**Potential interactions and aggregation in low-diversity monogenean and endohelminth communities in *Pseudoxiphophorus bimaculatus* (Teleostei: Poeciliidae) populations in a neotropical river.**

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**ABSTRACT**

1. The role of interspecific interactions in structuring low-diversity helminth communities is a controversial topic in parasite ecology research. Most parasitic communities of fish are species poor; thus, interspecific interactions are believed to be unimportant in structuring these communities.

2. We explored the factors that might contribute to the richness and coexistence of helminth parasites of a poecilid fish in a neotropical river.

3. Repeatability of community structure was examined in parasitic communities among 11 populationsof *Pseudoxiphophorus bimaculatus* in the La Antigua River Basin, Veracruz, Mexico. We examined the species saturation of parasitic communities and explored the patterns of species co-occurrence. We also quantified the associations between parasitic species pairs and analysed the correlations between helminth species abundance to look for repeated patterns among the study populations.

4. Our results suggested that interspecific competition could occur in species-poor communities, aggregation played a role in determining local richness, and intraspecific aggregation allowed the coexistence of species by reducing the overall intensity of interspecific competition.

**INTRODUCTION**

Parasitic systems enable us to explore the essential aspects of ecology (Poulin & Morand, 2004; Poulin, 2007). It is important to understand how local communities are configured and the interactions among species within a region. Holmes & Price (1986) recognised both interactive and isolationist parasitic communities. In the former, between-species interactions are important for structuring the community. In the latter, inter-species interactions play non-detectable roles and the influence of competition is negligible (Poulin, 2007). Thus, the presence of any species is independent of the presence of other species in isolationist communities (Rohde, 1979; Price, 1980). In species-rich parasitic communities that have high abundances, such as bird communities, both interspecific and intraspecific interactions among parasites are important forces that structure the community (e. g. Bush & Holmes, 1986; Stock & Holmes, 1988). In some fishes, mostly elasmobranchs, rich helminth communities have been described and interactions between species have been documented (e. g. Randhawa, 2012; Agrawal et al., 2017).

The importance of interspecific interactions for community structuring in low-diversity helminth communities remains a controversial topic. Caswell (1976) suggested that non-interactive communities lack saturation and species can coexist in the community because space is not a limiting factor. Most parasitic communities of fish are species poor and unsaturated with species; therefore, interspecific interactions are not important in structuring such communities (e. g. Gotelli & Rohde, 2002; Muñoz et al., 2006). Low-diversity parasitic assemblages are mostly structured by intraspecific, rather than interspecific, interactions (e. g. Haukisalmi & Henttonen, 1993; Morand et al., 1999). However, Kennedy (1992) suggested that interspecific competition can occur in species-poor isolationist communities. Additional empirical support by Vidal-Martínez & Kennedy (2000) showed that even relatively small numbers of acanthocephalans can produce a displacement of phylogenetically unrelated intestinal helminths (trematodes and nematodes) in a tropical cichlid fish, *Cichlasoma synspilum*. We recently described the potential interactions in low-diversity monogenean parasitic communities in a tropical freshwater fish, *Astyanax aeneus*, and showed (Salgado-Maldonado et al., 2019) that interspecific interactions can be an important factor for structuring low-diversity ectoparasitic helminth communities. Thus, the extent that interspecific interactions are important structuring factors for low-diversity fish ecto- and endoparasite communities remains uncertain.

Interspecific interactions may lead to species exclusion; however, there are several ways in which species can coexist (Morand et al., 1999). Aggregated resource use may reduce the overall competition intensity and is key to local parasite (monogeneans) richness in fish populations (Morand et al., 1999; Simková et al., 2000, 2001; Agrawal et al., 2017). Intraspecific aggregation allows the coexistence of species that would otherwise be excluded. More parasitic species can coexist in the same host population when the distributions between individual hosts are aggregated (Ives, 1988, 1991). The host population represents a collection of patches of resources among which the parasites are heterogeneously distributed. Some patches (i.e. hosts) have many individuals (parasites), whereas others have only a few. Aggregation thus refers to the degree to which individuals are added between patches (Ives, 1991). Generally, parasitic populations are distributed in an aggregated manner among individual hosts (i. e. the majority of hosts have a few parasites and most parasites are concentrated in a few hosts) (Poulin, 1998; Poulin & Morand, 2004). Aggregation is the most common feature of metazoan parasitic infections (Poulin, 1993; Simková et al., 2000).

To assess the repeatability of community structure in space, we examined parasitic community organisation among 11 populationsof *Pseudoxiphophorus* *bimaculatus* (Heckel, 1848) (Teleostei: Poeciliidae). We examined species richness patterns and whether the parasitic communities were species saturated and explored patterns of species co-occurrence. We quantified the associations between parasitic species pairs (e.g. Haukisalmi & Henttonen, 1993; Dezfuli et al., 2001), considering that positive or negative associations between parasitic species suggested a departure from random co-occurrence (Poulin, 2001, 2007). Further, we analyed the correlations between the abundance of different helminth species and whether the observed patterns were repeated across the study populations. Our study model consisted of many patches that were identical in resources (hosts) and sustained several helminth populations.

The level of competition that a helminth experiences depends on the number and species of helminths sharing the same patch (host), the distribution of helminths in those patches, and the number of hosts available to invade in each locality, i.e. the density of host species. We assessed the level of aggregation of helminth populations to test their influence on determining the local parasite richness within a host population. Further, we assessed whether intraspecific aggregation exceeded interspecific aggregation (e. g. Salgado-Maldonado et al., 2019). Our goal was to explore the factors that might contribute to the richness and coexistence of helminth parasites of *P*. *bimaculatus* across 11 localities in the La Antigua River, a neotropical system in Veracruz, Mexico. Populations of the poecilid *P*. *bimaculatus* in the La Antigua River were chosen for the present study because some aspects of their parasite community structure have been described previously, ng information on common and rare species (Salgado-Maldonado et al., 2014); however, information on community saturation, intraspecific and interspecific aggregation, and consistency of pairwise species associations is limited.

**METHODS**

**Study area**

The study was conducted at 11 sites located between 42 and 1245 m above sea level (a.s.l.) within the La Antigua River Basin (Fig. 1). The La Antigua River is a high-gradient foothill river originating from the Cofré de Perote volcano and adjacent mountains from the Sierra Madre Oriental (altitude 4200 m) in the states of Puebla and Veracruz, Mexico. Typical of rivers in hilly terrain, numerous headwater streams combine to form montane and piedmont canyons. The upper watershed of the La Antigua River covers a wide altitudinal range, from 480 to 4200 m a.s.l. before the river arrives at the coastal plain. The river runs approximately 100 km east of the Gulf of Mexico (Mercado-Silva et al., 2012).

