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Methodological issues affecting the study of fish parasites. II. Sampling method affects ectoparasite studies

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ABSTRACT: In this study, we assessed the impact of sampling method on the results of fish ectoparasite studies. Common roach *Rutilus rutilus* were sampled from the same gravel pit in the River Dyje flood plain (Czech Republic) using 3 different sampling methods, i.e. electrofishing, beach seining and gill-netting, and were examined for ectoparasites. Not only did fish caught by electrofishing have more of the most abundant parasites (*Trichodina* spp., *Gyrodactylus* spp.) than those caught by beach seining or gill-netting, they also had relatively rich parasite infracommunities, resulting in a significantly different assemblage composition, presumably as parasites were lost through handling and 'manipulation' in the net. Based on this, we recommend electrofishing as the most suitable method to sample fish for parasite community studies, as data from fish caught with gill-nets and beach seines will provide a biased picture of the ectoparasite community, underestimating ectoparasite abundance and infracommunity species richness.

KEY WORDS: Parasite community · Fish sampling method · Methodology · Parasitological examination · *Rutilus rutilus*

INTRODUCTION

Parasites can often act as biological tags of fish populations (Ihssen et al. 1981, Templeman 1983, MacKenzie & Abaunza 1998) and are widely used as indicators for different aspects of fish biology and ecology (Williams et al. 1991, Kuchta et al. 2009). The results of parasitological examination can be affected by a range of methodological issues, however, including the length of time the fish is held alive (Kvach et al. 2016) or the method used to preserve dead fish (Grutter 1995) prior to dissection. Preservation methods commonly used when there is no time to undertake immediate parasitological dissection include freezing (Aguirre-Macedo et al. 2007, Alarcos & Timi 2012) or storage in 10% Kohrsolin® (Zander et al. 2000, Zander 2003), 4% formaldehyde (Zander et al. 1993, Zander 2005), or 96% ethanol (Sokolov et al. 2015). Live fish that are stressed and/or injured (e.g. during sampling or fixation) can release parasites from the alimentary canal (Williams et al. 1991) or release catecholamines and corticosteroids that alter skin mucous-secretion homeostasis (Pottinger 2008, Pankhurst 2011, Tacchi et al. 2015), thereby changing the fish's first layer of defence against ectoparasites (Davis et al. 2002). Furthermore, the parasites themselves (especially ectoparasites such as *Gyrodactylus* sp.) may be difficult to identify on a preserved fish due to physical damage (Zander 2003, 2004). Finally, ectoparasite number and community composition will also depend on post-collection handling techniques and the method of ectoparasite removal (Grutter 1995). If parasite communities (including ectoparasites) are to be described completely, therefore, parasitological examinations should be undertaken on live fish as soon as possible after catching (i.e. within 3 d; Kvach et al. 2016).

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Fish for parasite studies are usually caught using active-nets, e.g. deep-nets (Zander 2003, 2004), trawls and beach seines (Rokicki & Rolbiecki 2002, Kuchta et al. 2009, Alarcos & Timi 2012, Gendron et al. 2012), or passive netting techniques, e.g. gill- and fyke-nets (Byrne et al. 2000, Aguirre-Macedo et al. 2007), though electrofishing is also commonly used in freshwaters (Esch et al. 1988, Mierzejewska et al. 2012). As all of these methods involve some degree of physical manipulation (either by the handler or by the net itself), it is also possible that the method used to catch the fish may have affected the parasite community. Fish caught by gill-nets, for example, have been found to be less infected with parasitic copepods than those caught with longlines (Nagasawa 1985). Furthermore, as different collection methods will have been used in different studies, it may be impossible to compare the results, as they will have been subject to different levels of bias. There are even cases where different methods have been used within the same study (e.g. Esch et al. 1988, Rokicki & Rolbiecki 2002), with no assessment of sampling method in the data analysis and no comparison between data gathered using different catching techniques.

The aim of this study was to evaluate the influence of a number of commonly used sampling techniques (electrofishing, beach seining and gill-netting) on a fish's ectoparasite community. In each case we consider not only the effect of the sampling method but also that of manipulation during sampling.

