathead chub, and identified dynamics of reproduction and recruitment relative to flow and thermal regimes in Fountain Creek. That information will assist with identification of conservation actions for this state-listed Species of Special Concern, related to ongoing and future management. Results clarified that flow and temperature regimes have important effects on spawning, hatching, and recruitment of flathead chub in Fountain Creek.

Because spawning and recruitment can each influence population dynamics, thermal and hydrologic regimes that promote success of both facets of flathead chub reproductive ecology should be incorporated into management considerations aimed at conservation of this species. Water temperature was found to be most important for initiation and continuation of spawning. Mean daily temperatures of 15ºC coincided with the onset and conclusion of spawning, and peaked at 15-21°C. The months of May and June proved crucial for recruitment as the majority of age-0 individuals captured in late summer and early fall hatched during these months. Low flow conditions during these months and throughout the reproductive season were associated with the greatest proportion of recruitment, while single high magnitude flow events proved capable of greatly reducing or eliminating recruitment. Additionally, results indicated that an increased frequency of high magnitude flows can further reduce recruitment capability if aforementioned low flow intervals are reduced or eliminated. Inter-annual frequency of high magnitude flows during the peak recruitment months of May and June also bears consideration as increasing the interval at which they occur between years could have long-term negative impacts on flathead chub populations given their short generation time (Bestgen et al. 1989, Bonner 2000). High magnitude flow events that occur during peak recruitment months, like the event observed in June 2012, have not occurred in more than four consecutive years dating back 28 years in Fountain Creek, and consecutive years with more than one day of high magnitude flows from 15 May to 15 July never occurred (Figure 18). Operation of the SDSP has potential for future alteration of flow and temperature regimes in Fountain Creek, therefore, guidelines to promote high flathead chub reproduction and recruitment are offered below:

1. Flows of 1-2 m^3/s and increasing water temperature (15-21°C) in late spring and summer (May and June) maximize reproduction and recruitment. Very low-flows (< 1 m^3/s), high or frequent flow spikes ($\geq$ 20 m^3/s), or declining or colder thermal regimes during peak reproductive months may reduce reproduction and recruitment.
2. Periods of high flathead chub recruitment associated with relatively constant flow (1-2 m^3/s) over at least three-week intervals during the reproductive season are desirable, particularly in later May and June.
3. Consecutive years with frequent and high magnitude SDSP flow releases equal to or exceeding instantaneous peaks of approximately 20 m^3/s, especially those in May and June, should be minimized to the extent possible to promote recruitment of young flathead chub and avoid potential population reductions over time.
4. Frequent (daily if possible) sluicing of the Owens-Hall diversion dam structure may convey live eggs downstream that would otherwise be trapped and die in the upstream diversion pool.

Flathead chub are a declining species in Colorado and throughout North America. Though listed as a Species of Special Concern in Colorado, a strong population exists in Fountain Creek. The greater understanding of reproduction and recruitment dynamics of flathead chub related to flow and water temperature regimes revealed by this study will help promote the persistence of flathead chub in Fountain Creek and Colorado.

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Table 1. Sampling details by location and year, including number of eggs collected, sampling time, and total water volumes (m^3) sampled with Moore egg collectors at each station (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

Year	River kilometer	Samples	Samples with eggs	Sample dates	First, last occurrence	Total eggs	Sampling time (hr)	Eggs/hr	Volume (m^3)	Density (eggs/m^3)
2012	58.0	385	71	5/10-9/21	5/16, 7/13	149	53.8	2.8	12,672	0.012
	50.9	200	110	5/23-9/21	5/23, 8/2	279	13.9	20.1	2,722	0.103
	43.4	262	152	5/22-9/21	5/22, 8/15	850	16.3	52.1	3,007	0.283
2013	58.0	499	70	3/20-10/4	4/24, 8/26	197	41.4	4.8	12,123	0.016
	50.9	381	161	4/8-10/4	4/24, 8/29	462	16.1	28.7	5,742	0.080
	43.4	324	95	4/8-10/4	4/8, 8/30	272	15.4	17.7	3,992	0.068

Table 2. Species composition of reared eggs collected with Moore egg collectors at drift net sampling stations in Fountain Creek, Colorado, 2012 and 2013.

		2012 (n = 1,278)		2013 (n = 931)		Total (n = 2,209)	
Common name	Scientific name	n	%	n	%	n	%
flathead chub	*Platygobio gracilis*	97	99.0	206	74.6	303	81.0
longnose dace	*Rhinichthys cataractae*	1	1.0	9	3.3	10	2.7
creek chub	*Semotilus atromaculatus*	-	-	9	3.3	9	2.4
longnose sucker	*Catostomus catostomus*	-	-	1	0.4	1	0.3
unidentified specimens	-	-	-	51	18.5	51	13.6
Total		98	100.0	276	100.0	374	100.0

Table 3. Sampling details by location and year, including number of eggs collected, sampling time, and total water volumes (m^3) sampled with drift nets at each station (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

Year	River kilometer	Samples	Samples with eggs	Sample dates	First, last occurrence	Total eggs	Sampling time (hr)	Eggs/hr	Volume (m^3)	Density (eggs/m^3)
2012	58.0	99	94	5/10-9/21	5/10, 9/7	6,212	72.4	85.8	33,671	0.184
	50.9	44	30	5/23-9/21	5/23, 9/21	507	25.4	20.0	10,660	0.048
	43.4	43	32	5/22-9/21	5/24, 8/9	433	20.1	21.5	8,086	0.054
2013	58.0	121	111	3/20-10/4	4/4, 9/10	6,473	51.7	125.2	27,602	0.235
	50.9	62	52	4/8-10/4	4/24, 10/4	301	19.7	15.3	10,725	0.028
	43.4	63	54	4/8-10/4	4/24, 9/11	728	18.6	39.1	12,632	0.058

Table 4. Sampling details by location and year, including number of larval flathead chub collected, sampling time, and total water volumes (m³) sampled with drift nets at each station (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

Year	River kilometer	Samples	Samples with larvae	Sample dates	First, last occurrence	Total larvae	Sampling time (hr)	Larvae/hr	Volume (m^3)	Density (larvae/m^3)
2012	58.0	99	32	5/10-9/21	6/4, 8/14	56	72.4	0.8	33,671	0.002
	50.9	44	17	5/23-9/21	6/15, 8/6	34	25.4	1.3	10,660	0.003
	43.4	43	17	5/22-9/21	6/14, 9/7	67	20.1	3.3	8,086	0.008
2013	58.0	121	37	3/20-10/4	5/21, 9/11	142	51.7	2.7	27,602	0.005
	50.9	62	18	4/8-10/4	5/22, 8/31	89	19.7	4.5	10,725	0.008
	43.4	63	19	4/8-10/4	5/23, 9/9	42	18.6	2.3	12,632	0.003

Table 5. Species composition of larval fish captured with drift nets at each station (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

		Rkm 58.0		Rkm 50.9		Rkm 43.4		Total	
Common name	Scientific name	N	%	N	%	N	%	N	%
flathead chub	*Platygobio gracilis*	198	33.1	123	36.8	109	40.1	430	35.7
longnose dace	*Rhinichthys cataractae*	208	34.8	114	34.1	67	24.6	389	32.3
*sand shiner	*Notropis stramineus*	7	1.2	9	2.7	48	17.6	64	5.3
fathead minnow	*Pimephales promelas*	4	0.7	3	0.9	9	3.3	16	1.3
red shiner	*Cyprinella lutrensis*	1	0.2	1	0.3	7	2.6	9	0.7
creek chub	*Semotilus atromaculatus*	1	0.2	2	0.6	1	0.4	4	0.3
central stoneroller	*Campostoma anomalum*	6	1.0	4	1.2	-	0.0	10	0.8
longnose sucker	*Catostomus catostomus*	97	16.2	32	9.6	12	4.4	141	11.7
white sucker	*Catostomus commersonii*	52	8.7	21	6.3	6	2.2	79	6.6
brook stickleback	*Culaea inconstans*	1	0.2	2	0.6	3	1.1	6	0.5
northern plains killifish	*Fundulus kansae*	3	0.5	-	0.0	2	0.7	5	0.4
unidentified specimens	-	20	3.3	23	6.9	8	2.9	51	4.2
Total		598	100.0	334	100.0	272	100.0	1,204	100.0

*bigmouth shiner *Notropis dorsalis* documented in drainage, identification of shiner species at larval stage is difficult

Table 6. Sampling details by year, including number of flathead chub collected (N), sample dates, mean and total length (TL) range, and mean catch per hour of sampling at seining stations (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

Year	Sample	Sample dates	N	Mean TL (mm)	Time (hr)	N/hr
2012	1	7/12	110	25 (18-33)	2.3	47.1
	2	7/24-7/26	202	30 (19-44)	3.3	62.2
	3	8/7-8/10	150	37 (21-49)	3.3	45.2
	4	8/24	289	44 (28-58)	2.7	107.0
	5	9/7	117	45 (16-61)	1.7	68.2
	6	9/21	167	47 (19-61)	2.3	71.6
	7	10/5	368	48 (20-62)	2.3	160.0
2013	1	7/9-7/10	103	27 (12-40)	3.0	34.3
	2	7/22-7/23	218	29 (15-45)	3.6	60.8
	3	8/5-8/6	147	36 (21-50)	2.1	71.7
	4	8/19-8/22	175	37 (25-53)	1.8	100.0
	5	9/4-9/5	145	41 (28-58)	1.7	87.0
	6	9/20	176	45 (29-63)	2.4	73.8
	7	10/4	119	46 (16-66)	2.3	52.9

Table 7. Sampling details by year, including number of flathead chub (N), sample dates, mean and total length (TL) range, and mean and range of growth rates (GR, mm/day) of age-0 flathead chub aged by otoliths from Fountain Creek, Colorado, 2012 and 2013.

Year	Sample	Sample dates	N	Mean TL (mm)	Mean GR (mm)
2012	1	7/12	23	24 (18-35)	0.70 (0.55-0.78)
	2	7/24-7/26	42	31 (19-44)	0.61 (0.32-0.79)
	3	8/7-8/10	31	36 (21-49)	0.60 (0.46-0.68)
	4	8/24	59	42 (28-58)	0.54 (0.34-0.71)
	5	9/7	40	42 (16-61)	0.52 (0.39-0.62)
	6	9/21	45	45 (19-61)	0.46 (0.37-0.59)
	7	10/5	60	40 (20-62)	0.40 (0.33-0.47)
2013	1	7/9-7/10	30	26 (12-40)	0.53 (0.30-0.67)
	2	7/22-7/23	44	30 (15-45)	0.53 (0.38-0.64)
	3	8/5-8/6	44	35 (21-50)	0.50 (0.40-0.62)
	4	8/19-8/22	44	37 (25-53)	0.45 (0.33-0.54)
	5	9/4-9/5	44	42 (28-58)	0.41 (0.33-0.48)
	6	9/20	43	44 (29-63)	0.39 (0.31-0.44)
	7	10/4	45	45 (16-66)	0.36 (0.28-0.43)

Table 8. Mean water temperature, percentage of total eggs captured, percentage of total hatching date estimates of larvae and juveniles, and relative survival of juveniles by 10-day interval from 1 May-30 September, 2012 and 2013. Shaded relative survival values indicate a peak instantaneous flow of 20 m³/s or greater occurred during that interval.

