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Geographic Patterns of Genetic Differentiation among Collections of Green Sturgeon

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Abstract.—The population structure of green sturgeon *Acipenser medirostris* has not previously been evaluated, although commercial and bycatch harvests may be impacting multiple populations of this species. Molecular markers were used to distinguish between green and white sturgeon *A. transmontanus* and to examine the genetic variation among green sturgeon collected from four locations along the West Coast of the United States between San Pablo Bay, California, and the Columbia River, Washington. Verification that putative green sturgeon samples were not white sturgeon was accomplished with a diagnostic restriction fragment length polymorphism in the mitochondrial cytochrome *b* locus. Genetic variation in green sturgeon was investigated using six microsatellite loci developed from published primers. Allele frequencies, the fixation index F_{ST}, and observed heterozygosities were determined at three disomic and three tetrasomic loci amplified from fin tissue samples. Pairwise F_{ST} values of 0.005 and −0.002 indicated no genetic differentiation between the Klamath and Rogue River collections or the Columbia River and San Pablo Bay collections. All other F_{ST} values among pairs of collections ranged between 0.06 and 0.08 and indicated a significant difference between collections from the Rogue and Klamath rivers and those from San Pablo Bay and the Columbia River. These results and highly significant *G*-tests of genotypic differences between these pairs of collections support the existence of genetic structuring in this species along the Pacific coast. These data suggest that not all spawning populations have been identified and that additional genetic research will be necessary to adequately describe the population structure of green sturgeon before effective management plans can be developed.

The green sturgeon *Acipenser medirostris* is a long-lived, anadromous acipenserid inhabiting the Pacific Ocean between the Aleutian Islands, Alaska, and northern Baja California, Mexico (Moyle 2001; Figure 1). Little is known about green sturgeon life history (Emmett et al. 1991). Juveniles are believed to migrate out to sea after spending 1–3 years in freshwater (Nakamoto et al. 1995). During this time, they are believed to feed on benthic invertebrates (Moyle 2001) and to exhibit nocturnal behavior (Cech et al. 2000). The length of time that juvenile green sturgeon remain in coastal estuaries is unknown. During the late summer and early fall, subadult and nonspawning adult green sturgeon concentrate in Pacific coastal estuaries north of San Francisco Bay (Emmett et al. 1991), although the reason for this behavior remains unknown. Adult green sturgeon are uncommon in the San Francisco Bay system (Ayres 1854; Schaffter and Kolhorst 1999) and are infrequently encountered in freshwater on the Fraser and Skeena rivers of British Columbia (Scott and Crossman 1973). They appear to be the most common sturgeon species in Willapa Bay, Washington (Emmett et al. 1991). Columbia River green sturgeon concentrate in the estuary and have been observed up to Bonneville Dam, though no evidence exists for spawning in this system (Rien et al. 2000). The population of origin for these fish remains unknown. Miller (1972) tagged 54 San Pablo Bay green sturgeon in 1967–1968, and three of five recovered tags occurred in coastal estuaries, including two at the mouth of the Columbia River and one in Grays Harbor, Washington. Also, the recovery of a San Pablo Bay tagged green sturgeon in Willapa Bay in 2002 (Matt Howell, Washington Department of Fish and Wildlife, personal communication) supports the theory of a migratory connection between northern and southern green sturgeon aggregations.

