Parasitol Res (2011) 108:1501–1506
DOI 10.1007/s00436-010-2200-8

ORIGINAL PAPER

The European eel—the swim bladder–nematode system provides a new view of the invasion paradox

Carlos Martínez-Carrasco · Emmanuel Serrano · Rocio Ruiz de Ybáñez · José Peñalver · José Antonio García · Alfonsa García-Ayala · Sergé Morand · Pilar Muñoz

Received: 28 October 2010 / Accepted: 26 November 2010 / Published online: 24 December 2010
© Springer-Verlag 2010

Abstract It is widely assumed that the likelihood of invasion decreases with increased species richness in the recipient community. However, the invasion paradox supports a negative and a positive relationship between native biodiversity and the success of an invader. Here, we show that for a host–parasite system (*Anguilla anguilla* as host and *Anguillicoloides crassus* as parasitic invader), invasion increases with native micro- and macroparasitic species richness. In fact, about 30% of the *A. crassus* intensity in eels could be explained by the number of both micro- and macroparasite species. This pattern could be due to the fact that *A. crassus* exploits a niche (the swim bladder) that is unoccupied by native parasite species and by the Th1/Th2 trade-off between native microparasites and the invader. We conclude that the host–parasite system resistance to invasion may depend on both niche availability and the Th1/Th2 trade-off. As well, we encourage researchers to incorporate native parasite richness as a risk factor in epidemiological models of *A. crassus*.

Carlos Martínez-Carrasco and Emmanuel Serrano contributed equally to this work.

C. Martínez-Carrasco · R. R. de Ybáñez · P. Muñoz (✉)
Departamento de Sanidad Animal, Facultad de Veterinaria,
Universidad de Murcia,
E-30100 Murcia, Spain
e-mail: pilarmun@um.es

E. Serrano
Servei d'Ecopatologia de Fauna Salvatge (SEFaS),
Departament de Medicina i Cirurgia Animals,
Universitat Autònoma de Barcelona,
Barcelona, Spain

J. Peñalver
Dirección General de Ganadería y Pesca,
Comunidad Autónoma de la Región de Murcia,
Murcia, Spain

J. Antonio García
Departamento de Sanidad Animal,
Universidad Complutense de Madrid,
Madrid, Spain

A. García-Ayala
Departamento de Biología Celular e Histología,
Universidad de Murcia,
Murcia, Spain

S. Morand
Institut des Sciences de l'Evolution, Université de Montpellier 2,
Montpellier, France

Introduction

Biological invasions have a dramatic impact on global biodiversity (Rosenzweig 2001). It is generally accepted that species-rich communities are less susceptible to invasion (Case 1990) given that they offer fewer niche opportunities (e.g. resources and space), thereby reducing both the establishment possibilities and the success of invading species (Shea and Chesson 2002). The invasion paradox (Fridley et al. 2007) claims that positive associations between native and exotic species richness often occurs, especially at broad spatial scales, whereas negative associations occur at fine scales. However, the observation that fine-scale native–exotic richness relationships are negative does not hold as a simple and accurate generalisation based on current evidence (Fridley et al. 2007), and positive associations have been reported at fine scales in marine invertebrate communities (Dunstan and Johnson 2004; Stachowicz and Byrnes 2006).

In natural conditions, multiple simultaneous parasite infections are the norm in wildlife. In fact, a host species can be understood as a complete ecosystem with various niches (e.g. organs) exploited by different species of parasites controlled by

a single immune system (Pérez et al. 2006). Despite the fact that parasites also form complex communities, e.g. infracommunities (Poulin 2007) that are likely to modulate the establishment of parasite invaders, the relationship between these parasite communities and their role in resisting parasite invasion still remain unexplored.

Species in free-living communities differ from those in parasite communities in ways that influence their resistance to invasion; for example, invaders in free-living communities can be controlled by different kinds of predators, whereas, within a host, all parasite species are under the control of the same immune system (Pedersen and Fenton 2007). However, an infected host responds to infection in two main ways (depending on the parasite type) and mobilises T helper type 1 (Th1) responses against microparasites (viruses, bacteria, fungi and protozoa) or Th2 responses against macroparasites (myxosporeans, helminths and arthropods). These two responses may be antagonistic; in fact, one macroparasite species may stimulate a strong Th2 response and leads to a downregulation of the Th1 response (Graham 2008). This Th1/Th2 trade-off obliges hosts to choose how to allocate potentially limited resources and influences the course of a multiple micro/macroparasite co-infection (Fenton et al. 2008). A parasite invasion framework provides an exceptional opportunity for testing the invasion paradox at a fine scale (individual), that is, whether or not the composition of the host's native parasite community influences the success of a parasite invasion.