MATERIALS AND METHODS

The common roach *Rutilus rutilus* is a common cyprinid species across Europe and western Asia, and is one of the most numerous species at our study site (Halačka et al. 1998, Kottelat & Freyhof 2007). All roach for this study were taken on a single day in April 2015 from a gravel pit (Hvězda; 48.6436° N, 16.9325° E) in the River Dyje floodplain (South Moravia, Czech Republic). The gravel pit, which is approximately 150 m long, 80 m wide and 1.0–1.5 m deep (depth similar throughout the pit), was purposely chosen, as its small size and steep banks lacking a littoral zone would effectively reduce equipment sampling selectivity (e.g. selective sampling of pelagial sub-populations by gill-nets or littoral sub-populations by electrofishing). Three types of sampling equipment were used concurrently:

(1) Electrofishing — An ML3 petrol-powered electrofishing unit (fa. Bednář, Czech Republic; pulsed DC, 2 kW, 230 V, 1.5–2 A, 80 Hz), with a 40 × 20 cm elliptical anode and 4 mm mesh, was used for continual fish sampling from a boat along the pond's littoral zone. Sampling time: 09:30–10:00 h.

(2) Beach seining — A 30 m beach seine (1 cm mesh, 1 m minimum height) was used to sweep approximately 10 m along the bank, using a boat for spreading the net circumpolar to the bank. Sampling time: 10:10–10:40 h.

(3) Gill-netting — A 22 mm mesh benthic gill-net (15 m length, 1.5 m height) was installed across the lake (5 m from the bank) for 3 h during the day and controlled every 0.5 h. Sampling time: 11:00–12:00 h.

All fish were transported alive in aerated barrels to the laboratory of the Institute of Vertebrate Biology, Czech Academy of Sciences (Brno), where they were transferred to a 1 m^3 outdoor holding basin (separate basin for each sampling method). Before dissection, the standard length (SL) of each fish was determined and the fins, skin and gills were examined for ectoparasites. Unicellular parasites were studied alive using light microscopy. Monogeneans were preserved in glycerine-ammonium picrate (GAP) as semi-permanent slides (Malmberg 1957), and glochidia and crustaceans, in 4% formaldehyde; they were then identified under light microscopy. All fish were dissected within 48 h of sampling. Prevalence, mean intensity and abundance were then calculated (see Bush et al. 1997), and infracommunity richness was assessed as the number of parasite species in each host individual, with mean infracommunity characterised as the mean number of parasite species per host individual (Zander 2004). The 'importance' of each parasite species in the community was judged using the altered core-/satellite-species concept based on abundance (Holmes & Price 1986, Zander et al. 2000), whereby 2 = core species, 2–0.6 = secondary species, 0.6–0.2 = satellite species and 0.2 = rare species.

Any differences in parasite community attributable to sampling method (hereon in 'inter-gear differences') were assessed using permutational multiple analysis of variance (PERMANOVA; Anderson 2001). When calculating the distance matrix of samples (i.e. fish individuals), a PERMANOVA response variable, we used both Bray-Curtis (quantitative) and Jaccard (binary) dissimilarity as a distance measure. For Bray-Curtis distance, we first performed a 4^{th}-root transformation of parasite species abundances to equalise the weight of common and rare parasite species (Wood et al. 2009); p-values were obtained

after 999 permutations. Non-metric multidimensional scaling plots were used to visualise ranked within-group and between-group Bray-Curtis and Jaccard dissimilarities.

Inter-gear differences in infracommunity species richness and in abundance, prevalence and mean intensity of infection of the most common ectoparasite species were tested for using generalised linear models (GLM; Poisson distribution detected for species richness, abundance and mean intensity; binomial distribution used for prevalence; both corrected for over- or under-dispersion, i.e. quasi-Poisson and quasi-binomial). Given the possible effect of fish size on parasite abundance, fish SL was included in each GLM as a covariate.

As all statistical comparisons were conducted for each of 3 combinations of gear-pairs, α-level was Bonferroni corrected for these comparisons to 0.05/3 = 0.016. All statistical analyses were conducted using R v. 3.2.1 (R Core Team 2015).

