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Methodological issues affecting the study of fish parasites. I. Duration of live fish storage prior to dissection

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ABSTRACT: We tested the ability of parasite species to respond quickly to artificial conditions (e.g. by changing abundance or even decreasing to extinction) while host fish species were being held alive prior to dissection. Prussian carp *Carassius gibelio* were sampled by electrofishing from 2 ponds alongside the River Dyje (Czech Republic) during 'cold' and 'warm' seasons. All fish were transported to the laboratory in aerated pond water and kept in a 1 m^3 outdoor basin with aged tap water for 6 d. Twenty fish were dissected on consecutive days (total 120 fish for each site). Our results indicated that there was little change in parasite loading over the first 3 d of holding, suggesting no impact on parasitological studies undertaken over this period. From the fourth day, however, overall parasite abundance increased due to rapid reproduction of some parasite species, especially gyrodactylids in the cold season and dactylogyrids in the warm season. Parasite diversity appeared less stable in the warm season, with significant differences being registered as early as the second day. In addition to holding period, environmental conditions during fish holding will also play an important role in parasite community shifts.

KEY WORDS: Parasite community · Temporal changes · *Carassius gibelio* · Fish holding · Methodology · Parasitological examination

INTRODUCTION

The study of parasite communities is an important component of many ecological, evolutionary and epidemiological studies, as parasites can strongly affect not only host population dynamics but also the ecology and evolution of host behaviour (Mittelbach 2005, Kortet et al. 2010). Furthermore, changes in parasite community structure can often reflect environmental disturbances, such as habitat fragmentation, pollution, biodiversity loss, climate change or the introduction of new species (Gelnar et al. 1997, Lafferty & Kuris 2005). As such, changes in parasite community structure can be used as indicators of environmental change (Palm et al. 2011). For such studies to be meaningful, however, the methodology used must be as exact and as reproducible as possible.

Full parasitological dissection of fish ideally requires that the fish be kept alive prior to dissection. Nevertheless, many previous studies have used frozen (e.g. González & Poulin 2005) or preserved (e.g. formaldehyde: Timi & Poulin 2003; ethanol: Sokolov et al. 2015) fish for parasite examination. Furthermore, it is generally recommended that fish are dissected within the first 24 h of catching (maximum 48 h), as some parasites are known to proliferate following stress to the host caused by sampling, handling, transport and storage (Pritchard & Kruse 1982, Post 1983, Thoesen 1994). Occasionally, however, it may not be possible to dissect within 48 h, and fish will be stored for a longer period prior to dissection, especially when sampling at a significant distance from the place of dissection (Kvach et al. 2015). In many cases, the holding period prior to dissection

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is omitted from the method description (e.g. Poulin & Valtonen 2002).

To the best of our knowledge, all previous experimental studies concerning variation in parasite abundance caused by holding time have been undertaken using frozen fish only. This includes, for example, 2 studies that described migration of anisakid larvae in Baltic herring *Clupea harengus* (Smith & Wootten 1975, Horbowy & Podolska 2001) and another describing migration of *Eustrongylides* nematode larvae (Locke et al. 1964).

In this study, we test how a range of thermophilic and cryophilic parasite species respond while their live host is held under artificial storage conditions prior to dissection. The results will not only add to our knowledge of parasite ecology and behaviour but will also be of great importance when planning future fish parasite studies, thus improving the accuracy of results and of cross-study comparisons.

MATERIALS AND METHODS

Prussian carp *Carassius gibelio*, the test host species used in this study, were sampled from 2 closely situated gravel ponds (Rohlik, Mirafeldy) in the River Dyje floodplain (Czech Republic). Two ponds were sampled as insufficient host fish were available from the first pond (Rohlik). This subsequently allowed the study of 'seasonal' differences in parasite behaviour. Fish were sampled from Rohlik (48.6477° N, 16.9280° E) in November 2014 (henceforth the 'cold season'; morning water temperature 7°C) and from Mirafeldy (48.6753° N, 16.9362° E) in June 2015 (henceforth the 'warm season'; morning water temperature 19.3°C) using electrofishing equipment (Petrol Electrofishing gear ML3, Bednár; pulsed DC, 2 kW, 230 V, 1.5–2 A, 80 Hz) and transported to the Czech Academy of Sciences' Institute of Vertebrate Biology (Brno) in aerated pond water. Around 150 similarly sized fish were sampled each season (Table 1). At the institute, the fish were transferred to a 1 m^3 outdoor basin containing 3 d-aged tap water, where they were kept for the 6 d of the experiment. No mortality was observed in the basin throughout the study. Water temperature, which was measured at 8:00 and 15:00 h each day, ranged from 6.3 ± 0.9 to 6.5 ± 0.9°C in November and 22.5 ± 0.7 to 27.5 ± 0.5°C in June.

