Notes

Production of Nonadhesive Eggs by Flathead Chub and Implications for Downstream Transport and Conservation

Kevin R. Bestgen,* Harry J. Crockett, Matthew R. Haworth, Ryan M. Fitzpatrick

K.R. Bestgen, M.R. Haworth, R.M. Fitzpatrick
Department of Fish, Wildlife, and Conservation Biology, Larval Fish Laboratory, Colorado State University, Campus Delivery 1474, Fort Collins, Colorado 80523

H.J. Crockett, R.M. Fitzpatrick
Colorado Parks and Wildlife, Fort Collins Service Center, 317 West Prospect Road, Fort Collins, Colorado 80526

Abstract

Plains stream fishes in North America, including flathead chub Platygobio gracilis, are negatively affected by streamflow alterations and fragmentation, and limited information on egg type and reproductive strategy hinders their conservation. On the basis of several lines of evidence, including laboratory culture, observations of reproduction in captivity, and capture and rearing of eggs from Fountain Creek, Colorado, we report that flathead chub produce nonadhesive eggs. Flathead chub eggs are relatively small at 2.3 mm mean diameter, have a greater yolk-to-egg volume ratio and thus sink faster, and take longer to hatch, compared with nonadhesive eggs from pelagic spawning species. Flathead chub are also longer lived compared with pelagic spawning species and the wider variety of habitat types they occupy may influence upstream egg retention. Although spawning mode (e.g., pelagic, lithopelagic, other) is incompletely known for flathead chub, habitat needs in terms of flows and reach lengths suitable for reproduction and recruitment may vary with habitat type but may be similar to that for other pelagic spawning species. Accommodating specialized reproductive life histories of fishes, including egg type and transport characteristics, in stream conservation planning may assist with maintaining or enhancing populations of all Great Plains cyprinids, including increasingly rare flathead chub.

Keywords: Great Plains streams, semibuoyant eggs, reproduction, stream fragments, stream flow, diversions

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* Corresponding author: kbestgen@colostate.edu

Introduction

Understanding the reproductive ecology of fishes, particularly egg type and reproductive mode, assists with defining flows and river reach lengths needed for conservation of some imperiled plains stream cyprinids (Dudley and Platania 2007; Perkin and Gido 2011; Worthington 2014a; Perkin et al. 2015). Egg type and reproductive strategy for flathead chub Platygobio gracilis, an increasingly rare and mainly plains stream species, is not known (Perkin and Gido 2011), despite its overlapping distribution with many other better-understood cyprinids, including pelagic spawning species (Platania and Altenbach 1998; Durham and Wilde 2008). Flathead chub have a remarkably wide latitudinal distribution, ranging south to the lower Mississippi River main stem and the southern Great Plains, and north to the Saskatchewan and Mackenzie River systems of the Yukon Territory and Northwest Territories, Canada (Olund and Cross 1961; Kucas 1980; Rahel and Thel...
2004). Some populations, including many recognized as *P. g. gulo* (Olund and Cross 1961; but see Bailey and Allum 1962 for differing opinion), are now rare in many flow-altered streams dissected by numerous diversion dams and storage reservoirs (Bonner and Wilde 2000; Welker and Scarnecchia 2004; Hoagstrom et al. 2006, 2011; Perkin and Gido 2011; Perkin et al. 2015). For example, flathead chub in Kansas was historically present in most major river basins but now occurs only sporadically in the westernmost portion of the Arkansas River (Cross et al. 1985; Cross and Collins 1995; Haslouer et al. 2005; Eberle 2014; M. Eberle, Fort Hays University, Kansas, personal communication). Additionally, Perkin et al. (2015) found flathead chub so reduced in fish samples from central and southern Great Plains streams (four of 448 samples from 1993 to 2013) that they excluded it from their assessment of effects of stream dissection and flow reductions on plains fish distribution and abundance. In Colorado, flathead chub is native to the southeastern portion of the state in the Arkansas River basin, and in the Rio Grande where it was first documented in 2002 (Bestgen et al. 2003a), but is first reduced in distribution and abundance and listed as needing conservation (Nesler et al. 1999).

