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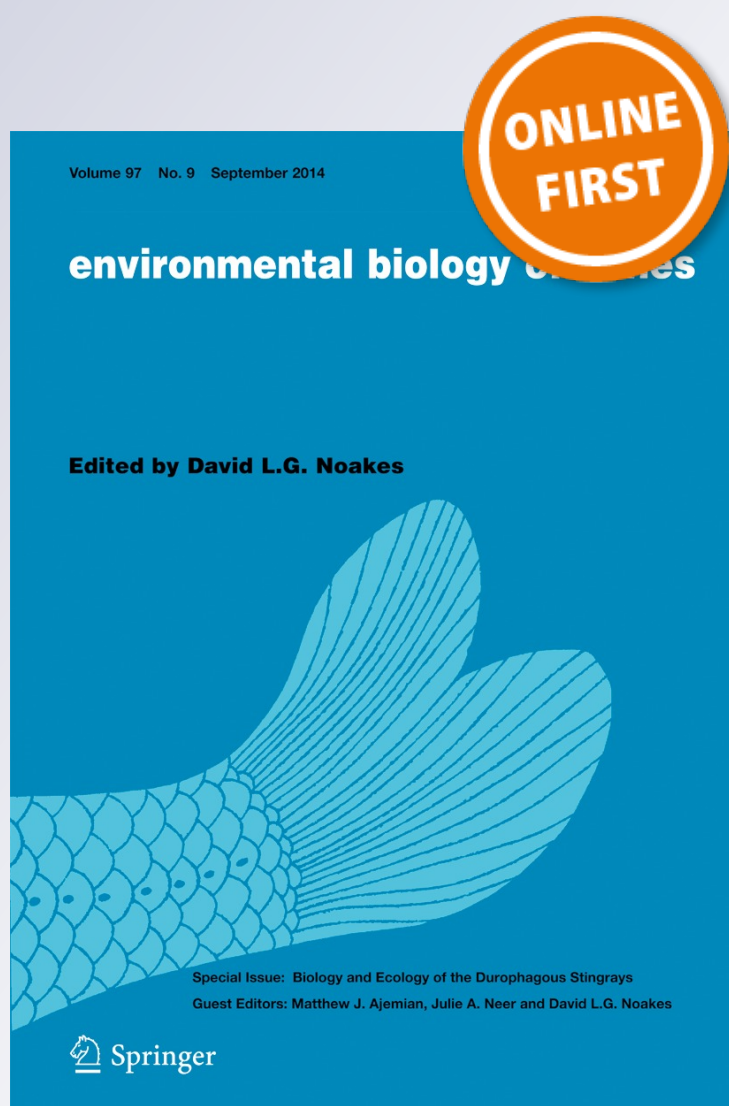
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# First documented spawning and associated habitat conditions for green sturgeon in the Feather River, California

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**Abstract** California's Sacramento River mainstem was previously the only known spawning area for the Southern Distinct Population Segment of North American green sturgeon, *Acipenser medirostris*. Our study provides the first documentation of green sturgeon spawning in the Feather River, a major tributary of the Sacramento River. Egg mats were used to sample two lower Feather River sites from April 12 to July 7, 2011, and we collected 13 green sturgeon eggs at one of those sites. Developmental stages of the eggs ranged from early gastrulation (Stage 15) to post-neurulation (Stage 27), which led us to estimate that four independent spawning events occurred between June 12 and June 19. Spawning occurred after a flow increase while water temperatures were at an optimum (<17.5 °C) for eggs. Results suggest that the area near Thermalito Afterbay Outlet may be important green sturgeon spawning habitat and that the lower Feather River has the potential to provide a second production area of Southern Distinct Population Segment green sturgeon. It should be noted that 2011 was a wet water year and supplemental

sampling is needed to understand if water-year type affects green sturgeon usage of the lower Feather River. Given this new information, future management decisions and water management strategies for the Feather River system should take green sturgeon life-history needs into consideration.

