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Selecting Monitoring Methods for Endangered Trout Populations

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Abstract: Endangered trout populations can be monitored with a variety of methods, the selection of which should consider social constraints and environmental variables known to affect method effectiveness. Here, we confront the effectiveness of four monitoring methods (removal with electrofishing, ELE; underwater camera survey, UCS; streamside visual survey, SVS; visual surveys with angling, VSA) to estimate the relative abundance of three populations of the endangered Mediterranean brown trout. The trout counts obtained via different methods were well correlated (r = 0.65–0.72), providing a coherent description of the relative pool abundance across the methods. However, the methods were differently affected by environmental variables, depending on the age classes of trout. Specifically, the adult and subadult counts provided by ELE and VSA were negatively and positively affected by the maximum pool depth, respectively; adult and subadult counts of VSA and the SVS were positively affected by pool area; the juvenile counts provided by the UCS were positively affected by pool shade and negatively affected by water turbidity; juvenile counts provided by VSA were positively affected by shade. Variables such as pool depth, area, shading, water turbidity and proportion of age classes can be hardly controlled in monitoring programs, and their bias could be modelled. Different sampling methods provided similar information about relative abundance and appeared equivalent. While ELE could be selected to collect samples and biometric data, monitoring relative abundance with the UCS, VSA, and SVS appears more suited and can also involve citizen scientists.

Keywords: adaptive monitoring; environmental heterogeneity; ethic and conservation; *Salmo trutta*; small streams; standard methods

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1. Introduction

Monitoring programs designed for species conservation should provide information about distribution, relative abundance, and environmental variables thought to affect the conservation status of populations [1]. Additionally, the operation of monitoring programs is affected by socio-ecological factors and decisions related to the human dimension. For example, when managing renewable resources of common interest [2], it is important to involve stakeholders in monitoring programs, so that relevant local ecological knowledge can be gathered with the support of non-professional researchers [3–5], and a consensus about resource conservation status and management can be possibly achieved [6,7]. Finally, when dealing with species of conservation concern, monitoring methods should be non-invasive [8–10], with ethical issues becoming dominant in selecting methods of investigation [11,12]. Therefore, selecting monitoring methods for endangered trout populations should consider issues such as management objectives, socio-ecological context, information needs, the available resources, and constraints related to animal welfare.

Freshwater fishes can be monitored with a variety of methods, each one with advantages and disadvantages [13]. Multiple-pass removal with backpack electrofishing is considered a standard method and commonly applied for counting salmonids in small streams, although it can be affected by several environmental variables [13,14]. Electrofishing can also cause injures, physiological stress and post-release mortality to fish, and it should be used with particular caution with populations of conservation concern [11,15]. Electrofishing also requires highly qualified field biologists, and it is difficult/expensive to perform in large-scale studies and in remote locations [13], where the transport of the electrofishing equipment is often difficult. Visual survey methods based on underwater cameras recordings [8,9] and streamside counts [13,16] are non-harmful alternatives to electrofishing that can provide information about distribution and relative abundance of salmonids in small streams with shallow and clear waters [10]. Visual surveys can also be used for large-scale monitoring programs involving non-professional researchers. However, visual surveys cannot provide information about individual covariates such as age, weight, length, morphology, and genetics, unless augmented with a collection technique. To obtain such information, streamside visual surveys can be performed by anglers during catch-and-release fishing activity [17]. Catch-and-release anglers are also the main salmonid users in freshwater habitats, particularly in remote location and natural parks, and they could contribute with their knowledge and skills to large-scale monitoring programs of fish and co-management [7].

The effectiveness of counting methods can vary with several environmental variables [13]. For example, boulder substrate, water depth, stream shading, and slow flow can negatively affect estimates of trout abundance provided by backpack electrofishing [14,18], while visual counts can be reduced by water turbidity, turbulence, depth, and glaring [18,19]. Several environmental variables cannot be controlled in monitoring programs, because monitoring aims at obtaining information about distribution, abundance, and threats to the species over space and time [1]. Sampling only in selected pools to maximize efficiency of given sampling methods would provide habitat-biased estimates. Instead, environmental variables thought to affect sampling can be included as covariates to address their biases on counts. Therefore, to develop local monitoring programs, it is important to evaluate methods that are thought to be useful, feasible, and effective by studying how provided counts can be affected by environmental variables.

Here, we apply the four methods mentioned above (removal via electrofishing, underwater camera survey, streamside visual survey, and visual surveys with angling) to study the abundance of three populations of the Mediterranean brown trout, *Salmo trutta* L., 1758 Complex (Osteichthyes: Salmonidae) [20], in Sardinia (Italy). The Mediterranean trout is considered critically endangered in the national Red List of the International Union for Conservation of Nature, as *Salmo cettii* [21] and is also listed as *Salmo macrostigma* in Annex II of the Habitats Directive (92/43/EEC). The main threats for the species are represented by habitat fragmentation, water pollution, invasive species, overexploitation of fish and water resources [21,22], and the introduction of domesticated trout [23,24]. The populations of conservation interest of this species are often confined in headwater streams, with shallow and clear waters, similarly to other endangered trout populations [11,25,26]. Therefore, it is important to study the reliability and effectiveness of monitoring methods that can be applied in remote headwater streams to gain information about the distribution and relative abundance of the Mediterranean brown trout, as well as the effect of environmental variables on abundance estimates.

By confronting trout counts obtained via repeating the four different methods in 10 randomly selected pools for each population, we attempt to evaluate their relative effectiveness in different environmental conditions, focusing on the following questions:

(1) Do trout counts differ among methods?
(2) How are sampling methods affected by environmental variables?

Considering the results of our comparison, we finally discuss the principles that could guide the development of locally adapted, feasible, and effective monitoring programs for endangered trout populations.

2. Methods

2.1. Study System

Sardinia (Italy), where we conducted this study, is characterized by a Mediterranean climate with hot dry summers and mild rainy autumn/winter seasons. The Mediterranean streams generally have an intermittent regime [27], with little surface flow and fragmented pools or stream stretches during summer. These streams are thus characterized by the presence of physical and hydraulic barriers that reduces river continuity and severely fragment fish populations.

Three headwater streams were selected for this study (the Piras, Furittu, and Flumineddu streams, Figure 1). The streams present viable populations of the Mediterranean native brown trout [24,28]. The headwater section of the Piras and Furittu streams have been designated as a genetic sanctuary (DR n.314/Dec.A9 07.02.2019) where fishing is not allowed, while in the Flumineddu stream, fishing is allowed.