**Host species and sampling**

*Pseudoxiphophorus* *bimaculatus* is distributed on the Atlantic slope, from the Misantla River, Veracruz, Mexico, southward to the Nombre de Dios ictio-province on the Caribbean side of Honduras (Matamoros et al., 2009). The species prefers well-shaded, slowly moving, fairly deep (up to 1.3 m) waters with fallen leaves and brush piles or overhanging riparian plants in creeks, lagoons, rivers, and swampy pools containing a variety of substrates. It feeds mainly on Culicidae (Diptera) (Trujillo-Jiménez & Toledo, 2007) and attains a maximum total length of 80 mm (Miller et al., 2005).

We examined 19 *P*. *bimaculatus* from Agua Bendita, 21 from Puente Nacional, and 20 from each of the other nine locations sampled in June 2016 (Fig. 1). Specimens were collected under collecting permit FAUT-0105. Fish were collected using DC backpack electroshockers, seines, and gill nets. The captured individuals were placed in plastic bags filled with water, transferred to the laboratory, and kept alive in aerated containers until subsequent examination for the presence of helminth parasites (within 8 h of capture). To complete the examination, the fish were euthanised with an overdose of anaesthetic with 2-phenoxyethanol (Sigma-Aldrich, St. Louis, Missouri), measured (total and standard lengths), and examined under a stereomicroscope in Petri dishes containing river water. Externally, the skin, scales, mouth, branchial cavity, anus, and fins of each host were examined. The branchial arches were removed, separated from the branchial cavity, and evaluated individually. All internal tissues, including the digestive tract, body musculature, and organs were examined for helminth parasites. The helminths that were obtained from the dissections were counted and recorded separately for each fish.

The overall parasite population structure was described using the following parameters: prevalence (percent of hosts infected), mean intensity (mean number of helminth individuals of a given species per infected host), and mean abundance (mean number of helminth individuals of a given species per examined host) as described by Bush et al. (1997). Analyses were conducted at two hierarchical community levels (Holmes & Price, 1986): 1) infracommunity that included the parasites of each fish examined and 2) component community that referred to the helminths of all hosts examined at each location and date. Because parasites inhabit different parts of the host and are not in contact with each other, we analysed the ectoparasitic monogeneans and endoparasites separately. Generally, ectoparasitic monogeneans and endoparasites are non-interactive communities because their transmission modes are different between the parasite groups. i.e. monogeneans infect a new fish directly (their life cycle does not require an intermediate host), whereas the transmission of endohelminths is mainly by the predation of infected intermediaries (Muñoz et al., 2006, however see Larsen et al., 2002).

**Data analysis**

(*a*) *Richness*

To assess the effectiveness of our sampling effort, all component communities were evaluated using species accumulation curves. In addition, we estimated the number of rare species that were not detected in each component community using the non-parametric Bootstrap estimator (see Supplementary information 1: Analyses of richness).

(*b*) *Saturation*

To explore local–regional richness relationships, we plotted the mean infracommunity parasite richness (local richness) versus the component community parasite species richness (regional richness) and calculated the function that best fit the data (Kennedy & Guégan, 1994; Cornell, 1996; Morand et al., 1999; Poulin, 2007). When local richness is regressed against regional richness and the relationship is linear, communities are unsaturated and exhibit proportional sampling of the regional species pool. If the relationship is somewhat curvilinear, the possibility of saturation may occur (Guégan et al., 2005). According to Morand et al. (1999), the dependence of infracommunity richness on the component community richness indicates non-saturation. The maximum observed infracommunity richness was examined because the co-occurrence of all species found in a component community in a single host individual is unlikely unless the prevalence is very high. A proportional relationship between maximum richness recorded in an infracommunity and the observed richness in the component community suggests that a maximum level of richness does not exist and is consistent with the absence of saturation in the communities (Morand et al., 1999).

(*c*) *Intraspecific aggregation*

We quantified the intra- and interspecific aggregation of helminths. We calculated the parameter J value for each helminth taxon (Table 1) as an intraspecific aggregation measurement that quantified the relative increase of conspecific competitors above the average number that a helminth experiences when infecting a new host. The J value is a measure of the proportional increase in the number of conspecific competitors that an individual helminth experiences from a random distribution. A value of J = 0 indicates that individual helminths are randomly distributed, whereas a value of J = 0.5 indicates a 50% increase in the average number of conspecific helminths expected in the patch (host) above what would be expected if the individuals were randomly distributed (Ives, 1988). In other words, J = 0.5 indicates a 50% increase in the aggregation of individuals of the same species in a host (Šimková et al., 2001).

(*d*) *Interspecific aggregation*

To measure the association between two species in each of the infracommunities, we calculated the C1,2 index (Table 1), which is a measure of the proportional increase in the number of heterospecific helminth competitors regarding a random association. C1,2 is the relative change in the average number of heterospecific helminths with which the helminths of species 1 has to compete when the species are not independently distributed (Ives, 1988). When C > 0, both species are positively correlated, and thus aggregated in the host (Ives, 1988). If C < 0, the species is negatively correlated and there is segregation between species. If C1,2 = 0.5, there is 50% of the expected number of heterospecific competitors in the host, above what one would expect if helminth species 1 and 2 were randomly distributed (Šimková et al., 2001).

(*e*) *Associations between pairs of parasite species and correlations*

The abundance of a parasite species in a host may depend on the presence or abundance of a second species. Identifying patterns of species co-occurrence and association can provide strong evidence of the importance of positive or negative interspecific interactions in structuring communities (Rohde, 1994; Dezfuli et al., 2001; Poulin, 2001, 2007). Pairwise analyses of species associations allow the identification of non-random patterns, with repeatability in space assessed across similar host populations to examine parasite community organisation (Poulin & Valtonen, 2002).

The quantification of associations between the pairs of parasite species represents a basic null model approach (Poulin and Valtonen, 2002). No association indicates that two parasite species are randomly distributed among hosts and a positive or negative association between parasite species suggests a departure from random occurrence (Vidal-Martínez & Kennedy, 2000; Dezfuli et al., 2001; Poulin, 2001, 2007; Poulin & Valtonen, 2002).

We used the Spearman’s rank correlation coefficient to evaluate the correlation between the intensities of two helminth species across hosts; we removed fish that were not infected by either of the two parasite species. In all cases, we indicated the statistical significance of the Spearman’s coefficient values with an asterisk: \* p < 0.05; \*\* p < 0.01, \*\*\* p < 0.001.

(*f*) *Decrease in competition*

To evaluate the decrease in competition owing to intraspecific aggregation, we compared the relative intensity of intraspecific aggregation versus interspecific aggregation in a pair of species, 1 and 2, by calculating the A1,2 (Table 1). If A1,2 > 1, intraspecific aggregation was greater than interspecific aggregation, and vice versa.

