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# Estimating abundance of the Stripeless tree-frog *Hyla meridionalis* by means of replicated call counts

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**Abstract.** The Stripeless tree-frog *Hyla meridionalis* reaches its eastern-most European distributional limit in NW Italy, and specifically in the Cinque Terre National Park. Here for two consecutive years, we estimated tree-frog population abundance by call surveys at 24 sites. Data were analysed in the framework of N-mixture open population models based on repeated counts of calling males. The results obtained by this statistical approach were effective in estimating population size together with annual recruitment and survival. The tree-frog male population size remained constant between years and site abundance was inversely related with altitude. On the bases of these findings, our application of N-mixture models to tree-frog calling males was successful and is a promising cost-effective method to obtain long-term monitoring data on this species over large geographic areas.

**Keywords.** Abundance estimation, call surveys, Cinque Terre National Park, detection probability, N-mixture models.

The Stripeless tree-frog *Hyla meridionalis* Boettger, 1874 is found in North-western Africa (Algeria, Morocco and Tunisa), South-western Europe (Portugal, Spain, South France and North-Western Italy) and the Canary and Balearic Islands (Sillero, 2010). On a portion of its distribution range the species is considered introduced, i.e., Canary and Balearic Islands (Sillero et al., 2014), and it is also possibly introduced for other European regions (Recuero et al., 2007). In Italy, the Stripeless tree-frog is common along the Mediterranean coast of Liguria (NW Italy), from the Province of Imperia to the province of La Spezia (Salvidio, 2007). Apart from morphometric and distributional data (Salvidio, 2007), little is known about the abundance and dynamics of Stripeless tree-frog populations in Italy, and quantitative data on populations size should be obtained to assess the species status and its ecological requirements, in particular near the species distribution limits, where a high population fragmentation is expected (Gaston, 2003).

Although photo-identification of Stripeless tree-frogs is possible (Crovetto unpublished data), the animals are arboreal and highly secretive during daytime. The use of PVC pipes may increase the probability of detection of tree-frogs (do Vale et al., 2018), however, in the CTNP the majority of the species' reproductive habitats are on private lands, and thus are not freely accessible (Romano et al., 2014). Therefore, the monitoring technique selected to estimate population size was based on nocturnal auditory surveys of calling males, because of the species highly distinctive mating call (Schneider, 1974; Márquez et al., 2005). Call survey is a relatively efficient technique for evaluating the distribution and diversity of anurans (Dorcas et al., 2009). Therefore, calling surveys are frequently used in large-scale amphibian monitoring programmes (e.g., Anthony, 2002; Weir and Mossman, 2005; Weir et al., 2005, 2009). However, the use of call surveys for estimating population abundances and trends suffers of the same problematic issues recognized in the case of repeated counts of individuals, because the detectability of anuran calling males is < 1 (i.e., not all males are calling in the same night; Schmidt and Pellet, 2005. Moreo-

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ver, anuran mating call activities display high variation in response to biotic and abiotic factors, that usually remain unknown and difficult to model (Royle and Link, 2005; Droege and Eagle, 2009). In fact, using raw counts of calling males or even scores derived from abundance indexes (i.e., indexes that group calling males by classes of relative abundance; Weir and Mossman, 2005) without accounting for detection probability may lead to relevant bias in abundance and trend estimates (Schmidt, 2004; Mazerolle et al., 2007). Therefore, to reliably estimate population abundance, the information derived from raw counts of calling males should always be corrected for species-specific detection probabilities (Schmidt and Pellet, 2005; Royle and Link, 2005). Recently, specific modelling approaches have been proposed for estimating anuran population abundances from the count of anuran calling indexes taking into account detection probabilities (Royle, 2004a; Royle and Link, 2005).

This study aimed to estimate the abundance of Stripeless tree-frog males together with some demographic parameters and ecological requirements in Italy, at the eastern limit of the species distribution. Moreover, we tried to establish a cost-effective monitoring protocol to provide future population trends. Because of the relatively small number of tree-frog males recorded per site, we had the opportunity to apply the open population generalization of Royle's (2004b) N-mixture model (Dail and Madsen, 2011) to count data derived from call surveys.