Table 1. Infestation parameters (prevalence, *P*; mean intensity, *I*; abundance, *A*) and diversity indices for parasite communities of fish caught using electrofishing, beach seine nets or gill-nets

	Electrofishing			Beach seine			Gill-nets		
	P	*I*	*A*	*P*	*I*	*A*	*P*	*I*	*A*
Parasite species									
Apiosoma spp.							2.4	10.0	0.2
Trichodina spp.	100	161.3	161.3	94.7	27.8	26.3	97.6	21.4	20.9
Gyrodactylus carassii	42.1	4.6	1.9	23.7	4.6	1.1	21.4	1.2	0.3
G. prostae/laevis group	10.5	21.0	2.2	2.6	58.0	1.5	4.8	1.5	0.1
G. rutilensis/vimbi group	89.5	3.8	3.4	47.4	5.2	2.4	54.8	4.8	2.6
Caligus lacustris juv.	2.6	1.0	0.03						
Neoergasilus japonicus	5.3	2.0	0.1	2.6	1.0	0.03	2.4	2.0	0.05
Unionicola sp.	2.6	1.0	0.03						
Anodonta spp. glochidia	52.6	2.5	1.3	44.7	2.0	0.9	40.5	4.2	1.7
Diversity indices									
Species richness		8			6			7	
Shannon index		0.280			0.701			0.697	
Species evenness		0.135			0.391			0.358	
Dominance		0.898			0.685			0.672	

RESULTS

In total, 118 roach were studied for parasites: 38 sampled by electrofishing (SL = 106.7 ± 8.9), 38 by beach seine (SL = 106.6 ± 7.1) and 42 by gill-nets (SL = 109.3 ± 6.1). We recorded 9 ectoparasite taxa, including 2 ciliates, 3 monogeneans, 2 parasitic crustaceans, 1 mite and 1 mollusc glochidia (Table 1). Five parasite taxa, comprising *Trichodina* spp., *Gyrodactylus carassii*, the *G. prostae/laevis* and *G. rutilensis/vimbi* groups and *Anodonta* sp. glochidia, were recorded as abundant on fish caught using all 3 methods. Other taxa occurred only sporadically. Two taxa, *Trichodina* spp. and the *Gyrodactylus rutilensis/vimbi* group, were registered as core-species for all 3 catching methods, while the *Gyrodactylus prostae/laevis* group was only recorded as a core taxon on fish caught by electrofishing (see Table 1). Secondary-species included glochidia (on fish sampled by all 3 methods), *Gyrodactylus carassii* (on fish caught by electrofishing and beach seine) and the *G. prostae/laevis* group (caught by beach seine). The satellite-species category comprised only *Apiosoma* spp. and *G. carassii* on fish caught by gill-nets. All other combinations occurred only rarely.

Ectoparasite communities differed significantly in fish caught by electrofishing and those caught by beach seine (PERMANOVA; df = 1,74, p = 0.002 for both Bray-Curtis and Jaccard distances) or gill-nets (PERMANOVA; df = 1,78, p = 0.001 for Bray-Curtis and p = 0.002 for Jaccard distances), but not between beach seine and gill-nets (PERMANOVA; df = 1,78, p = 0.082 for Bray-Curtis and p = 0.928 for Jaccard distances; Fig. 1). Similar results were recorded for parasite diversity. Fish collected by electrofishing had a significantly lower Shannon diversity index and evenness and higher dominance compared to beach seine or gill-net samples ($p < 0.001$ for all tests). No differences were observed between beach seine and gill-nets (Table 1).

The infracommunity of fish caught by electrofishing consisted mainly of 3–5 ectoparasite species (73.7% total), with the 3-species infracommunity dominating (39.5%; see Fig. 2). Uninfected fish were only caught using a beach seine (2.6%). One- and two-species infracommunities typically dominated in fish caught by beach seine and gill-nets (57.9% for beach seine, 61.9% for gill-nets), with 3-species infracommunities only occurring in 26.3% of fish caught by beach seine and 28.6% of gill-net caught fish (Fig. 2).