**RESULTS**

**Community composition**

A total of 18 helminth taxa were found in the present study (Table 2). Monogeneans were the most prevalent, abundant, and widely distributed group, being recorded in eight out of the 11 sampling locations. They occupied the highest number of patches (infracommunities and component communities) and were the most numerous in these patches (Table 3). Together, the four species of monogeneans found accounted for 43% (1048/2407) of all helminths collected in the study (Table S1 Supplementary information 1). One to four species of endohelminths were recorded from seven out of the 11 locations (Tables 3, S1). The four endohelminths (two adult trematodes and two adult nematodes) accounted for 16% (385/2407) of all helminth individuals. A third group of 10 taxa of helminths, including metacercariae and larval nematodes, accounted for 40% (974/2407) of all helminths. However, metacercariae of *Centrocestus* *formosanus* recorded from five localities accounted for 83% (811/974) of the larval helminths; the remaining nine taxa of this group were mostly rare and scattered in a few locations.

**Species richness and abundance**

A preliminary analysis of species richness (Supplementary information 1) indicated that our sampling effort was adequate because at least 70% of the helminth taxa available in each component were detected; only a few rare helminth taxa were likely to have been missed because of the number of hosts examined. The analyses allowed us to examine almost the entire composition of the helminth communities parasitising on the populations of *P*. *bimaculatus* along the La Antigua River Basin. Therefore, the patterns derived from the repeatability of community structure, species saturation, and species co-occurrence are based on the helminth species that were most characteristic in structuring the community.

Size ranges of the fish examined in the different localities varied from 30 mm to 100 mm total length (mean length of the 220 fish was 52.7 ± 13.4 SD mm). This variation was significant when the sizes of the fish were compared between localities (F = 6.1, p < 0.001). Tukey’s test showed that smaller fish were found in the Antigua Presa and Apazapán locations (Fig. S1 Supplementary information 2). However, the size class of fish remained consistent in each locality. Furthermore, neither helminth species richness nor abundance (total number of helminth individuals, monogeneans, or adult endohelminths, separately) correlated with the mean size of the fish examined in each locality (Figs. S2, S3 Supplementary information 2).

**Unsaturation of communities**

We did not find evidence of a curvilinear relationship between mean richness recorded in an infracommunity and component community richness for monogeneans or endohelminths (Fig. 2). For monogeneans, the proportion of variance in the distribution of observations that explained a curvilinear relationship was the same as that which explained a linear relationship (r2 = 0.15). For endohelminths, a high proportion of variance in the distribution of observations was explained by a linear relationship (r2 = 0.86). Thus, we did not find an upper limit of local species richness in the individual hosts in relation to the size of the regional pool of species.

The maximum richness of the infracommunities (in seven cases for the monogeneans and two cases for the endohelminths) was below that of the component communities (Table S1). However, we found a weak positive correlation between the observed richness of monogeneans (SOM) in the component community and the mean richness of monogeneans of the infracommunities (r = 0.38), as well as a very weak and negative correlation with the maximum richness recorded in an infracommunity (r = -0.13), i. e. increasing the monogenean richness of the component communities did not signify more species in the infracommunities. No correlation was found between the total number of individual monogeneans in the component community versus the observed richness of species of monogeneans in the component communities (r = 0.08) therefore, the populations of monogeneans may increase independently of richness. The maximum infracommunity richness in our study was limited only by the availability of species in the component communities.

We found a positive and very strong correlation between the observed richness of endohelminths (SOE) based on two trematode *Paracreptotrematoides heterandriae* and *Phyllodistomum inecoli* and two nematode species *Freitascapillaria moraveci* and *Spinitectus mexicanus* in the component community and the mean richness of the endohelminths in the infracommunities (r = 0.95\*\*). The correlation between the maximum richness recorded in an infracommunity was strong but not significant (r = 0.73 p = 0.07), suggesting, at least partially for endohelminths, that as the richness of the component communities increased, there were more species in the infracommunities. Our data also showed that the increase in the individual endohelminth species correlated positively and strongly with species richness (r = 0.91\*\*\*) and the mean endohelminth species richness in infracommunities increased with the total endohelminth individuals recorded in the component community (r = 0.93\*\*\*). These data suggest that the richness of endohelminths was density-dependent.

**Intraspecific aggregation of helminths**

Most (53/80) of the calculated J values were positive (Table 3, Fig. 3), (range 0.02 to 18.39). All endohelminth records showed aggregation. Species found in low numbers did not show aggregation (values J = 0 corresponded to records of a single parasitic specimen) (Table 3). Nine values of J < 0 belonged to a low number of infections by monogeneans (i.e. two to seven monogenean individuals distributed in approximately the same number of hosts, with J values ranging from -0.79 to -0.42).

J values for the three *Gyrodactylus* species suggested they were density-dependent. We identified significant and positive correlations between J values and the mean intensity of each species in the component communities where they were recorded (*G*. *xalapensis* r = 0.95\*\*\*, *G*. *takoke* r = 0.85\*\*, *Gyrodactylus* sp. r = 0.65\*). A very strong positive correlation between J values and the mean intensity of the invasive metacercariae *C*. *formosanus* was also found, r = 0.90\*\*. This pattern of density-ependence was not found in any of the other helminths studied.

**Interspecific aggregation of helminths**

A high proportion of interspecific association index C1,2 values were < 0, indicating between-species segregation (i. e. a high proportion of the analysed species pairs were negatively correlated). We calculated 77 values of interspecific aggregation between 16 pairs of species including all the registered monogeneans, *C*. *formosanus,* and the endohelminth taxa in each location (Table 4). Seventy seven % (47/61) of associations involving ectoparasitic monogeneans and metacercariae of *C*. *formosanus* were negative (C1,2 < 0) (Table 4). The calculated values of C1,2 in these cases suggested the presence of two species in the same component community and co-infections in a few infracommunities. The inclusion of *C*. *formosanus* in these calculations could be justified because they encyst in the gills and alter the tissues, as well as occupy space and negatively with monogeneans.

Interspecific aggregation C1,2 values were positively correlated with richness and abundance parameters. Considering the 61 calculated values of C1,2 for ectoparasites, monogeneans, and *C. formosanus*, we found moderate, significant positive correlations when regressed against the mean number of species per host (r = 0.49\*\*\*), the maximum number of species registered in an infracommunity (r = 0.56\*\*\*), the total number of individual monogeneans in the component community (r = 0.53\*\*\*), the mean number of individual monogeneans per host (r = 0.52\*\*\*), and the maximum number of monogeneans recorded in a host (r = 0.55\*\*\*). Density-dependence of C1,2 values was evident when each species pair of monogeneans was examined separately. From the analysis of all possible correlations of abundance and monogenean richness in the six possible pairs of monogenean species (Table 4), we found density-ependence in four of the pairs (Table 5). The species pairs *G*. *xalapensis* / *G*. *takoke* and *G*. *xalapensi* / *Gyrodactylus* sp. were recorded from each of the eight component communities; however, their calculated C1,2 values did not correlate with any of the richness or abundance parameters.

