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Fish catchability and comparison of four electrofishing crews in Mediterranean streams

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ABSTRACT

The knowledge of capture efficiency and side effects of electrofishing is essential for research and monitoring of stream fish populations. Differences amongst electrofishing crews have hardly been investigated and are particularly important given the on-going implementation of the Water Framework Directive and wide-ranging exchange of data worldwide. We aimed to assess fish catchability in Mediterranean streams and to compare four electrofishing crews (with minor differences in gears used) and their short-term effects on fish populations. In eight different sites, we compared two adjacent stations, one sampled with conventional single-pass catch-effort data and the other closed with block nets and with four-pass removal estimates. We used a Williams' cross-over design to estimate the independent effects of repeated sampling in four consecutive days, site and crew and also to assess a potential carry-over effect. We modelled capture probability and estimated population size using program MARK and an information-theoretic framework. Our results show that electrofishing was generally efficient in these reaches, with 50–100% of the species and of 40–60% of the individuals captured in a single pass. The CPUE was significantly higher at sites blocked with nets than at open sites, but observed richness was not significantly different. Capture probability was generally not constant along removal passes and increased with fish size. Observed fish richness and species composition did not depend on electrofishing crew and fishing day and there was no significant carryover effect. There were, however, significant differences in single-pass CPUE estimates amongst electrofishing crews, after accounting for other sources of variation. There was also a significant carry-over effect, surprisingly with increasing fish captured after fishing by specific crews. Overall, our results suggest that although capture probability depends heavily on a number of factors (such as species, size, and sampling site) and needs careful consideration, the effect of electrofishing crew is negligible for assessment of species richness and composition but considerable for fish abundance.

1. Introduction

Electrofishing is one of the most widely used sampling techniques for research and monitoring of freshwater fish populations, particularly in streams (Cowx, 1990). Electrofishing capture efficiency depends on a number of factors such as equipment, environmental features and fish attributes (Bohlin et al., 1989; Reynolds, 1993; Lobón-Cerviá et al., 1994; Peterson et al., 2004) and its understanding is thus essential for estimating and monitoring fish abundance and richness. For instance, electrofishing efficiency generally peaks at intermediate water conductivities and increases with fish size (Bohlin et al., 1989; Reynolds, 1993; Dolan and Miranda, 2003). Electrofishing can also be harmful to fish. Although mortality and population side effects of electrofishing have been poorly documented, spine injuries and haemorrhages are very frequent and not detected externally (Kocovsky et al., 1997; Snyder, 2003). Despite the frequency of injuries and that repeated electrofishing has been shown to decrease growth rates in some species (Gatz and Linder, 2008), it seems to have negligible population effects (Barrett and Grossman, 1988; Kocovsky et al., 1997). These side effects of electrofishing, however, are poorly known and need further assessment (Kocovsky et al., 1997; Snyder, 2003).

Fish sampling of European streams has recently been stimulated by the urgent need to implement the Water Framework Directive (WFD), which was approved in year 2000 and urges the

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achievement of good "ecological status" of European aquatic ecosystems before 2015. Many different crews equipped with different electrofishing gears have been sampling stream fish all over Europe and elsewhere to obtain data for the development and testing of biotic indices (e.g., Fame Consortium, 2004; Pont et al., 2006; Ferreira et al., 2007; Benejam et al., 2010), which according to the WFD for fish should be based on abundance, species composition and population structure (size or age). The European Committee for Standardisation (CEN) standards EN 14962 and EN 14011 specify the methods that should be used for sampling fish according to the WFD, which for wadable streams generally involve a single pass electrofishing, with the optional use of block or stop nets. Currently, many of the biotic indices developed with this kind of catch-effort data are being intercalibrated, i.e., analysed for their relationship and possible comparison. These intercalibration exercises and many other scientific fish studies generally assume that catchability does not vary amongst electrofishing crews and that capture efficiency of different equipments will not confound the relationship of these fish metrics with measures of anthropogenic perturbation and other abiotic factors. The European standard EN 14011 specifies that "when changing equipment, comparative results with old and new equipment shall be generated, to make it possible to compare new and old data". Nevertheless, very few studies have assessed this variability of capture efficiency amongst different electrofishing crews (e.g., Hardin and Connor, 1992) and equipments, particularly in a set of streams sampled simultaneously.

