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Population Ecology

Evaluating River Otter Demography Using Noninvasive Genetic Methods

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ABSTRACT The decline in river otter (*Lontra canadensis*) populations during the late nineteenth and early twentieth centuries throughout North America has led to protective status and strict harvest regulations. Despite sustained interest in protecting river otters, relatively few contemporary studies have evaluated the current status of populations, especially in California. An effective way to estimate river otter population sizes and facilitate monitoring is with noninvasive genetic methods. Our objective was to establish baseline demographic information for an understudied river otter population in northern California by estimating river otter abundance using noninvasive genetic sampling, supplemented with data obtained from an observation-based citizen science project focusing on river otters. We extracted DNA from scat and genotyped samples at 6 microsatellite loci. We used Program MARK to build mark-recapture models to estimate river otter abundance and pooled visual observations from a citizen science project maintained in Humboldt County, California to supplement population estimates with information regarding pups and group size. Between 41 and 44 river otters were in the study area based on noninvasive genetic samples, and group sizes ranged 1–7 or 2–12 based on observational and genetic data, respectively. The Humboldt Bay region had a high density of river otters (0.93 otters/km) as compared to other coastal systems; resource attributes of Humboldt Bay could contribute to this difference. Results reported herein demonstrate methods appropriate for establishing baseline river otter demographics. © 2013 The Wildlife Society.

KEY WORDS abundance estimate, citizen science, Humboldt Bay, *Lontra canadensis*, mark-recapture, noninvasive sampling, river otter.

Historically, river otter populations in North America and beyond have suffered dramatic declines due to habitat degradation and persecution (Polechla 1990, Lariviére and Walton 1998, Kruuk 2006). Precipitous declines resulted in protective legislation strictly regulating North American river otter (*Lontra canadensis*) harvest and led to reintroduction programs throughout the United States (Raesly 2001). However, in some state and provincial jurisdictions, managers have little recent information with which to assess population trends and evaluate the success of earlier protections (Raesly 2001, Melquist et al. 2003). Wildlife managers require accurate census estimates to determine the current status of river otter populations because management goals are articulated in terms of population abundance (Lancia et al. 2005). Reliable abundance estimates can also help identify factors that limit population growth when analyzed in association with other data, such as survival rates or habitat use (Monson et al. 2011, Guertin et al. 2012). This is particularly important for river otters because they suffer more from environmental degradation than other mammals because they move between terrestrial and aquatic landscapes, increasing their exposure to water pollutants and environmental contaminants (Bowyer et al. 2003). However, due to the elusive nature and semi-aquatic lifestyle of river otters, monitoring population demographics of this species is difficult (Melquist et al. 2003).

Frequently used monitoring and census techniques for river otter involve indirect counts of presence, such as track and scat surveys (Sulkava et al. 2008, Jeffress et al. 2011, Stevens et al. 2011, Crowley et al. 2012), which provide useful information regarding aspects of demography. When these methods are applied to population estimates, however, individual identification is not possible, which reduces accuracy (Arrendal et al. 2007, Gallant et al. 2007). Radiotelemetry studies provide valuable demographic data as well but only on a limited number of individuals, as trapping river otters is difficult and recapture events rare, reducing their utility for population estimates (Serfass et al. 1996, Blundell et al. 1999). An alternative method for estimating population abundance is noninvasive genetic sampling in which scat is used to establish individual identification via molecular genotyping and applied in a mark-recapture model framework (Taberlet and Luikart 1999). Noninvasive genetic techniques are especially appropriate for evaluating river otter abundance because otters regularly deposit scat and other obvious markings (intestinal mucus referred to as jellies) that may be used as genetic samples at latrines (Serfass

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et al. 1996, Stevens and Serfass 2008, Oldham and Black 2009), as demonstrated by a number of studies (Hansen and Jacobsen 1999, Dallas et al. 2003, Hung et al. 2004, Polechla et al. 2004, Mowry et al. 2011, Guertin et al. 2012).

A region where evaluating river otter abundance is warranted is coastal northern California, where since 2000, a citizen science project that records river otter observations has demonstrated long-term persistence of river otters throughout the region (Black 2009, also see Shannon 2012). An assessment of the first 5 years of the project provided evidence of reproduction and semi-annual changes in group size (Black 2009). However, the observational nature of the data and lack of rigorous volunteer training was not conducive to estimating population size, and past efforts to quantify California's river otter populations relied on relatively few sightings, making it difficult to assess status, trends, or population size (Kirk 1975, Gould 1977, Schempf and White 1977). A more rigorous noninvasive genetic study would establish baseline population estimates for evaluating river otter conservation status in the region. However, current noninvasive genetic techniques cannot reliably provide age structure (although see Pauli et al. 2011) and are limited in their ability to assess reproductive rates and group sizes. Combining noninvasive genetic population estimates with observational data from the citizen science project could provide a more complete picture of river otter demographics in northern California. For example, group size may vary depending on river otter social structure, so observational data can contribute additional information on sociality.