**Keywords** Green sturgeon · Spawning · Developmental stage · Feather River · Artificial substrate

## Introduction

In 2006, the National Marine Fisheries Service listed the Southern Distinct Population Segment (sDPS) of American green sturgeon, *Acipenser medirostris*, as a threatened species under the Endangered Species Act. One of the primary factors for listing the species was because the Sacramento River, in California, contained the only known green sturgeon spawning area for this DPS (NMFS 2006). However, the Feather River, the largest tributary to the Sacramento River, was designated critical habitat for the sDPS of green sturgeon in 2009 (NMFS 2009) and has the potential to be a key area for safeguarding the sDPS because the river contains suitable spawning habitat that may present a second river location capable of supporting green sturgeon reproduction.

Suitable spawning habitat consists of fast flow at optimal temperatures of 14–17 °C with small to medium sized gravel (Dettlaff et al. 1993; Van Eenennaam et al. 2005; Gessner et al. 2007; Poytress et al. 2009, 2010). Van Eenennaam et al. (2005) showed in laboratory

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experiments that temperatures above 20 °C are detrimental to green sturgeon embryos and that they experience 100 % mortality at 23 °C. In addition, both hatching rates and the size of embryos decreased at 11 °C. Green sturgeon spawning surveys in the Sacramento River by Poytress et al. during 2009–2012 indicate that sDPS green sturgeon spawn from early April through early July, in water temperatures ranging from 10.1 to 17.6 °C at depths ranging from 0.6 to 7.6 m. Water turbidity during those four years ranged from 1.7 nephelometric turbidity units (NTU) to 187.0 NTU.

Green sturgeon use of the lower Feather River could be important to the conservation of the sDPS. In general, very little information exists on green sturgeon in the Feather River. Prior to our study, no green sturgeon early life stages had been documented in the Feather River (Beamesderfer et al. 2004). Previously, it was believed that green sturgeon used the Feather River opportunistically (Beamesderfer et al. 2004). However, recent observations and anecdotal information gathered by the Department of Water Resources biologists since 2002 indicate that green sturgeon are found in the river annually and throughout most months of the year (DWR, unpubl. data). During 2006, about a dozen sturgeon were observed from early April through July either breaking the surface or as by-catch to spring-run Chinook Salmon, *Oncorhynchus tshawytscha*, and white sturgeon, *Acipenser transmontanus*, angling.

The Feather River is impounded by Oroville Dam as it leaves California's Sierra Nevada foothills. Lake Oroville, Oroville Dam, and its associated facilities (Oroville Facilities) present an absolute barrier to upstream sturgeon migration and impede access to about 16 km of habitat (Mora et al. 2009). Located downstream of Lake Oroville, the lower Feather River is a large, low-gradient river located within the Central Valley (Fig. 1; Seesholtz et al. 2004). Under normal operations, a portion of the flow from Lake Oroville is diverted into the historic river channel. However, the majority of water released from Lake Oroville is diverted into the Thermalito Forebay and Afterbay for the purposes of producing electricity, delivering water to local agricultural users, and to provide additional storage and operational flexibility. Excluding local water diversions, the water is returned to the Feather River through the Thermalito Afterbay Outlet (TAO; river kilometer (rkm) 95) into the river and then flows southward to the confluence with the Sacramento River at

Verona (rkm 0). The flow and temperature regime resulting from hydropower generation and water delivery managed with the Oroville Facilities may have an important influence on the migratory and spawning behavior of sturgeon in the lower Feather River.