The objectives of this paper are: (i) to compare capture efficiencies and standard fish metrics obtained by four independent fishing crews in a set of sampling sites in Mediterranean streams; and (ii) to assess if sampling repeatedly in four consecutive days induced changes in fish abundance or assemblage structure (e.g., due to short-term mortality or avoidance). We aimed to compare the abundance, observed fish richness and simple measures of species composition derived from the catch-effort data of single pass electrofishing, which is a routine method used throughout Europe and elsewhere, with four-pass removal estimates (using block nets) to estimate the capture efficiency of the typical protocols applied in Mediterranean streams. We also aimed to assess the differences observed in these metrics by four independent crews, while controlling for site and repeated visits. The four crews used shore-based electrofishing equipments, of two different brands, representative of the most widely used methods throughout Europe and elsewhere. Rather than only testing specific gear differences, we were interested in assessing the overall differences in capture efficiency that can be expected amongst crews in general and secondarily in examining whether fishing repeated in the same reaches during a few days revealed any apparent short-term mortality.

2. Materials and methods

2.1. Study area

The experiment was conducted during June 2008 at eight sampling sites of two tributaries (Rigard and Llémena) of the Ter River (Catalonia, NE Spain) (Fig. 1). The Ter River rises in the Pyrenees Mountains and has a drainage area of 2955 km^2, a mean annual water yield of 845 million m^3 $year^{-1}$, and a mean discharge of ca. 10 m^3 s^{-1} (Benejam et al., 2010; Boix et al., 2010). The Rigard is a headwater tributary of the Ter River, rising at 2200 m a.s.l. and with a snow-fed regime (Table 1). The four sampling sites in Rigard stream had 4.5 m of average wetted width, with abundant riffles and brown trout (*Salmo trutta*) as the only fish species present. Llémena is a typical Mediterranean stream that rises in littoral mountains at 800 m a.s.l., with a fish assemblage mostly consisting of three native species: Mediterranean barbel (*Barbus meridionalis*), chub (*Squalius laietanus*) and European eel (*Anguilla anguilla*). The four sampling sites in Llémena stream had 7.8 m of average wetted width and no riffles present. In both streams the riparian habitat was well preserved and the predominant land use was forest, with some agricultural and urbanised areas. We consider that the two streams are representative respectively of: (i) headwater streams, widely present throughout Europe, where brown trout is often the only species present (Mediterranean zone) or the most abundant one; and (ii) middle courses of Mediterranean streams, with often less than five species present, mostly endemic cyprinids (Ferreira et al., 2007; Benejam et al., 2008, 2010).

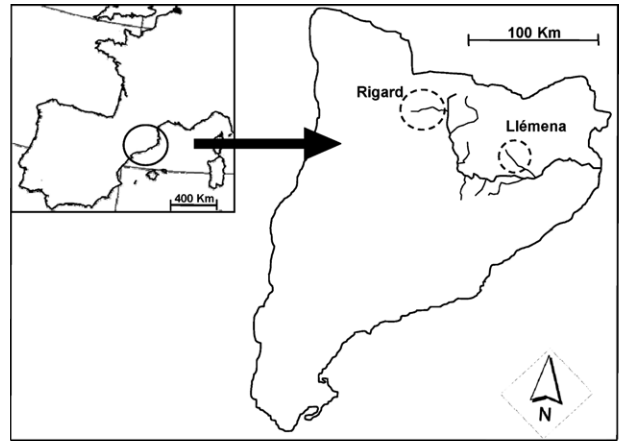

Fig. 1. The two tributaries of Ter river basin studied (Rigard and Llémena streams).

2.2. Experimental design

Similarly to Simonson and Lyons (1995), each site was divided into two contiguous stations, each being 100 m in length, and one was assigned to a CPUE station and the other to a removal estimate. The aim of this procedure was to provide catchability rates independently of the CPUE station and to assess the effect of using block nets by comparing the first passes at the two stations. Sites were chosen to have relatively uniform habitat conditions so that paired stations were as similar as possible: unpublished data on habitat assessment (Methods in Benejam et al., 2008, 2010) suggest negligible differences amongst stations within sites. In each stream, the removal station was randomly assigned upstream of the CPUE station for two of the sites and downstream of the CPUE station for the other two.

