

Eurasian otter (*Lutra lutra*) density estimate based on radio tracking and other data sources

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Abstract Estimating animal population size is a critical task in both wildlife management and conservation biology. Precise and unbiased estimates are nonetheless mostly difficult to obtain, as estimates based on abundance over unit area are

frequently inflated due to the “edge effect” bias. This may lead to the implementation of inappropriate management and conservation decisions. In an attempt to obtain an as accurate and conservative as possible picture of Eurasian otter (*Lutra lutra*) numbers, we combined radio tracking data from a subset of tracked individuals from an extensive project on otter ecology performed in Southern Portugal with information stemming from other data sources, including trapping, carcasses, direct observation of tagged and untagged individuals, relatedness estimates among genotyped individuals, and a minor contribution from non-invasive genetic sampling. In 158 km of water network, which covers a sampling area of 161 km² and corresponds to the minimum convex polygon constructed around the locations of five radio-tracked females, 21 animals were estimated to exist. They included the five radio-tracked, reproducing females and six adult males. Density estimates varied from one otter per 3.71–7.80 km of river length (one adult otter per 7.09–14.36 km) to one otter per 7.67–7.93 km² of range, depending on the method and scale of analysis. Possible biases and implications of methods used for estimating density of otters and other organisms living in linear habitats are highlighted, providing recommendations on the issue.

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Introduction

Estimating wildlife population size is a major goal for conservation biologists, who are often called upon to provide policy makers with pertinent data regarding rare or endangered species. Inaccurate estimates, especially overestimations, represent a serious threat to wildlife conservation, in that they may lead to less conservative management practices and conservation strategies. This may result in local extirpations or reserve sizes too small to support viable populations. Estimating

carnivore density is particularly challenging, as most of these animals are elusive, trap-shy, with low population density, and commonly display large home ranges (Powell 2012). Due to the last characteristic, carnivore density estimates based on abundance per unit area, with no boundary data, are frequently inflated due to the “edge effect” bias (Dice 1938; for carnivores see Garshelis 1992, Soisalo and Cavalcanti 2006, and Obbard et al. 2010). Similarly, density figures which are potentially inflated and not biologically sound may arise when distinction between resident and non-resident (i.e., transients and dispersers) individuals cannot be clearly defined.

Telemetry techniques provide an opportunity to obtain data on ranging behavior and residency, thus eliminating problems related to the edge effect bias. Indeed, the use of telemetry has been indicated as a valuable advancement in estimating wildlife population size already long time ago (Seber 1986; White and Garrott 1990), allowing accurate estimates of animal density when used alone (White and Shenk 2001) or in combination with other methods (e.g., capture-mark-recapture/resight—Garshelis 1992; Miller et al. 1997; Powell et al. 2000; non-invasive genetic sampling (NGS)—Solberg et al. 2006; camera trapping—Soisalo and Cavalcanti 2006). The use of telemetry, however, requires capturing, handling, and following marked animals, all activities being costly and invasive. This explains why the potential of telemetry to help obtaining reliable population estimates still remains largely unexplored, with few, notable exceptions (e.g., studies cited above).

Besides sharing all aforementioned carnivore characteristics, Eurasian otters (*Lutra lutra*) are mostly nocturnal and express little-to-no individual distinctiveness (Kruuk 2006). Due to this lack of individualization, commonly used methods for estimating population size such as capture-recapture/resight models (e.g., Seber 1986; Sinclair et al. 2006; Kelly et al. 2012; Pollock et al. 2012) or, even better, the combination of telemetry and mark-recapture (see Miller et al. 1997; Powell et al. 2000; Pollock et al. 2012), are not easily applicable with this species. Spraint (otter feces) surveys (e.g., Mason and Macdonald 1986; Reuther et al. 2002) are certainly suited for assessing otter presence or monitoring purposes, but not necessarily recommendable for estimating density, as spraint/sprainting sites' abundance may not depend on otter density (cf. Kruuk et al. 1986; Kruuk and Conroy 1987; Gallant et al. 2007; but see Mason and Macdonald 1986, 1987; Guter et al. 2008; Calzada et al. 2009). While certainly appealing for being practical and cheap, indeed, the use of spraint surveys for otter density estimation still needs to be adequately addressed in its reliability. Snow or mud tracking (Erlinge 1968; Ruiz-Olmo et al. 2001, 2011; Arrendal et al. 2007; Sulkava et al. 2007; Hájková et al. 2009) and direct observations (e.g., Kruuk et al. 1989; Ruiz-Olmo et al. 2001, 2011) are limited by suitability of climatic conditions, substrate type, work force constraints, observers' experience/capability, otter

detectability during the day, and subjectivity in the choice of observation sites, scale, and sampling hours (see also Hájková et al. 2009 and references therein). Moreover, these methods report otter density as a point estimate, including all individuals present at some time in the study area but with no data on individual boundaries, likely resulting in overestimates. When repeated over time, NGS offers the potential to overcome such constraints, allowing identification of individuals and estimations based on the capture-mark-recapture framework (e.g., Dallas et al. 2003; Arrendal et al. 2007; Hájková et al. 2009). Nonetheless, NGS is limited by genotyping errors, high costs, and laboratory expertise/facilities (Taberlet et al. 1999; Paetkau 2003; Bonin et al. 2004; Waits and Paetkau 2005; Lampa et al. 2013). In otters specifically, NGS also suffers from a low success rate of DNA typing (Ferrando et al. 2008; Hájková et al. 2009; Bonesi et al. 2013; Lampa et al. 2013), and can yield both underestimates (Mills et al. 2000) and overestimates (Creel et al. 2003) in population size. As a result, accurate reference estimates of Eurasian otter densities are rare throughout the species' range.