Small-bodied cyprinids in streams of the arid Great Plains use a host of reproductive strategies (Fausch and Bestgen 1997), but a subset has a specialized pelagic spawning strategy (Worthington et al. 2014b; Perkin et al. 2015). Pelagic spawning in fishes, which is common in marine systems but relatively rare in streams, occurs when nonadhesive ova are released into the water column, fertilized (ova then become eggs), and transported in prevailing currents (Balon 1975; Platania and Altenbach 1998). Most pelagic spawning stream fishes, which are perhaps more appropriately termed potamopelagic spawners, to acknowledge their lotic (not lentic) environment, occur in the Great Plains and reproduce during stable or increasing flows in spring or summer (Moore 1944; Bottrell et al. 1964; Taylor and Miller 1990; Platania and Altenbach 1998; Hoagstrom and Turner 2015). Ova released in the water column typically absorb large volumes of water after fertilization, expanding two to three times their original size. Because the eggs are nearly buoyant (specific gravity only slightly greater than water; Dudley and Platania 1999) and nonadhesive, they remain suspended in the water column with only minimal vertical turbulence and are easily transported downstream by prevailing stream flows. Although flathead chub have been speculated as producing nonadhesive, semibuoyant eggs, like other known pelagic-spawning species (e.g., Bonner and Wilde 2000, and Durham and Wilde 2005, citing Platania and Altenbach 1998, as the source), egg type and spawning strategy for flathead chub have not been verified. Platania and Altenbach (1998) did not study flathead chub, so claims of nonadhesive, semibuoyant eggs for the species on the basis of their work are unsubstantiated.

Understanding the reproductive life history of stream fishes is essential to strategies to increase abundance and survival of critical early life stages. This may be especially important if fishes are short lived, because conditions suitable for reproduction and recruitment must occur often to maintain populations (Fausch and Bestgen 1997; Perkin et al. 2015). Accommodating the egg type and transport characteristics of flathead chub propagules in conservation planning may assist with maintaining or enhancing fish communities in Great Plains streams. On the basis of several lines of evidence, including laboratory culture, observations of reproductive behavior in captivity, and capture and rearing of eggs from Fountain Creek, we describe egg type and egg size characteristics and speculate on reproductive mode for flathead chub.

**Study Area and Methods**

Adult flathead chubs (*n* = 21) used for 2009 and 2010 experiments were from Fountain Creek, Colorado, which flows south through Colorado Springs, Colorado, for about 100 km to its confluence with the Arkansas River near Pueblo, Colorado. The reach of Fountain Creek unimpeded by dams extends for 58 km downstream of the Owens–Hall Diversion near Fountain, Colorado, downstream to the confluence of the Arkansas River. The Arkansas River then flows unimpeded for another 35 km, for a total of 93 km of unimpeded length for both streams, and is short relative to mean occupied segment length for flathead chubs in other portions of its range (Perkin and Gido 2011). Fountain Creek exhibits high seasonal and annual flow fluctuations due to snowmelt runoff and thunderstorm-induced precipitation, but base flows < 2 m³/s are uncommon because of effluent releases downstream of Colorado Springs. The stream channel is typically 20–50-m wide, water depths at base flow are usually < 30 cm, and substrate is mainly shifting sand and small gravel. Additional details describing Fountain Creek are in Edelmann et al. (2002), Hawthorn (2015), and Haworth and Bestgen (2016).

Specimens were collected with fine-mesh seines or electrofishing and were used in swimming performance tests (Ficke et al. 2012) before our experiments. Flathead chubs were held in captivity for up to 18 mo in 340-L circular tanks containing flow-through well water that ranged from 10 to 22°C (Ficke et al. 2012). Photoperiod for all tests was 14-h light:10-h dark. All chubs were about 8 and 22 cm total length (TL), and were fed commercial flake food one or more times per day until satiated.

**2009 experiments**

In spring 2009, increased girth and gonadal development of flathead chub were observed, so we attempted to spawn fish indoors to obtain a known-identity-reared series of early life-stage specimens for description and illustration. Fish were held in a circular tank at 17.5°C for 1 wk and then allowed to acclimate at 20°C in 76–302-L aquaria in mid-June for one more week, after which we attempted to strip fish of gametes. Males were tuberculate on the head, pectoral fins, and dorsal surface of the body and released milt with slight abdominal
pressure, but similarly handled females did not expel ova. Females were then injected once on 25 June 2009 in the intraperitoneal space with a 0.0044 mg/g solution of ground acetone-dried carp pituitary (Argent Chemical) and water (Bestgen and Compton 2007). Male and female flathead chubs were held in separate aquaria overnight at 20°C, after which three females and three males were stripped of gametes to produce embryos for incubation and were returned to circular tanks. Ova were dry stripped into glass petri dishes and combined with milt from two or more males (Strawn and Hubbs 1956). A few milliliters of water was added to each petri dish, and eggs were gently stirred with a feather. At no point did the eggs stick to the feather or petri dish, nor were they adhesive when transferred to incubation baskets. Diameters of several fresh, unfertilized ova, as well as 2-h postfertilization eggs and developing embryos, were measured to the nearest 0.1 mm under a dissecting microscope (×10) fitted with an ocular micrometer.