Known green sturgeon spawning is limited to

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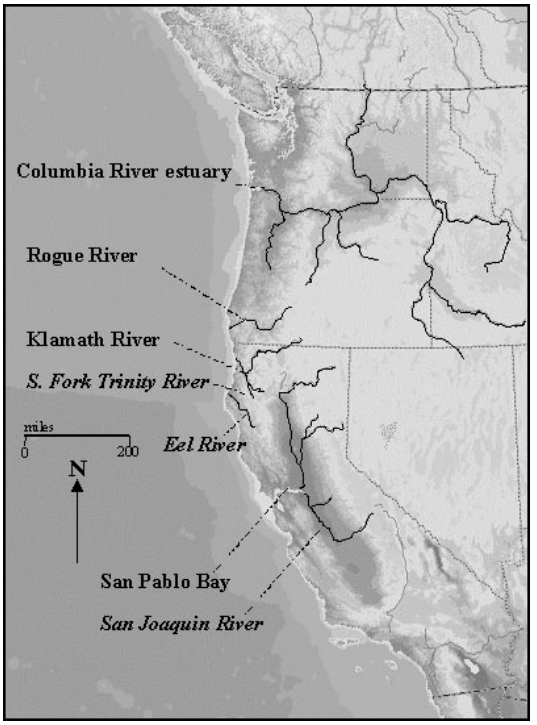

FIGURE 1.—Locations of the collection sites of green sturgeon samples analyzed in this study. Green sturgeon are believed to have been extirpated from the river systems whose names are in italics (Moyle 2001).

the Sacramento and Klamath rivers in California and the Rogue River in Oregon (Moyle 2001). Spawning populations are believed to be extirpated on the Eel, South Fork Trinity, and San Joaquin rivers in California (Moyle 2001). Adults are believed to spawn every 3–5 years and to reside in marine water between spawning runs (Adams et al. 2002), which occur during the summer. Estimated ages of reproductive female green sturgeon from the Klamath River ranged from 25 to 32, while those of the male green sturgeon used in the same artificial spawning ranged from 18 to 30 (Van Eenennaam et al. 2001).

In Canada, green sturgeon are recognized as rare by the Committee on the Status of Endangered Wildlife (Houston 1988) and were recently listed as a candidate species under the U.S. Endangered Species Act by the National Marine Fisheries Service (NMFS 2003). A substantial decline in the total annual catch of these fish from estuaries and the ocean has been observed over the last 15 years due to regulatory changes, yet the potential for differential impacts on multiple populations due to harvesting has not been evaluated (Adams et al. 2002). Sturgeons are known to have strong homing capabilities and spawning site fidelity (Bemis and Kynard 1997), but the natal rivers of nonspawning green sturgeon aggregations remain unknown.

No prior studies have evaluated the potential for stock structure in green sturgeon, although molecular analyses have demonstrated that population structure exists in other North American sturgeons, including lake sturgeon *A. fulvescens* (McQuown et al. 2002), Atlantic sturgeon *A. oxyrinchus* (King et al. 2001), and white sturgeon *A. transmontanus* (Smith et al. 2002). Clearly, insight into potential population structure in green sturgeon is necessary for the formulation of adequate management strategies. Unfortunately, work has been hindered by the inability to distinguish green sturgeon from the sympatric white sturgeon at all life history stages and by limited knowledge of green sturgeon spawning habitat and migration patterns. This study was undertaken with two main objectives in mind. The first was to develop an interspecific molecular marker to differentiate green and white sturgeons at all life history stages. The second was to test the hypothesis that nonspawning aggregations and spawning populations of green sturgeon comprise a single reproductive population. To accomplish the second objective, we used microsatellite DNA markers to investigate genetic differentiation among samples collected from two nonspawning aggregations of green sturgeon (San Pablo Bay and the Columbia River) and two of the three known spawning populations (the Klamath and Rogue rivers).

Methods

Samples.—Green sturgeon samples were collected from four locations along the western coast of the United States (Figure 1). Throughout the paper, the term ''sample'' is used to identify a tissue sample collected from a single green sturgeon. The word ''collection'' is reserved for a group of samples that were are taken from the same place over the same period of time. The term ''population'' describes green sturgeon collections containing samples from reproductively mature fish from rivers with known spawning activity. Adult fish were captured in freshwater from the Klamath and Rogue River populations. Table 1 shows the collection location, collecting organization, year of collection, collection size, and life history stage of green sturgeon samples included in this analysis. Tissue samples included dry fin clips and wet tissue samples preserved in 95% solutions of ethanol stored at room temperature.