Most (11/16, 68%) interactions between the four species of endohelminths, two trematodes *Paracreptotrematoides heterandriae* and *Phyllodistomum inecoli* and two nematodes *Freitascapillaria moraveci* and *Spinitectus mexicanus*, were negative (value of C1,2 < 0), although 32% (5/16) had positive C1,2 values (Table 4). Co-infections of endohelminth taxa were recorded in one to five infracommunities. C1,2 values calculated for endohelminths were not density-dependent, i.e. they were not correlated with richness and abundance parameters in either component communities or infracommunities.

**Association between pairs of parasite species**

Associations among monogeneans were consistently recorded in several locations. A total of 78% (48/61) of the calculated correlations between 61 pairs of ectoparasitic monogeneans and *C*. *formosanus* metacercariae were negative Of the 12 pairs of endohelminths in the component communities, 83% (10/12) had negative correlations in the intensity of species; 10 of these comparisons for the ectoparasites and five for the endoparasites were significant (Tables 6, 7). Significant negative interactions were detected between the three species pairs of monogeneans, *Urocleidoides* *vaginoclaustroides* / *G*. *xalapensis*, *U*. *vaginoclaustroides* / *G*. *takoke*, and *U*. *vaginoclaustroides* / *Gyrodactylus* sp., and between *U*. *vaginoclaustroides* / *C*. *formosanus*; which were repeated in more than one system (Table 6). However, only one species pair of endohelminths *Phyllodistomum* *inecoli*/*Paracreptotrematoides* *heterandriae* showed significant negative interactions in more than one system (Table 7). Only one significant positive interaction was found (i.e., *P*. *inecoli* / *F*. *moraveci* in Agua Bendita r = 0.54\*). No correlation was found between the abundance of any of the species listed above with the size of the hosts (total length) when from each component community was analysed (Tables 6 and 7).

**Decrease in competition**

Most (42/77) A1,2 values calculated between the 16 pairs of species, including all monogeneans, *C*. *formosanus,* and endohelminth taxa in each location were > 1 (Table 4, Fig. 4). Therefore, intraspecific aggregations were stronger than interspecific aggregations. No correlation was found between the values of A1,2 and richness parameters (number of observed species in the community component, and mean and maximum of observed species per host) or with abundance parameters (total number of helminths recorded, and mean and maximum number of helminths per infracommunity). Therefore, the increase in diversity or abundance did not correlate with an increase in intraspecific aggregation compared to interspecific aggregation.

**DISCUSSION**

**Low diversity of helminth communities**

Our results showed that interspecific interactions play an important role in structuring the low-diversity helminth communities of a tropical freshwater fish and that interspecific competition can occur in species-poor, non-saturated communities. on is an important factor for determining the local richness of parasites in fish populations. Intraspecific aggregation allows the coexistence of species in the same host population by decreasing the overall competition intensity.

Low species richness and abundance of helminths were most evident in our study system. For the endohelminth communities, we recorded a mean species richness between 0.1 ± 0.2 and 1.55 ± 1.05 species among the 11 sampled localities and a mean infracommunity abundance between 0.1 ± 0.2 and 9.4 ± 9.8 for the total individuals. These are relatively low numbers compared to values for intestinal infracommunities of other tropical or subtropical fish species such as *C. pearsei* (3.6 ± 0.7 species; 353 ± 27 individuals, Pineda-López, 1994), *C*. *urophthalmus* (2.2 ± 0.65 species; 76.8 ± 66.0 individuals, Salgado-Maldonado & Kennedy, 1997), and *C. synspilum* (2.4 ± 1.2 species; 34 ± 52 individuals, Vidal-Martínez & Kennedy, 2000).

There is little data for comparisons of ectoparasite richness and density in freshwater fishes. Bellay et al. (2012) counted 6650 individual monogeneans from 13 taxa in 61 specimens of the piranha *Serrasalmus marginatus* from the Paraná River, Brazil. The mean number of monogeneans ranged from 64.4 to 156.9 individuals per fish. Agrawal et al. (2017) counted 10920 individual monogeneans of five species of *Thaparacleidus* parasites in 72 specimens of the Indian freshwater shark *Wallago attu*, with a mean of 151.6 monogeneans per examined fish. Our numbers are comparatively low ( 1048 monogeneans of four species from 220 examined hosts, with a mean between 1.45 ± 0.93 and 18.6 ± 23 monogeneans per infected host).

**Non-saturated communities**

No limitation was found in the number of species for either monogenean or endohelminths in the infracommunities, which was in agreeance with findings from previous studies (Rohde et al., 1995; Morand et al., 1999; Salgado-Maldonado et al., 2019). Therefore, the infracommunities were not saturated by local residents, rather, the infracommunity richness (local richness) was dependent on the size of the species pool of the component community (regional richness). Two additional observations pointed towards non-saturation. First, empty niches were observed because the maximum richness of the infracommunities was lower than that the component community.

Therefore, a maximum potential infracommunity richness was less likely in the studied communities, as expected if interspecific interactions among parasites were important and led to species saturation. Second, evidence was found that increasing the monogenean richness of component communities did not signify more species in the infracommunities. The proportional relationship between endohelminth richness in the component community and richness in the infracommunities also suggests that a maximum level of richness did not exist, which was consistent with the absence of saturation in the endohelminth communities. The tendency towards non-saturation in infracommunities was more obvious for the endohelminths than for the monogeneans; therefore, species interactions might be negligible. Rohde (1991) suggested that most gill parasite species live in low-density populations in resource-rich habitats and that sections of available niches for ectoparasites remain empty. However, an alternative explanation for our observations was that infracommunities appeared non-saturated owing to species exclusion following interspecific interactions.

**Interspecific relationships**

Contrary to the expectations for the impoverish, low-density, non-saturated communities, our results on species associations (expressed as negative associations between pairs of helminth species and the C1,2 index of interspecific aggregation values) provide overall support for the role of negative, probably competitive, interactions in shaping the helminth communities, especially among the monogeneans. We found consistent, although not always significant, negative correlations between the numbers of helminth pairs of helminth species. Five of the species pairs of monogeneans and one species pair of endohelminths yielded a significant negative correlation in more than one location.

Consistent negative interactions are strong evidence of competitive interactions between species (Poulin, 2001, 2007; Dezfuli et al., 2001; Poulin & Valtonen, 2002). We contend that these are not spurious covariances for three main reasons. First, most statistical methods that are used to detect species covariances are more sensitive to positive associations than they are to negative ones (Haukisalmi & Henttonen, 1998). Second, our data include more common species with high prevalence, which could lead to a high number of positive associations (Lotz & Font, 1994). Third, we did not include rare species recorded in the component community, which could have produced spurious negative associations (Lotz & Font, 1994). The role of host size as a potential confounding factor creating spurious covariances can also be dismissed because our results showed that the number of monogeneans in each species pair that exhibited significant negative correlations was not correlated with the size of *P*. *bimaculatus* examined at any given site. Therefore, the recorded negative covariances were independent of the possible accumulation of monogeneans in a larger host.