We used noninvasive genetic mark-recapture modeling methodologies to determine river otter abundance and observational data from a citizen science project to supplement demographic data. These estimates established baseline otter population abundance and structure, which can be compared to other similar systems to elucidate ecologically relevant factors that influence river otter abundance, a necessary first step in designing future management plans for a sentinel wetland species such as the river otter.

STUDY AREA

Humboldt Bay is California's second largest coastal estuary (62.4 km^2; Barnhart et al. 1992), and within California, the abundance and diversity of coastal organisms is greater only in San Francisco Bay (Chamberlain and Barnhart 1993). The northern tip of the study area was approximately 145 km south of the Oregon–California border and ranged in latitude from 41°1′33.53°N to 40°41′26.25°N. Humboldt Bay supports a vast number of fish and bird species, the preferred diet of Humboldt County river otters (Penland and Black 2009, Cosby 2013). Several crab species (*Cancer* spp.) and over 30 fish species have been detected in the Bay (Chamberlain and Barnhart 1993), and Pacific salmon (*Oncorhynchu* spp.) and steelhead (*Oncorhynchus mykiss*) spawn in the coastal rivers (Nehlsen et al. 1991). The region is also a major wintering and stopover site for thousands of migratory shorebirds along the Pacific flyway (Colwell 1994), and more than 200 wetland bird species regularly feed, rest, or nest around the Bay throughout the year. The Humboldt Bay region's monthly mean air temperature varied by only 5.2°C, with lows in January (8.5°C) and highs in August (13.7°C; Barnhart et al. 1992). Precipitation was highest from late October to early May, averaging 97.8 cm annually (Barnhart et al. 1992).

The study area covered approximately 45 km of linear coastal habitat. We chose latrine collection sites to encompass all major watersheds with consistent river otter activity around the Bay. River otter groups ranging in size from 1 to 9 individuals and breeding activity have been documented at each site (Black 2009). Each site had 4–6 latrines and included, from north to south, Little River estuary, Mad River estuary, Arcata Marsh, Mad River Slough, Freshwater Creek, Woodley Island, Elk River estuary, King Salmon Marina, and the Humboldt Bay National Wildlife Refuge complex (Fig. 1). These sites encompassed a myriad of wetlands differing in salinities: brackish estuaries and marshes, freshwater sloughs, fresh water creeks, and salt marshes. Vegetation varied by habitat but was generally a combination of salt water and brackish plants such as saltgrass (*Distichlis spicata*), salt rush (*Juncus lesueurii*), and tufted hairgrass (*Deschiampsia cespitosa*; Barnhart et al. 1992).

METHODS

Noninvasive Field Sampling

Trained biologists collected scat samples from latrine sites 2 consecutive mornings each week during the dry season, from 18 May to 31 October 2008. We established the sampling period as part of a larger study evaluating river otter ecology in Humboldt Bay; we only used scat samples collected from August and September in mark-recapture models to adhere to closed-population assumptions associated with abundance estimates (Otis et al. 1978). August to September was a reasonable timeframe to assume a closed population given river otter longevity (15 yr in the wild), pups are born in early spring, and dispersal generally begins in October (Harris and Ogan 1997). Using 2 months of mark-recapture data also provided the ability to evaluate sampling duration and accuracy of abundance estimates by comparing model results based on 2 months of sampling to the total genotypes detected from an exhaustive 5-month sampling regime. We pooled sampling occasions and recorded a single detection for the week if multiple detections of an individual occurred during 2 consecutive days. We ranked all scat and jelly samples with freshness scores determined visually by moistness and odor: new (<24 hr), old (>24 hr), and recorded the primary diet content of each scat at time of collection. We placed collected scat and jellies into sterile 50-ml centrifuge tubes with sterile tongue depressors and stored them at −20°C until DNA extraction (Arrendal et al. 2007, Lampa et al. 2008).