The first fishing day, the crew assigned to the site conducted a four-pass electrofishing in the removal station (blocked with 4-mm mesh nets) and a single pass (without nets) in the CPUE station, starting with the downstream station. The following days the other crew performed a single pass electrofishing in the CPUE station. All fish collected were kept on shore in large buckets with battery-powered aerators and identified, counted, and measured (fork length in mm) separately for each pass, and then returned to the same reach where they were captured.

We also aimed to compare catchability amongst the four fishing crews and to assess short-time depletion effects of the passes. Therefore, we used a Williams' cross-over design (with crew as experimental treatment and site and day as blocking factors), and randomly assigned the four crews amongst sites and days, so that: (i) each of the four crews were once and only once in each order (fishing day) and site, (ii) all sequences of crew were equally distributed (see Table 4.4 in Jones and Kenward, 2003). Cross-over designs are distinguished by each experimental subject (site in our case) receiving a sequence of experimental treatments (crew in our

Table 1
Selected limnological features of the eight sampling sites from the Ter River tributaries during the intercalibration experiment.

Stream	Stretch number	Altitude (m a.s.l.)	UTM coordinates (31 T)	Mean wetted width (m)	Temperature (°C)	Conductivity (μS cm^{-1})	Oxygen concentration (mg L^{-1})	Phosphate concentration (mg L^{-1})
Llémena	1	250	470662/4654324	4.73	17.30	605	8.68	0.20
Llémena	2	150	475837/4651834	6.54	17.35	499	13.05	0.10
Llémena	3	110	478105/4650149	12.55	18.70	512	10.28	0.10
Llémena	4	100	478895/4649020	7.48	22.67	514	13.71	0.50
Rigard	5	1480	418103/4685990	2.07	11.20	259	10.50	0.00
Rigard	6	1230	421850/4686433	4.39	10.40	193	10.30	0.05
Rigard	7	1120	424060/4686220	4.91	10.47	157	13.55	0.00
Rigard	8	1060	425750/4685184	6.49	12.02	177	14.37	0.00

study) instead of a single treatment (Jones and Kenward, 2003). Williams' designs are a special cross-over design, which are similar to a Latin square design (i.e. every treatment appears once and only once in each the two block units, order and site in our case), with the additional restriction that every treatment follows every other treatment the same number of times (Williams, 1950; Jones and Kenward, 2003). Williams' designs allowed in our case to: (i) distinguish the effects of crew, site, and order; and (ii) detect if there is an effect of a specific crew which spreads the following days (carry-over effect). A carry-over effect of a specific crew might be expected for instance if one of the electrofishing equipments produced a stronger mortality in fishes and is tested in the analysis by coding a carry-over factor in a specific way (Jones and Kenward, 2003). We also checked analyses using a design with river factor (Llémena and Rigard) and a site factor nested within river but the qualitative conclusions were identical and we report separate analyses by site for simplicity. Analyses removing the nonsignificant carryover effects also provided the same conclusions.

All sites were shallow enough (depth < 1.5 m) to be easily sampled by wading. Fish were sampled by electrofishing the whole wetted width of the 100-m stations (fully rectified triphasic DC, generator-based equipments) in an upstream direction, following the CEN standards EN 14962 and EN 14011. Two of the crews (A and D in Table 2) used two Electracatch WFC4 electrofishing gears (Electracatch International, Wolverhampton, UK) with anode rings of 41 cm of diameter, whereas the other two used two Hans Grassl ELT62II GI Honda GCV160 equipments (Hans Grassl GmbH, Schönau am Königssee, Germany) with anode rings of 30 (crew B) and 32 cm of diameter. Each crew adapted their fishing method to the reach features according to their own experience: voltage used ranged 80–600 V and current 0.8–1.8 A. All crews consisted of one person operating the anode and two people netting. Although there is no formal training or certification in electrofishing in Spain, these twelve people all had many years of experience with these and other equipments, so we judge that the differences observed are representative of regular research or monitoring crews. One of the crews had an additional person for shore support. All fishes stunned were collected with nets, placed at the shore in buckets (one of the crews with aerators) and later identified to species, counted, measured (fork length in mm; total length for eel) and returned to the same station. One of the crews used MS-222 anaesthetic for measuring the fish.

