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7   **Straying and life history of adult steelhead in a small California coastal stream revealed by**

8   **otolith natural tags and genetic stock identification.**

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26   Running head: Steelhead straying

27   **Abstract**

28   Straying has been difficult to study directly in natural steelhead *Oncorhynchus mykiss*

29   populations. We analyzed an opportunistic sample of seven adult steelhead from a small basin

30   on the Big Sur coast of California to determine life-history traits including whether fish were

31   strays. Otolith natural tags ($^{87}Sr/^{86}Sr$) and genetic stock identification (GSI) indicated that all

32   seven adults were strays from at least six different sources. Three adults strayed from nearby

33   streams (<72 km) on the Big Sur coast, while three strayed from distant sources including the

34   Klamath River (680 km to the north); the source of one stray could not be identified. Six strays

35   were progeny of steelhead mothers but one was the offspring of a nonanadromous mother. Six

36   were female and one was male, and all that could be genotyped were homozygous (*n* = 4) or

37   heterozygous (*n* = 2) for the anadromy-associated haplotype in a migration-associated genomic

38   region. While the opportunistic nature and small size of the sample prevents us from inferring

39   the rate of straying into the basin, our study nonetheless demonstrates that steelhead may

40   stray across greater distances than generally appreciated and that nonanadromous females can

41   produce anadromous offspring that stray and hence provide connectivity among basins.

42   **Introduction**

43   Steelhead *Oncorhynchus mykiss* populations at the southern end of their range in

44   central and southern California have declined, and two distinct population segments (DPSs) of

45   winter-run steelhead in this region are currently listed as threatened or endangered under the

46   U.S. Endangered Species Act (U.S. Office of the Federal Register 2014). Many populations exist

47   in small coastal basins and are composed of both anadromous and nonanadromous individuals

48   (i.e., exhibit partial migration; Jonsson and Jonsson 1993). Among the important life-history

49   questions for understanding the persistence and dynamics of these populations include

50   identifying interactions among populations (through dispersal or "straying"), as well as factors

51   that influence anadromy versus residency (Boughton et al. 2007). Population genetic analyses

52   of coastal California *O. mykiss* populations indicate that there is some level of exchange among

53   populations that generally declines with distance (Garza et al. 2014), but there is very little

54	direct evidence of straying in natural populations due to the difficulty of sampling adult

55	steelhead, distinguishing natural-origin strays from returning fish, or identifying the natal

56	source of strays. In a review of the straying literature using Google Scholar and citations within

57	studies to identify all available data for steelhead on the West Coast of North America, we

58	found 25 papers with data on straying, but most were from hatchery populations, and there

59	were only five papers that examined natural populations: four for interior summer-run

60	steelhead in the Columbia River basin (Keefer et al. 2005, 2008; Marsh et al. 2012; Pearsons

61	and O'Connor 2020) and only a single study for coastal winter-run steelhead (Shapovalov and

62	Taft 1954), which found a low level of straying between two nearby streams (8 km apart) in

63	central California. Studies of hatchery-origin *O. mykiss* suggest donor straying rates are

64	generally low and most straying appears to be among populations from neighboring streams

65	(Westley et al. 2013; Keefer and Caudill 2014), although long-distance straying (100 to 450 km)

66	may occur less frequently (Schroeder et al. 2001).

67	Within *O. mykiss* populations that exhibit partial migration, some members of a

68	population appear more likely to migrate or to produce progeny that migrate than others.

69	There is growing evidence that expression of anadromy is influenced by both sex and genetics,

70	where anadromy is more common in females (Rundio et al. 2012; Ohms et al. 2013; Kendall et

71	al. 2015) and, at least for southern populations, in individuals with the migration-associated

72	haplotype of a large multi-gene complex on chromosome *Omy05* (Kelson et al. 2019; Pearse et

73	al. 2019). Anadromy is also associated with maternal life history, with progeny of anadromous

74	females more likely to migrate than progeny of nonanadromous females, although progeny of

75	both forms can express the alternative life history (Zimmerman and Reeves 2000; Thrower and

76	Joyce 2004; Courter et al. 2013).

77	Here we analyzed an opportunistic sample of seven adult steelhead from a small coastal

78	basin in central California to determine life-history traits including whether fish were strays,

79	maternal life history, sex, and genotype in the migration-associated region (*MAR*) of

80	chromosome *Omy05* (Leitwein et al. 2017). We used otolith natural tags (strontium isotopes

81	^{87}Sr/^{86}Sr) and genetic stock identification (GSI) to identify strays and their origins, taking

82	advantage of their ability to discriminate populations at local and regional spatial scales,

83 respectively (Barnett-Johnson et al. 2010; Miller et al. 2010; Johnson et al. 2016). We also

84 examined maternal life history, sex, and *MAR* genotype to identify traits that might be

85 associated with which members of the population migrate, stray, and potentially provide

86 connectivity among populations. Our purpose was simply to document life-history traits of

87 these fish given the paucity of data from natural-origin adult *O. mykiss* in coastal populations;

88 due to the nature and small size of our sample, inferences about rates of straying or

89 proportions of life-history traits for the population were not appropriate. While our sample was

90 small, natural-origin steelhead are difficult to sample after spawning for many reasons including

91 iteroparity, where post-spawning adults can leave the stream as kelts, so collections like ours,

92 and the insights they offer, are rare.

93

94 **[A] Methods**

95   [C] *Study area*.—Our study of adult steelhead focused on Big Creek, a small basin (58

96 km^2) located along the Big Sur coast (BSC) region of south-central California. We also sampled

97 water and juveniles from basins to the north and south of Big Creek, from the Carmel River

98 (Monterey County) to Santa Rosa Creek (San Luis Obispo County), to identify potential sources

99 of strays (Figure 1, Table 1). Steelhead populations in this region are winter run, with adults

100 maturing in the ocean and spawning shortly after returning to streams in winter to spring. This

101 section of the coast is relatively undeveloped and includes 37 named basins and numerous

102 smaller unnamed streams. Most basins are small (70% are <30 km^2), steep, and do not

103 penetrate the coastal Santa Lucia Range; the one exception is the Carmel River, which drains

104 the northeastern side of the Santa Lucia Range and is much larger than other basins along this

105 section of the coast. Natural waterfall migration barriers on Big Creek and many other regional

106 streams restrict anadromous *O. mykiss* to small portions of the lower basin (e.g., 7 stream km in

107 Big Creek), while nonanadromous forms occur upstream and downstream of these barriers. The

108 geology of the region is heterogeneous (Figure 1). The northern portion is composed of granitic

109 and older metamorphic rocks, while the southern portion is dominated by a mix of sedimentary

110 and metamorphic rocks known as the Franciscan complex (Henson and Usner 1993).

111   [C] *Field collections.*—A total of seven anadromous adult *O. mykiss* was collected

112   opportunistically in the lower reaches of Big Creek (<2.5 km from the ocean) over 12 years from

113   2006 to 2017 (Table 2). Six of the seven adults were found dead in the stream or on the stream

114   bank during the spring, presumably after they had spawned. Five of the six were collected as

115   carcasses and one was a dried skeleton still containing fins. One adult (Fish 7) was an incidental

116   mortality during a juvenile electrofishing survey in the fall.

