

# Further investigation of green sturgeon (*Acipenser medirostris*) distinct population segment composition in non-natal estuaries and preliminary evidence of Columbia River spawning

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**Abstract** Green sturgeon (*Acipenser medirostris*) is a highly migratory, marine oriented species that congregates in non-natal estuaries during summer and early fall. Individuals from the threatened Southern Distinct Population Segment (SDPS) and non-listed Northern Distinct Population Segment (NDPS) regularly co-occur in non-natal estuaries including the Columbia River estuary, Willapa Bay, and Grays Harbor in relative proportions not explained by abundance or distance from natal river. We used genetic markers to assign green sturgeon sampled in these estuaries from 2010 to 2012 to distinct population segments (DPS). We then examined interannual differences in DPS composition among estuaries. Fork length distributions were compared between SDPS and NDPS green sturgeon to determine whether size varied within and among DPSs and estuaries. The majority of green sturgeon sampled in the Columbia River estuary and Willapa Bay were

assigned to the SDPS, while we assigned nearly even DPS proportions to our Grays Harbor samples. NDPS green sturgeon were significantly smaller than those originating from the SDPS within and among estuaries. We used these findings to develop several hypotheses about the mechanisms that may lead to specific patterns of non-natal estuary use. Genetic markers also assigned a single age-0 green sturgeon sampled in the Columbia River to the NDPS, although our analyses suggest that this individual's parents may not have originated from known NDPS spawning populations. Because the Columbia River may serve as alternative spawning habitat for green sturgeon as climate change occurs, we recommend monitoring the Columbia River more closely for further evidence of green sturgeon spawning.

**Keywords** Green sturgeon · Pacific northwest · Estuary · Distinct population segment · Population composition

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## Introduction

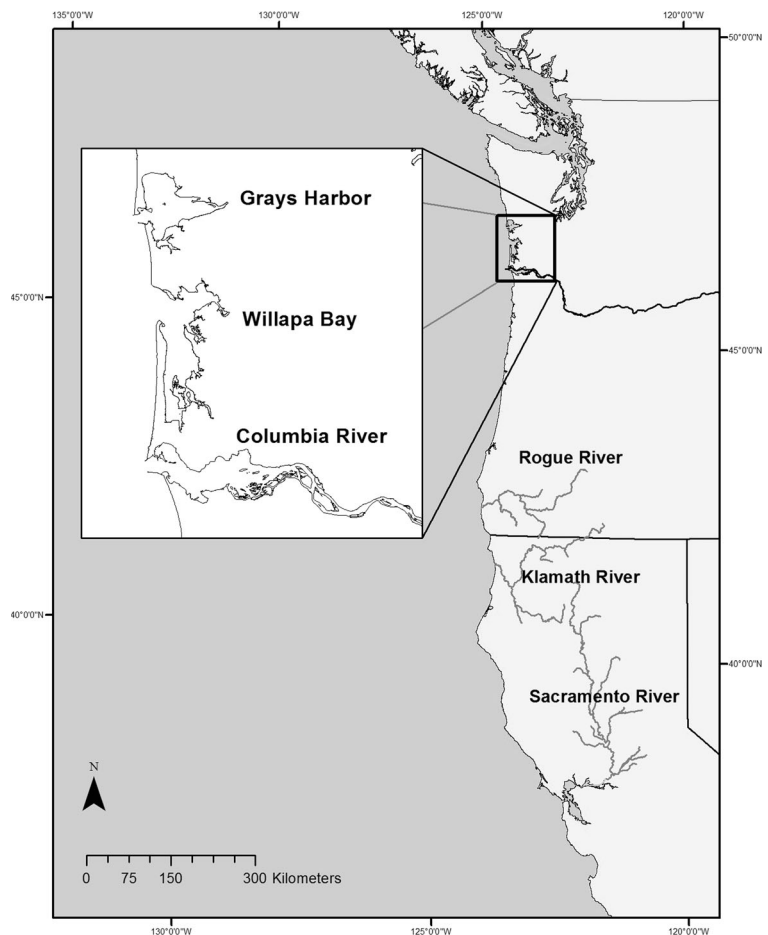
The green sturgeon, *Acipenser medirostris*, is a large bodied anadromous sturgeon that ranges from the El Socorro Coast of Baja California, Mexico (Miller and Lea 1972; Moyle 2002; Rosales-Casián and Almeda-Jáuregui 2009), to the Bering Sea, Alaska (Wilimovsky 1964; Miller and Lea 1972; Colway and Stevenson 2007). Federal listing of green sturgeon has recognized two genetically distinct populations (Israel et al. 2004; Israel et al. 2009). The federally threatened Southern Distinct Population Segment (SDPS) spawns in the

Sacramento River while the non-listed Northern Distinct Population Segment (NDPS) spawns in both the Klamath and Rogue rivers in California and Oregon, respectively (Adams et al. 2002; Fig. 1). At non-reproductive times, subadult and adult green sturgeon typically are found in shallow (< 200 m) coastal marine waters (Huff et al. 2012). During the late summer and early fall, subadult and adult green sturgeon from each DPS aggregate together in non-natal estuaries (estuaries not connected to known spawning rivers; Adams et al. 2007; Lindley et al. 2011). Because individuals from the SDPS spend a significant portion of their life far from the Sacramento River and often occupy the same habitats as the non-listed NDPS, much effort has been devoted to documenting and understanding DPS specific patterns of migration and non-natal estuary use.

