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ORIGINAL PAPER

Parasitism may alter functional response comparisons: a case study on the killer shrimp *Dikerogammarus villosus* and two non-invasive gammarids

Corentin Iltis · Thierry Spataro · Rémi Wattier · Vincent Médoc

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Abstract The Ponto-Caspian freshwater amphipod *Dikerogammarus villosus* has colonized most of the water bodies of continental Europe where it causes strong structural alterations in recipient communities that can lead to changes in ecosystem-level processes, mainly because of a strong predatory behaviour. Most of the *D. villosus* populations from the invaded range have been found infected with the co-introduced microsporidian parasite *Cucumispora dikerogammari*, known to decrease the predation rate of its host. Infection might thus mitigate the ecological impact of *D. villosus* and we wanted to test this assumption using the comparative functional response approach. We compared the relationship between resource use and resource availability (i.e. the functional response, FR) of *D. villosus*, either with infected individuals or not, to that of two non-invasive gammarids: *Gammarus pulex* and *Echinogammarus berilloni*. With infected individuals included, *D. villosus* displayed a higher FR than the two non-invasive gammarids. Although this effect was not significant, *C. dikerogammari* infection tended to alter the FR of *D. villosus* with a slight decrease in attack rate and handling time, resulting in a less steep initial slope and a higher asymptote, respectively. Removing infected *D. villosus* from the dataset did not affect the FR comparison with *G. pulex* but suppressed the difference in FR with *E. berilloni*. Although we cannot exclude the role of sample size reduction in this effect, this suggests that *C. dikerogammari* infection might increase the predation pressure on local prey populations in case of species replacement between *D. villosus* and *E. berilloni*. From a more general perspective, our study illustrates how parasites may alter our capacity to predict invasive species impacts from FR comparisons.

Keywords Biological invasions · Co-introduced parasite · *Cucumispora dikerogammari* · Freshwater amphipods · Functional response · Predation

C. Iltis · T. Spataro · V. Médoc
Institute of Ecology and Environmental Sciences – Paris (UMR CNRS 7618), Paris, France

C. Iltis · R. Wattier
Université de Bourgogne Franche-Comté, Dijon, France

T. Spataro
AgroParisTech, Paris, France

R. Wattier
Laboratoire Biogéosciences (UMR CNRS-UBFC 6282), Dijon, France

V. Médoc (✉)
Université Pierre et Marie Curie, Sorbonne Universités, 4 place Jussieu, tour 44-45, office 520, case 237, 75252 Paris Cedex 05, France
e-mail: vincent.medoc@upmc.fr

Introduction

Biological invasions are a major driver of biodiversity loss. Invasive species (species introduced outside their native range that establish self-sustaining populations and spread beyond their point of introduction with deleterious impact on the recipient ecosystem, Kolar and Lodge 2001) threaten native species through direct predation (Paolucci et al. 2013), competition (Riley and Dybdahl 2015), habitat degradation (Wallentinus and Nyberg 2007), disease emergence (Tompkins et al. 2003) or through more subtle trait-mediated indirect interactions (Byers et al. 2010; Mattos and Orrock 2010).

There is growing interest in the role of parasites in biological invasions (Prenter et al. 2004; Dunn 2009; Dunn and Hatcher 2015; Médoc et al. 2017). Parasites may contribute to invasion success and mediate ecological impacts in many ways. For instance, introduced free-living species can become hosts for native parasites, leading either to an increase in prevalence in native species if they are competent hosts that amplify transmission (spillback, Kelly et al. 2009), or to a decrease in prevalence in native species if they act as sink or poor reservoir (dilution, Norman et al. 1999; Ostfeld and Keesing 2000). Invasive species may gain advantage from parasite spillback through direct or apparent competition whereas parasite dilution may benefit native species. Regarding the parasites of introduced species, although the enemy release hypothesis predicts that most of them should be lost during introduction (Keane and Crawley 2002; Torchin et al. 2003), there are many examples of co-introductions (Lymbery et al. 2014). Co-introduced parasites may down-regulate the density of their exotic host through increased mortality and/or reduced fecundity, and modulate their predation rate or competitiveness through behavioural changes. Co-introduced parasites may also become invasive when they spread among native species (spillover, Power and Mitchell 2004; Strauss et al. 2012) and act as biological weapons, facilitating invasion and amplifying impact via apparent competition.

Due to their high connectivity and the development of commercial and recreational shipping, freshwater ecosystems are highly vulnerable to biological invasions (Sala et al. 2000; Carpenter et al. 2011). Many Ponto-Caspian fish, molluscs and crustaceans are successful invaders in Europe and North America (Ojaveer et al. 2002). Among them, the amphipod *Dikerogammarus villosus* (Sowinski 1894) is regarded as one of the 100 worst invasive species in Europe according to the experts of the Delivering Alien Invasive Species in Europe (DAISIE) project (see www.europe-aliens.org). It has colonized most of the water bodies of continental Europe in less than 20 years after the opening of the Rhine-Main-Danube canal in 1992 (see Rewicz et al. 2014 and references therein for a detailed invasion history), was reported in several localities across the United Kingdom in September 2010 (MacNeil et al. 2010), and is predicted to invade the North American Great Lakes (U.S. EPA 2008). Invaded communities are often extensively restructured with the extirpation of native invertebrates (Dick and Platvoet 2000; van der Velde et al. 2000; MacNeil et al. 2013), which can lead to changes in ecosystem-level processes like leaf-litter processing (MacNeil et al. 2011; Piscart et al. 2011; Truhlar et al. 2014). The ecological impacts of *D. villosus* are often attributed to its predatory behaviour, leading it to be referred to as the "killer shrimp". Indeed, marked predation on various animal food sources (conspecifics, other amphipods, isopods, insect larvae, juvenile crayfish, fish eggs and small fish) has been reported from laboratory studies (Dick et al. 2002; Krisp and Maier 2005; MacNeil and Platvoet 2005; Casellato et al. 2007; Buřič et al. 2009) and the high trophic position of *D. villosus* has been partially confirmed in the field (Van Riel et al. 2006; Bacela-Spychalska and Van der Velde 2013, but see Hellmann et al. 2015; Koester et al. 2016).

