Notes

Confirmed Observation: A North American Green Sturgeon *Acipenser medirostris* Recorded in the Stanislaus River, California

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Abstract

Two sturgeon species are native to the San Francisco Estuary watershed in California: White Sturgeon *Acipenser transmontanus* and North American Green Sturgeon *Acipenser medirostris*. The San Francisco Estuary has two main tributaries, the Sacramento and San Joaquin rivers. Recent studies have shown that the San Joaquin River is used by Green and White Sturgeon and that at least a small number of White Sturgeon spawn there when environmental conditions allow. However, records of Green Sturgeon in the San Joaquin River and its tributaries are rare and limited to information from angler report cards. In 2006, the National Marine Fisheries Service listed the southern distinct population segment of North American Green Sturgeon as threatened under the Endangered Species Act. Federally designated critical habitat for the southern distinct population segment of Green Sturgeon does not extend upstream of the San Joaquin River's confluence with the Stanislaus River. We recently confirmed an adult Green Sturgeon holding in a deep pool near Knights Ferry, California in the Stanislaus River. We observed and recorded the fish using a GoPro® video camera and used environmental deoxyribonucleic acid sampling techniques to confirm species identification. This paper provides the first confirmed record of Green Sturgeon in any tributary of the San Joaquin River, which is beyond the designated critical habitat area. Future well-designed research focused on the San Joaquin River and its tributaries is expected to improve our understanding regarding the importance of these rivers for the various life stages of North American Green Sturgeon.

Keywords: Green Sturgeon; Stanislaus River; eDNA; critical habitat; *Acipenser medirostris*

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Introduction

Two sturgeon species are native to the San Francisco Estuary (SFE) watershed: North American Green Sturgeon *Acipenser medirostris* and White Sturgeon *Acipenser transmontanus*. Although the two species appear to be sympatric in the SFE, White Sturgeon are more abundant and support a large sport fishery (Heublein et al. 2017). The Sacramento and San Joaquin rivers are the two main tributaries to the SFE. Recent studies suggest that White Sturgeon are spawning in the San Joaquin River system (Jackson et al. 2016). However, in the absence of historical or recent accounts in the Stanislaus River, Green Sturgeon data are limited to information from angler report cards in the San Joaquin River (DuBois and Danos 2017). To date, annual dual-frequency identification sonar (DIDSON) surveys have been the primary tool for monitoring spawning adult Green Sturgeon in the Sacramento River (Heublein et al. 2017).

In 2006, the National Marine Fisheries Service (NMFS) listed the southern distinct population segment of North American Green Sturgeon (hereafter Green Sturgeon) as threatened under the U.S. Endangered Species Act (ESA 1973 as amended; NMFS 2006). The species also has a northern distinct population segment, which is currently listed as a species of concern under the ESA (NMFS 2006). In contrast, White Sturgeon in California are not listed under the federal ESA or the State of California's Endangered Species Act (Heublein et al. 2017). White Sturgeon (Hildebrand et al. 2016) and Green Sturgeon both have large geographic ranges (Moyle 2002; NMFS 2015; Moser et al. 2016), with both species occupying areas from the Bering Sea (Colway and Stevenson 2007) south to Baja, Mexico (Rosales-Casian and Almeda-Juaregui 2009). In California, the southernmost Green Sturgeon spawning population is currently thought to occur in the Sacramento River (NMFS 2015).