2.3. Statistical analyses

We used generalised linear models (GLMs) to test for all these effects, with Poisson errors and log link functions for species richness (observed number of species) and abundances, and binomial errors and logit link functions for proportional abundances. These statistical analyses were performed with SPSS 15.

To obtain estimations of the true number of species richness present in the area sampled, we computed the second-order jackknife richness estimator (hereafter Jack2), using the software EstimateS (Colwell, 2010). Jack2 is a nonparametric resampling measure which is amongst the most recommended measures (Palmer, 1991; Brose et al., 2003). Jack2 was computed in two different ways: (i) using the data of the removal station (hereafter "Removal Jack2"); and (ii) using the data of each crew at the CPUE station ("CPUE Jack2").

We used program MARK (White and Burnham, 1999; available at http://warnercnr.colostate.edu/~gwhite/mark/mark.htm; last accessed October 2011) to estimate population sizes and capture probability ($\hat{p}$) for each site and species using the four-pass data of removal stations (i.e., recapture parameter c set at zero). We evaluated four different multinomial models ("Huggins Closed Capture" in MARK) of removal estimates: P, model with constant catchability between different electrofishing passes; P1, model with non-constant catchability between electrofishing passes; P1L, model with non-constant catchability between passes and a linear effect of fish length; and P1L2, model with non-constant catchability between passes and a quadratic function of fish length (Cooch and White, 2010). Models in program MARK are estimated by maximum likelihood and compared with an information theoretical framework (Burnham and Anderson, 2002), using the corrected Akaike's Information Criterion (AICc), which combines fit and parsimony (number of parameters) of models, with the best fitting model having the lowest AICc. The relative plausibility of each candidate model was assessed by calculating Akaike weights (w_i), which range from 0 to 1 and are interpreted as the probability that the model is the best amongst those evaluated given the data.

3. Results

3.1. Capture efficiencies

A single electrofishing pass was in general efficient to detect species richness, although observed richness ranged 53–100% of the richness estimated species with Jack2 (Table 2). In the headwater stream (Rigard), the single species present was always detected (no fish was ever captured in site no. 6), whereas in the downstream tributary the species detected in a single pass were always more than 50% of the species present and often close 100%. "Removal Jack2" was always equal or lower than "CPUE Jack2" because it does not include variability in capture probability amongst crews and days, explaining the apparent detectabilities larger than 100%.

A single electrofishing pass was also efficient to capture a high percentage of the population, on average 57.1% of the brown trout estimated population size in Rigard stream and 44.9% of Mediterranean barbel in Llémena stream (Table 3). Electrofishing was thus more efficient in the headwater stream and in sites with lower fish abundance. The models estimated with program MARK (Tables 4 and 5) suggested a number of points, namely: (i) only 3 of the 12 cases modelled indicated that a model with constant capture probability was the most adequate; (ii) the rest of models suggested variable catchabilities, with higher capture probabilities in the first pass; and (iii) eight of the models with highest

Table 2
Average number of species captured in a single electrofishing pass. "Removal Jack2" is the second-order jackknife richness estimator based on the four-passes at the removal station, and "CPUE Jack2" is the same estimator based on the different fishing days at the CPUE station.

Crew	Stream	Sampling site	Average number of species captured in a single pass	Removal Jack2	Detectability (%)	CPUE Jack2	Detectability (%)
A	Llémena	1	2.2	3.9	57	4.2	53
B	Llémena	2	2.7	3.9	70	4.9	56
C	Llémena	3	4.0	3.0	133	4.7	86
D	Llémena	4	3.5	3.0	117	3.7	96
A	Rigard	5	1.0	1.0	100	1.0	100
B	Rigard	6	0.0	–	0	–	–
C	Rigard	7	1.0	1.0	100	1.0	100
D	Rigard	8	1.0	1.0	100	1.0	100

Table 3
Number of fish captured (average of a single electrofishing pass by the different crews) and estimated abundance (based on the four-pass model with the highest AIC weight) for Mediterranean barbel in Llémena stream and brown trout in Rigard stream.