In many populations all over Europe, *Dikerogammarus villosus* is found infected with the microsporidian parasite *Cucumispora dikerogammari*, whose prevalence can reach 72% (Wattier et al. 2007; Ovcharenko et al. 2010). This co-introduced parasite infects muscles and is transmitted mainly horizontally during the consumption of infected conspecifics (Bacela-Spychalska et al. 2012). Spillover from *D. villosus* to native amphipods has been observed in the laboratory, but is expected to be limited in the field to ecological situations of high prevalence and where *D. villosus* is not the dominant species of the assemblage. Furthermore, *D. villosus* being a high-rank predator, it is unlikely to be preyed upon by other amphipods (Bacela-Spychalska et al. 2012). Although *D. villosus* show reduced survival in the later stages of infection, infected females reproduce earlier as compensatory

response, which could reduce the population-dynamic effect of *C. dikerogammari* (Bacela-Spychalska et al. 2012). *D. villosus* at a late stage of infection (i.e. with visible signs of infection) also display reduced predation compared to healthy individuals, while those at an early stage of infection (i.e. asymptomatic individuals) show an intermediate behaviour (Bacela-Spychalska et al. 2014). Infection might thus limit the predation pressure of *D. villosus* and hence its ecological impact on lower trophic level species.

Our aim was to investigate further the effect of *C. dikerogammari* infection on the ecological impact of *D. villosus*. For this, we used the functional response (FR), namely the relationship between resource use and availability (Solomon 1949). Typically, the *per capita* consumption rate of a predator increases with prey density and the relationship can be linear (type I), decelerating with an asymptote at higher prey densities (type II), or S-shaped with acceleration first at low densities and then deceleration towards satiation (type III, Holling 1959a). The decrease of *per-capita* predation risk with prey density in type II FRs can lead to unstable boom-burst population dynamics whereas the S-shape of type III FRs offers low-density refugia for prey, which prevents such unstable dynamics (Murdoch and Oaten 1975; Juliano 2001; Gentleman and Neuheimer 2008; Kalinkat et al. 2013). Comparative FRs may provide useful insights on the ecological impact of invasive predators on local prey populations, strong impacts being expected if they display a higher (i.e. rising more steeply and/or reaching a higher asymptote) or a more destabilizing FR (i.e. type II instead of type III) than the trophically analogous natives they (may) replace (Dick et al. 2014). The FR of *D. villosus* was found to be of type II and to rise more rapidly to a higher asymptote than that of other gammarids, which corroborates its known ecological impacts (Bollache et al. 2008; Dodd et al. 2014; Bovy et al. 2015). We wanted to know whether the presence of *C. dikerogammari* may alter this difference.

We compared the FR of *D. villosus* for either healthy or for naturally *C. dikerogammari*-infected individuals to that of two non-invasive amphipods: *Gammarus pulex* and *Echinogammarus berilloni*. We also tested whether infection shaped the FR of *D. villosus*. Based on the previous FR investigations on *D. villosus* and other gammarids (Bollache et al. 2008; Dick et al. 2010; Kestrup et al. 2011; Médoc et al. 2013; Dodd et al. 2014), a type-II FR was expected for the three amphipod species with a curve rising more steeply and/or reaching a higher asymptote for uninfected *D. villosus*. In addition, following the results of Bacela-Spychalska et al. (2014), we expected a negative effect of *C. dikerogammari* infection on the *per capita* predation rate of *D. villosus*, which might shape its FR and alter the between-species difference in FR.

Materials and methods

Sampling and maintenance of the gammarids

In March–April 2015, we collected the three amphipod species from the Seine river (Seine et Marne, 48°38′N and 2°79′E), where they coexist. Infection with *C. dikerogammari* was confirmed by PCR-based detection (see Wattier et al. 2007 for further details) with a prevalence around 25% in *D. villosus*. We did not look for the presence of *C. dikerogammari* in *G. pulex* and *E. berilloni* as spillover from *D. villosus* is very unlikely at this level of prevalence and also because *D. villosus* is not the dominant gammarid species at the study site (Bacela-Spychalska et al. 2012; Grabner et al. 2015). To avoid sex effect and reduce within-species variability in body length, we selected large-sized males (approximately 17, 14 and 13 mm in total length for *D. villosus*, *G. pulex* and *E. berilloni*, respectively). For each gammarid species, we excluded macroparasite-infected individuals in which mature parasitic larvae are visible through the cuticle as macroparasites at this developmental stage can alter gammarids' FR (Dick et al. 2010). At the Foljuif field station (UMS 3194 Ens CNRS CEREEP, Seine et Marne, 48°11′N and 2°14′E), gammarids were kept separately in aerated Seine-river water at 15 °C in a 13L:11D light regime with macrophytes as shelters and conditioned alder leaves (i.e. previously soaked for five days in the Seine-river water to create a surface biofilm) as food source for 5 days before the experiments.