The number of negative covariances we found was notable because variance tests on binary presence–absence data for parasitic species in infracommunities (Schluter, 1984) indicate that the number of positive covariances equal the number of negative covariances if infracommunities are random assemblages, which was assumed in the present study as a null model when testing for pairwise associations. Thus, we assumed that the high number of negative correlations we recorded was indicative of the role of negative, probably competitive, interactions in shaping the helminth communities. However, the observed patterns of species associations must be tested against other adequate null models in future studies (Simberloff, 1990; Lotz & Font, 1994; Simberloff & Moore, 1997; Poulin, 1997).

We reported a high proportion of values of interspecific association index C1,2 < 0, indicating that there was segregation and that a high proportion of the analysed 16 pairs of species were negatively correlated in our communities. Given that the C1,2 index is a measure of the proportional increase in the number of heterospecific helminth competitors regarding a random association (Ives, 1988, 1991), both the C1,2 < 0 values and negative correlations between the actual parasite numbers of pairs of species as strong evidence for interaction in our communities. Therefore, the present results support previous conclusions by Kennedy (1985, 1992) and Vidal-Martínez and Kennedy (2000) that interspecific competition can occur in species-poor, isolationist, and non-saturated communities. Interspecific competition, and thus its detectability, may vary among locations with the abundance of species because the prevalence and intensity of infection affect the magnitude and direction of pairwise associations as well as their detectability (Lotz & Font, 1994; Poulin & Valtonen, 2002).

The observed negative associations of the species pairs of monogeneans in the communities might be caused by the transmission of monogeneans in clumps from fish to fish, which could lead to a transfer of associations (Lotz et al., 1995; Dezfuli et al., 2001). This was noted recently in a different host-parasite system, suggesting it could be a general pattern (Salgado-Maldonado et al., 2019). Therefore, associations between species were transferred from the existing associations by passive transportation of monogeneans from fish to fish. However, this also highlighted the role of competition in the monogenean community structure with interspecific interactions occurring in the actual fish host. When monogeneans effectively disperse and colonise free patches, they compete with one another (Slatkin, 1974; Ives, 1988). Given that we recorded a high consistency in the distribution of individuals of different species, we assumed that the transmission of some species of monogeneans may be combined so that the colonisation of new fish within a component or between components faces the problem of the arrival of two or more heterospecific individuals simultaneously. This spreading from a common source and joint colonisation suggests that an initial interspecific interaction would work to build these communities. This is because when the transmission of propagules is multiple or linked, these species will have to compete even at low population densities (Ives, 1988).

We proposed that the negative association recorded in the two different locations for the endohelminth adult trematodes the Gorgoderidae *Phyllodistomum inecoli* (from the urinary bladder) and the Allocreadiidae *Paracreptotrematoides heterandriae* (from the intestine) reflected the interactions among metacercariae in intermediate hosts and might have nothing to do with species interactions operating in *P. bimaculatus*, the definitive host. Considering the general biology of the families, both these trematodes might infect *P. bimaculatus* similarly. Therefore, the recruitment of one species may not be independent of the other species. Both these families display a three host or abbreviated life cycle. The first intermediaries are usually bivalves (clams of the genera *Pisidium*, *Sphaeridium*, and *Musculium*), while metacercariae generally encyst in damselflies, trichopteran, or chironomid larvae or the larvae of diving beetles (Yamaguti, 1975). The definitive host *P. bimaculatus* becomes infected after ingesting infected intermediaries. Thus, the observed association among these trematodes might have originated from when the fish prey on an intermediate host, which may contain larvae of more than one helminth species (Bush et al., 1993; Lotz et al., 1995). This structure of larval helminth communities can then be transferred to adult helminth communities (Poulin, 2001).

Our results concerning endohelminths agreed with previous studies that found that pairwise associations between gastrointestinal species of helminths of freshwater fishes were erratic and unpredictable, including studies on *Salmo trutta* in Italy (Dezfuli et al., 2001), of *Perca fluviatilis* and *Rutilus rutilus* in Finland (Poulin & Valtonen, 2002), marine fish species *Epinephelus morio*, and the freshwater *Cichlasoma urophthalmus* in Mexico (Vidal-Martínez & Poulin, 2003). No pairwise association was observed consistently among the localities sampled and random patterns in the structure of parasite communities were observed only sporadically (Dezfuli et al., 2001; Poulin & Valtonen, 2002; Vidal-Martínez & Poulin, 2003). Local factors or short-term influences could mask or eliminate any competitive interaction.

**Intra- and interspecific aggregation**

Both monogeneans and endohelminths showed high population aggregation. A fundamental difference between them is that the interspecific association C1,2 values increased with monogenean richness and number of individuals, whereas the aggregation of endohelminths did not show this density-dependence. Therefore, intraspecific aggregation could have distinct origins in both subgroups. We recorded higher intraspecific aggregation rates than that of interspecific aggregation rates in both subgroups, thus facilitating species coexistence. The extent to which intraspecific aggregation will be high enough for interspecific aggregation to be important for coexistence can only be determined with planned experiments on particular communities (Ives, 1988). However, these communities can only be fully understood by examining how new helminths are recruited and improved knowledge regarding the biology of helminth species, including modes of transmission and host infection, and experimental studies are urgently required.

In conclusion, while we reported here on species-poor non-saturated communities with vacant niches, our study documented numerical effects elicited by the presence of one helminth species on the abundance of another species, especially between the monogeneans. Therefore, interspecific competition is likely to occur in isolationist communities. Our data provided empirical evidence that high aggregation levels of these helminths contributed to species richness within a population of hosts because intraspecific and interspecific aggregations would facilitate contact between individual parasites and the coexistence of the most frequent species.

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**AUTHOR CONTRIBUTIONS**: GSM, JMCM and EFMF conceived the ideas and designed methodology; GSM, JMCM, EFMF, MRG, AGV, NMS and IGV collected the data and identified the species; GSM, WO and NMS analysed the data and lead the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**DATA ACCESIBILITY:** Data supporting the results will be archived in the Dryad Digital Repository.