Year	Date Range	Mean temp (°C)	% eggs	% larvae	% juveniles	Relative survival (% eggs/% juveniles)
2012	5/1-5/10	15.5	2.0	0.0	0.0	0.0
	5/11-5/20	15.3	9.1	0.0	0.0	0.0
	5/21-5/30	17.4	16.1	0.0	0.6	0.04
	5/31-6/9	19.1	29.5	0.6	8.2	0.3
	6/10-6/19	19.7	4.3	54.8	53.0	12.2
	6/20-6/29	21.1	22.4	19.8	27.4	1.2
	6/30-7/9	21.5	9.5	1.3	4.4	0.5
	7/10-7/19	22.4	1.4	14.7	1.4	1.0
	7/20-7/29	22.3	1.8	0.6	0.9	0.5
	7/30-8/8	22.0	1.4	2.6	2.0	1.4
	8/9-8/18	21.0	2.1	2.6	1.8	0.9
	8/19-8/28	20.3	0.0	0.0	0.4	*
	8/29-9/7	19.9	0.1	0.0	0.0	0.0
	9/8-9/17	17.6	0.0	0.0	0.0	0.0
	9/18-9/30	16.1	0.2	0.0	0.0	0.0
2013	5/1-5/10	11.5	0.5	0.0	0.1	0.2
	5/11-5/20	15.7	0.5	1.1	2.0	4.3
	5/21-5/30	17.2	43.4	42.9	15.8	0.4
	5/31-6/9	17.4	11.5	30.8	28.2	2.4
	6/10-6/19	19.4	7.7	8.1	33.2	4.3
	6/20-6/29	20.6	13.4	10.3	17.9	1.3
	6/30-7/9	21.0	4.7	0.0	2.6	0.6
	7/10-7/19	21.7	4.8	0.0	0.0	0.0
	7/20-7/29	21.1	2.3	0.0	0.0	0.0
	7/30-8/8	21.4	1.5	0.0	0.0	0.0
	8/9-8/18	20.1	7.8	0.0	0.0	0.0
	8/19-8/28	21.5	1.0	2.2	0.2	0.2
	8/29-9/7	22.1	0.5	4.4	0.0	0.0
	9/8-9/17	17.9	0.1	0.4	0.0	0.0
	9/18-9/30	15.6	0.4	0.0	0.0	0.0

* Indicates interval where relative survival could not be calculated due to absence of eggs, but presence of hatching date estimates.

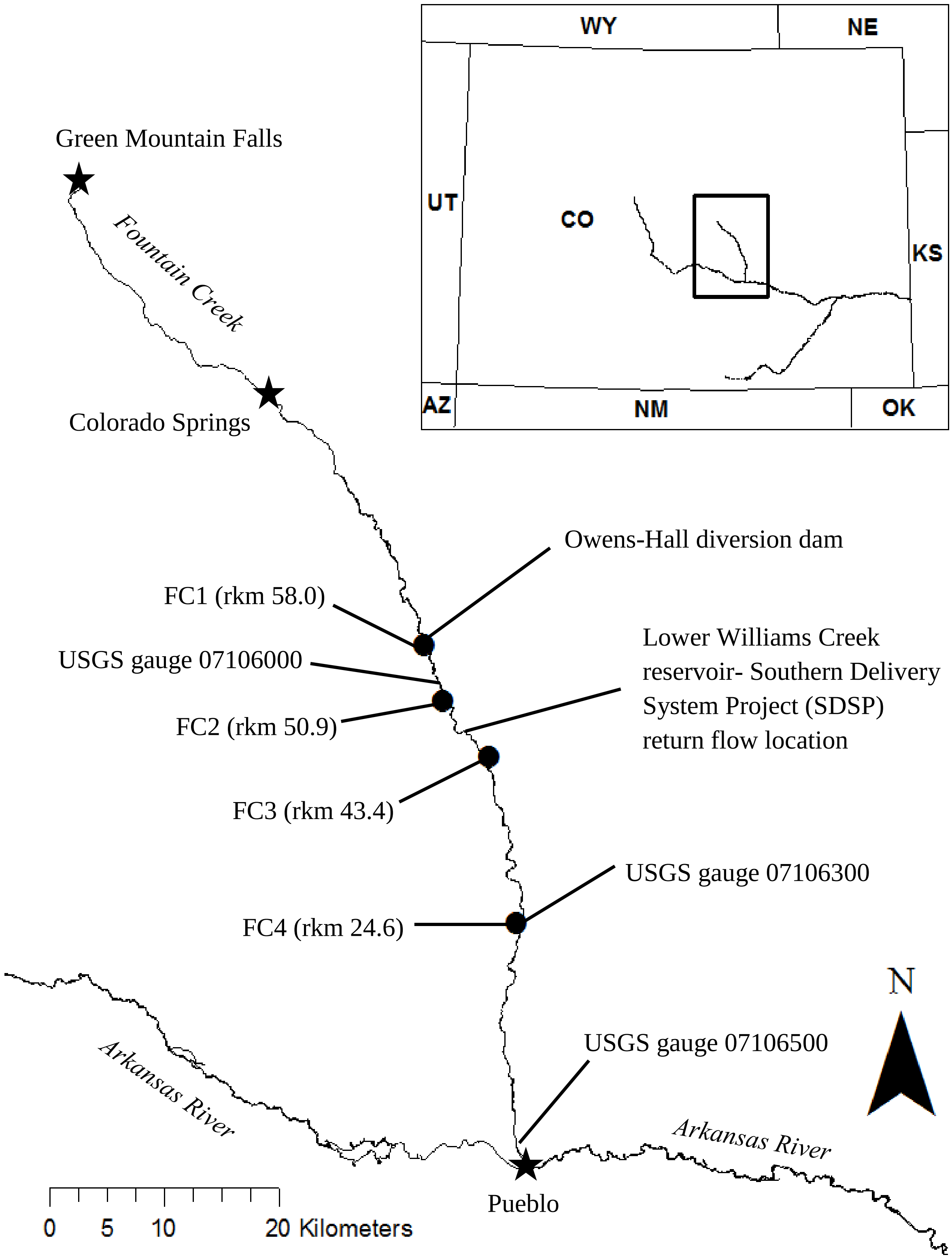

Figure 1. Map of Arkansas River basin in southeastern Colorado and sampling stations (FC) in the Fountain Creek drainage during 2012 and 2013.

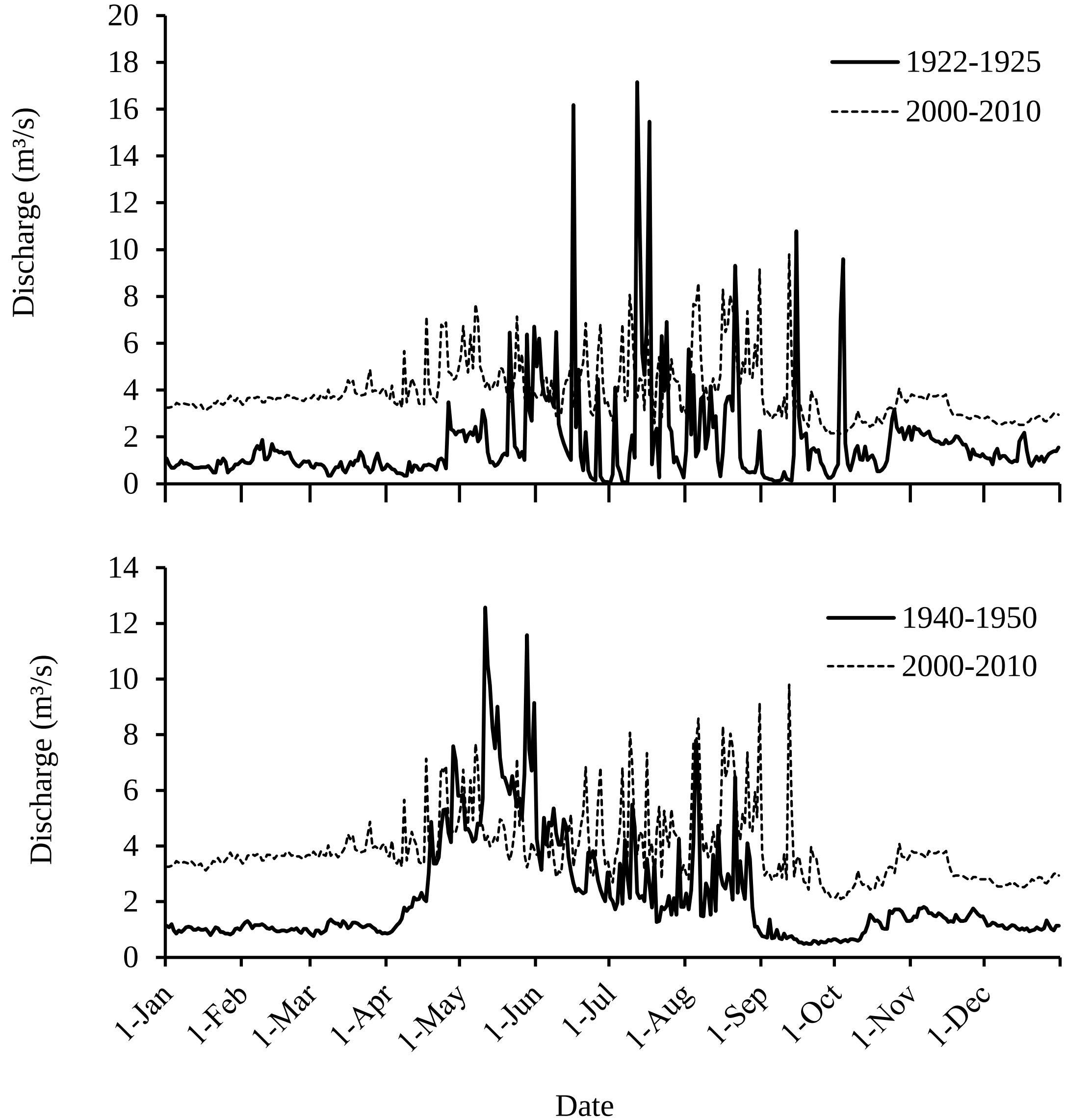

Figure 2. Mean daily discharge of Fountain Creek near Pueblo, Colorado, 1922-1925 and 1940-1950 compared to 2000-2010 (U.S. Geological Survey gage 07106500: period of record 1922-2013). Time periods spaced over the period of record illustrate loss of historical zero or low-flow conditions and more recent elevated base flows.

