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16. ABSTRACT

Rangewide monitoring of fish species is critical for determining status and trends in abundance and distribution; however, implementations of large-scale distribution surveys have generally been constrained by time and cost. This study uses environmental DNA (eDNA) to monitor the presence or absence of two endangered tidewater goby species, the northern tidewater goby (Eucyclogobius newberryi) and the southern tidewater goby (Eucyclogobius kristinae), across their combined geographic range that encompasses the entire California coast (1,350 km). A multi-scale occupancy model designed specifically for eDNA methods was used to account for imperfect detection and to estimate true site occupancy. A total of 209 sites were surveyed in coastal California from Del Norte to San Diego counties between May and September 2016. Among these sites, 12 were dry during the survey and assigned a status of non-detection. Among the 197 sites with water present, 430 water samples were collected, filtered, and tested for the presence/absence of northern and southern tidewater goby, using species-specific quantitative PCR (qPCR) assays. The number of water samples collected per site ranged between one and six. Northern tidewater goby were detected at 81 out of 175 sites and southern tidewater goby were detected at 4 out of 22 sites, resulting in a combined naïve occupancy of 0.43. In contrast, the multi-scale occupancy model estimated site occupancy at 0.55 (95% CRI 0.46–0.64), indicating that tidewater goby were present but not detected at 23 additional sites. Even though eDNA typically has higher detection probabilities than traditional field approaches, these findings indicate that imperfect detection needs to be accounted for in eDNA surveys. Tidewater goby were detected at seven sites where they have previously not been detected or were thought to be extirpated, including one site in San Francisco Bay.

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RANGEWIDE TIDEWATER GOBY OCCUPANCY SURVEY USING ENVIRONMENTAL DNA

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ABSTRACT

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Rangewide monitoring of fish species is critical for determining status and trends in abundance and distribution; however, implementations of large-scale distribution surveys have generally been constrained by time and cost. This study uses environmental DNA (eDNA) to monitor the presence or absence of two endangered tidewater goby species, the northern tidewater goby (*Eucyclogobius newberryi*) and the southern tidewater goby (*Eucyclogobius kristinae*), across their combined geographic range that encompasses the entire California coast (1,350 km). A multi-scale occupancy model designed specifically for eDNA methods was used to account for imperfect detection and to estimate true site occupancy. A total of 209 sites were surveyed in coastal California from Del Norte to San Diego counties between May and September 2016. Among these sites, 12 were dry during the survey and assigned a status of non-detection. Among the 197 sites with water present, 430 water samples were collected, filtered, and tested for the presence/absence of northern and southern tidewater goby, using species-specific quantitative PCR (qPCR) assays. The number of water samples collected per site ranged between one and six. Northern tidewater goby were detected at 81 out of 175 sites and southern tidewater goby were detected at 4 out of 22 sites, resulting in a combined naïve occupancy of 0.43. In contrast, the multi-scale occupancy model estimated site

occupancy at 0.55 (95% CRI 0.46–0.64), indicating that tidewater goby were present but not detected at 23 additional sites. Even though eDNA typically has higher detection probabilities than traditional field approaches, these findings indicate that imperfect detection needs to be accounted for in eDNA surveys. Tidewater goby were detected at seven sites where they have previously not been detected or were thought to be extirpated, including one site in San Francisco Bay. As a covariate, salinity was found to have a strong negative effect on qPCR detection probability and tidewater goby DNA availability in a water sample. This finding implies that when using eDNA methods for species detection, more water samples and qPCR replicates might be needed at high salinity sites to achieve the desired level of detection. This study illustrates the power of eDNA for generating point-in-time snapshots of a species' entire geographic distribution. The distributional information generated herein is critical for management as it will serve as a baseline for determining site occupancy and if tidewater goby are expanding or contracting in the number of sites occupied.

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INTRODUCTION

Understanding habitat requirements and the geographic distribution of species in the face of climate change and continued human habitat alterations is vital for making appropriate conservation and management decisions (Hernandez et al. 2006). Marine and freshwater ecosystems alike are under various anthropogenic pressures, including overfishing, pollution, habitat fragmentation, invasive species introductions, and the rise of both sea level and water temperatures (Jackson et al. 2001; Dudgeon et al. 2006; Pimm et al. 2014; Valenti et al. 2016). For example, increasing trends of northward dispersal (Cheung et al. 2015), southern range constrictions (Reid and Goodman 2016), and southern range extirpations (Augerot and Nadel Foley 2005) of various species have been observed. Monitoring species distributions at the local and rangewide levels is critical for understanding and preserving biodiversity, since many species are either migratory across large ranges (Israel et al. 2009; Port et al. 2016; Starks et al. 2016), invasive and spreading (Goldberg et al. 2013; Schneider et al. 2016) or existing in fragmented populations with limited or no dispersal (Lafferty et al. 1999; Kinziger et al. 2015; Swift et al. 2016).

The tidewater goby (*Eucyclogobius newberryi*), which is listed as endangered under the US Endangered Species Act, has experienced a reduction in the number of isolated estuarine sites it inhabits due to coastal developments, droughts, and invasive species introductions (Swift et al. 1989; USFWS 2005). Tidewater goby are small (< 60 mm total length), cryptic, annual fish that inhabit lagoons, sloughs, and estuaries that are

separated from each other by distances of 1 to 20 kilometers (Dawson et al. 2002). Tidewater goby are endemic to California and their historic distribution spanned the entire coastline from Tillas Slough in Del Norte County to Agua Hedionda Lagoon in San Diego County (Swift et al. 1989; USFWS 2005, 2014). Dispersal between sites is thought to be rare, especially in the northern part of the species' range (McCraney et al. 2010; Kinziger et al. 2015). Consequently, tidewater goby show some of the highest levels of genetic differentiation among populations of all vertebrates along the California coast (McCraney et al. 2010; Earl et al. 2010; Kinziger et al. 2015). Although tidewater goby have been studied extensively in select local habitats, the rangewide occupancy status of habitats is poorly known (USFWS 2014). Out of the 135 historically documented populations, 16% are believed to be extirpated and about 50% of the remaining populations are considered vulnerable to extinction (USFWS 2005, 2014).

The tidewater goby recovery plan divided the original species into six recovery units and 26 sub-units, defined by genetic, morphological, and environmental variables (USFWS 2005). As an annual species, individual tidewater goby populations experience large fluctuations in abundance from year to year (Swift et al. 1989; Lafferty et al. 1999, USFWS 2005; Hellmair and Kinziger 2014). Thus, the fundamental units of conservation are not individual fish, but each population (Lafferty et al. 1999; USFWS 2005). According to the recovery plan, downlisting of tidewater goby can be considered when threats to the species have been addressed and results of a metapopulation viability analysis indicate that sub-units within a recovery unit have a 75% or better chance of persistence for a minimum of 100 years, indicating viability of each recovery unit.

Pending completion of the metapopulation viability analysis, consistent occupancy of habitat capable of sustaining viable tidewater goby populations has been set as a temporary recovery objective (USFWS 2005).

Until recently, the tidewater goby was considered a single species that occurred along the entire coast of California. However, morphological and genetic assessments suggest that separation into two species, the southern tidewater goby, *Eucyclogobius kristinae*, and the northern tidewater goby, *Eucyclogobius newberryi* is warranted. The Palos Verdes Peninsula constitutes a geographic barrier that separates the northern and southern tidewater goby. Southern tidewater goby are currently known to exist in only three small sites on Marine Corps Base Camp Pendleton (Swift et al. 2016).

Environmental DNA (eDNA) methods have been used increasingly over the last decade for aquatic species detection (Goldberg et al. 2016). Environmental DNA is DNA that is shed or excreted into the environment by an organism in the form of epidermal cells, urine, or feces (Thomsen et al. 2012b). The use of eDNA for monitoring the presence or absence of a species is advantageous because it can be employed over large spatial scales more easily and cost-effectively than traditional methods (Port et al. 2016; Thomsen et al. 2016; Wilcox et al. 2016). Notably, it has been shown that eDNA surveys require less sampling effort and cost for rare species, especially when size and age data are not required (Evans et al. 2017). In addition, eDNA surveys are less invasive than traditional methods (Thomsen et al. 2016); generally more sensitive at detecting the species of interest (Pilliod et al 2013; Schmelzle and Kinziger 2016; Wilcox et al. 2016); able to capture all life stages simultaneously (Dijean et al. 2012; Thomsen and Willerslev

2015); and do not involve direct handling of the study organism. For these reasons, eDNA approaches are particularly useful for surveying rare and cryptic species (Rousell et al. 2015; Wilcox et al. 2015; Goldberg et al. 2016). Environmental DNA approaches have been successfully applied in lentic (Eichmiller et al. 2014; Moyer et al. 2014), lotic (Jane et al. 2015; Bergman et al. 2016; Wilcox et al. 2016), and marine systems (Thomsen et al. 2012a, 2016; Brandl et al. 2015; Port et al. 2016). More specifically, eDNA has been applied in the diverse lagoon, slough, and estuarine habitats of the northern California coast where it was shown that the detection probability for tidewater goby was nearly twice that of seining when analyzed with a multimethod occupancy approach (Schmelzle and Kinziger 2016).

Applying eDNA as a surveying tool requires an understanding of the processes and challenges associated with it. The amount of eDNA released depends on the species, its size, metabolism, density, and diet (Klymus et al. 2015; Strickler et al. 2015; Wilcox et al. 2016). Some of the DNA released is transported through water currents and diluted, while a large proportion of DNA degrades (Barnes et al. 2014; Pilliod et al. 2014). Degradation depends on UV-B levels, temperature, pH, salinity, and microbial activity and occurs over a period ranging from days to weeks (Thomsen et al. 2012a, Dejean et al. 2011). Any DNA remaining in the system therefore indicates recent species presence (Strickler et al. 2015; Goldberg et al. 2016). Although studies have found correlations between eDNA concentration and species abundance (Pilliod et al. 2013; Schmelzle and Kinziger 2016; Baldigo et al. 2017), it is important to account for covariates that

influence the release, transport, and degradation of DNA when using eDNA as a proxy for abundance (Goldberg et al. 2016; Baldigo et al. 2017).

Assays for species detection that are applied in eDNA surveys need to be both specific enough so that only the species of interest is detected and general enough so that they can be used across the entire range of the study species (Wilcox et al. 2015). Careful validation is paramount in order to minimize false positive and false negative detections (Wilcox et al. 2013). However, as with traditional surveys, imperfect detection of a species is likely (MacKenzie et al. 2006; Schmidt et al. 2013). In other words, non-detection of a species does not necessarily mean that the species is in fact absent (Moyer et al. 2014; Rousell et al. 2015). Regardless of sampling technique, these false negatives can be due to reasons related to the proximity of the species to the specific sampling location, cryptic behavior or coloration of a species, occurrence in low numbers, as well as habitat complexity and accessibility (e.g. Fiske and Chandler 2011; Kroll et al. 2015). For eDNA surveys, false negatives could also be the result of the assay not being sensitive enough to detect the target species (Roussel et al. 2015; Wilcox et al. 2015; Goldberg et al. 2016) or inhibition from certain chemicals found in the water (Hedman and Rådström 2013; Jane et al. 2015; Goldberg et al. 2016). False positive detections could be the result of contaminations that occurred during the sampling process, target DNA being deposited by a predator via fecal matter or carcass deposition at the sampling site (Roussel et al. 2015; Goldberg et al. 2016), or the assay not being specific enough to only detect the target species (Wilcox et al. 2013).

Not addressing imperfect detections in ecological research can result in misleading conclusions and management decisions (MacKenzie et al. 2006; Schmidt et al. 2013; Guillera-Arroita et al. 2014). Imperfect detections likely lead to underestimation of species distribution (Schmidt et al. 2013), but imperfect detection can be addressed by using occupancy models that consider uncertainties at various levels of the detection process (Wilcox et al. 2015; Goldberg et al 2016; Schmelzle and Kinziger 2016; Dorazio and Erickson 2017). Specifically, occupancy models are used for determining the proportion of sites where a species is present, given imperfect detection. Using occupancy models, it is also possible to determine the availability probability of DNA from a species in water samples given that they are present at the site and the detection probability of DNA from a species in qPCR replicates given that DNA is present in the water sample. The relationships between the occurrence of a species, the probability of detecting the species, and environmental variables can also be investigated (Mackenzie et al. 2006). Moreover, fewer samples are needed to reliably estimate presence or absence of a species when using occupancy models in comparison to when occupancy models are not used (Schmidt et al. 2013).

This study aims to illustrate the utility of eDNA as a standardized monitoring tool for rangewide species surveys. Most eDNA surveys to date have been conducted at much smaller geographical scales (e.g. Dejean et al. 2011; Goldberg et al. 2013; Bergman et al. 2016; Baldigo et al. 2017). This study encompassed the entire California coastline of approximately 1350 kilometers. Overall, more than 200 lagoons, sloughs, and estuaries were surveyed for the presence or absence of tidewater goby. This research included four

key objectives. The first objective was to generate a baseline of the geographic distribution of tidewater goby. The second objective was to evaluate concordance between eDNA and traditional field surveys. The detection results of this study were compared to the most recent seining surveys at 122 sites where data were available for both methods. The third objective was to determine occupancy and detection probabilities as indicated by covariates. To account for imperfect detection issues, the eDNA detection data were analyzed using a Bayesian multi-scale occupancy model that was developed specifically for eDNA (Dorazio and Erickson 2017). This approach provided the ability to specifically account for non-detection issues at several hierarchical levels. Finally, given that previous surveys suggest eDNA concentration is related to overall tidewater goby abundance (Schmelzle and Kinziger 2016), the fourth objective was to examine eDNA concentration among sites at a rangewide scale as well as the relationship between tidewater goby eDNA concentration and covariates.

MATERIALS AND METHODS

Field Methods

Between May 12 and September 20, 2016, a total of 430 eDNA water samples were collected at 197 sites along the entire California coast. An additional 12 sites were visited but were dry and therefore not sampled during the survey. Sites ranged from Gilbert Creek, located 1.5 miles south of the Oregon border, to Los Peñasquitos Lagoon, located about 30 miles north of the United States/Mexico border (Figure 1). Sites encompassed lagoons, sloughs, and estuaries ranging in size from a few square meters to several square kilometers and environments ranging from freshwater to hypersaline. Sites were defined as being demographically independent (as in Kinziger et al. 2015) based on (1) geographic isolation, as most sites were separated by at least one kilometer, and (2) supported by previous genetic analyses that indicate significant differences in allele frequency between geographically isolated tidewater goby locations (Kinziger et al. 2015). Sites included 186 locations that have been previously surveyed using seine nets (USFWS 2005; Kinziger et al. 2015; Schmelzle and Kinziger 2016; B. Spies, pers. comm., 2016; Swift et al. 2016) and 23 additional locations that appeared to have suitable habitat (e.g., muted tidal influence and slow currents (see Chamberlain 2006)) (B. Spies, pers. comm., 2016). Ten sites had to be visited twice during the survey due to clogging of filters, resulting in limited filtration volume at first visit (see Appendix A). Gaps in collection coverage included steep rocky coasts, such as the Lost Coast and Big Sur

areas, where lagoons or estuaries are not formed and where tidewater goby are believed to be absent (USFWS 2005). Both of those areas were inaccessible during this study. Collection was also not possible on sites with restrictions such as snowy plover nesting sites, private land, or government properties where collection permits could not be obtained.

Water samples at a given site were assumed to be independent replicates of a single population at the site and water sampling locations were chosen non-randomly based on access to the site. The distance between water sampling locations was generally larger for larger sites (200 – 2000 meters) and smaller for smaller sites (50 – 100 meters). Criteria determining sampling locations included adequate site coverage, completion of sampling within a manageable timeframe, and decreasing the probability of sampling transported tidewater goby DNA from a nearby sampling location. As a result, the number of water samples collected per site ranged from one to six, with generally more water samples collected at larger sites and fewer samples at smaller sites. See Appendix A for a list of all collection sites, collection dates, and the number of water samples collected per site. Appendix B shows a map of all sites.

At each water sample location, two liters of water were collected, by pulling a sterile Whirl-Pak™ Stand-up sample bag through the water near the surface. Whenever possible, water collection was conducted from shore to reduce the risk of contamination between sites (Laramie et al. 2015). Stirring up of sediment during collection was avoided because water samples that include sediment can lead to difficulty in filtration (Laramie et al. 2015) and inhibition during qPCR (Eichmiller et al. 2014). Also,

degradation rates of DNA in sediment are much slower than in the water column and DNA in sediment can be detected months after species absence (Turner et al. 2015). Therefore, to get a more accurate estimate of recent species presence, resuspension of sedimentary DNA was avoided. In addition, water collection near the surface has been shown to improve eDNA detection (Moyer et al. 2014). When sampling at a stream, downstream locations were sampled before upstream locations (Carim et al. 2015). At each water sampling location, geographic coordinates, date, time, water depth (ft), temperature (°C), salinity (‰), dissolved oxygen (mg/l), aquatic vegetation (*Ruppia maritima* in particular), substrate type (sand, mud, gravel), and tidal influence (open or closed to tidal flow at the time of sampling) were recorded.

To detect contamination associated with field practices and/or field equipment, at least one field blank per day was exposed to the sampling environment. A total of 65 field blanks were collected. The field blank consisted of 250 ml store-bought drinking water that was poured into a sterile Whirl-Pak™ Stand-up sample bag in the field. Field blanks were handled the same way as all water samples through all stages of processing to provide comprehensive negative controls (Goldberg et al. 2016).

Water samples were either filtered in the field immediately after collection or within 12 hours of collection to reduce DNA degradation (Goldberg et al. 2016; Yamanaka et al. 2016). If not filtered immediately, water samples were stored on wet ice in a cooler. All water samples were filtered over a 47mm diameter 3.0 µm polycarbonate, track-etched filter membrane (Takahara et al. 2013, 2015; Schmelzle and Kinziger 2016). Each filter was placed on a separate sterilized filter funnel and the water was pulled

across the filter membrane using an electric vacuum pump. If filtration occurred in the field, a portable generator was used as a power source. Filtration time for each filter was recorded and served as an indicator of turbidity. Filters were placed in a 2.0ml Eppendorf™ DNA LoBind microcentrifuge tube and stored in a portable freezer at -18°C until they could be transferred to a lab freezer at -20°C. Filters were stored at -20°C until extraction. Standard operating procedures for water collection and water filtration are provided in appendices C and D.