The swim bladder (hereafter, sb) nematode *Anguillicoloides crassus* (hereafter, Sbn), formerly known as *Anguillicola crassus*, is regarded as an excellent and successful model of a parasite invader since it has colonised three continents without the previous naturalisation of its natural host, *Anguilla japonica* (Rahhou et al. 2005; Taraschewski 2006). Despite the fact that Sbn is well tolerated by its natural host, the presence of Sbn in the sb lumen of the European eel (*Anguilla anguilla*, its new host in European ecosystems) results in inflammation, fibrosis and necrosis, which can lead to the impaired functioning or even total degeneration of this organ (Würtz and Taraschewski 2000) although few studies have investigated the immune responses of eels infected by Sbn (but see Fazio et al. 2009). In fact, Sbn infection has been identified as one of the major threats to *A. anguilla*, a vertebrate that has recently been classified as Critically Endangered by the IUCN (Freyhof and Kottelat 2008).

In this work, we aim to test whether or not the native adapted parasite diversity within the host protects against parasite invasion. Specifically, our objective was to test the following hypothesis: in several natural systems, native species richness acts as a barrier to invasions (Shea and Chesson 2002), and so, hosts (*A. anguilla*) harbouring a species-rich infracommunity may be less susceptible to Sbn infection. However, according to the Th1/Th2 trade-off, the success of a parasite invasion may well depend on the parasite type. Hence, eels showing high microparasite richness could be more susceptible to Sbn infection (alternative hypothesis).

Materials and methods

Study site and sampling procedure

In November 2008, 48 eels were collected from L'Albufera de Valencia (39° 20′ N, 0° 20′ W), an oligohaline (up to 2 g/l) lagoon situated in the western Mediterranean (Valencia, Spain). Eels were killed with an overdose of anaesthetic, weighed (to the nearest 0.01 g) and measured (to the nearest 0.1 cm). Skin scrapes and gill wet mounts were obtained. Kidney and spleen samples were obtained for bacteriological studies. Gill and intestine samples were fixed in 10% neutral buffered formalin. The remains of the intestinal tract were stored at −20°C until examination. The sb was examined macroscopically for the presence of adult Sbn in the lumen and then frozen for subsequent examination of Sbn larval stages located in its wall.

Parasite sampling procedure

Skin scrapes and gill wet mounts were microscopically examined to identify external parasites. Spleen and head kidney samples were cultured in Tryptone Soya Agar and Thiosulfate Citrate Bile Sucrose Agar for bacteriological analysis following standard procedures. Fixed tissues were embedded in paraffin blocks, cut and stained following standard procedures and observed microscopically. Intestinal mucosa and enteric contents were thawed and examined under a stereomicroscope. Parasites were stored in 70% ethanol and stained with Semichon's carmine for morphometric identification (Schmidt 1986).

Sbn parasites, removed during the macroscopic observation, were preserved in 70% ethanol and later examined under a light microscope for sex determination according to Moravec and Taraschewski (1988). For larval detection in the Sb wall, Sbs were thawed, digested at 40°C for 1 h and gentle shaken in freshly prepared 1.5% (*w*/*v*) pepsin (1:10,000 activity) and 1.5% (*w*/*v*) clorhidric acid in distilled water. Digested material was washed by centrifugation at 500×*g* for 5 min. The number of Sbn larval stages was counted in a Favatti chamber by microscopy, based on morphometric criteria (Rolbiecki 2008).

Statistical analysis

Prevalence, mean intensity and range of both micro- and macroparasite species in the eels were estimated according

to Bush et al. (1997). Using only the Sbn-infected eels, we explored whether or not the native macro- and microparasite species richness (that is, the number of parasite species in an individual host, an excellent proxy of infracommunity structure; see Bordes and Morand 2009) in the captured eels influenced the success of Sbn infection ('intensity of parasitation', number of specimens of a species of parasite in a single host). We fitted a set of generalised linear models (with a Poisson-linked error structure) in which the intensity of parasitation (considered as the total number of both adult and L4 larvae in the sb) was explained by the single effect of macroparasite (represented by cestodes and Myxozoa in our study case, see Table 1) and microparasite (represented by Coccidea and Bacteriae) species richness and their sum (Table 2). Due to the fact that the age of the eels was unknown and to avoid the potential confounding effect of age on the intensity of parasitation, we checked the relationship between body length (proxy of age) and both macro- and microparasite intensities of parasitation.