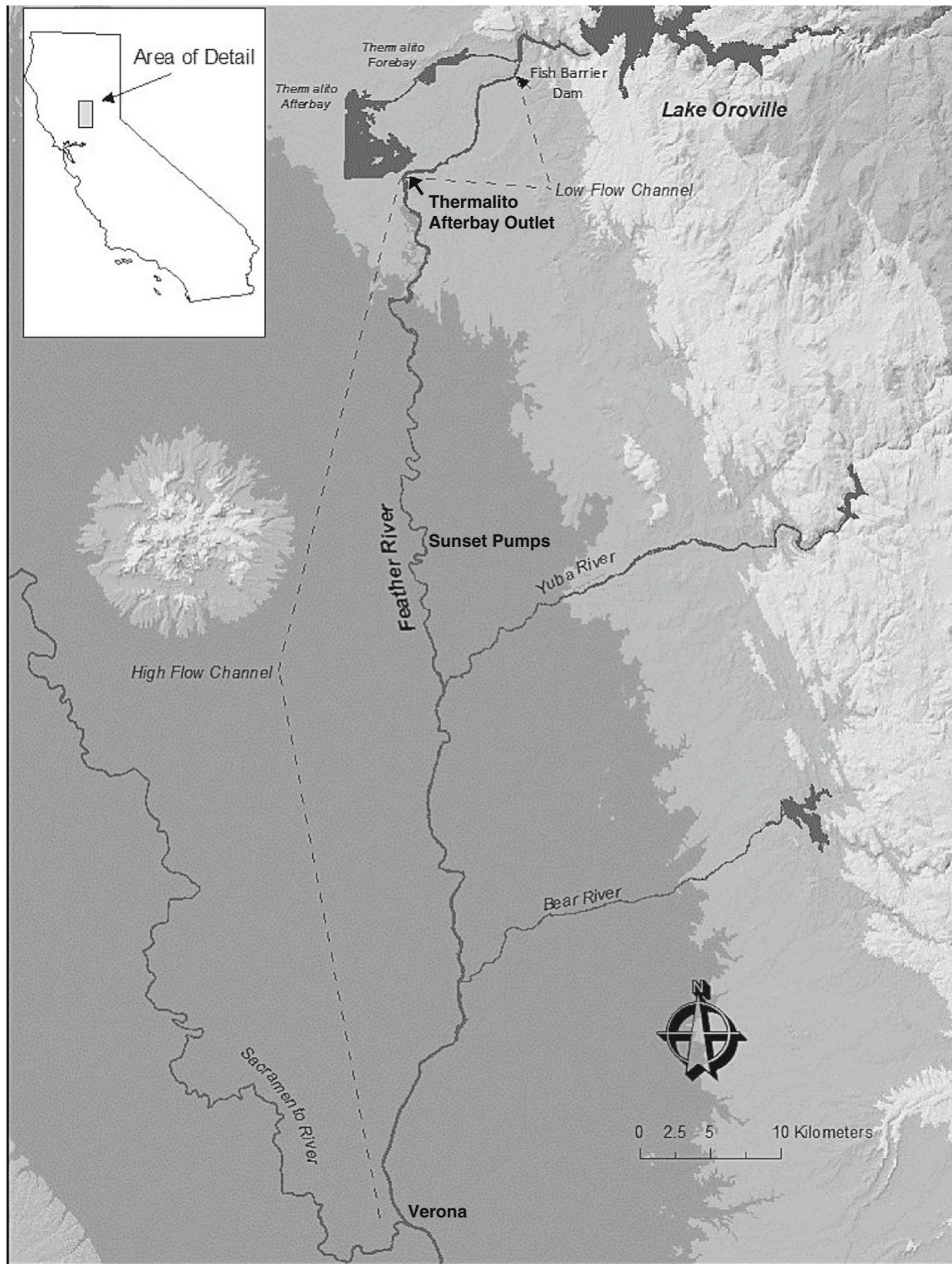
The purpose of our study was to determine if green sturgeon spawn in the lower Feather River. By sampling suspected spawning areas for eggs, we attempted to confirm that green sturgeon were successfully spawning in the Feather River. Other objectives were to collect habitat information to evaluate if the river conditions were suitable for spawning sDPS green sturgeon and to determine the developmental stage of embryos to estimate spawning dates and potential number of spawning events.

## Methods

We identified potential spawning locations as areas where multiple sturgeon were detected with a Dual Identification Frequency Sonar (DIDSON). The DIDSON is an effective and non-invasive sampling technique that enabled us to visually confirm the presence of sturgeon in deep, turbid water (Auer and Baker 2007; Crossman et al. 2011). Observations of angler-caught green sturgeon or in-field observations of breaching sturgeon also defined potential sampling sites.