TABLE 1.—Green and white sturgeon samples used in this study.

Sturgeon species	Location	Collecting agency	Year of collection	Samples used in analysis	Life history stage
Green	San Pablo Bay	California Department of Fish and Game	2001	46	Subadult, adult
Green	Klamath River	Yurok tribal fisheries program	1998	66	Adult
Green	Rogue River, Oregon	Oregon Department of Fish and Wildlife	2000	34	Adult
Green	Columbia River estuary	Washington Department of Fish and Wildlife	1995	32	NA[a]
White	Sacramento River	California Department of Fish and Game	1998	8	Subadult

[a] Not reported.

DNA extraction.—Samples from the Klamath and Columbia rivers were extracted by means of a standard phenol-chloroform procedure (Sambrook et al. 1989). DNA from the Rogue River and San Pablo Bay were isolated using a Qiagen DNeasy tissue kit. Two μL of each sample were evaluated for degradation by electrophoresis on a 0.7% agarose gel for 50 min at 100 V. Gels were fluorescently labeled with 1% Gelstar (BioWhitaker Molecular Applications, Rockland, Maine) and visualized on a Molecular Dynamics 595 FluorImager.

Species identification study.—A restriction map was created using DNA Club (Molecular Biology Shortcuts [www.justbio.com]) from cytochrome *b* sequences in green and white sturgeon (Birstein and DeSalle 1997) and examined for restriction site polymorphisms between the two species. Green and white sturgeon samples from multiple locations were amplified via polymerase chain reaction (PCR) with primers H15149 (Kocher et al. 1989) and L14724 (Meyer et al. 1990). Each reaction mixture contained 2.0 μL of DNA (~15 ng/μL), 2 μL of Promega 10× buffer (Madison, Wisconsin), 2 μL each of forward and reverse primers (10 μM), 1.6 μL of MgCl (25 mM), 2 μL of deoxynucleotide triphosphates (dNTPs; 2.5 mM), and 0.08 μL of Promega *Taq* polymerase. Filtered, deionized water was added to each mixture to bring the total volume to 20 μL. After amplification (Table 2), 10 μL of product were digested at 40°C for 6 h with 0.1 μL of *Ssp*1 restriction

TABLE 2.—Conditions of the different polymerase chain reaction (PCR) protocols used for amplification of microsatellite and restriction fragment length polymorphism (RFLP) loci used in this study. As shown in the lower part of the table, modifications were made to the standard protocols in order to amplify specific loci. The standard protocols utilized 25 mM of MgCl and annealing temperatures of 65, 56, and 52°C; the modified protocols utilized the quantities of MgCl and annealing temperatures shown in the lower part of the table.

Protocol			
Touchdown	Promega *Taq*	Faststart *Taq*	RFLP
Standard PCR			
95°C for 1 min 15 cycles of 95°C for 30 s 65°C for 1 min[a] 72°C for 1 min 15 cycles of 95°C for 30 s 50°C for 1 min 72°C for 1 min	95°C for 90 s 35 cycles of 95°C for 1 min 56°C for 45 s 72°C for 2 min 72°C for 5 min	95°C for 4 min 30 cycles of 95°C for 30 s 56°C for 30 s 72°C for 1 min 72°C for 5 min	94°C for 90 s 35 cycles of 95°C for 30 s 52°C for 30 s 72°C for 1 min 72°C for 5 min
Modifications for specific loci			
1.25 mM (*Aox 27*)	1.75 mM; 59°C (*AfuG 43*) 2.0 mM; 52°C (*AfuG 135*) 54°C (*Spl 101b*)	2.0 mM (*spl 120b*)	

[a] Less 1°C per cycle.

enzyme (New England BioLabs, Beverly, Massachusetts), 1.2 μL of 10× buffer, and 0.7 μL of distilled H_2O. Both undigested and digested PCR samples were run on a 5% acrylamide denaturing gel for 1.5 h at 35 W. Gels were stained with a fluorescein-agarose overlay (Rodzen et al. 1998) and visualized with a Molecular Dynamics 595 FluorImager.