The selection of the described models was performed under an information-theoretical approach using the Akaike Information Criterion corrected for small sample sizes (AICc). Briefly, for each of the candidate models,

Table 1 Macro- and microparasite prevalence and intensity in Sbn-infected (I; n=32) and Sbn-uninfected (U; n=16) eels from L'Albufera de Valencia, Spain

Parasite species	Eel type	Prevalence (%)	Mean intensity±SE	Range
Microparasites				
Coccidea				
Eimeria anguillae	U	0	–	–
	I	6.25 (n=2)	–	–
Bacteriae			–	–
Micrococcus spp.	H	6.25 (n=1)	–	–
	I	3.12 (n=1)	–	–
Rhodococcus spp.	U	6.25 (n=1)	–	–
	I	6.25 (n=2)	–	–
Kocuria spp.	U	0	–	–
	I	6.25 (n=2)	–	–
Edwarsiella tarda	U	12.5 (n=2)	–	–
	I	3.12 (n=1)	–	–
Vibrio alginolyticus	U	6.25 (n=1)	–	–
	I	3.25 (n=1)	–	–
V. vulnificus	U	18.75 (n=3)	–	–
	I	0	–	–
Pseudomonas spp.	U	12.5 (n=2)	–	–
	I	6.25 (n=2)	–	–
Aeromonas movil	U	62.5 (n=10)	–	–
	I	40.62 (n=13)	–	–
Macroparasites				
Myxozoa				
Myxidium giardi	U	68.7 (n=11)	–	–
	I	81.2 (n=26)	–	–
Nematodes				
A. crassus	U	0	–	–
	I	100	4.35±5.87	1–33
Cestodes				
Bothriocephalus claviceps	U	0		
	I	9.37 (n=3)	1±0	1
Proteocephalus macrocephalus	U	0		
	I	6.25 (n=2)	1±0	1

Table 2 Model selection for the effects of the native parasite community on the success of Sbn infection (n=32) in eels from L'Albufera de Valencia, Spain

Models for explaining the Sbn success of infection	*K*	AICc	Δi	wi
Microparasite species richness+macroparasite species richness	3	213.85	0	1
Microparasite species richness	2	227.87	14.02	0
Macroparasite species richness	2	252.67	38.81	0
Mo	1	260.59	46.74	0

Selected models are in bold

K number of estimated parameters, *AICc* Akaike's Information Criterion corrected for small sample size, *Δi* difference of AICc between each model and the most parsimonious one, *Wi* Akaike's weight of the model, *Mo* the null model

we estimated the AICc, selecting the model with the lowest AICc value. We then ranked the remaining competing models according to their AICc values and subsequently estimated their Akaike differences (Δi) with respect to the best model (lowest AICc) and the Akaike weight (wi) of each model (Burnham and Anderson 2002). Finally, to rule out the possibility that changes in microparasite species richness (Coccidea and Bacteriae) were due to a secondary infection in the Sbn-infected eels, we performed a non-parametric comparison (Mann–Whitney *U* test) of microparasite species richness between the Sbn-infected and non-infected eels. All statistical analyses were performed using R software version 2.10.1 (R Development Core Team 2009).

Results

Table 1 summarises the parasite diversity found in our sample. We identified a single species of both Myxozoa and Coccidea, eight bacteria species and two species of cestodes. Sbn prevalence was 66.6%, with a mean intensity of 4.35 (range, 1–33); two and three were the maximum number of micro- and macroparasites found in a Sbn-infested eel, respectively. No individual had more than four parasite species.

The lack of relationship between body length and both micro- (β=0.001, SE=0.002, p>0.05) and macroparasite intensity (β=0.008, SE=0.02, p>0.05) suggests two things; first, eels from our sample were in the same range of age or, second, that older eels from our sample were not more parasitized than the young ones. On the other hand, micro- and macroparasite diversities were the main factors explaining Sbn intensity and also explained 30.2% of the observed variability in the intensity of Sbn infection ($wi_{\text{microparasite species richness+macroparasite species richness}}$=1, $\beta_{\text{microparasite species richness}}$=0.72, SE=0.11; $\beta_{\text{macroparasite species richness}}$=0.52, SE=0.12; Fig. 1). On the other hand, a post hoc comparison revealed that microparasite species richness in Sbn-infected (median=1, min=0, max=2) and non-infected eels (median=1, min=0, max=2) were similar (U=307, df=1, p=0.238).