117   To construct a reference baseline of $^{87}Sr/^{86}Sr$ ratios for streams along the Big Sur coast,

118   juvenile *O. mykiss* and water samples were collected from Big Creek and 16 other streams

119   (Table 1). Juvenile *O. mykiss* were collected from Big Creek in nine years from 2004–2017.

120   These fish were incidental electrofishing mortalities from a series of long-term ecological

121   studies. In addition, ten Coastrange Sculpin *Cottus aleuticus* were collected from Big Creek in

122   2017 to compare otolith $^{87}Sr/^{86}Sr$ to water collections during the same period. Juvenile *O.*

123   *mykiss* were collected by electrofishing from nine streams neighboring Big Creek in 2005–2006

124   (Table 1). These collections were limited because *O. mykiss* on the Big Sur coast are listed as

125   threatened under the Endangered Species Act. A small number of juveniles were collected from

126   two other streams (Carmel and Big Sur rivers) from 2001 to 2016. These fish also were

127   incidental mortalities from a variety of juvenile electrofishing surveys conducted by National

128   Oceanic and Atmospheric Administration personnel that had been archived and stored in an

129   ultralow (-80 C) freezer.

130   Water samples were collected from Big Creek and eight other streams to supplement

131   the juvenile sampling and expand the spatial and temporal range of the reference set of

132    $^{87}Sr/^{86}Sr$ values. One or two water samples were collected from seven basins with few or no

133   juvenile samples (Table 1). Because of the watershed size, water was sampled at five sites on

134   the Carmel River to document potential variation among tributaries; sites were in the middle

135   basin and included the mainstem, as well as San Clemente and Tularcitos creeks. Water was

136   collected from Big Creek at roughly monthly intervals from both the north (upper Big Creek)

137   and south (Devils Creek) forks (the two main branches) from August 2016 to September 2017 to

138   document seasonal and spatial variation in $^{87}Sr/^{86}Sr$ ratios within the focal study stream. All

139   water samples were stored in the dark until analysis.

140   [C] *Analysis of $^{87}Sr/^{86}Sr$ ratios.*—Otoliths of adults and juveniles were removed, cleaned

141   with Milli-Q water, stored dry, and later mounted in thermoplastic resin (Crystalbond, Aremco

142   Products), then polished in the sagittal plane to the level of the primordia. Otoliths were

143   individually prepared then transferred to petrographic slides for analysis using the method of

144   Donohoe and Zimmerman (2010).

145   Strontium isotopic ($^{87}Sr/^{86}Sr$) ratios of water and otoliths were measured on a Nu

146   Plasma multi-collector inductively coupled plasma mass spectrometer (MC-ICPMS) at the

147   University of California, Davis Interdisciplinary Center for Plasma Mass Spectrometry using

148   methods similar to Courter et al. (2013) and Sturrock et al. (2015). The $^{87}Sr/^{86}Sr$ ratios of water

149   samples were measured on the MC-ICPMS in solution mode. Water samples were first

150   concentrated by evaporation then purified by passing the sample through a column of

151   strontium specific resin (Eichrom Technologies). The $^{87}Sr/^{86}Sr$ ratios in otoliths were measured

152   by ablating with a 213 nm Nd:YAG UV laser (New Wave Research) operated at a pulse

153   frequency of 10 hertz and beam diameter of 40 µm. Otoliths of adults and juveniles were first

154   ablated using a single line transect from the core to the mid-dorsal margin with the laser

155   moving at 5 µm sec^{-1}. The resulting $^{87}Sr/^{86}Sr$ profiles provided an isotopic life history that

156   spanned an inner maternal region, a freshwater juvenile region, and, in adults, an outer marine

157   region (Kalish 1990, Donohoe et al. 2008, Brennan et al. 2015). Profiles were inspected to

158   locate the transition from the maternal region to the juvenile freshwater region. This transition

159   was distinct in most profiles and typically occurred at a radius of 200–300 µm. For adult

160   otoliths, the juvenile region was re-sampled using a series of discrete spots spaced 40 µm apart

161   to increase precision of the measured $^{87}Sr/^{86}Sr$ ratio. Each spot was ablated for 25 sec.

162   Raw strontium isotope data from the MC-ICPMS were corrected for interferences,

163   instrument bias and drift (Barnett-Johnson et al. 2005, Sturrock et al. 2015). Isotope voltages

164   from the MC-ICPMS were first integrated over 0.2 sec intervals, the 0.2 sec integrations were

165   further aggregated into 1 sec blocks, outliers (>2 SD) were discarded, and remaining data were

166   used to calculate the $^{87}Sr/^{86}Sr$ ratio for each ablation. Interferences from krypton (Kr) were

167   blank subtracted by measuring background voltages for 30 sec prior to each set of ablations.

168   Mass bias of the instrument was corrected by monitoring the instantaneous $^{86}Sr/^{88}Sr$ ratio and

169   normalizing to the natural $^{86}Sr/^{88}Sr$ ratio of 0.1194. Interferences from ^{87}Rb (rubidium) were

170   removed by monitoring ^{85}Rb. Instrument bias and drift was monitored and corrected for by

171   measuring $^{87}Sr/^{86}Sr$ ratios on the NIST SRM 987 standard (for water) or a marine otolith

172   standard at regular intervals during the analysis day and normalizing to the standard value.

173   The mean $^{87}Sr/^{86}Sr$ ratio of the otolith natal region ($^{87}Sr/^{86}Sr_{NATAL}$) was calculated using

174   either the individual spot analyses (adults) or the portion of the line transect (juveniles)

175   collected within the early juvenile region of the otolith, as identified from profiles. Spots or

176   portions of the profile in later juvenile regions were included in the calculation of the mean if

177   the $^{87}Sr/^{86}Sr$ ratio in the early juvenile region remained stable into the late juvenile period. The

178   included data extended up to 920 μm from the otolith core in adults and up to 700 μm in

179   juveniles.

180   [C] *Genetic analyses and GSI.*—Tissue samples (fin clips) were taken from adult carcasses

181   and then air dried for later analysis. Total DNA was extracted from dried fin clips using the

182   DNeasy 96 filter-based nucleic acid extraction system on a BioRobot 3000 (Qiagen), following

183   the manufacturer's protocols. DNA extractions were diluted 2:1 with distilled water and used

184   for polymerase chain reaction pre-amplification. Genotyping of the 96 single nucleotide

185   polymorphism (SNP) locus panel described by Abadía-Cardoso et al. (2013) was conducted

186   using TaqMan (Applied Biosystems) and SNP Type (Fluidigm) assays and 96.96 SNP Genotyping

187   Dynamic Arrays on an EP1 system (Fluidigm). Two negative controls were included in each array

188   and genotypes were called using Fluidigm SNP Genotyping Analysis software. The SNP loci

189   included a sex identification assay (Brunelli et al. 2008) and two SNPs on *O. mykiss* chromosome

190   05 (Omy114448 and R04944) that were used to infer *MAR* genotypes (AA, AR, and RR, where A

191   and R are the haplotypes associated with anadromous and resident/nonanadromous life

192   histories, respectively; Leitwein et al. 2017; Pearse et al. 2019).