Microsatellite population structure study.—Samples from at least two of the geographically distinct green sturgeon collections were amplified with a touchdown PCR protocol (Table 2) using a total of 86 microsatellite primer pairs originally developed in shovelnose sturgeon *Scaphirhynchus platorynchus* (McQuown et al. 2000), lake sturgeon (May et al. 1997; Welsh et al. 2003), or Atlantic sturgeon (King et al. 2001; T. L. King, U.S. Geological Survey, personal communication). Each reaction mixture contained 4.8 μL of filtered, deionized H_2O, 1.0 μL of DNA (~15 ng/μL), 1 μL of Promega 10× buffer, 1 μL each of forward and reverse primers (10 μM), 0.3 μL of MgCl (25 mM), 0.8 μL of dNTPs (2.5 mM), and 0.075 μL of Promega *Taq* polymerase. Of the 86 primer pairs attempted, four promising systems (*Aox 27* [King et al. 2001], *AfuG 43* and *AfuG 135* [Welsh et al. 2003], and *Spl 106* [McQuown et al. 2000]) were selected that were readily amplifiable, did not amplify multiple loci or difficult-to-interpret stutter bands, and did not contain null alleles. To increase the number of usable markers, primers were redesigned to increase sequence specificity in two additional promising loci (*Spl 120* and *Spl 101* [McQuown et al. 2000]). Bands were cut out and sequenced (Davis Sequencing, Davis, California), sequences were compared for homology, and primers were designed for distinct locus sequences. A modified forward primer (5′-TTA AAG AGG ATT GAA TAG CCT AAT-3′) was used with the *Spl 120* reverse primer (McQuown et al. 2000) to amplify a disomic locus. The original *Spl 101* locus (McQuown et al. 2000) was modified with primers of the following sequences: forward (5′-GGA AAT TTG ACA AAT CAC ACC C-3′) and reverse (5′-AAA GCA CAT CAG TTA AAA GGT CAA-3′) to yield a scorable locus. These loci were renamed *Spl 120b* and *Spl 101b*, respectively, for this study. The PCR conditions and mixtures were optimized for each microsatellite system (Table 2). Locus *Spl 106* did not require modification of the PCR reaction mixture, which contained 4.7 μL of filtered, deionized H_2O, 1.0 μL of DNA (~15 ng/μL), 0.8 μL of Promega 10× buffer, 1 μL each of forward and reverse primers (10 μM), 0.5 μL of MgCl (25 mM), 0.8 μL of dNTPs (2.5 mM), and 0.1 μL of Promega *Taq* polymerase. The PCR reaction mixtures with Faststart *Taq* polymerase (Roche, Indianapolis, Indiana) contained 4.8 μL of filtered, deionized H_2O, 1.0 μL of DNA (~15 ng/μL), 1.0 μL of Roche 10× buffer with MgCl, 1 μL each of forward and reverse primers (10 μM), 1.0 μL of dNTPs (2.5 mM), and 0.1 μL of Faststart *Taq* polymerase. The PCR products were run on a 5% acrylamide denaturing gel for 50–90 min at 50 W. The gels were stained with a fluorescein-agarose overlay (Rodzen et al. 1998) and scanned with a Molecular Dynamics 595 FluorImager. Allele sizes were designated with FragmeNT Analysis 1.1 (Molecular Dynamics, Sunnyvale, California) with a 400-base-pair (bp) commercial ladder (Gel Company, San Francisco) and an allelic ladder.