Functional response experiments

During the FR experiments, single gammarids were starved for 24 h to standardise hunger and presented to *Chaoborus* sp. larvae (dipteran, 11 ± 1 mm in total

length) at five densities ($N = 4, 6, 8, 12, 20$, with 24 replicates per density for *D. villosus* and 12 replicates for *G. pulex* and *E. berilloni*) in black plastic dishes (11.5 cm diameter) filled with 200 mL of aerated Seine-river water. *Chaoborus* were collected from a pond of the field station (Seine et Marne, 48°11′N and 2°14′E). We already used *Chaoborus* in previous experiments (Médoc et al. 2013, 2015), allowing comparison of results. Controls were five replicates of each prey density without gammarids. The experiments were run from 9 am to 5 pm along 18 consecutive days during which they were distributed in a quasi-systematic way to avoid time effect. During the course of the experiments, we recorded the number of killed prey and replaced them to avoid prey depletion, new *Chaoborus* being introduced gently with a small plastic pipette so as to minimize disturbance. We recorded the number of killed prey and not the amount of biomass consumed because we were interested in the effect of the predator on the dynamic of the prey population, and not the reverse. Throughout this paper, we indiscriminately use the terms "predation" and "killing" to describe the ecological impact of the gammarid species on the prey population. At the end of the experiments, gammarids were euthanized in warm water, measured for total length to the nearest millimeter and dissected to confirm the absence of macroparasites. *D. villosus* individuals were stored individually in 1.5 mL tubes filed with pure ethanol for subsequent PCR analysis to identify those infected with *C. dikerogammari*. We found an overall prevalence of 33.6%, with 40 infected *D. villosus* over the 119 individuals in which infection has been diagnosed (because of a problem during storage, infection for one of the 24 replicates made with $N = 8$ has not been diagnosed and this replicate was excluded from the analyses). The prevalence ranged from 25 to 47.8% depending of the prey density ($N = 4$: 33.3%, $N = 6$: 25%, $N = 8$: 47.8%, $N = 12$: 29.2%, $N = 20$: 33.3%). We did not look for the presence of microparasites in *G. pulex* and *E. berilloni.* As explained above, spillover of *C. dikerogammari* is very unlikely and although we cannot exclude the presence of some vertically-transmitted microsporidians in *G. pulex* and *E. berilloni,* these parasites are not expected to alter gammarids' predatory behaviour (Dunn and Smith 2001; Terry et al. 2004; Grabner et al. 2015).

Statistical analyses

Statistical analyses were performed using the R software (version 3.2.0, R Development Core Team 2013) and the *pgirmess* package (version 1.6.5). As data did not meet the homoscedasticity assumption, we used a Krukal-Wallis test followed by multiple non-parametric pairwise comparison tests (*kruskalmc* function of the *pgirmess* package) to compare body length between species. A *t* test with heterogeneous variance was used to compare body length between uninfected and infected *D. villosus*. Three prey-dependent FR models (type I, II or III, Table 1) were fitted to the observed killing rates, the non-linear regressions being performed with the *nls* function. Because the residuals did not meet the normality assumption, we used a non-parametric bootstrap method to obtain confidence intervals of the parameter estimates. The candidate models were compared with the second order Akaike criterion (AIC_c). When the difference in AIC_c values between two models (ΔAIC_c) is less or equal to the value of two, then it is generally accepted that the two models perform as well (Nakaya et al. 2005). To test whether *C. dikerogammari* infection alters the between-species difference in FR, we made pairwise FR comparisons between *D. villosus* and either *G. pulex* or *E. berilloni* under two scenarios: in a first one with all *D. villosus* individuals (i.e. both infected and uninfected individuals) and then in a second one with only uninfected *D. villosus*. We also made a FR comparison between uninfected and *C. dikerogammari*-infected *D. villosus*. Because the type-II FR model was the best model for the three species (Table 1), the FR comparisons came down to a comparison of the estimates of its two parameters: the attack rate a (also called searching efficiency), and the handling time h, which is defined as the time during which the predator stops searching for prey after a capture (Holling 1959b) and therefore includes successive stages like prey handling and ingestion. We used a two-step procedure to compare two type-II FRs (between two gammarid species or between uninfected and infected *D. villosus*). In a first step, we performed a Likelihood Ratio Test (LRT) between the most complex model assuming different FR parameter values for the two curves (the 4-parameter model) and the simplest model considering the same parameter values for both curves (the 2-parameter model). If the fitting improvement provided by the

Table 1 Set of the candidate models used to investigate the functional response of the three gammarid species: *Dikerogammarus villosus*, *Gammarus pulex* and *Echinogammarus berilloni* feeding on dipteran larvae

Model	Instantaneous expression	Parameters	References
Type I	$g(N) = aN$	a	Lotka-Volterra
Type II	$g(N) = \frac{aN}{1 + ahN}$	a, h	Holling (1959a)
Type III	$g(N) = \frac{(b + cN)N}{1 + (b + cN)hN}$	b, c, h	Hassell et al. (1977)

g is the rate of prey consumption, N the prey density, a a measure of the searching efficiency, h the handling time, b and c some constants

additional parameters was significant, then we considered the two FRs as significantly different. In that case, a second step consisted in testing whether one parameter of the FR (either a or h) could nevertheless be considered identical for the two FRs by comparing (with LRTs) the fitting of the 4-parameter model with the fitting of the two 3-parameter models: the one with a common value for attack rate (a) and two distinct values for handling time (h), and the other one with a common value for h and two distinct values for a.

In the second scenario (with uninfected *D. villosus* only), removing the 40 infected *D. villosus* from the whole dataset also reduced sample size. Consequently, a difference in the results between the two scenarios (with and without infected *D. villosus* in the FR comparison) could be the artefact of sample size reduction rather than being due to parasitism. To test this effect in the case where the results for a given FR comparison (*D. villosus* vs. *G. pulex* or *D. villosus* vs. *E. berilloni*) differ between the two scenarios, we randomly removed 40 individuals from the whole dataset in the proportions (to the nearest integer) of uninfected and infected *D. villosus* we had per prey density ($N = 4$: 5 uninfected and 3 infected individuals removed, $N = 6$:6 and 2, $N = 8$:4 and 4, $N = 12$:6 and 2, $N = 20$:5 and 3) and we applied the first step of the model selection analysis described above (i.e. a comparison between the 2-parameter and the 4-parameter models). We repeated the whole procedure 1000 times.