No fish were dissected on the day of sampling (D0) due to lack of time. On each subsequent day (D1–D6), 20 similarly sized Prussian carp (i.e. 120 fish in total; Table 1) were sacrificed, measured (standard length, SL) and subjected to a full standardised parasitological dissection of fins, skin, gills, muscles and internal organs (Pritchard & Kruse 1982). Living unicellular parasites were studied using an Olympus CX41 light microscope. Monogeneans were preserved in GAP (glycerine-ammonium-picrate) and prepared as semi-permanent slides (Malmberg 1957). Digeneans, cestodes and nematodes were preserved in hot 4% formaldehyde, stained using iron acetic carmine, dehydrated in ethanol of increasing concentration and mounted in Canada balsam as permanent slides (Georgiev et al. 1986, Cribb & Bray 2010). Acanthocephalans were pressed between 2 slides, fixed in 70% ethanol and mounted in glycerol as temporary slides for light microscopy. Glochidia and crustaceans were preserved in 4% formaldehyde and identified under light microscopy. All parasites were identified to species level or to the highest possible taxa. Data for each species are presented as prevalence, mean intensity and mean abundance (see Bush et al. 1997).

Table 1. Mean ± SD standard length (SL) and total weight (W_t) of Prussian carp *Carassius gibelio* dissected over 6 consecutive days during cold and warm seasons. Sample size: 20 fish per day each season

Day	Cold		Warm	
	SL (cm)	W_t (g)	SL (cm)	W_t (g)
D1	41.3 ± 8.8	2.6 ± 1.6	91.2 ± 30.8	26.1 ± 20.9
D2	43.4 ± 4.9	2.8 ± 0.9	86.9 ± 27.2	23.6 ± 17.3
D3	46.7 ± 7.6	3.3 ± 1.5	79.8 ± 25.9	22.2 ± 17.8
D4	43.6 ± 7.2	2.9 ± 1.6	90.9 ± 19.7	26.1 ± 15.2
D5	47.7 ± 8.4	4.0 ± 1.8	84.7 ± 25.3	21.5 ± 15.2
D6	42.9 ± 6.5	2.9 ± 1.4	83.2 ± 23.6	22.9 ± 15.9

We used generalised linear models (GLM; Poisson distribution for abundance and binomial for prevalence, both corrected for overdispersal as quasipoisson and quasibinomial) in order to test whether there was a significant linear increase/decrease in parasite abundance or prevalence with storage time (continuous predictor). In a second step, we assessed whether there was a temporal threshold at which changes in parasite assemblage characteristics were significantly different from a reference value (we chose D1 values as those closest to the state at catching). For this, we calculated composite community species richness for each day's sample (D1–D6), as well as Jaccard (Ja) and Bray-Curtis (B-C) similarity for composite community between D1 and D2–D6. Differences from D1 were compared visually for all 3 composite community measurements. We tested for further differences between D1 and D2–D6 (treated as categorical variables) using 3 diversity indices (Shannon, Simpson's domi-

nance and evenness), infracommunity species richness, parasite assemblage composition and abundance of the most common species/groups. PAST software (Hammer et al. 2001) was used to calculate diversity index permutation tests to compare the diversity indices of different parasite communities. Differences in infracommunity species richness and parasite abundance were tested using GLM (quasi-poisson distribution). Differences in parasite assemblage composition were tested using permutational multiple analysis of variance (PERMANOVA), using both Ja and B-C distances (see Anderson 2001 for details). All tests were conducted using R 3.2.1 (R Core Team 2015). Discriminant analysis using StatSoft 2013 — Statistica for Windows 12, was also applied in order to provide a further measure of assemblage composition similarity and to assess any differences between parasite assemblages on fish dissected on different days. Abundance data for each parasite species on each host individual was used as source data for the discriminant analysis and day of dissection as the grouping parameter. The Mahalanobis distance was calculated between infestations (abundance data) on fishes dissected on different days.

RESULTS

While fish collected in June (warm season) were significantly larger than those collected in November (cold season) (Mann-Whitney *U*-test, $Z = 11.8$, $p < 0.001$), SL did not differ between sample days (D1–D6) for fish collected in either the cold (ANOVA, $F = 2.2$, $p > 0.05$) or warm (Kruskal-Wallis ANOVA, $H = 3.5$, $p > 0.05$) seasons.

