

Comparison of parasite communities in native and introduced populations of sexual and asexual mollies of the genus *Poecilia*

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The parasite communities of two molly species, the sexual *Poecilia latipinna* and the clonal *Poecilia formosa*, were investigated in four populations using a novel method applicable under field conditions. In two native populations from south Texas and two introduced populations from central Texas, four species of microparasites and eight species of macroparasites were recorded. Virtually no differences in the parasite diversity and species composition could be detected between the populations. Mollies from south Texas had a higher individual parasitization index of macroparasites. There was a negative correlation between the relative number of oocytes in gravid females and their individual macroparasite load.

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Key words: fitness consequences of parasites; gynogenetic reproduction; *Poecilia formosa*; *Poecilia latipinna*; spatial variation in parasite infection.

INTRODUCTION

Parasites are widely recognized as an important factor in evolution (Bush *et al.*, 2001). The selective force of parasites is thought to be a driving force for the evolution and maintenance of sexual reproduction. According to the Red Queen hypothesis (Van Valen, 1973), sexual reproduction is an adaptation to the constant selection pressure of fast evolving parasites on common host genotypes (Hamilton, 1980; Seger & Hamilton, 1988; Hamilton *et al.*, 1990). Parasites therefore could play a role in the stability and coexistence of asexual and sexual species complexes.

In fishes, there are several examples of sexual species coexisting with asexual sister species, most of which reproduce by gynogenesis (Vrijenhoek *et al.*, 1989).

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All-female, gynogenetic forms are asexual but require sperm of heterospecifics as a stimulus to trigger embryogenesis. Theoretically, the gynogenetic form should outcompete the sexual form from which it gets sperm due to the two-fold advantage of asexual reproduction: asexual females produce only daughters, whereas sexual females produce 50% dispensable males. Because the gynogens are sperm dependent both forms will finally become extinct (Maynard Smith, 1978; Ladle, 1992). The benefit of recombination for sexuals in the presence of parasites, however, might outweigh the two-fold cost they face.

Mollies are small livebearing fishes with internal fertilization. The Amazon molly *Poecilia formosa* (Girard) is one of the few gynogenetic vertebrates (Balsano *et al.*, 1989; Schlupp *et al.*, 1998). *Poecilia formosa* originated from a natural hybridization of a *Poecilia latipinna* (LeSueur) (sailfin molly) -like male and *Poecilia mexicana* Steindachner (Atlantic molly) -like female (Avise *et al.*, 1991; Schartl *et al.*, 1995). The Amazon molly occurs in syntopy with at least one of its sexual parental species and uses the males as sperm donors. In the northern part of its distribution in south Texas, the Amazon molly lives together with the sailfin molly whereas in the southern part (northern Mexico), it occurs together with the Atlantic molly (Schlupp *et al.*, 1998, 2002). Morphologically, *P. formosa* is intermediate to its parental species, but it cannot be distinguished ecologically. The Amazon molly lives in the same microhabitats and forms mixed groups together with the corresponding parental species (Hubbs, 1964; Balsano *et al.*, 1989; Schlupp *et al.*, 1998). Therefore, the exposure to parasites is assumed to be more or less identical.

Poecilia latipinna and *P. formosa* occur naturally in the same habitats in south Texas, but in central Texas, they were introduced (Brown, 1953). *Poecilia latipinna* was introduced from Louisiana in the 1940s and established a thriving population, whereas *P. formosa* was introduced from Brownsville, south Texas in the 1950s (Schlupp *et al.*, 2002). The introductions in central Texas can be viewed as a natural experiment since the *P. latipinna* that now serve as sperm donors in these habitats did not co-evolve with *P. formosa*. The situation is also interesting parasitologically and raises the question whether the parasite communities differ between native, co-evolved populations and introduced molly populations of recent sympatry.

The aim of the present study was to survey the composition and diversity of the parasite community of *P. latipinna* and the asexual *P. formosa* in their natural habitats. Two native and two introduced molly populations from south and central Texas were compared. Furthermore, a novel method to quantitatively assess the individual parasite load in the field is presented.