Results

The three gammarid species significantly differed in their body length (Kruskal–Wallis test, $\chi^2 = 178.59$, ddl $= 2$, $p < 10^{-5}$; Fig. 1). *D. villosus* was significantly larger than both *E. berilloni* and *G. pulex* (pairwise comparison tests, both $p < 0.01$) and *G. pulex* was significantly larger than *E. berilloni* (pairwise comparison test, $p < 0.05$). However there was no difference in size between uninfected and infected *D. villosus* (t test, $p = 0.34$).

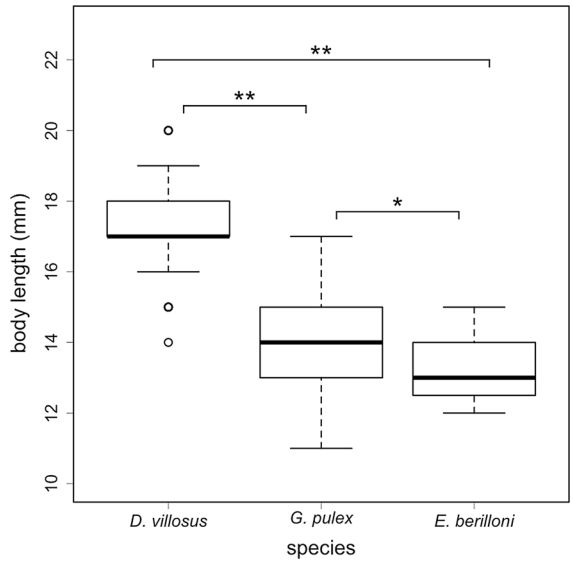


Fig. 1 Body length (median and interquartile range) of the *Dikerogammarus villosus* ($N = 119$), *Gammarus pulex* ($N = 60$) and *Echinogammarus berilloni* ($N = 60$) individuals used in the functional response experiments. * and ** a significant difference with respectively $p < 0.05$ and $p < 0.01$

No mortality was observed in control groups, suggesting that death of the *Chaoborus* larvae during the FR experiments was due to gammarid predation.

For all the amphipod species, the lowest AIC_c values were obtained with the type II FR model (Table 2). However, the type I FR model for *G. pulex* and the type III FR model for *D. villosus* performed as well as the type II FR model as suggested by the

corresponding ΔAIC_c values, which were below the threshold of two (Table 2). In the previous FR experiments on gammarids carried out in similar conditions (i.e. no shelter provided), the FR was always found to be of type II (Bollache et al. 2008; Dick et al. 2010; Kestrup et al. 2011; Médoc et al. 2013; Dodd et al. 2014). It is therefore reasonable to assume that the type II FR is also the best model to describe gammarid predation in the present study (Figs. 2, 3, 4). Parameter estimates with 95% confidence intervals for the type II FR model are given in Table 3.

When comparing *G. pulex* and *E. berilloni* with all *D. villosus* individuals (Figs. 2a, 3a), the model selection procedure indicated significantly different FRs because of a significant fitting improvement between the 2-parameter model and the 4-parameter models (Figs. 2c, 3c). In both cases, the 4-parameter model did not fit significantly better than the two 3-parameter models, suggesting that one of the two FR parameters could nevertheless be considered identical between *D. villosus* and the other species (Figs. 2c, 3c). However, for both comparisons (*D. villosus* vs. *G. pulex* and *D. villosus* vs. *E. berilloni*) and because of the very close AIC_c values of the two 3-parameter models, we were not able to discriminate between these two models and therefore to known which FR parameter could be considered identical (Figs. 2e, f, 3e, f). Anyway, all the selected models indicated a higher killing rate for *D. villosus* at intermediate and high prey densities.

When considering uninfected *D. villosus* individuals only (Fig. 2b), again, the model selection procedure selected indistinctly the two 3-parameter models for the FR comparison with *G. pulex* (Fig. 2d, g, h). By contrast, in the FR comparison with *E. berilloni* (Fig. 3b), the procedure did not detect any significant difference as the additional parameters of the 4-parameter model did not bring any fitting improvement compared to the 2-parameter model (Fig. 3d, g).