193   In order to identify the genetic origins of adults, we compiled a reference dataset

194   representing 57 *O. mykiss* populations in California. The dataset included population samples

195   from the 16 coastal and 30 Central Valley *O. mykiss* populations examined by Pearse and Garza

196   (2015), and supplemental population samples collected from Big Creek and 10 other BSC

197   streams (Little Sur River and San Jose, Garrapata, Rocky, Partington, Limekiln, Mill, Prewitt,

198 Willow, and Salmon creeks). The total north–south extent of the dataset was from

199 approximately 40 km south of Big Creek (Salmon Creek) to more than 700 km north of Big Creek

200 (Smith River, near the border with Oregon). The combined genotype data overlapped for a total

201 of 90 SNP loci that were included in the final dataset for GSI.

202   Sufficient genotype data were obtained for GSI for five of the seven adults. Of the two

203 fish that did not yield data for population assignment, one adult (Adult 6) was genotyped

204 repeatedly but failed at 30–55% of the loci and was completely homozygous at all loci that

205 successfully amplified, but the sex identification and *MAR* SNPs amplified and produced data

206 for those traits. The other adult (Adult 1) was a dried skeleton (still containing fins) and was not

207 successfully genotyped for any genetic data.

208   [C] *Identification of strays and their origins*.—Adults were classified as a stray if all or a

209 substantial portion of the $^{87}Sr/^{86}Sr$ profile did not overlap the $^{87}Sr/^{86}Sr$ signature for Big Creek.

210 The $^{87}Sr/^{86}Sr$ signature of Big Creek was defined by the minimum and maximum $^{87}Sr/^{86}Sr$ ratios

211 for all water samples and otolith $^{87}Sr/^{86}Sr_{NATAL}$ for all juvenile *O. mykiss* and Coastrange Sculpin

212 collected from Big Creek.

213   The source of strays was identified using GSI combined with the natural otolith tags. The

214 five adults that were successfully genotyped for GSI were assigned to a geographic region of

215 California using the R package *Rubias* (Anderson et al. 2008; Moran and Anderson 2019) and

216 the reference baseline of 57 California populations, where assignment probabilities to

217 reference populations within a region were summed to yield a regional assignment probability.

218 Strays assigned to the BSC region by GSI were then evaluated by otolith natural tags for

219 possible specific sources streams within the BSC by comparing the otolith $^{87}Sr/^{86}Sr_{NATAL}$ and

220 $^{87}Sr/^{86}Sr$ profile for overlap with the $^{87}Sr/^{86}Sr$ signature (minimum and maximum) of individual

221 BSC streams. To evaluate overlap for the seven BSC streams with only one or two samples, we

222 estimated a minimum and maximum $^{87}Sr/^{86}Sr$ ratio using the sample mean and a range of

223 0.00046, the average range in the $^{87}Sr/^{86}Sr$ ratio for ten BSC streams with nine or more samples

224 (Table 1). For strays not assigned to the BSC, including those without genetic data, $^{87}Sr/^{86}Sr_{NATAL}$

225 and $^{87}Sr/^{86}Sr$ profiles were compared to $^{87}Sr/^{86}Sr$ ratios of BSC streams and published $^{87}Sr/^{86}Sr$

226 values for other California streams to identify potential sources of strays within the assigned

227   genetic region or within California (Ingram and Weber 1999; Barnett-Johnson et al. 2008;

228   Hodge et al. 2016).

229   [C] *Determination of life-history traits of adults.*—The sex of adults was determined from

230   field and laboratory examination of the carcass based on external morphology (coloration,

231   presence/absence of kype jaw, etc.) and dissection in a couple cases; sex of the skeleton sample

232   was determined by presence of kype jaw. Phenotypic sex determinations were confirmed by

233   genetic analysis of the sex identification SNP locus for the six adults that could be genotyped for

234   that locus. *MAR* genotype (AA, AR, or RR) was assigned as described above. Otolith $^{87}Sr/^{86}Sr$

235   profiles of adults were examined to determine the migratory life-history form of the mother.

236   Otolith $^{87}Sr/^{86}Sr$ ratios in the core ($^{87}Sr/^{86}Sr_{CORE}$) of *O. mykiss* reflect the $^{87}Sr/^{86}Sr$ ratio of the

237   water where the mother matured (Kalish 1990; Miller and Kent 2009). Consequently, adults

238   were classified as the progeny of a nonanadromous mother if $^{87}Sr/^{86}Sr_{CORE}$ was similar to

239    $^{87}Sr/^{86}Sr_{NATAL}$ and the $^{87}Sr/^{86}Sr$ profile was relatively constant across the maternal and early

240   juvenile regions. Adults were classified as the progeny of an anadromous mother (steelhead) if

241    $^{87}Sr/^{86}Sr_{CORE}$ and inner profile were shifted towards the marine value of 0.70917 relative to

242    $^{87}Sr/^{86}Sr_{NATAL}$.

243

244   **[A] Results**

245   [B] $^{87}Sr/^{86}Sr$ ratios of BSC streams

246         $^{87}Sr/^{86}Sr$ ratios varied among Big Creek and the other BSC streams (Table 1, Figure 2).

247    $^{87}Sr/^{86}Sr$ ratios were higher for northern streams and lower for southern streams, with a

248   distinct shift between Prewitt and Willow creeks (Figure 2). The north-south pattern in $^{87}Sr/^{86}Sr$

249   ratios coincided with a broad geographic pattern in geology, with northern basins composed of

250   larger regions of older rock types, and southern basins composed primarily of younger rock

251   types (Figure 1). Although $^{87}Sr/^{86}Sr$ signatures varied considerably from north to south,

252   signatures of neighboring streams often overlapped (Figure 2).

253   Analysis of water samples showed that $^{87}Sr/^{86}Sr$ ratio of Big Creek was stable across

254   months and seasons, but differed between the two forks (Table 1, Figure 2). The $^{87}Sr/^{86}Sr_{WATER}$

255   from the north fork was consistently higher than the south fork. The difference in mean

256    $^{87}Sr/^{86}Sr_{WATER}$ between the two forks explains the larger variation and greater range in

257    $^{87}Sr/^{86}Sr_{NATAL}$ among juveniles from Big Creek because they were collected from both forks. The

258    $^{87}Sr/^{86}Sr$ ratios of water collected in 2017 and otolith $^{87}Sr/^{86}Sr$ ratios of sculpins collected in

259   2017 did not differ from those of juvenile *O. mykiss* collected from 2004–2016 (ANOVA: df =

260   2,71; *P* = 0.63).