Artificial substrates (egg mats), similar to those used in other sturgeon spawning studies (McCabe and Beckman 1990; Schaffter 1997; Paragamian et al. 2001; Brown 2007), were used to sample potential spawning sites. An egg mat consisted of a 76×107×5-cm rectangular stainless steel frame with a hog hair furnace filter inset. The egg mats, weighing 23.6 kg fully rigged, were heavy enough to keep them stationary on the bottom of the river in most flow conditions. During high flow conditions (>283 m<sup>3</sup>/s), the egg mats were anchored with a 6.8-kg anchor with three offset 30.5-cm iron grabbing tines to ensure they stayed moored.

Egg mats were deployed at two sites from April 12 through July 7, 2011. Initially 10 egg mats were deployed at TAO, a sturgeon fishing area, on April 12; two more were added April 18. Eight egg mats were deployed just below Sunset Pumps (rkm 62) on April 20 after observing an estimated 12–15 unidentified sturgeon with the DIDSON just downstream of the rock weir at this agricultural diversion on April 19.



**Fig. 1** The green sturgeon spawning survey was conducted in the lower Feather River, delineated as the reach downstream of Lake Oroville to the confluence with the Sacramento River near Verona, California

Egg mats were deployed and retrieved every three to four days. Egg mats were approached from downstream for retrieval and, once on-board, were placed in a custom-made stand that allowed for easy inspection and cleaning. Sturgeon eggs were measured with digital calipers ( $\pm 0.1$  mm) directly on the egg mat and then were placed in a solution of 95 % ethanol.

Water quality parameters were recorded using a Eureka Environmental Manta 2™ Water Quality Multiprobe at the site of each egg mat. The averages of all water quality parameters, collected every 15 s for a 3-min period, were calculated in the field using the Manta software and recorded for each specific mat. Daily flows were obtained from the California Data Exchange Center website (<http://cdec4gov.water.ca.gov/selectQuery.html>) using the Feather River below Thermalito (THA) and Feather River near Gridley (GRL) gauging stations.

Sturgeon eggs were identified to species and stage of embryonic development in the lab. Species identification was determined by egg size and chorion thickness since egg diameter is larger and chorion width is significantly thinner in green sturgeon than in white sturgeon (Van Eenennaam et al. 2008), and the development stage was classified using methods described in Dettlaff et al. (1993). The maximum length and width of the eggs were measured ( $\pm 0.01$  mm) using a dissecting scope with camera lucida and a digital image-analyzing tablet (Nikon Microplan II). Since the rates of embryonic development and hatching time at temperatures of 15–16 °C are similar in green and white sturgeon (Deng et al. 2002), estimated spawn date for each egg was back-calculated using an exponential function, based on water temperature and embryonic development described for white sturgeon (Wang et al. 1985, 1987).

## Results

We surveyed 19 locations with the DIDSON and identified two potential sturgeon spawning sites in 2011—TAO and Sunset Pumps. Multiple sturgeon were observed at both of these locations and each site appeared to have suitable spawning habitat. Supported by an absence of adult sturgeon detections with the DIDSON after mid-May, sampling at Sunset Pumps was terminated on May 24, because the egg mats were consistently embedded with sand and small gravel, making them

difficult to lift from the river bottom. Egg mats were pulled at TAO on July 7 after 15 consecutive days without collecting an egg.