Various factors have been found to influence genotyping success and subsequent capture probabilities in other noninvasive studies including daily time of collection,

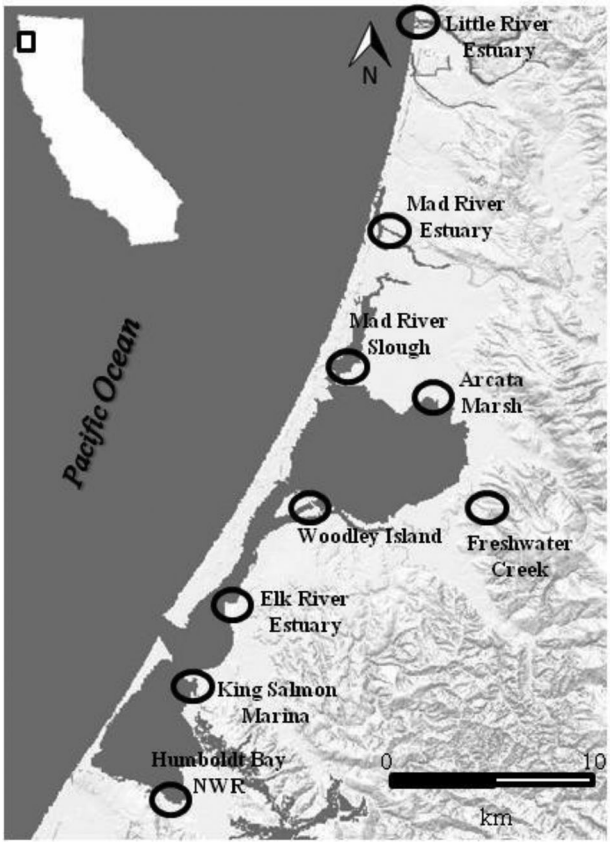

Figure 1. Focal latrine sites sampled noninvasively for river otter scat from 18 May to 31 October 2008, Humboldt Bay, California, USA.

weather conditions, diet, and sample type and age (Murphy et al. 2003, Nsubuga et al. 2004, Hajkova et al. 2006). Daily time of collection was consistent throughout sampling and the Pacific Northwest is a temperate region with comparatively little temporal variation in temperature, so these factors should not have significantly influenced genotyping success. We used contingency tables to assess if diet, sample type (scat or jelly), and sample age (new or old) influenced genotyping success in this study. We determined prey type through visual inspection of scat; we did not include samples with unidentifiable content in diet analyses since they were not informative in discerning if prey type influenced genotyping success.

Microsatellite Genotyping

We extracted DNA from scat and jelly samples using QIAmp® DNA Stool Mini Kit (Qiagen, Inc., Valencia, CA) per manufacturer's instructions or with a standard phenol–chloroform extraction protocol (Ausubel et al. 2003), and executed negative controls in all extractions and polymerase chain reactions (PCR) to detect any possible contamination. We genotyped river otters at 6 microsatellite loci (Lut453, Lut733, Rio08, Lut701, Rio18, and Lut604; Dallas and Piertney 1998; Beheler et al. 2004, 2005) in 10-μL reaction volumes: 2 μL genomic DNA, 10× PCR Gold Buffer, 1 unit Amplitaq Gold, 1.5 mM $MgCl_2$, 400 μM dNTPs, 0.15 μM reverse and forward M13-tailed primers, 0.30 μM unlabeled forward primer, 0.7 μM licor M13 labeled primer, and 0.3 μM bovine serum albumin (BSA). Thermal cycling temperatures and times followed published conditions (see above) except for Rio08 and Rio18, which we modified to 40 cycles to increase amplification. We read products using an LI-COR DNA 4300 Analyzer Gene Readir (LI-COR Biosciences, Lincoln, NE), and used GENE PROFILER (Scanalytics, Inc., Rockville, MD) imaging software to estimate allele sizes; only 1 person scored gels to maintain consistency.

We determined river otter sex using PCR/RLFP (restriction length fragment polymorphism) analysis of the zink-finger protein gene (ZFX/ZFY). This method has been successful in sex-typing Eurasian otters (*Lutra lutra*) and recently North American river otters (Mucci and Randi 2007, Mowry et al. 2011). It is more robust in eliminating genotyping errors associated with degraded DNA since it yields visible fragments for both sexes, unlike the commonly used SRY marker, which only yields 1 male band making it more sensitive to allelic dropout (Statham et al. 2007). We conducted PCRs in 10-μL reaction volumes: 2 μL genomic DNA, 10× PCR Gold Buffer, 1 unit Amplitaq Gold, 1.5 mM $MgCl_2$, 400 μM dNTPs, 0.15 μM forward and reverse primers, and 0.3 μM BSA. We digested PCR product using restriction enzyme $Taq^{\alpha}I$ at the following volumes: 10 μL PCR product, 5 units $Taq^{\alpha}I$, 2 μL Buffer E, 0.2 μL BSA, and 7.3 μL ddH_2O (New England Biolabs, Ipswich, MA), and incubated the digestion at 65°C for 3 hours followed by 80°C for 20 minutes. We visualized products on a 2% agarose gel, run at 85 V for 3 hours, stained with ethidium bromide, and photographed.