Once old enough to enter marine habitat, green sturgeon from both DPSs generally undertake a northward migration along the Pacific coast, with only

a few observations where individuals have traveled south of their spawning rivers (Roedel 1941; Adams et al. 2002; Erickson and Hightower 2007; Rosales-Casián and Almeda-Jáuregui 2009). Northward migrations are likely associated with the California Undercurrent and therefore may be energetically favorable for green sturgeon (Huff et al. 2012). All documented non-natal estuaries used by green sturgeon are located north of both the SDPS and NDPS spawning rivers (Lindley et al. 2011). Although some non-natal estuaries may be favored by a single DPS, many commonly contain an aggregate of green sturgeon from both DPSs. Israel et al. (2009) and Lindley et al. (2011) found SDPS and NDPS aggregating together in estuarine habitats of the Umpqua River (above the town of Winchester Bay), the Columbia River, Willapa Bay, and Grays Harbor. Heublein et al. (2009) observed SDPS sturgeon in the Sacramento River that had been tagged in the Columbia River estuary and Willapa Bay. Genetic and biotelemetry studies have

**Fig. 1** Map of North American green sturgeon natal rivers showing the Sacramento River where the Southern Distinct Population Segment (SDPS) sturgeon spawn, and the Klamath and Rogue rivers where the Northern Distinct Population Segment (NDPS) sturgeon spawn. The insert map shows the study area (Grays Harbor, Willapa Bay, and the Columbia River estuary) where the sampled green sturgeon were caught and released



shown that certain estuaries tend to be dominated by green sturgeon from a particular DPS although these patterns show interannual variation. Israel et al. (2009) documented that the majority of green sturgeon sampled in the Columbia River estuary in 1995, 1999, and 2004 and Willapa Bay in 2003 originated from the SDPS, although Lindley et al. (2011) report a more equitable DPS distribution in the Columbia River estuary from 2005 to 2006. Distance from the spawning river did not explain aggregation patterns in non-natal estuaries, as SDPS green sturgeon are more frequent than NDPS green sturgeon in estuaries closer to the NDPS spawning rivers (Israel et al. 2009). Therefore uncertainty remains about what biotic or abiotic factors are associated with the distribution of DPSs among non-natal estuaries.

The purpose of this study was to expand on previous investigations of green sturgeon use of the three largest non-natal estuaries in the Pacific Northwest (Columbia River, Willapa Bay, and Grays Harbor) and generate hypotheses about ecological factors contributing to asymmetrical patterns of estuary use. We use genetic data to assign individual green sturgeon to DPS and compare DPS composition across the three estuaries over two years. We examine size distributions of DPS assigned fish and identify possible explanatory mechanisms for distinct size differences observed between DPSs within and among estuaries. Finally, we genetically analyze an age-0 green sturgeon sampled in the Columbia River (river km 209), providing the first known detection of green sturgeon spawning in the Columbia River.

## Methods

### Samples

Washington Department of Fish and Wildlife (WDFW) and Oregon Department of Fish and Wildlife (ODFW) captured green sturgeon in the Columbia River estuary, Willapa Bay, and Grays Harbor from June through September in 2010, 2011, 2012 (Fig. 1; Langness et al. 2014). This period was targeted because green sturgeon are known to be most abundant in these estuaries during summer and early fall (Moser and Lindley 2007; Adams et al. 2007; Lindley et al. 2011).

Gillnets have proven to be the most effective gear with which to capture green sturgeon, based on many years of

commercial fisheries (Bluestein 1986) and previous research and monitoring studies in estuarine waters (Farr and Kern 2005; O. Langness, unpublished data; C. Chapman, ODFW, pers. comm.). Commercial fishers were contracted to deploy sinking type gillnets set stationary and perpendicular to the current whenever possible. These research gillnets were made of two or three 91.4 m X 9 m panels of 6–8 strand monofilament net of different stretch mesh sizes (18.4, 21.5 or 24.8 cm), joined to form a net up to 274 m in length. Nets were laid out in the estuary channel network where green sturgeon are known to congregate (Moser and Lindley 2007; Dumbauld et al. 2008; Langness et al. 2014) and fished for ~30 mins, most often at or close to the time of low slack tide. The net was hung even or loose to a depth of approximately 9 m (25–35 meshes, depending on mesh size), fishing most of the water column but allowing surface oriented salmonids to avoid the net. This net configuration and sampling approach allowed us to capture a broad range of green sturgeon size classes.

