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Temporal dynamics of species associations in the parasite community of European eels, *Anguilla anguilla*, from a coastal lagoon

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A R T I C L E I N F O

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A B S T R A C T

The resilience of biological communities is of central importance in ecology, but is difficult to investigate in nature. Parasite communities in individual hosts provide good model systems, as they allow a level of replication usually not possible with free-living communities. Here, using temporal data (2005–2017) on the communities of endohelminth parasites in European eels, *Anguilla anguilla*, from brackish-water lagoons in Italy, we test the resilience of interspecific associations to changes in the abundance of some parasite species and the disappearance of others. While most parasite species displayed changes in abundance over time, three trematodes that were present in the early years, two of which at high abundance, completely disappeared from the parasite community by the end of the study period. Possibly other host species required for the completion of their life cycles have declined in abundance, perhaps due to environmental changes. However, despite these marked changes to the overall community, pairwise correlations in abundance among the three most common parasite species (all trematodes) were stable over time and remained mostly unaffected by what happened to other species. We explore possible reasons for these resilient species associations within a temporally unstable parasite community inhabiting a mostly stable host population.

1. Introduction

The stability and resilience of ecological communities in the face of changes, such as species extinction or invasion, has long been and remains a central topic in modern ecological research (Holling, 1973; Stone et al., 1996; Thébault and Fontaine, 2010; Kawatsu and Kondoh, 2018). Species loss due to environmental changes is problematic in aquatic ecosystems (e.g., Ward, 1998; Lepori and Hjerdt, 2006). Are species interactions resilient to changes in the abundance, or even the disappearance, of other species in the community? If not, how do they respond? Answers to these questions are crucially important to understand, anticipate and mitigate the consequences of species loss. Although answers can be obtained from simplified artificial communities (e.g., Boyer et al., 2009; O'Gorman and Emmerson, 2009; Downing et al., 2014), they are difficult to validate in actual natural communities where replicated contrasts of pre- and post-change communities are generally not possible.

Communities of gastrointestinal parasitic worms in their vertebrate definitive hosts provide good model systems to test for the persistence of community structure in response to changes in the abundance, or even total disappearance, of certain species. Gastrointestinal helminths occur in a clearly defined physical space where all individuals of all species can easily be fully censused (Esch et al., 1990). They generally form communities with relatively few species, often belonging to the same higher taxonomic group, and with known colonization routes, i.e. the prey species ingested by the host and serving as intermediate hosts for the parasites. Importantly, communities of gastrointestinal helminths come in multiple replicates, since each individual host harbours an independent community (Esch et al., 1990; Poulin, 2001). This allows for tests of general patterns across replicate natural communities, a luxury not always possible with communities of free-living organisms.

Pairwise associations between the abundance of different helminth species across individuals sampled from the same host population are widely used to infer processes structuring parasite communities (e.g., Bush and Holmes, 1986; Haukisalmi and Henttonen, 1993; Dezfuli et al., 2001; Poulin, 2001; Lello et al., 2004; Dallas et al., 2019). Covariation between two helminth species can occur even when they inhabit different organs within the host, as seen in studies of fish parasites (Morley and Lewis, 2017, 2019). Negative correlations between the abundances of two species may result from direct competition, whereby high abundance of one leads to reduced establishment success, or even exclusion, of a second (Poulin, 2001). Alternatively, negative

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Table 1
Species of helminths found in eels, with information on their site of infection, intermediate hosts, and prevalence (percentage of infected hosts, with 95% confidence intervals for the most common species) and intensity of infection (mean number of parasites per infected host) in each of the three time periods.

Species	Site of infection[a]	Intermediate host[b]	2005–2006 (N = 140)		2010–2013 (N = 131)		2015–2017 (N = 30)	
			Prevalence, % (95% CI)	Mean intensity (range)	Prevalence, % (95% CI)	Mean intensity (range)	Prevalence, % (95% CI)	Mean intensity (range)
NEMATODES								
Anguillicoloides crassus[c]	swimbladder	Copepods (fish paratenic hosts?)	2.1	1.7 (1–3)	7.6	1.5 (1–2)	6.7	3.5 (2–5)
Contracaecum rudolphii (larvae)	stomach, intestine P (encysted)	Copepods	24.3 (17.4–32.2)	12.2 (1–45)	22.1 (15.4–30.2)	9.6 (3–38)	46.7 (28.3–65.7)	14.4 (1–100)
DIGENEANS								
Deropristis inflata[c]	intestine A,M	Polychaetes (e.g. *Nereis diversicolor*)	37.1 (29.1–45.7)	6.1 (1–89)	29.0 (21.4–37.6)	8.1 (1–53)	43.3 (25.5–62.6)	9.7 (1–44)
Helicometra fasciata	intestine M,A	Decapods (e.g. *Hippolyte inermis*)	60.0 (51.4–68.2)	16.0 (3–276)	55.7 (46.8–64.4)	25.5 (3–279)	0 (0–11.6)	0 (0)
Bucephalus anguillae[c]	intestine A,M	Fish (e.g. *Aphanius fasciatus*)	47.9 (39.3–56.5)	7.9 (1–135)	44.3 (35.6–53.2)	12.3 (2–114)	0 (0–11.6)	0 (0)
Lecithochirium musculus[c]	stomach, intestine A	Small fish species	9.3 (5.0–15.4)	13.6 (1–50)	13.7 (8.4–20.8)	8.3 (2–31)	0 (0–11.6)	0 (0)
CESTODES								
Tetraphyllidean larvae	intestine P	Unknown	16.4 (10.7–23.6)	4.4 (1–34)	6.9 (3.2–12.6)	6.4 (2–16)	3.3 (0.1–17.2)	5 (5)
Proteocephalus macrocephalus[c]	intestine M	Copepods (fish paratenic hosts?)	0	0 (0)	3.8	4.2 (1–7)	3.3	1 (1)
ACANTHOCEPHALANS								
Telosentis exiguous (larvae)	stomach, intestine A (encysted)	Amphipods (e.g. *Gammarus insensibilis*)	0	0 (0)	0	0 (0)	3.3	4 (4)