MATERIAL AND METHODS

FISH COLLECTION AND FIELD SITES

Fishes were collected from four populations in Texas, U.S.A., during spring 2004. Only adult individuals with a standard length (L_S) of >30 mm were included in this study. Mollies were collected from native populations in a ditch near Weslaco (WES) and in Brownsville (Lincoln Park, LKP) in south Texas (Fig. 1). The field site in Weslaco was part of an irrigation system and the one in Lincoln Park an oxbow lake of the Rio Grande. Both water bodies had very muddy ground, standing water, were



FIG. 1. Map of the collection sites in south (LKP and WES) and central Texas (Co101 and SMA). In central Texas, both molly species have been introduced.

highly turbid and heavily affected by human activities such as settlements and agriculture.

About 600 km north of the south Texan field sites, introduced mollies were collected at two sites of the San Marcos River, near San Marcos (County Road 101, Co101 and Martindale, SMA; Fig. 1) in central Texas. The collection site at County Road 101 was a section of the San Marcos River a few kilometres downstream from its spring. The ground consisted of bedrock and the current was relatively strong. The field site at Martindale was a small stream feeding into the San Marcos River. The ground was muddy, current was low and the whole area was densely vegetated. Both habitats were relatively undisturbed.

The populations in south Texas were sampled once. The populations from central Texas were sampled repeatedly for 7 weeks. All fishes were caught with small seines and then placed together into a previously prepared and temperature-isolated container with water from the collection site. A portable air pump provided oxygen.

DATA COLLECTION

To avoid artefacts of maintenance, the collected mollies were killed using an overdose of MS-222 (tricaine methanesulphonate) and checked immediately for parasites. Most specimens were processed within 10 h and all were inspected within 30 h after collecting. No laboratory was available in south Texas, thus all equipment necessary was installed in a motel room so that all fishes could be processed immediately. In central Texas, the collected fishes were transported within 1 h to a laboratory of the University of Texas in Austin.

Fishes were examined using a standardized procedure. Methods suggested by Amlacher (1992) and Roberts & Smail (2001) were modified as follows: (1) the L_S of the fish was measured and the body was controlled for signs of parasite infections. Externally visible parasites were counted; (2) a skin smear was prepared by scraping it with a scalpel from the edge of the operculum to the beginning of the caudal fin. This preparation was examined with a compound microscope for 210 s per sample; (3) two gill arches were selected at random and the gill filaments were gently separated using micro-scissors and placed on a slide. The gill filaments were also examined with a compound microscope for 210 s; (4) the body cavity was opened, the intestine was straightened to its full length and intestinal contents were squeezed out using a preparation needle. The intestine as well as its contents were examined with a compound microscope for 210 s. Parasites were identified using Untergasser (1989), Amlacher (1992), Bunkley-Williams & Williams (1994), Roberts (2001) and Baur & Rapp (2003) as references and documented using an Olympus C-3020 digital camera. The reproductive output of females was estimated as the number of oocytes or developing embryos in females with fully developed ovaries from central Texas.

DATA ANALYSIS

The prevalence and the mean intensity of all parasite species found in the samples of *P. latipinna* and *P. formosa* were calculated (Bush *et al.*, 1997). For the comparison of micro- and macroparasite diversity of each population, the Shannon-Wiener diversity index (H) and the evenness index (J) were calculated (Begon *et al.*, 1996).

To compare the total parasite burden between populations, an individual parasitization index (I_{PI}) was separately calculated for micro- and macroparasites following Kalbe

et al. (2002): $I_{PI} = \sum_{i=0}^{i=n_p} (10s_{mi}^{-1} \cdot n_i s_{ti}^{-1})$, where n_i is the individual number of a parasite i , n_p is the number of parasites entering the index, s_{ti} is the s.d. of parasite i in all fish present in the data set and s_{mi} is the maximum of the term $n_i s_{ti}^{-1}$ for parasite species i . Because of the infrequent and rare occurrence of some parasites, only the most prevalent species (overall prevalence >5%) were included in I_{PI} . The I_{PI} was then analysed using GLM with I_{PI} as a dependent factor, species as a fixed factor, population as a random factor and L_S as a covariate.

To test whether there was a correlation between I_{PI} and the reproductive output of females, a non-parametric correlation analysis (Spearman's rank correlation) with oocyte number and I_{PI} was conducted. To correct for size effects, residuals were calculated using a linear regression with L_S as an independent factor and number of oocytes as a dependent factor. Significance tests were one-tailed in these analyses. Alpha levels were corrected according to the number of multiple comparisons using Bonferroni adjustments [$\alpha' = 0.05$ (number of multiple comparisons) $^{-1}$].