We used a comparative multi-tube PCR approach to create consensual microsatellite genotypes (Frantz et al. 2003, Hansen et al. 2007). We determined genotypes by at least 2 positive PCR reactions for heterozygotes and 3 for homozygotes. We differentiated identical multilocus genotypes using GIMLET (Valière 2002), and also confirmed sex-typing using the multi-tube approach. We manually grouped incomplete multilocus genotypes (<5 confirmed loci) based on the most informative loci genotyped, sex, and collection location, similar to methods used by Frantz et al. (2003).

We calculated genotyping errors due to allelic dropout and false allele rates following Broquet and Petit (2004). Allelic dropout occurred when 1 allele of a heterozygous individual did not amplify during a PCR, which resulted in a recordable allele. False alleles were defined as a PCR-generated allele artifact that was a result of replication slippage. We calculated allelic dropout and false allele weighted averages of all loci as an indication of overall data quality. We calculated probability of identity (P_{ID}) to evaluate the power of the marker set to identify individuals. P_{ID} is the probability that 2 individuals drawn at random from a population will have the same genotype at multiple loci (Valière 2002). We used program GIMLET (Valière 2002) to calculate $P_{IDunbiased}$ (P_{ID} corrected for small sample size) and $P_{(ID)sib}$ (P_{ID} among a population of siblings). In general, more loci improve P_{ID} but also introduce genotyping error, especially with poor

quality DNA, as obtained from scat (Waits and Leberg 2000).

Abundance Estimates

We ran closed population models in Program MARK to estimate population size. Heterogeneity in capture probability among individuals can be difficult to evaluate in noninvasive sampling studies and can bias population estimates (Otis et al. 1978, Lukacs and Burnham 2005). The shadow effect, where 2 individuals have genotypes indistinguishable from each other, can also attribute to population estimate biases; Mills et al. (2000) demonstrated that modeling capture heterogeneity can decrease bias associated with shadow genotypes. We formulated 5 models to account for shadow effect, unequal detection probabilities, and time variation in capture probabilities. We included a behavioral response model with unequal encounter and recapture probabilities since a behavioral response could be an indication of heterogeneity. We ranked models using Akaike's Information Criterion corrected for small sample sizes (AIC_c). A relatively extended sampling regime for closed population models (i.e., longer than a few days or weeks) was necessary to capture temporal variation and accurately model detection probability. Given this, we examined Pradel models (Pradel 1996) to evaluate demographic and geographic population closure similar to methods in Harris et al. (2010). By systematically constraining or relaxing survival and recruitment terms in Pradel models, we were able to simulate open and closed population scenarios and rank subsequent models to evaluate if a closed system best fit the data.

Citizen Science Data Collection

The citizen science project coordinator (J. M. Black) solicited river otter observations in Humboldt and Del Norte counties since 2000 by yearly advertisement of the project and record requests by providing e-mail, postal addresses, and a website where information could be obtained and submitted (Black 2009). Observations reported herein were only from the greater Humboldt Bay study region recorded from January to December 2008 to correspond to the same year as the noninvasive genetic sampling. We invited regional wildlife and fisheries students and professionals to participate in distribution of information and collection and collation of observations. We placed signs at public access points to water bodies and replaced them as needed, and sent e-mail reminders to network participants each year.

We asked citizen volunteers to record date, time, location, and number of river otter adults and pups. We provided no official training for volunteers and they had little direct contact with the project manager as they submitted observations online or via e-mail. However, to ascertain submitted observations were reliable, the project coordinator visited each location to confirm habitat characteristics were consistent with river otter activity, responded via e-mail to most submitted observations, requested additional information if volunteer information was unclear, and identified volunteers as scientist or naturalist; wildlife, fish, or biology student; or layperson. We requested additional information including habitat features, tracks, slides, scat, dens, prey items, general behavior, and social interactions and used this as a means to confirm river otter behavior as opposed to other animals; we removed observational records if they did not clearly reflect river otter behavior. Several species in California could be confused for river otters including beaver (*Castor canadensis*), muskrat (*Ondatra zibethicus*), and harbor seal (*Phoca vitulina*), but only harbor seals were regularly detected in and around Humboldt Bay (Barnhart et al. 1992, Reid 2006), thus making the likelihood of misidentifying other aquatic mammals as river otters rare.

We determined group size by the total number of animals reported in an observation and classified pups according to size relative to adults: 0.25, 0.50, or 0.75. We assessed pup records using information about their body size and date of observation (see Black 2009 for details). The observational dataset was comprised of sightings that were clustered in 9 discrete areas encompassing water bodies or sections of water bodies that corresponded to sites in this study. We separated clusters by gaps where no sightings occurred; distance between adjacent gaps ranged from 3 km to 11 km. Although gaps were small compared to reported distances that river otters move (Reid et al. 1994), we assumed river otter groups in these discrete areas were comprised of different individuals for the purpose of generating group size, which was based on the maximum number of otters observed in each of the 9 areas.