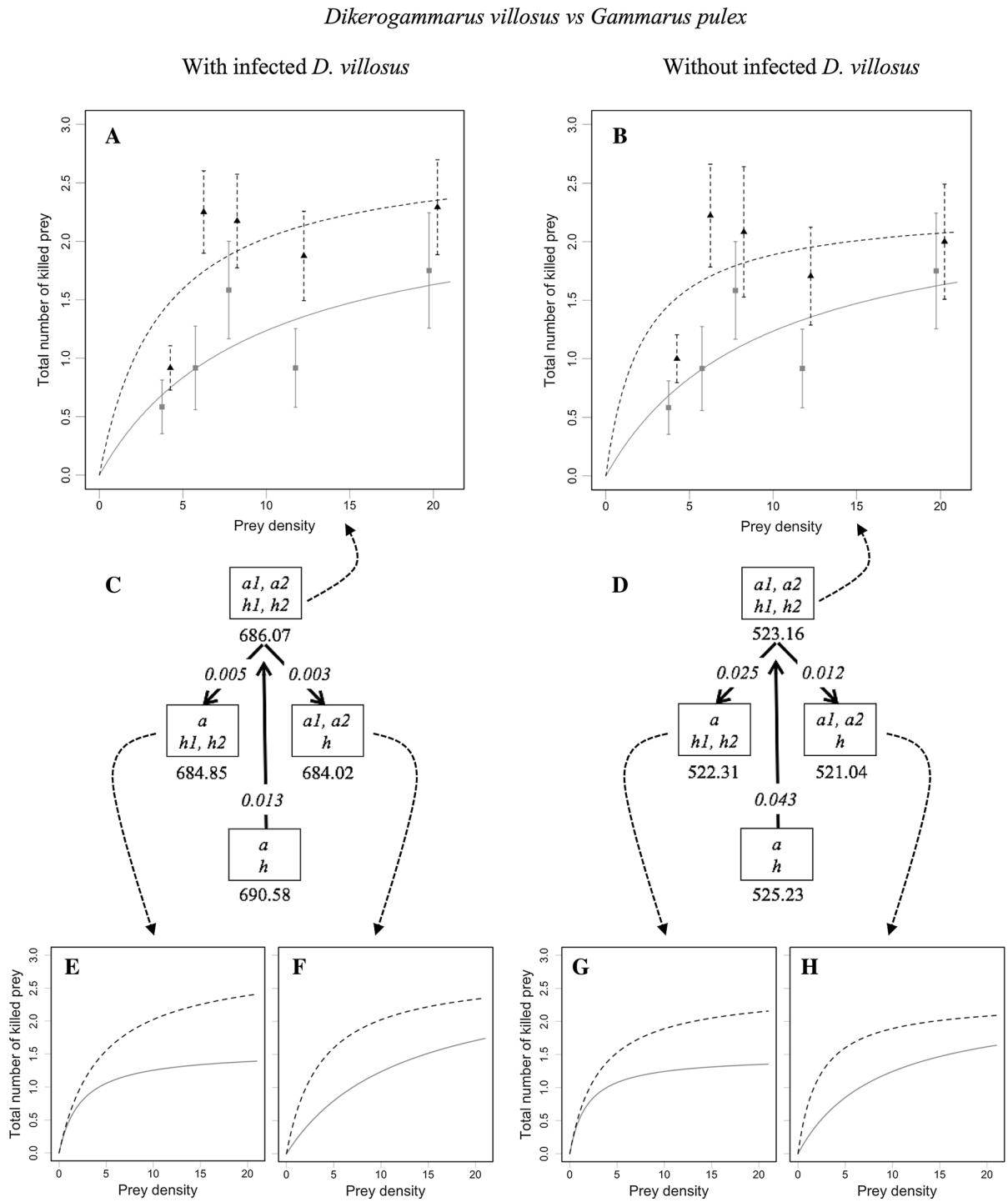
The results of the FR comparison between *D. villosus* and *E. berilloni* differed between the two scenarios (Fig. 3c, e, f vs. Fig. 3d, g). The random removal of 40 *D. villosus* individuals from the whole dataset to test whether this difference could be due to sample-size reduction resulted in the selection of the 4-parameter model over the 2-parameter model in 490 of the 1000 comparisons.

Fig. 2 Functional response (FR) comparison between two gammarid species: the invasive *Dikerogammarus villosus* and the native *Gammarus pulex*, feeding on dipteran larvae. The FR comparison was made under two scenarios: with or without *D. villosus* individuals infected by *Cucumispora dikerogammari* at a natural prevalence in the dataset. **a**, **b** The figurative elements are direct observations of the total number of killed prey (mean ± SD) and the curves are the fits of the "per-species" type II FR models for *D. villosus* (triangles and dashed curve) and *G. pulex* (squares and grey curve). **c**, **d** Results of our model selection procedure comparing the simplest model assuming similar parameter values for both species (the 2-parameter model *a*, *h*) to the most complex model assuming different parameter values for each species (the 4-parameter model *a*1, *a*2, *h*1, *h*2) (first step), and if necessary comparing the most complex model to the two intermediate models assuming similar values for one of the two parameters (the 3-parameter models *a*, *h*1, *h*2 and *a*1, *a*2, *h*) (second step, see text for further details). The numbers below the models are the Akaike criterion (AIC_c) values and the italic numbers on the arrows indicate the *p* values of the likelihood ratio tests. From **e** to **h** FR curves resulting from the model selection procedure, the dashed arrows indicating the corresponding model