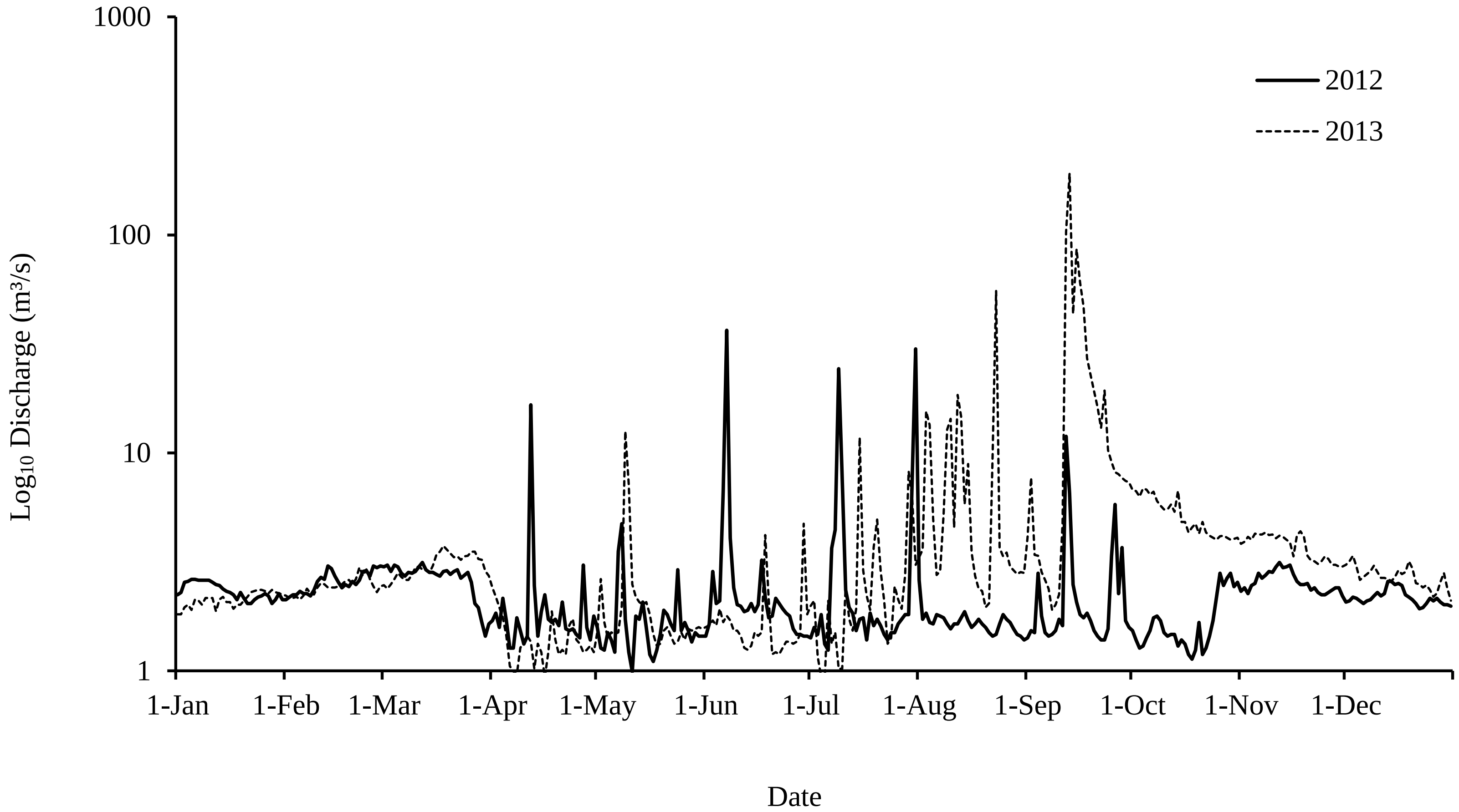

Figure 3. Mean daily discharge of Fountain Creek near Fountain, Colorado, 2012-2013 (U.S. Geological Survey gage 07106000). Discharge values are $\log_{10}$ transformed to allow comparison of elevated discharge events between years.

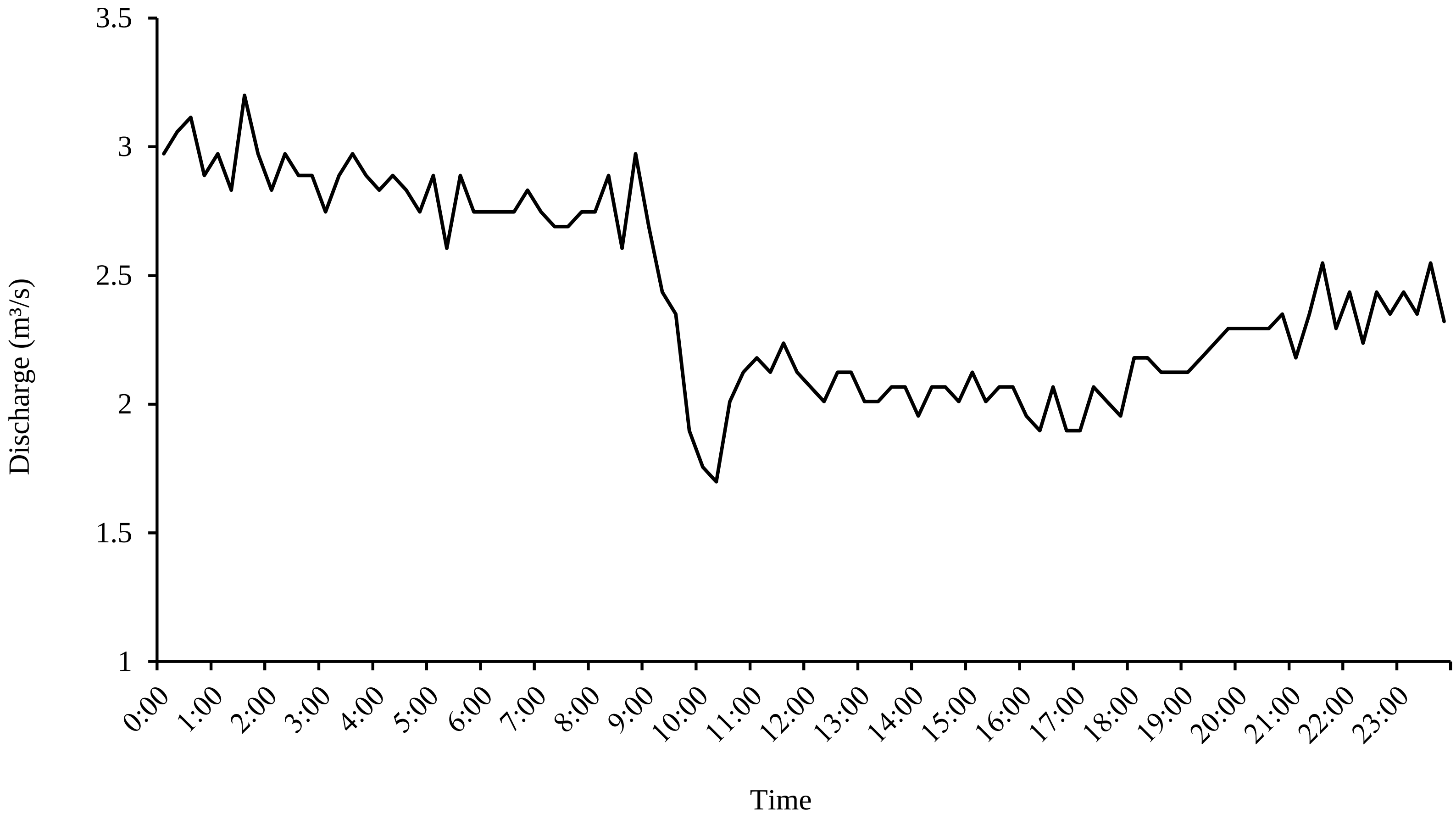

Figure 4. Typical instantaneous daily discharge variation during base flow conditions for Fountain Creek near Fountain, Colorado, 1 April 2013 (U.S. Geological Survey gage 07106000).

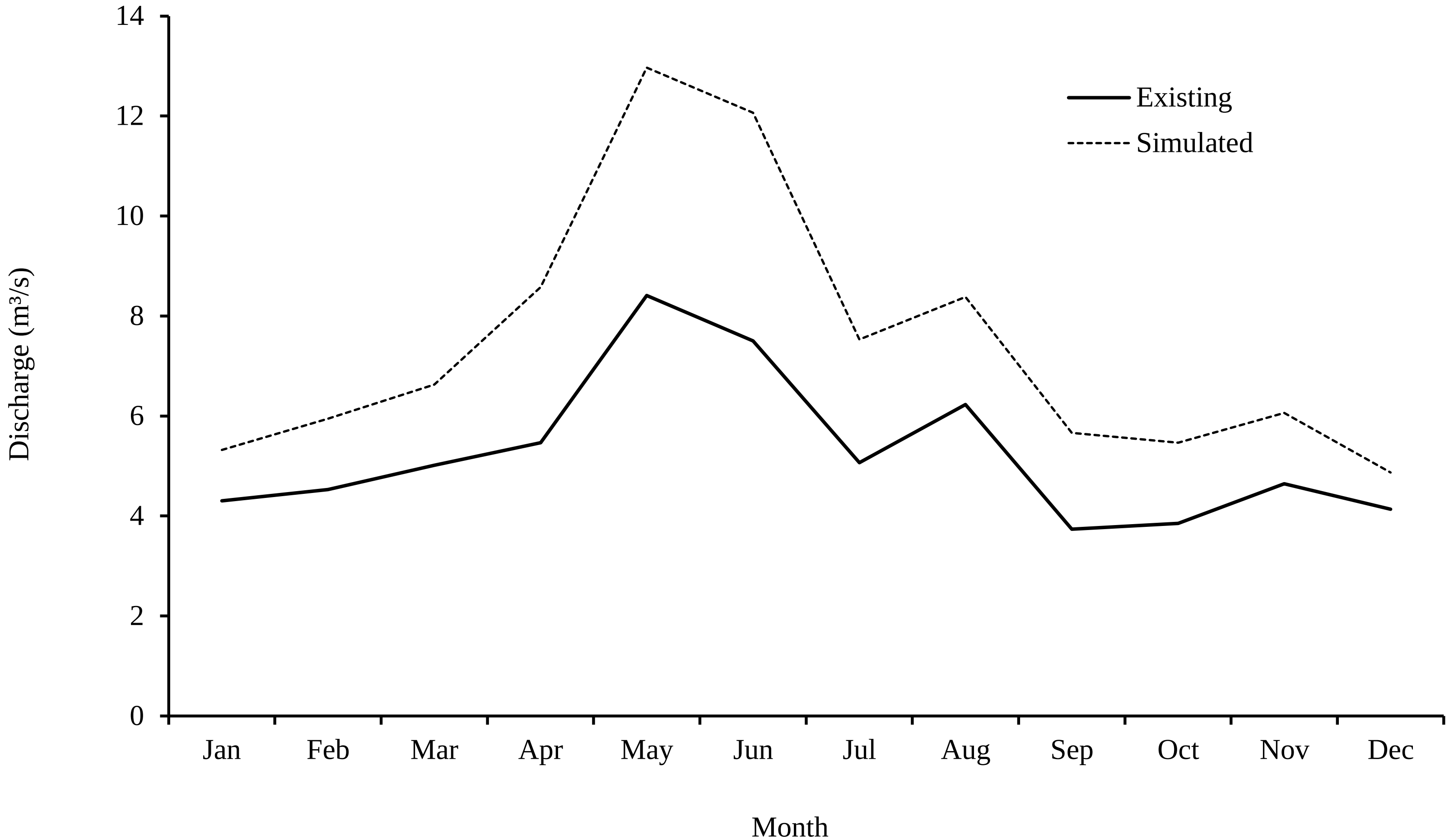

Figure 5. Existing and simulated average monthly streamflow conditions for Fountain Creek near Piñon, Colorado (U.S. Geological Survey gage 07106300). Conditions were taken from direct effects model for the most likely action alternative in the Environmental Impact Statement (U.S. Dept. of Interior 2008). Gage represents first station downstream of Southern Delivery System Project (SDSP) return flow location.