A total of 174, 533, and 640 green sturgeon were captured (or recaptured) in 167 Columbia River estuary net sets, 98 Willapa Bay net sets, and 101 Grays Harbor net sets, respectively. The mean fork lengths for these groups were 119.7 cm ( $n = 172$ ,  $SD = 21.6$ ), 134.5 cm ( $n = 492$ ,  $SD = 19.5$ ), and 130.3 cm ( $n = 636$ ,  $SD = 20.9$ ), respectively. The fork lengths of individuals captured ranged from 45 to 201 cm (~49–218 cm total length), almost completely encompassing the range of known sizes for sub-adult and adult green sturgeon (Moyle 2002). Nearly all of these fish were injected in musculature near the dorsal fin insertion (Kahn and Mohead 2010) with a 12.5 mm × 2.12 mm FDX-B 134.2 kHz ISO 11784 and 11,785 compliant passive integrated transponder (PIT) tag (Biomark) as part of a mark-recapture study. Uniquely coded acoustic transmitters (VEMCO models V13 ( $n = 1$ ), V13P ( $n = 4$ ), V16 ( $n = 291$ ), V16TP ( $n = 19$ ), or V9AP ( $n = 4$ )) were surgically implanted in the body cavity of 319 of those fish in order to track movement behavior (Langness et al. 2014). From all of these acoustic tagged fish, we systematically selected 180 for genetic analysis of fin clips. All fin clips collected were placed in a microcentrifuge tube containing 95 % ethanol and shipped to the Genomic Variation Laboratory at UC Davis. The mean fork lengths of the fish that underwent laboratory tests were 128.3 cm ( $n = 60$ ,  $SD = 20.9$ ), 133.4 cm ( $n = 60$ ,  $SD = 22.9$ ),

and 133.0 cm ( $n = 60$ ,  $SD = 28$ ), for the Columbia River estuary, Willapa Bay and Grays Harbor respectively (Table 1). We performed a Kolmogorov-Smirnov test to determine whether the fork lengths of the samples selected for genetic analysis deviated from the fork lengths of all fish sampled (in each estuary and overall). We found there was no significant difference in fork length between the total sample set and the subset of samples used for this study (Columbia River estuary,  $P = 0.105$ ; Willapa Bay,  $P = 0.604$ ; Grays Harbor,  $P = 0.233$ ; across all estuaries,  $P = 0.208$ ). Therefore, we conclude that the green sturgeon included in the genetic analysis were reasonably representative of those present in the three estuaries during the study period.

We also conducted genetic analysis on a tissue sample from an age-0 green sturgeon captured November 10, 2011 by ODFW on the Columbia River. It was captured during an overnight small mesh gillnet (~ 5 cm) set upstream of Rooster Rock, Oregon (river km 209; Joint Columbia River Research Staff 2012) as part of an unrelated juvenile white sturgeon sampling effort. In that effort, juvenile white sturgeon were sampled in the Columbia River from October through November with a gillnet 91.4 m in length and 3.7 m in height that was fished for ~24 h at a time (C. Chapman, ODFW, pers. Comm.). A small cross-section of pectoral fin spine was removed from 22 cm FL fish and aged following methods outlined in Brennan and Cailliet (1989) to confirm it was age-0.

**Table 1** Collection locations for green sturgeon tissue, years of collection, and sample sizes (N)

Location	Year	N
Columbia River estuary	2010	0
	2011	37 <sup>†</sup>
	2012	24
Willapa Bay	2010	10
	2011	29
	2012	21
Grays Harbor	2010	8
	2011	35
	2012	17
Total		181

<sup>†</sup> Includes 2011 age-0 sample collected by ODFW

## DNA extraction and microsatellite genotyping

DNA was extracted from tissue using a PureGene DNA extraction kit (Qiagen). Samples were quantified on a FLA 5100 fluorimeter (Fujifilm) and normalized to 20 ng. Polymerase chain reactions (PCRs) were conducted with fluorescently labeled primers for ten microsatellite loci AfuG 41, AfuG 43, AfuG 135, AfuG 247, An76, Aox 27, As 007, Spl 101, Spl 106, and Spl 120 using previously published protocols (Israel et al. 2009; Israel and May 2010). A single microliter of PCR product was combined with 0.2  $\mu$ l of Liz 600 size standard (Life Technologies; LT) and 8.80  $\mu$ l of formamide (The Gel Company) and denatured for three minutes at 95° C. Capillary electrophoresis was conducted on an ABI 3730xl Genetic Analyzer (LT) and fragment analysis was performed in GeneMapper v.4.0 (LT). Previous green sturgeon genetic studies used relative peak heights to score dosage for tetrasomic loci (AfuG 41, AfuG 43, AfuG 135, AfuG 247, An76, As 007, Spl 101, Spl 106) and create codominant genotypes (Israel and May 2010). In the course of data collection for this study, we discovered variability in allele dosage calls among independent readers. To reduce uncertainty in individual genotypes, we treated each microsatellite allele as a present/absent dominant locus (Rodzen and May 2002; Israel et al. 2004).