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**Table 1**. Measures, equations, parameters key and references used in data analyses.

|  |  |  |  |
| --- | --- | --- | --- |
| Measure | Equation | Parameter key | Reference |
| Intraspecific aggregation |  | *n1i*, = number of helminths of species 1 in the host *i*  *m1*= mean number of helminth individuals of species 1 per host, *V1* = variance in the number of helminth species 1. | Ives, 1988 |
| Interspecific aggregation |  | *n1i* and *n2i*= numbers of helminths of species 1 and 2 in the host i  *m1i* and *m2i*= mean number of helminths per host of species 1 and 2  *P* = number of hosts  *Cov*= co-variability between a pair of species. | Ives, 1988, 1991 |
| Decrease in competition |  | all variables apply as described above | Morand et al. (1999) |

**Table 2.** Helminth parasites of *Pseudoxiphophorus bimaculatus* collected in June 2016 from 11 localities of La Antigua river basin, Veracruz, Mexico.

|  |  |
| --- | --- |
| Parasite species | Microhabitat |
| MONOGENEA |  |
| Dactylogyridae Bychowsky, 1933 |  |
| *Urocleidoides vaginoclaustroides* Mendoza-Franco, Caspeta-Mandujano, Salgado-Maldonado and Matamoros, 2015 | Gills |
| Gyrodactylidae van Beneden and Hesse, 1863 |  |
| *Gyrodactylus takoke* García-Vásquez, Razo-Mendivil and Rubio-Godoy, 2015 | Fins |
| *G*. *xalapensis* Rubio-Godoy, Paladini, García-Vásquez and Shinn, 2010 | Fins |
| *Gyrodactylus* sp. | Fins |
| TREMATODA |  |
| Gorgoderidae Looss, 1901 |  |
| *Phyllodistomum inecoli* Razo-Mendivil, Pérez Ponce de León and Rubio-Godoy, 2013 | Urinary bladder |
| Allocreadiidae Looss, 1902 |  |
| *Paracreptotrematoides heterandriae* (Salgado-Maldonado, Caspeta-Mandujano and Vazquez, 2012) | Intestine |
| Metacercariae |  |
| Echinostomatidae Looss, 1899 |  |
| *Echinochasmus leopoldinae* Scholz, Ditrich and Vargas-Vázquez, 1996 | Intestinal mucosa |
| Heterophyidae Odhner, 1914 |  |
| *Centrocestus formosanus* (Nishigori, 1924) | Gills |
| *Ascocotyle* (*Leighia*) *megalocephala* Price, 1932 | Intestinal mucosa |
| *A*. (*Phagicola*) *macrostoma* (Robinson, 1956) | Gills |
| Clinostomidae Lühe, 1901 |  |
| *Clinostomum* cf. *marginatum* Rudolphi, 1819 | Mesenteries |
| Diplostomidae Poirier, 1886 |  |
| *Uvulifer ambloplitis* (Hughes, 1927) | Skin |
| *Posthodiplostomum* cf. *minimum* (MacCallum, 1921) | Mesenteries |
| NEMATODA |  |
| Capillariidae Railliet, 1915 |  |
| *Freitascapillaria moraveci* Caspeta-Mandujano, Salgado-Maldonado and Vázquez, 2009 | Gall bladder |
| Cystidicolidae Skrjabin, 1946 |  |
| *Spinitectus mexicanus* Caspeta-Mandujano, Moravec and Salgado-Maldonado, 2000 | Intestine |
| Nematode larvae |  |
| Dioctophymatidae Railliet, 1915 |  |
| *Eustrongylides* sp. | Mesenteries |
| Anisakidae Railliet and Henry, 1912 |  |
| *Contracaecum* sp. | Mesenteries |
| Rhabdochonidae Travassos, Artigas and Pereira, 1928 |  |
| *Rhabdochona* sp. | Intestine |

**Table 3**. Parasite taxa of *Pseudoxiphophorus bimaculatus* collected in 2016 from 11 localities at the Río la Antigua basin, Veracruz, Mexico. Data are percent of infection / and mean abundance±Sd of infections; total no. of helminth individuals collected/J (aggregation) values.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parasite taxa  No. of host examined | Pixquiac  20 | Xico  20 | Agua Bendita  19 | Teocelo  20 | Baxtla  20 | Jalcomulco  20 |
| *U*. *vaginoclaustrumoides* | 35/1±1.6; 19/1.6 |  | 74/6.9±7.0; 131/0.9 | 75/7.5±8.3; 150/1.1 | 80/14.0±23.0; 270/2.8 | 55/2.6±4.1;52/2.1 |
| *G*. *takoke* | 5/0.1±0.2; 1/0 | 30/0.4±0.6; 7/- 0.04 | 16/0.2±0.4; 3/- 0.7 | 30/0.6±1;12/1.3 | 35/0.8±1.2; 15/1.2 | 25/0.3±0.6; 6/0.3 |
| *G*. *xalapensis* | 25/0.4±0.7; 7/0.8 |  | 10/0.1±0.3; 2/ - 0.5 | 35/1.1±1.8; 21/2.1 | 35/0.8±1.4; 16/1.6 | 5/0.1±0.2; 1/0 |
| *Gyrodactylus* sp. | 50/1.2±0.7; 23/0.5 | 15/0.4±0.9; 7/3.3 | 21/0.2±0.4; 4/ - 0.8 | 80/5.1±4.1; 101/0.4 | 90/3.6±1.8; 71/- 0.02 | 15/0.2±0.4; 3/ - 0.7 |
| *P*. *inecoli* |  | 25/0.9±2.0; 17/4.4 | 26/1.3±2.8; 25/3.6 | 40/1.5±2.2; 29/1.6 | 20/1.5±3.1; 29/3.8 | 15/1.0±2.7; 20/6.0 |
| *P*. *heterandriae* | 5/0.7±0.7; 1/0 |  |  | 10/0.2±0.5; 3/3.9 | 15/0.2±0.5; 4/1.8 | 45/4.4±8.9; 88/3.8 |
| *E*. *leopoldinae* |  |  |  |  |  | 25/1.9±6.7; 38/11.8 |
| *C*. *formosanus* |  |  |  |  |  | 85/38±80; 750/4.5 |
| *A*.(*Leighia*) *megalocephala* |  |  |  |  |  | 5/0.2±0.2: 1/0 |
| *A*.(*Phagicola*) *macrostoma* |  |  |  |  |  |  |
| *C*. cf. *marginatum* |  |  |  |  |  | 10/0.2±0.5; 3/3.9 |
| *U*. *amblopitis* |  |  |  |  | 5/0.2±0.7;3/13.3 | 60/2.0±2.1; 40/0.6 |
| *P*. cf. *mínimum* |  |  |  |  | 5/0.1±0.2; 1/0 | 45/2.0±4.4;39/4.6 |
| *S*. *mexicanus* |  |  | 31/0.4±0.7; 8/0.3 | 30/0.5±1; 10/2 |  | 35/1.2±1.9; 23/1.8 |
| *F*. *moraveci* |  | 5/0.1±0.4; 2/10 | 79/1.9±1.7; 36/0.3 |  |  | 60/3.0±3.1; 57/0.7 |
| *Eustrongylides* sp. |  |  | 5/0.1±0.2; 1/0 |  |  |  |
| *Rhabdochona* sp. |  |  |  |  |  |  |
| *Contracaecum* sp. |  |  |  |  |  |  |