Fish species	Electrofishing crew	Sampling site	Number of fish captured	Estimated abundance
Barbus meridionalis	A	1	52.25	267.4
Barbus meridionalis	B	2	79.25	256.8
Barbus meridionalis	C	3	84.75	221.9
Barbus meridionalis	D	4	131.25	143.9
Salmo trutta	A	5	3.25	5.0
Salmo trutta	B	6	0.00	32.7
Salmo trutta	C	7	20.50	28.5
Salmo trutta	D	8	10.00	29.0

Akaike weights (w_i) included a linear or quadratic effect of fish length. Mean estimated capture probabilities ranged from 34–44% in brown trout and 36–52% in *B. meridionalis* to 50–62% in *Barbus graellsii*, being more variable for eel (18–74%) (Tables 4 and 5). However, capture probability clearly increased, rather nonlinearly, with fish size, varying for instance from ca. 60 to over 80% in the size range observed for *B. meridionalis* (Fig. 2).

The comparison of the first pass in the removal station with the catches in the CPUE by the same crew (Design 1 in Table 6) indicated significant effects of using block nets on CPUEs and species composition (% abundance of the most common fish) but not on observed species richness. Although overall effects of block nets were often nonsignificant, the significant interaction indicated an effect of block nets, dependent on site. CPUEs were higher at sites blocked with nets than in open sites, with estimated total abundances of 115 vs. 80 fish per reach in Llémena stream and 10 vs. 11 in Rigard stream (i.e. ca. 40% and 10% more with block nets). The catches at the CPUE station were, however, related to the removal estimates at the same site, with Pearson correlations

Table 4
Modelling of the capture probability ($\hat{p}$) of *Barbus meridionalis* at the four sites of Llémena stream. The four models compared are: P, model with constant catchability between different electrofishing passes; P1, model with non-constant catchability between electrofishing passes; P1L, model with non-constant catchability between passes and a linear effect of fish length; P1L2, model with non-constant catchability between passes and a quadratic function of fish length. The top-ranked models (highest AICc weights, w_i) are highlighted in bold.

Sampling site	Model	AICc	ΔAICc	w_i	$\hat{p}$
1	P	585.15	3.98	0.117	0.427
	P1	588.67	7.50	0.020	0.426
	P1L	**581.17**	**0.00**	**0.857**	**0.546**
	P1L2	591.15	9.97	0.006	0.433
2	**P**	**551.62**	**0.00**	**0.645**	**0.501**
	P1	552.89	1.26	0.343	0.519
	P1L	559.83	8.20	0.011	0.010
	P1L2	564.98	13.36	0.001	0.503
3	P	476.97	2.03	0.157	0.362
	P1	**474.95**	**0.00**	**0.433**	**0.388**
	P1L	477.67	2.72	0.111	0.002
	P1L2	475.69	0.74	0.299	0.366
4	P	332.05	10.31	0.005	0.583
	P1	327.47	5.74	0.049	0.589
	P1L	326.63	4.90	0.075	0.559
	P1L2	**317.94**	**0.00**	**0.978**	**0.797**

Table 5
Modelling of the capture probability ($\hat{p}$) of *Barbus graellsii* and European eel (*Anguilla anguilla*) in Llémena stream and brown trout (*Salmo trutta*) in Rigard stream. See Table 4 for explanation of the models and further details.

Species	Sampling site	Model	AICc	ΔAICc	w_i	$\hat{p}$
B. graellsii	3	P	60.79	14.97	0.001	0.539
		P1	64.15	18.33	0.000	0.500
		P1L	61.10	15.27	0.000	0.524
		P1L2	**45.82**	**0.00**	**0.999**	**0.573**
B. graellsii	4	P	25.48	6.61	0.035	0.660
		P1	28.83	9.96	0.007	0.615
		P1L	**18.87**	**0.00**	**0.953**	**0.035**
		P1L2	29.15	10.28	0.006	0.000
A. anguilla	1	P	7.80	135.8	0.000	0.343
		P1	14.77	142.8	0.000	0.484
		P1L	**−128.0**	**0.00**	**1.000**	**1.000**
		P1L2	−38.40	89.60	0.000	1.000
A. anguilla	2	**P**	**6.87**	**0.00**	**0.401**	**0.740**
		P1	9.15	2.28	0.128	0.667
		P1L	6.98	0.11	0.379	0.000
		P1L2	9.81	2.94	0.092	0.000
A. anguilla	3	P	23.91	7.55	0.022	0.183
		P1	27.99	11.63	0.003	0.313
		P1L	26.33	9.97	0.007	0.434
		P1L2	**16.36**	**0.00**	**0.968**	**0.374**
A. anguilla	4	P	17.18	4.87	0.079	0.637
		P1	21.26	8.95	0.010	0.625
		P1L	21.38	9.07	0.010	0.670
		P1L2	**12.31**	**0.00**	**0.901**	**1.000**
S. trutta	6	P	76.27	8.75	0.012	0.403
		P1	79.44	11.92	0.003	0.441
		P1L	**67.52**	**0.00**	**0.976**	**1.000**
		P1L2	76.94	9.42	0.009	1.000
S. trutta	7	**P**	**66.24**	**0.00**	**0.799**	**0.343**
		P1	70.25	4.02	0.107	0.390
		P1L	70.59	4.36	0.090	0.654
		P1L2	77.57	11.33	0.003	0.654