## Data analysis

### DPS assignment

Before conducting analyses, we modified the existing genetic baseline datasets, originally created from SDPS and NDPS green sturgeon captured in natal drainages (Israel et al. 2009). We added 19 new alleles detected on the capillary electrophoresis platform that had not been previously detected on a polyacrylamide gel electrophoresis platform. The new alleles were given a zero frequency in the baseline datasets. Prior to evaluating DPS composition of the non-natal estuaries, we wished to confirm the genetic distinctiveness of the NDPS and SDPS baselines developed by J. Israel (unpublished data) with the Bayesian program Structure (Pritchard et al. 2000; Falush et al. 2007). We conducted 6 replicate runs in Structure testing the likelihood of one ( $K = 1$ ) to four ( $K = 4$ ) populations in a dataset containing the NDPS and SDPS baseline samples. Burn-in was 500,000 MCMC

iterations followed by 500,000 iterations. We assumed correlated allele frequencies and applied the admixture model. We used the mean likelihood  $\ln \Pr(X|K)$  and  $\Delta K$  (Evanno et al. 2005) to interpret the Structure results. The estimate  $\ln \Pr(X|K)$  indicated that the likelihood of our genetic dataset was greatest when two populations of green sturgeon ( $K = 2$ ) were assumed. The  $\Delta K$  estimate, which uses the second order rate of change in  $\ln \Pr(X|K)$  to indicate the most likely number of populations also provided evidence for  $K = 2$ . The populations identified by Structure corresponded to the two DPSs, confirming the accuracy of the genetic baselines for NDPS and SDPS.

Next, we took a maximum likelihood approach in the program AFLPOP (Duchesne and Bernatchez 2002) to allocate the green sturgeon sampled in the non-natal estuaries from 2010 to 2012 to DPS genetic baselines. Only samples with no missing genotype data ( $n = 170$ ) could be analyzed with AFLPOP. We first conducted simulations with baseline datasets in AFLPOP to assess the accuracy with which known individuals could be re-allocated to the correct baseline and to identify the most appropriate minimum likelihood difference (MLD) threshold. An optimal MLD threshold maximizes the number of accurate allocations while minimizing the number of individuals that could not be allocated to either baseline. We tested a range of MLDs (0.7–1.2) and found that the most appropriate MLD threshold was 0.8, meaning that an individual had to be  $10^{0.8}$  ( $= 6.3$ ) times more likely to belong to one DPS than the other in order to be allocated to it. Next, we allocated unknown individuals to the NDPS and SDPS genetic baselines using the 0.8 MLD threshold and a zero frequency threshold of 0.006. Significance was determined with 1000 permutations.

Finally, we conducted two Structure population assignment tests (500,000 burn-in, 500,000 replicates;  $K = 2$ ) to corroborate the DPS assignments made in AFLPOP. The first test used only prior information for the NDPS and SDPS baseline samples and treated estuary samples as unknowns to be assigned. The second test used AFLPOP DPS designations for estuary samples as prior information to improve individual assignment to a baseline. In each case we examined Q values, which indicate the likelihood an individual originated from a particular population, from both assignment tests to assign a DPS origin to each individual. Structure and AFLPOP results were examined together to make final DPS designations.

### *Estuary composition*

We did a two-tailed Fisher's exact test using the Freeman-Halton extension (Freeman and Halton 1951; Vassarstats) to determine whether there was a significant difference in how SDPS and NDPS green sturgeon were distributed among the Columbia River estuary, Willapa Bay, and Grays Harbor, excluding the age-0 individual from the Columbia River estuary. The null hypothesis was NDPS and SDPS green sturgeon were distributed evenly throughout the three estuaries. To determine whether the proportions of SDPS and NDPS green sturgeon detected within estuaries changed across years, we performed a Cochran-Mantel-Haenszel test for repeated  $2 \times 2$  tests of independence with a continuity correction for small sample size (Cochran 1954; Mantel and Haenszel 1959; McDonald 2014). Because we observed opposite direction interannual changes across estuaries, we also performed individual Fisher's exact tests for each estuary with a sequential Bonferroni correction (Rice 1989) to control for familywise error rate. Tests of interannual change included only 2011 and 2012 samples due to low sample size in 2010.

### *DPS length distributions*

We wished to determine whether length distributions between SDPS and NDPS fish differed within and among the three estuaries. Fork length frequency distributions were created for each estuary and all samples across estuaries. Welch's unequal variance t-tests were used to make pairwise comparisons in mean fork length between SDPS and NDPS within estuaries and in the overall dataset. Welch's t-test is the preferred statistical approach when making pairwise comparisons of central tendencies of two groups with unequal sample size and variance (Ruxton 2006). Next, a one-way ANOVA was conducted for each DPS to examine differences in mean fork length among estuaries.

## **Results**

### *DPS assignment*

The program Structure confirmed significant genetic divergence between the SDPS and NDPS baselines ( $K = 2$ ; Mean  $\ln \Pr(X|K) = -19,268$ ). Using AFLPOP, we were able to allocate all but five non-natal estuary

samples to a DPS and the initial Structure analysis (prior population information from baseline only) identified 95 % of individuals as belonging to either SDPS or NDPS. We observed high correspondence between AFLPOP and Structure DPS designations. In the Structure population assignment test using AFLPOP DPS allocations as prior population information, green sturgeon assigned to the SDPS or NDPS with high probability (SDPS mean  $Q = 0.97$ , range 0.54–0.99; NDPS mean  $Q = 0.94$ , range 0.72–0.99). In total, 175 estuary samples received designations, while five individuals had assignments and allocations considered too ambiguous to designate a DPS. Those ambiguous individuals, classified as “unknown” either could not be allocated by AFLPOP, had low  $Q$  values in one of the two Structure analyses, or had conflicting assignments across methods (Table 2). Unknown individuals were found in each estuary. The age-0 individual captured in the Columbia River estuary in 2011 assigned to the NDPS with high likelihood in Structure ( $Q = 0.97$ ), although AFLPOP could not assign it to either the NDPS or SDPS with confidence.