Table 3. Continue

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parasite taxa  No. of hosts examined | Apazapan  20 | Los Pescados  20 | El Carrizal  20 | Puente Nacional  21 | Antigua Presa  20 |
| *U*. *vaginoclaustrumoides* | 65/2.5±3.4; 50/1.5 | 25/0.4±0.7; 7/0.8 | 10/0.3±0.8; 5/5.8 | 25/0.3±0.6; 6/0.4 | 25/0.9±1.7; 17/2.7 |
| *G*. *takoke* |  | 20/0.3±0.6; 5/0.8 | 5/0.1±0.2; 1/0 | 10/0.1±0.5; 3/4.2 | 5/0.1±0.2; 1/0 |
| *G*. *xalapensis* |  | 10/0.1±0.3; 2/ - 0.5 | 25/0.3±0.6; 6/0.2 | 15/0.2±0.5; 4/2.0 |  |
| *Gyrodactylus* sp. |  | 35/0.6±0.9; 12/0.8 | 10/0.1±0.3; 2/ - 0.5 | 15/0.1±0.4; 3/ - 1.0 |  |
| *P*. *inecoli* | 10/0.2±0.5; 3/3.9 |  |  |  |  |
| *P*. *heterandriae* | 35/0.7±1.1; 13/1.5 |  |  |  |  |
| *E*. *leopoldinae* |  |  |  |  |  |
| *C*. *formosanus* | 20/0.3±0.7; 6/1.4 |  | 10/1.9±8.3; 38/18.4 | 5/0.1±0.2; 1/0 | 30/0.8±1.5; 16/2.3 |
| *A*.(*Leighia*) *megalocephala* | 10/0.3±0.9; 5/9.2 |  |  |  |  |
| *A*.(*Phagicola*) *macrostoma* | 5/0.1±0.2; 1/0 |  |  |  |  |
| *C*. cf. *marginatum* | 5/0.1±0.4; 2/10 |  |  |  |  |
| *U*. *amblopitis* | 5/0.1±0.4; 2/10 | 5/0.1±0.2; 1/0 | 5/0.2±0.7; 3/13.3 |  |  |
| *P*. cf. *mínimum* |  |  | 5/0.2±0.7; 3/13.3 |  | 5/0.1±0.2; 1/0 |
| *S*. *mexicanus* |  |  |  |  |  |
| *F*. *moraveci* | 56/0.9±0.9; 17/0.02 | |  |  |  |
| *Eustrongylides* sp. |  |  |  |  |  |
| *Rhabdochona* sp. | 5/0.1±0.2; 1/0 |  |  | 5/0.1±0.2; 1/0 |  |
| *Contracaecum* sp. |  |  |  |  | 45/0.9±1.2; 17/0.8 |

**Table 4**. Number of positive and negative interspecific aggregation values C1,2 (+/-) within fish infected by both species of helminth pair. Below diagonal number of values of A1,2 > 1 within fish infected by both species of helminth pair. A. Ectoparasitic monogeneans and metacercariae of *C*. *formosanus*, B. Endohelminths

A. Monogeneans and *C*. *formosanus*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | *U*. *vaginoclaustroides* | *G*. *xalapensis* | *G*. *takoke* | *Gyrodactylus* sp. | *C*. *formosanus* |
| *U*. *vaginoclaustroides* |  | 0/8 | 2/6 | 1/8 | 0/4 |
| *G*. *xalapensis* | 3 |  | 3/5 | 4/4 | 0/2 |
| *G*. *takoke* | 4 | 3 |  | 4/5 | 0/2 |
| *Gyrodactylus* sp. | 4 | 4 | 3 |  | 0/3 |
| *C*. *formosanus* | 4 | 1 | 1 | 1 |  |

B. Endohelminths

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *P*. *inecoli* | *P*. *heterandriae* | *F*. *moraveci* | *S*. *mexicanus* |
| *P*. *inecoli* |  | ½ | 2/2 | 0/3 |
| *P*. *heterandriae* | 1 |  | 0/2 | 1/1 |
| *F*. *moraveci* | 4 | 2 |  | 1/1 |
| *S*. *mexicanus* | 3 | 2 | 2 |  |

**Table 5.** Spearman’s rank correlation coefficients obtained when comparing C1,2 values versus several density parameters of four monogenean species pairs. The species pairs *G*. *xalapensis* / *G*. *takoke* and *G*. *xalapensis* / *Gyrodactylus* sp. each were recorded from eight component communities, however any correlation between their values of C1,2 and the richness density parameters were recorded.

|  |  |  |
| --- | --- | --- |
|  | No. of component communities  in which was recorded | Spearman’s rank correlation between C1,2 values and |
| *G*. *takoke* / *Gyrodactylus* sp. | 9 | Maximum richness of monogenean species per host r = 0.76\*\* |
|  |  | Maximum no. of monogeneans in an infracommunity r = 0.68\* |
|  |  | Total # of *G*. *takoke* r = 0.95\*\*\* |
| *U*. *vaginoclaustroides* / *Gyrodactylus* sp. | 9 | Maximum richness of monogenean species per host r = 0.67\* |
|  |  | Mean richness of monogenean species per host r = 0.83\*\* |
|  |  | Maximum no. of monogeneans in an infracommunity r = 0.72\* |
|  |  | Total no. of monogeneans in the component community r = 0.74\* |
|  |  | Mean no. of monogeneans per host r = 0.72\* |
|  |  | Total # of *Gyrodactylus* sp. r = 0.84\* |
| *U*. *vaginoclaustroides* / *G*. *takoke* | 8 | Maximum richness of monogenean species per host r = 0.96\*\*\* |
|  |  | Total no. of monogeneans in the component community r = 0.82\*\* |
|  |  | Mean no. of monogeneans per host r = 0.83\*\* |
|  |  | Maximum no. of monogeneans in an infracommunity r = 0.79\* |
|  |  | Total # of *U*. *vaginoclaustroides* r = 0.79\* |
|  |  | Total # of *G*. *takoke* r = 0.97\*\*\* |
| *U*. *vaginoclaustroides* / *G*. *xalapensis* | 8 | Maximum richness of monogenean species per host r = 82\* |
|  |  | Mean richness of monogenean species per host r = 0.81\* |
|  |  | Total no. of monogeneans in the component community r = 0.84\* |
|  |  | Maximum no. of monogeneans in an infracommunity r = 0.85\* |
|  |  | Mean no. of monogeneans per host r = 0.85\* |

p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001

**Table 6.** Matrix of pairwise associations (Spearman’s rank correlation coefficients) between the intensity of infection of ectohelminth parasites of *P*. *bimaculatus* from 11 localities of La Antigua River basin, Veracruz, Mexico. Fish not harbouring worms from either species in a pairwise association (double zeros) were excluded; actual sample sizes are the numbers of fish harbouring at least one of the two species in a pair, and are given below the diagonal.