Discussion

In this work, we tested the invasion paradox and, above all, the expected negative association between the native parasite species richness and the success of an invasion of a host–

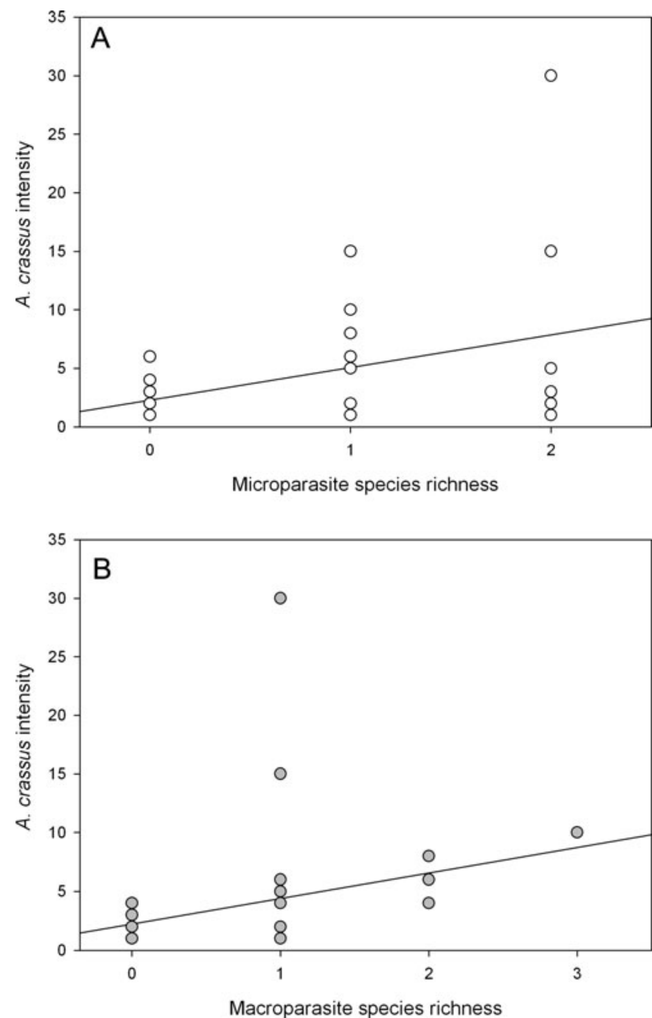

Fig. 1 Relationship between **a** micro- and **b** macroparasite species richness and the number of larvae (L4) and adult Sbn in eels from L'Albufera de Valencia, Spain

parasite system (*A. anguilla–A. crassus*). As indicated by our model selection procedure (and contrary to our main hypothesis), the Sbn intensity was higher in eels having both greater micro- and macroparasite species richness. In theory, within-host parasite interactions are especially intense when species share the same niche (Pedersen and Fenton 2007), a fact that could explain the lack of exclusion between native macroparasites (that infest the digestive tract in the case of Cestoda and the gills in the case of *Myxidium giardi*) and the invasive Sbn (that occupies the sb). In fact, macroparasite communities in eels are typically characterised by their low densities, poor species diversity, high dominance and vacant niches (Kennedy and Guégan 1996; Fazio et al. 2008). On the other hand, the fact that eels with high Sbn intensities had better body conditions (results not shown) suggests that, in eels with the most active feeding habits, there are better opportunities to predate intermediate or paratenic hosts.

Sbn intensities also increased in eels with higher micro-parasite species richness, perhaps due to the typical Th1/Th2 trade-off occurring between micro- and macroparasites in fishes (Joerink et al. 2006, Álvarez-Pellitero 2008) and particularly for *A. anguilla* facing Sbn infection (Fazio et al. 2009). Interestingly, the fact that both Sbn-infected and non-infected eels had similar microparasite richness suggests that, after infection, Sbn maduration is enhanced rather than slowed down in eels infected by several species of microparasites.

Despite the moderate sample size, this study provides evidence of another exception to the negative associations between native and exotic species richness predicted by the invasion paradox. In the European eel–swim bladder nematode system, native community richness can increase the success of an invasion. Further research is required to improve our understanding of the role of infracommunity parasite species richness in the success of parasite invasion in different host–parasite systems. However, given the Th1/Th2 trade-off, we would expect to find a positive association between native microparasite species richness and the success of macroparasite invaders, and vice versa. In addition, we would like to encourage other researchers to consider the use of native parasite diversity as a risk factor in epidemiological studies of the Sbn invasion.

Acknowledgements This work was supported by Fundación Séneca, Coordination Centre for Research (grant 04538/GERM/06). The authors wish to thank E. Romero for his assistance with the eel sampling and D. Riquelme for his technical assistance with parasite processing. E. Serrano is supported by the Juan de la Cierva postdoctoral programme of the MICINN, Spain.