RESULTS

From 18 May to 31 October 2008, we collected 362 river otter scats, 83 jellies, and 45 mixed scat-jelly samples from the 9 collections sites. Among the 490 collections, we successfully genotyped 124 (25%) samples. Primary prey types identified in scat were fish (from bones and scales), crab (carapace, legs), invertebrate (chitinous exoskeletons), bird (feathers), mixed (more than 1 prey type identified), and unidentifiable scat content. Prey type did not influence genotyping success of scat (Fisher's exact test, $P = 0.74$). Genotyping success rate of jellies (52%) was almost 3 times greater than successful scat (18%); mixed scat-jelly samples were intermediate in genotyping success (36%; $\chi^2_2 = 43.99$, $P < 0.001$). Fresh scat samples had a significantly greater genotyping success rate (28%) than older samples (13%; $\chi^2_1 = 7.13$, $P = 0.008$). All 6 microsatellite loci were polymorphic; the number of alleles ranged from 2 to 4. Allelic dropout rates ranged from 22.6% to 32.5%, with a weighted average over all 6 loci of 28.6%. False alleles occurred in 22 of 2,200 PCR reactions and ranged from 0.6% to 1.5% among loci. When evaluated for all 6 microsatellites, P_{ID} and $P_{(ID)sib}$ were 3.7×10^{-4} and 2.6×10^{-2}, respectively. All loci met Hardy–Weinberg equilibrium assumptions; observed heterozygosity ranged 0.36–0.69 and expected heterozygosity ranged 0.44–0.65 (Table 1).

Among the 124 scat samples genotyped, we identified 40 unique microsatellite genotypes. Furthermore, 2 individuals were identical at all 6 loci but differed in sex typing, providing a minimum count of 41 river otters. Of these 41

Table 1. The number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), tests for conformance to Hardy–Weinberg equilibrium (P), probability of identity (P_{ID}), sibling probability of identity ($P_{(ID)sibs}$), allelic dropout rates (ADO), and false allele rates (FA) at 6 microsatellite loci for river otters in the Humboldt Bay region, California, USA, from 18 May to 31 October 2008.

Locus	No. of alleles	H_O	H_E	P	P_{ID}	$P_{(ID)sibs}$	ADO (%)	FA (%)
Lut453	4	0.692	0.651	0.675	0.19	0.47	30.9	1.0
Lut733	3	0.650	0.587	0.683	0.24	0.52	28.2	1.2
Rio08	3	0.485	0.606	0.150	0.22	0.50	32.5	1.5
Lut701	4	0.541	0.537	0.480	0.26	0.55	29.8	1.1
Rio18	3	0.487	0.444	0.565	0.35	0.62	22.6	0.6
Lut604	2	0.361	0.453	0.222	0.40	0.62	24.1	0.7
Mean	3.17	0.536	0.546	0.462			28.6[a]	1.0[a]
Probability					0.0004[b]	0.026[b]		

[a] The weighted mean, which is the ratio of observed ADO or FA over all loci to the number of heterozygous genotypes.
[b] Probability value for all 6 loci.

individuals, 22 were males, 16 females, and 3 were of unknown sex (Table 2). The number of subsequent detections ranged from 0 to 12 (mean = 3.0 ± 0.39 [SE]) and we found no difference in number of subsequent detections between males (mean = 3.3 ± 0.65) and females (mean = 2.7 ± 0.38; $t_{37} = 0.83$, $P = 0.42$). Overall density, using minimum genotypes detected (41) over 45 km of coastline was 0.93 river otters/km. We detected new genotypes every month of the extensive 5-month sampling regime except October, the last month of sampling (Fig. 2). Eight individuals were detected at more than 1 sampling location, of which 7 were male and 1 was female. All other individuals were detected only within 1 sampling site. Since most individuals were detected only within 1 location, we estimated crude linear home range estimates based on average distance between neighboring sites; this was approximately 7 km. Few samples were found at Freshwater Creek ($n = 4$) and King Salmon Marina ($n = 2$), none of which yielded viable DNA for genotyping; every other site had multiple individual detections, although individuals sampled at Woodley Island were all more often detected at Elk River (Table 2). Eight individuals were identified only by jelly samples and 16 detected only from scat; 17 river otters were detected through both jelly and scat samples.