#### Estuary composition

When considering all assigned individuals from all three non-natal estuaries, 62 % (108) originated from the SDPS while only 38 % (67) assigned to the NDPS. Unknown individuals, found in each estuary, made up only 3 % of the total sample and were excluded from further statistical analyses. SDPS green sturgeon made up the majority of the samples from the Columbia River estuary and Willapa Bay while there were more NDPS than SDPS green sturgeon in Grays Harbor (Fig. 2). The two-tailed Fisher’s exact test on SDPS and NDPS

assigned fish revealed that there was a significant difference in DPS composition among the three estuaries ( $P = 0.00016$ ). We plotted the proportions of SDPS and NDPS green sturgeon detected in each estuary in 2011 and 2012 and found that in 2012, the proportion of SDPS green sturgeon decreased in the Columbia River estuary but increased in both Willapa Bay and Grays Harbor (Fig. 3). The Cochran-Mantel-Haenszel test for repeated  $2 \times 2$  tests of independence did not reveal a significant difference between estuaries and years ( $P = 0.649$ ). However, an erroneous non-significant result can be obtained with the Cochran-Mantel-Haenszel test if the direction of the change differs between comparisons (e.g., decrease in SDPS over years in the Columbia River estuary, increase in SDPS over years in Willapa Bay, Grays Harbor; McDonald 2014). Individual two-tailed Fisher’s exact tests for each estuary found a significant decrease in the proportion of SDPS individuals in the Columbia River Estuary between 2011 and 2012 ( $P = 0.001$ ; corrected  $\alpha = 0.017$ ) but no significant difference in the proportion of SDPS individuals detected between 2011 and 2012 in Willapa Bay and Grays Harbor ( $P = 0.338$  and  $0.366$ , respectively).

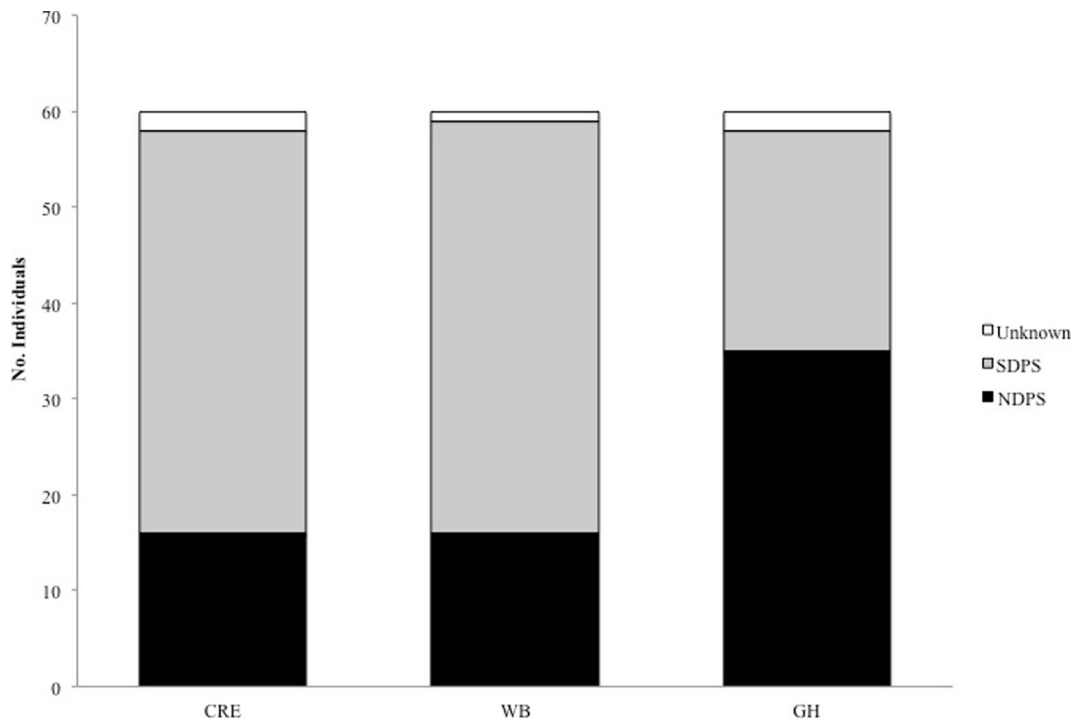
#### DPS length distributions

Mean fork length ranged from 134.4–142.2 cm for SDPS and 112.2–127.5 cm for NDPS green sturgeon. Fork length tended to increase moving northward along the coast (Columbia River estuary to Willapa Bay to Grays Harbor), but when we compared mean sizes of for each DPS across the three estuaries, there were no significant differences (SDPS  $F = 1.22$ ,  $P = 0.29$ ; NDPS  $F = 1.86$ ,  $P = 0.164$ ). In other words, the mean size of SDPS green sturgeon was consistent across

**Table 2** AFLPOP and Structure results for individuals classified as “unknown.” Year refers to the year sampled and Estuary provides the sampling location. The Structure 1 analysis incorporated no prior while the Structure 2 analysis used AFLPOP allocation as a prior. The results can be interpreted as follows. Individual