After water hardening (about 1 h) and presumptive fertilization, eggs were placed into fine-mesh (0.5 mm) baskets suspended in an aquarium at 20–22°C to incubate. Aeration was from an air stone, which provided only minimal water current that was insufficient to suspend eggs in the water column. Observations of egg development were made under the microscope every few hours the first day and daily afterward. Eggs were gently stirred with a feather. At no point did the eggs stick to the feather or petri dish, nor were they adhesive when transferred to incubation baskets. Diameters of several fresh, unfertilized ova, as well as 2-h postfertilization eggs and developing embryos, were measured to the nearest 0.1 mm under a dissecting microscope (×10) fitted with an ocular micrometer.

Two distended female and four ripe male flathead chub that were treated similarly as above, except that their gametes had not been stripped, were placed in a 302-L aquarium to observe behavior of ripe fish (also on 25 June 2009). Equal thirds of the aquarium bottom (46 cm wide × 122 cm long) were covered with cobble (particle diameter about 6–9 cm), gravel (2–3-cm diameter, middle section), or sand, in approximately equivalent-sized squares. At the cobble-covered end, a submersible pump attached to the narrow side of the aquarium issued a water velocity jet at about 30 cm/s, and 10 cm above the substrate. A 30-cm-long air stone placed on the sand on the opposite narrow side of the aquarium produced a vigorous vertical bubble stream and circulating current. A yarn mop 7.5 cm in diameter with 10-cm-long strands was placed on each of the three substrate sections such that strands were suspended in the water column. Overhead illumination of the aquarium was from two 34 watt, 122-cm-long cool white fluorescent bulbs placed over the glass-covered tank, but the room was otherwise dark to allow for easier observations. Observations of flathead chubs were made continuously for 3 h the first day and daily after that for three more days.

2010 experiments

After holding flathead chub in circular tanks since summer 2009, males were again tuberculate and females were distended in late May 2010. We held chubs at 20°C for 1 wk in smaller aquaria and then on 11 June 2010 placed adult fish (four females and eight males) in the large aquarium previously described; fish were not injected with carp pituitary. At that time, females did not produce ova with slight abdominal pressure, whereas males expressed milt. Observations were made every few hours the first day and daily afterward. Eggs were subsequently discovered in gravel and cobble particle interstices on the tank bottom, and these were collected and removed for incubation in separate aquaria at 20°C with a ± 2°C daily fluctuation, which simulates field conditions in Fountain Creek. Larvae produced from those eggs were subsequently preserved at intervals in 100% ethanol (> 95% ethanol by volume, no denaturing agents present) for a known-age series of fish to use for otolith age validation (Haworth 2015; Haworth and Bestgen 2016), or were preserved in 5% formalin for illustrations.

Field sampling

Field sampling was conducted in 2010–2013 in Fountain Creek in late spring and summer during low flows, using 500-μm-mesh drift nets as well as Moore egg collectors constructed with 0.3-mm-mesh fiberglass window screening (Altenbach et al. 2000; Haworth 2015). In 2010–2011, only occasional, experimental drift-net samples were collected, but in 2012 and 2013, Moore egg collection and drift-net sampling were conducted nearly daily from May to August in Fountain Creek for 5 to 60 min, depending on volume of suspended material in drift nets (Haworth 2015). We sampled the fastest portion of the thalweg, which was typically > 30 cm/s velocity. Both net types sampled the entire water column. Drift-net samples were preserved in ethanol and eggs and larvae picked from debris were retained. Diameters of field-captured and preserved eggs were measured as described above, and larvae were measured to the nearest 0.1 mm TL. Live eggs were sorted from Moore egg collector samples, transferred to the laboratory, and hatched and reared. Eggs from all experiments were incubated in standing water in glass trays (no aeration; water surface transfer maintained dissolved oxygen at 5 mg/L or higher at 20°C), or in minimally aerated water in aquaria in which fine mesh nets were suspended. Larvae were fed live brine shrimp nauplii, Artemia, ad libitum once per day. Glass trays were cleaned and water refreshed as needed, up to once per day, until larvae from field-collected eggs were of sufficient size (about 10–20 mm TL) for accurate identification. Larvae then were preserved in 5% formalin. All specimens are housed at the Larval Fish Laboratory, Colorado State University.