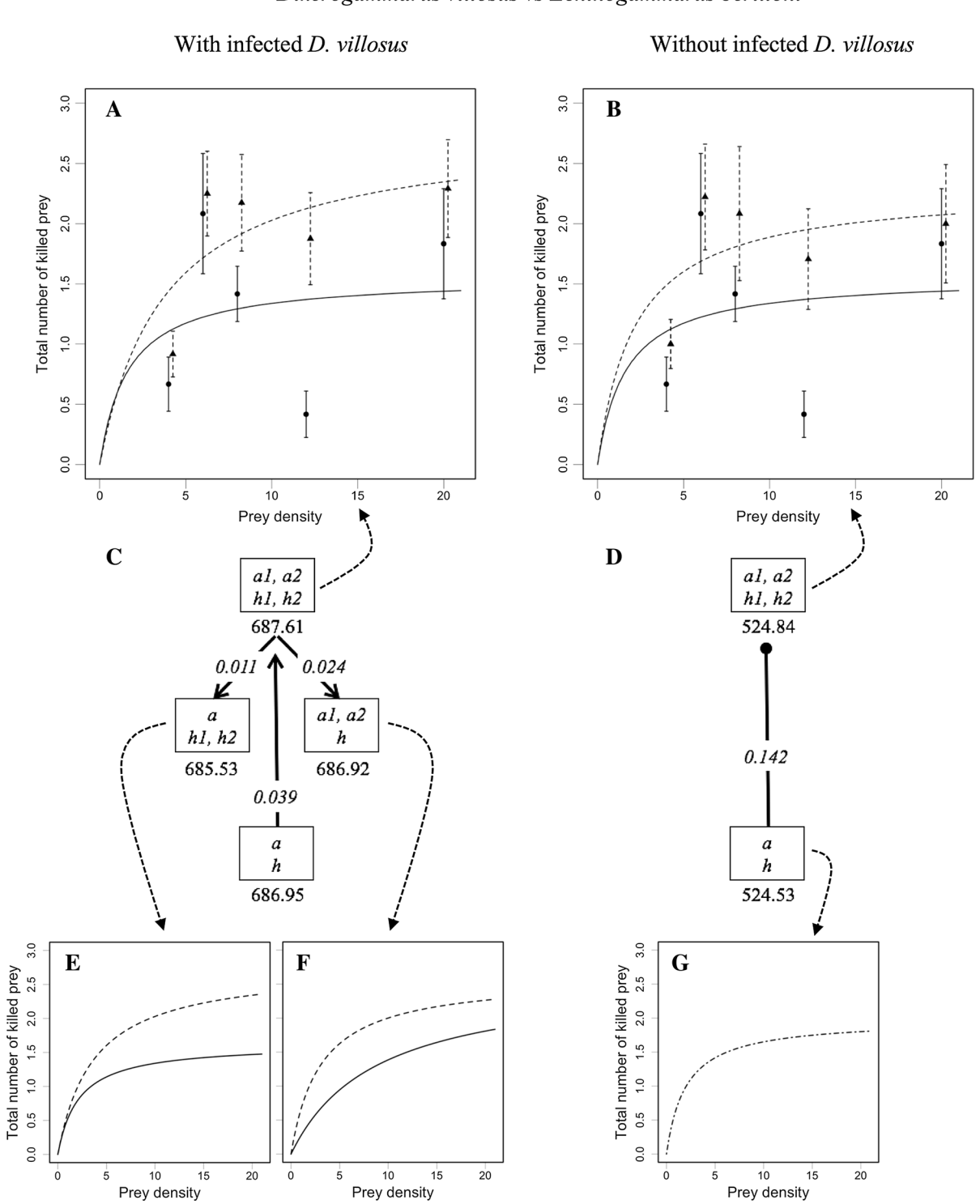
Table 2 Akaike criteria (AIC_c) for the three candidate functional response models and the three gammarid species: *Gammarus pulex*, *Echinogammarus berilloni* and *Dikerogammarus villosus* with uninfected individuals only, *Cucumispora dikerogammari*-infected individuals only, or both

Model	*G. Pulex*	*E. berilloni*	*D. villosus* (uninfected only)	*D. villosus* (infected only)	*D. villosus* (total)
Type I	207.69	215.18	326.61	167.91	492.07
	(1)	(6.15)	(13.04)	(2.46)	(17.21)
Type II	**206.69**	**209.03**	**313.57**	**165.45**	**474.86**
Type III	208.84	211.25	315.08	166.39	475.71
	(2.15)	(2.22)	(1.51)	(0.94)	(0.85)

Values in brackets correspond to ΔAIC_c: the difference between the AIC_c value for the current model and the lowest AIC_c value (in bold) for the given species. See Table 1 for details on the functional response models and their parameters



Although infected *D. villosus* tended to have a smaller attack rate and a smaller handling time than their uninfected counterparts (Table 3), the model selection procedure led to the simplest model with only two parameters (LRT, $p = 0.348$), indicating no significant difference in FR (Fig. 4).


Dikerogammarus villosus vs Echinogammarus berilloni
With infected D. villosus
Without infected D. villosus
A
B
Total number of killed prey
Prey density
C
D
a1, a2
h1, h2
687.61
0.011
0.024
a
h1, h2
685.53
a1, a2
h
686.92
0.039
a
h
686.95
524.84
0.142
524.53
E
F
G

◀**Fig. 3** Functional response (FR) comparison between two gammarid species: the invasive *Dikerogammarus villosus* and the non-invasive *Echinogammarus berilloni*, feeding on dipteran larvae. The FR comparison was made under two scenarios: with or without *D. villosus* individuals infected by *Cucumispora dikerogammari* at a natural prevalence in the dataset. **a**, **b** The figurative elements are direct observations of the total number of killed prey (mean ± SD) and the curves are the fits of the "per-species" type II FR models for *D. villosus* (triangles and dashed curve) and *E. berilloni* (dots and black curve). **c**, **d** Results of our model selection procedure comparing the simplest model assuming similar parameter values for both species (the 2-parameter model *a*, *h*) to the most complex model assuming different parameter values for each species (the 4-parameter model *a*1, *a*2, *h*1, *h*2) (first step), and if necessary comparing the most complex model to the two intermediate models assuming similar values for one of the two parameters (the 3-parameter models *a*, *h*1, *h*2 and *a*1, *a*2, *h*) (second step, see text for further details). The numbers below the models are the Akaike criterion (AIC_c) values and the italic numbers on the arrows indicate the *p* values of the likelihood ratio tests. From **e** to **g** FR curves resulting from the model selection procedure, the dashed arrows indicating the corresponding model