Finally, considering the importance of the study scale, which is known to explain significant variation in carnivore density (Smallwood and Schonewald 1998), we note that previous research estimating otter density did not pay the necessary attention to the scale of study and analysis, or at least authors failed to report them in their articles. For instance, information on the scale of hydrography layers used to estimate density seems to be missing in all published otter density studies. Using hydrography layers of different scales—which most likely differ in the extent of rivers encompassed by the same area (see for instance Peterson et al. 2013 for a recent, comprehensive introduction to the dendritic nature of river networks)—may very well lead to contrasting density results. In addition, otter density is often estimated in small areas or short river extensions (e.g., 0.20 and 1.49 km² in García et al. 2009, 9–12 km in Ruiz-Olmo et al. 2001, and 10–12 km up to a single test at 30 km in Ruiz-Olmo et al. 2011), but this creates a possible source of bias (overestimation), since small areas or short river extensions could very well correspond to the home range (or a portion of it) of a single adult otter (cf. Kruuk 2006), and otter males are known to encompass the range of several females (Erlinge 1968).

Utilizing multiple data sources (e.g., Bellemain et al. 2005; Boulanger et al. 2008; Bischof and Swenson 2012) may help to improve the reliability of carnivore density estimates, by reducing biases associated with single techniques. We combined radio tracking, other field data (trapping, carcasses, and direct observations of tagged and untagged individuals), and molecular data (relatedness estimates regarding genotyped individuals) concerning a wild, native, resident population of Eurasian otters in Southern Portugal, to provide the first, formal assessment of otter population size in Portugal. This density estimate refers to a study area of 161 km², corresponding

to the minimum convex polygon constructed around the locations of five radio-tracked females. In addition, we compared density results obtained using two scales (of different resolution levels) of hydrography layers, to address the influence of scale of analysis on density estimates of animals inhabiting linear environments. Finally, we estimated the density within each individual female range, to assess possible biases in estimations based on short river extensions.

Material and methods

Study area

Field and molecular data used as a baseline for computing otter density were collected within an extensive project on otter ecology and behavior performed in Southern Portugal (38° 40' 0" N, 8° 0' 0" W) from 2007 to 2010 (Quaglietta

2011) (Fig. 1). Otters in Portugal are widely distributed (Trindade et al. 1998). The climate is typically Mediterranean; annual rainfall is between 600–700 mm on average, mostly occurring from October to April (<http://www.cge.uevora.pt/>). The study area is mostly flat, averaging 200 m above sea level, and includes a rather continuous water network belonging to the Sado River basin. The basin consists of several streams, the majority of which average 5–10 m in width. Lentic systems are also abundant, including several widely scattered artificial ponds and 15 reservoirs (median area of 34 ha; interquartile range=52) used primarily for irrigation. The landscape is dominated by “montado,” traditional Mediterranean woodlands, consisting of cork oak (*Quercus suber*) and/or holm oak (*Quercus ilex*) stands, interspersed with extensive agriculture, forestry, and livestock grazing. Human settlements are mostly concentrated in cities and small villages.