The eastern-most limit of the species' range in Europe is the village of Riomaggiore (Province of La Spezia), in the Cinque Terre National Park (CTNP), a protected area where the Stripeless tree-frog reproduces in streams and in artificial water tanks used for irrigation (Salvidio, 2007; Romano et al., 2014). In this area water streams display short and steep courses, with relatively long summer drying periods, due to the lack of precipitations (Olivari et al., 2013). Among many possible land use of rural areas, agriculture is the only one in the CTNP, and from the sea level up to the hill tops vineyards and orchards are cultivated on strips of arable land, or "terraces", sustained by dry-stone walls. Irrigation is provided by means of water stored in tanks, often colonised by amphibians (Olivari et al., 2013; Romano et al., 2014). The survey sites were selected during both daytime and nocturnal preliminary surveys. During the day, streams and water reservoirs were inspected and selected as potential reproductive sites if adults, larvae or eggs of some amphibian species were observed. During the night, sites were located by perceiving the calls of Stripeless three-frog males. In total 24 sites were surveyed in the municipalities of Levanto, Monterosso and Riomaggiore (from West to East): 6 streams and 18 artificial water reservoirs in agricultural lands or urban settings (Table 1; Fig. S1).

All surveys began after sundown and after hearing the first tree-frog calls. In 2017 three nocturnal surveys were performed, from the end of March to May, by two operators that counted the number of males calling at each site during a two minute period. In 2018, three nocturnal surveys were performed, from the beginning of May to the beginning of June, with the same observers and procedure of 2017. In addition in 2018, a fourth survey was performed by a single operator that tallied calling males for 4 minutes. The asynchrony and the different tonalities of calls permitted to count with confidence the minimum number of males per site that, in all cases, was ≤ 6 (Table S1). All sites were surveyed during the same overcast or rainy night, but never during heavy showers that could hinder a clear hearing of frog calls. Four climatic variables were obtained from the meteorological station of Levanto: rainfall during the 24 h preceding the survey (RAIN), air temperature (TEMP), relative humidity (RH) and wind speed (WIND), recorded during the last hour of survey. These weather variables were selected, because they are known to influence anuran calling behaviour (e.g., Walls et al., 2011). Finally, for each site three variables were considered: altitude above the sea level (ELEV), a categorical variable for the municipality of the site (CITY) and if the water body was a stream or an artificial site (SITE).

Repeated count data were analysed using the Dail-Madsen (2011) model, which is a generalization of the Royle's (2004b) N-mixture model, capable of relaxing the closure assumption by considering the population closed to immigration/births and emigration/deaths during a short period (i.e., three/four survey nights performed each year), while considering the population demographically open between years, in a robust design-similar approach. This model estimates four parameters, two of which are in common with the Royle's (2004b) N-mixture original formulation: individual detection prob-

**Table 1.** Continuous variables included in the N-mixture open population models (Dail and Madsen, 2011) used to estimate *Hyla meridionalis* abundance, in the Cinque Terre National Park.

| Variable | Description | Sample size (N) | Mean (SD) | min | max |
|---|---|---|---|---|---|
| ELEV | Site altitude (m) - | 24 | 35.04 (29.66) | 8 | 83 |
| TEMP | Air temperature (°C) | 7 | 17.14 (3.44) | 12 | 21 |
| WIND | Wind speed (m/s) | 7 | 3.57 (1.27) | 2 | 5 |
| RH | Relative humidity (%) | 7 | 66 (13.55) | 45 | 85 |

**Table 2.** Candidate N-mixture open population models (Dail and Madsen, 2011) used to estimate *Hyla meridionalis* abundance, ranked by AICc. γ = recruitment rate; λ= initial site abundance; p = individual detection probability; ω = survival; AICc*WT* = model weights. In model list t stands for time dependence. For covariate abbreviations see table 1.