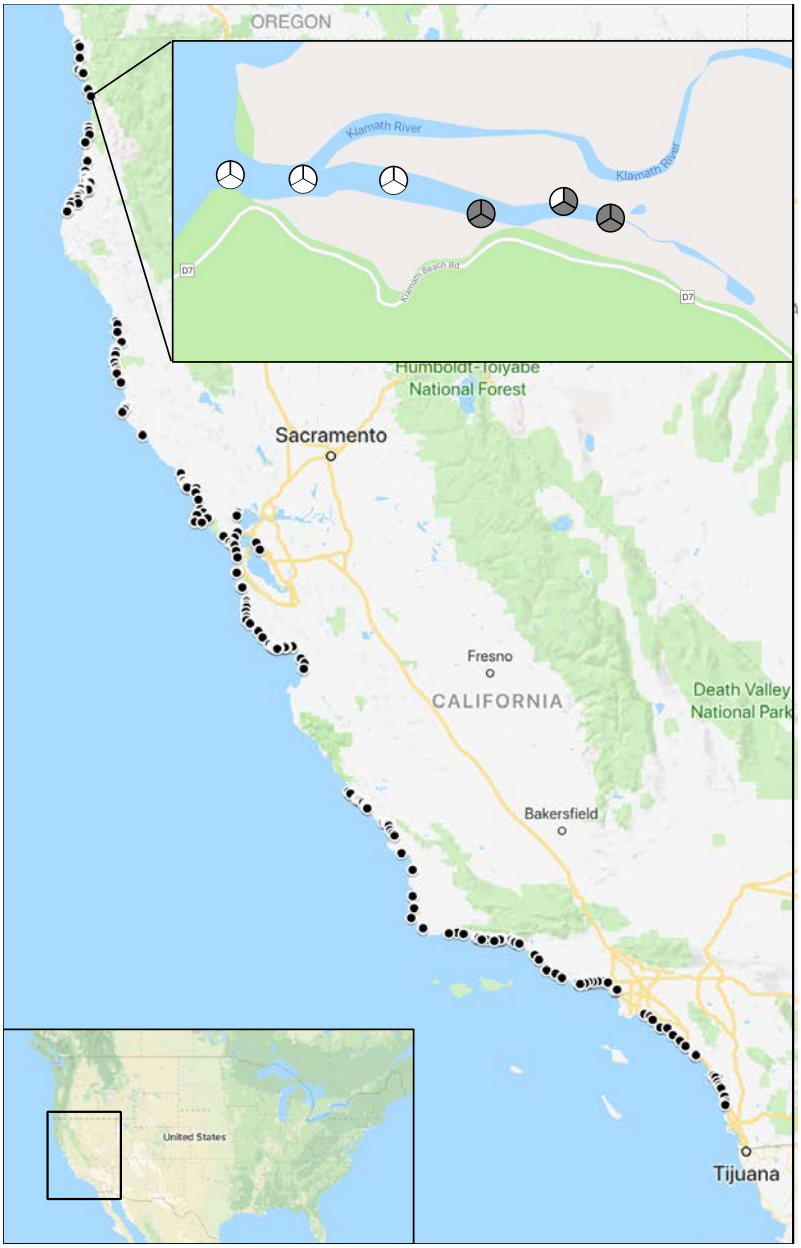

Figure 1: Map of the 209 survey sites (black dots) for tidewater goby, that encompassed the entire geographic range of the species and 1350km of California coastline. Top inset map depicts the Klamath River site to illustrate water-sampling locations at a site and the hierarchical nature of sampling, consistent with the multi-scale occupancy analysis used in this study. Each pie wedge represents a qPCR replicate with a gray wedge indicating tidewater goby qPCR detection and a white wedge tidewater goby qPCR non-detection. Map was created with Google maps (©2018 Google).

Lab Methods

All eDNA extractions were conducted in a dedicated laboratory, that is not used for high copy number samples. Workstations were treated with UV light before each use. Bench spaces, pipettes, centrifuges, and racks were wiped with RNase AWAY™ or 20% bleach before and after each extraction. The eDNA was extracted from filters using a QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's instructions, except that 4µl of RNase was added to the lysate after overnight incubation and QIAGEN's QIAshredder was used for lysate homogenization. Lysis buffer ATL volume was increased to 360µl and proteinase K to 40µl to allow for complete filter submersion (Schmelzle and Kinziger 2016). For the final elution step, only 100µl of Buffer AE was used to increase the final DNA concentration in the elute. All extractions were completed within two months of water collection and elute was stored at -20°C. The standard operating procedure for DNA extraction procedure is provided in appendix E.

For this study, a northern tidewater goby assay (NC10) was used for northern tidewater goby (north of Palos Verdes) and a southern tidewater goby assay (NC10-2) was used for southern tidewater goby (south of Palos Verdes). Both quantitative PCR (qPCR) assays target the same 119-base pair region of mitochondrial cytochrome *b* gene. They consist of forward and reverse primers as well as a minor groove binding (MGB) probe. The primer sets differ between northern and southern tidewater goby to ensure sensitivity across the range. However, the probe is the same for both species since no base pair mismatches are observed across the range. The probe includes a FAM-reporter

dye attached to the 5' end and a non-fluorescent quencher (NFQ) attached to the 3' end. Primer and probe base pair sequences of both assays are shown in Table 1.

The northern tidewater goby assay (NC10) was validated for sensitivity and specificity to ensure target species detection at low eDNA copy numbers and exclusion of non-target species (Schmelzle and Kinziger 2016). Appendix F lists sympatric species that NC10 was tested against for specificity. The northern tidewater goby mitochondrial target sequence of the NC10 primers was shown to be conserved across all tidewater goby populations in Del Norte, Humboldt and Mendocino counties (Schmelzle and Kinziger 2016). NC10 target sequences south of those counties are either conserved or show one to two combined base pair mismatches. The exception is one haplotype (EN_288) found in San Simeon Creek of San Luis Obispo county and the southern tidewater goby (Haplotypes EN_168 and EN_167) that exhibit mismatches of four base pairs in the target region. Testing of NC10 (Appendix G) showed reduced sensitivity for the EN_168 and EN_167 haplotypes and no sensitivity loss for all other haplotypes. EN_288 was not tested, but equal sensitivity loss based on shared target sequence with EN_168 and EN_167 is presumed. Because of that sensitivity loss, the southern tidewater goby assay (NC10-2) was designed specifically for this study, to ensure sensitivity of the assay across the range of tidewater goby. NC10-2 showed improved sensitivity for detection of haplotypes EN_168 and EN_167 (Appendix G).

NC10-2 was tested for specificity against arrow goby (*Clevelandia ios*) and bay goby (*Lepidogobidus lepidus*) (Appendix F), which are considered the two phylogenetic most closely related species to tidewater goby (Ellingson et al. 2014). NC10-2 failed to

amplify any DNA of tissue extracts from the two species. In addition, NC10-2 was also tested against a total of ten closely related or sympatric species to the southern tidewater goby (Ellingson et al. 2014; B. Spies, pers. comm., 2017) by sequence alignment with the software Mega 7 (Kumar et al. 2016). Total base pair mismatches ranging from 15 to 26 (Appendix H), suggest a low likelihood of non-target species DNA amplification (Wilcox et al. 2013). Table 1 shows base pair mismatches between tidewater goby and arrow goby.

Quantitative PCR setup was performed in the eDNA extraction lab, but in a separate qPCR workstation with UV hood and HEPA filter. The qPCR workstation was treated with UV light and all surfaces and lab equipment were wiped with RNase AWAY™ before each use. Total reaction volume was 25µl, including 10µl nuclease free water (Promega Corporation, P1193), 10µl of TaqMan™ Environmental Master Mix 2.0 (Applied Biosystems™, 4396838), 1µl of each primer (10µM), 1µl of probe (2.5µM), and 2µl of DNA template. As in Schmelzle and Kinziger 2016, the TaqMan™ Environmental Master Mix 2.0 was used to reduce effects of inhibition in qPCR reactions.

Quantitative PCR reactions were performed on an Applied Biosystems 7300 Real-Time PCR System in a dedicated high copy laboratory space. Cycling conditions consisted of 50° C for 5 minutes, 95° C for 10 minutes, and 55 cycles of 95° C for 30 seconds and 61° C for 1 minute. Quantitative PCR reactions were run in triplicate. If only one out of three qPCR reactions indicated tidewater goby presence, three additional qPCR reactions were run. Each qPCR included triplicate reactions of a positive control,

consisting of tidewater goby tissue extract, and triplicate reactions of a negative control, consisting of nuclease free water.

Table 1: Primer and probe sequences for quantitative PCR assays for northern and southern tidewater goby. The mitochondrial cytochrome *b* gene holds the 119-base pair target region for amplification for both assays. Bases bolded and underlined represent mismatches in the DNA sequence with tidewater goby's sister species, the arrow goby (*Clevelandia ios*). Note, the probe was the same for both assays, but the primer sequences differed.

Species	**Primer/ Probe**	**Primer/Probe sequence (5' to 3')**
Northern tidewater goby (*Eucyclogobius newberryi*)	NC10-F	C**C**TCAAT**TC**T**C**GT**T**CTACT**AG**TTGT
	NC10-R	**C**CT**A**GT**A**GCAGA**C**GTACTTATTCT**C**
	NC10-P	6FAM-ACGTGC**A**CT**G**AC**C**TTCCG**G**CC**T**TT**C**TC**C**-MGBNFQ
Southern tidewater goby (*Eucyclogobius kristinae*)	NC10-F2	C**C**TCAAT**TC**T**C**GT**T**CT**G**CT**AA**TTGT
	NC10-R2	**C**CT**G**GT**A**GCAGATGTACTTATTCT**C**
	NC10-P	6FAM-ACGTGC**A**CT**G**AC**C**TTCCG**G**CC**T**TT**C**TC**C**-MGBNFQ

Detection

To determine the limit of detection (LOD) for qPCR reactions and to quantify concentration in positive detections, standard curves were constructed for both the northern and southern tidewater goby assays (Appendix I). The LOD, which determines the highest allowable cycling threshold (Ct) values that will be considered positive detections, was determined separately for the northern and southern assay. The Ct value represents the inverse value of eDNA concentration in a qPCR reaction. For the northern assay, DNA of vouchered northern tidewater goby tissue (Humboldt State University Fish Collection number 4955, Big Lagoon) and for the southern assay, DNA of southern tidewater goby tissue (EN_167, Dave Jacobs, UCLA, San Onofre Creek), were extracted as described above. The DNA target region was amplified with a touchdown PCR procedure and amplified DNA presence was verified with gel electrophoresis. The amplified DNA was purified using a QIAquick gel extraction kit according to the manufacturer instructions and the DNA concentration was determined using a ND-1000 spectrophotometer (NanoDrop Technologies). Ten-fold serial dilutions, including ten replicates of each concentration, were analyzed on an Applied Biosystems 7300 real-time PCR system with cycling conditions identical to all eDNA water samples.

The LOD for both the northern and southern assay were set to five target DNA copies per qPCR reaction and the corresponding Ct values, based on the standard curves, were determined. Quantitative PCR detection in one out of six replicates with a Ct value at or below the LOD was considered indicative of tidewater goby presence.

To explore effects of covariates on detection, water samples with tidewater goby detection were compared to water samples with tidewater goby non-detection for the covariates dissolved oxygen, salinity, temperature, and turbidity. Comparisons were conducted using a Welch two sample t-test. If necessary, data were log-transformed to improve normality of predictors or evaluated with a Mann-Whitney test.

Seining versus eDNA

The results of eDNA detections provided by this study were compared to the results of field survey detections from seining. The eDNA data was from 2016 whereas the field surveys were from the years 2014 and 2015 (B. Spies unpublished data; Schmelzle and Kinziger 2016). Data was available for a total of 122 sites where both methods were used. A Pearson's chi-square analysis without continuity correction ($\alpha = 0.05$) tested agreement in detection between seining and eDNA methods. Although a direct comparison of seining versus eDNA, like in Schmelzle and Kinziger 2016, was not possible, comparison to the most recent known seining events should nonetheless provide an indication of the reliability of the two methods. The eDNA water samples were collected at the same geographic coordinates as those from the seining studies.

Occupancy Analysis

Data was analyzed using a Bayesian multi-scale occupancy model because it provided an approach to account for imperfect detection and generate an estimate of true site occupancy. Bayesian multi-scale occupancy models take advantage of the nested

design employed herein (Figure 1) that are common for eDNA based occupancy surveys (Schmidt et al. 2013; Kroll et al. 2015). The nested levels of survey design included: (i) the site occupancy probability (Ψ_i) defined as the probability of tidewater goby eDNA occurrence at site *i,* (ii) the availability probability (θ_{ij}) defined as the probability of tidewater goby eDNA being available for detection in water sample *j* given that it is present at site *i,* and (iii) detection probability (ρ_{ijk}) defined as the probability of tidewater goby eDNA detection in qPCR replicate *k* given that it is present in the water sample *j* and site *i*.

The main objectives were to estimate the parameters Ψ, θ, and ρ, identify environmental covariates that would impact model fit, and determine the effects of those environmental covariates on the parameters Ψ, θ, and ρ. Posterior mean estimates of Ψ, θ, and ρ were reported including a 95% credible interval (95% CRI) and the posterior distributions of the parameters β, α, and δ were used to describe the effects they have on Ψ, θ, and ρ, respectively (Dorazio and Erickson 2017). Additionally, the equations $1 - (1 - \hat{\theta})^n = 0.95$ and $1 - (1 - \hat{p})^n = 0.95$ (Schmidt et al. 2013) were used to determine the number of water samples and qPCR replicates required to achieve detection probabilities at or above 0.95.

Model assumptions include a closed system with no changes in occupancy status, independence of detection between sites and within sites, and no false positive detections (Donovan and Hines 2007). Since sites were only visited once during the survey, the assumption of a closed system is met, as the occupancy status of a site did not change

during time of sampling. Independence of detection between sites was not considered problematic because of distance, isolation, and limited tidewater goby dispersal between sites. However, non-independence of detections between water sample locations within a site cannot be ruled out, because transport of eDNA between water sample locations is theoretically possible, but water collections were spaced far apart in an attempt to reduce these effects (see above). The chances of false positive detections occurring were minimized by assay validation, careful decontamination procedures in the field and lab (see above), as well as by including comprehensive negative controls. Further, only those Ct values at or below the limit of detection, which was set at a conservative level of five target DNA copies per qPCR reaction, were considered detections for occupancy modeling.

A literature survey was conducted to identify covariates that were likely to have effects at different hierarchical levels of the nested survey design employed herein (Table 2). It was hypothesized that habitats without tidal influence at time of sampling and habitats with the aquatic plant *Ruppia maritima* present would have a higher site occupancy probability (Ψ). Availability probability (θ) was hypothesized to decrease with dissolved oxygen, salinity, and temperature, and increase with turbidity present. Lastly, higher amounts of salinity and turbidity were hypothesized to result in inhibition at the qPCR level and therefore decrease detection probability (ρ).

For model selection, all possible covariate combinations expected to influence site occupancy, water sample availability and qPCR detection probability (Table 2) were fitted by running the Markov chain Monte Carlo (MCMC) algorithm for 11,000 iterations

and then retaining the last 10,000 for posterior value estimation. Models with different covariate combinations were ranked according to the posterior-predictive loss criterion under squared error loss (PPLC) (Gelfand and Ghosh 1998) and the widely applicable information criterion (WAIC) (Watanabe 2010, 2013). Covariates that impacted model fit were identified and models with lower values for both criteria and fewer predictors were favored, according to the principle of parsimony. Once the best model was identified, it was fitted by running the MCMC algorithm for 110,000 iterations and retaining the last 100,000 iterations for posterior value estimation. All covariates included in the analysis revealed no collinearity ($r_{Pearson}$ = -0.082 – 0.264). Models were fitted using the package eDNAoccupancy (Dorazio and Erickson 2017) for the statistical program R (R Core team 2017).

Table 2: Environmental covariates expected to influence site occupancy, water sample availability and qPCR detection probability in the occupancy modeling analysis.

Covariate	**Description of hypotheses**	**Literature citation(s)**
Tidal influence (present or absent)	Habitats without tidal influence (closed) at time of sampling have a higher site occupancy probability (Ψ) than habitats with tidal influence (open) at time of sampling. This is based on the finding that habitats with only sporadic tidal fluctuation seem to have a higher probability of encountering tidewater goby.	Chamberlain (2006)
Ruppia maritima (present or absent)	Habitats with *Ruppia maritima* present provide cover for tidewater goby and have a higher site occupancy probability (Ψ) than habitats without vegetation present.	Moyle (2002) McGourty et al. (2008)
Dissolved oxygen (miligrams per liter)	Higher dissolved oxygen will result in lower availability probability in the water sample (θ) because of faster degradation.	Weltz et al. (2017)
Salinity (‰)	Higher salinity will result in lower availability probability in the water sample (θ) because of faster degradation.	Thomsen et al. (2012a)
	Higher salinity will result in lower detection probability in qPCR replicate (ρ) because of inhibition.	Foote et al. (2012)
Temperature (Celsius)	Higher temperature will result in lower availability probability in the water sample (θ) because of faster degradation.	Barnes et al. (2014)
Turbidity (filtration time)	Higher turbidity will result in higher availability probability in the water sample (θ) due to suspended sediments binding eDNA molecules and inactivating extracellular nucleases.	Barnes et al. (2014)
	Higher turbidity will result in lower detection probability in qPCR replicate (ρ) because of inhibition.	Williams et al. (2017)

eDNA Concentration

Concentration of eDNA was determined using standard curve approaches for all detections with Ct values below the LOD. Non-detections were assigned concentration values of zero and were used for the calculation of average DNA concentration values per water sample and site (following Ellison et al. 2006). Quantifications were reported as DNA concentration in copy number per 2-liter water sample (Goldberg et al. 2016).

To assess the relationship between eDNA concentration and environmental covariates per site, a multiple linear regression analysis was conducted. To account for dependence of errors typical with nested survey designs, average covariate and eDNA concentration values per site were used for analysis. In addition, to meet the assumptions of normality and homogeneity of variance and to improve model fit, only sites with at least one qPCR detection were included in the analysis and all eDNA concentrations were $\log_{10}$ transformed. Covariates considered were dissolved oxygen, the presence or absence of the aquatic plant *Ruppia maritima*, salinity, temperature, the presence or absence of tidal influence, and turbidity. Due to an insufficient number of observations, interactions between covariates were not considered in model selection. Models with all possible covariate combinations were fitted (using the regsubsets function R-package 'leaps', R Core team 2017) and ranked according to their Bayesian Information Criterion (BIC) scores, with a lower score indicating better model fits.

RESULTS

Detection

The limit of detection (LOD), defined as the Ct value corresponding to five target eDNA copies per qPCR reaction, corresponded to a Ct value of 40.87 for the northern tidewater goby assay and 40.04 for the southern tidewater goby assay. These LOD values encompassed 90% of the Ct values observed in this study (370 out of 409) (Figure 2). Based on these detection criteria, northern tidewater goby were detected from 137 of 379 water samples and southern tidewater goby from 4 of 51 water samples. Detection in a water sample was indicated by at least one positive qPCR. When considered on a per site basis, northern tidewater goby were detected at 81 of 175 sites and southern tidewater goby were detected at 4 of 22 sites (Appendix A, Appendix B). These estimates exclude the 12 dry sites for northern tidewater goby that were encountered during the survey. Two water samples collected at one site (Pismo Creek) were excluded from all analyses, owing to contamination. A total of 64 out of 65 field blanks tested negative for contamination. Contamination occurred in one field blank during lab procedures associated with processing the Pismo Creek water samples.

Tidewater goby were detected at dissolved oxygen levels from 0.8 to 24.9 milligrams per liter, salinities from zero to 44 parts per thousand, temperatures from 13 to 30.4 degrees Celsius and filtration time (an indicator of turbidity) from 29 to 802 seconds. Comparison of tidewater goby detections and non-detections in water samples

with a Welch two sample t-test (dissolved oxygen, temperature, log of turbidity) and a Mann-Whitney test (salinity) revealed that tidewater goby were generally detected in water samples with cooler water temperatures ($t = 2.24$, $df = 310.50$, p-value = 0.03) and lower salinities ($W = 24238$, p-value = 0.001) (Figure 3). No significant difference was revealed for dissolved oxygen and log of turbidity between detection and non-detection water samples.

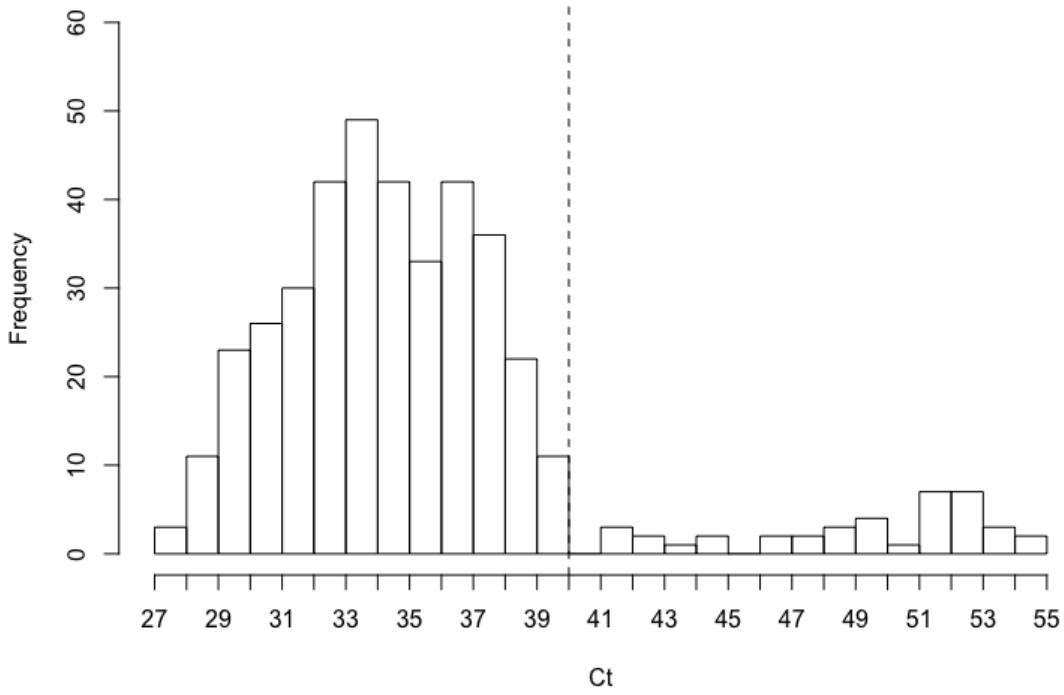

Figure 2: Frequency distribution of qPCR cycling threshold (Ct) values for both northern and southern tidewater goby assays (n=409), the dashed vertical line represents the limit of detection set for this study at a Ct-value of 40, which is equivalent to approximately 5 target eDNA copies per qPCR reaction.