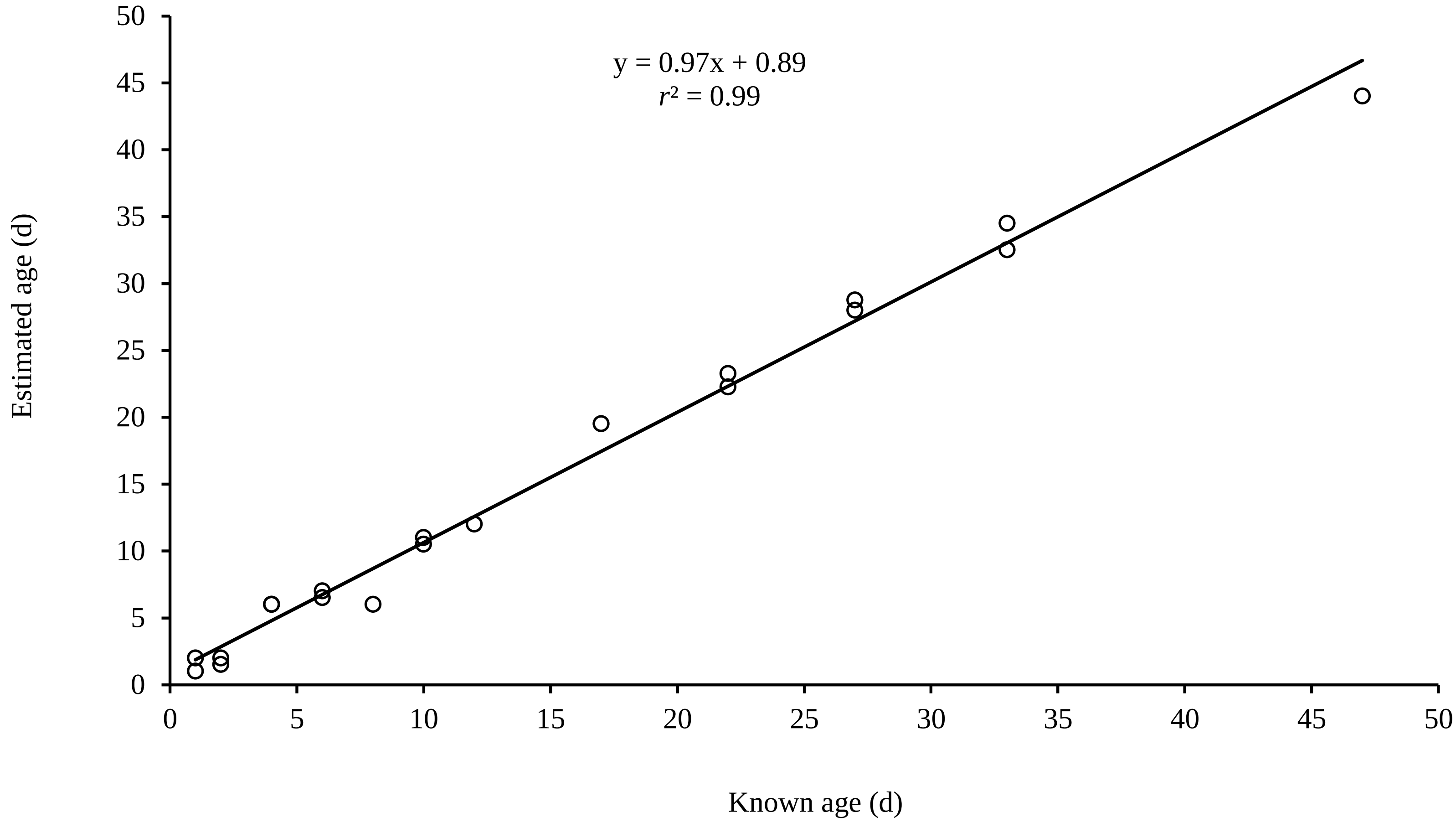

Figure 6. Linear regression of estimated age of flathead chub as a function of known age for otolith daily increment deposition validation, where y = estimated age in days and x = known age in days. Solid line represents a 1:1 ratio of estimated to known age.

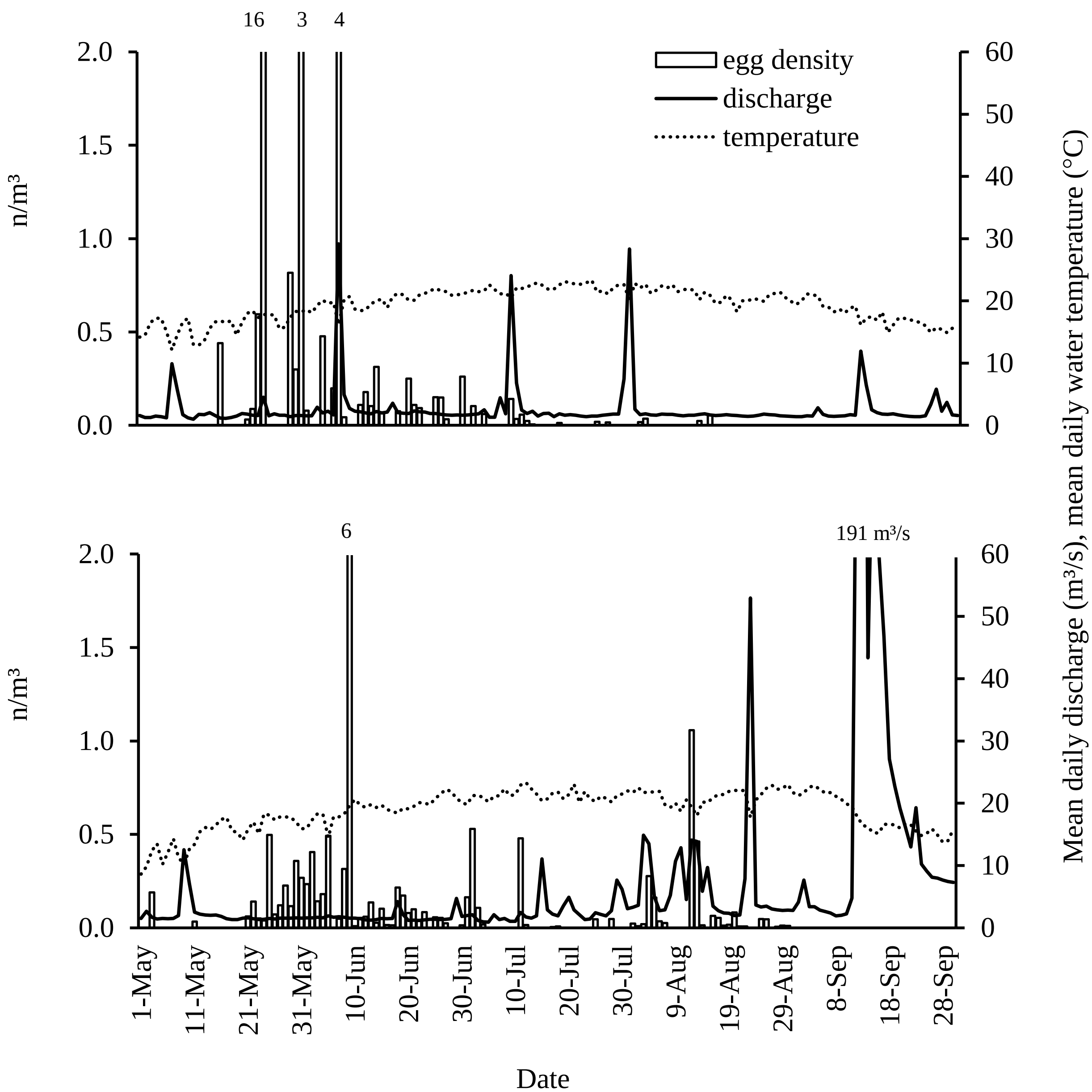

Figure 7. Density of eggs (n/m³ water sampled) captured by Moore egg collector as a function of mean daily discharge and water temperature (U.S. Geological Survey gage 07106000) in Fountain Creek near Fountain, Colorado, 1 May - 30 September, 2012 (top panel) and 2013 (bottom panel). Numbers above bars are shown to increase resolution of smaller egg density values, and describe peak discharges.

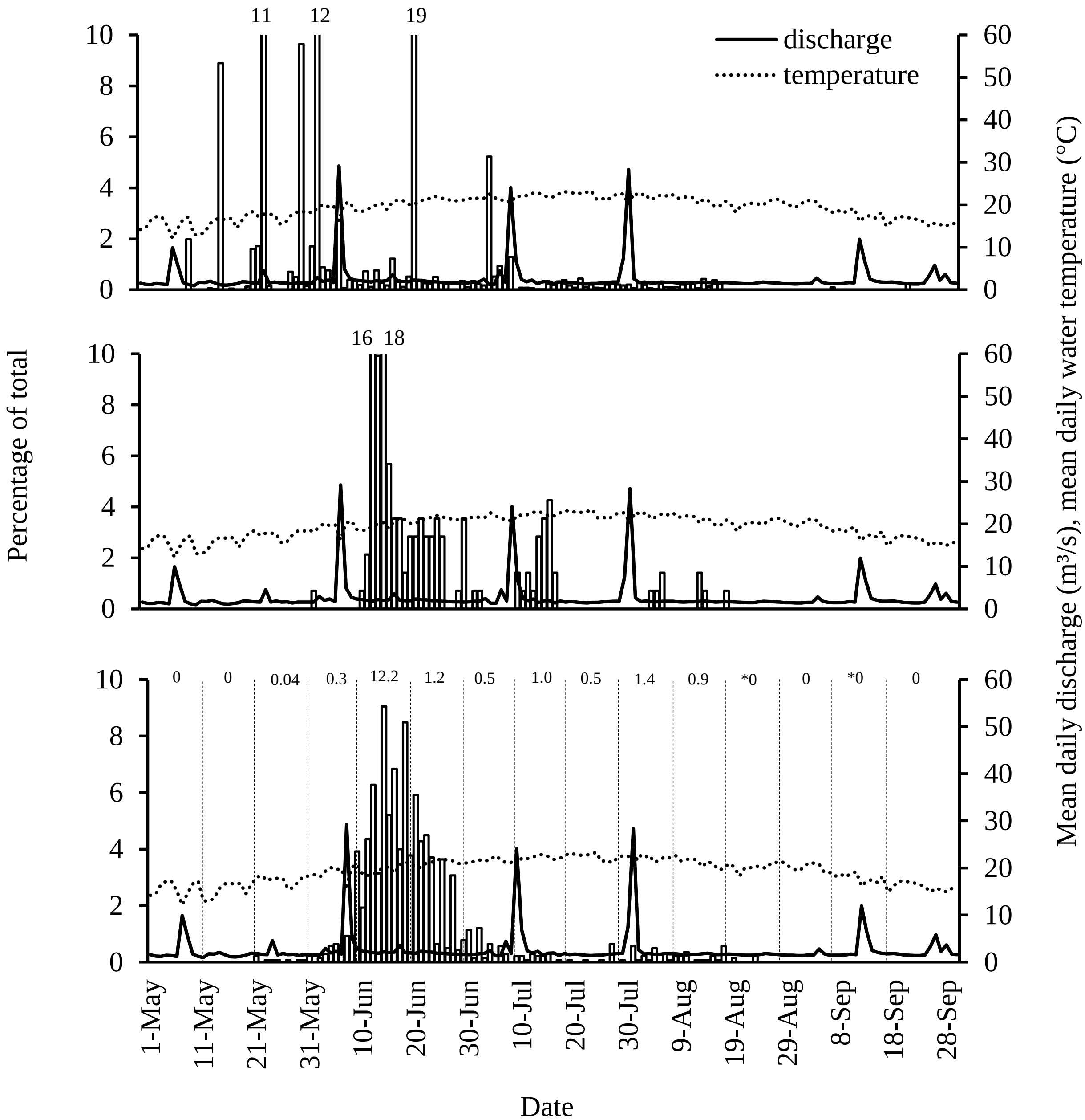

Figure 8. Percentage of drift net captured eggs (top panel), distribution of hatching dates for drift net-captured flathead chub larvae (middle panel), and distribution of hatching dates for seine-captured age-0 juvenile flathead chub (bottom panel) as a function of mean daily discharge and water temperature (U.S. Geological Survey gage 07106000) in Fountain Creek near Fountain, Colorado, 1 May – 30 September, 2012. Numbers above bars are shown to increase resolution of smaller egg and larval hatching date percentages. Dotted vertical lines on bottom panel depict 10 day intervals with corresponding relative survival values presented above. * indicates an interval in which zero eggs were captured in drift nets.