261

262   [B]Straying and origin of adults

263   Otolith natural tags revealed that all seven adult *O. mykiss* collected from Big Creek

264   were strays. Five adults had otolith $^{87}Sr/^{86}Sr$ profiles that did not overlap the $^{87}Sr/^{86}Sr$ signature

265   of Big Creek, clearly indicating they were strays (Table 2, Figures 2, 3). For the other two adults,

266   some areas of the $^{87}Sr/^{86}Sr$ profiles overlapped the $^{87}Sr/^{86}Sr$ signature of Big Creek but

267   substantial portions of the profiles were outside the range for Big Creek, indicating these two

268   adults could not have reared in Big Creek and also were strays. All seven adults had $^{87}Sr/^{86}Sr$

269   ratios in the outer otolith that converged on the marine value of 0.70917, confirming that all

270   had migrated to sea. Results of GSI combined with otolith natural tags showed that the seven

271   adults had reared in at least six different streams or sources. As described below in detail, three

272   adults strayed from nearby streams on the BSC, three strayed from distant sources, and the

273   source of one was uncertain.

274   Three adults were assigned by GSI to the BSC region with high probabilities (≥ 0.95;

275   Table 2). They had otolith $^{87}Sr/^{86}Sr_{NATAL}$ that matched either of two different sets of streams on

276   the BSC, which showed that among them they had strayed from at least two different streams

277   and possibly three. One stray (Adult 2) had otolith $^{87}Sr/^{86}Sr_{NATAL}$ and $^{87}Sr/^{86}Sr$ profile that

278   overlapped the $^{87}Sr/^{86}Sr$ signatures of a group of four streams 36 to 72 km south of Big Creek

279   (Figure 2). The other two adults (Adults 3 and 4) had otolith $^{87}Sr/^{86}Sr_{NATAL}$ that were higher than

280   the signature of Big Creek, and $^{87}Sr/^{86}Sr$ profiles most closely matched the $^{87}Sr/^{86}Sr$ signatures

281   of the Carmel and Little Sur rivers located 65 and 42 km north of Big Creek, respectively (Table

282   2, Figure 2).

283   Two adults were assigned by GSI to a population in the Klamath River region (Table 2).

284   Otolith $^{87}Sr/^{86}Sr_{NATAL}$ differed for the two adults indicating they strayed from two different

285   sources. One of these (Adult 5) had otolith $^{87}Sr/^{86}Sr_{NATAL}$ that did not match any BSC stream,

286   which is consistent with the genetic assignment to a more distant source. The other stray (Adult

287   7) had otolith $^{87}Sr/^{86}Sr_{NATAL}$ and $^{87}Sr/^{86}Sr$ profile that overlapped the $^{87}Sr/^{86}Sr$ signatures of

288   several BSC streams, but streams with these $^{87}Sr/^{86}Sr$ values are also found in the Klamath-

289   Trinity River basin. Notably, Adult 7 had no adipose fin and one otolith was composed largely of

290   vaterite, suggesting it had reared in a hatchery (Brown et al. 2013; Reimer et al. 2016). The

291      $^{87}Sr/^{86}Sr_{NATAL}$ of this fish overlapped the predicted $^{87}Sr/^{86}Sr$ signature of Trinity River Hatchery,

292   after correcting the published $^{87}Sr/^{86}Sr$ ratio of the stream for the effects of marine-derived

293   hatchery food on otolith $^{87}Sr/^{86}Sr$ ratios (Barnett-Johnson et al. 2008; Hodge et al. 2016).

294   For two adults, no genetic data were available for GSI assignment, but otolith values

295   indicated that one was from a distant source while the source of the other was ambiguous. For

296   Adult 6, otolith $^{87}Sr/^{86}Sr_{NATAL}$ was very low (0.70490) and the $^{87}Sr/^{86}Sr$ profile decreased to

297   0.70450 in the late juvenile region of the otolith. These values are much lower than any stream

298   on the BSC, indicating this adult strayed from a more distant source. In California, streams with

299      $^{87}Sr/^{86}Sr$ ratios in this range are most typical of basins dominated by young volcanic deposits

300   such as those in northeastern California (Barnett-Johnson et al. 2008; Bataille and Bowen 2012;

301   Hodge et al. 2016). This region is drained by tributaries of the Klamath and Sacramento rivers,

302   which enter the ocean 680 km and 210 km north of Big Creek respectively. For the other fish

303   (Adult 1), otolith $^{87}Sr/^{86}Sr_{NATAL}$ and $^{87}Sr/^{86}Sr$ profile overlapped the $^{87}Sr/^{86}Sr$ signatures of two

304   BSC streams but also with streams in other regions of California, so without GSI we could not

305   determine whether it had strayed from a nearby versus a more distant stream.

306

307   [B] Life-history traits of adults

308   Field and laboratory examinations showed that six adults were females and one was

309   male (Adult 1). Genetic analysis of the sex and *MAR* loci confirmed that the six adults that were

310   successfully genotyped for these markers were females, and were either homozygous (n = 4) or

311   heterozygous (n = 2) for the anadromy-associated (A) haplotype in the *MAR* (Table 2). Otolith

312      $^{87}Sr/^{86}Sr_{CORE}$ and $^{87}Sr/^{86}Sr$ profiles showed that six of seven adults were the progeny of

313   anadromous females and one (Adult 4) was the progeny of a nonanadromous female (Table 2,

314   Figure 3).

315

316   **[A] Discussion**

317   Our small, opportunistic sample of steelhead adults provided several important insights

318   about straying and life history of *O. mykiss*. Otolith natural tags combined with genetic markers

319   revealed that all seven of the steelhead adults collected in Big Creek were strays. These adults

320   strayed from multiple sources that included two or three nearby streams on the Big Sur coast,

321   two distant streams, and a distant hatchery, indicating that straying may occur across much

322   greater distances than has been generally appreciated. Further, all but one were female, and at

323   least six carried a haplotype associated with the anadromous life-history form. One stray was

324   the progeny of a nonanadromous mother, which provided limited but direct evidence that

325   nonanadromous females contribute to connectivity among *O. mykiss* populations on the BSC.

326   Although our sample was small, natural-origin steelhead are particularly difficult to sample

327   after spawning compared to semelparous salmon because post-spawning adults can leave the

328   stream as kelts, reducing the availability of carcasses for sampling, so studies like ours are rare.

329   Our primary finding was that steelhead strayed into Big Creek from multiple sources

330   that included both near and distant populations. The only other study of straying in natural,

331   winter-run steelhead was Shapovalov and Taft (1954), who found low rates of straying (< 3%)

332   between two small basins 8 km apart on the central California coast. Their study was based on

333   fish marked as juvenile outmigrants (natural-origin in one basin but hatchery-origin in the

334   other) and was only able to detect straying at a small spatial scale between the two streams.