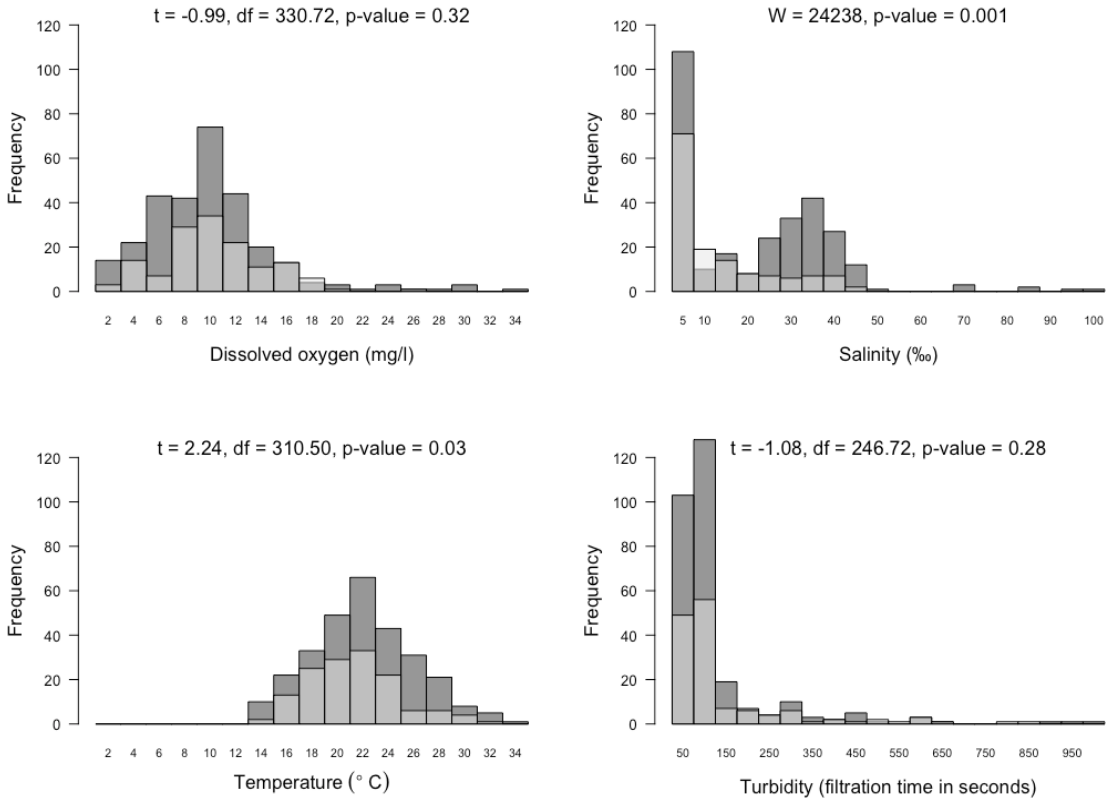

Figure 3: Overlapping histograms of measured covariate values showing the effects of covariates on tidewater goby detection (light gray bars) and non-detection (dark gray bars) in water samples (n = 430). Welch two sample t-test results ($\alpha = 0.05$) for dissolved oxygen, temperature and log of turbidity as well as Mann-Whitney test results ($\alpha = 0.05$) for salinity are depicted.

Seining versus eDNA

Comparing results from seining detections of the years 2014 and 2015 with eDNA detections from this study (2016) at 122 sites revealed that both methods agreed in 86% of cases (Table 3). Tidewater goby were detected with both methods at 68 sites and not detected by both methods at 37 sites. On the contrary, seining detected tidewater goby at six sites where eDNA was not successful at detection and eDNA detected tidewater goby at 11 sites where seining failed to detect tidewater goby. Results of a Pearson's chi-square analysis without continuity correction ($\alpha = 0.05$) rejected the null hypothesis of there being no agreement between seining and eDNA methods ($\chi^2 = 60.69$, df = 1, p-value < 0.001).

Table 3: Comparison of detections and non-detections from 122 sites that were sampled in the years 2014 or 2015 with seining methods (B. Spies, unpublished data; Schmelzle and Kinziger 2016) and in the year 2016 with eDNA methods. Results show the number of sites and the proportion of sites in parentheses.

	eDNA detection	**eDNA non-detection**
Seine detection	68 (0.56)	6 (0.05)
Seine non-detection	11 (0.09)	37 (0.30)

Occupancy Analysis

Excluding the 12 dry sites and the Pismo Creek site that was removed due to contamination, 85 out of 197 sites were occupied, resulting in a naïve occupancy estimate of 0.43. Fitting the occupancy model without covariates (Ψ(.),θ(.),ρ(.)), resulted in a posterior mean site level occupancy (Ψ) of 0.55 (95% CRI 0.46–0.65), which translates into an estimated number of sites occupied by tidewater goby of 108 (95% CRI 90–128). Given site presence, the probability of tidewater goby eDNA availability in the water sample (θ) was 0.61 (95% CRI 0.52–0.69). Based on the equation $1 - (1 - \hat{\theta})^{n} = 0.95$, if tidewater goby eDNA is available at a site, four water samples are needed to achieve a detection probability equal to or greater than 0.95. Given tidewater goby eDNA presence in a water sample, the probability of detecting it in a qPCR replicate (ρ) was 0.71 (95% CRI 0.66–0.75). Three qPCR replicates were therefore sufficient to achieve a detection probability equal to or greater than 0.95, when using the equation $1 - (1 - \hat{p})^{n} = 0.95$.

Examining detection probabilities for northern and southern tidewater goby separately results in a nave occupancy estimate of 0.46 for northern tidewater goby and 0.18 for southern tidewater goby. When fitting a model without covariates, a site occupancy level (Ψ) of 0.60 (95% CRI 0.50–0.70) is estimated for northern tidewater goby. In other words, 105 out of 175 sites were estimated to be occupied. For southern tidewater goby, however, the site occupancy level cannot accurately be determined since only 4 single qPCR detections were recorded for all 22 sites.

In the model selection analysis, a total of 256 models were examined, including all possible covariate combinations listed in Table 2 (Ψ: ruppia, tide; θ: dissolved oxygen, salinity, temperature, turbidity; ρ: salinity, turbidity). Plots of the model selection criteria (PPLC and WAIC) as a function of model rank indicated some distinct breaks in model fit that corresponded with specific covariates being included or excluded from models (Figure 4). The best fit models with the lowest PPLC scores (ranging from ~381 to ~385) all included the covariate salinity for ρ and θ, and the addition or subtraction of all other covariate combinations had only minor effect on the overall PPLC score (Figure 4). Application of the widely applicable information criterion (WAIC) for model selection indicated that the best models were identical to those identified by PPLC (Figure 4), and these all included salinity as a covariate for θ and ρ (Figure 4). Exploration of additional covariate effects not identified by the hypotheses, including all combinations of salinity, temperature, and turbidity as covariates of Ψ, did not reveal any models with better fit (2000 iterations).

The covariate salinity, when included for ρ and θ in model selection, always resulted in the lowest PPLC and WAIC scores, no matter what other covariates were included (Figure 4). Thus, based on the principle of parsimony, the best model was considered to be Ψ(.),θ(sal),ρ(sal). Results of fitting the model (Ψ(.),θ(sal),ρ(sal)) with 100,000 MCMC iterations suggests that the availability of tidewater goby eDNA in water samples decreases with salinity (α = -0.50, 95% CRI -0.76 to -0.21) (Figure 5). Similarly, the probability of detecting tidewater goby eDNA in qPCR decreases with salinity

(δ = -0.53, 95% CRI -0.71 to -0.35) (Figure 5). The posterior mean site level occupancy of 0.55 (95% CRI 0.46–0.64) as estimated with the null model remained unchanged. Fitting the null model resulted in a PPLC score of 425.4 and a WAIC score of 0.639, while fitting the best model resulted in a PPLC score of 381.1 and a WAIC score of 0.603.

Posterior median estimates of water sample availability probabilities (θ) ranged from 0.04 to 0.79 across sites and posterior median estimates of qPCR detection probabilities (ρ) ranged from 0.04 to 0.83 across sites (Appendix J). Fitting the model with 100,000 iterations did not alter the outcome of estimates compared to fitting the model with 10,000 iterations except for slight reductions in the estimates of standard error. In addition, analyses with and without outlier data of salinity (Figure 5) did not alter any conclusions in regard to model selection and model performance.

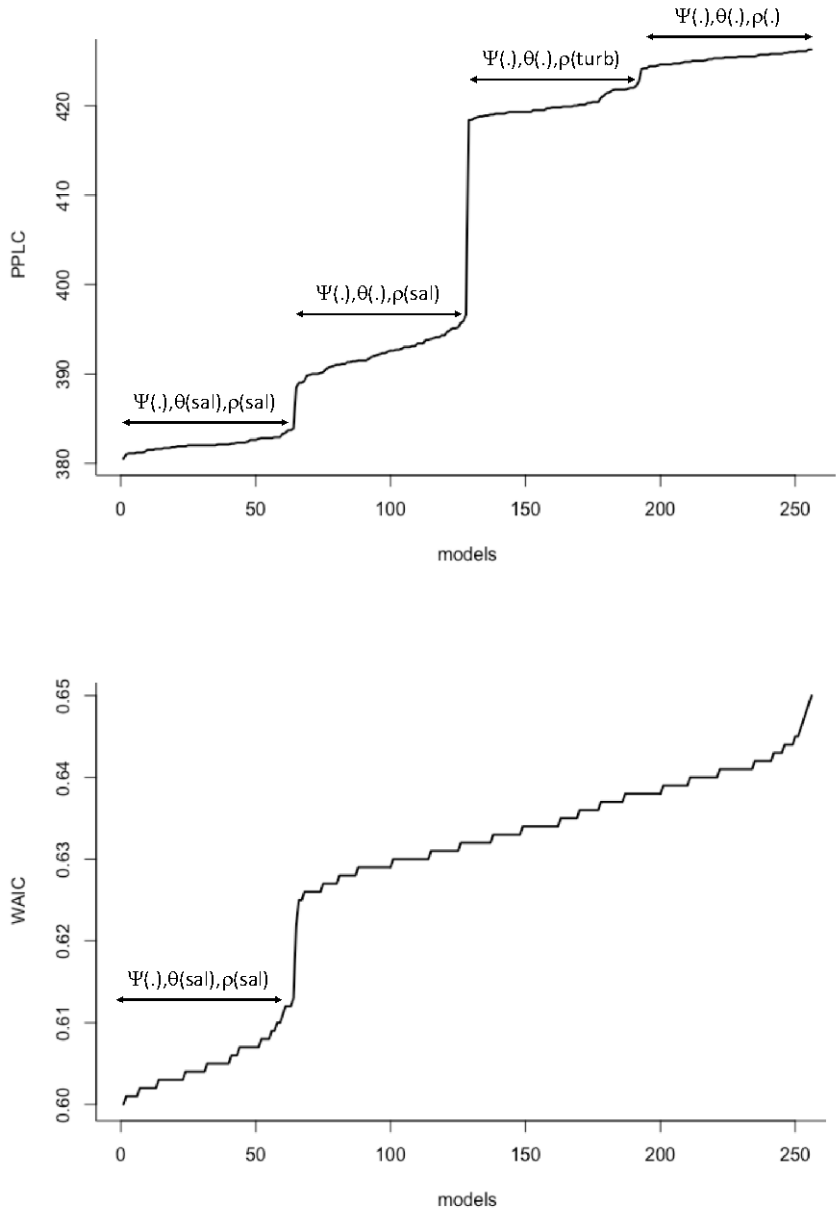

Figure 4: Plot of posterior-predictive loss criterion (PPLC; top) and widely applicable information criterion (WAIC; bottom) for all models evaluated in the occupancy analysis, including all 256 possible covariate combinations listed in Table 2 (Ψ: ruppia, tide: θ: dissolved oxygen, salinity, temperature, turbidity; ρ: salinity, turbidity). Models are ranked from best fit (lower PPLC and WAIC values) to worse fit. Results are for 10,000 iterations of the Markov chain Monte Carlo (MCMC) algorithm (initial 1000 iterations discarded as burnin). Double arrows indicate the range of ranked models that all have the depicted covariates as a commonality.

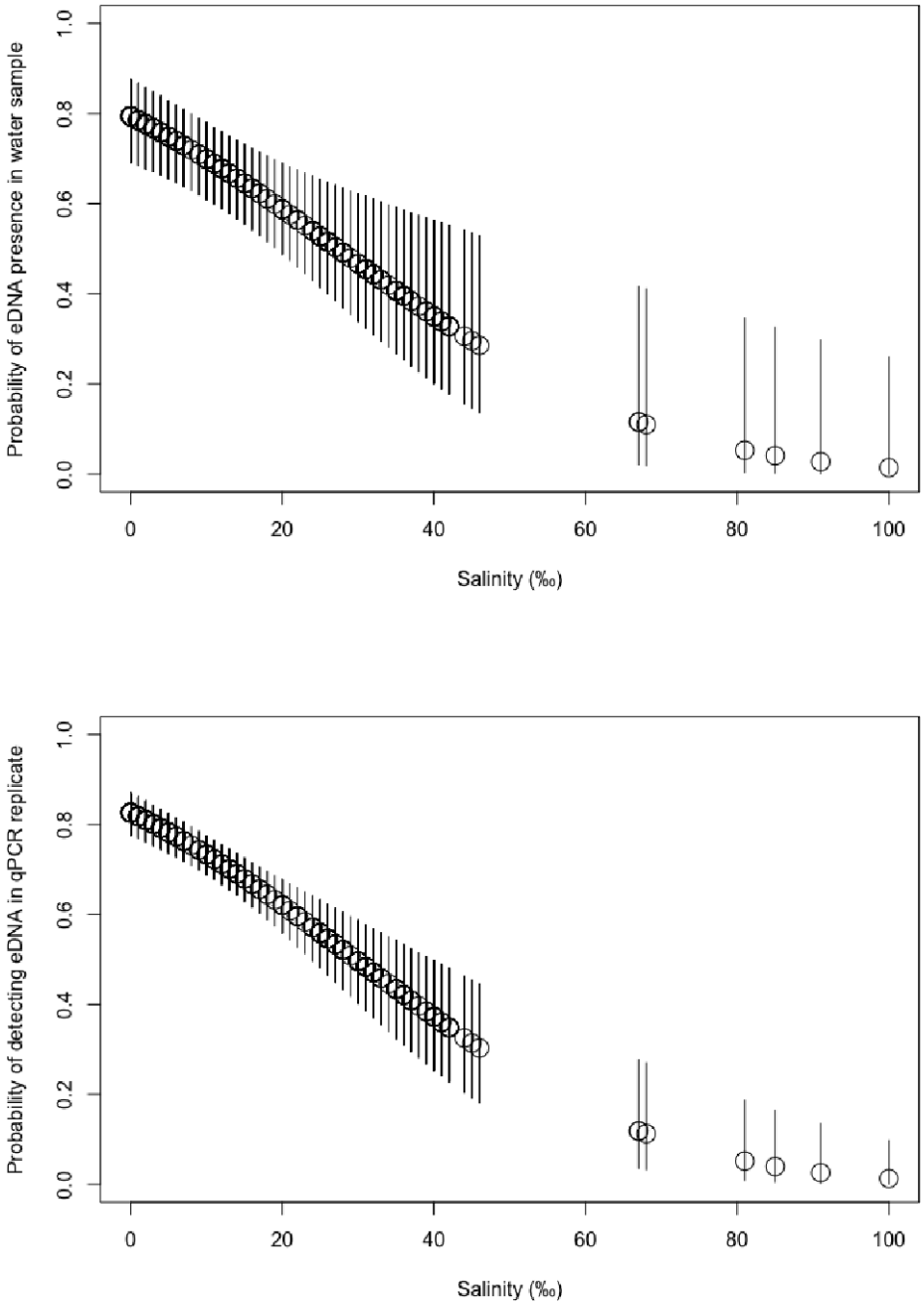

Figure 5: Effects of salinity on the estimated probabilities of tidewater goby eDNA availability in water samples (θ; top) and the estimated probability of tidewater goby eDNA detection in qPCR replicates (ρ; bottom). The circles represent estimates of posterior medians and the bars 95% credible intervals. Values are based on the best fit model (Ψ(.),θ(sal),ρ(sal)).

eDNA Concentration

Average concentrations of tidewater goby DNA in the positive samples, as estimated from the regression equations from the standard curve analysis (Appendix I), ranged from 110 to 1.41*10^6 copies per two-liter water sample. Average DNA concentrations per site are depicted in Figure 6. The goodness of fit (R^2) values for standard curve analyses were 0.985 for northern and 0.993 for southern tidewater goby (Appendix I).

The relationship between eDNA concentration and environmental covariates, based upon average site values, identified tidal influence as the only covariate of significance (at an α level = 0.05) in predicting $\log_{10}$ of the eDNA concentration ($y = 4.27 - 1.08x$, $R^2 = 0.19$, p-value < 0.001). Sites closed to tidal influence had on average 8.9*10^4 more eDNA copies than sites that were subject to tidal flow.