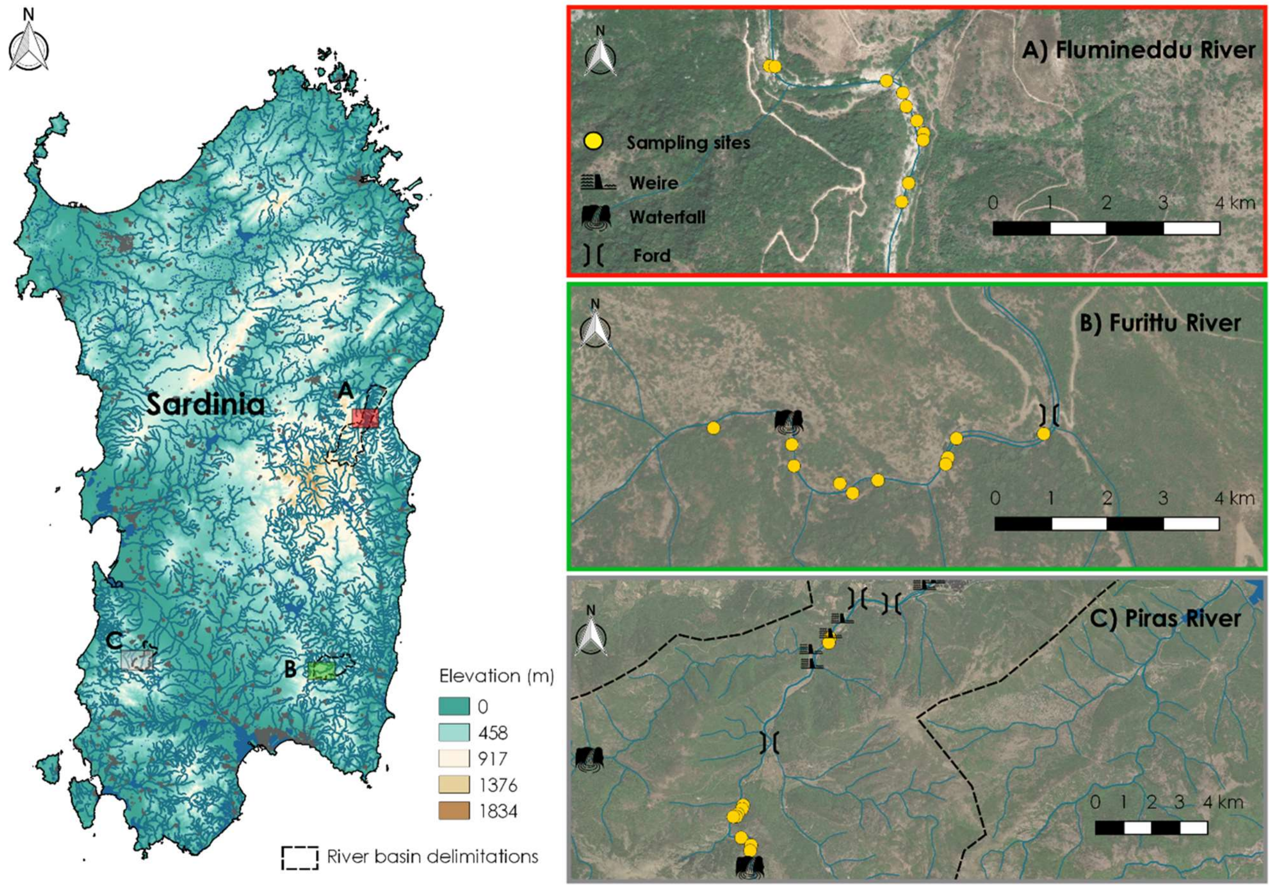

Figure 1. Study areas and sampling stations of the investigated pools. The color of the highlighted area on the left corresponds with the outline color of the inset on the right.

2.2. Sampling Design

This study was performed during June and July 2022, when dry weather and slow-flow regime provided optimal conditions for sampling, as shown in a comparison of underwater camera surveys and streamside visual surveys in detecting pool occupancy during high- and low-flow regimes [10]. Sampling occurred during sunny mornings when weather conditions remained stable (no wind, clouds, or rain). Within each population (stream), 10 consecutive pools were selected. Pools are defined as habitat units with water depths of at least 20 cm, separated by narrow stretches or small waterfalls, and with a substantial decrease in water velocity. During periods of minimal water flow, such discontinuity should minimize dispersals of individuals between adjacent pools. Considering that sampling in each stream was carried out over three consecutive days, fish dispersal between pools was assumed to be negligible and pools were thus considered independent. To minimize the potential impact of previous sampling efforts on counts, we started with less invasive methods such as streamside visual surveys and underwater camera surveys. Visual surveys with angling were conducted on the second day and removal with electrofishing was performed on the third day, as described below.

Streamside visual surveys (SVSs) were performed on the first day by two observers simultaneously walking along the stream on opposite banks. Both observers cautiously approached the pool and remained stationary while counting trout from selected viewpoints. The observers were not independent and, communicating with each other, they identified positions of all the observed fish to avoid double counts as much as possible. After approximately 3–5 min, depending on pool size, the number of different observed trout were recorded. Trout were visually categorized by size, with specimens divided into juveniles (J), subadults (SA), and adults (AD) based on total lengths (TL, cm) defined for the Mediterranean trout in Sardinia [29] (TL_J below 9 cm; TL_{SA} from 8 to 12 cm; and TL_{AD} more than 12 cm). Following the SVSs, one underwater camera was placed in the pool (see below) and habitat variables were measured. The observers then walked upstream to the next pool to repeat the observation process up to the 10th pool.

Underwater camera surveys (UCSs) were performed using one action camera per pool (Apeman Action Cam A100, Shenzhen, China). The camera was mounted on a stable stand (Sabrent magnetic support), placed to maximize the field of view and face upstream. The camera recorded video for a minimum of 30 min (1080p video resolution, 30 frames per second, ultra-wide frame of view, and 1920 × 1080 screen resolution). A recording time of 30 min has been considered sufficient for obtaining reliable counts in small streams [8–10]. The cameras were recovered at the end of the sampling session, when walking backstream and after an interruption of at least 30 min from SVS sampling of last pool. All videos were reviewed to count the maximum number of individuals observed in each single frame of 30 s, and the maximum possible observed count (MaxN) was used to estimate the number of individuals observed in the pool [8]. The counts for adults (A), subadults (SA), and juveniles (J) were recorded as described above.