Adult Green Sturgeon typically migrate into California river systems between February and July, with peak Sacramento River system spawning reported to occur between mid-April and mid-June (Brown 2007; Heublein et al. 2009; Poytress et al. 2015). Alternatively, White Sturgeon in SFE spawn between mid-February and early June (Miller 1972; Kohlhorst 1976; Schaffter 1997; Jackson et al. 2016). Although Green Sturgeon typically spawn at least 80 km upstream from White Sturgeon (Schaffter 1997; Poytress et al. 2015), the majority of spawning for both species occurs in the main-stem Sacramento River in deep turbulent channel areas (Heublein et al. 2017). Recent Sacramento River studies have shown that Green Sturgeon prefer depths ranging from 3 to 12 m and velocities from 0.8 to 1.3 m/s (Wyman et al. 2018). Preferred Sacramento River water temperature for Green Surgeon during dry and wet years averaged 13.5°C (Poytress et al. 2015). After spawning, Green Sturgeon hold in the river for varying periods of time and typically leave the system the following fall (Heublein et al. 2009). Although extended occupancy of spawning and holding habitats has been observed and may be related to hydrologic cues or food availability, continuous occupancy of these habitats for over a year remains unexplained (Heublein et al. 2017). Adams et al. (2007) reported that Green Sturgeon may have spawned in the habitat that existed historically in the San Joaquin River system. Critical habitat was designated for southern distinct population segment Green Sturgeon in 2009 (NMFS 2009), and is a term defined and used in the ESA that refers to "geographic areas that contain features essential to the conservation of an endangered or threatened species and that may require special management and protection" (USFWS 2015). However, federally designated critical habitat for this species in California currently does not extend upstream of the San Joaquin River–Stanislaus River confluence (NMFS 2009, 2015).

On a recreational rafting trip we observed what appeared to be an adult sturgeon occupying a deep pool in the Stanislaus River near Knights Ferry, California, approximately 86 river km (rkm) upstream from the federally designated critical habitat for Green Sturgeon. Because of the potential significance of this observation, our objective was to return to the site to more rigorously investigate this observation. Upon returning we conducted a snorkel survey, captured the fish on camera, and used a gridded environmental deoxyribonucleic acid (eDNA) sampling regime to verify the species of the observed fish. Before this confirmed observation, limited Green Sturgeon use of the San Joaquin River upstream of the Stanislaus River confluence was supported only by angler report card records (Dubois and Danos 2017). The timing of this observation suggests that this fish was not actively involved in seasonal reproductive migration or spawning activities. This paper provides the first confirmed record of Green Sturgeon in any tributary of the San Joaquin River, which was also beyond the current geographic range of federally declared critical habitat.

Study Area

The Stanislaus River is a tributary to the San Joaquin River in California's Central Valley (Figure 1). The river drains a watershed of approximately 2,400 km^2 and has north, middle, and south forks that originate in the Sierra Nevada mountain range. The watershed has a Mediterranean climate that is characterized by dry summers, with roughly 90% of the annual precipitation occurring between November and April. Watershed elevations range from 3,675 m at the crest of the Sierra Nevada mountains to 15 m at the confluence with the San Joaquin River (Kondolf et al. 2001). Goodwin Dam (37°51′46″N, 120°37′48″W) is located 94.0 rkm upstream from its confluence with the San Joaquin River and is an upstream barrier to anadromous fish in the Stanislaus River. Historically, anadromous salmonids traversed the river and its three forks upstream to their headwaters to hold in cold-water pools before spawning. Since at least the mid-1800s, the geomorphology of the Stanislaus River has been affected by agriculture, gravel mining, and regulated flow and sediment delivery regimes (Kondolf et al. 2001). Agricultural land-use changes, in-stream gravel mining, and flow regulation are three