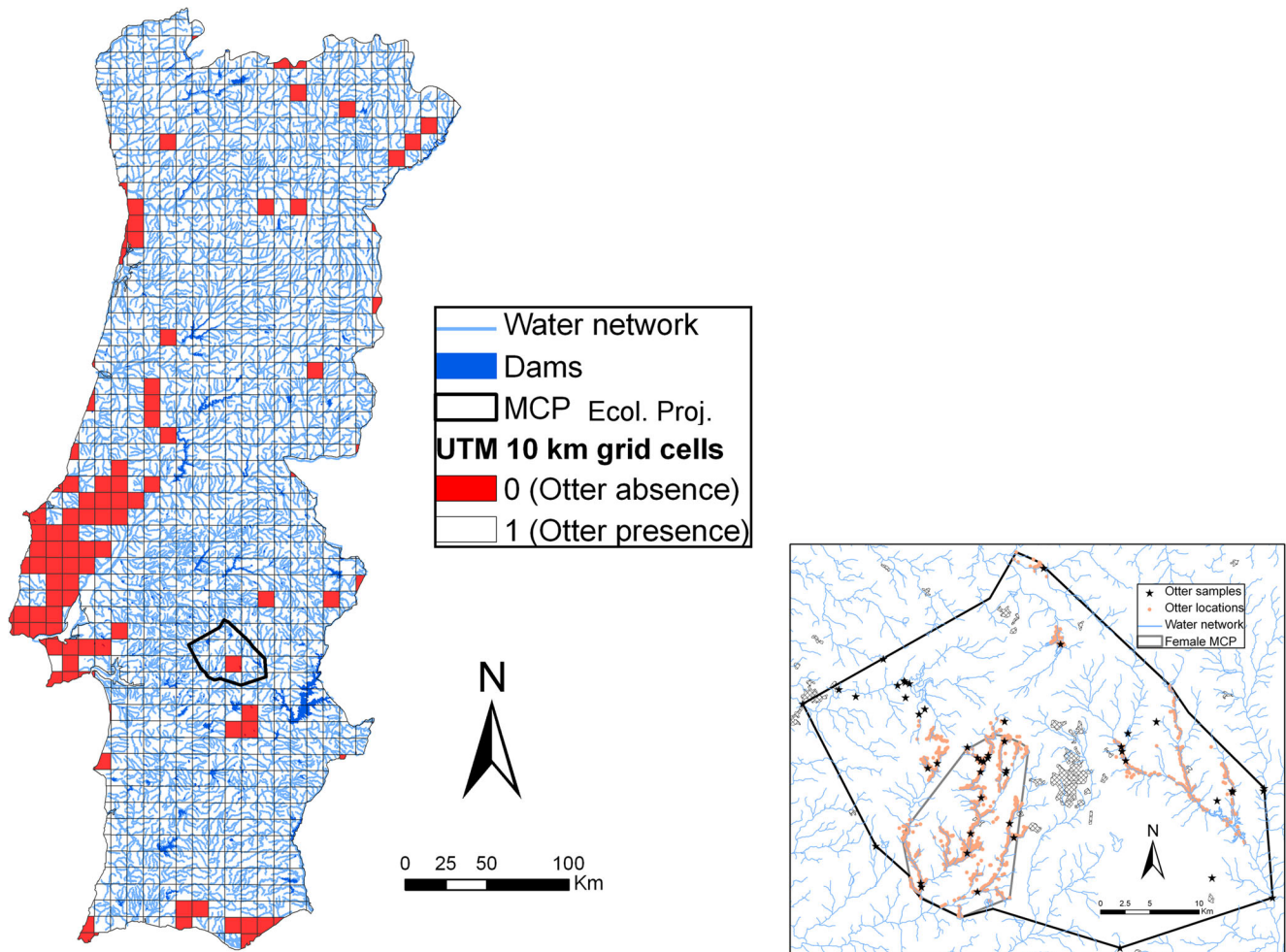


Fig. 1 Portuguese otter range (adapted from Trindade et al. 1998), contour of the study area of the extensive ecological project performed in Alentejo region (black line), and, within it, MCP describing the area used for the density estimate reported in this article (gray line). Stars

indicate all the genotyped individuals known in the larger study area (Quaglietta et al. 2013), while small pink dots indicate the locations of radio-tracked animals

Otter trapping

Otters were live-trapped and radio-transmitters (IMP 300/L 38 g, 8.1×2.3 cm and 400/L 95 g, 9.7×3.3 cm—Telonics Inc., Mesa, Arizona) were implanted in the peritoneal cavity. Traps (approximately 2–4 traps per 1×1 m² site) were preferentially set in water (when water depth was low, mainly during the dry season) and along otter paths (mainly during the wet season). They were moved along the river network as soon as the first individuals were caught at each trapping site (and sometimes set at the same trapping sites of captured otters, in an attempt to capture new individuals or recapture tagged ones). Traps were coupled with trap-alarms that warned us of capture, in order to minimize otter stress and injuries (cf. Ó Néill et al. 2007). Procedures regarding the surgical implantation mostly followed Ó Néill et al. (2008), with only slight modifications detailed in Quaglietta (2011). The tags weighed less than 1–2 % of otters' weight and had been used in previous studies on otter species (e.g., Melquist and Hornocker 1983; Somers and Nel 2004), including *L. lutra* (e.g., Ruiz-Olmo et al. 2001). Although the majority of previous studies held otters in captivity for days before and after surgery (e.g., Melquist and Hornocker 1983; Serfass et al. 1996; Saavedra 2002), we opted for an immediate release after recovery from anesthesia. This approach, previously proven successful (Ó Néill et al. 2008), was chosen in an attempt to limit the amount of time adult otters were kept away from their territories and to avoid separating families (see also Ó Néill et al. 2008). Handling procedures were approved by the Portuguese Institute for Nature and Biodiversity Conservation.

Sample collection and processing

Samples of blood and hair from live-caught animals, muscle and hair from carcasses were collected within the mentioned project, along one year (December 2008–December 2009). Two non-invasive samples (i.e., fresh spraints—cf. Prigioni et al. 2006) opportunistically collected within the study area during a negligible effort were also used in this study, as one of them resulted in one genetically identified presence in the study area (see “Results”). For genotyping, 19 polymorphic microsatellite loci were used. Details on sample storage and genetic analyses may be found in Online Resource 1, as well as in Quaglietta (2011), and Quaglietta et al. (2013), while information on spatial locations of trapping sites and sampling criteria is available in Quaglietta (2011) and Quaglietta et al. (2013). We estimated pairwise relatedness between individual otters via the coefficient of relatedness “R” (Queller and Goodnight 1989), using the software SPAGeDI v.1.3 (Hardy and Vekemans 2002). We assessed specific relationships using the “Specific Hypothesis Test” implemented in ML-Relate software (Kalinowski et al. 2006), as also reported in Quaglietta et al. (2013). Kin

relationships were distinguished as low (not of first order) relationship ($R < 0.25$), half-sibling ($0.25 < R < 0.5$), and parent/offspring or full-sibling ($R > 0.5$) (Queller and Goodnight 1989). The probability of identity values (PI, PIsibs) was calculated using the software GenAlEx v.6.3 (Peakall and Smouse 2006). Age classes (cub ≤ 6 –8 months, juvenile 8 months–2 years, adult ≥ 2 years—Quaglietta et al. 2014) were estimated by tooth-wear, body dimensions, and development of sexual characters from captured individuals and (when possible) carcasses. From the latter, a canine was extracted in order to allow a more accurate estimate by the count of cementum annuli, performed by the Norwegian Institute for Nature Research (Trondheim, Norway). Sex was determined by direct observation of external anatomy in captured otters and by marker Lut-Sry (see Dallas et al. 2000) on genotyped individuals.