On the second day, visual surveys with angling (VSA) were performed with the same 10 pools by a single observer (angler). The angler involved in this study (F. Curreli) has a degree in Natural and Environmental Science and was also the second observer during the SVS. To minimize the impact on trout, fishing attempts were made using low-impact catch-and-release equipment [17]. The angler moved from downstream to upstream ensuring minimal disturbance. Upon reaching the casting distance, the angler cast from 5 to 20 times, gradually moving from downstream towards the upstream limit of the pool. The goal was not to catch trout, as this could potentially startle other individuals, but rather to attract as many trout as possible out of their refuges for counting purposes. Therefore, efforts were made to reduce the likelihood of trout attacking the bait, such as quickly recovering the bait upon sensing the readiness of the fish to bite. If trout inadvertently bit the bait, they were promptly unhooked, keeping them in the landing net to minimize alerting other individuals. A spin-fishing tackle, consisting of a 180 cm ultralight rod with a casting range of 2–8 g, a size-2000 fishing reel filled with a 0.06 mm braided line and a 0.20 mm fluorocarbon leader, and a 3 g handmade spinner lure armed with a single barbless hook were used for fishing. After about 2–10 min of fishing attempts and observations, depending on pool size, the number of observed and captured adults (A), subadults (SA), and juveniles (J) were recorded.

On the third day, multiple-pass removal with backpack electrofishing (ELE) was performed in the same 10 pools sampled on previous days. Trout were sampled using a backpack electrofisher unit (Helt 60II with Honda engine 4 stroke, 49 ccm, power 1.8 KW/7000 rpm; average voltage and current values applied: 5.2–2.8 Å, 230–400 V, 1300 W) with an electrode diameter of 30 cm and a cathode section of 5 mm. Three operators were involved, one using the electrofisher and two others with landing nets to capture stunned trout. Water conductivity was assessed before sampling (Piras 182.71 ± 41.38 μS; Furittu 324.76 ± 13.81 μS; Flumineddu 406.26 ± 11.49 μS) and the electrofisher was tuned accordingly. All the trout captured were immediately measured and weighed on-site, including total length (TL, to the nearest 0.1 cm) and total weight (TW, nearest 0.1 g). The specimens were categorized as adults (A), subadults (SA), and juveniles (J). After processing, the trout were released back into the pool. Electrofishing operations were considered complete when two consecutive passes failed to capture further trout.

2.3. Environmental Variables

Environmental variables thought to affect counts provided by the different methods were recorded in each sampled pool, as below.

Pool depth may negatively affect counts obtained from ELE and the SVS, as pools deeper than 1 m are difficult to explore and observe [13,16]. The UCS and VSA could be less affected by pool depth, as cameras can be strategically placed, and angling may attract trout from refuges. To capture these effects, we recorded the maximum pool depth (*MDepth*) as a proxy for unsamplable pool extent. Pool length (*Length*), maximum width (*MWidth*), and surface area (*Area* = *Length*MWidth*) could negatively affect counts [but see 10]. The UCS counts could be affected by the pool extension due to its fixed field of view [30], while ELE, the SVS, and VSA allow observers to adapt their sampling efforts and move, potentially reducing the effect on counts. Variables describing pool size were measured using a roll meter (Table 1).

Table 1. Mean values and range of variation in environmental variables measured at the pool level.

Variable Name and Code	Unit	Min	Max	Mean	SD
Pool maximum depth (MDepth)	meters	0.5	2.5	1.09	0.49
Pool length (Length)	meters	2	31	13.36	7.72
Pool maximum width (MWidth)	meters	2	10	4.77	2.23
Pool area (Area)	m^2	8	140	63.87	44.85
Turbidity (Turb)	NTU	0.2	1.85	0.65	0.45
Pool shading (Shade)	% cover	20	100	79	28.93
Refuges (Refuge)	% cover	5	60	21.83	14.17
Water temperature (WTemp)	°Celsius	16.20	25.53	21.59	3.01
Trout count (N)	N°individuals	0	10	2.53	2.34

High levels of turbidity could reduce visibility and negatively affect counts provided by the four methods [13,16,31], perhaps with effects more evident on the SVS. Turbidity (*Turb*) was recorded using a turbidity meter (NTU) (AQUALYTIC® AL255T-IR, Pretoria, South Africa).

Direct light and glaring can negatively affect visual observation and electrofishing [13,16], as well as shading, which reduces light and vision. To capture such effects, pool shading (*Shade*) was measured as the percent of pool surface covered by shade. Sampling was performed in the morning and in valleys, where shade was often the result of the sun being covered by mountains or rocky outcrop, depending on the hour.

During sampling, trout often hide under submerged vegetation, roods, roots, and boulders [13], reducing their detectability. Refuges (*Refuge*) were visually estimated as the percent cover of the pool bottom that could not be seen through, under which trout could potentially hide. VSA could reduce this effect by attracting adult trout out from refuges. By estimating the percent cover of refuges, we aimed to account for their potential impact on trout counts and understand how different methods might be affected by refuge quantity.

Sampling was performed during summer, with high water temperatures (max T = 25°). Trout, in response to high temperatures, can seek thermal refuge in deeper and fresher parts of a pool [32], negatively affecting counts. Given the range of temperature observed (Table 1), low temperature effects could not be studied. Water temperature (*WTemp*, °C) was measured using a multiparameter probe (InSitu smarTROLL Multiparameter Handheld, Fort Collins, CO, USA).

Trout abundance should positively affect counts. Abundance was estimated in each pool by using the average value of counts for a given pool i, as below:

$$\overline{N}_i = (\mathrm{Ci(x)} + \mathrm{Ci(y)} + \mathrm{Ci(z)})\,/3);$$

where Ci(x, y, or z) is the number of trout counted in pool i with method x, y, or z. Indeed, by averaging across all the four methods, the counts provided by any given method could not be independent from $\overline{N}_i$. To address this, for each method, pool density was estimated by using counts provided by the other three methods, x, y, and z. A positive correlation between counts provided by a given method and $\overline{N}_i$ can be thus interpreted as support for the ability of the method to capture variation in density between pools. Indeed, the absence of correlations would suggest that variation in counts across different methods is random and not related to abundance. This variable was also used to distinguish variation in counts due trout abundance from variation in counts due to environmental variables or method effects.

2.4. Statistical Analysis

The data were analyzed by means of generalized linear models (GLMs) [33]. Variance of counts can be described by Poisson or negative binomial distributions [34]. The fit of the two distribution was assessed by means of the R package *fitdistrplus* [35]. Estimates of overdispersion were based on deviance, using the functions *glm* of software R 4.2.2 [36] and *glm.nb* of R package MASS [37].

The negative binomial distribution ($\mathrm{AIC_{NB}}$ = 507.32) showed a better fit compared to the Poisson distribution ($\mathrm{AIC_{Poisson}}$ = 640.28). The Poisson model applied to evaluate fit, considering the effect on counts of location, mean density of trout and sampling method (Counts(Tot) ~ Stream + $\overline{N}$ + Method, family = poisson) showed overdispersion (chat = 2.14). Residual deviance (241.8) and sum of Pearson (245.3) of the Poisson model were also higher than the five-percent critical value for a chi-squared test (138.81). Conversely, the residual deviance (130.7) and sum of Pearson (126.1) of the equivalent negative binomial model were lower than the critical value, showing a better fit of the negative binomial distribution. Model selection was thus performed using the glm function with family = negative.binomial implemented in software R 4.2.2 (see Supplementary Materials: data and R script).