Mean ectoparasite infracommunity species richness was significantly higher in fish caught by electrofishing than in those caught by beach seine (GLM, df = 1,73, $p < 0.001$) or by gill-net (GLM, df = 1,77, p = 0.001), but not between beach seine and gill-net (GLM, df = 1,77, p = 0.422). Fish captured by electrofishing also hosted significantly higher num-

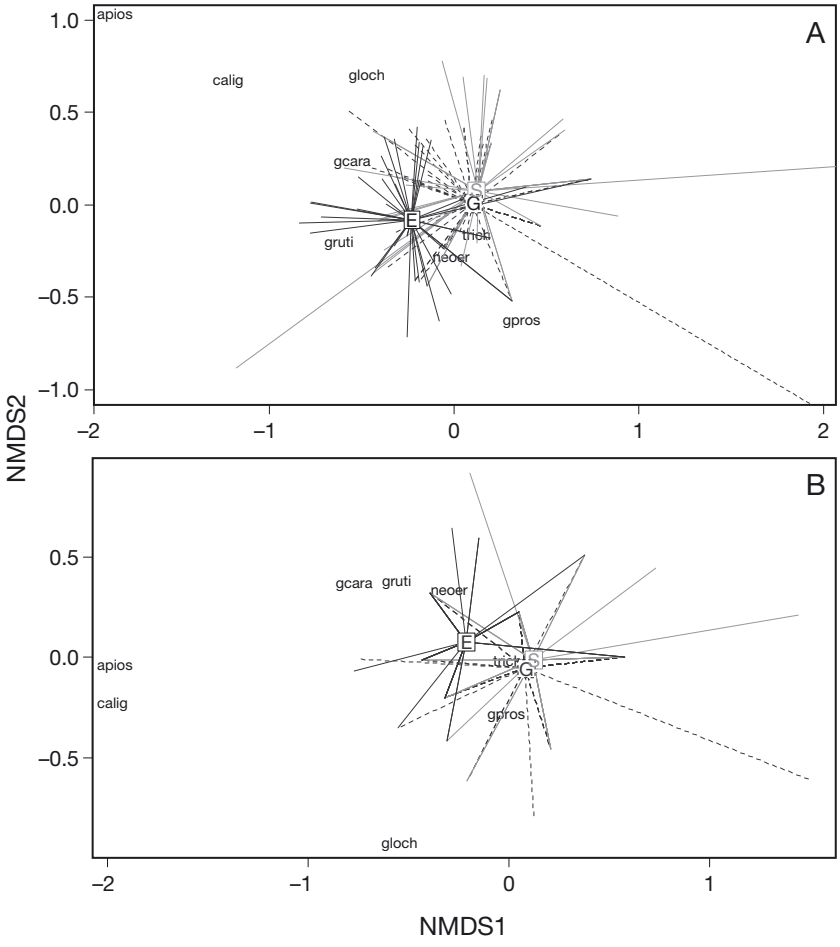

Fig. 1. Ordination results for non-metric multidimensional scaling (NMDS) based on (A) Bray-Curtis and (B) Jaccard distances. Lines connect each sample (fish) with the centroid of the respective group. E (solid black line) electrofishing; S (solid grey line): beach seine; G (dashed black line): gill-nets; apios: *Apiosoma* spp.; trich: *Trichodina* spp.; gcara: *Gyrodactylus carassii*; gpros: *G. prostae/laevis* group; gruti: *G. rutilensis/vimbi* group; calig: *Caligus lacustris* juv.; neoer: *Neoergasilus japonicas*; gloch: *Anodonta* spp. glochidia

bers of ectoparasites compared to both gill-net and beach seine fish (GLM, df = 1,77, df = 1,73, both $p < 0.001$), with no significant difference observed between gill-net and beach seine (GLM, df = 1,77, $p = 0.734$).

In fish caught by electrofishing, *Trichodina* spp. prevalence, mean intensity and abundance was significantly higher than that for fish caught by beach seine, and mean intensity and abundance were higher than in fish caught by gill-net (Table 2). None of the *Trichodina* spp. infection parameters differed between fish caught by beach seine and gill-net (Table 2, Fig. 3).

G. rutilensis/vimbi group prevalence was significantly higher in fish caught by electrofishing compared with those caught by beach seine or gill-net, with no difference between beach seine and gill-net and no significant inter-gear difference in abundance or mean intensity of species (Table 2).