Geographic coordinates of dead animals, individuals released because they were unsuited to be tagged (i.e., cubs), and fresh spraints were recorded via a portable GPS receiver (Garmin eTrex[®] H) at the field collection site under the assumption that their positions were situated inside individuals' home ranges (see “Discussion”). Otters were radio-tracked by triangulation, with an average frequency between two successive radio-locations of 36 h, covering in equal parts the night and day light hours. Home ranges of radio-tracked animals were estimated through the deterministic method adopted by Melquist and Hornocker (1983), and we checked that they reached an asymptote as signal of home range stability (i.e., site fidelity—cf. Powell 2012) (see also next paragraph). Location data of all known otter individuals were then projected in a geographic information system (GIS), using the software ArcGis[™] v.9.3.

Estimation of population size

We focused our estimate of otter population size on an area corresponding to the minimum convex polygon (MCP) enclosing the radio-locations of five monitored adult females (Fig. 1) for which we had the most accurate information available (see below), and on a period of 1 year (December 2008–December 2009). The choice to focus on females, shared by other authors who recently estimated otter population size in Ireland (Marnell et al. 2011), was based on the fact that female otters generally exhibit a more stable home range than males (Kruuk 2006; Marnell op. cit.), and keep a more constant use of their ranges, while males may be less constant in the use of portions of their ranges (Quaglietta et al. 2014).

For this area (161 km²), we had precise information either from field or molecular data, which allowed us to accurately estimate the minimum number of animals residing there. This approach is similar to the “known-to-be-alive” (e.g., Sinclair et al. 2006: 141), utilized in an

extensive study on *Lontra canadensis* ecology (Melquist and Hornocker 1983), but integrated by different data sources. Radio tracking provided data on stability and boundaries of individual home ranges. Boundaries were estimated using the deterministic method of the river extension commonly used in literature (e.g., Melquist and Hornocker 1983) and thought to be stable when they reached either the asymptote or $\geq 70\%$ of their final estimate, and did not vary with the inclusion of the successive 15 single fixes (equivalent to ~ 14 days) (see Quaglietta 2011 and Quaglietta et al. 2014 for further details).

We also reported a separate estimate (we called it our “best estimate”), which included individuals estimated to live in the same area—and period—of those with known spacing patterns and known to be residents. These individuals were: cubs belonging to the five females identified (whose geographical location had been defined) either through captures (i.e., they were captured but released untagged), direct observation, or genotyped carcasses; one male of unknown age genotyped from a fresh spraint, and three mature males whose presence (at least sporadic) within the ranges of females with offspring that were not genetically related to known (tracked or genotyped) males was inferred (see “Results” for further details). Although the cubs’ spacing patterns were unknown, we assumed the cubs were a constant presence within their mothers’ range, since they depend on their mother until 1 year of age (Kruuk 2006).

Estimates are reported as minimum number of otters per linear extension of river (km) and per sampling area (km²). In addition, we computed densities using both a 1:25,000 and a 1:100,000 scale hydrography layer, respectively, named Fine Network (FN) and Coarse Network (CN), in order to verify if the choice of the map scale influences density results, and to increase the comparativeness of our estimates (coarser layers being more common than finer layers). The main difference between the two layers is that FN is very accurate, and included several tributaries and ponds/dams (whose perimeters were included in the computation) which were absent in the CN. The CN is basically limited to principal watercourses, resulting in almost the double of the FN (the two being, respectively, extended 78 and 158 km).

Density values within individual female ranges were also computed. For the estimates expressed in terms of area, the minimum convex polygon used as sampling area for the overall density estimate was replaced by the minimum convex polygon of each female range. Since we had accurate data for the females’ range boundaries, we measured river width along watercourses they frequented. This was achieved by ad hoc surveys, performed to collect several measures of water availability

for a companion study on the influence of droughts on otter spacing patterns (Quaglietta 2011; L. Quaglietta unpublished). The information on river width was here used as a way to report the area of water effectively used by each radio-tracked female, with the intent of providing a better picture of river widths within our study area, and therefore facilitating future comparisons with other regions.