Statistical analyses.—Allele and genotype frequencies, along with genotypic disequilibrium at the three disomic loci, were calculated using the program GENEPOP 3.1 (Raymond and Rousset 1995). The significance of the observed genotypic differentiation was tested with an unbiased estimate of the *P*-value of a log-likelihood-based (*G*) exact test (Goudet et al. 1996). Observed genotype frequencies were tested for the deviation of each collection from Hardy-Weinberg expectations for the three disomic loci using Genes in Populations (program designed by B. May and C. Krueger and written in C by W. Eng and E. Paul; unpublished). Significance was determined by the log-likelihood *G*-test and a chi-square test ($P < 0.05$; Sokal and Rohlf 1981). Observed heterozygosities were found for data from three loci displaying disomic banding patterns. The number of alleles sampled and pairwise genetic differentiation (F_{ST}) tests for differentiation were computed with FSTAT (Goudet 2001). Pairwise F_{ST} values were evaluated for significance with the log-likelihood *G*-test (Goudet et al. 1996). This test statistic evaluated significance based on the *P*-value of all loci estimated with a standard Bonferroni correction (Rice 1989) following 120 permutations.

Results

Species Identification Study

A 467-bp fragment was amplified in the cytochrome *b* locus of putative green sturgeon. The restriction enzyme *Ssp*1 was determined to distinguish between green and white sturgeon by producing a single unique cut in green sturgeon mitochondrial DNA that resulted in two fragments,

one of 296 bp and the other of 195 bp. This marker was used to confirm the identity of putative green sturgeon in the collections. As a result, one white sturgeon in the San Pablo Bay collection and two in the Rogue River collection were identified and eliminated from the study.

Microsatellite Population Structure Study

Three of the microsatellite loci used in this study (*AfuG 43*, *Spl 101b*, and *Spl 106*) displayed banding patterns characteristic of tetrasomic loci. The remaining three loci (*Spl 120b*, *AfuG 135*, and *Aox 27*) displayed disomic banding patterns. Table 3 shows sample sizes, observed and expected heterozygosities, and allele frequencies for the six microsatellite loci and four geographically distinct collections used in this study. The number of alleles per locus ranged from six (*Spl 106* and *Aox 27*) to 20 (*Spl 101b*), and heterozygosities from three loci varied among collections from 0.577 (the Columbia River) to 0.783 (the Klamath River). The three disomic loci met Hardy-Weinberg expectations in all collections at all loci except for the Columbia River collection, which had an excess of heterozygotes at the *AfuG 135* locus. No significant linkage disequilibrium was detected among the three disomic loci in each of the four collections or across loci. The Klamath River collection had the greatest number of alleles (53) at all loci, while the Columbia River collection had the lowest overall number of alleles (44). Of the 65 alleles observed, 27 occurred at frequencies of 0.05 or less in all collections. *Aox 27* showed the least variability, the most common alleles ranging in frequency from 0.43 to 0.75. *Spl 101b* displayed the greatest variability, the most common alleles ranging in frequency from 0.28 to 0.35.

The *G*-tests of genotypic differentiation among collections indicated highly significant ($P \leq 0.0001$) differences at all six loci between the Klamath and Rogue River samples and the San Pablo Bay and Columbia River samples. Likewise, significant pairwise F_{ST} differences ($P \leq 0.008$) existed between the Klamath and Rogue River collections and those from the Columbia River and San Pablo Bay (Table 4). Significant differences were not detected between the Klamath and Rogue River collections or the Columbia River and San Pablo Bay collections.

Discussion

Six microsatellite loci showed significant differentiation between green sturgeon collections along the West Coast of the United States. Pairwise F_{ST} values showed a moderate level of differentiation between collections from the Columbia River and San Pablo Bay and those from the Klamath and Rogue rivers. These data also indicated a highly significant difference in genotype frequencies between the San Pablo Bay and Columbia River collections vis-à-vis the Klamath and Rogue River populations. The analysis does not support the hypothesis that the San Pablo Bay and Columbia River collections are derived from the Klamath and Rogue River populations. The lack of significant linkage disequilibrium and the observation of Hardy-Weinberg equilibrium in the San Pablo Bay collection suggests that this group of samples is not a mixed stock. Linkage disequilibrium due to an excess of heterozygotes at one of three loci in the Columbia River collection suggests that these samples represent a mixed stock that is not solely derived from the same population as the one in San Pablo Bay. These data indicate the likely existence of one or more additional spawning populations that is responsible for this aggregation. Tag and recapture data also support the proposition that green sturgeon from the Columbia River and San Pablo Bay are not different. Further, recapture data support the genetic distinctiveness of Klamath and Rogue River green sturgeon from these estuarine aggregations because no tag recoveries have indicated the exchange of fish between these locations. Since samples of juvenile and adult green sturgeon from freshwater areas of the Sacramento River were not analyzed, the unique variation observed in the San Pablo Bay and Columbia River collections cannot be attributed to the Sacramento River spawning stock. Our results support the existence of distinct populations of green sturgeon but do not offer clear insight into the reasons for the observed variation.