Results

2009 experiments

Flathead chub ova produced in vitro from injected females were only slightly adhesive before fertilization, on the basis of observations of ova accidentally spilled on a
Table 1. Species composition of reared eggs collected with Moore egg collectors in Fountain Creek, Colorado, May–August, 2012 and 2013, and subsequently hatched in the laboratory.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>2012 (N = 1,278)</th>
<th>2013 (N = 931)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Flatehead chub</td>
<td>Platygobio gracilis</td>
<td>97</td>
<td>99.0</td>
</tr>
<tr>
<td>Longnose dace</td>
<td>Rhinichthys cataractae</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>Creek chub</td>
<td>Semotilus atromaculatus</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Longnose sucker</td>
<td>Catostomus catostomus</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Unidentified specimens</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>98</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table top and into a glass petri dish, but were never adhesive after fertilization despite deliberate placement onto various rock, glass, plastic, or wood surfaces. Eggs absorbed water after fertilization, but remained essentially the same size throughout development and had a clear to golden perivitelline space and a yellow yolk. Within 2 h postfertilization, a blastodisc had formed. Fresh, unfertilized ova averaged 1.6 mm in diameter (1.5–1.8, standard deviation [SD] = 0.11, n = 5), and were smaller than the 2-h postfertilization eggs that averaged 2.3 mm (1.8–2.8 mm, SD = 0.09, n = 18). The yolk diameter of fertilized eggs averaged 1.4 mm (1.3–1.5, SD = 0.07, n = 10). Eggs remained in suspension when disturbed with a bubbling air stone in incubation baskets, but sank quickly in the absence of water currents. Eggs hatched in 6–7 d at 20–22°C. Some eggs acquired fungus and died but >75% hatched (about 650 eggs total). Fish grew 0.3–0.5 mm/d (measured three to five individuals on each of 12 occasions over a 47-d period; Haworth and Bestgen 2016).

Observations of ripe and injected flathead chubs in the large aquarium suggested spawning behavior, as males immediately swam aggressively around females in the middle of the water column. Males bumped or nudged females in abdominal and vent areas but females showed no apparent reproductive behavior, and gametes were not observed. Presumptive male spawning behavior abated after 90 min and was not observed subsequently. Unobserved spawning occurred, as a single larva was collected 14 d after fish were first placed in the tank, having apparently survived in the substrate.

2010 experiments

Noninjected adult flathead chub placed in the large aquarium with 22°C water temperature did not exhibit reproductive behavior over the first 2 d. On day 3 (morning, 14 June), several adults were found vertical in the water, aggressively probing the cobble substrate with their snouts. Subsequent siphoning in substrate interstices with a hollow glass tube (6.4 mm) and flexible hose produced nonadhesive eggs from among the cobble (n = 110) and gravel (n = 83) particles. No eggs were observed or in the sand and close inspection of several cobble and gravel particles did not reveal attached eggs, nor were any found in spawning mops, or on the sides of the aquarium.

Examination of siphoned eggs revealed at least two distinct stages, one relatively abundant and newly fertilized stage with blastodiscs, and fewer eggs that were more developed, with a trunk visible over the yolk. On the basis of 2009 observations of developing eggs, the early-stage 2010 eggs were 2–4 h postfertilization, whereas the others were likely 2–3 d postfertilization. Siphoned eggs were placed in a 7.6-L aquarium with a bubbling air stone to provide modest current. Eggs were easily suspended in the water column but sank in 5–10 s in 15 cm of water, after the air stone was removed. Eggs were never adhesive during development and all hatched within 6–7 d at 20–22°C, although the more-developed eggs hatched 2–3 d sooner.