11FA33 exhibited proportional ancestry of 0.69 in the SDPS (69 % of genome originates from SDPS), was allocated to the SDPS by AFLPOP, but assigned to the SDPS with a posterior probability of 0.23 when membership in SDPS was used as a prior. NA = no allocation possible in AFLPOP

Individual	Year	Estuary	Structure 1	AFLPOP	Structure 2
11FA33	2011	Willapa Bay	SDPS ( $Q = 0.69$ )	SDPS	SDPS ( $Q = 0.23$ )
11FB120	2011	Grays Harbor	NDPS ( $Q = 0.61$ )	NA	NA
11FB17	2011	Grays Harbor	SDPS ( $Q = 0.56$ )	NDPS	NDPS ( $Q = 0.81$ )
11FC016	2011	Columbia River estuary	SDPS ( $Q = 0.73$ )	SDPS	SDPS ( $Q = 0.11$ )
12FP023	2012	Columbia River estuary	NDPS ( $Q = 0.60$ )	NDPS	NDPS ( $Q = 0.54$ )



**Fig. 2** The Columbia River Estuary (CRE) and Willapa Bay (WB) contain a higher proportion of SDPS individuals than Grays Harbor (GH)

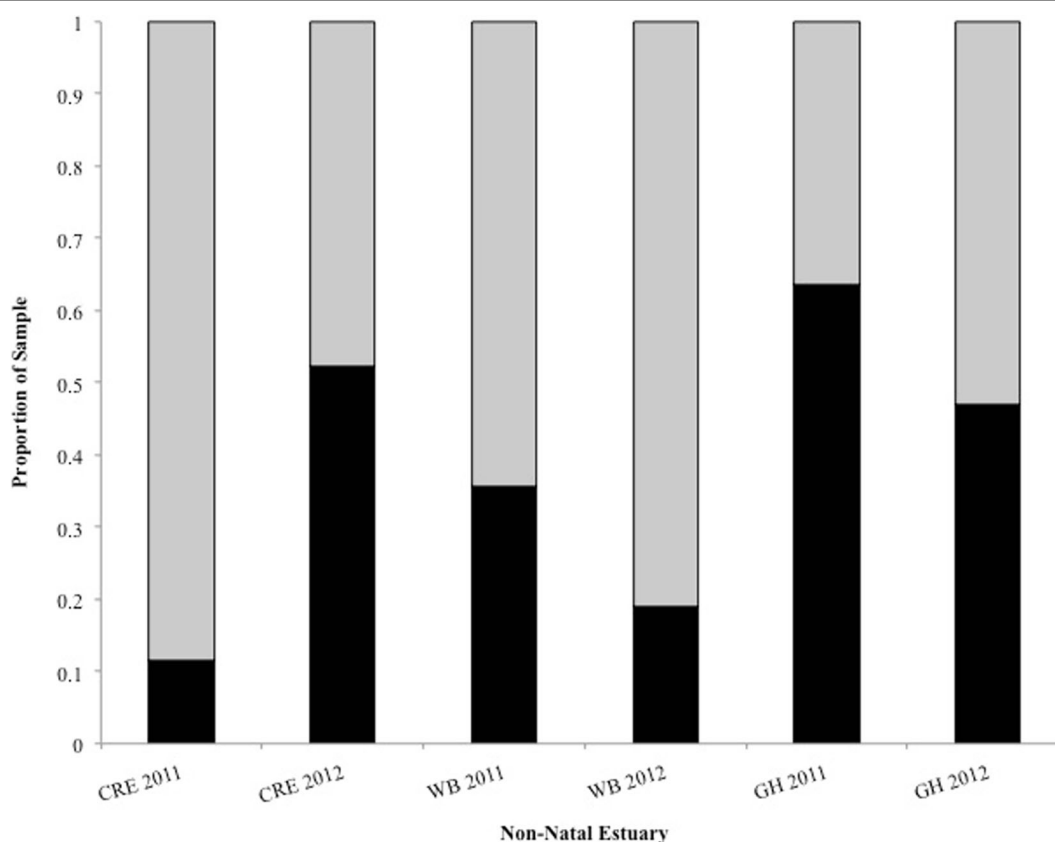
estuaries as was the mean size of NDPS green sturgeon. Fork length comparisons between DPSs showed that NDPS green sturgeon dominated the smaller size classes. Welch's t-test revealed that NDPS individuals had significantly smaller mean fork length than SDPS within all estuaries and in the overall collection (Table 3; Fig. 4).