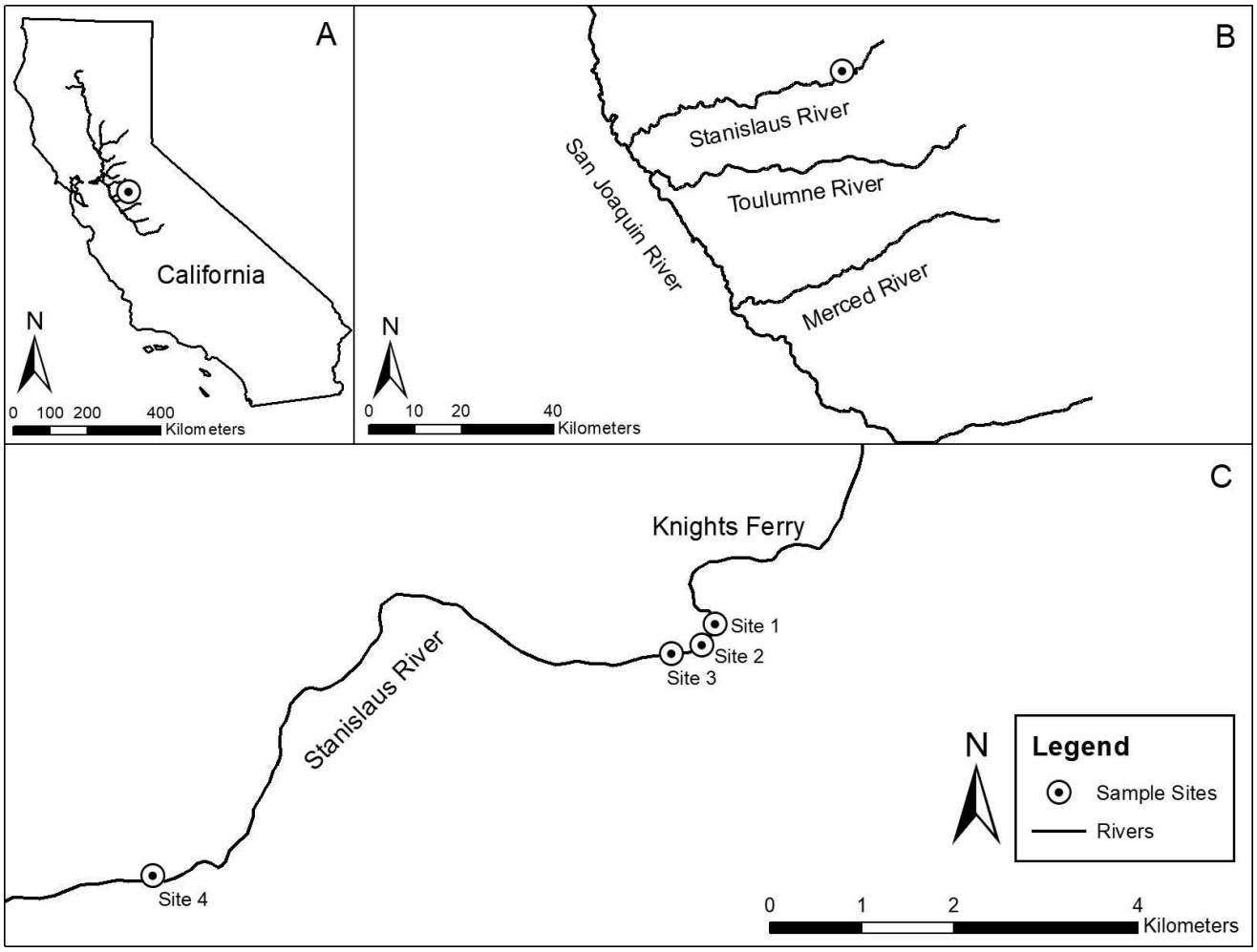

Figure 1. Sample site locations where we collected environmental deoxyribonucleic acid water samples on 9 October 2017 to test for the presence of North American Green Sturgeon *Acipenser medirostris* and White Sturgeon *Acipenser transmontanus* in relation to California (**A**); the San Joaquin River and its three tributaries (**B**); and the town of Knights Ferry, California (**C**).

major perturbations that have directly affected the geomorphic structure and physical habitat of the river. Overall, mining is thought to have extracted a considerable amount of coarse and fine sediment relative to the natural watershed supply (Kondolf et al. 2001; Schneider et al. 2003). Historically, relatively low-magnitude flow pulses occurred from late autumn until early spring in response to rainfall in the lower watershed, followed by an annual high-elevation snowmelt pulse during spring and early summer. During the 20th century, more than 40 dams were constructed on the Stanislaus River for flood protection, power generation, irrigation, and municipal water supply. Collectively, these dams can store up to 240% of the average annual runoff in the catchment, reducing the amount of riverine habitat available to anadromous fishes and affecting their migratory patterns (Zeug et al. 2014).