Models were compared by means of small-sample correction of the Akaike Information Criterion *AICc* [38]. When the difference of *AICc* between models (Δ*AICc*) was lower than 2, a likelihood ratio test (LRT) implemented in the R package *lmtest* [39] was applied.

Models' goodness of fit was estimated as deviance explained ($DE = (dev(M0) - dev(Mx))/dev(M0))$), where *M0* is the null model specifying no effects and *Mx* is the tested model.

2.4.1. Do Trout Counts Differ among Methods?

The effectiveness of sampling methods can vary with environmental variables and age classes [40,41]. Therefore, method effects were evaluated on total (Tot = J + SA + A), subadult and adult (SA + A) and juvenile (J) counts. The whole dataset (n = 30 pools × 4 methods = 120) was used, and fixed effects on counts of location (Stream) and pool trout density ($\overline{N}$) were assessed. However, method effects could be confounded with time effects (*Time*), i.e., the effect of the temporal repetition of sampling for four consecutive times (SVS = 1, UCS = 2, VSA = 3, and ELE = 4). Although we started from less invasive methods, such a sequence could alert trout and result in negative temporal trends of counts. As an attempt to distinguish between sampling method (category) and time effects (trend), model selection started from the general models *Counts ~ Stream +* $\overline{N}$ *+ Method* or *Counts ~ Stream +* $\overline{N}$ *+ Time*. By modeling method effects as category and time effect as trend, we attempt to distinguish if effects were relative to the method applied or to the sampling sequence. Since method and time effects are described by the same data (where method = sequence number), a simultaneous evaluation of method and time effects could not be performed. All possible combinations of simplified models were generated by means of automated model selection implemented in the R package *glmulti* [42]. To reduce the number of models run on a small dataset and facilitate the interpretation of results, simplified models were built allowing no interactions (*level* = 1).

2.4.2. How Are Methods Affected by Environmental Variables?

To assess the effect of environmental variables (Table 1) on the total (Tot = J + SA + A), subadult and adult (SA + A) and juvenile (J) counts, the whole dataset was analyzed (n = 30 pools × 4 methods = 120). Model building started from the best model selected with the analysis presented in the previous section, specifying fixed effects of location (*Stream*) and pool trout density ($\overline{N_i}$). Considering the small dataset, each variable was evaluated individually using univariate screening [43]. Additionally, given that the effects of environmental variables on counts could be method-dependent, method effects and interactions between method and variables were allowed. Univariate screening thus confronted two null models (*NullA: Counts* ~ *Stream* + $\overline{N}$ and *NullB: Counts* ~ *Stream* + $\overline{N}$ + *Method*) with the effect model *Counts* ~ *Stream* + $\overline{N}$ + *Method* + *Method:Variable*. If a variable affects counts provided by a given method, this effect should be captured by the model part *Method* + *Method:Variable*. One univariate model was created for each habitat variable (*Depth*, *Length*, *Width*, *Area*, *Turb*, *Shade*, *Refuges*, and *Temp*) and confronted with null models. The Pearson correlation coefficient showed that environmental variables were not collinear [44], except for *Length* vs. *Area* (r = 0.8465).

Environmental variables that were supported by the univariate screening were included into a general multivariate model, *Counts* ~ $\overline{N}$ + *Stream* + *Method* + *Method:Variable1* + *Method:Variable2* + . . . The structure of the model depended on the specific variables that were supported by each dataset (Tot, A + SA or J). The general model was then simplified by taking out each *Method:Variable* part at a time (see Supplementary Materials: data and R script).

3. Results

3.1. Do Trout Counts Differ among Methods?

The distribution of total (Tot), adult and subadult (ASA), and juvenile (J) counts provided by the different methods can be seen in the boxplot presented in Figures 2–4, respectively.

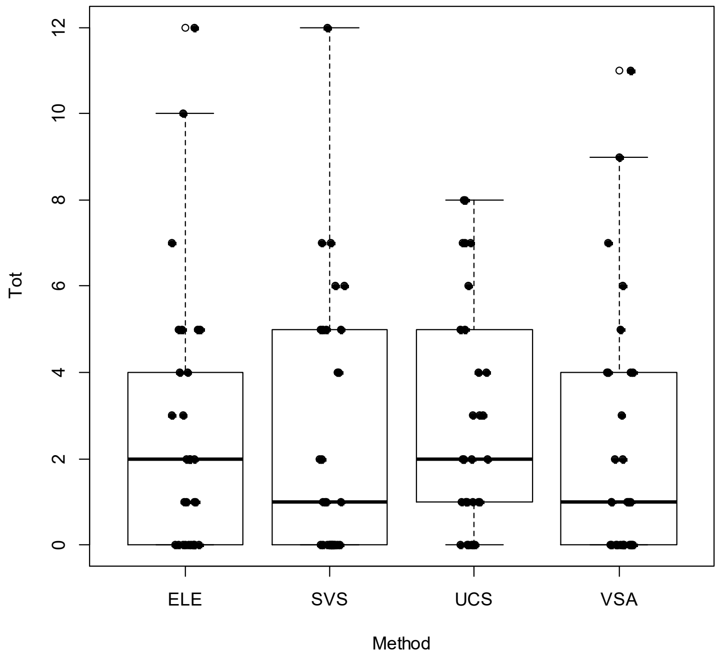

Figure 2. Distribution of total counts (Tot) provided by the different methods (backpack electrofishing, ELE; streamside visual survey, SVS; underwater camera survey, UCS; visual surveys with angling, VSA). Black circles show observed counts while white circles show counts that are classified as outliers.