Multiple factors may contribute to the observed differences between the Klamath and Rogue River populations and those from San Pablo Bay and the Columbia River. Multiple studies have confirmed the fidelity to spawning sites of other sturgeon species (Auer 1996; Hildebrand et al. 1999; Hatin et al. 2002) . These studies and limited information on green sturgeon (Erickson et al. 2002; Kurt Brown, California Department of Fish and Game, personal communication) suggest that green sturgeon spawning habitat is distinctive within the river systems they use for spawning. Because of the distances between the mouths of the rivers supporting the reproducing populations of the Sacramento River and Klamath Mountains Province, the genetic differentiation observed between col-

lections may be caused by geographic isolation. This explanation is also supported by the proximity of and nondifferentiation between the Rogue and Klamath River populations.

Reproductive populations of green sturgeon may represent distinct ecotypes. The Klamath and Rogue rivers drain coastal mountains of the Pacific mid-coastal ecoregion (Abell et al. 2000), while the Sacramento River empties interior tributaries of the Sierra Nevada through the Central Valley ecoregion (Omernik 1987; Abell et al. 2000). Artyukhin and Andronov (1990) suggested that green sturgeon evolved in association with ''reproduction in fast-flowing small rivers with a relatively short lowland reach and with spawning grounds quite close to the river mouth.'' This qualitatively describes the Klamath and Rogue rivers but not the Sacramento River. On the Klamath and Rogue rivers, habitats in a constrained channel with a bedrock or large-cobble substrate and swift velocities that are probably preferred for green sturgeon spawning occur throughout the lower 160 km of these rivers. However, on the Sacramento River, this type of habitat is limited throughout the entire system and spawning adults must ascend more than 320 km of river to find small reaches of this type of habitat (near Red Bluff, California). The Klamath and Rogue rivers have been noted for their high productivity (Kesner and Barnhardt 1972; Erickson et al. 2002), and this factor contributed to the presence of the unique ''half-pounder'' life history in steelhead *Oncorhynchus mykiss* (Kesner and Barnhardt 1972) found in these rivers. For this reason, juvenile green sturgeon may be rearing in freshwater portions of the Klamath and Rogue rivers instead of their small estuaries (311 and 254 ha, respectively). Conversely, juvenile green sturgeon from the Sacramento River may be using the highly productive San Francisco Estuary as a nursery ground. The San Francisco Estuary (of which San Pablo Bay is a part) is 1,037 km^2 and the Columbia River estuary is 41,208 ha; both are characterized by extensive shallow-water habitats. Age-to-growth curves are different between green sturgeon from the Klamath River and San Pablo Bay (Adams et al. 2002) and may signify a difference in growth that is influenced by feeding or migratory behavior. If juvenile green sturgeon become specialists for resources distinct to their natal drainages, their migration patterns may reflect a desire to find similar sources of food. This may contribute to the lack of differentiation between green sturgeon samples collected from the Columbia River and San Pablo Bay.