Thirty-one parasite species/taxa were recorded during the cold season (Table 2), of which 13 (41.9%) were ectoparasites. Three ectoparasite taxa were highly abundant: 2 ciliates (*Trichodina* spp., *Apio-*

Table 2. Measures of parasite infection (% prevalence/mean intensity/mean abundance) in Prussian carp *Carassius gibelio* over the 6 d holding period (D1–D6) in the cold season. met: metacercariae; L3: third stage larvae

Parasite species	D1	D2	D3	D4	D5	D6
Microsporidia gen. sp.					5/3.0/0.15	
Apiosoma spp.				5/30.0/1.5	20 /47.5/9.5	10/1.0/0.1
Trichodina spp.	35/21.8/7.65	50/8.7/4.35	65/10.7/7.0	25/72.4/18.1	40 /13.8/5.5	30/5.7/1.7
Dermocystidium sp.				20/1.5/0.3		15/1.3/0.2
Myxobolus sp.	10/5.5/0.55					
Myxosporidia gen. sp.				5/10.0/0.5		
Myxosporidia sp. 1					10/4.5/0.45	
Myxosporidia sp. 2				15/3.7/0.55	5/1.0/0.05	25/4.6/1.15
Dactylogyrus anchoratus			5/1.0/0.05			
Dactylogyrus baueri				5/1.0/0.05		
Dactylogyrus dulkeiti	10/2.0/0.2					
Dactylogyrus formosus	5/1.0/0.05		20/1.25/0.25	25/1.6/0.4	15/1.7/0.25	10/1.0/0.1
Dactylogyrus inexpectus	15/1.3/0.2			15/1.7/0.25		5/1.0/0.05
Dactylogyrus intermedius	15/1.7/0.25		15/1.3/0.2	20/1.75/0.35	20/1.5/0.3	10/1.0/0.1
Dactylogyrus vastator				10/1.0/0.1	5/2.0/0.1	10/1.0/0.1
Gyrodactylus longoacuminatus	50/3.0/1.5	80/1.7/1.35	65/2.3/1.5	75/2.9/2.2	75/3.2/2.4	95/3.2/3.05
Gyrodactylus schulmani		5/1.0/0.05	15/1.3/0.2			
Gyrodactylus vimbi	5/1.0/0.05	5/1.0/0.05				
Apharyngostrigea cornu met	5/24.0/1.2		5/1.0/0.05		5/2.0/0.1	5/1.0/0.05
Bucephalus polymorphus met			5/1.0/0.05	5/1.0/0.05		
Cyathocotyle prussica met	5/2.0/0.1	5/1.0/0.05	5/1.0/0.05	5/1.0/0.05		5/1.0/0.05
Holostephanus dubinini met	10/3.5/0.35			5/1.0/0.05		
Diplostomum spp. met	10/2.0/0.2	15/1.0/0.15		5/1.0/0.05	5/1.0/0.05	
Posthodiplostomum cuticola met				5/1.0/0.05		
Tylodelphys clavata met			5/1.0/0.05	15/4.0/0.6	5/4.0/0.2	
Digenea gen. sp. met	15/1.3/0.2	15/4.0/0.6	5/1.0/0.05	5/1.0/0.05	20/1.75/0.35	
Pomphorhynchus laevis	5/1.0/0.05					
Schulmanela petruschewskii	20/1.0/0.2	5/1.0/0.05				
Philometroides sanguineus				5/1.0/0.05		
Contracoecum rudolphii L3						10/1.0/0.1
Glochidia gen. sp.					5/1.0/0.05	
Total	90/14.2/12.8	95/7.0/6.7	95/9.9/9.5	100/25.3/25.3	85/22.9/19.5	95/7.1/6.8
Species number	16	9	13	21	16	14

soma spp.) and *Gyrodactylus longoacuminatus* (Table 2). The parasite community during the warmer season was relatively poor, consisting of just 10 taxa (Table 3), including 5 (50%) ectoparasites. Of these, *Dactylogyrus formosus* was the most abundant, with *D. anchoratus* and *Trichodina* spp. at relatively high prevalence (Table 3). Endoparasite abundance was relatively low during the cold season and almost negligible (6 individuals) during the warm season. During the cold season, the majority of endoparasites were tissue parasites, represented mainly by metacercariae. Gut parasites were represented by a single species during both the cold (*Pomphorhynchus laevis*) and warm (*Pseudocapillaria tomentosa*) seasons, with very low abundance in both cases (Tables 2 & 3, respectively).