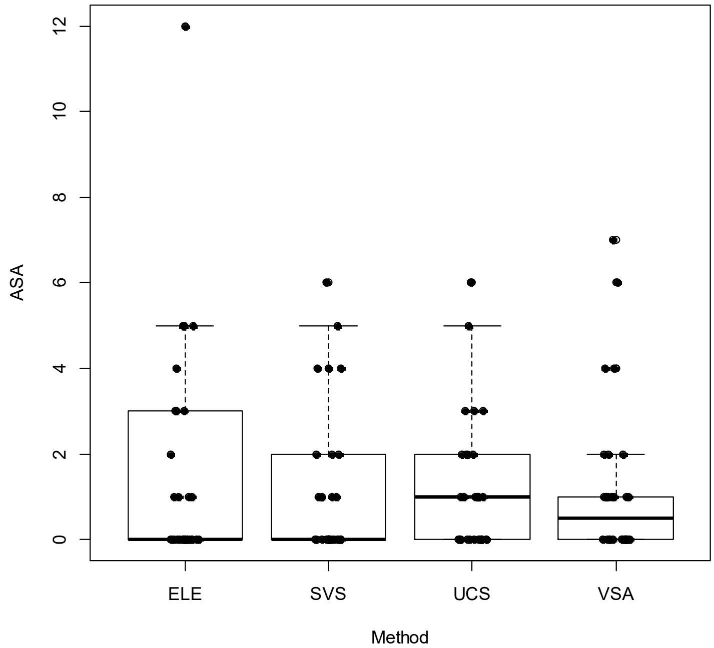

Figure 3. Distribution of adult and subadult counts (ASA) provided by the different methods (backpack electrofishing, ELE; streamside visual survey, SVS; underwater camera survey, UCS; visual surveys with angling, VSA). Black circles show observed counts while white circles show counts that are classified as outliers.

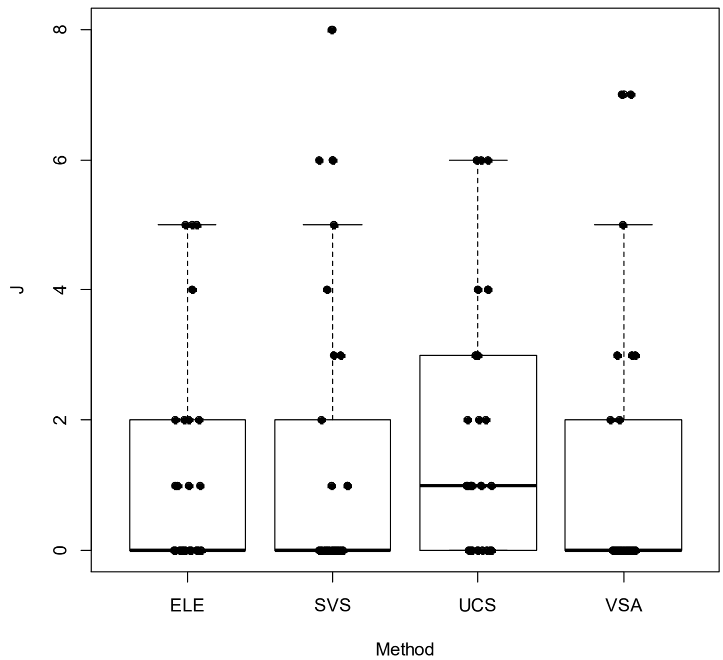

Figure 4. Distribution of juvenile counts (J) provided by the different methods (backpack electrofishing, ELE; streamside visual survey, SVS; underwater camera survey, UCS; visual surveys with angling, VSA). Black circles show observed counts while white circles show counts that are classified as outliers.

The best model selected with total trout counts (J + SA + A) was *Tot* ~ *Stream* + $\overline{N}$ (Table 2). This result was obtained via both model selections starting from the general model *Tot* ~ *Stream* + $\overline{N}$ + *Method* or *Tot* ~ *Stream* + $\overline{N}$ + *Time* (not shown in Table 2). Similarly, the best model selected with adult + subadult counts (ASA) supports the same effects

($ASA \sim Stream + \overline{N}$), with both model selections starting from the general model $ASA \sim Stream + \overline{N} + Method$ or $ASA \sim Stream + \overline{N} + Time$ (Table 2). Additionally, model weights (w_i) and $\Delta AICc$ values show that the second (for Tot data) and third (for ASA data) ranked models, which specify method effects, have fundamentally no support. In both cases, no method or time effects were found, while the effects of location (Stream) and pool density (N) were well supported. Deviance explained by the best models were $DE_{Best(Tot)} = 0.3709$ and $DE_{Best(ASA)} = 0.1808$, respectively.

Table 2. Model selection results for location (Stream), pool density (N) and method effects on total (Tot), adult + subadult (A + SA), and juvenile (J) trout counts (no interactions among covariates allowed).

Data	Rank	Model Structure	AICc	ΔAICc	w_i
Tot (J + SA + AD)	1	Tot ~ 1 + Stream + N	453.5055	0	0.8763
	2	Tot ~ 1 + Stream + N + Method	457.4694	3.964	0.1208
	3	Tot ~ 1 + N	465.6514	12.146	0.002
	4	Tot ~ 1 + Stream	468.6184	15.1129	0.0005
	5	Tot ~ 1 + N + Method	469.0052	15.4997	0.0004
	6	Tot ~ 1 + Stream + Method	473.2471	19.7416	0
	7	Tot ~ 1	525.9969	72.4914	0
	8	Tot ~ 1 + Method	531.3003	77.7949	0
A + SA	1	ASA ~ 1 + Stream + N	351.7343	0	0.7429
	2	ASA ~ 1 + N	354.3142	2.5799	0.2045
	3	ASA ~ 1 + Stream + N + Method	357.7945	6.0602	0.0359
	4	ASA ~ 1 + N + Method	359.4606	7.7263	0.0156
	5	ASA ~ 1 + Stream	364.9632	13.2289	0.001
	6	ASA ~ 1	370.038	18.3038	0.0001
	7	ASA ~ 1 + Stream + Method	371.0232	19.2889	0
	8	ASA ~ 1 + Method	375.3934	23.6591	0
J	1	J ~ 1 + Stream + N + Method	296.397	0	0.3394
	2	J ~ 1 + Stream + N	296.9249	0.5279	0.2606
	3	J ~ 1 + Stream + Method	297.4489	1.0519	0.2006
	4	J ~ 1 + Stream	297.4607	1.0637	0.1994
	5	J ~ 1 + N + Method	347.4382	51.0412	0
	6	J ~ 1 + N	348.3466	51.9497	0
	7	J ~ 1	408.5618	112.1648	0
	8	J ~ 1 + Method	410.9305	114.5335	0

With juvenile counts (J), no time effects were found, while method effects were supported by the general model $J \sim Stream + \overline{N} + Method$, ranked first (Table 2, J data). The model's weight (w_i) and $AICc$ value were very similar to those of the second ranked model, which specifies no method effects. However, the LRT showed that the first two models were significantly different (p_{LRT} first vs. second = 0.0664), supporting weak method effects on juvenile counts. Similarly to the analysis of total and adults + subadults counts, there was again support for the effects of location (Stream) and pool density (N). Deviance explained by the best model was $DE_{Best\ J} = 0.5477$.