RESULTS

One hundred and seventy six mollies were examined (66 *P. latipinna* and 110 *P. formosa*). In 18 *P. latipinna* (27%) and 34 *P. formosa* (31%) no parasites could be detected. The proportion of unparasitized individuals was significantly higher

TABLE I. Prevalence (P) and mean intensity (MI) ± s.d. of parasites in four populations of *Poecilia latipinna* and *Poecilia formosa* in central and south Texas (see Fig. 1). Parasite location on the host: G, gills; I, intestine; M, mesenteries; S, skin

Parasite species	Location on host	CO 101 (Central Texas)						SMA (Central Texas)						LKP (South Texas)						WES (South Texas)						
		<i>P. latipinna</i>		<i>P. formosa</i>		<i>P. latipinna</i>		<i>P. formosa</i>		<i>P. latipinna</i>		<i>P. formosa</i>		<i>P. latipinna</i>		<i>P. formosa</i>		<i>P. latipinna</i>		<i>P. formosa</i>		<i>P. latipinna</i>		<i>P. formosa</i>		
		N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	N	P (%)	MI ± s.d.	
Flagellata																										
<i>Ichthyobodo</i> sp.	G	5	1-00			4	4-00 ± 2-65																			
(<i>Costia</i> sp.)																										
<i>Oodinium</i> sp.	S	5	1-00	15	5-00 ± 2-65	7	2-50 ± 0-71	10	3-14 ± 2-19																	
Ciliates																										
<i>Ambipyrva</i> sp.	G	5	1-00	20	3-50 ± 1-29	14	2-50 ± 1-29	18	4-08 ± 2-90	40	5-00 ± 4-24	50	2-00 ± 0-00	57	7-13 ± 7-49	21	2-00 ± 1-00									
(<i>Scyphidia</i> sp.)																										
<i>Trichodina</i> sp.	S, G	32	2-33 ± 2-42	55	10-82 ± 9-86	39	5-27 ± 5-39	46	6-70 ± 5-75	100	4-80 ± 3-83	50	1-00	57	5-38 ± 5-07	43	2-33 ± 1-97									
Monogenean trematodes																										
<i>Dactylogyrus</i> sp.	G	5	1-00	40	1-25 ± 3-54			7	4-20 ± 2-17																	
Digenean trematodes																										
<i>Echinochasmus donaldsoni</i>	G			4	12-00					100	5-40 ± 1-14	75	8-33 ± 6-66													
<i>Posthodiplostomum minimum</i>	M	5	8-00	5	3-00			7	3-20 ± 2-76	60	1-67 ± 1-16															
<i>Uvulifer ambloplitis</i>	S	16	1-33 ± 0-58	5	1-00	21	1-50 ± 0-84	19	1-50 ± 0-65	20	1-00	75	1-67 ± 1-16	36	2-20 ± 2-17	71	4-90 ± 3-93									
Unidentified trematode cyst	I	11	1-50 ± 0-71	10	1-00	18	7-60 ± 9-76	15	8-27 ± 6-33	60	4-67 ± 1-53	75	3-00 ± 1-00	7	4-00											
Other helminth parasites																										
Unidentified nematode	I	5	4-00																							
<i>Acanthocephalus cf. alabamensis</i>	I																									
Copepoda																										
<i>Lernaea</i> sp.	S																									

TABLE II. Range of number of parasite species on each host (N), Shannon–Wiener diversity index (H) and the evenness index (J) for the sampled populations (see Fig. 1)

Population	N	Microparasites		Macroparasites	
		H	J	H	J
CO 101	0–3	0.65	0.47	1.11	0.69
SMA	0–6	0.82	0.59	1.17	0.73
LKP	0–2	0.65	0.93	1.01	0.73
WES	0–3	0.73	0.67	1.12	0.57

in central Texan habitats (65% at County Road 101, 55% at Martindale) than in those from south Texas (0% at Lincoln Park, 8% at Weslaco; χ^2 , d.f. = 3, $P = 0.003$). Twelve parasite species were recorded. Prevalence and mean intensity of all parasites as well as their location on the host are listed in Table I. Depending on the population, the hosts harboured from 0–2 to 0–6 parasite species per individual (Table II). The Shannon–Wiener diversity and the evenness indices for macroparasites and microparasites were similar in all four populations (Table II).