Parasite response to holding conditions

During the cold season, both abundance and prevalence of *G. longoacuminatus* increased significantly with time of storage (GLM, $p = 0.005$ and $p = 0.008$, respectively; Fig. 1a, Table 2). There was no significant linear trend in either abundance or prevalence of *Trichodina* spp. (GLM, $p = 0.676$ and $p = 0.307$; Fig. 1a, Table 2), with both varying widely.

During the warm season, both abundance and prevalence of *D. formosus* increased significantly over time (GLM, both $p < 0.001$; Fig. 1b, Table 3), with *D. anchoratus* marginally non-significant (GLM, $p = 0.081$; Fig. 1b, Table 3). Both abundance and prevalence of *Trichodina* spp. decreased significantly over time (GLM, $p = 0.025$ and $p = 0.001$, respectively; Fig. 1b, Table 3).

Searching for a threshold

While parasite assemblage composition at the component community level remained relatively similar over the first 3 d (D1–D3) in both cold and warm seasons, there was an apparent decrease in assemblage similarity between D4 and D6 (both Ja and B-C, Table 4). In both seasons, the assemblage shift was facilitated by several species appearing for the first time at D4 (7 of 31 species — cold season, 3 of 10 species — warm season; Tables 2 & 3), including some abundant species such as *Apiosoma* spp. during the warm season (Table 2). There was also a peak in both component community species richness and mean infracommunity species richness at D4 in both seasons (Table 4), and there appeared to be a peak in the abundance of the most numerous ectoparasites (*Trichodina* spp. — cold season, *D. formosus* — warm season; Table 4, Fig. 1) at D4.

Significant differences (from D1) in parasite assemblage composition at the infracommunity level were also first noted at D4 (Ja-based PERMANOVA only — cold season; both Ja- and B-C-based PERMANOVA — warm season; Table 4), with a further significant difference at D6 in both seasons (both Ja- and B-C-based PERMANOVA; all $p < 0.05$; Table 4). These results are consistent with those of discriminant analysis, which showed a clear difference between D1 and D4–D6 in the cold season and D1, D4 and D6 in the warm season (Table 4, Fig. 2).

Diversity indices first show a significant difference at D5 in the cold season, when dominance decreased and evenness and Shannon indices increased, the first 2 differences remaining at D6 (Table 4). Similarly, the first significant decrease in *Trichodina* spp. abundance was observed at D6 in the cold season

Table 3. Measures of parasite infection (% prevalence/mean intensity/mean abundance) in Prussian carp *Carassius gibelio* over the 6 d holding period (D1–D6) in the warm season. met: metacercariae

Parasite species	D1	D2	D3	D4	D5	D6
Trichodina spp.	25/4.6/1.2	40/3.25/1.3	30/1.0/0.3	10/1.0/0.1	10/5.5/0.6	
Myxosporidia sp. 1				5/1.0/0.05		
Dactylogyrus anchoratus	35/2.3/0.8	20/2.0/0.4	30/1.2/0.4	65/1.9/1.3	40/1.5/0.6	65/2.1/1.4
Dactylogyrus formosus	55/2.4/1.3	85/3.9/3.4	70/2.4/1.7	90/8.4/7.6	80/5.1/4.1	90/6.8/6.1
Dactylogyrus inexpectatus	5/1.0/0.1	10/1.0/0.1	5/1.0/0.1		5/2.0/0.1	10/1.0/0.1
Diplostomum spp. met				5/1.0/0.05		
Philometra sp.				5/1.0/0.05		5/1.0/0.05
Philometroides sanguineus						5/1.0/0.05
Pseudocapillaria tomentosa		5/1.0/0.05		5/1.0/0.05		
Caligus lacustris		5/1.0/0.05				
Total	80/4.1/3.3	95/5.5/5.3	85/2.8/2.4	90/9.8/8.8	85/6.3/5.4	90/8.5/7.7
Species number	4	6	4	7	4	5