| Model | Parameters | AICc | ΔAICc | AICc*WT* |
|---|---|---|---|---|
| λ(ELEV) p(.) ω(.) γ(.) | 5 | 451.35 | 0.00 | 0.40 |
| λ(ELEV) p(.) ω(ELEV) γ(.) | 6 | 453.45 | 2.1 | 0.14 |
| λ(CITY) p(.) ω(.) γ(.) | 6 | 453.98 | 2.63 | 0.11 |
| λ(ELEV) p(.) ω(CITY) γ(.) | 6 | 454.95 | 3.61 | 0.07 |
| λ(.) p(.) ω(.) γ(.) | 4 | 454.96 | 3.61 | 0.07 |
| λ(SITE) p(.) ω(.) γ(.) | 5 | 455.05 | 3.70 | 0.06 |
| λ(ELEV) p(.) ω(SITE) γ(.) | 7 | 456.15 | 4.80 | 0.04 |
| λ(.) p(TEMP) ω(.) γ(.) | 5 | 456.81 | 5.46 | 0.03 |
| λ(.) p(.) ω(SITE) γ(.) | 5 | 456.93 | 5.59 | 0.02 |
| λ(.) p(RH) ω(.) γ(.) | 5 | 457.14 | 5.80 | 0.02 |
| λ(.) p(WIND) ω(.) γ(.) | 5 | 475.54 | 6.20 | 0.02 |
| λ(.) p(RAIN) ω(.) γ(.) | 5 | 458.03 | 6.69 | 0.01 |
| λ(.) p(.) ω(ELEV) γ(.) | 5 | 459.15 | 7.80 | 0.01 |
| λ(.) p(.) ω(CITY) γ(.) | 6 | 459.35 | 8.01 | 0.01 |
| λ(.) p(t) ω(.) γ(.) | 11 | 483.07 | 31.73 | 0.00 |

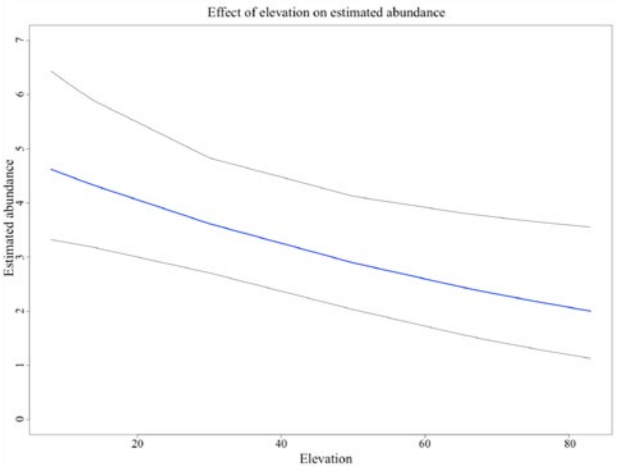

**Fig. 1.** Effect of elevation on site specific abundance of *Hyla meridionalis* in the Cinque Terre National Park, with 95% confidence intervals, obtained by N-mixture open population modelling (Dail and Madsen, 2011).

ability (p) and mean initial abundance for each site (λ). The Dail-Madsen (2011) model estimates two additional parameters: the recruitment rate (γ), comprehensive of births and immigrations, and the apparent survival probability (ω), comprehensive of deaths and emigrations. In our study, we built models with Poisson error distribution, since Negative Binomial distribution could lead to identifiability issues and may produce infinite abundance estimates (Barker et al., 2017; Link et al., 2018). Furthermore, in order to avoid truncated estimates of abundance (Knape et al., 2018), we set the upper limit for integration (K) to 50 (i.e., we checked estimate stability at incremental values of K). We then began the model building procedure by fitting a global model (i.e., the most complex model on which other models are nested) and assessing the fit of this model in two ways: i) by means of a Pearson chi-square test (MacKenzie and Bailey, 2004), using a parametric bootstrap procedure (5000 re-samplings), ii) by inspecting residuals (Knape et al., 2018). In order to avoid overfitting and creating too many models, deriving from the combinations of covariates for each of the four parameters of the Dail-Madsen (2011) model, which can lead to uninformative and biologically unsound models, we preferred to build fewer models in a stepwise approach, considering one parameter at a time, and building biologically informative models.