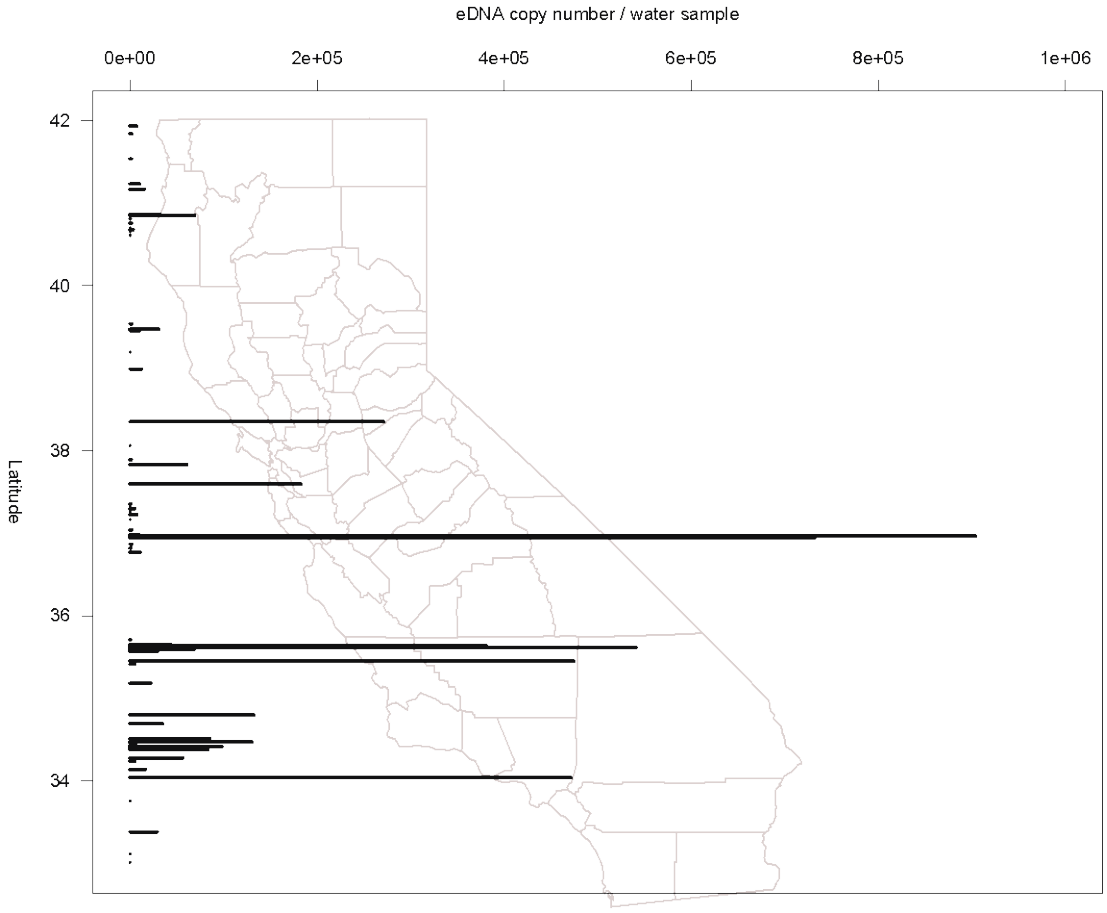

Figure 6: Barplot showing average concentration of tidewater goby eDNA per site for all 85 sites with positive eDNA detection. Sites are separated by latitude and barplot is overlaid onto map of California.

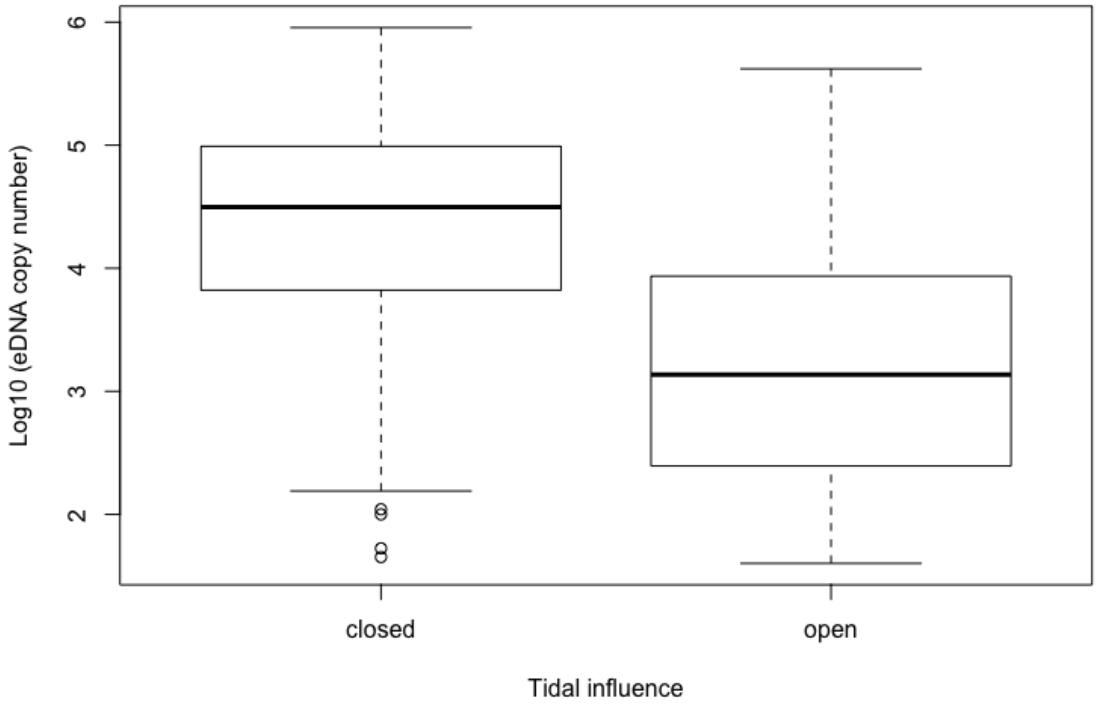

Figure 7: Boxplot of $\log_{10}$ transformed average eDNA concentration values in sites that are closed versus open to tidal flow at the time of field collection. Only sites with at least one qPCR detection were included. Median values are depicted with bold lines, the box represents the middle 50% of data, whiskers represent the upper and lower 25% of data, and circles represent outliers.

DISCUSSION

Detection

The determination of a Ct threshold indicative of a detection versus a non-detection in a qPCR reaction has varied among studies, ranging from 300 copies per qPCR reaction (Eichmiller et al. 2014), to 1 copy per qPCR reaction (Williams et al. 2017), to including all qPCR amplifications as positive detections (Schmelzle and Kinziger 2016). The decision about what Ct value to use as the cutoff value, or limit of detection (LOD), determines which sites are considered occupied and, especially for endangered species, needs to be carefully considered. Setting the cutoff value too high can result in overestimation of sites being occupied and could also result in false positive detections. Setting the cutoff value too low can result in underestimation of sites being occupied and therefore false negative detections. Setting the Ct cutoff values at 40.87 for northern tidewater goby and 40.04 for southern tidewater goby, which is based on 5 copies per qPCR reaction, included 90% of qPCR detections in this study. Raising the LOD to 1 copy per qPCR reaction would likely have resulted in an increased number of positive water sample detections at four sites (San Gregorio Creek, Scott Creek, Ocean Ranch North, and Aptos Creek), but would not have resulted in any changes to the number of sites occupied overall.

Northern tidewater goby were detected using eDNA methods at four sites where they have not previously been detected, including Navarro River, Mill Creek, San Pedro

Creek, and Arroyo De La Cruz. Among the three water samples collected from the Navarro River, tidewater goby were only detected in one of 12 qPCR replicates with a Ct value of 39. The only previous field surveys at the Navarro River were conducted in 1975 and once in 2014, and during these surveys tidewater goby were not detected. At Mill Creek, all three qPCR replicates (one water sample) detected tidewater goby with Ct values of 38. Mill Creek is a tributary of San Francisco Bay where tidewater goby have not been detected since 1961 (Swift et al. 2016). San Pedro Creek showed a strong signal of tidewater goby presence with Ct values averaging 31 among three qPCR replicates (one water sample). No recent survey records are available for San Pedro Creek, which lies just 13 miles south of San Francisco Bay. At Arroyo De La Cruz, one out of six qPCR replicates detected tidewater goby with a Ct value of 37. Arroyo De La Cruz was last surveyed in 2014 and tidewater goby were not detected (B. Spies, unpublished data). Mill Creek, San Pedro Creek, and Arroyo De La Cruz have all been listed as potential re-introduction sites in the U.S. Fish and Wildlife Service's recovery plan for the tidewater goby (USFWS 2005).

Sites where southern tidewater goby were detected using eDNA but have not been documented from previously consisted of Alamitos Bay, Canyon De Las Encinas, and Escondido Creek – San Elijo Lagoon. At all three locations, detections were indicated by a single qPCR replicate and in all cases the Ct value was 38. At Alamitos Bay and Canyon De Las Encinas, two water samples with nine qPCR replicates were analyzed, and at San Elijo Lagoon a total of three water samples and 12 qPCR replicates were examined. Alamitos Bay is fully exposed to tidal action and does not represent a natural

system. Tidewater goby presence appears unlikely based on the habitat preferences of the species(Swenson 1999; Chamberlain 2006). Canyon De Las Encinas is a small site (250 – 300 square meters) that is dominated by mosquitofish and thought to have little suitable habitat for tidewater goby (B. Spies, pers. comm., 2017). San Elijo Lagoon is a large (approximately 3.8 square kilometer) tidal marsh system dominated by arrow goby (B. Spies, pers. comm., 2017). Interestingly, a historic record indicates possible tidewater goby presence (Swift et al 2016) and follow up eDNA sampling in 2017 confirmed positive detection for tidewater goby (C. Martel, unpublished data). Since all three locations lie in highly populated areas, one hypothesis is that tidewater goby DNA may have been introduced from nearby aquaria or wastewater facilities, but this has not been confirmed.

Sites where tidewater goby are thought to be present (Swift et al. 2016) but were not detected by the eDNA approaches employed in this study included Estero Americano, Estero San Antonio, Yankee Jim, Waddell Creek, Soquel Creek, Arroyo del Oso, and Malibu Lagoon. Sites that were inaccessible due to sampling restrictions, but with potential tidewater goby presence (Swift et al. 2016), are listed in Appendix K.

Seining versus eDNA

The high agreement of 86 percent between seining and eDNA, as demonstrated by comparing the two methods at 122 sites, indicates that both methods are valid survey tools for detecting tidewater goby. The fact that tidewater goby were detected with eDNA methods but not with seining at 11 sites (Table 4), was likely due to the high sensitivity

of eDNA methods (Schmelzle and Kinziger 2016) that would be advantageous relative to seining especially when tidewater goby are present at very low numbers. The six sites where tidewater goby were detected with seining but not with eDNA methods (Table 5) can be attributed to insufficient water sampling. If more water samples were collected at these sites it would have allowed an adequate detection probability at those sites. Except for Waddell Creek where two water samples were collected, only one water sample was collected for the remaining five sites. However, this study shows that the probability of eDNA availability in a water sample is dependent on salinity and with high salinity sites such as Estero Americano, Estero De San Antonio, and Papermill Creek, up to 8 water samples per site would have been necessary to achieve a detection probability of at least 0.95. The sites Waddell Creek, Soquel Creek, and Willow Creek are low salinity sites where only two water samples should have been sufficient for detection, given tidewater goby presence. Disagreement in detection between the two methods could be the result of local extinction and/colonization events (Lafferty et al. 1999), or issues associated with non-detection that likely influence both seining and eDNA approaches (Schmelzle and Kinziger 2016).

Table 4: List of sites (north to south), out of 122, where tidewater goby were detected with eDNA for this study, but not with seining in 2014 or 2015 (B. Spies, unpublished data; Schmelzle and Kinziger 2016). The column qPCR lists the number of qPCR detections out of the total numbers of qPCR per site. Sites are listed north to south.

Site Name	**qPCR**	**last seine detection**	**historic presence**
McDaniel Slough West	6/12	2011	yes
Gannon slough / pond	4/12	2010	yes
Jacoby creek	1/6	2010	yes
Hwy 101 ditch	3/12	2004	yes
Ocean Ranch South	1/15	unknown	unknown
Navarro river	1/12	never	no
Arroyo De La Cruz	1/6	never	no
Goleta Slough	1/18	unknown	yes
Devereux slough	1/24	2013	yes
Santa Clara River	6/12	unknown	yes
Canyon de las Encinas	1/9	never	no

Table 5: List of sites, out of 122, where tidewater goby were detected with seining in 2014 or 2015 (B. Spies, unpublished data; Schmelzle and Kinziger 2016), but not eDNA for this study. The column qPCR lists the number of qPCR detections out of the total numbers of qPCR per site. Sites are listed north to south.

Site Name	**qPCR**	**last seine detection**	**historic presence**
Estero Americano	0/3	2015	yes
Estero De San Antonio	0/3	2015	yes
Papermill Creek	0/3	2014	yes
Waddell Creek	0/6	2015	yes
Soquel Creek	0/3	2015	yes
Willow Creek	0/3	2015	yes

Occupancy Analysis

Despite the fact that species detection is often imperfect, accounting for imperfect detection in ecological research is still far from common (Kellner and Swihart 2014). Assuming that detection is perfect can lead to misleading conclusions about animal abundance, distribution, extinction-colonization processes, and ultimately management decisions that are based on faulty data (MacKenzie et al. 2006, Kellner and Swihart 2014, Kinziger et al. 2015).

By addressing imperfect detection through the use of a multi-scale occupancy model that takes into account imperfect detection at the site, the water sample, and the qPCR replicate, site occupancy (Ψ) was estimated as 0.55 (95% CRI 0.46–0.64). By contrast, the naïve estimate of occupancy, estimated without accounting for imperfect detection, was only 0.43. Thus, if imperfect detection is accounted for, there are 23 additional sites where tidewater goby are present, but not detected in this study. Given the higher detection sensitivities of eDNA methods compared to seining (Schmelzle and Kinziger 2016), tidewater goby might go undetected at an even higher number of sites with seining than with eDNA approaches. This result highlights two important concepts that should be considered when surveying for relatively rare and cryptic species like the tidewater goby. First, occupancy models should be used to account for imperfect detection, and second, non-detection at a site does not necessarily imply extirpation of the species at the site. These findings suggest that extinction-colonization dynamics in tidewater goby might be happening less frequently on a rangewide basis than previously

suggested for a small subset of populations examined in southern California (Lafferty et al. 1999).

Historically, tidewater goby were estimated to occupy 135 sites (USFWS 2005), with the most recent finding of 114 known tidewater goby locations (USFWS 2014). This estimate comes remarkably close to the occupancy models' estimate of 108 sites being occupied generated herein. One reason for the difference was likely due to the inability to access a number of sites with potential tidewater goby presence (Appendix K). Another reason for the difference is due to the fact that previous estimates were based upon site occupancy data compiled across multiple years whereas the eDNA survey conducted herein was completed over four months. Because the previous estimates were based upon data from longer periods, occupancy status could have changed whereas the eDNA estimates were conducted over a sufficiently restricted time period such that they represent a point estimate of occupancy.

Consistent with previous studies (Foote et al. 2012; Thomsen et al. 2012a), salinity was found to have a strong negative effect on DNA availability in the water sample and detection in the qPCR replicate; however, site occupancy was not affected by salinity. Implications of this finding are that more water samples and more qPCR replicates are needed at high salinity sites to achieve the desired level of species detection. At sites with low salinity only two water samples and qPCR replicates might be sufficient to achieve a detection probability equal to or greater than 0.95, but for sites with hypersaline conditions, up to 8 water samples and 8 qPCR replicates might be needed. This finding is illustrated at the Devereux Slough site that was visited twice

during the study (Appendix A). On June 3, 2016, salinity measurements were recorded as 39, 40, and 40 ‰, while on August 14, 2016 the values were 67, 67, 68, and 91 ‰. The only qPCR detection out of a total of 24 replicates for all seven water samples at the site stems from the water sample with the lowest salinity value. While Schmelzle and Kinziger (2016) determined that the availability of eDNA in a water sample and the detectability of eDNA in a qPCR replicate is affected by habitat type (lagoons, estuaries, sloughs), or tidal influence (open, closed), the primary factor identified herein was salinity. Despite exploration of additional variables identified as important in the literature (Table 2), this analysis did not resolve significant effects of these covariates on occupancy, availability, or detection.

eDNA concentration

Out of the 85 sites where tidewater goby were detected, about two thirds (54 sites) experienced no tidal flow at time of sampling. Furthermore, the presence versus absence of tidal flow was found to explain a significant amount of variability in eDNA concentration. Sites exposed to tidal influence had significantly lower eDNA concentrations than sites without tidal influence. This is consistent with the findings by Schmelzle and Kinziger (2016), that related the increased eDNA concentration at sites without tidal influence to increased tidewater goby catch per unit effort in seine hauls. Moreover, the fact that tidal influence was found to be the only covariate of significance in predicting eDNA concentration, is reflected by the conclusion of Chamberlain (2006), that attributed the presence of tidewater goby at a site to limited tidal action rather than

environmental covariates. Thus, the relationship between the absence of tidal flow and tidewater goby eDNA concentration identified in this study is probably the result of two factors, the preference of tidewater goby for perched habitats that do not regularly experience tidal turn-over and the increased concentration of eDNA at sites that do not experience tidal flushing.

Examining average tidewater goby eDNA concentration per site separated by latitude (Figure 6) conveys two main points: (1) Tidewater goby along the California coast exhibit patchy distributions: there are some regions with a high number of tidewater goby sites (e.g. Santa Cruz, San Simeon, and Santa Barbara localities), but other sites are geographically isolated. (2) Assuming the relationship between eDNA concentration and tidewater goby abundance found by Schmelzle and Kinziger (2016) holds, the abundance of tidewater goby at any given site appears largely independent from neighboring sites, supporting the idea of demographic independence. In other words, sites may be more dependent upon local birth-death processes rather than immigration or emigration for determination of site abundance.

Management Implications and Future Research

Section 7 of the Endangered Species Act (ESA) requires consultation with the U.S. Fish and Wildlife Service (USFWS) for activities involving endangered species so as to not cause harm to listed species or their habitats. A survey protocol using seining, dip netting, and minnow traps to determine the presence or absence of tidewater goby was developed and is currently being used by USFWS (USFWS 2005); however,

sampling is time and labor intensive, requires entering, and potentially disturbing, goby habitat, and can result in the unavoidable take of tidewater goby and other non-target species. In addition, permitting due to capture and handling of an endangered species is extensive and may involve several agencies (USFWS 2005).

This study demonstrates that there is a high agreement between seining and eDNA methods, not only by comparing the two methods at 122 sites with recent field efforts from 2014 and 2015, but also compared to the most recent estimates of tidewater goby occupied habitats on a rangewide scale (Swift et al. 2016). The reliability of eDNA combined with the advantages of being non-invasive to habitat and species (Thomsen and Willerslev 2015, Thomsen 2016); being more cost effective (Baldigo et al. 2017; Evans et al. 2017); involving fewer permitting requirements and safety issues (Pilliod et al. 2013); and being able to be employed over large spatial scales during a relatively short time period, as demonstrated by >200 sites surveyed in four months for this study, would justify increased use of eDNA methods when surveying for tidewater goby and other aquatic species. The eDNA protocols described herein can easily be implemented by the California Department of Transportation to determine tidewater goby presence/absence using a validated, rapid, and inexpensive approach. The included standard operating procedures provide step-by-step instructions and equipment lists (part numbers and vendors) for conducting tidewater goby eDNA surveys.

Regardless of the surveying method used, future studies investigating the status of tidewater goby should use occupancy models to account for imperfect detection. For species like the tidewater goby that are relatively rare and cryptic, it is unlikely that

presence will always result in successful detection (Mackenzie et al. 2006). Accounting for imperfect detection is vital to avoid introducing measurement error and bias and to lead to better policy making regarding species conservation (Fiske and Chandler 2011; Kellner and Swihart 2014). In addition, when using eDNA methods, the number of water samples taken per site and the number of qPCR replicates per water sample should be adjusted depending on the salinity of the sample. This study has shown that more water samples and qPCR replicates are needed for high salinity sites.

Despite detecting tidewater goby at 85 sites and the occupancy model suggesting an even higher number of sites being occupied, it is important to note that occupancy cannot be equated to viability of a population. Although tidewater goby might be present at a site, the population might be too small or the site might be too degraded to guarantee continued persistence. Studies determining tidewater goby abundance, genetic diversity, habitat suitability, and persistence of individual populations are required to determine the long-term potential of those populations.

Future studies could incorporate spatial components to their analyses. For example, evaluations could determine if a site is more likely to be occupied if it is closer to an occupied site, or if it is less likely to be occupied if closer to a disturbance, like heavily populated areas. Other spatial analyses could include the effects of topographical features like rocky headlands or sandy beaches on tidewater goby occupancy. To increase our understanding and aid in preservation of both tidewater goby species, having access to and monitoring of restricted sites (Appendix K) should be considered. This is especially true for southern tidewater goby that have been documented in only three

small sites on Marine Corps Base Camp Pendleton; all three sites come with a high risk of extinction (Swift et al. 2016). Elimination of those last remaining southern sites would result in a southern range constriction and could lead to the extinction of the southern species.

Efficient rangewide monitoring of a species is increasingly important in habitats that are dominated by anthropogenic impacts. This work highlights the power of eDNA combined with occupancy modeling as a capable tool for rangewide distribution monitoring to support species conservation. This study can serve as a baseline for examining the status and trends in occupancy for tidewater goby. In addition, presence/absence data of this study will be used to supplement a metapopulation viability analysis. Lastly, this study can help evaluate range expansion or contraction of the species and contribute to a better understanding of metapopulation dynamics in tidewater goby.