References

Álvarez-Pellitero P (2008) Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Vet Immunol Immunopathol 126:171–198

Bordes F, Morand S (2009) Parasite diversity: an overlooked metric of parasite pressures? Oikos 118:801–806

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: A practical information-theoretic approach. Springer, New York

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Case TJ (1990) Invasion resistance arises in strongly interacting species-rich model competition communities. Proc Natl Acad Sci USA 87:9610–9614

Dunstan PK, Johnson CR (2004) Invasion rates increase with species richness in a marine epibenthic community by two mechanisms. Oecologia 138:285–292

Fazio G, Sasal P, Lecomte-Finiger R, Da Silva C, Fumet B, Moné H (2008) Macroparasite communities in European eels, *Anguilla anguilla*, from French Mediterranean lagoons, with special reference to the invasive species *Anguillicola crassus* and *Pseudodactylogyrus* spp. Knowl Managt Aquatic Ecosyst 1:390–391

Fazio G, Mone H, Da Silva C, Simon-Levert G, Allienne JF, Lecomte-Finiger R, Sasal P (2009) Changes in gene expression in European eels (*Anguilla anguilla*) induced by infection with swim bladder nematodes (*Anguillicola crassus*). J Parasitol 95:808–816

Fenton A, Lamb T, Graham AL (2008) Optimality analysis of Th1/Th2 immune responses during microparasite-macroparasite co-infection, with epidemiological feedbacks. Parasitology 135:841–853

Freyholf J, Kottelat M (2008) *Anguilla anguilla* .IUCN Red List of Threatened Species, version 2010. www.iucnredlist.org, accesed 28/10/2010

Fridley JD, Stachowicz JJ, Naeem S, Sax DF, Seabloom EW, Smith MD, Stohlgren TJ, Tilman D, von Holle B (2007) The invasion paradox: reconciling pattern and process in species invasions. Ecology 88:3–17

Graham AL (2008) Ecological rules governing helminth-microparasite coinfection. Proc Natl Acad Sci USA 105:566–570

Joerink M, Forlenza M, Ribeiro CMS, de Vries BJ, Savelkoul HFJ, Wiegertjes GF (2006) Differential macrophage polarisation during parasitic infections in common carp (*Cyprinus carpio* L.). Fish Shellfish Immunol 21:561–571

Kennedy CR, Guégan J-F (1996) The number of niches in intestinal helminth communities of *Anguilla anguilla*: are there enough spaces for parasites? Parasitology 113:293–302

Moravec F, Taraschewski H (1988) Revision of the genus *Anguillicola* Yamaguti 1935 (Nematoda: Anguillicolidae) of the swimbladder of eels, including descriptors of two new species, *A. novaeze-landiae* sp. N., and *A. papernai* sp. N. Folia Parasitol 35:125–146

Pedersen AB, Fenton A (2007) Emphasizing the ecology in parasite community ecology. Trends Ecol Evol 22:133–139

Pérez JM, Meneguz PG, Dematteis A, Rossi L, Serrano E (2006) Parasites and conservation biology: the "Ibex-ecosystem". Bio-divers Conserv 15:2033–2047

Poulin R (2007) Are there general laws in parasite ecology? Parasitology 134:763–776

R Development Core Team 2.10.1 (2009) A language and environ-ment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org, accessed 28/10/2010

Rahhou I, Morand S, Lecomte-Finiger R, Sasal P (2005) Biogeo-graphical relationships of the eel parasite *Anguillicola crassus* revealed by random amplified polymorphic DNA markers (RAPDS). Bull Fr Pêche Piscic 378–379:87–98

Rolbiecki L (2008) New data on the biology of the introduced exotic nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974

in the eel *Anguilla anguilla* in Lake Wdzydze (Polish waters). Int J Oceanogr Hidrobiol 37:37–48
Rosenzweig ML (2001) The four questions: what does the introduction of exotic species do to diversity? Evol Ecol Res 3:361–367
Schmidt GD (1986) Handbook of tapeworm identification. CRC Press, Boca Raton
Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Trends Ecol Evol 17:170–176
Stachowicz JJ, Byrnes JE (2006) Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. Mar Ecol Prog Ser 311:251–262
Taraschewski H (2006) Hosts and parasites as aliens. J Helminthol 80:99–128
Würtz J, Taraschewski H (2000) Histopatological changes in the swimbladder wall of the European eel *Anguilla anguilla* due to infections with *Anguillicola crassus*. Dis Aquat Org 39:121–134