[a] Portion of the intestine most frequently occupied: A, anterior; M, middle; P, posterior.
[b] Hosts from which eels become infected via trophic transmission.
[c] Eel specialist, i.e. uses mostly eels as definitive hosts.

Table 2

Mean infracommunity species richness in eels, and frequency of pairwise co-occurrences in the same fish host among the three most abundant helminth species, in each of the three time periods. See Table 1 for full species names.

	Period 2005–2006	Period 2010–2013	Period 2015–2017
Infracommunity richness (range)	2.85 (1–6)	2.53 (1–5)	1.45 (1–3)
Deropristis & *Helicometra* (no. with both/no. with at least one)	52/84 (62%)	37/74 (50%)	–
Bucephalus & *Helicometra* (no. with both/no. with at least one)	67/84 (80%)	58/73 (79%)	–
Deropristis & *Bucephalus* (no. with both/no. with at least one)	50/74 (67%)	35/61 (57%)	–

correlations can also arise between the abundances of two helminths that use different intermediate hosts when individual definitive hosts show marked differences in dietary specialization: some definitive hosts will accumulate much more parasites of one species than another because they preferentially feed on the intermediate hosts of the former (Poulin, 2001; Knudsen et al., 2014). Positive correlations between the abundances of two helminth species across individual hosts can also have several causes. If the two parasites use the same intermediate host, then their trophic transmission to the definitive host will occur in close parallel (Bush et al., 1993; Poulin and Valtonen, 2001). The two species will then, almost inevitably, accumulate in the same individual definitive hosts at comparable rates (Lotz et al., 1995; Vickery and Poulin, 2002). In contrast, if they use different intermediate hosts but still show a positive correlation in abundance across definitive hosts, then other processes are involved. For instance, one species may cause immunosuppression of the host, thereby indirectly facilitating the establishment of a second species, leading to an association between their abundances (Poulin, 2001; Lello et al., 2004). The various mechanisms underlying statistical associations between helminth species in their definitive hosts are best resolved using experimental infections (e.g., Benesh and Kalbe, 2016). However, given the logistical difficulties associated with the controlled infection of wild vertebrate species, observations from naturally infected hosts can also yield insights into the processes shaping helminth communities. For instance, if the colonization routes (i.e. intermediate hosts) of different helminth species are known, and if the role of host age/size (parasites of different species may all simply increase in numbers with increasing host age) is taken into account, one can narrow down the potential causes of interspecific correlations in abundance.

The helminth community of the European eel, *Anguilla anguilla*, has served as a model system for studies of temporal dynamics in parasite communities (e.g., Kennedy, 1997, 2001; Kennedy et al., 1998; Kennedy and Moriarty, 2002; Schabuss et al., 2005). In general, helminth communities of eels have been found to be mostly stable in terms of their species richness and composition, with only modest changes in the relative abundances of different species over time. In the few cases where more significant perturbation such as loss of core species or colonization of new species occurred over time (e.g., Kennedy, 1997; Kennedy et al., 1998), no rigorous quantification of the consequences for pairwise associations among remaining species was conducted.

Several studies have investigated the spatial predictability and repeatability of helminth community structure in fish populations (e.g. Poulin and Valtonen, 2002; Timi et al., 2010; Braicovich and Timi, 2015), but little attention has been paid to the temporal stability of these communities. The objectives of the present study are to (i) document temporal changes in the occurrence or abundance of helminth parasite species in an eel population over a span of 12 years, and (ii) test the impact of these changes on the pairwise associations among other key species in the community. In contrast to previous studies (cited above) on eel parasites that were all conducted in freshwater ecosystems, ours investigates eels from a coastal brackish lagoon. Our findings provide an assessment of the resilience of a helminth community to species loss, measured through the persistence of stable pairwise interactions among the remaining members of this relatively species-poor community.

2. Methods

2.1. Study site

Comacchio Lagoons (12°11′29″E, 44°40′18″N), on the Italian coast of the northern Adriatic Sea, consist of shallow, interconnected brackish lagoons within the regional park of the Po Delta. They are home to a wide variety of shore birds, and are also the site of one of the oldest and most important eel fisheries in the Mediterranean. Previous studies on helminth parasites of eels in these lagoons (Di Cave et al., 2001; Dezfuli et al., 2014) reported some temporal changes in the relative abundances of certain species; however, they focused mostly on comparisons of the helminth community with that from eels in other localities, and did not cover more recent years during which more important changes occurred in the helminth community (see Results). The focus of these earlier study was also at the component community level (i.e. all parasites of all species in a host population), whereas the present study examines patterns in the species composition of infracommunities (i.e. all parasites of all species in a host individual).