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APPENDIX A

Appendix A: GPS coordinates (WGS 84 datum), site names, location of site within the range of northern tidewater goby, *Eucyclogobius newberryi* (N) or southern tidewater goby, *Eucyclogobius kristinae* (S), collection dates, number of water samples collected per site, and qPCR detection (1) or non-detection (0). Sites with no water present at time of visit are listed as dry. An asterisk indicates that site was visited twice due to clogging of filters, resulting in limited filtration volume at first visit. The site Pismo Creek could not be evaluated with qPCR because of contamination during lab procedures. Sites are listed north to south.

Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
41.98142	-124.20453	Gilbert Creek	N	07/07/16	1	0
41.97347	-124.20434	Unnamed Creek - Kamph Memorial	N	07/07/16	1	0
41.96011	-124.20621	Lopez Creek	N	07/07/16	1	0
41.93345	-124.19090	Tillas Slough	N	09/09/16	5	1
41.84268	-124.20577	Lake Earl	N	07/07/16	5	1
41.75049	-124.19341	Elk Creek	N	07/07/16	2	0
41.74063	-124.16389	Crescent City Marsh Outlet	N	07/07/16	2	0
41.72569	-124.15113	Endert Beach Pond	N	09/09/16	dry	-
41.60483	-124.10052	Wilson Creek	N	09/09/16	dry	-
41.59463	-124.10240	Lagoon Creek	N	09/09/16	2	0
41.53680	-124.07581	Klamath River	N	07/04/16	6	1
41.29394	-124.08816	Redwood Creek	N	06/27/16	6	0
41.26671	-124.09694	Freshwater Lagoon	N	09/09/16	2	0
41.23176	-124.08442	Stone Lagoon	N	06/13/16	5	1
41.16971	-124.12916	Big Lagoon	N	06/13/16	5	1
41.01910	-124.10644	Little River	N	07/11/16	3	0
40.93240	-124.12770	Mad River	N	07/11/16	4	0
40.89799	-124.13498	Mad River Slough	N	07/11/16	4	0
40.87971	-124.13051	Liscom Slough	N	07/11/16	3	0
40.86689	-124.10298	McDaniel Slough East	N	09/20/16	4	0
40.86408	-124.09571	McDaniel Slough Area 13 (Freshwater East)	N	09/20/16	1	0
40.86362	-124.09635	McDaniel Slough Area 10 (Freshwater West)	N	09/20/16	1	1

Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
40.86132	-124.09345	McDaniel Slough Area 11.1 (Brackish Pond)	N	09/20/16	1	0
40.85748	-124.12351	McDaniel Slough West	N	07/11/16	4 + 1 dry	1
40.85631	-124.09060	Butcher's Slough (Jolly Giant Creek)	N	07/12/16	2	0
40.85463	-124.09261	Klopp Lake	N	07/12/16	2	0
40.85378	-124.09128	Arcata Wastewater Pond 1	N	09/20/16	1	0
40.85306	-124.09124	Arcata Wastewater Pond 3	N	09/20/16	1	0
40.85262	-124.09129	Arcata Wastewater Pond 4	N	09/20/16	1	0
40.85303	-124.09162	Arcata Wastewater Raceway 1	N	09/20/16	1	1
40.85271	-124.09176	Arcata Wastewater Raceway 2	N	09/20/16	1	0
40.84575	-124.08122	Gannon Slough / Pond	N	05/15/16	2	1
40.84352	-124.08163	Jacoby Creek	N	05/15/16	1	1
40.80988	-124.11276	Hwy 101 Ditch	N	05/15/16	2	1
40.80381	-124.10606	Fay Slough	N	07/12/16	2	0
40.79754	-124.12288	Dead Mouse Marsh	N	07/21/16	3	0
40.78771	-124.18626	Palco Marsh	N	07/21/16	3	0
40.78662	-124.09178	Freshwater Slough	N	07/12/16	3	0
40.78543	-124.10050	Wood Creek	N	07/21/16	2	0
40.75743	-124.17127	Martin Slough	N	05/15/16	2	1
40.75731	-124.18822	Elk River At Hwy 101	N	07/21/16	2	0
40.75684	-124.19314	Elk River Estuary Area 1	N	07/27/16	5	0
40.75556	-124.19456	Elk River Estuary Area 2	N	07/27/16	4 + 1 dry	0
40.74918	-124.18850	Elk River Wildlife Area	N	07/28/16	2	0
40.73522	-124.21411	King Salmon Marsh	N	07/28/16	4	0
40.70141	-124.21374	HBNWR North	N	09/11/16	5	1
40.67832	-124.20697	HBNWR South	N	09/11/16	5	1
40.68835	-124.27833	Ocean Ranch North	N	07/21/16	6	1
40.65381	-124.29306	Ocean Ranch South	N	07/27/16	4	1
40.61901	-124.31166	Salt River	N	09/11/16	5	1
40.60590	-124.32656	Eel River Estuary Preserve	N	07/28/16	5	1
39.70288	-123.80330	Juan Creek	N	06/22/16	1	0
39.67789	-123.79047	Howard Creek	N	06/22/16	1	0
39.61328	-123.78260	Chadbourne Gulch (Breaking Bad Beach)	N	06/22/16	1	0
39.53936	-123.74568	Ten Mile River	N	06/22/16	3	1
39.47145	-123.80415	Virgin Creek	N	06/22/16	2	1
39.45757	-123.80730	Pudding Creek	N	06/22/16	2	1

Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
39.42768	-123.80809	Noyo River	N	06/23/16	3	0
39.37690	-123.81753	Jug Handle Creek	N	06/23/16	2	0
39.36171	-123.81585	Caspar Creek	N	06/23/16	2	0
39.35958	-123.81698	Doyle Creek	N	06/23/16	1	0
39.32878	-123.80474	Russian Gulch	N	06/23/16	2	0
39.30224	-123.78787	Big River	N	06/23/16	3	0
39.27393	-123.79121	Little River	N	06/23/16	1	0
39.19666	-123.74738	Navarro River	N	06/23/16	3	1
39.00385	-123.69596	Alder Creek	N	06/24/16	2	0
38.99119	-123.70180	Davis Lake / Pond	N	06/24/16	4	1
38.97613	-123.71128	Brush Creek	N	06/24/16	2	0
38.95192	-123.73282	Garcia River	N	06/24/16	3	0
38.75942	-123.52151	Gualala River	N	06/24/16	2	0
38.43550	-123.10238	Russian River	N	08/17/16	1	0
38.38513	-123.08318	Scotty Creek	N	08/17/16	1	0
38.36985	-123.07368	Marshall Gulch	N	08/17/16	1	0
38.35068	-123.06336	Salmon Creek	N	08/17/16	2	1
38.33433	-123.04995	Johnson Gulch - Bodega Bay	N	08/17/16	1	0
38.31846	-123.03601	Cheney Gulch - Bodega Bay	N	08/17/16	3	0
38.30966	-122.93577	Estero Americano	N	08/17/16	1	0
38.27755	-122.94832	Estero De San Antonio	N	08/17/16	1	0
38.22233	-122.92042	Walker Creek	N	08/16/16	3	0
38.13862	-122.89559	Tomales Bay	N	08/16/16	4	0
38.13616	-122.89816	Indian Beach - Tomales Bay	N	08/16/16	2	0
38.11002	-122.49504	Petaluma Creek	N	05/22/16	1	0
38.09154	-122.92897	Schooner Creek	N	08/16/16	1	0
38.08378	-122.50641	Novato Creek	N	05/22/16	1	0
38.07062	-122.81193	Papermill Creek	N	08/15/16	1	0
38.06270	-122.81968	Lagunitas Creek	N	08/15/16	2	1
38.03268	-122.95433	Horseshoe Cove	N	08/16/16	2	0
38.02735	-122.88257	Limantour Slough	N	08/16/16	3	0
37.94195	-122.49968	San Rafael Bay – Corte Madera Channel	N	05/22/16	1	0
37.90612	-122.65068	Bolinas Lagoon	N	08/15/16	4	0
37.89195	-122.52388	Mill Creek	N	05/22/16	1	1
37.86021	-122.57750	Redwood Creek Lagoon	N	08/15/16	2	0
37.85125	-122.30025	San Francisco Bay - Berkeley	N	05/22/16	1	0

Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
37.84154	-122.55131	Tennessee Valley Lagoon	N	08/15/16	3	0
37.83195	-122.52590	Rodeo Lagoon	N	08/15/16	3	1
37.79872	-122.25906	Lake Merrit	N	05/22/16	1	0
37.77958	-122.51404	Cliff House Lagoon (Sutro Baths)	N	05/23/16	1	0
37.72574	-122.49829	Lake Merced	N	05/23/16	1	0
37.61167	-122.49602	Laguna Salada	N	05/23/16	1	0
37.59593	-122.50550	San Pedro Creek	N	05/23/16	1	1
37.48083	-122.45111	Frenchmans Creek	N	05/23/16	2	0
37.47458	-122.44765	Pilarcitos Creek	N	05/23/16	2	0
37.35687	-122.39967	Tuniitas	N	05/24/16	1	1
37.32137	-122.40378	San Gregorio Creek	N	05/24/16	2	1
37.29924	-122.40521	Pompino Creek	N	05/24/16	1	1
37.26544	-122.40822	Pescadero Creek	N	05/24/16	2	1
37.22399	-122.40620	Bean Hollow	N	05/25/16	1	1
37.19295	-122.39814	Yankee Jim	N	05/25/16	1	0
37.16532	-122.36157	Gazos Creek	N	05/25/16	1	1
37.09711	-122.27823	Waddell Creek	N	05/25/16	2	0
37.04064	-122.22875	Scott Creek	N	05/25/16	2	1
36.98364	-122.15426	Laguna Creek	N	05/25/16	1	1
36.97197	-121.95293	Soquel Creek	N	05/27/16	1	0
36.96924	-121.90646	Aptos Creek	N	08/14/16	2	1
36.96675	-122.12386	Baldwin Creek	N	05/26/16	2	1
36.96581	-122.01319	San Lorenzo River	N	05/26/16	2	1
36.96332	-121.99684	Schwan Lagoon	N	05/26/16	1	0
36.96258	-122.11260	Lombardi Creek	N	05/26/16	1	1
36.96060	-121.98412	Corcoran Lagoon	N	05/27/16	2	1
36.95676	-121.97757	Moran Lake	N	05/27/16	1	1
36.95478	-122.09140	Old Dairy Creek	N	05/26/16	1	1
36.95231	-122.05834	Moore Creek	N	05/26/16	1	1
36.94941	-122.06759	Younger Lagoon	N	08/15/16	3	1
36.86836	-121.81722	Watsonville Slough	N	05/27/16	1	1
36.82114	-121.78505	Bennet Slough	N	05/27/16	1	1
36.77192	-121.78961	Mojo Cojo / Salinas Irrigation Channel	N	05/27/16	1	1
35.70861	-121.30431	Arroyo De La Cruz	N	05/28/16	1	1
35.69253	-121.29041	Arroyo Del Oso	N	05/28/16	1	0

Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
35.65124	-121.21990	Oak Knoll Creek / Arroyo Laguna	N	05/28/16	2	1
35.64708	-121.21167	Arroyo De Tortuga	N	05/28/16	1	1
35.64434	-121.18901	Arroyo Del Puerto	N	05/29/16	1	1
35.64215	-121.18275	Broken Bridge Creek	N	05/29/16	1	1
35.63402	-121.16339	Little Pico Creek	N	05/29/16	2	1
35.61578	-121.14941	Pico Creek	N	05/29/16	2	1
35.59578	-121.12576	San Simeon Creek	N	05/28/16	2	1
35.58212	-121.11870	Leffingwell Creek	N	05/29/16	dry	-
35.56718	-121.10903	Santa Rosa Creek	N	05/29/16	2	1
35.45012	-120.90744	Cayucos Creek	N	05/30/16	2	1
35.44827	-120.90388	Little Cayucos Creek	N	05/30/16	1	1
35.44808	-120.93398	San Geronimo Creek	N	05/30/16	1	1
35.43529	-120.88754	Old Creek	N	05/30/16	dry	-
35.42816	-120.88236	Willow Creek	N	05/30/16	1	0
35.41279	-120.87347	Torro Creek	N	05/30/16	2	1
35.37611	-120.86268	Morro Creek	N	05/30/16	dry	-
35.35079	-120.83140	Chorro Creek - Morro Bay	N	05/30/16	2	0
35.33256	-120.81819	Oso Creek - Morro Bay	N	05/31/16	2	0
35.18031	-120.73881	San Luis Obisbo Creek	N	05/31/16	2	1
35.13120	-120.63857	Pismo Creek	N	05/31/16	2	-
35.03111	-120.62052	Oso Flaco Lake	N	05/31/16	2	0
34.79664	-120.62055	San Antonio Creek	N	06/01/16	3	1
34.69142	-120.60069	Santa Ynez River	N	06/01/16	3	1
34.60820	-120.63606	Canada Honda	N	06/01/16	dry	-
34.51222	-120.50220	Jalama Beach	N	06/01/16	1	1
34.47376	-120.14132	Arroyo Hondo	N	06/02/16	1	0
34.47132	-120.22647	Gaviota Creek	N	06/02/16	2	1
34.46331	-120.06969	Refugio Creek	N	06/02/16	1	1
34.43554	-119.92946	Eagle Canyon	N	06/02/16	dry	-
34.43214	-119.91774	Tecolote Canyon	N	06/02/16	dry	-
34.42954	-119.91250	Winchester / Bell Canyon*	N	06/02/16	1	0
34.42954	-119.91244	Winchester / Bell Canyon	N	08/13/16	dry	-
34.42191	-119.65824	Andre Clark Bird Refugee*	N	06/04/16	2	1
34.42191	-119.65824	Andre Clark Bird Refugee	N	08/13/16	3	0
34.42175	-119.87905	Phelps Creek	N	08/14/16	1	0
34.41779	-119.82986	Goleta Slough*	N	06/03/16	2	0

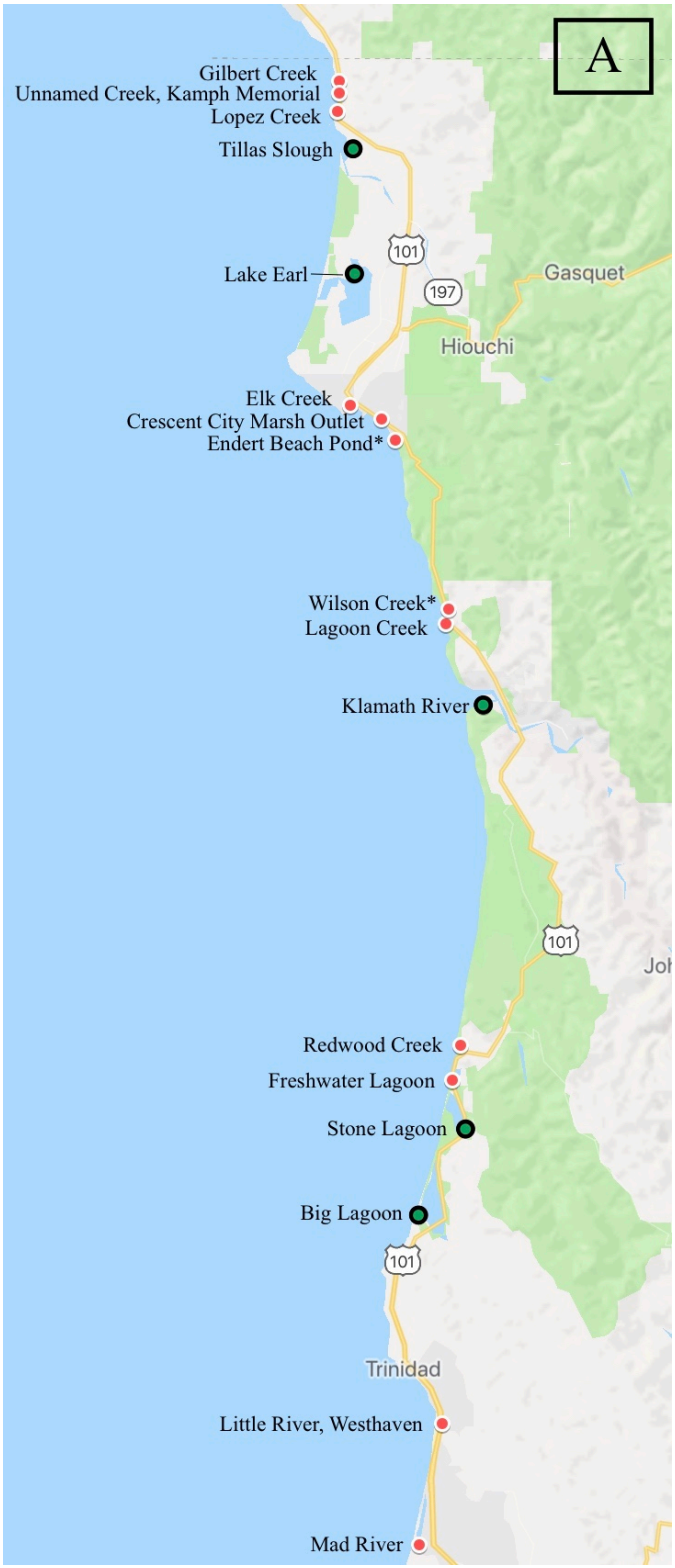
Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
34.41779	-119.82986	Goleta Slough	N	08/13/16	3	1
34.41717	-119.66674	Sycamore Creek	N	06/04/16	1	1
34.41717	-119.66674	Sycamore Creek	N	08/13/16	1	1
34.41345	-119.68537	Laguna Channel*	N	06/03/16	1	1
34.41345	-119.68537	Laguna Channel	N	08/13/16	1	1
34.41323	-119.55838	Arroyo Paredon*	N	06/04/16	1	1
34.41323	-119.55838	Arroyo Paredon	N	08/13/16	1	1
34.41258	-119.68817	Mission Creek*	N	06/03/16	1	1
34.41258	-119.68817	Mission Creek	N	08/13/16	1	1
34.40971	-119.87973	Devereux Slough*	N	06/03/16	3	1
34.40971	-119.87973	Devereux Slough	N	08/14/16	4	0
34.40273	-119.74267	Arroyo Burro	N	06/03/16	2	1
34.39741	-119.52663	Carpinteria Salt Marsh*	N	06/04/16	3	0
34.39741	-119.52663	Carpinteria Salt Marsh	N	08/13/16	3	0
34.39086	-119.51953	Carpinteria Creek*	N	06/03/16	2	1
34.39086	-119.51953	Carpinteria Creek	N	08/13/16	2	1
34.37413	-119.47696	Rincon Creek*	N	06/04/16	1	1
34.37413	-119.47696	Rincon Creek	N	08/12/16	1	1
34.27727	-119.30706	Ventura River Lagoon	N	08/12/16	2	1
34.23661	-119.25669	Santa Clara River	N	08/12/16	4	1
34.13751	-119.18349	Ormond Lagoon	N	08/12/16	3	1
34.13194	-119.07944	Revolon Slough - Calleguas Creek	N	08/12/16	2	0
34.07166	-119.01472	Sycamore Canyon	N	08/11/16	dry	-
34.03855	-118.58327	Topanga Creek	N	08/11/16	1	1
34.03706	-118.63659	Las Flores Canyon	N	08/11/16	1	0
34.03367	-118.73415	Corral Canyon	N	08/11/16	dry	-
34.03319	-118.68543	Malibu Lagoon	N	08/11/16	3	0
34.03305	-118.74242	Solstice Canyon	N	08/11/16	dry	-
34.03001	-118.84189	Trancas Canyon	N	08/11/16	1	0
34.02778	-118.51948	Santa Monica Canyon - Rustic Creek	N	08/11/16	1	0
34.02587	-118.76584	Escondido Canyon	N	08/11/16	dry	-
34.01448	-118.82075	Zuma Lagoon	N	08/11/16	1	0
33.96673	-118.42758	Ballona Freshwater Marsh	N	08/11/16	1	0
33.96424	-118.45022	Ballona Wetlands	N	08/11/16	2	0
33.96195	-118.45134	Del Rey Lagoon	N	08/11/16	1	0