## Discussion

Our results allow us to draw some general conclusions about DPS-specific patterns of non-natal estuary use. As in previous studies (Israel et al. 2009; Lindley et al. 2011), we find that SDPS green sturgeon predominate collections from the Columbia River estuary and Willapa Bay, despite evidence from spawning river surveys suggesting that SDPS fish were less abundant than NDPS fish in 2010–2012 (NMFS 2015; Mora 2016). We also find that NDPS green sturgeon are more common in Grays Harbor than in the other two estuaries, similar to observations in Israel et al. (2009). Unlike Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) estuarine aggregations, which are often dominated by the most geographically proximate spawning population

(Dunton et al. 2012; Wirgin et al. 2012; Waldman et al. 2013), it appears that non-natal estuary use in green sturgeon is not merely a function of geographic distance. Factors such as stage or state specific needs and social learning could influence non-natal estuary composition (Lindley et al. 2011). It is also possible that our study estuaries represent only a portion of the species' over-summer range and NDPS green sturgeon also utilize smaller, less studied coastal estuaries in Oregon or migrate further north of Grays Harbor. Several acoustic tagged green sturgeon were detected off the northwest coast of Vancouver island during the study period (O. Langness, unpublished data). Monitoring small, Oregon estuaries and alleged green sturgeon winter aggregation sites including the inlets along the Hecate Strait and waters off the north end of Vancouver Island (Lindley et al. 2008) may reveal additional over-summer sites for the NDPS. An increased migratory range for NDPS green sturgeon would explain why this population exhibits lower proportions in the Columbia River Estuary and Willapa Bay despite its greater adult abundance in 2010–2012 spawning river surveys (NMFS 2015; Mora 2016).

Annual differences in estuarine composition between 2011 and 2012 were similar in magnitude to changes



**Fig. 3** Annual changes in Distinct Population Segment (DPS) composition for the Columbia River Estuary (CRE), Willapa Bay (WB), and Grays Harbor (GH). NDPS coded in black and SDPS in grey. Unknown individuals are not included

observed in Umpqua River estuary (Winchester Bay) between 2000 and 2002 (Israel et al. 2009). Interestingly, the proportion of SDPS green sturgeon in the Columbia River estuary decreased in the same year (2012) that SDPS were captured more frequently in Willapa Bay and Grays Harbor. Our data don't allow us to determine whether these phenomena are directly related but future monitoring of these acoustic tagged fish

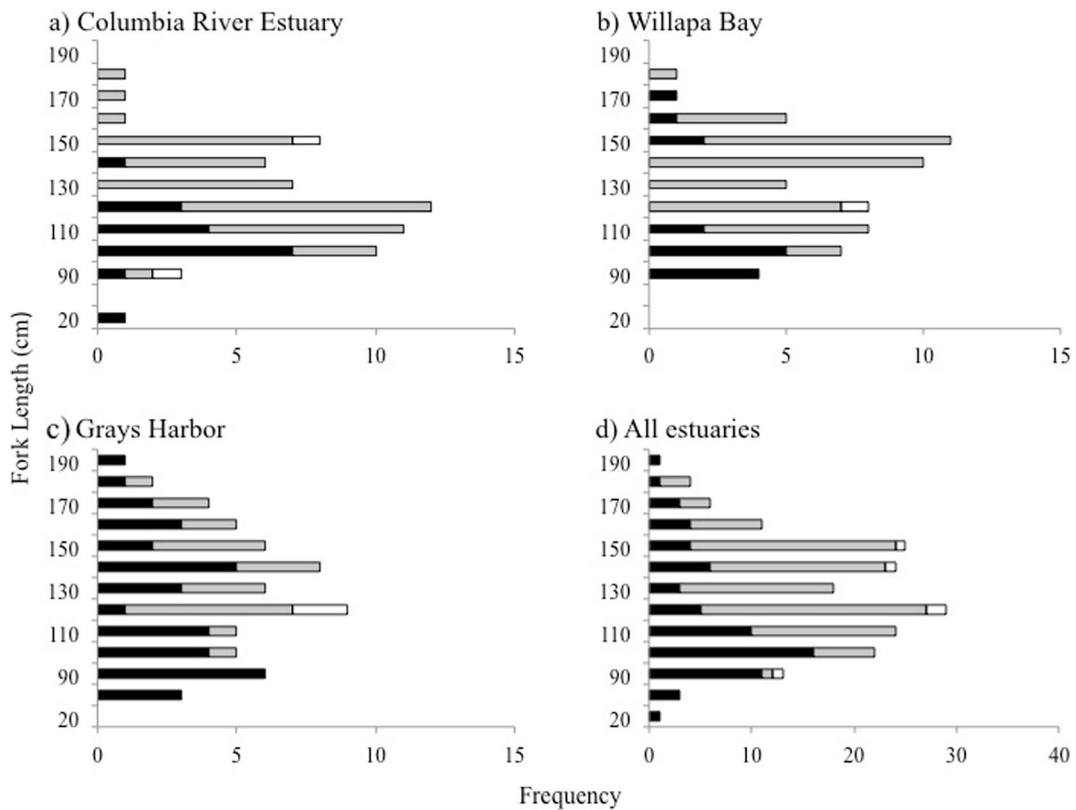
could identify whether individuals favor different estuaries in different years. Factors that could contribute to interannual variation in non-natal estuarine use include changes in freshwater, estuarine or marine conditions and possibly DPS specific fluctuations. For example, SDPS spawning adults often over-summer in the Sacramento River or the San Francisco Bay-Delta rather than directly re-entering marine habitat after spawning (Heublein et al. 2009). In years where many adults spawn in the Sacramento River, fewer SDPS adults would enter non-natal estuaries if many chose to over-summer in their natal drainage. Future studies should pair DPS occurrence data over additional years with estuarine condition factors (e.g., water temperatures, salinity, tributary discharge, prey availability, predator abundance), ocean condition factors (e.g., Columbia River plume, upwelling, PDO), and DPS specific demographic variables (e.g., annual spawner abundance in natal rivers), to identify abiotic and biotic variables contributing to non-natal estuary selection.