Parameter estimates from the best models showed a positive sign of the slope parameter (β) describing the relationship between counts and pool density: $\beta N_{Tot} = 0.1897 \pm 0.0471$ (SE); $\beta N_{ASA} = 0.2781 \pm 0.0712$; $\beta N_J = 0.0938 \pm 0.0579$ (taken from the best models, *Tot ~ 1 + Stream + N*, *ASA ~ 1 + Stream + N*, and *J ~ 1 + Stream + N + Method*, respectively). The Pearson correlation coefficients between total trout counts obtained via each method and relative pool density were, in decreasing order, $r_{VSA} = 0.7199$, $r_{SVS} = 0.6564$, $r_{UCS} = 0.5841$, and $r_{ELE} = 0.57059$. The best model selected with juvenile counts showed significantly higher values of juvenile counts obtained via the UCS ($p = 0.0345$). Finally, the best models selected with Tot, ASA, and J data all showed significant and well-supported location effects (*Stream*). In fact, there were substantial differences among average stream trout counts ($N_{Furittu(Tot)}$ = 4.625 ± 2.88(SD); $N_{Piras(Tot)}$ = 2.200 ± 2.74; $N_{Flumineddu(Tot)}$ = 0.750 ± 1.42 and $N_{Furittu(J)}$ = 3.150 ± 2.18(SD); $N_{Piras(J)}$ = 0.550 ± 1.30; $N_{Flumineddu(J)}$ = 0.125 ± 0.33).

3.2. How Are Methods Affected by Environmental Variables?

Univariate screening of environmental variables with total trout counts (Tot), supported the effect of *Depth*, *Area*, and *Length*, depending on method (Table 3, data = Tot).

Table 3. Univariate model selection of environmental variables affecting total (Tot), adult + subadult (A + SA) and juvenile (J) trout counts obtained via different sampling methods.

Data	Rank	Model Structure	K	AICc	ΔAICc	w_i
Tot (J + SA + A)	1	Tot ~ N + Stream + Method + Method:Depth	12	452.8375	0	0.5243
	2	Tot ~ N + Stream + Method + Method:Area	12	454.8241	1.9866	0.1942
	3	Tot ~ N + Stream + Method + Method:Length	12	455.6747	2.8372	0.1269
	4	Tot ~ N + Stream (Null A)	5	455.6778	2.8403	0.1267
	5	Tot ~ N + Stream + Method (Null B)	8	459.7729	6.9354	0.0164
	6	Tot ~ N + Stream + Method + Method:Refug	12	461.8712	9.0336	0.0057
	7	Tot ~ N + Stream + Method + Method:Turb	12	463.7103	10.8728	0.0023
	8	Tot ~ N + Stream + Method + Method:Shade	12	464.5323	11.6947	0.0015
	9	Tot ~ N + Stream + Method + Method:Temp	12	465.1169	12.2794	0.0011
	10	Tot ~ N + Stream + Method + Method:Width	12	465.6282	12.7907	0.0009
A + SA	1	ASA ~ N + Stream + Method + Method:Depth	12	348.2256	0	0.6509
	2	ASA ~ N + Stream + Method + Method:Area	12	349.9593	1.7336	0.2736
	3	ASA ~ N + Stream (Null A)	5	353.9072	5.6815	0.038
	4	ASA ~ N + Stream + Method + Method:Length	12	354.1168	5.8912	0.0342
	5	ASA ~ N + Stream + Method (Null B)	8	360.0974	11.8717	0.0017
	6	ASA ~ N + Stream + Method + Method:Shade	12	362.4088	14.1831	0.0005
	7	ASA ~ N + Stream + Method + Method:Width	12	362.6081	14.3825	0.0005
	8	ASA ~ N + Stream + Method + Method:Turb	12	364.2744	16.0488	0.0002
	9	ASA ~ N + Stream + Method + Method:Refug	12	364.3348	16.1091	0.0002
	10	ASA ~ N + Stream + Method + Method:Temp	12	365.3122	17.0866	0.0001
J	1	J ~ N + Stream + Method + Method:Shade	12	293.5737	0	0.6262
	2	J ~ N + Stream + Method + Method:Turb	12	296.2197	2.646	0.1668
	3	J ~ N + Stream + Method + Method:Width	12	297.4444	3.8707	0.0904
	4	J ~ N + Stream + Method (Null B)	8	298.6943	5.1206	0.0484
	5	J ~ N + Stream (Null A)	5	299.1034	5.5297	0.0394
	6	J ~ N + Stream + Method + Method:Area	12	300.5745	7.0009	0.0189
	7	J ~ N + Stream + Method + Method:Depth	12	302.982	9.4084	0.0057
	8	J ~ N + Stream + Method + Method:Temp	12	304.8883	11.3146	0.0022
	9	J ~ N + Stream + Method + Method:Length	12	305.6803	12.1066	0.0015
	10	J ~ N + Stream + Method + Method:Refug	12	307.6453	14.0716	0.0006

Specifically, the first model (Depth) has a Δ*AICc* value higher than two compared to *NullA* (fourth). The second (Area) and third (Length) models have Δ*AICc* values smaller than two but are significantly different from *NullA* according to the LRT (p_{LRT} *NullA* vs. second = 0.0159; p_{LRT} *NullA* vs. third = 0.0218). Therefore, multivariate model selection started form the general model *Tot ~ N + Stream + Method + Method:Depth + Method:Area + Method:Length* and supported again the effect of Depth, Area or Length on total counts (Table 4). Indeed, the first model and third models ranked in Table 4 (data = Tot) are significantly different from the second model, specifying only Depth effects (p_{LRT} first vs. second = 0.0338; p_{LRT} third vs. second = 0.0417), thus supporting the presence of either Area or Length effects. However, given the correlation between Length and Area, only the effect of Area was considered with Depth, as it was supported by the lower *AICc* value of the first model *Tot ~ N + Stream + Method + Method:Depth + Method:Area*. Deviance explained by this first multivariate model was $DE_{1st\ M(Tot)} = 0.5120$.

Table 4. Multivariate model selection of environmental variables affecting total (Tot), adult + subadult (A + SA) and juvenile (J) trout counts obtained via different sampling methods.