Methods

During a 23 September 2017 rafting trip on the Stanislaus River, we observed a fish (visually estimated 1.5 m total length) occupying a deep pool (estimated depth 3–6 m) downstream of Knights Ferry, California (rkm 86.1). On 5 October 2017, we returned to the same pool where the fish was first observed and performed a snorkel survey (Sellheim et al. 2016) to relocate the fish. During our snorkel survey we located an adult sturgeon and recorded its image and location using a GoPro® HERO 4 (GoPro, Inc., San Mateo, CA) video camera (Video S1, *Supplemental Material*) and a Trimble® GeoXT (Trimble, Inc., Sunnyvale, CA) handheld global positioning system unit. During this time we also visually scanned the fish for any signs of external tags or marks at close range ($<$ 1 m) in relatively clear water (Figure 2).

Because considerable effort is presently expended on acoustically tracking Central Valley sturgeon (see Klimley et al. 2015), on 9 October 2017, we also used standard telemetry detection methods to potentially gather additional data on the fish to aid in species identification (Adams et al. 2012). We used a Vemco® (Bedford, NS, Canada) VR100 mobile acoustic receiver to determine if the fish was previously tagged with an acoustic transmitter. Additionally, we collected filtered water

Figure 2. Screen-captured image of the adult North American Green Sturgeon *Acipenser medirostris* from video footage recorded on 5 October 2017 in the Stanislaus River near Knights Ferry, California.

samples for eDNA analysis to determine which sturgeon species was observed.

Water sample collection

We collected a total of 10 water samples (2 L per sample) from an inflatable raft at four sites in the Stanislaus River: three samples upstream of the fish observation site, three near the tail of the pool where the sturgeon was observed, and a total of four samples from two different downstream sites (Figure 1; Table 1). We collected water samples using methods similar to those recently published by Bergman et al. (2016). For each sampling event we directly filtered 2 L of water from the Stanislaus River at $\leq$ 0.2 m below the surface using sterile Saint Gobain XL-60 silicon tubing (Tygon®, Malvern, PA; internal diameter 6.3 mm), and a portable Masterflex® L/S Easy-Load II peristaltic pump (Cole-Parmer®, Vernon Hills, IL) powered by a cordless hand drill. We filtered water samples through a Millipore Sterivex™ GP 0.45-μm sterile filter unit (EMD Millipore, Burlington, MA). We performed all filtration from our raft at each sampling site. We captured and measured sample filtrate in graduated flasks to verify the volume of each sample. Once we completed filtering, we poured the filtrate back into the river so no water was otherwise removed or transported from the Stanislaus River. We used new tubing for each sample to eliminate potential cross-contamination among sites. Sterile filters were for single use and individually wrapped from the manufacturer, and we only opened them immediately before use. After we completed filtration, we capped the filters, labeled them with sample-specific identification numbers, placed them into a secondary sterile container, and stored them on ice for transport to the lab (Genidaqs, West Sacramento, CA). We kept all filters on ice in a cooler on the raft for the duration of the sampling event until we transferred them to a −20°C freezer in the Genidaqs lab, where we stored them in individually sealed secondary containers at −20°C until DNA extraction.

Table 1. Environmental deoxyribonucleic acid testing results for the presence of North American Green Sturgeon *Acipenser medirostris* and White Sturgeon *Acipenser transmontanus* from water samples collected from the Stanislaus River near Knights Ferry, California on 9 October 2017.