2.2. Sampling and parasite recovery

Eels were sampled from the lagoons with different capture methods based on size/stage of eels: silver eels were captured using V-shaped screens, called lavorieri, and yellow eels using modified fyke nets, called cogolli; both methods are widely used for eel fishing in the shallow lagoons of the northern Adriatic. Eels were obtained mainly in spring and autumn, during three time periods: 2005–2006 (from October 2005 to May 2006; $N = 140$ eels), 2010–2013 (from March 2010 to December 2013; $N = 131$), and 2015–2017 (from March 2015 to December 2017; $N = 30$). The fish could not be sampled in exactly the same seasons in the three time periods, a factor we took into account in our analyses (see below). The seasonal numbers of eels examined per time period were: 103 in autumn, 15 in winter and 22 in spring for 2005–2006; 78 in autumn, 3 in winter, 39 in spring, and 11 in summer for 2010–2013; 8 in autumn, 2 in winter, 13 in spring, and 7 in summer for 2015–2017. Eels were brought live to a laboratory at the University of Ferrara, anesthetized with MS-222 (150 mg/L for 20 min) (Sandoz), and killed by severing the spinal cord. Total body length (cm) and weight (g) were recorded for each fish. The entire digestive tract and associated organs were removed, the intestine was sliced open longitudinally and searched for helminths. In addition, the swim bladder was also examined carefully for adult nematodes. All parasites (except larval cestodes) were identified based on morphology (through light and electron microscopy; representative specimens sent to experts for confirmation) to species level and counted. Crucially, across all years, eel sampling was conducted in the same manner in the same locations within the lagoons, and all parasites were recovered, identified and counted by the same two researchers (B. Sayyaf Dezfuli and L. Giari), making data from different years comparable.

2.3. Statistical analysis

Values for both total length and weight of eels caught overlapped extensively among the three time periods. However, there were

Table 3
Results of generalized linear mixed models testing for the effect of host total length, time period, and their interaction on abundance (number of individuals per eel host) of helminth parasites, for each of the six most abundant parasite species. Season was included as a random factor; significant effects are indicated in bold.

Predictor	Coefficient estimate[a]	Standard error	z value	P
Contracaecum rudolphii				
Intercept	**0.834**	**0.092**	**9.075**	**< 0.001**
Total length	**0.490**	**0.079**	**6.216**	**< 0.001**
Period 2010–2013	**−0.298**	**0.105**	**−2.834**	**0.005**
Period 2015–2017	−0.096	0.195	−0.492	0.623
Length[a] Period 2010–2013	**0.617**	**0.108**	**5.699**	**< 0.001**
Length[a] Period 2015–2017	**1.897**	**0.252**	**7.524**	**< 0.001**
Deropristis inflata				
Intercept	**0.934**	**0.246**	**3.798**	**< 0.001**
Total length	**0.212**	**0.074**	**2.870**	**0.004**
Period 2010–2013	0.043	0.088	0.487	0.626
Period 2015–2017	0.052	0.163	0.323	0.747
Length[a] Period 2010–2013	**0.202**	**0.092**	**2.204**	**0.028**
Length[a] Period 2015–2017	**1.075**	**0.213**	**5.056**	**< 0.001**
Helicometra fasciata				
Intercept	**2.485**	**0.285**	**8.713**	**< 0.001**
Total length	**0.169**	**0.035**	**4.779**	**< 0.001**
Period 2010–2013	**0.340**	**0.040**	**8.527**	**< 0.001**
Period 2015–2017	−23.582	35.501	−0.664	0.507
Length[a] Period 2010–2013	**0.215**	**0.042**	**5.093**	**< 0.001**
Length[a] Period 2015–2017	−0.238	38.593	−0.006	0.995
Bucephalus anguillae				
Intercept	**1.546**	**0.321**	**4.815**	**< 0.001**
Total length	0.105	0.059	1.789	0.074
Period 2010–2013	**0.353**	**0.065**	**5.413**	**< 0.001**
Period 2015–2017	−22.643	80.954	−0.280	0.780
Length[a] Period 2010–2013	**0.420**	**0.069**	**6.065**	**< 0.001**
Length[a] Period 2015–2017	−0.172	181.020	−0.001	0.999
Lecithochirium musculus				
Intercept	0.563	0.394	1.426	0.154
Total length	**0.252**	**0.107**	**2.352**	**0.019**
Period 2010–2013	−0.184	0.131	−1.403	0.161
Period 2015–2017	−21.674	148.318	−0.146	0.884
Length[a] Period 2010–2013	0.240	0.136	1.761	0.078
Length[a] Period 2015–2017	−0.019	148.318	−0.001	0.999
Tetraphyllidean larvae				
Intercept	**−0.947**	**0.429**	**−2.208**	**0.027**
Total length	0.051	0.165	0.309	0.757
Period 2010–2013	**−0.607**	**0.241**	**−2.523**	**0.012**
Period 2015–2017	−0.992	0.538	−1.846	0.065
Length[a] Period 2010–2013	**1.192**	**0.241**	**4.943**	**< 0.001**
Length[a] Period 2015–2017	−0.121	0.752	−0.161	0.872

[a] Period 2005–2006 included in the intercept.