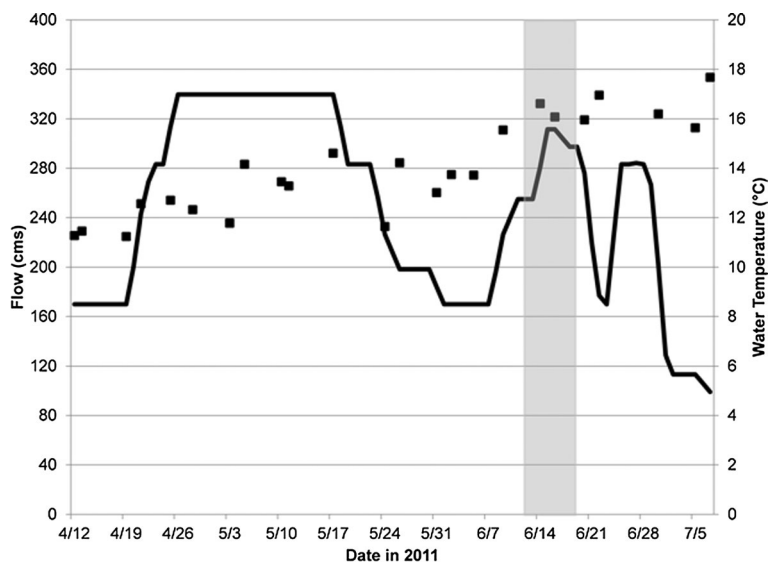
A total of 13 sturgeon eggs were collected during the 2011 sampling season. All eggs were collected in the TAO vicinity between June 14 and June 22. The majority of the sturgeon eggs were sampled on the downstream edge of the TAO outfall; one egg was recovered near a mid-river gravel bar just upstream of the TAO outfall. Eight eggs were attached to the top side of the mats and five were found on the bottom of the mats (side facing the river bottom). The only other eggs sampled (TAO only) in this survey included 64 Sacramento sucker eggs (*Catostomus occidentalis*), which are also deposited in gravel in a similar manner to green sturgeon (Reyes 2011). The artificial substrates, particularly the regions around the corners, were used as habitat by some juvenile and adult species we also collected at TAO that included 1 riffle sculpin (*Cottus gulosus*), 25 prickly sculpin (*Cottus asper*), and 13 crayfish that were not differentiated but both *Pacifastacus leniusculus* and *Procambarus clarkii* are common at this site (DWR unpubl. data).

All 13 eggs were measured in the field and the mean maximum diameter (length) and width were 4.5 and 4.2 mm, respectively. In the laboratory, we were able to measure the egg diameter for 9 of the 13 eggs and the mean maximum length and width were 3.90 and 3.78 mm, respectively. Several eggs were broken or compressed during field handling, and accurate size could not be measured in the laboratory.

All 13 eggs were positively identified as green sturgeon based on egg size, color, and thickness of the egg chorion. The eggs collected were likely spawned from four different spawning events based on date and time of capture, water temperature, stage of development, and estimated spawn date and time (Table 1), and the assumption that it takes a female up to 21 h to release all of her eggs (Van Eenennaam et al. 2012). Eleven of the eggs were viable and two eggs may have been fertilized and then died during embryogenesis, based on the observation that one was marbled in color and the other was covered in fungus.

Measured water temperatures throughout the sampling period at TAO, April 11 to July 7, ranged from 11.2–17.7 °C ( $\bar{x}$  = 14.0 °C; Fig. 2). Water temperatures throughout the sampling period at Sunset Pumps, April 20 to May 24, ranged from 11.8 to 14.3 ( $\bar{x}$  = 13.0 °C).

**Fig. 2** Flow and temperature near the Thermalito Afterbay Outlet sampling site in the lower Feather River from April 12–July 7, 2011. The black line represents flow and the black squares represent discrete water temperature measurements. The gray box indicates the estimated spawning dates of the green sturgeon eggs collected



Eggs were collected from the mats at TAO when water temperatures were 16.0–17.0 °C ( $\bar{x}$  = 16.5 °C; Fig. 2). Other water quality parameters measured during periods when eggs were sampled at TAO included: specific conductance of 71.1 to 80.8 microsiemens per centimeter (uS/cm) ( $\bar{x}$  = 74.6 uS/cm); turbidity of 2.1 to 14.1 NTU ( $\bar{x}$  = 6.5 NTU); and dissolved oxygen levels of 10.1 to 10.6 mg/l ( $\bar{x}$  = 10.3 mg/l) and saturation of 104.7 to 110.5 % ( $\bar{x}$  = 106.9 %).