Table 6
Statistical analysis of the main fish metrics: total CPUE in the two streams and species richness and % abundance of the most common fish (*Barbus meridionalis*) in the Llémena stream. Design 1 compares electrofishing catches in the first pass with or without block nets, whereas Design 2 corresponds to Williams' crossover design and tests for differences amongst crew, fishing day (order), site and a carry-over effect. Shown are the *P* values of Wald's chi-square tests of the corresponding generalised linear model, with binomial errors for % dominance and Poisson errors otherwise (see Section 2 for further details).

	Rigard stream	Llémena stream		
Source of variation	Total CPUE	Total CPUE	Species richness	% dominance
Design 1				
Block nets	0.71	***	0.49	0.86
Site	0.002	0.22	0.98	***
Block nets × site	0.009	0.012	0.98	0.025
Design 2				
Order	0.97	***	0.52	***
Crew	0.17	***	0.98	0.42
Carry-over	0.73	***	0.87	0.54
Site	***	***	0.42	***

*** $P < 0.0005$.

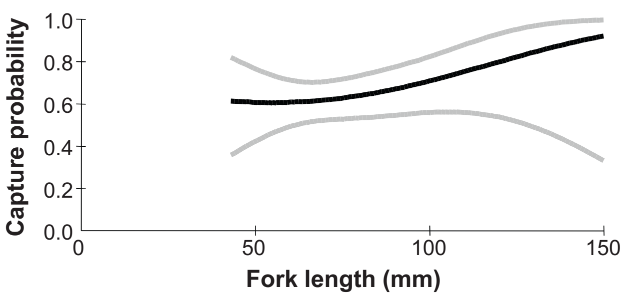

Fig. 2. Relationship of estimated capture probability (with 95% confidence intervals) of *B. meridionalis* with fish length at the first electrofishing pass (Llémena stream, site number 4), using the model with non-constant catchability between passes and a quadratic function (polynomial regression) of fish length (see Table 5).

for the species captured in at least 3 sites, ranging from 0.62 for *B. meridionalis* to 0.86 for trout and 0.92 for eel.

3.2. Effects of crew and repeated sampling

The results of Williams' design (Design 2 in Table 6) showed that despite the strong differences amongst sites (highly significant site effects) there were no significant differences amongst crews except for the observed CPUE in Llémena stream (crews A and B had higher catch rates, Fig. 4). The observed species richness always coincided for the four crews in Rigard stream (0 or 1 depending on site) and varied slightly in the other stream (Fig. 3) but did not show any significant source of variation (Table 6). Independently of other factors, there was a significant effect of order (electrofishing day) on CPUE and species composition in Llémena stream, because catch rates tended to increase after the first visit (Fig. 4) and the dominant species (*B. meridionalis*) had higher catch rates in the first day, whereas other species such as *B. graellsii* and eel had higher catch rates in the following days (Fig. 5). There was a significant carry-over effect only for CPUE in Llémena stream, because after accounting for other factors catch rates were slightly higher in the day after the visit of the most efficient teams A and B (Fig. 4).