The truncated mark-recapture dataset based on genotypes detected in August and September yielded 29 genotypes. The top closed population model held encounter probability and recapture probabilities equal but varied by time (Table 3). The 95% confidence intervals around the top model's abundance estimate was 30–44 individuals, with N equal to 33 (Table 3). Encounter probability estimates (p) and associated standard errors were reasonably small and varied widely between capture occasions (p ranged 0.06–0.39, SE ranged 0.04–0.09). The top model encompassed the minimum number of genotypes (41) detected from the extensive 5-month sampling period and reported more than the number of genotypes detected in the 2 months alone. Heterogeneity models, included here because they have been shown to limit error in noninvasive population estimate studies (Solberg et al. 2006, Ruell et al. 2009), were the lowest ranking models in our analysis. This may have been because of strong temporal differences in capture probabilities, masking individual heterogeneity within capture histories. The top-fitting Pradel model simulated a closed system indicating demographic closure was met (Table 4).

We cataloged 107 river otter sightings in the citizen science database within the Humboldt Bay study area from January to December 2008 from 51 volunteer observers. We discarded 2 records as they were likely not river otters.

Table 2. Total number of scat and jelly samples collected, river otters genotyped via scat or jelly, and number of males (M), females (F), and unknown sex (U) river otters detected in 9 areas within the Humboldt Bay region, California, USA, from 18 May to 31 October 2008. We derived river otters and pups observed, as well as mean and maximum observed groups' sizes from the citizen science project's recorded observations from January to December 2008.

Sampling site[a]	Samples collected	River otters genotyped (M, F, U)	Mean group size	Max. group size	Max. pups observed	River otter observations
Little River Estuary	35	4 (2, 1, 1)	1	1	0	5
Mad River Estuary	48	8 (4, 3, 1)	2	5	2	21
Arcata Marsh	89	9 (3, 5, 1)	1.1	2	0	9
Mad River Slough	48	3 (0, 3, 0)	2.4	7	3	52
Freshwater Creek	4	0 (0, 0, 0)	1	1	0	4
Woodley Island	24	2[b] (2, 0, 0)	1	1	0	2
Elk River Estuary	146	12 (9, 3, 0)	2.7	3	2	6
King Salmon Marina	2	0 (0, 0, 0)	1.5	3	1	2
HBNWR	94	3 (2, 1, 0)	1.7	4	0	6
Totals	490	41 (22, 16, 3)	14.3	27	8	107

[a] Abbreviated site name Humboldt Bay National Wildlife Refuge (HBNWR).
[b] Individuals more often detected at Elk River site then Woodley Island site.

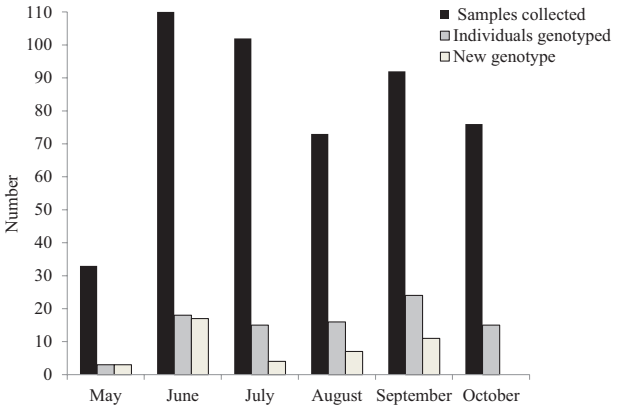

Figure 2. Number of river otter scat and jelly samples collected, number of individuals successfully genotyped, and newly detected genotypes in the Humboldt Bay region, California, USA, from 18 May to 31 October 2008.

Reported group sizes ranged from 1 to 7 river otters (mean = 2.0 ± 1.3 [SD]), and pups were observed at 4 of the 9 sites, with 8 pups observed in total (Table 2).

DISCUSSION

We estimated between 41 and 44 river otters in the study area based on minimum genotypes detected and the top mark-recapture model's upper 95% confidence interval. Mark-recapture estimates from a 2-month sampling regime based on 29 genotypes encompassed the total number of genotypes detected during the rigorous 5-month study, suggesting the top model accurately estimated river otter abundance. Future studies focused on abundance could sample for several summer months to provide sufficient data for population estimates. Given the lack of new genotype detections in October, most river otters in the Humboldt Bay area were sampled before the end of the study. This may have been because of the ease of sampling at latrines, which serve as sites for olfactory communication integral in social interactions of river otters and are therefore frequently visited and marked by local individuals (Kruuk 1992, Melquist et al. 2003, Rostain et al. 2004, Oldham and Black 2009).