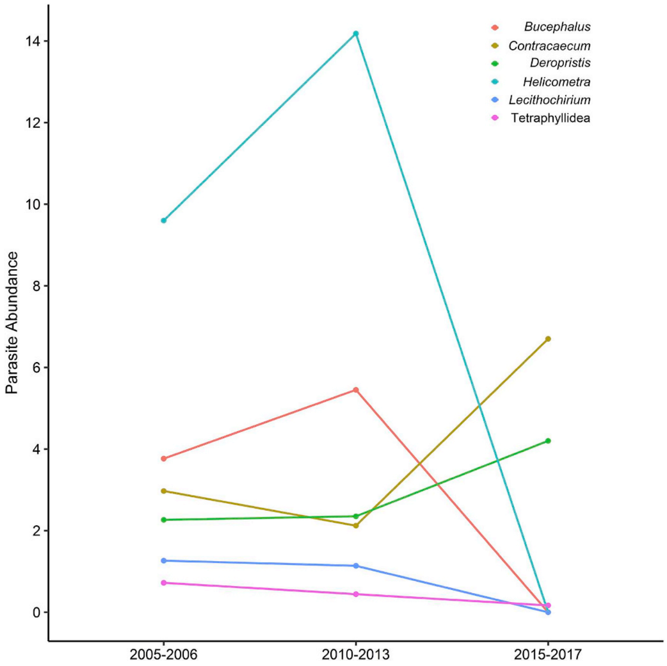

Fig. 1. Abundance (mean number of parasites per host, including non-infected hosts) of the six most common helminth parasites of eels, *Anguilla anguilla*, in Comacchio Lagoons, during three sampling periods: 2005–2006 ($N = 140$ eels), 2010–2013 ($N = 131$), and 2015–2017 ($N = 30$). Note that some values for the time period 2015–2017 are based on very few fish; see Table 1 for actual numbers and for full species names.

significant differences in both length (one-way ANOVA: $F_{2,298} = 16.148$, $P < 0.0001$) and weight ($F_{2,298} = 17.248$, $P < 0.0001$) of eels among time periods; eels tended to be a little smaller in the middle time period 2010–2013 (mean length ± SE, 60.2 ± 1.4 cm) than in the early (70.9 ± 1.3 cm) or late period (68.6 ± 2.9 cm). Total length and weight were strongly positively correlated among all 301 eels examined ($r = 0.948$, $P < 0.0001$), therefore we chose to include only total length as a measure of eel body size in the analyses described below. Also, no distinction was made between yellow and silver eels (two developmental stages of eels) in the analyses, as they overlapped extensively in size and did not have distinct helminth communities.

To test for temporal changes in infection levels across the three time periods, while simultaneously accounting for the potential influence of host size, we ran separate generalized linear mixed models (GLMMs) with Poisson distribution as error structure, for each of the 6 most abundant parasite species. All GLMMs were run with the package *lme4* in R (version 3.5.3; R Core Development Team, 2019), using the *glmer* function. The number of parasites per host was the response variable, and the predictors were host size, the time period (2005–2006, 2010–2013 and 2015–2017), and the interaction between host size and time period; season of sampling (winter, spring, summer or autumn) was included as a random factor, to account for the lack of independence among fish caught in the same season, and also for possible variation among seasons in general infection levels (see Chubb, 1979, 1980). Because certain species were absent from certain time periods (see Results), in some models the excess of zeros led to aberrant results that did not correspond to visual inspection of the data. Therefore, comparisons of the number of parasites per host among time periods were validated using the non-parametric Kruskal-Wallis test, with post-hoc pairwise comparisons between time periods performed using Dunn-Bonferroni tests, implemented with the *dunn.test* package.

To test for temporal changes in the covariation of infection levels between pairs of parasite species, we ran other GLMs (function *glm* in R) with quasi-Poisson distribution as error structure, for different combinations of the three species with the highest overall prevalence (*Deropristis inflata, Helicometra fasciata, Bucephalus anguillae*). Double zeros, i.e. fish not infected by either parasite species, were removed from each analysis; this reduced the excess of zero values and allowed the data to be fitted with a quasi-Poisson distribution. The three parasite species considered are all digeneans inhabiting the host intestine,

Table 4
Results of Kruskal-Wallis tests comparing the abundance (number of individuals per eel host) of helminth parasites among the three time periods, with post-hoc pairwise comparisons (Dunn-Bonferroni tests) where necessary (i.e. when the main test is significant). Different letters indicate significant differences between time periods.

Parasite species	Chi-squared (df = 2)	P	Post-hoc contrasts between time periods		
			2005–2006	2010–2013	2015–2017
Contracaecum rudolphii	6.833	0.033	a	a	b
Deropristis inflata	2.480	0.289	–	–	–
Helicometra fasciata	30.573	< 0.001	a	a	b
Bucephalus anguillae	21.652	< 0.001	a	a	b
Lecithochirium musculus	5.096	0.078	–	–	–
Tetraphyllidean larvae	7.630	0.022	a	b	b