335   Our study was able to detect straying across both small and large spatial scales, and we found

336   strays from both local populations, similar to Shapovalov and Taft (1954), and also very distant

337   populations. Three adults strayed from nearby basins 36 to 72 km away, although we could not

338   identify the exact source stream of immigrants due to overlap in the ^{87}Sr/^{86}Sr signatures of

339   neighboring streams. These results provide the first direct evidence of current straying among

340   basins on the Big Sur coast and are consistent with, and complement, indirect estimates of

341   straying based on genetic population structure. Basins on the Big Sur coast were genetically

342 similar and coastal populations showed a general pattern of isolation-by-distance (IBD) within

343 the central coast region and across California, suggesting gene flow was generally higher among

344 nearby populations (Garza et al. 2014). Straying among nearby streams also is consistent with

345 tagging studies of hatchery-origin salmonids that show straying rate decreases with distance

346 from the natal stream, with the majority of straying to neighboring streams, often within 10 to

347 50 km (Keefer and Caudill 2014). The other three adults that we could identify to source strayed

348 from distant locations: two from sources on the Klamath River (680 km north) and a third likely

349 from either the Klamath or Sacramento River (210 km north) basin, demonstrating that

350 steelhead in coastal basins of California can stray from considerable distances. Although long-

351 distance straying has not been reported in natural winter-run *O. mykiss* populations, it has been

352 observed in hatchery-origin winter-run *O. mykiss* along the Oregon coast where 24% of fish

353 strayed > 100 km (and up to 456 km), although the majority entered neighboring rivers

354 (Schroeder et al. 2001).

355   Although we were surprised that all adults in our sample were strays, the opportunistic

356 nature and small size of our sample make it inappropriate to infer the actual rate of straying

357 into the population. Our sample of seven fish was collected over 12 years and likely represents

358 a small percentage of the total run over that period. For example, the steelhead run in Big

359 Creek in 2007 was estimated at 22–33 adults based on sonar (DIDSON) surveys (Pipal et al.

360 2010), but our sample only included three fish from that year. Although we did not conduct

361 spawner surveys in other years, we saw eight or fewer spawners per winter during limited

362 observations. If we assume the average number of spawners was four to five adults per winter

363 during these years, our sample would represent 10% or less of the total return over the study

364 period. In addition, it is not clear whether our sample was representative or biased relative to

365 the entire population. For example, although we are not aware of any data, it seems plausible

366 that long-distance strays might have lower post-spawning survival than non-strays, either due

367 to lack of local adaption or greater migration distance, which could make them more likely to

368 be recovered as carcasses rather than leave the stream as kelts. Finally, we have detected adult

369 returns of fish that were PIT tagged in Big Creek as juveniles, so we know that not all adult

370 steelhead during those years were strays. Therefore, given uncertainty of how our small, non-

371 random sample reflects the entire population of returned adults, we cannot draw any

372 conclusions about the rate of straying into the population. Nevertheless, our study does

373 provide direct evidence that straying into Big Creek does occur and that the spatial scale of

374 movements by adult steelhead between basins ranges from tens to hundreds of kilometers

375 including across DPS boundaries.

376 Our results about the life-history traits of adult steelhead are consistent with recent

377 studies showing that anadromy in *O. mykiss* is associated with sex, genetics, and maternal life

378 history (Kendall et al. 2015) but they also provide new insights. First, our finding that all but one

379 adult steelhead in our sample were female is consistent with a female bias for anadromy in

380 many *O. mykiss* populations (Rundio et al. 2012; Ohms et al. 2013; Kelson et al. 2019).

381 Iteroparity also is strongly female biased in steelhead (Keefer et al. 2008; Copeland et al. 2019),

382 although we did not attempt to determine whether any of the fish in our sample were repeat

383 spawners. Second, the fact that all adult steelhead that could be genotyped were either

384 homozygous or heterozygous for the migratory *Omy05 MAR* haplotype is consistent with recent

385 studies that have found a strong association between this haplotype and expression of

386 anadromy in southern *O. mykiss* populations (Pearse et al. 2014; Leitwein et al. 2017; Kelson et

387 al. 2019; Pearse et al. 2019). Our results complement prior studies that were based primarily on

388 juveniles and nonanadromous adults. Finally, six of the adults in our study were progeny of

389 anadromous mothers but one had a nonanadromous mother, demonstrating that non-

390 migratory forms can produce migrants that stray and provide connectivity among basins on the

391 Big Sur coast. Previous studies have shown that although *O. mykiss* often express the same

392 migratory type as their mother, both forms can produce offspring of the alternative life history

393 (e.g., Zimmerman and Reeves 2000; Thrower and Joyce 2004; Courter et al. 2013). However,

394 our study is the first, to our knowledge, to document straying by an anadromous offspring of a

395 nonanadromous mother into a neighboring basin. This is an important finding that highlights an

396 additional aspect of life-history diversity that may promote resilience and persistence of small,

397 partially migratory populations of *O. mykiss*.

398 Combining otolith natural tags and genetic stock identification allowed us to identify

399 strays from both local and distant sources, which would not have been possible for most adults

400   in our sample using either technique alone. Like other studies that have used this approach

401   (Barnett-Johnson et al. 2010; Miller et al. 2010; Johnson et al. 2016), GSI provided information

402   about genetic origins of adults at a regional spatial scale (reflecting population structure of *O.*

403   *mykis*s in California; Garza et al. 2014; Pearse and Garza 2015) while otolith natural tags

404   provided complementary information at a finer scale (reflecting local differences in geology and

405   water chemistry). For example, adults that assigned by GSI to the BSC could only be identified

406   as strays with the addition of otolith $^{87}Sr/^{86}Sr$ data (Adults 2, 3, and 4). Conversely, otolith

407   natural tags alone could not identify the natal source of most adults because $^{87}Sr/^{86}Sr$ ratios of

408   BSC streams overlap those in other parts of California, including northern California and the

409   Central Valley (Ingram and Weber 1999; Barnett-Johnson et al. 2008; Sturrock et al. 2015;

410   Hodge et al. 2016). For example, one fish (Adult 7) had otolith $^{87}Sr/^{86}Sr$ values that overlapped

411   several streams within both the BSC and the Klamath –Trinity River basin, but GSI assigned it to

412   the Klamath, clarifying that it was a distant rather than local stray (vaterite in the otolith and a

413   clipped adipose fin further indicated that it was hatchery fish and consequently had to be from

414   a distant source). There was one exception that could be identified as a distant stray by otolith

415   natural tag alone without GSI: Adult 6 had otolith $^{87}Sr/^{86}Sr_{NATAL}$ that was extremely low and

416   matched stream $^{87}Sr/^{86}Sr$ ratios that appear to be limited to one volcanic region in northeastern

417   California as the nearest possible source.

418   Although we were successful at combining otolith natural tags and GSI to identify the

419   adult steelhead in our sample as strays from local versus distant sources, this approach had

420   several limitations in our study. First, it was not possible to identify the sources of strays to the

421   level of individual streams. As mentioned above, GSI assignments were limited to regional

422   population groups, and otolith natural tags identified two or more possible source streams for

423   each fish based on similar $^{87}Sr/^{86}Sr$ ratios (Figure 2, Table 2); overlapping $^{87}Sr/^{86}Sr$ ratios

424   occurred among BSC streams, between BSC streams and other regions, and among streams in

425   other regions. Second, although they did not occur in our sample, adults from several streams

426   in the BSC would be impossible to identify as strays into Big Creek by this approach because of

427    $^{87}Sr/^{86}Sr$ ratios that overlap with Big Creek (e.g., Carmel River, San Jose Creek, and Mill Creek;

428   Table 1 and Figure 2). Third, both GSI and otolith natural tags are dependent on the available

429 baselines used as reference datasets to match adult samples. In this study, our genetics

430 baseline was geographically extensive across California but did not include all potential source

431 populations (e.g., coastal California basins south of the BSC, or populations in Oregon and

432 Washington). Given that two fish assigned to the northern limit of our baseline, and two fish

433 (Adults 1 and 6) could not be genotyped for GSI, it is possible that that fish may have strayed

434 from even greater distances than we were able to detect. Likewise, our reference set of

435 ^{87}Sr/^{86}Sr values was fairly complete for the BSC but sparse for other regions. For example,

436 ^{87}Sr/^{86}Sr signatures of many tributaries in the Klamath and upper Sacramento river basins are

437 not known and prevented us from identifying potential source streams for Adults 5 and 6.