Data	Rank	Model Structure	K	AICc	ΔAICc	w_i
Tot (J + SA + A)	1	Tot ~ N + Stream + Method + Method:Depth + Method:Area	16	452.7768	0	0.275
	2	Tot ~ N + Stream + Method + Method:Depth	12	452.8375	0.0608	0.2667
	3	Tot ~ N + Stream + Method + Method:Depth + Method:Length	16	453.2807	0.5039	0.2137
	4	Tot ~ N + Stream + Method + Method:Area	12	454.8241	2.0473	0.0988
	5	Tot ~ N + Stream + Method + Method:Length	12	455.6747	2.8979	0.0646
	6	Tot ~ N + Stream	5	455.6778	2.9011	0.0645
	7	Tot ~ N + Stream + Method	8	459.7729	6.9962	0.0083
	8	Tot ~ N + Stream + Method + Method:Depth + Method:Area + Method:Length	20	460.273	7.4962	0.0065
	9	Tot ~ N + Stream + Method + Method:Area + Method:Length	16	462.6188	9.842	0.002
A + SA	1	ASA ~ N + Stream + Method + Method:Depth	12	348.2256	0	0.5054
	2	ASA ~ N + Stream + Method + Method:Depth + Method:Area	16	349.6221	1.3965	0.2514
	3	ASA ~ N + Stream + Method + Method:Area	12	349.9593	1.7336	0.2124
	4	ASA ~ N + Stream	5	353.9072	5.6815	0.0295
	5	ASA ~ N + Stream + Method	8	360.0974	11.8717	0.0013
J	1	J ~ N + Stream + Method + Method:Shade	12	293.5737	0	0.4416
	2	J ~ N + Stream + Method + Method:Shade + Method:Turb	16	294.6245	1.0509	0.2611
	3	J ~ N + Stream + Method + Method:Turb	12	296.2197	2.646	0.1176
	4	J ~ N + Stream + Method + Method:Width	12	297.4444	3.8707	0.0638
	5	J ~ N + Stream + Method	8	298.6943	5.1206	0.0341
	6	J ~ N + Stream + Method + Method:Turb + Method:Width	16	299.0925	5.5188	0.028
	7	J ~ N + Stream	5	299.1034	5.5297	0.0278
	8	J ~ N + Stream + Method + Method:Shade + Method:Width	16	300.0327	6.4591	0.0175
	9	J ~ N + Stream + Method + Method:Shade + Method:Turb + Method:Width	20	301.481	7.9073	0.0085

Univariate screening of environmental variables with adult and subadult trout counts supported the effect of Depth and Area, depending on method (Table 3, data = ASA). The first (Depth) and second (Area) models have Δ*AICc* values higher than two compared to *NullA* (third). Therefore, multivariate model selection started form the general model ASA ~ *N + Stream + Method + Method:Depth + Method:Area* and again supported the effect of Depth and Area. Indeed, the first (Depth), second (Depth + Area), and third (Area) models ranked in Table 4 (data = ASA) are significantly different according to the LRT (p_{LRT} first vs. second = 0.0619; p_{LRT} third vs. second = 0.0301), thus supporting the presence of both Depth and Area effects. Deviance explained of the model *ASA ~ N + Stream + Method + Method:Depth + Method:Area* was $DE_{2nd\ M(ASA)} = 0.4108$.

Univariate screening of environmental variables with juvenile trout counts supported the effect of pool shading (Shade), water turbulence (Turb), and pool width (Width), depending on method (Table 3, data = J). The first (Shade) and second (Turb) models have Δ*AICc* values higher than two compared to *NullB* (fourth). Confirming the results of the previous section, focusing on general method effects, the best null model here also specifies method effects. The third model (Width) has a Δ*AICc* value smaller than two but is significantly different from model *NullB* according to the LRT (p_{LRT} *NullB* vs. third = 0.0281), supporting Width effects. The sixth model, specifying Area effect is not significantly different form model *NullB* according to the LRT (p_{LRT} *NullB* vs. sixth = 0.1016), i.e., Area effects are not supported. Therefore, multivariate model selection started form the general model J ~ N + *Stream* + *Method* + *Method:Shade* + *Method:Turb* + *Method:Width* and supported the effect of Shade and Turb. Indeed, the first (Shade) and second (Shade + Turb) models ranked in Table 4 (data = J) have $\Delta AICc$ values lower than two but are significantly different according to the LRT (p_{LRT} first vs. second = 0.0537), thus supporting the presence of both Shade and Turb effects. Deviance explained of model J ~ N + *Stream* + *Method* + *Method:Shade* + *Method:Turb* is $DE_{2nd\ M(J)}$ = 0.6530.

Parameter estimates taken from the best multivariate models selected for Tot, ASA, and J data (Table 5) show that counts provided by each method are differently affected by environmental variables, depending on age classes. Specifically, adults and subadults counts provided by ELE are negatively affected by the maximum pool depth (Table 5, Best Tot and Best ASA). Adult and subadult counts provided by VSA are positively affected by the maximum pool depth (Best ASA). A positive effect of pool area is shown on VSA and SVS adult and subadult counts (Best Tot and Best ASA). Instead, juvenile counts provided by the UCS are positively affected by pool shade and negatively affected by water turbidity (Best J). Additionally, juvenile counts provided by VSA appears also positively affected by Shade.

Table 5. Parameter estimates (standard errors) taken form the best models selected on total (Tot), adult + subadult (A + SA) and juvenile (J) trout counts (significance: + $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Significant effects of environmental variables in bold.

Parameter	**Best Model Parameter Estimates (Standard Error)^{Significance}**		
	Best Tot	**Best ASA**	**Best J**
Intercept	0.088 (0.510)	0.210 (0.693)	−4.213 (1.111) ***
N	0.227 (0.046) ***	0.309 (0.064) ***	0.088 (0.066)
Streamfurit	1.116 (0.304) ***	−0.030 (0.413)	3.486 (0.537) ***
Streampiras	0.697 (0.282) *	0.592 (0.356) +	1.944 (0.556) ***
MethodVSA	−1.956 (0.659) **	−3.793 (0.974) ***	−0.169 (1.401)
MethodSVS	−1.808 (0.654) **	−3.329 (0.958) ***	0.548 (1.308)
MethodUCS	0.215 (0.608)	−1.099 (0.863)	0.912 (1.313)
MethodELE × Depth	**−1.232 (0.411)** **	**−1.588 (0.592)** **	
MethodVSA × Depth	0.538 (0.357)	**1.144 (0.490)** *	
MethodSVS × Depth	0.302 (0.352)	0.653 (0.494)	
MethodUCS × Depth	−0.486 (0.369)	−0.319 (0.515)	
MethodELE × Area	**0.008 (0.004)** *	0.007 (0.005)	
MethodVSA × Area	0.006 (0.004)	**0.013 (0.006)** *	
MethodSVS × Area	**0.010 (0.004)** *	**0.015 (0.006)** *	
MethodUCS × Area	−0.003 (0.004)	0.002 (0.006)	
MethodELE × Shade			0.015 (0.010)
MethodVSA × Shade			**0.018 (0.010)** +
MethodSVS × Shade			0.007 (0.009)
MethodUCS × Shade			**0.026 (0.010)** **
MethodELE × Turb			0.047 (0.514)
MethodVSA × Turb			−0.007 (0.514)
MethodSVS × Turb			0.346 (0.472)
MethodUCS × Turb			**−1.475 (0.564)** **

Focusing on the more significant negative effects, Figure 5 shows the magnitude of the effect of increasing pool depth on ELE adult and subadult counts and increasing turbidity on UCS juvenile counts. Model predictions were obtained using parameters taken from best models selected with ASA and J counts.