4. Discussion

4.1. Capture probability

Our results agree well with previous literature. First, similarly to Simonson and Lyons (1995) we observed increased catch rates (10–40%) in a single pass with the use of block nets (compared to a single-pass without block nets) but no clear effects on observed

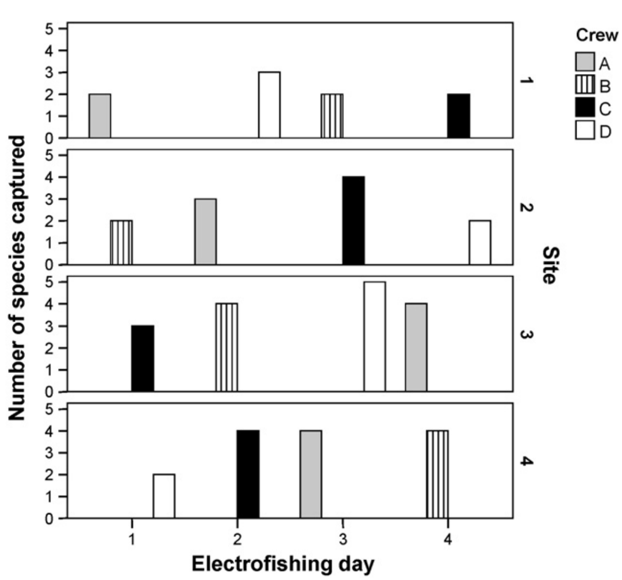

Fig. 3. Number of species observed in the four sampling sites of Llémena stream by each of the electrofishing crews. The visit order (electrofishing day) was interspersed following a Williams' design, see Section 2.

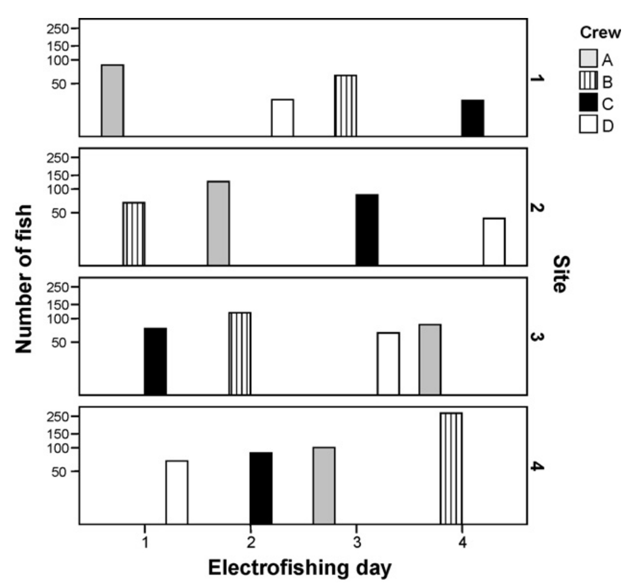

Fig. 4. Number of Mediterranean barbel (*Barbus meridionalis*) captured in the four sampling sites (reach length of 100 m) of Llémena stream by each of the electrofishing crews.

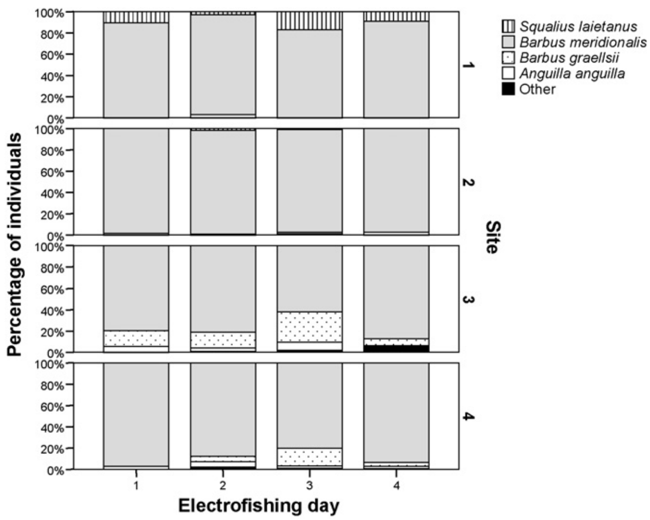

Fig. 5. Percentage of fish individuals by species captured in the four sampling sites of Llémena stream by electrofishing day.

richness and relative abundance. Although a single pass without nets may sometimes be appropriate to estimate abundance (given the correlation with removal estimates) and richness, block nets are necessary for removal estimates (Peterson et al., 2005) and differences amongst reaches may introduce biases in abundance comparisons (Pusey et al., 1998; Rosenberger and Dunham, 2005). The use of block nets is probably not cost effective in standard surveys (Kruse et al., 1998; van Liefferingere et al., 2010) and impossible to apply in large rivers or during high flows, making it difficult to correct for capture efficiency along rivers.