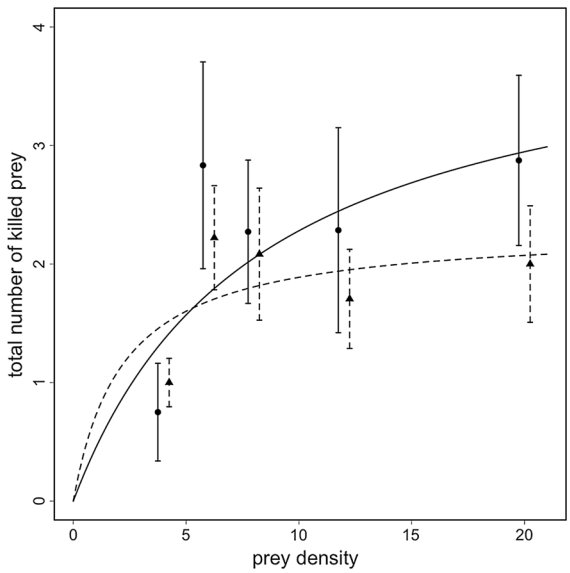


Fig. 4 Relationship between the total number of killed prey and the prey density for the invasive gammarid *Dikerogammarus villosus* feeding on dipteran larvae. The figurative elements are direct observations (mean ± SD) and the curves are the fits of the type II functional response (FR) models for either uninfected individuals (triangles and dashed curve) or individuals infected by *Cucumispora dikerogammari* (dots and solid curve). Because the model selection procedure (not shown) indicated no significant difference between the two FRs, the FR curve that best describes *D. villosus* predation is the one depicted in Figs. 2a and 3a (triangles and dashed curve)

Discussion

Although the enemy release hypothesis predicts the loss of natural enemies during the introduction process (Keane and Crawley 2002), co-introduced parasites are common (Lymbery et al. 2014). As an example, the microsporidian parasite *Cucumispora dikerogammari* has invaded Western Europe along with its gammarid host *Dikerogammarus villosus* (Wattier et al. 2007; Ovcharenko et al. 2010). Because infected gammarids were found to display reduced predation compared to uninfected specimens (Bacela-Spychalska et al. 2014), the presence *C. dikerogammari* may modulate the ecological impact of *D. villosus* in invaded areas. To test this assumption, and also to test whether parasitism may represent a confounding factor when assessing the ecological impact of an invasive species, we compared the functional response (FR) of *D. villosus* infected or not with *C. dikerogammari* with that of two non-invasive gammarids.

As expected and consistently with previous findings on gammarids' FR (Bollache et al. 2008; Dick et al. 2010; Kestrup et al. 2011; Médoc et al. 2013, 2015; Dodd et al. 2014; Bovy et al. 2015), the three gammarid species displayed a type II FR in which predation rate rises to an asymptote as a function of prey density. Compared to the less destabilizing type III FR, the type II FR is common in simple environments with a single prey species and no predator-free space, implying that the predator is efficient even at low prey densities (Barrios-O'Neill et al. 2015).

For both non-invasive gammarid species, we found a significant difference in FR with *D. villosus* when all individuals were included (first scenario). However, as the model selection procedure led indiscriminately to the two 3-parameter models, we cannot know whether the difference in FR is because *D. villosus* displayed a smaller handling time resulting in a FR with a higher asymptote (Figs. 2e, 3e), or a higher attack rate resulting in a FR with a steeper initial slope (Figs. 2f, 3f). From a behavioural perspective, *G. pulex* and *E. berilloni* actively search for their prey whereas *D. villosus* behaves like a sit-and-wait predator, attacking free-swimming animals from a steady position (Platvoet et al. 2009). Attack rate directly depends on predator–prey encounter rate, and therefore on predator's activity level. So, although we did not record activity during our experiments, we may expect that given its hunting strategy, it is quite unlikely for

Table 3 Estimates of the functional response parameters (attack rate *a* and handling time *h*) of the type II model for the three gammarid species: *Gammarus pulex*, *Echinogammarus berilloni* and *Dikerogammarus villosus* with uninfected individuals only, *Cucumispora dikerogammari*-infected individuals only, or both

Functional response parameter	*G. pulex*	*E. berilloni*	*D. villosus* (uninfected only)	*D. villosus* (infected only)	*D. villosus* (total)
a	0.032	0.119	0.132	0.063	0.093
[95% CI]	[0.014; 0.230]	[0.023; 1.42]	[0.038; 1.54]	[0.026; 0.468]	[0.040; 0.660]
h	3.35	5.14	3.48	1.92	2.87
[95% CI]	[0.506; 6.50]	[1.91; 7.07]	[1.74; 4.75]	[0.363; 3.60]	[1.63; 4.12]

95% confidence intervals were obtained by bootstrapping (see the "Materials and methods" section for further detail)

D. villosus to have a higher attack rate than *G. pulex* and *E. berilloni*. From a morphological perspective, *D. villosus* is bigger and therefore potentially stronger, and displays adaptations to a more predatory life style including well-developed gnathopods, second antennae and mouthparts (Mayer et al. 2008; Platvoet et al. 2009; van der Velde et al. 2009). This may facilitate prey handling and ingestion, and thus reduce handling time. So, the 3-parameter model that includes similar attack rates and a smaller handling time for *D. villosus* (Figs. 2e, 3e) probably better explains the FR difference between *D. villosus* and the two non-invasive gammarids. Overall, the higher FR of *D. villosus* compared to *G. pulex* and *E. berilloni* suggests a stronger predatory impact, which corroborates the documented field impacts of *D. villosus* and is consistent with the already published FR comparisons between *D. villosus* and other gammarids (Bollache et al. 2008; Dodd et al. 2014; Bovy et al. 2015, but see Médoc et al. 2015 for predation tests with multiple predators).