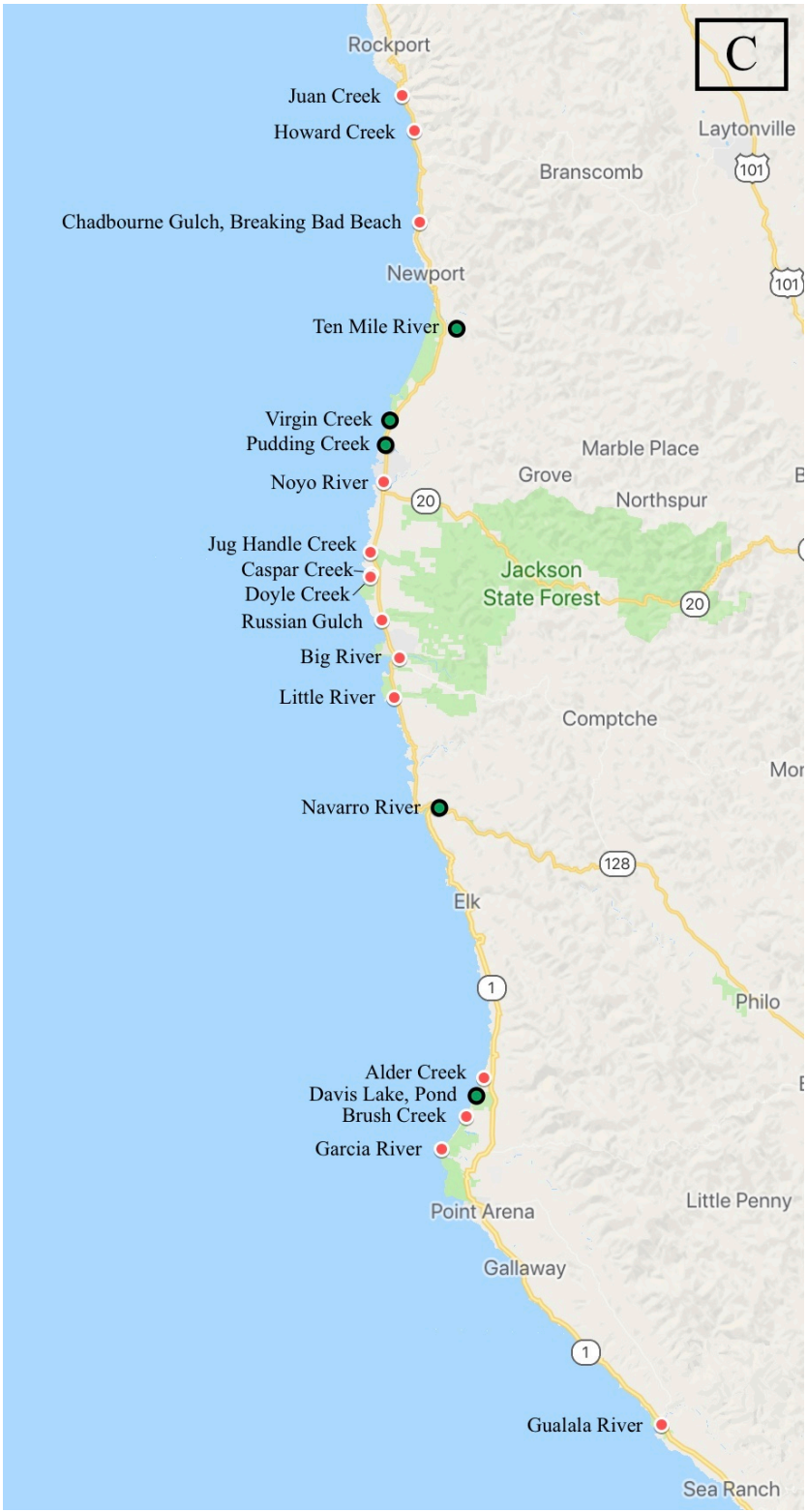
Latitude	Longitude	Site Name	N/S	Collection	Water Samples	qPCR
33.77035	-118.13213	Colorado Lagoon - Alamitos Bay	S	08/10/16	2	0
33.75384	-118.13134	Alamitos Bay	S	08/10/16	2	1
33.72950	-118.06983	Bolsa Chica Channel	S	08/10/16	1	0
33.69671	-118.04472	Bolsa Chica	S	08/10/16	4	0
33.63337	-117.95807	Talbert Marsh	S	08/10/16	1	0
33.63105	-117.95655	Santa Ana River	S	08/10/16	2	0
33.62539	-117.88476	Upper Newport Bay	S	08/10/16	4	0
33.56435	-117.82796	Muddy Creek - Crystal Cove	S	08/10/16	1	0
33.51076	-117.75286	Aliso Creek	S	08/09/16	2	0
33.46261	-117.68406	San Juan Creek	S	08/09/16	2	0
33.46203	-117.68914	Puerto Creek	S	08/09/16	1	0
33.38674	-117.59411	San Mateo Creek Lagoon	S	08/09/16	2	0
33.38143	-117.57864	San Onofre Creek	S	08/09/16	1	1
33.20343	-117.39123	San Luis Rey	S	08/09/16	3	0
33.17974	-117.34136	Buena Vista Lagoon	S	08/09/16	3	0
33.17720	-117.36912	Loma Alta Creek	S	08/09/16	2	0
33.14439	-117.34226	Agua Hedionda Lagoon	S	08/08/16	3	0
33.11582	-117.32449	Canyon de las Encinas	S	08/08/16	2	1
33.08767	-117.31232	San Marcos Creek - Batiquitos Lagoon	S	08/08/16	4	0
33.01192	-117.27270	Escondido Creek - San Elijo Lagoon	S	08/08/16	3	1
32.96369	-117.25571	San Dieguito Lagoon	S	08/08/16	3	0
32.93411	-117.26024	Los Penasquitos Lagoon	S	08/08/16	3	0

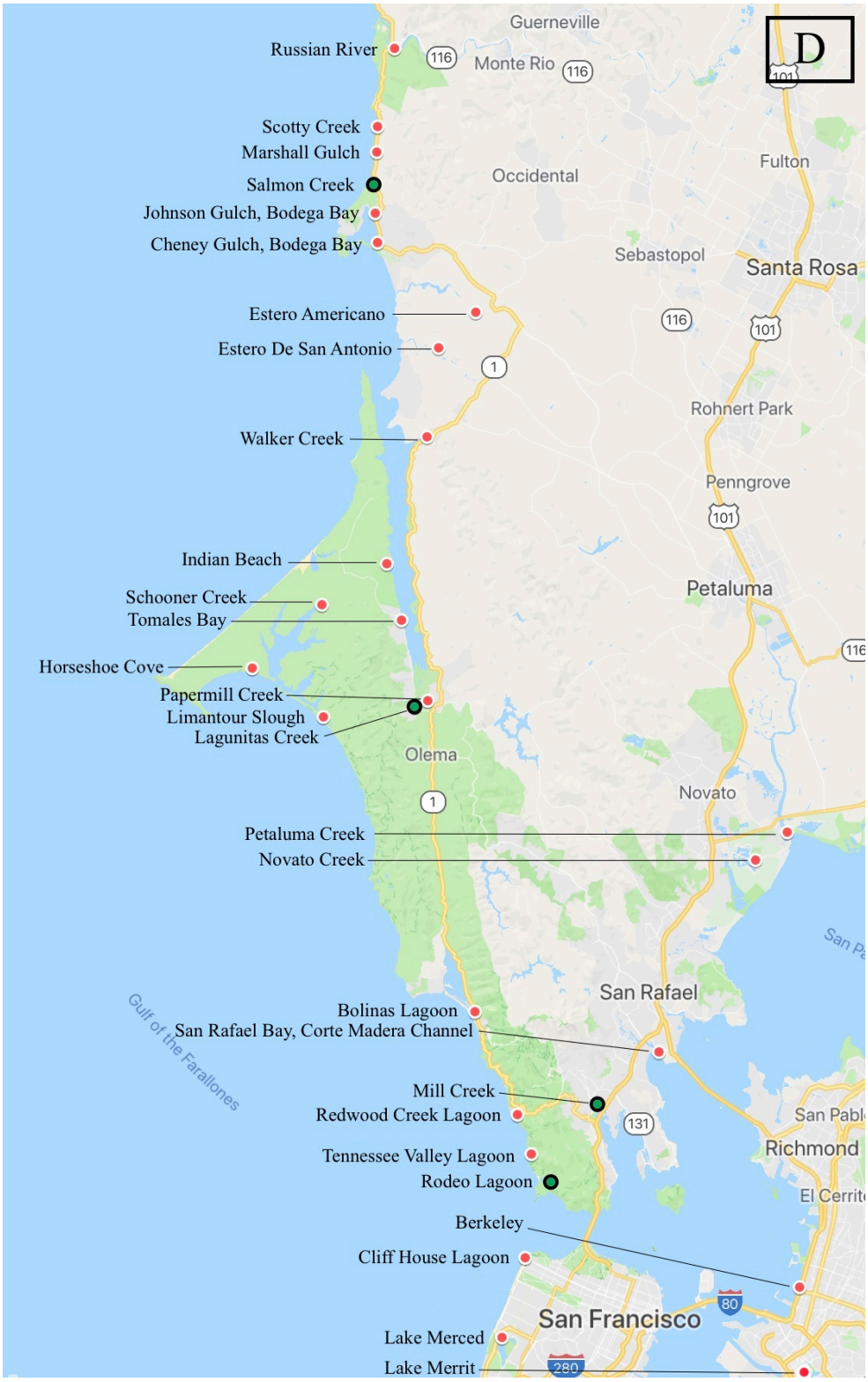
APPENDIX B

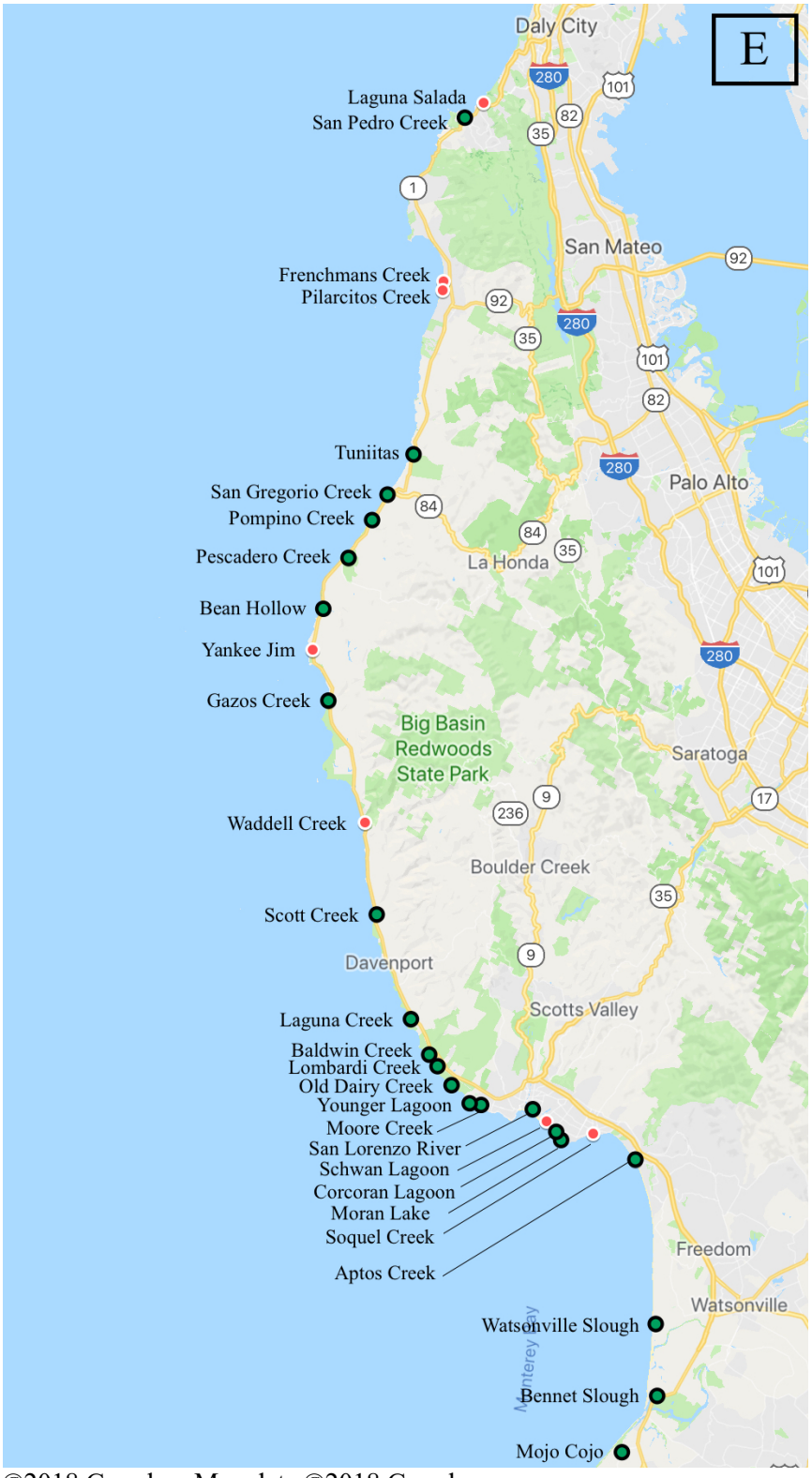
Appendix B: Overview maps of the United States and California followed by regional maps of all sites listed from north to south. Maps A through G encompass the range of northern tidewater goby (*Eucyclogobius newberryi*) and map H depicts all southern tidewater goby (*Eucyclogobius kristinae*) collection sites. There is a single dot for each site, individual water collection locations are not indicated. All sites are marked with black dots on the overview maps. For the regional maps green dots with black circles indicate tidewater goby detection in at least one qPCR replicate and red dots with white circles indicate tidewater goby qPCR non-detection. An asterisk next to a site name indicates that the site was dry during time of visit. The number of water samples collected at each site is listed in Appendix A. All maps are created with Google maps (©2018 Google).

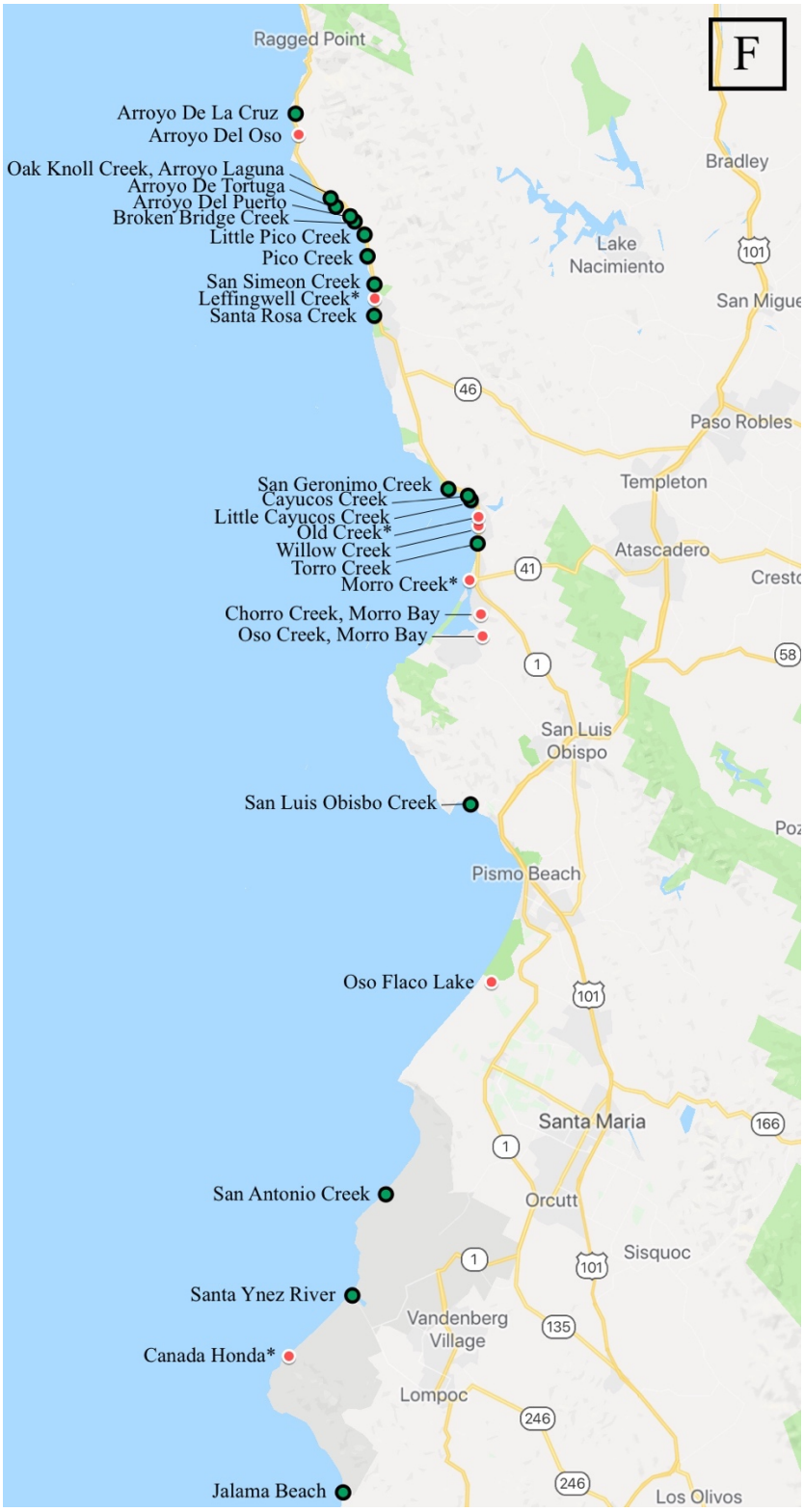
©2018 Google – Map data ©2018 INEGI, Google