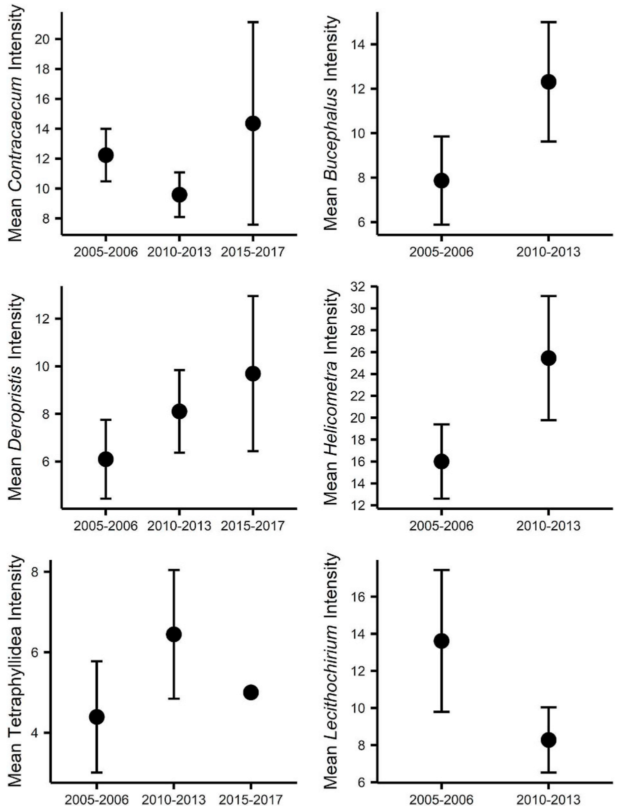

Fig. 2. Intensity of infection (mean ± SE number of parasites per host, including infected hosts only) of the six most common helminth parasites of eels, *Anguilla anguilla*, in Comacchio Lagoons, during three sampling periods. Graphs on the right-hand side do not include the 2015–2017 period, as these species were not found during that period. See Table 1 for full species names.

therefore direct interactions, or at least coincidental associations, are more likely among them. The number of parasites per host for the most prevalent species in a pair was always used as a predictor, with the other species used as a response in the models. For any species pair, only the time periods when at least one was present were considered in the GLM. Other predictors were host size and the time period, as well as the interactions between the abundance of the predictor species and the time period, since we are specifically looking for changes in how two species covary as a function of the time period in which the hosts were sampled.

3. Results

Nine species of helminths (two nematodes, four digeneans, two cestodes and one acanthocephalan) were recovered from the 301 eels examined (Table 1). All parasite species are marine except *Proteocephalus macrocephalus* (very rarely found) and *Anguillicoloides crassus* which is generally considered freshwater but able to survive high salinities. They varied greatly in prevalence and abundance, with some of these helminth species not found in certain time periods. In particular, three digenean species that were relatively abundant among eels caught in the first two time periods were completely absent from eels caught in the most recent time period (Table 1). The disappearance of these species has resulted in a reduction in mean infracommunity species richness in the most recent time period (Table 2).

Table 5
Results of generalized linear models testing for the effect of host total length, the more abundant trematode species, and time period on abundance (number of individuals per eel host) of trematode parasites, for all pairwise associations among the three most abundant species. Significant effects are indicated in bold.

Predictor	Coefficient estimate[a]	Standard error	t value	P
Deropristis inflata (response) (N = 171 fish)				
Intercept	**0.804**	**0.133**	**6.034**	**< 0.001**
Total length	−0.035	0.091	−0.387	0.699
***Helicometra* abundance**	**0.014**	**0.001**	**15.942**	**< 0.001**
Period 2010–2013	−0.167	0.220	−0.761	0.448
Period 2015–2017	**1.482**	**0.209**	**7.107**	**< 0.001**
Period x *Helicometra*	−0.0004	0.001	−0.311	0.756
Bucephalus anguillae (response) (N = 157 fish)				
Intercept	**1.310**	**0.151**	**8.687**	**< 0.001**
Total length	0.079	0.099	0.797	0.427
***Helicometra* abundance**	**0.013**	**0.001**	**13.258**	**< 0.001**
Period 2010–2013	0.350	0.222	1.579	0.116
Period x *Helicometra*	−0.001	0.001	−1.044	0.298
Deropristis inflata (response) (N = 143 fish)				
Intercept	**1.042**	**0.134**	**7.778**	**< 0.001**
Total length	−0.017	0.093	−0.180	0.858
***Bucephalus* abundance**	**0.026**	**0.002**	**14.403**	**< 0.001**
Period 2010–2013	−0.125	0.216	−0.581	0.562
Period 2015–2017	**1.237**	**0.213**	**5.804**	**< 0.001**
Period x *Bucephalus*	0.003	0.003	1.095	0.276

[a] Period 2005–2006 included in the intercept.

Generalized linear mixed models indicate that for four of the six most abundant helminth species, the number of parasites per eel was significantly and positively affected by host size, and almost significantly in a fifth species (Table 3). There were also significant interactions between host size and time period in many cases (Table 3), indicating that the strength of the association between host size and parasite abundance was not consistent across time periods. However, this appears mostly due to the absence of several species in the most recent time period, resulting in no size-abundance correlation in that period in contrast to a weak positive one in earlier periods (Supplementary Fig. S1). With respect to differences among time periods, the results of the generalized linear models are not always fully