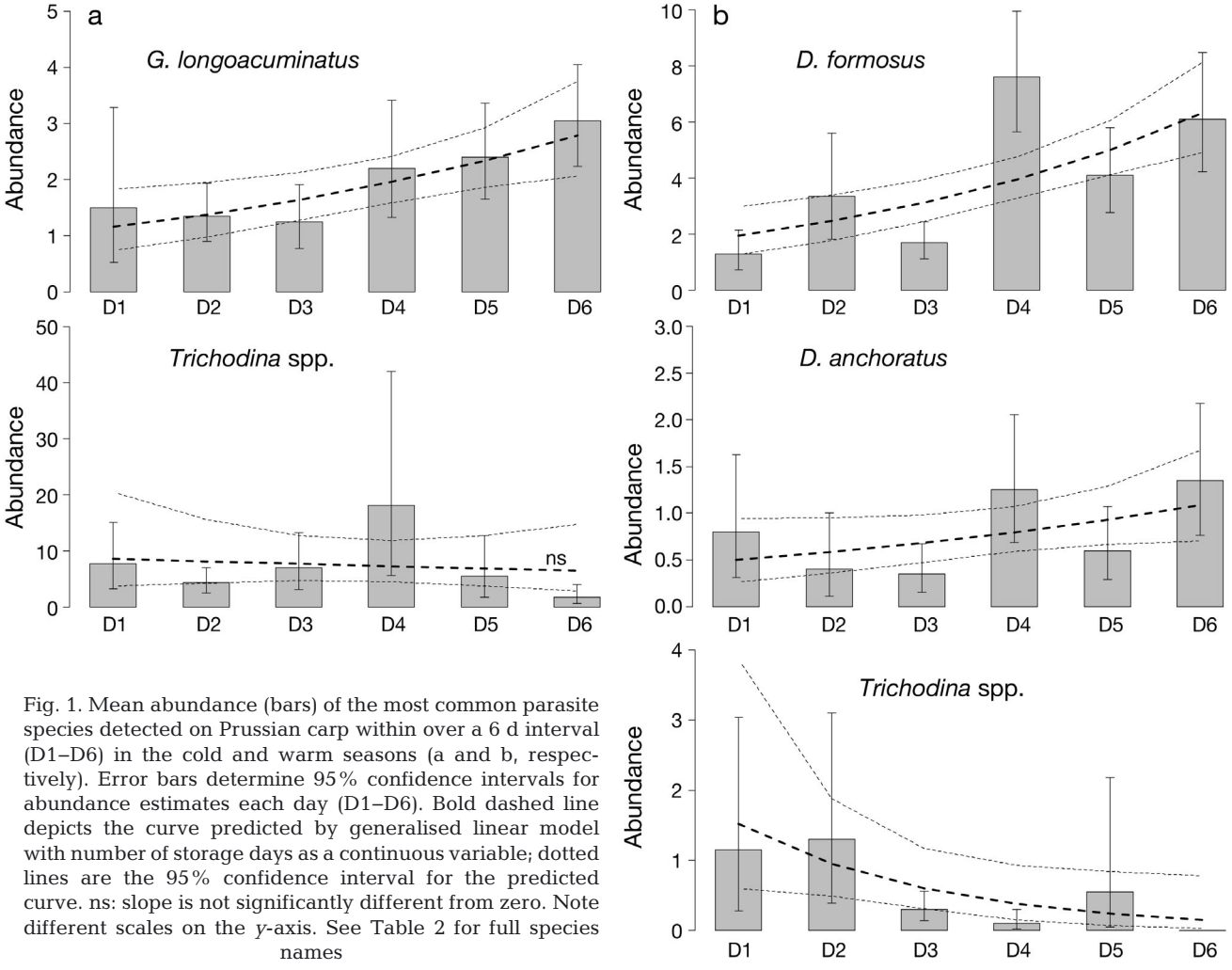

Fig. 1. Mean abundance (bars) of the most common parasite species detected on Prussian carp within over a 6 d interval (D1–D6) in the cold and warm seasons (a and b, respectively). Error bars determine 95% confidence intervals for abundance estimates each day (D1–D6). Bold dashed line depicts the curve predicted by generalised linear model with number of storage days as a continuous variable; dotted lines are the 95% confidence interval for the predicted curve. ns: slope is not significantly different from zero. Note different scales on the *y*-axis. See Table 2 for full species names

(GLM, p = 0.028; Table 4), though no such threshold was detected in abundance of *G. longoacuminatus*, total ectoparasite abundance or infracommunity species richness (GLM, all p > 0.05; Table 4). The first decrease in endoparasite abundance was observed at D3, however, with a further significant decrease observed at D6 (GLM, p = 0.024 and 0.017; Table 4).

During the warm season, significant differences were observed in all 3 diversity indices from D2 onward, with an opposite trend to that in the cold season, i.e. an increase in dominance and a decrease in the evenness and Shannon indices (Table 4). The first significant increase in *D. formosus* abundance was observed at D2, with D4–D6 also being significantly different from D1 (GLM, p = 0.032, p < 0.001, p = 0.001 and p < 0.001, respectively; Table 4). A significant increase in both total ectoparasite and *Trichodina* spp. abundance was first observed at D4, with D6 also being significantly different from D1 in both cases (GLM, p = 0.039 and p = 0.008 for *Trichodina* and p < 0.001 and p = 0.007 for ectoparasites; Table 4). No significant difference was observed in either *D. anchoratus* abundance or infracommunity species richness (GLM, all > 0.05; Table 4).