Ambiphyra sp., *Oodinium* sp. and *Trichodina* sp. were the most prevalent microparasite species (prevalence >5% in the whole dataset) included into the I_{PI} for microparasites. *Dactylogyrus* sp., *Echinochasmus donaldsoni*, *Posthodiplostomum minimum*, *Uvulifer ambloplitis* and unidentified trematode cysts were included into the I_{PI} for macroparasites. Analysing I_{PI} using GLM showed that neither the three-way nor the two-way interactions were significant, so that only the main effects were analysed. The main effects had no significant influence on I_{PI} for the microparasite species (Table III). The I_{PI} of the macroparasites, however, was significantly influenced by the factor population (Table III). The populations in south Texas (mean \pm s.d.; LKP: 2.095 ± 1.351 ; WES: 1.123 ± 1.394) had a higher I_{PI} for macroparasites than the populations in central Texas (CO101: 0.662 ± 1.804 ; SMA: 0.410 ± 1.226). The factor species had no influence on I_{PI} for micro- and macroparasites (Table III).

Eighty three females (20 *P. latipinna* and 63 *P. formosa*) from central Texas had ripe oocytes or developing embryos in their ovaries. The number of oocytes increased significantly with L_S (Fig. 2; $r = 0.338$, $P < 0.001$; $\alpha' = 0.025$). This

TABLE III. Comparison of the individual parasitization index (I_{PI}) using GLM

Factor	Microparasites			Macroparasites		
	d.f.	F	P	d.f.	F	P
Population	3	0.910	0.437	3	5.722	0.001
Species	1	1.341	0.249	1	1.402	0.238
L_S	1	0.911	0.341	1	0.181	0.671

L_S , standard length.

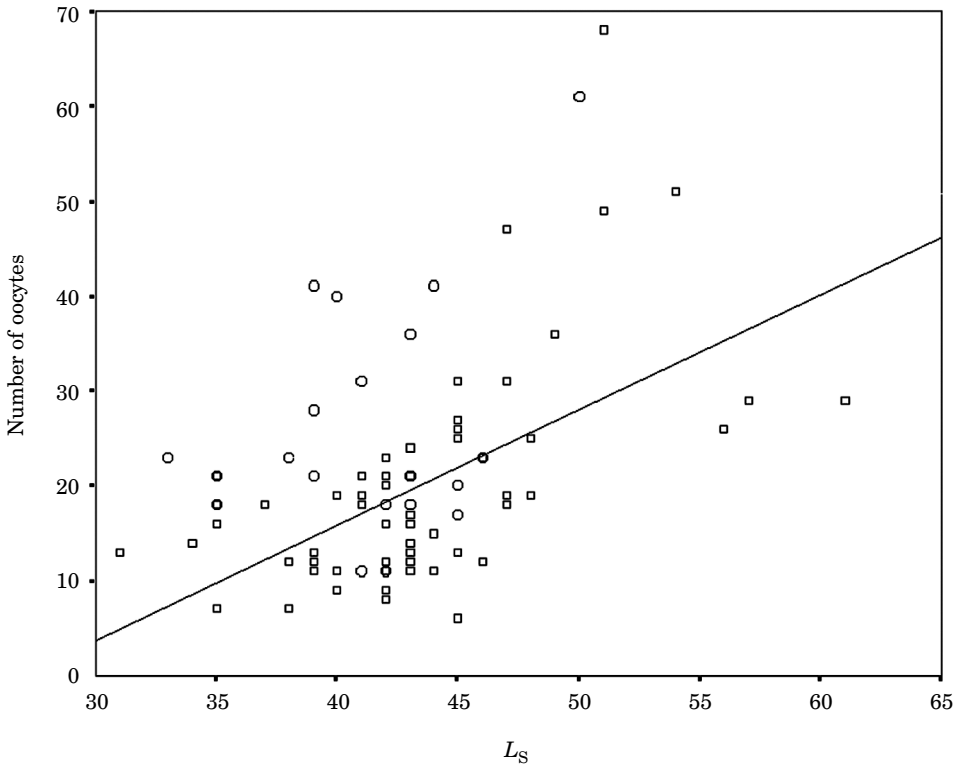


Fig. 2. The number of oocytes or developing embryos correlated positively with the standard length of a female. In *Poecilia formosa* (□—) this correlation was significant ($y = 1.21x - 32.60$; $r = 0.545$, $P < 0.001$), but not in *Poecilia latipinna* ($r = 0.039$, $P = 0.436$; ○).