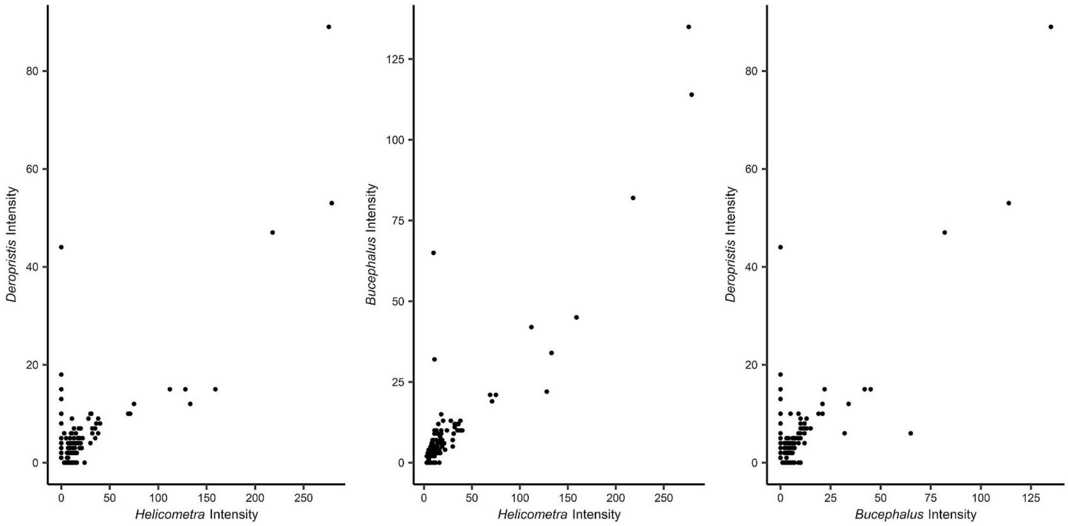

Fig. 3. Scatterplots of pairwise relationships between numbers of parasites per host for the three most common digenean parasites of eels, *Anguilla anguilla*, in Comacchio Lagoons, across all three sampling periods combined. See Table 1 for full species names.

congruent with the visible trends in the data, which show three digenean species (*Helicometra fasciata, Bucephalus anguillae*, and *Lecithochirium musculus*) disappearing from eels in 2015–2017, while the digenean *Deropristis inflata* and the encysted larvae of the nematode *Contracaecum rudolphii* appear to increase in abundance during that time period (Table 1, Fig. 1). In contrast, results of Kruskal-Wallis tests indicate that *H. fasciata*, *B. anguillae* and *C. rudolphii* indeed had different abundances in the 2015–2017 period, with the difference for *L. musculus* being marginally significant (Table 4). The Kruskal-Wallis tests did not fully support the apparent disappearance of *L. musculus* probably because it had relatively low infection levels in the previous periods, nor did they support the rise in abundance of *D. inflata* in the most recent period (but see below), possibly because of much more variable infection intensities recorded for this parasite in eels caught in 2015–2017 (Fig. 2). Nevertheless, these latter results confirm that the helminth community underwent significant changes in the abundance of some of its species, including the disappearance of some of them, or at least their reduction of abundance below detection levels.

The other set of generalized linear models testing for temporal changes in the pairwise covariation of infection levels among the three species with highest overall prevalence (*Deropristis inflata, Helicometra fasciata,* and *Bucephalus anguillae*) did support an increase in the abundance of *D. inflata* in the most recent time period (Table 5), possibly due to the elimination of several zero values in these analyses. In contrast to the previous analyses, these generalized linear models did not find an effect of host size on numbers of parasites per fish, again most likely because of the exclusion of many zero values (uninfected hosts), which probably correspond to smaller fish in general. More importantly, the results indicate significant positive associations between all pairs of species among these three digenean species (Table 5). Independently of host size (its effects are accounted for), eels with many individuals of one helminth species tend to harbour also many individuals of the other two species (Figs. 3 and 4). Also, the frequency of pairwise co-occurrences in the same infracommunity remained roughly consistent among the time periods in which both species in a pair were present (Table 2). The specific objective of the GLM analyses was to look for changes in how two species covary as a function of the time period in which the hosts were sampled. Significant interactions between the abundance of the predictor species and the time period would be evidence that the effect of the abundance of one species on the abundance of the other species depends on the time period. However, there was no significant interaction between these factors in any of the generalized linear models (Table 5), indicating that species associations remained unchanged through time despite some species in the community disappearing and others undergoing fluctuations in abundance.

4. Discussion

The results of the present study reveal two aspects of the temporal dynamics of the helminth community of eels, *Anguilla anguilla*, in Comacchio Lagoons. Firstly, we demonstrate that, in terms of species composition, the helminth community is unstable and has changed over time. Three species of digenean appear to have disappeared in the most recent time period. Although fewer eels were sampled during that period ($N = 30$) than in previous periods ($N = 140$ and 131), the relatively high prevalence of these species in earlier periods makes it unlikely that they were still present but simply missed through inadequate sampling. Secondly, we show that among the most common species in the community, pairwise associations have remained unchanged over time. Thus, despite changes in relative abundance of some species, the community shows resilience in terms of patterns of associations among species.