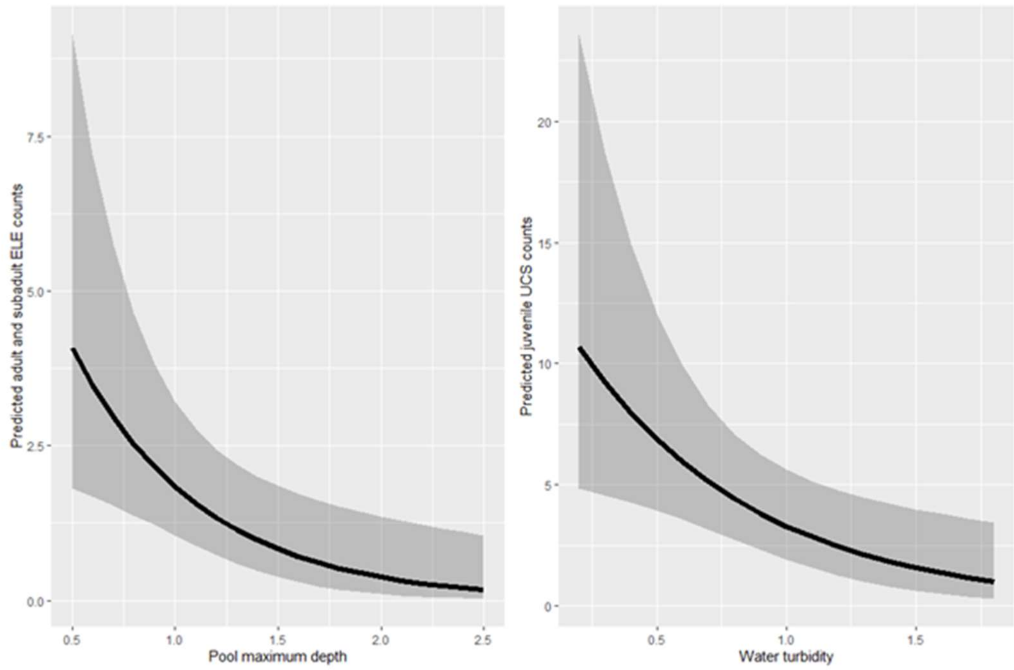

Figure 5. Effect of increasing pool depth on adult and subadult counts provided by backpack electrofishing (ELE; **left**) and effect of increasing water turbidity on juvenile counts provided by underwater camera surveys (UCSs; **right**). Counts and 95% confidence intervals (shaded area) were predicted from best models selected on adult + subadult counts and juvenile trout counts, respectively.

4. Discussion

The results show that trout counts obtained by sampling from the same pools with removal with electrofishing (ELE), underwater camera surveys (UCSs), streamside visual surveys (SVSs), and visual surveys with angling (SVA) are well correlated, providing a coherent description of relative pool abundance across the methods. However, the results show that the counts obtained via the different sampling methods are affected by different environmental variables measured at the pool level, depending on the age classes of the trout. Indeed, the methods' effects emerged clearly when interactions between the method and environmental variables were considered, while a general method effect on counts was weakly shown only with juvenile counts. From a practical perspective, it is important to note that the effectiveness of the applied methods varied differently depending on environmental features that cannot be controlled, such as the pool features and age structure of trout populations, thus affecting the reliability of counts in heterogeneous environments.

The shown negative effect of the maximum pool depth on counts provided by backpack electrofishing (Figure 5) appears important. Indeed, despite the well-known limitations of backpack electrofishing in deep pools, this method is considered a standard method for monitoring trout populations for conservation and management purposes [13]. Apart from ethic and conservation concerns [9–11], backpack electrofishing does not emerge here as a superior method, showing the weakest correlation with estimated pool density. Therefore, the application of electrofishing should be based more on practical reasons, such as the speed of sampling in selected stretches of homogeneous streams and a need to collect adequate numbers of genetic samples and detailed biometric data (e.g., length, weight, and scales to assess age). When seeking information about distribution and relative abundance over longer stretches of heterogeneous streams, other methods such as the UCSs, VSA, and SVSs may be more practical, similarly reliable (i.e., affected by environmental features but proportional to abundance to some extent), and can involve stakeholders and non-professional researchers [5–7].

The absence of meaningful negative effects of environmental variables on counts emerged with SVS and VSA methods also suggests their utility in a larger set of environmental conditions, which is a good attribute of methods selected for large-scale monitoring. On the other hand, the weak positive effects observed for the SVS and VSA (Depth, Area, and Shade) are unlikely to be due to increased detectability of trout and could be simply related to higher abundance in larger and deeper pools [45], with larger pools also representing thermal refuges where fish aggregate during warmed periods [32].

The negative effect of water turbidity on counts provided by the UCS with juvenile counts (Figure 5) is well known [9,46,47] and was expected considering the smaller size of juveniles and the reduced visibility of turbid waters. Although this effect could be controlled by monitoring during clear water conditions, monitoring for conservation often requires covering large spatial scales, limiting resources available for sampling only with optimal conditions.

In general, it is important to address biases in the counts provided by different methods in given conditions with statistical modeling. To do that, information about environmental features thought to affect pool abundance and the sampling process should be gathered. Indeed, monitoring programs need to be informed by scientific hypotheses about factors affecting variation in detectability [48] and population dynamics through space and time, so that some understanding about determinants of population trends can be gained [1].

5. Conclusions

Our results suggest that, in small Mediterranean streams, different sampling methods can provide similar information about the relative abundance of trout populations and are in this sense equivalent. However, sampling methods selected for monitoring are seldom perfectly standardizable in heterogeneous environments, because they can be significantly affected by uncontrollable environmental variables. Therefore, selecting monitoring methods for conservation can be based on several criteria, among which reliability (proportionality with what is measured), applicability on large spatial scales and wide sampling conditions, the type of information needed, the social context and resources available (e.g., interest of anglers or other volunteers in monitoring), and ethics and conservation issues (unwillingness to risk mortality or disturb endangered populations). Instead of searching for the best standard method, monitoring programs could be based on a set of locally tested methods, with their performance adaptively evaluated [1,49] so that variation in method effectiveness within heterogeneous contexts can be addressed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16080442/s1, The data used in the analysis (DataConfr2.txt) and R script (R Script.txt) to repeat the analysis are provided.

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