The 25% genotyping success reported herein was similar to other published otter fecal DNA studies (20%, Dallas et al. 2003; 24%, Kalz et al. 2006; 12.3%, Guertin et al. 2010; 24%, Mowry et al. 2011). Overall genotyping error rates were also comparable to other noninvasive genetic studies and considered acceptable for individual identification (Broquet and Petit 2004), although we were only able to differentiate between 2 genetically similar individuals with sex-typing alone. We considered this a less costly mistake than adding ghost individuals due to genotyping error associated with processing additional microsatellite loci (Mills et al. 2000, Waits and Leberg 2000). Because we found agreement between genotypes detected and mark-recapture estimates, as well as followed strict adherence to a multi-tube approach, we do not believe shadow or extraneous genotypes were common in our data.

Recently, Mowry et al. (2011) reported in a study conducted in south-central Missouri, USA, that older river otter scat had greater genotyping success than new scat and that although jellies genotyped better than scat, they only added 2 additional genotypes and were perhaps not as useful as scat when monitoring river otters. Interestingly, our results were not in concordance with some of their findings. We

Table 3. Abundance estimate model rankings based on corrected Akaike's Information Criterion (AIC_c), AIC_c weight (w_i), and number of parameters (K), as well as corresponding population estimates (N), standard error (SE), and 95% confidence intervals (CI) for river otters in the Humboldt Bay region, California, USA, August–September 2008.

Model[a]	Description	ΔAIC_c	w_i	K	N	SE	95% CI
$\{(N,p(t) = c(t))\}$	Time varying p	0.00	0.85	10	33.4	3.1	30.3–44.1
$\{(N,p(.),c(.))\}$	Behavioral response	4.37	0.10	3	61.3	46.5	33.0–286.6
$\{(N,p(.) = c(.))\}$	Constant p	6.36	0.04	2	34.1	3.4	30.6–45.5
$\{(N,\pi,p_a(.) = c_a(.),p_b(.) = c_b(.))\}$	Heterogeneous p	8.40	0.01	3	34.2	3.4	30.6–45.6
$\{(N,\pi,p_a(t) = c_a(t),p_b(t) = c_b(t))\}$	Heterogeneous time varying p	10.85	0.00	20	32.1	2.8	29.7–42.9

[a] N = abundance parameter, p = capture probability, c = recapture probability, t = temporal variation, (.) = constant, π = heterogeneity model, $_a$ = heterogeneity period 1, $_b$ = heterogeneity period 2.

Table 4. Pradel models with varying parameter constraints to simulate geographic and demographic closure scenarios for river otters detected via noninvasive genetics from August to September 2008, in the Humboldt Bay region, California, USA. Model rankings based on corrected Akaike's Information Criterion (AIC_c), AIC_c weight (w_i), and number of parameters (K). Fully closed models were constrained by fixing the survival term (φ) to 1 (no death or emigration) and the recruitment term (f) to zero (no births or immigration).

Model[a]	Description	ΔAIC_c	w_i	K
$\{\varphi(1),p(t),f(0)\}$	Fully closed	0.00	0.51	11
$\{\varphi(1),p(t),f(.)\}$	No subtractions, possible recruitment	2.19	0.17	11
$\{\varphi(.),p(t),f(0)\}$	Possible subtractions, no recruitment	2.52	0.14	11
$\{\varphi(.),p(.),f(.)\}$	Open, fully constrained	3.09	0.11	3
$\{\varphi(.),p(t),f(.)\}$	Open, time varying p	4.00	0.07	11
$\{\varphi(t),p(t),f(t)\}$	Open, temporal variation	34.09	0.00	25

[a] p = capture probability, t = temporal variation, (.) = constant.

found that fresh scat samples genotyped significantly better than older samples and jelly samples contributed a substantial number of new genotypes (approx. 20% of total detected). Although environmental conditions could contribute to the observed differences between Missouri and northern California, in general our results highlight the variability of noninvasive genetics where factors influencing genotyping success may change not only by species but among or within populations (Beja-Pereira et al. 2009). Given this, we suggest testing river otter scat and jellies of all ages to determine the most efficient samples to target within a population of interest.

The Humboldt Bay river otter density estimate of 0.93 river otter/km was high compared to other coastal studies (0.28–0.60 river otter/km in Alaska, Testa et al. 1994; 0.26–0.46 river otter/km in Alaska, Bowyer et al. 2003; 0.37–0.63 river otter/km in British Columbia, Guertin 2009); however, the methodologies of Testa et al. (1994) and Bowyer et al. (2003) were different than those of the present study. Reported otter densities in inland systems were also much lower (0.17–0.37 river otter/km, Melquist and Hornocker 1983; 0.069–0.511 river otter/km, Mowry et al. 2011), likely a result of narrow riparian habitats typical of inland systems compared to wider coastal systems. This is similar to what Kruuk (1995) describes for the Eurasian otter, where densities were higher at coastal compared to inland riparian habitats. Two factors may have contributed to high river otter density in Humboldt Bay. First, the abundance of river otter prey in the Humboldt Bay region (Penland and Black 2009, Cosby 2013) may create an environment conducive to supporting a large river otter population. Blundell et al. (2000) found river otters maintained smaller home ranges in resource rich marine environments compared to freshwater habitats with fewer or less dense prey. If this is a general trend, we would expect a higher density of river otters in the Bay compared to adjacent inland river otter populations, reflective of differences in food availability between coastal and riparian systems (sensu Kruuk 1995). Second, because we were unable to discriminate juveniles from adults via noninvasive genetic methods our estimates included some juveniles that would inevitably disperse out of the area; census estimates of only resident adults may have been lower.