G. carassii mean intensity was significantly lower in fish caught by gill-net, compared to both electrofishing and beach seine, with differences in abundance following the trend in mean intensity (though only significant between electrofishing and gill-net, beach seine/gill-net difference being marginally non-significant; Table 2). No significant difference in abundance or mean intensity of *G. carasii* was observed between electrofishing and beach seine, nor was there any inter-gear difference in the species' prevalence (Table 2).

No significant inter-gear differences were detected in abundance, prevalence, or mean intensity of the *G. prostae/laevis* group or of glochidial infection (Table 2).

DISCUSSION

Our results show a clear difference in the parasite community between fish caught by electrofishing and those caught by beach seining or gill-netting, with fish caught by electrofishing typically displaying a higher abundance of the most abundant parasites (*Trichodina* spp., *Gyrodactylus* spp.), and relatively rich parasite infracommunities, with a significantly different assemblage composition. This strongly suggests that data obtained using net-caught fish are biased due to a loss of parasites (mainly ectoparasites) during the netting procedure, either through physical abrasion by the nets, manipulation during retrieval, or through stress-related factors. By implication, therefore, fish caught by electrofishing are most likely to provide parasite assemblage data closest to reality.

Use of nets had a particularly strong influence on the ectoparasite community and on the abundance of particular species. Similar results were also observed by Nagasawa (1985), who registered lower infestation by copepods *Lepeophtheirus salmonis* on chum salmon *Oncorhynchus keta* that had been caught using gill-nets rather than longlines.

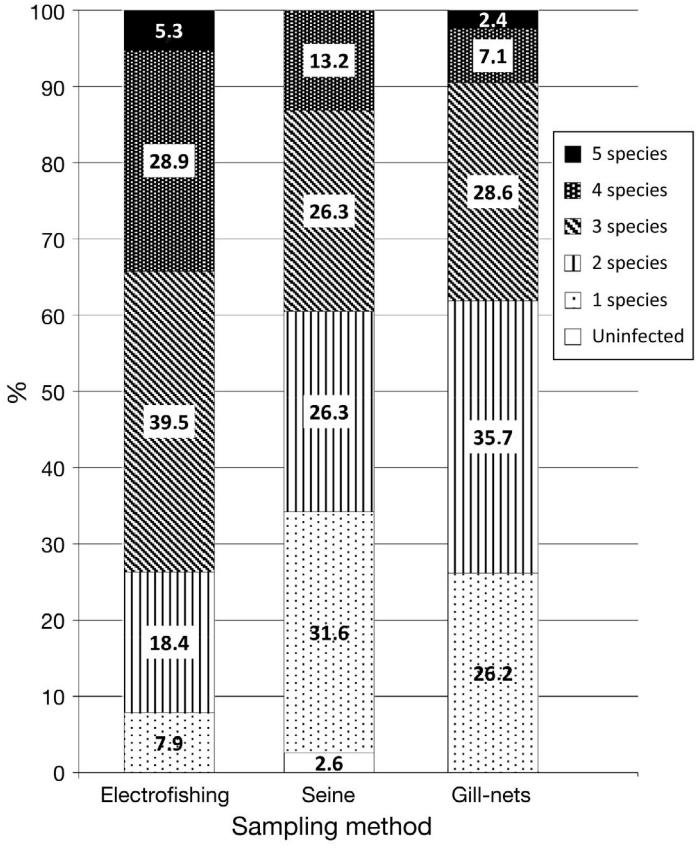

Fig. 2. Proportions of ectoparasites in parasite infracommunities infecting fishes caught using electrofishing, beach seine nets or gill-nets

Our fish were sampled in April, when *Trichodina* numbers are normally at their maximum (Migała 1971, 1978, Pojmańska 1995). As such, they were the most abundant species in the parasite community of roach and showed greatest differences between the catching methods. Like all trichodinids, the *Trichodina* are armed with an adhesive disc for attaching themselves to a substrate — usually an aquatic organism (Hausmann & Hausmann 1981, Lom & Dyková 1992). As trichodinids are a non-obligate parasite (essentially commensal), they occur not only on fish but also aquatic invertebrates and vegetation (Lom & Dyková 1992, Babko & Kuzmina 2004). This more 'temporary' form of attachment means that trichodinids can easily be detached from their 'host' through mechanical contact. Indeed, our study showed that *Trichodina* ciliates were highly sensitive to manipulation by nets.