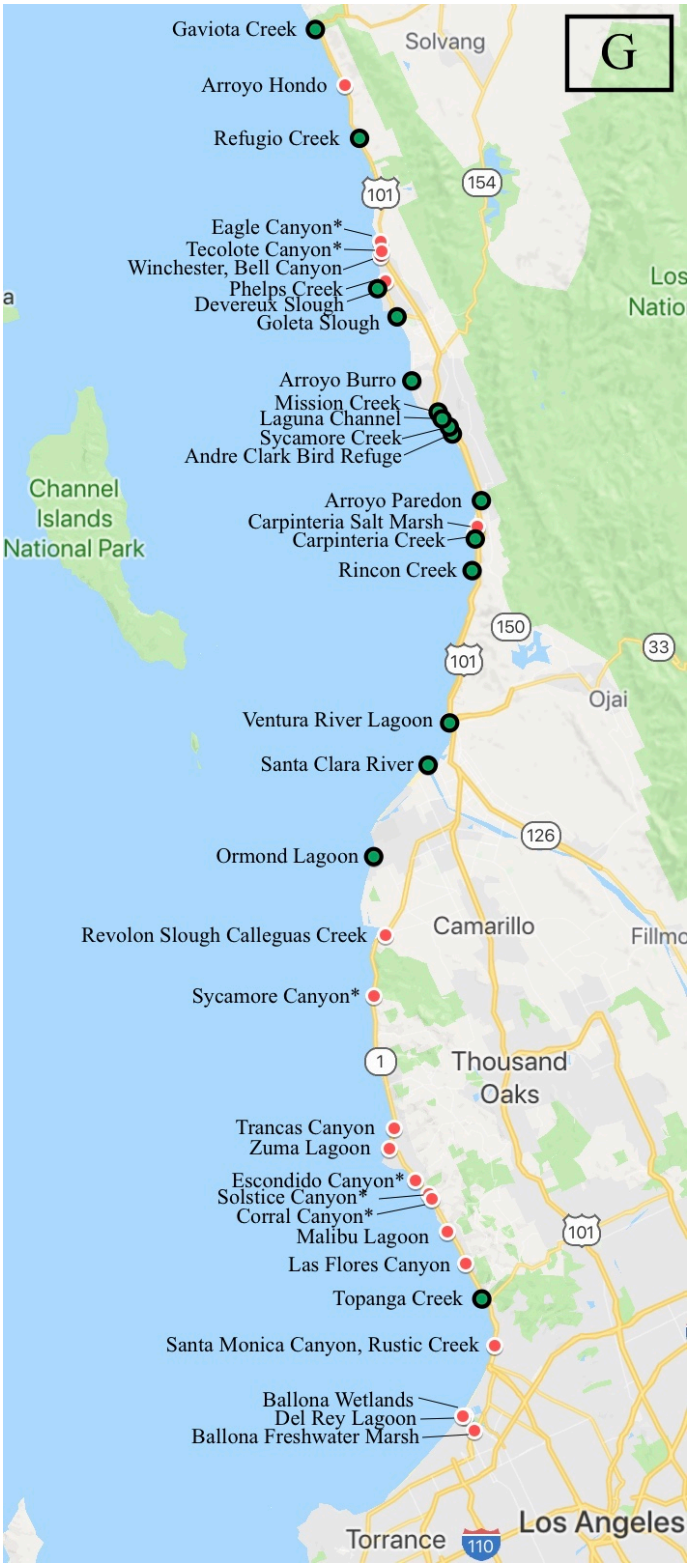


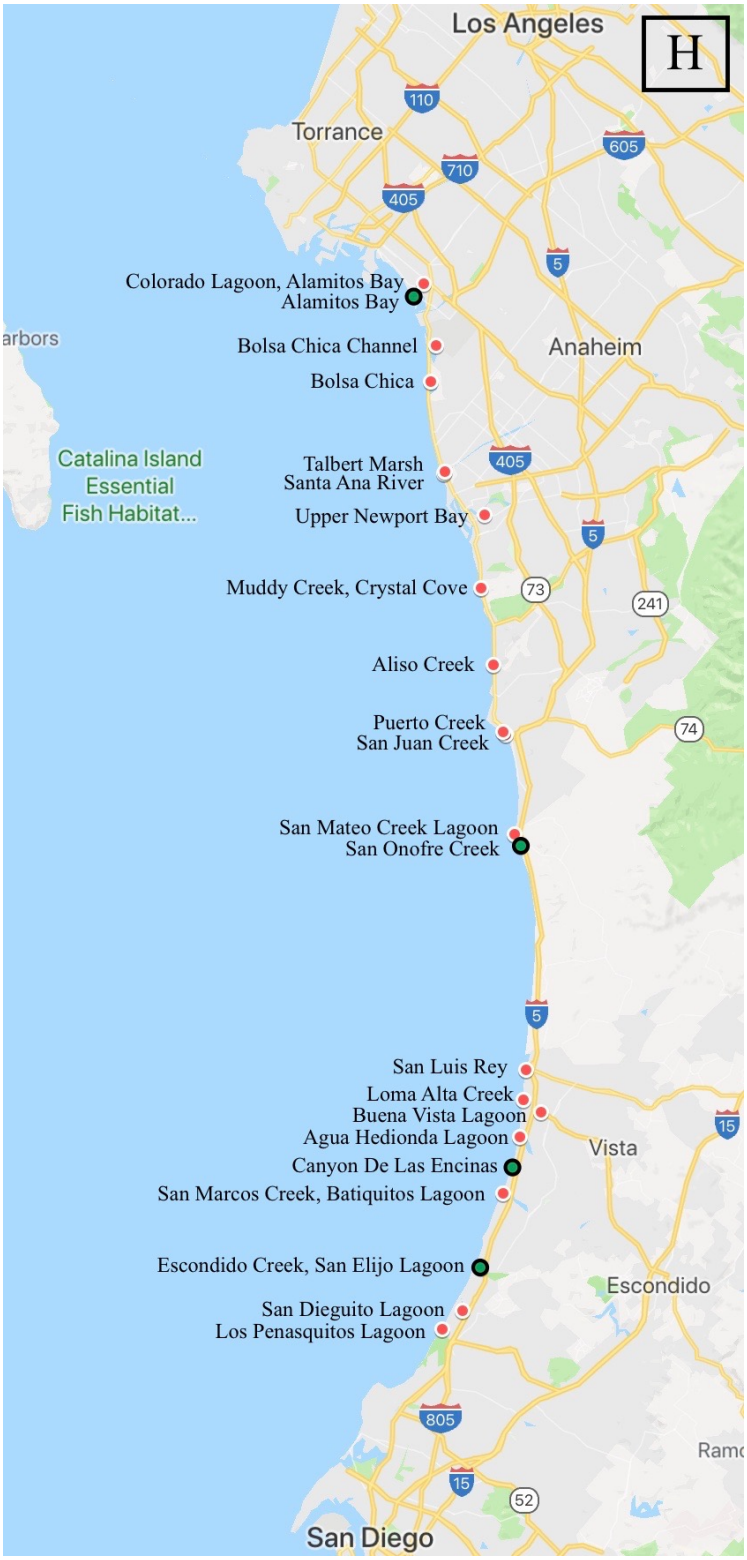












APPENDIX C

Appendix C: Environmental DNA water collection procedure.

Water collection:

1) Wear a separate set of disposable gloves for each water sample collected.
2) Collect from shore and avoid stirring up the sediment when collecting the water sample.
3) Submerge a 69oz Whirl-Pak sample bag and collect two liters of water near the surface by pulling the bag through the water.
4) Close the Whirl-Pak bag by rolling the opening at least five times and securing it with the integrated tabs.
5) Label the bag with collection number that corresponds to the collection number on the field collection list.
5) Spray the outside of the bag with 20% bleach and wipe dry with paper towels to avoid contamination between water samples.
6) Store used gloves and paper towels in a separate sealed bag.
7) Filter water as soon as possible to avoid any degrading of eDNA present. If necessary, store water samples on wet ice until filtration (no longer than 16h, USFWS 2015).

For each water sampling location record the following:

1) Site Name and number
2) GPS coordinates
3) Date and time of sampling
4) Water depth
5) Temperature (°C), salinity (‰), dissolved oxygen (mg/L)
   Make sure to record these water quality measurements after water collection to avoid contamination between sites.
6) Substrate (gravel, sand, etc.)
7) Aquatic vegetation
8) Sea connection/tidal influence (open or closed)
9) Weather/weather events (recent floods, storms, lagoon breaches)

Field blank:

In order to detect any contamination that could result from handling of water samples during collection and field filtration, it is necessary to use a field blank. Use at least one field blank per sampling day and every time water is filtered.

1) Wear a new set of disposable gloves.
2) At sampling or filtration site pour 250ml reverse osmosis water (or store-bought drinking water) into a 24oz Whirl-Pak sample bag.
3) Close the field blank sample bag by rolling the opening at least five times and securing it with the integrated tabs.
4) Label the bag with collection number that corresponds to the collection number on the field collection list.
4) Spray the outside of the bag with 20% bleach and wipe dry with paper towels to avoid any contamination.
5) Store used gloves and paper towels in a separate sealed bag.
6) Filter field blank water as soon as possible. If necessary, store field blank on wet ice until filtration (no longer than 16h, USFWS 2015).

Decontamination procedures:

1) If water could not be collected from shore, clean boots or waders with 20% bleach to avoid contamination between sites.

2) If water could not be collected from shore, clean boots or waders with ROCCAL®-D Plus (Pfizer) disinfectant to avoid the spread of New Zealand mudsnails between sites.

Materials:

Whirl-Pak sample bags:
Nasco Whirl-Pak™ Stand-up sample bags (sterile), 69oz, Fisher Scientific catalog # 01-812-129
Nasco Whirl-Pak™ Stand-up sample bags (sterile), 24oz, Fisher Scientific catalog # 01-812-125

Dissolved oxygen meter/thermometer:
YSI, ProODO

Refractometer:
Sper Scientific, Model # 300011

APPENDIX D

Appendix D: Environmental DNA field water filtration procedure.

Water filtration in the field:

1) Find a clean and level area away from hazards to set up generator and vacuum pump. Plug pump into generator.
2) Screw jar lid, with #8 rubber stopper and air nozzle connector (Image 1), onto a one-gallon glass jar (Image 2). Connect vacuum pump with air nozzle on jar lid with silicone tubing.
3) Put on a new set of disposable gloves. Also, change gloves or wipe with RNase AWAY™ if contamination is suspected.
4) Securely fit a sterilized 250 ml filter funnel with base (Image 3) into the rubber stopper hole.
5) Separate filter funnel from base and with sterilized forceps dedicated for filter placement, place a Milipore 47 mm diameter 3.0 µm polycarbonate filter membrane onto filter funnel base. Take extreme care when placing new filters onto the filter funnel base. Only take one filter at a time and be mindful of static electricity. If you drop a filter, use a new one to avoid contamination.
6) Securely replace the 250ml funnel on top of the base and filter. Ensure that it is snug and leak proof. Make sure not to touch the inside of the filter funnel.
7) Turn on generator.
8) Invert and swirl water sample (be careful when inverting Whirl-Pak sample bags that they do not accidentally open). Spray the outside of the bag with 20% bleach or RNAse AWAY™ and wipe dry with paper towels to avoid any contamination.
9) Unroll the Whirl-Pak sample bag and slowly pour approximately 250ml of collected water into the filter funnel.
10) Start the vacuum pump and a timer. (Filtration time will be used as a proxy for sampling location turbidity.)
11) Slowly pour the remainder of the water sample into the filter funnel. Swirl the last 300 – 500 ml of remaining sample to recapture any DNA on walls of sample bag.
12) Once all sample water has passed through the filter and into the glass jar, stop the timer, and vacuum pump. Remove the filter funnel by slowly twisting up and off.
13) With sterilized forceps dedicated for filter removal, carefully fold the filter membrane in half and then in half again.
14) Place filter membrane into a labelled 2ml Eppendorf™ LoBind microcentrifuge tube. Place forceps into 50% bleach followed by reverse osmosis water (or store-bought drinking water) rinse x3 before reuse.
15) Store microcentrifuge tubes with filter membranes in a portable freezer at -18°C until they can be placed in the lab freezer at -20°C.

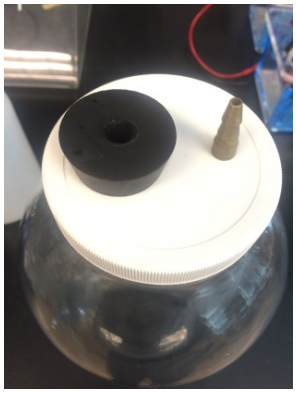


Image 1: Glass jar lid with #8 rubber stopper and air nozzle connector.

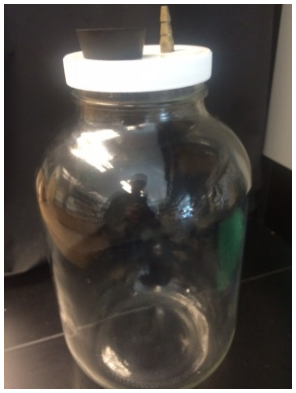


Image 2: 1-gallon glass jar with lid attached.

Image 3: Whatman filter funnel with base attached.

Cleaning of reusable Whatman filter funnels:

1) Rinse filter funnels with tap water.
2) Soak filter funnels in 20% bleach for at least one hour.
3) Thoroughly rinse filter funnels with reverse osmosis or store-bought distilled water.
4) Let filter funnels dry.
5) Autoclave filter funnels in sterilization pouch with a small dry (gravity) cycle at a temperature of 132.0°C and a sterilization time of 30 minutes.

Materials:
(all catalog numbers are Fisher Scientific catalog numbers unless otherwise noted)

Whirl-Pak sample bags:
Nasco Whirl-Pak™ Stand-up sample bags (sterile), 69oz, catalog # 01-812-129
Nasco Whirl-Pak™ Stand-up sample bags (sterile), 24oz, catalog # 01-812-125

RNAse AWAY™:
Thermo Scientific 7002, RNAse AWAY™, Spray bottle, 475ml, catalog # 21-402-178

Vacuum Pump:
Welch Model No. 2522B01, catalog # 01-051-1A

Silicone tubing:
Cole Parmer Masterflex (Platinum) L/S 15, catalog # 13-310-110

Filter Funnels:
Whatman, 250ml, catalog # 1920-7001 (discontinued)

Filters:
EMD Millipore Isopore™ Polycarbonate Membrane Filters, 47mm diameter, 3µm pore size,catalog # TSTP04700

Microcentrifuge tubes:
Eppendorf™ DNA LoBind microcentrifuge tubes 2.0ml, catalog # 13-698-792

Sterilization pouch:
Fisherbrand Instant Sealing Sterilization Pouch, 25x38cm, catalog # 01-812-57

Generator:
Honda EU2000i (Honda: #EU2000iT1A1)

Portable Freezer:
ARB fridge freezer 37 QT (ARB: #10800352)

APPENDIX E

Appendix E: Environmental DNA extraction procedure.

eDNA extractions are based on the QIAGEN DNeasy Blood and Tissue Kit. Periodically change gloves or clean them with RNAse AWAY™ or when touching DNA contaminated surfaces.

1) Expose DNA extraction room (Science C, 111) and hood to ultraviolet light prior to use.
2) Wipe down workspace and instruments to be used with RNAse AWAY™ solution or 20% bleach.
3) Preheat incubator to 56°C. Re-dissolve precipitates in buffer ATL by placing bottle on incubator for a few minutes. Equilibrate frozen filters to room temperature. Wipe outside of the tubes with RNase AWAY™ solution.
4) Add 360 µl Buffer ATL and 40 µl proteinase K to microcentrifuge tubes containing the filters. Vortex and make sure the filter is completely submerged in the lysis solution.
5) Incubate the lysis solution with filter paper at 56°C overnight.
6) Vortex and centrifuge lysed samples for 5 minutes at 13'000 rpm. This should force the filter to the bottom of the tube and the solution containing the DNA to the top.
7) Add 4 µl RNase to the lysate and incubate for 2 minutes at room temperature.
8) Pipette lysate into a labelled QIA shredder spin-column tube.
9) Centrifuge for 2 minutes at > 20'000 rpm. Discard QIA shredder spin-column and keep collection tube with lysate.
10) Add 200 µl of Buffer AL and 200 µl of Ethanol (96-100%), close tube with lid, vortex for 15 seconds. Remove collection tube lid and discard (use scissors to peel of lid).
11) Transfer the lysis solution into a Qiagen DNeasy Blood and Tissue spin-column with the collection tube provided.
12) Centrifuge the spin-column at 8000 rpm for 1 minute. Discard flow-through and collection tube.
13) Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 min at 8000 rpm. Discard flow-through and collection tube.
14) Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 14,000 rpm to dry the DNeasy membrane. Discard flow-through and collection tube.

15) Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 8000 rpm to elute.
16) If not used immediately, store elute at -20°C

Materials:
(all catalog numbers are Fisher Scientific catalog numbers unless otherwise noted)

Master mix:
TaqMan Environmental 2.0 Master Mix (Life Technologies), Cat No: 43-968-38

QIAshredder:
QIAGEN, QIAshredder (250), QIAGEN catalog # 79656

DNeasy:
QIAGEN, DNeasy Blood & Tissue Kit (250), QIAGEN catalog # 69506

RNase:
RNAsecure RNase Inactivation Reagent, Invitrogen catalog # AM7005

RNAse AWAY™:
Thermo Scientific 7002, RNAse AWAY™, Spray bottle, 475ml, Catalog # 21-402-178

Pipette tips:
eppendorf LoRetention Dualfilter, 10ul, Catalog # 02-717-340
eppendorf LoRetention Dualfilter, 100ul, Catalog # 02-717-343
eppendorf LoRetention Dualfilter, 300ul, Catalog # 02-717-342
eppendorf LoRetention Dualfilter 1000ul, Catalog # 02-717-344

Microcentrifuge tubes:
Eppendorf DNA LoBind microcentrifuge tubes, 1.5ml, catalog # 13-698-791

APPENDIX F

Appendix F: List of sympatric species to tidewater goby for which the quantitative PCR genetic assays NC10 and NC10-2 were tested (*) against to ensure specificity at the mitochondrial cytochrome b sequence. NC10 was designed and tested for northern tidewater goby (*Eucyclogobius newberryi*) specificity (Schmelzle and Kinziger 2016). NC10-2 was designed to improve sensitivity for southern tidewater goby (*Eucyclogobius kristinae*) and was tested against arrow goby (*Clevelandia ios*) and bay goby (*Lepidogobidus lepidus*), which are considered the two phylogenetic most closely related species to tidewater goby (Ellingson et al. 2014). None of the species listed were amplified with the assays that they were tested against, indicating specificity to tidewater goby.

Sympatric species	**NC10**	**NC10-2**
Arrow goby (*Clevelandia ios*)	*	*
Bay goby (*Lepidogobius lepidus*)	*	*
Bay pipefish (*Syngnathus leptorhynchus*)	*	
Northern anchovy (*Engraulis mordax*)	*	
Pacific lamprey (*Entosphenus tridentatus*)	*	
Pacific staghorn sculpin (*Leptocottus armatus*)	*	
Prickly sculpin (*Cottus asper*)	*	
Steelhead (*Oncorhynchus mykiss*)	*	
Threespine stickleback (*Gasterosteus aculeatus*)	*	
Topsmelt (*Atherinops affinis*)	*	

APPENDIX G

Appendix G: Comparison of the northern (NC10) and southern (NC10-2) tidewater goby primers on tissue extractions obtained throughout the range of northern tidewater goby (N) and southern tidewater goby (S) (Dave Jacobs, UCLA). The probe used is the same for both species since it shows no base pair mismatches. ID numbers are from Dave Jacobs's extractions. Cycling threshold (Ct) values shown are averaged out of three qPCR reactions. A lower Ct value indicates higher sensitivity of the assay. Sites are listed north to south.