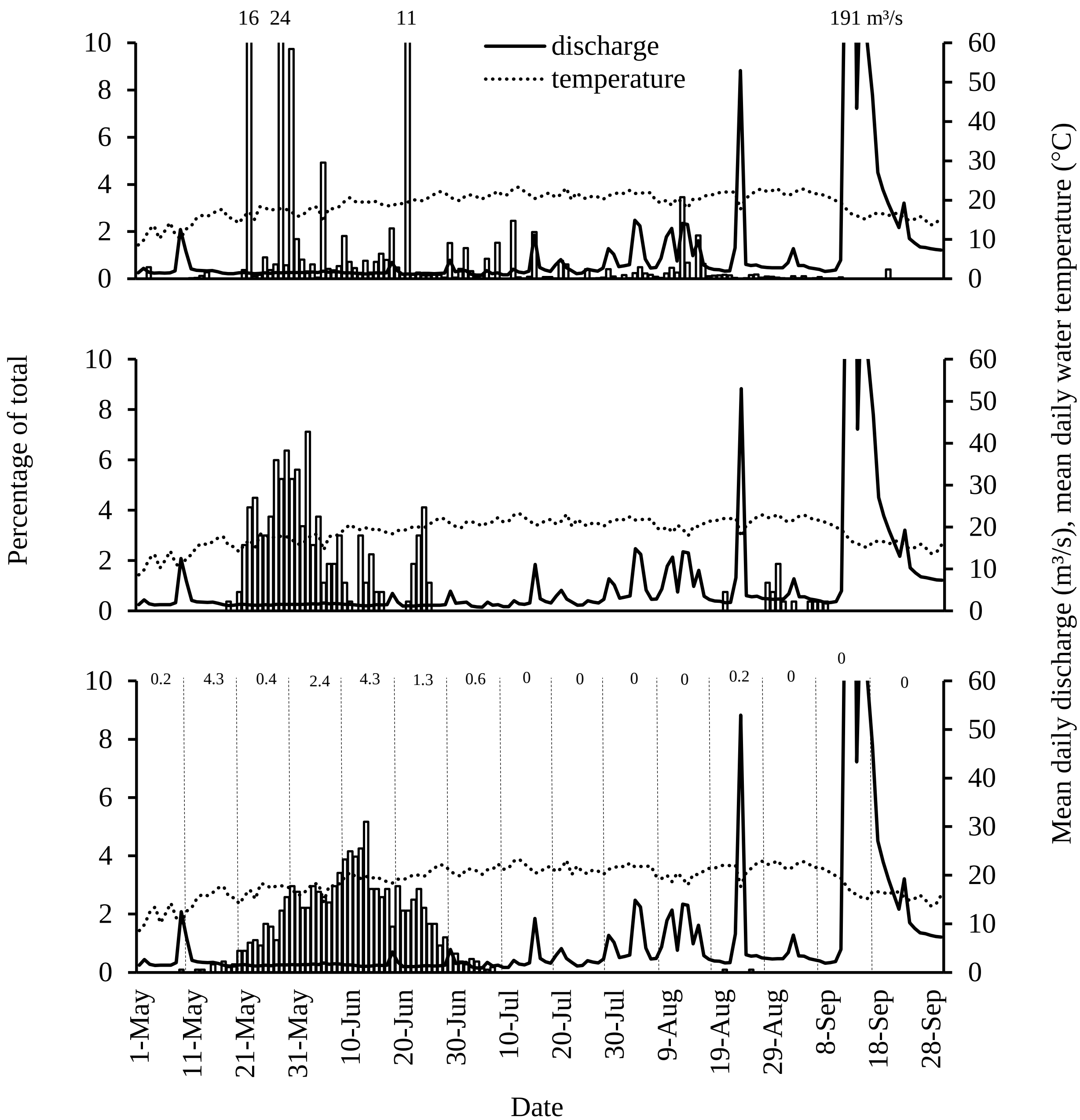

Figure 9. Percentage of drift net captured eggs (top panel), distribution of hatching dates for drift net-captured flathead chub larvae (middle panel), and distribution of hatching dates for seine-captured age-0 juvenile flathead chub (bottom panel) as a function of mean daily discharge and water temperature (U.S. Geological Survey gage 07106000) in Fountain Creek near Fountain, Colorado, 1 May – 30 September, 2013. Numbers above bars are shown to increase resolution of smaller egg percentages, and describe peak discharges. Dotted vertical lines on bottom panel depict 10 day intervals with corresponding relative survival values presented above.

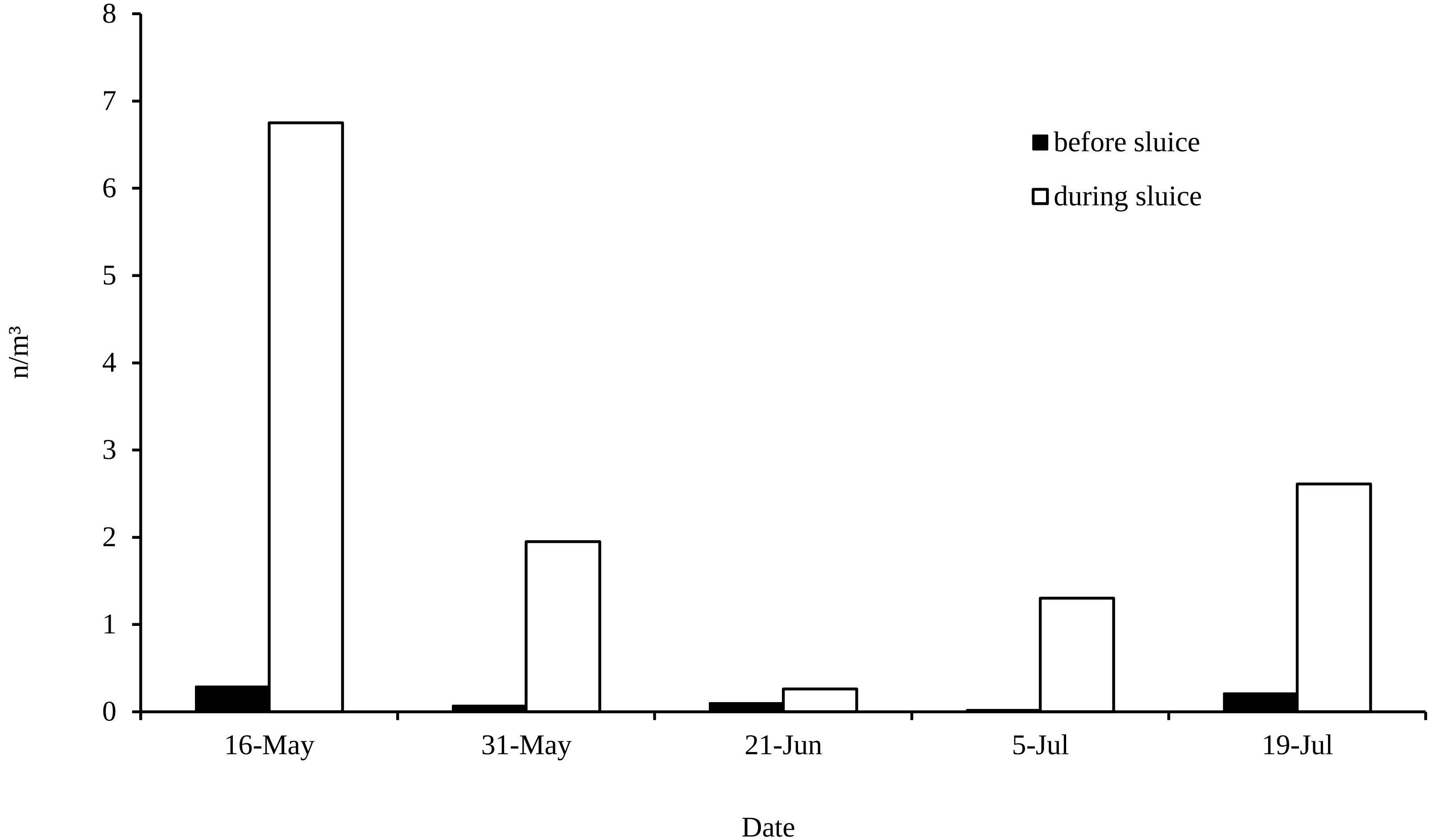

Figure 10. Drift net egg densities (n/m³ water sampled) in samples taken on five occasions immediately before and during sediment sluicing just downstream Owens-Hall diversion dam structure (Figure 1) in Fountain Creek, Colorado, 2012.

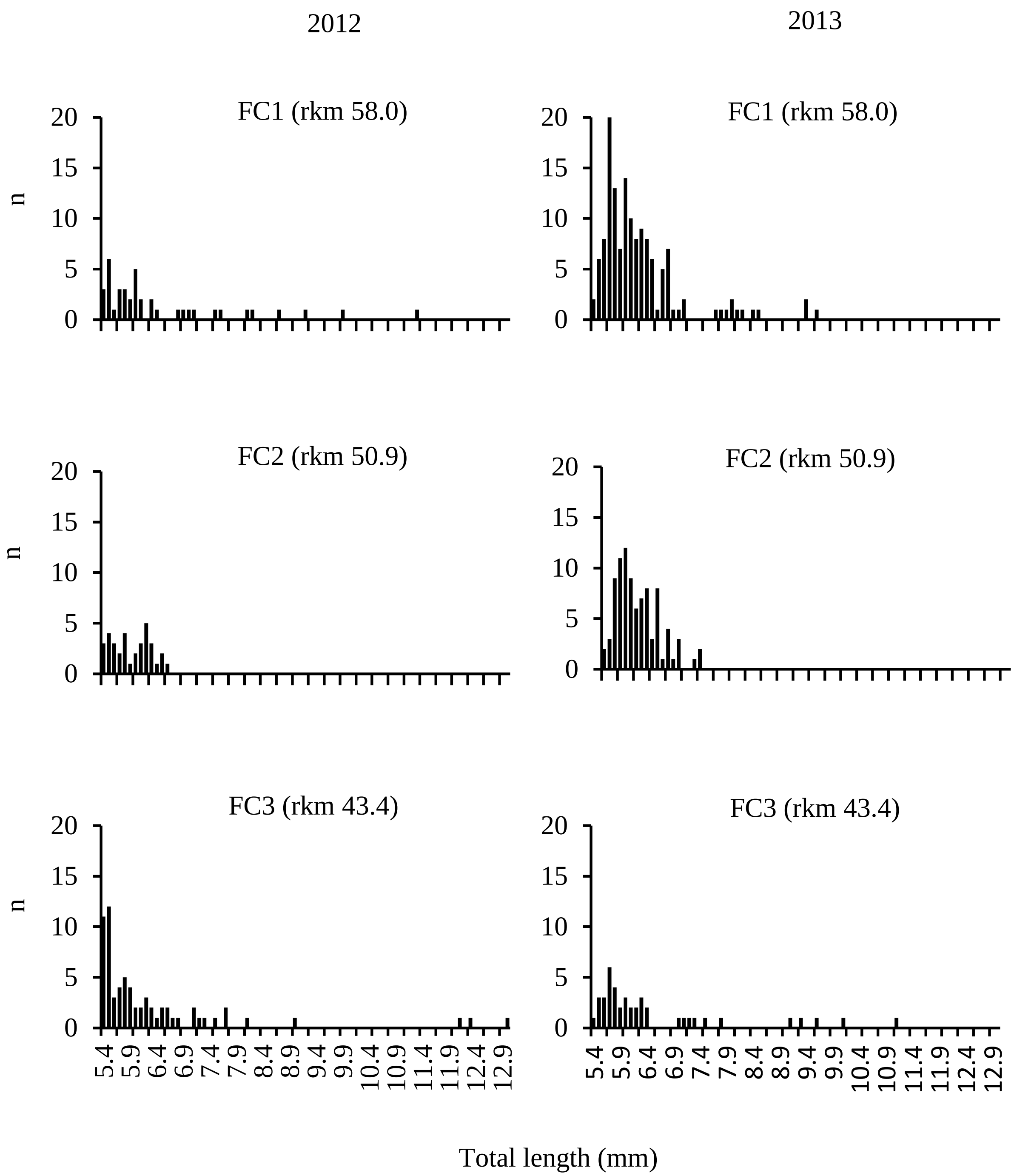

Figure 11. Length frequency of larval flathead chub collected in drift nets at all sampling stations (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