Results

Overall density estimates

Our best estimate of number of otters living within the 161-km² sample area during 1 year (with one exception—see “Discussion”) was 21 (see below and next paragraph for details on how we obtained this estimate). This area contained 78 km of Coarse Network, or 158 km of Fine Network. Total density estimates were thus one otter per 3.71 km of CN or one otter per 7.52 km of FN, and expressed in terms of area, one otter per 7.67 km². Five reproductive females and six adult males were estimated to live in the area, resulting in a density of one reproductive female per 15.6 km of CN and one adult male per 13 km of CN, or one adult individual per 7.09 km considering both sexes. Total density estimates could be made even more conservative by including in the computation other 5.8 km² (or 5.8 km of FN or 3.3 km of CN) which, although falling outside the study area boundary, are actually part of some (M2 and M4) males’ ranges (Fig. 2). This would result in a density of one otter per 3.87 km of CN, or 7.8 km of FN, or in 7.93 km². The minimum number of known, resident individuals (excluding carcasses and assumed fathers—see below and next paragraph) was 15, including 5 reproductive, tagged females (and their cubs) and 2 adult, tagged males, resulting, respectively, in a total density of one otter per 5.2 km of CN, or 10.53 km of FN, or in 10.73 km². Considering only adult individuals, the estimate is of one reproductive female per 15.6 km of CN and one adult male per 39 km of CN (or one adult individual per 11.14 km considering both sexes).

Out of the 21 individuals, 11 were live caught: 6 females (adult females F1, F3, F5, F6, F13, and the released untagged cub F11) and 5 males (adult males M2 and M4, and sub-adult males M3, M5, M8) (Table 1). Of these, 10 were tagged (the 5 selected females and the 5 above reported males), corresponding to the 48 % of overall individuals in the area (Table 1), being radio-tracked for an average of 15 ± 7 months. Among the other 10 individuals, 4 were untagged, directly observed cubs (19 % over the total), 2 were carcasses (1 sub-adult male and 1 female cub) (Table 1), and 4 were untagged adult males (19 % over the total)—including one inferred by NGS

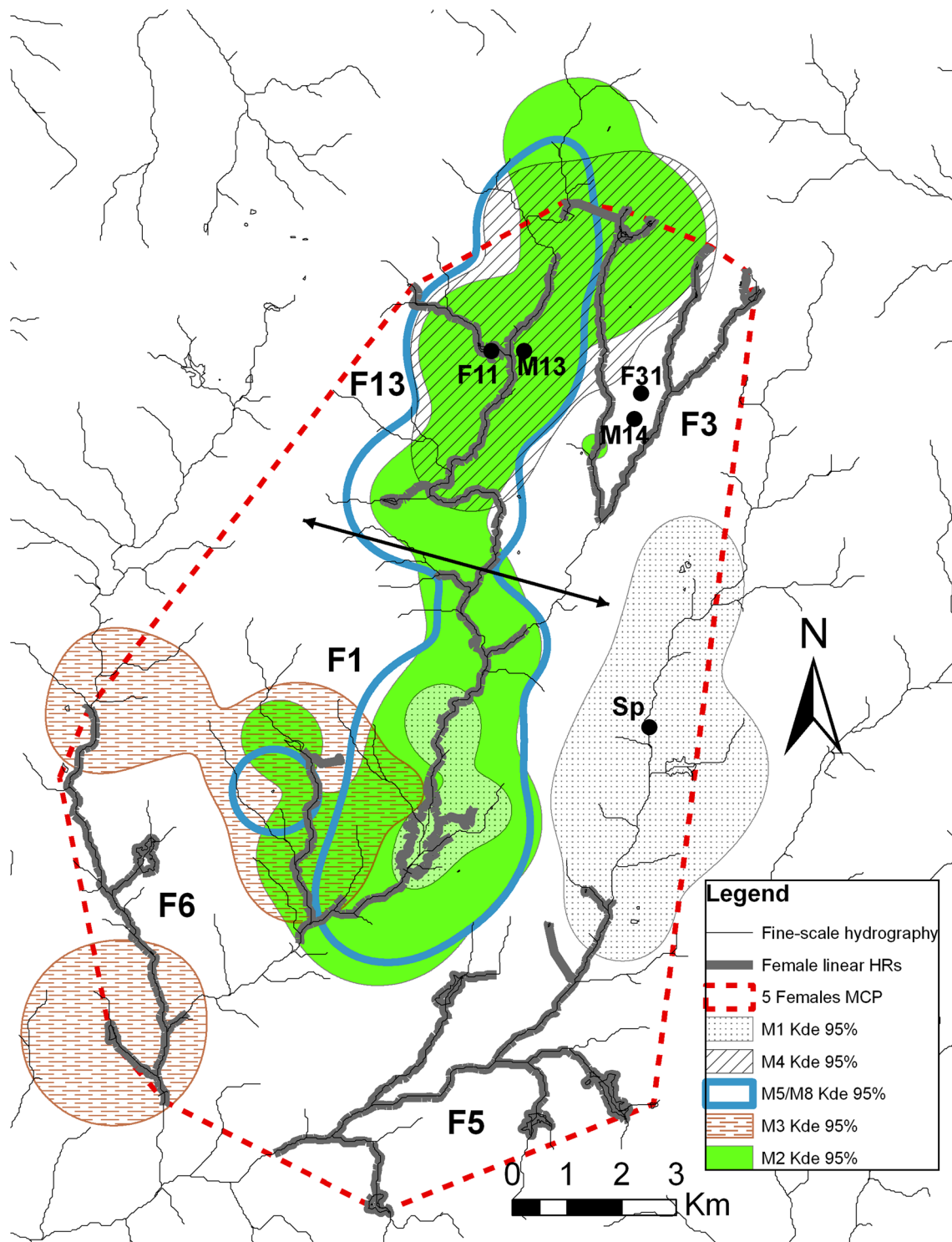


Fig. 2 MCP encompassing the individual linear home ranges of five adult females (F1, F3, F5, F6, F13) residing in a small, accurately known area of 161 km², encompassing 158 km of fine-scale water network (FN—shown in the figure) or 78 km of only principal watercourses (CN—see “Methods”). The *double arrow* indicates the end of F1’s home range and the beginning of F13’s; Sp, M13, F11, M14, and F31 non-