Green sturgeon of Klamath and Rogue River origin may have a migratory pattern different from those in San Pablo Bay and the Columbia River. The potential for green sturgeon to learn migrational patterns may play a role in explaining the observed nonclinal structuring of the collections. The longevity, seasonal aggregation in estuaries, and electrosensory capacity of green sturgeon may support behavior that would allow informational transfer between different age-groups. Lucas and Baras (2001) suggested that informational transfer is important for spatially mapping and learning migration routes, particularly if the knowledge of an older individual may influence foraging movements. If green sturgeon follow learned migratory patterns, as is exhibited by other fish species (Lucas and Baras 2001), that would support the idea that the subadult and adult green sturgeon in the Columbia River are primarily from a reproductive population common to the San Pablo Bay samples. If learned behavior is an important factor in explaining the observed structuring of green sturgeon populations, managers have yet to identify the migration pattern of green sturgeon from the Klamath and Rogue rivers.

Geological events in the Klamath and Rogue rivers may have eliminated free passage to spawning habitats in these coastal basins and contributed to the differentiation of reproductive populations. As recently as the 1980s, green sturgeon faced migration obstacles that limited access at low flows beyond a debris slide near Coon Creek Falls on the lower Klamath River (Adair et al. 1985). If ancient or unrecorded barriers existed for long periods, the Sacramento River may have constituted the single spawning population. As green sturgeon recolonized unoccupied coastal drainages, they found habitat that favored high survival and rapid population growth by a small number of colonizers. Genetic drift from a small pool of founding genotypes and gene flow between the newly established Klamath and Rogue River populations could (because of their proximity) explain the observed nonsignificant differentiation of these collections and their significant differentiation from the San Pablo Bay samples. The size of the Columbia River's mouth and estuary permitted accessibility from the ocean and probably kept the river open during periods when smaller coastal basins to the south were inaccessible (McPhail and Lindsay 1986), allowing migratory green sturgeon to concentrate in the early summer and late fall in an estuary similar to the one in their natal system.

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TABLE 3.—Allele frequencies, sample sizes (n), and number of gene doses at six microsatellite loci in four collections of green sturgeon. Observed (H_o) and expected (H_e) heterozygosities are given at the three microsatellite loci displaying disomic banding patterns.

		Collection			
Locus	Alleles (bp)	Klamath River	Rogue River	San Pablo Bay	Columbia River
Spl 101b (4 doses)	163			0.006	
	167			0.051	0.056
	171				0.016
	175	0.005	0.016		0.008
	179	0.009		0.019	0.032
	183	0.186	0.172	0.038	0.04
	187	0.1	0.094	0.103	0.04
	191	0.282	0.328	0.353	0.315
	195	0.105	0.062	0.122	0.081
	199	0.114	0.141	0.09	0.161
	203	0.045	0.031	0.051	0.032
	207	0.091	0.086		0.024
	211	0.023	0.031	0.051	0.065
	215	0.027	0.031	0.038	0.048
	219	0.014	0.008	0.006	0.056
	223			0.013	
	229			0.019	
	231			0.026	0.008
	235			0.006	0.016
	239			0.006	
	H_o	0.718	0.750	0.747	0.787
	H_e	0.840	0.820	0.832	0.820
	n	55	32	40	32
Spl 106 (4 doses)	230			0.012	
	234	0.076	0.098	0.018	0.023
	238	0.237	0.288	0.024	0.032
	240	0.004	0.030	0.024	
	242	0.679	0.583	0.888	0.921
	246	0.004		0.035	0.024
	n	56	33	43	32
Aox 27 (2 doses)	124			0.012	
	132	0.473	0.439	0.679	0.758
	136	0.268	0.394	0.048	0.048
	140	0.205	0.121	0.262	0.194
	144	0.045	0.045		
	160	0.009			
	H_o	0.589	0.727	0.429	0.452
	H_e	0.660	0.635	0.469	0.386
	n	56	33	42	31
AfuG 43 (4 doses)	192	0.035	0.029	0.011	
	196		0.007		
	204	0.395	0.309	0.344	0.375
	208	0.281	0.412	0.506	0.453
	212	0.096	0.041	0.078	0.055
	216	0.004	0.015		
	220	0.066	0.059	0.044	0.07
	224	0.088	0.118	0.017	0.039
	228	0.035	0.008		0.008
	n	57	34	46	32
AfuG 135 (2 doses)	220		0.015	0.013	0.018
	224	0.017			
	228	0.095	0.258	0.488	0.614
	232	0.034			
	236	0.069	0.03		
	240	0.034	0.015	0.013	
	244	0.095	0.121		
	248	0.164	0.076	0.087	0.07
	252	0.25	0.152	0.188	0.175
	256	0.147	0.167	0.162	0.088