Second, although overall capture efficiency was ca. 40% for brown trout and 35–60% for cyprinids we observed non-constant catchability with multiple passes, with higher capture probability in the first pass and clear, nonlinear increases of capture probability with fish size. These results, often implying underestimation of population sizes, have also been found in a number of previous studies (Bohlin and Cowx, 1990; Libosvárský, 1990; Riley and Fausch, 1992; Peterson et al., 2004; Rosenberger and Dunham, 2005; Dauwalter and Fisher, 2007) and have many methodological implications. When multiple passes are performed, more than two passes seem advisable to allow estimating models with non-constant catchability and to avoid introducing biases in population size estimates. Models with non-constant catchability should preferably be used. Modern programs such as MARK (White and Burnham, 1999), although complex to use, provide a valuable framework to model capture probabilities, with multiple advantages over less comprehensive software. MARK advantages include allowance of: non-constant catchability amongst passes, effects of covariates such as fish size or environmental features that affect catchability, and an information-theoretic framework, which does not rely on hypothesis testing for instance for testing if capture probabilities are constant. Finally, other methods such as mark-recapture techniques are desirable, if possible, to check and reinforce removal estimates (Peterson et al., 2004).

Similarly to Sály et al. (2009), although the observed species richness in a single pass generally underestimated the species present in a specific stream reach, richness and % composition metrics (such as % of the most dominant species used in this paper) displayed much less methodological variation and are much easier to estimate precisely than CPUE and population size. However, CPUEs probably have a unique indicator value in fish bioassessments, particularly in headwater streams where richness and species composition vary less, reinforcing the need of understanding factors mediating capture efficiency and accounting for them in statistical analyses and models. Overall, our results suggest that the electrofishing methods used, which complied with CEN standards and are probably the most widely used technique for sampling European stream fish, are adequate to estimate abundance, species composition and richness in headwaters and middle courses of the Mediterranean region.

4.2. Differences amongst electrofishing crews

Our study is probably the first to compare electrofishing crews with an experimental design such as Williams' cross-over design and to assess the effects of multiple passes in four consecutive days. The order (electrofishing day) and carry-over effects found were rather surprising because they suggested higher catch rates after the first day and following specific crews, instead of the short-term mortality or avoidance that we hypothesised for such a sampling intensity. We handled, measured and returned fish to the station of capture and handling has been suggested to have a greater impact on cumulative mortality than repeated DC electrofishing (Snyder, 2003). Various non-mutually exclusive mechanisms might explain these higher catch rates after the first day and the differences in crew efficiency: reduced ability of fish to escape electrofishing with repeated shocking, change in microhabitat or behaviour of fish, and removal of fish from refuges in previous passes. Indeed, Mesa and Schreck (1989) observed that cutthroat trout *Oncorhynchus clarki* reduced their activity during several hours after being electrofished. Nevertheless, most studies have noticed no clear effects of repeated electroshocking on movement behaviour of various fish species (Gowan and Fausch, 1996; Dunham et al., 2002; Gatz and Linder, 2008). Therefore, further studies are needed to understand how frequent these side effects of electrofishing are, how do they vary amongst species, and what are the mechanisms involved. Our results are, however, comforting because they suggest no extensive short-term fish mortality or avoidance of the sites despite intensive sampling, as also found in other studies (Barrett and Grossman, 1988; Kocovsky et al., 1997).

Overall, our results are also encouraging because they show that although CPUE estimates depended on crew (and are likely to be difficult to calibrate), observed species richness and species composition were barely affected. These results have important implications because electrofishing surveys in most regions are generally conducted by multiple electrofishing crews and fish data are and will be extensively shared for intercalibration exercises and further implementation of the Water Framework Directive. Personal observations suggest that the differences in CPUE estimates were due to time devoted to the pass by the different crews rather than gear differences. When multiple crews share fish monitoring schemes or data, the time devoted to the electrofishing passes and the rest of field methodology should probably be more standardised to obtain comparable data. Although many methodological factors affecting electrofishing capture efficiency have been investigated, such as length of the reach, number of passes, or differences amongst gears (e.g., Cowx, 1990), our results suggest that the "human factor" has been underestimated and merits further investigation and standardisation.

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