Field sampling

Sampling in Fountain Creek with drift nets (2010–2013) or Moore egg collectors (2012–2013) showed that eggs were abundant in the drift (reported in part in Haworth 2015), with several to >200 captured in ≤10 min of sampling during maximum-density egg drift times. Diameter of preserved eggs captured in drift nets averaged 2.0 mm (1.8–2.2 mm, from four 30-egg samples collected in both June and August, 2012 and 2013, mean sample SD = 0.086 mm). Live eggs captured in Moore egg collectors and transferred to the laboratory hatched in 2–7 d, depending on their state of development at capture. Specimens reared from field-collected live eggs were predominantly flathead chubs, 99% in 2012, and 74% in 2013 (Table 1). The few eggs of other taxa collected, including typically demersal and adhesive eggs from longnose dace Rhinichthys cataractae (McPhail and Lindsey 1970; Balon 1975; Cooper 1980), were assumed displaced from upstream. The relatively large number of unidentified specimens (n = 51) reared in 2013 was difficult to identify with certainty because development was incomplete and they died before reaching adequate size. Examination of approximately 200 substrate particles (about 5–15-cm diameter) in Fountain Creek, immediately after Moore egg collector samples revealed many eggs in the water column in the vicinity of capture locations of ripe adults, did not reveal presence of eggs attached to substrate.

Discussion

On the basis of multiple lines of evidence, we found flathead chub produce nonadhesive eggs that sink in standing water but remain suspended in the water.
column with a slight bubble current; exacting buoyancy characteristics of eggs were not measured and may vary within populations (Bergey et al. 2003). Nonadhesive eggs were produced in the laboratory from fish that were injected with hormones as well as those that were not, and eggs were also captured in samples from Fountain Creek. Whether flathead chub have a potamoipelagic (pelagic per Platania and Altenbach 1998), lithopelagic (deposing eggs on or over larger rock or gravel and sand substrate [Balon 1975]), or some other spawning mode is not known. However, spawning mode is inconsequential when considering downstream transport of nonadhesive eggs in flowing water, because such propagules are swept downstream either continuously or in an interrupted, saltataory pattern (Gilbert 1914), or are deposited in low-velocity locations in the channel (e.g., margins) or substrate interstices until hatching occurs. Downstream transport distance of most eggs and weak-swimming larvae may be considerable, especially during high flows, even though hatching times for several pelagic-spawning taxa with nonadhesive eggs are as short as 1–2 d (Moore 1944; Bottrell et al. 1964; Dudley and Platania 2007; Durham and Wilde 2008). Transport distances are potentially long for flathead chub early-life stages, given the buoyancy of eggs when in current, the longer 6–7-d incubation period, and the small size of larvae at hatching (5.5 mm TL; Haworth 2015). If downstream transport of nonadhesive eggs and larvae is substantial, subsequent dispersal upstream by adults may be needed to replenish populations if suitable numbers of propagules are not retained in spawning reaches (Dudley and Platania 2007; Hoagstrom et al. 2008; Bestgen et al. 2010). Below we discuss aspects of the reproductive ecology of flathead chub as it relates to differences from pelagic spawning cyprinids. We further discuss Great Plains stream habitat for persistence of fishes that produce nonadhesive eggs, including pelagic spawning cyprinid taxa and nuances of Fountain Creek habitat that make it suitable for flathead chub.

**Laboratory and field observations**

Eggs produced in the laboratory aquarium by spawning flathead chub likely settled in substrate immediately after fertilization or were carried for some time in the water column by submersible pump currents before settling in gravel and cobble. We found no eggs over sand substrate, nor were any attached to other surfaces, suggesting that eggs may have been swept off the sand by water currents, or that they were quickly consumed by adult fish, given observations of flathead chubs aggressively seeking and apparently consuming eggs in interstices of larger substrate. Egg eating is not an uncommon behavior in aquarium-spawned cyprinids (Platania and Altenbach 1998; Bestgen et al. 2003b; Bestgen and Compton 2007) and may also occur in the wild.

Flathead chub eggs were dissimilar to those produced by pelagic broadcast spawning cyprinids in at least three ways (Platania and Altenbach 1998). The first difference involved egg size, as flathead chub ova expanded only slightly after fertilization and water hardening, averaging 2.3 mm in diameter (1.8–2.8) and had a small perivitelline space relative to the yolk. Mean diameters of eggs of pelagic spawning species are typically 2.9–3.4-mm diameter (Platania and Altenbach 1998), so flathead chub eggs were, on average, 21–32% smaller. Volumetrically, differences between the yolk and egg diameters of the two egg types were much greater, as volume of an average flathead chub egg was about 2.7 times greater than that of the yolk (yolk and egg diameters = 1.4 and 2.3 mm, respectively, yielding volumes of 6.14 mm$^3$ and 16.61 mm$^3$, respectively; 16.61 mm$^3$/6.14 mm$^3$ = 2.7). In comparison, egg volume of pelagic spawning Rio Grande silvery minnow was about 10.3 times greater than the yolk (yolk and egg diameters = 1.0 and 3.2 mm respectively, yielding volumes of 3.14 mm$^3$ and 32.3 mm$^3$ respectively [R. Dudley, American Southwest Ichthyological Researchers, Albuquerque, New Mexico, unpublished]).