Monogeneans, on the other hand, are obligate parasites that attach to their hosts securely using hooks. The *G. rutilensis/vimbi* group and *G. carassii* occurred mainly on the fins and body surface, and these were the locations where differences in infestation parameters were most obvious. In particular, fish caught by electrofishing showed a higher mean intensity and abundance of *G. carassii* than those caught with gill-nets, while the *G. rutilensis/vimbi* group showed lower prevalence in fish caught with both types of nets (Table 2). In comparison, infestation by the *G. prostae/laevis* group, which occurred most often on the gills, showed little change in intensity and prevalence between catching methods, again suggesting external losses due to mechanical displacement.

Our results also showed that loss of parasites (and particularly ectoparasites) from the fins and body surface influenced infracommunity species richness. Intuitively, if an ectoparasite is represented by just a few individuals on a fish then there is an increased probability that the whole infrapopulation could be removed from the fish during net manipulation. This was clearly reflected in our results, with >70% of fish caught by electrofishing hosting 3–5 parasite species, compared to just 40% of fish caught by gill-net or beach seine (Fig. 2).

The importance of particular species in the community also appeared to differ based on the sampling method used, with *G. carassii* classified as a secondary species on fish caught with electrofishing and

Table 2. Test statistics (p-values resulting from the generalised linear model) inferring inter-gear differences in prevalence, mean intensity and abundance of the most common ectoparasites on fish caught using electrofishing (E), beach seine nets (S), or gill-nets (G). p-values suggesting significant differences ($p < 0.016$) are in **bold**. na: test statistic not available

Parasite species	Prevalence			Mean intensity			Abundance		
	E vs. S	E vs. G	S vs. G	E vs. S	E vs. G	S vs. G	E vs. S	E vs. G	S vs. G
Trichodina spp.	**0.002**	0.102	0.288	**0.001**	**0.001**	0.713	**<0.001**	**<0.001**	0.786
Gyrodactylus carassii	0.097	0.076	0.837	0.957	**0.005**	**0.005**	0.231	**<0.001**	0.027
G. prostae /laevis group	0.160	0.370	0.478	na	0.383	na	0.773	0.054	0.150
G. rutilensis/vimbi group	**0.001**	**0.001**	0.376	0.173	0.392	0.591	0.302	0.268	0.848
Anodonta spp. glochidia	0.497	0.365	0.984	0.018	0.123	0.090	0.046	0.520	0.050