438 Despite these limitations, this can be a powerful approach for identifying origins and

439 movements of fishes across multiple spatial scales.

440

441 [B] Management implications

442   One of the key challenges of recovery planning for *O. mykiss* along the south-central and

443 southern California coasts has been assessing the long-term viability of ESA-listed populations

444 within these regions. Two main sources of uncertainty in assessing viability are the lack of data

445 on (1) the rate and pattern of straying among basins (i.e. the degree of demographic

446 independence among populations) and (2) the extent that nonanadromous forms contribute to

447 and potentially re-establish the anadromous life-history form, which is considered critical for

448 long-term viability of these populations. Straying and life-history exchange that provide

449 connectivity among basins or population segments are expected to stabilize population

450 dynamics and thereby lower the risk of extinction. Boughton et al. (2007) noted that depending

451 on the rate and pattern of straying, small coastal basins in these regions may range from

452 isolated populations (low straying), to local multi-basin metapopulations, to sink populations

453 dependent upon dispersal from more distant sources. Although our results do not provide

454 rates, they demonstrate that *O. mykiss* stray among neighboring coastal basins within the Big

455 Sur coast and also stray from distant sources. While these findings appear to suggest that *O.*

456 *mykiss* populations in Big Sur operate as a local metapopulation that also receives distant

457 strays, and hence may fall on the continuum between the local metapopulation and long-

458 distance sink models outlined by Boughton et al. (2007), they also highlight the need for further

459 studies on connectivity to improve viability models. In particular, further research on the

460 recipient straying rate and reproductive success of strays is needed to reconcile our finding of

461 long-distance straying with the IBD pattern in population genetic structure in coastal *O. mykiss*

462 in California (Garza et al. 2014). This information is critical for understanding the ultimate

463 demographic and population consequences of straying, which can have both positive and

464 negative effects that are likely to be especially influential in small recipient populations (Bett et

465 al. 2017). Our finding of long-distance straying also suggests that distant management actions

466 (e.g., hatchery production) may influence local populations, and that *O. mykiss* on the south-

467 central coast may be capable of straying to distant basins, such as those in southern California.

468 Our study provides direct evidence that nonanadromous females on the Big Sur coast do

469 produce steelhead progeny that stray, but the importance of nonanadromous forms in

470 maintaining or re-establishing the anadromous life-history form in populations within the study

471 region remains unclear.

472 In conclusion, our study provided important insights into straying and life history of

473 anadromous *O. mykiss* by applying otolith microchemistry and genetic analyses to a small,

474 opportunistic sample of adults from a natural population. Most notably, we found that straying

475 may occur across much greater distances than generally appreciated, and that offspring of

476 nonanadromous females may stray into different basins as adult steelhead. While it would be

477 difficult to replicate or scale-up our study to larger sample sizes because the approach requires

478 otoliths collected from adult carcasses, our study demonstrates that opportunistic samples,

479 despite their limitations, can provide valuable information and encourages the analysis of

480 similar rare, small collections when they are available.

481

482 **[A] Acknowledgments**

495   **[A] References**

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Table 1. $^{87}Sr/^{86}Sr$ ratios of Big Creek and 16 other sampled streams on the Big Sur coast of California based on the analysis of water (W) or otoliths (O) of juvenile *O. mykiss* (and for Big Creek, 10 Coastrange Sculpins) collected in each stream.

Stream	Code	Km from Big Creek	Sample type	Years	N	$^{87}Sr/^{86}Sr$ ratios Min	Max	Mean	SD
Carmel River	CAR	65	O	2002–16	13	0.71062	0.71119	0.71095	0.00019
			W	2017	5	0.70987	0.71102	0.71055	0.00043
San Jose Creek	SJ	64	O	2006	11	0.70973	0.71029	0.70995	0.00016
Garrapata Creek	GARR	55	W	2005	1			0.70976	
Little Sur River	LSUR	42	O/W	2006/17	1/1	0.71057	0.71099	0.71078	0.00029
Big Sur River	BSUR	34	O/W	2001/17	1/1	0.71174	0.71209	0.71191	0.00025
Partington Creek	PAR	15	O	2006	9	0.70923	0.70982	0.70960	0.00021
Big Creek	BIG	0	O	2004–17	38	0.70989	0.71061	0.71021	0.00018
north fork			W	2014–17	16	0.71043	0.71053	0.71048	0.00003
south fork			W	2014–17	17	0.70989	0.71014	0.70999	0.00007
Limekiln Creek	LIM	11	O	2006	10	0.70935	0.70974	0.70953	0.00014
Mill Creek	MIL	15	O	2005–06	12	0.70957	0.71006	0.70981	0.00017
Prewitt Creek	PRW	19	O	2005–06	10	0.70928	0.70948	0.70936	0.00007
Willow Creek	WIL	23	O	2005–06	10	0.70710	0.70734	0.70725	0.00008
Villa Creek	VIL	30	O	2005–06	28	0.70682	0.70728	0.70714	0.00009
Salmon Creek	SMN	36	O	2005–06	23	0.70760	0.70794	0.70777	0.00009
San Carpoforo Creek	SCRP	42	W	2017	1			0.70787	
Arroyo de la Cruz	ADLC	48	W	2017	1			0.70785	
San Simeon Creek	SSIM	70	W	2017	2	0.70732	0.70742	0.70737	0.00007
Santa Rosa Creek	SROS	72	W	2017	1			0.70771	

Table 2. Life-history traits and genetic and natal assignments of the seven adult steelhead strays collected from Big Creek, California. Genetic assignment to geographic region was based on analysis of single nucleotide polymorphisms (SNPs). Natal assignments to region and stream were made by comparing $^{87}Sr/^{86}Sr_{NATAL}$ to $^{87}Sr/^{86}Sr$ signatures of streams on the Big Sur coast (BSC) and published values (see Table 1 for abbreviations). Also shown are collection date, fork length (mm), otolith $^{87}Sr/^{86}Sr$ ratios in the core and natal regions, maternal life history form (A=anadromous, NA=nonanadromous), migratory life history genotype (*Omy05 MAR*; A=anadromous, R=resident), and whether the adipose fin had been clipped. (Fish 1 was a skeleton with fins present and FL was estimated from otolith size and skeleton length.)