The egg mats were set in water depths that ranged from 1.1 to 11.0 m ( $\bar{x}$  = 4.1) in depth at TAO and from 2.3 to 9.0 m ( $\bar{x}$  = 3.8 m) at Sunset Pumps; however, eggs were sampled at depths between 1.6 and 5.5 m ( $\bar{x}$  = 3.8 m). Flows at TAO ranged from 99 to 340 m<sup>3</sup>/s ( $\bar{x}$  = 243 m<sup>3</sup>/s; Fig. 2). Flows at Sunset Pumps ranged from 194 to 339 m<sup>3</sup>/s ( $\bar{x}$  = 296 m<sup>3</sup>/s). Eggs were collected from the mats at TAO when flows ranged from 172 to 312 m<sup>3</sup>/s ( $\bar{x}$  = 253 m<sup>3</sup>/s; Fig. 2).

## Discussion

The 13 green sturgeon eggs collected at TAO provide the first substantiated evidence that sDPS green sturgeon spawn in the Feather River, or any river other than the mainstem Sacramento River. Back-calculation, using developmental stage of the eggs and water temperature at time of retrieval, indicated the eggs were spawned between June 12 and June 19. These estimated spawning dates fall in the later part of the spawning

season when compared to those recorded in the Sacramento River (Brown 2007; Poytress et al. 2012).

Green sturgeon spawn in the Klamath and Sacramento rivers from April through June (Van Eenennaam et al. 2005; Poytress et al. 2010, 2011). While our DIDSON surveys detected sturgeon in the Feather River throughout these months, eggs were only sampled during mid-June. No eggs were collected during a flow increase and cooler water temperatures, which occurred in mid-April and lasted for a month. It is possible that we missed earlier spawning events because of the limited number of mats we deployed or because egg mat placement within the spawning area did not adequately sample the patchiness of egg deposition by sturgeon (Caroffino et al. 2010).

We did not detect sturgeon eggs while sampling near Sunset Pumps even though DIDSON surveys indicated this area had higher numbers of sturgeon than TAO and contained habitat characteristics found in other rivers that support green sturgeon spawning. Both TAO and Sunset Pumps are highly affected by anthropogenic alterations to the system. The TAO pool is a deep scour hole that is formed from water diverted through the Thermalito Afterbay and then rerouted back into the river. Sunset Pumps pool is the result of scouring that occurs downstream of a large rock weir that extends across the river to create hydraulic head for irrigation pumps. However, unlike TAO, Sunset Pumps pool had a high sediment load that, as suggested from studies on other sturgeon species (Fox et al. 2000; Kock et al.



**Table 1** Date eggs were collected during the 2011 sampling season in the lower Feather River. Data are egg length and width (in lab), stage of embryonic development, and estimated spawn date. Eggs not viable (NV) and broken (B) could not be staged. Spawning event number (#) was based on spawn date, date egg

mats were removed, and the assumption it takes up to 21 h to complete oviposition. Estimated spawn date and time for each egg was back-calculated using an exponential function based on water temperature and developmental stage

Date collected	Time collected	Egg L(mm)	Egg W(mm)	Stage	Spawning event # <sup>a</sup>	Estimated spawn date	Estimated spawn time
6/14/2011	13:12	4.197	4.147	19	1	6/12/2011	13:00
6/14/2011	13:24	–	–	19	1	6/12/2011	13:00
6/14/2011	13:24	3.977	3.793	19	1	6/12/2011	13:00
6/14/2011	13:41	–	–	19	1	6/12/2011	13:00
6/16/2011	13:35	4.078	3.756	15	2	6/15/2011	11:35
6/16/2011	12:37	3.995	3.942	NV	2	unknown	unknown
6/20/2011	15:14	3.633	3.578	20	3	6/18/2011	11:14
6/20/2011	15:14	3.722	3.654	20	3	6/18/2011	11:14
6/20/2011	15:32	3.682	3.594	23	3	6/18/2011	7:14
6/22/2011	8:34	–	–	B	4	unknown	unknown
6/22/2011	8:34	4.108	3.965	NV	4	unknown	unknown
6/22/2011	8:34	3.687	3.619	27	4	6/19/2011	20:34
6/22/2011	8:34	–	–	B	4	unknown	unknown

<sup>a</sup> Each spawning event had a minimum of one participating female because it is possible that more than one female was spawning in the area at the same time and location

2006; Chiotti et al. 2008), could be suboptimal or detrimental to sturgeon eggs spawned in this area and may be a factor influencing whether or not the area is used for spawning.