DISCUSSION

To the best of our knowledge, this is the first study to empirically demonstrate that the length of time that live fish are held prior to dissection results in changes in parasite abundance and a shift in parasitological characteristics. In our experiment, the main changes in parasite assemblage were revealed on D4 of storage, in both the warm and cold seasons, with some indication of more rapid changes (starting on D2) during the warm season and delayed effects in the cold season. Overall, our results demonstrate

Table 4. Basic parasitological characteristics of Prussian carp *Carassius gibelio* kept for 1 to 6 d (D1–D6) before dissection. *S:* species richness; B-C: Bray-Curtis similarity; Ja: Jaccard similarity; *F: F*-statistics of PERMANOVA based on B-C or Ja distances; MD: squared Mahalanobis distances; *D:* dominance index; *H:* Shannon diversity; *E:* evenness; ecto: ectoparasites; *Trich: Trichodina* spp.; *G. longo: Gyrodactylus longoaccuminatus*; endo: endoparasites; *D. form: Dactylogyrus formosus*; *D. anch: D. anchoratus*. Significant differences from D1 ($p < 0.05$; in **bold).** No tests were conducted on *S*, B-C or Ja; –: not applicable

Component community				Diversity			Infracommunity				Abundance			
Cold season														
	S	B-C	Ja	*D*	*H*	*E*	Mean *S*	*F* B-C	*F* Ja	MD	Ecto	*Trich*	*G. longo*	Endo
D1	16	–	–	0.62	0.79	0.41	2.15	–	–	–	9.9	7.7	1.5	2.3
D2	9	0.64	0.47	0.62	0.64	0.46	1.8	1.90	1.96	4.79	5.8	4.4	1.4	0.9
D3	13	0.80	0.40	0.61	0.80	0.45	2.1	2.06	2.00	6.06	9.2	7.0	1.3	**0.3**
D4	21	0.52	0.38	0.64	0.81	0.39	2.8	1.77	**1.84**	**10.36**	23.0	18.1	2.2	1.0
D5	16	0.48	0.35	**0.39**	**1.14**	**0.59**	2.35	1.40	1.34	**6.83**	18.1	5.5	2.4	0.7
D6	14	0.36	0.38	**0.45**	1.03	**0.53**	2.3	**4.37**	**3.54**	**7.41**	5.2	**1.7**	3.1	**0.2**
Warm season														
	S	B-C	Ja	*D*	*H*	*E*	Mean *S*	*F* B-C	*F* Ja	MD	Ecto	*Trich*	*D. form*	*D. anch*
D1	4	–	–	0.34	1.14	0.82	1.2	–	–	–	3.3	1.2	1.3	0.8
D2	6	0.68	0.71	**0.49**	**0.90**	**0.65**	1.65	1.96	2.22	1.39	5.2	1.3	**3.4**	0.4
D3	4	0.70	1.00	**0.54**	**0.87**	**0.62**	1.35	0.17	0.11	0.52	2.4	0.3	1.7	0.4
D4	7	0.35	0.50	**0.73**	**0.50**	**0.36**	1.8	**4.38**	**4.12**	**5.5**	**9.0**	**0.1**	**7.6**	1.3
D5	4	0.58	1.00	**0.61**	**0.76**	**0.55**	1.35	1.63	1.50	1.2	5.4	0.6	**4.1**	0.6
D6	5	0.39	0.57	**0.69**	**0.54**	**0.49**	1.75	**3.9**	**4.69**	**2.74**	**7.6**	**0.0**	**6.1**	1.4

no significant variation in host fish parasite assemblage during the first 3 d of holding. A number of ectoparasite species showed major shifts in abundance from the fourth day after sampling, however, and this was consistent in both cold and warm seasons. Note that the fish taken as a reference in our study (dissected on D1) could have been affected by stress induced by sampling, transport and overnight storage. For logistic reasons, however, it was not possible to confirm this possible bias. Thus, although we are convinced our approach provides the most accurate results available, they should be treated with some caution regarding this possible bias.