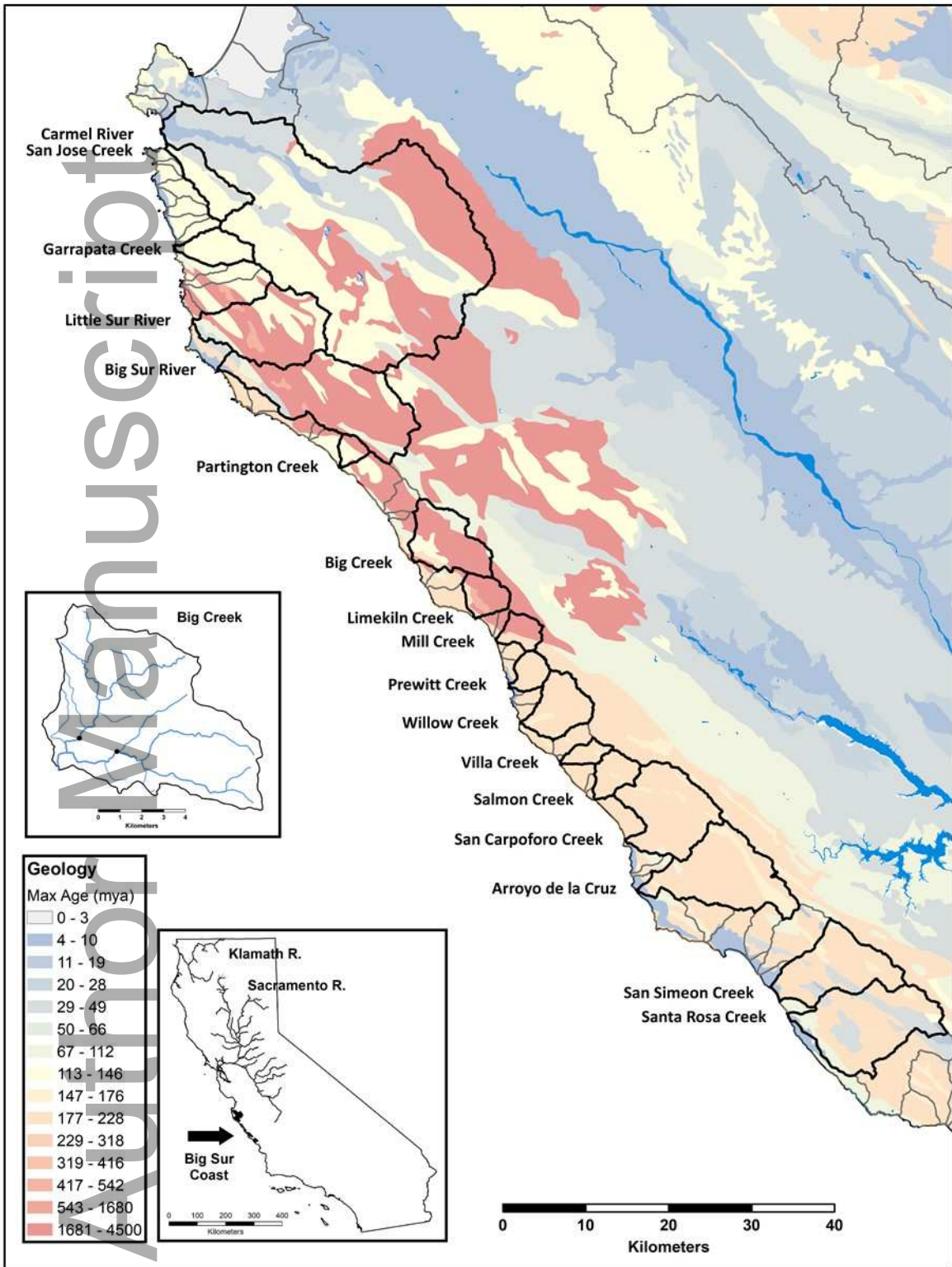|				Otolith $^{87}Sr/^{86}Sr$					Genetic assign.		Natal assign.		
Fish	Date	Sex	FL	Core	Natal	Mat LH	*MAR*	Ad clip	Region	Prob.	Match BSC?	Possible BSC stream	Source
1	5/31/06	M	600	0.70887	0.70713	A	-	N	-	-	Y	VIL, WIL, SSIM	ambiguous
2	3/27/07	F	660	0.70896	0.70794	A	AA	N	BSC	1.00	Y	SMN, SCRP, ADLC, SROS	BSC
3	4/12/07	F	740	0.70936	0.71065	A	AA	N	BSC	0.99	Y	CAR, LSUR	BSC
4	5/01/07	F	550	0.71082	0.71083	NA	AA	N	BSC	0.95	Y	CAR, LSUR	BSC
5	3/18/09	F	600	0.70845	0.70630	A	AR	N	Klamath R.	0.97	N	-	Klamath R.
6	5/22/13	F	425	0.70843	0.70490	A	AA	N	-	-	N	-	volcanic stream
7	9/20/17	F	348	0.70842	0.70782	A	AR	Y	Klamath R.	0.99	Y	SMN, SCRP, ADLC, SROS	Klamath R. (hatchery)

Figure captions

Figure 1. Geologic map of the Big Sur coast of California showing the locations of Big Creek and 16 other sampled basins (thick black outlines) and the maximum age of the underlying major rock type (Saucedo et al. 2000). Inset shows locations of migration barriers (black dots) on the two major forks of Big Creek, California.