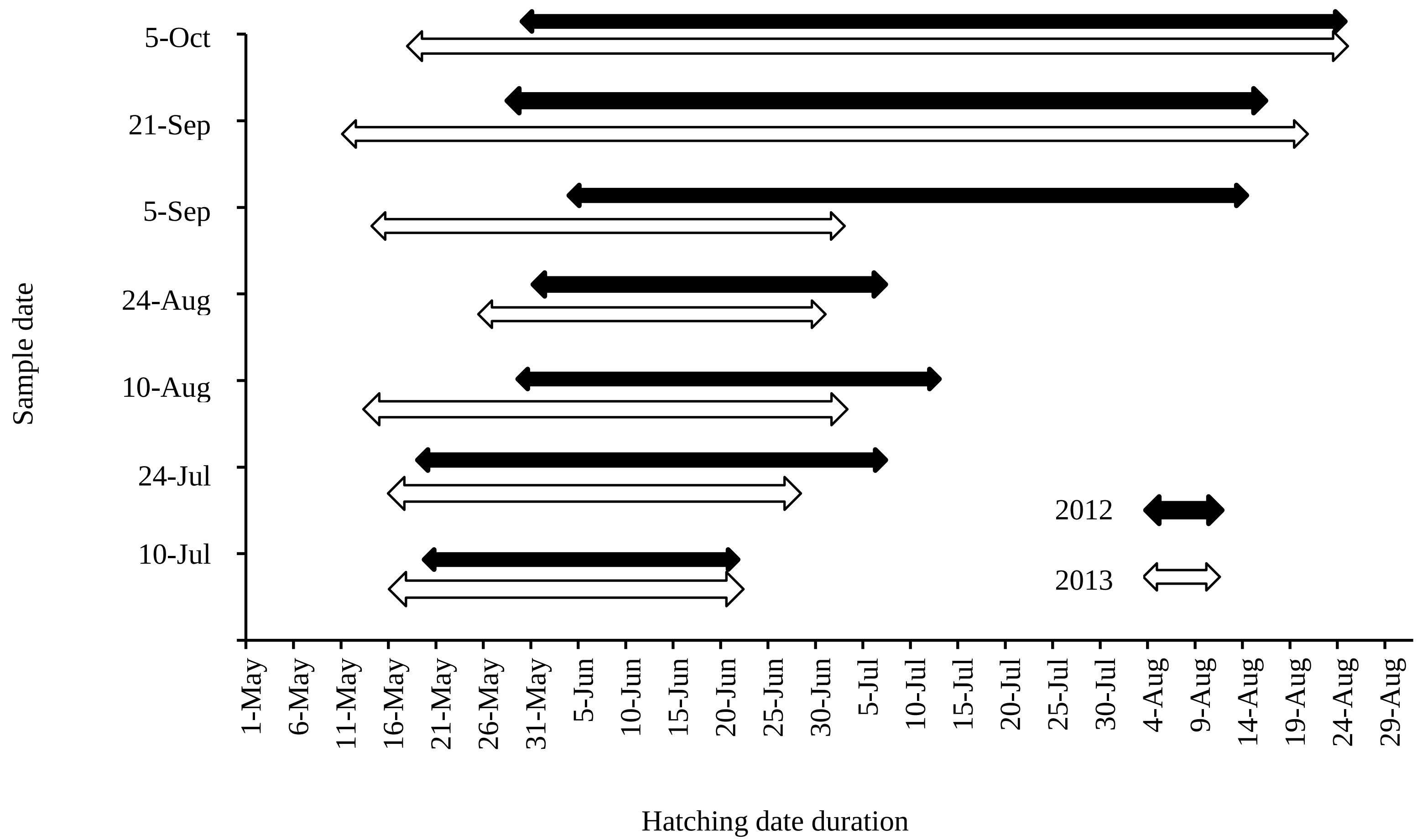

Figure 12. Hatching date range for age-0 flathead chub collected by seining over seven sampling dates in Fountain Creek, Colorado, 2012 and 2013. Hatching date ranges were derived from counts of daily increments in otoliths.

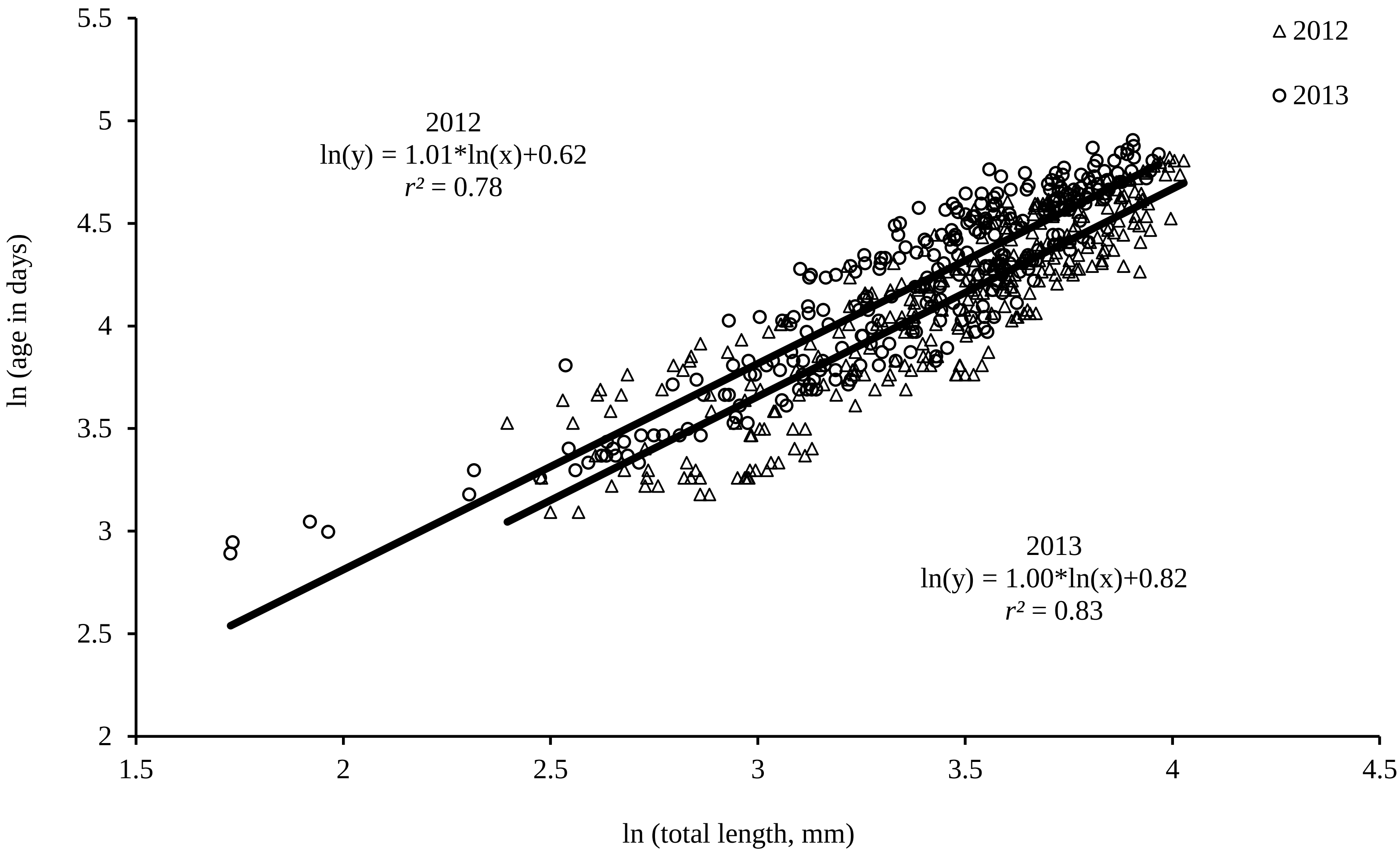

Figure 13. Linear regression of natural ln (age in days) as a function of natural ln (total length, mm) for age-0 flathead chub captured by seining in Fountain Creek, Colorado, 2012 and 2013, where y = ln (age in days) and x = ln (total length in mm).

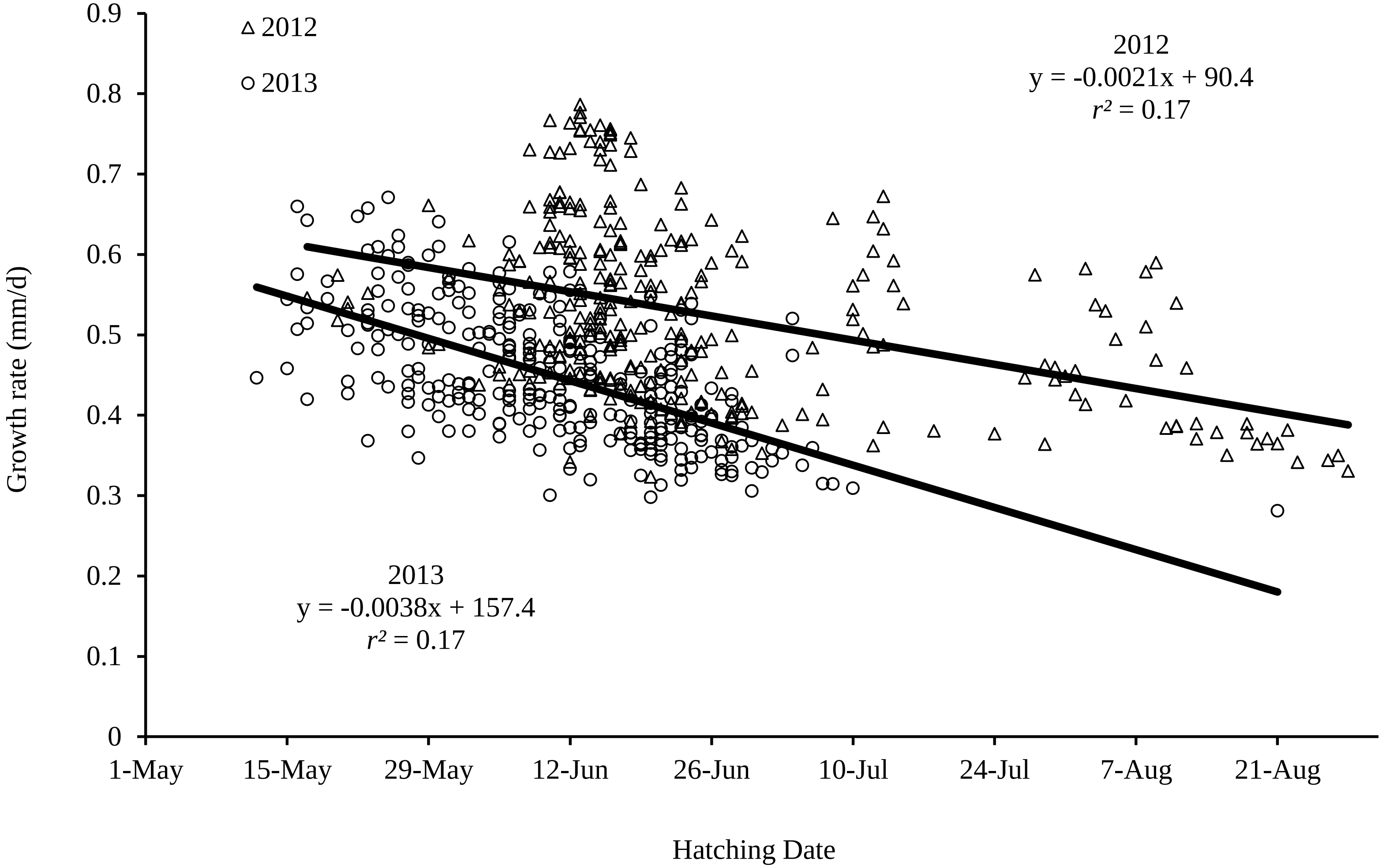

Figure 14. Linear regression of daily growth as a function of hatching date for age-0 flathead chub captured by seining in Fountain Creek, Colorado, 2012 and 2013, where y = growth rate (mm/d) and x = hatching date.