Citizen volunteer observations provided evidence that river otters successfully bred in the Humboldt Bay area, although reproduction may vary among sites given pups were observed at only 4 of 9 sites (also see Black 2009). Describing how reproductive performance varies could be instructive in evaluating river otter habitats, such as comparing habitat attributes of Arcata Marsh, a restored coastal marsh where litters have been reported consistently (although not in 2008), to Elk River estuary, the mouth of a watershed surrounded by harvested forests, agriculture, and residential areas where litters were reported infrequently (Black 2009). Citizen volunteer observations also contributed supplemental data for regions where noninvasive genetic sampling was not as informative. Such was the case with Freshwater Creek and King Salmon Marina where citizen volunteers observed 1 and 3 otters, respectively, but we were unable to generate genotypes from scat samples collected at these sites. Pairing noninvasive genetic analyses with citizen volunteer programs produced a more informed picture of river otter demography, which provides useful information as feedback for public participants.

Noninvasive genetic data indicate Humboldt Bay river otters may live in social groups as multiple individuals were detected repeatedly within the same geographical area; observational data corroborate these findings as groups of river otters were observed together multiple times within sites. River otter sociality has been reported to range from solitary individuals to social groups as large as 9 (Shannon 1989, Rock et al. 1994, Blundell et al. 2002, Black 2009). Observed group size ranged 1–7 river otters in our study area based on direct observations and 2–12 individuals based on total number of genotypes detected at sites over the 5-month sampling period. In our study area, large group sizes have been anecdotally observed to be comprised of female–female social units including a matriarchal female, older helper daughters, and young of the year (Shannon 1989, 2012); alternatively, they could be comprised of males related to the resident female and unrelated males from adjacent families (Melquist and Hornocker 1983, Rock et al. 1994). In coastal Alaska, social otters tended to have smaller home ranges than solitary otters, potentially because group living allowed for greater foraging efficiency (Blundell et al. 2002). Linear home ranges of coastal river otters have a large degree of spatial variation, but average (linear) home ranges for female and male coastal river otters range between 8–20 km and 21–45 km, respectively (Bowyer et al. 1995, Blundell et al. 2001). Crude home range estimates in Humboldt Bay averaged roughly 7 km, placing Humboldt river otter ranges on the smaller end of the spectrum, consistent with social living and dense food resources, as found in Alaska (Blundell et al. 2000). In our study, 7 of the 8 river otters detected at multiple locations were males, possibly reflecting larger male home ranges or alternative social strategies exhibited between the sexes (Blundell et al. 2004, Gorman et al. 2006). We also observed a male sex bias where 57.9% of river otters of known sex were male. Reports of male-biased river otter populations are common and the bias may be due to larger male home ranges, male biased birth rates, or behavioral differences between the sexes resulting in greater male detection rates (Hamilton and Eadie 1964, Melquist and Hornocker 1983, Mowry et al. 2011). Further investigation of genetic relatedness may provide insights into the social behavior and structure of Humboldt Bay river otter groups and contribute to our understanding of river otter space utilization and intraspecific tolerances.

MANAGEMENT IMPLICATIONS

Overall, methods and results reported herein demonstrate the usefulness of noninvasive genetic surveys for estimating population abundance of river otters. Both scat and jellies provided a substantial number of new genotypes and newer samples amplified better than old samples. Based on the

differences between these results and other river otter fecal studies, we would suggest a pilot project to identify the most informative samples for a specific region. Population estimates derived from just 2 months of data encompassed the total number of genotypes detected from 5 months of sampling, indicating future studies could collect sufficient data for accurate population estimates in a much shorter sampling period. The inclusion of citizen science data provided information on pups and group size in the Humboldt Bay region, data not available through genetic analyses. Prior to these efforts, no rigorous demographic study of river otters had been conducted in northern California. Assessing how river otter abundance and density change over time will inform managers about the health of an apparently productive coastal system. We recommend these methods and models to others interested in examining populations of river otters for management of wetland ecosystems in a changing world.

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