Figure 2. Comparison of otolith $^{87}Sr/^{86}Sr_{NATAL}$ of the seven adult steelhead collected from Big Creek, California (horizontal lines, numbers 1 to 7) and the $^{87}Sr/^{86}Sr$ signatures (mean and range) of Big Creek and 16 other streams on the Big Sur coast of California. Values for otoliths
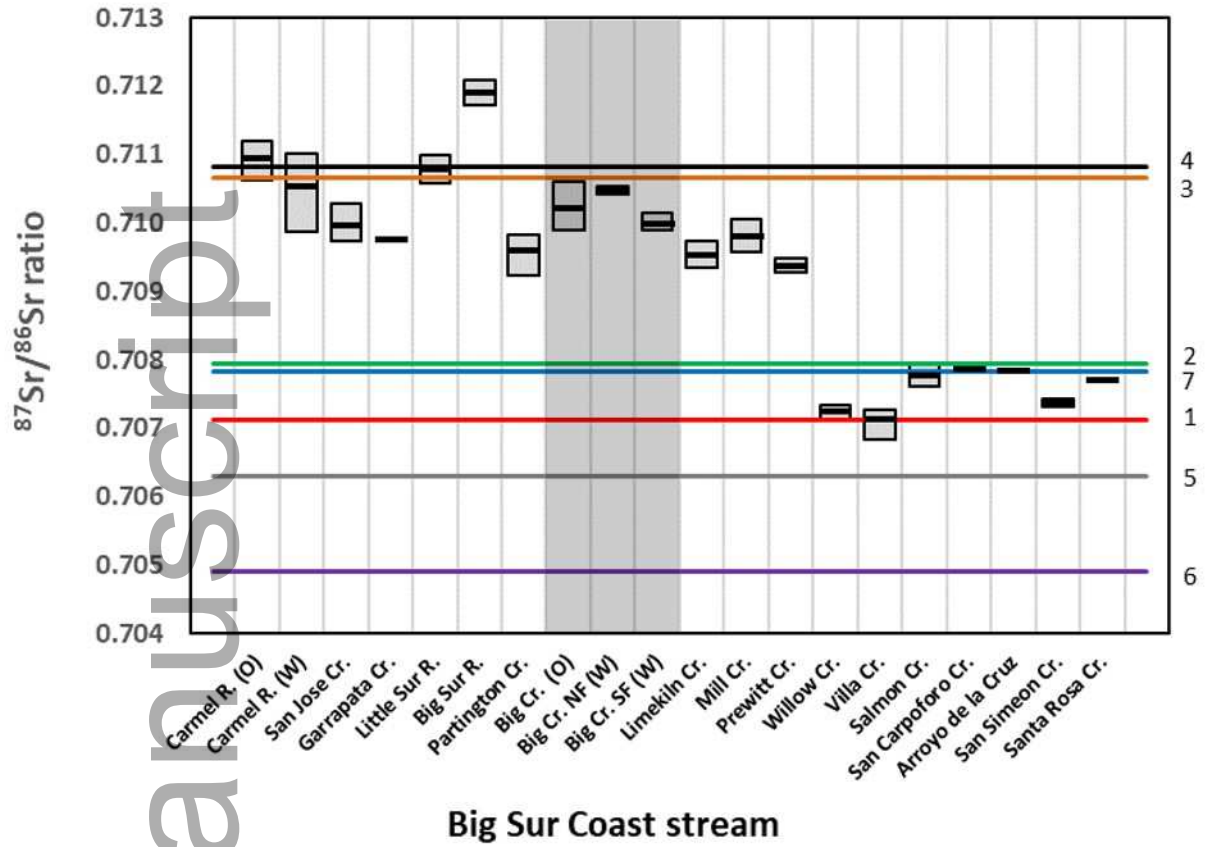
(O) and water (W) are shown separately for Big Creek (north and south forks) and the Carmel River. Streams are ordered from north to south.

Figure 3. Otolith ^{87}Sr/^{86}Sr profiles of seven adult steelhead (number 1-7) collected from Big Creek, California, from 2006-2017. Profile lines are smoothed ^{87}Sr/^{86}Sr ratios from a line transect (5 µm sec^{-1}) from core to edge. Circles are discrete 40 µm spot analyses primarily in the freshwater region. Regions near the core (<300 microns) are influenced by maternal life history, while rapid shifts in the outer otolith towards the ocean value of 0.70917 (solid line) represent seaward migration. Shaded region marks the minimum and maximum ^{87}Sr/^{86}Sr $_{NATAL}$ of juvenile *O. mykiss* and coastrange sculpins from Big Creek, California.

Carmel River
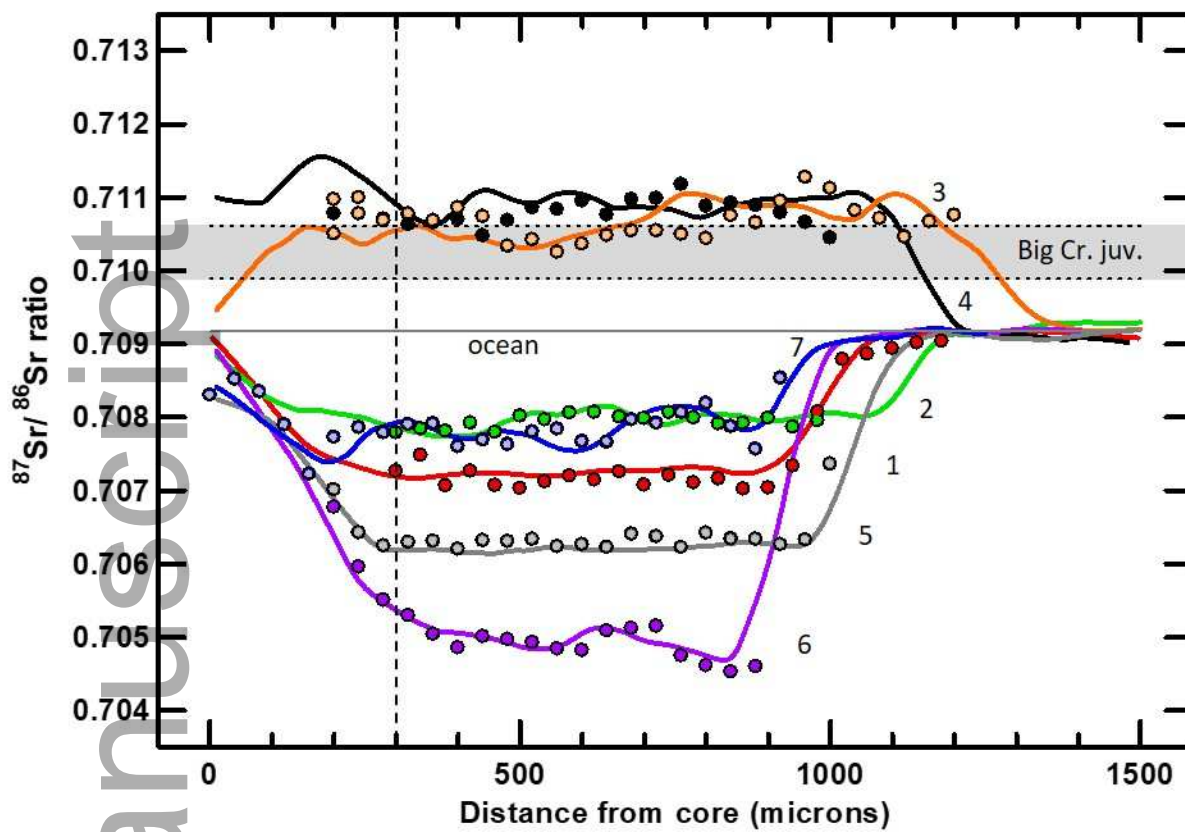San Jose Creek
Garrapata Creek
Little Sur River
Big Sur River
Partington Creek
Big Creek
Limekiln Creek
Mill Creek
Prewitt Creek
Willow Creek
Villa Creek
Salmon Creek
San Carpoforo Creek
Arroyo de la Cruz
San Simeon Creek
Santa Rosa Creek

Big Creek

Geology
Max Age (mya)
0 - 3
4 - 10
11 - 19
20 - 28
29 - 49
50 - 66
67 - 112
113 - 146
147 - 176
177 - 228
229 - 318
319 - 416
417 - 542
543 - 1680
1681 - 4500

Klamath R.
Sacramento R.
Big Sur
Coast

0   10   20   30   40
Kilometers

nafm_10577_f1.jpg

nafm_10577_f2.jpg

nafm_10577_f3.jpg