The apparent local disappearance of the digeneans *Helicometra fasciata, Bucephalus anguillae*, and *Lecithochirium musculus* from helminth communities in eels has no obvious explanation. They were present in 2005–2006 and 2010–2013 as well as in earlier surveys of eel parasites in the lagoons (Di Cave et al., 2001; Dezfuli et al., 2014). Decreases in the abundance of the second intermediate hosts of these parasites, and/or in the rate at which they are eaten by eels, could account for their absence in 2015–2017. The second intermediate hosts of *B. anguillae* and *L. musculus* are small fish species, for which they are probably not too host-specific, whereas *H. fasciata* uses decapod crustaceans as second intermediate hosts (Bartoli and Gibson, 2007). Although the Comacchio Lagoons are inhabited by a rich fish community, use of the lagoons by marine and brackish water fish species is strongly influenced by interconnected environmental variables including wind speed and direction, precipitation and dissolved oxygen levels (Milardi et al., 2019). Similarly, the lagoons harbour a rich community of crustaceans, whose distribution and abundance are greatly influenced by microhabitat characteristics, in particular macroalgal coverage (Mistri et al., 2000). This community has undergone significant changes in recent years, associated with rising average temperatures, leading to the disappearance of several invertebrate species, including crustaceans that may have acted as hosts to *H. fasciata* (Pitacco et al., 2018). It is therefore conceivable that changes in the availability of prey has

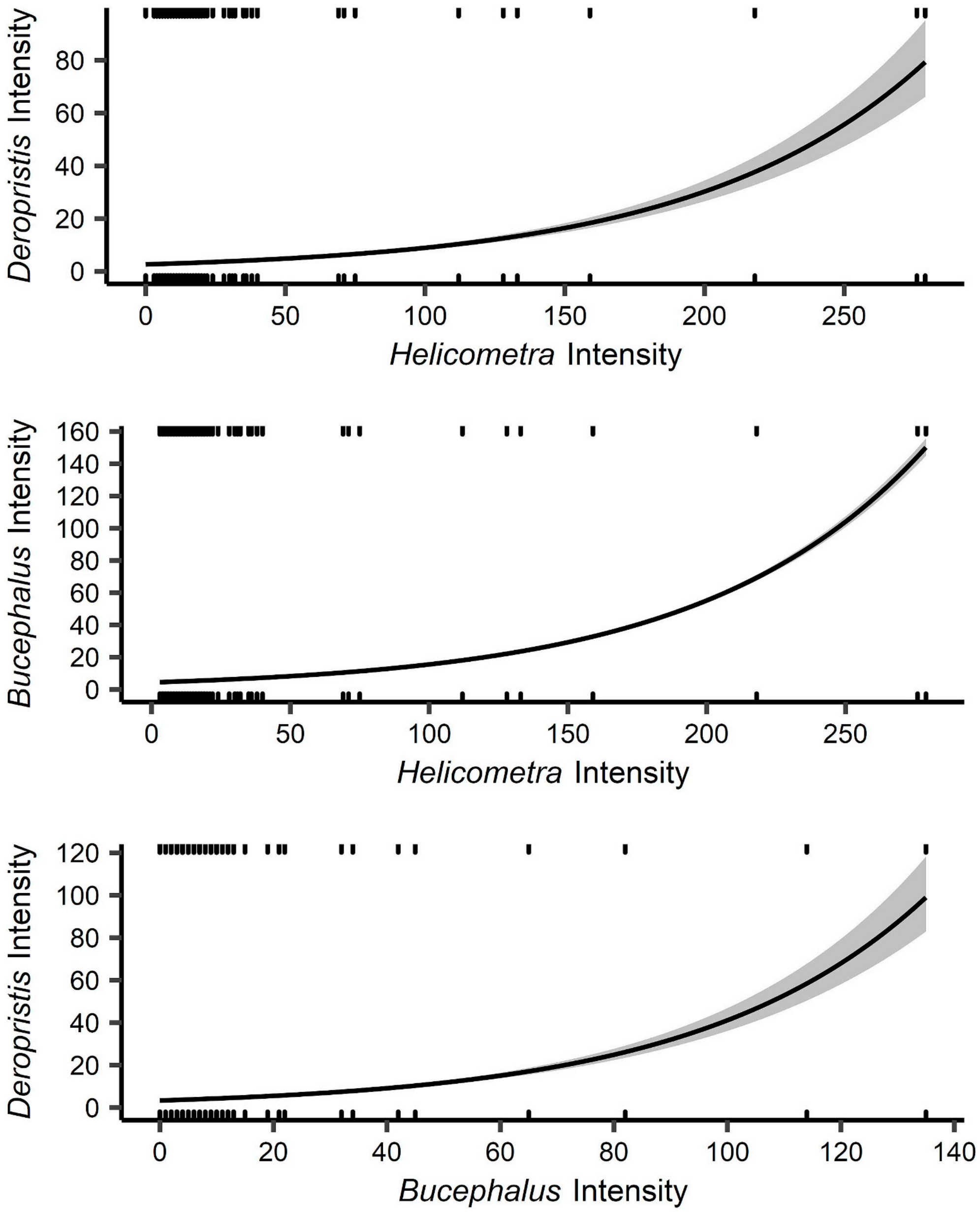

Fig. 4. Pairwise relationships between numbers of parasites per host for the three most common digenean parasites of eels, *Anguilla anguilla*, in Comacchio Lagoons, across all three sampling periods combined. The line represents the relationship (with 95% confidence intervals) predicted by the generalized linear model; see text. Tick marks indicate partial residuals with either positive (top) or negative values (bottom). See Table 1 for full species names.

resulted in a shift in the diet of eels, no longer exposing them to trophic infection by certain helminth species. The only information available on eel diet in Comacchio Lagoons indicates that the diet changes with length from amphipods and small shrimps in smaller eels, to larger shrimps, crabs and small fish in larger eels (Lanzoni et al., 2018). Perhaps the slightly smaller size of eels caught in 2010–2013 meant they fed more on shrimps, and were exposed to greater infections by *H. fasciata*; however, there are no data available on long-term changes in eel diet.