tagged but genotyped individuals. Full-siblings males M5 and M8 are shown with a unique kernel contour, because their ranges were rather similar during this study’s time frame and displaying the two would have resulted in a difficult-to-read figure. For the same reason (illustrative purposes), males’ ranges are shown as kernel contours and not as linear ranges

(Table 1), the assumed fathers who sired the cubs of females in whose ranges no tagged males were known to be present.

Probability of identity values were $PI=2.14 \times 10^{-12}$ and $PI_{sibs}=5.96 \times 10^{-6}$.

Table 1 Individual otters used for the density estimation

Code	Source	Age	Start date	End date	End cause	No. fixes	Fix lost (%)
F1	RT	Ad	06/15/2007	09/12/2008	End of battery?	315	5
M2	RT	Ad	07/21/2007	03/20/2008	Trapping	139	19
M3	RT	Sub	09/28/2007	06/20/2009	Road-kill	163	45
F3	RT	Ad	12/03/2007	03/01/2010	End of battery	191	18
M4	RT	Ad	05/10/2008	11/18/2008	Road-kill	97	10
M5	RT	Sub	09/04/2008	04/10/2010	End of project	215	4
M8	RT	Sub	11/20/2008	11/06/2009	Road-kill	155	2
F5	RT	Ad	11/26/2008	12/03/2009	End of battery?	132	12
F6	RT	Ad	12/02/2008	03/01/2010	End of battery?	140	16
F11	UCp	C	12/07/2009	–	–	–	–
F13	RT	Ad	03/17/2010	10/06/2010	End of project	49	18
F31	UC	C	10/28/2008	–	–	–	–
M14	UC	Sub	07/02/2008	–	–	–	–
MSp	NGS	U	December 2009	–	–	–	–
F5's cub	UDO	C	08/11/2009	–	–	–	–
F5's cub	UDO	C	08/11/2009	–	–	–	–
F5's cub	UDO	C	08/11/2009	–	–	–	–
F6's cub	UDO	C	08/20/2009	–	–	–	–

Their code (*M* and *F* indicate respectively males and females, *Sp* the unique spraint analyzed), source of data (*RT* radio tracking; *UF* untagged father, inferred from the presence of cubs of females in whose ranges no tagged fathers were known to be present; *UC* untagged carcasses; *UDO* untagged directly observed; *NGS* non-invasive genetic sample; *UCp* untagged captured), age (*Ad* adult, *Sub* sub-adult, *C* cubs; *U* unknown), date of first detection (may either be capture, collection, or visual observation), and, for radio-tracked individuals, end of monitoring and its reason, sampling effort, and estimate of lost fixes. *End of battery?* indicates cases of suspected end of batteries, while *End of battery* refers to those cases in which this was documented

Otter numbers within individual female home ranges

Within each individual range of the five selected females (F1, F3, F5, F6, F13), there were on average 4.4 ± 0.9 otters, including 1.6 ± 0.9 offspring (Table 2). The average family (consisting of mothers plus cubs) size was 2.6 (SD=0.9), the mean female home range was 11.2 km (SD=1.6) of CN, the mean proportion of males or untagged animals overlapped with females' ranges was 0.43 (9 animals out of 21 or 1.8 ± 0.8 animals per individual female range), and the average interstitial range (proportion of CN hydrography unused by any of the radio-tracked individuals) was 0.25. The number of otters within each range was positively correlated with the number of lentic systems present (R_s , 0.89; $P=0.041$; $N=5$). Individual (adult, resident) female range density estimates yielded one otter per 2.61 km of CN (± 0.47 ; CI=0.41) or 4.51 km of FN (± 0.88 ; CI=0.77) and one otter per 3.56 km² (± 1.55 ; CI=1.36) (Table 2). Density values calculated as linear river extent and water area did not correlate.

In detail, female F1's home range was stably occupied by her two tagged, sub-adult full-sibling male offspring (M5 and M8) and a tagged adult male (M2) frequently associated with her despite not being the cubs' father (Fig. 2). Thus, F1's home range has presumably been visited by one untagged, adult male (the cub's father). Known to exist within F3's range

were: her female cub (F31) and an untagged juvenile male, possibly in dispersal (M14, half-sibling of F13), both found separately road-killed in the area, one adult monitored male at a time (first, the adult M2, and, after his death, the young adult M4), and at least one untagged adult male (the cub's father—M2, M4, and M14 were not). Female F5 shared her range with her three (untagged) cubs (Fig. 1) and at least one untagged, adult male (the cubs' father, possibly the male of unknown age identified by fresh spraint sample "Sp" in Fig. 2). Sporadic visits by sub-adult, dispersing males M1 (resulted to be another F1's cub of prior generation—Quaglietta et al. 2013) and M3 to F1's range, and by male M1 to F5's range, were not included in the estimate because they were considered occasional sallies (Quaglietta 2011). In female F6's range, there were one observed, untagged cub (M. Bandini pers. obs.), assumed to be her offspring, the tagged sub-adult male M3 (unlikely to be the cub's father, based on the age estimate made at the time of capture) with which F6 intensively overlapped spatio-temporally, and there should be one untagged, adult male (the cub's father). Female F13 showed high space-use overlap with two tagged males (in two different periods) (Fig. 2), reproducing with both in two consecutive years. First, she had (at least) one cub (M13) with adult male M2, and, after M2's death, birthed at least one cub (F11) with the recently matured male M4. Due to the asynchronous nature of