**Table 3** Mean fork length for NDPS and SDPS green sturgeon within and across estuaries. X is mean fork length in cm.  $t_w$  refers to Welch's t statistic with degrees of freedom (df) in parentheses

Estuary	X <sub>NDPS</sub>	X <sub>SDPS</sub>	$t_w$ (df)	P value
Columbia River	106.9	134.4	4.24 (25)	< 0.001*
Willapa Bay	118.9	138.5	2.70 (21)	0.01*
Grays Harbor	127.5	142.2	2.18 (56)	0.03*
All	120.0	138.3	4.53 (104)	< 0.001*

\*Significant at  $\alpha = 0.05$





**Fig. 4** Fork length frequency histogram for the (a) Columbia River estuary, (b) Willapa Bay, (c) Grays Harbor, and (d) all estuaries. NDPS coded in *black*, SDPS in *gray*, and unknown individuals in *white*

Comparing size distributions for SDPS and NDPS green sturgeon revealed that SDPS green sturgeon were significantly larger than NDPS in all three estuaries. Lindley et al. (2011) also reported that green sturgeon using these three estuaries but not the San Francisco Bay, likely NDPS fish, were significantly smaller than green sturgeon using the same three estuaries in addition to San Francisco Bay, likely SDPS fish. In contrast, no significant differences in size distributions were found between DPSs contributing to non-natal estuary aggregations of Atlantic sturgeon in Long Island Sound (Waldman et al. 2013). We do not have sufficient data to explain size differences between DPSs in non-natal estuaries but we can develop some hypotheses. This predominance of larger SDPS fishes could reflect a greater abundance of adult SDPS fish than NDPS fish, although this is not supported by observations of riverine adult abundance (NMFS 2015; Mora 2016). Alternatively, size asymmetry between DPSs raises the possibility that movements into non-natal estuaries may

be influenced by age or size. One hypothesis is that SDPS and NDPS green sturgeon differ in their ages of saltwater entry as observed in Atlantic sturgeon, where ages of marine entry range from 2 to 6 years of age across the species' range (Dovel and Berggren 1983). NDPS green sturgeon from the Klamath River prefer saltwater (34 ppt) by seven months of age (~40 cm fork length) in lab experiments (Poletto et al. 2013) and enter marine habitat by 2.5–3.5 years of age (Allen et al. 2009) but the age of marine entry for SDPS green sturgeon is unknown. If SDPS green sturgeon entered marine habitat at older ages, they may be reaching non-natal estuaries at larger sizes. Another possibility is that migration ability is limited by age or size and only large SDPS green sturgeon are capable of reaching the Columbia River estuary, Willapa Bay, and Grays Harbor from the Sacramento River. Similarly, small NDPS green sturgeon would be found in nearer non-natal estuaries (Columbia River estuary, Willapa Bay) while larger individuals could migrate further

northwards to Grays Harbor and potential British Columbia aggregation sites. Our finding of a positive, though non-significant, trend in fork length from south to north among the three estuaries for each DPS is consistent with a relationship between age or size and migration distance. However, a test of these hypotheses would require age data for DPS assigned fish, knowledge of when SDPS green sturgeon first enter marine habitat, and more monitoring of putative green sturgeon aggregation sites north of Grays Harbor.

The finding of an age-0 (22 cm) green sturgeon in a tidal freshwater area of the Columbia River in 2011 provides strong biological evidence of green sturgeon spawning in the Columbia River, either due a straying event or existence of a spawning population there. This individual is smaller than the smallest juveniles detected in a beach seine survey of NDPS green sturgeon in the Klamath River (32–66 cm; estimated ages 1–4 years; Nakamoto et al. 1995). The size of this individual is also below the mean size ( $26.6 \pm 2.4$  cm TL) at which Allen et al. (2011) observed seawater tolerance in laboratory experiments that gradually acclimated age-0 (134 days post hatch) NDPS green sturgeon. Therefore, it is unlikely that an individual of this size could have survived a marine migration from the most proximate known spawning rivers, the Rogue and Klamath, to the Columbia River. Although small NDPS green sturgeon 80–100 cm in the Columbia River might be juveniles yet to undertake a marine migration, we note that SDPS individuals from 90 to 100 cm were recorded in the Columbia River estuary, Willapa Bay, and Grays Harbor, suggesting sturgeon in this size range are capable of marine migrations.

Although the age-0 sturgeon assigned to the NDPS with high probability in population assignment testing, it could not be allocated confidently to either DPS with AFLPOP suggesting uncertainty regarding the origin of its parents. We suggest that although this individual belongs to the NDPS, its parents may not originate from the Klamath or Rogue rivers. Israel et al. (2009) reported that NDPS green sturgeon sampled from the Columbia River estuary and Grays Harbor exhibit empirical cumulative density functions that differ from the Klamath River and Rogue River genetic baseline, indicating that a third spawning population may be contributing to those collections. We recommend genetic analysis of any adults or additional age-0 green sturgeon captured in the Columbia River to better characterize population genetic diversity within this segment of the NDPS.

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