correlation, however, was only significant for *P. formosa* when species were treated independently (Fig. 2; $r = 0.039$, $P = 0.436$ for *P. latipinna*; $r = 0.545$, $P < 0.001$ for *P. formosa*; $\alpha' = 0.025$). The I_{PI} for microparasites did not correlate with the relative number of oocytes of a female ($r = -0.133$, $P = 0.116$; $\alpha' = 0.025$; if species were treated separately: $r = 0.530$, $P = 0.339$ for *P. formosa*; $r = -0.395$, $P = 0.042$ for *P. latipinna*; $\alpha' = 0.025$). In contrast, females with a high I_{PI} for macroparasites had significantly fewer oocytes than females with a low I_{PI} (Fig. 3, $r = -0.249$, $P = 0.012$; $\alpha' = 0.025$). If both species were treated separately, this correlation was only weakly significant in *P. formosa* (Fig. 3; $r = -0.001$, $P = 0.498$ for *P. latipinna*; $r = -0.239$, $P = 0.030$ for *P. formosa*; $\alpha' = 0.025$).

DISCUSSION

Up to six different parasite species were detected in individuals of the molly species *P. latipinna* and *P. formosa* in nature. The L_S of a molly had no influence on the I_{PI} indices in the analysis. If males were excluded from the dataset, however, L_S had a significant influence on the index indicating that macroparasites are acquired and accumulated with increasing size in females (Tobler &

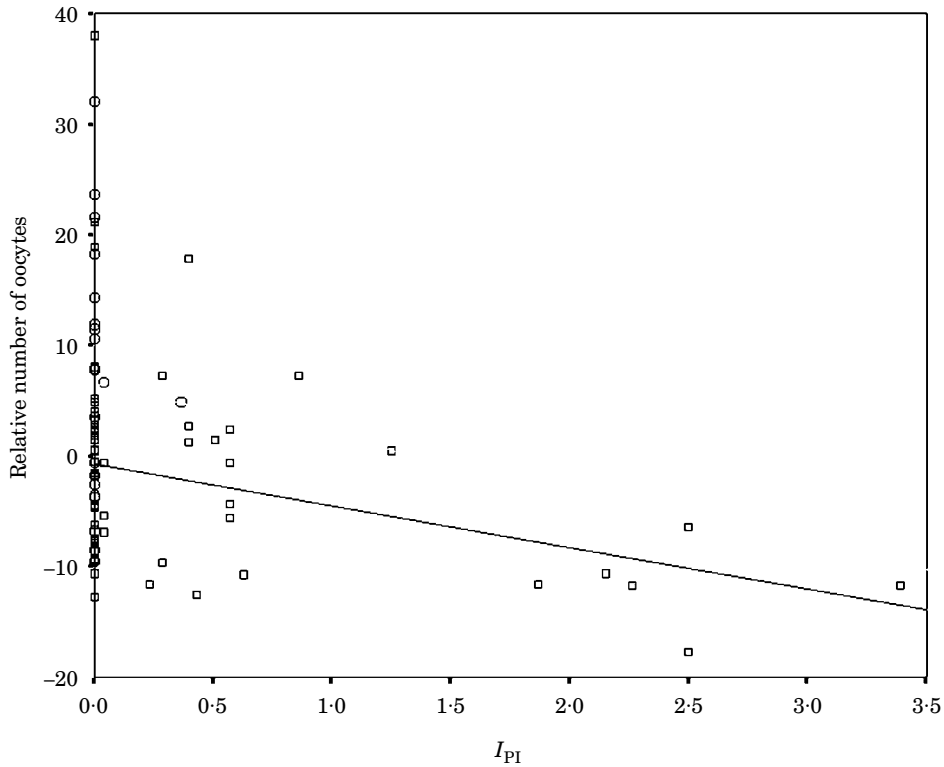


FIG. 3. The relationship between relative number of oocytes and the individual parasitization index (I_{PI}) for macroparasites. In *Poecilia formosa* (□ —), females with a high I_{PI} had significantly fewer oocytes than females with a low I_{PI} ($y = -3.78x - 0.69$; $r = -0.239$, $P = 0.030$). In *Poecilia latipinna* (○), however, this correlation was not significant ($r = -0.001$, $P = 0.498$).