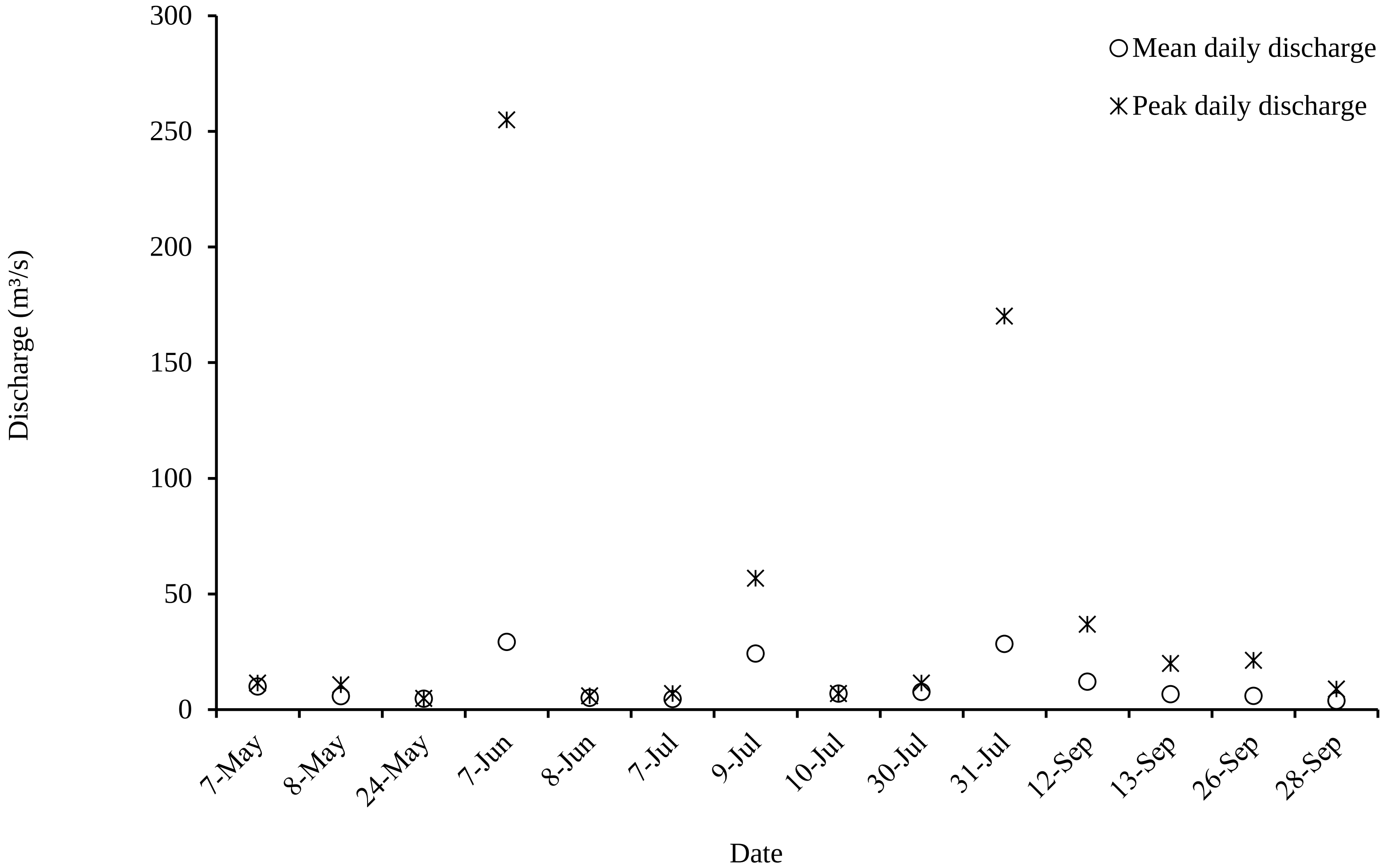

Figure 15. Comparison of daily mean and peak instantaneous discharge for days with mean discharge greater than 4 m^3/s from 1 May – 30 September in Fountain Creek near Fountain, Colorado, 2012 (U.S. Geological Survey gage 07106000).

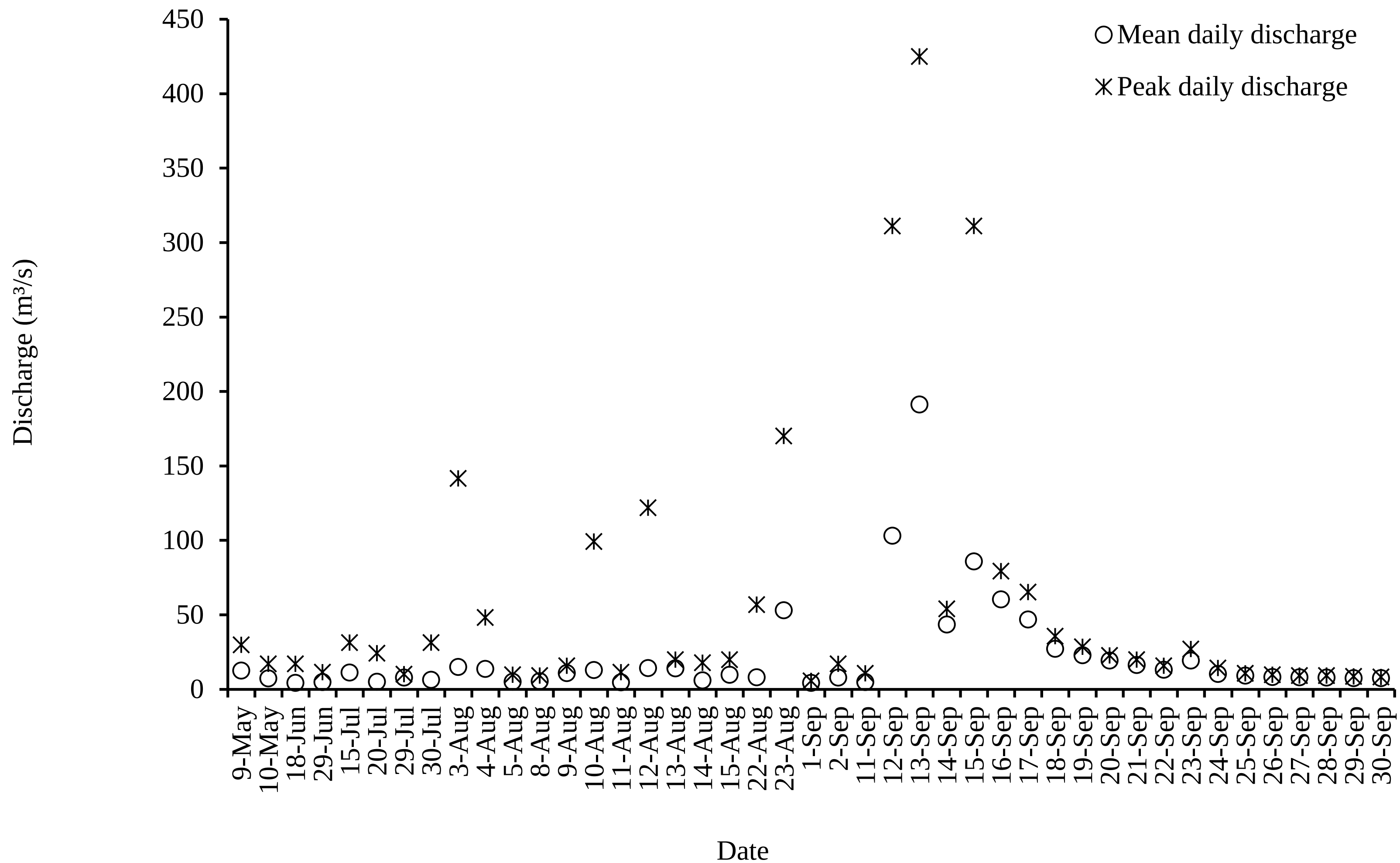

Figure 16. Comparison of daily mean and peak instantaneous discharge for days with mean discharge greater than 4 m^3/s from 1 May – 30 September in Fountain Creek near Fountain, Colorado, 2013 (U.S. Geological Survey gage 07106000).

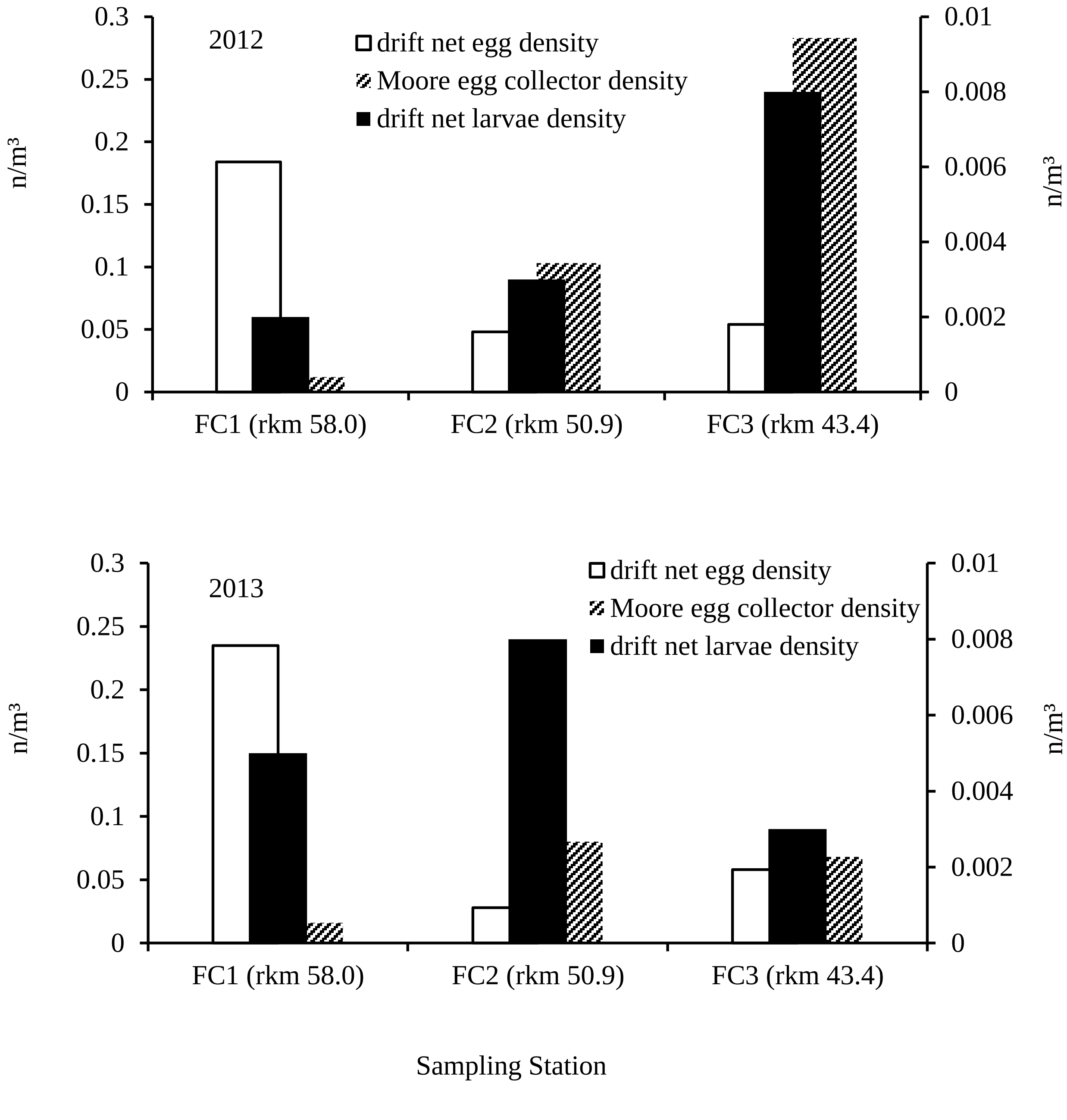

Figure 17. Drift net egg density (left axis), Moore egg collector density (left axis), and larval flathead chub density (right axis) at each station (Figure 1) in Fountain Creek, Colorado, 2012 and 2013.