TABLE 3.—Continued

Locus	Alleles (bp)	Collection			
		Klamath River	Rogue River	San Pablo Bay	Columbia River
	260	0.06	0.121	0.013	
	264	0.034	0.045	0.038	0.035
	H_o	0.897	0.909	0.625	0.500
	H_e	0.859	0.844	0.691	0.585
	n	58	33	40	28
Spl 120b (2 doses)	228		0.029		
	232	0.017	0.029		0.031
	236	0.025	0.088	0.023	
	240	0.042	0.015	0.307	0.344
	244	0.195	0.176	0.057	0.094
	248	0.042	0.015	0.034	0.016
	252	0.102	0.132	0.227	0.125
	256	0.314	0.309	0.284	0.266
	260	0.059	0.059	0.068	0.078
	264	0.136	0.132		0.031
	268	0.042	0.015		
	272	0.017			0.016
	288	0.008			
	H_o	0.864	0.765	0.750	0.781
	H_e	0.825	0.825	0.764	0.778
	n	59	34	44	32
Average H_e		0.782	0.768	0.601	0.583
Average H_o		0.783	0.794	0.601	0.577

Fisheries Service to recognize distinct northern and southern green sturgeon population segments is an important first step toward delineating green sturgeon stocks; however, the complexities of the species' migration and population structure are not adequately reflected in these initial delineations (NMFS 2003). Our data suggest that the detection of population structure is possible with a limited number of microsatellites. However, additional microsatellite loci should be used to complete a genetic stock identification program and quantify the potential contribution of distinct population segments to mixed stocks found in the ocean and estuaries. This would provide managers with information on migratory patterns and give them a more comprehensive understanding of the stock complexity of green sturgeon. Additional samples from the Sacramento River and other putative breeding locations will be required to understand the composition of green sturgeon aggregations like those found in San Pablo Bay and the Columbia River. The marker described here, which distinguishes green and white sturgeon samples, will allow us to accurately assess the presence or absence of green sturgeon from larval samples as well as spawning activity in basins where both species are sympatric. This marker could also have forensic uses in verifying whether caviar or meat samples are from white or green sturgeon.

TABLE 4.—Population pairwise F_{ST} values for four collections of green sturgeon are given above the diagonal; below are the associated P-values (Bonferroni adjusted; $\alpha = 0.008$). Significant values are marked with asterisks.

Population	Population			
	Klamath River	Columbia River	San Pablo Bay	Rogue River
Klamath River		0.074	0.061	0.005
Columbia River	0.008*		−0.002	0.078
San Pablo Bay	0.008*	0.992		0.065
Rogue River	0.017	0.008*	0.008*	

Currently, the management of green sturgeon sport fisheries along the West Coast are based on the status and population estimates of white sturgeon (B. James, Washington Department of Fish and Game; T. Rien, Oregon Department of Fish and Wildlife; and R. Schaffter, California Department of Fish and Game, personal communications). Until the status of green sturgeon spawning populations is better understood, fishing regulations should discriminate their harvest from that of white sturgeon. Development of additional microsatellite markers will allow evaluation as to whether the distinct populations of green sturgeon have been impacted in the recent past by population bottlenecks, estimation of the effective population sizes of reproducing populations, and quantification of gene flow between drainages.

This information will be essential for understanding the biology of the green sturgeon, identifying the need for adaptive management to adequately protect the genetic diversity of these fish, and developing management plans that reflect genetic considerations.

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