C. dikerogammari-infected *D. villosus* displayed smaller attack rate and handling time than uninfected *D. villosus*, although this difference was not statistically significant probably because of a too small sample size. The two FR curves crossed over because the higher attack rate of uninfected *D. villosus* resulted in a steeper initial slope whereas their higher handling time resulted in a lower asymptote (Fig. 4). This suggests that the difference in predation pressure between uninfected and infected *D. villosus* might vary with prey density, with uninfected individuals being more predatory in low prey densities and vice versa for high prey densities. A decrease in both attack rate and handling time induced by infection may lead to antagonistic predictions in terms of predatory impact, but we can try to find an explanation based on infection costs. Parasites often rely on hosts' resources, which imposes a metabolic cost that can be compensated for by increased foraging. This has been advanced to explain why infection with the acanthocephalan parasite *Echinorhynchus truttae* increased the FR of *G. pulex* (Dick et al. 2010; Laverty et al. 2017). In our study, the predation rate of infected *D. villosus* might have saturated at higher prey densities compared to uninfected individuals to cope with the parasite-induced increase in metabolic demand, which would have resulted in a FR reaching a higher asymptote. Handling time being the inverse of the asymptote, this could explain why *C. dikerogammari* infection tended to decrease the handling time of *D. villosus*. To investigate further the metabolic cost induced by infection, it would be interesting to record the biomass of prey consumed in addition to the number of killed prey and to compare the FRs obtained with both metrics for infected and uninfected *D. villosus*. A parasite-induced increase in the metabolic demand could increase the biomass of prey consumed without necessarily changing the number of killed prey. At low prey densities, a smaller attack rate might reflect the negative effect of *C. dikerogammari* infection on the overall performance of *D. villosus*, resulting in a reduced capacity to find prey in poor environments. Additional FR experiments with more infected individuals and behavioural measurements could help understand our results.

Using classical predation tests (i.e. with a single prey density) with live chironomid larvae, Bacela-Spychalska et al. (2014) found that *C. dikerogammari* infection made *D. villosus* less predatory. This reduction in predation rate was significant for symptomatic individuals (i.e. at a stage of infection close to septicaemia) but not for asymptomatic individuals (i.e. at an early stage of infection). In the present study,

infected *D. villosus* were mostly asymptomatic and a PCR analysis was needed to identify infection. Our results therefore provide precision to those of Bacela-Spychalska et al. (2014) involving asymptomatic amphipods. The way the FR curves of uninfected and *C. dikerogammari*-infected *D. villosus* intersect (Fig. 4) suggests that infection tends to decrease predation towards low prey densities and to increase predation towards high prey densities. Such a shift in predation rate was not detected by Bacela-Spychalska et al. (2014), which illustrates the usefulness of the FR approach compared to predation tests with a single prey density. However, the use of different prey species makes it difficult to go further in the comparison of results between our study and that of Bacela-Spychalska et al. (2014).

Accounting for parasitism through the removal of infected *D. villosus* individuals from the dataset (second scenario) did not alter the result of the FR comparison with *G. pulex* (Fig. 2c, e, f vs. Fig. 2d, g, h), although this tended to reduce the magnitude of the difference between the two FR curves (Fig. 2a vs. Fig. 2b). As in the first scenario, the model selection procedure led indiscriminately to the two 3-parameter models. On the contrary, with *E. berilloni*, removing infected *D. villosus* suppressed the difference in FR that we found in the first scenario (Fig. 3c, e, f), the simplest FR model with similar values of attack rate and handling time being the one chosen by the model selection procedure (Fig. 3d, g). This is consistent with the tendency of *C. dikerogammari* to decrease attack rate and handling time that we found when comparing the FR of infected and uninfected *D. villosus*. Removing infected individuals from the comparison probably slightly increased the overall handling time of *D. villosus* and caused its FR to saturate to a lower asymptote, which might have suppressed the significant difference in FR. However the random removal of *D. villosus* individuals from the dataset led to the 2-parameter FR model in 510 over the 1000 comparisons that we made. It follows that the between-scenario difference in the results of the FR comparison involving *D. villosus* and *E. berilloni* was not necessarily due to parasitism but might result from sample size reduction.

C. dikerogammari was found in most of the introduced *D. villosus* populations all over Europe with low prevalences in recently-colonized sites, high prevalences sometimes reaching peaks of 72% in sites sampled three to five years after initial colonization, and medium prevalences in sites colonized for a long time (Wattier et al. 2007). Our results on the FR comparison between *D. villosus* and *E. berilloni* illustrate how this co-introduced parasite might mediate the ecological impact of its invasive host, more particularly in a rich environment (i.e. at high prey densities when the predator saturates). Indeed, *D. villosus* displayed a significantly higher FR, suggesting a stronger predatory impact, only in the presence of *C. dikerogammari*. It follows that the predation impact of *D. villosus* might be lower in uninfected populations, or at the beginning of the invasion process when the prevalence of *C. dikerogammari* is low. Infection might increase the predation pressure on local prey populations in case of invasion with species replacement between *E. berilloni* and *D. villosus*. Our investigation also suggests that what can be inferred from a given pairwise FR comparison cannot be extrapolated to other species, as *C. dikerogammari* infection did not change the results of the FR comparison between *D. villosus* and *G. pulex*. In addition to parasitism, other environmental factors likely to shape FR differences such as predator density, habitat complexity, super-predation (reviewed in Médoc and Spataro 2015) must be taken into account to fully characterize the predation impact of an invasive species.

In terms of impact prediction, our study illustrates how ignoring parasitism might alter our ability to predict invasive species impact based on FR comparisons. This is an issue for the parasites whose detection needs molecular investigations and it would be interesting to known to what extent *C. dikerogammari* infection contributes to the results of the previously published FR comparisons involving *D. villosus* (Bollache et al. 2008; Dodd et al. 2014; Bovy et al. 2015; Médoc et al. 2015).

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