Site	Sample identification	White Sturgeon	Green Sturgeon
Site 1	UPSTRM1	ND[a]	ND
Site 1	UPSTRM2	ND	ND
Site 1	UPSTRM3	ND	ND
Site 2	OBSRV1	ND	(+)
Site 2	OBSRV2	ND	(+)
Site 2	OBSRV3	ND	ND
Site 3	DWNSTRM1	ND	ND
Site 4	BB1	ND	ND
Site 4	BB2	ND	ND
Site 4	BB3	ND	ND
Control	NTC	ND	ND
Control	WST (+)	(+)	ND
Control	GST (+)	ND	(+)
Control	EC	ND	ND

[a] ND: no detection.

Genetic analysis

We performed extraction of DNA using a PowerWater® Sterivex™ DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) following the manufacturer's recommended guidelines. We processed a DNA extraction negative control in parallel to ensure sample integrity throughout the extraction procedure. The DNA extraction negative control consisted solely of Sterivex filtered ultrapure water. We processed field and extraction DNA controls using the same equipment used to collect samples as reported by Bergman et al. (2016).

We analyzed each sample in triplicate for the presence of the Green Sturgeon *COI* mitochondrial and the *CytB* genes using a quantitative polymerase chain reaction primer and probe set as described by Brandl et al. (2015). Each quantitative PCR replicate consisted of a 5-μL reaction volume. Each 5-μL quantitative PCR reaction was composed of 1× Applied Biosystems TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems™, Foster City, CA), 900-nm final primer concentration, 60-nm final probe concentration, and 1-μL DNA template. We performed thermocycling using a Bio-Rad CFX 96 real-time system (Bio-Rad Laboratories, Inc., Hercules, CA) with the following profile: 10 min at 95°C, 40 cycles of 15-s denaturation at 95°C, and 1-min annealing–extension at 60°C. We ran six template control reactions on the plate with the samples. Template controls consisted of 1 μL of ultrapure water replacing DNA template within the reaction volume. We also tested three positive control reactions consisting of 20 ng/μL Green Sturgeon genomic DNA template in parallel to ensure consistent PCR performance. We made all PCR master mixes inside an ultraviolet PCR enclosed work-

station. We added the DNA template to the master mix outside of the ultraviolet PCR workstation on a dedicated PCR setup workbench. We conducted all PCR reactions on instruments located outside of the main lab in a separate portion of the building. We analyzed results of the quantitative PCR reactions using Bio-Rad CFX manager v3.1 (Bio-Rad). We considered a sample positive for the presence of Green or White Sturgeon DNA if any one of the three replicates showed logarithmic amplification within 40 cycles.

Results

We confirmed the fish observed and filmed on 5 October 2017 as an adult Green Sturgeon, with an estimated total length of 1.5 m on the basis of recorded footage from the GoPro video camera (Figure 2; Video S1, *Supplemental Material*). We did not observe any external tags or marks at close range (< 1 m). The lack of tag detection with the Vemco VR100 receiver suggested that the fish was not previously tagged with an acoustic transmitter. Analysis of replicated eDNA samples collected from the river at the fish location site confirmed the presence of Green Sturgeon. We detected Green Sturgeon eDNA in two samples collected at the deep pool (site 2) where the sturgeon was visually recorded on 5 October 2017 (Table 1). Samples from the upstream site (site 1) and both downstream sites (sites 3 and 4) were negative for Green Sturgeon eDNA. Additionally, all White Sturgeon eDNA samples were negative.

Discussion

We positively identified Green Sturgeon occupancy of the Stanislaus River near Knights Ferry, California using a combination of visual observation, recorded close-up video footage, and genetic techniques. Positive species identification was difficult to confirm even with the aid of high-quality short-range video footage. In this case, identification of important external characteristics used to differentiate Green Sturgeon and White Sturgeon (e.g., dorsal scute shape and number) could not be definitively confirmed by imagery, although they suggest that the photographed fish was a Green Sturgeon (McGinnis 2006). However, as shown here, eDNA can increase the ability of resource managers to positively identify and better understand and manage rare, sensitive, or cryptic species (Barnes and Turner 2016). Combining traditional survey techniques and systematic eDNA sampling regimes provides a means to increase the efficiency of monitoring the spatial and temporal distribution of Green Sturgeon (Bergman et al. 2016).