Site Name	County	ID	Latitude	Longitude	Collection	N/S	NC10 Ct	NC10-2 Ct
Salmon Creek	Sonoma	CCS_99-76-037	38.35500	-123.06667	10/19/99	N	16.59	37.59
Lagunitas / Papermill Creek	Marin	CCS_03-86-05	38.08917	-122.83250	10/03/04	N	16.79	41.57
Arroyo de los Frijoles	San Mateo	EN_374	37.22500	-122.40667	06/12/05	N	17.68	34.10
Baldwin Creek	Santa Cruz	EN_358	36.96639	-122.12194	06/12/05	N	16.80	34.54
Corcoran Lagoon	Santa Cruz	EN_328	36.96167	-121.98056	06/12/05	N	18.65	32.36
Corcoran Lagoon	Santa Cruz	EN_323	36.96167	-121.98056	06/12/05	N	18.66	33.73
Moore Creek	Santa Cruz	CCS_99-66-044	36.95000	-122.05750	10/17/99	N	19.68	36.60
Aptos Creek	Santa Cruz	CCS_99-65-021	36.96972	-121.90500	10/17/99	N	17.51	35.42
Bennett Slough	Monterey	CCS_99-63-013	36.82278	-121.77750	10/17/99	N	16.68	35.47
San Onofre Creek	San Diego	EN_168	33.38028	-117.57750	06/12/05	S	27.75	21.69
San Onofre Creek	San Diego	EN_167	33.38028	-117.57750	06/12/05	S	27.39	21.10

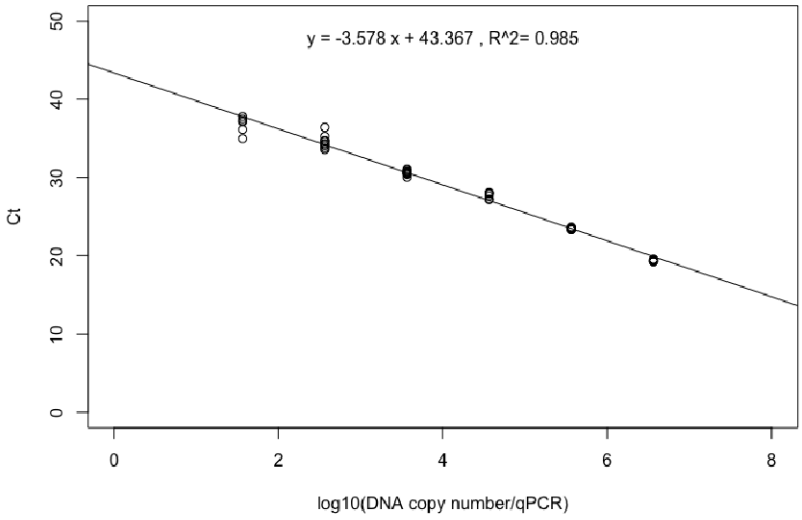
APPENDIX H

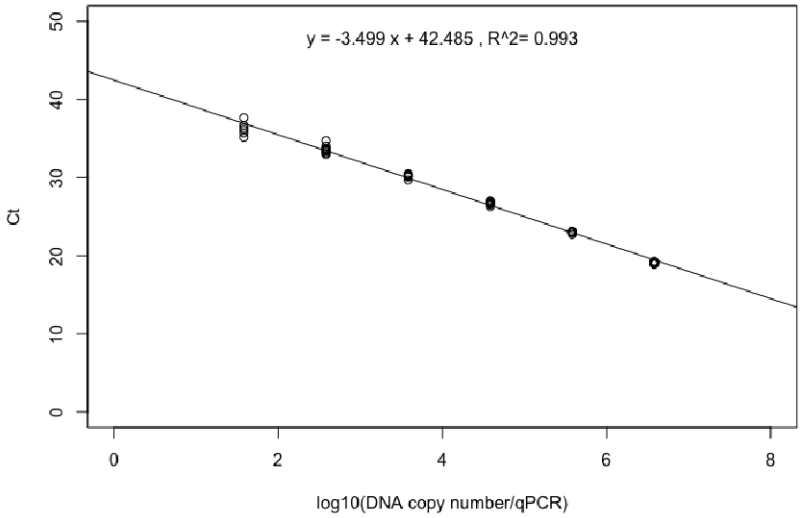
Appendix H: List of closely related or sympatric species to the southern tidewater goby (Ellingson et al. 2014; B. Spies, pers. comm., 2017) for which mitochondrial cytochrome *b* sequences were aligned and evaluated for base pair mismatches with the southern tidewater goby assay NC10-2. Number of base pair mismatches for forward primer, reverse primer, and probe are listed, as well as total number of base pair mismatches of the assay.

Species	**Forward primer**	**Reverse primer**	**Probe**	**Total**
Arrow goby (*Clevelandia ios*)	8	4	7	**19**
Bay goby (*Lepidogobius lepidus*)	10	6	8	**24**
Bright goby (*Ilypnus cf. luculentus*)	9	5	8	**23**
Cheekspot goby (*Ilypnus gilberti*)	7	5	7	**19**
Delta mudsucker (*Gillichthys detrusus)*	8	4	10	**22**
Guaymas goby (*Quietula guaymasiae*)	6	5	7	**18**
Longjaw mudsucker (*Gillichthys mirabilis*)	9	5	11	**25**
Shadow goby (*Quietula y-cauda)*	6	3	6	**15**
Shortjaw mudsucker (*Gillichthys seta*)	5	7	10	**22**
Yellowfin goby (*Acanthogobius flavimanus*)	9	8	9	**26**

APPENDIX I

Appendix I: Standard curves with ten-fold serial dilutions in replicates of ten for northern tidewater goby tissue extract, amplified with northern tidewater goby assay NC10 (top graph) and southern tidewater goby tissue extract amplified with southern tidewater goby assay NC10-2 (bottom graph). Graphs show cycling threshold (Ct) values plotted against $\log_{10}$ transformed DNA copy numbers per qPCR reaction and the corresponding regression equations for each serial dilution.





APPENDIX J

Appendix J: Posterior median estimates of site occupancy (Ψ), water sample availability (θ), and qPCR replicate detection (ρ) probabilities, from fitting the model (Ψ(.),θ(sal),ρ(sal)). The model was fitted by running the Markov chain Monte Carlo (MCMC) algorithm for 110,000 iterations and retaining the last 100,000 for posterior value estimation. Sites are listed north to south.

Site Name	**Ψ**	**θ**	**ρ**
Gilbert Creek	0.545883927	0.794099355	0.826433288
Unnamed Creek, Kamph Memorial	0.545883927	0.794099355	0.826433288
Lopez Creek	0.545883927	0.794099355	0.826433288
Tillas Slough	0.545883927	0.688825978	0.722916903
Lake Earl	0.545883927	0.758513483	0.791972626
Elk Creek	0.545883927	0.678170939	0.712199028
Crescent City Marsh Outlet	0.545883927	0.794099355	0.826433288
Lagoon Creek	0.545883927	0.699379186	0.733379087
Klamath River	0.545883927	0.794099355	0.826433288
Redwood Creek	0.545883927	0.794099355	0.826433288
Freshwater Lagoon	0.545883927	0.78547863	0.818180015
Stone Lagoon	0.545883927	0.699379186	0.733379087
Big Lagoon	0.545883927	0.758513483	0.791972626
Little River, Westhaven	0.545883927	0.776655921	0.809701042
Mad River	0.545883927	0.794099355	0.826433288
Mad River Slough	0.545883927	0.515414746	0.546494151
Liscom Slough	0.545883927	0.419006163	0.445974077
McDaniel Slough Area 13, Freshwater East	0.545883927	0.78547863	0.818180015
McDaniel Slough East	0.545883927	0.431043936	0.458518795
McDaniel Slough Area 10, Freshwater West	0.545883927	0.515414746	0.546494151
McDaniel Slough Area 11.1, Brackish Pond	0.545883927	0.372495293	0.396548902
McDaniel Slough West	0.545883927	0.306322496	0.325715883
Butcher's Slough, Jolly Giant Creek	0.545883927	0.431043936	0.458518795
Klopp Lake	0.545883927	0.407207974	0.433514214
Arcata Wastewater Pond 1	0.545883927	0.454932053	0.483605806
Arcata Wastewater Pond 3	0.545883927	0.454932053	0.483605806

Site Name	Ψ	θ	ρ
Arcata Wastewater Raceway 1	0.545883927	0.395428463	0.421114977
Arcata Wastewater Raceway 2	0.545883927	0.78547863	0.818180015
Arcata Wastewater Pond 4	0.545883927	0.515414746	0.546494151
Gannon Slough, Pond	0.545883927	0.645168464	0.679013174
Jacoby Creek	0.545883927	0.78547863	0.818180015
Hwy 101 Ditch	0.545883927	0.78547863	0.818180015
Fay Slough	0.545883927	0.527516396	0.559029323
Dead Mouse Marsh	0.545883927	0.454932053	0.483605806
Palco Marsh	0.545883927	0.442926803	0.471033921
Freshwater Slough	0.545883927	0.767682413	0.800914803
Wood Creek	0.545883927	0.467046615	0.496219819
Elk River Estuary, Area 1	0.545883927	0.454932053	0.483605806
Martin Slough	0.545883927	0.794099355	0.826433288
Elk River At Hwy 101	0.545883927	0.599194283	0.632369383
Elk River Estuary, Area 2	0.545883927	0.327833369	0.348787542
Elk River Wildlife Area	0.545883927	0.53952804	0.571464611
King Salmon Marsh	0.545883927	0.407207974	0.433514214
HBNWR North	0.545883927	0.38392899	0.408770673
Ocean Ranch North	0.545883927	0.467046615	0.496219819
HBNWR South	0.545883927	0.395428463	0.421114977
Ocean Ranch South	0.545883927	0.467046615	0.496219819
Salt River	0.545883927	0.395428463	0.421114977
Eel River Estuary Preserve	0.545883927	0.739503322	0.773317656
Juan Creek	0.545883927	0.794099355	0.826433288
Howard Creek	0.545883927	0.794099355	0.826433288
Chadbourne Gulch, Breaking Bad Beach	0.545883927	0.794099355	0.826433288
Ten Mile River	0.545883927	0.767682413	0.800914803
Virgin Creek	0.545883927	0.794099355	0.826433288
Pudding Creek	0.545883927	0.491318888	0.521392485
Noyo River	0.545883927	0.467046615	0.496219819
Jug Handle Creek	0.545883927	0.794099355	0.826433288
Caspar Creek	0.545883927	0.776655921	0.809701042
Doyle Creek	0.545883927	0.794099355	0.826433288
Russian Gulch	0.545883927	0.794099355	0.826433288
Big River	0.545883927	0.442926803	0.471033921

Site Name	Ψ	θ	ρ
Little River	0.545883927	0.794099355	0.826433288
Navarro River	0.545883927	0.794099355	0.826433288
Alder Creek	0.545883927	0.794099355	0.826433288
Davis Lake, Pond	0.545883927	0.794099355	0.826433288
Brush Creek	0.545883927	0.794099355	0.826433288
Garcia River	0.545883927	0.749138642	0.78274852
Gualala River	0.545883927	0.794099355	0.826433288
Russian River	0.545883927	0.656324516	0.690219751
Scotty Creek	0.545883927	0.729758804	0.763650607
Marshall Gulch	0.545883927	0.729758804	0.763650607
Salmon Creek	0.545883927	0.622461021	0.655974947
Johnson Gulch, Bodega Bay	0.545883927	0.515414746	0.546494151
Cheney Gulch, Bodega Bay	0.545883927	0.349848807	0.372419102
Estero Americano	0.545883927	0.327833369	0.348787542
Estero De San Antonio	0.545883927	0.349848807	0.372419102
Walker Creek	0.545883927	0.56353508	0.596145006
Tomales Bay	0.545883927	0.349848807	0.372419102
Indian Beach	0.545883927	0.407207974	0.433514214
Petaluma Creek	0.545883927	0.587361901	0.620410604
Schooner Creek	0.545883927	0.295806829	0.314373095
Novato Creek	0.545883927	0.587361901	0.620410604
Papermill Creek	0.545883927	0.395428463	0.421114977
Lagunitas Creek	0.545883927	0.678170939	0.712199028
Horseshoe Cove	0.545883927	0.040817396	0.040020206
Limantour Slough	0.545883927	0.349848807	0.372419102
San Rafael Bay, Corte Madera Channel	0.545883927	0.527516396	0.559029323
Bolinas Lagoon	0.545883927	0.407207974	0.433514214
Mill Creek	0.545883927	0.56353508	0.596145006
Redwood Creek Lagoon	0.545883927	0.758513483	0.791972626
Berkeley	0.545883927	0.527516396	0.559029323
Tennessee Valley Lagoon	0.545883927	0.767682413	0.800914803
Rodeo Lagoon	0.545883927	0.729758804	0.763650607
Lake Merrit	0.545883927	0.527516396	0.559029323
Cliff House Lagoon	0.545883927	0.78547863	0.818180015
Lake Merced	0.545883927	0.794099355	0.826433288

Site Name	Ψ	θ	ρ
Laguna Salada	0.545883927	0.794099355	0.826433288
San Pedro Creek	0.545883927	0.794099355	0.826433288
Frenchmans Creek	0.545883927	0.794099355	0.826433288
Pilacritos Creek	0.545883927	0.794099355	0.826433288
Tuniitas	0.545883927	0.794099355	0.826433288
San Gregorio Creek	0.545883927	0.776655921	0.809701042
Pompino Creek	0.545883927	0.749138642	0.78274852
Pescadero Creek	0.545883927	0.749138642	0.78274852
Bean Hollow	0.545883927	0.794099355	0.826433288
Yankee Jim	0.545883927	0.794099355	0.826433288
Gazos Creek	0.545883927	0.794099355	0.826433288
Waddell Creek	0.545883927	0.794099355	0.826433288
Scott Creek	0.545883927	0.794099355	0.826433288
Laguna Creek	0.545883927	0.794099355	0.826433288
Soquel Creek	0.545883927	0.794099355	0.826433288
Aptos Creek	0.545883927	0.667301565	0.701292272
Baldwin Creek	0.545883927	0.78547863	0.818180015
San Lorenzo River	0.545883927	0.678170939	0.712199028
Schwan Lagoon	0.545883927	0.794099355	0.826433288
Lombardi Creek	0.545883927	0.78547863	0.818180015
Corcoran Lagoon	0.545883927	0.442926803	0.471033921
Moran Lake	0.545883927	0.527516396	0.559029323
Old Dairy Creek	0.545883927	0.776655921	0.809701042
Moore Creek	0.545883927	0.749138642	0.78274852
Younger Lagoon	0.545883927	0.361136425	0.38438937
Watsonville Slough	0.545883927	0.699379186	0.733379087
Bennet Slough	0.545883927	0.645168464	0.679013174
Mojo Cojo	0.545883927	0.749138642	0.78274852
Arroyo De La Cruz	0.545883927	0.794099355	0.826433288
Arroyo Del Oso	0.545883927	0.78547863	0.818180015
Oak Knoll Creek, Arroyo Laguna	0.545883927	0.758513483	0.791972626
Arroyo De Tortuga	0.545883927	0.767682413	0.800914803
Arroyo Del Puerto	0.545883927	0.78547863	0.818180015
Broken Bridge Creek	0.545883927	0.749138642	0.78274852
Little Pico Creek	0.545883927	0.78547863	0.818180015

Site Name	Ψ	θ	ρ
Pico Creek	0.545883927	0.78547863	0.818180015
San Simeon Creek	0.545883927	0.776655921	0.809701042
Santa Rosa Creek	0.545883927	0.78547863	0.818180015
Cayucos Creek	0.545883927	0.610943875	0.64427114
Little Cayucos Creek	0.545883927	0.758513483	0.791972626
San Geronimo Creek	0.545883927	0.749138642	0.78274852
Willow Creek	0.545883927	0.776655921	0.809701042
Torro Creek	0.545883927	0.776655921	0.809701042
Chorro Creek, Morro Bay	0.545883927	0.527516396	0.559029323
Oso Creek, Morro Bay	0.545883927	0.515414746	0.546494151
San Luis Obisbo Creek	0.545883927	0.749138642	0.78274852
Oso Flaco Lake	0.545883927	0.78547863	0.818180015
San Antonio Creek	0.545883927	0.776655921	0.809701042
Santa Ynez River	0.545883927	0.699379186	0.733379087
Jalama Beach	0.545883927	0.794099355	0.826433288
Arroyo Hondo	0.545883927	0.794099355	0.826433288
Gaviota Creek	0.545883927	0.729758804	0.763650607
Refugio Creek	0.545883927	0.78547863	0.818180015
Winchester, Bell Canyon	0.545883927	0.467046615	0.496219819
Phelps Creek	0.545883927	0.699379186	0.733379087
Goleta Slough	0.545883927	0.407207974	0.433514214
Andre Clark Bird Refugee	0.545883927	0.739503322	0.773317656
Sycamore Creek	0.545883927	0.527516396	0.559029323
Arroyo Paredon	0.545883927	0.794099355	0.826433288
Laguna Channel	0.545883927	0.699379186	0.733379087
Mission Creek	0.545883927	0.587361901	0.620410604
Devereux Slough	0.545883927	0.349848807	0.372419102
Arroyo Burro	0.545883927	0.749138642	0.78274852
Carpinteria Salt Marsh	0.545883927	0.53952804	0.571464611
Carpinteria Creek	0.545883927	0.587361901	0.620410604
Rincon Creek	0.545883927	0.729758804	0.763650607
Ventura River Lagoon	0.545883927	0.758513483	0.791972626
Santa Clara River	0.545883927	0.767682413	0.800914803
Ormond Lagoon	0.545883927	0.667301565	0.701292272
Revolon Slough, Calleguas Creek	0.545883927	0.794099355	0.826433288

Site Name	Ψ	θ	ρ
Topanga Creek	0.545883927	0.794099355	0.826433288
Las Flores Canyon	0.545883927	0.794099355	0.826433288
Malibu Lagoon	0.545883927	0.667301565	0.701292272
Trancas Canyon	0.545883927	0.38392899	0.408770673
Santa Monica Canyon, Rustic Creek	0.545883927	0.776655921	0.809701042
Zuma Lagoon	0.545883927	0.78547863	0.818180015
Ballona Freshwater Marsh	0.545883927	0.794099355	0.826433288
Ballona Wetlands	0.545883927	0.587361901	0.620410604
Del Rey Lagoon	0.545883927	0.503330152	0.533991974
Colorado Lagoon, Alamitos Bay	0.545883927	0.467046615	0.496219819
Alamitos Bay	0.545883927	0.491318888	0.521392485
Bolsa Chica Channel	0.545883927	0.454932053	0.483605806
Bolsa Chica	0.545883927	0.515414746	0.546494151
Talbert Marsh	0.545883927	0.527516396	0.559029323
Santa Ana River	0.545883927	0.53952804	0.571464611
Upper Newport Bay	0.545883927	0.776655921	0.809701042
Muddy Creek, Crystal Cove	0.545883927	0.794099355	0.826433288
Aliso Creek	0.545883927	0.794099355	0.826433288
San Juan Creek	0.545883927	0.794099355	0.826433288
Puerto Creek	0.545883927	0.794099355	0.826433288
San Mateo Creek Lagoon	0.545883927	0.794099355	0.826433288
San Onofre Creek	0.545883927	0.794099355	0.826433288
San Luis Rey	0.545883927	0.794099355	0.826433288
Buena Vista Lagoon	0.545883927	0.794099355	0.826433288
Loma Alta Creek	0.545883927	0.776655921	0.809701042
Agua Hedionda Lagoon	0.545883927	0.527516396	0.559029323
Canyon De Las Encinas	0.545883927	0.527516396	0.559029323
San Marcos Creek, Batiquitos Lagoon	0.545883927	0.527516396	0.559029323
Escondido Creek, San Elijo Lagoon	0.545883927	0.454932053	0.483605806
San Dieguito Lagoon	0.545883927	0.454932053	0.483605806
Los Penasquitos Lagoon	0.545883927	0.678170939	0.712199028

APPENDIX K

Appendix K: List of sites with known or potential northern (N) or southern (S) tidewater goby presence (Swift et al. 2016) that could not be accessed during this study due to sampling restrictions. Hollister Ranch sites are on private property and were last surveyed in the early 2000s (B. Spies, pers. comm., 2018). No permit could be obtained for the Marine Corp Base Camp Pendleton sites listed. Sites are listed north to south.

Latitude	Longitude	Site Name	Species	Status	Notes
36.95362	-122.07722	Wilder Creek	N	Present	no public access
35.68473	-121.28638	Arroyo de Corral	N	Present	no access (fenced off)
35.46111	-120.97000	Villa Creek	N	Present	no access (Snowy Plover)
35.09944	-120.62916	Arroyo Grande Creek	N	Present	no access (fenced off)
34.96972	-120.64305	Santa Maria River	N	Present	no access (Snowy Plover)
34.84472	-120.59555	Shuman Canyon	N	Present	site not located
34.45025	-120.42638	Damsite Canyon	N	Unknown	Hollister Ranch site
34.45333	-120.41611	Canada del Cojo	N	Unknown	Hollister Ranch site
34.45916	-120.35416	Arroyo San Augustine	N	Unknown	Hollister Ranch site
34.45970	-120.34027	Canada de las Agujas	N	Unknown	Hollister Ranch site
34.46273	-120.33361	Arroyo El Bulito	N	Unknown	Hollister Ranch site
34.46555	-120.31472	Canada del Agua	N	Unknown	Hollister Ranch site
34.46742	-120.30638	Canada de Santa Anita	N	Unknown	Hollister Ranch site
34.46916	-120.27194	Canada de Alegria	N	Unknown	Hollister Ranch site
34.46833	-120.25222	Canada de Agua Caliente	N	Unknown	Hollister Ranch site
33.27554	-117.45166	Hidden Lagoon	S	Present	Camp Pendleton site
33.25027	-117.43138	Cockleburr Canyon	S	Present	Camp Pendleton site