Diameter of laboratory-reared flathead chub eggs corresponded well with samples of preserved eggs from drift nets in 2012 and 2013 (mean = 2.0 mm), which may have been slightly smaller because of shrinkage in ethanol preservative (Snyder 1983). Gould (1985) noted that preserved mature ova in flathead chub collected in the Musselshell River, Montana were 1.0–1.4 mm in diameter, similar to the 1.4-mm mean diameter for fresh, unfertilized flathead chub ova we observed. One other pelagic spawning fish, peppered chub Machybythopsis tetranema, also had relatively small ova (2.5-mm diameter; Bottrell et al. 1964). The egg diameter and yolk volume differences among flathead chub and potamopelagic species such as Rio Grande silvery minnow noted above should result in eggs with differing buoyancy characteristics (sensu Dudley and Platania 1999). This constituted a second and related difference, as our laboratory observations showed that flathead chub eggs were more demersal and sank in 5–10 s in 15 cm of water in the absence of aeration. In contrast, observations showed that plains minnow Hybognathus placitus eggs, which are larger and similar in size and characteristics to other pelagic-spawning species (mean diameter = 3.2 mm, Sliger 1967; Platania and Altenbach 1998), sank more slowly, remaining in suspension for 30 s or more in aquaria when vertical turbulence ceased (observations of plains minnow eggs cultured for toxicity testing of larvae, used in Beyers 1995).

A third difference was that incubation times were consistently longer for flathead chub, at 6–7 d at 20–22°C rather than 1–2 d for eggs from other typical pelagic spawning species, albeit those species’ incubation temperatures were warmer at 22–27°C (Moore 1944; Bottrell et al. 1964; plains minnow egg observations, from those used in Beyers 1995). Although our cooler water temperatures may have increased time to hatching slightly, it is unlikely that those differences entirely explain the slower development of flathead chub eggs that we observed. For example, mean hatching times of
eggs of another cyprinid, Colorado pikeminnow *Ptychocheilus lucius*, differed by less than a day when incubated at comparably different temperatures (4.6 vs. 4.0 d to hatching, respectively, at 22°C and 26°C; Bestgen and Williams 1994).

Egg type differences may also be expected on the basis of other life-history differences that exist between flathead chub and other pelagic spawning species. For example, flathead chub live longer at 5–7 y of age or more, and have maximum body size (300 mm TL) greater than most other pelagic spawning Great Plains fishes, which are typically <100 mm TL and live for 1–2 y (Bishop 1975; Martyn and Schmulbach 1978; Gould 1985; Bestgen et al. 1989; Scarneccia et al. 2000; Hoagstrom et al. 2008, 2014; Hayer et al. 2008). Thus, flathead chub have more reproductive opportunities, and possibly more time to return upstream and a greater swimming capacity to do so, compared with short-lived species with lesser body length and associated reduced swimming ability (Bestgen et al. 2010; Ficke et al. 2012; Walters et al. 2014). Flathead chubs also occur at higher latitudes or altitudes (Kucas 1980), locations that may offer different habitat types. For example, downstream distribution of flathead chub in the Pecos River, New Mexico, which is characterized by higher gradient and mixed sand, gravel, and cobble substrate, only minimally overlaps with upstream distribution of more typical pelagic spawning species that occur in lower gradient and mainly sand-bedded reaches (Sublette et al. 1990; C. Hoagstrom, Weber State University, Ogden, Utah, personal communication).