This confirmed observation of an adult Green Sturgeon in the Stanislaus River near Knights Ferry (rkm 86.1) is an important discovery that extends the previously accepted geographical range for the species by an additional ~86 rkm upstream into the Stanislaus River. The timing of this fish observation (23 September and 5 October 2017) suggests that it was not involved in reproductive migration (typically occurring from February to April), or spawning, which typically occurs in the Sacramento River system from mid-April to mid-June (Brown 2007; Heublein et al. 2009; Poytress et al. 2015). However, if this fish migrated up the Stanislaus River with the intent to find suitable spawning habitat it might have been able to do so during the known migration period (i.e., February–April) when river flows are typically elevated. Base summer and early fall river flows might make it difficult for a fish of this size to successfully navigate its way out of the river until flows increase in late October. Additional research is needed to better understand limitations to passage of these fish relative to river flow and depth levels.

This finding represents the second known case of documenting the presence of Green Sturgeon using eDNA techniques during the past 2 yr (see Bergman et al. 2016) and provides an example of a cost-effective, noninvasive sampling method to complement traditional survey methods for rare, cryptic species (Rees et al. 2014; Lugg et al. 2017). Consistent with this positive species identification, we recommend continued refinement and the combined application of various fish location technologies, including eDNA, for providing data needed to better identify and manage rare, threatened, and endangered species. Additional well-designed research focused on the San Joaquin River and its tributaries is expected to improve our understanding of the ecological importance of these rivers for natural production and life-cycle completion of southern distinct population segment Green Sturgeon.

Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content or functionality of any supplemental material. Queries should be directed to the corresponding author for the article.

Video S1. GoPro video footage of an adult Green Sturgeon *Acipenser medirostris* observed on the Stanislaus River near Knights Ferry, California on 5 October 2017.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S1 (5.43 MB MOV).

Reference S1. DuBois J, Danos A. 2017. 2016 Sturgeon fishing report card: preliminary data report. Stockton, California: California Department of Fish and Wildlife.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S2 (1.6 MB PDF); also available at https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentId=141241.

Reference S2. Heublein JC, Bellmer R, Chase RD, Doukakis P, Gingras M, Hampton D, Israel JA, Jackson ZJ, Johnson RC, Langness OP, Luis S, Mora E, Moser ML, Rohrbach L, Seesholtz AM, Sommer T, Stuart JS. 2017. Life history and current monitoring inventory of San Francisco Estuary Sturgeon. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-589.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S3 (421 KB PDF); also available at https://doi.org/10.7289/V5/TM-SWFSC-589.

Reference S3. Klimley AP, Chapman ED, Cech JJ Jr, Cocherell DE, Fangue NA, Gingras M, Jackson Z, Miller EA, Mora EA, Poletto JB, Schreier AM. 2015. Sturgeon in the Sacramento–San Joaquin Watershed: new insights to support conservation and management. San Francisco Estuary and Watershed Science.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S4 (1.64 MB PDF); also available at https://doi.org/10.15447/sfews.2015v13iss4art1.

Reference S4. [NMFS] National Marine Fisheries Service. 2015. Southern distinct population segment of the North American Green Sturgeon (*Acipenser medirostris*); 5-year review. West Coast Region, Long Beach, CA.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S5 (580 KB PDF); also available at https://repository.library.noaa.gov/view/noaa/17034.

Reference S5. Rosales-Casian JR, Almeda-Juaregui C. 2009. Unusual occurrence of a green sturgeon (*Acipenser medirostris*) at El Socorro Bay, Baja California, Mexico. California Cooperative Oceanic Fisheries Investigations Report 50:169-171.

Found at DOI: https://doi.org/10.3996/012018-JFWM-006.S6 (646 KB PDF); also available at https://www.researchgate.net/publication/227854967_Unusual_occurrence_of_a_green_sturgeon_acipenser_medirostris_at_el_socorro_Baja_California_Mexico.

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