The differences in mean egg size in the field compared to the laboratory measurements were due to differences in accuracies because of storage of eggs in ethanol, which resulted in some shrinkage. Green sturgeon eggs fixed in buffered formalin were reported to average 4.33 mm maximum length (Van Eenennaam et al. 2006) compared to white sturgeon eggs, which are approximately 3.6×3.3 mm (Conte et al. 1988).

We wanted to determine if all 13 eggs were from a single spawning event to provide insight into the size and genetic variability of the spawning population. We concluded that the spawning population consisted of at least four distinct spawning events. Considering that green sturgeon are single batch spawners (Van Eenennaam et al. 2012) and that spawning event #3 was likely completed on June 19 around 07:00, the one egg collected on June 22 (Table 1) that could be staged was assessed to be fertilized on June 19 at 20:34 from a potential fourth spawning event. The eggs collected on June 22 were not recovered during the June 20 collection period, as suggested by the one egg with a

June 19 spawn date, because these eggs were on an egg mat that had been unrecoverable from May 10 until June 22. Until genetic-parentage analyses can be completed on the embryo samples, each spawning event is assumed to be from at least one female because it is possible that more than one female was spawning in the area at the same time and location. Currently, a protocol to reliably extract DNA from individual early-staged sturgeon eggs is in development (Zachary Jackson, pers. comm.). Once the extraction process is validated, genetic analyses will be conducted to verify the parentage and the minimum number of spawning adults that contributed to reproduction at TAO (Israel et al. 2011).

The sturgeon congregated at Sunset Pumps did not behave like the sturgeon farther upstream at the TAO; sturgeon were detected at Sunset Pumps for a little over a week rather than months. There are two other possibilities that may explain the large number of adults concentrated at Sunset Pumps pool: 1) it was used for a holding or staging area or 2) the water levels at the rock weir created a passage impediment to upstream movement. However, the Sunset Pumps area does not fit the holding site criteria described by Erickson et al. (2002) which found green sturgeon holding sites generally were

in low-gradient reaches of the main channel or off-channel coves, close to sharp bends in the river, greater than 5-m deep, and had low to no current. DIDSON surveys in this area suggest that the rock weir may have affected migration since the sturgeon were no longer detected in the area after flows increased from 168 m<sup>3</sup>/s to 330 m<sup>3</sup>/s (DWR, unpubl. data).

The first known spawning event at TAO was estimated to occur five days after a second flow increase in which water temperatures rose from about 14 to 16 °C. In laboratory studies, Van Eenennaam et al. (2005) found that the greatest hatching success and low incidence of deformities of green sturgeon embryos occurred at 16 °C. Fertilization of a green sturgeon egg to hatch takes about six to eight days so the latest hatch date of the eggs sampled was likely June 27 (Deng et al. 2002). Water temperatures at TAO stayed within the optimal range for embryo development (Van Eenennaam et al. 2005) until after the first week of July, so any eggs that were spawned at the same time as the ones we collected would have had favorable water temperatures during the most sensitive early embryonic stages for sturgeon (Dettlaff et al. 1993).

The 13 eggs collected provide the first evidence that sDPS green sturgeon do spawn outside of the Sacramento mainstem and substantiates that green sturgeon spawn in the Feather River. However, 2011 was designated a wet water year in the basin and many still believe that green sturgeon will only utilize the system in wet water years. Supplemental sampling is needed to determine if spawning occurs regularly in the lower Feather River and to understand if water-year type affects green sturgeon usage of the lower Feather River. Furthermore, continued monitoring in this system will help us elucidate whether spawning occurs earlier in the year than we detected, if additional spawning sites exist, and which biotic and abiotic factors are important to the species in the Feather River. Additional data are needed to evaluate the existing quality and quantity of habitat in the lower Feather River and to determine if the Feather River is a second reliable spawning location for sDPS green sturgeon. Current research and conservation efforts for sDPS green sturgeon should focus on river management, particularly the protection and improvement of spawning and rearing habitats.

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