|  | *U*. *vaginoclaustroide* | *G*. *takoke* | *G*. *xalapensis* | *Gyrodactylus* sp. | *C*. *formosanus* |
| --- | --- | --- | --- | --- | --- |
| **Pixquiac** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | -0.58 | -0.86\*\*\* | - 0.48 |  |
| *G*. *takoke* | 8 |  | - 0.70 | - 0.57\* |  |
| *G*. *xalapensis* | 12 | 6 |  | 0.24 |  |
| *Gyrodactylus* sp. | 13 | 11 | 12 |  |  |
| **Xico** |  |  |  |  |  |
| *G*. *takoke* |  |  |  | 0.21 |  |
| *Gyrodactylus* sp. |  | 7 |  |  |  |
| **Agua Bendita** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | -0.47\* | - 0.20 | - 0.03 |  |
| *G*. *takoke* | 16 |  | - 1.00 | - 0.70 |  |
| *G*. *xalapensis* | 15 | 5 |  | - 0.25 |  |
| *Gyrodactylus* sp. | 14 | 6 | 6 |  |  |
| **Teocelo** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | - 0.18 | - 0.005 | 0.26 |  |
| *G*. *takoke* | 16 |  | 0.59 | 0.02 |  |
| *G*. *xalapensis* | 15 | 9 |  | - 0.09 |  |
| *Gyrodactylus* sp. | 18 | 18 | 18 |  |  |
| **Baxtla** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | - 0.21 | - 0.18 | - 0.27 |  |
| *G*. *takoke* | 17 |  | - 0.32 | 0.36 |  |
| *G*. *xalapensis* | 18 | 9 |  | 0.28 |  |
| *Gyrodactylus* sp. | 18 | 19 | 19 |  |  |
| **Jalcomulco** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | - 0.22 | - 0.48 | -0.72\*\* | - 0.60\*\* |
| *G*. *takoke* | 13 |  | - 0.25 | 0 | - 0.49 |
| *G*. *xalapensis* | 12 | 5 |  | 0 | 0.12 |
| *Gyrodactylus* sp | 14 | 6 | 3 |  | - 0.23 |
| *C*. *formosanus* | 19 | 19 | 17 | 18 |  |
| **Apazapan** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  |  |  |  | - 0.54\* |
| *C*. *formosanus* | 15 |  |  |  |  |
| **Río de los Pescados** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | - 0.81 | - 0.66 | - 0.70\*\* |  |
| *G*. *takoke* | 7 |  | 0 | - 0.08 |  |
| *G*. *xalapensis* | 8 | 5 |  | - 0.66 |  |
| *Gyrodactylus* sp | 11 | 8 | 8 |  |  |
| **El Carrizal** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | - 0.86 | -0.86\*\* | - 0.94 |  |
| *G*. *takoke* | 3 |  | - 0.77 | - 1.00 |  |
| *G*. *xalapensis* | 7 | 6 |  | 0 |  |
| *Gyrodactylus* sp | 4 | 3 | 6 |  |  |
| **Puente Nacional** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  | -0.86\* | - 0.90\*\* | - 0.72 | - 0.25 |
| *G*. *takoke* | 7 |  | 0.33 | - 0.96 | - 0.86 |
| *G*. *xalapensis* | 8 | 4 |  | - 0.94 | - 0.81 |
| *Gyrodactylus* sp | 7 | 5 | 5 |  | - 1.00 |
| *C*. *formosanus* | 5 | 3 | 3 | 3 |  |
| **Antigua Presa** |  |  |  |  |  |
| *U*. *vaginoclaustroides* |  |  |  | - 0.66 | - 0.22 |
| *Gyrodactylus* sp | 6 |  |  |  | - 0.61 |
| *C*. *formosanus* | 8 |  |  | 7 |  |

p < 0.05; \* p < 0.01; \*\* p < 0.001\*\*\*

**Table 7.** Matrix of pairwise associations (Spearman’s Rank correlation coefficients) between the intensity of infection of endohelminth parasites of *P*. *bimaculatus* from four localities of La Antigua River basin, Veracruz, Mexico. Fish not harbouring worms from either species in a pairwise association (double zeros) were excluded; actual sample sizes are the numbers of fish harbouring at least one of the two species in a pair, and are given below the diagonal.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | *P*. *inecoli* | *P*. *heterandriae* | *F*. *moraveci* | *S*. *mexicanus* |
| **Agua Bendita** |  |  |  |  |
| *P*. *inecoli* |  |  | 0.54\* | - 0.86\*\* |
| *F*. *moraveci* | 15 |  |  | 0.10 |
| *S*. *mexicanus* | 8 |  | 17 |  |
| **Teocelo** |  |  |  |  |
| *P*. *inecoli* |  | - 0.46 |  | - 0.51 |
| *P*. *heterandriae* | 9 |  |  | - 0.48 |
| *S*. *mexicanus* |  | 6 |  |  |
| **Baxtla** |  |  |  |  |
| *P*. *inecoli* |  | - 0.87\* |  |  |
| *P*. *heterandriae* | 7 |  |  |  |
| **Jalcomulco** |  |  |  |  |
| *P*. *inecoli* |  | 0.24 | - 0.37 | - 0.39 |
| *P*. *heterandriae* | 9 |  | - 0.58\*\* | - 0.08 |
| *F*. *moraveci* | 14 | 17 |  | - 0.54\*\* |
| *S*. *mexicanus* | 8 | 10 | 16 |  |
| **Apazapan** |  |  |  |  |
| *P*. *inecoli* |  | - 0.75\* | - 0.44 |  |
| *P*. *heterandriae* | 9 |  | - 0.38 |  |
| *F*. *moraveci* | 13 | 15 |  |  |

p < 0.05; \* p < 0.01; \*\* p < 0.001\*\*\*

**FIGURE CAPTIONS**

**Fig. 1** The río La Antigua basin at Veracruz, Mexico. Sampled localities are: 1, Pixquiac (Coord: UTM 14Q 0715115, 2154905; altitude 1245 m a.s.l.), 2. Xico (0709328, 2148062; 1438 m), 3. Agua Bendita (0708849, 2147130; 1278 m), 4. Teocelo (0712295, 2143510; 1115 m), 5. Baxtla (0712154, 2142160; 1105 m), 6. Jalcomulco (0725770, 2144871; 617 m), 7. Apazapan (0738583, 2139350; 328 m), 8. Río de Los Pescados (0741490, 2137128; 282 m), 9. El Carrizal (0748702, 2138013; 211 m), 10. Puente Nacional (0764574, 2138651; 78 m), 11. Antigua Presa (770702, 2140755; 42 m)

**Fig. 2** Relationship between component community species richness (SO) and mean infracommunity species richness; A) monogeneans; B) endohelminths.

**Fig. 3.** Intraspecific aggregations. Eighty values of J calculated for each parasite species. Note 56 values J > 0 (range 0.02 to 18.39), 9 values J < 0 (range – 0.79 to – 0.01) and 15 values J = 0

**Fig. 4.** Interspecific aggregations. Seventy-seven values of A1,2 calculated for each of 16 pair of parasite species. Note 64 values A1,2 > 1 (range 1.1 to 52.8).