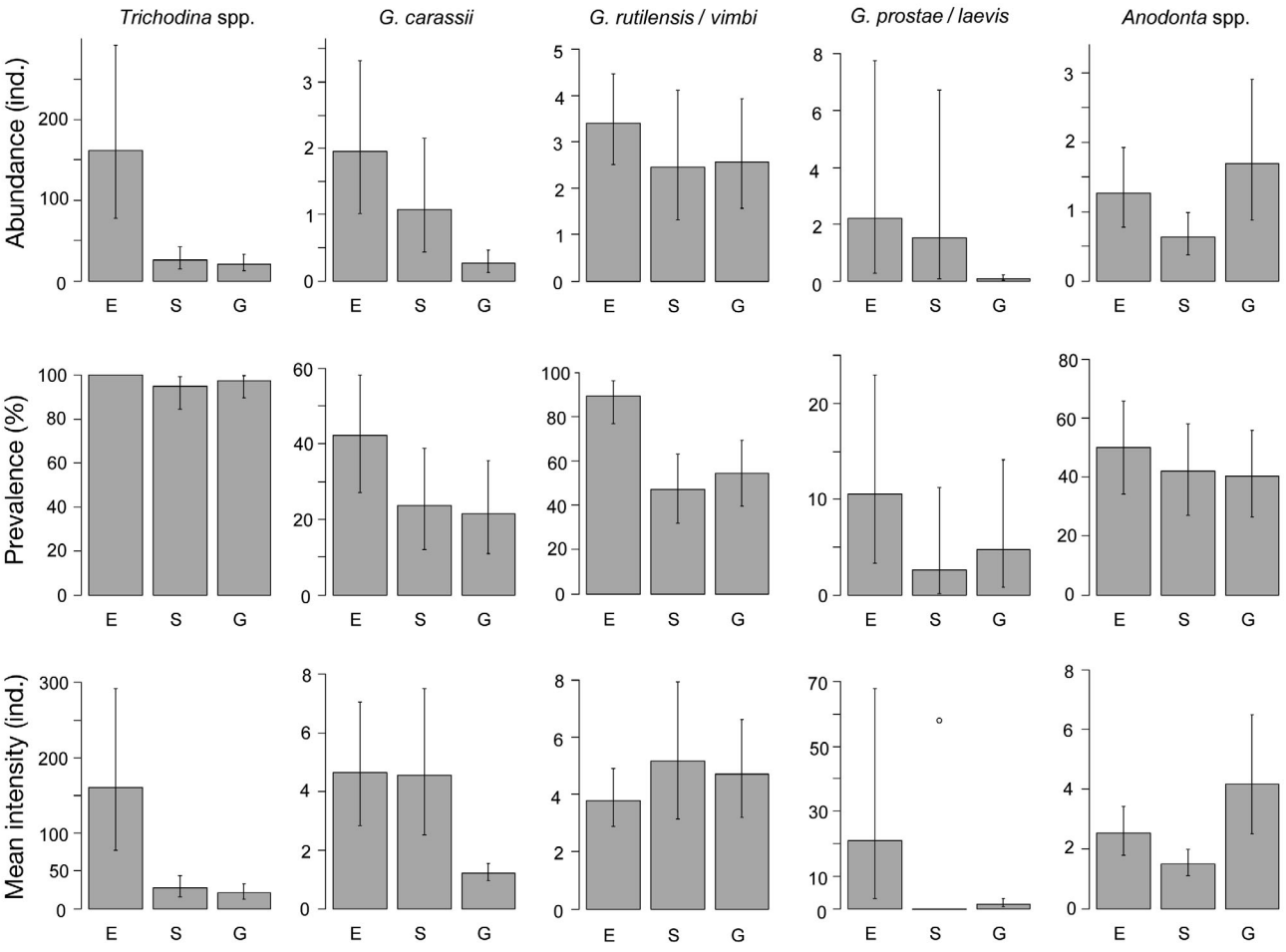

Fig. 3. Abundance, prevalence and mean intensity of infection for the most common ectoparasites on fish caught using different methods (E: electrofishing; S: beach seine; G: gill-net), estimated from the generalised linear model, including 95% confidence intervals (whiskers). No estimate of mean intensity was possible for the *G. prostae/laevis* group on fish caught by seine netting as only a single fish hosted the parasite (a point is shown instead)

beach seine but a satellite species on fish caught with gill-nets. Likewise, the *G. prostae/laevis* group was recorded as a core species on fish caught by electrofishing, as a secondary species on beach-seine-caught fish and as rare on gill-net-caught fish. While differences in infestation by the *G. prostae/laevis* group on fish caught using different methods were not significant, there was a tendency for fewer of these parasites to appear on gill-net-caught fish. As this group concentrates mainly on the gills, it is likely that the gill-nets had a higher impact on these species, despite the higher degree of protection provided by the gills and operculae. According to the core–satellite hypothesis (Hanski 1982), core species are abundant and well-spaced in niche space, while satellite species are rare and widely spaced. The taxa classified as core and secondary species in fish caught by electrofishing, can be recognised as well-spaced in the locality. But the usage of both nets for the sampling reduced the abundance of these parasites, therefore affecting the results of the study. The importance of particular parasite taxa was maximally reduced in fish caught by gill-nets.

Overall, this study demonstrated a significant effect on ectoparasite abundance and infracommunity species richness in fish caught by gill-nets or by beach seining due to losses caused during the netting procedure, through physical abrasion by the nets, manipulation during retrieval, or through stress-related factors. In conclusion, we recommend electrofishing as the most suitable method for sampling fish for parasite community studies, as it is most likely to provide a representative and unbiased picture of local parasite assemblages. We further recommend that the host sampling method be carefully considered in future comparative studies on ectoparasite communities when using previously published data.

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