Table 2 Density estimates and minimum number of otters estimated to live in an area of 161 km² (“Sampling area”) and within individual female ranges (“HRs”)

HR	FN (km)	CN (km)	MCP (km ²)	U-FN (km ²)	U-CN (km ²)	No. otter	Km per 1 otter (FN)	Km per 1 otter (CN)	Km ² per 1 otter (MCP)
F1	20.9	12.3	15.9	0.63	0.62	5 (2–2)	4.18	2.46	3.18
F3	20	13.4	11.7	0.08	0.08	5 (1–2)	4.00	2.68	2.34
F5	28.7	10.1	29.1	0.41	0.41	5 (3–1)	5.74	2.02	5.82
F6	14.2	10.2	8.3	0.19	0.18	4 (1–1)	3.55	2.55	2.08
F13	15.2	10.0	13.2	0.08	0.07	3 (1–1)	5.07	3.33	4.40
Mean F	19.8 (±5.8)	11.2 (±1.6)	15.6 (±8.0)	0.28 (±0.24)	0.27 (±0.24)	4.4 (±1)	4.51 (±0.88)	2.61 (±0.47)	3.56 (±1.55)
Sampling area	158	78	160.8			21	7.52	3.71	7.66

The sampling area corresponds to the minimum convex polygon (“MCP”) built around the radio-locations of five adult females. In parentheses are reported the number of cubs (first number on the left) and adult males (second number). Estimates are expressed both as linear extent (km) of the water network and area (km²), and referred to a fine-scale (1:25,000) hydrography network (“FN”), which includes tributaries and ponds/reservoirs’ perimeters, and a coarser network (1:100,000), comprising only the main principal watercourses (“CN”). For the individual female ranges the water area effectively used by the otters (as measured in water transects—see “Methods”) is reported (“U-FN” and “U-CN”)

these events, only one male and one cub were included in F13’s estimate. The individual identified by the other fresh spraint sample resulted to be F13. In one case, the same tagged male (M2) simultaneously encompassed the range of two individual females (F1 and F3). This individual has been computed twice, one for each individual female range, but only once for the overall density estimate.

Discussion

Evaluation of the obtained density estimates

Several non-invasive methods commonly used to estimate wildlife density at local scale (e.g., capture-recapture-based studies by molecular scatology) often lack any information on animals’ actual range, and are therefore prone to yield overestimates due to edge effect (Dice 1938; Garshelis 1992; Soisalo and Cavalcanti 2006; Obbard et al. 2010). Besides this type of bias, NGS results may also appear inflated due to genotyping errors (Taberlet et al. 1999; Creel et al. 2003; Hájková et al. 2009; Lampa et al. 2013). Our study is based on the assumption that all animals in the area have been caught, or detected and identified, or that there is an estimated proportion of non-identified otters. We think these assumptions are reasonable. In fact, all ($N=16$) otters radio-tracked in the area exhibited site fidelity to their annual home ranges and no vagrant animals were detected (Quaglietta 2011; Quaglietta et al. 2014). Furthermore, the study of Ó Néill et al. (2009) has showed that live trapping may be successful in capturing almost all otters living in an area. This is further supported by our relatively high number of re-captures (5 out of 22, or 23 % of all the captures performed in the area of the present study—Quaglietta 2011). Finally, the interstitial spaces among radio-tracked female individuals’ ranges were

not large enough to host other conspecifics of the same sex (considering the intra-sexual territoriality of the species also in the study area—Quaglietta et al. 2014). We note, however, that we were unsuccessful in capturing all mature male otters living in (or passing through) the area (the four assumed fathers). Capturing adult females seemed to have higher probability than adult males, possibly because of more regular and restricted spatial usage by females (Kruuk 2006; Quaglietta et al. 2014).

Ideally, populations to be estimated should be closed (Otis et al. 1978). However, the assumption of geographic closure of populations is rarely achieved in research conducted on carnivores (Solberg et al. 2006; Obbard et al. 2010). Our study length was 1 year, thus covering only one reproductive event (Kruuk 2006; Quaglietta 2011 and L. Quaglietta unpublished for the study area), therefore complying with the closure assumption. An exception to the population’s temporal closeness was represented by the inclusion (in the estimate) of female F13, which was not monitored in the exact time frame of this study, but captured a couple of months after the upper time limit (Table 1). We decided to add this female because of evidence, stemming from field and molecular data from a companion study, of her coexistence with two males (M2 and M4) in the study area during different periods—prior to her capture (Quaglietta et al. 2014). This gives us confidence she was living in the same area during the period of this study (see also Quaglietta et al. 2014), also considering that otters commonly display high site fidelity to home ranges along years (Ruiz-Olmo pers. comm.; Quaglietta 2011). We were uncertain as to the appropriateness of including two inferred untagged males in F1 and F3’s range estimate, due to the observed presence of other males in these females’ ranges. We opted to include them, as we knew adult male and female otters in the area spend considerable time together, intensively and extensively overlapping each other’s, regardless of which

male fathered the cubs (Quaglietta et al. 2014). This is compatible with the sporadic presence of more than one adult male within a singular female's range. Also, females were rather stable in their space use; thus, untagged males were indeed more plausible than putative, occasional sallies by females (in search of mates) for explaining parenthood.