Schlupp, 2005). Other than females, males cease growing when they reach sexual maturity. Thus the presence of males in the data set abolished the correlation of age and I_{PI} .

Most importantly, the two fish species studied here were parasitized by the same parasite species and did not differ consistently in their parasite load, although one is an asexual species. The Red Queen hypothesis predicts differences in how well a species can deal with parasites and generally asexuals are predicted to have higher parasite loads (Van Valen, 1973; Hamilton, 1980; Seger & Hamilton, 1988; Hamilton *et al.*, 1990), a pattern that was not found in this study. Due to the presence of males in the dataset, however, this analysis is not suitable for a direct test of the Red Queen hypothesis (Tobler & Schlupp, 2005). Testosterone is assumed to have a negative effect on immune function so that in adult fishes there are often differences in intensity and species composition of parasites and their impact on the host physiology between the sexes (Alexander & Stimson, 1988; Folstad & Karter, 1992). Sex differences in parasitization remain to be studied in this system. Furthermore, the even degree of parasitization might also be explained by parasite induced mortality (McKeown & Irwin, 1997). It was not possible to exclude this hypothesis based on field data.

There were no major differences in the parasite diversity between the parasite communities of the populations examined. Moreover, the parasite species recorded in the native molly populations from south Texas were the same as those from central Texas, where they were introduced in the mid 20th century. The few exceptions (*Acanthocephalus* cf. *alabamensis* and *Lernaea* sp. were not recorded in central Texas, *Oodinium* sp. not in south Texas) were rare parasites that occurred only with low prevalences in the other populations and thus might have been missed due to chance. The parasite communities were likely to be similar because almost all recorded parasite species have a wide geographical distribution and infect a vast variety of fish species. More specific parasites (like the dactylogyrid and the trichodinid species) could have been introduced to central Texas together with the founder populations of mollies.

In the GLM, the factor population had a significant influence only on the I_{PI} for macroparasites. Mollies from the native populations in south Texas had more macroparasites than those from central Texas. Furthermore, in central Texan populations, a higher proportion of the molly population was unparasitized. Differences in parasite loads between different habitats have also been documented for other fishes (Kalbe *et al.*, 2002; three-spined sticklebacks *Gasterosteus aculeatus* L.). This difference may be explained by several hypotheses, which are not mutually exclusive and cannot be verified on the basis of field data. Most likely, the higher I_{PI} in mollies of south Texas was due to environmental differences. The habitats in south Texas were more disturbed. The water was very turbid and temperature as well as oxygen levels were often extreme with strong seasonal fluctuations of the abiotic conditions (K.U. Heubel & I. Schlupp, unpubl. data). Furthermore, differences in the habitat structure, standing water in south Texan and flowing water in central Texan habitats, may be relevant for the transmission rate of many infective parasite stages. In this case, however, environmental factors cannot be separated from the 'residence status' (*i.e.* native *v.* introduced) of the molly populations. The parasites in south Texas might be better adapted to the mollies since there has been a long co-evolutionary history between the parasites and their hosts. Contrary, in central Texas where the mollies have been introduced only recently, the local parasites may be less adapted to the new hosts.

Host reproduction can be affected in many ways by parasitism and often the host's reproductive success is decreased. The negative effects range from castration to reduced levels of reproductive output (Hurd, 1993; Poulin, 1998; Lafferty *et al.*, 2000; Bush *et al.*, 2001). In the gravid mollies, there was a negative correlation between the macroparasitization I_{PI} and the numbers of oocytes, indicating a possible fitness cost for females. In future studies, not only egg number but also relative ovary mass must be taken into account since parasites might affect also size of eggs or embryos. In three-spined sticklebacks for example, egg size is reduced in females infected with a cestode parasite (Heins & Baker, 2003).

The method applied may underestimate the actual parasite diversity and load compared with classical parasitological methods, but is nonetheless suitable to examine a reasonable number of individuals in the field and the results gathered in this way can be quantitatively analysed for comparative studies. In future, more detailed studies should investigate the effects of parasites on the host's life

history, seasonal variation of parasitization and environmental effects on parasitization. Furthermore, this system is suited to investigate the role of parasites for the stability of gynogenetic systems and the maintenance of sexual reproduction in general.

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