We proceeded modelling the detection probability, considering it to be constant, time-dependent, or to be affected by climatic variables. Then we modelled the initial abundance as a function of site specific covariates (ELEV, CITY and SITE) or constant over sites. Finally, we considered the survival to be influenced by the same site covariates as abundance, or constant over sites. For each model we considered recruitment as constant. We ranked all models with Akaike's Informative Criterion corrected for small samples (AICc). We conducted model selection and considered only models with ΔAICc > 2 (Burnham and Anderson, 2002). Modelling was conducted in the R environment with package Unmarked (Fiske and Chandler, 2011) and AICcmodavg (Mazerolle, 2017).

In 2017 we counted a total of 131 male frog calls during three surveys (44; 45; 42; respectively), while in 2018 we counted 129 male frogs during four surveys (33; 37; 32; 27; respectively, Table S1). The global model had a good fit (goodness-of-fit, P = 0.34; c-hat overdispersion = 1.12, and visual inspection of residuals). Model building procedure produced a total of 15 models (Table 2). The most supported model included elevation as a covariate on the initial abundance, highlighting a negative effect of elevation ($\beta_{ELEV}$ = -0.331; 95% CI = -0.59 to -0.08; Figure 1). The estimated mean frog abundance per site was 3.4 (95% CI = 2.5 – 4.6). Individual detection probability for this model was constant, and estimated as p = 0.53 (95% CI = 0.42 – 0.63). Survival probability between years was considered constant among sites and resulted ω = 0.71 (95% CI = 0.50 – 0.86). Finally, the recruitment rate was constant across sites γ = 0.11 (95% CI = 0.00 – 12.10). From this best model we also obtained, as a derived parameter from the posterior distribution of the latent abundance, the total abundance of surveyed sites, which resulted of 89 frogs in 2017 (95% CI = 81 – 147) and 64 frogs in 2018 (95% CI = 60 – 109).

Our study showed that N-mixture modelling applied to individual frog calls can be successfully used to estimate male population size together with demographic parameters and ecological understandings. In the CTNP, where *H. meridionalis* is a species of high conservation concern, the male tree-frog population size showed no significant change between years, and site abundance was negatively related with altitude (Salvidio, 2007; Sillero, 2010). Moreover, the usefulness of N-mixture approach may be appreciated by comparing population estimated corrected by detectability to raw counts that, in the present case, underestimated the total number of males by about 45%, in both years. Another important application of N-mixture population open models (Dail and Madsen, 2011) relies on the possibility of estimating temporal variations in inter-annual population size, this information being of interest in conservation and management programmes concerning protected species characterised by low or variable detection probabilities (Ficetola et al., 2018). Conversely, the major limits of our study were that the occurrence of calling males does not always assure for the presence of a breeding site, while no data on population structure (i.e., population sex ratio and proportion of juveniles) can be provided (Dorcas et al., 2009). In any case, N-mixture models are cost-effective alternatives to mark-recapture and removal sampling methods (Kéry and Royle, 2015; Kéry, 2018), and they have been used to estimate population size and temporal trends of many species in very different ecological contexts (e.g., Priol et al., 2014; Romano et al., 2017; Kéry, 2018; Costa et al., 2019). However, to our knowledge there are few applications of N-mixture modelling to anuran call counts, because of the difficulties in correctly counting calling males in large frog choruses when dozens of calls are synchronous (Weir and Mossman, 2005). Nevertheless, when few individual males are calling at each site the application of the N-mixture modelling seems useful and can be preferred to other methods that estimate population abundance because there is no need to mark and recapture the focal individuals (Royle, 2004a; Royle and Link, 2005).

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## SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www.unipv.it/webshi/appendix> manuscript number 25342.

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