Ectoparasite abundance, and especially that of ciliates and monogeneans, is dependent on the environmental conditions of their hosts (Lester 1990). Stress suffered by fish during sampling, transport and holding can induce the release of catecholamines and corticosteroids and increase glucose levels in blood, which influences skin mucosal homeostasis (Pottinger 2008, Pankhurst 2011, Tacchi et al. 2015). Skin epithelium and mucous secretions are passive physical barriers and form part of a fish's innate protective mechanism (Davis et al. 2002). Ectoparasites, therefore, such as ciliates and monogeneans, are the first to reflect stress-mediated changes in host physiology. In addition, high fish densities in holding tanks will support parasite transmission between individuals, resulting in an increase in prevalence and parasite reproduction, also contributing to the increase in abundance. Just such an increase in prevalence was observed in this study for *G. longoacuminatus* (Table 2), *D. formosus* and *D. anchoratus* (Tables 2 & 3).

Trichodinid ciliates (*Trichodina*) occur on a range of aquatic invertebrates, as well as on the gills, skin and fins of fish (van As & Basson 1989). The abundance of *Trichodina* cells depends mainly on season, being most abundant in March to April (Migała 1971, 1978, Pojmańska 1995), and sometimes up to May (Özer & Erdem 1998, 1999, Özer 2000). In our study, trichodinids decreased significantly during the warm season from D2 onwards, most probably due to the higher temperature. During the cold season, there was a general trend for trichodinid ciliate abundance to peak on D4, followed by a rapid decrease (see Fig. 1). This variation in the abundance of a single parasite group strongly affected the parasite community in general, resulting in a significant increase in total parasite abundance on D4 (see Table 4).

Monogenean parasites were represented by 2 genera, *Gyrodactylus* and *Dactylogyrus*, with 3 species most abundant. Whilst *G. longoacuminatus* and *D. anchoratus* are common parasites of Prussian carp in the study area, *D. formosus* is relatively rare in adults (Šimková et al. 2013). This species can reach high abundances in juvenile forms (Ondračková & Jurajda 2000), however, as was the case in this study. During the cold season, of *G. longoacuminatus* numbers gradually increased, reaching maximum abundance and prevalence values on D6 of holding (see Fig. 1;