Conservation implications

There are several conservation implications for flathead chub given verification of the nonadhesive egg type. Nonadhesive eggs like those of flathead chub may remain suspended in the water column and likely represent an adaptation over other egg types that may get exposed to shifting and grinding sand substrates that may bury or destroy eggs. Alternatively, flathead chub eggs deposited over cobble or gravel in natural streams may successfully develop in substrate interstices, given our finding of live eggs beneath cobble and gravel in aquaria with high-velocity water moving over substrate. Thus, flathead chub eggs can likely successfully reproduce in a variety of stream environments. Our observations also indicate that it may not be essential for eggs to remain in the water column for hatching to occur, per Hoagstrom (2014, 2015; but see Wilde and Urbanczyk 2013, 2014, for a different perspective). Indeed, successful hatching of hundreds of wild-captured flathead chub eggs from Fountain Creek occurred in glass dishes, without current velocity or aeration, and supports that contention (Haworth 2015). However, high sediment loads sometimes present in turbid plains streams may suffocate fish eggs deposited in low-velocity areas such as floodplains or large pools. For example, most flathead chub eggs deposited in the low-velocity pool upstream of the Owens–Hall Diversion Dam on Fountain Creek were dead upon capture in drift nets (Haworth 2015).

Streams having substrate interstices to hold eggs likely also allow greater retention of developing eggs and recruitment near spawning locations. Egg retention nearer spawning areas may explain continued presence of abundant flathead chubs in reaches of stream that are short relative to mean stream lengths suggested for their persistence in other plains streams (Perkin and Gido 2011, Bestgen et al. in review). Such reaches include the Purgatoire River, Colorado, Fountain Creek, and upstream locations in the Rio Grande and Pecos, Canadian, and Chama rivers, New Mexico, where larger substrate particles in riffles and runs are common (Bestgen and Platania 1990, 1991; Platania 1991; Bramblett and Fausch 1991; Haworth 2015). The high fecundity of flathead chub, coupled with a long reproductive season (Haworth 2015) and highly fluctuating plains stream habitat conditions (Perkin et al. 2015), suggest the concept of sweepstakes reproductive success may be relevant (Hedgecock and Pudovkin 2011). An outcome of this idea is that most recruitment of older life stages of fishes results from a relatively small proportion of larvae produced in a season, which may also derive from few adults. A better understanding of survival patterns of young flathead chub would assist with determining whether sweepstakes reproductive success is operating, and pinpoint what environmental conditions are required for successful recruitment.

Regardless of potentially reduced downstream transport of eggs in the presence of larger stream substrate, and documented retention of early life stages of flathead chub through the juvenile life stage in upstream reaches, large numbers of flathead chub eggs were transported downstream in the predominantly sand-bedded Fountain Creek (Haworth 2015). Such egg transport indicated that longer and connected stream reaches with reliable flow may be more likely to support flathead chub, given that much of the reproductive output was swept downstream. Without such connectivity and suitable flows, upstream populations, particularly those in relatively short reaches dissected by diversion dams, may be susceptible to extirpation if adults are not able to swim back upstream to replenish populations (Bestgen et al. 2010; Perkin and Gido 2011; Perkin et al. 2015). An alternative view is that if upstream retention of eggs and larvae is adequate for local recruitment, short-term population consequences of downstream egg transport may be unimportant and simply a byproduct of the spawning strategy of these species (Byers and Pringle 2006; Hoagstrom 2014, 2015). Our understanding of recruitment processes for flathead chub, and whether juveniles derive from local or distant locations, is just beginning to emerge (Haworth 2015). Regardless, connected reaches that have sufficient flow are demonstrably premium locations for conservation of all plains cyprinids including pelagic spawning species (Falke et al. 2010; Wilde and Urbanczyk 2013, 2014;
Hoagstrom also support many other important ecological functions as well (Johnson 2002; Sabo et al. 2012). We have better defined the egg type, reproductive mode, and environmental conditions under which successful reproduction by flathead chub can occur. Substrate type and flow-needs information may be useful to conserve or recreate environmental conditions and habitat in streams to support flathead chub. That information, coupled with ongoing studies of reproduction, recruitment, and movement dynamics of flathead chub, may assist managers in providing appropriate flow and habitat so declining plains stream fish assemblages can be stabilized.

**Supplemental Material**

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**Data S1.** Flathead chub *Platygobio gracilis* ova (unfertilized by definition) and egg diameter data (fertilized by definition) from laboratory studies in 2009 and 2010 and field studies in 2012 and 2013. Adult flathead chubs were captured from Fountain Creek, Colorado, for use in laboratory studies. Found at DOI: http://dx.doi.org/10.3996/022016-JFWM-018.S1 (12 KB XLSX).


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