In contrast, the rise in abundance of the nematode *Contracaecum rudolphii* in the helminth communities of eels may reflect an increase in the local abundance of piscivorous birds, such as cormorants, which act as the nematode's definitive hosts. However, no long-term data are available on changes in bird abundance in Comacchio Lagoons to confirm this explanation.

Digeneans also require a molluscan first intermediate host in their life cycle. The first intermediate host of *L. musculus* is unknown, whereas in other localities it is known that *B. anguillae* uses bivalves (such as *Abra tenuis*) and *H. fasciata* uses snails (*Gibbula* spp.) (Bartoli and Gibson, 2007). Although the first intermediate host species they use in Comacchio Lagoons have not been ascertained, several mollusk species have also experienced declines in recent years in the lagoons (Pitacco et al., 2018). Therefore, the disappearance of these digeneans from helminth communities in eels may be driven by declines, possibly even local extinction, of their first intermediate host. The digenean *D. inflata*, which was still found in eels in the most recent time period, uses different snail species (*Hydrobia* spp.) as first intermediate hosts (Vaes, 1978; Bartoli and Gibson, 2007), and therefore could have persisted despite changes to other digenean life cycles.

Whatever the reasons for the decline and disappearance of the three digenean species from the helminth communities in eels, and for the changes in abundance of other species, this has had little impact on the pairwise associations among the three most common species in the community. Of those three species, *D. inflata* was found in eels from all time periods, whereas *H. fasciata* and *B. anguillae* were no longer observed in 2015–2017. Across the total temporal extent of our samples, these three species were always positively associated with each other. In helminth communities in general, positive associations between pairs of species generally outnumber negative ones (see Bush and Holmes, 1986; Lotz and Font, 1991, 1994). To some extent, this may be because statistical methods to detect associations are slightly more sensitive to positive covariance between species than to negative ones of the same absolute magnitude (Haukisalmi and Henttonen, 1998; Vickery and Poulin, 2002). However, the associations we observed are relatively strong, and unlikely to arise as statistical artefacts. In addition, the three species investigated all share the same microhabitat within the host (i.e. the intestine), a situation in which negative, possibly competitive, associations are more frequently observed (see Dallas et al., 2019). The fact we observed positive associations in this situation, and that they persisted over time despite other substantial changes to the community, strongly suggest they arise from biological processes and not as statistical artefacts.

So what mechanisms may lead to resilient and persistent positive associations among these digenean species? First, we can probably rule out co-transmission and co-accumulation in the same host individuals, since the three digeneans do not all use the same intermediate hosts through which they are transmitted trophically to eels. Of course, positive associations between two helminth species may result from many fish preying on the very different intermediate hosts of both helminth species, as suggested in other systems (e.g. Kuhn et al., 2016), although this seems unlikely given the changes to the community of invertebrates and small fish mentioned above. Second, we can probably also rule out simple parallel accumulation of different species over time as the hosts become larger and older, because our analyses accounted for the influence of host size. Third, it is possible that positive associations arise through indirect immune-mediated interactions, whereby one parasite species suppresses the immune response of the host and thus facilitates the establishment of other parasites within the same host. However, immune-mediated interactions are difficult to demonstrate using data from natural infections (e.g., see Behnke, 2008). Ultimately, experimental manipulation of one species and careful measurement of what happens to the abundance of other species are necessary to elucidate the mechanisms underpinning associations between parasite species sharing the same host (e.g. Knowles et al., 2013).

Changes in species interactions are not only measurable as altered correlations between abundance levels; functional responses are also possible (Poulin, 2001). Indeed, in many helminth communities some species can shift their position along the host's intestine without any change to their abundance, either expanding to new locations when other species are absent, or contracting their spatial niche in the intestine in the presence of certain competitors (Holmes, 1973; Haukisalmi and Henttonen, 1994; Friggens and Brown, 2005). Therefore, although the positive associations between digenean species in the eels studied here may have remained constant across time periods based on their abundance, it could be that one species changed its distribution along the host's intestine after the disappearance of the other species. However, no data were recorded to this effect and no obvious species-specific differences in site of occupation in the intestines of eels were noted during dissections and parasite recovery.

In summary, we present evidence for resilient species associations within a temporally unstable parasite community inhabiting a stable host population. Indeed, the eel population of Comacchio Lagoons has experienced a drastic decline over many decades until the early 2000s, but since then (i.e. during the time frame of our study) it appears to have remained mostly stable (Aschonitis et al., 2017a,b). There have been earlier reports of long-term persistence of species associations within helminth communities (e.g. Lello et al., 2004), but none from communities that have experienced apparent extinction of previously common species. The search for the mechanisms that allow this resilience will require in-depth experimental studies, but promises to reveal fundamental processes shaping and structuring parasite communities.

Data availability

The dataset generated and analysed during the current study is available from the corresponding author upon reasonable request.

Ethics compliance

All relevant national and institutional guidelines for the use of animals in research were followed.

Note

Supplementary material associated with this article.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.05.001.

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