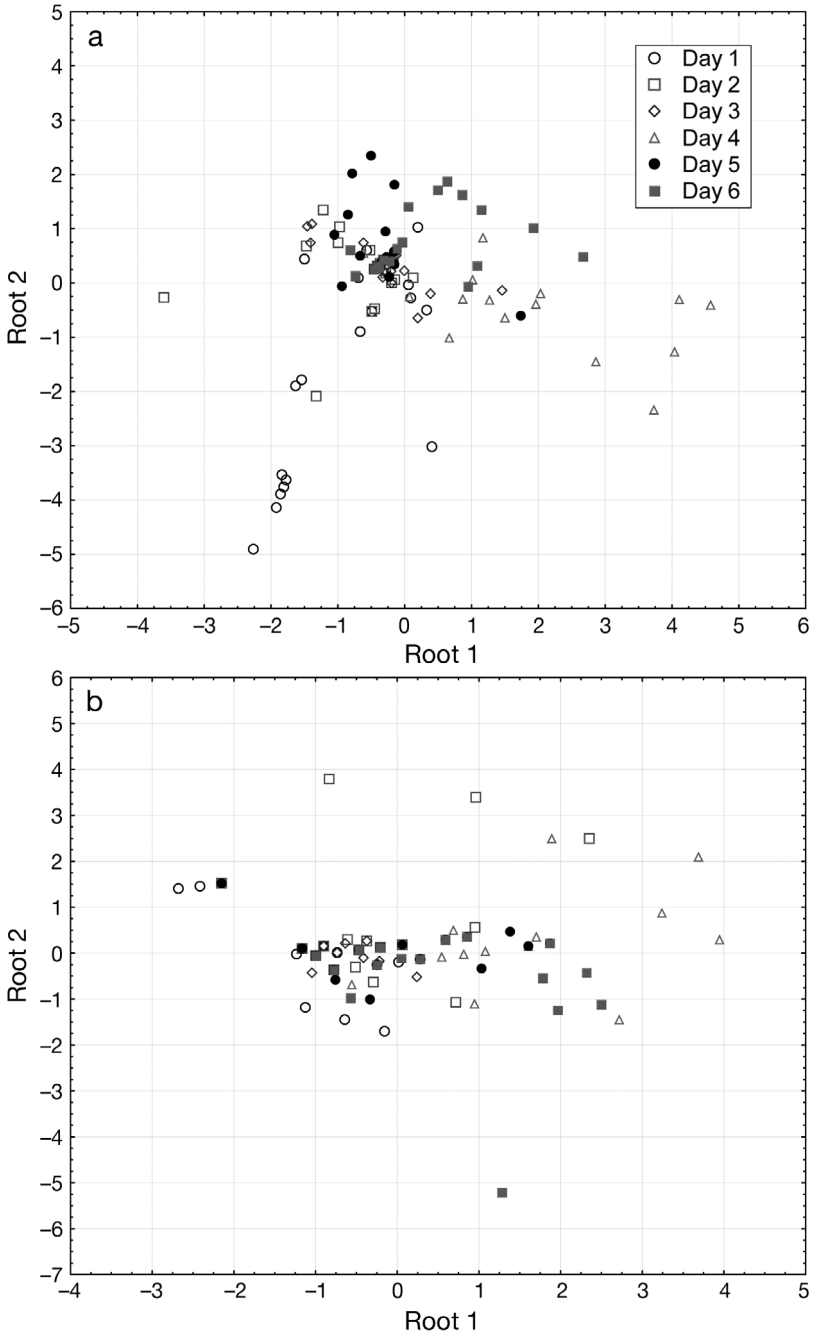

Fig. 2. Discriminant function plots for parasite community over the 6 d of the experimental holding period (a: cold season; b: warm season)

Tables 2 & 3). Similarly, *D. formosus* increased in both abundance and prevalence from D2 onward in the warm season, peaking by D4.

In addition to such factors as stress-induced immunosuppression, which affects the transmission and dissemination of monogeneans (Harris et al. 2000), or high fish density in the holding tanks, water temperature is also likely to have played an important role in the rapid reproduction of gyrodactylids in the cold season and dactylogyrids in the warm season. Throughout the experiment, all fish were held in outdoor tanks with natural levels of light and temperature. Any changes observed in the number of cryophilic and thermophilic parasite species, therefore, were seasonally dependent. Gyrodactylids are cryophilic parasites, increasing their numbers under cold conditions and decreasing with heat (Chubb 1977). *Apiosoma* are also cryophilic, increasing in number from October to January (Özer & Erdem 1998). This relationship with temperature explains the absence of these parasites in our samples during the warm season (see Table 3). Whilst gyrodactylids showed a gradual increase in reproduction during holding, sessile *Apiosoma* ciliates only appeared on D4, probably as their density in the storage basin increased beyond a threshold where they start to act as parasites. Hence, *Apiosoma* infection served as an example of infection caused directly by storage conditions, in contrast to other species that 'appeared' in our samples later than D1, a phenomenon attributable to stochasticity and high species turnover in the cold season (Table 2).

In the same way, the increase in temperature during the warm season accelerated development of thermophilic dactylogyrids (Chubb 1977, Koskivaara et al. 1992), as observed for *D. formosus* after 4 d of holding. According to Prost (1963), the temperature optimum for dactylogyrid reproduction (*D. anchoratus*) is 23°C. High June air temperatures during this study occasionally caused water temperatures to reach 27°C in the basin, thereby facilitating dactylogyrid reproduction.

Holding period significantly affected parasite community diversity in this study. Variation in parasite communities is caused by many factors, including niche competition between parasites (Kennedy & Bush 1994, Poulin 2001), host density and distribution (Rohde 2005) and temperature (Chubb 1977, Šimková et al. 2001). Accordingly, the parasite community in our study tended to be more stable during the

cold season, with diversity indices comparable for the first 4 d. On the other hand, high temperatures during the warm season led to rapid reproduction of dactylogyrids, resulting in a rapid increase in dominance and a decrease in the Shannon and species evenness scores by the second day of holding (D2).

Our results indicate that parasitological dissections of fish (especially in studies concerning ectoparasites) are best undertaken within the first 3 d after sampling. Thereafter, the abundance of some parasite species can differ significantly to those observed on D1, thereby affecting overall parasite community structure results. As such, we do not recommend parasitological dissection of fish held under artificial conditions longer than 3 d. Note also that the conditions fish are held under during this period (e.g. fish density and water temperature) will also play an important role in how the parasite community varies. We strongly recommend, therefore, that parasitologists, where at all possible, adjust the number of fish taken from the field to their capacities (staff number, storage capability) and to the presumed dissection period per fish (depending on size of fish and assumed number of parasites) such that all fish taken are processed within 72 h of catching (or 24 h during warmer periods). If the team's capacities are insufficient to guarantee timely dissection of all fish needed, then sampling should be spread over several days. In each case, this may involve a trade-off between an increase in expenditure and securing results unbiased by long fish storage.

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