Unfortunately, we could not use robust methods such as the combination of telemetry and mark-recapture (see Miller et al. 1997; Powell et al. 2000; Pollock et al. 2012); instead, we somehow opportunistically combined field and molecular data, detailed collection of dead animals, and direct observations (all focused on a small study area during the same period), which nevertheless allowed us to obtain what we believe is a rather conservative estimate. Importantly, this study is based on a substantial amount of data gathered on individual age, sex, and spacing patterns, allowing generating both a best guess estimate and an estimate based only on resident otters and thus eliminating inflation due to edge effect. Future research is encouraged to further assess the use of multiple sources for estimating otter density. The jointed use of telemetry and mark-recapture, either of tagged individuals or genotyped non-invasive samples, could in fact provide even more accurate estimates than ours and especially a repeatable as well as robust method. Critically, the use of multiple data sources to estimate otter numbers could allow to reduce biases and limitations associated with single techniques (e.g., Arrendal et al. 2007; Hájková et al. 2009), which could be particularly relevant, considering the oscillating nature of otter populations (Kruuk 2006, Ruiz-Olmo et al. 2011) and their vulnerability to climate change (Cianfrani et al. 2011; Quaglietta 2011; this study, see last paragraph). We recognize the use of telemetry is not always possible, because of associated costs and invasiveness. We nonetheless strongly advocate not neglecting to make use of the potential of telemetry to help obtaining reliable population estimates while performing telemetry studies. When, anyway, telemetry is not applicable, to avoid the risk of ignoring data on spatial usage and range boundaries of otters, biologists could attempt to identify resident individuals' range—which is possible using, for instance, NGS over a period of time—or apply approaches which aim to minimize the discussed biases (e.g., Obbard et al. 2010).

Our ability to compare our study to others has been limited by factors that will be discussed in the following section. We nonetheless believe that the population studied was rather dense, considering the limited carrying capacity of the aquatic habitat, as dictated by the small, average size of the study area's streams. Our linear estimates (0.27 otters per kilometer of CN and 0.13 otters per kilometer of FN) were indeed similar to or higher than those observed in other Mediterranean zones, such as Southern Italy (0.18–0.20—Prigioni et al. 2006) and Spain (0.14—García et al. 2009; 0.4—Ruiz-Olmo et al. 2011). Further evidence that otter density in our study area is relatively high stems from: (i) radio tracking data

displaying stable, annual home ranges for all otters monitored in the area (except dispersing individuals); (ii) all captured adult females mated; (iii) our relatively high trapping success rate (49 otters in 388 trap nights—Quaglietta 2011)—it should be noted, nevertheless, that we feel speculating otter densities based on trapping success rates is misleading, as trapping success of a territorial carnivore appears more related to field/habitat conditions and trappers' skills than actual population density.

Implications for estimating density of other animals living in linear habitats and with large ranges

Our density estimates varied greatly by method, from one otter per 3.71 km of CN to 7.52 km of FN. Although this remarkable, twofold difference was somehow expected (see "Introduction"), it highlights the influence of hydrography resolution on otter density results. Moreover, estimates referring to individual female ranges were always higher than the overall density estimate. This remarks how estimating density within short river extensions, as done in some case studies (cited in the "Introduction"), may significantly overestimate the true density, since short river extensions often correspond to the home range—or a portion of it—of a single adult otter (cf. Kruuk 2006), and otter males are known to encompass the range of several females (Erlinge 1968). Together, these findings demonstrate the effects of study scale and hydrography resolution on the results of density estimation of animals, such as otters, living in linear environments. They also reinforce the importance of choosing appropriate scale of analysis when estimating density of animals—such as carnivores (Powell 2012)—with large home ranges, providing further evidence that carnivore density estimation may be strongly scale-dependent (e.g., Smallwood and Schonewald 1998).

We urge authors aiming to estimate the density of otters or other animals living in linear habitats to report the scale of hydrography used for their estimations, and suggest to do the same also for river width and depth when possible, to increase comparativeness of results worldwide. Recognizing that coarser layers may be more readily available to researchers, we recommend caution in their use, since this may yield overly optimistic results, putting endangered populations at risk. Also, while waiting for future studies addressing this issue in detail, we recommend that researchers avoid estimating otter density in sampling areas less than 70–100 km²; this threshold is based on the largest area occupied by a single otter reported in the literature (66 km² corresponding to the minimum convex polygon built upon locations of an adult radio-tracked male otter, encompassing 68 km of FN—Quaglietta 2011), and should be viewed as dependent on the nutrient richness and complexity of waterways in the study area.

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