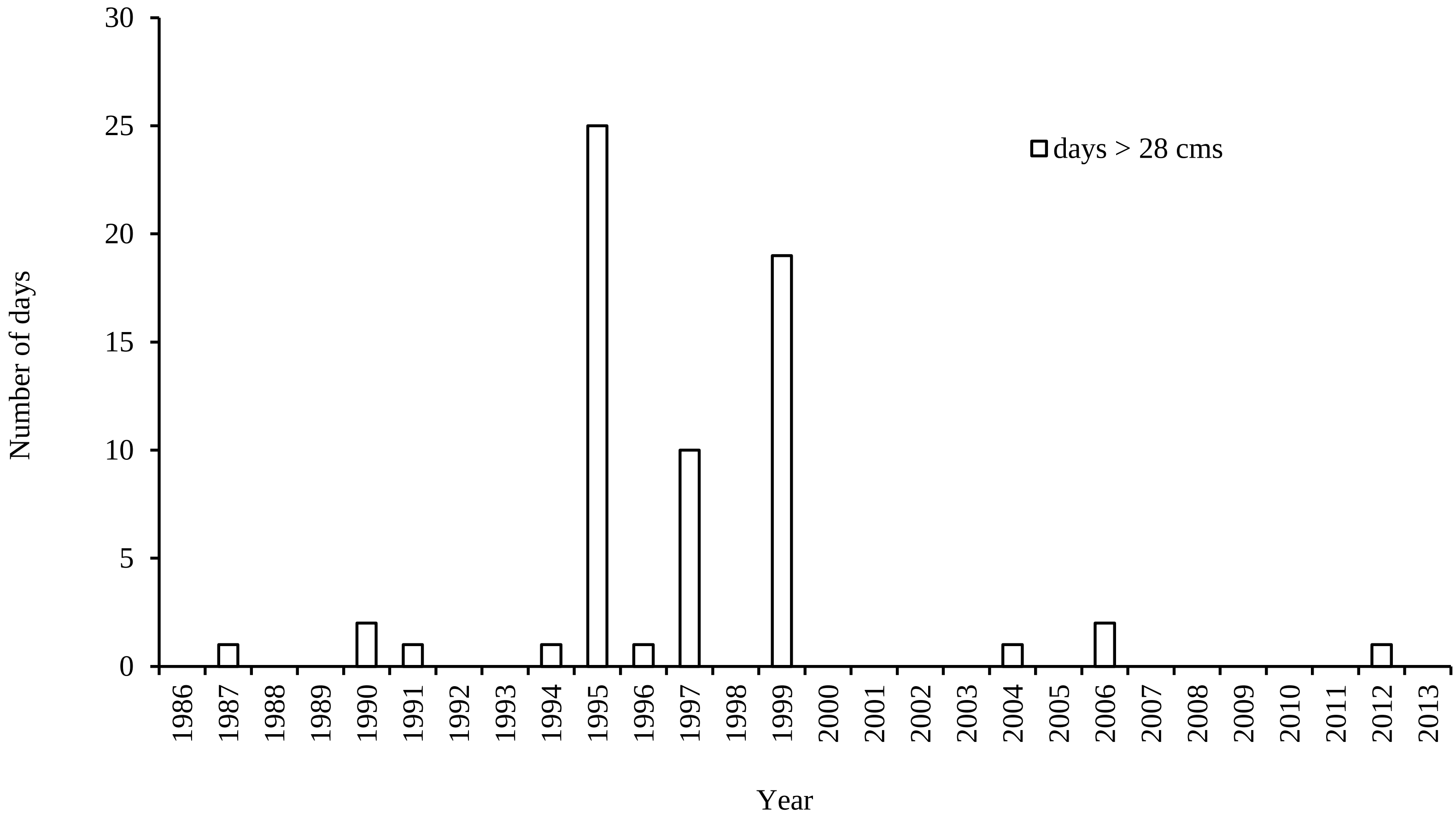

Figure 18. Frequency of mean daily discharges by year that exceeded 28 m³/s from 15 May - 15 July in Fountain Creek near Fountain, Colorado, 1986-2013 (U.S. Geological Survey gage 07106000).

APPENDICES

Appendix I. Number of eggs, larval fish by species (FHC=flathead chub, LND=longnose dace), hatching success, time sampled, and total water volume (m^3) sampled with Moore egg collectors at drift net sampling stations in Fountain Creek, Colorado, 2012.

Sample dates	Eggs	Larvae hatched	FHC	LND	% hatched	Time (hr)	Volume (m^3)
5/14-5/18	27	0	-	-	0	4.9	730
5/21-5/24	431	0	-	-	0	7.5	980
5/29-6/1	343	0	-	-	0	8.1	1,642
6/4-6/8	114	28	27	1	24.6	6.8	1,198
6/11-6/15	155	33	33	-	21.3	7.4	2,798
6/18-6/22	85	14	14	-	16.5	7.6	1,887
6/25-7/1	69	14	14	-	20.3	7.5	1,567
7/2-7/6	24	0	-	-	0	5.6	1,018
7/10-7/13	9	0	-	-	0	4.6	945
7/16-7/20	1	0	-	-	0	5.4	1,186
7/24-7/28	5	0	-	-	0	4.2	996
7/30-8/2	1	0	-	-	0	4.0	859
8/6-8/10	0	0	-	-	-	5.2	1,584
8/13-8/16	14	9	9	-	64.3	4.1	910
9/7	0	0	-	-	-	1.2	100
Total	1,278	98	97	1	-	84.0	18,400

Appendix II. Number of eggs, larval fish by species (FHC=flathead chub, LND=longnose dace, CRC=creek chub, LGS=longnose sucker, UNK=unidentified species), hatching success, time sampled, and total water volume (m^3) sampled with Moore egg collectors at drift net sampling stations in Fountain Creek, Colorado, 2013.

Sample dates	Eggs	Larvae hatched	FHC	LND	CRC	LGS	UNK	% hatched	Time (hr)	Volume (m^3)
3/20	0	0	-	-	-	-	-	-	0.2	82
4/8	8	0	-	-	-	-	-	0	0.5	165
4/24	93	10	-	-	9	1	-	10.8	0.2	96
5/3	9	0	-	-	-	-	-	0	0.5	158
5/11-5/14	1	0	-	-	-	-	-	0	1.8	620
5/21-5/26	139	36	1	-	-	-	35	25.9	6.4	2,370
5/27-6/2	185	98	98	-	-	-	-	53.0	6.3	1,953
6/3-6/9	209	67	47	5	-	-	15	32.1	5.8	1,766
6/10-6/16	42	9	9	-	-	-	-	21.4	6.2	1,645
6/17-6/23	72	13	11	2	-	-	-	18.1	4.6	1,427
6/24-6/30	19	9	9	-	-	-	-	47.4	4.4	1,379
7/1-7/7	54	11	10	1	-	-	-	20.4	4.3	1,038
7/8-7/14	11	4	4	-	-	-	-	36.4	2.4	564
7/15-7/21	2	0	-	-	-	-	-	0	3.3	1,103
7/22-7/28	2	1	1	-	-	-	-	50.0	3.9	1,041
7/29-8/4	12	5	5	-	-	-	-	41.7	3.1	1,009
8/5-8/11	13	2	1	1	-	-	-	15.4	3.6	1,041
8/12-8/18	32	0	-	-	-	-	-	0	3.0	794
8/19-8/25	21	11	10	-	-	-	1	52.4	4.3	1,003
8/26-9/1	7	0	-	-	-	-	-	0	4.7	1,501
9/2-9/8	0	0	-	-	-	-	-	-	2.6	860
9/9-9/15	0	0	-	-	-	-	-	-	0.9	245
Total	931	276	206	9	9	1	51	-	72.9	21,857

Appendix III. Sample date, station (Rkm=river kilometer) number of flathead chub collected, time sampled, and seining catch per hour of sampling by station (Figure 1) in Fountain Creek, Colorado, 2012.

Rkm	Sample	Sample date	N	Time (hr)	N/hr
50.9	1	7/12	65	1.2	55.7
	2	7/25	52	1.2	43.3
	3	8/8	54	0.8	67.5
	4	8/24	88	0.9	97.8
	5	9/7	51	0.8	63.8
	6	9/21	37	0.7	52.9
	7	10/5	144	0.8	172.8
43.4	1	7/12	45	1.2	38.6
	2	7/26	54	1.3	43.2
	3	8/7	30	1.4	21.2
	4	8/24	138	1.0	138.0
	5	9/7	66	0.9	72.0
	6	9/21	84	0.8	100.8
	7	10/5	130	0.7	195.0
24.6	1	-	-	-	-
	2	7/24	96	0.8	115.2
	3	8/10	66	1.1	60.9
	4	8/24	63	0.8	84.0
	5	-	-	-	-
	6	9/21	46	0.8	61.3
	7	10/5	94	0.8	125.3

Appendix IV. Sample date, station (Rkm=river kilometer) number of flathead chub collected, time sampled, and seining catch per hour of sampling by station (Figure 1) in Fountain Creek, Colorado, 2013.

Rkm	Sample	Sample date	N	Time (hr)	N/hr
50.9	1	7/9	43	1.5	28.7
	2	7/23	36	1.6	22.7
	3	8/6	58	0.6	105.5
	4	8/20	61	0.6	104.6
	5	9/4	43	0.6	78.2
	6	9/20	90	0.7	135.0
	7	10/4	39	0.7	58.5
43.4	1	7/10	60	1.5	40.0
	2	7/22	80	1.3	60.0
	3	8/5	53	0.5	106.0
	4	8/19	63	0.6	108.0
	5	9/5	51	0.6	87.4
	6	9/20	51	0.8	63.8
	7	10/4	53	0.6	90.9
24.6	1	-	-	-	-
	2	7/22	102	0.7	153.0
	3	8/5	36	1.0	36.0
	4	8/22	51	0.6	87.4
	5	9/4	51	0.5	95.6
	6	9/20	35	0.9	38.2
	7	10/4	27	1.0	27.0